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Article

Functional and Nutritional Properties of Grape By-Product Flours from Coahuila, Mexico as Potential Food Ingredients

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Abstract: Grape Pomace Flour (GPF) is a by-product of the wine industry that is currently underutilized. This research aimed to determine the functional and nutritional properties of GPF from Coahuila, Mexico. The experimental strategy included the evaluation of the color parameters in GPF with different particle sizes; subsequently, the determination nutritional parameters (moisture, ash, minerals, fat, protein, and carbohydrates) were determined and finally the functional parameters: water solubility index (WSI), swelling power (SP), water absorption index (WAI) and oil absorption index (OAI) in GPF, as well as the determination and identification of bioactive compounds by HPLC-MS. The results showed better color characteristics in particle size less than 180 μm . GPF is a good source of minerals such as potassium, magnesium, and calcium, highlighting that the magnesium content is higher than in other grape pomace flours. The results of the functional properties showed that GPF presented higher values of WAI (2.64 ± 0.06 g/g), WSI (5.75 ± 0.61 %), and SP (2.80 ± 0.05 mL/g) than commercial flours. Finally, GPF presented a high content of phenolic compounds; the HPLC analysis identified important bioactive phenolic compounds. In conclusion, GPF has exceptional functional and nutritional properties, surpassing many conventional flours and aligning with non-conventional ones. GPF's natural color enhances product appeal, making it a versatile, nutrient-rich ingredient that improves food quality.

Keywords: *Vitis vinifera*; grape waste; agro-industrial by-product; functional and nutritional potential; agri-food bioeconomy

1. Introduction

In 2023, 7.2 million hectares of vineyards were cultivated worldwide [1]. In the same year, 35,000 hectares were cultivated in Mexico [2]. Additionally, 455,000 tons of grapes were harvested, of which 73,000 tons were designated for wine production [3]. The state of Coahuila is one of the most

important wine regions in Mexico, and it is known for its winemaking tradition, which dates back several centuries. The ethnobiological significance of grapes in Parras de la Fuente, Coahuila, is notable; this fruit was present before the Spanish arrived and was utilized by indigenous tribes such as the Mexicas. Although the exact amount of wine produced in Coahuila can vary annually depending on the harvests and climatic conditions, Coahuila generally produces about 20% of Mexican wine, and Parras de la Fuente is the main producer, accounting for approximately 95% of the state's production [4].

Grape pomace is the primary by-product of the wine industry, representing approximately 30% of the total weight of the grapes used for wine production [5]; this means that approximately 21,900 tons of grape pomace are generated annually in Mexico, specifically in the region of Parras, Coahuila, around 4,380 tons of grape pomace are produced (empirical calculation based on data obtained from Ramirez, 2023 [4]).

Grape pomace is a waste discarded in municipal landfills, leading to significant environmental pollution as it decomposes. There is a strong emphasis on protecting the environment [6]. Therefore, it is crucial to find ways to reuse and develop new bioproducts made from grape pomace [7].

The generation of grape pomace waste in Mexico, particularly in Coahuila, has risen in recent years due to the influx of international wineries operating in the region over the past decade, totaling 22 wineries [8]. This growth makes Coahuila the second-largest wine-producing state in the country. The quality of the grapes produced in the region contributes to its reputation; however, this has also led to serious environmental pollution due to the massive accumulation of waste in fields and landfills, as there is no defined use for it. While a small portion of this waste is utilized as livestock feed, the majority poses an environmental challenge, as there are currently no waste recovery initiatives in the area. This situation presents an opportunity to add value to the primary by-product of the wine industry.

This by-product is gaining increasing attention in food research due to its rich content of bioactive compounds and functional properties [9–11]. Recent research has highlighted the significant potential of this by-product as a functional ingredient in food production, driven by its nutritional characteristics and beneficial effects on human health [12–14].

This study will explore the production of grape pomace flour (GPF) from this residue as an alternative for valorization [15]. GPF is produced by grinding dried pomace, and its inclusion in food products enhances their nutritional value, functionality, and sensory properties. The bioactive compounds found in GPF include polyphenols, anthocyanins, flavonoids, and dietary fiber. These compounds are linked to beneficial effects such as antioxidant, anti-inflammatory, and cardioprotective activities [14,16–18]. It has also been reported that gluten-free bioproducts can be formulated from grape pomace flour [18,19].

This study aimed to determine the nutritional and functional properties of grape pomace flour (GPF) from grapes cultivated in Parras de la Fuente, Coahuila, to predict its potential applications in the food industry.

2. Materials and Methods

2.1. Experiment Plan

The study was conducted in three experimental phases:

Phase 1: Grape Pomace Flour (GPF) was generated from the grape residue, and the effect of 4 particle sizes on color parameters was evaluated by a completely randomized design with one factor (particle size) and four levels (550, 450, 250, and <180 μm) and color parameters L^* , a^* , and b^* as response variables.

Phase 2: Nutritional properties of GPF were evaluated by a uni-factorial design with particle size <180 μm as the only level and Moisture, Ash, Minerals, Fat, Protein, and Carbohydrate as response variables.

Phase 3: The functional properties of GPF: Water Solubility Index (WSI), Swelling Power (SP), Water Absorption Index (WAI), and Oil Absorption Index (OAI) were evaluated using commercial wheat flour as control. Total phenolic content and compound identification were also performed.

2.2. Materials

2.2.1. Chemical Reagents

Cellulose filter paper (Whatman®, U.S.A.), petroleum ether (Sigma-Aldrich®, U.S.A.), distilled water (J.T. Baker®, U.S.A.), concentrated sulfuric acid, 0.1 N hydrochloric acid (Merk Millipore®, Germany), commercial wheat flour (HT) “Escudo Anaya” (Moldeo Anaya®, Mexico), whole wheat flour (HTi) “San Blas” (Molinos San Blas®, Mexico) and vegetable oil (Merk Millipore®, Germany), commercial wheat flour (HT) “Escudo Anaya” (Moldeo Anaya®, Mexico), whole wheat flour (HTi) “San Blas” (Molinos San Blas®, Mexico) and commercial edible vegetable oil “123” (Deli Max®, Mexico).

2.2.2. Plant Material

17 kg of wet grape pomace was kindly provided by the red wine production factory of the Technological University of Parras (UTP) (2023 campaign), located in Parras de la Fuente (Coahuila, Mexico); grape pomace was sourced from the production of wine made from *Tempranillo* grapes and had the following characteristics: total acidity of 6.7%, pH 3.8, and 25.6 °Bx. Grape pomace was immediately transported to the Research Center and Ethnobiological Garden (CIJE) food laboratory of the Autonomous University of Coahuila (UAdeC) located in Viesca, Coahuila, for future analysis.

2.3. Grape Pomace Flour (GPF)

The complete pomace (seeds, skins, and stems) was dried in an oven (ICB®, Mexico) for 96 hours at an average temperature of 60 °C. After drying, the grape pomace was milled using a paddle impact mill (RETSCH®, Germany) until a fine powder was obtained. The particle size of the GPF was determined using dry sieve analysis, a conventional method for assessing particle size distribution. For this purpose, stainless steel sieves (Fiicsa®, Mexico) with mesh sizes of 550, 450, 250, and <180 µm were employed. The GPF was vacuum-packed in transparent, food-grade bags with a capacity of 1 kg. Figure 1 illustrates the flow diagram of the process used to obtain grape pomace flour (GPF), and the bags were stored in a dark place at room temperature. The production yield of GPF was calculated using formula 1:

$$\text{GPF yield (\%)} = ((\text{amount of GPF obtained}) / (\text{amount of initial waste})) \times 100 \quad (1)$$

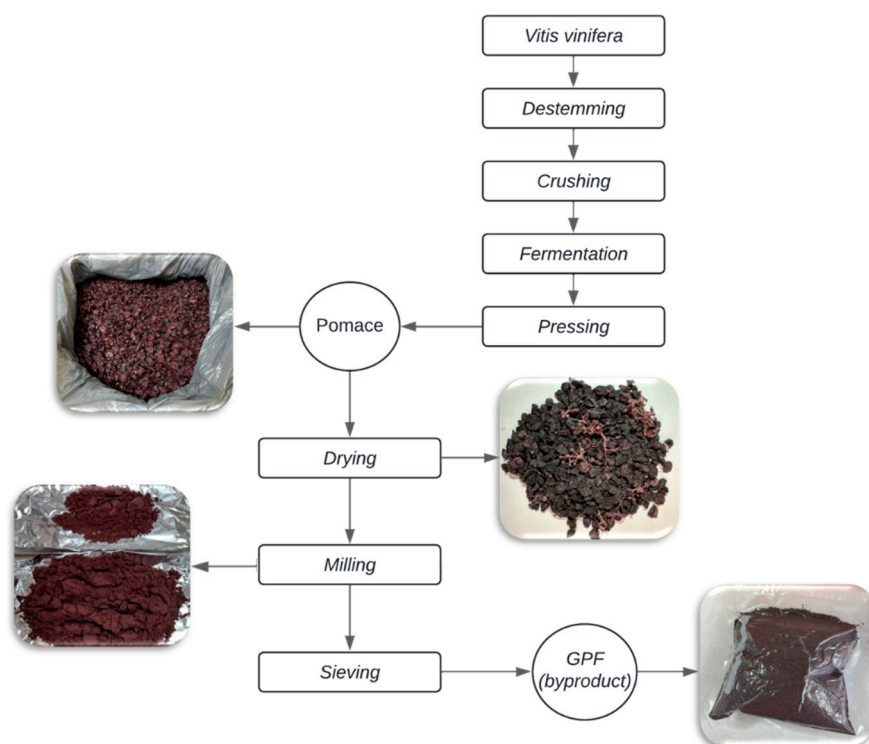


Figure 1. Flow diagram illustrating the process for obtaining grape pomace flour (GPF).

2.3.1. Color Analysis in GPF

Color analysis (WR-10QC colorimeter, FRU®, China) was performed on flours with different particle sizes to identify the effect of milling on color and possible applications in food development. Measurements were taken at three points for each sample to represent color accurately. The data were read directly from the equipment and expressed in terms of the parameters L^* , a^* , and b^* . L^* : Indicates the brightness of the sample, with values ranging from 0 (black) to 100 (white). a^* : Represents the color trend on the red-green scale. b^* : Indicates the color trend on the yellow-blue scale.

2.4. Nutritional Properties of GPF

2.4.1. Determination of Moisture, Ash, Minerals, Fat, Protein, fiber and Carbohydrate

Moisture content was determined using the AOAC 2005 method 925.09 [20]. A drying oven (ICB®, Mexico) was used to dry 1 g of the sample to a constant weight at 105 °C for 4 hours. The sample was then cooled in a desiccator for 30 minutes. The moisture percentage was calculated based on weight difference using an analytical balance (OHAUS Adventurer®, USA).

For ash determination, 5 g of the sample was weighed in pre-tared porcelain crucibles. The sample was pre-calcined on a grill and then fully calcined in a muffle furnace (Feliza®, Mexico) at 525 °C for 5-12 hours. After cooling, the samples were weighed to determine their ash percentage [21].

Determining minerals in grape pomace flour was conducted in triplicate using X-ray fluorescence equipment (Panalytical Epsilon 1, Netherlands) [22]. The samples were heat-treated at 600 °C for 1 hour to assess ignition losses in a muffle furnace (Thermolyne, USA). The weight percentages of the elements were calculated based on the ash percentage of the material.

Fat content was determined using a Soxhlet extraction system (Scorpion Scientific®, Mexico). 1 g sample was weighed on Whatman brand cellulose paper, and 8 mL of petroleum ether was added at a temperature range of 30-75 °C and allowed to stand for 3 hours. Afterward, the remaining ether was evaporated, and the change in weight of the flask was recorded and expressed as a percentage [23].

Protein content was determined using the Kjeldahl method for total nitrogen analysis [24]; 1 g sample was digested with sulfuric acid and then subjected to automatic distillation using a CTR Scientific® distiller (Mexico). After distillation, the sample was titrated with 0.1 N hydrochloric acid. A factor of 6.25 was applied, multiplying this factor by the percentage of nitrogen obtained to calculate the total protein content.

The fiber content was measured using the AOAC 991.43 acid and alkaline hydrolysis method [25]. A 2 g defatted sample was placed in a Berzelius glass with 100 mL of 0.225 N sulfuric acid (Fisher Scientific, USA) and connected to reflux equipment for 30 minutes. After boiling, it was dried, filtered with a linen cloth, washed with hot distilled water, and checked for acid traces with litmus paper (Whatman, England). The remaining fiber was then treated with 100 mL of 0.313 N sodium hydroxide (Fisher Scientific, USA) in reflux for 30 minutes, filtered, and washed, verifying the alkaline reaction. The fiber was dried in an oven (ICB®, Mexico, Germany) at 100 °C for 12 hours, weighed, and then incinerated in a muffle furnace (Feliza®, Mexico) at 550 °C for 3 hours, with the final weight recorded.

Finally, the carbohydrate content was calculated as the percentage difference based on the results of the other determinations [26].

2.5. Functional Properties of GPF

2.5.1. Determination of Water Solubility Index (WSI), Swelling Power (SP), Water Absorption Index (WAI), and Oil Absorption Index (OAI)

Three 0.5 g samples of GPF were weighed using an analytical balance (OHAUS Adventurer®, USA) and placed in centrifuge tubes. Then, 6 mL of distilled water at 30 °C was added to each tube, and the tubes were placed in a shaking incubator for 30 minutes (Lab Instruments®, Mexico). The samples were centrifuged at 5000 rpm for 20 minutes in a centrifuge (Lab Instruments®, Mexico). The supernatant was decanted, its volume was measured, and 2 mL of the filtrate was taken for drying at 90 °C for 4 hours. The gel retained in the tubes was weighed and recorded to calculate the Water Solubility Index (WSI), Swelling Power (SP), and Water Absorption Index (WAI) according to equations 2, 3, 4, and 5 [27]. Commercial vegetable oil was used instead of water to determine the Oil Absorption Index (OAI).

$$\text{WSI (\%)} = (\text{Dry weight of the supernatant (g)})/(\text{Sample weight (g)}) \times 100 \quad (2)$$

$$\text{SP} = (\text{Gel weight (g)})/(\text{Sample weight (g)} - \text{Dry weight of the supernatant (g)}) \quad (3)$$

$$\text{WAI} = (\text{Gel weight (g)})/(\text{Sample weight (g)}) \quad (4)$$

$$\text{OAI} = (\text{Gel weight (g)})/(\text{Sample weight (g)}) \quad (5)$$

2.5.2. Determination and Identification of Bioactive Compounds of GPF

2.5.2.1. Preparation of GPF Extracts

Methanol extracts were prepared with 25 mL of solution (methanol/ water) (80% v/v), and 2.5 g of GPF sample homogenized with the Ultra-Turrax apparatus (T18, Wilmington, USA) at 24,000 rpm for 1 min. The mixture was left under continuous stirring (300 rpm) at room temperature for 30 min. Each resulting sample was centrifuged (4480g for 15 min, 4 °C), and the supernatants were collected and filtered by 0.45 µm (Orange Scientific, Braine-l'Alleud, Belgium). Only 15 mL aliquot of the extracts were evaporated to dryness (30 °C) in the RCV 2-18 speed-vacuum evaporator (Chris. Osterode am Harz, Germany) and stored at -20 °C until their analysis.

2.5.2.2. Total Polyphenol Content

Total phenolic content (TPC) of the GPF extracts was determined by Folin–Ciocalteu method [28], 80 μ L of Folin–Ciocalteu reagent 10% (v/v) was added to 20 μ L of extract (previously dissolved in distilled water), followed by 100 μ L of sodium carbonate (7.5% (m/v)) and allowed to react in the dark at room temperature (23 °C) for 1 h. After that, absorbance was measured at 750 nm (Microplate Spectrophotometer, Thermo Fisher Scientific, USA) in a 96-well microplate (Thermo Fisher Scientific, USA). Gallic acid was used as a standard for the calibration curve, and the results were expressed as milligrams equivalent of gallic acid per gram of dry extract (mg GAE/g DE).

2.5.2.3. Phenolic Compounds Identification by HPLC

The quantitative profile of phenolic compounds in GPF extracts (previously dissolved in ultrapure water) was performed using a Waters Alliance e2695 separation module system interfaced with a photodiode array UV/Vis detector 2998 (PDA 190–600 nm) (Waters, USA). The compounds were separated in a reverse-phase C18 column (COSMOSIL 5C18-AR-II Packed Column–4.6 mm I.D. x 250 mm; Dartford, UK). The mobile phases and the gradient program were prepared according to Oliveira et al. [28] with some modifications. The mobile phase was composed of solvent A: Water/acetonitrile/TFA (94.9/5/0.1%) and solvent B: Acetonitrile/TFA (99.9/0.1%) with the elution gradient: 0–1 min 0% B; 1–30 min 21% B; 30–42 min 27% B; 45–55 min 58% B; 55–60 min 0% B, and kept another 1 min at 0% B. Flow rate was 1 mL/min, the oven temperature was set as 25 °C, and the injection volume was 20 μ L. Detection was performed at 280 nm, 320 nm, 360 nm, and 520 nm, while data acquisition and analysis were accomplished using Software Empower 3. Compounds were identified by comparing the retention times and spectra with pure standards (HPLC grade).

2.6. Statistical Analysis

Determinations were conducted in triplicate, and the results were expressed as percentages, means, and standard deviations (\pm). Statistical analysis was performed using Tukey's mean comparison test ($p < 0.05$) with Statistica® (Software, USA).

3. Results and Discussion

3.1. Grape Pomace Flour (GPF)









GPF production yielded 13 kg, resulting in % overall yield of 76.5%. The yield obtained in GPF was higher than that determined in Pinot noir grape pomace flour from white wine from Brazil (11%) [29].

3.1.1. Color Analysis in GPF

Visual perception typically serves as the first impression of food, and color influences acceptability [30]. The determination of the color parameters at different particle sizes is shown in Table 1. These findings reveal a relationship between particle size and color. An increase in the L^* value was observed as particle size decreased, while the a^* and b^* values decreased with smaller particle sizes of GPF. Furthermore, the table indicates that the color obtained from the size fraction below 180 μ m closely resembles the characteristic color of grapes. This aligns with the average particle size recommended for flours by the Mexican Official Standard NOM-247-SSA1-2008 [31], validating this particle size as appropriate for GPF. A smaller particle size also directly affects the digestion of the nutrients present in the flour [30]. Tukey's mean comparison test ($p < 0.05$) indicates that there are no significant differences in " L^* " values for particle sizes <180 μ m and 250 μ m; however, significant differences are observed for 425 μ m and >425 μ m. The results for parameter " a^* " revealed that all values are statistically different, while the results for parameter " b^* " exhibited a trend similar to that of parameter " L^* ". Based on the above, a particle size of less than 180 μ m was chosen for subsequent studies.

The color of GPF indicates antioxidant quality; the color of pomace is mainly attributed to phenolic compounds, which have important antioxidant properties. To continue this interesting topic, the final section of the manuscript will be the determination and identification of phenolic compounds.

Table 1. Relationship Between GPF Color and Particle Size.

Sieve size (μm)	L*	a*	b*	Calculated Color	Visual Evidence
> 425	24.8 ± 0.72 b	14.23 ± 0.65 d	15.18 ± 0.95 b		
425	25.36 ± 0.82 b	15.27 ± 0.13 c	14.17 ± 0.88 b		
250	29.76 ± 0.60 a	17.37 ± 0.39 b	1.13 ± 0.08 a		
< 180	30.34 ± 0.66 a	12.97 ± 0.14 a	1.08 ± 0.11 a		

Means with different letters are significantly ($p < 0.05$) different (Tukey's test). Mean value ± standard deviation.

3.2. Nutritional Properties of GPF

3.2.1. Determination of Moisture, Ash, Minerals, Fat, Protein, fiber and Carbohydrate

Results presented in Table 2 indicate that GPF has a moisture content of 4.4 ± 0.17 %, which is within the permissible limit set by CODEX STAN 152-1985, which states that moisture in flour should not exceed 15%. The moisture value determined in GPF is lower than those reported in other agro-industrial waste flours such as chontaduro flour (11%) [32], cocoa shell flour (10%) [33], and quinoa flour (10%) [34].

Table 2. Physicochemical Characterization of Grape Pomace Flour.

Analysis (%)	GPF
moisture	4.4 ± 0.17
ash	13.7 ± 0.08
protein	11.9 ± 0.71
fiber	14.9 ± 0.69
fat	9.4 ± 0.26
carbohydrates	39.43 ± 0.01

The ash content determined in GPF was 13.7%. As shown in Table 3, the main minerals found in Mexican grape pomace flour were potassium, magnesium, calcium, phosphorus, silicon, chlorine, and iron. Potassium, calcium, and phosphorus have been previously reported as the main minerals in European grape pomace [35]. However, in the case of Mexican grape pomace, magnesium was the

second largest mineral found after potassium, so it can be established that this residue is a good source of magnesium. Magnesium is essential in the human body; this mineral is essential in energy production, the construction of proteins and nucleic acids, insulin function, healthy nerve and muscle function, the immune system, heart rate stability, and blood circulation [36]. Potassium, the main mineral found, is considered an essential mineral to help maintain osmotic balance in humans and plays an essential role in reducing blood pressure and the risk of osteoporosis due to reduced urinary calcium excretion [37]. Calcium, another mineral in Mexican grape pomace in high concentrations, is essential for forming and maintaining bones and skeletal integrity [38]. Nutritional insecurity is associated with a deficiency of micronutrients that play an essential role in the normal metabolic functioning of the human body [39], and agro-industrial waste such as grape pomace can be a very good source of these micronutrients [40].

Table 3. Minerals of GPF via X-Ray Fluorescence.

K	Mg	Ca	P	S	Cl	Fe	Sr	Mn	Zn	*	**
49.31	22.26	20.10	4.00	2.56	0.54	0.45	0.19	0.09	0.08	<0.015	95.53

* Trace elements: Ti, Cu, Sn, Co, Ba, Te, Cs, Rb, Sb, I. ** Ignition losses.

The protein value determined in GPF (11.9%) (Table 2) was higher than that reported in grape pomace flour from ‘Vinhão’ variety from Portugal (9%) [41] and grape pomace from Quinta de Azevedo (9.8%) [42] and lower than that reported in Pinot noir grape pomace flour from white wine from Brazil (13%) [29]. The lower protein value in red wines compared to white wines is due to the alcoholic fermentation process [29].

Dietary fiber in GPF was 14.9%. Flours with 10 – 20 % fiber can help maintain intestinal health, reduce cholesterol, and regulate blood sugar levels [43]. The fiber content in GPF indicates that incorporating it into food products enhances their nutritional profile [44]. Overall, GPF has been recognized as an excellent opportunity to transform a waste product into a valuable bioproduct, thereby adding value to the wine industry.

The fat value obtained in this study for GPF was 9.4%. This result was higher than that reported for grape pomace flour from the cultivar ‘Vinhão’ from Portugal (3.3%) [41] and grape husk flour from the cultivar Marselan (5.1%) from Brazil [42]. The higher lipid content in Mexican grape pomace may be due to the greater presence of seeds.

3.3. Functional Properties of GPF

3.3.1. Determination of Water Solubility Index (WSI), Swelling Power (SP), Water Absorption Index (WAI), and Oil Absorption Index (OAI)

WAI reflects the amount of water that the flour can retain. As shown in Figure 2, GPF significantly exhibited the highest WAI value (2.64 ± 0.06 g/g), followed by whole wheat flour (HTi) (2.15 ± 0.01 g/g) and regular wheat flour (HT) (1.86 ± 0.02 g/g). The WAI value for GPF exceeds those reported for other non-conventional flours, such as rice flour (1.25 ± 0.01 g/g), amaranth flour (0.96 ± 0.01 g/g), quinoa flour (1.15 ± 0.04 g/g), and chickpea flour (1.92 ± 0.09 g/g) [45]. A higher water holding capacity is advantageous as it enhances the texture of the products, and these elevated values are attributed to the higher fiber content in the flour [46].

The determination of OAI revealed that GPF (2.04 ± 0.03 g/g) did not present a significant difference with respect to HT (2.02 ± 0.03 g/g) and HTi (2.08 ± 0.02 g/g) (Figure 2).

The OAI value of GPF (2.04 ± 0.03 g/g) is higher than the 1.4 ± 0.3 g/kg reported in quinoa seeds [47] and is comparable to values found in some cereals, such as rice [48]. This is advantageous, as a higher oil absorption capacity is desirable in bioproducts intended for baking applications [47].

In the present study, the determination of SP revealed that GPF (2.80 ± 0.05 mL/g) was significantly higher than the values obtained for HT and HTi flours (1.89 ± 0.02 mL/g and 2.17 ± 0.01

mL/g, respectively). The SP values indicate a strong ability of the flour to increase in volume by absorbing liquids; this characteristic enhances the overall texture of bioproducts and is crucial for preparing viscous foods such as doughs and baked goods, ensuring proper interaction between proteins and water [49].

Finally, in the essay on WSI, GPF (5.75 ± 0.61 %) was significantly higher than HT and HTi (1.75 ± 0.38 % and 1.92 ± 0.12 %, respectively). The WSI for GPF is comparable to that of banana flour (5.50 ± 0.38 %) [50] and blue corn flour (5.70 ± 0.04 %) [51]. These studies suggest that the solubility index is related to the soluble solids content in the flour, and the WSI measures the amount of dissolved matter in water, indicating the degradation of components within the food matrix [52].

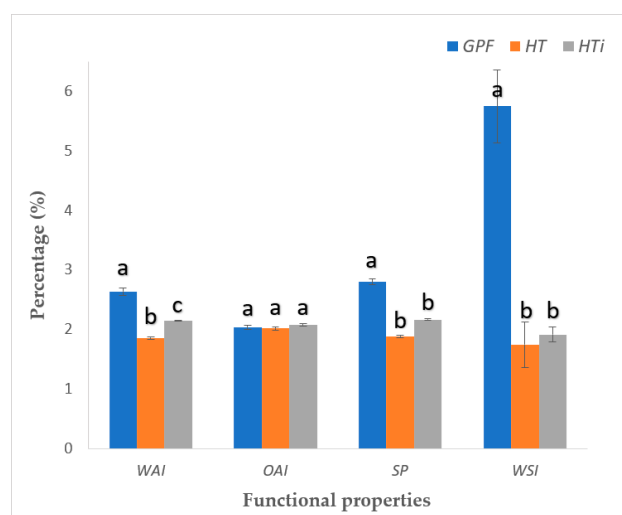


Figure 2. Results of the water solubility index (WSI), swelling power (SP), water absorption index (WAI), and oil absorption index (OAI) analyses for grape pomace flour (GPF), wheat flour (HT) and whole wheat flour (HTi). Means with different letters are significantly ($p < 0.05$) different (Tukey's test).

The moisture analysis of the evaluated flours revealed that GPF has a significantly lower moisture content (4.87%) compared to the two commercial flours: HT (11.04%) and HTi (11.03%). GPF is a bioproduct well within the maximum moisture limit (15%) for flours set by Mexican Official Standard NOM-247-SSA1-2008 [31]. Moisture content is critical in product quality and shelf life of flours and influences the preservation and susceptibility to deterioration by microorganisms [53].

3.3.2. Determination and Identification of Bioactive Compounds of GPF

3.3.3. Determination of Total Polyphenolic Content

The total polyphenol content of grape pomace flour (GPF) showed that it is a significant source of these compounds, with a value of 0.97 ± 0.03 mg GAE g^{-1} . The results obtained are higher than those of some studies that tested different extraction methodologies for bioactive compounds from grape pomace. Monteiro, 2021 [54] obtained 0.30 ± 0.261 mg GAE g^{-1} , using a mixture of reagents (methanol: water) for extraction, combined with ultrasound. Aresta et al., 2020 [55] obtained 0.57 ± 0.01 mg GAE g^{-1} for a supercritical fluid extraction using carbon dioxide and ethanol as a co-solvent to increase the low polarity of carbon dioxide and facilitate the extraction of polar compounds.

Grape pomace flour, a rich source of total polyphenols (TPC), is an excellent option for developing functional and nutraceutical foods due to its antioxidant, anti-inflammatory, and anticancer properties. Polyphenols are the most abundant antioxidants in the human diet, widely present in plant-based foods and beverages. Scientific evidence suggests that regular consumption of foods rich in these compounds protects against cardiovascular and degenerative diseases, cancer, and diabetes. In addition to its potential as a functional ingredient, including grape pomace flour in various food matrices would improve nutritional value and contribute to sustainability through the

utilization of agricultural by-products, enhancing its potential in the food industry. It is also important to highlight that polyphenols are rapidly absorbed and metabolized in the human body, with the gut microbiota playing a key role in this process.

3.3.4. Identification of Compounds by HPLC

The individual phenolic compounds of GPF extracts identified using HPLC are summarized in Table 4.

The chromatographic profile of GPF extracts indicated that phenolic acids and flavonoids are GPF’s most prevalent chemical classes. The phenolic compound identification analysis results showed that among the phenolic acids, gallic acid, protocatechuic acid, vinyl acid, syringic acid, and 4-caffeoylquinic acid were present. In the flavonoid class, rutin, kaempferol-3-O-glucoside, neodiosmin, and quercetin were identified. This result aligns with findings by Negro et al. (2021) [56], who reported that the primary polyphenols in grape pomace are phenolic acids and flavonoids. Additionally, Chakka (2022)[57] highlighted that grape pomace represents a significant source of bioactive, non-nutritive compounds, with phenolic acids being the most noteworthy.

The significance of phenolic acids in GPF is heightened by the wide array of functional compounds that offer health benefits. These compounds exhibit various functional properties, including antimicrobial, antioxidant, anticancer, anti-inflammatory, and antiviral activities [58,59], as well as neuroprotective, antibacterial, antiosteoporotic, analgesic [60,61], antivenom, cardioprotective, hepatoprotective, and free radical scavenging activities [62,63], alongside cardioprotective, antidiabetic, and antiendotoxic properties [64–66].

The functional properties provided by the presence of flavonoids in GPF include antioxidative, antihypertensive, antidiabetic, anti-inflammatory, cardioprotective activities, and improvements in metabolic function [68,69], as well as antirheumatic, antioxidant, neuroprotective, cardioprotective, anti-obesity, antiosteoporotic, anticancer, and antiulcer effects [59,70]; prevention of osteoporosis, antiviral [71,72]; and anti-allergy, blood pressure-lowering, and antiviral activities [73,74].

Table 4. Functional properties of the main compounds present in GPF, as identified by HPLC.

Compounds	Formula	Functional properties	References
<i>Phenolic acids</i>			
Gallic Acid	C ₇ H ₆ O ₅	Antimicrobial, antioxidant, anticancer, anti-inflammatory, and antiviral properties.	[58,59]
Protocatechuic Acid	C ₇ H ₈ O ₄	Antioxidant, anti-inflammatory, neuroprotective, antibacterial, antiviral, anticancer, antiosteoporotic, analgesia.	[60,61]
Vannillic Acid	C ₈ H ₈ O ₄	Anti-venom, anti-inflammatory, antimicrobial, cardioprotective, hepatoprotective, free radical scavenging, and antioxidant activities.	[62,63]

Syringic Acid	C ₉ H ₁₀ O ₅	Antioxidant, anti-inflammatory, hepatoprotective, cardioprotective, neuroprotective, antimicrobial, antidiabetic, and antiendotoxic	[64,65]
4-Caffeoylquinic acid	C ₁₆ H ₁₈ O ₉	Prevention/treatment of cancer, diabetes, cardiovascular, and inflammatory diseases.	[66,67]
<i>Flavonoids</i>			
Rutin	C ₂₇ H ₃₀ O ₁₆	Antioxidative, antihypertensive, antidiabetic, anti-inflammatory, and cardioprotective activities improve metabolic function.	[68,69]
Kaempferol-3-O-glucoside	C ₂₁ H ₂₀ O ₁₁	Antirheumatic, anti-inflammatory, antioxidant, neuroprotective, cardioprotective, antiobesity, antiosteoporotic, anticancer, antiulcer, and antidiabetic properties.	[56,70]
Neodiosmin	C ₂₁ H ₂₄ O ₁₃	Treatment of alcoholic liver disease, prevention of osteoporosis, antiviral.	[71,72]
Quercetin	C ₁₅ H ₁₀ O ₇	Anti-allergy, anticancer, blood pressure lowering, anti-inflammatory and antiviral activities.	[73,74]

3.4. Potential Applicability of Grape Pomace Flour (GPF)

The GPF could offer several potential applications within the value chain due to the combination of its nutritional characteristics and functional properties. Such applications include Food Industry as a functional ingredient for bakery and other cooked foodstuffs, highlighting its high dietary fiber, protein, and antioxidant-rich phenolic contents. GPF can absorb water and oil, enhancing the texture and stability of final products such as bread, cookies, and muffins while also reducing the need for fats and other additives in these formulations; in Healthy and Dietary Foods Developments, the inclusion of GPF could be beneficial, exploiting its low moisture and high fiber content as a promotor of gut health related to the potential prebiotic effect, also could control cholesterol, and aid in blood sugar management. Additionally, as a plant base by-product rich in natural antioxidants, it plays a significant role in the development of nutraceutical and functional foods, aiming the prevention of chronic diseases like obesity, hypertension, heart disease, diabetes, and cancer;

Natural Colorant and Flavor Substitute is another trend application; the natural color of GPF, derived from phenolic compounds, mostly anthocyanins, offers visual appeal for industries and final consumers and could be used as a natural additive in food products, especially in applications where a distinctive color is needed, avoiding synthetic additives; Gluten-Free Products, the GPF could be a sustainable attentive for gluten-free products production, making it suitable for people with gluten intolerance issues in products such as pasta, snacks, and cookies; Cosmetic and Pharmaceutical

Industries also can be reached due to the high antioxidant properties of GPF, which can boost natural skincare products and dietary supplements, exerting its anti-inflammatory and protective properties against oxidative damage.

4. Conclusions

Grape pomace flour (GPF) demonstrates exceptional functional and nutritional properties, positioning it as a promising ingredient for the food industry. Its ability to enhance texture and stability in baked goods and its high antioxidant and fiber content support its use in functional and health-oriented foods. Additionally, GPF provides aesthetic benefits as a natural colorant. This improves the nutritional profile of foods and promotes a circular bioeconomy by repurposing by-products from the wine industry.

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