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Antioxidant activity in banana peel extracts: Testing extraction conditions and related bioactive compounds

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ABSTRACT

Banana (*Musa acuminata* Colla AAA) peel extracts obtained in this work had a high capacity to scavenge 2,2-diphenyl-1-picrylhydrazyl (DPPH·) and 2,2′-azino-bis(3-ethylbenzothiazoline)-6-sulfonic acid ($ABTS^{\cdot+}$) free radicals, and they were also good lipid peroxidation inhibitors. Acetone:water extracts were considerably more effective (compared with methanol, ethanol, acetone, water, methanol:water or ethanol:water) at inhibiting the peroxidation of lipids in the β-carotene/linoleic acid system or scavenging free radicals. However, aqueous extracts had a high capacity to protect lipids from oxidation in the thiobarbituric acid reactive substances (TBARS) test, as well as in the β-carotene bleaching assay. In addition, acetone:water most efficiently extracted all extractable components (54 ± 4%), phenolic compounds (3.3 ± 0.8%), and anthocyanin compounds (434 ± 97 μg cyanidin 3-glucoside equivalents/100 g freeze-dried banana peel). Banana peel contained large amounts of dopamine and L-dopa, catecholamines with a significant antioxidant activity. However, ascorbic acid, tocopherols or phytosterols were not detected in the different extracts. The antioxidant activity of banana peel extracts from different cultivars was similar. However, the impact of extraction time or temperature should be studied in greater depth.

1. Introduction

From an environmental perspective it is vital that plant by-products produced by the agro-food industry be reused. Council Directive 1999/31/EC on landfill waste, requires that member states reduce biodegradable organic waste in landfills by 65% (compared to 1995 levels) by no later than 2016 (European Union, 1999). The main by-product of the banana processing industry is the peel, which represents approximately 30% of the fruit. This by-product constitutes an environmental problem because it contains large quantities of nitrogen and phosphorus and its high water content makes it susceptible to modification by microorganisms.

Potential applications for banana peel depend on its chemical composition. Banana peel is rich in dietary fibre (50% on a dry matter (DW) basis), proteins (7% DW), essential amino acids, polyunsaturated fatty acids and potassium (Emaga, Andrianaivo, Wathelet, Tchango, & Paquot, 2007). Attempts at the practical utilisation of banana by-products include the production of biomass, protein, ethanol, methane, pectins and enzymes (Clarke, Radnidge, Lai, Jensen, & Hardin, 2008; Emaga, Ronkart, Robert, Wathelet, & Paquot, 2008; Essien, Akpan, & Essien, 2005). Banana peel has also been used as food for livestock (Onwuka, Adetiloye, & Afolami, 1997) or as an adsorbent for water purification (Annadurai, Juang, & Lee, 2002).

Banana peel is rich in phytochemical compounds, mainly antioxidants. The total amount of phenolic compounds in banana (*Musa acuminata* Colla AAA) peel ranges from 0.90 to 3.0 g/100 g DW (Nguyen, Ketsa, & van Doorn, 2003; Someya, Yoshiki, & Okubo, 2002). Someya et al. (2002) identified gallocatechin at a concentration of 160 mg/100 g DW. Ripe banana peel also contains other compounds, such as the anthocyanins delphinidin and cyanidin (Seymour, 1993), and catecholamines (Kanazawa & Sakakibara, 2000). Furthermore, carotenoids, such as β-carotene, α-carotene and different xanthophylls, have been identified in banana peel in the range of 300–400 μg lutein equivalents/100 g (Subagio, Morita, & Sawada, 1996), as well as sterols and triterpenes, such as β-sitosterol, stigmasterol, campesterol, cycloeucalenol, cycloartenol, and 24-methylene cycloartanol (Knapp & Nicholas, 1969). To date, only Someya et al. (2002) have evaluated the antioxidant activity in banana peel, measured as the effect on lipid autoxidation, in relation to its gallocatechin content.

The polarity of the solvent and that of the different antioxidant compounds affects the efficiency of the extraction and the activity of the obtained extracts. Water, methanol, ethanol, acetone, aqueous solutions of the aforementioned solvents and ethyl acetate are commonly used as extraction solvents (Lafka, Sinanoglou, & Lazos, 2007; Pinelo, Rubilar, Jerez, Sineiro, & Núñez, 2005; Shui & Leong, 2006). It must also be kept in mind that certain complications arise

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when recovering phytochemical compounds from plant by-products due to their high enzyme activity. However, drying the plant by-product before extraction, immediately immersing the by-product in methanol (Arts & Hollman, 1998) and using an acid extraction medium protects the material from oxidation. The pH of the water can determine the degree of solubility of water-soluble compounds and also influence the possible solubilisation of the hydrolysable fraction (Lafka et al., 2007). Other factors, such as extraction temperature and time (Lafka et al., 2007; Pinelo et al., 2005; Shui & Leong, 2006), the liquid-to-solid ratio and the cultivar (González-Paramás, Esteban-Ruano, Santos-Buelga, de Pascual-Teresa, & Rivas-Gonzalo, 2004), also affect extraction with solvents.

Comparative studies must be carried out for each plant by-product to identify the extraction conditions that produce maximum antioxidant activity. This work is a first attempt at identifying those conditions for banana peels. To this end, various factors that could affect extraction efficiency were tested: the use of solvents with different polarities (methanol, ethanol, acetone, water and mixtures (1:1, v:v) of the organic solvents and water), the pH of the water when it is used as an extractant (3.0–8.0), the extraction time (1 or 120 min), the extraction temperature (25 or 55 °C) and the banana cultivar ("Grande Naine" and "Gruesa"). In addition, the bioactive compounds (phenolic compounds, anthocyanins, catecholamines, ascorbic acid, tocopherols and phytosterols) that may be responsible for antioxidant activity were characterised. This information is useful to help characterise the extracts obtained from banana peel.

2. Materials and methods

2.1. Chemical and reagents

Dopamine, L-dopa (3,4-dihydroxy-L-phenylalanine), ascorbic acid, gallic acid, α-tocopherol, δ-tocopherol, γ-tocopherol, β-sitosterol, stigmasterol and campesterol were purchased from Sigma (Madrid, Spain). (±) 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) and β-tocopherol were supplied by Aldrich (Madrid, Spain) and Supelco (Madrid, Spain), respectively. Methanol, ethanol, acetone, *n*-hexane and chloroform were purchased from Scharlau Chemie (Barcelona, Spain) and 1-pentanol was obtained from Merck (Darmstadt, Germany), all HPLC grade. Deionised water of 18 MΩ cm resistivity, purified with a Milli-Q system (Millipore, Bedford, MA), was used. *trans*-β-Carotene and phosphatidyl-choline (refined egg lecithin) were obtained from Aldrich and from Alfa Aesar (Karlsruhe, Germany), respectively. Linoleic acid, Tween 40, 2,2-diphenyl-1-picrylhydrazyl (DPPH·), 2,2′-azino-bis(3-ethylbenzothiazoline)-6-sulfonic acid (ABTS), hydrogen peroxide (30%), iron(III) chloride, trichloroacetic acid (TCA), 2-thiobarbituric acid (TBA), butylated hydroxytoluene (BHT) and Folin–Ciocalteu reagent (2 N) were supplied by Sigma. The concentration of the enzyme horseradish peroxidase (HRP) Type VI [RZ ($A_{403\ nm}/A_{275\ nm}$) = 2.8], obtained from Sigma, was determined by measuring the absorbance at 403 nm using an extinction coefficient of $\varepsilon_{403\ nm} = 100\ mM^{-1}\ cm^{-1}$ (Arnao, Cano, & Acosta, 2001). N,O-bis(Trimethylsilyl)trifluoroacetamide (BSTFA), trimethylchlorosilane (TMCS) and anhydrous pyridine were purchased from Fluka (Madrid, Spain). All other reagents used were of analytical grade. Ethrel®-48 (48% ethephon) was provided by Etisa (Barcelona, Spain).

2.2. Plant material

Two cultivars of banana (*Musa acuminata* Colla AAA), "Grande Naine" and "Gruesa" (Canary Islands Dwarf Cavendish selection), were obtained from the research fields of Instituto Canario de Investigaciones Agrarias in Tenerife (Canary Islands, Spain). Both cultivars were grown under the same agricultural practices and crop conditions. Banana bunches (n = 21) were harvested at physiological maturity stage with a similar calibre (33 ± 1 mm, measured in the middle finger of the outer whorl of the second hand from the distal end of the bunch). The second hand from the proximal end of each bunch (approximately 60 kg of banana per cultivar) was selected, dipped in a 1 ml/l solution of Ethrel®-48 for 1 min to accelerate ripening and stored at 18 °C and 80–90% relative humidity until full-ripeness or stage 6 in the von Loesecke banana colour scale was reached.

The ripeness stage of the bananas was characterised in the middle finger of the outer whorl of each banana hand. When fully ripe, both cultivars possessed similar peel and pulp colour, firmness, total soluble solids content (TSS) and pH. Banana peel lightness, hue angle and chromaticity were 81 ± 7, 97 ± 2 and 65 ± 5, respectively. The colour of the banana pulp was characterised by a lightness of 84 ± 9, a hue angle of 96 ± 2 and a chromaticity of 42 ± 4. Fruit and pulp firmness were 20 ± 2 N and 0.87 ± 0.14 N/g respectively. TSS (24 ± 1 Brix) and pH (4.6 ± 0.1) were similar for both cultivars; however, acid content was higher for "Gruesa" cultivar (317 ± 43 mg malic acid/100 g) than for "Grande Naine" cultivar (241 ± 21 mg malic acid/100 g).

Once the bananas were ripe, peels were manually separated (peel:banana ratio, 38 ± 4%), cut into small pieces, frozen in liquid nitrogen and freeze-dried at 50 mPa and −40 °C (Christ alpha-1-4 LSC, Osterode, Germany) to decrease the enzyme activity during storage. The moisture content of peel was found to be 88 ± 2%. The dried banana peel was ground to a fine powder, placed in plastic containers and stored at −20 °C until the extractions were carried out.

2.3. Solvent extraction

The freeze-dried banana peel (0.15 g) was extracted with 3 ml of different solvents: methanol, ethanol, acetone, water acidified with hydrochloric acid (pH 3.0) or mixtures (1:1, v:v) of the organic solvents and water. In order to determine the effect of the pH of the aqueous medium on the antioxidant activity of the extracts, the pH of the water was adjusted to between 3.0 and 8.0 with hydrochloric acid or sodium hydroxide. The mixture was homogenised with a Politron PT-6000 (Kinematica AG, Lucerne, Switzerland) high speed blender at 12,000*g* for 1 min. The extractions were carried out in sealed tubes in a water bath for 1 or 120 min (extraction temperature of 25 or 55 °C). Precautions were taken in order to perform all the operations under reduced light and at 4 °C. Extracts were centrifuged at 5000*g* for 20 min in a Jouan CR-312 centrifuge (Thermo Electron Corporation, Madrid, Spain). The extracts obtained were stored at −80 °C for less than three days, at which time the antioxidant potential, extraction yield and bioactive compounds were estimated. Each extraction process was done at least in triplicate (3–9 times).

2.4. Extract antioxidant activity

All measurements to determine antioxidant activity were made on a Shimadzu UV–visible 160A double-beam spectrophotometer (Kyoto, Japan) equipped with a Hellma (Jamaica, NY) cell (pathlength 10^{-2} m). Trolox (a water-soluble analogue of α-tocopherol) and/or ascorbic acid were used as standards to calibrate the methods. The type and polarity of the solvent used to extract antioxidants from banana peel can affect single electron transfer and hydrogen atom transfer, which are key aspects in the measurements of antioxidant capacity (Becker, Nissen, & Skibsted, 2004). For this reason, the calibration of each method was carried out in

each of the evaluated solvents (methanol, ethanol, acetone water or mixtures (1:1, v:v) of the organic solvents and water).

2.4.1. Extract capacity to inhibit lipid peroxidation: β-carotene bleaching assay

The β-carotene bleaching method is based on the capacity of antioxidants to decrease oxidative losses of β-carotene in a β-carotene/linoleic acid system. Forty milligrams of β-carotene, 200 mg of linoleic acid and 2 g of Tween 40 were dissolved in 10 ml of chloroform. One millilitre of this solution was then distributed into individual tubes and the organic solvent was removed at 40 °C (for 24 h) in a Heto VR-1 evaporator (Allerod, Denmark). Oxygenated water (50 ml) generated by bubbling air into deionised water for 60 min was added to the tubes with vigorous stirring. Aliquots (1.5 ml) of the aqueous emulsion formed were then added to tubes containing 75 μl of the extracts, diluted by a factor of 5, and mixed thoroughly. A control sample with the different solvents used in the extractions instead of extract was analysed for antioxidant activity. To induce autoxidation, the tubes were placed in an incubation block at 50 °C, until the β-carotene was decolourised (210 min).

The antioxidant activity was expressed as mg of Trolox equivalents (TE)/100 g DW banana peel. Solutions in the four assayed solvents of Trolox in the 0.1–7.5 mg/l range were used for calibration (r^2 = 0.927–0.989). The repeatability standard deviation of the procedure was always <5%. Antioxidant activity coefficient (*AAC*) was also estimated as the relative oxidation in the presence and absence of extracts:

$$AAC = \left(\frac{A_t - A_t^0}{A_0^0 - A_t^0}\right) \times 1000$$

where A_t and ${A_t}^0$ are extract and control absorbance after incubation for 210 min and ${A_0}^0$ is control absorbance measured at zero time.

2.4.2. Extract capacity to scavenge radicals

With the objective of evaluating the capacity of the extracts to scavenge free radicals, two methods were used, based on the formation of stable free radicals like 2,2-diphenyl-1-picrylhydrazyl (DPPH·) and 2,2′-azino-bis(3-ethylbenzothiazoline)-6-sulfonic acid ($ABTS^{\cdot+}$).

One millilitre of a methanolic solution of DPPH· (with an absorbance of approximately 1.000) was added to 25 μl of the different extracts, diluted by a factor of 5. The decrease in absorbance was recorded at 515 nm after 15 min (when absorbance reached a steady state).

Scavenging activity was also determined with a slightly modified version of the method described by Arnao et al. (2001). This method is based on enzymatic generation with 0.25 μM of the enzyme horseradish peroxidase HRP (in 50 mM sodium phosphate buffer pH 7.5) of the cationic radical $ABTS^{\cdot+}$ (2 mM ABTS) in the presence of hydrogen peroxide (45 μM), in a total volume of 1 ml. The antioxidant extracts (50 μl) were diluted by a factor of 5 and then added to 1 ml of the $ABTS^{\cdot+}$ radical once it was formed (15 min until stable absorbance), thereby preventing interference from compounds that can affect radical formation. The assay temperature was controlled at 25 °C using a water bath and the antioxidant inhibition was measured at 730 nm after 6 min.

In both methods, a control with the addition of the different solvents assayed (instead of extracts) was used. Results were expressed as g of TE or AE (ascorbic acid equivalents)/100 g DW banana peel. Calibration graphs for each of the solvents evaluated (r^2 = 0.940–0.997) were constructed by plotting the absorbance against the antioxidant concentration at seven concentration levels analysed in triplicate (50–500 mg Trolox/l and 25–250 mg ascorbic acid/l). The repeatability standard deviation of the procedure was <5%. The antioxidant activity (*AA*, %) was also calculated as scavenging percentage:

$$AA = \frac{A_0^0 - A_t}{A_0^0} \times 100$$

where ${A_0}^0$ is control absorbance measured at zero time and A_t is extract absorbance at the time when antioxidant activity is evaluated (15 min for DPPH· method and 6 min for $ABTS^{\cdot+}$ method).

2.4.3. Extract capacity to inhibit lipid peroxidation: TBARS assay

The capacity of the extracts to inhibit lipid peroxidation was also evaluated by using the modified assay of thiobarbituric acid reactive substances (TBARS) (González-Paramás et al., 2004). The method is based on the peroxidation of a liposome system (25 μl of 50 mg/ml phosphatidyl-choline in 1.5:1 (v:v) chloroform:ethanol) induced by 200 μl of 1 mM iron chloride containing 300 mM potassium chloride in the presence of the extracts (50 μl). Peroxidation was started by adding ascorbate (125 μl at 0.16 mM) and incubating at 37 °C for 24 h. The reaction was stopped by adding 0.75 ml of a mixture 1.5:1 (v:v) of 9.4% TCA in 0.47 N hydrochloric acid (pH 1.5) with 1% TBA and 50 μl of BHT (760 mg/l in ethanol). The production of TBARS, mainly malonaldehyde, as a secondary product of peroxidation, was measured spectrophotometrically at 535 nm after incubation at 95 °C for 60 min.

A control without the extracts (with the different solvents used in the extractions) was used to evaluate the phosphatidyl-choline peroxidation as inhibition ratio (*IP*, %):

$$IP = \left(1 - \frac{A_t}{A_t^0}\right) \times 100$$

where A_t and ${A_t}^0$ are extract and control absorbance after incubation for 60 min. The repeatability standard deviation of the procedure was always <10%.

2.5. Extract yield determination

The different extracts obtained were evaporated to dryness in a Heto VR-1 vacuum evaporator at 40 °C. The extract yield was defined as the amount of dried extract (g) obtained from 100 g of banana peel DW (Llorach, Espín, Tomás-Barberán, & Ferreres, 2002).

2.6. Bioactive compound determination

2.6.1. Total extractable phenols

Phenolic compound content was estimated by mixing 200 μl of deionised water, 50 μl of the extracts diluted by a factor of 5 and 50 μl of Folin–Ciocalteu reagent. After 6 min, 500 μl of 7% sodium carbonate solution were added to the mixture, which was adjusted to 1.3 ml with deionised water and allowed to stand at room temperature for 60 min. Then, the absorbance was read at 765 nm. Gallic acid (ranging from 15 to 250 mg/l) was used to construct the calibration curves (r^2 = 0.904–0.998), in the different solvents evaluated. The results were expressed as g of gallic acid equivalents (GAE)/100 g banana peel DW.

2.6.2. Monomeric anthocyanins

The total monomeric anthocyanin content of the banana peels was measured using a spectrophotometric pH differential protocol (Lee, Durst, & Wrolstad, 2005). To increase the detection limit the banana peel extracts were obtained from 300 mg of dried banana peel extracted with 2 ml of the different solvents. The extracts were mixed thoroughly with 0.025 M potassium chloride pH 1.0 buffer in 1:36 ratio of extract to buffer and the absorbance of the mixture was measured at 510 and 700 nm after 15 min. The

extracts were combined similarly with 0.4 M sodium acetate buffer pH 4.5 and the absorbance of these solutions was measured at the same wavelengths. The anthocyanin concentration in the extracts was calculated as follows:

$$\text{monomeric anthocyanins (mg/l)} = \left((A_{515} - A_{700})_{\text{pH1.0}} - (A_{515} - A_{700})_{\text{pH4.5}}\right) \times MW \times \left(\frac{1000}{\varepsilon \times 1}\right)$$

where A is absorbance, MW is molecular weight for cyanidin 3-glucoside (449.2 g/mol), ε is the molar extinction coefficient of cyanidin 3-glucoside (26,900 l/mol cm^{-1}) and l is path-length (cm), and expressed as mg of cyanidin 3-glucoside equivalents/100 g banana peel DW.

2.6.3. Catecholamines

All liquid chromatographic analyses were carried out on a Shimadzu (Kyoto, Japan) modular chromatographic system, equipped with a LC-10AD pump, a RF-10 spectrofluorometric (catecholamines) or a SPD-10AV UV–visible (ascorbic acid and tocopherols) detector and controlled *via* Shimadzu LC-Solution software. The injection valve was a Rheodyne 7725i (Cotati, CA) with an injection loop of 20 μl.

Catecholamines (dopamine and L-dopa) were analysed using an isocratic liquid chromatographic (LC) method with fluorescence detection (Zydrón, Baranowski, Bialkowski, & Baranowska, 2005). The analytical column was a Spherisorb ODS-2 RP-C_{18} (Alltech, Deerfield, IL), 5 μm particle size, 250 × 4.6 mm i.d. A 0.1 M acetate buffer (pH 4.66) and methanol (97:3%, v:v) was used as mobile phase at a flow rate of 0.8 ml/min. The excitation and emission detection wavelengths were set at 285 and 315 nm, respectively.

The catecholamines were identified by comparing their fluorescence spectral characteristics and retention time with those of commercial standards. The spectra (from 250 to 350 nm) were recorded for the peaks that could be identified as dopamine or L-dopa by retention time.

2.6.4. Ascorbic acid

Ascorbic acid in the extracts was determined (Gökmen, Kahraman, Demir, & Acar, 2000) using LC with UV–visible detection on a Spherisorb ODS-2 RP-C_{18} (Alltech) column (5 μm particle size, 250 × 4.6 mm i.d.). A 0.2 M potassium phosphate monobasic solution in Milli-Q water was used as mobile phase (pH adjusted to 2.4 with orthophosphoric acid) at a flow rate of 0.5 ml/min. Detection wavelength for the detector was set at 254 nm.

2.6.5. Tocopherols

Tocopherol separation (α-, β-, δ- and γ-tocopherols) was done by normal-phase LC using a 5 μm particle size Kromasil 60-Silica (Scharlab, Barcelona, Spain) column (250 × 4.6 mm i.d.) with an isocratic *n*-hexane:1-pentanol (98.5:1.5, v:v) mobile phase at a flow rate of 1.5 ml/min (Pyka & Sliwiok, 2001). Analytes were detected at 290 nm. In the case of extracts in methanol and water (solvents incompatible with the mobile phase), the solvent was evaporated to dryness in a Heto VR-1 vacuum evaporator at 25 °C and the residue reconstituted in ethanol.

The ascorbic acid and tocopherols were identified by comparing their UV–visible spectral characteristics and retention times with those of commercial standards. The spectra (from 190 to 400 nm) were recorded for the peaks that could be identified as ascorbic acid or a particular tocopherol by retention time, using a Shimadzu SPD-M6A UV–visible diode array detector.

2.6.6. Phytosterols

Phytosterols (β-sitosterol, stigmasterol y campesterol) were analysed using gas chromatography–mass spectrometry as their trimethylsilyl ether derivatives (Abidi, 2001). An aliquot (500 μl) of the extracts in the different solvents evaluated was evaporated to dryness in a Heto VR-1 vacuum evaporator at 25 °C. Then, 100 μl of anhydrous pyridine and 100 μl of the derivatisation reagent containing 99% BSTFA and 1% TMCS were added. The sample was incubated at 60 °C for 30 min and allowed to stand overnight at room temperature.

Analyses were carried out on a Shimadzu GC-17A gas chromatograph equipped with a TRB-5 fused-silica capillary column, 30 m × 0.25 mm i.d. (coated with a 0.25 μm thick film of 5% diphenyl–95% dimethylpolysiloxane) from Supelco. The chromatographic conditions were: an initial temperature 250 °C (1 min), followed by a gradient of 0.5 °C/min to 270 °C, which was held for 1 min. The injector temperature was 300 °C. Helium (6.0 grade; Air Liquide, Tenerife, Spain) was used as the carrier gas, at a flow rate of 1 ml/min.

The identities of eluted GC peaks were confirmed by comparing their mass spectra (from m/z 50 to 500 in the electron impact mode) and retention times with those of the sterol standards. To achieve this, the chromatograph was coupled to a Shimadzu QP-5000 mass spectrometer. The transfer line and source temperature were 250 and 220 °C, respectively; the ionisation energy was 70 eV. Samples (1 μl) were injected in the split mode (1:10 ratio).

2.7. Statistical analysis

Data analysis was carried out with Statgraphics-Plus software 5.1 (Statistical Graphics, Rockville, MD). Grubbs' test was applied to detect outliers in the data set and analysis of variance was used to evaluate the effect of the solvent, pH of the water used as extractant, extraction temperature, extraction time and banana cultivar on antioxidant activity, extraction yield and bioactive compound content in banana peel extracts. Fisher's least significant difference test, at the 5% significance level, was applied to experimental results to assess intra-pair differences.

3. Results and discussion

3.1. Extract antioxidant activity

Four different methods were used to determine the antioxidant properties of the banana peel extracts, to obtain information about the activity of those extracts in the different stages of the oxidation reaction. The methods were inhibition of β-carotene co-oxidation in a linoleate model system, scavenging of DPPH· and ABTS·$^+$ free radicals and inhibition of peroxidation of phosphatidyl-choline which is induced by the ascorbate/iron complex.

All the extracts assayed prevented the bleaching of β-carotene in carotene/linoleic acid mixtures (Table 1). However, banana peel extracts prepared by different solvents exhibited varying degrees of antioxidant capacity. When extraction was done for 1 min, the extracts obtained with acetone:water were the most effective in inhibiting the oxidation of linoleic acid and subsequent bleaching of β-carotene, in comparison with the control. The extracts obtained with methanol:water, ethanol:water, methanol, ethanol or water had lower antioxidant activity in the β-carotene/linoleic acid system, 40–75% of the inhibition of extracts obtained with acetone:water. The extracts obtained with acetone were the least effective in minimising the oxidation of lipids (around 20% of the efficiency of extracts obtained with acetone:water). The antioxidant activity coefficient remained constant in extracts where water pH ranged from 4.0 to 7.0 (269 ± 24), being slightly higher than when extraction was done with water at pH 3.0 (220 ± 10). However, antioxidant capacity decreased drastically when water pH was adjusted to 8.0. Only the antioxidant activity of extracts in

Table 1
Antioxidant activity of banana peel extracts in a β-carotene/linoleate model system.

Extraction solvent	"Grande Naine"				"Gruesa"			
	25 °C, 1 min	25 °C, 120 min	55 °C, 1 min	55 °C, 120 min	25 °C, 1 min	25 °C, 120 min	55 °C, 1 min	55 °C, 120 min
Antioxidant capacity (mg Trolox equivalents/100 g DW)								
Methanol	61 ± 2^{dB}	40 ± 1^{cC}	60 ± 1^{cB}	104 ± 1^{bA}	91 ± 18^{bA}	60 ± 4^{dB}	109 ± 17^{bA}	111 ± 6^{bA}
Ethanol	49 ± 4^{eB}	54 ± 1^{cB}	50 ± 3^{cdB}	75 ± 5^{dA}	87 ± 5^{bA}	54 ± 1^{deD}	63 ± 4^{dC}	80 ± 3^{dB}
Acetone	21 ± 1^{fC}	41 ± 8^{cB}	21 ± 1^{eC}	75 ± 4^{dA}	25 ± 3^{cC}	48 ± 3^{eB}	25 ± 3^{eC}	91 ± 16^{cdA}
Water	53 ± 2^{e}	48 ± 1^{c}	54 ± 1^{d}	52 ± 8^{e}	87 ± 18^{bA}	46 ± 1^{eB}	88 ± 3^{cA}	48 ± 1^{eB}
Methanol:water (1:1)	87 ± 4^{b}	85 ± 9^{b}	91 ± 8^{b}	94 ± 5^{c}	88 ± 1^{bB}	87 ± 5^{bB}	92 ± 2^{cB}	98 ± 2^{bcA}
Ethanol:water (1:1)	79 ± 3^{c}	79 ± 9^{b}	86 ± 1^{b}	90 ± 5^{c}	82 ± 7^{bB}	77 ± 3^{cB}	95 ± 2^{cA}	90 ± 3^{cdA}
Acetone:water (1:1)	116 ± 1^{a}	128 ± 15^{a}	133 ± 3^{a}	116 ± 4^{a}	132 ± 5^{aB}	108 ± 5^{aC}	128 ± 2^{aB}	145 ± 5^{aA}
Antioxidant activity coefficient, AAC								
Methanol	287 ± 8^{cB}	188 ± 7^{cC}	283 ± 8^{cB}	490 ± 4^{bA}	428 ± 84^{bA}	242 ± 18^{dB}	518 ± 79^{bA}	522 ± 29^{bA}
Ethanol	230 ± 19^{dBC}	216 ± 3^{cC}	254 ± 13^{cB}	350 ± 24^{dA}	408 ± 21^{bA}	216 ± 3^{deD}	295 ± 17^{eC}	377 ± 12^{deB}
Acetone	85 ± 5^{eC}	166 ± 31^{cB}	85 ± 5^{eC}	299 ± 18^{eA}	99 ± 13^{cC}	192 ± 14^{eB}	99 ± 13^{fC}	368 ± 66^{eA}
Water	216 ± 10^{d}	197 ± 3^{c}	220 ± 3^{d}	214 ± 31^{f}	358 ± 73^{bA}	188 ± 6^{eB}	345 ± 11^{dA}	203 ± 9^{fB}
Methanol:water (1:1)	412 ± 24^{b}	404 ± 44^{b}	434 ± 39^{b}	461 ± 19^{bc}	416 ± 6^{bB}	413 ± 23^{bB}	432 ± 11^{cB}	465 ± 8^{bcA}
Ethanol:water (1:1)	389 ± 5^{b}	381 ± 46^{b}	410 ± 2^{b}	432 ± 28^{c}	385 ± 26^{bC}	366 ± 9^{cC}	456 ± 3^{cA}	428 ± 5^{cdB}
Acetone:water (1:1)	560 ± 25^{a}	607 ± 79^{a}	628 ± 17^{a}	574 ± 17^{a}	627 ± 29^{aB}	517 ± 14^{aC}	606 ± 4^{aB}	681 ± 27^{aA}

Values are the mean ± standard deviation of at least three determinations (n = 3–9).
Within a column (a–f) or a row (A–D), different letters denote significant differences ($p < 0.050$) between extraction solvent or extraction conditions (temperature and time), respectively.

acetone showed a clear tendency to increase when extraction time and extraction temperature increased. So, the inhibition of the linoleic acid oxidation was 2–3.5 times higher when extraction was done for 120 min than for 1 min. Similarly, increasing the extraction temperature from 25 to 55 °C (for 120 min) nearly doubled antioxidant activity. However, increasing extraction temperature or time when using other solvents did not have a clear or significant effect on the antioxidant activity in the β-carotene/linoleic acid system. The antioxidant activity described for banana peel extracts in this work was similar to that reported by Lapornik, Prosek, and Wondra (2005) for red currant and grape marc and slightly lower than for blackcurrant by-products. While extraction time did not affect the antioxidant activity of currant by-product extracts, in the case of grape marc extracts, the inhibition of linoleic acid oxidation increased with the time of extraction.

Banana peel extracts showed strong scavenging activity against both DPPH· (Table 2) and $ABTS^{\cdot+}$ (Table 3) radicals. Extracts obtained with acetone:water had the highest antioxidant activity compared with the other solvents assayed by a factor ranging between 1.3–1.9 (methanol) and 25–35 (acetone) for the DPPH· assay and a factor ranging between 2–4 (methanol) and around 10–35 (acetone and water in "Grande Naine", and ethanol, acetone and water in "Gruesa") for the $ABTS^{\cdot+}$ assay. The radical-scavenging activity of the extracts obtained with acetone:water was superior to 1.8 g TE or AE/100 g of freeze-dried residue powder. Although ethanolic extracts showed higher DPPH· inhibition percentages than extracts obtained with acetone (4.5–5.5-fold greater), the activities against $ABTS^{\cdot+}$ radicals of the extracts obtained with these solvents were very similar. The capacity to scavenge $ABTS^{\cdot+}$ radicals was higher in "Gruesa" aqueous extracts than those obtained with ethanol and acetone. However, these aqueous extracts showed no activity against DPPH· radicals. This activity was similar to that obtained by Llorach et al. (2002) for artichoke by-products (0.15–0.95 g TE/100 g by-product), by Xu et al. (2008) for citrus fruits peel (0.6–0.9 g TE/100 g DW by-product) or by Shui and Leong (2006) for star fruit residue (3.4 g AE/100 g DW or 5.2 g TE/100 g DW). Water pH (ranging between 3.0 and 8.0) did not affect scavenging activity. Prolonging extraction times (from 1 to 120 min), at 25 °C, for extracts obtained using methanol, ethanol, acetone, methanol:water, ethanol:water or acetone:water did not lead to higher DPPH· scavenging. At 55 °C, the increase in extraction time doubled the antioxidant activity of the extracts obtained with acetone. An extraction time of 120 min did not change the antioxidant activity against $ABTS^{\cdot+}$ radicals, except for the "Gruesa" cultivar, for which an increase in extraction time led to the highest activity for the extracts obtained using ethanol or the lowest activity for the extracts obtained using acetone. The antioxidant activity of aqueous extracts decreased significantly (from 20% to 90%) when the extraction time was increased from 1 to 120 min, probably due to polyphenol oxidase, which was not inactivated in the aqueous extracts (the brown hue observed in those extracts increased with extraction time). Increasing the extraction temperature did not change the antioxidant activity of extracts obtained with methanol or ethanol:water. However, as extraction temperature increased (when the extraction was done at 120 min), the capacity of extracts from both cultivars obtained using acetone and that of the "Gruesa" cultivar in ethanol, acetone, methanol:water or acetone:water to scavenge DPPH· radicals increased. Also the scavenging of $ABTS^{\cdot+}$ radicals increased significantly when the extraction temperature was increased from 25 to 55 °C (120 min) in extracts of methanol, ethanol or acetone and in extracts of ethanol:water and acetone:water of the "Gruesa" cultivar. The antioxidant activity of aqueous extracts was also affected by extraction temperature. So, extraction at 55 °C (120 min) reduced the antioxidant activity of extracts by more than 50% (relative to extraction at 25 °C for 120 min). In aqueous extracts, at this extraction temperature (55 °C), polyphenol oxidase was not inactivated (thermal inactivation occurs at temperatures higher than 70 °C); this was evident from the brown hue observed in those extracts. The fact that this enzyme shows greatest activity at around 40 °C could explain the decrease in antioxidant activity that occurred when extraction temperature increased from 25 to 55 °C.

The highest antioxidant activity obtained using the TBARS method (Fig. 1) was found for aqueous extracts, followed by extracts in acetone:water. On the other hand, the lowest inhibition was observed for extracts obtained with the less polar solvents, ethanol and acetone and the mixtures of those solvents with water. The percentage of inhibition shown in this work for aqueous extracts is similar to values reported in the literature (Murthy, Singh, & Jayaprakasha, 2002; Singh, Murthy, & Jayaprakasha, 2002), although comparison is highly difficult because of the different extraction conditions used. The inhibition of lipid peroxidation tended to decrease when water pH was higher than 6.0. All the extracts obtained at 25 or 55 °C for 1 min were capable of preventing TBARS formation. However, when extraction time was increased, the antioxidant activity of methanolic and ethanolic extracts

Table 2
Comparison of the capacity of banana peel extracts to scavenge DPPH· radicals.

Extraction solvent	"Grande Naine"				"Gruesa"			
	25 °C, 1 min	25 °C, 120 min	55 °C, 1 min	55 °C, 120 min	25 °C, 1 min	25 °C, 120 min	55 °C, 1 min	55 °C, 120 min
Antioxidant capacity (g Trolox equivalents/100 g DW)								
Methanol	1.9 ± 0.4^{b}	2.4 ± 0.4^{b}	1.8 ± 0.1^{b}	2.0 ± 0.1^{b}	1.8 ± 0.5^{b}	2.2 ± 0.3^{a}	1.6 ± 0.1^{b}	2.0 ± 0.1^{b}
Ethanol	0.56 ± 0.02^{d}	0.45 ± 0.11^{de}	0.58 ± 0.07^{de}	0.59 ± 0.12^{d}	0.59 ± 0.01^{cB}	0.50 ± 0.09^{bB}	0.52 ± 0.02^{cdB}	0.80 ± 0.05^{cA}
Acetone	0.13 ± 0.01^{eB}	0.16 ± 0.03^{eB}	0.12 ± 0.01^{fB}	0.25 ± 0.02^{eA}	0.11 ± 0.01^{dB}	0.14 ± 0.02^{cB}	0.12 ± 0.01^{dB}	0.25 ± 0.02^{dA}
Water	0.22 ± 0.02^{deA}	0.040 ± 0.004^{eB}	0.23 ± 0.01^{efA}	0.018 ± 0.004^{eB}	–	–	–	–
Methanol:water (1:1)	0.95 ± 0.10^{c}	0.85 ± 0.11^{cd}	0.88 ± 0.05^{cd}	0.88 ± 0.06^{d}	0.80 ± 0.01^{cA}	0.52 ± 0.04^{bB}	0.88 ± 0.13^{cA}	0.94 ± 0.20^{cA}
Ethanol:water (1:1)	1.2 ± 0.2^{c}	1.1 ± 0.1^{c}	1.2 ± 0.1^{c}	1.3 ± 0.1^{c}	0.78 ± 0.03^{c}	0.70 ± 0.08^{b}	0.82 ± 0.13^{c}	0.85 ± 0.18^{c}
Acetone:water (1:1)	2.9 ± 0.4^{a}	3.1 ± 0.5^{a}	3.0 ± 0.6^{a}	3.1 ± 0.4^{a}	3.3 ± 0.3^{aA}	2.1 ± 0.2^{aB}	2.8 ± 0.6^{aAB}	3.2 ± 0.4^{aA}
Antioxidant capacity (g ascorbic acid equivalents/100 g DW)								
Methanol	1.6 ± 0.3^{b}	2.0 ± 0.4^{b}	1.5 ± 0.1^{b}	1.6 ± 0.1^{b}	1.5 ± 0.4^{b}	1.9 ± 0.2^{a}	1.3 ± 0.1^{b}	1.7 ± 0.1^{b}
Ethanol	0.43 ± 0.02^{d}	0.35 ± 0.09^{de}	0.45 ± 0.05^{de}	0.45 ± 0.09^{e}	0.45 ± 0.01^{cB}	0.38 ± 0.07^{bB}	0.41 ± 0.02^{cdB}	0.62 ± 0.04^{cA}
Acetone	0.093 ± 0.004^{eB}	0.11 ± 0.02^{eB}	0.092 ± 0.004^{fB}	0.18 ± 0.02^{fA}	0.080 ± 0.009^{dB}	0.10 ± 0.01^{cB}	0.084 ± 0.004^{dB}	0.18 ± 0.02^{dA}
Water	0.16 ± 0.02^{deA}	0.030 ± 0.003^{eB}	0.17 ± 0.01^{efA}	0.013 ± 0.003^{fB}	–	–	–	–
Methanol:water (1:1)	0.80 ± 0.08^{c}	0.72 ± 0.09^{cd}	0.75 ± 0.05^{cd}	0.74 ± 0.05^{d}	0.68 ± 0.01^{cA}	0.44 ± 0.03^{bB}	0.75 ± 0.11^{cA}	0.79 ± 0.17^{cA}
Ethanol:water (1:1)	1.0 ± 0.2^{c}	0.91 ± 0.07^{c}	1.0 ± 0.1^{c}	1.1 ± 0.1^{c}	0.66 ± 0.03^{c}	0.59 ± 0.07^{b}	0.69 ± 0.11^{c}	0.72 ± 0.15^{c}
Acetone:water (1:1)	2.4 ± 0.3^{a}	2.6 ± 0.4^{a}	2.6 ± 0.5^{a}	2.6 ± 0.4^{a}	2.8 ± 0.2^{aA}	1.8 ± 0.1^{aB}	2.3 ± 0.5^{aAB}	2.7 ± 0.3^{aA}
Scavenging percentage (%)								
Methanol	36 ± 7^{b}	44 ± 8^{b}	33 ± 2^{b}	36 ± 1^{b}	33 ± 8^{b}	42 ± 5^{a}	29 ± 3^{b}	37 ± 1^{b}
Ethanol	10 ± 1^{de}	8.0 ± 2.0^{de}	10 ± 1^{de}	10 ± 2^{e}	10 ± 1^{cB}	8.8 ± 1.6^{bB}	9.3 ± 0.4^{cdB}	14 ± 1^{cA}
Acetone	2.5 ± 0.1^{eC}	3.0 ± 0.5^{eB}	2.5 ± 0.1^{fC}	4.8 ± 0.4^{efA}	2.1 ± 0.2^{dB}	2.7 ± 0.4^{cB}	2.3 ± 0.1^{dB}	4.8 ± 0.4^{dA}
Water	2.8 ± 0.3^{eA}	0.52 ± 0.06^{eB}	3.0 ± 0.1^{efA}	0.23 ± 0.01^{fB}	–	–	–	-
Methanol:water (1:1)	18 ± 2^{cd}	16 ± 2^{cd}	17 ± 1^{cd}	17 ± 1^{d}	15 ± 1^{cA}	10 ± 1^{bB}	16 ± 2^{cA}	18 ± 4^{cA}
Ethanol:water (1:1)	24 ± 3^{c}	20 ± 2^{c}	23 ± 2^{c}	24 ± 2^{c}	14 ± 1^{c}	13 ± 1^{b}	15 ± 3^{c}	16 ± 3^{c}
Acetone:water (1:1)	55 ± 9^{a}	58 ± 9^{a}	56 ± 11^{a}	60 ± 9^{a}	61 ± 5^{aA}	39 ± 4^{aB}	51 ± 10^{aAB}	60 ± 7^{aA}

Values are the mean ± standard deviation of at least three determinations (n = 3–9).
Within a column (a–f) or a row (A–C), different letters denote significant differences ($p < 0.050$) between extraction solvent or extraction conditions (temperature and time), respectively.

Table 3
Comparison of the capacity of banana peel extracts to scavenge $ABTS^{\cdot+}$ radicals.

Extraction solvent	"Grande Naine"				"Gruesa"			
	25 °C, 1 min	25 °C, 120 min	55 °C, 1 min	55 °C, 120 min	25 °C, 1 min	25 °C, 120 min	55 °C, 1 min	55 °C, 120 min
Antioxidant capacity (g Trolox equivalents/100 g DW)								
Methanol	2.4 ± 0.6^{cB}	1.6 ± 0.1^{cC}	2.9 ± 0.3^{cAB}	3.1 ± 0.1^{dA}	2.7 ± 0.4^{bA}	1.7 ± 0.2^{cB}	2.4 ± 0.2^{cA}	1.9 ± 0.1^{dB}
Ethanol	0.40 ± 0.01^{dC}	0.35 ± 0.01^{dD}	0.46 ± 0.01^{dB}	0.75 ± 0.05^{eA}	0.31 ± 0.04^{dC}	0.49 ± 0.06^{dB}	0.21 ± 0.03^{eD}	0.59 ± 0.05^{eA}
Acetone	0.34 ± 0.01^{dB}	0.28 ± 0.01^{dB}	0.33 ± 0.01^{dB}	0.70 ± 0.06^{eA}	0.26 ± 0.01^{dA}	0.12 ± 0.01^{eC}	0.26 ± 0.03^{eA}	0.18 ± 0.01^{eB}
Water	0.31 ± 0.01^{dA}	0.16 ± 0.02^{dB}	0.11 ± 0.03^{dB}	0.025 ± 0.011^{fC}	0.72 ± 0.22^{cA}	0.086 ± 0.006^{fB}	0.68 ± 0.15^{dA}	–
Methanol:water (1:1)	3.6 ± 0.1^{b}	3.6 ± 0.1^{b}	3.7 ± 0.1^{b}	3.9 ± 0.1^{b}	2.7 ± 0.3^{b}	2.3 ± 0.1^{b}	2.8 ± 0.5^{c}	2.8 ± 0.1^{c}
Ethanol:water (1:1)	3.2 ± 0.2^{b}	3.2 ± 0.2^{b}	3.2 ± 0.1^{bc}	3.2 ± 0.1^{c}	2.7 ± 0.3^{bB}	2.4 ± 0.2^{bB}	3.7 ± 0.4^{bA}	4.4 ± 0.2^{bA}
Acetone:water (1:1)	7.3 ± 0.6^{a}	8.4 ± 1.3^{a}	7.1 ± 0.6^{a}	7.4 ± 0.1^{a}	9.6 ± 0.2^{aA}	6.5 ± 0.3^{aB}	8.5 ± 0.3^{aA}	9.3 ± 0.7^{aA}
Antioxidant capacity (g ascorbic acid equivalent/100 g DW)								
Methanol	1.8 ± 0.4^{cB}	1.2 ± 0.1^{cC}	2.1 ± 0.3^{cAB}	2.3 ± 0.1^{cA}	2.0 ± 0.3^{bA}	1.3 ± 0.1^{cB}	1.7 ± 0.2^{cA}	1.7 ± 0.1^{dA}
Ethanol	0.37 ± 0.01^{dC}	0.33 ± 0.01^{dD}	0.42 ± 0.01^{dB}	0.68 ± 0.04^{dA}	0.29 ± 0.04^{dC}	0.45 ± 0.05^{dB}	0.20 ± 0.02^{eD}	0.54 ± 0.04^{eA}
Acetone	0.32 ± 0.01^{dB}	0.26 ± 0.01^{dB}	0.31 ± 0.01^{dB}	0.67 ± 0.05^{dA}	0.24 ± 0.01^{dA}	0.11 ± 0.01^{eC}	0.24 ± 0.02^{eA}	0.18 ± 0.01^{fB}
Water	0.34 ± 0.01^{dA}	0.22 ± 0.04^{dB}	0.18 ± 0.02^{dB}	0.11 ± 0.02^{eC}	0.67 ± 0.18^{cA}	0.16 ± 0.05^{eB}	0.71 ± 0.12^{dA}	0.064 ± 0.004^{gC}
Methanol:water (1:1)	2.6 ± 0.1^{b}	2.7 ± 0.1^{b}	2.7 ± 0.1^{b}	2.8 ± 0.1^{b}	2.0 ± 0.2^{b}	1.7 ± 0.1^{b}	2.1 ± 0.3^{c}	2.1 ± 0.1^{c}
Ethanol:water (1:1)	2.3 ± 0.1^{b}	2.4 ± 0.1^{b}	2.4 ± 0.1^{bc}	2.4 ± 0.1^{c}	2.0 ± 0.2^{bB}	1.8 ± 0.1^{bB}	2.8 ± 0.3^{bA}	3.3 ± 0.1^{bA}
Acetone:water (1:1)	5.3 ± 0.4^{a}	6.3 ± 0.9^{a}	5.2 ± 0.4^{a}	5.4 ± 0.1^{a}	7.1 ± 0.2^{aA}	4.7 ± 0.2^{aB}	6.4 ± 0.2^{aA}	6.9 ± 0.4^{aA}
Scavenging percentage (%)								
Methanol	44 ± 10^{cB}	28 ± 1^{dC}	50 ± 6^{cAB}	56 ± 2^{cA}	48 ± 8^{bA}	29 ± 4^{cB}	42 ± 4^{bA}	45 ± 1^{cA}
Ethanol	11 ± 1^{dC}	7.2 ± 0.1^{eD}	16 ± 1^{dB}	19 ± 1^{dA}	9.7 ± 0.7^{cB}	11 ± 2^{dB}	8.6 ± 0.7^{cB}	16 ± 1^{dA}
Acetone	2.7 ± 0.6^{dB}	2.7 ± 0.2^{eB}	2.7 ± 0.2^{eB}	13 ± 2^{eA}	8.0 ± 0.1^{cA}	2.3 ± 0.1^{eC}	8.0 ± 0.4^{cA}	6.0 ± 0.1^{eB}
Water	8.8 ± 0.1^{dA}	5.8 ± 0.9^{eB}	8.4 ± 0.5^{eA}	3.2 ± 0.4^{fC}	8.3 ± 0.5^{cA}	6.8 ± 1.2^{deAB}	7.9 ± 0.3^{cA}	4.1 ± 1.0^{eB}
Methanol:water (1:1)	61 ± 1^{bB}	62 ± 1^{bB}	63 ± 2^{bB}	69 ± 4^{bA}	48 ± 5^{b}	40 ± 2^{b}	49 ± 7^{b}	50 ± 2^{b}
Ethanol:water (1:1)	58 ± 1^{b}	56 ± 4^{c}	56 ± 1^{bc}	58 ± 1^{c}	47 ± 4^{b}	44 ± 2^{b}	42 ± 4^{b}	50 ± 3^{b}
Acetone:water (1:1)	84 ± 5^{a}	89 ± 7^{a}	82 ± 7^{a}	86 ± 1^{a}	96 ± 1^{aA}	75 ± 4^{aB}	96 ± 1^{aA}	98 ± 1^{aA}

Values are the mean ± standard deviation of at least three determinations (n = 3–9).
Within a column (a–g) or a row (A–D), different letters denote significant differences ($p < 0.050$) between extraction solvent or extraction conditions (temperature and time), respectively.

decreased. This behaviour was more remarkable when ethanol was used as the extractant (70–100% decrease) as opposed to methanol (40–100%). Moreover, increasing the extraction temperature (from 25 to 55 °C for 120 min) induced a loss of antioxidant capacity of 100% for both solvents. High temperatures have been reported to improve the efficiency of extraction, due to the enhanced diffusion rate and solubility of analytes in solvents. However, at high temperatures bioactive compounds with antioxidant activity can react with other components of the plant material, thus impeding extraction. Moreover, it has been widely reported that extraction temperature affects the stability of phenolic compounds, due to chemical and enzymatic degradation, losses caused by volatilisation or thermal

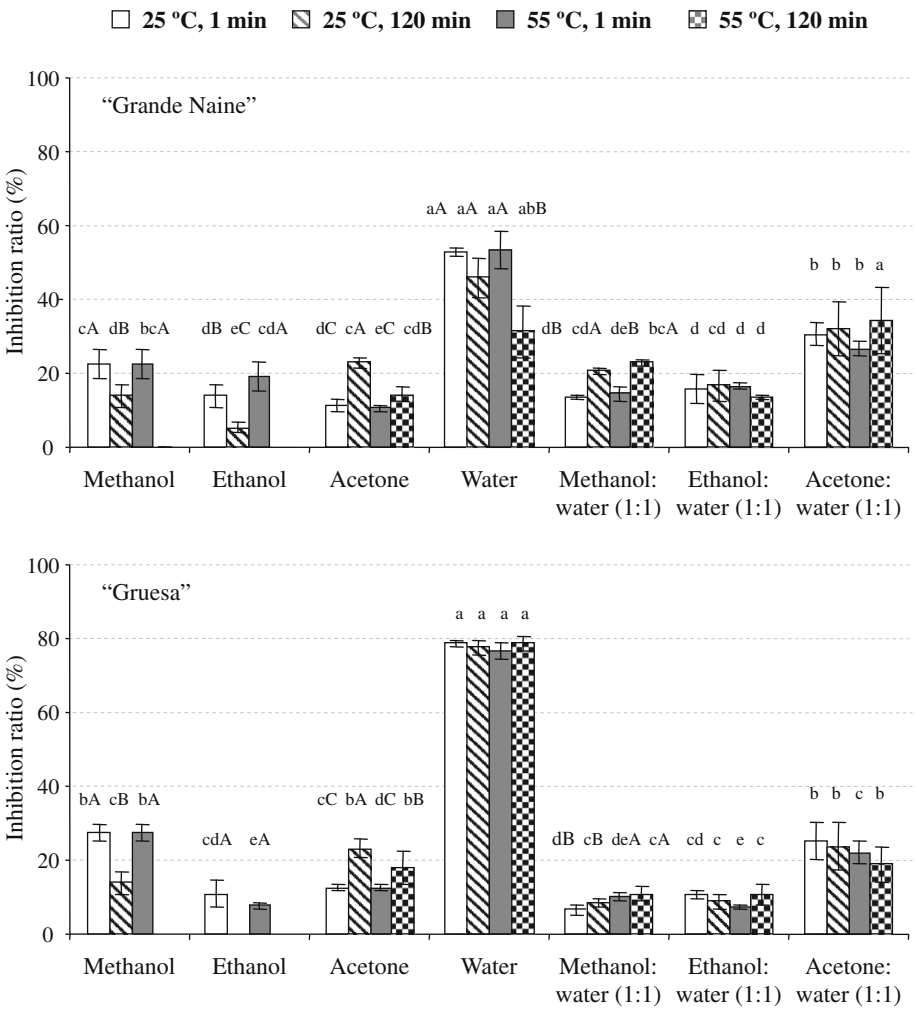

Fig. 1. Comparison of the capacity of banana peel extracts to inhibit the production of thiobarbituric acid reactive substances (TBARS). Each extraction process was done at least in triplicate (n = 3–9). Different lower or upper case letters denote significant differences ($p < 0.050$) between extraction solvent or extraction conditions (temperature and time), respectively.

decomposition. The temperature increase also diminished the antioxidant activity of extracts in acetone (20–40%) at 120 min. The antioxidant activity of aqueous extracts did not change when using different extraction times (25 °C, 1 or 120 min), while the activity of extracts in acetone tended to increase with longer time (inhibition ratio 2 times higher).

In general, banana peel extracts worked better as antiradical agents than as lipid peroxidation inhibitors, although high antioxidant activity was obtained using all the assays. The extracts prepared with different solvents and different extraction conditions exhibited varying degrees of antioxidant activity. This fact indicates that antioxidants or active compounds of different polarity were present in the extracts.

3.2. Extract yield and bioactive compound determination

Banana peel contains considerable amounts of extractable compounds (Fig. 2). Variation in the yields of various extracts is attributed to the polarities of different compounds present in the fruit by-products. The mixtures (1:1) of water with methanol, ethanol or acetone had a clear ability to extract substances from this by-product. When the extraction was done at 25 or 55 °C for 1 min, the extract yield ranged from 54 ± 4% (methanol:water and acetone:water extraction for "Grande Naine" cultivar and also for ethanol:water extraction for "Gruesa" cultivar) to 3.1 ± 0.6% (acetone extraction). The extraction efficiency of methanol increased with extraction time (22–86%) and with temperature (33%, for the "Grande Naine" cultivar only) when the extraction was done at 120 min. For ethanolic extracts in the "Gruesa" cultivar, the extract yield increased significantly (1.7 times) when the extraction time was increased from 1 to 120 min (at 25 °C). No significant effects of extraction temperature or time were found for the other solvents. Similar results were obtained by Murthy et al. (2002) in grape pomace, for which methanolic extracts contained 5.6 ± 0.5% of total solids. The contents reported by Singh et al. (2002) are similar to those obtained in this work in extractables from pomegranate peel or seeds (9.4% or 8.6%, respectively). Nevertheless, Pinelo et al. (2005) found a maximum total solid of 44% in ethanolic extracts from grape pomace, whereas values near 30% were found for methanol and water.

Acetone:water (1:1) was the most effective solvent in extraction of total phenolic compounds from banana peels (Table 4). When the extraction was done at 25 °C for 1 min the polyphenol content in acetone:water was 1.5–3.5 times higher than those in methanol, methanol:water or ethanol:water. The content of phenolic compounds in methanolic extracts was similar to what other authors found in banana peel. Someya et al. (2002) determined a phenolic compound content of 0.91 g/100 g DW. Moreover, Nguyen et al. (2003) found phenolic compound contents of 0.9 or 3.0 g/100 g DW in the "Kluai Khai" or "Kluai Hom Thong"

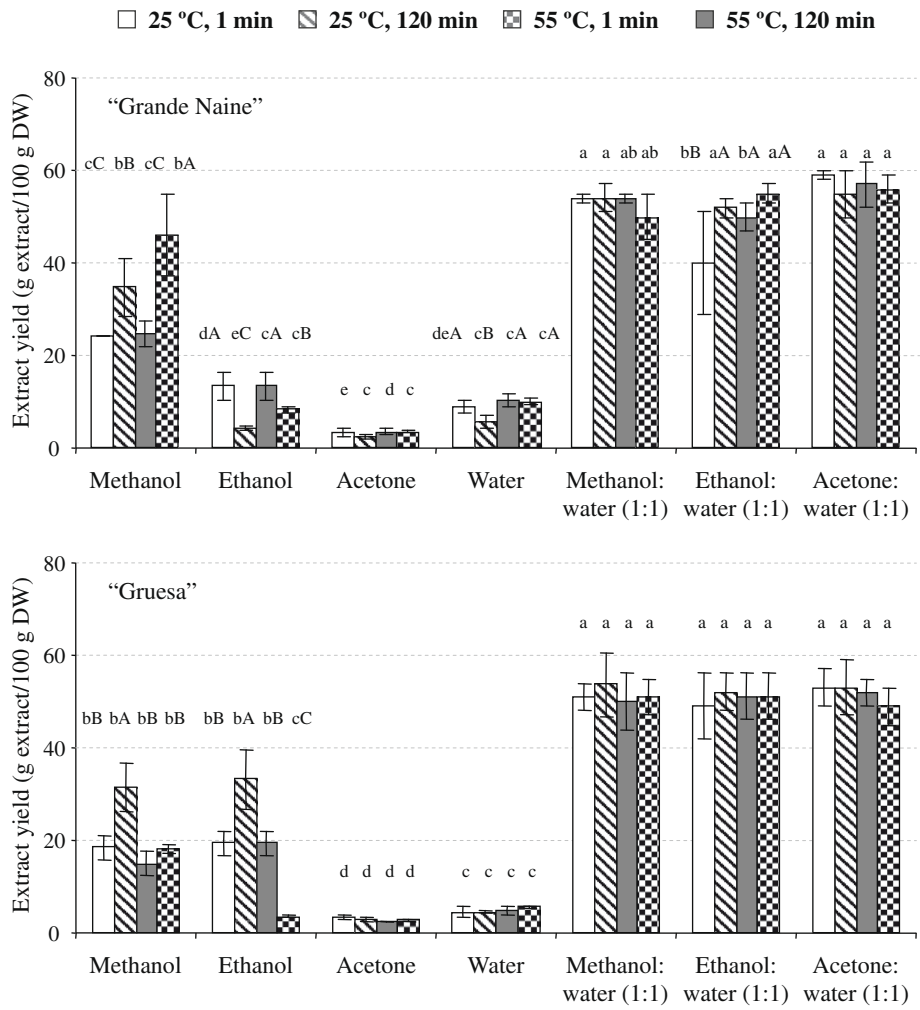

Fig. 2. Extract yield of banana peel extracts. Each extraction process was done at least in triplicate ($n = 3–9$). Different lower or upper case letters denote significant differences ($p < 0.050$) between extraction solvent or extraction conditions (temperature and time), respectively.

cultivars, respectively. Notwithstanding the fact that the extract yield was constant, an increase (around 50%) in the recovery of phenolic compounds was observed when the water pH was higher than 4.0. The extraction of phenolic compounds with methanol increased with extraction time (1.5 times) while extraction temperature did not improve the extraction. It has also been reported that raising the extraction temperature from 40 to 100 °C does not yield a higher content of phenolic compounds and stronger antioxidant capacity in extracts obtained from other fruit by-products (Xu et al., 2008). However, for ethanol and acetone the efficiency of the extraction of phenolic compounds increased with temperature (at 120 min). The amount of phenolic compounds increased 1.8–2.6 times when ethanol was used as extractant solvent and the temperature increased from 25 to 55 °C. Higher yields were obtained with acetone when the temperature was increased, increasing 5.5 times for "Grande Naine" and 1.8 times for "Gruesa" cultivar. The ratio of total phenolic compounds to the total extractable compounds ranged from 0.30% to 20%. This meant that in all cases between 80% and almost 100% of the extractable compounds were not phenolic compounds. Methanol, ethanol, acetone and mixtures of those solvents with water had similar efficiencies in extracting phenolic compounds from banana peel. Water extracted the least amount of phenolic compounds (0.30–2.0% of the extractable compounds).

Choosing the appropriate solvent is one of the most important factors in obtaining extracts with a high content of bioactive compounds. In general, the highly hydroxylated aglycone forms of phenolic compounds are soluble in alcohols such as ethanol and methanol (Arts & Hollman, 1998). Less polar solvents such as ethyl acetate, acetone and chloroform are used for the less polar and the highly methoxylated aglycone forms that are very common in fruit skin (Lafka et al., 2007). The most polar phytochemical compounds can be extracted using water. The results obtained coincide with reports that the mixture acetone:water is an effective solvent for extracting phenolic compounds from fruit by-products (Shui & Leong, 2006; Wolfe, Wu, & Liu, 2003). Moreover, phenolic compound content was comparable to the content described in the literature for other extracts of fruit by-products (Lafka et al., 2007; Shui & Leong, 2006). However, other authors have reported that the by-products of other plants such as artichoke (Llorach et al., 2002), pomegranate (Singh et al., 2002) and grape (Murthy et al., 2002) have 10–20 times more phenolic compounds. The low recovery of phenolic compounds obtained with water could be caused by the oxidation of phenolic compounds by polyphenol oxidase, whereas in methanol, ethanol and acetone the enzyme is inactivated. Therefore, aqueous extracts had a brown hue not observed in extracts obtained using the other solvents. The increase in temperature (from 25 to 55 °C) assayed was not sufficient to inactive polyphenol oxidase in aqueous extracts.

The extracts obtained with acetone:water contained anthocyanins. When acetone:water extractions were carried out for 1 min (25 and 55 °C), the amount remained between 404 ± 111 and

Table 4
Total phenolic compounds and dopamine and L-dopa content of banana peel extracts.

Extraction solvent	"Grande Naine"				"Gruesa"			
	25 °C, 1 min	25 °C, 120 min	55 °C, 1 min	55 °C, 120 min	25 °C, 1 min	25 °C, 120 min	55 °C, 1 min	55 °C, 120 min
Total extractable phenolic compounds (g gallic acid equivalents/100 g DW)								
Methanol	1.4 ± 0.3^{bB}	1.8 ± 0.2^{bA}	1.4 ± 0.1^{cB}	1.8 ± 0.1^{bA}	1.4 ± 0.3^{bBC}	1.9 ± 0.3^{bA}	1.2 ± 0.1^{cC}	1.6 ± 0.1^{cAB}
Ethanol	0.31 ± 0.07^{cB}	0.23 ± 0.01^{cB}	0.30 ± 0.04^{dB}	0.53 ± 0.01^{dA}	0.29 ± 0.06^{cB}	0.35 ± 0.01^{eB}	0.27 ± 0.06^{dB}	0.68 ± 0.06^{dA}
Acetone	0.11 ± 0.01^{cB}	0.16 ± 0.03^{cB}	0.12 ± 0.01^{dB}	0.88 ± 0.13^{cA}	0.10 ± 0.01^{cC}	0.13 ± 0.01^{eB}	0.10 ± 0.01^{dC}	0.24 ± 0.01^{eA}
Water	0.18 ± 0.01^{cA}	0.092 ± 0.019^{cB}	0.20 ± 0.01^{dA}	0.031 ± 0.006^{eC}	0.29 ± 0.02^{cA}	0.19 ± 0.01^{eB}	0.32 ± 0.03^{dA}	0.13 ± 0.01^{fB}
Methanol:water (1:1)	1.6 ± 0.1^{b}	1.6 ± 0.1^{b}	1.6 ± 0.1^{bc}	1.7 ± 0.1^{b}	1.4 ± 0.1^{bAB}	1.2 ± 0.1^{dB}	1.5 ± 0.1^{bA}	1.6 ± 0.2^{cA}
Ethanol:water (1:1)	1.7 ± 0.3^{b}	1.8 ± 0.1^{b}	1.7 ± 0.1^{b}	1.8 ± 0.1^{b}	1.7 ± 0.3^{bB}	1.5 ± 0.1^{cB}	1.6 ± 0.2^{bB}	2.9 ± 0.3^{bA}
Acetone:water (1:1)	2.6 ± 0.6^{a}	2.8 ± 0.7^{a}	2.6 ± 0.3^{a}	3.1 ± 0.4^{a}	4.7 ± 0.7^{aA}	3.2 ± 0.2^{aB}	3.8 ± 0.2^{aB}	3.8 ± 0.5^{aB}
Dopamine (mg/100 g DW)								
Methanol	265 ± 52^{aB}	334 ± 10^{aA}	373 ± 16^{aA}	381 ± 36^{aA}	288 ± 18^{aB}	342 ± 22^{aA}	325 ± 11^{aA}	339 ± 20^{aA}
Ethanol	157 ± 27^{bc}	148 ± 36^{bc}	161 ± 6^{c}	204 ± 53^{b}	150 ± 25^{b}	136 ± 15^{c}	148 ± 20^{b}	166 ± 13^{c}
Acetone	95 ± 13^{d}	110 ± 2^{d}	92 ± 18^{d}	120 ± 34^{c}	88 ± 4^{c}	96 ± 10^{c}	85 ± 9^{c}	101 ± 25^{c}
Water	4.2 ± 0.1^{e}	4.5 ± 0.5^{e}	4.7 ± 0.3^{e}	4.8 ± 0.3^{d}	3.8 ± 0.2^{d}	3.5 ± 0.3^{d}	4.0 ± 0.5^{d}	3.9 ± 0.4^{d}
Methanol:water (1:1)	153 ± 4^{cAB}	139 ± 10^{cB}	167 ± 9^{cA}	115 ± 9^{cC}	148 ± 2^{b}	106 ± 6^{c}	158 ± 4^{b}	143 ± 40^{c}
Ethanol:water (1:1)	203 ± 8^{bA}	173 ± 9^{bB}	217 ± 14^{bA}	195 ± 20^{bAB}	177 ± 35^{b}	173 ± 7^{b}	184 ± 27^{b}	239 ± 7^{b}
Acetone:water (1:1)	172 ± 11^{bc}	172 ± 7^{b}	190 ± 45^{bc}	197 ± 12^{b}	117 ± 26^{bB}	112 ± 30^{cB}	140 ± 13^{bAB}	201 ± 27^{bA}
L-dopa (mg100 g DW)								
Methanol	30 ± 1^{bA}	17 ± 1^{cC}	21 ± 1^{bBC}	28 ± 6^{cAB}	28 ± 2^{bA}	19 ± 5^{bB}	26 ± 3^{bA}	24 ± 3^{bA}
Ethanol	16 ± 3^{c}	12 ± 1^{d}	13 ± 2^{c}	12 ± 1^{d}	11 ± 4^{c}	13 ± 2^{b}	14 ± 5^{c}	13 ± 4^{c}
Acetone	12 ± 1^{dB}	11 ± 2^{dB}	9.4 ± 2.3^{cB}	18 ± 4^{dA}	9.3 ± 0.7^{c}	8.8 ± 0.5^{c}	7.9 ± 0.6^{d}	8.0 ± 0.4^{d}
Water	8.1 ± 0.5^{eA}	6.7 ± 0.6^{eB}	8.8 ± 1.4^{cA}	5.1 ± 0.5^{eC}	4.3 ± 0.5^{d}	4.3 ± 0.3^{d}	4.5 ± 0.6^{e}	4.8 ± 0.3^{e}
Methanol:water (1:1)	30 ± 1^{bB}	43 ± 1^{aA}	38 ± 5^{aA}	30 ± 1^{cB}	45 ± 4^{a}	35 ± 6^{a}	53 ± 6^{a}	53 ± 7^{a}
Ethanol:water (1:1)	33 ± 2^{aC}	35 ± 3^{bBC}	38 ± 3^{aB}	49 ± 1^{bA}	46 ± 4^{aB}	41 ± 2^{aB}	47 ± 6^{aB}	63 ± 1^{aA}
Acetone:water (1:1)	31 ± 3^{abB}	34 ± 1^{bB}	36 ± 4^{aB}	56 ± 5^{aA}	56 ± 4^{aBC}	43 ± 4^{aC}	58 ± 6^{aB}	88 ± 15^{aA}

Values are the mean ± standard deviation of at least three determinations ($n = 3$–9).
Within a column (a–f) or a row (A–C), different letters denote significant differences ($p < 0.050$) between extraction solvent or extraction conditions (temperature and time), respectively. The absence of lower case or capital letters after values indicates any significant differences.

465 ± 78 μg cyanidin 3-glucoside equivalents/100 g DW banana peel. However, the extraction of these compounds decreased (20–35%) when the extraction was done at 55 °C for a longer period of time (120 min). These amounts were much lower than those described by Lapornik et al. (2005) for redcurrant, blackcurrant and grape by-products and by Wolfe et al. (2003) for apple peels. An amount of 139 ± 37 μg cyanidin 3-glucoside equivalents/100 g DW banana peel was detected in the extracts in acetone when the extraction was done at 25 and 55 °C for 1 min. Moreover, a negligible amount of total monomeric anthocyanins of 27 ± 9 μg cyanidin 3-glucoside equivalents/100 g DW was detected in methanolic extracts. In the other extracts anthocyanins were not detected (detection limit (DL), 10 μg cyanidin 3-glucoside equivalents/100 g DW).

Banana peel contained large amounts of dopamine and L-dopa, catecholamines with a significant antioxidant activity (Table 4). This strong activity is related to their *o*-dihydroxy structure and its amino residue facilitates their hydrophilic character (Kanazawa & Sakakibara, 2000). Extracts obtained with methanol had the highest dopamine content, compared with the other solvents assayed by a factor ranging between 1.5–3.0 (methanol:water, ethanol:water, acetone:water or ethanol) and 65–98 (water). The dopamine content increased significantly when the extraction time was increased from 1 to 120 min (at 25 °C) for methanolic extracts and when the extraction temperature was increased from 25 to 55 °C for extracts obtained with acetone:water. Varying extraction temperature or time when using other solvents revealed no significant changes in dopamine content of the extracts. The metabolising precursor of dopamine (L-dopa) was less abundant than dopamine in banana peel; methanol:water, ethanol:water and acetone:water extracts contained the largest amounts of this catecholamine. On the other hand, the lowest L-dopa content was found in extracts obtained with water. The extraction of this compound increased (1.5–1.8 times) when the extraction temperature increased from 25 to 55 °C for 120 min, with ethanol:water and acetone:water. The catecholamine contents reported by Kanazawa & Sakakibara (2000) from the peel of ripe (*Musa acuminata* Colla AAA) bananas were much lower (80–560 mg dopamine/100 g fresh peel and 1.1–8.0 mg L-dopa/100 g fresh peel) than those obtained in this work.

Other bioactive compounds that were assayed as potentially responsible for the antioxidant activity of banana peels extracts, such as ascorbic acid (DL, 100 μg ascorbic acid/100 g DW), tocopherols (DL, 500–1000 μg/100 g DW) or phytosterols (DL, 300–400 μg/100 g DW), were not detected in the extracts under the extraction conditions used in this work (solvent, water pH, temperature, time and cultivar).

4. Conclusions

There is a great deal of antioxidant activity in banana peel and it could be a very inexpensive source of extracts rich in bioactive compounds, as previously suggested by Someya et al. (2002). Extracting banana peel with acetone:water was not only very efficient but also produced extracts with high antioxidant capacity, as confirmed by various model systems. This may be due to variation in the quality and quantity of phenolic compounds and other bioactive compounds present in the different extracts, such as catecholamines and anthocyanins (ascorbic acid, tocopherols and phytosterols were not detected). The antioxidant activities of banana peel extracts obtained from different cultivars ("Grande Naine" and "Gruesa") were similar. However, the impact of other extraction conditions, such as time or temperature, should be studied in greater depth. Further work is also required for the isolation and characterisation of individual phenolic compounds present in various extracts, to determine the mechanisms involved in the antioxidant capacity of these by-product extracts.

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