

Effect of microwave drying and oven drying on the water activity, color, phenolic compounds content and antioxidant activity of coconut husk (*Cocos nucifera* L.)

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Abstract The coconut (*Cocos nucifera* L.) husk is basically composed by fiber and pith material and remained under-utilized. This is an important source of phenolic compounds that could be used as functional ingredients. The aim of this study was to determine the effect of: oven-drying (OD) and microwave drying (MD), on the water activity, color, phenolic compound content and antioxidant activity of coconut husk. The OD was performed at 60 °C for 12 h and MD was performed at 900 W for 10 min. The total phenolic content (TPC) in fresh coconut husk was 64.2 mg GAE/g dry wt and significant higher than observed after OD and MD of 35.8 and 45.5 mg GAE/g dry wt, respectively. Ten phenols were identified in fresh and dehydrated coconut husks. The husk MD showed an increase in the content of gallic, 4-hydroxybenzoic, ferulic and syringic acids and epicatechin compared with the fresh; while coconut husk OD and MD, showed a decrease in the content of vanillic acid, vanillin, catequin and kaempferol. The antioxidant activity decreased after both OD and MD. However, MD resulted in a better antioxidant activity in husk than OD. MD of husk resulted into better retention of preserved color, TPC and TFC than OD.

Keywords Coconut husk · By-product · Microwave/oven drying · Phenolics · Antioxidant activity · HPLC

Introduction

The *Cocos nucifera* L. species of the *Arecaceae* family, is commonly known as coconut, and is considered an important fruit crop in tropical countries (Chakraborty and Mitra 2008). A mature coconut typically is composed by: coconut water (25 wt%), white meat (28 wt%) which is surrounded by a hard protective shell (12 wt%) and a thick husk (35 wt%). This husk is constituted of fiber (30 wt%) and pith material (70 wt%) (Van Dam et al. 2004). Coconut husk is one of the major agroindustrial by-products that are generated in developing countries each year (Dey et al. 2003; Van Dam et al. 2004). Nowadays, small amount of coconut fiber husk, is used for the production of yarns, mats, brushes, padding of mattresses and ropes. However, the majority remains under-utilized (Chakraborty and Mitra 2008; Dey et al. 2003; Rodrigues and Pinto 2007; Van Dam et al. 2004).

The composition of the husk includes complex carbohydrates and polyphenolic compounds such as: catechin, epicatechin, 4-hydroxybenzoic and ferulic acids and condensed tannin (Dey et al. 2003). In last years the study of the phenolic compounds has been increased due to their antioxidant properties (Balasundram et al. 2006). Some health benefits of phenols has been reported such as: anti-proliferative activity on leukaemic cells and normal blood lymphocytes (Kirszberg et al. 2003), anti-bacterial and anti-viral activity (Esquenazi et al. 2002) and analgesic and free radical-scavenging activities (Alviano et al. 2004).

In recent years, there has been a global trend toward the use of natural plant food components, such as: dietary fiber and phytochemicals as ingredients in functional foods

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(Bensadón et al. 2010). Coconut husk represents a good source of phytochemicals, however its moisture content (>80 %) reduces the husk shelf-life complicating the phytochemical extraction (Sagar and Kumar 2010). The drying of coconut husk could be an interesting and cheap alternative to preserve the phenolic compounds. Lately new and innovative drying methods that increase the drying rate and enhance product quality have achieved considerable attention. Microwave drying is a method that is gaining popularity due to meet the four major requirements in the drying of foods: speed of operation, energy efficiency, cost of operation, and quality of dried products (Zhang et al. 2006).

Therefore, the aim of this study was to determine the effect of two drying methods: oven-drying (OD) and microwave drying (MD), on the water activity, color, phenolic compound content and antioxidant activity of coconut husk.

Materials and methods

Materials

Folin–Ciocalteu reagent, potassium persulphate, 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), 2,4,6-Tri(2-pyridyl)-s-triazine (TPTZ), 2,2-diphenyl-1-picrylhydrazyl (DPPH), the acid standards (vanillic, trans-cinnamic, 4-hydroxybenzoic, protocatechuic, syringic, gallic, and ferulic acids), the flavonoids standards (quercetin, quercitrin, catechin, rutin, epicatechin and kaempferol) and vanillin were purchased from Sigma Chemical Co. (St. Louis, MO, USA). The organic reagents and acetic acid were of analytical or HPLC grade and purchased from JT Baker Chemical Co. (Phillipsburg, NJ, USA).

Plant material and drying processes

Whole young coconuts (3–4-months-old) from the Guerrero coast in Mexico that weighed approximately 1.6 kg were obtained during July 2015 from a local market in Mexico City. The fruits were washed, sanitized and rinsed with distilled water. Before drying, the husk materials (40 wt%) were manually sliced longitudinally following fiber arrangement into 11 cm lengths and 1.5 cm widths. The kernel and shell were separated from the husk and then discarded. The coconut husk slices were weighed in 100 g portions and placed on 20 cm round glass plates. Then, they were dried using a hot air electric oven or a microwave dryer. The oven drying was conducted in a cabinet dryer (Lindberg, Blue M, USA) that was adjusted to 60 ± 2 °C for 12 h. The microwave drying was conducted in a microwave oven (LG, MJ1481BP, China) at

700 ± 20 W for 10 min. All dried samples were grounded separately and were passed through a 40-mesh sieve. Then, they were stored in amber vials of 15 mL at -40 °C until further use.

Water activity and color analysis

The water activity (A_w) was measured using an Aqualab water activity meter (Decagon Devices, Model 4 TE, USA) at 25 ± 0.2 °C. The color of the fresh and dried coconut husks was determined using a CR-10 colorimeter (Konica Minolta, Japan), and the L^* , a^* , b^* , chroma, hue angle values and color difference (ΔE) were measured based on the CIE Lab* color space.

Evaluation of phenolic compounds

The extraction method used was performed according to Appaiah et al. (2014) with some modifications. The fresh and dried samples (100 mg) were homogenized with 5 mL of a methanol–water mixture (80:20, v/v) for 30 min. The sample supernatant was then recovered after centrifugation (Hermle, Z326, Germany) at 2500 rpm for 10 min at room temperature (24 ± 2 °C). This extraction was repeated four times, and the extracts were mixed. The extracts were analyzed immediately to evaluate the total phenol and flavonoid contents, the antioxidant activity and HPLC analyses.

The total phenolic content (TPC) was determined according to the method of Tan et al. (2014) with minor modifications. Two hundred and fifty microliters of the sample were added with 17.5 mL of water that was mixed with Folin–Ciocalteu reagent (diluted 1:10). The mixture was allowed to react for 5 min; then, it was added to 3.75 mL of sodium carbonate solution (7.5 %, w/v). The absorbance was measured at 765 nm using a Genesys 10-UV-Vis spectrophotometer. All values are expressed as mg gallic acid equivalents (GAE)/g dry wt.

The total flavonoid content (TFC) was evaluated using a colorimetric assay (Osorio-Esquivel et al. 2011). Briefly, 0.25 mL of the sample was mixed with 1.25 mL of distilled water in a test tube, followed by the addition of 75 μ L of a 5 % NaNO_2 solution. After 6 min, 150 μ L of a 10 % $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ solution was added and allowed to stand for another 5 min before 0.5 mL of 1 M NaOH was added. The mixture was brought to 2.5 mL using distilled water and was mixed well. The absorbance was measured at 510 nm. These values are expressed as mg catechin equivalents (CE)/g dry wt.

Chromatographic methods

The prepared coconut husk extracts were used to separate and quantify the phenolic compounds (Guzmán-

Maldonado et al. 2010) using an Agilent 1260 Infinity HPLC with a diode array detector (HPLC–DAD) and automatic injection. The HPLC was equipped with an acquisition system (Agilent OpenLAB CDS Ezchrom Edition ver. A.04.04) with a 150×4.5 mm i.d., $5 \mu\text{m}$ particle size, and Zorbax SB-C18 reverse-phase column. A linear gradient elution was performed using solvent A (acetic acid/water, 2:98, v/v) and solvent B (acetic acid/acetonitrile/water, 2:30:68, v/v/v). During the sample analysis, the solvent gradient was programmed to increase from 10 to 100 % B over 30 min with a flow rate of 1.5 mL/min. The UV detector was set at 280 nm. The compounds were identified and quantified by comparing the retention time (Rt) and absorption spectra of the sample chromatographic peaks with those of authentic standards obtained using the same HPLC operating conditions.

Antioxidant activity

The antioxidant activities of the coconut husk samples were determined using three antioxidant assays: DPPH, ABTS and FRAP (ferric-reducing antioxidant potential). The DPPH assay was performed according to the method by Chakraborty and Mitra (2008). Aliquots (0.1 mL) of the extracts were mixed with 2.9 mL of a methanolic solution of DPPH radical (60 μM). The decrease in the absorbance was measured at 515 nm when the reaction reached a plateau. The percentage inhibition was calculated against a control. The radical scavenging activities of the samples were expressed as the Trolox equivalent antioxidant capacity (TEAC) as μmol Trolox equivalent (TE)/g dry wt.

The ABTS assay was performed according to the modified method of Ozgen et al. (2006). For ABTS radical generation, 2.45 mM of potassium persulfate was reacted with 7 mM ABTS salt in 20 mM acetate buffer (pH 4.5) for 12–16 h at room temperature in the dark. The resultant ABTS radical cation was diluted in an acidic medium of 20 mM sodium acetate buffer (pH 4.5) to an absorbance of 0.700 ± 0.01 at 734 nm. For the spectrophotometric assay, 3 mL of the ABTS radical adjusted solution and 20 μL of the extract were mixed, and the absorbance was determined. The percentage inhibition was calculated against a control and compared to a Trolox standard curve. The results are expressed in terms of the Trolox equivalent antioxidant capacity (μmol TE/g dry wt).

The FRAP assay was performed following the procedure described previously (Chakraborty and Mitra 2008). The FRAP reagent was freshly prepared as follows: 10 mM of TPTZ solution, 12 mM of ferric chloride and sodium acetate buffer (pH 3.6) were mixed at a volume ratio of 1:1:10. Aliquots of the methanolic extract were added separately to 3 mL of the FRAP reagent and kept at 37 °C. The absorbance was recorded at 593 nm. The reducing

potential is expressed as the Trolox equivalent antioxidant capacity (μmol TE/g dry wt).

Statistical analysis

The experiments were performed in triplicate. The mean values, standard deviations and analyses of variance (ANOVA) were calculated by Sigma Stat software, version 3.5. The significant differences between the means were determined using the Student–Newman–Keuls (SNK) multiple range tests ($p \leq 0.05$).

Results and discussion

Effect of drying on water activity and husk color

The results of the moisture, water activity (a_w) and the color of the fresh and dried coconut husks are presented in Table 1. The moisture content of the fresh coconut husk was 83.2 %, and was reduced to 4.4 % after OD and to 8.5 % with MD. The a_w of fresh coconut husk was 0.98 and it was reduced up to 0.37 and 0.38 after OD and MD, respectively. The lightness (L^*) of the OD decreased significantly compared with fresh coconut husk. However, MD did not change lightness of husk significantly than that of fresh one. Redness (a^*) and yellowness (b^*) values were higher in OD and remained unchanged upon MD compared with the fresh coconut husk. The increase in the a^* value showed more red chroma, which is indicative of the browning reaction (Vadivambal and Jayas 2007). Thus, product dried by microwaves was less brown than the conventionally oven-dried product, maybe due to faster microwave heating (Horuz and Maskan 2015). These results have also been found by Arslan and Özcan (2010) who reported that microwave dried onion revealed better color values, compared with oven dried onion.

Effect of drying on phenolic compound contents (TPC)

The TPC of the fresh coconut husk was 64.2 mg GAE/g dry wt. This value was within the range reported by Bezerra dos Santos Oliveira et al. (2013) for husk fibers from four varieties of *C. nucifera* L. (58–531 mg GAE/g dry extract). However, TPC of the coconut husk was higher than those found by Dey et al. (2003) of 13.0 mg/g dry wt. After drying of coconut husk by both methods (OD and MD), the TPC decreased significantly ($p \leq 0.05$) from 64.2 mg GAE/g dry wt to 35.8 ± 1.0 for OD and to 45.5 ± 2.7 for MD. MD provided a higher retention (70.9 %) of phenolic compounds than OD (55.8 %). These results confirm the findings of (Arslan and Özcan 2010;

Table 1 Color parameters, A_w and moisture content in coconut husk

	Moisture (g/100 g)	A_w	L*	a*	b*	Chroma	ΔE
Fresh	83.2 ± 0.2 ^a	0.98 ± 0.004 ^a	67.3 ± 0.2 ^a	3.1 ± 0.2 ^a	20.9 ± 0.4 ^a	17.5 ± 0.4 ^a	
Oven drying	4.4 ± 0.1 ^b	0.37 ± 0.002 ^b	65.8 ± 0.4 ^b	3.6 ± 0.1 ^b	23.2 ± 0.4 ^b	23.5 ± 0.4 ^b	2.9
Microwave drying	8.5 ± 0.1 ^c	0.38 ± 0.002 ^c	67.4 ± 0.2 ^a	3.2 ± 0.1 ^a	20.4 ± 0.5 ^a	20.3 ± 0.2 ^c	0.6

Different letters (a, b or c) of the means of triplicates in the same column indicates significantly different at $p \leq 0.05$

Igual et al. (2012) who found a better TPC retention in fruits and vegetables using MD compared with OD method; also Chan et al. (2009), reported that oven, sun-drying and microwaves, caused significant decrease in the TPC in ginger leaves. Nevertheless, different studies have shown that drying processes may result in lower or higher levels of TPC depending on the type of phenolic compound present in the plant material and their location in the cell (Arslan and Özcan 2010; Hamrouni-Sellami et al. 2013; Roshanak et al. 2016; Verma et al. 2015). For instance: sage plants dried in a microwave oven (800 W) showed an increase of 4.2-fold in TPC after drying (Hamrouni-Sellami et al. 2013) while Zheng et al. (2015) reported a significant decrease in TPC during the drying of loquat flower by using freeze drying, microwave, vacuum and hot-air drying. The higher content phenolic compounds after MD, may be attributed to disruption in the plant tissue by microwave causing more release of the phenolic compounds (Hamrouni-Sellami et al. 2013).

Fresh coconut husk contains 49.4 ± 3.8 mg CE/g of dry weight of flavonoids, which represents 77 % of the total phenolic compounds. These results were comparable with those found by Bankar et al. (2011) in coconut endocarp, who reported a flavonoids content of 70 % of the total phenolic compounds. After drying, OD produced a significant ($p \leq 0.05$) decrease from 49.4 ± 3.8 to 41.6 ± 0.6 mg CE/g of dry weight and MD to 42.4 ± 1.2 . These results are comparable with the results reported Zainol et al. (2009) and Toor and Savage (2006) drying of *Centella asiatica* and tomatoes respectively. According with Zainol et al. (2009), the loss of flavonoids during the thermal processing, was the result of thermal breakdown of the cellular structure, leading to losses via various chemical reactions that involve enzymes, light and oxygen. Thus, flavonoids presents the same behavior with thermal processing that TPC, this means that it is difficult to predict the changes that may occur during the thermal processes because the change in chemical composition of the fruits during the drying is the result of complex mechanisms (Miletić et al. 2013).

The HPLC chromatogram of the phenolic compounds for the fresh coconut husk sample is shown in Fig. 1. Ten compounds were successfully identified based on their Rt.

The concentrations of gallic, 4-hydroxybenzoic, vanillic, ferulic and syringic acids, vanillin, catechin, kaempferol, epicatechin and rutin are shown in Table 2. The values founded for these compounds in this study are comparable with those reported by Chakraborty and Mitra (2008), Dey et al. (2003) and Dey et al. (2005). However, higher flavonoid concentrations were obtained than those reported by Bezerra dos Santos Oliveira et al. (2013) for coconut husk.

The two drying methods affected the individual phenols in different ways (Table 2), the most important variation was observed for epicatechin content, which showed a significant increased after MD from 0.15 mg/g dry wt to 1.99 and for OD to 0.19. This increase was reported by Igual et al. (2012), during the microwave drying of apricots. Esquenazi et al. (2002) observed that epicatechin and catechin were obtained by hydrolyzing a crude tannin extract of coconut husk. It is possible that the increase of epicatechin in the present work, could be related with the hydrolysis of husk matrix during the drying process (Arslan and Özcan 2010). Also we observed after MD an increase in gallic acid from 1.08 mg/g to 2.26 mg/g (Table 2). Such increase in gallic acid content after drying, was most likely due to the thermal degradation of phenolic compounds, such as gallotannins, which were reported to be the esters of gallic acid and sugars (Miletić et al. 2013). Also higher concentrations of 4-hydroxybenzoic and ferulic acids were found in the dried husk by MD, compared with the fresh husk. Previous studies show that ferulic acid is a derivate from the cinnamic acid, which plays an important role in altering the mechanical properties of cell walls by acting as a cross-link between polysaccharides and lignin (Dey et al. 2003). It may be inferred that the increase of 4-hydroxybenzoic and ferulic acids after the MD of coconut husk, could be the result of their release from the cell-wall phenolic bound forms. Conversely it was found a significant decline of kaempferol after OD from 2.19 ± 0.08 to 1.23 ± 0.01 and for MD to 0.28 ± 0.04 , of vanillic acid after OD from 1.55 ± 0.02 to 1.48 ± 0.02 and for MD to 0.97 ± 0.02 , of vanillin after OD from 1.22 ± 0.02 to 0.98 ± 0.04 and for MD to 1.06 ± 0.01 , of catechin after OD from 1.43 ± 0.02 to 1.17 ± 0.01 and for MD to 0.33 ± 0.01 . Zainol et al. (2009) and Hamrouni-Sellami

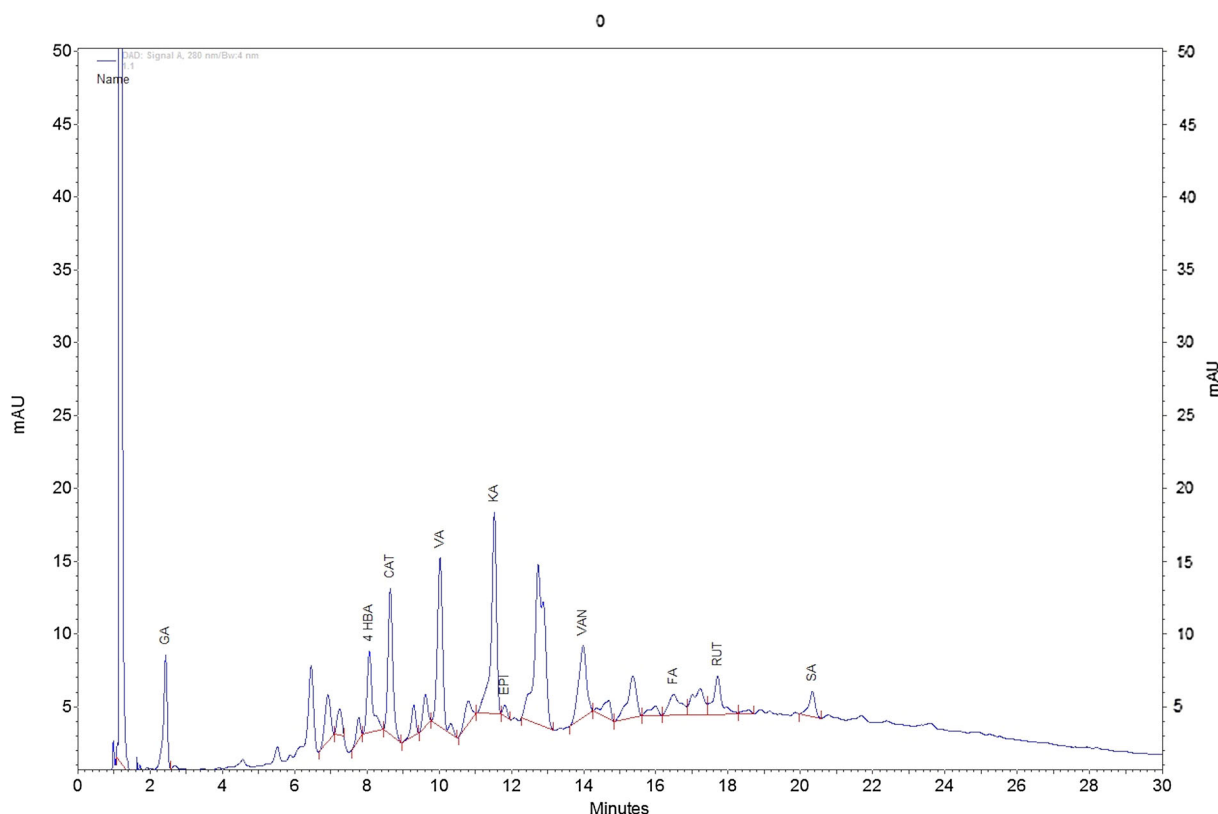


Fig. 1 HPLC chromatogram of the phenolic compounds detected in the fresh coconut husk. Wavelength program: 0–30 min, 280 nm. *GA* gallic acid, *4 HBA* 4-hydroxybenzoic acid, *CAT* catechin, *VA* vanillic acid, *KA* kaempferol, *EPI* epicatechin, *VAN* vanillin, *FA* ferulic acid, *RUT* rutin, *SA* syringic acid

Table 2 Content of phenolic compounds in coconut husk determined using HPLC analysis (mg/g dry wt of husk material)

Phenolic compound	Rt (min)	Fresh	Oven drying	Microwave drying
Gallic acid	2.433	1.08 ± 0.02 ^a	ND	2.26 ± 0.03 ^b
4-Hydroxybenzoic acid	8.067	1.37 ± 0.01 ^a	0.99 ± 0.03 ^b	1.94 ± 0.02 ^c
Catechin	8.640	1.43 ± 0.02 ^a	1.17 ± 0.01 ^b	0.33 ± 0.01 ^c
Vanillic acid	10.020	1.55 ± 0.02 ^a	1.48 ± 0.02 ^b	0.97 ± 0.02 ^c
Kaempferol	11.520	2.19 ± 0.08 ^a	1.23 ± 0.01 ^b	0.28 ± 0.04 ^c
Epicatechin	11.807	0.15 ± 0.01 ^a	0.19 ± 0.01 ^b	1.99 ± 0.02 ^c
Vanillin	13.980	1.22 ± 0.02 ^a	0.98 ± 0.04 ^b	1.06 ± 0.01 ^c
Ferulic acid	16.500	0.47 ± 0.01 ^a	0.44 ± 0.01 ^b	0.51 ± 0.01 ^c
Rutin	17.720	0.62 ± 0.03 ^a	0.70 ± 0.03 ^b	Traces
Syringic acid	20.340	0.39 ± 0.01 ^a	0.38 ± 0.02 ^a	0.43 ± 0.01 ^b
Total		10.47	7.56	9.77

The means of triplicates in the same row with different letters are significantly different at $p \leq 0.05$

et al. (2013) described the rapid degradation of phenolic compounds after drying as a consequence of the high temperatures (above 60 °C) and drying time. Heating in drying processes not only deactivates enzymes but also degrades phytochemicals due to the possibility of inducing oxidative condensation or the decomposition of thermolabile compounds.

Effect of drying on antioxidant activity

Since the antioxidant activity of plant extracts cannot be evaluated using only one method due to the complex composition of the phytochemical and oxidative processes (Hamrouni-Sellami et al. 2013), three different methods were used in this study: the DPPH radical scavenging

assay, the ABTS radical cation decolorization assay and the ferric-reducing antioxidant potential (FRAP).

The antioxidant activity values of the husk material extracts in TE using the DPPH were $858 \pm 31.3 \mu\text{mol TE/g dry wt}$ for fresh husk, 594.9 ± 19.2 after OD and 630.5 ± 6.4 after MD, for ABTS were 1850.4 ± 89.3 , 743.8 ± 23.4 after OD and 1226.6 ± 51.5 after MD and for FRAP were 493.7 ± 10.9 , 372.4 ± 6.4 after OD and 419 ± 13.8 after MD. These values are comparable with those reported by Bezerra dos Santos Oliveira et al. (2013), who using FRAP method found values from 125 to 3286 $\mu\text{mol TE/g}$ of antioxidant activity in dried husk ethanolic extracts of four coconut varieties. Both drying methods decreased the antioxidant activity of husk. This may be attributed to decrease in TPC and TFC in coconut husk after the drying process. The correlation between TPC and TFC and antioxidant activity was reported by Zheng et al. (2015) in loquat flower who found that the effect of all drying methods on DPPH and ABTS radical scavenging activities were almost in accordance with those on the TPC and TFC. TEAC in our study showed that hot-air dried samples presented the lowest antioxidant activity between the drying methods. The loss of antioxidant activity could be attributed to the drying time and temperature which open the cell matrix and facilitate the extractability and bioaccessibility of total phytochemicals; promoting the release of bound phenols in soluble forms of low-molecular weight that can be easily degraded by browning and oxidative reactions due to the heating process inducing a decrease in the TEAC (Mejia-Meza et al. 2010; Moure et al. 2001; Tian et al. 2016; Zheng et al. 2015).

Conclusion

Coconut husk is a potential source of phenolic compounds. Results of color, TPC and TFC retention indicated that MD of coconut husk, may contribute to the successive extraction of the phenolic compounds.

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