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Chromatographic techniques for the determination of alkyl-phenols, tocopherols and other minor polar compounds in raw and roasted cold pressed cashew nut oils

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ABSTRACT

Anacardium occidentale belongs to the family *Anacardiaceae* and is principally grown in tropical America (Mexico, Peru, Brazil, etc.) and India. Cashew nuts contain low amounts of hydroxy alkyl phenols that come from an oily liquid present in their shell and that is known as cashew-nut shell liquid. This paper reports the alkyl phenols composition of cold pressed raw and roasted cashew nut oil. First of all, cashew nut shell liquid was used for a basic fractionation of the alkyl phenol classes by preparative TLC and definitively identified by GC–MS and GC-FID. Anacardic acids were the major alkylphenols contained in both oils followed by cardol, cardanol and 2-methylcardol compounds, respectively. Raw and roasted oils did not show different compositions except for cardanols. The oil produced from roasted cashew nut reported a higher concentration of cardanols. Furthermore, tocopherols and other minor polar compounds were determined by HPLC-FLD and HPLC-DAD–MS, respectively. Tocopherol content varied in a range of 171.48–29.56 mg/100 g from raw to roasted cashew nut oil, being β-tocopherol the one which presented a higher decrease (93.68%). Also minor polar compounds in cashew oil decreased after roasting from 346.52 to 262.83 mg/kg.

1. Introduction

Anacardium occidentale is a tropical tree originated in tropical America (Mexico, Peru, Brazil, etc.) and that belongs to the family *Anacardiaceae*. Despite of that fact, nowadays it is widely cultivated in India and east Africa being India its largest producer.

Besides the tree, the generic name 'cashew' also applies to its fruit, its nuts, and the family to which it belongs. The cashew fruit is very peculiar and it is not really a fruit but a swollen peduncle that grows behind the real fruit that yields the cashew nut. This peduncle is usually considered as the cashew fruit or apple. At the end of each fruit is a kidney shaped ovary, the nut, with a hard double shell. The shell is 2–3 mm thick, with a leathery outer case and a thinner, harder inner case, between which is a honeycomb structure containing the phenolic cashew nut shell liquid (CNSL).

The *A. occidentale* as many other plant species has been used in the Amazonia region in traditional medicine since ancient times because of its therapeutic potential (anti-diarrheic, against topical diseases such as dermatitis and cephalalgia) [1]. Furthermore, cashew nut occupies a central position in the diets of the human population throughout the world and it has been proved that its consumption has a cardioprotective, antiobesity, anticancer and antioxidant effects [2]. In fact, generally nuts including cashew nuts have been suggested as a natural source of antioxidants such as phenolics, flavonoids, tocopherols and alkyl-phenols [3–5].

Alkyl-phenols have been found in the different parts of cashew (cashew apple, nut and shell liquid). It has been reported in literature the presence of anacardic acids and cardols in raw and roasted cashew nuts [4]. These compounds have an important antioxidant activity; specially anacardic acids [6] that have proved to possess a greater antioxidant capacity compared to a range of other known antioxidants such as 1-(+)-acetoxypinoresinol, hydroxytyrosol, tyrosol, salicylic acid and caffeic acid [4], most of them present in olive oil and also with a significant antioxidant activity. Anacardic acids are also known to inhibit enzymes such as prostaglandin synthase [7], tyrosinase [8] and lipoxygenase [9]. They also strongly inhibit digestive enzymes such as α-glucosidase, invertase and aldose reductase, whereas the structurally related salicylic acid lacking an alkyl phenyl side-chain, is a very weak inhibitor [10]. Even it has been observed that anacardic acids present a gastroprotective activity against gastric mucosal damage induced by ethanol [11] and show a high preservative efficacy

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against three important pathogens, which suggests that edible natural compounds have the potential to be used as an alternative to synthetic preservatives in food [12].

Tocopherols, α- and γ-tocopherols, have also been detected in raw cashew nuts in concentrations of 0.29 and 1.10 mg/100 g of dry matter, respectively [2]. It is well-known that tocopherols exhibit a protective role on lipid peroxidation of membrane lipids, lipoproteins, and depot fats [13] and, therefore, protect against atherosclerosis. It is also known the ability of vitamin E to induce apoptosis in tumor cells and modulate oncogenes [14]. Furthermore, tocopherols have also been found in cashew nuts, being β- and γ-tocopherols the predominant ones [6].

Phenolic compounds in cashew nuts have been studied nearly exclusively as total phenols by Folin–Ciocalteu [2] and as far as we concern the single phenolic compounds have never been identified in this kind of nuts before.

The aim of this study was to analyze the content of antioxidants: tocopherols, phenolic compounds and alkyl-phenols in cold pressed raw and roasted cashew nut oils. This matrix is particularly interesting because cashew nut is considered to be very promising for the development of synthetic and functional products and as a feedstock for production of fine chemicals and a wide variety of food materials. Moreover, cashew nut oil has been poorly studied and in most cases oil extracted with solvents has been used [2,15], while in this work the research has been carried out with cold pressed oil. The anacardic acids, cardols, cardanols and methyl-cardols were fractionated as classes by TLC, TLC-$AgNO_3$ and the individual alkyl phenols were identified by GC/MS, tocopherols were determined by HPLC-FLD and phenolic compounds were studied by HPLC-DAD–MS.

2. Materials and methods

2.1. Samples

Cashew nut shell liquid (CNSL) was purchased from the cashew nut processor.

Cold pressed oils from raw and roasted cashew nut (cv. Morgaon from India) were furnished by an Italian factory.

Roasting was taken at 160 °C for 15 min (personal communication by the factory). The samples (3× 100 mL of each oil sample) were furnished to the laboratory after one week from the oil production.

2.2. Identification of alkylphenols

To separate the different alkylphenols classes, 20 mg of CNSL was methylated with diazomethane [16] and was fractionated by using thin layer chromatography (TLC) (thickness 0.25 mm) (Merck, Darmstadt, Germany). Elution was performed with *n*-hexane:diethyl ether (3:2, v/v) in a glass developing chamber. Plate was then sprayed with a 0.2% (w/v) ethanolic solution of 2′,7′-dichlorofluorescein sodium salt to highlight the bands under an UV source (254 nm). The bands with Rf between 0.33 and 0.88 were separated and extracted from the silica gel with chloroform (2 + 2 + 2 mL). After removing the chloroform under vacuum, the residue was dissolved in 0.2 mL of *n*-hexane.

The bands of each class of alkylphenols were fractionated according to the number and geometry of double bounds by TLC silver. The TLC glass plates with silica gel were incubated with 10% aqueous solution of $AgNO_3$ for 1 h, were partially air dried, and were activated at 110 °C for 30 min. Therefore the extracts of the band from preparative TLC were loaded on the TLC silver. The plate was developed in a saturated chamber in hexane and diethyl ether (9:1, v/v) with 15 cm migration. At the end of chromatographic runs, the plates were air dried and sprayed with dichlorofluorescein, and the bands were visualized under UV light. The bands corresponding to the saturated and unsaturated alkylphenols, were scraped into a flask and were extracted with chloroform. The solvent was evaporated in a rotovapor and stream of nitrogen. After that alkyl-phenols were sylilated according to Sweeley et al. [17] and used for GC–MS and GC-FID analyses.

2.3. Alkyl-phenols extraction from cashew nut oil

In order to isolate the alkyl-phenolic fraction a solid phase extraction system was used. The extraction protocol was the following: a cartridge of silica (Si) was placed in a vacuum elution apparatus and conditioned by passing 3 mL hexane. Cashew nut oil (2 g) was dissolved in 2 mL hexane and was passed through the column. The cartridge was washed with 4 mL of hexane/diethyl ether 8/2 (v/v), which was then discarded in order to remove the nonpolar fraction of the oil. Finally, the sample was recovered by passing through of 4 mL of hexane/diethyl ether 1/1 (v/v) and than 4 mL of methanol/acetic acid 97/3 (v/v) and brought to dryness in a rotary evaporator under reduced pressure at a temperature of 35 °C. The residue was dissolved with 1 mL hexane/2-propanol (3/2, v/v).

2.4. Alkyl-phenols determination

The alkyl-phenols were analyzed by GC after sylilation [17]. The trimethylsilyl derivatives (TMS) of alkyl-phenols were analyzed by GC using a Clarus 500 instrument (Perkin Elmer, Norwalk, CT) equipped with a flame ionization detector (FID) using the following conditions: Rtx 65TG (65% diphenyl and 35% dimethylpolysiloxane) (Restek, Bellefonte, PA, 30 m × 0.25 mm i.d., film thickness 0.10 μm); helium carrier gas at 1.0 mL/min; split ratio 1:40; injector temperature: 330 °C; oven temperature: 240 °C to 325 °C at 2 °C/min. Analyses were performed in triplicate for each oil. Identification of alkyl-phenol compounds was based on mass spectral data obtained by GC–MS (VARIAN 3900 gas chromatograph equipped with a ion trap MS detector SATURN 2100T, Varian, Inc. Corporate Headquarters, Palo Alto, CA, USA) using a DB-5MS column (cross-linked/surface bonded, virtually identical to 5% phenyl and 95% dimethylpolysiloxane) (J&W scientific, Falsam, CA, USA, 30 m × 0.25 mm i.d., film thickness 0.25 μm).

2.5. Determination of tocopherols in oils

One gram of oil sample was dissolved in 10 mL of *n*-hexane and then extracts were filtered through a 0.45 μm nylon filter. The tocopherols were determined by HPLC (Jasco mod. PU-1580) equipped with a fluorimeter detector (Jasco mod. PU-1580). The excitation wavelength was 290 nm and the emission one was 325 nm. The used column was a Luna Hilic Phenomenex column (250 mm × 4.6 mm i.d., 5 μm particle size) in isocratic conditions as reported by Panfili et al. [18] with just one modification, the flow rate was 0.5 mL/min instead of 1.6 mL/min. The calibration curves were constructed with standard solutions of each compound and used for quantification.

2.6. Phenolic compounds extraction from cashew nut oil

Phenolic compounds were extracted from cashew nut oil according to Pirisi et al. [19]. After that, the dry extracts were dissolved in 0.5 mL of a methanol/water (50/50, v/v) solution and filtered through a 0.2 μm syringe filter.

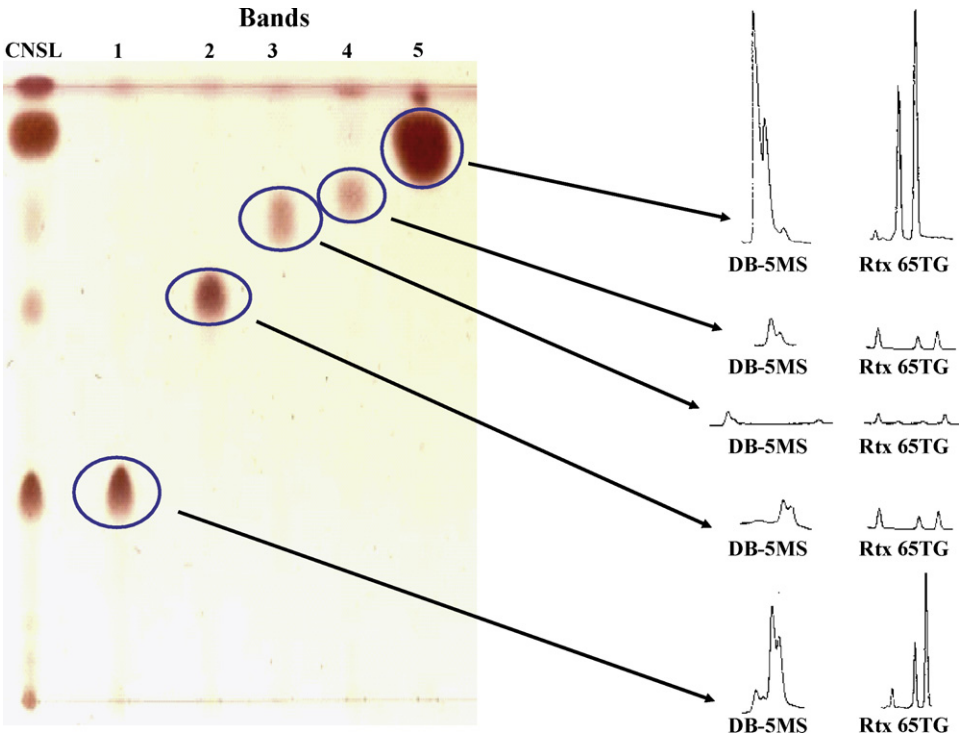

Fig. 1. TLC separation of methylated alkyl-phenols in CNSL and respective chromatograms obtained by the two different columns, respective retention times are reported in Table 1.

2.7. Determination of minor polar compounds fraction by HPLC-DAD–MSD

Determination of the minor polar compounds fraction was performed using an HPLC-DAD/ESI-MSD system according to Rotondi et al. [20]. The HPLC was an HP 1100 Series instrument (Agilent Technologies, Palo Alto, CA, USA) equipped with a binary pump delivery system, degasser and autosampler. The analytical HPLC column used was a C_{18} Luna column, 5 μm, 25 cm × 3.0 mm (Phenomenex, Torrance, CA, USA), with a C_{18} pre-column (Phenomenex) filter. The UV detector and the mass spectrometer were an HP diode array UV–vis detector (DAD, model G1315A) and a HP mass spectrometer detector (MSD, model G1946A), respectively; integration and data elaboration were performed using Chemstation software (Hewlett-Packard).

2.8. Statistical analyses

One-way analysis of variance, ANOVA (Tukey's honest significant difference multiple comparison) was evaluated using Statistica 6.0 software (2001, StatSoft, Tulsa, OK, USA). p values lower than 0.05 were considered statistically significant. All chemical analyses were carried out in triplicate, and the analytical data were used for statistical comparisons.

3. Results and discussion

3.1. Identification of alkyl-phenols

To determine the alkyl-phenols in cashew nut oil, a new method was developed. To this end the alkyl-phenols were determine in a CNSL sample. The CNSL was analyzed by gas–liquid chromatography with a medium polarity phase column (65% phenylmethyl silicon open tubular fused-silica capillary column) operating under optimal conditions and the alkyl compounds were separated according to both their alkyl-phenols structures. The trimethylsilyl derivatives of the alkyl-phenols were purely resolved with no interference from the sample matrix indicating the specificity of the method. The high bleeding of Rtx 65TG column does not permit their use on GC–MS and TLC preparative were developed in order to collect the single alkyl phenols that were analyzed by GC–MS equipped with a non-polar MS certified column.

To isolate the alkyl-phenols, methylated CNSL was separate by TLC. The separation revealed five different bands at Rf 0.33, 0.65, 0.76, 0.80 and 0.88, respectively (Fig. 1).

The results obtained were apparently in disagreement with literature [4,21] that reported only four classes of alkyl phenols in cashew and their products. For this reason, each TLC band was separate by TLC-$AgNO_3$ according to the number of double bounds. The bands were scraped off, extracted, sylilated and analyzed by GC-FID (Rtx TG 65) and GC–MS (DB 5MS).

The results of MS analyses are reported in Table 1. The GC–MS analysis of band 1 obtained from the TLC preparative and subsequently separated by $AgNO_3$-TLC showed the presence of cardols. The base peak ions of cardol saturated and mono-bi-tri unsaturated at m/z 464, 462, 460 and 458, respectively, were reported. The first fragment of each of them showed the loss of methyl group $(M-15)^{+\bullet}$, while the fragments at m/z 281, 283, 285 and 287 were obtained from the loss of O-TMS groups. Finally the ion at m/z 268 described the breakage in β of the aliphatic chain (Fig. 2A). This fragmentation pattern was in agreement with Trevisan et al. [4].

Analysis of band 2 showed two different bands in $AgNO_3$-TLC and the GC–MS analysis reported a molecular ions at 400 and 402 m/z. Their fragments (387 and 385 m/z) demonstrated the loss of a methyl group. The ions at m/z 371 and 369 showed the loss of a methoxy group and the base peak at m/z 209 confirmed the breakage of β bond in the aliphatic chain. These compounds were identified as *di* and *tri* unsaturated methoxy-cardanols. The identified compounds have not been described in literature and they were assigned as artefacts of methylation [22]. To confirm this, a CNSL extract was only sylilated and analyzed by GC–MS. The chromatogram confirmed the absence of these compounds, because of

Table 1
GC–MS and GC-FID data of the alkyl phenols in cashew nut shell liquid.

TLC band	Rt DB-5MS[a] (min)	Rt Rtx 65TG[b] (min)	*m/z* (relative intensity, %)		Compound
			$M^{+\bullet}$	Fragment ions	
3	17.53	5.95	370(30)	355(13), 180(100), 179(63)	Cardanol ($C_{15:3}$)
	17.68	5.84	372(48)	357(7), 180(100), 179(48)	Cardanol ($C_{15:2}$)
	17.96	4.97	374(22)	359(3), 180(100), 179(35)	Cardanol ($C_{15:1}$)
	18.17	4.86	376(80)	361(3), 180(100), 179(27)	Cardanol ($C_{15:0}$)
1	24.28	6.78	458(32)	443(5), 281(20), 268(100)	Cardol ($C_{15:3}$)
	24.45	6.67	460(40)	445(10), 283(23), 268(100)	Cardol ($C_{15:2}$)
	24.62	6.37	462(37)	447(10), 285(21), 268(100)	Cardol ($C_{15:1}$)
	24.71	6.18	464(39)	449(7), 286(20), 268(100)	Cardol ($C_{15:0}$)
4	24.80	12.53	472(25)	457(10), 281(100), 207(52)	Methylcardol ($C_{15:3}$)
	24.88	11.75	476(23)	461(5), 281(100), 207(43)	Methylcardol ($C_{15:1}$)
	25.06	11.54	478(20)	463(8), 281(100), 207(38)	Methylcardol ($C_{15:0}$)
5	25.75	9.25	428(3)	413(100), 396(69), 219(63)	Anacardic acid ($C_{15:3}$)
	25.90	9.03	430(3)	415(100), 398(63), 219(55)	Anacardic acid ($C_{15:2}$)
	26.10	8.71	432(2)	417(100), 400(60), 219(39)	Anacardic acid ($C_{15:1}$)
	26.45	8.66	434(3)	419(100), 402(55), 219(22)	Anacardic acid ($C_{15:0}$)

[a] Data obtained by GC–MS.
[b] Data obtained by GC-FID.

that the second band was not taken into account in our study. However, the methylation is indispensable to the correct separation and identification of alkyl-compounds by TLC, because only sylilation gives cause to the overlapping of the bands.

The third band produced four different bands in $AgNO_3$-TLC. The molecular ions were at *m/z* 370, 372, 374 and 376. The fragments with molecular weight 355, 357, 359, 361 confirmed the loss of a methyl group and the fragment at *m/z* 180 described the cleavage in β of the aliphatic chain of alkyl benzenes (Fig. 2B) according to Strocchi and Lercker [23]. These compounds were identified as *tri*, *di*, *mono*-unsaturated and saturated cardanol, respectively.

The silver nitrate TLC revealed three different bands for the band 4 with $M^{+\bullet}$ at *m/z* 478, 476 and 472. The mass spectra showed a loss of a methyl group and a subsequently cleavage in β of the aliphatic chain giving an ion at 281 *m/z*. The ion 207 *m/z* described the loss of a TMS group. The compounds of band 4 were identified as saturated methylcardol and *mono* and *tri* unsaturated methyl cardol (Fig. 2C).

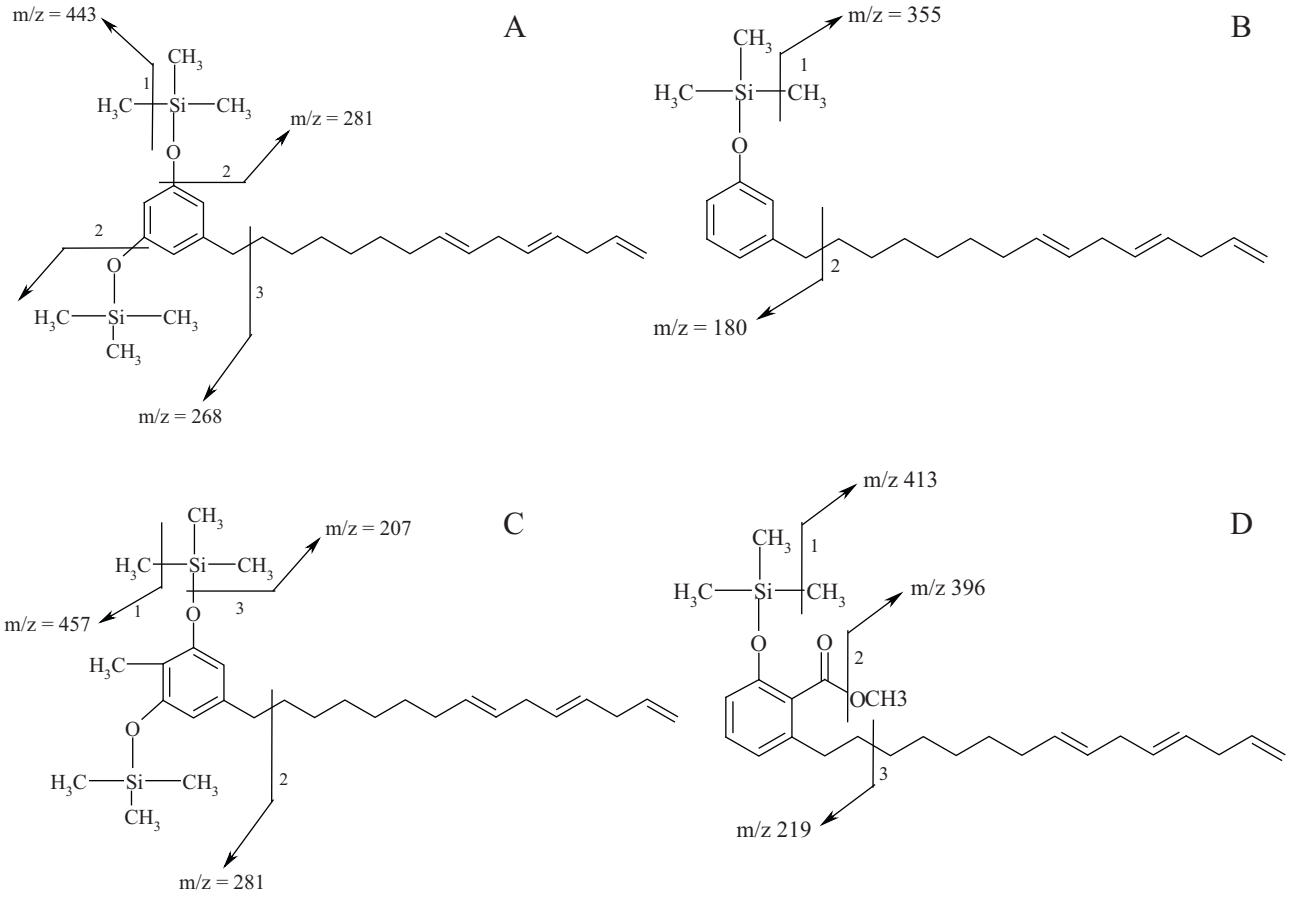

Fig. 2. Proposed fragmentation pattern of the alkyl-phenols. (A) Cardol ($C_{15:3}$), (B) cardanol ($C_{15:3}$), (C) methyl-cardol ($C_{15:3}$), and (D) anacardic acid ($C_{15:3}$).

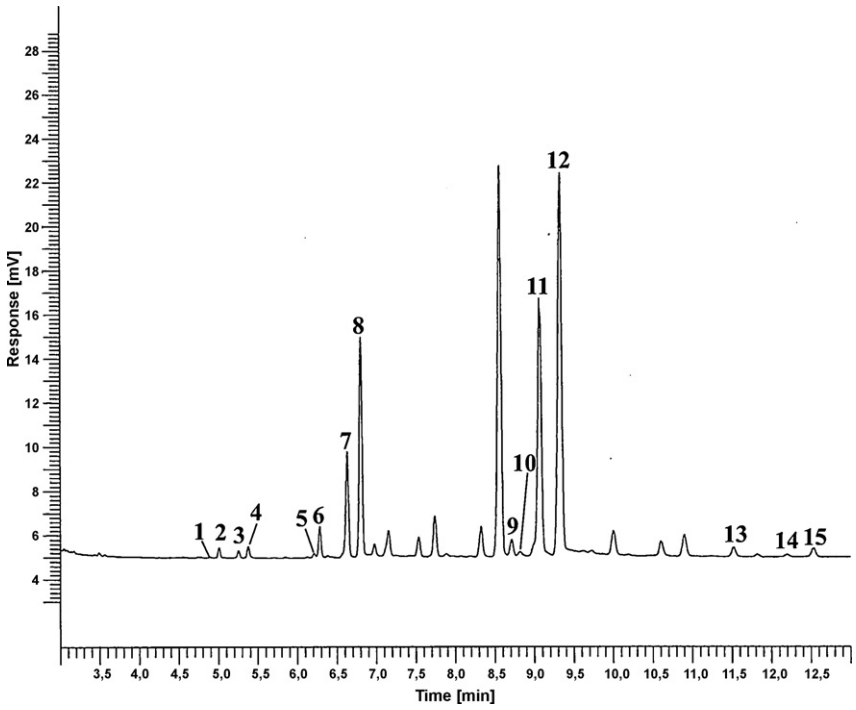

Fig. 3. Chromatogram of CNSL alkyl phenols obtained by Rtx-65TG column. (1) Cardanol ($C_{15:0}$); (2) cardanol ($C_{15:1}$); (3) cardanol ($C_{15:2}$); (4) cardanol ($C_{15:3}$); (5) cardol ($C_{15:0}$); (6) cardol ($C_{15:1}$); (7) cardol ($C_{15:2}$); (8) cardol ($C_{15:3}$); (9) anacardic acid ($C_{15:0}$); (10) anacardic acid ($C_{15:1}$); (11) anacardic acid ($C_{15:2}$); (12) anacardic acid ($C_{15:3}$); (13) methylcardol ($C_{15:0}$); (14) methylcardol ($C_{15:1}$); and (15) methylcardol ($C_{15:3}$).

The band 5 reported the same Rf of anacardic acid ($C_{15:3}$) that was used as standard. The $AgNO_3$-TLC revealed four different bands. The GC–MS analysis showed four peaks at *m/z* 434, 432, 430 and 428. The first fragment was obtained from the loss of a methyl group. The fragments with *m/z* 402, 400, 398 and 396 were obtained by the loss of a O–CH_3 group. The fragment with γ-cleavage of the side chain gave rise to the common fragment ion of 219 u; the same fragmentation pattern was obtained by Schötz [24] for the ginkgolic acids. The fragmentation pattern was confirmed analyzing the standard of anacardic acid ($C_{15:3}$) (Fig. 2D).

The bands obtained by silver-TLC and the CNSL were also injected in GC-FID with Rtx TG65 column and the isolate compounds were identified (Fig. 3).

This methodology of derivatization was only developed to the proper identification of alkylphenols. To quantify the alkylphenols, as it is described below, only sylilation was carried out.

3.2. Extraction and determination of alkylphenols of cashew oils

To isolate the alkylphenols of cashew oil, SPE extraction system was used. To verify the utility and the recovery of this method, a solution of standard of anacardic acid ($C_{15:3}$) was dissolved in soybean oil. The oil was diluted with *n*-hexane and the solution was eluted in silica cartridge.

Due to the polarity of alkyl phenols, three solutions were used to eluted them in the following order: hexane:diethylether 8:2 (v/v), hexane:diethylether 1:1 (v/v), methanol:acetic acid 97:3 (v/v). The three fractions collected were eluted by TLC to verify the presence of anacardic acid. TLC showed the presence of anacardic acid in the second and third fraction. This result was confirmed by GC–MS analysis. After collection, the second and third fractions were mixed and used for the analysis.

The recovery was evaluated spiking the solution of anacardic acid with dihydrocholesterol (used as internal standard). The recovery of anacardic acid was about 94%. LOQ was determined as the signal to noise ratio of 10:1 and LOD was determined as signal to noise ratio of 3:1. LOD and LOQ were 5 and 16.7 ng/L, respectively. Intraday repeatability of the method was evaluated by three preparations of cashew nut oil samples, and each preparation was analyzed in duplicate. Repeatability relative standard deviation (RSD, %) was in the range of 0.15–0.51%. Interday repeatability was obtained analyzing the three preparation for three days. Interday repeatability relative standard deviation was in the range of 1.25–1.76%.

Table 2 shows the content of alkyl phenols in cold pressed cashew nut oils. The oils did not show different compositions except for cardanols. The oil produced from roasted cashew nut reported a little increase of cardanols probably due to the decarboxylation of anacardic acids [23,25].

Anacardic acids were the major alkylphenols contained in both oils followed by cardol, cardanol and 2-methylcardol compounds, respectively.

Table 2
Composition of the alkyl phenols in cold pressed cashew oils. Different letters in the same line indicate significantly different values ($p < 0.05$).

Compound	Raw cashew nut oil (mg/100 g)	Roasted cashew nut oil (mg/100 g)
Cardanol ($C_{15:3}$)	0.35 (b)	0.75 (a)
Cardanol ($C_{15:2}$)	0.14 (b)	0.57 (a)
Cardanol ($C_{15:1}$)	0.21 (b)	0.35 (a,b)
Cardol ($C_{15:3}$)	0.26 (a)	0.23 (a)
Cardol ($C_{15:2}$)	7.00 (a,b)	9.17 (a)
Cardol ($C_{15:1}$)	6.70 (a,b)	9.08 (a)
Cardol ($C_{15:0}$)	1.46 (a)	1.89 (a)
Anacardic acid ($C_{15:3}$)	9.14 (a)	8.95 (a,b)
Anacardic acid ($C_{15:2}$)	7.77 (a)	6.42 (a,b)
Anacardic acid ($C_{15:1}$)	10.97 (a)	11.20 (a)
Anacardic acid ($C_{15:0}$)	0.63 (a)	0.59 (a)
2-Methylcardol ($C_{15:3}$)	0.36 (a)	0.41 (a)
2-Methylcardol ($C_{15:0}$)	0.43 (a)	0.40 (b)

Table 3
Tocopherol content in raw and roasted cashew nut oils (mg/100 g oil). Different letters in the same line indicate significantly different values ($p < 0.05$).

Compounds	Rt (min)	Raw cashew nut oil	Roasted cashew nut oil
α-Tocopherol	8.5	7.84 (a)	1.08 (b)
β-Tocopherol	9.4	132.98 (a)	8.41 (b)
γ-Tocopherol	9.9	30.03 (a)	20.12 (b)
δ-Tocopherol	10.4	0.63 (a)	0.30 (b)
Total		171.48 (a)	29.56 (b)

Narasimhan et al. [12] demonstrate that at a concentration of 0.014% the anacardic acid was active against both gram-positive and gram-negative bacteria and that can act as a potential preservative.

3.3. Tocopherols

Tocopherols were identified by comparing their retention times and fluorescence data provided by the fluorimeter with those of a standard mix of tocopherols. Each tocopherol was quantified using its own standard by building a calibration curve of each one, all of them presented good linearity between different concentrations and regression coefficients were higher than 0.990.

The chromatographic method used allowed to have results in less than 12 min.

The study of the different samples showed that roasted cashew nut oil had a significant level of reduction of tocopherols when compared with results obtained for raw cashew nut oil (Table 3). The decrease of total tocopherols was 82.76% from raw cashew nut oil to roasted cashew nut oil; the total tocopherol content varied in a range of 171.48–29.56 mg/100 g. These results are in agreement with those found in bibliography where it has been proved that heating treatments entail the degradation of tocopherols; however, this degradation occurs in a different way depending on the matrix [26,27].

In raw cashew nut oil, β-tocopherol appeared to be the highest tocopherol (132.98 mg/100 g) while δ-tocopherol presented the lowest concentration in this oil (0.63 mg/100 g).

After roasted β-tocopherol showed a noticeable decrease, 93.68%, from 132.98 in raw oil to 8.41 mg/100 g in roasted oil. Moreover, the roasted process also affected to α-, γ- and δ-tocopherols but their degradation was not as strong as in the case of β-tocopherol (86.27%, 33.00% and 52.66%, respectively). γ-tocopherol and δ-tocopherol showed higher thermal stability in agreement with Yoshida and Tagaki results [28]; they found that the thermal stability of tocopherols increases in the following order: α-tocopherol < β-tocopherol < γ-tocopherol ≈ δ-tocopherol.

Roasted cashew nut oil as raw cashew nut oil had a very low concentration of δ-tocopherol (0.30 mg/100 g), on the contrary, γ-tocopherol was the one presented in the highest concentration.

3.4. Minor polar compounds

The compounds found in the polar fraction were tentatively identified based on their MS spectra obtained by HPLC-DAD/ESI-MSD (Table 4).

The compound at retention time 17.1 min with a molecular ion at m/z 137 was identified as *p*-hydroxybenzoic acid. The identity was confirmed by co-elution with the relative standard.

The compound at 21.9 min showed a molecular ion at m/z 153 and two fragments at m/z 123 and 109. The same fragmentation pattern was reported by Bellés et al. [29], so this compound was identified as gentisic acid.

The peak at 23.4 min and m/z 121 was identified as benzoic acid.

The compound at retention time 25.0 min reported a molecular ion at m/z 185 and a strong fragment ion at m/z 141 (loss of carboxylic group). Lu et al. [30] obtained the same fragmentation pattern for the 1-naphtylacetic acid (NAA). This compound is an auxinic phylohormone.

The compound at retention time 26.7 min reported molecular ion at m/z 181 and two different fragments at m/z 153 $[M-Et]^-$ and 109 $[M-COOEt]^-$. This fragmentation pattern has been assigned to ethylprotocatechuate [31]. This compound has already been isolated in other kind of nuts [32].

The peak at retention time 27.1 min showed a molecular ion at m/z 193 and it was identified as ferulic acid; the identity was confirmed by co-elution with its relative standard.

The compound at retention time 39.4 min reported a molecular ion at m/z 187 and a fragment at m/z 159 ($[M-H-CO]^-$), Hsieh et al. [33] assigned this fragmentation pattern to plumbagin.

A peak at 45.0 min showed a molecular ion at 263 m/z and a fragment at 153 m/z, Izumi et al. [34] assigned this pattern at the abscisic acid.

The peak at retention time 46.6 min with molecular ion 203 was identified as 1,4-dihydroxy-2-naphthoic acid. Several authors [35,36] have reported that this compound is a precursor of menaquinone or vitamin K_2.

The compound at retention time 47.1 showed a molecular ion at m/z 171 and two major fragments at m/z 143 (−28 amu) and 115 (−56 amu). According to Binder et al. [37], this compound was assigned as 2-methyl-1,4-naphthoquinone and it has been already identified in walnut.

Table 4
Minor polar compounds in raw and roasted cashew nut oil (mg/kg oil). Different letters in the same line indicate significantly different values ($p < 0.05$).

Compounds	Rt (min)	$[M-H]^-$	Other fragments	Raw cashew nut oil	Roasted cashew nut oil
p-Hydroxybenzoic acid[a]	17.1	137	–	21.94 (a)	13.65 (b)
Gentisic acid[a]	21.9	153	123, 109	104.04 (a)	81.29 (b)
Benzoic acid[a]	23.4	121	–	31.77 (a)	26.81 (b)
Naphthylacetic acid[b]	25.0	185	141	10.38 (a)	11.41 (a)
Ethylprotocatechuate[a]	26.7	181	153, 109	13.76 (a)	7.45 (b)
Ferulic acid[a]	27.1	193	–	21.91 (a)	19.22 (a,b)
Plumbagin[b]	39.4	187	159	6.74 (a)	5.53 (a,b)
Abscisic acid[a]	45.0	263	153	22.71 (a)	19.18 (a)
1,4-Dihydroxy-2-naphthoic acid[2]	46.6	203	–	29.16 (a)	11.07 (b)
2-Methyl-1,4-naphthoquinone[b]	47.1	171	143, 115	10.75 (a)	7.52 (b)
Kaempferol pentoside[c]	55.2	417	285	73.36 (a)	59.70 (b)
Total				346.52 (a)	262.83 (b)

[a] Calculated using the calibration curve of ferulic acid at λ = 280 nm.
[b] Calculated using the calibration curve of juglone at λ = 240 nm.
[c] Calculated using the calibration curve of rutin at λ = 280 nm.

Finally, the compound at *m*/*z* 417 (retention time 55.2 min) showed a fragment at 285 *m*/*z*. Michodjehoun-Mestres et al. [38] reported the same fragmentation pattern for kaempferol pentoside and identified this compound in cashew apple.

The quantification was carried out after preparing the calibration curves. Three calibration curves were built: rutin, ferulic acid and juglone. The calibration curve of rutin at $\lambda = 280$ nm was used to quantify kaempferol pentoside; p-hydroxybenzoic, dihydroxybenzoic acid, benzoic acid, ethylprotocatechuate, ferulic acid were quantified using the calibration curve of ferulic acid at $\lambda = 280$ nm while naphthylacetic acid, 7-methyljuglone, abscisic acid, 1,4-dihydroxynaphthoate and 2-methyl-1,4-naphthoquinone were quantified using juglone at $\lambda = 240$ nm.

Table 4 summarizes the identified polar compounds and the quantification in the raw and roasted samples expressed as mg/kg oil.

Both raw and roasted cashew nut oil presented a high number of naphthoquinone derivates, compounds that have been identified previously in other nuts as walnuts [39,40]. Naphthoquinones have proved to possess cytotoxic effects against a wide range of microorganisms, including a variety of bacteria, filamentous bacteria, algae, and dermatophytes [41,42].

As far as we concerned, this is the first time that individual minor polar compounds have been identified in this matrix.

4. Conclusions

In this work three different classes of antioxidant compounds were studied in cold pressed cashew nut oils. The method for the determination of alkyl-phenols profile has been optimized on two different columns after two different TLC separations. The RtxTG65 column showed the higher resolution and the loss time analysis; however, the DB-5MS column was indispensable for the GC–MS analyses. Both raw and roasted cashew nut oils reported similar alkyl-phenols composition, although the roasting process increases the content of cardanols due to the decarboxylation of anacardic acid. Furthermore the roasting process also affects the tocopherol and minor polar compounds content.

As far as we concerned, this is the first time that a characterization of cold pressed cashew nut oil has been carried out. This matrix can be used in the future as common vegetable oil for the preservation of food due to its high content of alkyl-phenols and naphthoquinones. These compounds have been related to possess antimicrobial and cytotoxic effects against bacteria and to show high antioxidant power that allow to preserve the oil from oxidation.

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