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CHEMICAL PROFILE BY GC-MS OF THE UNSAPONIFIABLE FRACTION OF THE FAT FROM THE KERNELS OF *ANACARDIUM OCCIDENTALE* L. FROM CÔTE D'IVOIRE

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ABSTRACT

Objective: Despite the importance of cashew nut production in Côte d'Ivoire, data on the chemical composition of the unsaponifiable fraction of the fat in *Anacardium occidentale* L. kernels remain limited. The aim of this study was to determine the unsaponifiable content and characterize its chemical profile by gas chromatography-mass spectrometry (GC-MS).

Methodology: Fat extracted from cashew kernels from Bouaké, Côte d'Ivoire, was saponified with a KOH solution, and the unsaponifiable fraction was then extracted with diethyl ether. After derivatization, the constituents were analyzed by GC-MS and identified based on their retention indices and mass spectra, compared to data from NIST spectral libraries.

Results: The unsaponifiable fraction was 1.583 ± 0.003% of the fat content. Chromatographic analysis identified several constituents, primarily belonging to the phytosterol, terpene, and diterpene alcohol families. The main compounds identified were stigmastan-5-en-3-ol (25.07%), stigmasterol (17.87%), stigmasta-3,5-diene (10.05%), geranylgeraniol (3.90%), and phytol (1.15%).

Conclusion : Various minor compounds present in the unsaponifiable fraction of cashew nut fat were studied. This characterization provides a foundation for a better understanding and nutritional and cosmetic valorization of cashew nuts from Côte d'Ivoire.

Keywords: *Anacardium occidentale* L.; Cashew nut; Fat; Unsaponifiable fraction; Phytosterols; GC-MS; Côte d'Ivoire.

1 INTRODUCTION

Vegetable fats are complex matrices primarily composed of triacylglycerols, along with minor compounds found mainly in the unsaponifiable fraction. This fraction generally represents a small proportion of the fat, but it exhibits significant structural diversity and includes phytosterols, triterpenes, diterpenes, terpene alcohols, tocopherols, and hydrocarbons. These constituents contribute to the nutritional, functional, and biological properties of vegetable oils and are generating increasing interest in the food, cosmetics, and plant resource valorization sectors (1,2,3,4). Among the constituents of the unsaponifiable fraction, phytosterols occupy a special place due to their nutritional value and numerous biological properties reported in the literature (5,6). β-Sitosterol, campesterol, and stigmasterol are among the most frequently found phytosterols in vegetable oils. Other minor compounds, such as tocopherols, terpenes, and certain terpene alcohols, also contribute to the functional value and stability of vegetable fats. Characterizing these compounds is therefore crucial for assessing the quality and potential for valorizing oilseed resources.

Anacardium occidentale L. (Anacardiaceae), commonly known as cashew, is a species widely cultivated in tropical regions and constitutes an important agricultural and economic resource in Côte d'Ivoire. Its kernels are particularly rich in fat and are widely used for human consumption. Several studies have investigated the lipid composition and minor constituents of the fat in cashew kernels from different regions of the world. Ryan et al. (2006) (7) notably reported the presence of phytosterols, squalene, and tocopherols in cashew oil. Gomez-Caravaca et al. (2010) (8) also characterized the minor compounds in cold-pressed cashew nut oil. Other studies have highlighted the predominant presence of β-sitosterol as well as variability in phytosterol composition depending on geographical origin and extraction conditions (9,10). However, while the overall composition of cashew oil and some of its phytosterols are relatively well documented, data specifically dedicated to the chemical profile of the unsaponifiable fraction of the fat in A. occidentale kernels from Côte d'Ivoire remain limited. Detailed characterization of this fraction could contribute to a better understanding of the minor constituents of this oilseed resource and to the identification of compounds with potential applications. GC-MS, particularly after derivatization of the relevant constituents, is a particularly suitable approach for profiling the volatile and semi-volatile compounds of the unsaponifiable fraction.

In this context, the present study aimed to determine the unsaponifiable fraction content of the fat in *Anacardium occidentale* L. kernels from Bouaké, Côte d'Ivoire, and to establish its chemical profile using GC-MS.

2 MATERIAL AND METHODS

2.1 Plant Material and Fat Studied

The study material consisted of cashew nut kernels (*Anacardium occidentale* L.) from Bouaké, Côte d'Ivoire. The kernels were used as the raw material for fat extraction.

The fat, obtained from the kernels using a Soxhlet extractor, constituted the matrix used for the extraction and characterization of the unsaponifiable fraction. A representative sample of this fat is shown in Figure 1.

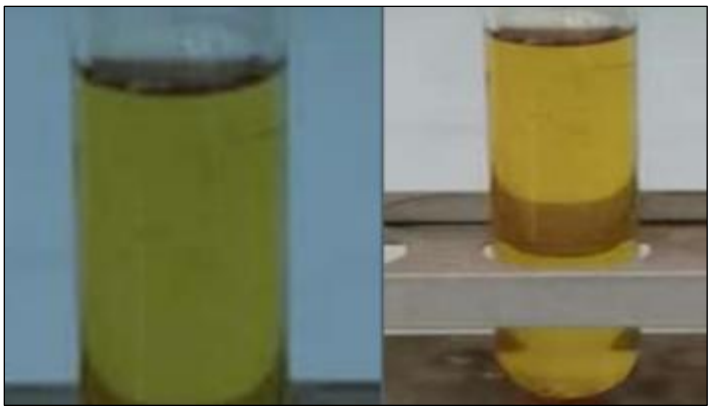

Figure 1: Fat extracted from the kernels of *Anacardium occidentale* L.

2.2 Extraction of the Unsaponifiable Fraction

The unsaponifiable fraction was extracted using a method adapted from Bamba et al. (2015) (11). A mass of 5.0 g of fat was introduced into a flask equipped with a reflux condenser. Saponification was carried out by adding a 2 N KOH solution. The reaction mixture was boiled under reflux for 1 h to allow hydrolysis of the saponifiable components. After cooling to room temperature, 100 mL of distilled water was added to the reaction mixture. The mixture was then transferred to a separatory funnel and extracted with 50 mL of diethyl ether (Et_2O). After decantation, the aqueous soapy phase was separated from the organic phase containing the unsaponifiable components. The diethyl ether extraction was repeated twice under the same conditions. The organic phases were combined and then washed successively with distilled water until a neutral pH was reached, in order to remove any alkaline residues and soaps that might be present. The organic phase was then dried over anhydrous sodium sulfate (Na_2SO_4). After filtration, the solvent was removed under reduced pressure using a rotary evaporator. The resulting residue corresponded to the unsaponifiable fraction, which was retained until chromatographic analysis.

The unsaponifiable fraction content was determined from the initial mass of fat used (m_2 in g) and the mass of unsaponifiable fraction obtained after extraction and evaporation of the solvent (m_1 in g). It was calculated using the following formula:

$$\textbf{Ins}\ (\%) = \left(\frac{\mathbf{m_1}}{\mathbf{m_2}}\right) \times \mathbf{100}$$

The results were expressed as a percentage of the fat analyzed.

2.3 Thin-Layer Chromatography (TLC) Analysis

A preliminary analysis of the unsaponifiable fraction was performed by TLC to obtain a qualitative fingerprint of the main families of compounds present. The unsaponifiable fraction was dissolved in 1 mL of hexane. A 2 µL volume of this solution was spot-deposited onto 60 F_{254} silica gel plates on an aluminum support (Merck). The spots were deposited approximately 1 cm from the edges of the plate, with a regular spacing of approximately 1 cm between spots. Chromatographic migration was performed with a hexane/ethyl acetate mixture (4:0.5; v/v) as the mobile phase. After migration, the chromatograms were examined directly under visible light and under ultraviolet radiation at 366 nm. Some plates were then developed using Liebermann-Bürchard and Godin reagents to highlight different families of metabolites. The distances traveled by the various spots and by the solvent front were measured, and then the retention factors (Rf) were calculated according to the equation:

$$Rf = \frac{\text{distance traveled by the compound}}{\text{distance traveled by the solvent front}}$$

The Rf values and colors observed after development were used as guidelines for identifying the main constituent families of the unsaponifiable fraction.

2.4 Preparation of the unsaponifiable fraction for GC/MS analysis

Prior to chromatographic analysis, the unsaponifiable fraction underwent derivatization by acetylation to improve the chromatographic properties of constituents containing hydroxyl groups. A 1 mg sample of the unsaponifiable fraction was treated with 2 mL of a pyridine/acetic anhydride mixture (1:1; v/v) at 70 °C for 45 min.

2.5 GC/MS Analysis of the Unsaponifiable Fraction

The qualitative analysis of the derivatized unsaponifiable fraction was performed by GC-MS, according to a method adapted from Bamba et al. (2015) (11). The analyses were carried out using an Agilent Technologies 6890N chromatograph coupled to an Agilent Technologies 5973 mass spectrometer. The chromatographic separation was performed on a fused silica capillary column measuring 30 m × 0.25 mm internal diameter × 0.25 µm film thickness. Helium (He) was used as the carrier gas at a flow rate of 1 mL/min. The furnace temperature program was as follows: an initial temperature of 50 °C maintained for 5 min, followed by a ramp of 3 °C/min up to 250 °C, and then held at 250 °C for 15 min. The injector and source/detector temperatures, depending on the exact instrument configuration, were 230 °C and 250 °C, respectively. The sample was injected in split mode. Mass spectrometry was performed with an ionization energy of 70 eV. Constituents were identified based on comparison of their retention indices (RI) and mass spectra with those of reference compounds available in the NIST Mass Spectral Library, associated with the GC-MS system. The relative composition of the identified constituents was expressed as a percentage of their relative area relative to the total area of the corresponding chromatogram.

2.6 Data Processing

Chromatographic data were processed using appropriate software associated with the GC-MS system. Results relating to the unsaponifiable fraction content and the proportions of identified constituents were organized and processed using Microsoft Excel 2016.

Unsaponifiable fraction results were expressed as mean ± standard deviation when multiple independent determinations were performed.

3 RESULTS AND DISCUSSION

3.1 Unsaponifiable Fraction Content

Saponification of the fat extracted from the kernels of *Anacardium occidentale* L. yielded an unsaponifiable fraction of 1.583 ± 0.003% of the fat studied (Table 1). This value indicates that, although a minor component compared to the saponifiable constituents of the fat, the unsaponifiable fraction represents a measurable proportion and constitutes a source of compounds belonging to different chemical families. The value obtained is of the same order of magnitude as that reported by Toschi et al. (1993) (12) for an *Anacardium occidentale* L. oil from Italy, whose unsaponifiable content was approximately 1.40%. This similarity suggests that the unsaponifiable content of cashew fat can fall within a relatively comparable range depending on the origin of the raw material and the extraction conditions. However, the unsaponifiable content can be influenced by several factors, including geographical origin, variety or genotype, degree of almond maturity, storage conditions, and the oil extraction process. Therefore, comparisons of this value with other cashew oils should be interpreted with caution when experimental conditions differ.

Table 1: Unsaponifiable fraction content of the oil content of *A. occidentale* L. almonds.

Species	***Anacardium occidentale* L.**
m_2 (g)	4.988
m_1 (g)	0.079
Content (%)	1.583 ± 0.003

*m_1: mass of the unsaponifiable fraction; m_2: mass of the fat

3.2 Chromatographic Profile of the Unsaponifiable Fraction by Thin-layer chromatography (TLC)

TLC analysis provided a chromatographic fingerprint of the unsaponifiable fraction of the fat from *Anacardium occidentale* L. With the hexane/ethyl acetate system (4:0.5; v/v), several spots were observed at different retention factors (Rf), indicating the presence of constituents with different polarities. Visualization with the Liebermann-Bürchard reagent revealed several spots associated with profiles characteristic of sterols and triterpenes. Similarly, visualization with Godin's reagent highlighted several constituents belonging primarily to the terpene, triterpene, and sterol families.

Table 2: TLC Profile of the Unsaponifiable Fraction Revealed by the Liebermann-Bürchard Reagent

Rf		**Color**	**Compounds**
Visible	**UV 366**		
	***A. occidentale* L.**		
0.7		Green	Sterol and Triterpene
	0. 65	Yellow	Sterol
0.58	0.58	Blue	Steroid
0.40	0.40	Green	Sterols
0.29	0.29	Yellow	Steroid
0.25		Green	Triterpene

Table 3: TLC profile of the unsaponifiable fraction revealed by Godin's reagent

Rf			**Color**		**Compounds**
Visible		**UV 366**			
	***A. occidentale* L.**		***A. occidentale* L.**		
	0.93			Violet	Polyterpene
			0.80	Blue	Triterpene
	0.65		0.66	Yellow	Trterpene
	0.40			Blue	Sterol
			0.25	Green	Triterpene
	0.17		0.17	Brown	Triterpene Genin

These observations are consistent with the expected nature of an unsaponifiable fraction of vegetable fat, generally composed of compounds such as phytosterols, triterpenes, diterpenes, terpene alcohols, and hydrocarbons. The diversity of spots observed by TLC thus suggests a chemically heterogeneous composition of the fraction studied.

However, TLC is essentially a qualitative screening method. Rf values and color reactions help guide the identification of chemical families, but do not, on their own, allow for the definitive establishment of the structural identity of individual compounds. Therefore, more precise characterization was performed by GC-MS.

3.3 Chemical profile of the unsaponifiable fraction of *A. occidentale* L

Analysis of the derivatized unsaponifiable fraction by GC-MS yielded a chromatogram with several peaks, reflecting the diversity of constituents present in this fraction (Figure 2).

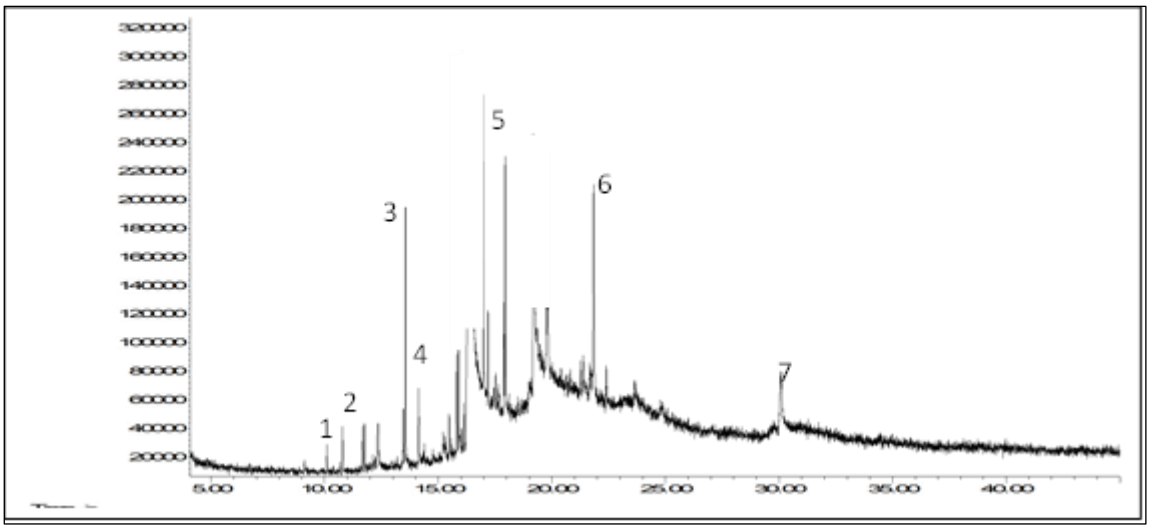

Figure 2: GC-MS of the unsaponifiable fraction of almond fat *Anacardium occidentale* L.

Seven major or characteristic constituents were identified based on their retention index and mass spectra. They belong primarily to the phytosterol, terpene alcohol, terpene, and hydrocarbon families.

Table 4: Main constituents identified in the unsaponifiable fraction

Pic	TR (min)	Compounds	Content (%)
1	10.80	Phytol	1.15
2	11.69	Géranylgeraniol	3.90
3	13.53	Octadecane	15.90
4	14.10	3,7-diméthyl-6-nonen-1-ol	5.24
5	18.06	Stigmastan-5-en-3-ol	25.07
6	21.90	Stigmasterol	17.87
7	30.00	Stigmasta-3,5-diene	10.05

3.3.1 Predominance of Phytosterols

The results show a significant presence of stigmastan-5-en-3-ol, stigmasterol, and stigmasta-3,5-diene, which are the main compounds identified in the analyzed fraction. Stigmastan-5-en-3-ol, corresponding to β-sitosterol, has the highest relative abundance among the identified compounds, at 25.07%, followed by stigmasterol (17.87%) and stigmasta-3,5-diene (10.05%). The predominance of β-sitosterol in the unsaponifiable fraction is consistent with data reported in the literature on cashew nut fats. Ryan et al. (2006) (7) notably reported the presence of phytosterols in cashew oil, while subsequent work confirmed the predominance of β-sitosterol among the sterols in this matrix. This observation has also been reported for cashew kernels from different geographical regions. The presence of stigmasterol is also an interesting finding. This phytosterol is commonly found in vegetable fats and can contribute, along with β-sitosterol and other sterols, to the composition of the unsaponifiable fraction. However, it is important to distinguish the relative abundance determined by GC-MS from the actual content in mg/kg. The percentages presented in this work correspond to the relative proportions of the constituents detected and identified by chromatographic analysis and should not be interpreted directly as absolute concentrations.

3.3.2 Presence of stigmasta-3,5-diene

Stigmata-3,5-diene, representing 10.05% of the identified constituents, warrants particular attention. This compound is a sterolic hydrocarbon that can be associated with the transformation or thermal dehydration of phytosterols, particularly β-sitosterol, under certain technological conditions. Its presence in the analyzed fraction could therefore be linked, at least in part, to the conditions of fat processing or sample preparation. However, this hypothesis cannot be confirmed based solely on the data obtained in this study. Further analyses would be necessary to determine the exact origin of this compound.

3.3.3 Terpene Alcohols and Other Constituents

In addition to phytosterols, GC-MS analysis revealed several terpene compounds or terpenoids. Phytol, detected at 1.15%, is a diterpene alcohol associated in particular with the phytol chain of chlorophyll. Its presence in plant matrices and oils may be linked to the degradation or transformation of chlorophyll pigments. Geranylgeraniol, representing 3.90%, also belongs to the diterpene alcohol family. The simultaneous presence of these compounds and phytosterols confirms the observations obtained by TLC and shows that the unsaponifiable fraction is not limited to sterols alone. Octadecane (15.90%) and 3,7-dimethyl-6-nonen-1-ol (5.24%) complete the profile of identified constituents. The presence of octadecane shows in particular that the analyzed fraction also contains aliphatic hydrocarbons.

Thus, the GC-MS profile reveals a structural diversity in the constituents of the unsaponifiable fraction, dominated by sterols but also including terpene alcohols and hydrocarbons.

3.4 Identification of compounds based on mass spectra

The mass spectra obtained allowed for the characterization of the main constituents based on their molecular ions and characteristic fragments (Table 5).

Table 5: Main spectral data of the compounds identified in the unsaponifiable fraction

Pic	TR (min)	%	Molecular formula	Molar mass (g / mol)	Fragmentation, m/z (%)	Identified compounds
1	10.80	1.15	$C_{20}H_{40}O$	296	296 $[C_{20}H_{40}O]^+$, 278 (18) $[M-H_2O]^+$, 123 $[C_9H_{15}]^{+\bullet}$, 71 $[C_5H_{11}]^{+\bullet}$, 57 $[C_4H_9]^{+\bullet}$, 263 $[M- H_2O- CH_3]^{+\bullet}$	Phytol
2	11.69	3.90	$C_{20}H_{34}O$	290	290 $[C_{20}H_{34}O]$+, 275 (15) [M-CH3]+, 257 [M-33] $^{+\bullet}$, 245 [M-45] $^{+\bullet}$, 231 [M-59] $^{+\bullet}$, 205 [M-85] $^{+\bullet}$, 177 [M-113] $^{+\bullet}$, 163 [M-127] $^{+\bullet}$	Géranylgeraniol
5	18.06	25.07	$C_{29}H_{50}O$	414	414 (1) $[C_{29}H_{50}O]^{\bullet+}$, 396 (18) $[M-H_2O]^+$, 381 [M-33]+, 329 [M-85]+, 255 [M-159]+, 145 [M-269]+, 81 [M-333]+,69 [M-345] $^{\bullet+}$	Stigmastan-5-en-3-ol
6	21.90	17.87	$C_{29}H_{48}O$	412	412 $[C_{29}H_{48}O]^{\bullet+}$, 394 (18) $[M-H_2O]^+$, 327 [M-85] $^{\bullet+}$, 327 (85) $[M- C_6H_{13}]^+$, 255 (157) $[M-C_9H_{17}+Cycle]^+$, 211 [M-201]+	Stigmasterol
7	30.00	10.05	$C_{29}H_{48}$	396	396 (1) [M] $^{\bullet+}$, 381 $[M-CH_3]^{\bullet+}$, 367 [M-C2H5] $^{\bullet+}$, 353 [M-	Stigmasta-3,5-diene

					C3H7] $^{\bullet+}$, 339 [M-C4H9] $^{\bullet+}$, 325 [M-C5H11] $^{\bullet+}$	

For phytol, the molecular ion observed at m/z 296 is consistent with the molecular formula $C_{20}H_{40}O$, while the fragment resulting from the loss of a water molecule at m/z 278 is consistent with the presence of a hydroxyl group. Geranylgeraniol has a molecular ion at m/z 290, corresponding to the formula $C_{20}H_{34}O$. Its spectrum shows several characteristic fragments resulting from cleavages of the terpene chain. Stigmastan-5-en-3-ol (β-sitosterol) has a molecular ion at m/z 414, while the fragment at m/z 396, corresponding to the loss of a water molecule, is consistent with the presence of a hydroxyl group. Stigmasterol has a molecular ion at m/z 412, consistent with its molecular formula $C_{29}H_{48}O$. Finally, stigmasta-3,5-diene has a molecular ion at m/z 396, corresponding to the formula $C_{29}H_{48}$.

These spectral data are consistent with the structures proposed for the identified compounds (Figure 3).

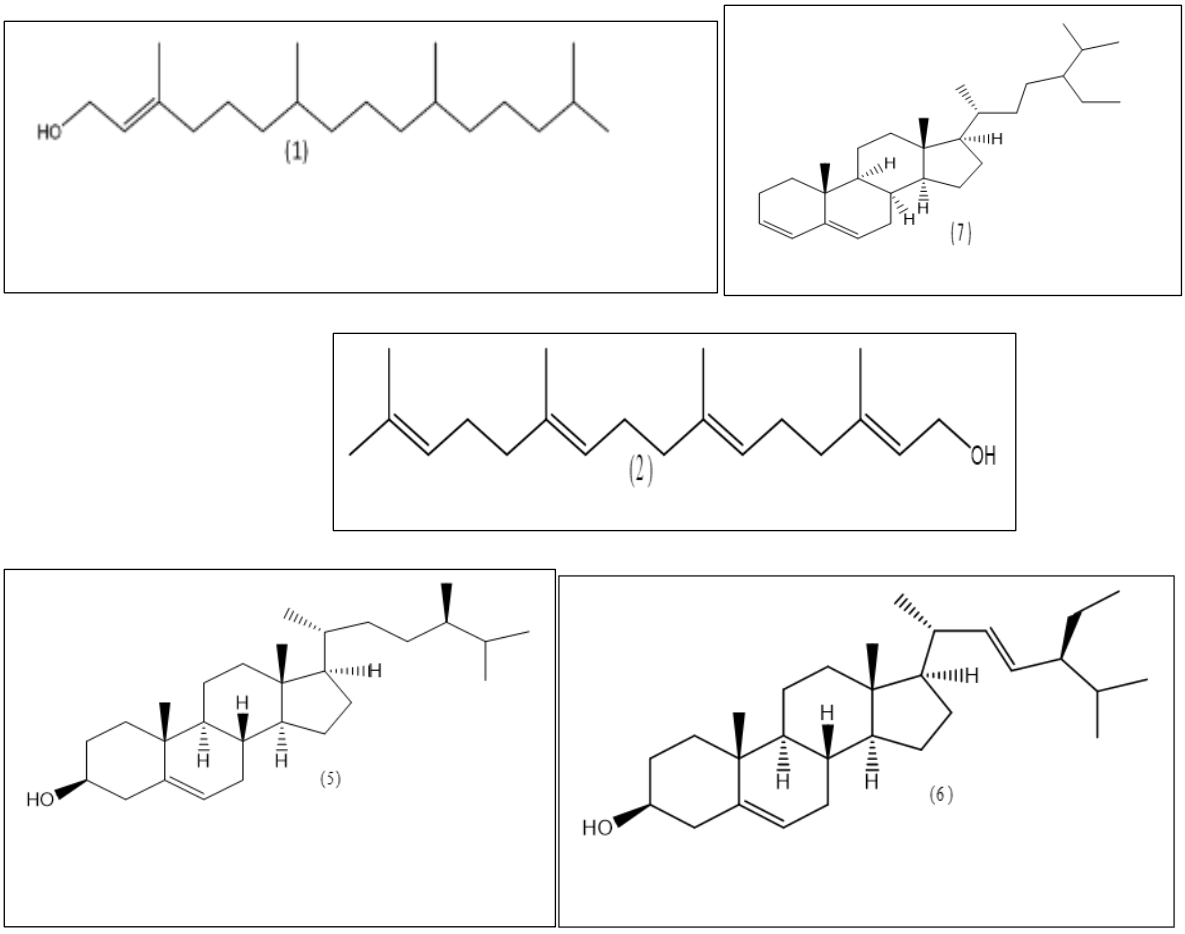

Figure 3: Structure of some phytoconstituents of the unsaponifiable fraction of *A. occidentale* L.

3.5 Importance of the unsaponifiable fraction for cashew nut valorization

The predominant presence of phytosterols in the studied fraction is of interest from the perspective of cashew kernel valorization. Phytosterols are natural constituents of plants and are widely studied for their nutritional role, particularly their ability to reduce intestinal cholesterol absorption when consumed in sufficient quantities as part of the diet (13). Previous studies have also reported various biological properties associated with phytosterols, including anti-inflammatory effects and other biological activities (14, 15, 16). However, these properties cannot be directly attributed to the fraction studied in this work, since no biological testing was conducted.

The identified terpene constituents, such as phytol and geranylgeraniol, also show potential interest due to their presence in various plant matrices and their applications being studied in the food, cosmetic, and pharmaceutical sectors (17, 18, 19).

Thus, the results obtained suggest that the unsaponifiable fraction of the fat in *Anacardium occidentale* L. kernels constitutes a source of compounds of interest, particularly phytosterols. However, further investigations are needed before considering specific applications.

4 CONCLUSION

The unsaponifiable fraction of the fat in the studied *Anacardium occidentale* L. kernels exhibits a diverse composition dominated by phytosterols. These results contribute to the chemical characterization of cashew kernels produced in Côte d'Ivoire and highlight their potential as a source of compounds of nutritional and cosmetic interest. Further studies focusing on the individual quantification of the major compounds and the evaluation of their biological properties will allow for a deeper exploration of their potential applications.

STATEMENTS ON COMPLIANCE WITH ETHICAL STANDARDS

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Disclosure of conflict of interest

The authors declare no conflicts of interest. The authors declare that this work was carried out in accordance with the regulations in force for scientific research.

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