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Costa Rican cashew (*Anacardium occidentale* L.): Essential oils, carotenoids and bromatological analysis

Jiménez Mata, Villegas-Castro, Granados-Chinchilla

Abstract
Considering cashew tree as economically relevant in Costa Rica, we extracted and characterized the essential oil from leaves and ripe cashew apple. Both oils were tested *in vitro* for antioxidant potential. The ripe cashew apple was also tested for carotenoid content. We performed nutritional analysis on the nutshell to assess its potential as a supplement for animal feed. Finally, the cashew nut'sfatty acid profile was also determined. Cashew tree leaves exhibited 2, 4-di-*tert*-butylphenol, 3-hydroxy-1, 3-diphenylpropan-1-one, and 5-methylpyrogallolin 14.14, 6.59, and 6.10 g/100 g, respectively while cashew apple volatile compounds included 2-hydroxy-4-methylvaleric acid, 1, 4-xylene, and 1-nonadeceneat relative concentrations of 13.89, 12.08, and 9.84 g/100 g, respectively. Cashew nut fat profile included oleic and linoleic acids as most prevalent with 56.26 and 14.50 g/100 g, respectively with monounsaturated fatty acids representing an average of (71.97 ± 1.49) g/100 g. Total carotenoids accounted for (2.93 ± 0.07) mg β-carotene/100 g fresh material. It was concluded that the pigment in cashews is imparted, by α-carotene and β-carotene representing 0.24 mg (8.2%) and 1.25 mg (42.5%) per 100 g sample, respectively. We also obtained average values of (2 291.2 ± 35.6) and (6 158.9 ± 136.8) µmol TE/100 g, for leaves and apple essential oils, respectively. Cashew outer shell exhibited a profile rich in fiber (NDF and ADF 38.46 and 27.67 g/100 g, respectively) and crude fat (16.00 g/100 g). Furthermore, we assessed the residue to input a 57.3% of digestible energy. We concluded essential oil to have antioxidant potential and the cashew nut shell to prove potential as a feed supplement.

Keywords: Cashew, anacardium occidentale l., leaves, fruit, receptacle, fatty acid profile, carotenoids, essential oils, animal feed

1. Introduction
Cashewis a tropical spreading, low-branched, medium-sized (may grow to a height of 5-8 m) evergreen tree [1, 2]. Has alternate oblong-, oval- or sometimes obovate-shaped leathery leaves, borne in terminal clusters that are pale green or reddish when young and become darker when maturing. The fruit is a grey or purple colored kidney-shaped nut consisting of a double-walled shell with a hard exo carp and a thin endocarp, and an edible kernel surrounded by a thin testa [1, 2]. The fruit does not split open at maturity, but once its fruit is fully grown but not ripe, its receptacle swells and becomes a fleshy, juicy, pear or apple-shaped edible hypocarp (or pseudo fruit) which possesses red or yellow coloration when mature [1, 2]. The kernel of the cashew nut, the pseudo fruit (cashew apple) and the leaves are edible [1, 2]. Harvesting areas of 2,742,167 ha have been reported, resulting in1, 600,002 metric ton cashew nut production, worldwide [3]. Cashew is economically relevant as its dry, roasted kernels are sold as a snack [4]. Some studies have been revolving cashew tree. For example, direct solvent extracted phytochemicals and nutritional analysis of cashew tree leaves have already been reported [5], and isolation of several phytochemicals (mostly phenols) have been isolated from cashew tree bark [6]. Several other biological activities have been reported for cashew structures [7] including antimicrobial [8], hypotensive and cardio-inhibitory [9], antioxidant [10]. Additionally, protein isolates and concentrates have been obtained from defatted cashew nut powder [11], and peptides with angiotensin-converting enzyme inhibitory have also been found [12]. Herein we report the extraction and characterization of the essential oil from leaves and ripe cashew apple (receptacle). Both oils were tested for *in vitro* antioxidant potential. The ripe cashew apple was tested for carotenoid content. As a way to repurpose a byproduct from cashew seed roasting processing, we performed nutritional analysis on the nutshell (seed's outer coating) to assess its potential as a supplement for animal feed. Finally, as the kernel is considered nutrient dense food [4, 11], we also determined its fatty acid profile. To our knowledge, cashew from Costa Rica has not been studied previously.

2. Materials and Methods

2.1 Sample collection sites

Cashew fruits are seasonal. Ripe cashew apples and nuts and leaves were collected in five different batches from January to February from trees grown along the central Pacific coast of the country (where it occurs more frequently, onaltitudes from 0 to 800 m, 9°54′3.54″N 84°31′46.98″W). In this region, during the recollection time, temperatures ranged from 22.0 to 32.0ºC, relative humidity varied78 to 81%, precipitation averaged from 29.0 to 57.6 mm with4 to 7 days of rain. Only whole leaves were collected. Mature fruits were collected directly from the tree when in season (between January and February).Specimens were identified and selected based on structural characteristics of their leaves and trunks by a biologist with botanical and taxonomical expertise and based on the guidelines previously described [5]. All samples were collected from adult trees and randomly from the tops. Each collection was formed by sampling five different specimens.

2.2 Leaves and cashew apple essential oil extraction

The essential oil was extracted by the process of steam distillation using an all-glass still and purified water. Briefly, crushed cashew apples and aerial parts of plant material (ca. 150 g in each case) were placed in a Clevenger type apparatus with 1000 mL flask, oil separator tube, and condenser. A total of 250 mL of purified water was added, and the mixture was vapor distilled (at 96°C at a rate of 20°C/minute and then kept at 96°C for 180 minutes) into a 125-mL Erlenmeyer, which was used to collect the aqueous distillate. The receiving receptacle was kept cold (0°C, using acetone-ice mixture) during the extent of distillation. Finally, liquid-liquid extraction was performed, with diethyl ether (309966, $(CH_3CH_2)_2O$, for HPLC, ≥ 99.9%, inhibitor-free, Sigma-Aldrich, St. Louis, Mo, USA) as the organic solvent, to recover volatiles. The organic fraction was dried in a rotatory evaporator until an oily substance (invariably, mixtures of volatile organic compounds) was obtained. Only ripened, healthy (without visible scarring), cashew apples were processed. In the samples of cashew apples, paraffin was added to avoid foaming of the non-volatile material in the flask during processing. Type III water with a final conductivity of <10 µScm-1 was obtained using a RiOS™ system (EMD Millipore, Billerica, MA, USA). Oil yields ranged between 0.13 and 0.74 mL oil per 100 grams fresh material; independent components were separated, identified, and quantified using gas chromatography (GC, Agilent 7820A) and mass-spectrometry (MS, Agilent 5977E mass spectrometer). The GC-MS system deployed was equipped with a J&W DBWAX microbore column of 10 m length, 0.1 mm diameter, 0.1 µm film thickness (Agilent Technologies). The carrier gas was helium at a constant flow of 0.3 mL min^{-1}. The column oven temperature was initially held at 50ºC for 0.34 min, then programmed to reach 200ºC at a rate increase of 72.51°C min^{-1} and held for 0.17 min and, a final temperature increment programmed to reach 230°C at a rate of 8.7°C min^{-1}, held for 7.9 minutes. The total run time was 13.93 min. The split ratio was adjusted at 30:1. The injector temperature was set at 250°C. The mass range was 50-450 m/z. Electron energy was set at 70 eV, 150°C, at positive polarity. Peaks were identified through comparison to spectra contained in the NIST library 14 (Scientific Instrument Services, Inc. ™, Ringoes, NJ, USA). Only hits with a match factor of 80% or above were considered. 2-Hydroxyisocaproic acid (99%, 219819, 3.26 min, M+ 132.1 m/z), 3-hydroxy-1,3-diphenylpropan-1-one(99%, 137731, 3.11 min, M+ 226.1 m/z), 1,4-xylene (≥ 99.5%, 95680, 1.34 min, M+ 107.0 m/z),1-nonadecene(≥ 97%, 284831, 4.19 min, M+ 266.1 m/z). Geraniol (≥99.0%, 48798, 5.85 min, M+ 154.1 m/z) was used as an internal standard. All reagents were acquiredfrom Sigma-Aldrich except 5-methylpyrogallol (Santa Cruz Biotechnology, sc-475292, M+ 140.0 m/z, Dallas, Texas, USA). An example chromatogram is shown (Figure 1A). Additionally, Kovats retention indices (RI) were calculated according to Van den Dool and Kratz [13] using as references a C_7 - C_{30} saturated alkanes (Sigma-Aldrich, 49451-U, 1 000 µg mL^{-1} each component in hexane, Table 1 and 2). Reference RI values for each compound were also tabulated when available. The pure solvent was injected and used as a blank and subtracted from the oil chromatograms to rule out artifacts.

Table 1: Essential oil composition obtained from cashew tree leaves

	Analyte	Mean ± SD (relative abundance, g/100 g)[a]	RI literature[b]	RI calculated[c]
	Major components			
1	2,4-di-*tert*-butylphenol	14.14 ± 2.47	2280	2277
2	3-Hydroxy-1,3-diphenylpropan-1-one	6.59 ± 1.84	-	1647
3	5-Methylpyrogallol	6.10 ± 0.78	-	1726
4	1,3-Xylene	5.91 ± 1.25	1132	1132
5	1-Nonadecene	5.71 ± 2.87	1938	1938
6	Methyl (*E*)-octadec-9-enoate	4.07 ± 0.68	2426	2420
7	1-Tridecene	3.75 ± 1.30	1337	1339
8	Methyl (*E*)-octadec-15-enoate	3.55 ± 0.18	-	2536
9	2-Phenylacetaldehyde	3.52 ± 0.47	1648	1649
10	1-Heptadecanol	3.13 ± 0.16	2451	2457
11	1-Docosene	2.96 ± 1.54	-	2219
12	1,4-Xylene	2.86 ± 1.95	1130	1133
13	(*E*)-5-Octadecene	2.46 ± 1.97	1811	1814
14	1-Undecanol	2.26 ± 0.80	1840	1836
15	6-Methylhept-5-en-2-one	1.80 ± 0.28	1341	1341
16	1-Octanol	1.78 ± 0.10	1565	1567
17	1-heptyl-2-methylcyclopropane	1.76 ± 0.13	-	1626
18	9-methyl-1-Decene	1.75 ± 0.28	-	1303
19	1-*O*-octadecyl 2-*O*-prop-2-enyl oxalate	1.54 ± 1.29	-	2992
20	2,6-bis(1,1-dimethylethyl)-4-hydroxy-4-methyl-2,5-cyclohexadien-1-one	1.44 ± 0.29	2117	2113
21	(*Z*)-Hex-3-en-1-ol	1.38 ± 0.46	1386	1387

22	2-(4-methoxyphenyl)propan-2-ol	1.34 ± 0.47	-	1626
23	Methyl Octadecanoate	1.25 ± 0.53	2419	2417
24	5,5-Dimethylhex-1-ene	1.21 ± 0.56	1204	1202
25	Cyclotetradecane	1.15 ± 0.06	2073	2074
26	Methyl hexadecanoate	1.14 ± 0.59	2202	2203
27	β-Caryophyllene	1.07 ± 0.12	1591	1590
28	(*E*)-pent-3-en-2-one	1.04 ± 0.30	1133	1130
29	1-Hexadecanol	0.99 ± 0.22	2363	2359
30	2,3 Pyridinedicarboxylic anhydride	0.95 ± 0.32	-	815
31	8-methyl-1-undecene	0.94 ± 0.33	1124	1123
32	Cyclopentyl 4-ethylbenzoate	0.85 ± 0.19	1835	1821
33	1-Decanol	0.85 ± 0.09	1760	1751
34	Nonylcyclopropane	0.84 ± 0.05	-	1075
35	Octyl propanoate	0.80 ± 0.25	1542	1540
36	Phenylmethanol	0.64 ± 0.05	-	1679
37	(*E*)-5-methylundec-4-ene	0.60 ± 0.08	-	1880
38	2-(4-methylcyclohexa-2,4-dien-1-yl)propan-2-ol	0.59 ± 0.13	1814	1810
39	1-Tridecyn-4-ol	0.57 ± 0.27	-	2494
40	3-Methoxypropane-1,2-diol	0.54 ± 0.23	-	1562
41	Bis(2-ethylhexyl) hexanedioate	0.46 ± 0.32	1892	1879
42	*N*-hydroxyacetamide	0.40 ± 0.49	-	1239
43	(*Z*)-3-Hexenyl benzoate	0.40 ± 0.39	2093	2081
44	3-Methylhex-1-ene	0.34 ± 0.08	-	1562
45	Oxalic acid, allyl hexadecyl ester	0.32 ± 0.09	-	1782
46	2-Methylbutan-1-ol	0.31 ± 0.04	1208	1203
47	5-methylidenetridecane	0.30 ± 0.11	-	940
48	1,1,3-Trimethylcyclopentane	0.28 ± 0.04	1219	1221
49	1-Methyl-2-(3-methylpentyl)cyclopropane	0.25 ± 0.08	-	1448
50	*N*-nitro-1-pentanamine	0.23 ± 0.06	1390	1384
51	1,3-dimethoxypropan-2-ol	0.22 ± 0.09	1403	1416
52	Octylcyclopropane	0.19 ± 0.11	-	794
53	Ethenyl-3-methyloxirane	0.15 ± 0.04	-	1001
54	Diphenylmethanone	0.15 ± 0.03	-	1313
55	1,1-dimethyl-cyclopentane	0.12 ± 0.05	869	866
56	1-methyl-cyclopentene	0.10 ± 0.12	782	770
		Total area sum, g/100 g		
Alkanes/Cycloalkanes		34.84 ± 1.88		
Carboxylic acids		34.57 ± 1.18		
Alcohols/Phenols		15.33 ± 1.56		
Ketones		11.02 ± 0.86		
Aldehydes		3.52 ± 0.20		

[a]Standard deviation (SD) reported as the result of the variation among five independent batches of leaves. [b]Kovats standard polar retention indices obtained from the NIST Standard Reference Database 1A v17. https://www.nist.gov/srd/nist-standard-reference-database-1a-v17. [c]Nonisothermal Kovats retention indices calculated as $RI_x = 100_n + 100(t_x\text{-}t_n)/(t_{n+1} - t_n)$.

Table 2: Essential oil profile and composition obtained from cashew apples

	Analyte	Mean ± SD (relative abundance, g/100 g)[a]	RI literature[b]	RI calculated[c]
Major compounds				
1	2-Hydroxy-4-methylvaleric acid	13.89 ± 0.63	-	2151
2	1,4-xylene	12.08 ± 1.30	1130	1137
3	1-Nonadecene	9.84 ± 4.89	1938	1942
4	1-Docosene	7.83 ± 4.24	-	2049
5	(*E*)-3-Octadecene	6.47 ± 2.03	1896	1904
6	2,4-Ditert-butyl-phenol	6.35 ± 0.39	2280	2291
7	(*E*)-5-Eicosene	6.20 ± 2.37	2047	2055
8	1-Tridecene	6.10 ± 2.75	1337	1338
9	Cyclotetradecane	1.89 ± 0.30	-	2249
10	Methyl 14-methylpentadecanoate	1.64 ± 0.47	2166	2166
11	1-Hexadecanol	1.63 ± 0.60	2363	2363
12	1-Decanol	1.54 ± 0.22	1760	1762
13	1-*O*-octadecyl 2-*O*-prop-2-enyl oxalate	1.54 ± 0.14	-	2393
14	1,1-Dimethylcyclopentane	1.53 ± 0.77	-	1506
15	1,1,3-Trimethylcyclopentane	1.44 ± 1.14	-	1481
16	Nonylcyclopropane	1.39 ± 0.85	-	861
17	Methyl (*Z*)-*N*-hydroxybenzenecarboximidate	1.34 ± 0.46	-	1184
18	2,3 Pyridinedicarboxylic anhydride	1.26 ± 0.57	-	653
19	3,4-Dimethylpentan-1-ol	1.26 ± 0.40	1412	1414
20	Methyl hexadecanoate	1.04 ± 0.05	2202	2204

21	3-Methoxypropane-1,2-diol	0.95 ± 0.20	-	1273
22	3,7-Dimethyloct-1-ene	0.92 ± 0.34	-	1044
23	2-methyloct-1-ene	0.92 ± 0.30	-	636
24	2-(1,2,4-triazol-1-yl) ethanol	0.90 ± 0.36	-	1481
25	Hexanedioic acid bis (2-ethylhexyl) ester	0.86 ± 0.17	1892	1893
26	5-methyl-(triazolylethanol)-1-hexanol	0.86 ± 0.06	1442	1438
27	Methyl octadecanoate	0.83 ± 0.28	2419	2419
28	2-Methylbutan-1-ol	0.80 ± 0.36	1208	1205
29	1-(3,4-dihydro-2H-pyrrol-5-yl) ethenone	0.72 ± 0.32	-	1273
30	Pentadecanal	0.69 ± 0.06	2016	2019
31	[(*Z*)-dodec-9-enyl] acetate	0.64 ± 0.40	1986	1988
32	2,2,4-Trimethylpentane	0.58 ± 0.24	698	698
33	4-Methylhexan-1-ol	0.47 ± 0.06	1414	1410
34	6-Methylhept-1-ene	0.44 ± 0.07	-	1044
35	(3S)-3,4-dimethylpentan-1-ol	0.43 ± 0.07	1412	1414
36	(*Z*)-dodec-2-en-1-ol	0.36 ± 0.17	-	1642
37	Octylcyclopropane	0.33 ± 0.18	-	636
38	4-methylhexan-2-ol	0.32 ± 0.22	-	819
39	2,2,3-trimethylcyclobutan-1-one	0.26 ± 0.08	-	1252
40	(*E*)-2,2-dimethyldec-3-ene	0.22 ± 0.05	-	1731
41	1-Hexanol	0.21 ± 0.12	1360	1360
42	*N*-hydroxyacetamide	0.21 ± 0.12	-	1413
43	(*Z*)-3-Hexen-1-ol	0.20 ± 0.05	1386	1376
44	3-hydroxybutan-2-one	0.18 ± 0.08	1289	1296
45	4,4-Dimethylpent-1-ene	0.18 ± 0.08	-	1672
46	(*Z*)-2-Butene-1,4-diol	0.15 ± 0.07	-	1667
47	1,3-Dimethoxypropan-2-ol	0.14 ± 0.12	-	942
		Total area sum, g/100 g		
Alkanes/Cycloalkanes		59.90 ± 3.35		
Carboxylic acids		22.66 ± 1.47		
Alcohols/Phenols		16.67 ± 1.27		
Ketones		1.16 ± 0.05		

[a]Standard deviation (SD) reported as the result of the variation among five independent batches of cashew apples.[b]Kovats standard polar retention indices obtained from the NIST Standard Reference Database 1A v17. https://www.nist.gov/srd/nist-standard-reference-database-1a-v17.[c]Non-isothermal Kovats retention indices calculated as $RI_x = 100_n + 100(t_x\text{-}t_n)/(t_{n+1} - t_n)$.

2.3 Cashew nut fatty acid profiling

Twenty-five nuts per batch (five different batches in total) were mechanically pressed using an oil expeller (PITEBA, Scheemb derzwaag, Scheemda, The Netherlands). Twenty µL of the resulting oil was diluted 100-fold using diethyl ether, after mild methanolysis, using an organic catalyst (tetramethyl ammonium hydroxide, 334901, 25 wt. % solution in methanol, Sigma-Aldrich, St. Louis, MO, USA), the resulting methyl-ester fatty acids were separated, identified, and quantified using gas chromatography using the conditions mentioned at 2.2.Tetradecanoic (6.16min; M+ 227.6 m/z), pentadecanoic (6.72 min; M+ 243.4 m/z), hexadecenoic (7.58 min; M+ 256.3 m/z), octadecanoic (9.70 min; M+ 285.5 m/z), 9*Z*-octadecenoic (7.78 min; M+ 284.1 m/z), and (*Z*,*Z*)-9,12-octadecadienoic (10.86 min; M+ 280.0 m/z) acids from Nu-Chek Prep (Elysian, MN, USA) were used as standards. An example chromatogram is shown (Figure 1B).

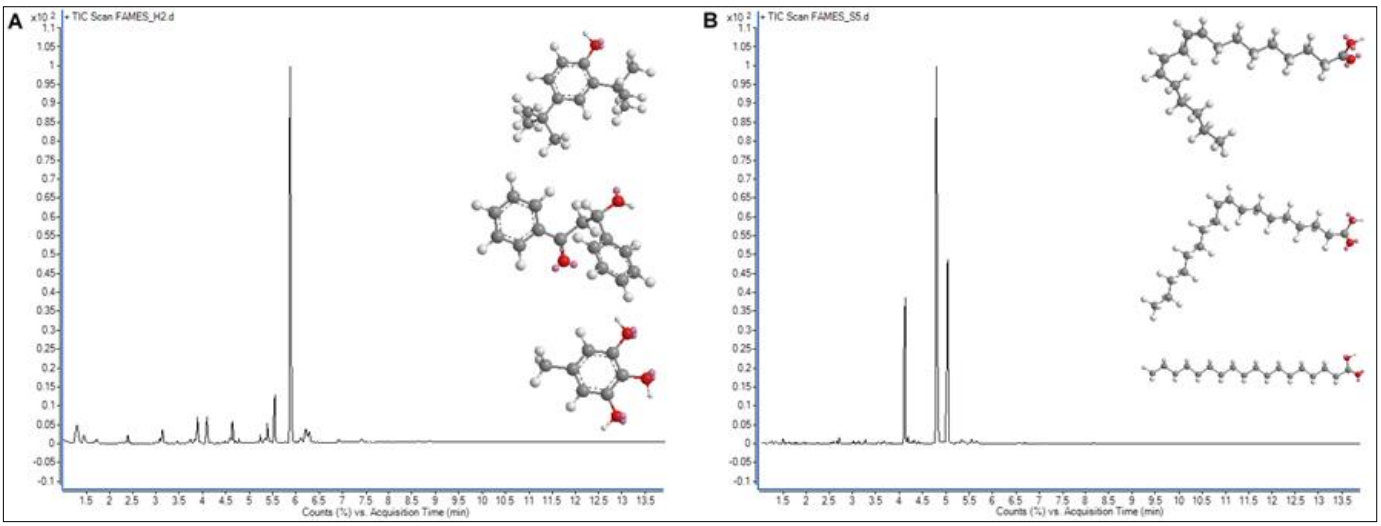

Fig 1: Example chromatograms of **A**. Fatty acid profile from cashew nut **B**. Essential oil (volatile) profile of cashew tree leaves. MM2 minimized 3D structures of the most abundant compounds found are shown for each panel

2.4 Total carotenoid content and HPLC carotenoid identification assay.

Carotene assays were performed according to Biehler and coworkers [14] with some modifications. Briefly, 5g of a thawed sample weighed into a 50-mL high-density polyethylene centrifuge tube (BD Biosciences, CA, and USA). Then, fivemL ofmethanol (chromatographic grade, J.T. Baker, Avantor Materials, PA, and USA) and 1 g magnesium carbonate (USP, M7179, Sigma-Aldrich, and St. Louis, MO, USA) were added. The mixture was forced into contact and homogenized using a digital Ultra-turrax® at 18 000 rpm (T25, IKA®WerkeGmbH& Co. KG, Staufen im Breisgau, Germany) during 1-3 min. Later, mixtures were incubated for 15min on ice; samples were centrifuged(Thermo Scientific ™ Sorvall ™ ST 16R Thermo Fisher Scientific, Inc. Waltham, MA, USA) at 10ºC for 5 min at 2500 × *g*. The supernatant was decanted into another 50-mL centrifuge tube, extraction was repeated twice with eight mL of a mixture of hexane: acetone (1:1, both chromatographic grade, J.T. Baker, Avantor Materials, PA, and USA) and organic fractions were combined. To the combined extracts, 25 mL of saturated aqueous sodium chloride (ACS grade, 1064045000, Sigma-Aldrich, St. Louis, MO, USA) solution was added, and the mixture was shaken. The supernatant hexane phase was transferred into a 50-mL centrifuge tube, and the lower aqueous phase was re extracted with eight mL of hexane and combined with the 1st extract. Hexane extracts were weighed exactly for volume determination. A 5-mL aliquot was then pipette from the combined extracts into a 12-mL glass vial, evaporated to dryness under vacuum at 10ºC (Centrivap, LABCONCO, Kansas City, MO, USA), and purged with argon (ultra-high purity, 99.999%, Praxair, Danbury, CT, USA), and sealed.

2.4.1 Spectrophotometric analyses

Dried extracts were reconstituted in 1 to 10 mL of hexane and sonicated for 2 min. Absorbance values are measured at 450 nm using a 1-mL quartz cuvette in a UV/Visible spectrophotometer (Shimadzu Pharmaspec UV-1700); concentrations achieved by comparing against a 0.5 to 10 mg β-carotene L^{-1} 7-point standard calibration curve.

2.4.2 Chromatographic analyses

All samples were stored at −70ºC until HPLC analysis; dried extracts were re dissolved using one mL MTBE and transferred to an HPLC 2 mL capacity vial. Chromatographic separation was achieved using a 150 mm x 4.6 mm, 5 µm analytical column (YMC Co. Ltd., Carotenoid C30, Kyoto Prefecture, Japan) and a solvent system that included MeOH (solvent A) and MTBE.A modular HPLC system (Shimadzu Prominence, Shimadzu Corporation, Kyoto, Kyoto Prefecture, Japan) equipped with a degasser (DGU-20A5), quaternary pump (LC-20AT), an autosampler (SIL-20A HT), a system controller (CBM-20A), a column oven (CTO-20A), and photodiode array detector (SPD-M20AV) was used for analysis. Chromatographic data management was performed using LC Solutions (Version. 5.2). Gradient elution was set as follow: 0-5 min 80% A (5 min), 5-7 min 73% A, 7-15 min 62.5% A, 15-20 62,5 A, 20-30 min 45% A, 30-35 10% A, 35-40 10% A, 40-45 min 80% A. Solvent flow and column compartment temperature, detector wavelength and sample injection volume were kept constant during elution at 0.6 mL min^{-1}, 30°C, 450 nm (using 472 nm as reference wavelength), and 3 µL, respectively.

2.5 Lipophilic $ORAC_{FL}$ assay

The determinationwas performed according to prior and co-workers [15]. Briefly, ten µL leaves and cashew apple essential oil was dissolved in 25 µL of acetone and then diluted with 65 µL of a seven g/100 g water and acetone (1:1) solution of randomly methylated β-cyclodextrin. All aliquots are mixed in a black flat-bottomed polystyrene 96-well micro liter plates (Thermo Scientific™ Nunc™ FluoroNunc™, Roskilde, Denmark). Forty µL of fluorescein solution was added by injectors in the micro plate reader, followed by 150 µL of 2, 2′-azobis (2-amidino-propane) dihydrochloride (17.2 mg mL^{-1}, 9.4 µmol $well^{-1}$); readings were initiated immediately. Fluorescence was measured with a Synergy™Biotek HT microplate reader at λ_{ex}= 485 nm and λ_{em}= 520 nm andthe Gen 5™software (BioTek Instruments Inc., Winooski, VT, USA).Analyses were performed in triplicate.

2.6 Proximate analysis of cashew nutshell

Dry matter (DM, loss of drying/moisture), crude protein (CP), fat (EE), fiber (CF), and ash, as well as calcium, phosphorus, neutral detergent fiber (NDF), Acid detergent fiber (ADF), lignin, and gross energy assayswere performed to assess the nutritional quality of each of the animal by-products meals collected. All tests were performed using ISO 17025 accredited methods based on AOAC 930.15, 990.03, 920.39, 962.09, 942.05, 968.08/975.03/985.35, 965.17/986.24, 935.13, 2002.04, 973.18 and ISO 9831:1998 respectively. Neutral and aciddetergent insoluble nitrogen (NDIN/ADIN) were determined as previously described [16].

3. Results and Discussion

3.1 Characterization of cashew tree leaves and apples essential oil

Few articles have described volatile components of cashew leaves or apple reports. Experiments have been conducted and reported from Brazil [17, 18], and Venezuela [19] with compounds such as palmitic/oleic acid, ethyl 2-hydroxy-4-methylpentanoate, and car-3-ene recounted for pseudo fruits, respectively. In our case, cashew tree leaves exhibited 2, 4-di-*tert*-butylphenol, 3-hydroxy-1, 3-diphenylpropan-1-one, and 5-methylpyrogallolin 14.14, 6.59, and 6.10 g/100 g, respectively. On a previous species, we already discussed the presence of 2, 4-di-*tert*-butylphenol in tree leaves [20]. However, in this case, cashew as an evergreen tree, the compound may be related to stress and UV injury as it grows near the coasts where temperatures are relatively high. Additionally, 3-hydroxy-1, 3-diphenylpropan-1-one may be responsible for some of the leaves yellow-tones coloration. As this is naturally a highly resinous tree, some of these aromatic compounds may be used as building blocks for gum-resins, then, the pyrogallol related compound may be a precursor for more complex plant structures such as hydrolyzabletannins [21]. Further, chalcone analogs have been described previously in plants as a response to different stimuli and have several described physiological functions [22]. On another hand, cashew apple volatile compounds included 2-hydroxy-4-methylvaleric acid,1,4-xylene, and 1-nonadeceneat relative concentrations of 13.89, 12.08, and 9.84 g/100 g, respectively.2-Hydroxy-4-methylvaleric acid has been isolated previously from wine [23], and a similar compound was also isolated from cashew apple [17]. Also, recently, 1, 4-xylene has been isolated from *Chrysophyllum albidum* G. Don [24]. Interestingly, 1-nonadecene has been described recently as a component of the solvent extracts from *Zygophyllum coccineum* L. leaves [25] and *Terminalia travancorensis* Wight

&Arn bark [25].

The sum of the four most abundant components represented 32.74% in tree leaves and 43.64% for cashew apple essential oil (Figure 2 A, B), and 96.3% of the fatty acid profile from mechanically extracted cashew nut oil (Figure 2 C).

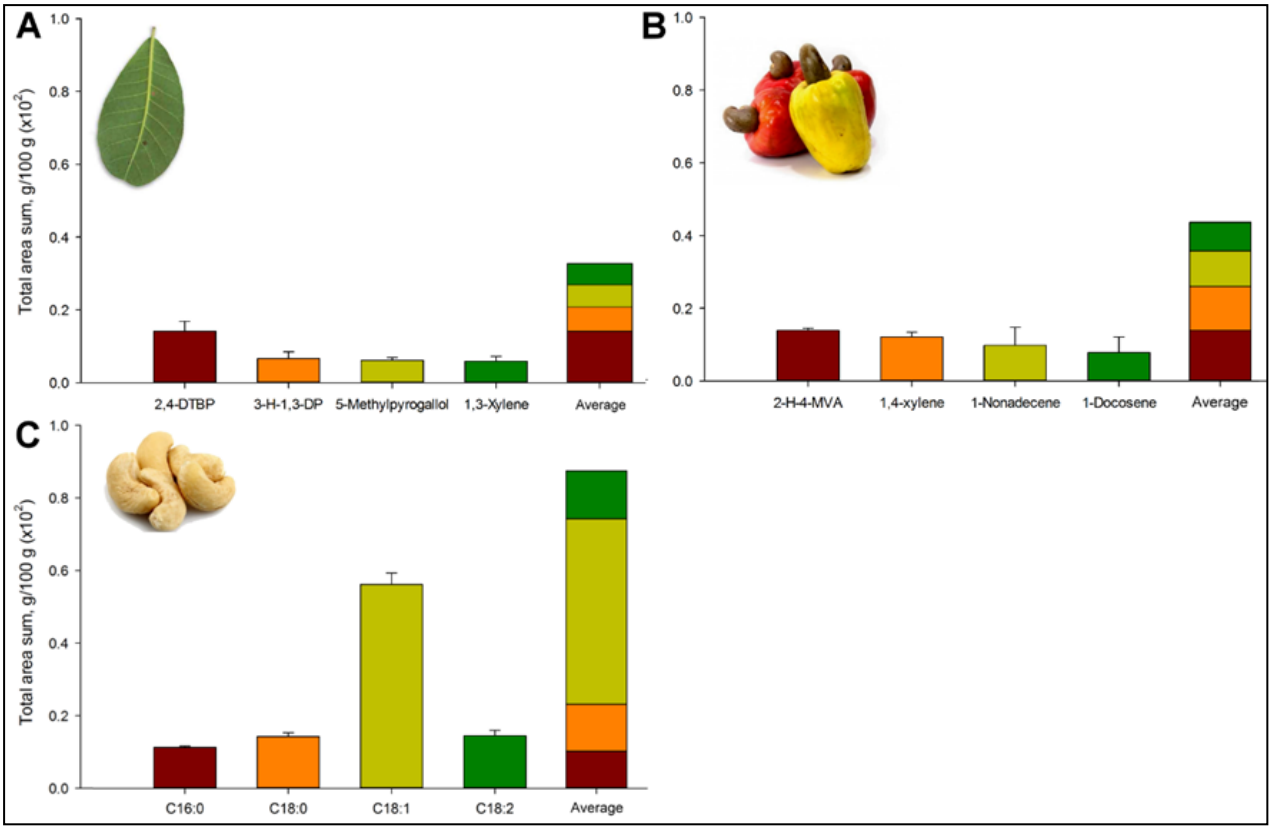

Fig 2: Distribution of the four most abundant compounds found in A. cashew tree leaves essential oil B. cashew apple essential oil and C. fatty acid profile from mechanically extracted cashew nut oil.Key:2,4-di-*tert*-butylphenol (2,4-DTBP), 3-hydroxy-1,3-diphenylpropan-1-one (3-H-1,3-DP), 2-hydroxy-4-methylvaleric acid (2-H-4-MVA)

3.2 Fat fatty profile of the cashew nut

We obtained a profile that included oleic and linoleic acids as most prevalent with 56.26 and 14.50 g/100 g, respectively. Monounsaturated fatty acids represent an average of 71.97 ± 1.49 (Table 3). These values are in line with those obtained previously [27]. The fatty acid composition containinga combination of palmitic ($C_{16:0}$) and oleic acids ($C_{18:1}$), hint toward a potential use of cashew nut oil as long chain fatty acid supplement for dairy cows [28].

Table 3: Fatty acid profile analysis of fresh cashew seeds

Fatty acid[a] (shorthand nomenclature)	Mean ± SD (total area sum, g/100 g)
$C_{18:1}$	56.26 ± 3.14
$C_{18:2}$	14.50 ± 1.48
$C_{18:0}$	14.28 ± 1.05
$C_{16:0}$	11.26 ± 0.41
Sum of saturated fatty acids	26.69 ± 1.13
Sum of monounsaturated fatty acids (MUFA)	71.97 ± 1.49
Sum of polyunsaturated fatty acids (PUFA)	1.34 ± 0.29

[a]Identified compounds with a total area sum ≤ 1 g/100 g also included $C_{7:0}$, $C_{8:0}$, $C_{9:0}$, 9:0-diacid, $C_{10:0}$, 9c-$C_{12:1}$,$C_{14:0}$, 11c-$C_{16:1}$, $C_{17:0}$, $C_{20:0}$, $C_{22:0}$ and $C_{24:0}$.

3.3 Carotenes

Total carotenoids were measured on three independent batches of colored cashew apples, (2.93 ± 0.07) mg β-carotene/100 g fresh material. Additionally, HPLC carotenoid analysis show six major pigment signals evidencing that, for the most part, the pigment in cashews is imparted, in fact, by α-carotene (retention time of 14.351, figure 3) and β-carotene (retention time of 17.111, figure 3) representing 0.24 mg (8.2%) and 1.25 mg (42.5%)per 100 g sample, respectively. This data is in line with carotenoid contents for another Anacardiaceae genus [29, 30]. β-Carotene esters can be observed24 to 28 min region (Figure 3). However, no conclusive identification was achieved for these signals. Other spectrometric approaches (e.g., NMR, MS) may be applied in the future, to characterize said components.

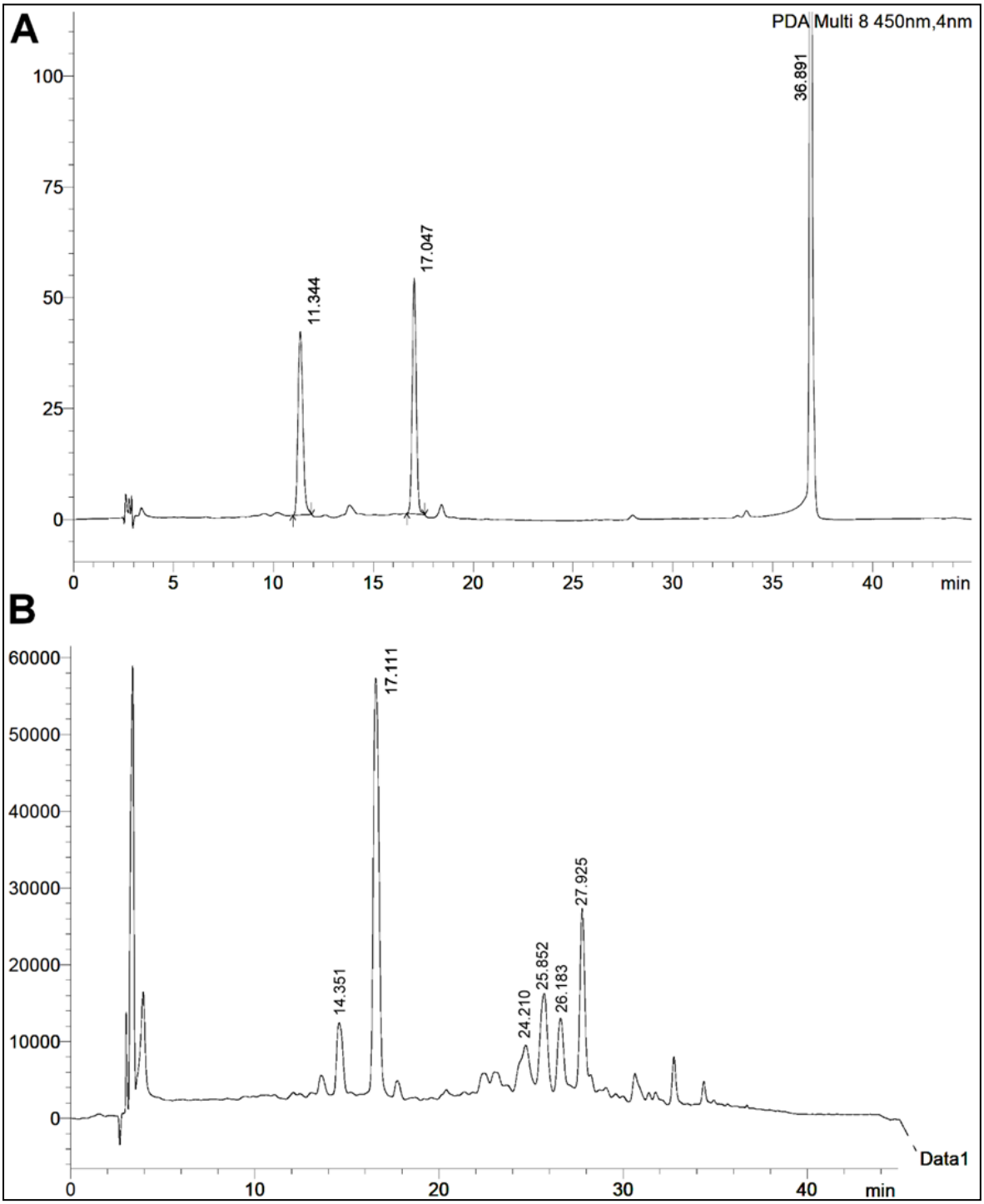

Fig 3: Chromatographs of **A**.Chloroform diluted standards of β-cryptoxanthin (R_t = 11.344 min), β-carotene (R_t = 17.047 min) and lycopene (R_t = 36.891 min). **B**. Cashew apple chloroform extract exhibiting α-carotene (R_t = 14.351 min), β-carotene (R_t = 17.111 min, area sum 8.20%), unknown signals at 24.201 (area sum 7.46%), 25.825 (area sum 11.94%), 26.183 (area sum 8.95%) and 27.925 min (area sum 20.89%)

3.4 Cashew apple and leaves essential oil antioxidant potential

Based on the compounds found during the profiling, we used a lipophilic ORAC using Trolox equivalents as a suggestive assay to assess both cashew apple and leaves essential oil potential for radical scavenging capabilities; we obtained average values of (2 291.2 ± 35.6) and (6 158.9 ± 136.8) µmol TE/100 g, respectively. Cashew apple has already been reported as a cellular mediated and direct scavenging potential [10]. Aromatic and phenol-based structures found in the leaves' oil account for the high *in vitro* potential for radical scavenging.

3.5 Cashew outer shell, potential as animal feed

As expected nutshell exhibits a profile rich in fiber (NDF and ADF 38.46 and 27.67 g/100 g, respectively). Also, crude fat content is considerable, i.e., 16.00 g/100 g (Table 4). The average energy of the cashew residue that is digestible corresponds to 57.3%, being moderately useful. Cashew nut shell is a remnant available in the country which operation units needed for processing are few, which means that meal production from this residue is cost-effective, thus making its use asanimal feed, especially ruminants, a feasible option. Cashewnutshell meal could be combined with other residues or forages to supplement the diet of the animal. However, an additional the elimination of the irritant anacardic acid/cashew

nut shell liquid may be necessary before hand. In this regard, other studies have included cashew nut shell liquid [31] and cashew apple essential oil into feed as an additive [32, 33] successfully. Cashew bagasse and pulp has already been considered as a feed ingredient for ruminants [34], and cashew nut testa has been considered as pig feed [35]. Using an evaluation of ration balancing system (National Research Council (NRC) Nutrient Requirements [36]), we concluded that a maximum of 4 kg (ration inclusion of ca. 8.4%) of cashew nut outer shell can be incorporated to a dairy cattle's diet if an animal of 450 kg with daily milk production of 16 kg per day and a ration that included 20 kg silage, 1.5 kg citrus pulp, 6 kg compound feed, 1 kg molasses and 10 kg star grass [*Cynodon dactylon* (L.) Pers.] are considered.

Table 4: Proximate analysis and bromatological data for cashew nutshell

Assay	Concentration, g/100 g[a]
Proximate analysis	
Crude fat	16.00 ± 0.24
Dry matter/Loss on drying	93.73 ± 0.30
Crude protein	5.31 ± 0.26
Crude ash	1.19 ± 0.08
Fiber and protein fractionation	
Neutral detergent fiber (NDF)	38.46 ± 2.07
Acid detergent fiber (ADF)	27.67 ± 1.55
Lignin	3.91 ± 0.61
Neutral detergent nitrogen (NDIN)	0.42 ± 0.06
Acid detergent nitrogen (ADIN)	0.29 ± 0.05
Mineral	
Calcium (mg kg^{-1})	533.01 ± 101.22
Phosphorus (mg kg^{-1})	415.23 ± 39.14
Energy input	
Gross energy (kJ kg^{-1})	4 760.51 ± 355.74

[a]Standard deviation (SD) reported as the result of the variation among five independent batches of cashew nut shells.

4. Conclusion

Essential oils fromleavesand cashew apple demonstrated the presence of phenolic and aromatic compounds and hence demonstrate a potential for radical scavenging, other applications or biological activities may further be investigated. As a colored fleshy product, cashew has a good source of provitamin a, which may improve its antioxidant potential drastically. Cashew nutshell is the residue left after the kernel has been removed for toasting and future consumption or oil extraction, we demonstrated that the residue has the potential of being repurposed. Fatty acid from nut oil has an excellent profile and the potential both as an animal feed supplement and oil for human consumption especially as it is highly monounsaturated. We contribute new comparative data on a tree that is common in the coastline of Costa Rica.

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