Supercritical Carbon Dioxide and Solvent Extraction of the Phenolic Lipids of Cashew Nut (*Anacardium occidentale*) Shells

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Supercritical carbon dioxide extraction of raw cashew nut shells yielded 18.7% by weight phenolic lipids in 17.5 h. Composition of the cashew nut shell liquid (CNSL) thus obtained was quantitatively analyzed using high-performance reversed-phase liquid chromatography. The analytical data were compared with those of solvent-extracted CNSL from India (both raw cashew nuts and steam-processed cashew nuts), Brazil, Kenya, and Mozambique. Indian CNSL is richer (by about 10%) in anacardic acids, and extraction by supercritical carbon dioxide yields a better quality product. Steam-processed cashew nut shells retain about 80% of the anacardic acids.

INTRODUCTION

Cashew nut shell liquid (CNSL) is an important agricultural byproduct of cashew nut (*Anacardium occidentale* L.; Anacardiaceae) production. The potential annual availability of this renewable material, which forms about 24% of the whole nut or about 32% of the shell, is roughly equal to the yield of the edible kernel itself and is estimated to be over 65 000 ton in India alone (on the basis of 1988–1989 production figures). The other important cashew nut producing countries are Brazil, Mozambique, Tanzania, and Kenya. Industrial applications of the CNSL-based products are numerous and include brake linings, paints and primers, foundry chemicals, lacquers, cements, and speciality coatings (Menon et al., 1985). Lately, the biological activities of the CNSL components have attracted considerable attention in the areas of, for example, molluscicidal activity (Kubo et al., 1986), antitumor activity (Itokawa et al., 1987), prostaglandin synthase inhibition (Kubo et al., 1987), and, most recently, antimicrobial activity (Himejima and Kubo, 1991). Much of the biological activity is attributed to the anacardic acids (1–4; Figure 1), which are the major constituents of the cashew nut shells, along with cardols (5–8). However, the major constituents of the technical CNSL, as it is obtained in the current industrial practice of roasting or hot oil bath methods (Tyman, 1980), are the cardols and cardanols (9–12).

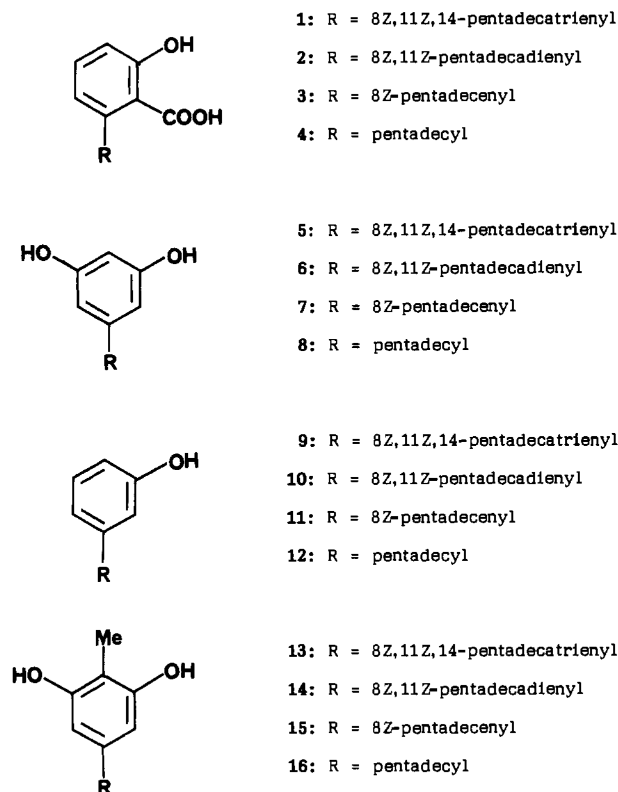

Figure 1. Structures of the phenolic lipids of CNSL.

The cardanols are obviously formed by thermal decarboxylation of the anacardic acids, and when required, anacardic acids are extracted by manual or mechanical shelling and solvent extraction of the shells. A technologically viable process, occasionally used in India, involves softening of the whole nuts by high-pressure steam (2–3 bar, 30 min) and cutting open the shells into halves; cashew nut shells thus obtained could possibly retain much of the valuable anacardic acids, but no analysis of the CNSL from the steam-processed cashew nut shells is available.

The extraction and chemical composition of the CNSL have been the subject of extensive investigation by Tyman et al. (1984, 1989), who concluded that while the efficiency of extraction (time and amount of solvent required) may vary, cold static extraction with diethyl ether or Soxhlet extraction with petroleum ether gave identical yield of CNSL. They have also noted considerable geographical variation in the composition of CNSL from the South American and African countries (see Table I). The present investigation is undertaken mainly for three reasons: (a) to determine the composition of the Indian cashew nut shells (which has not been done before); (b) to determine the changes occurring during steam-processing; and (c) to obtain the true composition of the natural CNSL by supercritical carbon dioxide extraction of the raw cashew nut shells. The last objective was necessitated by the facts that (a) anacardic acids are highly susceptible to decarboxylation to cardanols and the presence of cardanols in the natural CNSL has not been conclusively proved and (b) the presence of a large proportion of unsaturated lipids (particularly the 1*Z*,4*Z* dienes and 1*Z*,4*Z*,7*Z* trienes) indicated the possibility of autoxidation (Gunstone, 1986) during extraction and, to ascertain the true composition of the cashew nut shells, an extraction process was required that minimized the use of heat, light, and exposure to oxygen during extraction and removal of the solvent. The most favored method for

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Table I. Percentage Composition of the Phenolic Lipids in CNSL from Different Sources

structure	*a*	*b*	*c*	*d*	*e*	*f*	*g*
1	30.60	30.80	20.50		30.02	27.17	27.26
2	12.60	13.60	12.82		12.72	11.01	11.18
3	25.80	28.50	24.10		16.98	23.15	22.85
4	0.71				0.96	1.21	1.27
total 1–4	69.71	72.90	57.42		60.68	62.54	62.56
5	10.20	13.20	11.38	12.20	17.26	13.76	14.28
6	3.50	4.20	5.60	3.78	5.68	4.51	4.24
7	0.80	0.28	3.70	1.90	1.90	2.37	2.29
total 5–7	14.50	17.68	20.68	17.88	24.84	20.64	20.81
9	1.60	1.61	2.38	26.60	2.33	2.48	2.94
10	0.71		1.20	11.60	1.25	1.17	1.27
11	1.15	1.50	3.10	19.40	1.13	2.04	1.95
total 9–11	3.46	3.11	6.68	57.60	4.71	5.96	6.16

[a] Supercritical carbon dioxide extract of raw cashew nut shells. [b] Pentane extract of raw cashew nut shells. [c] Pentane extract of steamed cashew nut shells. [d] Indian technical CNSL. [e] Brazilian CNSL [from Tyman et al. (1984)]. [f] Kenyan CNSL [from Tyman et al. (1984)]. [g] Mozambiquan CNSL [from Tyman et al. (1984)].

such purposes, especially for lipids, is supercritical fluid extraction using carbon dioxide (McHugh and Krukonis, 1986).

MATERIALS AND METHODS

Cashew Nut Shells. Raw, unshelled cashew nuts were collected from the trees grown in the Visakhapatnam district (India) during January 1990. Prior to extraction, the whole cashew nuts were placed in liquid nitrogen when they cracked. The shells were then broken by light hammering and separated from the kernel and testa. Steam-processed cashew nut shells and technical CNSL were kindly supplied by K. V. Subraya Kamath and Sons, Mangalore, India.

Solvent Extraction. Solvent extraction was carried out by placing the freshly broken shells (50 g) in an Erlenmeyer flask and covering them with the pentane (100 mL). The extract was filtered off after 12 h, and the shells were again covered with the solvent. Five such extracts were combined and evaporated on rotary evaporator under reduced pressure, below 30 °C. Pentane used for the extraction was obtained by fractionating commercial pentane using a packed column and collecting the fraction distilling at 32 °C.

Supercritical Fluid Extraction. A Nova-Swiss 1.2 Ex supercritical fluid extractor, equipped with temperature, pressure, and flow regulators and monitors, was used in the study. Carbon dioxide, maintained at 40 °C and 250 bar, was used for the extraction; the flow rate of carbon dioxide was 4–5 kg/h. Freshly broken cashew nut shells (300 g) were charged into the extractor; the extract was collected every 2.5 h, and the extraction was continued for 17.5 h.

High-Performance Liquid Chromatography. The HPLC instrument used was a modular system consisting of two Waters 510 reciprocating pumps, a Waters U6K loop injector, an LKB variable-wavelength monitor at 280 nm, and a Waters 820 FC system controller and data processor. The mobile-phase systems used were (a) acetontrile–water–acetic acid (66:33:1) and (b) acetonitrile–water–acetic acid (80:20:1), both at 1.80 mL/min. A Waters Novapak C_{18} column (4.6 mm × 15 cm, packed with 4 μm of octadecylsilica) was used for the analysis. The plate count (n) was determined using acenaphthene (monitored at 254 nm) and acetonitrile–water (1:1) at a flow rate of 1.5 mL/min according to the formula

$$n = 5.54(t_R/w_h)^2$$

where t_R was the retention time and w_h the peak width at half-height.

Qualitative Analysis. Cardol (15:0) was used as internal standard. The cardol fraction (2 g; mixture of 5–7) obtained by solvent extraction and chromatography, essentially as described by Kubo et al. (1986), was hydrogenated in ethyl acetate (50 mL) for 4 h over 10% palladium–carbon (200 mg) using a Parr hydrogenator. The catalyst was filtered off and the solvent evaporated under vacuum to yield cardol (15:0), which was recrystallized from pentane. Anacardic acid (15:0) and cardanol (15:0) were similarly obtained. Waters Maxima 820 software was used for determination of the area percentage and quantitation.

Each analysis was carried out by taking an aliquot (25 mg) of the extract and adding the internal standard (5 mg). The mixture was dissolved in acetonitrile (1 mL) and passed through a C_{18} Sep-Pak cartridge (Waters Associates, Milford, MA). The Sep-Pak cartridge was eluted with a further 3 mL of acetonitrile, and the combined eluant was made up to 5 mL in a volumetric flask. The injection was 5 μL.

The linearity of the detector response (area units per microgram) was established by injecting five different concentrations of cardol (15:0) from 2 to 10 μg; the stability of the response factors of anacardic acid (15:0) and cardanol (15:0) relative to cardol (15:0) was similarly established, varying the analyte-to-standard ratio from 0.5 to 2.0. The response factors were determined from the linear graphs obtained in the usual manner. Each of the analytical results considered was the mean of three experimental values.

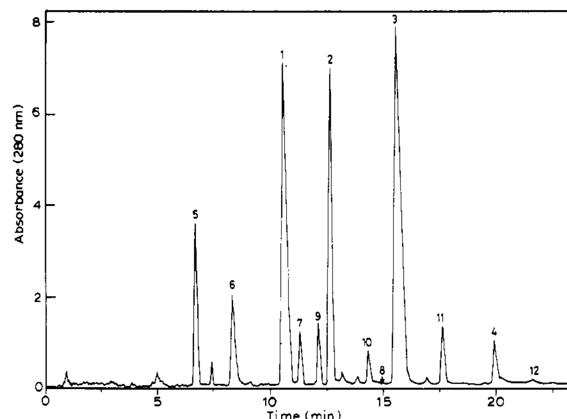

Figure 2. HPLC of the phenolic lipids of CNSL using Novapak C_{18} column and acetonitrile–water–acetic acid (66:33:1).

RESULTS AND DISCUSSION

Qualitative and quantitative analysis of CNSL has been the subject of detailed investigation by Tyman et al. (1984), who describe the high-performance liquid chromatography (HPLC) analysis using an octadecylsilyl-bonded stationary phase (5 μm of Mangnusphere and Spherisorb, packed in a 4.6 mm × 250 mm column), acetonitrile–water–acetic acid (64:33:2) as the mobile phase at 2.7 mL/min, and *p-tert*-butylphenol as the internal standard. The drawbacks of the cited procedure are that (a) each analysis required about an hour and (b) the wide differences in the retention times as well as detector responses (relative molar response factors) between the fast-eluting internal standard and the late-eluting analytes are potential sources of error. In the present study, we used a 4.6 × 150 mm (Waters Novapak C_{18}) column packed with 4 μm of octadecasilyl-bonded silica that generated 12 500 plates; the result is a fast analysis (about 20 min) and superior resolution. Figure 2 is the chromatogram obtained using nearly the same mobile phase as described by Tyman et al. (mobile-phase system a). It is increasingly recognized that the chromatographic characteristics of commercial stationary phases vary considerably from one brand to another. The need to optimize the stationary phase in reversed-phase liquid chromatography and the desirability of noting the plate count (using a standard method) in papers dealing with chromatographic separation are thus advocated.

For quantitative analysis of the large number of samples, it was convenient to use mobile-phase system b as it

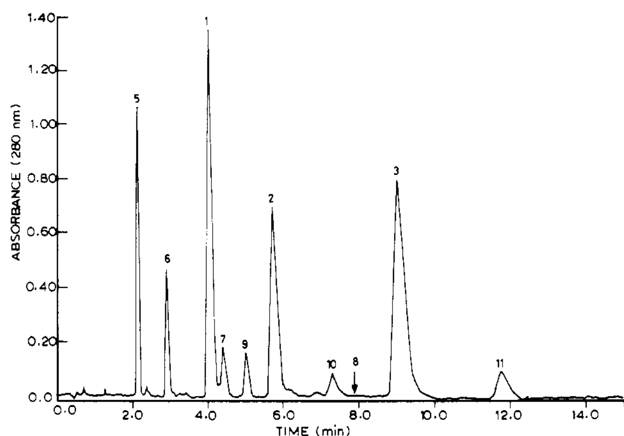

Figure 3. HPLC of the phenolic lipids of CNSL using Novapak C_{18} column and acetonitrile–water–acetic acid (80:20:1).

enabled faster analysis (12.5 min each) with adequate resolution of all the analytes (Figure 3); the last peak (t_R = 17.5 min) was absent in most samples, as shown by qualitative HPLC analysis. Cardol (15:0) (8) was preferred as the internal standard for the following reasons: (a) its retention time (7.75 min) was midway between the various peaks; (b) since it belongs to the same chemical class, its detector response was expected to be comparable with that of the CNSL constituents; and (c) it is not present in the natural CNSL to any significant extent (see Figures 2 and 3). A detector setting of 280 nm was found convenient as all the analytes had adequate absorbance at this wavelength. The relative response factor of anacardic acid (15:0) to cardol (15:0) was 0.97 and that of cardanol (15:0) to cardol (15:0) was 1.09. As it was difficult to obtain each of the analytes in a pure form for accurate determination of the response factors, the detector responses of the 15:0 analogues were taken as equivalent to those of 15:1, 15:2, and 15:3 enomers. This appeared reasonable as the groups of compounds have the same chromophore and comparable molecular weights. The CNSL is also known to contain 2-methylcardols (**13–16**), which have been ignored in the present study since their concentration is insignificantly low.

One of the main features of interest in the supercritical carbon dioxide extraction is the possibility of selective extraction and fractionation. The extracts were, therefore, withdrawn at 2.5-h intervals, weighed, and analyzed. The extraction rate was maximum between 5 and 10 h, the extraction efficiency rapidly tapering off after that time (Figure 4); the extract from 300 g of cashew nut shells was 56 g (18.7%) in 17.5 h, beyond which time the extraction rate was very slow. Though the yield was only about 60% of that obtainable by other methods (Tyman et al., 1989), the product was nearly colorless. Further extraction of the cashew nut shells with pentane yielded about 30 g (10%) more of the CNSL of nearly the same composition, although highly colored. It may be noted that the major problem hampering several of the industrial applications of CNSL is the very dark brown color of the solvent-extracted product; several attempts to obtain a colorless product are recorded in the patent literature (Tyman, 1980).

Each fraction of the supercritical carbon dioxide extract was analyzed by HPLC. The relative proportion of the enomers in each group was very similar, but the ratio of total cardols, cardanols, and anacardic acids varied appreciably, indicating selectivity of the extraction (Figure 5). Thus, much of the cardanols was extracted in the first two fractions (0–5 h) which contained much less of the

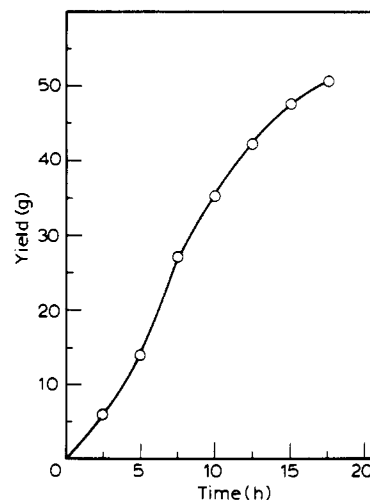

Figure 4. Supercritical carbon dioxide extraction profile of raw cashew nut shells.

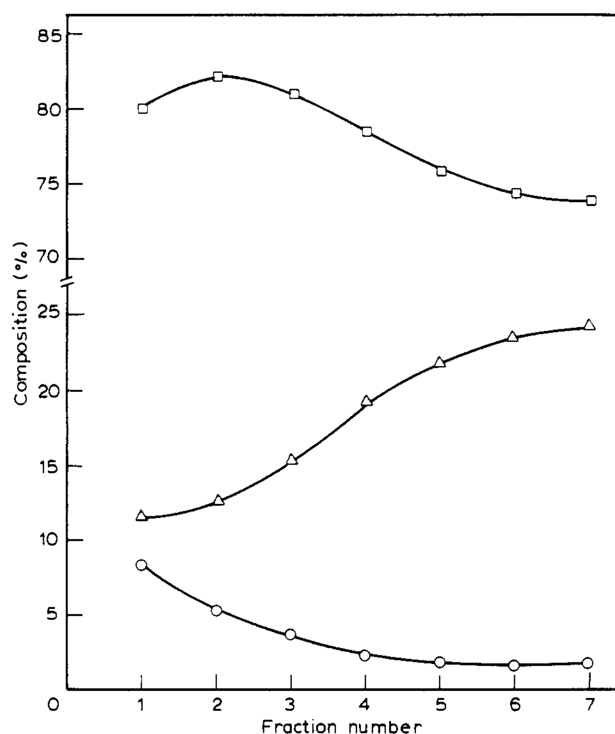

Figure 5. Selectivity of extraction of anacardic acids (□), cardols (△), and cardanols (○) from cashew nut shells by supercritical carbon dioxide.

cardols; the cardol proportion increased significantly beyond 7.5 h. However, for the purpose of comparison with other CNSL samples, the composition of the total extract could be considered.

Table I gives the composition of Indian CNSL obtained by (a) supercritical carbon dioxide extraction, (b) direct pentane extraction of raw cashew nut shells, (c) pentane extraction of steam-processed cashew nut shells, and (d) the composition of technical Indian CNSL. Also included in the table are the values recorded for the CNSL samples from Brazil, Kenya, and Mozambique (Tyman et al., 1984). An examination of the table would lead to the conclusion that while the compositions of the supercritical carbon dioxide and pentane extracted CNSL from the raw cashew nut shells were comparable, there is a considerable decrease in the proportion of anacardic acids (particularly the 15:3 enomer) in the steam-processed cashew nut shells and an expected increase in the proportion of the cardanols.

Interestingly, there is an increase in the proportion of cardols in the CNSL from both solvent-extracted and steam-processed cashew nut shells, suggesting the presence of labile compounds (possibly 4-hydroxyanacardic acids) in the cashew nut shells, which could yield cardols during steam-processing and/or solvent extraction. Also revealed in the table is the fact that the natural CNSL from Indian cashew nuts is richer in anacardic acids than the corresponding products from Brazil, Kenya, and Mozambique.

It may be noted that the 10 compounds considered here account for about 90% of the CNSL. The unaccounted portion is considered to be polymeric (Tyman et al., 1984) but may also be due to nonphenolic lipids in the supercritical carbon dioxide and pentane extracted CNSL from the raw cashew nut shells. Further work to identify the nonphenolic lipids and to confirm the presence of hydroxyanacardic acids in the cashew nut shells is in progress.

NOMENCLATURE

Anacardic acid, cardol, and cardanol are the common names of 2-hydroxy-6-pentadecylbenzoic acid, 5-pentadecylresorcinol, and 3-pentadecylphenol, respectively. While there is no accepted nomenclature for the individual unsaturated analogues, 15:1, 15:2 and 15:3 are suffixed in brackets following the names of the parent compounds for the mono-, di-, and trienes (see Figure 1) by analogy with commonly used fatty acid nomenclature.

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