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Functional and sensory evaluation of bread made from wheat flour fortified with wine byproducts

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Abstract

Grape pomace is the main byproduct of the wine industry and an important source of dietary fiber and phenolic compounds. Grape pomace powder (GPP) partially substituted 8, 10, 12, 15, and 25% of the wheat flour in bread formulations. The proximate composition, total dietary fiber content, phenolic compounds, texture profile, color, and bioaccessibility of phenolic compounds in vitro were measured in the bread. Bread sensory acceptance by consumers was determined using a 9-point hedonic scale. Compared with the control bread (CB), the 8% GPB-substituted bread presented the best results and exhibited an increase in total protein content (7.5%) and total dietary fiber content (6.1%). The total phenolic content was greater in GPB (5.1 mg GAE/g) than in CB (2.1 mg GAE/g). Adding GPP to the bread affected the color, and the color of the GPB-treated bread was darker than that of the CB-treated bread. Still, no significant differences were detected regarding the texture profile or consumer sensory acceptance between the GPB-treated and CB-treated bread. The in vitro *analysis* of phenolic compound bioaccessibility revealed no differences between the two samples during gastrointestinal digestion. GPP is an interesting byproduct that can be used in bakery. The replacement of 8% of the bread with GPP increased the nutritional content of the bread, particularly the protein, total dietary fiber, and total phenolic content, without affecting the texture or sensory acceptance of the bread. To understand the possible beneficial effect of GPB on consumers, further research on the bioavailability of phenolic compounds and the impact of dietary fiber increment needs to be assessed.

Keywords Grape pomace, Bread, Bioaccessibility, Sensory acceptance, Dietary fiber, Phenolic compounds

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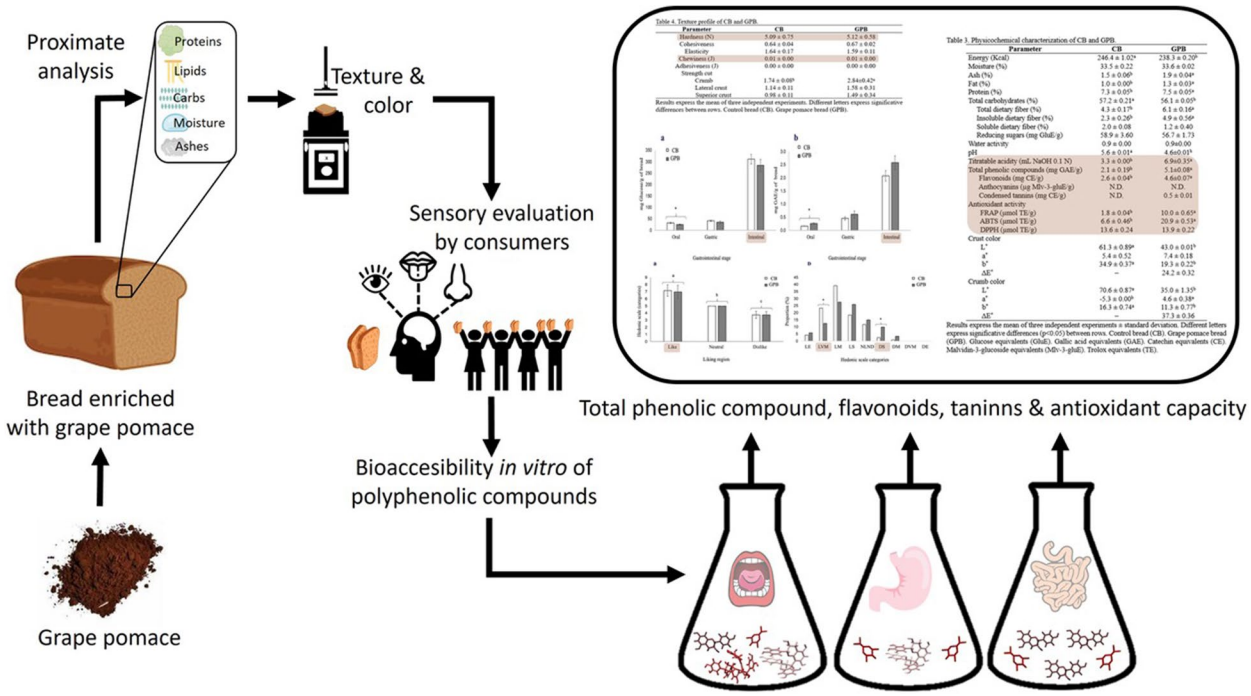
Graphical Abstract



Introduction

Grape pomace is the main byproduct generated during wine production and is composed of grape skins, seeds, small stalks, and yeasts from wine fermentation (García-Lomillo & González-SanJosé, 2017). Grape pomace is considered a functional ingredient due to its content of dietary fiber and phenolic compounds that can promote beneficial effects on health (Tolve et al., 2021). From an economic point of view, the grape pomace generated by the wine industry is used to produce distilled beverages, fertilizer, and animal food. Nevertheless, the disposal of grape pomace is costly for the industry and generates pollution problems (Antonić et al., 2020; Troilo et al., 2022). In this context, the circular economy, integrated into the 2030 UN agenda, proposes the use of by-products to maximize the use of primary resources by minimizing the generation of waste and promoting the integration of economic, social, and environmental prosperity (Rasera et al., 2024; United Nations, 2015).

The bioactive compounds in grape pomace have led food technologists to develop new food products that have health benefits for consumers. This is combined with the growing demand from consumers to obtain food products that offer the necessary nutrients and an aggregated value to health (Antonić et al., 2020; Hayta et al., 2014). In this sense, bakery goods have been one of the most explored food products for developing new functional foods, using grape pomace and other agricultural by-products, because they are consumed worldwide and because of their nutritional value (Boff et al., 2022; Chiranthika et al., 2024; Hayta et al., 2014; Zhang & Li, 2024). A high glycemic index characterizes bakery products since they are starch-based (Rocchetti et al., 2021). Partial substitution of wheat flour with nonconventional ingredients such as byproducts can be an option to decrease the glycemic index. Several studies have shown that byproducts from different fruits and vegetables are potential sources of dietary fiber and can be used as potential functional ingredients (Boff et al., 2022; González-Centeno et al., 2010; Guevara-Arauza et al., 2015; Ojukwu et al., 2013). Several baked products have been developed using grape pomace, such as muffins (Troilo et al., 2022), brownies (Walker et al., 2014), and white wheat bread (Tolve et al., 2021). However, integrating non-conventional ingredients presents a challenge regarding new products' sensory and textural characteristics. Developing new products requires a balance of incorporating dietary fiber and bioactive compounds without a loss of consumer acceptance (Czajkowska–González et al., 2021; Walker et al., 2014).

The fortification of white wheat bread with grape pomace is one of the most interesting options for designing a new functional food. White wheat bread is a starch source and has a low content of macro- and micronutrients (Tolve et al., 2021). Adding grape pomace to bread has been demonstrated to increase the content of phenolic compounds and total dietary fiber while increasing its antioxidant activity (Hayta et al., 2014; Tolve et al., 2021). Moreover, different percentages of grape pomace, ranging from 5 to 25%, were tested, and the results revealed that higher percentages of grape pomace modified the sensory and textural attributes of bread (Hayta et al., 2014; Tolve et al., 2021; Walker et al., 2014).

Different aspects need to be assessed to obtain a new functional food, such as the adequate percentage of grape pomace to minimize the detrimental effects on the texture, taste, and odor, among other characteristics, and consumer sensory acceptance. In addition, new functional foods must be developed to demonstrate that consumers can take up bioactive compounds incorporated in bread. This study aimed to produce a fortified bread with grape pomace with better nutritional value, potential prebiotic action, and an increase in bioactive compounds compared to the control bread and to evaluate the in vitro bioaccessibility of phenolic compounds in both bread samples.

Materials and methods

Collection of samples and preparation

Cabernet Sauvignon (*Vitis vinifera* L.) grape pomace from vintage 2018 was kindly donated by the winery Grupo Alximia, Baja California, Mexico. Grape pomace was collected immediately after fermentation. Grape pomace was stored at -20 °C in vacuum bags and transported to the Universidad Autónoma de Ciudad Juárez laboratory. Once in the laboratory, the samples were dried at 55 °C for 72 h until a constant weight was reached (Isotemp oven, Fischer Scientific®, Waltham, MA, USA). Dried grape pomace samples were ground and sieved to a particle size of 420 µm and stored in vacuum bags until use (grape pomace powder, GPP).

Bread formulation and preparation

All food-grade ingredients were purchased from local markets. The bread was produced according to the methodology described by Márquez Barraza (2016). The CB was formulated with wheat flour, water, vegetable oil, sugar, salt, and instant dry yeast. Five different enriched breads were prepared by replacing the wheat flour with 8, 10, 12, 15, or 25% of GPP. For each of these enriched breads, the amount of lipid coming from the GPP was considered, and the vegetable oil added was adjusted to keep the final lipid amount equal in all formulations (Table S1). The ingredients were added to a breadmaker machine (Model 29,881, Hamilton Beach®, Glen Allen, VA, USA) following the manufacturer's instructions. All the liquid ingredients were added, followed by dried ingredients. The breadmaker machine controlled the mixing, kneading, leavening, and baking processes. GPP was added after the first leavening (Figure S1). The breads were allowed to cool at room temperature for 1 h before analysis.

Consumer acceptance test

A preliminary acceptance test (9-point hedonic scale) was performed for CB and all the formulated GPB. Each preliminary test was executed with a total of 10 participants.

The preselected formulations of GPB (8%) and CB were evaluated in a consumer acceptance test by 120 healthy participants (21 ± 3 years, 60.8% males and 39.2% females). This protocol was approved by the institutional committee of ethics and bioethics of the Universidad Autónoma de Ciudad Juárez (CIEB-2019–1-051). We hereby certify that the study was performed following the 1964 Helsinki Declaration and comparable ethical standards. Before sensory evaluation, the participants were informed about the ingredients of each bread and asked about any allergy or intolerance (including sulfites) they might have to the ingredients. The test protocol was also explained, and the participants were requested to sign an informed consent letter. Each sample was evaluated in a single way. The participants were given 4 g of bread at room temperature. Each sample was placed in a plastic cup (2 oz) and labeled with three-digit random numbers. The position effect was reduced when half of the participants first evaluated the CB and the GPB, and the other half evaluated the GPB and then the CB. Participants were requested to rinse their mouths with purified water (Alaska®, Ciudad Juárez, Chihuahua, Mexico) before and between samples. They tasted each sample and used the hedonic scale to indicate the degree to which they liked it (Rodríguez-Tadeo et al., 2021).

Physicochemical characterization

After baking, the CB and GPB samples were cooled at room temperature for 1 h, ground in a food processor (Model NB-101S, NutriBullet®, Pacoima, CA, USA), and stored at 4 °C until further analysis. After cooling, the height of the samples was determined with a calibrated vernier.

The proximate composition and total dietary fiber content were determined following the AOAC (AOAC, 2000) methods: moisture by the oven method at 105 °C for 8 h (Model 1324, VWR®, Irving, TX, USA); ash in a muffle furnace (Model FE-340, Felisa®, Guadalajara,

Jalisco, Mexico) at 550 °C for 5 h; crude protein by the Kjeldahl method (Model RapidStill II, Labconco®, Kansas City, MO, USA) using nitrogen to protein conversion factor of 5.70 for bread and 6.25 for GPP; fat by the Soxhlet method (Model 2043, Soxtec™, Foss™, Hilleroed, Denmark); total carbohydrates by difference; dietary fiber by an enzymatic-gravimetric assay using an enzymatic kit TDF-100A-1KT (Merck®, St. Louis, MO, USA); and the insoluble dietary fiber fraction, which was determined, and the soluble dietary fiber fraction was calculated by difference. Water activity was determined with AQUALAB® (Model Serie 3, Meter Food, Washington, DC, USA) equipment, pH was determined with a potentiometric method (Model AB15 Plus, Accumet®, Westford, MA, USA), and titratable acidity was determined via a titration method. All determinations were carried out in triplicate (AOAC, 2000). The bread's energy (calorie) value was calculated according to the official Mexican standard (NOM-247-SSA1-2008), where carbohydrates provide 4 kcal/g, protein provides 4 kcal/g, and fat provides 9 kcal/g. Reducing sugars were determined by the 3–5, Dinitrosalycilic acid (DNS) reagent. In brief, 300 µL of sample or standard were mixed with 600 µL of DNS reagent (DNS 0.04 M, potassium sodium tartrate 1 M, sodium hydroxide 0.4 M). The reaction was incubated at 100 °C for 10 min in a water bath; after this period, the reaction was cooled at 25 °C. Then, 250 µL were transferred in 96 well-plate. The reaction was measured at 540 nm in a well-plate spectrophotometer (xMark, Biorad®, Hercules, CA, USA). Glucose was used as standard, and the results are expressed as mg glucose equivalents/g fresh weight (FW) (mg GluE/g FW) (Teixeira et al., 2012).

Textural analysis was performed by a texture analyzer (Model TAPlus, Lloyd Instrument®, Bognor Regis, UK) according to the protocol of Tamsen et al. (2018). Bread profile texture (TPA) was analyzed over the GPB, CB crumb, and crust. A cubic sample of crumb (2.0×2.0×2.0 cm) was compressed twice using a 5 cm flat probe with a 0.3 N load cell and a 70% compression ratio at a speed of 1.5 mm/s. The TPA parameters obtained from the force–deformation curve were used to determine the hardness, elasticity, cohesiveness, chewiness, and adhesiveness.

Cutting tests were performed on the crumb and crust. The sample dimensions were as follows: crumb, 4×2 cm (width×height); crust, 2×6 cm (width×length). The samples were placed at the base platform and cut to a depth of 30 mm using a 0.3 N load cell at a speed of 1.50 mm/s.

The cohesiveness and adhesiveness of the bread dough were measured. Samples of 2 g of bread dough were rounded into spheres and compressed using a 5 cm diameter flat probe with a 0.3 N load cell and a 70% compression ratio at a speed of 1.5 mm/s.

The color of the CB and GPB samples was measured using a colorimeter (Model CR-400, Konica Minolta®, Ramsey, NJ, USA). Ten measurements were taken at the surface of the bread crust and crumb for the GPB and CB. The values of L* (lightness), a* (-a* green, +a* red) and b* (-b* blue, +b* yellow) were registered. The color change (ΔE) was calculated using Eq. 1:

$$\Delta E = \sqrt{(L_0 - L)^2 + (a_0 - a)^2 + (b_0 - b)^2} \quad (1)$$

where L_0, a_0, and b_0 are the values obtained for the CB. L, a, and b are the values obtained for the GPB.

Phytochemical analysis

For the CB and GPB breads, freshly baked samples were cooled at room temperature for 1 h, ground, frozen at -80 °C, lyophilized (Model Freezone 6, Labconco®, Kansas City, MO, USA) for 72 h, powdered, and sieved to a particle size of 420 µm. GPP, CB, and GPB were defatted with hexane 1:10 $_{(w/v)}$, as previously reported by Muñoz-Bernal et al. (2023).

Phenolic compounds were extracted from the samples at a 1:25 $_{(w/v)}$ ratio. Two grams of sample was mixed with 50 mL of 0.2% HCl $_{(v/v)}$, acidified with 80% MeOH $_{(v/v)}$, sonicated (B5000, Branson®, Brookfield, CT, USA) for 30 min, and centrifuged at 2465 g for 15 min at 4 °C (3000 rpm in a TX-400 rotor, Model Sorvall 16R, Thermo Scientific®, Waltham, MA, USA). The supernatant was collected, and methanolic extraction was repeated once again. The resulting pellet was re-extracted with 70% $_{(v/v)}$ acetone, sonicated for 30 min, and centrifuged at 2465×*g* (3000 rpm) for 15 min at 4 °C. The methanolic and acetonic extract supernatants were stored at -20 °C until further analysis.

Spectrophotometric methods to determine phenolic compounds were carried out according to Muñoz-Bernal et al. (2023). The phenolic compound content was determined using the Folin-Ciocalteu method. In brief, 25 µL of sample or standard were poured into a 96-well plate, then 100 µL of sodium carbonate (7.5% $_{w/v}$) and 125 µL of Folin-Ciocalteu reagent (10% $_{v/v}$) were added. The well-plate spectrophotometer (xMark, Biorad®, Hercules, CA, USA) was set at 765 nm and 45 °C and programmed to read at 1 min intervals for 15 min. Gallic acid was used as standard, and the results are expressed as mg of gallic acid equivalents per g of FW (mg GAE/g FW). Flavonoids were quantified via complexation with aluminum chloride ($AlCl_3$). In a 96 well-plate, 31 µL of standard or sample were diluted with 125 µL of distilled water. Then, 9.5 µL of sodium nitrite (5% $_{w/v}$), 9.5 µL of aluminum chloride (10% $_{w/v}$), and 125 µL of hydroxide

sodium (0.5 M) were added to the plate. The reaction was incubated for 30 min at room temperature and measured at 510 nm in a well-plate spectrophotometer. Catechin was used as standard, and the results are expressed as mg of catechin equivalents per g of FW (mg CE/g FW). Condensed tannins were measured through the reaction with the *p*-dimethylamminocinnamaldehyde reagent (DMAC). In a 96 well-plate, 50 µL of standard or sample were mixed with 250 µL of DMAC reagent (0.1% $_{w/v}$ in acidified methanol (10% $_{v/v}$). The reaction was incubated for 10 min at room temperature and in light absence. The reaction was measured in a well-plate spectrophotometer at 640 nm. Catechin was used as standard, and the results are expressed as mg of catechin equivalents per g of FW (mg CE/g FW). Anthocyanins were quantified using the pH differential method. In a test tube, 250 µL of sample were mixed with 2 mL of potassium chloride (2 M; pH 1). The mixture was incubated for 30 min at room temperature and in light absence. In another test tube, 250 µL of the sample was mixed with 2 mL of sodium acetate (2 M; pH 4.5). The mixture was incubated for 20 min at room temperature and light absence. After incubation, 300 µL of each tube was placed in a 96 well-plate. Absorbance was measured at 520 and 700 nm. The results are expressed as mg of malvidin-3-glucoside equivalents g of FW (mg Mv-3-gluE/g FW).

Phenolic profile by UHPLC-MS/MS

The phenolic profiles of CB and GPB were assessed according to the methodology described by Muñoz-Bernal et al. (2023). Methanolic and acetonic extracts from bread samples (500 µL) were filtered through a 0.45 µm nylon syringe filter (Titan 3, Thermo Scientific®, Waltham, MA, USA). The samples were analyzed in a 1290 Infinity series high-performance liquid chromatography (HPLC) system (Agilent Technologies, Inc., Santa Clara, CA, USA). The system was equipped with a 1290 Infinity quaternary pump with a built-in degasser, a 1290 Infinity autosampler with temperature control, a 1290 thermostated column compartment, and a 1290 Infinity diode array detector. The detector was set at the following wavelengths: 220, 320, and 370 nm. A reversed-phase C_{18} column (ZORBAX®, Agilent Technologies, Inc., Santa Clara, CA, USA) separated the compounds at 25 °C. The mobile phase conditions were as follows: mobile phase A was composed of 0.1% formic acid in water, and mobile phase B was composed of 100% acetonitrile. The gradient conditions were as follows: 0–1 min, 10% B; 1–4 min, 30% B; 4–6 min, 38% B; and 6–8.5 min, 60% B; and 8.5–10 min, 10% B. The sample injection volume was 3 µL, and the flow rate was 0.4 mL/min. The mass spectrometer system was an Agilent 6530 Accurate-Mass Q-TOF–MS/MS instrument equipped with an electrospray ionization (ESI) source operated in negative mode. Nitrogen was used as the drying gas at 340 °C and 13 L/min, the nebulizer gas pressure was 30 psi, the capillary voltage was 4000 V, the fragmentor voltage was 175 V, the skimmer voltage was 65 V, and the mass-to-charge ratio (m/z) scan range was 100–1100 for MS and 100–1000 for MS/MS. Phenolic compound identification in the samples was carried out using the software Mass Hunter Qualitative version B.07.00 (Agilent Technologies Inc., Santa Clara, CA, USA) according to the methodology described by Muñoz-Bernal et al. (2022).

Antioxidant capacity

The methods used to assess the antioxidant activity of the samples were carried out according to the methodology described by Muñoz-Bernal et al. (2020). FRAP, $ABTS^+$, and $DPPH^*$ methods were used to determine the antioxidant capacity of the bread samples. In brief, to determine the antioxidant activity by FRAP method, 24 µL of sample or standard were mixed with 180 µL of FRAP reagent (TPTZ 10 mM in HCl 40 mM, iron chloride hexahydrate 20 mM, acetate buffer 0.3 M, pH 3 in a ratio 1:1:10, prepared daily) in a 96 well-plate. The reaction was incubated at 37 °C for 30 min and in light absence. Absorbance was measured in a well-plate spectrophotometer at 595 nm. Trolox was used as standard, and the results were expressed as micromole Trolox equivalent per g of FW (µmol TE/g FW). To measure the antioxidant activity of samples by $ABTS^+$, 12 µL of sample or standard were mixed with 285 µL of $ABTS^+$ reagent 45 µM (in phosphate buffer 0.1 M, pH 7.4, potassium persulfate 0.5 mM) in a 96 well-plate. The reaction was incubated for 5 min at room temperature. Absorbance was measured at 734 nm in a well-plate spectrophotometer. Trolox was used as standard, and the results were expressed as µmol TE/g FW. The antioxidant capacity measured by $DPPH^*$ was as follows: 25 µL of sample or standard were mixed with 180 µL of $DPPH^*$ reagent 6 mM (dissolved in methanol) in a 96 well-plate. The reaction was incubated for 10 min at room temperature and in light absence. Absorbance was measured at 517 nm in a well-plate spectrophotometer. Trolox was used as standard, and the results were expressed as µmol TE/g of FW.

In vitro bioaccessibility of bread phenolic compounds.

A static in vitro digestive model was used to predict the gastrointestinal releasability of phenolic compounds from CB and GPB samples (Kopf-Bolanz et al., 2012). *Oral phase*: 300 mg of ground sample was placed in an Erlenmeyer flask, and 2.25 mL of distilled water was added, followed by the addition of 3 mL of synthetic saliva (Table S2) and 62.7 µL of an enzymatic salivary mixture (Table S3). The flasks were incubated in a water

bath at 37 °C and 70 rpm for 5 min (Dubnoff Shaker Bath, Precision®, Winchester, VA, USA). *In the gastric phase*, 6 mL of synthetic gastric juice (Table S2) was added, followed by 168.9 µL of the enzymatic gastric mixture (Table S3). Flasks were incubated in a water bath at 37 °C and 70 rpm for 120 min. *Intestinal phase*: 6 mL of synthetic pancreatic juice (Table S2), 3 mL of synthetic bile juice (Table S2), 564.9 µL of enzymatic pancreatic mixture (Table S3), and 624.38 µL of bile enzymatic mixture (Table S3). The flasks were incubated in a water bath at 37 °C and 70 rpm for 120 min.

Once each digestion phase was completed, a 500 µL aliquot was taken. To avoid overestimating phenolic compound content, carbohydrates were removed using a reversed-phase C18 solid-phase extraction cartridge (Model 57,064, Supelclean® ENVI®-18, Sigma Aldrich, St. Louis, MO, USA). The cartridge was activated with 6 mL of methanol and then equilibrated with 6 mL of ultrapure water. 500 µL of the sample was poured into the cartridge. To elute the sugars, 6 mL of ultrapure water was passed through the cartridge. Finally, 6 mL of methanol was passed through the cartridge to recuperate the phenolic compounds. Phenolic content was determined in the sugar-free samples, and the bioaccessibility was determined using Eq. 2:

$$\%\ bioacessibility = \frac{CF_D}{CF_i} \times 100 \quad (2)$$

where CF_D is the phenolic content after the in vitro digestion process, and CF_i is the phenolic content before the in vitro digestion process.

Data analysis

The data were analyzed using Levene's test to assess homoscedasticity and subsequently analyzed using the Student's t-test. The chi-squared test analyzed data from the consumer acceptance test, comparison of two proportions, and one-way ANOVA with Fisher's least significant difference (LSD). A difference was considered statistically significant when P was ≤ 0.05. The statistical analysis was performed using the program XLSTAT version 2019.4.2 (Addinsoft®, Paris, Francia). The results are expressed as the mean ± standard deviation (SD).

Results and discussion

Grape pomace powder composition

Table 1 shows the physicochemical and phytochemical composition of the Cabernet Sauvignon grape pomace powder (GPP).

The values obtained for moisture, ash, lipids, protein, and total carbohydrates agree with those reported by previous authors for GPP (Difonzo et al., 2023; Nakov et al., 2020). Nakov et al. (2020) compared the physicochemical composition of GPP and wheat flour and observed higher values of ash, lipids, proteins, and total carbohydrates in GPP than in wheat flour. The ash content in GPP is explained by the presence of minerals in the grape skins (Difonzo et al., 2023). The GPP was greater for lipids than that reported previously for wheat flour (1.71 – 2.02%). Seeds from grape pomace are the main source of lipids (Difonzo et al., 2023). Grape seeds contain fatty acids such as linoleic, oleic, and palmitic acids (García-Lomillo & González-SanJosé, 2017; Ribéreau-Gayon et al., 2006). According to Difonzo et al. (2023), the protein content in grape pomace ranges between 6 and 15%. A previous study by Gazzola et al. (2014) reported that seeds from grapes are a source of globulins and albumins. These proteins differ from those observed in wheat flour, gliadin, and glutenin (Posner, 2009). GPP presented a lower total carbohydrate content than wheat flour (71.00 – 82.41%) (Eshak, 2016; Ojukwu et al., 2013). The total dietary fiber content of GPs was greater than that reported for wheat flour (10.37%) (Ojukwu et al., 2013). The total dietary fiber in GPP is related to wall cell polysaccharides from grape seeds, mainly cellulose, pectin, and cellulose. On the other hand, stems contain lignin and hemicellulose (González-Centeno et al., 2010; L. Zhang et al., 2017).

Phenolic composition in grape pomace is influenced by the cultivar, soil, climate, and the winemaking

Table 1 Physicochemical and phytochemical composition of the grape pomace powder (GPP)

Parameter	GPP
Energy (kcal/100 g)	189
Moisture (%)	3.8 ± 0.07
Ash (%)	7.0 ± 0.02
Lipids (%)	10.7 ± 0.30
Protein (%)	10.9 ± 0.13
Total carbohydrates (%)	67.6 ± 0.11
Total dietary fiber (%)	55.4 ± 0.65
Reducing sugars (mg GluE/g)	96.0 ± 5.0
Total phenolic compounds (mg GAE/g)	68.2 ± 0.65
Flavonoids (mg CE/g)	38.7 ± 2.44
Anthocyanins (µg Mav-3-gluE/g)	286.5 ± 6.48
Condensed Tannins (mg CE/g)	11.2 ± 0.71
Antioxidant activity	
FRAP (µmol TE/g)	292.4 ± 13.55
$ABTS^+$ (µmol TE/g)	503.2 ± 28.55
$DPPH^*$ (µmol TE/g)	448.1 ± 17.43

The values are expressed as the means of three independent samples ± standard deviations. Results are expressed per gram of grape pomace powder (g)

+ and * relect to positively charged and neutral ions

GluE Glucose equivalents, *GAE* Gallic acid equivalents, *CE* Catechin equivalents, *TE* Trolox equivalents

process (Difonzo et al., 2023). Differences in the phenolic content observed in GPP used in this study can be attributed to these agro-industrial conditions. The results showed that the main fraction of phenolic compounds in GPP was flavonoids, followed by condensed tannins. Contrary to what was expected, only insignificant amounts of anthocyanins were detected in GPP. This can be attributed to the drying process. According to Patras et al. (2010), anthocyanins are susceptible to thermal degradation at temperatures above 50 °C. In the present study, the drying process was conducted at 55 °C for 72 h, which can affect this phenolic fraction in GPP.

Furthermore, the antioxidant activity of GPP was determined by three different methods (Table 1). According to the antioxidant activity results, the antioxidant activity of GPP was greater in the $ABTS^{+}$ assay than in the $DPPH^{*}$ and FRAP assays. The mechanism by which phenolic compounds can function as antioxidants differs and depends on the compound structure, the solvent used, and the pH value (Shahidi & Zhong, 2015). According to Schaich et al. (2015), $ABTS^{+}$ is related to small phenolic compounds since, in complex phenolic compounds, the phenolic ring can interfere with electron transfer to the $ABTS^{+}$ radical. This can be related to the phytochemical content where condensed tannins and anthocyanins were present in lower amounts.

The results from the physicochemical composition of GPP showed that partial substitution in wheat flour bread can improve a bakery product by adding phenolic compounds and increasing its antioxidant activity. Moreover, fortifying a wheat flour bread with GPP can modify the fatty acids profile, increase protein content, and modify the total dietary fiber in the final product.

Bread formulation

Grape pomace bread (GPB) was prepared at five different GPP- wheat flour substitutions: 8, 10, 12, 15, and 25%. The various formulations presented changes in appearance, such as color, weight, and height. The breadmaking process was modified to allow proteins in the wheat flour to work correctly. GPP was added to the dough during the first leavening, just before the second kneading. Figure 1 shows the effect of adding GPP to the bread on the height and weight of the bread. The height of the bread decreased as the GPP percentage increased. This reduction in height can be attributed to interactions between phenolic compounds and dietary fiber from GPP with wheat flour proteins. According to Xu et al. (2019), phenolic compounds can interact with gluten proteins via covalent and non-covalent bonds with the hydroxyl groups of phenolic compounds. Also, the interaction between phenolic compounds and proteins can modify their secondary and tertiary structures (Xu et al., 2019). In a previous study by Pycia and Ivanišová (2020), the enrichment of bread with hazelnuts and walnuts reduced loaf volumes. Also, a significant linear correlation was reported between the bread volume and the total phenolic and flavonoid content. On the other hand, replacing flour with GPP can result in a reduced proportion of gluten proteins and, as a result, a weakened gluten network (Pycia & Ivanišová, 2020).

According to Posner (2009), bread volume is related to flour protein, and protein content also contributes to increased gluten in dough, contributing to water absorption and retention. The effect on water absorption and

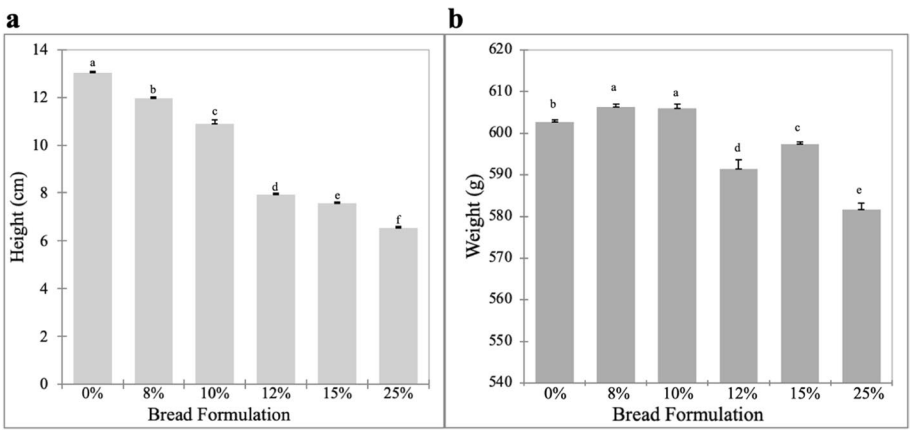


Fig. 1 **a** Height observed in each bread formulation. **b** Weight registered for each bread sample. Control bread (CB = 0%). Grape pomace powder addition expressed in %. The results are expressed as the mean ± standard deviation from three independent experiments. Different letters express significant differences ($P < 0.05$)

retention can also be related to the weight of the bread, as gluten networks are interrupted by the presence of phenolic compounds from GPP, and less water retention is observed in the bread. A previous study by Bock and Damodaran (2013) investigated the effect of adding bran to dough on water retention and gluten structure. They reported that wheat bran affects the dough's hydration by water redistribution and results in gluten dehydration.

A preliminary sensory analysis revealed that as GPP substitution increased, consumer acceptance decreased (data not shown). It has been reported that the range of substitution of GPP in different bakery products is between 5 and 10%, which is acceptable to consumers (Walker et al., 2014). Based on these preliminary results, 8% GPP was selected for subsequent experiments (GPB).

Microbiological analysis

Before any formulation development, a microbiological analysis was conducted on the GPP to assess food safety in the final product. All evaluated microorganisms (aerobic mesophiles, total coliforms, molds, and yeasts) presented values much lower than the maximum limit established in the Mexican Official Standard NOM-247-SSA1-2008 (Table S4). According to these results, GPP was safe for use in bakery products.

Acceptance testing of grape pomace bread

One of the most critical steps in developing functional foods is consumer acceptance of the food; the consumer's appreciation of foods is related to visual and sensorial impression (Nakov et al., 2020). Sensory acceptance testing was performed on 120 habitual consumers of bread. Of the total sample, 60.83% were men ($n=73$), and 39.17% were women ($n=47$). The average age was 21 ± 3 years. Before tasting, volunteers were given a consumption frequency questionnaire. The 9-point hedonic scale was separated into three acceptance regions: liking, neutral, and dislike. Figure 2a shows that control bread (CB) and GPB were in the liking region, and no significant difference was detected between the samples ($P<0.01$). Consumers accepted both samples. The results for the 9-point hedonic scale are shown in Fig. 2b. According to these results, the scale "like very much" presented a significant difference between CB and GPB ($P=0.03$), where CB was preferred over GPB. In addition, the scale "dislike slightly" presented a significant difference, where GPB was more disliked than CB ($P=0.01$).

In general, 71.7% of the participants evaluated the GPB on the scales of "like extremely," "like very much," and "like slightly." These results indicate that consumers accepted both bread samples and that a GPP of 8% in the GPB did not affect consumer acceptance, in contrast to CB. The results obtained in the present study are in accordance with those obtained by Smith and Yu (2015), where bread with a GPP from Cabernet Sauvignon at 5 and 10% presented a general consumer acceptance similar to that of a control bread. Nakov et al. (2020) evaluated the sensory acceptance of cakes enriched with GPP. The authors evaluated different addition levels (4, 6, 8, and 10%) and reported that the best evaluation was the cakes enriched at 4%. The authors justified this behavior by stating that lower quantities of addition impart better sensorial characteristics to the product. This effect can explain the results from this research; lower levels of enrichment with GPP (8%) may help to obtain a similar consumer acceptance in GPB than CB.

Physicochemical composition of bread

Table 2 shows the physicochemical compositions of CB and GPB. CB presented a greater energetic value than GPB ($P<0.01$). This decrease in the energetic value of GPB is related to its lower content of total carbohydrates ($P<0.01$) and higher content of total dietary fiber ($P<0.01$). The moisture content in both bread

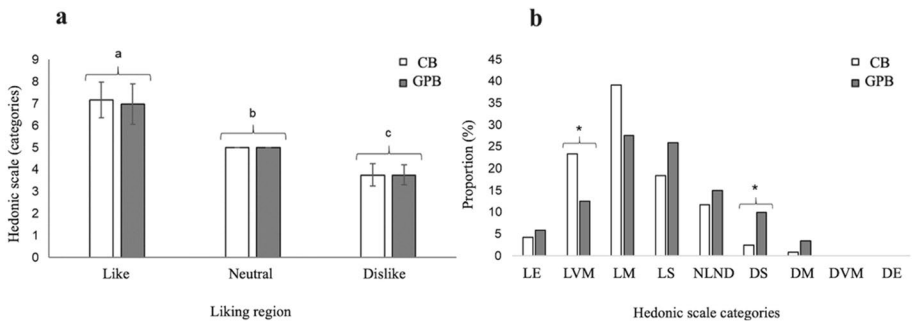


Fig. 2 **a** Acceptance region of the control bread (CB) and grape pomace bread (GPB). Different letters indicate significant differences between regions ($P<0.05$). **b** Hedonic scale results. LE = Like extremely; LVM = Like very much; LM = Like much; LS = Like slightly; NLND = Nor like nor dislike; DS = Dislike slightly; DM = Dislike much; DVM = Dislike very much; DE = Dislike extremely. * Indicates a significant difference between samples ($P<0.05$)

Table 2 Physicochemical characterization of CB and GPB

Parameter	CB	GPB
Energy (Kcal)	246.4 ± 1.02^{a}	238.3 ± 0.20^{b}
Moisture (%)	33.5 ± 0.22^{a}	33.6 ± 0.02^{a}
Ash (%)	1.5 ± 0.06^{b}	1.9 ± 0.04^{a}
Lipids (%)	1.0 ± 0.00^{b}	1.3 ± 0.03^{a}
Protein (%)	7.3 ± 0.05^{b}	7.5 ± 0.05^{a}
Water activity	0.9 ± 0.00^{a}	0.9 ± 0.00^{a}
pH	5.6 ± 0.01^{a}	4.6 ± 0.01^{b}
Titratable acidity (mL NaOH 0.1 N)	3.3 ± 0.00^{b}	6.9 ± 0.35^{a}
Total carbohydrates (%)	57.2 ± 0.21^{a}	56.1 ± 0.05^{b}
Total dietary fiber (%)	4.3 ± 0.17^{b}	6.1 ± 0.16^{a}
Insoluble dietary fiber (%)	2.3 ± 0.26^{b}	4.9 ± 0.56^{a}
Soluble dietary fiber (%)	2.0 ± 0.08^{a}	1.2 ± 0.40^{a}
Reducing sugars (mg GluE/g FW)	58.9 ± 3.60^{a}	56.7 ± 1.73^{a}
Crust color		
L^{*}	61.3 ± 0.89^{a}	43.0 ± 0.01^{b}
a^{*}	5.4 ± 0.52^{b}	7.4 ± 0.18^{a}
b^{*}	34.9 ± 0.37^{a}	19.3 ± 0.22^{b}
ΔE^{*}	–	24.2 ± 0.32
Crumb color		
L^{*}	70.6 ± 0.87^{a}	35.0 ± 1.35^{b}
a^{*}	-5.3 ± 0.00^{b}	4.6 ± 0.38^{a}
b^{*}	16.3 ± 0.74^{a}	11.3 ± 0.77^{b}
ΔE^{*}	–	37.3 ± 0.36
Total phenolic compounds (mg GAE/g FW)	2.1 ± 0.19^{b}	5.1 ± 0.08^{a}
Flavonoids (mg CE/g FW)	2.6 ± 0.04^{b}	4.6 ± 0.07^{a}
Anthocyanins (μg Mlv-3-gluE/g FW)	N.D	N.D
Condensed tannins (mg CE/g FW)	N.D	0.5 ± 0.01
Antioxidant activity		
FRAP (μmol TE/g FW)	1.8 ± 0.04^{b}	10.0 ± 0.65^{a}
$ABTS^{+}$ (μmol TE/g FW)	6.6 ± 0.46^{b}	20.9 ± 0.53^{a}
$DPPH^{*}$ (μmol TE/g FW)	13.6 ± 0.24^{a}	13.9 ± 0.22^{a}

The results are expressed as the mean of three independent experiments ± standard deviation. Different letters indicate significant differences ($P < 0.05$) between rows

L*, a*, and b* are the way in which they are commonly named these parameters. + and * relect to positively charged and neutral ions

CB Control bread, *GPB* Grape pomace bread, *GluE* Glucose equivalents, *GAE* Gallic acid equivalents, *CEs* Catechin equivalents, *Mlv-3-gluE* Malvidin-3-glucoside equivalents, *TE* Trolox equivalents

samples was similar ($P > 0.05$). This result differs from those observed in cakes enriched with GPP at 4, 6, 8, and 10% presented lower moisture values than the control cake (Nakov et al., 2020). According to Souza et al. (2023), the moisture retention potential is related to the interaction between amylose and lipids. In addition, GPB presented a slightly greater content of lipids ($P < 0.01$) and protein ($P < 0.01$) than CB. Higher lipids levels have been reported for fortified breads with GPP (Smith & Yu, 2015; Tolve et al., 2021). The increase in lipid content of GPB is the result of adding GPP into the bread since the grape seeds are rich in oils, particularly in unsaturated fatty acids (Nakov et al., 2020). The effect on protein content can be attributed to proteins in the GPP. Previous studies have reported that increasing GPP substitution (5 and 10%) in bread did not affect the protein content (Tolve et al., 2021). In contrast, the enrichment of cakes with GPP presented a higher protein content than the control cake (Nakov et al., 2020). This dissimilitude in the results depends on the GPP used and the physicochemical composition of GPP.

The total carbohydrate content was lower in GPB than in CB ($P < 0.01$). The total carbohydrate content was determined by difference. Since GPB presented a higher content of fat, proteins, and ash, a lower total carbohydrate content was reported. Although GPB had a lower total carbohydrate content, it presented greater total and insoluble dietary fiber than CB (Table 2). The soluble dietary fiber content was also greater in the GPB; however, this increase was not statistically significant. Walker et al. (2014) also reported an increase in total dietary fiber in bread supplemented with GPP, which showed a dose-dependent pattern. Similar results were reported by Mildner-Szkudlarz et al. (2011), who reported an increase in soluble and insoluble dietary fiber in rye bread fortified with 10% GPP. Insoluble dietary fiber is mainly composed of lignin, cellulose, and hemicellulose; this type of fiber can modify water retention and the viscosity and texture of foods (L. Zhang et al., 2017). Moreover, insoluble dietary fiber can interact with other nutrients in the food matrix and bioactive compounds such as phenolic compounds and regulate its release during digestion (Jakobek, 2015).

Regarding the titratable acidity and pH, GPB presented a greater titratable acidity than CB ($P = 0.045$) and, consequently, a lower pH. GPP is an important source of organic acids such as tartaric, malic, and citric acids. The presence of these organic acids in the GPP can explain the lower pH observed in the GPB than in the CB. This effect of adding GPP to bread on the pH value has been reported previously (Tolve et al., 2021). According to Tolve et al. (2021), bread's acidic conditions affect yeast activity during leavening and can impact the gluten network. This can explain why GPP needed to be added after the first leavening to increase the height of the bread.

The color results for both bread samples can be found in Table 2. The lightness (L^{*}) of GPB was lower than that of CB, indicating that GPB was darker than CB. The reduction in the parameter L^{*} can be attributed to the anthocyanins in the GPP. The stability of anthocyanins depends on different factors, such as pH and temperature (Hayta et al., 2014); at high temperatures, anthocyanins can be degraded, resulting in a brown color. According to

Nikolaou et al. (2022), the L* value is related to the pH; lower L* values are related to lower pH values and the integration of phenolic compounds into the dough. This agrees with the results observed in the present study.

The parameter a* (green/red) was greater in the GPB crumb than in the CB crumb, indicating that GPB presented a red color. Such an effect was observed previously by Nikolaou et al. (2022) in panettone enriched with GPP. The authors justified this behavior by stating that the phenolic compounds present in the enriched samples intensified the red color. Moreover, b* (blue/yellow) was lower in the crumb and crust from the GPB than in those from the CB, indicating that the GPB was bluer. Similar results were previously reported for bread supplemented with 10% GPP, which presented lower values of a* and b* than the control bread (Hayta et al., 2014; Tolve et al., 2021). The total color difference (ΔE) expresses the effect of the addition of GPP to the bread, and it can be observed that the crumb presented a higher ΔE value than the crust. According to Mikulec et al. (2019), when the ΔE values between two compared products are greater than 3, there are perceptible changes in the new product. This explains why the crust and crumb from GPB were different in color due to the addition of GPP. The enrichment of pizza crust with GPP at 15, 20, and 25% presented ΔE values greater than 5, representing significant color differences between the control (Difonzo et al., 2023). This phenomenon was justified by the values found in L* and a*. This agrees with the results observed in the present study, where L* and a* values presented significant differences between GPB and CB.

According to the results in Table 2, adding GPP increased the total phenolic compound and flavonoid contents in the bread. Vegetables and fruits are the principal source of polyphenols and flavonoid content (Sarker et al., 2022a, 2022b, 2022c; Sarker et al., 2022a, 2022b, 2022c). For this reason, polyphenol and flavonoid content were increased in GPB due to the presence of GPP. On the other hand, the condensed tannin content was only quantified in the GPB, indicating that condensed tannins were not present in wheat flour. Anthocyanins were absent in both bread samples. A previous study by Smith and Yu (2015) reported that adding GPP to bread increased the phenolic compound content dose-dependently and that the loss of phenolic compounds may occur during baking. This effect can be observed in the anthocyanin fraction missing in the final product (GPB). The presence of condensed tannins in GPB is attributed to GPP since the grape pomace used for this study contains stalks beside the skins and seeds from the grapes. An increase in phenolic compounds produced greater antioxidant activity in GPB than in CB.

As shown in Table 2, the antioxidant activity of GPB was greater than that of CB, according to the FRAP and $ABTS^+$ methods. Nevertheless, no significant difference between CB and GPB was observed with the DPPH* method. Previous studies have reported that fortifying bread with grape pomace increases antioxidant activity compared with wheat flour bread (Smith & Yu, 2015; Tolve et al., 2021). According to Mildner-Szkudlarz et al. (2011), these differences between the FRAP and DPPH* methods can be explained mainly by flavonoids in the bread due to their structural characteristics, which allow them to transfer electrons. On the other hand, Tolve et al. (2021) found a correlation between the total phenolic content of the bread and its antioxidant activity via FRAP and $ABTS^+$ methods. This agrees with the results observed in the present study.

Table S5 shows the phenolic profile of CB. The phenolic compounds identified in CB were *m*-hydroxybenzoic acid and caffeic acid. Other compounds identified were secoisolariciresinol and 2-hydroxyenterodiol. These results differ from those of Mildner-Szkudlarz et al. (2011). In the study, the authors used grape pomace to fortify sourdough bread. The bread's main phenolic compounds were gallic acid, catechin, p-coumaric acid, and trans-ferulic acid.

Table S6 shows the phenolic compounds identified in GPB. The main differences between CB and GPB were the detection of flavan-3-ols, catechin, epicatechin, and epigallocatechin-3-glucuronide in the GPB samples. Benzoic acids have important biological and pharmacological activities such as antioxidant, antimicrobial, anti-inflammatory, anticancer, cardioprotective, gastroprotective, and neuroprotective effects (Sarker & Ercisli, 2022; Sarker et al., 2022a, 2022b, 2022c). Catechin was detected in GPB due to the presence of GPP because vegetables and fruits are the principal sources of catechin (Sarker & Ercisli, 2022). Flavan-3-ols have been reported previously in bread samples enriched with grape pomace (Mildner-Szkudlarz et al., 2011). Moreover, quercetin-3-rhamnoside-7-glucoside (flavonol) and piceatannol (stilbene) were identified in the GPB samples. The presence of flavonols and stilbenes has been reported previously in a muffin partially substituted with grape pomace flour (Troilo et al., 2022).

Texture analysis

Bread's texture profile is an important characteristic that can be modified by adding GPP due to the phenolic compounds and dietary fiber present in its composition. Table 3 shows the hardness, cohesiveness, elasticity, chewiness, and adhesiveness of the CB and GPB samples.

No significant differences were detected ($P > 0.05$) for any parameters measured in the samples, except for the

Table 3 Texture profile of CB and GPB

Parameter	CB	GPB
Hardness (N)	5.09 ± 0.75[a]	5.12 ± 0.58[a]
Cohesiveness	0.64 ± 0.04[a]	0.67 ± 0.02[a]
Elasticity	1.64 ± 0.17[a]	1.59 ± 0.11[a]
Chewiness (J)	0.01 ± 0.00[a]	0.01 ± 0.00[a]
Adhesiveness (J)	0.00 ± 0.00[a]	0.00 ± 0.00[a]
Strength cut		
Crumb	1.74 ± 0.08[b]	2.84 ± 0.42[a]
Lateral crust	1.14 ± 0.11[a]	1.58 ± 0.3[a]
Superior crust	0.98 ± 0.11[a]	1.49 ± 0.34[a]

The results are expressed as the means of three independent experiments. Different letters indicate significant differences between rows

CB Control bread, *GPB* Grape pomace bread

crumb strength cut. Previous studies evaluating the addition of grape pomace flour to white wheat bread have reported that the hardness, chewiness, and cohesiveness increase as the amount of grape pomace added increases (Hayta et al., 2014; Mildner-Szkudlarz et al., 2011). The increase in hardness resulting from adding grape pomace was explained by the ability of hydroxyl groups from dietary fiber to interact with water molecules. The reduction of water in the dough can affect yeast activity, promoting less gas formation and a weakened gluten network (Mildner-Szkudlarz et al., 2011; Pycia & Ivanišová, 2020). These results differ from those obtained in the present study. Results from Table 2 indicate that the soluble fiber in GPB was similar to CB; according to Pycia and Ivanišová (2020), the soluble fiber fraction in bread can increase the hardness by interaction with other macromolecules such as proteins and lipids. The similar soluble fiber content in both samples can explain why hardness was not modified in GPB. On the other hand, the crumb strength cut was greater in the GPB than in the CB. Previously, adding dietary fiber from prickly pear cactus to white wheat bread resulted in a viscous dough that affected the crumbing of the bread. This effect was attributed to the interaction between cellulose, hemicellulose, lignin, and the gluten matrix, which increased their structural strength (Guevara-Arauza et al., 2015). These findings are similar to those observed in the crumb from GPB, where the crumb presented a greater strength cut due to its insoluble fiber content.

Bioaccessibility of phenolic compounds during in vitro gastrointestinal digestion

The nature of food matrix components ultimately determines food bioactive's liberation (bioaccessibility) during gastrointestinal processing. In particular, bakery products are an important source of highly (α-1,4-polysaccharides) and low-to-null (resistant starch or β-1,4-polysaccharides) digestible carbohydrates and proteins, whose structural organization modifies the releasability rate of bioactive phytochemicals, including phenolic compounds (Kan et al., 2020; Rochetti et al. 2021; Eshak et al., 2016). Here, grape pomace-enriched (GPB) and nonenriched (control, CB) bread samples differing in total dietary fiber/polyphenol content (GPB > CB) were evaluated in a widely accepted static in vitro digestion model (Kopf-Bolanz et al., 2012) to assess the rate of phenolic releasability and carbohydrate digestion rate as the main concurrent events.

As expected from highly digestible starchy food, sugar releasability due to amylase action was quite significant [Fig. 3a: simulated oral stage (CB > GBP) < simulated gastric stage (CB = GPB) < simulated intestinal stage (CB = GPB)], so the complex carbohydrate nature of both (CB, GPB) food matrices decreased at a similar rate. Additionally, considering that free sugars interfere with the Folin-Ciocalteu assay for quantifying total

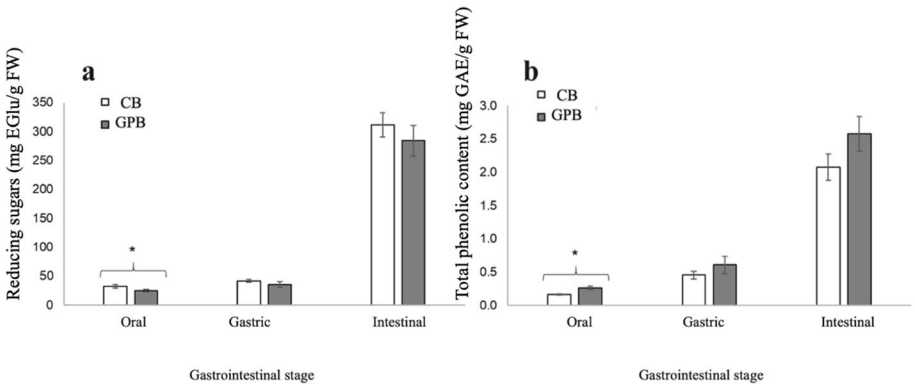


Fig. 3 **a** Glucose released during each gastrointestinal stage. CB = Control bread. GPB = Grape pomace bread. **b** Bioaccessibility of phenolic compounds during each stage of the gastrointestinal system. * Indicates a significant difference between samples ($P < 0.05$)

phenolic compounds in a given sample (Muñoz-Bernal et al., 2017), these compounds were removed before phenolic quantification. According to Fig. 3b, the in vitro bioaccessibility of phenolic compounds followed almost the same trend as that of free sugars [simulated oral stage (CB < GBP) < simulated gastric stage (CB = GPB) < simulated intestinal stage (CB = GPB)]. However, GBP had greater phenolic compound bioaccessibility during the simulated oral stage. Under simulated oral conditions, the bioaccessibility of phenolic compounds depends on both their glycosylated state and the interaction between phenolic compounds and salivary proteins (Velderrain-Rodríguez et al., 2014), while gastric pH and pepsin activity promote food matrix derangements (e.g., oligomer hydrolysis and deglycation events) and consequent phenolic bioaccessibility (Thakur et al., 2020; Velderrain-Rodríguez et al., 2014). Last, considering that the highest bioaccessibility of phenolic compounds was achieved under simulated intestinal conditions, which is closely related to starch/protein hydrolysis, it is quite clear that monomeric (molecularly entrapped) > polymeric (strongly bonded) phenolic compounds were released at this stage. Nonetheless, GPB has more phenolic compounds than CB; this was not reflected in a higher bioaccessibility. The neutral-to-alkaline intestinal environment causes significant structural instability of monomeric anthocyanins (Olivas-Aguirre et al., 2020; Thakur et al., 2020; Velderrain-Rodríguez et al., 2014), which are among the most significant phenolic subgroups in grape pomace, which may partially explain why the phenolic bioaccessibility of CB is similar to that of GBP.

According to the results shown in Fig. 3b, GPB showed slightly nonsignificant greater bioaccessibility during the simulated intestinal stage. The simulated intestinal stage has alkaline conditions due to the liberation of pancreatic and bile juices; this alkaline condition produces unstable phenolic compounds and may reduce their bioaccessibility (Thakur et al., 2020; Velderrain-Rodríguez et al., 2014). Recently, Rocchetti et al. (2021) studied the bioaccessibility of phenolic compounds from two bread formulations with GPP (5 and 10%). The authors reported a decrease in phenolic compounds' bioaccessibility as the addition of GPP increased in the bread. This effect was attributed to the increase in dietary fiber. The phenolic compounds from the GPP can interact with the dietary fiber and other macromolecules, such as lipids and proteins, limiting phenolic compounds' bioaccessibility (Rocchetti et al., 2021). These results agree with those reported in the present study. Still, the GPB had a high initial content of phenolic compounds; this was not reflected in its relatively high bioaccessibility. While CB showed 99% bioaccessibility, GPB was reported only to have 50.5% bioaccessibility. In other words, 2.5 mg GAE/g bread remained in the food matrix during the simulated intestinal stage. These results may be explained by phenolic compounds interacting with other molecules, such as proteins and dietary fiber (Jakobek, 2015). In this sense, dietary fiber needs to be considered, as observed in the results from chemical composition (Table 2). One of the main changes in GPB was the addition of dietary fiber; according to these results, the total dietary fiber in GPB was 6.1% and in CB 4.3%. In a previous study, it was observed that the bioaccessibility of phenolic compounds was modified according to the addition of GPP to bread (5 and 10%) (Rocchetti et al., 2021). The authors reported that the bioaccessibility of specific phenolic compounds, mainly flavones and other flavonoids, was modified mostly due to the addition of dietary fiber to the food matrix (Rocchetti et al., 2021). Another study in which bread was fortified with an extract of berries showed that anthocyanins' bioaccessibility increased while procyanidins' bioaccessibility decreased (Kan et al., 2020). This effect was attributed to interactions with the food matrix according to the structure of the phenolic compound (Kan et al., 2020). The hydroxyl groups in the dietary fiber structure allow them to interact with phenolic compounds (Jakobek, 2015); such interactions can attach phenolic compounds to dietary fiber and diminish the bioaccessibility of phenolic compounds from the GPB. Dietary fiber can form a gel during gastrointestinal digestion by encapsulating the phenolic compounds in the GPB and avoiding the action of enzymes that can release phenolic compounds from the food matrix (Bohn et al., 2015).

On the other hand, the bioaccessibility results indicate that a proportion of phenolic compounds remain in the GPB. The phenolic compounds bound to the food matrix can be metabolized during the colonic stage, increasing the bioaccessibility of phenolic compounds. During colonic fermentation, phenolic compounds such as flavonoids can be biotransformed into phenolic acids by the microbiota in this stage (Crozier et al., 2009). Nevertheless, in the present study, the colonic stage was not investigated, and there is no data on whether the phenolic compounds remaining in the GPB can increase their bioaccessibility. Moreover, a phenolic profile is necessary to determine the different phenolic compounds that can be released during every stage of the digestion process. However, according to a recently published review, it is known that during colonic fermentation phenolic compounds can be released and metabolized from insoluble bound phenolic compounds, being detected in plasma 3–4 days after the consumption of phenolic-rich foods (Rasera et al., 2024). In this context, it is expected that the phenolic compounds released from GPB will be higher.

Conclusions

The bread enriched with grape pomace powder had higher ash, lipids, proteins, soluble fiber, insoluble fiber phenolic compounds, and antioxidant activity than the control bread. The 8% enrichment with grape pomace did not affect consumer acceptance. This finding is important since the development of new products needs to improve the nutritional value and, at the same time, be accepted by the consumers. The increase in phenolic content and dietary fiber can catalog this bread as a new product with bioactive compounds. It also presents the grape pomace as an alternative flour to produce bakery products with better physicochemical proprieties. The bioaccessibility studies are essential to demonstrate the beneficial effects of the new product.

Regarding this aspect, phenolic compound bioaccessibility was similar in both bread samples. This behavior is mainly related to the dietary fiber content in the grape pomace bread. This can be useful for the microbiota using these compounds as prebiotics. Future studies should address the effect of the increase in fiber in the bread and the possible effects of prebiotics. Moreover, studies focused on the bioavailability of phenolic compounds need to be carried out.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s43014-024-00271-3.

Additional file 1
Additional file 2
Additional file 3
Additional file 4
Additional file 5
Additional file 6
Additional file 7

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Declaration of generative AI and AI-assisted technologies in the writing process
During the preparation of this work the authors used DeepL in order to spell check and grammar check, improving the English readability and quality. After using this tool/service, the authors reviewed and edited the content as needed and takes full responsibility for the content of the publication.

Authors' contributions
OAMB was involved in the study design, laboratory and data analysis, data interpretation, and manuscript drafting. AJCO worked on the conception of the study, sample preparation, laboratory analysis, data analysis, data interpretation, data presentation, and manuscript drafting. AAVF: sample methodology and manuscript drafting. CRSC was involved in laboratory analysis and data interpretation. MLRV and JRG were involved in the sample methodology analysis and manuscript review. LADIR was involved in the financial support and review of the manuscript. EAP and NRMR were involved in the conception of the study, study design, data interpretation, and manuscript review. All the authors have read and approved the final manuscript.

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Data availability
All data generated or analyzed during this study are included in this published article [and its supplementary information files].

Declarations

Ethics approval and consent to participate
This study was approved by the Institutional Ethics Committee of Universidad Autónoma de Ciudad Juárez (CIEB-2019–1-051).

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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