



Gelatin-structured jelly matrix enriched with black Isabella (*Vitis labrusca* L.) grape concentrate: bioaccessibility, sensory properties, and storage stability

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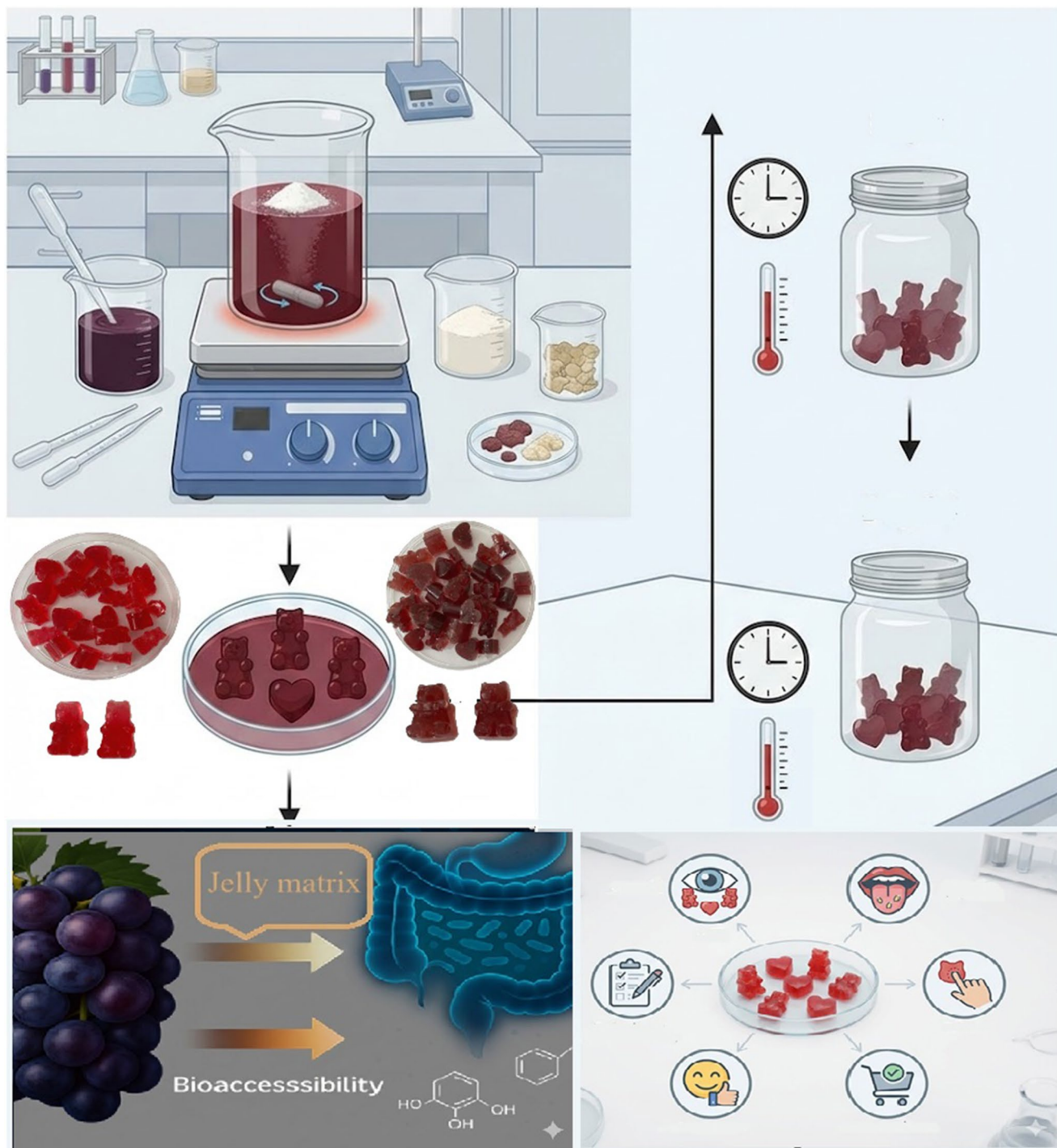
Abstract

Novel grape-derived products stand out as key components in health-oriented product development due to their richness in bioactive compounds. *Vitis labrusca* L. represents an underutilized grape-derived resource with substantial potential for value-added transformation, particularly in the development of nutritionally enhanced gel-based food systems. This study utilized black Isabella grape concentrate in a jelly formulation and assessed total phenolic content (TPC), ferric reducing antioxidant power (FRAP), 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, color, bioaccessibility, microbiological properties, storage stability along with sensory performance. Chlorogenic acid (229.56 µg/g) was the predominant compound in the black Isabella grape concentrate utilized for the jelly formulation, followed by rutin (161.08 µg/g) and trans-cinnamic acid (116.30 µg/g). While the black Isabella grape jelly formulation maintained microbial stability ($<1.0 \times 10^3$ CFU/g) over 30 days, the control sample stored at 25 °C showed visible mold development by day 10. The bioaccessibility of TPC, FRAP, and DPPH in black Isabella grape jelly was 48.61%, 26.28%, and 45.19%, respectively, whereas significantly lower values of 32.86%, 23.28%, and 20.24% were measured in the control sample. Black Isabella jelly exhibited favorable sensory scores and maintained higher overall acceptability throughout storage. These results suggest that black Isabella grape-derived resources exhibit strong potential for application in a shelf-stable, antioxidant-rich jelly matrix.

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Graphical Abstract



Keywords Black Isabella grape · Jelly · Bioaccessibility · Bioactivity · Storage · Sensory

Introduction

The grape of *Vitis labrusca* L. is characterized by its thick purplish-black skin, characteristic aroma, and a distinctive

“foxy” flavor [1]. Foxy is a specific term used in the literature to describe the sweet, slightly musky taste of *Vitis labrusca* grapes, which comes from compounds such as methyl anthranilate, o-aminoacetophenone, and furaneol [2]. This grape,

also known as black Isabella, is cultivated along the coastal regions of the Black Sea. The vines grow organically on host trees, including walnut, elm, mulberry, and maple, without requiring fertilizers or pesticides [3]. Although black Isabella grapes are traditionally consumed in various forms across different countries, including juices, jams, and molasses, and are also utilized in traditional gel-based products such as “pepeçura” in the Black Sea region of Türkiye, their potential as value-added ingredients in modern gel-based food systems remains relatively underexplored. In this context, gel-based confectionery systems may be regarded as modern adaptations of traditional gel-like products. Inspired by traditional grape-based desserts such as “pepeçura”, the present study aims to develop a gelatin-based jelly matrix incorporating *Vitis labrusca* L., thereby reinterpreting this concept within modern gel-based confectionery formulations.

Recent studies have focused on altering jelly candy formulations by substituting sweeteners or honey [4] and incorporating bioactive ingredients from fruits, vegetables, edible plants, and flowers [5]. Various plant-based materials such as carob [6], blueberry, strawberry, blackberry [7], pomegranate juice and aronia puree [8] have been incorporated into jelly systems. Moreover, grape-derived ingredients from different *Vitis* species, including the pomace of Kalecik Karası (*Vitis vinifera* L. cv. Kalecik Karası) [9] and the grape juice of the BRS Violeta hybrid species [5] have also been used in jelly candy formulations.

Previous research has reported that pectin and grape juice powders derived from black Isabella grapes can be used in jelly candy formulations, enhancing the stability of bioactive compounds and functional potential [10]. Jelly candies are structured using various gelling agents such as pectin, starch, carrageenan, and gums; however, gelatin is a primary gelling agent due to its ability to provide a characteristic soft and elastic texture [4, 11]. Despite the widespread use of gelatin in jelly formulations, studies on gelatin-based systems incorporating *Vitis labrusca* L. remain limited. Therefore, this study addresses a notable gap by incorporating black Isabella grape concentrate into a gelatin-based jelly formulation designed to incorporate, protect, and deliver grape-derived phenolic compounds. The developed product was analyzed for its bioactive, physicochemical, and sensory properties, as well as its storage stability and behavior under simulated in vitro digestion.

Materials and methods

Materials

Commercial black Isabella grape juice was purchased from Natuva, a local producer in the Black Sea Region of Türkiye.

Powdered bovine gelatin (CAS No. 9000-70-8, 240 bloom) and water-based liquid food colorants (E122 and E129) were supplied by Gusto Pastry Food Industry Trade Ltd. Co. (İstanbul, Türkiye). Honey was obtained from Balparmak (İstanbul, Türkiye), and sucrose (beet sugar) was purchased from Torku (Konya, Türkiye). Analytical- and laboratory-grade chemicals and solvents were purchased from Sigma Chemical Co. (Munich, Germany). Phosphate-buffered saline (Oxoid, BR0014), pepsin (Sigma, P7000), pancreatin (Sigma, P7545), and bile salts (Sigma, B8631) were employed for in vitro digestion experiments. Culture media used in the microbiological analyses were procured from Merck-Millipore.

Grapefruit juice concentration

The initial soluble solids content of the commercial juice was measured as 11.77 ± 0.06 °Brix. This product was concentrated to 30.00 ± 1.04 °Brix at 50 °C using a rotary evaporator under reduced pressure (600 mmHg) for 100 min. The concentrate was stored at −20 °C for one week prior to its incorporation into the jelly candy formulation.

Jelly candy production with grape juice concentrate

The jelly candy formulation was prepared by modifying the procedures described by Seremet et al. [12] and Ali et al. [13]. Sucrose and gelatin were each dissolved in water heated to 60 °C and stirred separately on a magnetic stirrer for 20 min. The two solutions were then combined and stirred for an additional 5 min. Once the mixture cooled to 40 °C, honey, grape (*Vitis labrusca* L.) concentrate, and citric acid were added sequentially. The formulation (100 mL) comprised 9 g of honey, 22 g of sucrose, 15 g of gelatin, 30 mL of water, 23 g of grape (*Vitis labrusca* L.) concentrate, 1 mL of citric acid (50%, w/w), and 10 µL of water-based food colorant. The control formulation was prepared in the same manner as the test samples, with all components included except the concentrate. The end point of the cooking process was determined using a hand-held refractometer, and the syrup was considered ready when it reached a °Brix value of 61–65%. The resulting syrup was poured into molds to complete the gelatinization process and allowed to set at 25 °C for 18–24 h. After demolding, the samples were stored in hermetically sealed glass jars at two different temperatures, + 4 °C and 25 °C, for 30 days.

Phenolic profile of grape juice concentrate

The pH of the black Isabella grape concentrate was adjusted to 2 using 1 M HCl. It was then subjected to liquid–liquid extraction with diethyl ether at room temperature, using 10

mL and 5 mL volumes for 15 min at 200 rpm. The same procedure was repeated with ethyl acetate, and after each extraction step, the collected organic phases were combined in a pre-weighed flask. The solvent in the organic phase was removed using a rotary evaporator at 40 °C, and the residue in the flask was dissolved in 2 mL of methanol, filtered through a 0.45 µm membrane, and injected into the RP-HPLC-PDA system [14]. RP-HPLC-PDA analysis was carried out using 25 phenolic standards. Calibration curves were obtained at compound-specific wavelengths as follows: ellagic acid, daidzein, meta-hydroxybenzoic acid, rutin, protocatechuic acid, and para-hydroxybenzoic acid (250 nm); chrysin, gallic acid, hesperetin, pinocembrin, syringic acid, epicatechin, and trans-cinnamic acid (280 nm); resveratrol, chlorogenic acid, apigenin, caffeic acid phenethyl ester, para-coumaric acid, caffeic acid, and ferulic acid (320 nm); and curcumin, luteolin, myricetin, kaempferol, and quercetin (360 nm). Chromatographic separation was performed on a reverse-phase C18 column (250 × 4.6 mm, 5 µm; GL Sciences) using a gradient elution system with acetic acid, acetonitrile, and water as the mobile phase components. The mobile phase consisted of solvent A (70:30, v/v, acetonitrile: ultrapure water) and solvent B (ultrapure water containing 2% acetic acid, v/v). The gradient elution program was applied as follows: 5% A and 95% B at 0–3 min; 15% A and 85% B at 8 min; 21% A and 79% B at 10 min; 52% A and 48% B at 20 min; 67% A and 33% B at 35 min; and 90% A and 10% B at 50 min. The HPLC system was then returned to initial conditions (5% A and 95% B) at 50.01 min and equilibrated until 60 min. An injection volume of 20 µL was used, with a flow rate of 1.0 mL/min, while the column temperature was maintained at 30 °C.

Physico-chemical analysis of jelly candies

Jelly candies were dissolved in distilled water (1:5, w/v), and the water-soluble solids were assessed using a handheld refractometer, with values reported in °Brix [15].

A 2.5 ± 0.05 g of the jelly candy was weighed and brought to 25 mL with distilled water. The mixture was dissolved in a water bath maintained at 50 °C with agitation at 240 rpm, and the pH was subsequently measured at 25 °C using a calibrated pH meter [15, 16].

The color determination of the jelly samples was performed using a Konica Minolta CR5 colorimeter, and the results were expressed as L^* , a^* , and b^* values.

Bioactivity assays

The bioactive properties of the jelly candy samples were determined using a modified extraction method given by Ben-Rejeb et al. [17]. 4 g of small pieces of samples were

mixed with 80% methanol at a 1:5 (w/v) ratio. The extraction was performed using a shaking incubator at 37 °C and 100 rpm for 3 h, then the mixture was filtered through Whatman No. 1 filter paper. The filtrate was centrifuged at 9400 g for 30 min, and the resulting supernatant was used for all subsequent bioactivity analyses.

The total phenolic content of the samples was determined using the Folin–Ciocalteu method with gallic acid as the standard [18]. 20 µL of sample, 680 µL of distilled water, and 400 µL of 0.2 N Folin–Ciocalteu reagent were added and mixed thoroughly, followed by incubation under dark conditions for 5 min. Then, 400 µL of 10% Na_2CO_3 solution was added, and the mixture was incubated in the dark for 2 h. After incubation, the absorbance was measured at 760 nm using a spectrophotometer against the concentration. A gallic acid standard calibration curve was constructed using six concentration levels ranging from 0.03125 to 1.0 mg/mL ($R^2 > 0.99$). The results were given as mg GAE (gallic acid equivalents) per g of sample on a fresh weight basis.

A modified version of the method developed by Benzie and Strain [19] was used to assess the ferric reducing antioxidant power (FRAP) analysis of the jelly samples. The principle of the method is based on the reduction of the Fe^{3+} -tripirydyltriazine complex to the blue-colored Fe^{2+} chelate upon the addition of an antioxidant-containing sample to an aqueous solution. The FRAP reagent was freshly prepared daily by mixing 2.5 mL of 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) prepared in 40 mM HCl, 25 mL of 0.3 M acetate buffer solution (pH 3.6), and 2.5 mL of 20 mM FeCl_3 solution. Following, 1.5 mL of FRAP reagent was mixed with 50 µL of sample, incubated at 37 °C for 4 min. The absorbance values were measured at 595 nm. A Trolox standard calibration curve was constructed in the range of 31.25–1000 µM ($R^2 > 0.99$). The results were calculated as µmol Trolox equivalents (µmol TE) per g of sample on a fresh weight basis.

The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity was determined colorimetrically according to the method of Floegel et al. [20] with some modifications. The absorbance of methanolic DPPH solution was adjusted to 0.8 using 80% (v/v) methanol. Then, 50 µL of sample extract or standard were mixed with 1.95 mL of DPPH solution and incubated in the dark for 50 min at room temperature. The absorbance was subsequently measured at 517 nm. The radical scavenging activity of each sample was expressed as micromoles of Trolox equivalents (µmol TE/g) using a Trolox calibration curve constructed over the concentration range of 31.25–1000 µM ($R^2 > 0.99$).

In vitro digestion of jelly candies

The method described by Ozkan et al. [21] and Sessa et al. [22] was used for the in vitro digestion simulation of jelly

candies. 10 g of sample were mixed with 10 mL of PBS (phosphate-buffered saline), incubated at 37 °C for 15 min to ensure proper dispersion of the samples. Since the jelly candy formulation consisted of a starch-free matrix, the addition of salivary α -amylase was omitted from the oral phase simulation. The pH was then adjusted to 2 using 1 M HCl to simulate gastric conditions [23]. Pepsin (727 U/mg) was then added to the mixture to obtain a final concentration of 1.3 mg/mL, followed by incubation at 37 °C and 100 rpm for 2 h. For the intestinal phase, the pH was adjusted to 5.8 with 1 M NaHCO₃, and a mixture of pancreatin (8× USP, United States Pharmacopeia) and bile salts was added to achieve final concentrations of 0.175 mg/mL and 1.1 mg/mL, respectively. After the addition of pancreatin and bile salts, the pH was further adjusted to 6.5 with NaHCO₃. The mixture was then incubated at 37 °C and 100 rpm for 2 h. The digestion process was terminated by placing the samples on ice and immediately centrifuging at 9400 g for 30 min. After intestinal digestion, all jelly candy samples were dissolved, and no residue was observed in the pellet after centrifugation. To eliminate possible interactions arising from the enzymes and buffer solutions used in the digestion procedure, a blank digestion medium was prepared without adding the sample and incubated under the same conditions. Bioactivity analyses were performed on the supernatants obtained after centrifugation. The purpose of the in vitro digestion application was to compare the bioaccessibility of phenolic compounds in the control jelly candies and those enriched with black Isabella grape concentrate, and to reveal the effect of concentrate addition on digestibility. The supernatants were stored at −20 °C until analysis. The bioaccessibility index of the samples was calculated using the following equation:

$$\text{Bioaccessibility index (\%)} = \frac{BC_{\text{post digestion}}}{BC_{\text{initial}}} \times 100$$

where $BC_{\text{post digestion}}$ represents the amount/activity measured in the supernatant obtained after in vitro digestion, and BC_{initial} represents the amount/activity determined before digestion.

Microbiological analysis

The total mesophilic aerobic bacterial and yeast–mold counts of the produced jelly candies were determined by plating appropriate serial dilutions of homogenized samples. 5 g of sample was aseptically homogenized with 45 mL of sterile Ringer solution using a stomacher. Serial decimal dilutions (10^{-1} – 10^{-5}) were prepared, and dilutions were inoculated onto Plate Count Agar (PCA). The total mesophilic aerobic bacterial counts of the produced jelly candies

were determined by counting the colonies on Plate Count Agar (PCA) after incubation at 35 °C for 48 h [24]. Potato Dextrose Agar (PDA) was used to enumerate the yeast and mold colonies, incubated at 25 °C for 3–5 days [25]. The quantification limit (LOQ) of the enumeration method was 1.0×10^3 CFU/g based on the lowest countable number of colonies per plate [24, 25].

Sensory analysis

Each 10 g jelly candy was provided to voluntary 20 semi-trained panelists (students and staff from the Food Processing Department of Maçka Vocational School, aged 18–37 years) and evaluated using a hedonic scale based on appearance, color, texture, aroma–taste, overall liking, and purchase intent [26]. The panelists were selected based on their ability to perceive and differentiate basic taste modalities (sweet, salty, sour, and bitter) at adequate sensitivity levels. Prior to the evaluation, they received a brief orientation on the typical characteristics and potential defects of fruit jelly products [27]. In addition, participants were informed about the procedure and provided their voluntary verbal consent before the sensory test. Samples stored under refrigerated conditions were allowed to equilibrate to room temperature (approximately 22–25 °C) for 30 min prior to evaluation, while samples stored at room temperature were served directly. All samples were presented in identical containers and coded with random three-digit numbers. A 9-point hedonic scale was used (1 = dislike extremely, 2 = dislike very much, 3 = dislike moderately, 4 = dislike slightly, 5 = neither like nor dislike, 6 = like slightly, 7 = like moderately, 8 = like very much, 9 = like extremely).

Storage stability analysis

Jelly candies in glass jars were stored at two different temperatures (+4 °C and 25 °C) for 30 days. pH, color parameters, antioxidant activity, and sensory attributes were performed on day 1 and day 30. Sample codes were simplified as follows: ‘C’ represents control samples and ‘BJ’ represents black Isabella grape jelly samples. The values following the sample code indicate the storage temperature and the corresponding storage day, respectively. The sample codes and their descriptions based on storage temperature and storage period are provided in the Table 1.

Statistical analysis

Statistical evaluations were performed using the SPSS 20.0 software package (SPSS Inc., Chicago, IL, USA). One-way ANOVA was applied to determine statistical differences among groups, and Duncan’s multiple range test was used

Table 1 Sample codes based on storage temperature and duration

Sample code	Sample type	Storage temperature and duration
C41	Control	+4 °C, Day 1
C430	Control	+4 °C, Day 30
C251	Control	25 °C, Day 1
C2530	Control	25 °C, Day 30
BJ41	Black Isabel jelly	+4 °C, Day 1
BJ430	Black Isabel jelly	+4 °C, Day 30
BJ251	Black Isabel jelly	25 °C, Day 1
BJ2530	Black Isabel jelly	25 °C, Day 30

Table 2 Phenolic profile of black Isabella grape concentrate

Phenolic compound	Concentration (µg/g)*
Protocatechuic acid	21.36±0.84
Chlorogenic acid	229.56±2.60
para-hydroxybenzoic acid	7.73±0.51
Syringic acid	29.55±0.96
Rutin	161.08±2.87
Quercetin	1.67±0.20
trans-cinnamic acid	116.30±3.82

* A total of 25 standards were analyzed; however, ellagic acid, daidzein, meta-hydroxybenzoic acid, chrysin, gallic acid, hesperetin, pinocembrin, epicatechin, resveratrol, apigenin, caffeic acid phenethyl ester, para-coumaric acid, caffeic acid, ferulic acid, curcumin, luteolin, myricetin, and kaempferol were below the detection limit

for comparisons involving at least three groups. Pairwise comparisons were performed using the *t*-test when two groups were compared. Paired *t*-tests were used to compare initial and post-digestion values, whereas independent samples *t*-tests were applied to evaluate differences between control and black Isabella jelly samples. A significance level of $p < 0.05$ was considered statistically significant. The jelly production process was carried out in two independent batches, and each batch was analyzed in triplicate (three parallel measurements).

Results and discussion

Phenolic profile

Phenolic composition of black Isabella grape concentrate was shown in Table 2. Chlorogenic acid was found in the highest amount (229.56 ± 2.60 µg/g), followed by rutin (161.08 ± 2.87 µg/g) and trans-cinnamic acid (116.30 ± 3.82 µg/g). Similar to results reported by Domingues Neto et al. [28], chlorogenic acid was highly abundant in Bordô and Isabel grape juices, which are *Vitis labrusca* cultivars, while Brazilian hybrids such as BRS Violeta and IAC 138 – 22 Máximo also exhibited elevated levels of this compound. Chlorogenic acid is a phenolic constituent also present in grape by-products [29]. Furthermore, the detection of

trans-cinnamic acid and rutin in black Isabella concentrate is consistent with previous research on Turkish black Isabella varieties that identified those compounds [30].

In a study examining grape juice produced from a blend of three grape varieties (Isabel Precoce – 50%, BRS Magna – 40%, and BRS Carmem – 10%), syringic acid, which was initially present at 6.05 mg/L in the whole fruit was found to be below the limit of detection following concentration processing [31]. The relatively low levels of para-hydroxybenzoic acid (7.73 ± 0.51 µg/g) and quercetin (1.67 ± 0.20 µg/g) in black Isabella grape concentrate suggest that certain phenolic compounds may have undergone degradation or been lost during processing. Previous studies have demonstrated that grape juice concentration can lead to significant losses in individual phenolic compounds, including hydroxycinnamic acids and flavonols [31, 32]. The phenolic profile of the concentrate studied can vary considerably depending on the grape variety, harvest season, and processing methods. Based on the present findings, black Isabella grape concentrate represents a significant source of phenolic compounds.

Bioaccessibility

Changes in TPC and antioxidant activity after in vitro digestion were shown in Table 3. The low TPC content and antioxidant activity measured in the control sample may be attributed to the presence of flower honey incorporated into the jelly formulation. Although honey is a recognized source of phenolic compounds, its contribution to the measured TPC and antioxidant activity in the control sample may have been limited by matrix effects and processing conditions. Digestion led to a loss in TPC, with a bioaccessibility index of 32.86%. Cianciosi et al. [33] reported a significant decrease in the amounts of phenolic compounds (ranging from 1.52% to 3.59% of the total phenolics in undigested honey) and flavonoids (ranging from 15% to 19% of the total flavonoids in undigested honey) following gastrointestinal digestion for Manuka honey. Losses may arise from the susceptibility of phenolic compounds in honey to heat, light, and jelly processing conditions as well as from their interactions with sugars and other macromolecules. The structure of the food matrix is one of the most important factors affecting the bioaccessibility of polyphenols. They can interact with certain food components, directly affecting their digestibility and thereby limiting their release into the free form. Protein–phenolic interactions can occur through covalent bonds, which are established via enzymatic or non-enzymatic reactions, or through reversible non-covalent interactions, including hydrophobic interactions, hydrogen bonding, van der Waals forces, and electrostatic forces [34]. Interactions can occur via a variety of methods, depending on the matrix composition, processing parameters, kind

Table 3 Bioaccessibility results of jellies after in vitro digestion

		Initial	Post-digestion	Bioaccessibility Index (%)
TPC (mg GAE/g)	Control	0.067±0.00 ^b	0.022±0.00 ^a	32.86±1.46 ^A
	Black Isabella jelly	1.463±0.08 ^b	0.710±0.01 ^a	48.61±2.58 ^B
FRAP (μmol TE/g)	Control	0.089±0.00 ^b	0.021±0.00 ^a	23.28±0.82 ^A
	Black Isabella jelly	3.672±0.10 ^b	0.964±0.03 ^a	26.28±0.94 ^B
DPPH (μmol TE/g)	Control	0.230±0.03 ^b	0.047±0.00 ^a	20.24±1.22 ^A
	Black Isabella jelly	0.982±0.00 ^b	0.444±0.05 ^a	45.19±2.37 ^B

Different lowercase letters within the same row and different upper-case letters within the same column indicate statistically significant differences for each parameter ($p < 0.05$)

of protein and phenolic molecule, and digestive processes [35]. Those interactions exert a significant influence on health-related and pharmacological properties of phenolics [36–38]. Black Isabella grape jellies showed a higher bioaccessibility of 48.61%. This result implies that black Isabella concentrate is better protected in the digestive environment. A study utilizing black Isabella grape peel as an alternative pectin source in jelly formulations indicated that jelly samples achieved a TPC recovery of 43–48%, with higher pectin concentrations promoting greater recovery of phenolic compounds [10]. In gelatin-based systems, phenolic compound degradation during digestion has been reported, with losses reaching approximately 34% in jelly candies [39] and a decline from 42.4% to 30.1% in gelatin-gellan gum gummies [40]. These findings support the role of gelatin as an effective carrier system, given that bioactive compounds decline during storage in both pectin- and gelatin-based formulations, while gelatin matrices tend to exhibit higher retention [41].

Antioxidant capacity was further characterized using FRAP and DPPH assays which are based on different reaction mechanisms and may respond differently to digestion-induced changes in phenolic compounds. Black Isabella jelly shows a significantly higher total antioxidant capacity compared to the control group. The absolute FRAP value post-digestion was roughly 46 times greater than that of the control, demonstrating the jelly formulation effectively carried the phenolic compounds. However, the bioaccessibility index (26.28%) is not as high as that in TPC (48.61%). A significant quantity of phenolics remained post-digestion, although the antioxidant activity exhibited a relatively larger decline. This suggests that although a considerable proportion of phenolic compounds remained after digestion, their antioxidant activity declined to a greater extent. This finding can be attributed to the structural transformation of phenolic

compounds. The smaller metabolites formed after digestion may exhibit lower reducing capacity—when measured by the FRAP method—compared to their parent compounds. Interactions with gelatin and the jelly matrix, a reduction in the functional groups that react with the FRAP reagent, oxidative losses occurring during digestion, as well as interactions between phenolic compounds and digestive enzymes or bile salts, may also be contributing factors.

Following in vitro digestion, a marked decrease in DPPH activity was also measured in both samples, with bioaccessibility indexes of 20.24% and 45.19% for the control and black Isabella jelly, respectively. In the DPPH assay, antioxidants can react through both rapid electron transfer and slower hydrogen atom transfer mechanisms. Consequently, differences between antioxidant assays arise because each method reflects a specific reaction pathway, and therefore, their results may not necessarily follow the same trend [42]. In samples with higher phenolic concentrations, such as black Isabella jelly, structural transformations during digestion have a more evident impact on antioxidant mechanisms, leading to a clearer divergence between FRAP and DPPH results. Structural transformations during digestion exert a stronger influence on antioxidant mechanisms in phenolic-rich samples, as evidenced by the more pronounced divergence between FRAP and DPPH responses in black Isabella jelly. This may be attributed to the bioactive compounds released from the matrix, which seem to be more effective in scavenging free radicals than in reducing metal ions, thereby resulting in higher DPPH values compared to FRAP [43].

Physicochemical, microbiological, sensory, and bioactive properties during storage

The control jelly exhibited a water-soluble dry matter content of 61.17 ± 0.41 °Brix, whereas black Isabella jelly reached 64.83 ± 0.12 °Brix. Incorporation of black Isabella grape concentrate was found to significantly enhance the soluble solids content of the samples compared to the control jelly. Similar to the °Brix values reported for jelly candies enriched with blueberry (61.0 °Brix) [44] and pomegranate (63–67 °Brix) [8] concentrates, jelly samples containing black Isabella grape concentrate exhibited similar water-soluble dry matter content. Although formulations with varying soluble solid contents can be produced, particularly for products containing concentrates, higher °Brix levels are often associated with enhanced flavor and greater product stability over its shelf life.

pH, color measurements, microbiological analyses, bioactivity tests, and sensory evaluations were performed on days 1 and 30 of storage. It was determined that mold growth began on day 10 of storage in the control sample stored at

25 °C. Consequently, further analyses for this sample were discontinued due to microbial spoilage, whereas the remaining seven samples were evaluated according to the experimental design. For both total mesophilic aerobic bacteria and yeast–mold counts, microbial levels in the remaining samples were consistently below the quantification limit of the method ($< 1.0 \times 10^3$ CFU/g) throughout the storage period, indicating very low microbial loads. Such microbial levels, typically below approximately 10^2 – 10^3 CFU/g, fall within the acceptable range for sugar-rich products and do not indicate spoilage or safety concerns [45, 46]. The incorporation of black Isabella grape concentrate into the jelly formulation reduced microbial growth under room temperature storage conditions (25 °C), resulting in improved stability compared to the control sample. The acidic nature of the grape concentrate caused a change in the pH of the final product, and this reduction contributed to a decrease in microbial load observed at 25 °C compared to the control. A key finding of this study is that the incorporation of black Isabella grape concentrate into jelly formulation limited yeast and mold growth during storage at 25 °C; a result primarily attributed to its low pH and content of bioactive compounds, consistent with previous reports on other fruit-based jelly products [47, 48].

pH and color values during storage period were given in Table 4. In the control sample, storage at +4 °C caused a slight decrease in pH, whereas in the sample containing black Isabella grape concentrate, both storage temperature and duration were found to have a statistically significant effect on pH values. As the temperature increased, a decrease in pH was observed in the samples. Organic acids originating from the grape, such as malic and tartaric acids, contributed to a more pronounced acidic character in the samples containing black Isabella grape concentrate compared to the control group, and this acidic character became more evident particularly at higher storage temperatures. Warm storage can accelerate anthocyanin degradation, promoting pH reduction and triggering browning reactions, which may partially explain the positive shift observed in the b^* value of the soft candy samples (indicating a transition from blue toward yellow). Mutlu et al. [4] developed soft candy formulations by using fruit concentrate and honey instead of sugar. Two different mixing methods and

three gelatin doses were tested, and the pH values varied depending on the mixing method and the amount of gelatin. Soft candies produced using the hot-mixing method at 115 °C were reported to have lower pH values than those prepared using the cold-mixing method. Increasing the gelatin content of the formulation also resulted in a decrease in pH. Mixing technique, temperature, and gelatin dosage were all identified as factors influencing the final pH of the products.

The brightness values of the samples containing concentrate were found to be markedly lower compared to the control group, which can be attributed to the presence of dark-colored pigments originating from the grapes, mainly anthocyanins. No significant differences were observed in the brightness values of the candies containing grape concentrate depending on storage temperature or duration, and the L^* values were preserved. At this point, anthocyanin stability governed by structural factors such as the degree of acylation, anthocyanidin type, cis–trans isomerism, and glycosylation pattern can be considered one of the factors contributing to the preservation of color values [2, 49–51]. Although *Vitis labrusca* species are generally characterized by a high content of diglycosylated anthocyanins, studies on Isabella grape have shown that the predominant anthocyanin in this cultivar is malvidin-3-O-glucoside, a monoglucoside form [52, 53]. The relatively high stability of malvidin compounds may contribute to the prolonged preservation of color in this grape. Nevertheless, color stability in anthocyanin-containing systems is a multifactorial phenomenon, influenced not only by pigment presence but also by pH, the chemical structure and form of anthocyanins, enzymatic activities, copigmentation interactions, and processing conditions such as temperature and time [54]. Interactions between anthocyanins and protein-based carriers such as gelatin may further contribute to pigment stability, as anthocyanins are capable of forming conjugates with proteins through free radical grafting reactions, resulting in covalent interactions that enhance their color stability [55, 56].

In the control sample, a statistically significant decrease in the L^* value was detected after 30 days of storage at +4 °C. While the control group exhibited a pronounced redness, the jelly samples containing grape concentrate were found to be less red due to the predominance of purple-blue

Table 4 pH and color values of samples during the storage period

Parameter	Sample	Day 1, +4 °C	Day 1, 25 °C	Day 30, +4 °C	Day 30, 25 °C
pH	Control	5.05±0.04 ^{abB}	5.05±0.03 ^{bB}	4.99±0.02 ^{aB}	nds
	Black Isabella jelly	4.66±0.02 ^{cA}	4.66±0.00 ^{cA}	4.63±0.01 ^{bA}	4.60±0.00 ^a
L^*	Control	36.16±1.00 ^{bB}	36.61±0.06 ^{bB}	34.09±0.04 ^{aB}	nds
	Black Isabella jelly	24.05±0.67 ^{aA}	24.15±0.01 ^{aA}	24.12±0.11 ^{aA}	23.59±0.41 ^a
a^*	Control	18.65±0.02 ^{aB}	18.66±0.03 ^{aB}	18.69±0.01 ^{aB}	nds
	Black Isabella jelly	7.46±0.06 ^{aA}	7.46±0.08 ^{aA}	7.45±0.05 ^{aA}	7.41±0.03 ^a
b^*	Control	4.92±0.04 ^{aB}	4.92±0.01 ^{aB}	4.95±0.02 ^{aB}	nds
	Black Isabella jelly	−0.42±0.02 ^{bA}	−0.42±0.01 ^{bA}	−0.41±0.03 ^{abA}	−0.35±0.05 ^a

Different lowercase letters within same rows and uppercase letters within same columns indicate significant differences in pH, L^* , a^* , and b^* values ($p < 0.05$). nds: Not determined due to visible mold growth on day 10 of storage

hues attributed to grape-derived anthocyanins (Table 4). Storage temperature and duration had no statistically significant effect on the redness values of the samples.

Positive b^* values represent yellowness, whereas negative b^* values represent blueness. Black Isabella grape jellies demonstrated significantly more pronounced blue hues than the control sample. After 30 days of storage at 25 °C, a shift of the b^* value toward the positive direction—indicating a decrease in blueness—was observed. However, no significant change occurred in the b^* values during storage at +4 °C. The anthocyanins present in black Isabella grape are responsible for the blue-purple hues. Products containing anthocyanins are prone to color changes during processing and storage [57], particularly under elevated temperatures, where these pigments may undergo degradation or structural transformation, leading to color loss and a shift from bluish tones toward yellowish hues, which is consistent with the observed changes in b^* values. However, anthocyanin degradation was not directly quantified in this study, and the observed changes in b^* values should be interpreted as indirect indicators of possible structural or compositional modifications in anthocyanin compounds during storage. When a natural colorant is used in a food product, the resulting color is not solely dependent on the pH of the mixture; it is also directly influenced by the chemical forms of the anthocyanins present in the bioactive ingredient used in the formulation, the presence of co-pigmentations, and the changes these compounds undergo during the production process [58].

The sensory analysis scores of the jelly samples are presented in Table 5 and further visualized in Fig. 1. The jelly samples containing grape concentrate and stored at room temperature had the most preferred appearance at the beginning of storage, and its appearance score was similar to that of the sample stored at +4 °C on day 1. The appearance score of the control sample stored under refrigeration for 30 days was 6.00, while the sample containing concentrate and stored at +4 °C for the same duration had a score of 6.50, which was statistically similar.

The jelly candy sample containing grape concentrate and stored at room temperature received an average score of 7.80 for both flavor and texture, indicating that it was

highly appreciated by the panelists. Storage at room temperature and under refrigeration was found to affect the texture and flavor scores, leading to decreased liking scores for the samples. The lowest texture score belonged to the control sample stored under refrigeration for 30 days. The sample containing grape concentrate was the most preferred in nearly all sensory parameters—including appearance, flavor, texture, purchase intention, and overall acceptability. In contrast, the control sample stored at +4 °C for 30 days received the lowest scores across all evaluated sensory attributes and showed significantly lower liking scores for most criteria. Considering the panelists' sensory evaluation scores, the incorporation of black Isabella grape into the soft candy formulation provides not only bioactive advantages but also contributes positively to consumer acceptability.

The total phenolic content and antioxidant activity during storage were measured, and the results are presented in Table 6. The phenolic compounds present in the control sample originate from the honey used in the product formulation, and the addition of black Isabella grape concentrate had a statistically significant effect on the total phenolic content of the produced jelly candy samples. In the control sample, phenolic compounds were preserved at the end of the 30-day storage period under low-temperature conditions. Similarly, the total phenolic content and antioxidant activity of the jelly candy samples containing black Isabella grape concentrate stored at +4 °C was comparable on days 1 and 30 of storage. However, storage at 25 °C for 30 days resulted in decreases of 19%, 12%, and approximately 10% in total phenolic content, FRAP, and DPPH radical scavenging activity, respectively. DPPH radical scavenging activity decreased markedly in the control sample during storage, particularly at +4 °C, whereas the black Isabella jelly exhibited relatively lower losses, with only a slight reduction observed after 30 days at 25 °C. The more pronounced decline in the control sample indicates that the incorporation of grape concentrate contributed to the improved antioxidant stability during storage which may be attributed to its phenolic compounds that neutralize reactive free radicals and interfere with oxidative chain mechanisms. During storage, phenolic compounds are susceptible to oxidative degradation due to environmental factors such as oxygen, pH, and

Table 5 Sensory analysis scores of the jelly samples

Sample Code	Appearance	Color	Flavor	Texture	Purchase Intention	Overall Acceptability
C41	6.70±0.48 ^b	7.10±0.74 ^{ab}	6.20±0.63 ^b	6.30±0.48 ^b	5.90±0.32 ^b	6.00±0.67 ^{ab}
C430	6.00±0.47 ^a	6.50±0.71 ^a	5.50±0.53 ^a	5.60±0.52 ^a	5.20±0.42 ^a	5.60±0.70 ^a
C251	6.60±0.70 ^b	7.40±0.84 ^b	6.30±0.67 ^b	6.50±0.53 ^{bc}	6.20±0.63 ^{bc}	6.40±0.84 ^{bc}
BJ41	7.40±0.70 ^{cd}	6.90±0.74 ^{ab}	7.60±0.70 ^{de}	7.20±0.42 ^d	7.40±0.52 ^{de}	7.40±0.52 ^c
BJ430	6.50±0.53 ^{ab}	6.50±0.97 ^a	6.80±0.63 ^{bc}	6.10±0.32 ^b	6.50±0.53 ^c	6.70±0.67 ^{cd}
BJ251	7.70±0.67 ^d	7.30±0.48 ^b	7.80±0.92 ^e	7.80±0.63 ^c	7.90±0.74 ^c	7.80±0.63 ^c
BJ2530	7.10±0.74 ^{bc}	6.90±0.57 ^{ab}	7.10±0.57 ^{cd}	6.80±0.63 ^{cd}	7.30±0.67 ^d	7.30±0.67 ^{de}

Different lowercase letters within the same column indicate statistically significant differences ($p < 0.05$)

Fig. 1 Radar chart representing the sensory properties of jelly samples

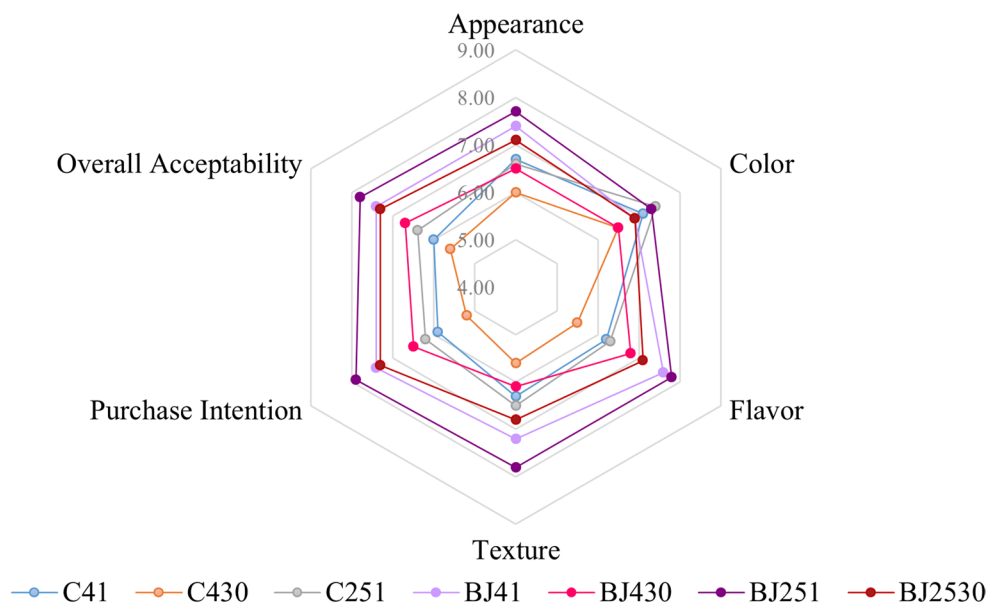


Table 6 TPC and FRAP values during storage

Different lowercase letters within same rows and uppercase letters within same columns indicate significant differences in TPC and FRAP values ($p < 0.05$). nds: Not determined due to visible mold growth on day 10 of storage

Parameter	Sample	Day 1–+4 °C	Day 1–25 °C	Day 30–+4 °C	Day 30–25 °C
TPC (mg GAE/g)	Control	0.07 ± 0.01^{aA}	0.07 ± 0.00^{aA}	0.06 ± 0.01^{aA}	nds
	Black Isabella jelly	1.48 ± 0.11^{bB}	1.46 ± 0.08^{bB}	1.47 ± 0.19^{bB}	1.18 ± 0.06^a
FRAP ($\mu\text{mol TE/g}$)	Control	0.10 ± 0.0^{bA}	0.09 ± 0.00^{abA}	0.08 ± 0.02^{aA}	nds
	Black Isabella jelly	3.72 ± 0.17^{bB}	3.67 ± 0.10^{bB}	3.68 ± 0.04^{bB}	3.22 ± 0.06^a
DPPH ($\mu\text{mol TE/g}$)	Control	0.24 ± 0.01^{bA}	0.23 ± 0.03^{bA}	0.14 ± 0.00^{aA}	nds
	Black Isabella jelly	0.99 ± 0.00^{bB}	0.98 ± 0.00^{bB}	0.98 ± 0.01^{bB}	0.88 ± 0.00^a

light [59]. However, the bioactive compounds of black Isabella grape concentrate may create an antioxidant synergy that delays this loss. In addition to acting as radical scavengers, phenolic compounds may interact with gelling agents to form more stable complexes, thereby enhancing resistance to oxidative degradation and contributing to structural stability [60, 61]. In a study aimed at developing jelly candy products and their low-calorie alternatives enriched with anthocyanin pigments, formulations were created using two different anthocyanin sources (pomegranate juice and aronia purée) and two different sweeteners (sucrose and erythritol/sucralose), based on a pomegranate concentrate matrix. In this study, it was determined that the anthocyanin content of two sucrose-containing control formulations (23% pomegranate concentrate + 38% pomegranate juice) and (23% pomegranate concentrate + 38% aronia concentrate) decreased by approximately 68% and 54%, respectively, after 30 days of storage at 20 °C. In the low-calorie formulations developed in the same study, decreases of 46–50% were reported. On the other hand, storage at +4 °C significantly slowed the degradation of anthocyanins in all products [8]. Storage at lower temperatures exhibited a similar

protective effect when black Isabella grape concentrate was used. Moreover, both TPC and antioxidant activity showed lower losses compared to the low-calorie jelly candy formulations prepared with pomegranate juice and aronia purée throughout storage. This may suggest that phenolics derived from black Isabella grape are relatively more stable during storage, whereas anthocyanins from pomegranate and aronia are more susceptible to degradation, particularly under elevated temperature conditions.

Conclusion

The incorporation of black Isabella grape concentrate into a gelatin-based jelly matrix enhanced the bioaccessibility of phenolic compounds and improved storage stability, as evidenced by enhanced microbiological quality and radical scavenging capacity. The findings of the present study further demonstrated that black Isabella jelly showed favorable performance in terms of sensory properties and color stability. This study highlights its potential as a promising ingredient in gel-based systems, offering a novel pathway

for product development. From the perspective of industrial scalability, future studies establishing a robust framework integrating textural parameters, storage kinetics, and in vivo digestion behaviour are essential to provide critical insights into the practical applicability of this approach. Future research focusing on different gelling agents, various forms of black Isabella grapes—including their by products—and the development of low-calorie formulations, would further highlight the industrial potential of this work.

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Declarations

Ethics declarations All participants involved in the sensory analysis were informed about the study procedures, and their voluntary verbal informed consent was obtained prior to participation. Participation was entirely voluntary, and participants were free to withdraw at any time. The authors confirm that all samples used in the study were food-grade and safe for consumption. The sensory analysis was conducted under appropriate food safety and hygiene conditions.

Competing interests The authors declare no conflict of interest.

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