An Integrated Analysis of the *Musa Paradisiaca* Peel, Using UHPLC-ESI, FT-IR and Confocal Microscopy Techniques

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Abstract

When the banana (*Musa paradisiaca*) peel was subject of enzymatic hydrolysis with cellulase and hemicellulase, only glucose was obtained. Images from banana peel, using confocal laser scanning microscopy, demonstrate that the cellulose is the main structural compound. Fatty acids, phenolic and other compounds were detected from the organic residual extract, and characterized by means of NMR, FT-IR and UHPLC-MS techniques. The presences of these compounds were corroborated through a steam distillation. Under this condition, banana peel could have potential applications in the food field, where could be used to improve some procedures such as the obtaining of banana vinegar.

Introduction

Bananas and plantains (*Musa spp.*) are two of the world's most important food crops [1], and the economies of some banana producing countries are partially or entirely dependent on the cultivation, trade, processing and consumption of these fruits [2]. Worldwide bananas and plantains are harvested year round and play a major role in the nutrition and cultural life of millions of people in tropical and subtropical regions [3].

Banana plants consist of leaf, pseudostem, root and banana fruits. Banana fruits have been used for dietary purposes as well as local therapies [4]. Some of the pharmaceutical applications of banana fruit and stem include: use as a remedy for constipation; curing diarrhea and dysentery; to heal the intestine lesions; useful in stomach upset and diabetes [5]. Banana fruits are generally conserved during transportation by washing thoroughly and possible soaking in fungicide prior to packaging into cartons for transportation. Banana residue generated after harvesting of banana fruit are leaf, pseudo stem, roots and rotten banana. Since the banana selected for exportation requires high quality standards such as dimensions and peel with good characteristics, there are high quantities of banana that are rejected. Moreover, some of the rejected banana is not properly used and is disposed outdoors allowing its decomposition thus creating an environmental problem. Rejected banana is an interesting source of polysaccharides in the form of starch (in the pulp) and lignocellulose (in the peel) that can be transformed physically, chemically, enzymatically or microbiologically into value-added products [5-7].

Mexico is in tenth place of banana production, with a little more than three percent of global production. The annual production of banana represents 1.4 percent of the value of domestic agricultural production, mainly from the states of Tabasco, Chiapas, Veracruz and Colima [8,9]. Of the total production, 92 % was for domestic consumption and the remaining 8% is for exportation.

Generally, peels from consuming bananas are used in the animal feeding, as organic fertilizer or they are simply discarded. Disposal of these peels may cause environmental problems. Currently, there are reports on literature describing the usage of these peels, e.g., production of ethanol [10], methane [11,12], feed for livestock [13], or as adsorbents for water purification [14]. These peels could be converted into other natural products or raw material for secondary process which can also be used directly as functional compounds in human nutrition and prevention and healthcare.

This paper addresses the use of enzymatic reactions and analytical techniques such as UHPLC-ESI, FT-IR, NMR and microscopy analysis to study the banana peel and generated compounds that could be valued as raw materials for food industrial applications.

Materials and Methods

Methanol, Acetonitrile and Water HPLC grade were purchased from Sigma-Aldrich (St Louis, MO, USA). Common inorganic reagents and solvents used in the study were analytical grade. Silica

gel coated TLC plates (silica gel 60, 0.25 mm thickness) and 70–230 mesh silica gel for chromatography were also obtained from Sigma-Aldrich.

Enzymatic hydrolysis of cell-wall model substrates

Banana peel from agro residual wastes were obtained from local market and then were subject of an enzymatic treatment with *Aspergillus niger* pectinase (EC 3.2.1.15), present at 1 mg/mL (0.9 units/mg), shaking in 50 mM sodium acetate buffer (pH 4) at 31°C for 2 days. After this, the reaction was filtered and treated with *Aspergillus niger* cellulase (EC3.2.1.4), present at 1.0 mg/mL (5.1 units/mg) and hemicellulase (10 mg/mL, 0.051 unit/mg) suspended in 50 mM sodium acetate buffer (pH 5) at 37 °C, for 2 days. Reactions were carried out in a thermostatically controlled incubator shaker (New Brunswick Instruments, New Brunswick, NJ), using a stirbar for mechanical agitation. Dewaxing was carried out by successive Soxhletextractions with methanol: chloroform (1:1, v/v). From 1 Kg of banana peel wastes, 1.2 gr of lignocellulosic insoluble material was obtained.

Steam distillation

The steam generator flask was filled out with distilled water and heated with a heating mantle. As the water vaporized, the steam passed to the distillation flask containing 500 gr of banana peel, then, through the cooled tube where it was condensed. The distillate (520 mL) was collected in the receiving flask after 3 h distillation, and extracted with hexane (3 X 50 mL). The hexane was evaporated in a rotaevaporator and the extract was analyzed by means of NMR and UHPLC-ESI (-).

NMR spectroscopy

Soluble products were characterized by ¹H- and ¹³C-NMR (Varian NMR System, 500 MHz) (Palo Alto, CA). The NMR spectra were recorded in deuterated chloroform (CDCl₃) or methanol (CH₃OD).

FT-IR spectroscopy

FTIR Spectra were recorded with an FTIR module IR2 equipped with an Indium Gallium Arsenide (InGaAs) detector, coupled to a Horiba JobinYvonLabRam HR800 spectrometer. The spectra were recorded in the region of 4000–400 cm⁻¹ with a spectral resolution of 4 cm⁻¹ and 32 scans per measurement, using an ATR contact objective.

UHPLC-ESI analysis

Electrospray Ionization (ESI) analysis was done on a Bruker micrOTOF-QII (BrukerDaltonics, Billerica, MA). Samples were dissolved in methanol and were injected directly to the spectrometer. The polymer related peaks were found in positive and negative ion mode (ESI+ or ESI-). The capillary potential was -4.5 kV, the dry gas temperature 200°C and the drying gas flow 4 L/min. Total ion chromatograms from m/z 500 to 3,000 were obtained. MS data were processed using PolyTools 1.0 (BrukerDaltonics, Billerica, MA).

The chemical characterization of the aqueous extract was carried out by UHPLC-MS (ultra-high performance liquid chromatography mass spectroscopy) analysis.

An Ultimate 3000 Ultra-High Performance Liquid

Chromatography (UHPLC) system (Dionex Corp., CA, USA) with Photodiode Array Detection (PAD), was coupled to a Bruker micrOTOF-QII system with an Electrospray Ionization (ESI) interface (BrukerDaltonics, Billerica, USA) for chromatographic and Mass Spectrometric (MS) analysis. For chromatography separation, a Luna-NH₂ column (5.0 μm, 150 X 4.60 mm) (Phenomenex) was used. An isocratic system consisted of a mobile phase of water (A) and acetonitrile (B) (3:7, v/v) was used. The solvent flow rate was 0.4 mL/min, the column temperature was set to 40°C. The conditions of MS analysis in the negative ion mode were as follows: drying gas (nitrogen), flow rate, 8 L/min; gas temperature, 180°C; scan range, 50–3000 m/z; end plate offset voltage, 500 V; capillary voltage, 4500 V; nebulizer pressure, 2.5 bar.

The accurate mass data of the molecular ions were processed through the software Data Analysis 4.0 (BrukerDaltonics), which provided a list of possible elemental formulas using Generate Molecular Formula Editor, as well as a sophisticated comparison of the theoretical with the measured isotope pattern (σ value) for increased confidence in the suggested molecular formula (Bruker Daltonics Technical Note 008, 2004). The widely accepted accuracy threshold for confirmation of elemental compositions was established at 5 ppm. During the development of the UHPLC method, external instrument calibration was performed using a 74900-00-05 Cole Palmer syringe pump (Billerica, MA, USA) directly connected to the interface, with a sodium Formate cluster solution. The calibration solution was injected at the beginning of each run and all the spectra were calibrated prior to the compound identification.

Confocal Laser Scanning Microscopy

For CLSM analysis, each sample was mounted on glass slices and observed under CLSM (LSM 710 NLO, Carl Zeiss, Jena, Germany) with objective EC Plan-Neofluar 10x/0.3. The laser wavelength excitation was 405, 488, 561 y 633 nm, simultaneous. This capture mode used was a spectral imaging technique that automatically outputs separated channels of multiple labeled samples. This tool detects the autofluorescence signal of banana skin and was compared experimentally with patrons (cellulose) between 420 to 720 nm. The z-stack images (3D images) were captured by means the software ZEN 2010 (Carl Zeiss, Germany), at 512 x 512 pixels in RGB color and stored in TFF format at 8bits.

Results and Discussion

It is well known that banana peel is mainly composed of cellulose, lignin and other compounds such as phenolic, sterols, carotenoids and fatty acids. However, all those studies have been done separately according with different analysis, especially for biological activities.

Enzymatic treatment of the banana peels with pectinase gave a small loses in cuticular material. However, when cellulase and hemicellulase were used, most of the organic material was hydrolyzed. Typically, from 1 kg of banana peel, approximately 2 gr of insoluble residues are obtained. These residues were washed in a soxhlet extractor with a mixture of MeOH: Chloroform (1:1, v/v), to

give 1.2 gr of lignocellulosic insoluble material and 600 mg of soluble compounds (aromatic and aliphatic compounds).

Analysis of the enzymatic hydrolysis of the banana peel

The aqueous residual of the enzymatic reaction was analyzed with UHPLC-ESI (-) in order to know the monosaccharide's composition. As we can see in Figure 1, the analysis shows only the presence of glucose. Enzymatic hydrolysis was carried out using cellulose and hemicelluloses, to remove these polysaccharides from the banana

peel. However, the enzymatic process hydrolyzed completely the peel giving glucose, as the main product, and only a few amorphous components (residues of cellulose with lignin components).

Other enzymes, such as the xylanase, have been used to produce cellulose nanofibers from banana peel [7]. With this enzyme, most of the hemicellulose and lignin can be removed and, cellulose banana nanofibers can be obtained and used as reinforcing elements in composites. On the other hand, the use of cellulase and hemicellulases could be used to hydrolyze the banana peel prior to use it in the food industry such as in the banana vinegar preparation.

To complete the analysis, a microscopy analysis was done. When the banana peel is removed, there is a white remaining material in the inner skin. This was analyzed with Confocal Laser Scanning Microscopy (CLMS), and according with Figure 2a, 2b and 2e, was characterized as cellulose (green fluorescence in the 3D images). When this material was removed mechanically, the signal for the lignin components is gone and the cellulose remains in two forms: crystalline cellulose and amorphous cellulose (Figure 2c and 2d). However, when the peel was enzymatically hydrolyzed, the signals for lignin components increases (red fluorescence) and the cellulose decrease. This was corroborated by FT-IR (Figure 3). The ATR FT-IR obtained from the insoluble lignocellulosic material (Figure 3) shows all the expected functional groups of ligninocellulosic derivatives. The adsorption bands at 1605 cm^{-1} and 1517 cm^{-1} were characteristic of phenyl ring skeletal vibrations of lignin macromolecules [15]. This observation confirmed that part of the core of lignin polymer did not change significantly during the enzymatic reaction. The broad band at 3413 cm^{-1} was due to the presence of hydroxyls, mainly from the part

of the cellulose. The band at 2940 cm^{-1} was contributed to methylene and methyl groups. The adsorption of aromatic methoxy group was located at 2850 cm^{-1} . The absorption at 1711 cm^{-1} was characteristic for the presence of an aldehyde or ketone carbonyl group. As to the fingerprint region: the vibration of methylene and methyl groups were at 1461 cm^{-1} and 1420 cm^{-1} , and methyl groups also at 1375 cm^{-1} , methylenes and hydroxyls at 1330 cm^{-1} and aromatic rings near 910 cm^{-1} .

Analysis of the organic soluble compounds

Compounds obtained from the soxhlet extraction were analyzed by direct injection in the spectrometer and UHPLC-ESI (-) (Table 1). Main identified compounds were fatty acids: palmitic acid, linoleic acid and linolenic acid. Only ferulic acid, caffeic acid and 7-Phenylheptyl 4-hydroxybenzoate were present in less than 1%.

The complete extract was analyzed by ^1H NMR in order to correlate the signals with the compounds. According to Figure 4, phenolic are present in very small amount (δ 7.00 – 7.8 ppm), and aliphatic compounds are the main compounds present (δ 0.5 – 2.5 ppm) corroborating the presence of the compounds previously identified with mass spectrometry.

In order to analyze the possible oil compounds in the banana peel, a steam distillation was done, and the extract obtained was analyzed and compared with the organic extract previously analyzed. In both

Table 1: Compounds identified from the organic fraction by UHPLC and direct injection (ESI-).

Compound	Formula	[M-H] ⁻ Calc.	[M-H] ⁻ Obsv.	Err [ppm]	mSigma	Abundance (%)
Dodecanoic acid	$\text{C}_{12}\text{H}_{23}\text{O}_2$	199.1704	199.1738	-18.0	75.2	0.24
Hexadecanoic acid	$\text{C}_{16}\text{H}_{31}\text{O}_2$	255.2330	255.2377	-18.0	2.2	18.52
Linolenic acid	$\text{C}_{18}\text{H}_{30}\text{O}_2$	277.2179	277.2218	-16.2	77.1	12.55
Linoleic acid	$\text{C}_{18}\text{H}_{32}\text{O}_2$	279.2330	279.2376	-18.5	42.9	15.86
18-hydroxyoleic acid	$\text{C}_{18}\text{H}_{34}\text{O}_3$	297.2435	297.2478	-13.8	82.8	7.90
Ferulic acid	$\text{C}_{10}\text{H}_{10}\text{O}_4$	193.0495	193.0403	-26.6	38.4	0.38
Caffeic acid	$\text{C}_9\text{H}_8\text{O}_4$	179.0338	179.0572	-19.7	55.2	0.06
7-Phenylheptyl 4-hydroxybenzoate	$\text{C}_{20}\text{H}_{23}\text{O}_3$	311.1653	311.1742	-14.6		0.85
Unknown*	$\text{C}_{23}\text{H}_{34}\text{O}_6$	405.2283	405.2266	4.7	37.8	

cases, a compound present in the extract was very similar (Figure 5). An additional compound with m/z 405.2266, corresponding to $\text{C}_{23}\text{H}_{34}\text{O}_6$ (m/z 405.2283) was identified from the steam distillation extract.

Conclusion

Disposal of Banana peel may cause environmental problems. However, the use of these agro-residual wastes could be successfully

used in different areas, such in the production of bioethanol, methane and others. Here, we analyze the banana peel using confocal laser scanning microscopy to determine the presence of cellulose as the principal structural component. This was corroborated with an enzymatic hydrolysis to detect only the presence of glucose. Some compounds like fatty acids, phenolics and other are present in minor amounts. These compounds were extracted through a steam distillation and were in agreement with those obtained from the

enzymatic hydrolysis. The enzymatic hydrolysis could be used to incorporate banana peel into the food processing industry, such as the banana vinegar production.

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