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Characterization of Benitaka Grape Pomace (*Vitis vinifera* L.): An Analysis of Its Properties for Future Biorefinery Applications

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Abstract: This study investigates the properties of Benitaka grape pomace (*Vitis vinifera* L.), a byproduct of the wine industry, focusing on its potential for applications in the circular economy and biorefinery processes. The analysis covers a range of physical, chemical, and structural characteristics, including the composition of proteins, moisture, lipids, ash, sugars, fiber fractions (such as neutral-detergent fiber, cellulose, lignin, and hemicellulose), pH, acidity, gross energy, as well as bioactive compounds such as total phenolics, flavonoids, anthocyanins, and antioxidant capacity. Advanced characterization techniques, such as nitrogen adsorption/desorption isotherms, Fourier-transform infrared spectroscopy, differential scanning calorimetry, scanning electron microscopy, and high-performance liquid chromatography coupled with mass spectrometry, were employed. The results revealed an acidic pH of 4.05 and a titratable acidity of 1.25 g of tartaric acid per 100 g. The gross energy was 3764 kcal kg^{-1}, indicating high energy capacity, similar to wood chips. The pomace exhibited high hygroscopicity (31 to 50 g of moisture per 100 g), high levels of fiber, cellulose, and lignin, as well as bioactive compounds with significant values of total phenolics (5956.56 mg GAE 100 g^{-1}), flavonoids (1958.33 mg CAT 100 g^{-1}), and anthocyanins (66.92 mg C3G 100 g^{-1}). Antioxidant analysis showed promising results, with DPPH and FRAP values of 20.12 and 16.85 µmol TEAC g^{-1} of extract, respectively. This study not only validates existing data but also provides new insights into the composition of hemicellulose and lignocellulosic phase transitions, highlighting grape pomace as a promising resource for sustainability in industry and biorefinery processes.

Keywords: grape pomace; anthocyanins; polyphenols; acids; antioxidant compounds

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1. Introduction

The grape pomace of Benitaka (*Vitis vinifera* L.) represents an intriguing agricultural byproduct with vast untapped potential. As the world grapples with the challenges of sustainable resource utilization and waste reduction, exploring the multifaceted properties of grape pomace has emerged as a promising avenue for multidisciplinary research and application development. Often marginalized as a wine production or grape residue, it harbors a potential source of bioactive compounds, fibers, and other components that hold value beyond the vineyard [1–3].

As global demand for sustainable and eco-friendly materials continues to rise, attention has turned to agricultural byproducts such as brewers' spent grains [4], jabuticaba peel [5], açaí processing waste [6], chicken feather [7], corn straw [8], and grape pomace [9]. Scientific research recognizes the value of grape residue (pomace) as a versatile resource

that serves various industries, ranging from the food and pharmaceutical sectors to materials science and environmental engineering. Examples include its use in enriching the nutritional profile of food products [10–12], obtaining bioactive compounds [13,14], formulating films [15,16], developing adsorbent materials [17], researching its anticancer properties, animal feed, and biofertilizer [18], as well as biofuel production [19].

A multidisciplinary research approach is paramount in an era marked by the convergence of scientific disciplines and the quest for innovative solutions [20]. Grape pomace provides an ideal platform for this interdisciplinary exploration. This study aims to bridge the gaps between traditionally isolated fields of study, promoting collaboration among researchers in chemistry, biology, materials science, and engineering. This resource's comprehensive characterization lays the foundation for scientists and professionals to explore its potential in many applications. This entails systematically examining its physical, chemical, potential bioactive, and structural properties, encompassing composition, texture, thermal behavior, and surface morphology.

The study of grape pomace characterization has several important purposes and applications in several areas, including enology, the food industry, scientific research, and health. The complete characterization of waste from the wine industry can contribute to greater wine production as the grape pomace is a fundamental part. The knowledge of the chemical composition of grape pomace expands its application possibilities, driving the development of new food products and bringing significant benefits to various industries. The quantification of bioactive compounds present allows its use as raw material for obtaining extracts with antioxidants, anti-aging, and other beneficial properties, positively impacting health and the environment. These properties are increasingly valued by the pharmaceutical and cosmetic industries, which are seeking natural and sustainable solutions.

This study aimed to conduct a comprehensive characterization of grape pomace. The report involved an exhaustive analysis of its structural, physical, thermal, chemical, morphological, bioactive, and surface properties. It also aimed to establish a comprehensive database specifically focused on this material, providing valuable information about its characteristics and application potential.

2. Materials and Methods

2.1. Preparation of Specimens

Approximately 5 kg of Benitaka's grape pomace (BGP) samples was generously provided by a wine production cooperative in the State of Paraná, Brazil. These samples were promptly stored at 4 °C upon collection for future use (initial moisture content of 75.7%). Before utilization, the BGP samples were dried at 40 °C for 36 h (final moisture content of approximately 10.5%). Subsequently, they were packed into plastic bags and frozen for storage until their use. Figure 1 illustrates both the raw and dry BGP samples.



Figure 1. Benitaka's grape pomace visual appearance.

2.2. Characterization of Raw Materials

2.2.1. Physical and Chemical Characteristics

Physical and chemical parameters refer to measurable properties or characteristics that define an object or substance. The titrimetric method was used to determine titratable acidity [20]. pH measurements were conducted with a digital pH meter (model LUCA-210, Lucadema®, São José do Rio Preto, Brazil). Hygroscopicity was evaluated using the method adapted from Cai et al. [21] in different temperatures and relative humidity levels. The gravimetric method was employed with an oven-determined moisture and ash content (model SSDcr-110L, SolidSteel®, Piracicaba, Brazil) set at 105 °C and a muffle furnace (model LUCA2000G, Lucadema®, São José do Rio Preto, Brazil) set at 575 °C. Protein content was assessed using the Kjeldahl method, while lipid composition was determined through hot extraction using hexane via the refluxing technique [20]. The Somogyi–Nelson method is considered a reliable and well-established technique for sugar analysis, and the reducing, non-reducing, and total sugars were determined [22]. The Weende method is a classic and well-established laboratory technique used to determine the crude fiber content of a sample, often used in the analysis of food products, and the crude fiber content was determined [23]. In contrast, neutral-detergent fiber and acid-detergent fiber were quantified using the Van Soest method [24,25]. Additionally, the lignocellulosic composition was determined following the method by Van Soest and Wine [26] methodology.

2.2.2. Determination of Bioactive Compounds

To quantify the bioactive compounds present in the grape pomace at a ratio of 1:50 (w/v), BGP extracts were prepared using a hydroethanolic solution as the extracting solvent (40:60, ethanol/water (v/v)), following the adapted methodology by Ribeiro et al. [27].

Total phenolic compounds were determined following the method of Swain and Hillis with modifications [28]. The total anthocyanin content of the BGP was analyzed by the pH differential method [29]. The total flavonoid content was quantified following Meyers et al.'s methodology [30]. The antioxidant activity was determined by scavenging the radical DPPH (1,1-difenil-2-picrilhidrazil) [31] and by the ferric reduction antioxidant power (FRAP) that was determined according to the methodology proposed by Benzie and Strain [32].

2.2.3. Identification of Anthocyanins by UPLC-PDA-MS/MS

The anthocyanins present in Benitaka Grape Pomace were identified using mass spectrometry (UPLC-PDA-MS/MS). Initially, analysis was performed by flow injection analysis (FIA) using a Thermo Fisher Scientific ion trap mass spectrometer (San Jose, CA, USA) equipped with an electrospray ionization source. Subsequently, MS and MS/MS analysis was carried out in positive ionization mode (100–1500 Da) with a flow rate of 0.5 mL.min^{-1}, capillary voltage ranging from −25 to −35 V, spray voltage of 5 kV, tube lens offset of 75 V, capillary temperature between 250 and 300 °C, and sheath gas (N_2) flow set to 8 (arbitrary units). The data were processed using Xcalibur software (version 2.2 SPI.48).

2.2.4. Thermal Properties

Finally, the crude energy from BGP was determined following ASTM D5865 [33] using a bomb calorimeter (model C5000, IKA®, Staufen, Germany) calibrated previously with benzoic acid. The thermogravimetric analysis (TGA) is a highly versatile analytical technique conducted using a thermal analyzer (model STA6000, PerkinElmer®, Waltham, USA) to study the thermal decomposition and stability of materials over a wide range of temperatures. The nitrogen (N_2) was the carrier gas (flow rate of 20 mL min^{-1}), the BGP samples were positioned in a sample holder, and the temperature spanned from 50 to

900 °C, with a heating rate of 10 °C min^{-1}. DSC (differential exploratory calorimetry) is a powerful and versatile technique for understanding the thermal behavior of materials and was used in an ultramicrobalance (model AD-6, PerkinElmer®, Waltham, MA, USA) and an aluminum pan. N_2 was used as the carrier gas at a flow rate of 20 mL min^{-1}, and the analysis was conducted between 50 and 350 °C, using a consistent heating rate of 10 °C min^{-1}.

2.2.5. Morphometric Properties, Structural Attributes, and Surface Characteristics

The morphology of the BGP samples was examined using scanning electron microscopy (SEM) with energy dispersive spectroscopy (EDS) (model VEGA3, Tescan®, Brno, Czech Republic and model PentaFET Precision, Oxford Instruments®, Abingdon, UK). Fourier-transform infrared spectroscopy (FTIR) was performed to study the materials' structures, measuring transmittance from 750 to 4000 cm^{-1} (model Tensor 37, Bruker®, Billerica, MA, USA). The material surface characteristics were investigated using N_2 adsorption/desorption isotherms at 77K in a BET Surface Area and Pore Size Analyzer (model NOVA 2000e, Quantachrome Instruments®, Boynton Beach, FL, USA).

2.3. Statistical Analysis

The results were expressed as mean ± standard deviation, calculated using the Minitab® software (version 19, 2020) to ensure accuracy and reliability. This approach provided the average value of a set of data points and the standard deviation to indicate the variability or uncertainty in the data. The tests were conducted in triplicate and statistically analyzed by ANOVA and the Tukey test.

3. Results and Discussion

3.1. Physical Parameters

Table 1 shows cases of the outcomes acquired in measuring the physical characteristics of the BGP sample. The pH of the BGP sample appeared to be around 4, which aligns with expectations, given that the pH of grape wort during wine fermentation typically falls within the range of 3.5 to 4. This pH range is optimal for yeast metabolism, as it promotes the conversion of sugars in the wort into ethanol, transforming it into wine [34,35]. Previous studies have reported similar pH values for grape pomace, ranging from 3.32 to 3.8 [36,37].

The acidity value of the BGP was approximately 1.25 g tartaric acid 100 g^{-1} dry BGP, a relatively high value expected due to the sample's nature, containing acidic compounds like aminocarboxylic acids, phenol derivatives, triglycerides, and carboxylic acids (e.g., malic acids tartaric, succinic, and citric) [9,38,39]. Other studies in the literature have reported similar acidity values for grape pomace. For instance, Castro et al. [9] working with Benitaka's grape pomace, found a titratable acidity value of 1.1 g tartaric acid 100 g^{-1} grape pomace, while Ribeiro et al. [27] when working with red grape pomace, found a value of approximately 0.62 g tartaric acid 100 g^{-1} grape peel.

These pH and acidity characteristics of grape pomace enable various practical interpretations and applications. The acidic environment is particularly advantageous for the stability of bioactive compounds, such as phenolics, which are more prone to degradation under alkaline conditions [40]. Additionally, the acidity enhances microbiological integrity, reducing the need for extensive post-drying treatments to preserve the product [41]. Expanding this perspective, the natural acidity of grape pomace supports hydrolytic and fermentative processes in sustainable bioprocesses [42]. The slightly acidic medium facilitates the hydrolytic breakdown of sugars. It provides optimal conditions for the activity of various yeasts and enzymes, enhancing the production of fermentable sugars, bioethanol, and other high-value bioproducts [43,44].

Table 1. Physical parameters of Benitaka's grape pomace (BGP) samples.

Parameters		Values
pH		4.05 ± 0.10
Titratable acidity (g tartaric acid 100 g^{-1} dry BGP)		1.25 ± 0.02
Energy (kcal kg^{-1} of dry BGP)		3764.00 ± 57.00
Hygroscopicity analysis		
Room temperature (°C)	Relative humidity (%)	Ḣ * (g of moisture 100 g^{-1} of dry sample)
10	11	31.31 ± 0.21 ^{aA}
	43	34.80 ± 0.39 ^{aA}
	75	39.34 ± 0.13 ^{bA}
	98	42.86 ± 0.51 ^{bA}
25	11	34.61 ± 0.03 ^{aB}
	43	36.95 ± 0.27 ^{abA}
	75	40.58 ± 0.74 ^{bAB}
	98	49.44 ± 0.21 ^{cB}
35	11	36.71 ± 0.10 ^{aB}
	43	39.33 ± 0.21 ^{aB}
	75	45.26 ± 0.45 ^{bB}
	98	50.23 ± 0.33 ^{cB}

* Ḣ indicates the hygroscopicity value obtained for the dry BGP samples. Identical lowercase letters do not differ between the different relative humidity at the same temperature, and identical uppercase letters do not differ between the same relative humidity at different temperatures ($p \leq 0.05$) [ANOVA and Tukey's test].

The crude energy of the dehydrated BGP sample was approximately 3764 kcal kg^{-1}, a content in line with other materials commonly employed for generating electrical energy, such as wood chips, which possess an energy value of 3801 kcal kg^{-1} [45]. Additionally, other studies have evaluated the energy generation of grape pomace. For example, Vasileiaduo [46] when working with Marc grape pomace, obtained an energy of approximately 3300 kcal kg^{-1}, while Malatak et al. [47] found an energy of approximately 3776 kcal kg^{-1} when working with Riesling grape pomace. The results suggest that BGP could generate power via direct combustion [48,49].

The hygroscopicity measurements for the BGP sample ranged from 31.31 to 50.23 g of moisture 100 g^{-1} of BGP. These results revealed a clear correlation between environmental humidity levels and the sample's hygroscopicity, with higher humidity leading to increased moisture absorption. This behavior is due to the elevated concentration of water molecules in the air, which enhances the sample's capacity to retain moisture. Additionally, a notable trend was observed with changes in temperature: as the room temperature increased, the hygroscopicity of the BGP samples also rose. This phenomenon is likely linked to the influence of temperature on the mass diffusion process between air moisture and the material's porous structure. At higher temperatures, water molecules exhibit increased kinetic energy, facilitating their movement and interaction with the material, which results in a slight but measurable increase in hygroscopicity.

In general, for the same temperature, higher humidity levels statistically significantly differ from lower levels, where the hygroscopicity values are marked with different lower-

case letters (^{b}), indicating a higher moisture absorption capacity at these higher humidity levels. However, for the same humidity level at different temperatures, there is no significant statistical difference between the samples exposed to specific humidity levels. For example, at 75% relative humidity, the samples tended to have the same hygroscopic profile regardless of temperature, which corroborates the previous discussion that, at higher temperatures, hygroscopicity tends to be higher, but also at higher relative humidity.

The hygroscopic profile of BGP underscores the need for careful storage strategies to maintain its stability and quality. For instance, the use of moisture-barrier packaging is crucial to protect the dried grape pomace from undesirable moisture uptake, preserving its functional properties.

On the other hand, the pronounced water absorption capacity of BGP opens up exciting possibilities for its utilization in various applications. For example, its hygroscopic nature could be leveraged in moisture control systems, such as desiccants or humidity-regulating components in packaging. Furthermore, this property could be explored in purification processes, where BGP might serve as an absorbent material for removing moisture or specific impurities from liquids or gases. Such applications align with sustainability goals, as BGP represents a value-added use of an agricultural residue, contributing to circular economy principles and reducing waste.

3.2. Determination of Chemical Parameters

Table 2 shows the chemical characteristics of BGP in different cases and the outcomes derived from the assessment. The moisture and ash levels observed in the grape pomace align with information available in the existing literature. For example, Castro et al. [9] reported 10.3 g 100 g^{-1} for moisture and 7.1 g 100 g^{-1} for ash content. Ribeiro et al. [28] evaluated grape pomace's moisture and ash content and found values of 13.6 g 100 g^{-1} and 9.02 g 100 g^{-1}, respectively. Pereira and collaborators [50] reported values of 3.37 g 100 g^{-1} and ashes of 4.35 g 100 g^{-1}. These variations are expected, as factors like geographical location, grape variety, soil composition, and rainfall levels can all influence the chemical properties of grape peel. In addition, the water source chosen for winemaking can influence grape pomace's mineral composition [20,51].

Table 2. Chemical parameters of Benitaka's grape pomace (BGP) samples.

Parameters	Values (g 100 g^{-1} Dry BGP)
Moisture	9.70 ± 0.39
Ash	6.79 ± 0.33
Protein	12.78 ± 0.92
Lipid	6.98 ± 0.57
Total sugar	20.35 ± 1.05
Reducing sugar	3.82 ± 0.25
Non-reducing sugar *	18.70 ± 1.26
Crude fiber	22.78 ± 2.12
Neutral-detergent fiber	75.21 ± 1.57
Acid-detergent fiber	52.33 ± 2.88
Cellulose	24.95 ± 1.60
Lignin	21.37 ± 1.33
Hemicellulose *	27.38 ± 2.02

* Value determined by difference.

It is worth noting that moisture content directly correlates with hygroscopicity measurements. When the moisture content of BGP samples is low, an increase in hygroscopicity is expected. This is because water released during sample vaporization tends to be reabsorbed into the dry substrate, especially when there is high environmental humidity. This reabsorption primarily occurs through mass diffusion mechanisms [20,52].

The determination of ash content holds substantial importance due to its pivotal role in various applications. For instance, in industries such as civil construction, where it is used in cement production or the synthesis of zeolitic materials, the ash composition becomes a critical factor [53,54]. Furthermore, when BGP is used as a source for electricity generation (biofuel), the amount and characteristics of ashes play a significant role in system design, operation, and environmental compliance because the combustion process can significantly affect the design and operation of electrical energy production systems. Some aspects of ash, such as alkali metals and chlorine, can lead to harmful gases and environmental concerns about inadequate disposal practices [55,56]. In the case of grape pomace, the ash content found can be considered moderate. This value is reasonable for industrial applications such as cement production and zeolitic materials, where the ash content can be advantageous, provided the composition is suitable [57]. However, for biofuel production, the ash content may pose a challenge, as higher values can lead to ash accumulation in the combustion system, impairing energy efficiency and requiring more frequent maintenance [58]. The ash composition should be analyzed more thoroughly, as the presence of alkali metals and other compounds can affect system operation and cause environmental issues, such as the emission of harmful gases. Therefore, the ash found is acceptable for some applications, but its impact should be assessed based on the specific characteristics of the process.

The protein content observed in this study closely resembled the protein levels previously documented in the literature for grape peel samples. Nakov and collaborators [12] found a protein content of approximately 15.5 g 100 g^{-1}, while Rainero et al. [59] reported a value of 11.5 g 100 g^{-1} for protein content in Cabernet grape pomace. Bravo and Saura-Calixto [60] found protein content values ranging from 12 to 14 g 100 g^{-1} while working with red grape pomace. As for the lipid content, which was around 6.98 g 100 g^{-1}, this value varies compared to other studies. Nakov et al. [12] reported a lipid content of approximately 14.95 g 100 g^{-1} for the grape pomace sample, and Sousa et al. [61] found a value of 8.16 g 100 g^{-1} when working with Benitaka's grape pomace.

Yet, there is a notable absence of current data in the literature regarding the levels of reducing and non-reducing sugars in this specific grape variety (Benitaka). This shortage of information could be attributed to the scarcity of extensive studies that have thoroughly explored the carbohydrate composition of this substance. This scarcity is likely because a significant portion of these sugars is typically extracted during fermentation, leaving only minimal quantities available for quantification. Even so, using HPLC, Sousa et al. [61] reported a value of approximately 7.95 g 100 g^{-1} of glucose, slightly higher than the one found in this work, which is 3.82 g 100 g^{-1} of reducing sugars.

Regarding the fibrous component, the observed levels closely matched those documented in the existing literature. For example, Winkler et al. [62] reported values ranging from 21.4 to 29.6 g 100 g^{-1} of crude fiber and 34.6 to 44.8 g 100 g^{-1} of acid-detergent fiber. The substantial concentration of lignocellulosic fibers offers significant potential for the food supplements industry, where they can be utilized to extract dietary fibers for human supplementation [2,62]. Additionally, materials with significant fiber content find application in creating composite films for packaging [63,64]. These applications contribute to creating environmentally friendly, biodegradable materials and promote sustainability in packaging solutions by reducing reliance on synthetic polymers. These fibers' versa-

tile properties offer innovation opportunities in both the food and packaging industries, aligning with current trends in eco-conscious product development.

Concerning the cellulosic component, the quantities of cellulose and lignin closely resembled those reported in the prior literature. When working with grape pomace, Castro et al. [9] reported values of 23.96 g 100 g^{-1} for cellulose content and 26.65 g 100 g^{-1} for lignin content. Madadian et al. [65], while working with red grape pomace, found values of 19 g 100 g^{-1} for cellulose content and 38 g 100 g^{-1} for lignin content, and Pedras et al. [66] reported a value of 30.3 g 100 g^{-1} for acid lignin. Based on the observed cellulose and lignin contents, the BGP can be used in several sustainable applications. Cellulose can produce biofuels [67], composite materials [68], paper, and pulp [69]. At the same time, lignin can be exploited in biofuels, biodegradable materials, natural absorbents, and cosmetic products due to its antioxidant properties [70–72]. Studies have already been conducted with other raw materials rich in lignin and cellulose, such as sugarcane bagasse for ethanol and paper production [73,74], and corn and soybean residues for biopolymer production [73]. Similarly, residues like coconut husks have been explored to produce biofuels and biodegradable materials [75,76]. These uses highlight the potential of grape pomace as a valuable source of lignocellulosic fibers for several industries, promoting the circular economy and innovation in bioproducts.

Nevertheless, comparable values to those observed in this study were not discovered in the existing literature for BGP concerning the neutral-detergent fiber and hemicellulose contents. This difference arises due to the interplay between these constituents, with neutral-detergent fiber encompassing the collective remnants of cellulosic components within the sample, encompassing cellulose, lignin, and hemicellulose. The variation in these values can be attributed to factors like winemaking parameters, the treatment of grapes during fermentation, and regional factors like soil and climate, which differ from country to country [34,66,77].

3.3. Determination of Bioactive Compounds

The BGP extract's total phenolic compounds (TPCs) were approximately 5956 mg GAE 100 g^{-1} (Table 3). This value may vary from recent literature findings. For instance, Eyiz et al. [78] reported TPC values ranging from 1600 to 2400 mg GAE 100 g^{-1} when working with red grape pomace extract. However, Castrica et al. [79], when working with grape marc residue, found a TPC value of 4480.5 mg GAE 100 g^{-1}, and Theagarajan et al. [80] achieved values of approximately 28,060.0 mg GAE 100 g^{-1} of TPC when working with Muscat grape pomace. Such wide variation in values can be attributed to several factors, including different extraction methods, solvents, grape varieties, non-standardized assays, and various environmental aspects, such as cultivation practices, plant age, maturity, and postharvest processing.

However, it is crucial to highlight the significance of TPC in today's context. These compounds are valued for their strong antioxidants and health benefits, defending against oxidative stress and reducing the risk of chronic diseases [79,81]. Beyond their importance in human health, phenolic compounds also influence food products' taste, color, and shelf life, making them essential components in the food industry [82–84]. In essence, total phenolic compounds are integral to both our health and the food systems that sustain us, underscoring their profound importance in modern society [13,85,86].

As for total anthocyanin (TA) content, this study reached a value of 67 mg C3G 100 g^{-1} BGP extract. These results are relatively low compared to other grape varieties identified in the literature. For instance, Eyiz et al. [78] found TA values of up to 120 mg C3G 100 g^{-1}, and Sousa et al. [61] reported a TA value of approximately 131 mg C3G 100 g^{-1}. However, some works have reported values of TA similar to this study. Arboleda Mejia et al. [87]

found a TA value of 49 mg C3G 100 g^{-1} when working with red grape pomace. In summary, the level of anthocyanins in grape varieties is influenced by a complex interplay of genetic, environmental, and viticultural factors. Understanding these factors is essential for grape growers and winemakers to produce wines with desired color, flavor, and antioxidant properties [6,88,89].

Table 3. Bioactive compounds of Benitaka's grape pomace (BGP) extracts.

Parameters *	Values
Total phenolic compounds (mg GAE 100 g^{-1} BGP extract)	5956.56 ± 573.30
Total anthocyanins (mg C3G 100 g^{-1} BGP extract)	66.92 ± 5.29
Total flavonoids (mg CAT 100 g^{-1} BGP extract)	1958.33 ± 102.33
DPPH (µmol TEAC g^{-1} BGP extract)	20.12 ± 2.53
FRAP (µmol TEAC g^{-1} BGP extract)	16.85 ± 1.22

* GAE = gallic acid equivalent; C3G = cyanidin-3-glucoside content; CAT = catechin equivalent; TEAC = trolox equivalent antioxidant capacity.

The total flavonoids (TF) present in the BGP extracts in this study were close to 1960.0 mg CAT 100 g^{-1}. This high value of TF has potential benefits for applying this extract in various products. Flavonoids can act as powerful antioxidants, combat inflammation, and support cardiovascular health by reducing blood pressure and improving blood vessel function [90,91]. Additionally, flavonoids may help prevent cancer, protect brain health, regulate blood sugar levels, promote skin health, aid in weight management, alleviate allergy symptoms, and support gut health by nourishing beneficial gut bacteria [92,93]. Similar works in the literature have reported high values for TF when working with grape pomace, such as Nakov et al. [12], which reached a value of approximately 1461.5 mg CAT 100 g^{-1}, while Cui et al. [94] found values around 2854.0 mg CAT 100 g^{-1}, and Mollica et al. [81] reached values of approximately 2276.0 mg CAT 100 g^{-1}. These results demonstrate that grape peel extracts contain a higher concentration of flavonoids [90].

Finally, the BGP's antioxidant capacity was assessed through DPPH and FRAP measurements, and the values obtained were 20.12 and 16.85 µmol TEAC g^{-1} BGP extract, respectively. Monteiro et al. [95] reported DPPH values ranging from 10.22 to 14.32 µmol TEAC g^{-1} in red hybrid grape pomace extract. Szabo et al. [96] reported FRAP values of approximately 40.0 µmol TEAC g^{-1} in Cabernet Sauvignon grape pomace extract, while de la Cerda-Carrasco et al. [97] found DPPH values between 50.0 and 110.0 µmol TEAC g^{-1} in grape pomace when working with four different grape cultivars. The differences in grape pomace antioxidant capacity stem from variances in grape type, ripeness, and the vinification process [98]. These variables influence the types and concentrations of antioxidants, impacting overall antioxidant potential [99,100]. Additionally, processing methods and extraction techniques affect the retention of antioxidant compounds, leading to variations in antioxidant capacity among different grape pomace extracts [101,102].

Given the rich content of phenolic compounds, flavonoids, and anthocyanins in BGP, its potential applications in various industries are vast. The high levels of TPC and flavonoids make it a valuable resource for the food industry, where it can be used as a natural antioxidant to enhance the shelf life, flavor, and color of food products [103–106]. Furthermore, its health-promoting properties, such as anti-inflammatory, anticancer, and

cardiovascular benefits, position BGP as an ideal candidate for functional food supplements [107,108]. In addition to food products, BGP can be explored for the development of natural preservatives in cosmetics and pharmaceuticals due to its antioxidant capacity. By focusing on efficient extraction methods, grape pomace can be leveraged to create high-value products, fostering sustainability and innovation within the food, health, and cosmetics industries.

Table 4 presents the identification of eight anthocyanins detected by HPLC/MS/MS, along with their respective retention times, $[M]^+$—m/z, and MS/MS data. These compounds have been previously identified in other studies in the literature. Mesquita et al. [109] reported the presence of Delphinidin-3-O-glucoside, Peonidin-3,5-O-diglucoside, Malvidin-3-O-glucoside, and Malvidin-3-O-(6-O-p-coumaryl)-glucoside. Similarly, Ribeiro et al. [27] identified different anthocyanins, including Cyanidin-3-O-glucoside, Petunidin-3-O-glucoside, and Peonidin-3-O-glucoside.

Table 4. Anthocyanins identified in the extract of Benitaka grape pomace: Retention time, Molecular ion, MS/MS fragmentation pattern, Aglycone, and Putative identification.

Retention Time (min)	Molecular Ion $[M^+]$ (m/z)	MS/MS	Aglycone	Putative Identification *
3.25	611	449, 253	Cyanidin	Cyanidin-3,5-*O*-diglucoside
3.53	465	303	Delphidin	Delphidin-3-*O*-glucoside
3.71	625	463, 301	Peonidin	Peonidin-3,5-*O*-diglucoside
3.93	449	287	Cyanidin	Cyanidin-3-*O*-glucoside
4.18	479	317	Petunidin	Petunidin-3-*O*-glucoside
4.74	463	301	Peonidin	Peonidin-3-*O*-glucoside
4.89	493	331	Malvidin	Malvidin-3-*O*-glucoside
6.48	625	463, 317	Malvidin	Malvidin-3-*O*-(6-*O*-p-coumaryl)-glucoside

* Reference: Mesquita et al. (2023) [109].

In summary, the bioactive compounds in grape pomace offer significant societal and environmental benefits [110]. They can be harnessed for health-promoting supplements and functional foods, reducing the risk of diseases [111]. Additionally, these compounds have industrial applications in cosmetics, pharmaceuticals, and winemaking processes, enhancing product quality and sustainability [112,113]. Furthermore, the utilization of grape pomace reduces food waste. It contributes to a more environmentally friendly and economically viable grape industry, highlighting their importance in [114].

3.4. Thermal Analysis

Figure 2 presents the outcomes of the thermal analysis conducted on the BGP. In Figure 2a, the TG curve of the grape pomace displays two notable stages of mass loss. The initial decline from around 50 to 150 °C is ascribed to moisture evaporation, leading to a roughly 10% reduction in mass [114]. The following phase, occurring within the temperature range of 300 to 450 °C, signifies the liberation of various organic substances, encompassing waxes, fats, glycosides, and alkaloids. These substances originate from the thermal decomposition of the lignocellulosic structure, leading to a reduction in mass of roughly 70% [115,116]. Beyond the 700 °C mark, a final residual char, making up roughly 20% of the total mass, is obtained from the BGP. This significant residue can be attributed to non-combustible minerals within the biomass [117].

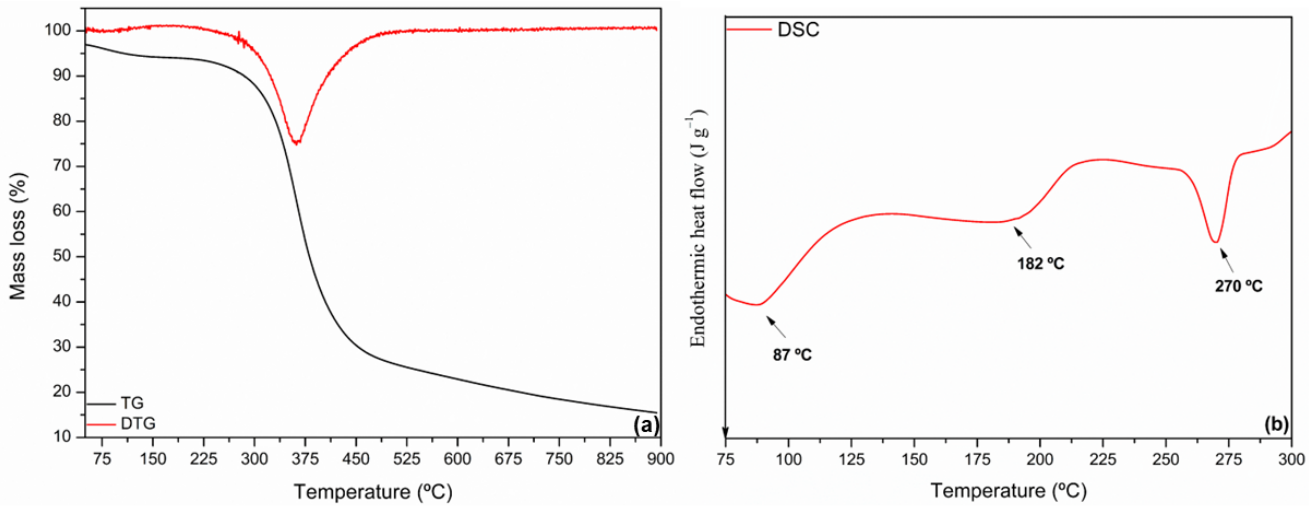


Figure 2. Thermal characterization of Benitaka's grape pomace: (**a**) TGA and (**b**) DSC.

We can observe three prominent endothermic peaks within the DSC curve presented in Figure 2b for the BGP sample. The initial peak, around 80 °C, potentially correlated with the lignin glass-to-rubber transition, resembles transitions observed in other plant-based materials [118]. The second peak, occurring at around 182 °C, can be attributed to the melting point of tartaric acid, the primary organic acid found in grape pomace [9,119]. Finally, the peak at 270 °C indicates the possible thermal decomposition of the present pectin within the grape pomace's structure [36,120].

Thermal analysis provides valuable information on the behavior of BGP under different temperature conditions, revealing how it behaves in decomposition and compound release processes. For example, knowledge about the decomposition temperature of compounds such as lignin, pectin, and tartaric acid can guide extraction and conversion processes, such as the production of biofuels or antioxidant compounds [121–123]. In addition, information on residue formation and thermal absorption capacity helps optimize the use of BGP in composite materials, bioplastics, or adsorbents [124,125]. In short, understanding these thermal aspects allows for a more efficient and targeted use of BGP, maximizing its value in various industrial applications and promoting sustainable solutions.

3.5. Morphological Analysis

Figure 3 showcases the micrograph captured for the BGP sample using SEM. The micrographs depict a textured surface with well-organized granular particles, as evidenced by the 1000× magnification. Moreover, when magnified to 10,000×, the material reveals a porous and granular surface. The material's porous composition hints at its possible utility in crafting porous substances, like adsorbents, zeolites, or nanocomposites, which hold potential in various adsorption-based and catalytic applications [20,126]. Adsorbents are used in purification and substance separation [127], and zeolites are important in catalysis and ion exchange [128], while nanocomposites, with their combination of nanoscale materials, are applied in catalysts, energy storage, and electronic devices [129]. The versatility of these porous substances supports areas such as water treatment, biotechnology, renewable energy, and sustainability.

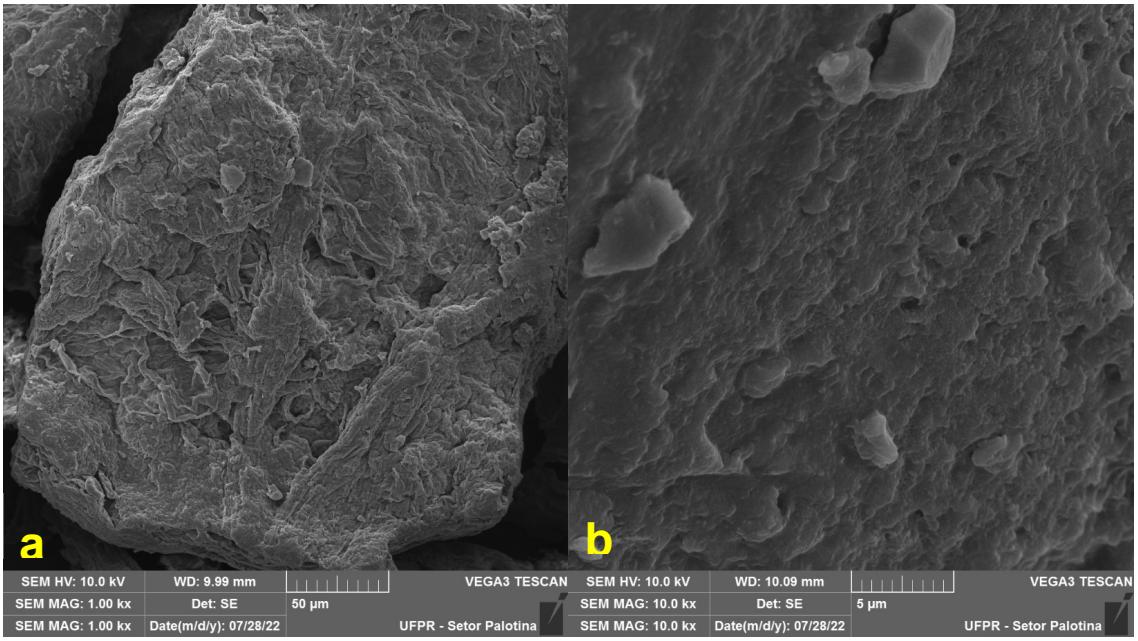


Figure 3. SEM images of Benitaka's grape pomace: (**a**) 1000× magnification and (**b**) 10,000× magnification.

The EDS examination provided the subsequent results for the elemental content: C (63.94 ± 3.55%), O (32.95 ± 3.24%), Mg (0.29 ± 0.03%), K (2.70 ± 0.12%), and Ca (0.18 ± 2.70%). The major constituent of the BGP composition is carbon, constituting roughly 64% of the overall composition, with oxygen accounting for approximately 33%. This outcome aligns with the sample's inherent traits and the chemical analysis findings (Table 2). The elevated carbon content is anticipated because of the prevalence of carbohydrates in BGP, with these essential nutrients primarily consisting of carbon–hydrogen (C-H) bonds [113].

The lower concentrations of elements like magnesium, potassium, and calcium can be ascribed to the inherent composition of grapes used to create grape pomace during the winemaking procedure [130,131].

The high carbon content (63.94%) in BGP is advantageous for applications such as biofuels, biochar, and adsorption in purification processes. This high carbon content makes BGP worthwhile for energy conversion processes such as combustion and bioconversion to bioethanol [132,133]. The significant presence of oxygen also favors fermentation processes [134]. The low concentration of minerals such as magnesium, potassium, and calcium indicate that BGP is more suitable for these energy and environmental applications than for use as a fertilizer [135,136].

3.6. Structural Analysis

Figure 4 illustrates the FTIR spectra for the BGP raw material. The spectrum is divided into four distinct regions. Spanning wavenumbers from 3125 to 2750 cm^{-1} (Region 1), it is possible to observe characteristic vibration signals related to the alkane, alkene, and alkyne bonds (-CH_3, -CH_2, and -CH) present in the cellulosic composition of BGP [137]. Region 2, ranging from wavenumbers 2375 to 2000 cm^{-1}, is characterized by the vibrations of -C-H and C=C bonds within the ring structure of BGP. This implies the potential existence of lignocellulosic substances like cellulose, lignin, and hemicellulose [138]. Moving on to Region 3, which extends from 1750 to 1250 cm^{-1}, we can identify distinctive features, including C=O stretching vibrations of carbonyl and carboxyl groups, CH bond bending bands of alkane groups, C-C stretching bands, and -C-O stretching bands. These features all indicate the lignocellulosic structure of grape pomace [64]. Finally, Region 4, covering

wavenumbers from 1250 to 750 cm^{-1}, is characterized by the -C-O stretching band and CO-O-CO stretching band of the organic acids present in the material's structure, as well as the -CH bond stretching bands of alkane groups found in the sample [9,20].

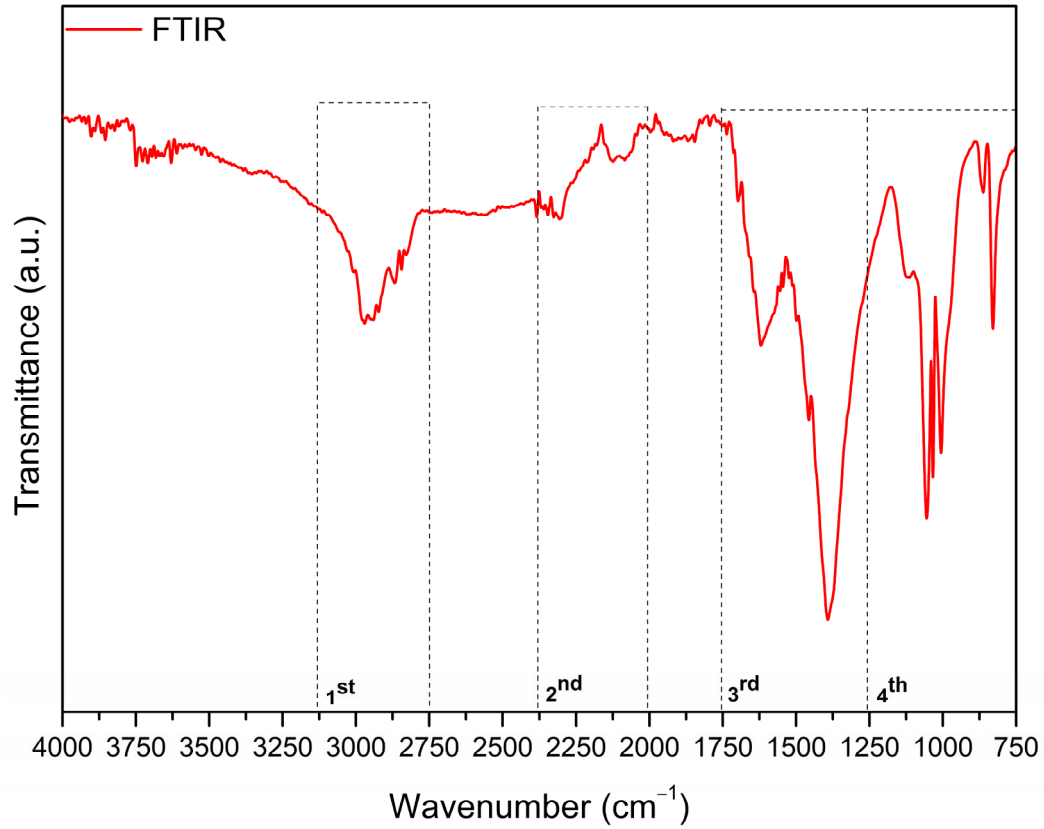


Figure 4. Infrared spectrum of Benitaka's grape pomace.

3.7. Superficial Analysis

The N_2 adsorption/desorption isotherms are depicted in Figure 5. The isotherm exhibited in Figure 5a strongly resembles a Type 4 isotherm following IUPAC guidelines. This pattern signifies the existence of materials displaying microporous attributes. The inflection point on the isotherm represents establishing the initial adsorbed layer that coats the entire material surface, a phenomenon extensively documented in the existing literature [20,139]. Furthermore, upon closer examination of Figure 5a, it becomes apparent that there is an H2 hysteresis gap, as defined by IUPAC standards. This observation indicates the presence of pores with an "ink-bottle" shape [140].

Turning our attention to pore distribution, as depicted in Figure 5b, it becomes apparent that most pores fall within 1.8 nm. In Table 5, you can find information regarding the BET surface area (S_o), pore volumes (V_p), mean pore diameter (d_p), and BJH diameter (d_{BJH}) for the BGP sample. Upon referencing Table 5, it becomes evident that the BGP sample exhibits a substantial surface area and an average pore size of 3.99 nm. These measurements indicate the material showcases a porous structure firmly situated within the mesoporous range (with pore diameters between 2 and 50 nm). These findings align with the conclusions drawn from the isotherms showcased in Figure 5.

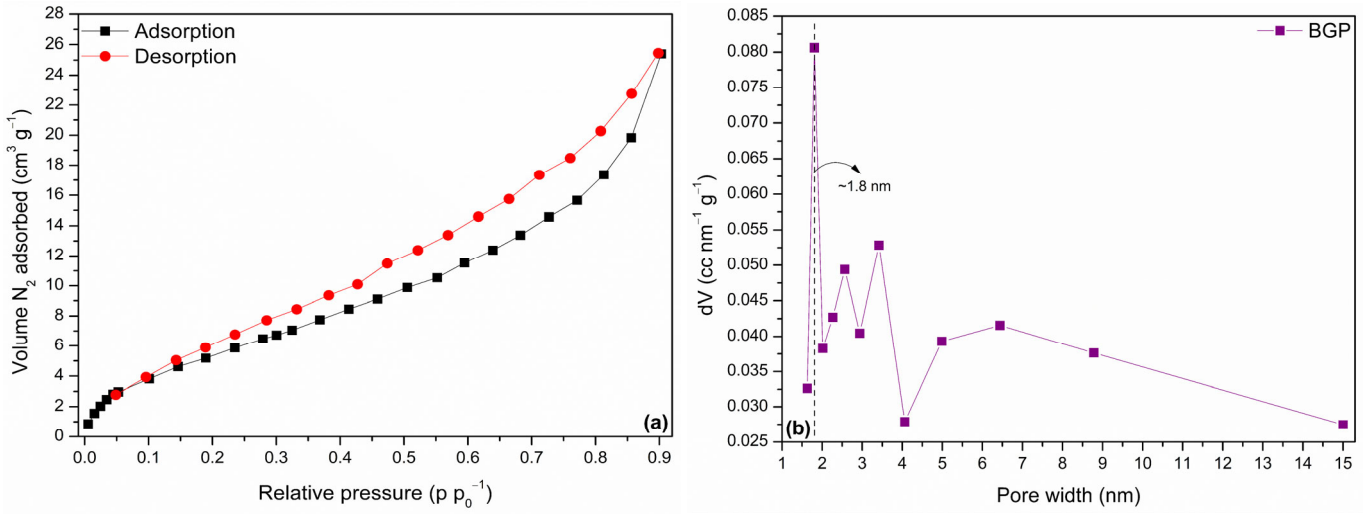


Figure 5. Benitaka's grape pomace surface characterization: (**a**) N_2 isotherm and (**b**) pore diameter distribution.

Table 5. Superficial properties of Benitaka's grape pomace (BGP).

Sample	S_o (m^2 g^{-1})	V_p (cm^3 g^{-1})	d_p (nm)	d_{BJH} (nm)
BGP	120.44 ± 0.87	0.05 ± 0.001	3.99 ± 0.03	1.81 ± 0.01

The data regarding the surface attributes of Benitaka's grape pomace hold significant importance for prospective research, especially in crafting adsorbent materials. The inherent characteristics of the BGP material inherently include favorable adsorption properties, a significant surface area, and a porous structure [126,141]. These features suggest that BGP could be effectively utilized in water treatment, waste management, and pollutant removal applications [142]. Its porous structure efficiently captures organic compounds, heavy metals, and other contaminants, making it an ideal candidate for environmental remediation.

4. Conclusions

In conclusion, Benitaka's grape pomace sample was characterized efficiently, yielding results that align with the existing literature across various dimensions, including physical, chemical, bioactive compounds, thermal, morphological, structural, and surface parameters. The chemical composition of grape pomace indicates the presence of anthocyanins, which are natural pigments (red, purple and blue colors), tannins, polyphenols, acids (such as resveratrol, quercetin, and catechins), sugars, acidic pH, and antioxidant compounds. Furthermore, this study has contributed novel findings not typically encountered in other research, potentially positively influencing the scientific community. These results can serve as valuable resources for fellow researchers as they consult databases for information to inform their work in diverse scientific fields. Based on the results obtained, we can conclude that grape pomace has great potential to be used as an adsorbent due to its porous structure and the presence of bioactive compounds such as anthocyanins, polyphenols, and acids. Additionally, this material can be used as a raw material to produce biofuels, sustainable materials, and high-value-added products, such as antioxidant and biodegradable compounds. Its versatility, low cost, and promising characteristics make it an effective alternative for various industrial applications, contributing to the advancement of the circular economy and the development of environmental solutions.

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