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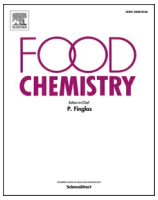

Green banana (*Musa* ssp.) mixed pulp and peel flour: A new ingredient with interesting bioactive, nutritional, and technological properties for food applications

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ABSTRACT

This study aimed to characterize and evaluate the *in vitro* bioactive properties of green banana pulp (GBPF), peel (GBPeF), and mixed pulp/peel flours M1 (90/10) and M2 (80/20). Lipid concentration was higher in GBPeF (7.53%), as were the levels of free and bound phenolics (577 and 653.1 mg GAE/100 g, respectively), whereas the resistant starch content was higher in GBPF (44.11%). Incorporating up to 20% GBPeF into the mixed flour had a minor effect on the starch pasting properties of GBPF. GBPeF featured rutin and trans-ferulic acid as the predominant free and bound phenolic compounds, respectively. GBPF presented different major free phenolics, though it had similar bound phenolics to GBPeF. Both M1 and M2 demonstrated a reduction in intracellular reactive oxygen species (ROS) generation. Consequently, this study validates the potential of green banana mixed flour, containing up to 20% GBPeF, for developing healthy foods and reducing post-harvest losses.

1. Introduction

Banana (*Musa* spp.) is one of the most widely produced and commercialized fruits globally. It plays a crucial role in food security and the global economy due to its high nutritional value and substantial consumption (Khoozani, Bekhit, & Birch, 2019). Despite this, significant quantities of bananas are lost in the market primarily due to inadequate post-harvest practices (Angelis-Pereira, Barcelos, Pereira, Pereira, & Sousa, 2016).

Considering the losses of bananas in the supply chain, utilizing green (unripe) fruits could be a practical solution to reduce waste, lower management costs, and mitigate environmental risks (Khoozani, Bekhit, & Birch, 2019). At early maturity stages, the fruit's high levels of resistant starch, dietary fiber, antioxidants, and minerals make it a suitable raw material for producing flour with potential health benefits (Angelis-Pereira et al., 2016; Pereira, Malairaj, Brohi, Boateng, & Zhang, 2020). Various studies have highlighted the efficacy of green banana pulp flour (GBPF) in preventing intestinal disorders and reducing glycemic indices and inflammation (Almeida-Junior, Curimbaba, Chagas, Quaglio, & Di Stasi, 2017; Angelis-Pereira et al., 2016). Dietary

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interventions incorporating 5% and 10% GBPF have been shown to increase the concentration of short-chain fatty acids in the colon by two to five times (Almeida-Junior et al., 2017).

Pulp and peel flours can be derived from unripe bananas; however, GBPF is the most commonly utilized green banana flour for food applications, thus attracting substantial interest in the food industry primarily for its high concentrations of resistant starch and functional properties, such as thickening (Pereira et al., 2020). Green banana peel flour (GBPeF) is rich in minerals, bioactive compounds, and dietary fiber (Angelis-Pereira et al., 2016), yet it remains under-researched and underutilized. Furthermore, there is a notable absence of studies on the phenolic compounds' characterization in green banana flours, including their phenolic profiles by UPLC-MS^E and the in vitro antioxidant properties of their phenolic extracts. Consequently, additional research into the chemical, technological, and bioactive characteristics of GBPF and GBPeF is warranted.

Additionally, the composite green banana pulp and peel flour could serve as a promising raw material for developing novel, healthy food products and industrial applications, potentially replacing conventional flours due to the wide availability, low processing cost, and nutritional characteristics of GBPF and GBPeF, as well as their capacity to reduce waste through full fruit utilization. Mixed flours offer several advantages, as evidenced by Martinez et al. (2020), who demonstrated the health benefits of mixed sorghum and quinoa flour. A more detailed characterization of GBPeF and GBPF would be beneficial for proposing mixed flours for various food applications. Therefore, this study aimed to evaluate the nutritional, bioactive, and technological properties of green banana pulp and peel flours, as well as their mixtures (composite flours).

2. Materials and methods

2.1. Raw materials

Bunches of 'Prata' cultivar green bananas (Musa paradisiaca, AAB group) were harvested from May to July 2021 in the rural district of Rua Nova in Viçosa, Minas Gerais, Brazil. The fruits were harvested at maturity stage 1 (completely green), 90 to 120 days after flowering, and were immediately processed.

2.2. Production of green banana pulp and peel flours

After harvesting the bunches, the clusters were manually detached using a stainless-steel knife. The clusters were weighed and disinfected in water containing 100 mg-L^{-1} chlorine for 20 min. Subsequently, the fruits were manually peeled, and their respective parts were treated in different solutions. The pulps were immediately immersed in drinking water, whereas the peels were submerged in a 0.5% (*w/v*) citric acid solution. The sliced materials were maintained in their respective solutions for 20 min to prevent enzymatic browning (Angelis-Pereira et al., 2016).

Regarding the drying process, the banana pulps and peels were first sliced using a CL 50 Robot Coupe food processor equipped with a 2 mm slicing disc and then spread on aluminum mesh trays. Drying occurred at 55 °C in a gas-heated tray dryer (Polidryer PD 150). The drying airspeed was set to 55 °C using a tray dryer with gas heating (Polidryer PD 150). The drying airspeed was adjusted to 1.5 m.s^{-1} using a portable digital hot-wire anemometer (Instrutherm® TAFR-180). The drying procedure lasted approximately 9 h, which was the time needed for the samples to reach a constant weight. After drying, the samples were ground in a knife mill (Brabender, WI) equipped with a stainless-steel sieve of 1.0 mm aperture and kept in airtight plastic bags at −18 °C. The mixed flours were prepared by manually mixing green banana pulp and peel flours in ratios of 90:10 (M1) and 80:20 (M2) (pulp:peel) for 1 min in a plastic container.

2.3. Physical and chemical characterization of green banana flours

2.3.1. Flour yield

The flour yield was determined by calculating the final weight of the flour after drying and dividing it by the initial weight of the fresh bananas. The results were then expressed as g flour/kg of fresh bananas.

2.3.2. Particle sizing test

To assess the particle size distribution of GBPF and GBPeF, 100 g of flour was sieved using a Ro-Tap Sieve Shaker (Cleveland, USA). The procedure was performed in triplicate and involved sieving for 5 min using vibrating sieves with apertures of 0.84, 0.42, 0.25, 0.21, 0.177, 0.149, and 0.00 mm (Method 66–20.01; AACC, 2009). The material retained on each sieve was weighed, and the results were reported as percentages.

2.3.3. Instrumental color

The color of the flours was measured using a Minolta Color Reader CR-10 colorimeter. Measurements were taken through direct readings of the samples under illuminant D65 (daylight, 6500 K) with an observation angle of 10°. The CIELab system (coordinates L*, a*, b*) was employed. Readings were conducted on flours evenly distributed in Petri dishes, in triplicate, at different parts of the sample. The whiteness index (WI) (Eq. 1) and the browning index (BI) (Eq. 2 and 3) were calculated as follows:

$$WI = 100 - \sqrt{(100 - L)^2 + a^2 + b^2} \quad (1)$$

$$BI = \frac{100.(X - 0.31)}{0.172} \quad (2)$$

$$X = \frac{(a + 1.75.L)}{(5.645.L) + (a - 3.021.b)} \quad (3)$$

2.3.4. Proximate composition

The moisture, protein, lipid, and ash contents of GBPF and GBPeF were determined using standard methods prescribed by AOAC (2005). The moisture content was measured in a fan oven at 105 °C for 4 h (method 925.40); protein content (expressed as % N × 6.25) was determined by the Kjeldahl method (method 955.04); total lipids were extracted using the Soxhlet method with petroleum ether at 40–60 °C (method 920.39); and ash content was quantified by incineration in a muffle furnace (method 923.03). Total carbohydrates were calculated by difference [100 - (moisture + ash + protein + lipids)]. The results were expressed in grams per 100 g of flour.

Total dietary fiber concentrations, including soluble and insoluble fibers, were quantified using the enzymatic-gravimetric method (TDF 100 A, Sigma®) according to AOAC (2005).

2.3.5. Minerals

The concentration (mg/100 g) of the major minerals in the flours—including phosphorus (P), calcium (Ca), magnesium (Mg), sulfur (S), boron (B), zinc (Zn), copper (Cu), manganese (Mn), iron (Fe), and sodium (Na)—was determined using the method described by Oliveira et al. (2024).

2.3.6. Fatty acid composition of the flours

The fatty acid profile of the green banana flours was analyzed by gas chromatography (GC) according to the methods described by Guihéneuf, Schmid, and Stengel (2015) and Ichihara and Fukubayashi (2010). The chromatographic analyses were conducted using a GC-2010 (Shimadzu, Kyoto, Japan) equipped with a flame ionization detector (FID) and an SGE BPX70 capillary column (25 m × 0.5 mm × 0.25 μm).

2.3.7. Determination of the lipid classes by thin-layer chromatography

The lipid classes in GBPF and GBPeF were determined using thin-

layer chromatography (TLC) as described by Yao, Gerde, Lee, Wang, and Harrata (2015). Sample solutions along with lipid standards (monolein, oleic acid, and triolein) were poured onto a chromatographic plate coated with silica gel layers (20 × 20 cm) (Macherey-Nagel, GmbH & Co, KG, Germany).

2.3.8. Concentration of total starch, amylose, and resistant starch

The concentrations of total starch (TS) and resistant starch (RS) in the flours were determined using methods 76–13 and 32–40, respectively, as prescribed by AACC (2009). These analyses were conducted using kits from Megazyme International (Wicklow, Ireland). Additionally, the amylose concentration in GBPF and GBPeF was measured using the amylose/amylopectin kit, also from Megazyme International.

2.3.9. Determination of free and bound phenolic compounds and condensed tannins

Phenolic extracts were obtained from the green banana flours using an 80% ethanol (*v*/v) solution, as described by Santos et al. (2019). The concentrations of free and bound phenolic compounds were determined using the Folin-Ciocalteu method, as detailed by Singleton and Rossi (1965). The phenolic content was expressed as mg of gallic acid equivalents per 100 g of flour (mg GAE/100 g).

The condensed tannin content of the flours was estimated as described by Price, Van Scoyoc, and Butler (1978) with modifications. Flour (3.0 g) was mixed with 8.0 mL of 1% HCl in methanol, incubated in a water bath at 30 °C for 60 min, and then centrifuged at 6000 rpm for 15 min. Aliquots from the supernatant were used for further analysis, where tubes for the blank (4% HCl in methanol) and sample (vanillin reagent) were prepared. The concentration of condensed tannins was expressed as mg catechin equivalent (CE)/g sample.

2.3.10. Phenolic profile determination of green banana flour by UPLC-ESI-QTOF-MS^E

The extracts from green banana pulp, peel, and mixed flours (section 2.3.9) were analyzed to evaluate their phenolic profile. A total of 5 μL of each sample or a mixed solution containing 33 commercial standards of phenolic compounds was filtered through a 0.22 μm hydrophilic PTFE syringe filter (Analitica, Diadema, SP/Brazil) and injected into a UPLC Acquity system (Waters Co., Milford, MA) coupled with a XEVO G2S Q-TOF (Waters Co., Manchester, UK). The system was equipped with an electrospray ionization source operating in negative mode (Santos et al., 2019). A UPLC HSS T3 C18 column (100 × 2.1 mm, 1.8 μm particle diameter; Waters) was maintained at 30 °C with a flow rate of 0.5 mL/min using mobile phases A (ultrapure water with 0.3% formic acid and 5 mM ammonium formate) and B (acetonitrile with 0.3% formic acid). The gradient was set as follows: 0 min – 97% A; 11.80 min – 50% A; 12.38 min – 15% A; 14.23 min – 15% A; 14.70 min – 97% A. Data were acquired using the MS^E DIA (data-independent acquisition) method.

To monitor the instrument, assess data quality, and ensure proper alignment, pooled quality control samples (QC samples) containing all extracts were injected after every nine sample analyses. Raw data processing was performed using Progenesis QI software (Waters, Nonlinear Dynamics), evaluating parameters such as isotopic distribution of neutral mass, exact mass, retention time, and MS/MS fragment spectra. Untargeted identification utilized a custom database built from the PubChem and Phenol-Explorer 3.6 databases (http://phenol-explorer.eu/), employing the MetaScope search engine for theoretical fragmentation. Criteria applied included an exact mass error of <10 ppm, isotopic similarity above 80%, a score above 30, and the highest fragmentation score, all generated by the software. Additionally, tentative identification was conducted after applying an RSD filter of <30% in compounds from QC samples. MS/MS experimental spectra were compared with data from the NORMAN MassBank (https://massbank.eu/MassBank). Phenol-Explorer, literature data, and the chemical characteristics of the matrix were also employed to assist in the tentative identification of phenolic compounds, following the levels of identification described by Sumner et al. (2007). Multivariate data analysis was performed using MetaboAnalyst, considering sample normalization by median, data transformation by cube root, and Pareto scaling.

2.4. In vitro bioactive properties

2.4.1. Chemicals, green banana flour phenolic extracts, and cell lines

Dichloro-dihydro-fluorescein diacetate (DCFH-DA), 3–4,5-dimethylthiazol-2, and diphenyl tetrazolium bromide (MTT), along with Dulbecco's Modified Eagle's Medium/Nutrient Mixture F-12 Ham (DMEM), were sourced from Sigma-Aldrich (São Paulo, Brazil). Aqueous solutions were prepared using ultrapure water (Millipore, São Paulo, Brazil). Cell lines A549 (lung adenocarcinoma epithelial cells), HepG2 (human hepatoma carcinoma cells), and HCT8 (human ileocecal adenocarcinoma cells) were obtained from the Rio de Janeiro Cell Bank (Rio de Janeiro, Brazil).

The weight of the flours (pulp, peel, and mixed) for phenolic extraction was based on a total phenolic concentration of 38.191 μg/mL, requiring 140 mg of flour for each 1 mL of an 80% ethanol (*v*/v) solution used as the solvent. The mixtures were stirred for 2 h and then centrifuged at 3000 ×*g* for 10 min. The supernatants containing the free phenolics were freeze-dried and resuspended in distilled water for biological assays. The aqueous extracts were filtered prior to determining biological activity in cell cultures.

2.4.2. In vitro cell-based cytotoxicity evaluation

The cytotoxic effects of the extracts (GBPF, GBPeF, 90:10 pulp: peel, and 80:20 pulp: peel) were assessed on lung adenocarcinoma epithelial cells (A549), human ileocecal adenocarcinoma cells (HCT8), and human hepatoma carcinoma cells (HepG2). Briefly, cells were plated in 96-well plates at densities of 2×10^4 cells/well (HepG2) and 1×10^4 cells/well (HCT8, A549). After treatment with extract concentrations ranging from 5 to 100 μg/mL for 48 h, MTT (0.5 mg/mL) was added to each well and incubated for 4 h at 37 °C. The metabolically active cells converted the MTT to blue formazan crystals, which were dissolved in DMSO. The absorbance was then measured at 570 nm. Parameters including IC_{50} (50% cell viability inhibition), GI_{50} (50% growth inhibition), and LC_{50} (50% lethal concentration) were determined following the methodology outlined by do Carmo, Pressete, Marques, Granato, and Azevedo (2018).

2.4.3. Reactive oxygen species (ROS) generation

Intracellular ROS generation was evaluated using the DCFH-DA assay. Malignant cells (A549, HCT8, and HepG2) were seeded at a density of 6×10^4 per and treated for 1 h with varying concentrations of green banana flour extracts (5–25 μg/mL) or 22.5 μMol H_2O_2 as a positive control, or culture medium as a negative control. After the treatment, 22.5 μMol H_2O_2 was added to the wells, and the fluorescence intensity (λ_emission = 538 nm and λ_excitation = 485 nm) was measured as described by Escher et al. (2018).

2.5. Chemometric analysis

Principal component analyses (PCA) were conducted in three independent stages using (i) the phenolic profile results from the analyzed samples (extracts used in the biological assays—section 2.4); (ii) the cellular viability values (IC_{50}, IG_{50}, and LC_{50}) against HCT8, HepG2, and A549 cell lines; and (iii) the ROS detection values in the presence of H_2O_2 than the positive control. All data inputs were neither normalized nor auto-scaled, as they were of the same unit and scale. The analyses were performed using the Chemoface software (Nunes, Freitas, Pinheiro, & Bastos, 2012), incorporating score plots to understand the relationship of samples based on the given variables and loading plots to analyze the contribution of each variable in explaining the behavior of the samples.

2.6. Technological properties of the green banana flours

2.6.1. Starch pasting properties

The analysis was conducted using a Rapid Visco-Analyzer (RVA-4500, Warriewood, Australia) following standard methodology 2 (STD2) with 3.5 g of sample containing 14% moisture. The starch suspensions were placed in aluminum vials and subjected to agitation at 160 rpm in the RVA. The SD2 profile is as follows: initially, the RVA is held at 50 °C for 1 min, then heated at a rate of 6 °C/min to 95 °C, maintained at this temperature for 5 min, then cooled to 50 °C at 6 °C/min, and held at 50 °C until the analysis concludes at 23 min. The results were analyzed using Thermocline software for Windows. All measurements were performed in duplicate, and the parameters evaluated included maximum viscosity, minimum viscosity, breakdown, final viscosity, setback, and pasting time and temperature.

2.6.2. Gel strength

The samples from the RVA analysis were evaluated for gel strength using a TA.XT2i texture analyzer (Stable Microsystems, Surrey, England). The cooked paste was maintained in the aluminum vial of the RVA, capped with PVC film, allowed to cool, stored at 2 ± 5 °C for 16 h, then brought to room temperature (25 °C) for 1 h before testing. The test conditions included an acrylic cylindrical probe with a diameter of 25 mm, a test speed of 0.5 mm/s, a distance of 10 mm, and a load cell of 50 kg.

2.7. Statistical analysis

The software SISVAR, version 5.6 (Ferreira, 2011), was utilized for statistical analysis. All tests were conducted in three replicates. Significant differences between treatments were analyzed using analysis of variance (ANOVA), and the means were compared using Tukey's test at a significance level of $p < 0.05$.

3. Results and discussion

3.1. Physical characterization of the green banana flours

GBPF exhibited higher yields than GBPeF, with average values of 290.70 and 78.80 g/kg of fresh fruit, respectively. The particle size distribution results for GBPF and GBPeF are detailed in Table S1. GBPF retained a higher percentage of particles on the 0.177 mm sieve (61%), resulting in a greater proportion of fine particles compared to GBPeF. Conversely, GBPeF exhibited higher particle retention on the larger aperture sieves (0.42 and 0.25 mm), notably on the 0.25 mm sieve (25.67%). The increased concentration of insoluble fiber in the peel flour contributes to its larger particle size, which is a key factor directly influencing water retention capacity. This factor affects the viscosity of starch pastes and, subsequently, influences the mixing time and sensory characteristics of the final products, including appearance, flavor, and texture (Brito, Carrajola, Gonçalves, Martelli-Tosi, & Ferreira, 2019).

Significant differences ($p < 0.05$) were observed in the instrumental color of the green banana flours (Fig. S1; Table S2). GBPF exhibited higher brightness (L*) and whiteness index (WI), and a lower browning index (BI), with values of 73.27, 68.09, and 30.24%, respectively. In contrast, GBPeF displayed lower L* and WI values and higher BI, with values of 44.57, 41.28, and 61.43%, respectively. Visual observations also confirmed this difference, as the peel flour appeared much darker than the pulp flour. This difference in coloration may be attributed to the presence of chlorophylls, which are responsible for the characteristic green color of the peel.

The a* coordinate value of the peel flour was 5.67, while the pulp flour registered an a* value of 4.97, indicating a tendency toward red chromaticity (+a*) for both samples (Table S2). Concerning the b* coordinate, a dominance of yellow (+b*) was noted, with values of 18.53 for the peel and 16.77 for the pulp flours, respectively. Although the pulps were only immersed in water before drying, the GBPF displayed a lighter color (higher L* value) than those from other studies where flours underwent chemical pretreatments to prevent enzymatic browning, such as the application of dilute acids and sulfites (Anyasi, Jideani, & Mchau, 2017; Bakare, Ogunbowale, Adegunwa, & Olusanya, 2017). The drying process in this study, conducted at mild temperatures of 55 °C for a shorter duration of 9 h, likely contributed minimally to the browning of the banana pulp flour during processing. Higher drying temperatures ranging from 50 °C for 7 h to 110 °C for 2 h have been shown to result in darker flours (Khoozani, Birch, & Bekhit, 2019), which corroborates our findings. Our choice of a lower drying temperature helped maintain the flour's light color and prevented starch gelatinization, optimizing the naturally present resistant starch content in the flour.

3.2. Proximate composition

The proximate composition of the GBPF and GBPeF is presented in Table 1. Significant changes were observed in the moisture content: the GBPF had lower moisture (6.15%) than the GBPeF (7.40%), which was sufficiently low to increase shelf life and facilitate the milling process of both flours.

The GBPeF displayed significantly higher lipid content than the GBPF, with values of 7.53% and 0.61%, respectively (Table 1). This interesting finding motivated us to further investigate the lipid profile in the samples (section 3.4). We observed low protein contents for all samples, with higher values for GBPeF (5.8%) than GBPF (3.33%) (Table 1). We also noted a difference in the ash content between the flours, with significantly higher levels for the peel flour (8.41%) than the pulp flour (2.23%) (Table 1). Generally, fruit peels contain higher levels of minerals and fibers than their respective edible parts, highlighting their importance as an alternative nutrient source and a means of preventing food waste.

Regarding the dietary fiber content, GBPF contained 8.63%, while GBPeF had almost five times higher dietary fiber levels (39.57%) (Table 1). GBPeF was particularly notable for its high amounts of insoluble fiber (34.68%), while GBPF showed lower but relevant amounts of insoluble (4.89%) and soluble (2.39%) fibers. Therefore, the green banana mixed pulp and peel flour could potentially increase dietary fiber content and diversify the fiber profile in foods. The high dietary fiber content of GBPeF (39.57%) compares to those reported for mango and prickly pear peels, 39.25% and 39.33%, respectively (Garcia-Amezquita, Tejada-Ortigoza, Heredia-Olea, Serna-Saldívar, & Welti-Chanes, 2018).

3.3. Minerals

The concentrations of the major minerals identified in the green

Table 1
Proximate composition of the green banana pulp and peel flours.

Proximate composition (g/100 g)	Flours	
	GBPF	GBPeF
Moisture	6.15 ± 0.15^{b}	7.40 ± 0.13^{a}
Lipids	0.61 ± 0.05^{b}	7.53 ± 0.02^{a}
Proteins	3.33 ± 0.48^{b}	5.80 ± 0.00^{a}
Ash	2.23 ± 0.04^{b}	8.41 ± 0.11^{a}
Total carbohydrates	87.68 ± 0.18^{a}	70.86 ± 0.09^{b}
Dietary fiber		
TDF	8.63 ± 0.62^{b}	39.57 ± 1.23^{a}
SDF	2.39 ± 0.65^{a}	6.24 ± 0.64^{a}
IDF	4.89 ± 0.03^{b}	34.68 ± 0.59^{a}

GBPF: green banana pulp flour; GBPeF: green banana peel flour TDF: total dietary fiber; SDS: soluble dietary fiber; IDF: insoluble dietary fiber. Results were expressed as means ± standard deviation. Tests were performed in three replicates. Means followed by the same letter in the rows (GBPF versus GBPeF) do not differ at 5% probability by the F-test (ANOVA).

banana flours are listed in Table S3. Potassium (K) was the predominant mineral, followed by magnesium (Mg), phosphorus (P), calcium (Ca), sulfur (S), and manganese (Mn).

We observed high mineral contents in both green banana flours, with GBPeF showing higher concentrations than GBPF, except for copper (Cu), demonstrating that the peel can enhance the mineral content of foods (Table S3). Iron (Fe) and zinc (Zn) concentrations in the GBPeF were nearly three and five times higher, respectively. Micronutrients like Fe and Zn are crucial for proper body function. Additionally, the calcium (Ca) and manganese (Mn) levels were significantly higher in the GBPeF than in the GBPF, exceeding a tenfold difference. Therefore, products incorporating GBPeF can serve as a valuable source of calcium and manganese, essential for maintaining health.

3.4. Lipid classification and fatty acid profile of the lipids extracted from the flours

The main lipid classes found in the GBPF and GBPeF are shown in Fig. S2. The monoacylglycerols (MAGs) and polar lipids constituted the highest percentage among the lipid fractions from the GBPeF, while the lipids from the GBPF primarily comprised triacylglycerols (TAGs) and MAGs.

Polar lipids, such as phospholipids, were detected in the GBPeF, appearing in the lower band, while sterol esters appeared in the upper band, positioned above the TAGs band (Guihéneuf et al., 2015; Ichihara & Fukubayashi, 2010). As Hamed and Abo-Elwafa (2012) and Beevi and Sukumaran (2015) reported, the band between the free fatty acids (FFAs) and TAGs might indicate the presence of diacylglycerols (DAGs) in the peel flour. Lipids like MAGs, DAGs, and phospholipids are known for their excellent emulsifying properties. Interactions between lipids and other compounds in the green banana peel and pulp may have hindered a complete separation of the lipid fractions by the methodology used in this study, complicating the identification of FFAs in the flours.

The composition of the main fatty acids in the GBPF and GBPeF is illustrated in Table 2 and Fig. S3. The average content of saturated fatty acids ranged from 56.11% in the peel to 60.66% in the pulp flours. Palmitic acid was the most abundant saturated fatty acid in both the green banana pulp and peel flours, with concentrations of 41.19% and 47.81%, respectively (Table 2). Palmitic acid is known to form strong complexes with amylose, thereby enhancing the concentration of resistant starch in foods (Hasjim et al., 2010). Oleic acid was the principal monounsaturated fatty acid, comprising 16.42% in GBPeF and 27.98% in GBPF. The levels of polyunsaturated fatty acids in the green banana pulp and peel flours varied between 10.63% and 26.61%. Within this group, linoleic acid, or omega-6 (C18:2), was the dominant compound, with higher concentrations in GBPeF (13.40%) compared to GBPF (8.84%). Additionally, GBPeF exhibited high levels of linolenic acid (3.77%) and eicosapentaenoic acid (EPA) (6.09%). The intake of foods rich in monounsaturated fatty acids, such as oleic acid, and polyunsaturated fatty acids from the omega-3 family, including EPA and linolenic acid, aids in reducing plasma low-density lipoprotein (LDL-cholesterol) and total cholesterol, thereby offering health benefits by lowering the risk of cardiovascular diseases (Saini & Keum, 2018).

Table 2
Fatty acid composition of green banana pulp and peel flours.

Fatty acid profile	% fatty acid methyl esters (FAMEs)	
	GBPF	GBPeF
C14:00 (Myristic acid)	11.96 ± 0.32	2.62 ± 0.24
C16:0 (Palmitic acid)	41.19 ± 1.17	47.81 ± 0.39
C18:0 (Stearic acid)	7.51 ± 0.13	5.68 ± 0.35
C18:1n9c (Oleic acid)	27.98 ± 0.86	16.42 ± 0.15
C18:2n6c (Linoleic acid)	8.84 ± 0.88	13.40 ± 0.53
C18:3n3 (Linolenic acid)	nd	3.77 ± 0.12
C20:5n3 (Eicosapentaenoic acid- EPA)	1.79 ± 0.13	6.09 ± 0.27
C20:4n6 (Arachidonic acid)	nd	3.35 ± 0.19
C24:1n9 (Nervonic acid)	nd	0.33 ± 0.11
Total saturated fatty acid (%)	60.66	56.11
Total monounsaturated fatty acid (%)	27.98	16.75
Total polyunsaturated fatty acid (%)	10.63	26.61

nd: not detected.
GBPF: green banana pulp flour; GBPeF: green banana peel flour.
Tests were performed in three replicates.

3.5. Total starch, amylose, and resistant starch contents

The total starch content (TS) of the flours was 69.54 ± 0.75% for GBPF and 24.04 ± 0.01% for GBPeF, while the amylose content was 22.78 ± 0.29% for GBPF and 25.97 ± 1.43% for GBPeF. The resistant starch (RS) concentration was 44.11 ± 0.66% in GBPF and 17.89 ± 0.40% in GBPeF. The resistant starch present in both flours is characterized as type II starch (RS2), which is known for its type B crystallinity that makes it more resistant to hydrolysis (Fuentes-Zaragoza, Riquelme-Navarrete, Sánchez-Zapata, & Pérez-Álvarez, 2010). These findings suggest that both GBPF and GBPeF exhibit low starch digestibility, potentially associated with a lower glycemic index. Consequently, diets incorporating green banana mixed pulp and peel flour could positively impact blood glucose control and help manage diabetes.

3.6. Phenolic profile, total bound and free phenolics, and tannins

The results of the concentration of total phenolics (free and bound) are presented in Table S4. This is the first study to report on the bound phenolics in green banana pulp and peel flours. The total phenolic concentration (free and bound) was significantly higher in the GBPeF than in the GBPF, highlighting an advantage in processing the peel into flour. Additionally, the concentration of bound phenolics was higher than that of free phenolics in both GBPF and GBPeF flours. Both free and bound phenolics are significant, as free phenolics can be released in the body and act as antioxidants, while bound phenolics are fermented in the large intestine and modulate the gut microbiota (Rocchetti et al., 2022).

A total of 89 phenolic compounds were identified and annotated by UPLC-ESI-QTOF-MS^E in the green banana flours, as per the level annotations of the Metabolomics Standards Initiative (MSI) described by Sumner et al. (2007). The identified classes include phenolic acids, flavonoids, lignans, and other polyphenols (Table S5, Figs. S4-S9). Most identified phenolic compounds (free and bound) were flavonoids, followed by phenolic acids. The relative abundance of phenolic compounds in free and bound phenolic extracts was determined by total ion counting in mass spectrometry analysis. Fig. 1 shows the ten most abundant phenolic compounds found in the GBPF, GBPeF, and mixed flours M1 (green banana mixed pulp 90% and peel 10% flour) and M2 (green banana mixed pulp 80% and peel 20% flour). Detailed information about each phenolic compound indicated in the graphs from Fig. 1 (e.g., PC53, PC14, PC52) is provided in Table S5. Of the 25 most abundant compounds in each extract, six were identified based on Level 1 (confirmed with standards according to the MSI). Notably, these include rutin (609.1451 *m/z*), trans-ferulic acid (193.0497 *m/z*), p-coumaric acid (163.0393 *m/z*), myricetin (317.0290 *m/z*), gallic acid (169.0131 *m/z*), quercetin (301.0343 *m/z*), and caffeic acid (179.0338 *m/z*).

GBPeF exhibited rutin as the most abundant free phenolic compound, followed by hydroxybenzoic acid isomer 2, kaempferol-3-O-galactoside-7-O-rhamnoside, and epicatechin. In contrast, trans-ferulic acid was the dominant bound phenolic compound, followed by p-coumaric acid. In GBPF, the major free phenolics included hydroxybenzoic acid isomer 2, epicatechin, dihydroxyphenylacetic acid, and eriodictyol. Notably, the major bound phenolics in GBPF, such as 4-*O*-methyl gallic acid, trans-ferulic acid, and p-coumaric acid, were the same as those in GBPeF, albeit in lower abundance. Phenolic acids, primarily bound in

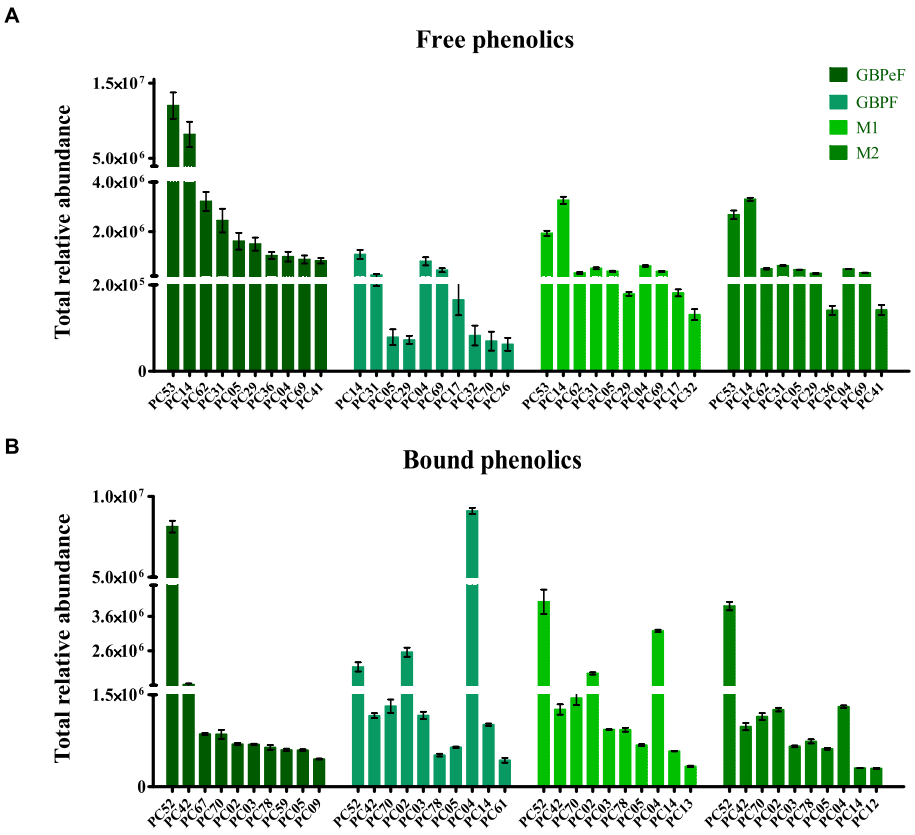

Fig. 1. The ten most abundant phenolic compounds (free and bound) found in the green banana flours. For detailed information on the identified phenolic compounds (e.g., PC53, PC14, PC62), refer to Table S5.
GBPF: green banana pulp flour; GBPeF: green banana peel flour. M1: green banana mixed pulp (90%) and peel (10%) flour. M2: green banana mixed pulp (80%) and peel (20%) flour. Analysis was performed in three replicates. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

grains, have been linked to numerous health benefits, including antioxidant properties and gut microbiota modulation (Rocchetti et al., 2022; Santos et al., 2019). M1 and M2 exhibited a blend of the phenolic compounds (free and bound) found in both GBPeF and GBPF. In the subsequent sections (3.7 and 3.8), we will explore the potential bioactive properties of the free phenolics in green banana flours, including chemometric analysis.

As expected, GBPF showed a lower tannin content (44.70 ± 2.02 mg CE/g) compared to GBPeF, which displayed a significantly higher value (76.00 ± 3.20 mg CE/g). Tannins, protective metabolites in plants, are naturally present in higher concentrations on the outer parts of vegetables. Besides their robust antioxidant capacity, condensed tannins can bind with starch, reducing its digestibility and thereby increasing the content of resistant starch, which functions as dietary fiber (Barros, Awika, & Rooney, 2012).

3.7. In vitro bioactive properties

3.7.1. Cell viability and proliferation

Herein, the in vitro tests with extracts of green banana flour (GBPF, GBPeF, M1–90:10 pulp: peel, and M2–80:20 pulp: peel) containing free phenolics were conducted to explore their biological activities against three cancer cell lines (A549, HCT8, and HepG2) as illustrated in Fig. S10. Analyzing results across all cell lines, the extracts exhibited cytotoxic effects at varying concentrations depending on the cell type. Notably, the M2–80:20 (pulp: peel) extract demonstrated less activity than the others, as indicated by high values of IC_{50}, GI_{50}, and LC_{50} (>100 μg/mL), suggesting the need for higher concentrations to inhibit half the cells' proliferation, indicating low cytotoxicity and cell inhibition. Conversely, GBPeF (100% banana peel) showed a greater capacity to inhibit cell growth, with lower GI_{50} values (10.24 to 24.59 μg/mL), and was the only extract to exhibit lethality at a concentration of 88.2 μg/mL in the HCT8 cell line (Fig. S10).

In related studies, Kumar, Durgadevi, Saravanan, and Uma (2019) demonstrated the cytotoxic activity of aqueous methanol extract of Nendran banana peel against MCF-7 breast cell lines. Dahham, Mohamad, Tabana, and Majid (2015) found that banana peel extract prepared from hexane solvent exhibited the highest toxicity toward HCT-116 (colorectal carcinoma cell line from humans) with 64.02% inhibition of cell proliferation. Vijayakumar et al. (2017) showed that banana peel crude extract could also be used to synthesize gold nanoparticles that inhibited the biofilm formation of the Gram-positive bacterium *Enterococcus faecalis* and were cytotoxic to human lung cancer cells. These results support the chemopreventive potential of phenolic compounds in green banana flour by inhibiting cancer cell growth.

3.7.2. Measurement of intracellular ROS

Oxidative damage caused by ROS, which includes radical species and non-radical hydrogen peroxide, plays a crucial role in metabolism, oxidative stress, signal transduction, and apoptosis in both cancerous and non-cancerous cells and may contribute to the initiation of cytotoxicity in cancer cells (Azevedo et al., 2016). In this study, we measured ROS production in cell lines exposed to various concentrations of green banana flour phenolic extracts using DCFH-DA. Here, H_2O_2, an oxidizing agent, was used, and the ROS levels in the positive control were higher than in both the negative control and the treatment groups treated with green banana flour extracts (Fig. 2). These results provide direct evidence of significant ROS scavenging by the green banana flour extracts

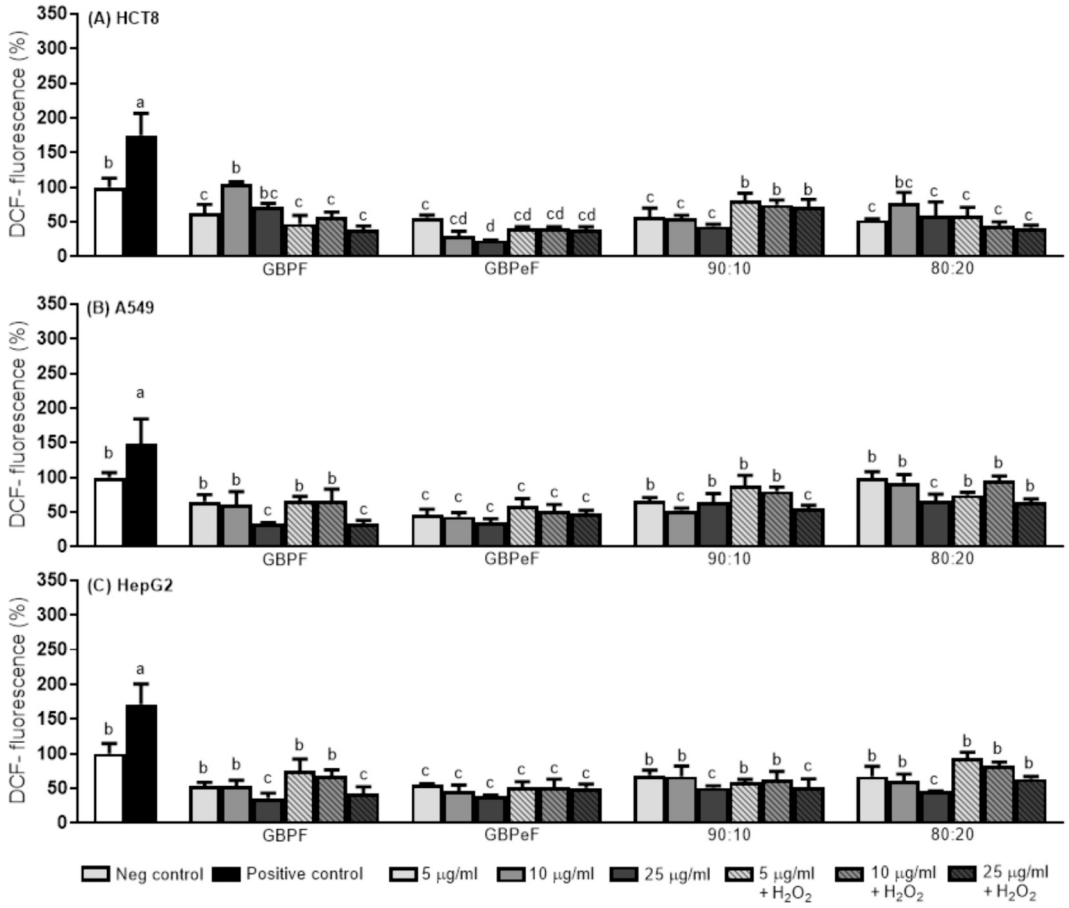

Fig. 2. Results of intracellular ROS measurement in HCT8 (A), A549 (B), and HepG2 (C) cells via spectrofluorimetric analysis. Quantitative data are presented as mean ± standard deviation. One-way ANOVA was performed within the same extract and related controls ($p \leq 0.05$). GBPF: green banana pulp flour; GBPeF: green banana peel flour; 90:10 = M1: green banana mixed pulp (90%) and peel (10%) flour; 80:20 = M2: green banana mixed pulp (80%) and peel (20%) flour. Tests were performed in three replicates. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

against H_2O_2-induced damage, indicating a protective effect in A549, HCT8, and HepG2 cell lines.

Among the extracts, GBPeF proved to be the most effective at reducing intracellular ROS generation in all cells and at all concentrations tested, achieving levels lower than those spontaneously generated, thereby indicating antioxidant activity. According to the literature (do Carmo, Granato, & Azevedo, 2021; Fidelis et al., 2021), this antioxidant effect can be attributed to certain phenolic compounds, including tannins, which vary according to each extract's chemical profile and cellular adaptations. Notably, GBPeF exhibited the highest concentration of total free phenolic compounds, both in the pulp and their respective mixtures (refer to Tables S4 and S5; see Fig. 1).

Cancer cells are characterized by an increased rate of localized ROS generation compared to normal cells. This heightened ROS activity helps hyper-activate signaling pathways that promote cell proliferation, survival, and metabolic adaptation to the tumor environment (do Carmo et al., 2021). When ROS generation is suppressed below optimal levels by the effects of antioxidant phenolic compounds, it disrupts cell signaling, resulting in the loss of homeostasis control. Various types of physical-chemical stress stimuli can then trigger necrosis or apoptosis, including cell membrane damage, mitochondrial dysfunction, and destabilization (do Carmo et al., 2021). Therefore, the cell death observed in the cell viability tests may involve mechanisms related to the scavenging or neutralizing ROS in cancer cells by phenolic compounds.

3.8. Chemometric analysis

The first PCA using the chemical profile values (total relative abundance of free phenolics in the samples) as input (Fig. 3A and B) revealed that the first principal component (PC1) captured 97.8% of the total information in the dataset. Consequently, the GBPF, M1, and M2 samples exhibited proximity on the X-axis, indicating their similarity (Fig. 3A). The chemicals primarily responsible for distinguishing between the four analyzed samples are rutin, hydroxybenzoic acid isomer 2, kaempferol-3-O-galactoside-7-O-rhamnoside, epicatechin, and dihydroxyphenylacetic acid, in order of importance (Fig. 3B). This pattern aligns with the concentration rankings of the top 10 phenolic compounds present in these samples (Fig. 1).

The second PCA, predominantly interpreted through PC1 (which accounted for 95.15% of the total variance), suggested that the M1, M2, and GBPF samples promote higher cellular viability in all assays than GBPeF (Fig. 3C and D). Furthermore, GBPeF demonstrated a greater capacity to reduce ROS levels in the presence of a positive control during the cellular assays (Fig. 3E and F), according to PC1 (99.02% of the information). Although all samples reduced ROS values, GBPF, and the mixtures exhibited better cytotoxic profiles, suggesting that despite their lesser potency in antioxidant activity, they could be considered for future studies due to their favorable potency/safety balance.

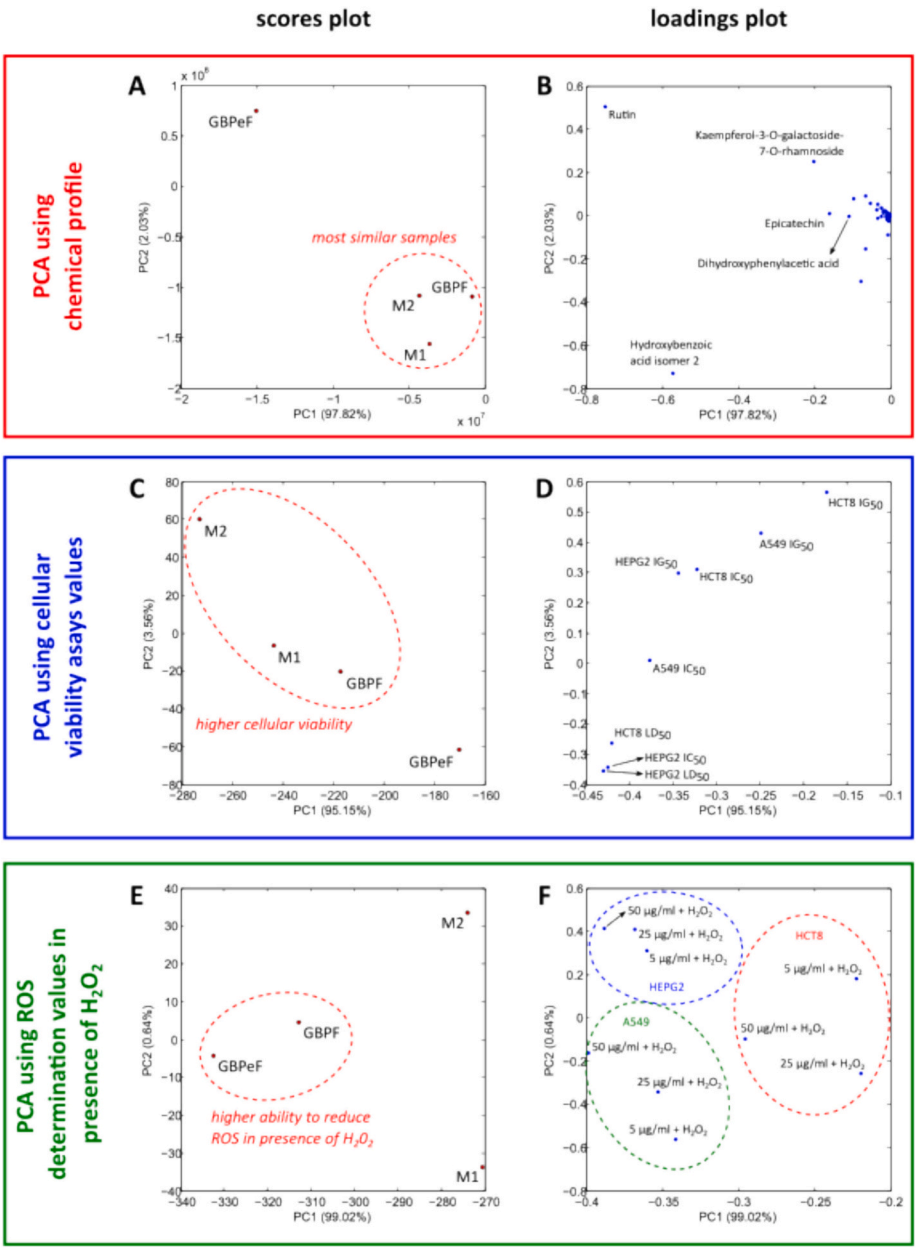

Fig. 3. Score values (A, C, and E) and loading values (B, D, and F) for PCA using chemical profile (red rectangle), cellular viability (blue rectangle), and ROS assays (green rectangle). GBPF: green banana pulp flour; GBPeF: green banana peel flour; M1: green banana mixed pulp (90%) and peel (10%) flour;M2: green banana mixed pulp (80%) and peel (20%) flour. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 3
Pasting properties and gel strength of the green banana pulp and peel flours and their blends.

Flours	Parameters							
	Pasting temperature (°C)	$Visc_{max.}$ (cP)	$Visc_{mín.}$ (cP)	*Breakdown* (cP)	$Visc_{final}$ (cP)	*Setback* (cP)	Pasting time (min.)	Gel strength (N)
GBPF	69.92 ± 0.38^{a}	5971.00 ± 25.12^{a}	2278.00 ± 32.08^{a}	3693.00 ± 43.97^{a}	3803.00 ± 22.54^{c}	1525.00 ± 54.06^{b}	7.04 ± 0.10^{b}	7.21 ± 0.51^{a}
M1	69.92 ± 0.01^{a}	5936.33 ± 257.93^{a}	2661.00 ± 27.71^{a}	3275.33 ± 230.27^{b}	4239.33 ± 90.59^{b}	1578.33 ± 72.54^{ab}	7.34 ± 0.12^{b}	5.34 ± 0.20^{b}
M2	69.65 ± 0.01^{a}	6006.33 ± 26.16^{a}	2667.00 ± 83.16^{a}	3339.33 ± 68.53^{ab}	4395.00 ± 32.00^{a}	1728.00 ± 53.70^{ab}	7.82 ± 0.10^{b}	5.78 ± 0.39^{b}
GBPeF	70.05 ± 0.22^{a}	1851.67 ± 158.70^{b}	1631.67 ± 305.21^{b}	220.00 ± 148.54^{c}	3579.33 ± 57.19^{d}	1947.67 ± 294.75^{a}	10.89 ± 1.14^{a}	1.62 ± 0.01^{c}

GBPF: green banana pulp flour; GBPeF: green banana peel flour; M1: mix of green banana pulp (90%) and peel (10%) flours; M2: mix of green banana mixed pulp (80%) and peel (20%) flours. Results were expressed as means ± standard deviation. Tests were performed in three replicates. Means followed by the same letter in the columns do not differ at 5% probability by the Tukey test.

3.9. Technological properties of the green banana flours

3.9.1. Pasting properties

The pasting properties of the GBPF and GBPeF, along with their mixtures, M1 and M2, are detailed in Table 3. The pasting temperature values did not differ significantly between the samples, all maintaining a consistent temperature of 69.89 °C. GBPF exhibited a higher peak viscosity at 5971.0 cP than GBPeF, which registered 1851.67 cP. This variance is attributed to the higher fiber content and lower starch levels in the peel flour than the pulp flour. Additionally, fiber competes with starch for water absorption, resulting in a lower apparent viscosity for the peel flour. Similar observations were made by Bertolini, Bello-Pérez, Méndez-Montealvo, Almeida, and Lajolo (2010) for green banana (*Musa acuminata* var. 'Nanicão') flours, where pulp flours showed higher paste viscosity than peel flours, at 3470 cP and 1210 cP, respectively. Comparing the maximum viscosity values between GBPF and the mixtures (M1 and M2), no significant differences were noted, with readings of 5971.00 cP, 5936.33 cP, and 6006.33 cP for the pulp flour, M1, and M2 respectively. Thus, incorporating up to 20% of peel flour into the pulp flour has minimal impact on the starch pasting properties of the GBPF.

The breakdown values of the samples increased significantly with the starch concentrations (Table 3). The pulp flour and the mixed flours (M1 and M2) exhibited higher breakdown levels compared to the peel flour. The lower breakdown viscosity of the peel flour likely results from differences in particle size distribution, as well as its high dietary fiber and lipid contents. According to Zheng, Liu, Liu, Xing, and Jiang (2021), high lipid content in whole wheat flour can enhance the shear strength of the material during heating, thereby improving the strength of the starch gel structure post-extrusion.

As indicated in Table 3, the mixed flours M1 and M2 showed higher final viscosity ($_{final}$Visc) results than both the peel and pulp flours. The lower final viscosity of the GBPeF may be attributed to the type of fiber, which likely reduces the availability of free water in the system, impairing starch gelatinization, in addition to its lower starch content compared to M1 and M2. Regarding the setback values, a significant range from 1525.00 to 1947.67 cP was observed between GBPeF and GBPF. The lower setback value noted for GBPF (22.78% amylose content) compared to GBPeF (25.97% amylose content), with values of 1525.00 and 1947.67 cP, respectively, suggests that the pulp flour maintains its technological characteristics longer, likely due to its lower amylose and dietary fiber contents, thus reducing syneresis (Felisberto et al., 2020). Lower setback values are critical for applications requiring low syneresis, such as frozen and baking products.

Therefore, it is advisable to incorporate only lower levels of GBPeF (up to 20%, as demonstrated in this study) to maintain the similar technological properties of GBPF, which is predominantly the banana flour used in food applications.

3.9.2. Gel strength

The hardness of the gels derived from the pasting properties of GBPF, GBPeF, and their mixtures (M1 and M2) was measured and reported in Table 3. The gel from the GBPeF exhibited a lower hardness (1.62 N) compared to that of the pulp flour (7.21 N) and the respective mixtures, M1 and M2, which recorded hardness values of 5.34 N and 5.78 N, respectively. This difference was anticipated based on the final viscosity results from the RVA data (Table 3).

The variance in gel strength can be explained by the differing concentrations of total starch and dietary fiber in the flours. GBPF contained a higher percentage of total starch (69.54%) and the lowest percentage of total dietary fiber (8.63%), whereas GBPeF had considerably lower starch content (24.04%) and higher dietary fiber content (39.57%). As Felisberto et al. (2020) have noted, gel formation is influenced by the interaction between amylose and amylopectin, which together develop networks to retain water. The substantial disparities in starch and fiber contents among the samples likely led to the formation of gels with varying strengths, with the high fiber content in GBPeF impeding the ability of starch molecules to trap water despite its higher amylose content (25.97%).

Furthermore, the lipid content in GBPeF, which was significantly higher than in GBPF and the mixtures (Table 1), also contributed to the softer gels observed. The higher lipid content interferes with the gel structure, resulting in lower gel hardness in GBPeF (1.62 N) compared to the gels formed by GBPF (7.21 N) and the mixed flours with 10% (5.34 N) and 20% (5.78 N) peel flour.

4. Conclusions

The present study showed that GBPeF is a significant source of dietary fiber, total phenolics (free and bound), minerals, and fatty acids such as oleic, linoleic, and eicosapentaenoic (EPA). GBPF is rich in resistant starch and exhibits high gel strength and paste viscosity when heated. Replacing up to 20% of GBPF with GBPeF yielded blends rich in resistant starch, dietary fiber, and antioxidants, with minimal impact on the technological properties of GBPF. Both types of banana flour contain similar bound phenolic compounds, including trans-ferulic acid, 4-*O*-methyl gallic acid, and p-coumaric acid. Flour extracts from GBPeF, GBPF, and their mixtures (M1 and M2), which contain free phenolic compounds, demonstrated cytotoxic effects against cancer cell lines (A549, HCT8, and HepG2) and reduced intracellular ROS formation. These effects may be attributed to phenolic compounds, such as rutin, kaempferol-3-O-galactoside-7-O-rhamnoside, epicatechin, and dihydroxyphenylacetic acid, present in the extracts.

These promising findings pave the way for further studies to evaluate the in vivo bioactive properties of green banana mixed pulp and peel flour, an ingredient with significant potential for developing functional foods. Furthermore, this study promotes the utilization of green banana peel flour, an underused, readily available material with bioactive properties, as a strategy to reduce waste and minimize management costs.

CRediT authorship contribution statement

Leonara Martins Viana: Formal analysis, Investigation, Methodology, Data curation, Writing – original draft, Writing – review & editing, Visualization. **Fabiana Silva Rocha Rodrigues:** Methodology, Formal analysis. **Millena Cristina Barros Santos:** Methodology, Formal analysis, Data curation. **Amanda dos Santos Lima:** Methodology, Formal analysis, Data curation. **Elizabeth Harumi Nabeshima:** Methodology, Formal analysis, Writing – review & editing. **Mauricio de Oliveira Leite:** Methodology, Formal analysis, Writing – review & editing. **Márcio Arêdes Martins:** Methodology, Writing – review & editing. **Carlos Wanderlei Piler de Carvalho:** Methodology, Formal analysis, Writing – review & editing. **Vinícius Gonçalves Maltarollo:** Data curation, Writing – review & editing. **Luciana Azevedo:** Methodology, Writing – review & editing. **Mariana Simões Larraz Ferreira:** Methodology, Writing – review & editing. **Hércia Stampini Duarte Martino:** Methodology, Supervision, Writing – review & editing. **Mária Herminia Ferrari Felisberto:** Methodology, Supervision, Writing – review & editing. **Frederico Augusto Ribeiro de Barros:** Funding acquisition, Conceptualization, Methodology, Project administration, Resources, Supervision, Writing – review & editing, Visualization.

Declaration of competing interest

The authors declare no conflict of interest.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2024.139506.

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