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Extraction Solvent Effects on Banana Blossom Bioactive Compounds: Enhanced Bioaccessibility via Gastrointestinal Digestion

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Abstract

Banana blossoms are often discarded as agricultural byproducts, yet they contain bioactive compounds with potential health-promoting properties. The transformation of these underutilized materials into functional food ingredients represents an important circular economy strategy. Banana blossoms from the *Musa* AAA group and *M.* × *paradisiaca* cultivars were selected for analysis. Bioactive compounds and antioxidant capacity were evaluated in different anatomical parts (bracts, florets and core) through aqueous and 50% ethanol extraction. The extracts were assessed for bioaccessibility using *in vitro* gastrointestinal digestion following the INFOGEST protocol to determine their potential for developing functional foods. Bioactive content and antioxidant activities varied significantly across cultivars, anatomical parts, and extraction solvents. *Musa* AAA group showed superior performance, with aqueous core extracts achieving the highest phenolic content (1,033.57 ± 20.20 mg GAE/100 g) and aqueous bract extracts achieving the highest flavonoid content (1,529.23 ± 27.20 mg QE/100 g). Aqueous extraction was more effective than ethanol extraction. Gastrointestinal digestion produced contrasting effects: phenolic content increased substantially in certain extracts (9.1-fold in *M.* × *paradisiaca* bracts), while antioxidant activities decreased dramatically (77.4% - 99.6% reduction, $p < 0.001$). PCA confirmed that *in vitro* digestion resulted in standardized bioactive compound profiles regardless of source materials. PC-1 variance increased from 63.00% before digestion to 96.44% after digestion, demonstrating uniform bioaccessibility patterns across all samples. This study highlights banana blossoms as a promising source of bioactive compounds for functional food development, with aqueous extraction being effective and digestion ensuring uniform bioaccessibility. Their use can contribute to sustainable agricultural waste valorization.

Keywords: Banana blossoms, Bioactive compounds, Phenolic content, Flavonoid content, Aqueous extraction, Bioaccessibility, Gastrointestinal digestion, Agricultural byproducts

Introduction

Banana (*Musa* spp., family Musaceae) is a native plant of the southwestern Pacific region. It is one of the most important tropical fruits in the global market, being inexpensive yet highly nutritious [1]. Globally, banana cultivation includes nearly 1,000 varieties grown across more than 150 countries [2]. The FAO reported that global banana production in 2020 reached approximately 120 million tons, with a trend of increasing annual consumption [2]. Increasing banana

consumption has also led to larger amounts of banana blossoms being discarded as agricultural waste during harvesting. Converting banana blossoms into food products or sources of bioactive compounds, therefore, enhances the value of agricultural waste. This utilization strategy for agricultural residues supports circular economy principles within the food industry, prioritizing waste reduction, resource recycling, and optimized material usage [3].

Banana blossoms contain essential bioactive components, including phenolic compounds, flavonoids, and β-sitosterol, some of which exhibit antioxidant properties [4]. Additionally, these compounds may be responsible for the traditional galactagogue effects of banana blossoms in promoting milk production among breastfeeding mothers [5,6]. The amount of bioactive compounds varies depending on the species, cultivation source, and extraction method [4]. However, measuring only the content of bioactive compounds in banana blossoms is insufficient for evaluating their absorption in humans. Bioaccessibility studies provide a solution to understand the absorption capacity of these bioactive compounds [7,8]. These analyses reveal how food matrices influence the release and absorption of active compounds. Generally, bioactive compounds in food are often trapped within plant cell structures or possess complex structures that limit their release in the human gastrointestinal tract.

The composition of the food matrix, together with pH, temperature, and processing methods, can strongly influence bioaccessibility [9]. Processing methods, especially thermal treatments, can greatly affect accessibility, sometimes enhancing phytochemical extractability. However, it adversely affects heat-labile substances, causing the decomposition of compounds such as vitamins and certain polyphenols [10]. Moreover, digestion conditions influence stability and absorption, particularly regarding phenolic substances and flavonoids [11]. This investigation therefore employs *in vitro* gastrointestinal digestion to comprehensively assess the bioaccessibility of bioactive compounds from banana blossom extracts. These data provide essential information for guiding the development of functional foods. Despite extensive research on banana blossom bioactive compounds, no studies have systematically evaluated the bioaccessibility of these compounds through gastrointestinal digestion. The findings from this research will help fill this knowledge gap in functional food product development.

This study investigated the content of phenolic compounds, flavonoids, and antioxidant activities in banana blossoms from *Musa* AAA group (Cavendish) and *Musa* × *paradisiaca* (ABB group). *Musa* AAA group, with its AAA genome, is the most consumed and exported banana in the world [12]. *M.* × *paradisiaca* is a hybrid between *M. acuminata* and *M. balbisiana* and is popularly consumed in Southeast Asia [13]. Previous studies reported that *Musa* AAA group florets and bracts contained total phenolic contents of approximately 90 and 60 μg GAE/g when extracted with aqueous solvent, and 170 and 75 mg GAE/g with 95% ethanol extraction, respectively. Flavonoid contents were approximately 75 and 70 mg QE/g for aqueous extracts, and 110 and 30 mg QE/g for 95% ethanol extracts, respectively [4]. Similarly, the 95% ethanol extracts of *Musa* ABB CV. Kluai "Namwa" demonstrated higher phenolic contents of approximately 1,091.30 ± 156.98 and 742.42 ± 120.42 μg GAE/g in floret and bract parts, respectively [14].

Previous studies have used various solvents to extract bioactive compounds from banana blossoms, including water, ethanol, and methanol [4]. The polarity differences between water and ethanol-water mixtures influence the solubility and extractability of different bioactive compound classes [15]. This study selected water and 50% ethanol as solvents due to their complementary properties [16]. Aqueous extraction offers a safe and environmentally friendly approach, while 50% ethanol effectively extracts moderately polar phenolic and flavonoid compounds.

The bioaccessibility of bioactive compounds from different anatomical parts of banana blossoms using various extraction solvents remains unexplored. Therefore, this study aimed to investigate (1) total phenolic and flavonoid content, (2) antioxidant activity (DPPH, ABTS, and FRAP), and (3) bioaccessibility and antioxidant retention after *in vitro* gastrointestinal digestion in different anatomical parts (bracts, florets, and core) from *Musa* AAA group and *M.* × *paradisiaca* using aqueous and 50% ethanol extraction.

Based on existing research findings, the following hypotheses were formulated: (1) *Musa* AAA group banana blossoms would exhibit higher bioactive

compound concentrations than *M.* × *paradisiaca* banana blossoms; (2) different extraction solvents would show varying efficiency for specific compound classes; (3) anatomical parts would display distinct bioactive profiles; and (4) *in vitro* digestion would significantly modify compound bioaccessibility and antioxidant activity.

Materials and methods

Sample preparation

Banana blossoms from the *Mus*a AAA group and *M.* × *paradisiaca* were collected from Pak Tong Chai district, Muang Nakhon Ratchasima, Thailand, in November 2024, approximately 30 days after inflorescence emergence. Three individual banana plants from each cultivar were independently selected for sample collection to ensure biological replication. Banana blossoms were cleaned and separated into bracts, florets, and core, then sliced (0.3 - 0.5×2.5 - 3.0 cm^2) and immersed in 1 %(w/v) citric acid solution for 15 min to minimize enzymatic browning, rinsed with distilled water, and drained completely. Samples were then dried in a tray dryer (HS-169, O.V.D. Series 10 Trays, AT Packing, Thailand) at 50 °C to achieve a moisture content of 8 ± 2%. Dried samples were ground using a Waring 7009S grinder (Waring, Japan) and sieved through a 60-mesh (250 μm) sieve to obtain uniform powder. The powdered samples were stored in foil bags at room temperature in the dark until analysis, which was conducted within 30 days of collection.

Sample extraction

Each anatomical part of banana blossoms was extracted using 2 solvents: Distilled water (65 ± 2 °C) and 50 %v/v ethanol. Extraction was performed using 1 g powder with 25 mL solvent at 65 °C for 30 min in a digital water bath (TSGP20, Thermo Fisher Scientific, US) under intermittent stirring. Mixtures were cooled to room temperature (25 ± 2 °C) and then filtered through Whatman No. 1 filter paper. Filtered extracts were freeze-dried using a modified method from ElNaker et al. [17]: frozen at −80 °C (24 h), then freeze-dried at −40 °C and 0.1 mbar for 48 h. The freeze-dried powders (yield: 11.2%) were stored in airtight containers at 4 °C until analysis. For the assays, powders were reconstituted in their original extraction solvent at a concentration of 1 g per 25 mL. Extract codes were assigned as follows: *Musa* AAA group - CBW/CFW/CCoW (bracts/florets/core with aqueous); CBE/CFE/CCoE (bracts/florets/core with 50% ethanol). *M.* × *paradisiaca* - MBW/MFW/MCoW (bracts/florets/core with aqueous); MBE/MFE/MCoE (bracts/florets/core with 50% ethanol).

Determination of total phenolic content

Total phenolics were determined using the Folin-Ciocalteu method [18] with modifications. Extract samples (2.5 mL) were mixed with 200 μL Folin-Ciocalteu reagent (Sigma-Aldrich, USA) and incubated in darkness at room temperature (25 ± 2 °C) for 5 min. Following this, 2 mL of 7 %(w/v) Na_2CO_3 solution (Loba Chemie, India) was added, and samples were further incubated in darkness for 90 min. Absorbance measurements were taken at 760 nm using a UV-Vis spectrophotometer (UH5300, Hitachi, Japan). Gallic acid standards (0 - 100 μg/mL) were used for calibration. Results were calculated as mg gallic acid equivalents (GAE) per 100 g dry weight (dw).

Determination of total flavonoid content

The total flavonoid content was analyzed using a modified method of Zhishen *et al.* [19]. Approximately 1.5 mL of the extracted sample was mixed with 2.8 mL of distilled water, 100 μL of a 10 %w/v aluminum chloride in methanol (Loba Chemie, India), and 100 μL of a 1 M potassium acetate buffer solution. The mixture was incubated at room temperature in the dark for 10 min. Afterward, the absorbance was measured at 415 nm. Quercetin was used as a standard solution to exhibit a standard curve, and the total content of flavonoids was expressed as mg quercetin equivalents (QE) per 100 g of dry weight (dw).

DPPH assay

The DPPH assay was performed following Brand-Williams *et al.* [20] with modifications. The extract (300 μL) was mixed with 1.5 mL of 0.2 mM 2,2-diphenyl-1-picrylhydrazyl (DPPH) in ethanol (Sigma-Aldrich, USA) and maintained in darkness at ambient temperature for 40 min. Absorbance was measured at 517 nm, and antioxidant activity was expressed as μg Trolox equivalents (TE) per 100 g dry weight (dw), based on a Trolox calibration curve (0 - 100 μg/mL).

ABTS assay

The ABTS assay was performed following Re *et al.* [21] with modifications. The ABTS working solution was prepared by combining 7 mM 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) (Sigma-Aldrich, USA) with 2.45 mM $K_2S_2O_8$ (QRëC, New Zealand) at a 1:1 ratio and storing it in darkness at room temperature for 12 h. The solution was diluted with 95% methanol to achieve an absorbance of 0.700 ± 0.05 at 734 nm, which served as the working solution. Subsequently, 20 µL of extract was added to 1.98 mL of the ABTS working solution and incubated for 5 min in the dark at ambient temperature. Absorbance readings were obtained at 734 nm employing a UV-Vis spectrophotometer (UH5300, Hitachi, Japan). Trolox (0 - 100 µg/mL) (Sigma-Aldrich, USA) served as the standard for calibration. Results were expressed as µg Trolox equivalents (TE) per 100 g dry weight (dw).

FRAP assay

A modified FRAP protocol based on Benzie and Strain [22] was used to evaluate the antioxidant capacity. FRAP reagent preparation involved combining 300 mM sodium acetate buffer (pH 3.6), 20 mM $FeCl_3$, and 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) in 40 mM HCl using a 10:1:1 volumetric ratio. Subsequently, 1.5 mL of FRAP reagent was added to 150 µL of extract and incubated for 20 min in the dark at room temperature. Absorbance determination was performed at 593 nm employing a UV-Vis spectrophotometer (UH5300, Hitachi, Japan). Trolox (0 - 100 µg/mL) (Sigma-Aldrich, USA) served as the standard for calibration. The antioxidant capacity was expressed as µg Trolox equivalents (TE) per 100 g dry weight (dw).

Bioaccessibility analysis

Bioaccessibility was evaluated using a modified static *in vitro* gastrointestinal digestion model based on the INFOGEST protocol [8,23], which simulates sequential oral, gastric, and intestinal phases under physiological conditions. In the oral phase, extract (5 mL) was mixed with simulated salivary fluid (5 mL, pH 7.0, α-amylase 75 U/mL final concentration) and maintained at 37 °C for 2 min under gentle agitation to obtain an oral bolus.

The entire oral bolus (10 mL) was then mixed with simulated gastric fluid (10 mL, pH 3.0) containing porcine pepsin (2,000 U/mL final concentration) and $CaCl_2$ (0.075 mM final concentration). The solution pH was modified to 3.0 by adding 1 M HCl, then the mixture was held at 37 °C for 2 h using continuous gentle stirring to produce gastric chyme. Subsequently, the complete gastric chyme (20 mL) was combined with simulated intestinal fluid (20 mL, pH 7.0) containing pancreatin (trypsin 100 U/mL final concentration), fresh porcine bile (10 mM bile salts final concentration), and $CaCl_2$ (0.3 mM final concentration). The solution pH was modified to 7.0 by adding 1 M NaOH and maintained at 37 °C for 2 h with continuous gentle shaking.

At the end of the intestinal phase, the digesta was centrifuged (5000× g, 10 min, 4 °C), followed by filtration of the supernatant through a 0.45 µm membrane filter. The bioaccessible fraction was analyzed for total phenolics, flavonoids, and antioxidant capacity using previously described methods. Bioaccessibility was expressed as the percentage of compounds recovered in the bioaccessible fraction.

Statistical analysis

Experiments were performed using biological replicates (n = 3 individual banana plants per cultivar) and technical replicates (n = 3). Data were analyzed using ANOVA followed by Duncan's Multiple Range Test ($p < 0.05$). PCA was employed to examine relationships between bioactive compounds before and after *in vitro* gastrointestinal digestion using PAST 4.13 (University of Oslo, Norway). All statistical tests were conducted using SPSS Statistics version 27 (IBM Corp., USA).

Results and discussion

Total phenolic content

The total phenolic content (TPC) in different parts of banana blossoms from the *Musa* AAA group and *M.* × *paradisiaca* before and after *in vitro* gastrointestinal digestion is presented in **Figure 1**.

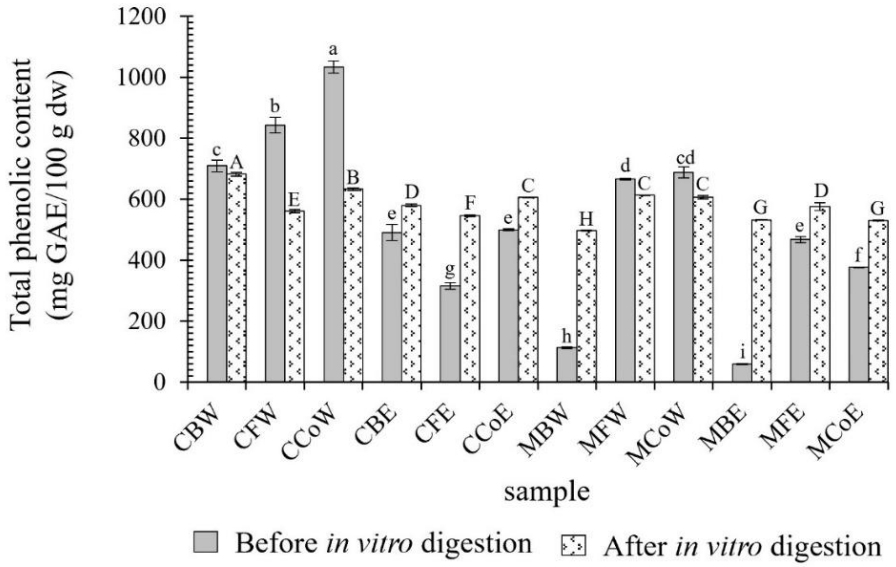

Figure 1 Total phenolic content (TPC) in different parts of banana blossoms before and after *in vitro* gastrointestinal digestion. Values are expressed as mg GAE/ 100 g dw (mean ± standard deviation, n = 3). Different superscripts (a-i, A-H) within bars of the same pattern indicate significant differences ($p < 0.05$) as determined by Duncan's multiple range test. Sample codes: C = *Musa* AAA group, M = *M.* × *paradisiaca*, B = bracts, F = florets, Co = core, W = aqueous extraction, E = 50% ethanol extraction.

Before digestion, CCoW exhibited the highest TPC (1,033.57 ± 20.20 mg GAE/ 100 g), while MBE showed the lowest (58.43 ± 1.62 mg GAE/ 100 g). *Musa* AAA group consistently demonstrated higher TPC than *M.* × *paradisiaca*, and aqueous extraction yielded superior results compared to 50% ethanol for both cultivars. For *Musa* AAA group, aqueous extraction yielded TPC in the order of core (highest), florets, and bracts (lowest), while 50% ethanol extraction demonstrated core (highest), bracts, and florets (lowest). After digestion, ethanol extracts generally showed increased TPC, while aqueous extracts decreased, except for MBW. The most pronounced increase occurred in MBE, whereas the largest decrease was observed in CCoW. The 9.1-fold increase in MBE following digestion suggests enhanced liberation of bound phenolic compounds from cellular matrices under simulated gastrointestinal conditions, as previously observed in complex plant matrices [8]. Post-digestion, TPC distribution patterns differed between cultivars: *Musa* AAA group showed reduced TPC in florets compared to other anatomical parts, while *M.* × *paradisiaca* exhibited enhanced TPC specifically in florets. The TPC values obtained in this study were considerably higher than our previous reference [14], which may be attributed to differences in cultivar (*Musa* AAA group vs. *Musa* ABB), extraction methodology (aqueous and 50% ethanol vs. 95% ethanol), and environmental growing conditions. These research findings contribute to the growing body of evidence that gastrointestinal digestion can enhance the bioaccessibility of phenolic compounds from various plant sources [8,24]. The increased phenolic content in 50% ethanol extracts following digestion indicates that digestive mechanisms can release more phenolic compounds compared to aqueous extracts. This finding demonstrates enhanced absorption potential. The phenolic profile variations reflect diverse compounds, including extractable polyphenols and hydrolyzable polyphenols, as documented in banana tissues [25].

Higher TPC in the *Musa* AAA group may result from genetic differences in phenolic biosynthesis pathways [26]. The higher polarity of water favors extraction of hydroxyl-rich phenolics, explaining the superior performance of aqueous extraction [27]. The results reveal contrasting bioaccessibility patterns, suggesting different release mechanisms. Phenolic compounds from 50% ethanol extraction likely consist

of protein-lipid-bound phenolics liberated during digestion, whereas aqueous-extracted phenolic compounds undergo degradation or form complex structures [28]. These findings align with studies on citrus peels, apple peels, and grape pomace, which have shown digestion-induced profile modifications [9]. The substantial increase in ethanol extract bioaccessibility suggests enhanced bioaccessibility potential, while aqueous extract stability concerns may require protective formulation strategies.

Total flavonoid content

The total flavonoid content (TFC) in different parts of banana blossoms before and after *in vitro* gastrointestinal digestion is presented in **Figure 2**.

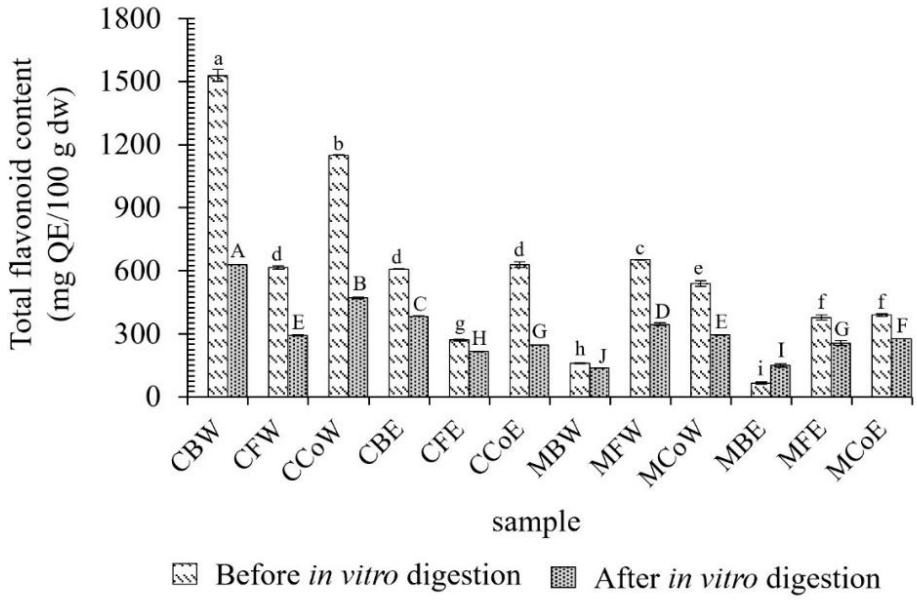

Figure 2 Total flavonoid content (TFC) in different parts of banana blossoms before and after *in vitro* gastrointestinal digestion. Values are expressed as mg QE/ 100 g dw (mean ± standard deviation, n = 3). Different superscripts (a-i, A-J) within bars of the same pattern indicate significant differences ($p < 0.05$) as determined by Duncan's multiple range test. Sample codes: C = *Musa* AAA group, M = *M.* × *paradisiaca*, B = bracts, F = florets, Co = core, W = aqueous extraction, E = 50% ethanol extraction.

TFC was significantly influenced by banana cultivar, anatomical part, and digestion process ($p < 0.05$). Before digestion, CBW exhibited the highest TFC (1,529.23 ± 27.20 mg QE/ 100 g dw), while MBE showed the lowest (66.15 ± 4.90 mg QE/ 100 g dw). *Musa* AAA group consistently demonstrated superior TFC compared to *M.* × *paradisiaca*, with aqueous extracts yielding higher flavonoid content than ethanol extracts in both cultivars. Particularly striking was that aqueous extraction of *Musa* AAA group bracts yielded approximately 9.5-fold higher TFC. Following *in vitro* digestion, TFC patterns exhibited both similarities and differences compared to those of phenolic compounds. Most samples exhibited a decreased content, except for MBE, which demonstrated a remarkable increase from 66.15 ± 4.90 to 150.00 ± 8.16 mg QE/ 100 g dw (a 2.3-fold increase). CBW showed the highest reduction (59% decrease), while ethanol extracts typically retained higher TFC levels than aqueous extracts.

Flavonoids appeared slightly more stable during digestion than total phenolics, reflecting differences in their degradation and release dynamics. The intestinal-mediated increase in flavonoid bioaccessibility parallels observations across diverse botanical sources, where digestive conditions facilitate cellular disruption [8,24]. The pronounced 2.3-fold elevation in MBE highlights unique matrix-compound associations within banana blossom tissues. These cultivar differences can be attributed to genetic variations affecting secondary metabolite production and flavonoid biosynthetic

pathway expression [29]. The superior performance of aqueous extraction aligns with the water-soluble nature of many flavonoid glycosides present in banana blossoms. Flavonoids in plant tissues often exist as sugar-bound forms (glycosides), which enhance their solubility in polar solvents like water [30]. This explains the significantly higher TFC observed in aqueous extracts compared to 50% ethanol extracts across both cultivars.

Antioxidant activity

DPPH radical scavenging activity

The antioxidant activity measured by DPPH radical scavenging of banana blossom extracts before and after *in vitro* gastrointestinal digestion is presented in **Figure 3**.

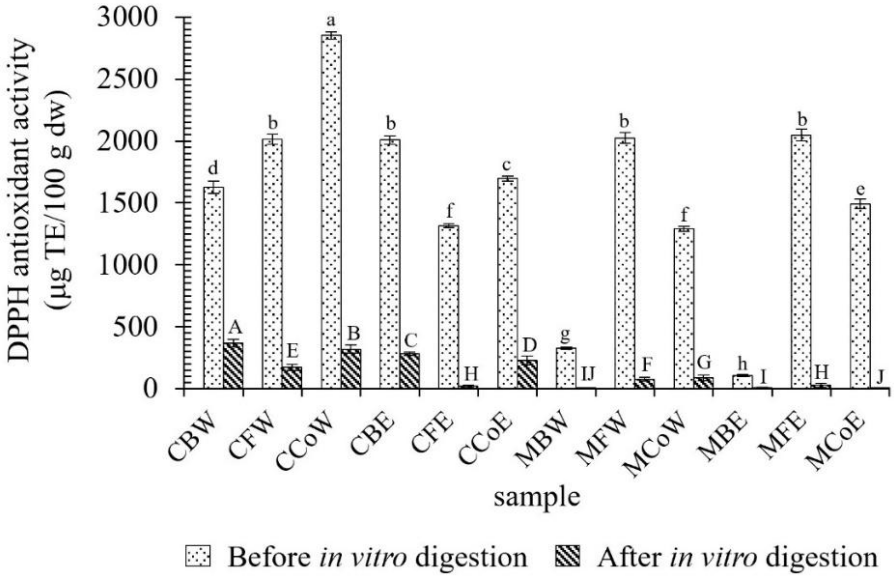

Figure 3 DPPH radical scavenging activity in different parts of banana blossoms before and after *in vitro* gastrointestinal digestion. Values are expressed as μg TE/ 100 g dw (mean ± standard deviation, n = 3). Different superscripts (a-h, A-J) within bars of the same pattern indicate significant differences ($p < 0.05$) as determined by Duncan's multiple range test. Sample codes: C = *Musa* AAA group, M = *M.* × *paradisiaca*, B = bracts, F = florets, Co = core, W = aqueous extraction, E = 50% ethanol extraction.

DPPH activity was significantly influenced by cultivar, anatomical part, and extraction solvent ($p < 0.001$). Before digestion, CCoW exhibited the highest activity (2,851.75 ± 29.70 μg TE/100 g), while MBE showed the lowest (104.78 ± 8.40 μg TE/100 g), representing a 27-fold difference. *Musa* AAA group extracts consistently demonstrated superior activity compared to *M.* × *paradisiaca*, with aqueous extracts generally outperforming ethanol extracts. Within the *Musa* AAA group, the order of activity was core (highest), florets (intermediate), and bracts (lowest) for both extraction solvents, consistent with the distribution pattern observed in phenolic and flavonoid content. Following *in vitro* digestion, DPPH activity decreased dramatically in all samples, with a 77.4% - 99.6% reduction ($p < 0.001$). CBW retained the highest residual activity (366.42 ± 32.79 μg TE/100 g), while several samples showed over 95% reduction. These losses likely result from compound degradation under acidic and alkaline conditions, oxidation, and interactions with digestive enzymes or bile salts [31].

The dramatic reduction in DPPH activity represents one of the most severe losses observed among antioxidant assays, significantly exceeding the moderate decreases seen in ABTS activity (13% - 72%). This stark difference suggests that DPPH-reactive compounds, particularly those involving hydrogen atom transfer mechanisms, are highly susceptible to degradation under gastrointestinal conditions [31]. The pH fluctuations from acidic gastric conditions (pH 2.0) to alkaline

intestinal environment (pH 7.0 - 8.0) appear to affect the electron-donating capacity measured by DPPH assay disproportionately. Similar patterns have been observed in other fruit matrices, where DPPH activity showed greater sensitivity to digestive conditions compared to other antioxidant assays [32].

ABTS radical scavenging activity

The antioxidant activity measured by ABTS radical scavenging of banana blossom extracts before and after *in vitro* gastrointestinal digestion is presented in **Figure 4**.

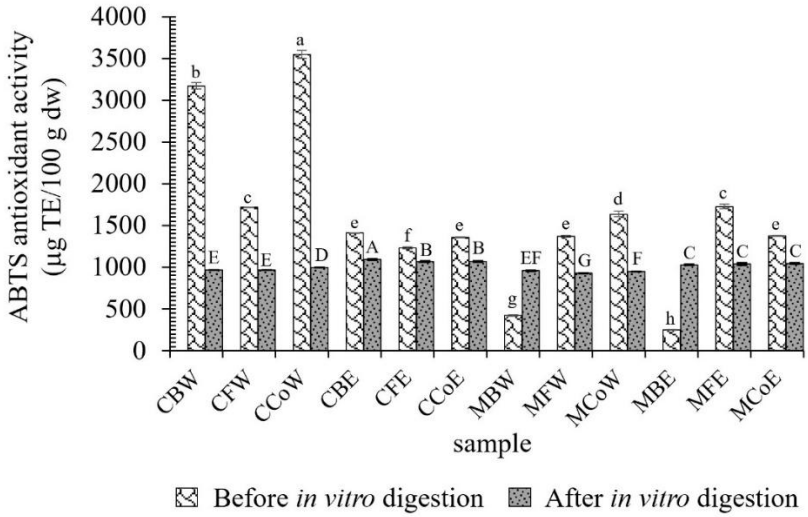

Figure 4 ABTS radical scavenging activity in different parts of banana blossoms before and after *in vitro* gastrointestinal digestion. Values are expressed as μg TE/ 100 g dry weight (mean ± standard deviation, n = 3). Different superscripts (a-h, A-G) within bars of the same pattern indicate significant differences ($p < 0.05$) as determined by Duncan's multiple range test. Sample codes: C = *Musa* AAA group, M = *M.* × *paradisiaca*, B = bracts, F = florets, Co = core, W = aqueous extraction, E = 50% ethanol extraction.

Before digestion, CCoW exhibited the highest activity (3,550.16 ± 48.63 μg TE/ 100 g), while MBE showed the lowest (248.59 ± 7.12 μg TE/100 g). *Musa* AAA group consistently demonstrated superior ABTS activity compared to *M.* × *paradisiaca*, particularly in aqueous extracts, which is consistent with the DPPH results. Following digestion, most samples exhibited significant decreases in ABTS activity, except for MBW and MBE, which showed remarkable increases (4.1-fold and 2.3-fold, respectively). CCoW and CBW exhibited the greatest reductions (71.9% and 69.5%, respectively, $p < 0.001$). ABTS showed better retention compared to DPPH activity, with moderate losses (13% - 72%) versus the dramatic DPPH reductions (77.4% - 99.6%), suggesting that ABTS-reactive compounds are more stable under gastrointestinal conditions.

The significant reduction in ABTS activity can be attributed to structural modifications under varying pH conditions, enzymatic degradation, and the formation of complexes with bile salts. Comprehensive reviews have also documented that gastrointestinal digestion significantly reduces the antioxidant potential of polyphenols due to protein-polyphenol interactions, with reductions typically ranging from 40% to 80%, depending on the plant material composition [33]. Notably, post-digestion ABTS values converged within a narrow range (927.08 - 1,091.98 μg TE/100 g), suggesting a standardizing effect where gastrointestinal processing equalizes antioxidant profiles across different sources [33]. This finding supports the PCA results showing reduced variance post-digestion and indicates that the choice of banana blossom source may be less critical for final antioxidant delivery.

Ferric reducing antioxidant power

The antioxidant activity measured by FRAP of banana blossom extracts before and after *in vitro* gastrointestinal digestion is presented in **Figure 5**.

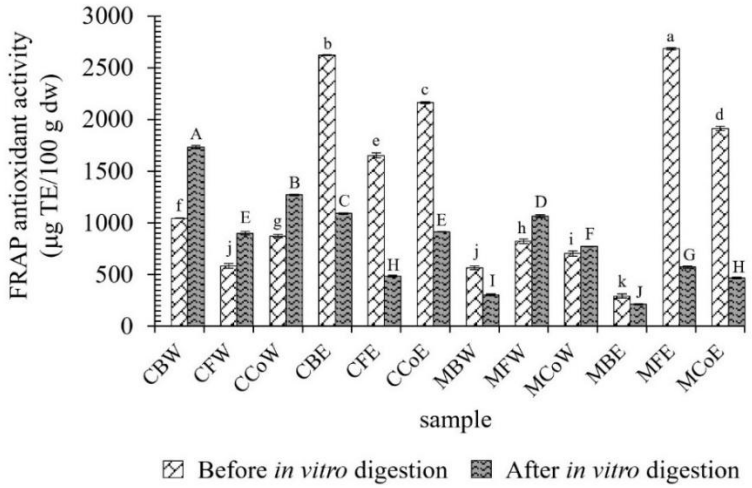

Figure 5 FRAP antioxidant activity in different anatomical parts of banana blossoms before and after *in vitro* gastrointestinal digestion. Values are expressed as μg TE/100 g dry weight (mean ± standard deviation, n = 3). Different superscripts (a-k, A-J) within bars of the same pattern indicate significant differences ($p < 0.05$) as determined by Duncan's multiple range test. Sample codes: C = *Musa* AAA group, M = *M.* × *paradisiaca*, B = bracts, F = florets, Co = core, W = aqueous extraction, E = 50% ethanol extraction.

Before digestion, MFE exhibited the highest FRAP activity (2,684.29 ± 10.10 μg TE/100 g), while MBE showed the lowest (293.29 ± 21.21 μg TE/100 g). Unlike DPPH and ABTS results, 50% ethanol extracts demonstrated superior FRAP values, as this method measures electron transfer capacity, where compounds with high electron-donating ability are better extracted in water-ethanol mixtures [27]. Following digestion, distinct patterns emerged between solvent types. Aqueous extracts showed increased FRAP values, with CBW exhibiting the highest increase (65.7% to 1,732.72 ± 15.15 μg TE/100 g). Conversely, ethanol extracts decreased significantly, with MFE showing the greatest reduction (78.6%). This suggests that acidic gastric conditions favor the release of bound phenolics from aqueous extracts, whereas alkaline intestinal conditions may destabilize ethanol-extracted phenolics through oxidation [34].

The magnitude of FRAP changes was intermediate between DPPH (77.4% - 99.6% reduction) and ABTS (13% - 72% changes), indicating that electron-donating compounds demonstrated moderate stability during gastrointestinal digestion compared to hydrogen atom transfer mechanisms. From a functional food development perspective, the enhanced FRAP activity in aqueous extracts post-digestion suggests that electron-donating antioxidants exhibit improved bioaccessibility. These results suggest that water-based extractions may be preferable for developing banana blossom-derived functional products, especially beverages. The contrasting responses between aqueous and ethanol extracts in the FRAP assay (increases vs. decreases post-digestion) highlight the complex nature of polyphenol-matrix interactions during digestion [35]. This differential behavior suggests that extraction solvent choice significantly influences the liberation mechanisms of electron-donating compounds during gastrointestinal processing.

The 3 antioxidant assays provided complementary validation. DPPH and ABTS confirmed the superior capacity and aqueous extraction efficacy of the *Musa* AAA group. However, FRAP revealed ethanol extraction advantages for electron-donating compounds. All assays demonstrated significant digestion-induced changes, with FRAP showing divergent solvent-dependent responses. The dramatic reduction in antioxidant activities reflects patterns documented across diverse plant materials during gastrointestinal processing [24,33]. These results confirm that bioactive content and biological activity respond differently to digestive conditions and underscore the need for multiple assays to comprehensively evaluate antioxidant bioaccessibility.

Principal component analysis of bioactive compounds

Multivariate analysis using the Principal component analysis (PCA) identified the relationships between bioactive compounds and their response to gastrointestinal digestion (**Figure 6**).

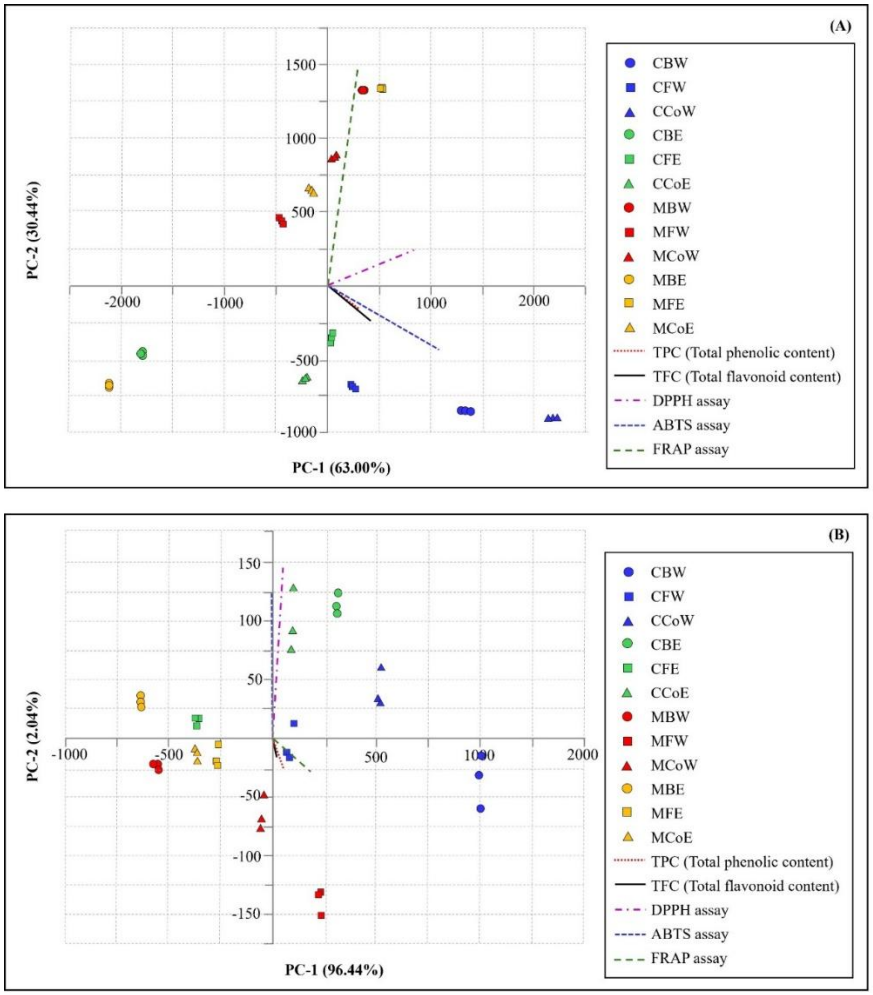

Figure 6 Principal component analysis (PCA) biplots showing relationships between bioactive compounds and antioxidant activities in banana blossom extracts before (A) and after (B) *in vitro* gastrointestinal digestion. Samples represent 3 anatomical parts (bracts, florets, core) from *Musa* AAA group and *M.* × *paradisiaca* extracted using distilled water and 50% ethanol. Sample codes: C = *Musa* AAA group, M = *M.* × *paradisiaca*, B = bracts, F = florets, Co = core, W = aqueous extract, E = ethanol extract. PC-1 and PC-2 explain 93.44% (A) and 98.48% (B) of the total variance, respectively. Vector arrows represent variable loadings with lengths proportional to their contribution to each principal component. All data derive from 3 replicate analyses per sample type.

Before digestion, PC-1 and PC-2 explained 93.44% of the total variance (63.00% and 30.44%, respectively), showing well-defined clustering patterns among samples. The PCA biplot revealed clear separation between *Musa* AAA group and *M.* × *paradisiaca* cultivars, as well as distinct groupings based on extraction solvents and anatomical parts. FRAP demonstrated the strongest loading on PC-2, while TPC and TFC showed similar vector directions, indicating a strong positive correlation. Following *in vitro* gastrointestinal digestion, a dramatic shift in data structure occurred, with PC-1 explaining 96.44% of total variance compared to PC-2's 2.04%. This convergence suggests that digestive processes resulted in more

uniform patterns of bioaccessibility across samples. These findings are consistent with previous studies, which have shown that gastrointestinal processing can reduce inter-sample variability in plant materials [24]. The PCA results demonstrate that gastrointestinal digestion acts as a "great equalizer," minimizing initial differences in bioactive compound profiles [33]. These findings support the hypothesis that digestion significantly modifies compound bioaccessibility regardless of source characteristics. This convergent behavior enhances the practical utility of banana blossom byproducts for functional food applications regardless of initial processing parameters.

Conclusions

Our findings demonstrate that *Musa* AAA group contains significantly higher phytochemical levels than *M.* × *paradisiaca.* Water-based extraction yielded better recovery of both phenolics and flavonoids compared to 50% ethanol. The core of banana blossoms contained the highest phenolic content, while bracts had the highest flavonoid levels. *In vitro* digestion significantly enhanced phenolic bioaccessibility, with a notable 9.1-fold increase in MBE extracts (ethanolic extraction of bracts part of *M.* × *paradisiaca*). PCA analysis revealed that digestive processing standardized bioaccessibility patterns regardless of source material, indicating that cultivar selection becomes less critical for functional food development. These findings highlight the potential of banana blossom byproducts as a sustainable source of bioactive compounds for functional food applications, though further in vivo validation is required.

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Generative AI tools (Claude) were used to improve the readability and language clarity of this manuscript. All content remains under full author responsibility and oversight.

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