International Journal of Food Science and Technology 2021

Original article

A potential of banana flower and pseudo-stem as novel ingredients rich in phenolic compounds

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(Received 28 January 2021; Accepted in revised form 27 April 2021)

Keywords Banana flower, banana pseudo-stem, extractable polyphenols, non-extractable polyphenols.

Introduction

World banana production in 2017–2019 reached 116 million tonnes, making it the most important fruit production worldwide (FAO, 2020). Due to the way bananas are harvested, the amount of waste generated in the plantations reaches 80% of the total biomass (Padam et al., 2014), constituting both an environmental problem related to soil toxicity and elevated carbon print (Adsal et al., 2020) and a food safety problem derived from food waste management (Campos et al., 2020). Interest in banana residues has been renewed in recent times mostly due to their variety of bioactive compounds (Lau et al., 2020), apart from the emerging uses of banana plant fibre in several industries (Ortega et al., 2016; Rodríguez et al., 2020); all these usages and its processes generate waste as a secondary byproduct after processing. The utilisation of these secondary by-products fit with the current FAO ODS actions on the Circular Economy (FAO, 2018).

Polyphenols are the most common bioactive compounds in plant residues (Singh *et al.*, 2016; Lau *et al.*, 2020). They are secondary metabolites with biological activities such as radical scavenging ability or microbiota modulation, which results in health benefits related to glucose homeostasis, obesity, type II diabetes, systemic inflammation or lipid metabolism, besides their use as a natural preservative (Cao *et al.*, 2019; Fraga *et al.*, 2019; Lau *et al.*, 2020). Interest in polyphenols have been traditionally focussed on the so-called extractable polyphenols (EPP), that is, low

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molecular weight compounds present free in vegetal cells, although increasing evidence is showing the relevance of non-extractable polyphenols (NEPP) or macromolecular antioxidants, which are high molecular weight polyphenols or small ones associated with macromolecules such as protein or dietary fibre (Pérez-Jiménez *et al.*, 2013).

Among agricultural residues from banana production are the banana flowers, which arise upon the bracts axis of the inflorescence (Ram et al., 1962) and cut down when they reach a specific size so that they do not interfere with the ripening and growth of the fruit (Lau et al., 2020), and the whole pseudo-stem, which is left on the ground of the plantation. These by-products have been widely used in traditional medicine (Mathew & Negi, 2017; Lau et al., 2020). There are currently several studies on the inclusion of banana by-products in animal and human diets. For instance, banana leftovers were used to replace up to 75% of the cornmeal in lamb diets without causing adverse effects (Menezes et al., 2018). Likewise, several studies with pseudo-stem show that it can be considered as an alternative source to traditional livestock fodder (Wang et al., 2016). The banana flower has been studied for its antioxidant and anti-diabetic capacity (Lau et al., 2020); thus Bhaskar et al. (2012) showed that both, banana pseudo-stem and banana flower used as a dietary supplement in patients with diabetes may reduce associated complications.

Nevertheless, studies on the phytochemical characterisation of banana flower or banana pseudo-stem are still scarce. Thus, Bashkar *et al.* (2012) is the only found study with these two banana by-products

doi:10.1111/ijfs.15072

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from *Musa sp. elakki bale*, where HPLC analysis was performed. Also, Schmidt *et al.* (2015) have reported some characterisation of banana male flower and bracts from *Musa cavendish* in which EPP content by spectrophotometry was measured. Moreover, most of the studies performed with banana residues do not differentiate the banana flower from the inflorescence being the composition and effects indistinctly attributed to both parts of the plant (Lau *et al.*, 2020).

The present study aims to perform a preliminary approach to evaluate the potential of the use of banana flower and pseudo-stem as novel food ingredients, for which a nutritional characterisation of both materials was performed, including HPLC-MS analysis of polyphenol fractions.

Material and methods

Chemicals

The Folin-Ciocalteu reagent was from Panreac (Castellar del Vallés, Barcelona, Spain). Gallic acid, kaempferol, myricetin, ferulic acid, 2-hydroxybenzoic acid, protocatechuic acid, 1-caffeoylquinic acid, *p*-coumaric acid, (+)-catechin and (-)-epicatechin were all obtained from Sigma-Aldrich (St. Louis, MO). All the reagents used for the preparation of phenolic compounds fractions and spectrophotometric determinations were of analytical grade, while for MS analysis, they were of MS grade. Condensed tannin concentrate from Mediterranean carob pods (*Ceratonia siliqua L*) was supplied by Nestlé Ltd. (Vers-chez-les Blancs, Switzerland).

Samples

Pseudo-stem and flowers were supplied by local producers of banana cultivars (*Musa accuminata cavendish*) from the 2017 and 2018 harvests. Pseudo-stem was processed at University of Las Palmas de Gran Canaria facilities to mechanically separate the external long fibre for other studies (patent: WO2014/174115), the residue that remained in the machine was the pseudo-stem fibre considered for the present study. Dried banana flower (DBF) and dried pseudo-stem (DBPS) were freeze-dried and ground in a mill (Ultra Centrifugal Mill ZM200, Retsch, Germany) to a particle size of 0.5mm. All the analysis were performed in triplicates for each harvest; results are given on a dry weight (dw) basis and are expressed as the mean of the two harvest \pm standard deviation.

Proximate composition and fatty acids profile

Moisture, crude protein and ash content were determined according to AOAC (1995). Lipids were determined according to Folch *et al.* (1957). The total carbohydrate content was calculated by the difference method. FAMEs (Fatty Acid Methyl Esters) were obtained by transmethylation of total lipids following Christie (1982) protocol and separated and quantified by liquid chromatography (Izquierdo *et al.*, 1989).

Extractable polyphenols (EPP)

0.5 g of sample was extracted by shaking at room temperature with 20 mL of methanol/water (50:50 v/v, pH 2) and then with 20 mL of acetone/water (70:30, v/v) for 1 hour. The two supernatants were combined, which corresponded to the extractable phenolic compounds fraction (Pérez-Jiménez *et al.*, 2008).

Hydrolyzable polyphenols (HPP)

The residue from the extractable phenolic compounds extraction was treated with 20 mL of methanol and 2 mL of sulfuric acid (12 M) at 85 °C for 20 h. After washing with distilled water, an aliquot of the extract was adjusted to pH 5.5 (Hartzfeld *et al.*, 2002; Arranz *et al.*, 2009).

Nonextractable proanthocyanidins (NEPA)

The residue from the extractable phenolic compounds extraction was treated with butanol/HCl (97.5:2.5, v/v) with 0.1% FeCl3 at 100 °C for 1h (Pérez-Jiménez *et al.*, 2009).

Determination of EPP, HPP and NEPA content

The total content of phenolic compounds was determined by spectrophotometry. The Folin-Ciocalteau assay (Singleton *et al.*, 1999) was applied to EPP and HPP, the results being expressed as g of gallic acid equivalents/100 g dw. NEPA content was determined by measuring the sum of absorbance at 450 and 555 nm (Zurita *et al.*, 2012) and the results were expressed as mg NEPA/100g dw, using a standard curve from carob pod concentrate. The total percentage of non-extractable polyphenols was determined by the formula (HP+NEPA)/total polyphenols x100. All these measurements were carried out in a 96-well plate reader (Synergy MX, Bio Tek, Winooski, Vermont, USA).

Phenolic profile by HPLC-ESI-QTOF

The EPP and HPP fractions from DBF, as well as HPP fraction of DBPS, were concentrated (6:1) with an N_2 stream; EPP fraction of pseudo-stem was discarded after previous evaluation of spectrophotometry results. For separation, the HPLC apparatus (Agilent

1200, Agilent Technologies, Santa Clara, CA) with DAD (Agilent G1315B) and a QTOF mass analyzer (Agilent G6530A) with an atmospheric pressure electrospray ionization (ESI) was used. The column used was a 100A 50 mm × 2 mm i.d., 5 µm, Luna C18 (Phenomenex, Torrance, CA) at 25°C. Gradient elution was performed with a binary system consisting of 0.1% aqueous formic acid (solvent A) and 0.1% formic acid in acetonitrile (solvent B). The following gradient was applied at a flow rate of 0.4 mL/min: 0 min, 8% B; 10 min, 23% B; 15 min, 50% B; 20 min, 50% B; 23 min, 100% B; followed by a re-equilibration step. The injection volume was 20 uL. Data were acquired using negative ion mode with a mass range of 100 - 1200 Da and using a source temperature of 325 °C and a gas flow of 10 L/h. Peak identity was established by comparison with the retention times of commercial standards when available. Also, the molecular formula proposed by the MassHunter Workstation software version 4.0 for the different signals obtained in the MS experiments were compared with previously reported phenolic compounds in banana and other vegetal materials, and a maximum error of 10 ppm was accepted. For MS/MS experiments, the auto MS/MS acquisition mode was used; the main fragments were compared with the fragmentation patterns reported for phenolic compounds.

A relative quantitation was performed based on UV signals, using the calibration curve of a commercial standard for each class of phenolic compounds. Hydroxybenzoic acid was monitored at 280 nm and directly quantified, other hydroxybenzoic acids were monitored at 280 nm and quantified with protocate-chuic acid, *p*-coumaric acid was monitored at 320 nm and directly quantified, other hydroxycinnamic acids were monitored at 320 nm and quantified with ferulic acid, flavonols were monitored at 365 nm and quantified with kaempferol.

Statistical analysis

The distribution of the results obtained in each batch was analysed with Rcmdr: R Commander (R package version 2.6-2, Fox J, Bouchet-Valat M, 2020) with Shapiro-- test to determine the normality and also the descriptive statistics were performed.

Results and discussion

This study aims to explore the potential of DBF and DBPS as possible new functional ingredients, as a way to valorise these by-products in a circular economy context. For this, the proximate composition of these materials was obtained, completing previous data and, especially, a comprehensive analysis of polyphenols was performed.

The proximate composition of DBF and DBPS are provided in Table 1. There were some differences with previous studies, which reported lower values in proteins and ash content (Bhaskar *et al.*, 2012; Wang *et al.*, 2016) in pseudo-stem, or proteins, lipids and ash in the flower (Sheng *et al.*, 2010). This may be related to the differences between varieties and the samples that were taken; for instance, the part of the pseudostem that was analysed in the present study was the remain fibrous pulp after the extraction of most of the fibre, instead of the whole pseudo-stem.

The detailed composition of the fat fraction of each material was evaluated (Table 2). The two samples presented marked different fatty acid profile. The overall profile for DBF agreed with that described in another study (Lau et al., 2020), although other authors described a lower proportion of unsaturated fatty acids (Vilela et al., 2014). DBPS composition differs from the one described by Ramu et al. (2017), where palmitic acid content was lower while linoleic acid was higher than in this preliminary study. Regarding the potential benefits of fatty acids present in these residues, since DBPS exhibits a low amount of lipids in this product, the actual impact on health would be irrelevant. On the contrary, the DBF does present an appreciable quantity of lipids that can influence health. Thus, a portion of 20g of DBF can provide 197.3 mg of linoleic acid, which is 18% of the recommended dietary amount; DHA quantity present in 20g of DBF is the equivalent of half DHA present in 100g of salmon; also, the content of n-3 fatty acid is similar to the recommended portion which is 450 mg/day (Kris-Etherton et al., 2009; Ytrestøyl et al., 2015). Furthermore, eicosadienoic acid, a precursor of DHA (Lupette & Benning, 2020), is present with 157.2 mg/portion. The benefits of MUFA and PUFA are widely described in the literature, such as mediation in the innate immune system, reduction of cardiovascular disease risk and the redistribution of adipose tissue (Tvrzicka et al., 2011; Lau et al., 2020).

Therefore, these results show that both DBF and DBPS may have an interesting nutritional

 Table 1
 Proximate composition of dried banana flower and dried banana pseudo-stem

	Banana flower	Banana pseudo-stem		
(% dw)				
Lipids	$\textbf{8.66} \pm \textbf{0.23}$	1.01 ± 0.32		
Ash	18.07 ± 0.76	15.97 \pm 2.67		
Protein	$\textbf{13.59}\pm\textbf{0.41}$	$\textbf{7.25} \pm \textbf{2.48}$		
Moisture	$\textbf{7.23} \pm \textbf{1.89}$	$\textbf{8.97}\pm\textbf{1.01}$		
Carbohydrates	59.68 ± 1.26	$\textbf{76.09} \pm \textbf{5.58}$		

(mean \pm sd deviation of the two harvest).

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 Table 2
 Fatty acid profile from dried banana flower and dried banana pseudo-stem

FAA (%)	Banana flower	Banana pseudo-stem		
14:00	0.16	1.55		
14:1n-5	0.06	0.77		
15:00	0.26	2.29		
15:1n-5	0.03	0.11		
16:00	12.90	39.89		
16:1n-7	0.21	1.04		
16:1n-5	0.03	0.08		
16:2n-6	0.62	0.00		
17:00	0.04	0.30		
16:3n-4	0.03	0.21		
16:3n-3	0.02	0.05		
16:3n-1	0.03	0.10		
16:4n-3	0.26	0.16		
18:00	2.19	6.17		
18:1n-9	2.04	9.21		
18:1n-7	0.73	3.93		
18.1n-5	0.04	0.18		
18:2n-9	0.03	0.09		
18:2n-6	11.47	15.46		
18:2n-4	1.02	0.25		
18:3n-6	0.46	0.90		
18:3n-3	5.68	0.22		
18:4n-3	0.37	8.39		
20:00	1.15	1.92		
20:1n-9	0.12	0.39		
20:11-5 20:1n-7	1.55	0.48		
20.1n-5	0.20	0.48		
20:2n-9	0.09	0.03		
20:211-9 20:2n-6	9.14	0.46		
20:211-6 20:3n-6	9.14 1.24			
	0.00	1.02		
20:4n-6		0.46		
20:3n-3	0.41	0.07		
20:4n-3	0.23	0.12		
20:5n-3	0.17	0.16		
22:1n-11	0.41	0.25		
22:1n-9	19.11	1.10		
22:4n-6	0.79	0.15		
22:5n-6	0.31	0.40		
22:5n-3	0.36	0.00		
22:6n-3	26.04	0.94		
∑SFA	16.70	52.12		
∑MUFA	24.47	16.86		
∑PUFA	58.77	29.68		
n-3	33.54	10.11		
n-6	24.03	18.85		
n-9	21.39	10.86		

MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid.

composition, especially regarding proteins and lipids, which could make them suitable to be tested in different formulations.

From phenolic compound analysis (Table 3), DBF emerged as a potentially relevant source of these

Table 3 Total content (mg/100 g dw) for extractable polyphenols (EP), hydrolysable polyphenols (HP) and non-extractable proanthocyanins (NEPA), and percentage of non-extractable polyphenols (NEPP) in dried banana flower and dried banana pseudo-stem

	Banana flower	Banana pseudo-stem		
Fraction				
EP	1075.02 ± 20.60	nd		
HP	735.23 \pm 103.91	150.75 ± 53.54		
NEPA	11200.00 \pm 3357.61	nd		
%NEPP	91.73	100		

(mean \pm sd. deviation of the two harvest).

compounds. Regarding EPP fraction (not detected in DBPS), the number of phenolic compounds in the flower agreed with that reported by Padam et al. (2012) and, interestingly, was much higher than that described in the fruit (Pico et al., 2019). In agreement with this higher phenolic compound content in DBF than in DBPS, HPP fraction was between 6-fold and 8-fold higher in the latter. Previous data showed that a relevant fraction of phenolic compounds in DBF was associated with hemicellulose A (Bhaskar et al., 2012), thus corresponding to the HPP fraction. But the most relevant fact of phenolic compounds analysis in these by-products was the determination, by the first time, of NEPA content. A very high content (about 10% dw) was determined in DBF, indeed constituting 90% of the total phenolic compound content in this material. This novel result is in concordance with previous descriptions of a very high NEPA content in banana fruit (Pérez-Jiménez & Saura-Calixto, 2015) and banana peel (Pérez-Jiménez & Saura-Calixto, 2018), being commonly underestimated. The difference observed between the spectrophotometry and the HPLC-MS determination was within the ranges observed in other characterisations of vegetal materials (Pérez-Ramírez et al., 2018), where the spectrophotometry determinations were higher due to the unspecific of the technique and are not directly comparable techniques.

Detailed phenolic compounds profile was evaluated by HPLC-ES-QTOF MS technique analysis in EPP fraction of DBF (Table 4), and HPP fractions in DBF (Table 5) and DBPS (Table 6). NEPA, due to the butanol medium, exhibit problems for HPLC analysis and, besides, due to the depolymerization process information about original structures would be completely lost (Pérez-Jiménez & Torres, 2011).

The identity of individual phenolic compounds in DBF and DBPS was performed for the first time by MS.

In BBF EPP fraction (Table 4) and both HPP fractions (Table 5, Table 6), phenolic acids were the most

ID	Compound	Formula	(M-H)-teo	(M-H)-cal	Diff	Concentration (mg/100 g dw)
Hydrox	ybenzoic acids					
1	3,5-Dihydroxybenzoic acid	C ₇ H ₆ O ₄	153.0187	153.0195	2.17	$\textbf{0.44}\pm\textbf{0.02}$
	Gentisic acid	C ₇ H ₆ O ₄	153.0187	153.0195	2.17	$\textbf{0.44} \pm \textbf{0.02}$
2	Protocatechuic acid*	C ₇ H ₆ O ₄	153.0187	153.0185	4.28	$\textbf{0.43}\pm\textbf{0.04}$
3	Hydroxybenzoic acid*	C ₇ H ₆ O ₃	137.0238	137.0237	4.86	$\textbf{6.61} \pm \textbf{2.74}$
Hydrox	ybenzaldehydes					
4	4-Hydroxybenzaldehyde	C ₇ H ₆ O ₂	121.0289	121.0291	2.98	$\textbf{0.24}\pm\textbf{0.02}$
	Benzoic acid	C ₇ H ₆ O ₂	121.0289	121.0291	2.98	$\textbf{0.24}\pm\textbf{0.02}$
Hydrox	ycinnamic acids					
5	Caffeic acid	C ₉ H ₈ O₄	179.0344	179.0345	2.78	10 ± 3.38
6	<i>p</i> -Coumaric acid*	C ₉ H ₈ O ₃	163.0398	163.0395	1.54	$\textbf{0.38}\pm\textbf{0.05}$
7	Ferulic acid*	C ₁₀ H ₁₀ O ₄	193.0507	193.0500	4.11	1.64 ± 0.57
Flavono	ols					
8	Quercetin 3,4'-O-diglucoside	C ₂₇ H ₃₀ O ₁₇	625.1404	625.1440	-4.24	1.01 ± 0.83
	Quercetin 3-O-sophoroside	C ₂₇ H ₃₀ O ₁₇	625.1404	625.1440	-4.24	1.01 ± 0.83

 Table 4
 Phenolic compounds identified by HPLC-ESI QTOF MS analysis in the extractable polyphenols (EPP) fraction of dried banana flower of the two harvest

*Identified by standard. cal, calculated; exp, experimental.

 Table 5
 Phenolic compounds identified by HPLC-ESI-QTOF MS analysis in the hydrolizable polyphenols (HPP) fraction of dried banana flower of the two harvest

ID	Compound	Formula	(M-H)-teo	(M-H)-cal	Diff	Concentration (mg/100g dw)
Hydroxyb	penzoic acids					
2	Protocatechuic acid*	C ₇ H ₆ O ₄	153.0187	153.0195	1.16	$\textbf{48.68} \pm \textbf{0.62}$
9	Gallic acid 3-o-gallate	C ₁₄ H ₁₀ O ₉	321.0246	321.0227	7.68	$\textbf{0.43} \pm \textbf{0.01}$
Hydroxyc	cinnamic acids					
7	Ferulic acid*	C ₁₀ H ₁₀ O ₄	193.0500	193.0498	4.4	$\textbf{0.71} \pm \textbf{0.17}$
10	Isoferulic acid	$C_{10}H_{10}O_4$	193.0500	193.0505	0.64	$\textbf{5.74} \pm \textbf{0.16}$

*Identified by standard. cal. calculated; exp. experimental. nd, non-detected.

Table 6 Phenolic compounds identified by HPLC-ESI-QTOF MS analysis in the hydrolizable polyphenols (HPP) fraction of dried banana pseudo-stem of the two harvest

ID	Compound	Formula	(M-H)-cal	(M-H)-exp	Diff	Concentration (mg/100g dw)
Hydroxy	benzoic acids					
2	Protocatechuic acid*	C ₇ H ₆ O ₄	153.0187	153.0191	4.24	$\textbf{49.02} \pm \textbf{27.64}$
Hydroxy	cinnamic acids					
6	<i>p</i> -Coumaric acid*	C ₉ H ₈ O ₃	163.0395	163.0395	3.34	$\textbf{0.48}\pm\textbf{0.12}$
7	Ferulic acid*	$C_{10}H_{10}O_4$	193.0500	193.0508	-1.4	$\textbf{1.26}\pm\textbf{0.29}$
11	<i>p</i> -Coumaric acid ethyl ester	$C_{11}H_{12}O_3$	191.0708	191.0720	-3.76	0.05 ± 0.01

*Identified by standard; cal. calculated; exp. experimental. nd, non-detected.

relevant constituents, although some flavonol was also detected, which agrees with previous results in banana fruit and peel (Tsamo *et al.*, 2015). Previous studies also identified epicatechin and gallic acid in the banana flower associated with hemicellulose and in green banana flour (Bhaskar *et al.*, 2012; Pico *et al.*,

2019) or protocatechuic acid in the banana rhizome (Kandasamy & Aradhya, 2014). Nevertheless, for most detected phenolic compounds, identity could not be assigned, since they corresponded to structures not present in common databases nor literature on banana. In the EPP fraction from DBF, 45 non-

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identified compounds were detected, and 28 compounds in HPP fraction. In the HPP fraction from DBPS 12 non-identified were detected (data not shown). The wide diversity of structures in the phenolic compounds family makes that new structures are still being identified (Nguyen *et al.*, 2019); this indicates the need for further structural analysis in DBF and DBPS.

Due to the total concentration and profile of individual phenolic compounds in DBF, this product, as an additional source of phenolic compounds in the context of the whole diet, might have health benefits. For instance, protocatechuic acid, for which health effects related to cancer, type I and II diabetes and neuroprotection have been reported (Adedara et al., 2019; Al Olayan et al., 2020), was detected in DBF at a concentration higher than that observed in most common foods, according to the Phenol-Explorer database (Neveu et al., 2010). Although the potential effects would be mostly derived from the combination of all phenolic compounds present in this product, as shown in a study in diabetic rats where phenolic extracts of banana pseudo-stem and flowers modulated the antioxidant defence enzymes, decreasing the complications associated with diabetes (Bhaskar et al., 2011). Also, regarding the high NEPA content detected in DBF, it should be remarked that other products rich in this phenolic compound fraction, such as grape pomace, have shown several health-related properties, for instance regarding fasting insulin (Martínez-Maqueda et al., 2018).

In conclusion, this study provides preliminary new data on proximate composition and, especially, phenolic compounds profile, in DBF and DBPS, two byproducts from the banana industry currently neglected. In the light of the results obtained, future comprehensive studies need to be performed to establish an accepted range of the main constituent of these byproducts, taking into account the possible difference between batches of the same species due to environmental growing conditions (Vinson *et al.*, 2018; Lau *et al.*, 2020); and thus, the real potential of the DBF and DBPS as novel food ingredients as well as the technological aspects for their incorporation into foods and their potential health effects.

Acknowledgment

This research was partially funded by the EU Environment and Climate Action LIFE Programme (European Union), under the LIFEBAQUA project (code: LIFE15 ENV/ES/000157). We are grateful to the ICTAN Analysis Services Unit for providing facilities for chromatography analysis and in particular to Dr Inmaculada Álvarez-Acero for technical assistance.

Author Contribution

Sara Ramírez-Bolaños: Data curation (lead); Formal analysis (lead); Investigation (equal); Writing-original draft (lead). Jara Pérez-Jiménez: Conceptualization (equal); Resources (equal); Supervision (lead); Validation (lead); Writing-review & editing (lead). Sara Díaz: Formal analysis (supporting). Lidia Robaina: Conceptualization (equal); Funding acquisition (equal); Project administration (lead); Resources (equal); Supervision (supporting); Writing-review & editing (supporting).

Conflict of interest

We declare that we do not have any commercial or associative interest that represents a conflict of interest in connection with this manuscript.

Ethical Approval

Ethics approval was not required for this research.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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8 Banana by-products as novel ingredients S. Ramírez-Bolaños et al.

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