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Synergistic Action of Multiple Enzymes Resulting in Efficient Hydrolysis of Banana Bracts and Products with Improved Antioxidant Properties

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Abstract: This study investigated the effect of enzymatic hydrolysis of banana bracts from different varieties (Maçã, Nanica and Prata) using pectinase, protease and cellulase (singly or in combinations) on their antioxidant properties. The results showed that the antioxidant properties and total phenolic compounds (TPC) of extracts increased after the enzymatic treatment with a clear synergistic effect between the different enzymes. The ternary mixture of pectinase, protease and cellulase resulted in increases of 458% and 678% in TPC content for extracts obtained from Maçã and Nanica varieties and up to 65% in antioxidant properties of those produced from Prata variety compared to the non-hydrolyzed samples. In general, the extracts obtained from the Prata variety showed the highest levels of TPC, as well as antioxidant activity, as follows: 14.70 mg GAE g^{-1} for TPC, 82.57 μmol TE g^{-1} for ABTS, 22.26 μmol TE g^{-1} for DPPH and 47.09 μmol TE g^{-1} for FRAP. Phenolic compounds identified by HPLC in extracts included ρ-coumaric, ferulic, sinapic and vanillic acids and the flavonoid rutin. This study reported for the first time the enzymatic treatment applied to banana bracts as a promising method to release antioxidant compounds, offering a new opportunity to explore these residues as a source of molecules with high added value through an environmentally friendly and safe process.

Keywords: banana flower; green extraction methods; agricultural waste; natural antioxidants

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1. Introduction

Banana (*Musa acuminata*) is considered one of the most consumed fruits in the world due to its highly nutritional value and is hence associated with economic interest. In 2020, 119 million tons of banana were produced in more than 150 countries of which India, China, Brazil, Ecuador and Philippines are the major producers [1].

Concerning this chain of production, banana industry generates large amounts of agricultural waste with high value-added. All banana plant structures such as fruit, pseudostem, leaves, inflorescences and peel have been reported to have medicinal properties [2]. Bract, a part of banana inflorescence, also known as flower or blossom, is typically treated as agricultural waste, although it is widely used in different regions for culinary purposes and in folk medicine [3]. Banana inflorescence has gained scientific relevance due to its variety of bioactive compounds and its associated beneficial health properties, including antioxidant, anti-diabetic, anti-microbial, anti-inflammatory and cardiovascular-protective activities [4–10].

In the last few years, the demand for functional ingredients has increased as well as the interest in agricultural wastes due to its great source of bioactive compounds [11]. Several studies have shown that the daily intake of plant-based foods such as vegetables, fruits and whole grains can be an essential factor to protective effects on human health. The antioxidant properties of these foods have been related to the presence of bioactive compounds, such as polyphenols, which may have preventive effects for obesity, cancer, cardiovascular and neurodegenerative diseases [12,13]. The protective role of phenolic compounds can be

attributed to their antioxidant properties that prevent the formation of reactive species and stabilizes free radicals through the transfer of electrons and hydrogen ions [14].

Bioactive compounds in plants are available in low concentrations; thereby, the development of a proper method is one of the main challenges to extract these biomolecules [13,15]. Several strategies have been investigated to encourage sustainable methods, as an alternative to conventional extraction using organic solvents due to low environmental impact and higher efficiency [16,17]. Examples of these methods include high pressure, pressurized liquids, pulsed electric fields, microwaves and enzyme-assisted extraction (EAE). EAE consists of the disruption of structures of the cell wall (cellulose, hemicellulose, lignin, pectin and proteins) by hydrolysis using an enzyme as a catalyst with optimum experimental conditions, in order to liberate the intracellular constituents, such as phenolic compounds [18]. The proximate composition of the banana inflorescence is dependent on banana species and cultivars, but in general, the high levels of carbohydrates (ranging from 10.18 to 95.61%) and proteins (ranging from 1.43 to 19.30%) deserve to be highlighted [19]. Thus, the addition of specific hydrolytic enzymes in the system allows the recovery of several components. The use of enzymes, such as cellulases, pectinases and proteases, has already been reported to be efficient for hydrolysis of plant substrates and recovery of bioactive compounds [20–22]. Furthermore, it is a technology with low environmental impact and high efficiency, due to the specificity and regioselectivity of the enzymes [23,24].

In this context, the aim of this study was to investigate the single and combined use of different enzymes on the hydrolysis of three varieties of banana bracts (Maçã, Nanica and Prata) and their effects on the recovery of compounds with antioxidant properties.

2. Materials and Methods

2.1. Material and Reagents

Banana bracts from Maçã and Prata varieties were collected from cultivated local farmland (20°33′56.5″ S, 46°05′21.1″ W) in Capitólio, state of Minas Gerais, Brazil. The Nanica variety was donated by Magário company, Jaíba, Minas Gerais, Brazil.

ABTS ([2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)]), DPPH (2,2-diphenyl-1-picrylhydrazyl), Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), TPTZ (2,4,6-tripyridyl-s-triazine), Folin & Ciocalteau's phenol reagent, gallic acid, catechin, vanillin and the commercial enzymes Flavourzyme™ (protease), Celluclast™ 1.5 L (cellulase) and Pectinex™ Ultra Pulp (pectinase) were acquired from Sigma-Aldrich (São Paulo, Brazil). All other chemicals were purchased as analytical grade.

2.2. Enzymatic Hydrolysis and Obtaining Banana Bract Extracts

Banana bract was separated from inflorescence, cleaned, ground, frozen and freeze-dried; the powdered sample was stored in vacuum packs at −18 °C. For enzymatic hydrolysis, the substrate solution was prepared using 0.25 g of banana bracts and 25 mL of phosphate buffer (100 mmol L^{-1}, pH 5). Three different commercial enzymes preparations namely: Pectinex™ Ultra Pulp (blend of pectinases, hemicellulases and beta-glucanases), Flavourzyme™ 500 L (proteases from *Aspergillus oryzae*) and Celluclast™ 1.5 L (cellulase from *Trichoderma reesei* ATCC 26921) were applied singly or in binary/ternary mixtures on substrate solution at a final concentration of 1% (*v*/*v*) (Table 1). Non-hydrolyzed samples (control) were also prepared as mentioned above, without the addition of enzymes.

All the mixtures were kept at 50 °C for 2 h under agitation at 100 rpm, followed by cooling for 10 min for enzymatic activity cessation. The samples were collected and then centrifuged at 8000× *g* for 15 min at 5 °C. The supernatants containing the antioxidant compounds were frozen and stored at –20 °C for further analysis.

Table 1. Matrix of the simplex centroid design used for extraction of antioxidant compounds of banana bracts from Maçã, Prata and Nanica varieties using different enzymatic preparations.

Run	Independent Variables					
	Coded Variables			Real Variables (mL)		
	x_1	x_2	x_3	Pectinex™	Flavourzyme™	Celluclast™
Control	0	0	0	0	0	0
1	1	0	0	0.250	0	0
2	0	1	0	0	0.250	0
3	0	0	1	0	0	0.250
4	1/2	1/2	0	0.125	0.125	0
5	1/2	0	1/2	0.125	0	0.125
6	0	1/2	1/2	0	0.125	0.125
7	1/3	1/3	1/3	0.083	0.083	0.083
8	2/3	1/6	1/6	0.166	0.042	0.042
9	1/6	2/3	1/6	0.042	0.166	0.042
10	1/6	1/6	2/3	0.042	0.042	0.166

To determine the most adequate enzymes or mixtures for maximum antioxidant compound recovery from banana bracts, a simplex centroid design was employed [24]. Each enzyme preparation was evaluated at six levels: 0 (0%), 1/6 (16%), 1/2 (50%), 1/3 (33%), 2/3 (66%) and 1 (100%), totalizing 10 runs (extracts) (Table 1). All the runs were evaluated comparatively with non-hydrolyzed extracts (control). For this study, quadratic and/or cubic models were employed to fit variations of all investigated responses ($p \leq 0.10$) as a function of interaction effects between the proportions of pectinase, protease and cellulase, with acceptable determination coefficients greater than 70% ($R^2 > 0.70$) represented by Equation (1):

$$Yi = \sum_{i=1}^{q} \beta_i X_i + \sum \sum_{i<j}^{q} \beta_{ij} X_i X_j + \sum \sum_{i<j<k}^{q} \sum \beta_{ijk} X_i X_j X_k \quad (1)$$

where '*Yi*' corresponds to predicted response (total phenolic compounds and antioxidant properties-ABTS, DPPH and FRAP); '*q*' represents the independent variables (components) in the system; "*Xi*, *Xj*, *Xk*" represent the coded components; and "*βi*", "*βij*" and "*βijk*" corresponds to the regression coefficients (binary and ternary interaction, respectively) [25,26]. The software Statistica 13.3 TIBCO Software Inc. (Palo Alto, CA, USA) was used for Student's t-test ($p \leq 0.10$) and model building by analysis of variance (ANOVA) ($p \leq 0.10$).

2.2.1. Determination of Total Phenolic Compounds (TPC)

The total phenolic compounds were determined according to the method described by Magro and De Castro [27]. Aliquots of 25 µL of diluted extracts (25 mg mL^{-1}), 25 µL of Folin–Ciocalteau solution (50% *v*/*v*) and 200 µL of sodium carbonate (5% *w*/*v*) were mixed and then, incubated at 40°C in the dark for 20 min. The absorbance was measured at 760 nm on a spectrophotometer (Multiskan GO, Thermo Fisher Scientific, Vantaa, Finland). For the calibration curve, gallic acid was used with a concentration range from 0 to 10 mg mL^{-1} and the results were expressed as mg of gallic acid equivalent per gram of dry weight of sample (mg GAE g^{-1}).

2.2.2. Measurement of the Antioxidant Properties

ABTS-Radical Cation Scavenging Activity

The ABTS-radical cation scavenging activity was determined as described by Neta and De Castro [28]. The ABTS aqueous solution (7 mmol L^{-1}) and potassium persulfate (140 mmol L^{-1}) were mixed and maintained for 16 h at room temperature, without light exposure, to generate free radicals. After this incubation period, the absorbance was ad-

justed to 0.70 ± 0.02 with deionized water. Aliquots of 20 µL of extracts (25 mg mL^{-1}) were mixed with 220 µL of ABTS solution and the absorbance measurements were determined after 6 min of reaction at 734 nm using a spectrophotometer (Multiskan GO, Thermo Fisher Scientific, Finland). Trolox was used for the calibration curve (0–400 µmol L^{-1}) and the results were expressed as µmol of Trolox equivalents per g of sample (µmol TE g^{-1}).

DPPH-Radical Scavenging Activity

The DPPH assay was performed according to the method described by Rasera et al. [29]. Aliquots of 134 µL of DPPH ethanolic solution (150 µmol L^{-1}) were added to 66 µL of diluted extracts (25 mg mL^{-1}) or standard (Trolox). After 45 min of the reaction without light exposure at room temperature, the absorbance measurements were determined at 517 nm against a blank (ethanol) using a spectrophotometer (Multiskan GO, Thermo Fisher Scientific, Finland). Trolox was used as the standard with a calibration curve ranging from 0 to 125 µmol L^{-1}. The results were expressed in µmol TE g^{-1}.

Ferric Reducing Antioxidant Power (FRAP)

The FRAP assay was performed according to the method described by Firuzi et al. [30] with some modifications proposed by Aguilar et al. [31]. The FRAP solution was composed of acetate buffer (300 mmol L^{-1}, pH 3.6), ferric chloride hexahydrate (20 mmol L^{-1}) dissolved in distilled water and TPTZ (2,4,6-tris(2-pyridyl)-s-triazine) (10 mmol L^{-1}) dissolved in HCl (40 mmol L^{-1}) (10:1:1, *v*:*v*:*v*). Aliquots of 25 µL of banana bract extracts (25 mg mL^{-1}) and 175 µL of freshly prepared FRAP solution were mixed and absorbance measurements were collected after 30 min of reaction at 595 nm using a spectrophotometer (Multiskan GO, Thermo Fisher Scientific, Finland). Trolox (0–250 µmol L^{-1}) was used as the standard, and the results were expressed in µmol TE g^{-1}.

2.2.3. Identification of Phenolic Compounds by HPLC

Phenolic compounds identification was performed according to the method described by Silva et al. [32] with slight modifications. The separation of compounds by HPLC was performed using a Shimadzu ODS-A column (4.6 mm, 250 mm, 5 µm) in a thermostated oven at a constant temperature of 30 °C, an injection volume of 20 µL and a photodiode array detector (SPD-M10AVp, Shimadzu Co., Kyoto, Japan). The mobile phase consisted of eluent A, water/formic acid (99.75/0.25, *v*/*v*) and eluent B, acetonitrile/formic acid/water (80/0.25/19.75, *v*/*v*) at a flow rate of 1.0 mL min^{-1}. The gradient conditions were as follows: 10% (B), increased to 20% (B) at 10 min, 30% (B) at 20 min, 100% (B) at 30 min and 10% (B) at 35 min. The analysis was completed in 40 min and the chromatograms were analyzed with Class-VP® software. Each compound peak was identified based on a comparison of the retention time, spectral data and peak area. Phenolic acid standards (gallic, 3,4-dihydroxybenzoic, vanillic, caffeic, coumaric, ferulic and sinapic acids) and flavonoids (rutin and quercetin) were used to quantify the individual compounds. The standard curves (0.03–0.20 µg mL^{-1} concentrations) were determined using the chromatographic parameters previously mentioned. The limit of detection (*LOD*) and limit of quantification (*LOQ*) (Equations (2) and (3)) were calculated as follows:

$$LOD = 3.3 \times s \div S \quad (2)$$

$$LOQ = 10 \times s \div S \quad (3)$$

where "*s*" is the standard deviation of the linear coefficient of the equation and "*S*" is the slope of the standard curve.

2.2.4. Calculations and Statistics

The results were reported as the mean values ± standard deviation (n = 3) and Minitab software, version 19 (Minitab Inc., State College, PA, USA) was used to verify if there was a

significant difference (p-value ≤ 0.05) between the means calculated by analysis of variance (ANOVA) followed by Tukey's test.

3. Results and Discussion

3.1. Effect of Enzymatic Hydrolysis on the Recovery of Antioxidant Compounds

The experimental values under distinct combinations of enzymatic preparations are displayed in Table 2. The highest TPC content (15.87 mg GAE g^{-1}) was detected for the extract obtained from the Maçã variety (run 10). For antioxidant properties, the highlights were the extracts obtained from bracts of the Prata variety, that reached 82.57 µmol TE g^{-1} for ABTS (run 9) and 47.09 µmol TE g^{-1} for FRAP (run 4), while the extract from Maçã variety showed the greatest ability to inhibit DPPH radicals (30.52 µmol TE g^{-1}-run 7).

Ternary mixture of the enzymes (Pectinex™, Flavourzyme™ and Celluclast™, in equal proportions, run 7) was responsible by obtaining of banana bract extracts from Maçã and Prata varieties with maximum values of TPC and antioxidant activities. Extracts from Maçã variety showed increases ranging from 30% to 458% in all analysis when compared to the control (non-hydrolyzed). Similar results were obtained for banana bract extract from Prata variety, in which increases greater than 65% were detected for TPC and antioxidant activities compared to the control. For the Nanica variety, the ternary mixture was also very efficient, however the proportion between the three enzymes was different; in this case, the mixture of Pectinex™ (1/6), Flavourzyme™ (2/6) and Celluclast™ (1/6) (run 9) resulted in the highest TPC content and antioxidant properties, reaching increases ranging from 13% to 678% compared to the control (Table 2).

Most of the mathematical models proposed for Nanica and Prata varieties showed R^2 values greater than 0.74, and the F values calculated were higher than F-tabulated with statistical significance ($p \leq 0.10$) according to ANOVA (Table 3). For responses in which the statistical parameters were not satisfactory, the mathematical models (equations) were not generated (Table 4). The variations in TPC content and antioxidant assays of the analyzed banana bract extracts were also represented by mixture contour plots (Figures 1 and 2).

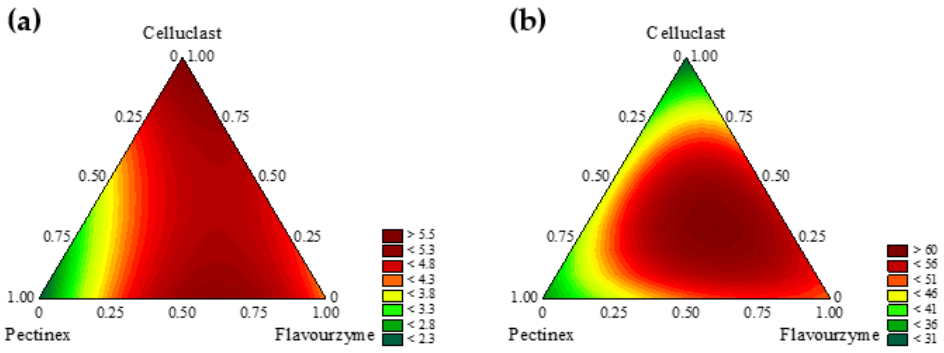

Figure 1. Contour plots for total phenolic compound (TPC) (**a**) and antioxidant activity evaluated by ABTS (**b**) method for banana bract extracts (Nanica variety).

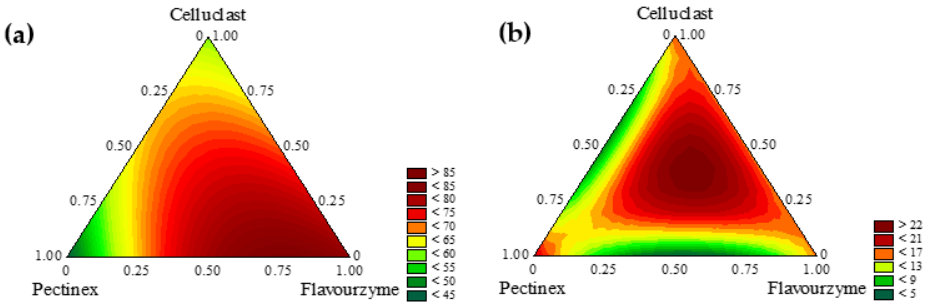

Figure 2. Contour plots for antioxidant activity evaluated by ABTS (**a**) and DPPH (**b**) methods, respectively, for banana bract extracts (Prata variety).

The interpretation of the contour plots just confirmed that the ternary combination of Pectinex™, Flavourzyme™ and Celluclast™ was the most suitable to obtain extracts from banana bracts with higher concentration of TPC and antioxidant properties. According to the Figure 1a, we can see a synergistic effect improving the TPC content of Nanica variety when the enzymes Pectinex™ and Flavourzyme™ were used in binary combination (run 4) with increases between 24 and 130% as compared to the single enzymes (runs 1 and 2), respectively. A synergistic effect between the ternary mixture of Pectinex™ (1/6), Flavourzyme™ (2/3) and Celluclast™ (1/6) (run 9) was also detected; under this condition, increases of 80, 27 and 107% in ABTS assay were reached when compared to the extracts obtained by the use of single enzymes (runs 1, 2 and 3), respectively (Figure 1b). Similar synergistic effect was observed between the binary mixture composed of Pectinex™ and Flavourzyme™ (run 4) for Prata variety, in which increases between 3% and 85% in ABTS assay compared to the extracts produced by use of single enzymes (runs 1 and 2), respectively, were observed (Figure 2a). On the other hand, the ternary combination of Pectinex™ (1/3), Flavourzyme™ (1/3) and Celluclast™ (1/3) (run 7) showed a synergistic effect and increased the DPPH-radical scavenging of the extracts in 20, 29 and 34% when compared to those produced using single enzymes (run 1, 2 and 3), respectively (Figure 2b).

Plant cell wall consisted of a well-organized matrix of carbohydrates (cellulose, hemicellulose and pectin) composed of sugar–alcohol bonds. The majority of bioactive compounds are enclosed to the cell wall polysaccharides by hydrogen bonds and hydrophobic linkages. Phenolic acids, for example, are associated with the cell wall by hydrogen and hydrophobic linkages and by ether bonds between proteins and carbohydrates [33,34]. Banana bracts, for instance, have cell wall rich in cellulose (5.48–13.19%), hemicellulose (14.36–18.83%), pectin (3.97–5.31%) and other macronutrients such as proteins (2.06–2.55%) and a fiber-rich content (61.13–62.22%). To release this complex, enzyme-assisted extraction was applied to facilitate the recovery of compounds through the degradation of plant cell structure and, consequently, biomolecules will be more available for extraction [18]. For better yields in the recovery of bioactive compounds, the enzymatic preparation mixtures were used due to their broad spectrum of activity that enables the hydrolysis of distinct constituents of the plant cell wall [35].

In view of the complexity of the composition of banana bracts, it is evident why the enzyme combinations and their significant interaction effects were more efficient in the hydrolysis and recovery of antioxidant compounds from this material. Pectinex™ is composed of pectinases, hemicellulases and beta-glucanases and acts mainly on hydrolyze the pectic substances and also glycosidic bonds along the carbon chain [36]. It also has been proved to be particularly efficient for phenolic compounds extraction [22]. The use of hemicellulase together with pectinases allows the complete disintegration of the cell wall and improve the recovery of biomolecules [37]. Flavourzyme™ is a blend of proteases which broader specificity and has two mechanisms of action: the endopeptidase activity is responsible for the cleavage of peptide bonds inside the polypeptide chain and the exopeptidase activity catalyzes the hydrolysis of a peptide bond from the N- or C-terminal of a polypeptide chain [38]. Celluclast™, is mostly constituted by cellulases (endo-glucanases) that hydrolyses the cellulosic chain and converts them to oligosaccharides, cellobiose and glucose [17].

Therefore, the cooperative effects between enzyme preparations with different specificities resulted in cell wall degradation and consequent release of bioactive compounds. Thus, the diversity of compounds is greater when enzyme blends are used, increasing the antioxidant properties of the extracts in relation to the extracts produced from non-hydrolyzed samples (control).

Table 2. Results for total phenolic compounds (TPC) and antioxidant activities (ABTS, DPPH and FRAP assays) in banana bract extracts obtained by use of different enzymes (pectinase, protease and cellulase) and a combination of them.

Run	TPC (mg GAE g^{-1})	ABTS (μmol TE g^{-1})	DPPH (μmol TE g^{-1})	FRAP (μmol TE g^{-1})
		Maçã Variety		
Control	2.69 ± 0.76 ^{f}	Not detected	17.43 ± 0.09 ^{ef}	3.38 ± 0.25 ^{f}
1	5.49 ± 0.71 ^{de}	9.45 ± 3.97 ^{d}	23.65 ± 2.19 ^{bc}	3.70 ± 0.31 ^{def}
2	8.89 ± 0.33 ^{c}	3.50 ± 0.38 ^{d}	18.71 ± 1.39 ^{def}	3.67 ± 0.28 ^{def}
3	10.24 ± 0.24 ^{c}	37.30 ± 0.31 ^{abc}	15.55 ± 1.66 ^{f}	3.64 ± 0.30 ^{ef}
4	6.83 ± 0.13 ^{d}	8.84 ± 0.74 ^{d}	20.03 ± 1.57 ^{cde}	3.91 ± 0.46 ^{cdef}
5	4.68 ± 0.05 ^{e}	47.25 ± 3.33 ^{a}	20.01 ± 1.03 ^{cde}	3.75 ± 0.27 ^{def}
6	6.55 ± 0.29 ^{d}	31.05 ± 1.29 ^{bc}	24.88 ± 0.68 ^{b}	5.10 ± 0.43 ^{b}
7	15.00 ± 0.36 ^{a}	37.58 ± 2.60 ^{abc}	30.52 ± 1.86 ^{a}	4.40 ± 0.03 ^{bcde}
8	12.98 ± 0.57 ^{b}	39.80 ± 1.59 ^{ab}	22.26 ± 0.75 ^{bcd}	4.48 ± 0.19 ^{bcd}
9	6.54 ± 0.17 ^{d}	36.11 ± 3.13 ^{abc}	20.46 ± 1.58 ^{cde}	4.66 ± 0.08 ^{bc}
10	15.87 ± 0.59 ^{a}	24.48 ± 4.74 ^{c}	19.66 ± 0.47 ^{de}	7.42 ± 0.09 ^{a}
		Nanica variety		
Control	0.77 ± 0.22 ^{d}	14.54 ± 0.65 ^{f}	14.61 ± 0.14 ^{b}	18.21 ± 1.23 ^{abc}
1	2.03 ± 0.06 ^{d}	34.73 ± 1.44 ^{de}	15.87 ± 1.47 ^{ab}	16.02 ± 0.18 ^{c}
2	3.76 ± 0.17 ^{c}	49.16 ± 0.23 ^{bc}	16.72 ± 0.75 ^{bc}	16.70 ± 0.72 ^{bc}
3	4.77 ± 0.25 ^{abc}	30.15 ± 1.45 ^{ef}	10.63 ± 0.75 ^{ab}	18.30 ± 2.28 ^{abc}
4	4.66 ± 0.04 ^{bc}	49.56 ± 1.37 ^{bc}	12.62 ± 2.42 ^{b}	17.24 ± 0.84 ^{abc}
5	4.28 ± 0.52 ^{c}	44.94 ± 1.06 ^{cd}	14.96 ± 0.54 ^{ab}	17.97 ± 0.33 ^{abc}
6	4.59 ± 0.17 ^{bc}	53.02 ± 1.89 ^{abc}	13.08 ± 0.10 ^{ab}	16.64 ± 0.68 ^{bc}
7	5.00 ± 0.27 ^{abc}	59.73 ± 2.60 ^{ab}	14.52 ± 0.04 ^{ab}	19.53 ± 0.35 ^{abc}
8	3.81 ± 0.36 ^{c}	49.47 ± 0.90 ^{bc}	22.02 ± 3.05 ^{ab}	17.47 ± 0.50 ^{abc}
9	5.99 ± 0.80 ^{a}	62.44 ± 4.43 ^{a}	24.10 ± 0.43 ^{a}	20.62 ± 1.18 ^{a}
10	5.67 ± 0.39 ^{ab}	51.46 ± 0.78 ^{bc}	13.37 ± 0.51 ^{ab}	19.85 ± 1.02 ^{ab}
		Prata variety		
Control	*Not detected*	20.77 ± 2.41 ^{d}	11.81 ± 1.01 ^{d}	28.06 ± 3.84 ^{bcd}
1	8.51 ± 0.38 ^{e}	43.41 ± 3.58 ^{cd}	18.54 ± 1.63 ^{abc}	43.15 ± 4.72 ^{ab}
2	14.70 ± 0.23 ^{a}	77.92 ± 0.89 ^{ab}	17.30 ± 0.53 ^{abc}	34.43 ± 2.83 ^{abc}
3	1.68 ± 0.25 ^{g}	52.95 ± 6.84 ^{bc}	16.60 ± 1.34 ^{bcd}	13.37 ± 3.07 ^{d}
4	1.81 ± 0.15 ^{g}	80.18 ± 2.51 ^{ab}	4.29 ± 3.43 ^{e}	47.09 ± 0.79 ^{a}
5	0.75 ± 0.12 ^{h}	63.78 ± 2.81 ^{abc}	5.36 ± 0.75 ^{e}	38.27 ± 5.07 ^{ab}
6	12.56 ± 0.44 ^{b}	79.17 ± 7.07 ^{ab}	17.85 ± 1.98 ^{abc}	19.85 ± 1.78 ^{cd}
7	9.51 ± 0.22 ^{d}	66.22 ± 8.39 ^{abc}	22.26 ± 0.92 ^{a}	46.29 ± 3.60 ^{a}
8	9.40 ± 0.41 ^{de}	73.93 ± 1.34 ^{ab}	20.51 ± 1.58 ^{ab}	38.08 ± 4.02 ^{ab}
9	5.20 ± 0.06 ^{f}	82.57 ± 1.21 ^{a}	15.32 ± 1.27 ^{cd}	18.39 ± 5.64 ^{cd}
10	10.85 ± 0.13 ^{c}	76.76 ± 11.87 ^{ab}	19.44 ± 1.14 ^{abc}	33.28 ± 3.01 ^{abc}

Values were expressed as the mean (triplicate) ± standard deviation. Different lowercase letters in the same line indicate statistical difference ($p < 0.05$) between the results by Tukey test.

Studies regarding banana inflorescence have been reported since 1979, however, the potential applications of enzymatic hydrolysis are still underexplored. The only study found in the literature, to our knowledge, reported a comparative analysis between the enzyme-assisted extraction (EAE) and ultrasonic-assisted alkaline extraction (UAE) of proteins from banana inflorescence. The greatest protein yield was reached with UAE (252.25 mg g^{-1}) under the following conditions: 30 min extraction time, 50 °C, 1 mol L^{-1} NaOH and 24 kHz. These extracts generated high protein content when compared to EAE (102.98 mg g^{-1}) after 6 h incubation using pepsin. The UAE-extracted proteins were characterized and showed the presence of tryptophan, tyrosine and amide bonds with antibacterial and anti-microbial effects [3].

Table 3. Analysis of variance (ANOVA), including models, R^2 and probability values for the final reduced models for TPC and antioxidant activity (ABTS and DPPH) from banana bract extracts (Nanica and Prata varieties).

Responses	Model	Equations	Fcalculated	Ftabulated	R^2	*p*-Value
		Nanica Variety				
TPC	Quadratic	$Y = 2.20x_1 + 4.18x_2 + 5.51x_3 + 7.95x_1x_2$	5.67	3.29	0.74	0.035
ABTS	Cubic	$Y = 34.18x_1 + 50.10x_2 + 30.25x_3 + 31.25x_1x_2 + 49.10x_1x_3 + 55.54x_2x_3 + 214.21x_1x_2x_3$	24.49	5.28	0.98	0.012
		Prata variety				
ABTS	Quadratic	$Y = 50.46x_1 + 81.48x_2 + 64.86x_3 + 66.92x_1x_2$	4.09	3.26	0.77	0.070
DPPH	Cubic	$Y= 19.91x_1 + 16.38x_2 + 16.77x_3 - 54.14x_1x_2 - 46.28x_1x_3 + 423.52x_1x_2x_3$	6.67	4.05	0.89	0.040

The coded values in model equations represent the independent variables and their interactions: x_1 = Pectinex™; x_2 = Flavourzyme™ and x_3 = Celluclast™.

Table 4. Statistical parameters including F-test, R^2 and probability values for TPC and antioxidant activities (ABTS, DPPH and FRAP) from the extract of banana bract extracts (Maçã, Nanica and Prata varieties), that did not generate statistically valid models.

Responses	Fcalculated	Ftabulated	R^2	*p*-Value
		Maçã variety		
TPC	3.40	3.29	0.63	0.09
ABTS	1.13	5.28	0.68	0.49
DPPH	2.74	3.29	0.58	0.13
FRAP	0.46	3.26	0.11	0.65
		Nanica variety		
DPPH	1.28	3.26	0.26	0.330
FRAP	0.66	3.26	0.15	0.540
		Prata variety		
TPC	1.64	3.29	0.45	0.270
FRAP	4.17	3.26	0.54	0.070

The coded values in model equations represent the independent variables and their interactions: x_1 = Pectinex™; x_2 = Flavourzyme™ and x_3 = Celluclast™.

In more recent studies, this technology was applied in other banana agroindustrial wastes such as peel, peduncle and pseudostem. The cellulose fiber from dried banana peel was investigated and the product obtained was a potential prebiotic fiber. For this, hemicellulose and lignin were removed by alkaline pre-treatment followed by two methods: an enzymatic hydrolysis with Celluclast™ 1.5 L and diluted acid hydrolysis. The results showed that enzymatic treatment was better than dilute-acid hydrolysis due to higher content of water-soluble cellulose and cellodextrins, which promoted the growth of probiotics [39].

The effects of xylooligosaccharides (XOS) from banana pseudostem xylan were investigated and evaluated for their prebiotic activity. Xylan was solubilized with 6% H_2O_2 followed by an enzymatic treatment performed using endoxylanase from *Aspergillus versicolor* and the obtained xylan was alkali extracted. The results showed a good yield and content of XOS (61% and 11 g L^{-1}, respectively). The media with high degree of polymerization XOS evidenced their prebiotic property [40].

The extraction of cellulose from banana peduncle by a combination of mild acid treatment followed by enzymatic hydrolysis for glucose production was studied. Results showed that the pre-treatment facilitated the cellulose yield (with low degree of polymerization) and increasing the enzymatic hydrolysis with a commercial cellulase enzyme from *Aspergillus niger.* The maximal glucose yield (97%) was detected with 50 mg mL^{-1} of substrate, 30 FPU g^{-1} (filter paper units per gram of enzymatic solution) of enzyme,

5 mg mL^{-1} of surfactant and 96 h incubation time. Under these conditions, the process also eliminated lignin and hemicellulose [41].

In general, further studies related to banana inflorescence are encouraged due to its higher concentration of bioactive compounds (polyphenols) than other fractions of banana plantain [19]. Previous studies reported this substantial difference in nutritional composition of the banana inflorescence (*Musa* sp. *cv. Nanjangud rasa bale*) and pseudostem. Phytochemical constituents as phenols showed values of 2.01 and 1.88 mg g^{-1} and flavonoids exhibited values of 0.83 and 0.78 mg g^{-1} for banana inflorescence and pseudostem, respectively [5]. In another study, banana inflorescence (*Musa* sp. *cultivar Elakki bale*) was found to be a better and rich source of dietary fiber (65.6%) when compared to banana pseudostem (28.8%) [6].

3.2. Identification of Phenolic Compounds by HPLC

The identification of phenolic compounds was carried out with the extracts that showed the best results for antioxidant properties, as follows: (i) for the Maçã and Prata varieties, the extracts produced using the ternary mixture of Pectinex™, Flavourzyme™ and Celluclast™, in equal proportions (run 7) and (ii) for the Nanica variety, the extracts produced using the ternary mixture of Pectinex™ (1/6), Flavourzyme™ (2/6) and Celluclast™ (1/6) (run 9).

According to our results, most of the runs revealed an increase in recovery of phenolic compounds after the enzymatic hydrolysis compared to the control (non-hydrolyzed extracts). Five compounds were detected in the extracts: ρ-coumaric acid, ferulic acid, sinapic acid, vanillic acid (phenolic acids) and rutin (flavonoid) (Table 5). Coumaric acid was the main compound identified in banana bract extracts, while sinapic acid was observed only in hydrolyzed extracts from the Nanica variety and rutin was found only in hydrolyzed and non-hydrolyzed extracts from the Prata variety (Table 5).

Table 5. Quantification by HPLC of phenolic compounds from banana bracts extracts after enzymatic hydrolysis using the ternary mixture of pectinase, protease and cellulase.

Compound	Variety	Concentration (μg g^{-1})		Validation Parameters		
		Non-Hydrolyzed Samples (Control)	Hydrolyzed Samples	LOD (μg) [1]	LOQ (μg) [2]	Linearity (R^2)
Coumaric acid	Maçã	n.d.	n.d.	0.0023	0.0070	0.9994
	Nanica	n.d.	76.46 ± 5.38			
	Prata	n.d.	46.97 ± 0.09			
Ferulic acid	Maçã	n.d.	36.17 ± 0.60	0.0015	0.0046	0.9985
	Nanica	7.20 ± 0.18 [b]	44.22 ± 2.48 [a]			
	Prata	n.d.	78.98 ± 0.24			
Sinapic acid	Maçã	n.d.	n.d.	0.0262	0.0792	0.9907
	Nanica	n.d.	22.77 ± 0.13			
	Prata	n.d.	n.d.			
Vanillic acid	Maçã	4.64 ± 0.31 [b]	6.81 ± 0.62 [a]	0.0022	0.0066	0.9979
	Nanica	n.d.	n.d.			
	Prata	5.85 ± 0.11 [b]	13.07 ± 0.18 [a]			
Rutin	Maçã	n.d.	n.d.	0.0014	0.0042	0.9745
	Nanica	n.d.	n.d.			
	Prata	27.01 ± 0.01 [b]	33.56 ± 0.01 [a]			

Values were expressed as the mean (triplicate) ± standard deviation. Different lowercase letters in the same line indicate statistical difference ($p < 0.05$) between the results by Tukey test. n.d: not detected [1] LOD: detection limit. [2] LOQ: limit of quantification.

These increment in recovery of biomolecules is associated with the increase of phenolic acids (available in insoluble form) that are bounded covalently with constituents of cellular

walls (cellulose, hemicellulose, lignin, pectin and proteins). Thus, enzymes (and their combinations) aid cell wall solubilization, releasing the bioactive compounds [18].

EAE has been considered as an excellent method for obtaining extracts with higher biological activities. Among them, it is important to highlight that those compounds detected in banana bract extracts (ρ-coumaric acid, ferulic acid, sinapic acid, vanillic acid and rutin) were reported to promote several health benefits associated to their antioxidant properties. The beneficial effects of antioxidant are related to reduction of oxidative stress where they act as free radical scavengers and reducing agents [42].

Hydroxycinnamic acids, such as ρ-coumaric acid, ferulic acid and sinapic acid, have a wide array of biological activities including antioxidant, anti-inflammatory, anticancer and neuroprotective effects. Among these, the mechanism of the antioxidant effect is based on the ability for neutralization of free radical with generation of more stable phenoxyl radicals [43]. Vanillic acid, belonging to hydroxybenzoic acids, also has numerous biological activities including antioxidant, anti-inflammatory, anticancer and neuroprotective activities [44,45].

The biological properties of rutin, a flavonol glycoside, include anti-inflammatory, antimicrobial, anticancer, neuroprotective and cardioprotective activities which are mainly associated to its antioxidant activities as being a free radical scavenger [46]. The antioxidant capacity is due to the presence of phenolic rings and free hydroxyl groups which can donate hydrogen atoms to prevent further oxidation [47].

The ethanolic extract from banana inflorescence (*Musa* sp. *cv. Nanjangud rasa bale*) was investigated for its antihyperglycaemic effects. The results showed that ethanol extract of banana inflorescence inhibited α-glucosidase enzyme in comparison with drug acarbose (positive control) with IC_{50} values of 7.79 μg mL-^{1} and 9.68 μg mL^{-1}, respectively. The main compounds present in extracts were umbelliferone (7.08 μg mL^{-1}) and lupeol (7.18 μg mL^{-1}), belonged to the coumarin and triterpenoids, respectively. These compounds isolated from banana inflorescence proved to be α-glucosidase inhibitors and they showed a higher free radical scavenging activity. Umbelliferone is recognized for its bioactivities including antirheumatic, analgesic and antipyretic effects, whereas, lupeol was reported to have anti-inflammatory and anticancer effects [48].

Colorimetric methods, such as the ABTS, DPPH and FRAP assays, are widely used for first-level screening to assess the potential bioactivity of plant substrates [49]. However, it is important to recognize that these methods have some limitations and that further analyses involving the understanding of the antioxidant action of bioactive compounds are crucial to confirm their biological effects. Therefore, the determination of bioactive compounds by chromatographic techniques to establish the structure–activity relationship and application in at least one in vitro biological test (i.e., cell lines and simulated digestion) or, preferably, in vivo evaluation using animal models are strongly encouraged as complementary analyses to prove the effects of these compounds [50].

4. Conclusions

This study provided information about the most suitable enzyme combinations for better release of bioactive compounds from banana bracts. The use of the ternary mixture of Pectinex™, Flavourzyme™ and Celluclast™ enzymes resulted in extracts with higher phenolic content and antioxidant properties. In addition, HPLC analysis detected the presence of five polyphenolic compounds, the most prominent being coumaric acid (76.46 μg g^{-1}) and ferulic acid (78.98 μg g^{-1}) for banana bract extracts obtained from Nanica and Prata varieties, respectively, after enzymatic hydrolysis. Thus, it was possible to correlate the presence of these polyphenols with the antioxidant potential of the extracts. The results obtained in our study open new possibilities for the development of functional ingredients for the food industry through fast, efficient and safe processes, such as enzymatic hydrolysis using banana bracts, a substrate that has not been explored with this process until now.

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References

1. FAO. Food and Agriculture Organization of the United Nations. Banana—Production Quantity. 2022. Available online: https://www.fao.org/faostat/en/#data/QCL (accessed on 17 March 2022).
2. Mathew, N.S.; Negi, P.S. Traditional Uses, Phytochemistry and Pharmacology of Wild Banana (Musa acuminata Colla): A Review. *J. Ethnopharmacol.* **2017**, *196*, 124–140. [CrossRef] [PubMed]
3. Sitthiya, K.; Devkota, L.; Sadiq, M.B.; Anal, A.K. Extraction and Characterization of Proteins from Banana (*Musa sapientum* L.) Flower and Evaluation of Antimicrobial Activities. *J. Food Sci. Technol.* **2018**, *55*, 658–666. [CrossRef]
4. Basumatary, S.; Nath, N. Assessment of Chemical Compositions and in Vitro Antioxidant Properties of *Musa balbisiana Colla* Inflorescence. *Int. J. Pharm. Res.* **2018**, *10*, 80–85.
5. Ramu, R.; Shirahatti, P.S.; Anilakumar, K.R.; Nayakavadi, S.; Zameer, F.; Dhananjaya, B.L.; Nagendra Prasad, M.N. Assessment of Nutritional Quality and Global Antioxidant Response of Banana (*Musa* sp. CV. Nanjangud Rasa Bale) Pseudostem and Flower. *Pharmacogn. Res.* **2017**, *9* (Suppl 1), S74–S83. [CrossRef]
6. Bhaskar, J.J.; Chilkunda, N.D.; Salimath, P.V. Banana (Musa Sp. Var. Elakki Bale) Flower and Pseudostem: Dietary Fiber and Associated Antioxidant Capacity. *J. Agric. Food Chem.* **2012**, *60*, 427–432. [CrossRef]
7. Padam, B.S.; Tin, H.S.; Chye, F.Y.; Abdullah, M.I. Antibacterial and Antioxidative Activities of the Various Solvent Extracts of Banana (Musa Paradisiaca Cv. Mysore) Inflorescences. *J. Biol. Sci.* **2012**, *12*, 62–73. [CrossRef]
8. Prakasan, N.; Saraswathy, M. Flavanoid Rich Ethyl Acetate Fraction of Musa Paradisiaca Inflorescence Down-Regulates the Streptozotocin Induced Oxidative Stress, Hyperglycaemia and MRNA Levels of Selected Inflammatory Genes in Rats. *J. Funct. Foods* **2013**, *5*, 1838–1847. [CrossRef]
9. Arun, K.B.; Thomas, S.; Reshmitha, T.R.; Akhil, G.C.; Nisha, P. Dietary Fibre and Phenolic-Rich Extracts from Musa Paradisiaca Inflorescence Ameliorates Type 2 Diabetes and Associated Cardiovascular Risks. *J. Funct. Foods* **2017**, *31*, 198–207. [CrossRef]
10. Sheng, Z.; Dai, H.; Pan, S.; Wang, H.; Hu, Y.; Ma, W. Isolation and Characterization of an α-Glucosidase Inhibitor from *Musa* spp. (Baxijiao) Flowers. *Molecules* **2014**, *19*, 10563–10573. [CrossRef] [PubMed]
11. Bandara, N.; Chalamaiah, M. Encyclopedia of Food Chemistry. In *Encyclopedia of Food Chemistry*; Varelis, P., Melton, L., Shahidi, F., Eds.; Elsevier: Amsterdam, The Netherlands, 2019; p. 2194.
12. Cory, H.; Passarelli, S.; Szeto, J.; Tamez, M.; Mattei, J. The Role of Polyphenols in Human Health and Food Systems: A Mini-Review. *Front. Nutr.* **2018**, *5*, 87. [CrossRef]
13. Marathe, S.J.; Jadhav, S.B.; Bankar, S.B.; Singhal, R.S. Enzyme-Assisted Extraction of Bioactives. In *Food Bioactives: Extraction and Biotecnhonology Applications*; Elsevier: Chatswood, Australia, 2017; p. 326.
14. Olszowy, M. What Is Responsible for Antioxidant Properties of Polyphenolic Compounds from Plants? *Plant Physiol. Biochem.* **2019**, *144*, 135–143. [CrossRef]
15. Azmir, J.; Zaidul, I.S.M.; Rahman, M.M.; Sharif, K.M.; Mohamed, A.; Sahena, F.; Jahurul, M.H.A.; Ghafoor, K.; Norulaini, N.A.N.; Omar, A.K.M. Techniques for Extraction of Bioactive Compounds from Plant Materials: A Review. *J. Food Eng.* **2013**, *117*, 426–436. [CrossRef]
16. Soquetta, M.B.; Terra, L.D.M.; Bastos, C.P. Green Technologies for the Extraction of Bioactive Compounds in Fruits and Vegetables. *CYTA—J. Food* **2018**, *16*, 400–412. [CrossRef]
17. De la Peña-Armada, R.; Villanueva-Suárez, M.J.; Rupérez, P.; Mateos-Aparicio, I. High Hydrostatic Pressure Assisted by Celluclast® Releases Oligosaccharides from Apple By-Product. *Foods* **2020**, *9*, 1058. [CrossRef] [PubMed]
18. Nadar, S.S.; Rao, P.; Rathod, V.K. Enzyme Assisted Extraction of Biomolecules as an Approach to Novel Extraction Technology: A Review. *Food Res. Int.* **2018**, *108*, 309–330. [CrossRef] [PubMed]
19. Lau, B.F.; Kong, K.W.; Leong, K.H.; Sun, J.; He, X.; Wang, Z.; Mustafa, M.R.; Ling, T.C.; Ismail, A. Banana Inflorescence: Its Bio-Prospects as an Ingredient for Functional Foods. *Trends Food Sci. Technol.* **2020**, *97*, 14–28. [CrossRef]
20. Gligor, O.; Mocan, A.; Moldovan, C.; Locatelli, M.; Crișan, G.; Ferreira, I.C.F.R. Enzyme-Assisted Extractions of Polyphenols—A Comprehensive Review. *Trends Food Sci. Technol.* **2019**, *88*, 302–315. [CrossRef]

21. Casas, M.P.; Domínguez González, H. Enzyme-Assisted Aqueous Extraction Processes. In *Water Extraction of Bioactive Compounds: From Plants to Drug Development*; Elsevier: Amsterdam, The Netherlands, 2017; pp. 333–368. [CrossRef]
22. Danalache, F.; Mata, P.; Alves, V.D.; Moldão-Martins, M. Enzyme-Assisted Extraction of Fruit Juices. In *Fruit Juices: Extraction, Composition, Quality and Analysis*; Academic Press: Cambridge, MA, USA, 2018; pp. 183–200. [CrossRef]
23. Mu, R.; Wang, Z.; Wamsley, M.C.; Duke, C.N.; Lii, P.H.; Epley, S.E.; Todd, L.C.; Roberts, P.J. Application of Enzymes in Regioselective and Stereoselective Organic Reactions. *Catalysts* **2020**, *10*, 832. [CrossRef]
24. Liu, J.J.; Gasmalla, M.A.A.; Li, P.; Yang, R. Enzyme-Assisted Extraction Processing from Oilseeds: Principle, Processing and Application. *Innov. Food Sci. Emerg. Technol.* **2016**, *35*, 184–193. [CrossRef]
25. Neto, B.B.; Scarminio, I.S.; Bruns, R.E. *Como Fazer Experimentos: Aplicações Na Ciência e Na Indústria*, 4th ed.; Bookman: Porto Alegre, Brazil, 2010.
26. de Castro, R.J.S.; Cason, V.G.; Sato, H.H. Binary Mixture of Proteases Increases the Antioxidant Properties of White Bean (*Phaseolus vulgaris* L.) Protein-Derived Peptides Obtained by Enzymatic Hydrolysis. *Biocatal. Agric. Biotechnol.* **2017**, *10*, 291–297. [CrossRef]
27. Magro, A.E.A.; de Castro, R.J.S. Effects of Solid-State Fermentation and Extraction Solvents on the Antioxidant Properties of Lentils. *Biocatal. Agric. Biotechnol.* **2020**, *28*, 101753. [CrossRef]
28. Neta, I.M.R.; de Castro, R.J.S. Enzyme-Assisted Extraction of Biocomponents of Lentils (Lens Culinaris L.): Effect of Process Parameters on the Recovery of Compounds with Antioxidant Properties. *Biocatal. Biotransform.* **2020**, *38*, 15–23. [CrossRef]
29. Rasera, G.B.; Hilkner, M.H.; de Alencar, S.M.; de Castro, R.J.S. Biologically Active Compounds from White and Black Mustard Grains: An Optimization Study for Recovery and Identification of Phenolic Antioxidants. *Ind. Crops Prod.* **2019**, *135*, 294–300. [CrossRef]
30. Firuzi, O.; Lacanna, A.; Petrucci, R.; Marrosu, G.; Saso, L. Evaluation of the Antioxidant Activity of Flavonoids by "Ferric Reducing Antioxidant Power" Assay and Cyclic Voltammetry. *Biochim. Biophys. Acta* **2005**, *1721*, 174–184. [CrossRef]
31. Aguilar, J.G.; de Castro, R.J.S.; Sato, H.H. Optimization of the Enzymatic Hydrolysis of Rice Protein by Different Enzymes Using the Response Surface Methodology. *3 Biotech* **2018**, *8*, 372. [CrossRef] [PubMed]
32. Silva, A.P.; Rosalen, P.L.; de Camargo, A.C.; Lazarini, J.G.; Rocha, G.; Shahidi, F.; Franchin, M.; de Alencar, S.M. Inajá Oil Processing By-Product: A Novel Source of Bioactive Catechins and Procyanidins from a Brazilian Native Fruit. *Food Res. Int.* **2021**, *144*, 110353. [CrossRef]
33. Marathe, S.J.; Jadhav, S.B.; Bankar, S.B.; Kumari Dubey, K.; Singhal, R.S. Improvements in the Extraction of Bioactive Compounds by Enzymes. *Curr. Opin. Food Sci.* **2019**, *25*, 62–72. [CrossRef]
34. Patil, P.D.; Patil, S.P.; Kelkar, R.K.; Patil, N.P.; Pise, P.V.; Nadar, S.S. Enzyme-Assisted Supercritical Fluid Extraction: An Integral Approach to Extract Bioactive Compounds. *Trends Food Sci. Technol.* **2021**, *116*, 357–369. [CrossRef]
35. Costa, J.R.; Tonon, R.V.; Cabral, L.; Gottschalk, L.; Pastrana, L.; Pintado, M.E. Valorization of Agricultural Lignocellulosic Plant Byproducts through Enzymatic and Enzyme-Assisted Extraction of High-Value-Added Compounds: A Review. *ACS Sustain. Chem. Eng.* **2020**, *8*, 13112–13125. [CrossRef]
36. Liu, X.; Kokare, C. Microbial Enzymes of Use in Industry. In *Biotechnology of Microbial Enzymes: Production, Biocatalysis and Industrial Applications*; Academic Press: Cambridge, MA, USA, 2017; pp. 267–298. [CrossRef]
37. Mehta, P.K.; Sehgal, S. Microbial Enzymes in Food Processing. In *Biocatalysis: Enzymatic Basics and Applications*; Springer: Singapore, 2019; pp. 255–275. [CrossRef]
38. Ohara, A.; Cason, V.G.; Nishide, T.G.; Miranda de Matos, F.; de Castro, R.J.S. Improving the Antioxidant and Antidiabetic Properties of Common Bean Proteins by Enzymatic Hydrolysis Using a Blend of Proteases. *Biocatal. Biotransform.* **2021**, *39*, 100–108. [CrossRef]
39. Phirom-on, K.; Apiraksakorn, J. Development of Cellulose-Based Prebiotic Fiber from Banana Peel by Enzymatic Hydrolysis. *Food Biosci.* **2021**, *41*, 101083. [CrossRef]
40. de Freitas, C.; Terrone, C.C.; Masarin, F.; Carmona, E.C.; Brienzo, M. In Vitro Study of the Effect of Xylooligosaccharides Obtained from Banana Pseudostem Xylan by Enzymatic Hydrolysis on Probiotic Bacteria. *Biocatal. Agric. Biotechnol.* **2021**, *33*, 101973. [CrossRef]
41. Baruah, J.; Bardhan, P.; Mukherjee, A.K.; Deka, R.C.; Mandal, M.; Kalita, E. Integrated Pretreatment of Banana Agrowastes: Structural Characterization and Enhancement of Enzymatic Hydrolysis of Cellulose Obtained from Banana Peduncle. *Int. J. Biol. Macromol.* **2022**, *201*, 298–307. [CrossRef] [PubMed]
42. Leichtweis, M.G.; Oliveira, M.B.P.P.; Ferreira, I.C.F.R.; Pereira, C.; Barros, L. Sustainable Recovery of Preservative and Bioactive Compounds from Food Industry Bioresidues. *Antioxidants* **2021**, *10*, 1827. [CrossRef] [PubMed]
43. Torrisi, C.; Morgante, A.; Malfa, G.; Acquaviva, R.; Castelli, F.; Pignatello, R.; Sarpietro, M.G. Sinapic Acid Release at the Cell Level by Incorporation into Nanoparticles: Experimental Evidence Using Biomembrane Models. *Micro* **2021**, *1*, 120–128. [CrossRef]
44. Ullah, R.; Ikram, M.; Park, T.J.; Ahmad, R.; Saeed, K.; Alam, S.I.; Rehman, I.U.; Khan, A.; Khan, I.; Jo, M.G.; et al. Vanillic Acid, a Bioactive Phenolic Compound, Counteracts LPS-Induced Neurotoxicity by Regulating c-Jun N-Terminal Kinase in Mouse Brain. *Int. J. Mol. Sci.* **2021**, *22*, 361. [CrossRef]
45. Punvittayagul, C.; Chariyakornkul, A.; Jarukamjorn, K.; Wongpoomchai, R. Protective Role of Vanillic Acid against Diethylnitrosamine- and 1,2-Dimethylhydrazine-Induced Hepatocarcinogenesis in Rats. *Molecules* **2021**, *26*, 2718. [CrossRef]

46. Gullón, B.; Lú-Chau, T.A.; Moreira, M.T.; Lema, J.M.; Eibes, G. Rutin: A Review on Extraction, Identification and Purification Methods, Biological Activities and Approaches to Enhance Its Bioavailability. *Trends Food Sci. Technol.* **2017**, *67*, 220–235. [CrossRef]
47. Chua, L.S. A Review on Plant-Based Rutin Extraction Methods and Its Pharmacological Activities. *J. Ethnopharmacol.* **2013**, *150*, 805–817. [CrossRef] [PubMed]
48. Ramu, R.; Shirahatti, P.S.; Zameer, F.; Ranganatha, L.V.; Nagendra Prasad, M.N. Inhibitory Effect of Banana (Musa sp. Var. Nanjangud Rasa Bale) Flower Extract and Its Constituents Umbelliferone and Lupeol on α-Glucosidase, Aldose Reductase and Glycation at Multiple Stages. *S. Afr. J. Bot.* **2014**, *95*, 54–63. [CrossRef]
49. De Camargo, A.C.; Biasoto, A.C.T.; Schwember, A.R.; Granato, D.; Rasera, G.B.; Franchin, M.; Rosalen, P.L.; Alencar, S.M.; Shahidi, F. Should We Ban Total Phenolics and Antioxidant Screening Methods? The Link Between Antioxidant Potential and Activation of NF-Kb Using Phenolic Compounds from Grape By-Products. *Food Chem.* **2019**, *290*, 229–238. [CrossRef] [PubMed]
50. Granato, D.; Shahidi, F.; Wrolstad, R.; Kilmartin, P.; Melton, L.D.; Hidalgo, F.J.; Miyashita, K.; van Camp, J.; Alasalvar, C.; Ismail, A.B. Antioxidant activity, total phenolics and flavonoids contents: Should we ban in vitro screening methods? *Food Chem.* **2018**, *264*, 471–475. [CrossRef] [PubMed]