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Using drying treatments to stabilise mango peel and seed: Effect on antioxidant activity

Eva Dorta, M. Gloria Lobo, Mónica González*

Post-harvest and Food Technology Laboratory, Department of Tropical Fruit Crops, Instituto Canario de Investigaciones Agrarias, Apdo. 60, 38200 La Laguna, Spain

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ABSTRACT

The aim of this work was to study the effect of different drying treatments on the antioxidant activity of mango peel and seed. Freeze-drying allowed the peel (when extraction was carried out with ethanol:-water) and seed to be stabilised without diminishing their antioxidant activity. In addition, this treatment improved mango peel's antiradical capacity against $ABTS^{\bullet+}$ (in ethanol:water), as well as the capacity of the seed to scavenge free radicals and to inhibit the lipid peroxidation. Oven-drying at 70 °C (with static or forced air) was the treatment that had the most negative effect on the antioxidant capacity of mango peel (when extraction was carried out with ethanol) and seed. Because the effect of drying methods on the phenol and anthocyanin content of mango peel or seed is related to antioxidant activity (with a moderately strong significant correlation, $p < 0.003$), it can be concluded that the phenol content of both materials is largely responsible for their antioxidant activity.

1. Introduction

The amount of bio-waste produced by the food industry annually in the European Union is estimated at 37,000,000 mT (Commission of the European Communities, 2008). These bio-wastes constitute an environmental problem because they contain large quantities of nitrogen and phosphorous and they also have high water content which makes them susceptible to modification by micro-organisms, with leachate formation and gas emission. The European Union is requiring Member States to reduce organic biodegradable waste in landfills by 65% compared to 1995 levels by 2016 (European Union, 1999) and to take measures ensuring that bio-waste undergoes recovery operations (European Union, 2008).

The main by-products of processing mangos (*Mangifera indica* L.) are the peel and the seed, which represent approximately 35–60% of the fruit (Larrauri, Rupérez, Borroto, & Saura-Calixto, 1996). According to FAO estimates (FAOSTAT database), in 2008 world production of mango, alongside mangosteen and guava, was 34,343,083 mT. Approximately, 0.5% of world mango production is used to obtain derived products; therefore the amount of bio-waste produced by processing industries is estimated to be around 75,000 mT worldwide.

Mango peel and seed have a great deal of antioxidant activity (Ajila, Naidu, Bhat, & Prasada-Rao, 2007a; Maisuthisakul & Gordon, 2009; Soong & Barlow, 2004) because they are rich in bioactive compounds such as phenolic compounds (quercetin, quercetin *O*-glycosides, isoquercetrin quercetrin galactoside, 3,4-dihydroxy benzoic acid, ellagic acid, mangiferin, isomangiferin, homomangiferin, mangiferin 3-*C*-6-*O*-*p*-hydrybenzoic acid, xanthones), carotenoids, tocopherols (α-, γ-) and sterols (β-sitosterol, Δ-avenasterol, campesterol, stigmasterol) (Abdalla, Darwish, Ayad, & El-Hamahmy, 2007; Ajila, Bhat, & Prasada-Rao, 2007b; Ribeiro, Barbosa, Queiroz, Knödler, & Schieber, 2008; Schieber, Berardini, & Carle, 2003).

In mango bio-waste processing, drying may be an essential step to inactivate enzymes responsible for degrading many active compounds and to decrease the rate of microbial growth. However, drying temperature and time affect the activity and stability of bioactive compounds due to chemical and enzymatic degradation, losses by volatilisation and/or thermal decomposition. The effect of drying temperature on the polyphenol content and antioxidant activity of red grape pomace peels was studied by Larrauri, Rupérez, and Saura-Calixto (1997). When drying temperature was 100 and 140 °C, a significant reduction in both total extractable polyphenols and condensed tannins was observed, as well as a decrease of 28 and 50% in the antioxidant activity of the bio-wastes, respectively. Radical scavenging activity and levels of polyphenolic compounds in mulberry leaves air-dried at 60 °C or below were not different

* Corresponding author. Fax: +34 922 476303.
E-mail addresses: glez.glez.monica@gmail.com, mongonza@ull.es (M. González).

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from those of freeze-dried leaves, whereas both values in mulberry leaves air-dried at 70 °C and over decreased significantly (Katsube, Tsurunaga, Sugiyama, Furuno, & Yamasaki, 2009). Wolfe and Liu (2003) highlighted that the air-dried and freeze-dried apple peel retained much better their phenols, flavonoids and anthocyanins (with similar contents to those of the fresh apple peel) than the oven-dried samples at 40, 60 or 80 °C.

Therefore, drying conditions play an important role in determining the quality of the final product, especially in terms of its antioxidant activity. The chemical and biochemical changes that take place during this process should be studied; however, no information about the bioactive compounds' stability and antioxidant activity of mango peel or seed was found. The aim of this work was to study the effect of drying methods (freeze-drying and oven-drying with static or forced air) on the polyphenol and chlorophyll content and antioxidant activity of mango peels and seeds. Moreover, statistical methods were used to identify which of the natural antioxidants are related to the antioxidant activity measured (simple lineal correlation) and to ensure that the quality variables analysed can explain how the different drying treatments affect the quality of the mango peel and seed (cluster analysis and principal component analysis).

2. Materials and methods

2.1. Plant material

Mango (*M. indica* L., cv. 'Keitt') was obtained from fields located in La Gomera (Canary Islands, Spain). Fruit (around 20 kg) was harvested at physiological maturity stage (mature-green) and allowed to ripen (full-ripeness or consumption stage) at 18 °C and 80–90% relative humidity. Once the mangos were ripe, the peel or the seed (shell of fibrous endocarp, testa and embryo) was manually separated (peel:mango ratio, 31 ± 1%; seed:mango ratio, 4.2 ± 2.1%), cut into small pieces (0.5 × 1 cm) and frozen in liquid nitrogen. The mango peel or seed was placed in plastic containers and stored at −80 °C, to decrease the enzyme activity during storage, until the drying treatments were carried out.

2.2. Drying of mango by-products

The mango peel or seed were freeze-dried or oven-dried at 70 °C with static or forced air. Non-dried by-products from mango (fresh, frozen in liquid nitrogen and stored at −80 °C) were used as reference to evaluate the effect of drying method on sample quality. The freeze-drying of peels and seeds was carried out in a Christ Alpha 1-4 LSC freeze-dryer (Osterode, Germany). The condenser temperature was −40 °C, the shelf temperature was set at 25 °C and the vacuum was 50 mPa for five days. The oven-dried by-products were placed in a P-Selecta serie 2000 (Barcelona, Spain) or P-Selecta S-374 oven with static or forced air, respectively at 70 °C for 24 h. Each drying trial was performed in triplicate. The dried or non-dried mango peels and seeds were ground to a fine powder by impact grinding with an IKA A11 mill (Staufen im Breisgau, Germany), granulometrically characterised and stored at −20 °C or at −80 °C, respectively, until the extractions were carried out.

To determine water lost during drying, mango peel and seed were weighed before (ca. 100 g) and after the drying processes (in three independent replicates of the drying process). Then, the weight loss was used to calculate the water losses during drying. Chromatic attributes (L*, h° and C*) were measured with a Minolta Chroma Meter model CR-300 (Wheeling, IL, USA) colour difference metre, using ca. 30 g of the ground sample placed in a glass flask (in three independent replicates).

2.3. Obtaining extracts with antioxidant activity

The extracts from mango peel or seed were obtained by microwave-assisted extraction (ETHOS 1, Milestone SRL, Sorisole, Italy) for 60 min and at a potency of 500 W. The extraction conditions have been previously optimised (Dorta, González-Montelongo, Lobo, & González, 2009): ethanol:water (1:1, v:v) or ethanol at 75 °C were used as extractants for the peel and acetone:water (1:1, v:v) at 50 °C for the seed. The amount of plant material used in each extraction process varied depending on the water content of the bio-waste (400 mg of dried peel or seed and 1.6 g or 800 mg of non-dried peel or seed, respectively), all of them extracted with 20 ml of extractant. Extracts were centrifuged and stored at −80 °C until the analyses were carried out. Each extraction process was done at least in triplicate (3–9 times).

2.4. Bioactive compound determination

All determinations were made on a Shimadzu UV–visible 160A double-beam spectrophotometer equipped with a Hellma cell (path-length 10^{-2} m).

Phenolic compound content was estimated with the method described by González-Montelongo, Lobo, and González (2010) and the results were expressed as g of gallic acid equivalents (GAEs)/100 g DW mango peel or seed.

The total monomeric anthocyanin content of the mango bio-wastes was measured using a spectrophotometric pH differential protocol (Lee, Durst, & Wrolstad, 2005). The anthocyanin concentration in the extracts was calculated as follows:

$$\text{monomeric anthocyanins (mg/l)} = \left[(A_{515} - A_{700})_{\text{pH 1.0}} - (A_{515} - A_{700})_{\text{pH 4.5}}\right] \cdot \text{MW} \cdot \frac{1000}{\varepsilon \cdot l}$$

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) and l is path-length (cm). The amount of anthocyanin in the plant material was expressed as mg cyanidin 3-glucoside equivalents/100 g DW.

Chlorophyll determination was done at two characteristics wavelengths, 647 and 664 nm, which are the maximum absorption wavelengths for chlorophyll *b* and chlorophyll *a*, respectively (Cubas, Lobo, & González, 2008). To increase the detection limit, both in the anthocyanin and chlorophyll determination, the mango peel and seed extracts were obtained from 2.0 g of dried by-product or from 4.0 or 8.0 g of non-dried peel or seed, all of them extracted with 20 ml of extractant. Calibration graphs were obtained by using multiple linear regression and the results were expressed as mg/100 g DW mango peel or seed.

2.5. Extract yield determination

The different extracts obtained were evaporated to dryness in a vacuum Heto VR-1 evaporator (Allerod, Denmark) at 40 °C. The extract yield was defined as the amount of dried extract (g) obtained from 100 g of DW mango by-product.

2.6. Extract antioxidant activity

The antioxidant activity (understood as the capacity of antioxidants to inhibit or prevent the oxidation of different molecules by being oxidised themselves) of the extracts were evaluated by using different methods to obtain information about their activity during different stages of the oxidation reaction: by their capacity to inhibit lipid peroxidation (the antioxidants terminate chain

reactions of fat oxidation); or by their antiradical capacity (the antioxidants scavenge free radicals generated in the chain reaction).

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 (González-Montelongo et al., 2010; Miller, 1971). The antioxidant activity was expressed as antioxidant activity coefficient (AAC). The capacity of the bio-wastes to react with secondary oxidation products that were formed in the advanced stages of this reaction was evaluated by using the modified assay of thiobarbituric acid reactive substances (TBARS) described by González-Montelongo et al. (2010) and expressed as inhibition ratio (IP, %).

The capacity to scavenge the DPPH$^{\bullet}$ radical was monitored according to a slightly modified version of the method used by Brand-Williams, Cuvelier, and Berset (1995) at 515 nm after 15 min. The scavenging activity against the ABTS$^{\bullet+}$ radical was determined by a method (Arnao, Cano, & Acosta, 2001) based on enzymatic generation of the radical by reaction of the ABTS with the horseradish peroxidase in sodium phosphate buffer pH 7.5, in the presence of hydrogen peroxide. Results were expressed as g of TE or AE (trolox or ascorbic acid equivalent antioxidant capacity, respectively)/100 g mango bio-waste on a dry matter basis (DW) and as scavenging percentage (antioxidant activity AA, %) (González-Montelongo et al., 2010).

2.7. Statistical analysis

Data analysis was carried out with the Statgraphics-Plus software 5.1 (Statistical Graphics, Rockville, MD, USA). Grubbs' test was applied to detect outliers in the data set and analysis of variance to evaluate the effect of the different drying methods on antioxidant activity, extraction yield and bioactive compound content in mango peel and seed. Fisher's Least-Significant-Difference test, at the 5% significance level, was applied to experimental results to assess intra-pair significant differences. Simple linear correlation analysis was used to measure the correlation between the extract yield or the bioactive compounds and the antioxidant activity of the bio-wastes. Two multivariate techniques were used to characterise the quality of mango peel and seed dried by using different treatments on the basis of their antioxidant activity and their bioactive content: cluster analysis (CA), selecting the Euclidean distance as similarity measurement and Ward's method as amalgamation rule, and principal component analysis (PCA) with all variables being mean centred and scaled to unit variance prior to analysis.

3. Results and discussion

The water lost by the seed, during drying, was similar for all the evaluated treatments. However, in peel, there were significant differences between drying treatments, showing the oven-dried peel a higher water loss than the freeze-dried (Table 1).

The evaluation of the peel or seed colour enabled to distinguish the influence of the drying treatment on some quality characteristics of these dried bio-wastes when compared to non-dried material; moreover the colour modification of the plant material could be related to a modification of the bioactive compounds contained in it. Drying treatments changed peel or seed colour: freeze-dried peel had the highest lightness and the peel dried in a static-air oven the lowest chromaticity. The highest hue angle was found for fresh peel (non-dried). In the case of seed, the freeze-dried one showed highest lightness and hue angle and lowest chromaticity that the oven-dried seed and non-dried, treatments which affected to a lesser extent seed colour.

Table 1
Effect of the method used to dry the mango peel and seed on the water lost during the drying and on the colour of these bio-wastes. Mango peel and seed were dehydrated by using three different methods: freeze-drying (FD) and oven-drying at 70 °C with static air (OS) or with forced air (OF). Non-dried (ND) bio-wastes were used as a control.

Drying treatments	W (%)	L	H	C
Peel				
ND	–	50 ± 1^{d}	86 ± 2^{a}	26 ± 2^{a}
FD	71 ± 2^{b}	64 ± 1^{a}	77 ± 1^{c}	24 ± 1^{b}
OS	74 ± 2^{a}	53 ± 1^{b}	75 ± 2^{d}	24 ± 2^{ab}
OF	72 ± 5^{a}	52 ± 1^{c}	79 ± 1^{b}	19 ± 1^{c}
Seed				
ND	–	65 ± 1^{d}	89 ± 1^{b}	19 ± 1^{a}
FD	51 ± 6^{a}	76 ± 1^{a}	91 ± 1^{a}	14 ± 1^{d}
OS	47 ± 1^{a}	72 ± 1^{b}	87 ± 1^{c}	18 ± 1^{b}
OF	54 ± 4^{a}	69 ± 1^{c}	84 ± 1^{d}	16 ± 1^{c}

Values are the mean ± standard deviation of at least 3 determinations. Different letters (a–d) denote significant differences ($p < 0.050$) between drying treatments. W, Water lost; L, Lightness; H, Hue angle; C, Chromaticity.

3.1. Effect of drying method on the extract yield and bioactive compound

The drying treatment influenced the extraction yield in peel and seed, for any extraction solvent, improving in all cases over 1.5–4.0 times the yield obtained for the non-dried bio-waste (Table 2). In peel as well in seed, the extraction yield was highest for the freeze-dried by-product. Therefore, the drying treatment may cause an enhancement of the extractability of different compounds.

Regarding the content of bioactive compounds, responsible for the antioxidant activity of peel or seed, considerable amounts of phenolic compounds were detected, being their content higher in peel than in seed; however, anthocyanin compounds and chlorophylls were only detected in peel (Table 2). In the extracts obtained from peel with ethanol:water, the total phenol content was equal in all drying treatments to that of non-dried peel. On the other hand, freeze-drying and oven-drying with forced air led to an increase (1.6 times) in anthocyanin content compared to non-dried peel. Drying treatments also favoured the chlorophyll *a* and total chlorophyll extraction from mango peel (Table 2), possibly because the water removal from plant material favours the extraction of these liposoluble compounds. Peel drying induced a loss of phenolic compounds in ethanolic extracts; therefore, those obtained from oven-dried peel had the lower content: around 25–40% of the content of non-dried material and 50% of that of freeze-dried peel. However, anthocyanin or chlorophyll content was greater in freeze-dried or oven-dried (with static air) peel extracts, respectively.

The content of phenolic compounds in the freeze-dried mango seed was higher (1.2–2.1 times) than that of the other treatments.

3.2. Effect of drying method on the capacity of mango peel or seed to inhibit lipid peroxidation and scavenge free radicals

The capacity of mango peel to protect lipids from oxidation (Fig. 1), in the TBARS test as well as in the β-carotene bleaching assay, and to scavenge the DPPH$^{\bullet}$ free radical (Table 3) was not affected by the drying treatment compared to non-dried material, for extracts obtained with ethanol:water. However, the extracts obtained with ethanol, from mango peel freeze-dried or dried in a static-air oven showed higher scavenging capacity against ABTS$^{\bullet+}$ radical (around 1.5 times higher) than the non-dried peel (Table 3). When the extraction was done with ethanol, peel drying (freeze- and oven-drying) minimised the capacity to inhibit lipid peroxidation (Fig. 1) and to scavenge DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ free radicals (Table 3). In both cases, the treatment that had the most negative

Table 2
Total phenolic compound, anthocyanin compound and chlorophyll content of mango peel and seed. Mango peel and seed were dehydrated by using three different methods: freeze-drying (FD) and oven-drying at 70 °C with static air (OS) or with forced air (OF). Non-dried (ND) bio-wastes were used as a control.

Drying treatment	Extract yield (g/100 g DW)	Phenolics[e]	Anthocyanins[f] (mg/100 g DW)	Chlorophyll *a*	Chlorophyll *b*	Total chlorophyll
Peel; extractant, ethanol:water						
ND	$5.9 \pm 1.4^{c/A}$	$7.7 \pm 0.5^{a/B}$	$1.5 \pm 0.2^{b/A}$	$0.31 \pm 0.17^{c/B}$	$1.0 \pm 0.6^{b/B}$	$1.3 \pm 0.7^{b/B}$
FD	$24 \pm 1^{a/A}$	$9.2 \pm 0.5^{a/A}$	$2.6 \pm 0.4^{a/A}$	$0.72 \pm 0.10^{bc/B}$	$2.6 \pm 0.6^{a/A}$	$3.3 \pm 0.7^{a/B}$
OS	$20 \pm 1^{b/A}$	$8.5 \pm 0.7^{a/A}$	$1.5 \pm 0.1^{b/A}$	$0.83 \pm 0.23^{b/B}$	$3.0 \pm 0.8^{a/B}$	$3.8 \pm 1.0^{a/B}$
OF	$20 \pm 1^{b/A}$	$7.9 \pm 0.5^{a/A}$	$2.2 \pm 0.4^{a/A}$	$3.8 \pm 0.4^{a/B}$	$0.86 \pm 0.10^{b/B}$	$4.6 \pm 0.5^{a/B}$
Peel; extractant, ethanol						
ND	$6.0 \pm 0.3^{c/A}$	$13 \pm 1^{a/A}$	$1.9 \pm 0.3^{b/A}$	$3.6 \pm 1.0^{d/A}$	$3.5 \pm 1.0^{b/A}$	$7.1 \pm 0.7^{c/A}$
FD	$16 \pm 1^{a/B}$	$9.8 \pm 0.3^{b/A}$	$3.3 \pm 0.1^{a/A}$	$6.3 \pm 0.6^{c/A}$	$3.3 \pm 0.9^{b/A}$	$9.6 \pm 0.7^{c/A}$
OS	$14 \pm 1^{b/B}$	$4.6 \pm 0.2^{c/B}$	$1.6 \pm 0.2^{b/A}$	$15 \pm 1^{a/A}$	$6.7 \pm 1.4^{a/A}$	$21 \pm 2^{a/A}$
OF	$14 \pm 1^{b/B}$	$4.6 \pm 0.3^{c/B}$	$2.0 \pm 0.2^{b/A}$	$10 \pm 2^{b/A}$	$3.0 \pm 0.9^{b/A}$	$13 \pm 3^{b/A}$
Seed; extractant, acetone:water						
ND	4.0 ± 0.4^{d}	3.8 ± 0.1^{c}	–	–	–	–
FD	12 ± 1^{a}	7.4 ± 0.4^{a}	–	–	–	–
OS	7.7 ± 0.4^{b}	6.0 ± 0.5^{b}	–	–	–	–
OF	5.3 ± 0.6^{c}	3.5 ± 0.3^{c}	–	–	–	–

Values are the mean ± standard deviation of at least 3 determinations; n.c., non-quantifiable. Different lower or upper case letters denote significant differences ($p < 0.050$) between drying treatments (a–d) or between extraction solvents used to extract the peel (A–B), respectively.
[e] GAEs, gallic acid equivalents.
[f] cyanidin 3-glucoside equivalents.

effect on the antioxidant capacity was oven-drying. Moreover, the capacity to scavenge radicals and prevent TBARS formation of the extracts obtained from dried peel, with ethanol:water, was better than the capacity of the extracts obtained with ethanol.

Freeze-drying improved the seed capacity to prevent the bleaching of β-carotene in carotene/linoleic acid mixtures (Fig. 1) and to scavenge DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ radicals (Table 3), compared to non-dried seed. Thus, the antioxidant capacity of freeze-dried seed

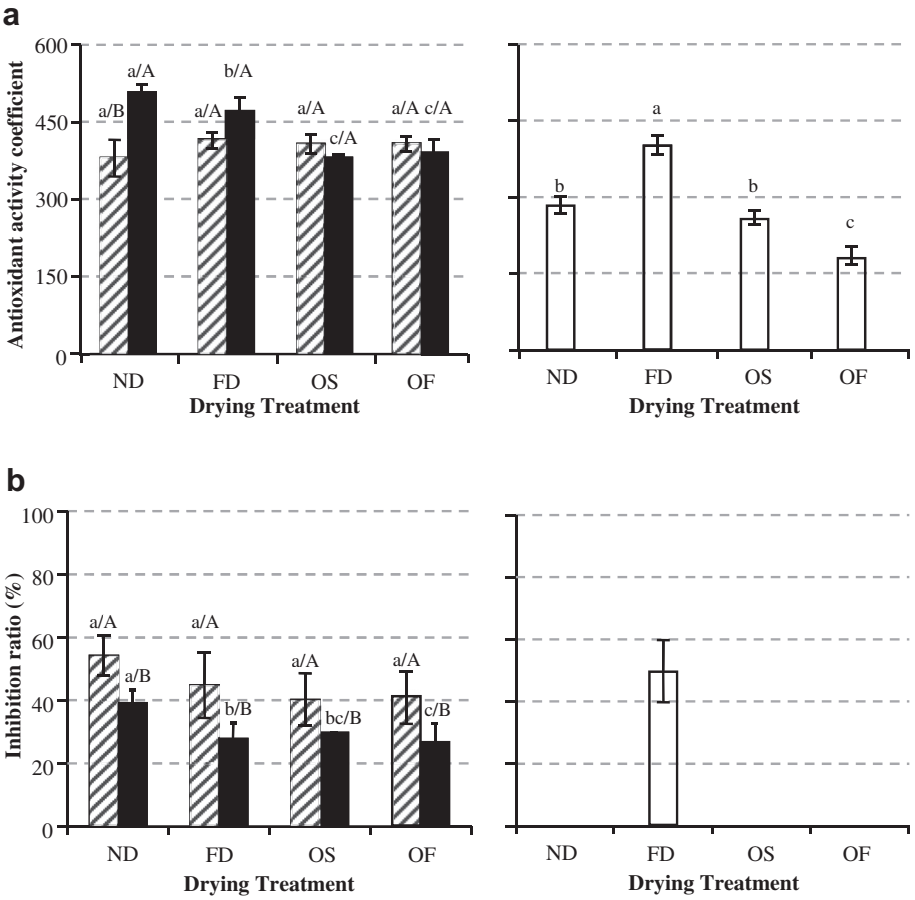

Fig. 1. Effect of drying treatments on the capacity of mango peel and seed to inhibit lipid peroxidation. The inhibition of the lipid peroxidation was measured by using the β-carotene bleaching (a) and the thiobarbituric acid reactive substances (TBARS) (b) assays. Striped bars represent peel extracts obtained with ethanol:water; black bars represent peel extracts obtained with ethanol; and white bars represent seed extracts obtained with acetone:water. Mango peel and seed were dehydrated by freeze-drying (FD) and oven-drying at 70 °C with static air (OS) or with forced air (OF). Non-dried (ND) bio-wastes were used as a control. Each process was done in triplicate. Different lower or upper case letters denote significant differences ($p < 0.050$) between drying treatments (a–c) or between extraction solvents used to extract the peel (A–B), respectively.

Table 3
Effect of drying method on the capacity of mango peel or seed to scavenge free radicals. Mango peel and seed were dehydrated by using three different methods: freeze-drying (FD) and oven-drying at 70 °C with static air (OS) or with forced air (OF). Non-dried (ND) bio-wastes were used as a control.

Drying treatment	DPPH•(g/100 g DW)		ABTS•+ (g/100 g DW)	
	TE	AE	TE	AE
Peel; extractant, ethanol:water				
ND	$56 \pm 9^{a/A}$	$41 \pm 7^{a/A}$	$25 \pm 3^{b/A}$	$28 \pm 2^{b/A}$
FD	$48 \pm 1^{a/A}$	$35 \pm 1^{a/A}$	$37 \pm 4^{a/A}$	$38 \pm 4^{a/A}$
OS	$44 \pm 3^{a/A}$	$32 \pm 2^{a/A}$	$39 \pm 2^{a/A}$	$39 \pm 1^{a/A}$
OF	$49 \pm 4^{a/A}$	$36 \pm 3^{a/A}$	$34 \pm 2^{a/A}$	$34 \pm 1^{a/A}$
Peel; extractant, ethanol				
ND	$33 \pm 3^{a/B}$	$26 \pm 2^{a/B}$	$27 \pm 2^{a/A}$	$24 \pm 1^{a/A}$
FD	$22 \pm 1^{b/B}$	$17 \pm 1^{b/B}$	$22 \pm 1^{b/B}$	$19 \pm 1^{b/B}$
OS	$12 \pm 1^{c/B}$	$8.9 \pm 0.9^{c/B}$	$11 \pm 1^{c/B}$	$9.7 \pm 0.6^{c/B}$
OF	$11 \pm 1^{c/B}$	$8.9 \pm 0.4^{c/B}$	$12 \pm 1^{c/B}$	$10 \pm 1^{c/B}$
Seed; extractant, acetone:water				
ND	18 ± 1^{b}	18 ± 1^{b}	14 ± 1^{b}	13 ± 1^{b}
FD	34 ± 2^{a}	34 ± 2^{a}	32 ± 2^{a}	30 ± 2^{a}
OS	14 ± 1^{c}	14 ± 1^{c}	14 ± 1^{b}	13 ± 1^{b}
OF	7.8 ± 0.9^{d}	7.8 ± 0.9^{d}	8.6 ± 1.1^{c}	8.2 ± 1.0^{c}

Values are the mean ± standard deviation of at least 3 determinations. Different lower or upper case letters denote significant differences ($p < 0.050$) between drying treatments (a–d) or between extraction solvents used to extract the peel (A–B), respectively. TE or AE, trolox or ascorbic acid equivalent antioxidant capacity.

was 1.5–2.5 times higher than that of non-dried seed or dried in a static-air oven and 2.0–4.5 times higher than that of seed dried in a forced-air oven. However, oven-drying with forced air decreased the antioxidant activity of the seed compared to non-dried (Table 3). Only the freeze-dried seed showed capacity to prevent the TBARS formation (Fig. 1).

The differences in the extract yield of dried and non-dried peel that were described were not correlated with the changes that took place in antioxidant activity; consequently, it is possible that some substances without antioxidant activity are co-extracted from mango peel. On the other hand, the increase in the yield of dried seed was correlated with an increase in the antioxidant activity of the extracts obtained from dried material.

Because the effect of drying methods on the phenol content of mango peel and seed seems to be related to its effect on antioxidant activity, it can therefore be concluded that the phenol content of both materials is largely responsible for their antioxidant activity.

The reduced levels of the polyphenolic compounds found in ethanolic extracts obtained from oven-dried mango peel correlated well to the antioxidant activity, an indication that the decrease of antioxidant activity resulted from the degradation of phenolic compounds at high temperatures, due to chemical, enzymatic or thermal decomposition (Nicoli, Anese, & Parpinel, 1999). At high temperatures bioactive compounds with antioxidant activity can react with other components of the plant material, thus impeding extraction. Moreover, in forced-air drying ovens, phytochemical compounds can oxidise because the plant material has greater contact with oxygen. Larrauri et al. (1997) did not notice differences in antioxidant activity between freeze-dried grape peel and dried at 60 °C in a forced-air oven; however, when drying temperature increased (100 or 140 °C) a decrease of 28 and 50%, respectively in the antioxidant activity of the samples was observed. Moreover, polyphenol content diminished when oven-drying temperature increased. This behaviour was accompanied by a loss in lightness and hue angle, compared to freeze-dried grape peel. Wojdylo, Figiel, and Oszmianski (2009) highlighted that, in general, the capacity to scavenge the DPPH• and ABTS•+ radicals and the phenolic and anthocyanin content was higher in freeze-dried strawberry than in convective-dried (70 °C, air velocity 1 m/s) or vacuum-dried (50 °C, 100 Pa). Higashi-Okai, Kamimoto, Yoshioka, and Okai (2002) found that extracts from dried peels of Satsuma mandarin (*Citrus unshiu* Marcorv.) had stronger antioxidant activity than those obtained from fresh peel. Extracts from dry heat-treated *Tamarindus indica* seed coat exhibited higher antioxidant activity (scavenging activity and inhibition of peroxidation) than extracts obtained from raw samples (Siddhuraju, 2007). In mulberry leaves, Katsube et al. (2009) established that the DPPH• radical scavenging activity and the phenolic compounds in plant material air-dried at 40 or 60 °C were not significantly different from those of freeze-dried leaves; however, both values decreased significantly in leaves air-dried at 70 °C. Freeze-dried or air-dried apple peel (Wolfe & Liu, 2003) also had a phenol and flavonoid content similar to non-dried peel (500–400 mg GAEs/100 g and 300 mg catequin equivalents/100 g, respectively). However, in the peel that was oven-dried in a mechanical convection oven, at temperatures between 40 and 80 °C, the phenol and flavonoid content was 1.5 times lower. Piga, Del Caro, and Corda (2003) and Piga et al. (2009) established that the plum and peach drying in air-flow cabinet, at temperatures between 55 and 85 °C, reduced polyphenol, anthocyanin and ascorbic acid content compared to fresh fruit. In all cases, the lower amounts of bioactive compound content on the vegetable samples, as a consequence of drying treatment, were attributed to thermal and oxidative degradation of these compounds.

The freeze-dried seed contained significantly higher phenol content than the fresh seed and exhibited higher antioxidant activity. In recent studies, it has been established that plant material stabilisation by drying not only favours its conservation but improves the antioxidant activity of the extracts and, in some cases, the bioactive compound content. Therefore, Wojdylo et al. (2009) compared four methods to dehydrate strawberry fruits: freeze-drying, convection- or vacuum-drying and vacuum-microwave drying. Freeze-drying improved the content of some phenols, such as kaempferol 3-*O*-glycoside and (+)-catechin in 'Elsanta' variety and of cyanidin 3-*O*-glucoside in 'Kent', around 1.3 times compared to non-dried strawberry. The phenolic compound content of the freeze-dried mulberry leaves (Katsube et al., 2009) and the anthocyanin content of the freeze-dried apple peel (Wolfe & Liu, 2003) was much higher than the content of the non-dried material. This behaviour may be due to the fact that a large percentage of phenolic compounds are bound to cellular structures and drying treatments release bound phytochemicals from the matrix to make them more accessible in extraction. Another possible explanation is that the polyphenols in an intermediate state of oxidation can exhibit higher radical scavenging efficiency than the non-oxidised ones, although, a subsequent loss in the antioxidant properties has been found for advanced enzymatic oxidation steps (Nicoli et al., 1999).

3.3. Correlations and multivariate statistical analysis

With all the data obtained for the different conditions evaluated ($n = 36$), statistical analysis based on regression lines was carried out in order to analyse the complex relationship between the extract yield or the phytochemical compounds and their antioxidant capacity. The correlation coefficients indicated a moderately strong relationship ($p = 0.000$) between the total extractable compounds and the capacity of the bio-wastes to scavenge ABTS•+ radicals ($r > 0.600$) and the content of anthocyanin compound ($r = 0.629$) and a relatively weak relationship with the capacity to scavenge DPPH• radicals ($r = 0.363$, $p = 0.017$) and the inhibition of TBARS formation ($r = 0.451$, $p = 0.006$). The correlations between antioxidant activity and the extract yield or the phytochemical compounds illustrate that could exist other non-phenolic compounds in the extracts that affect the ability to prevent lipid peroxidation and to scavenge free radicals, probably fibre which

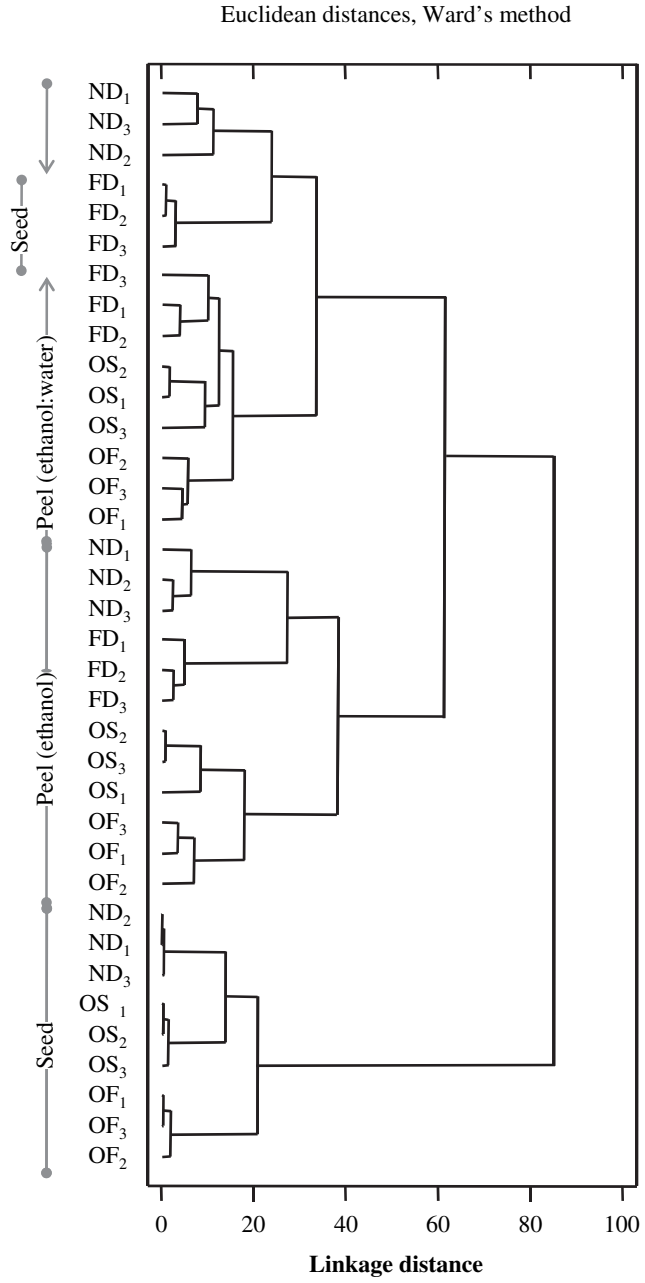

Fig. 2. Dendogram resulting from a cluster analysis selecting the Euclidean distance as similarity measurement and Ward's method as amalgamation rule, applied to all mango peel and seed studied. Mango peel and seed were dehydrated by freeze-drying (FD) and oven-drying at 70 °C with static air (OS) or with forced air (OF). Non-dried (ND) bio-wastes were used as a control. Three independent replicates of each treatment were analysed: subscripts indicate the number of the sub-sample.

antioxidant capacity has been previously reported (Larrauri et al., 1996, 1997).

A moderately strong significant correlation ($p = 0.000$) also existed between the phenolic compound content and the antiradical capacity of the bio-wastes ($r > 0.585$ for DPPH$^{\bullet}$ and $r > 0.658$ for ABTS$^{\bullet+}$ radicals) and between phenols and the capacity to inhibit lipid peroxidation ($r = 0.802$ for β-carotene bleaching and $r = 0.591$ for TBARS tests). The relationship between anthocyanin compounds and the inhibition of the β-carotene bleaching and the TBARS formation was also moderately strong ($r = 0.703$, $p = 0.000$ and $r = 0.528$, $p = 0.002$, respectively) and between anthocyanin

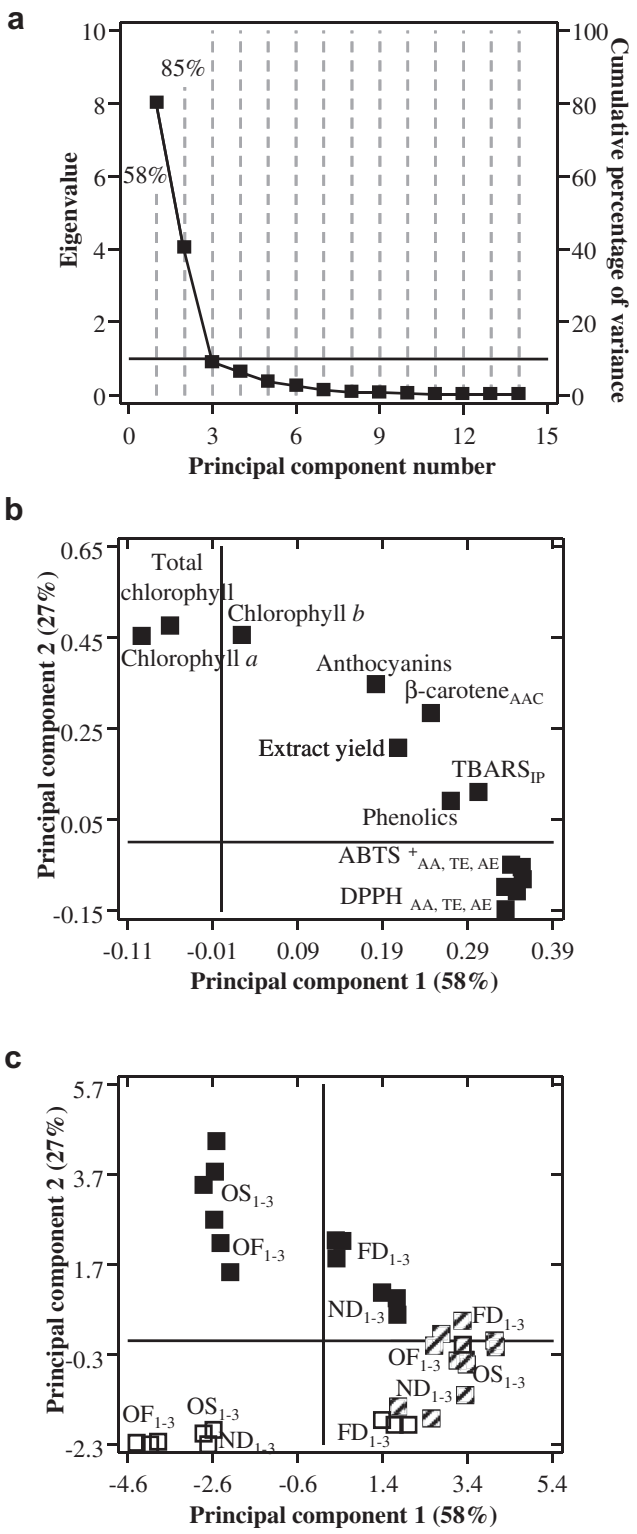

Fig. 3. Score plot of the principal components (a) and distribution of samples of mango peel and seed (b) and plot of variable loadings (c) on the plane formed by the first two principal components, where striped bars represent peel extracts obtained with ethanol:water; black bars represent peel extracts obtained with ethanol; and white bars represent seed extracts obtained with acetone:water. Mango peel and seed were dehydrated by freeze-drying (FD) and oven-drying at 70 °C with static air (OS) or with forced air (OF). Non-dried (ND) bio-wastes were used as a control. The antioxidant activity was expressed as: AAC, antioxidant activity coefficient; IP, inhibition ratio (%); AA, scavenging percentage (%); TE or AE, trolox or ascorbic acid equivalent antioxidant capacity (g/100 g DW). Three independent replicates of each treatment were analysed: subscripts indicate the number of the sub-sample.

compounds and the antiradical capacity of the bio-wastes was relatively weak ($r > 0.432$, $p < 0.012$ for DPPH$^{\bullet}$ and $r > 0.412$, $p < 0.017$ for ABTS$^{\bullet+}$ free radicals). The relationship between chlorophylls and the capacity to inhibit β-carotene decolouration was moderately strong for chlorophyll *b* ($r = 0.535$, $p = 0.001$) and relatively weak for chlorophyll *a* ($r = 0.322$, $p = 0.055$). However, there was not a statistically significant relationship between chlorophylls and the capacity to prevent TBARS formation or the antiradical capacity of bio-wastes. Therefore, the correlations between antioxidant activity and the phenolic and anthocyanin compounds seem confirm that these compounds played a major role in the antioxidant capacity of mango peel or seed.

To ensure that the quality variables analysed (antioxidant activity and phytochemical compounds) can achieve a separation that explain how the drying treatment affect the quality of the mango peel and seed, multivariate analysis was carried out.

The results of the cluster analysis (CA) made over all studied mango bio-wastes are presented as dendogram in Fig. 2. Taking as an arbitrary cut-off point a similarity level < 50, three main clusters can be visualised, one of them consists of mango peel extracted with ethanol:water and the freeze-dried seed, the second cluster contains the mango peel extracted with ethanol and the third one the non-dried and oven-dried seed.

Due to its unsupervised character, CA is not conclusive in and of itself as it merely gives information on the similarity of the different samples. For this reason, principal component analysis (PCA), a technique that illustrates which variables account for most of the variability in the data, was employed. PCA showed two interpretable components, chosen on the basis of Kaiser's criterion (eigenvalues higher than 1.0 are chosen), explaining together 85% of the total variance in dried samples quality (Fig. 3a). Fig. 3b shows the plot of loadings by selecting the two principal components as axes. The first principal component that explains the higher percentage of variance (58%) is mainly related antioxidant activity and to the phenolic and anthocyanin compounds and the second principal component (27% of the variance) is related to the total chlorophyll and chlorophyll *a* content (both with a positive correlation). On the other hand, Fig. 3c also shows the corresponding scores onto the two first principal components. As can be seen, samples are grouped according to their quality in three distinct groups: the mango peel extracted with ethanol:water and the freeze-dried seed, the mango peel extracted with ethanol and the mango seed (non-dried and oven-dried). The variables at the bottom right correspond with mango peel, extracted with ethanol:water, and with freeze-dried seed; the variables at the top left or right of the loading plot correspond with mango peel, extracted with ethanol, that was oven-dried or freeze-dried and non-dried, respectively; and the variables at the bottom left of the plot correspond with the non-dried and oven-dried mango seed. Thus, the plant material with the highest antioxidant capacity (mango peel, extracted with ethanol:water, and freeze-dried seed) was correlated with antioxidant activity (especially with the capacity to scavenge free radicals) and with the phenolic compound content. Therefore, it seems to confirm that the quality of these extracts, in terms of antioxidant activity, is largely related to the polyphenol content of mango peel. Oven-dried peel, extracted with ethanol, scored high for chlorophyll *a* and total chlorophyll, which is in agreement with the results previously obtained.

4. Conclusions

Mango bio-wastes can be stabilised through freeze-drying without reducing its antioxidant activity. This drying method can even increase mango peel's capacity to trap the ABTS$^{\bullet+}$ free radical (when it was extracted with ethanol:water) and also favour mango seed's capacity to inhibit lipid peroxidation and scavenge free radicals. This behaviour may be due to the fact that a large percentage of phenolic compounds are bound to cellular structures and drying treatments release bound phytochemicals from the matrix to make them more accessible in extraction.

When compared to non-dried material, none of the drying methods evaluated modified the ability of mango peel extracts (obtained from ethanol:water) to inhibit lipid peroxidation and scavenge the DPPH$^{\bullet}$ free radical. However, when the skin was extracted with ethanol, all of the drying treatments evaluated caused the antioxidant activity to decrease, when compared to non-dried material. Oven-drying with forced air also decreased the antioxidant activity of mango seed, compared to non-dried material, in both cases, possibly due to thermal and oxidative degradation of phenolic compounds.

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