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Neutral Lipid Characterization of Non-Water-Soluble Fractions of *Carica Papaya* Latex

Nathalie Barouh · Slim Abdelkafi · Benjamin Fouquet · Michel Pina · Frantz Scheirlinckx · Frédéric Carrière · Pierre Villeneuve

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Abstract The non-water-soluble fraction of *Carica papaya* latex (CPL) constitutes a waste material from papain production; very little information exists regarding its chemical composition. The non-water-soluble fraction of CPL was fractionated by liquid chromatography into neutral lipids, glycolipids and phospholipids. The most abundant compounds were found to be the polar lipids, accounting for 79.2% (w/w) of the total extractible matter, while the total amount of neutral lipids was only around 20%. It was composed of free fatty acids, sterols and triterpenic alcohols, but no glycerides were detected. A high content of saturated fatty acids was measured; these saturated fatty acids were represented by very long chains with C24:0, C26:0 and C28:0 accounting for 6.3, 11.0 and 6.3%, respectively, in the total extractible matter and 7.3, 9.0 and 3.9% in the FFA fraction. The monounsaturated fatty acids were about 23–25% in both samples, with oleic acid (C18:1) being the most abundant. The polyunsaturated fatty acids that were 25.1% in the total matter and 21.6% in the FFA fraction were mainly represented by linoleic acid (C18:2n-6). Finally, a very interesting characteristic of the FA composition of this latex concerns the presence of odd-numbered fatty acids in significant amounts (around 22% in the total extract and 24.3% in the FFA fraction).

Keywords *Carica papaya* · Latex · Lipids · Fatty acids, sterols · Triterpenic alcohols

N. Barouh · M. Pina · P. Villeneuve (✉)
CIRAD Dept PERSYST, Ingénierie des Agro-Polymères et Technologies Emergentes, Montpellier, France
e-mail: villeneuve@cirad.fr

S. Abdelkafi · F. Carrière
CNRS, Aix-Marseille Université, Enzymologie Interfaciale et Physiologie de la Lipolyse, Marseille, France

B. Fouquet · F. Scheirlinckx
Biohainaut, Z A E de Ghislenghien, Ghislenghien, Belgium

Introduction

The papaya tree (*Carica papaya*) is cultivated in many tropical areas under appropriate temperatures (21–33 °C) and mildly acid soil (pH 6.0–6.5). Its latex is generally collected by tapping 1–3 mm of the surface of the unripe green fruit or stems and trunk of papaya [1] using optimal conditions to avoid its oxidation [2, 3]. The obtained *Carica papaya* latex (CPL) is well known for containing proteases; one of these is papain (E.C. 3.4.22.2), a thiol protease with many industrial applications in the food and pharmaceutical industry as a meat tenderizer, digestive aid, clarifying agent in breweries, contact lens cleaner or bloodstain remover in detergents [4]. Therefore, CPL is the most important plant extract exploited in various industries for its proteasic activity. In fact, the term papain corresponds to the commercial name given to the spray-dried latex obtained by tapping. Proteinases are then usually extracted as water-soluble proteins from the latex [5]. Consequently, the resulting non-water-soluble material is generally considered as waste, and in comparison to the water soluble fraction, little is known regarding its chemical composition. Recently, the presence of hydrolytic enzymes such as GDSL-motif carboxylester hydrolase [6] or lipase has been demonstrated [7]. For the latter, an important characterization of its biocatalytic activities has been carried out [8–11], and some potential applications were also described in the field of racemic mixture

resolution [12–14], oil and fat restructuration [15–17], and enzyme replacement therapy [18]. Regarding other molecular species naturally present in the non-water-soluble fraction of CPL, very little information exists, particularly for lipids, for which little if any characterization can be found in the literature. Indeed, from a general point of view, complete studies of lipids naturally present in latex species are very scarce. This is probably due to the difficulty encountered when trying to extract them from such complex matrices as latex [19]. Not surprisingly, the latex from hevea (*Hevea brasiliensis*) is the most characterized. As early as 1946, Altman [20] observed the ether-soluble fraction of fresh latex was made mainly of free fatty acids (53–63%), phytosterols (11–15%) and fatty alcohols (7–8%). Similarly, Hasma and Subramaniam [21] analyzed lipids from fresh latex of *Hevea brasiliensis* clone RRIM501 and found that natural lipids were the most abundant (53.6% of total lipids) for a lipid content close to 1.6%. However, in this case, triacylglycerols were the main components of neutral lipids (63.3%). Very interestingly, some unusual fatty acids can be found in this latex. For example, for RRIM 501 clone, 98% of FAs were shown to be a C18 furanoid fatty acid identified as 10,13-epoxy-11-methyl-octadecan-10,12-dienoic acid [22, 23]. For other fatty acids, palmitic, stearic, oleic and linoleic acids were also found [21]. It is believed that fatty acids in this latex are either linked to the rubber polymer itself or are present under their free form [24]. When linked, they may play a role in the stability and colloidal behavior of the latex.

Concerning polar lipids, Smith [25], showed that phospholipids were also present with a wide distribution among them as phosphatidyl inositol, phosphatidyl ethanolamine and phosphatidyl choline were detected. Finally, glycolipids were found to account for 33% of total lipids in clone RRIM 501 and consisted of free and esterified steryl glucosides, mono- and digalactosyl diglycerides [20].

When considering other latex species, it is very difficult to find any bibliographic reference describing the lipids present in the studied latex extensively. However, when such literature exists, unusual lipid profiles are often found, especially with the presence of rare fatty acids. For example, Warnaar [26] demonstrated the presence of conjugated decadienoic and decatrienoic fatty acids in latex from *Euphorbia lathyris*. As mentioned above, the non-water-soluble fraction of CPL, to date, constitutes a waste material from papain production. Therefore, there is an interest in trying to characterize its lipid profile in search of any unusual molecules that may be of interest for further industrial use. To the best of our knowledge, this paper is the first attempt to analyze the neutral lipids of CPL.

Materials and Methods

Plant Material and Chemicals

Dried CPL was obtained from Biohainaut (Ghislenghien, Belgium). Fresh latex was collected from the Congo after incisions on the epidermis of the fruit. This latex is usually dried and roughly crushed in order to obtain a crude powder called P3. Monoclonal *Hevea brasiliensis* latex was collected from RRIM 600 trees (Union Rubber Co. Ltd., Chanthaburi, Thailand) and filtered through a metallic sieve (pore size of 2 mm). Sodium methylate, acetyl chloride, phospholipids, sterols and sterol esters were from Sigma-Aldrich (Saint Quentin, France). Potassium hydroxide, sodium sulfate, HCl, phenolphthalein, methanol, BHT, octanol, ethanol, 2-methyl 2-propanol, chloroform, THF and hexane, all of analytical grade, were purchased from Carlo Erba (Montpellier, France). Steryl glucoside (SG) and acylated steryl glucoside (ASG) were from Larodan Fine Chemical (Sweden).

Methods

Lipid Extraction and Total Lipid Content

The total lipids were extracted by the method of Folch [27]; 7.5 g of dried latex in 20-fold volume of chloroform-methanol (2: 1 v/v) was magnetically stirred for 2 h. Potassium chloride 0.8% (31.5 ml) was added, and the mixture was stirred for 5 min, then centrifuged. The aqueous phase was removed and substituted by a mixture of chloroform-methanol-potassium chloride 0.8% (3:48:47 v/v/v). The washing was repeated twice. The organic phase was isolated and evaporated to dryness. The lipid extract was solubilized with chloroform-methanol (2:1 v/v) (10 mg/ml). The sample was stored at −20 °C before analytical procedures.

Quantification of Polyisoprene

Size exclusion chromatography with refractive index and multi-angle laser light scattering detectors (SEC-MALS) was used to quantify the polyisoprene [28]. The measurements were carried out at 23 °C. One gram of latex in 40 ml of cyclohexane was incubated at 30°C for 2 weeks. The solution was then filtered through a 1-µm filter membrane and evaporated to dryness. Sample solution [3 mg/ml in THF stabilized with BHT (100 mg/l)] was then injected into the SEC-MALS system. The system consisted of an ERMA ERC-3112 solvent degasser, a Waters 717plus autosampler with a loop of 50 µl, a Waters 515 pump, a RI-Waters 2414 refractive index

detector at 30 °C (Waters Corp., St Quentin en Yvelines, France), a Dawn DSP DDL detector (Wyatt technology, Santa Barbara, CA) set to 633 nm, and three columns in series (45 °C): two Waters Styragel columns (300 mm by 7.8 mm) with a porosity of 20 μm and a PLgel mix column (300 mm by 7.8 mm) with a porosity of 20 μm (Varian, Les Ullis, France). Control and acquisition software was Maxima (Waters, Corp., St Quentin en Yvelines, France). The mobile phase was 100% THF stabilized with BHT (100 mg/l), and the flow rate was 0.65 ml/min. The concentration of polyisoprene was determined using a polyisoprene standard calibration curve.

Quantification of Nitrogen by Elemental Analysis

The amount of elemental nitrogen was quantified by combustion using a prescribed procedure (AFNOR NF ISO 13 878, 1998).

Fractionation by Liquid Chromatography

Lipid extracts were fractionated into neutral lipids, glycolipids and phospholipids using Companion silica gel plastic column flash chromatography (Serlabo Technologies, Avignon, France). About 1 g of lipid extract was added to the top of the chromatography column (12 g) that had already been conditioned with chloroform. The flow was 30 ml/min. Neutral lipids were eluted with chloroform (about 500 ml). Glycolipids were eluted with a mix of chloroform-acetone as follows: chloroform (100%) for 5 min, chloroform to acetone (100–0%) for 25 min and acetone (100%) for 15 min. Phospholipids were eluted with a mix of acetone-methanol as follows: acetone (100%) for 5 min, acetone to methanol (100–80%) for 15 min and the mix (80–20%) for 20 min. To assess if elution of each fraction was complete, all fractions where characterized by thin layer chromatography (TLC).

Lipid Identification by Thin Layer Chromatography

Neutral lipids were separated by TLC on silica gel 60 plates using hexane/diethyl ether/acetic acid (70:10:1 v/v/v); polar lipids were eluted with chloroform/methanol/water (95:20:2.5 v/v/v). The lipids separated by TLC were identified by comparing R_f values with those of pure standard compounds. For phospholipids, sterols and glycolipids, the identity was guaranteed using respectively specific sprays of molybdenum blue, ferric chloride [29] and orcinol-sulfuric acid reagent confirmed with ferric chloride for the SG and ASG.

Quantification of Neutral Lipids by TLC-FID

Neutral lipids were quantified using an Iatroscan MK6 TLC-FID system (Bionis, Paris, France); 1 μl of each sample in $CHCL_3$ (10 mg/ml) was deposited on silica Chromarods using a semi-automatic applicator SES 3202. Chromarods were developed in toluene-chloroform-acetid acid (60:24:0.8 v/v/v). After development, chromarods were dried and analyzed by Iatroscan MK6 equipped with Chromstar 2Ea quantification software. The reported data correspond to mean values of three determinations (area %).

Transformation into FAMES and GC Analysis of Fatty Acid (FA) Composition

In 25-ml round-bottom flasks, 10 mg of samples was added to 3 ml sodium methylate solution with phenolphthalein. The reaction medium was refluxed for 10 min; 3 ml chlorhydric methanol was added to phenolphthalein discoloration. The mixture was refluxed again for 10 min and then cooled to ambient temperature; 8 ml hexane and 10 ml water were added, and the organic phase was recovered, dried over anhydrous sodium sulfate and filtered for subsequent GC analysis: Agilent 6890 series using a Supelcowax 10 capillary column (SGE, Courtaboeuf, France) with the following characteristics: length, 30 m; internal diameter, 0.32 mm; film thickness, 0.25 μm. Fatty acid methyl esters were directly injected into the GC. Carrier gas: helium flow rate 1 ml/min, splitting ratio: 1/80, injector temperature: 250 °C, FID detector temperature: 270 °C. The temperature settings were as follows: 185–225 °C at 5 °C/min, 225 °C for 35 min.

Free Fatty Acid (FFA) Composition

FFAs were isolated on a preparative TLC. After development of the plate, the spots were visualized by spraying with 2,7-dichlorofluorescein. The desired compound was scraped off the plate and transformed into FAMES according to the procedure described above.

Phytosterols and Triterpenic Alcohol Analysis

The determination of phytosterols was carried out in accordance with the corresponding AFNOR Method NF ISO 6799. Sterols were extracted from 500 mg of lipid extract. The sample was saponified using 5 ml alcoholic potassium hydroxide (0.5 N) under reflux for 15 min. The unsaponifiable components were separated from the soaps on alumina columns and washed with diethyl ether. The

solvent was evaporated, and the residue was taken up in 1 ml of chloroform. The separation of sterols from other unsaponifiable components was carried out by thin-layer chromatography. The plates were developed with a hexane/diethyl ether/acetic acid solution (80:20:1 v/v/v). The desired compound was scraped off the plate and desorbed with chloroform. The individual sterol species were resolved using a focus Thermo Fisher Scientific GC with a FID and a focus Thermo Fisher Scientific GC connected to a mass spectrometer DSQ II. Both were fitted with a SAC5 column (length 30 m, internal diameter 0.25 mm, film thickness 0.25 μm) that allowed the direct analysis of sterols. The injector was set to 260 °C with a split ratio of 1/15. The carrier gas was helium at a flow rate of 1.0 ml/min, and the oven temperature isotherm was set at 285 °C. First, the detector was set to 310 °C. Second, the mass spectrometer (MS) was operated in the electron impact ionization mode, the source temperature was programmed from 250 °C, the energy of ionization was 70 eV, and the parameters of acquisition of the spectra were 50–450 uma for the range of mass. Two GC analyses of products from three experiments were made.

Tocopherol and Tocotrienol Analysis

Tocopherols are analyzed by HPLC in accordance with the AFNOR method ISO 9936. The HPLC consisted of modules provided by Thermo Fisher Scientific (France): a quaternary pump (P1000XR), an auto sampler (AS1000) and a six-way injection valve with a loop of 20 μl, a spectrofluorimetric detector (FL3000) and software for data processing (PC 1000). The column was a silica hypersil with a porosity of 5 μm (25 cm by 0.4 cm). The mobile phase consisted of a mixture of hexane-dioxane (97:3 v/v), with a flow of 1 ml/min. The wavelength of excitation and emission were 290 and 330 nm, respectively. Analyses were run in triplicate.

Results and Discussion

Lipid Extraction and Lipid Class Profiles

The analysis of lipids naturally found in various latexes requires an initial solvent extraction step that may be problematic owing to the possible presence of polyisoprene chains. Indeed, these chains may be co-extracted with the lipids, resulting in an incorrect estimation of the real lipid content, or some neutral lipids could also be trapped during coagulum [30]. Concerning *Hevea brasiliensis*, which is particularly rich in polyisoprene, various extraction techniques have been tested using the method of Folch et al. [27]. In their extensive study of the optimization of solvent extraction for polar lipids of *Hevea brasiliensis*, Bonfils et al. [30] followed by Liengprayoon et al. [19] showed that hot Soxhlet extraction led to lower extraction rates and low repeatability, which could be attributed to the fact that natural rubber fragments were progressively compacted during the extracting process, a factor that hindered the effectiveness of solvent extraction. These authors concluded that the cold-immersion technique using a chloroform-methanol mixture was optimal, especially when considering phospholipid and glycolipid extraction. Before selecting any lipid extraction procedure for CPL, we checked for the presence of polyisoprene chains using SEC-MALS methodology and compared it with *Hevea brasiliensis* clone PB235 (Fig. 1). With the method used, only the polymers with molecular weight above 1 kDa were quantifiable. Whereas the presence of such polymers was clearly observed for *clone PB 235* (massive peak between 28 and 40 min corresponding to the molar mass between 50,000 and 1.10^7 g/mol), on the contrary, for *Carica papaya* no polyisoprene chain was detected at this molecular weight.

Therefore, we estimated that the subsequent lipid extraction would not be hindered by the presence of such polymeric chains in large amounts and that the classical Folch method could be employed with no loss of extracted lipids [19]. Accordingly, dried latex from *Carica papaya* was extracted with chloroform-methanol (2: 1 v/v), and the resulting extractable matter was found to be around 25%. This appears very high compared to other latexes. For example, in *Hevea brasiliensis*, lipids account for 1–2% of dry matter. In order to check for possible concomitant extraction of protein-derived compounds, which can be encountered when using the Folch method, we carried out elemental analysis of the dried latex and the extractable matter. In dried latex, the nitrogen content was 2.5%, while it was only 0.2% in the extractable matter, which showed that the presence of co-extracted proteins was improbable.

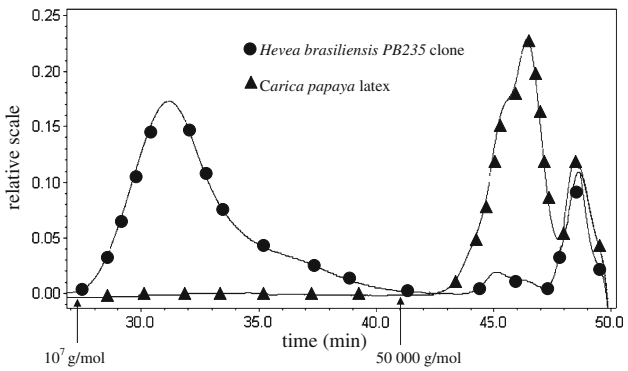


Fig. 1 Chromatograms showing the refractive index (RI) as a function of time of *Hevea Brasiliensis* PB235 clone and *Carica Papaya* latex. *Filled circles: Hevea Brasiliensis* PB235 clone; *filled triangles: Carica papaya* latex

However, the 0.2% value suggests that some traces of lipoproteic compounds may be present in the extractible matter from Folch extraction. Concerning its lipid class profile, the extract was fractionated by liquid chromatography into neutral lipids and polar lipids, which represented around 20 and 79% of the total extractible matter, respectively (Table 1). The neutral fraction separated and quantified by TLC-FID (Fig. 2) was mainly composed of free fatty acids, sterols and triterpenic alcohols representing 6.1, 5.5 and 0.4%, respectively, by considering that the response coefficient was similar for all compounds. However, when using the calibration curve to quantify FFA, sterols and triterpenic alcohols, their contents were respectively 4.4, 3.4 and 1.2%, representing a total of 9% compared to 12% obtained by TLC-FID. The results showed that the analysis by TLC-FID area led to overestimation (3%).

Moreover, the presence of a very apolar band was detected, accounting for 8.8% area of the total lipids. Although it was not possible to identify this compound, its corresponding TLC band was revealed positively by molybdenum blue, suggesting the presence of a phosphate function. Therefore, it may be possible that this unknown product corresponds to short polyisoprene chains that would be linked by covalent bonding to phospholipid molecules, as suggested by Tarachiwin et al. [31] for *Hevea brasiliensis* latex. However, further analytical work has to be carried out in order to identify this compound.

Table 1 Mass % neutral lipid by TLC-FID

Compounds	Mass %
Unknown	8.8 ± 1.5
FFAs	6.1 ± 0.3
Triterpenic alcohols and methylsterols	0.4 ± 0.0
Sterols	5.5 ± 0.4
Polar lipids	79.2 ± 1.7

All given values are means of three determinations

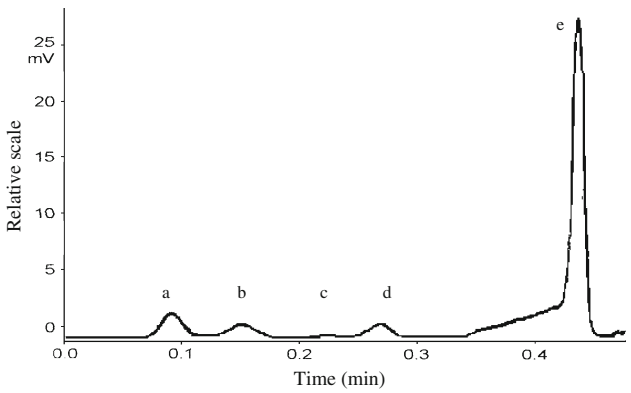


Fig. 2 TLC-FID of the neutral lipids. The peaks are as follows: *a* unknown, *b* FFA, *c* triterpenic alcohols, *d* sterols, *e* polar lipids

Finally, it is worth noting that no glycerides at all were detected by TLC and TLC-FID as triacylglycerol, diacylglycerol or monoacylglycerol forms. By analogy with other latex species, this absence of glycerides seems very surprising; however, it may be explained by the strong lipolytic activity found in CPL [7], which would then lead to a full hydrolysis of all glycerides within the latex.

Fatty Acid Composition

The fatty acid composition of the total extractible matter and of the free fatty acid fraction was evaluated, and the results are summarized in Table 2. Similar profiles were

Table 2 Fatty acid composition of total extractible matter and free fatty acid fraction (values are mean ± SD of triplicate determinations)

Fatty acid	Mass %	
	Total extractible matter	Free fatty acid fraction
C14:0	0.4 ± 0.3	0.6 ± 0.0
C15:0	0.5 ± 0.1	0.9 ± 0.0
C15:1	0.1 ± 0.1	0.0 ± 0.0
C16:0	2.4 ± 0.5	4.2 ± 0.0
C16:1	0.4 ± 0.2	0.5 ± 0.1
C17:0	4.2 ± 0.5	7.0 ± 0.0
C17:1	0.7 ± 0.1	0.6 ± 0.0
C18:0	3.0 ± 0.3	5.1 ± 0.0
C18:1	15.3 ± 0.7	11.7 ± 0.0
C18:2	25.1 ± 0.6	21.6 ± 0.1
C19:0	0.6 ± 0.5	1.4 ± 0.0
C19:1	2.5 ± 0.1	1.9 ± 0.2
C20:0	1.1 ± 0.1	1.7 ± 0.0
C20:1	0.7 ± 0.1	1.0 ± 0.0
C21:0	0.8 ± 0.1	1.2 ± 0.0
C22:0	1.6 ± 0.1	2.3 ± 0.0
C22:1	0.6 ± 0.1	0.8 ± 0.1
C23:0	1.8 ± 0.1	2.5 ± 0.1
C24:0	6.3 ± 0.1	7.3 ± 0.0
C24:1	1.7 ± 0.1	2.3 ± 0.1
C25:0	5.1 ± 0.2	4.9 ± 0.1
C25:1	0.7 ± 0.1	0.4 ± 0.4
C26:0	11.0 ± 0.6	9.0 ± 0.1
C26:1	0.5 ± 0.4	1.0 ± 0.1
C27:0	4.9 ± 0.3	3.5 ± 0.2
C28:0	6.3 ± 0.9	3.9 ± 0.2
C28:1	1.7 ± 0.6	2.7 ± 0.0
Σ saturated FA	50.0 ± 0.3	55.4 ± 0.5
Σ monounsaturated FA	24.9 ± 0.3	23.1 ± 0.4
Σ polyunsaturated FA	25.1 ± 0.3	21.6 ± 0.1
Σ odd FA	22.0 ± 0.3	24.3 ± 1.0

found in both samples for which fatty acid composition ranged from C14:0 to C28:1. A high content of saturated fatty acids was measured with 50% for the total matter and 55.4% for the free fatty acid fraction. Interestingly, these saturated fatty acids were represented by very long chains with C24:0, C26:0 and C28:0 accounting for 6.3, 11.0 and 6.3%, respectively, in the total extractible matter and 7.3, 9.0 and 3.9% in the FFA fraction. The monounsaturated fatty acids were about 23–25% in both samples, with oleic acid (C18:1) being the most abundant among them with 15.3% in the total matter and 11.7% in the FFA fraction. The polyunsaturated fatty acids (PUFAs), which were 25.1% in the total matter and 21.6% in the FFA fraction, were mainly represented by linoleic acid (C18:2n-6), which alone represented half of the total PUFAs. Finally, a very interesting characteristic of the FA composition of this latex concerns the presence of odd-numbered fatty acids in significant amounts (around 22% in the total extract and 24.3% in the FFA fraction). The main ones were C25:0 and C27:0 with 5.1 and 4.9%, respectively, in the total extractible extract. The presence of such odd-number long-chain fatty acids in significant amounts is very rare in the plant kingdom [32].

Tocopherol and Tocotrienol Compositions

Tocopherols and tocotrienols are antioxidant molecules naturally found in most vegetable oils. Besides their stabilizing effect on unsaturated fatty acids oxidation, they are also of great nutritional and economic interest because of their vitamin E activity. Therefore, it was interesting to check for the presence of such molecules in the lipid extract from CPL. However, according to our LC analysis, only traces were found, with α tocopherol being the predominant one (only 2 ppm).

Phytosterol Composition

Phytosterol components were identified by the comparison of both their retention times and mass spectra with those of commercial standards. Results, given in Table 3, showed that the extractible matter contained about 3.08% (w/w) of

Table 3 Phytosterol composition and repartition (values in brackets indicating the centesimal proportion of each phytosterol)

Total amount (% w/w)	3.08 ± 1.57
Stigmasterol	0.31 ± 0.02 (9.4)
5α stigmasta 7.16 dien 3ß ol	0.28 ± 0.01 (8.7)
Δ5 avenasterol	1.00 ± 0.18 (31.1)
5α stigma 7 en 3ß ol	0.11 ± 0.02 (3.9)
Δ7 avenasterol	1.38 ± 0.04 (46.9)

All values given are means of three determinations

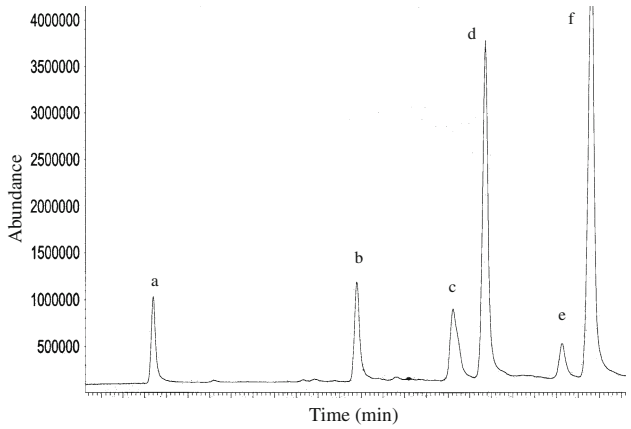


Fig. 3 Chromatogram of phytosterols by GC-MS. The peaks are as follows: *a* internal standard: cholesterol, *b* stigmasterol, *c* 5α stigmasta 7.16 dien 3ß ol, *d* Δ5 avenasterol, *e* 5α stigma 7 en 3ß ol, *f* Δ7 avenasterol

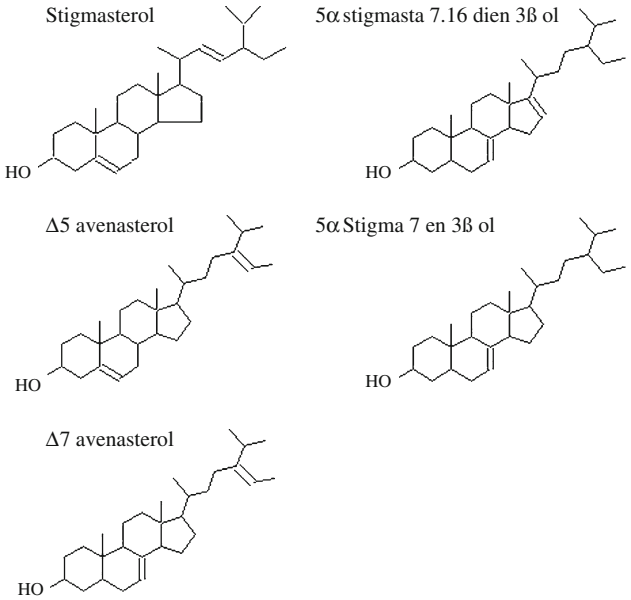


Fig. 4 Structures of phytosterols

Table 4 Triterpenic alcohol and methyl sterol composition relative % by GC-MS

Triterpenic alcohol and methyl sterol	(%)
5α Cholesta 8.24 dien 3β 4α 14 dimethyl	Tr
Unknown	4.8
Ergosta 8.24 (28) dien 3 ol 4 14 dimethyl. 3β 5α 5α	8.9
Ergosta 7.24 (28) dien 3 ol 4α methyl 5α	3.6
Cycloeucalenol	39.7
Cycloartenol	16.6
24 Methylene cycloartenol	12.9
Citrostadienol	13.5

phytosterols. Five different sterols were quantified. Among them, Δ7 avenasterol was the most present (46.9%), while Δ5 avenasterol was detected at 1% (w/w), accounting for 31.1% of total sterols. The common sterol stigmasterol was present at 9.4%. Some rare sterols were also found, such as 5α stigmasta 7.16 dien 3ß ol (8.7%) and 5α stigma 7 en 3ß ol (3.9%) (Figs. 3, 4).

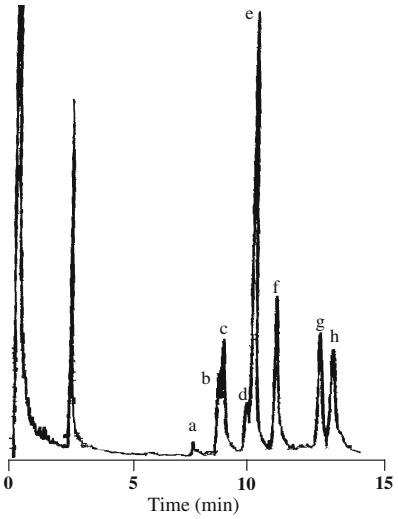


Fig. 5 Chromatogram of triterpenic alcohols and methyl sterols by GC-FID. The peaks are as follows: *a* 5α cholesta 8.24 dien 3*β* 4α 14 dimethyl, *b* unknown, *c* ergosta 8.24 (28) dien 3 ol 4 14 dimethyl. 3*β* 5α 5α, *d* ergosta 7.24 (28) dien 3 ol 4α methyl 5α, *e* cycloeucalenol, *f* cycloartenol, *g* 24 methylene cycloartenol, *h* Δ7 citrostadienol

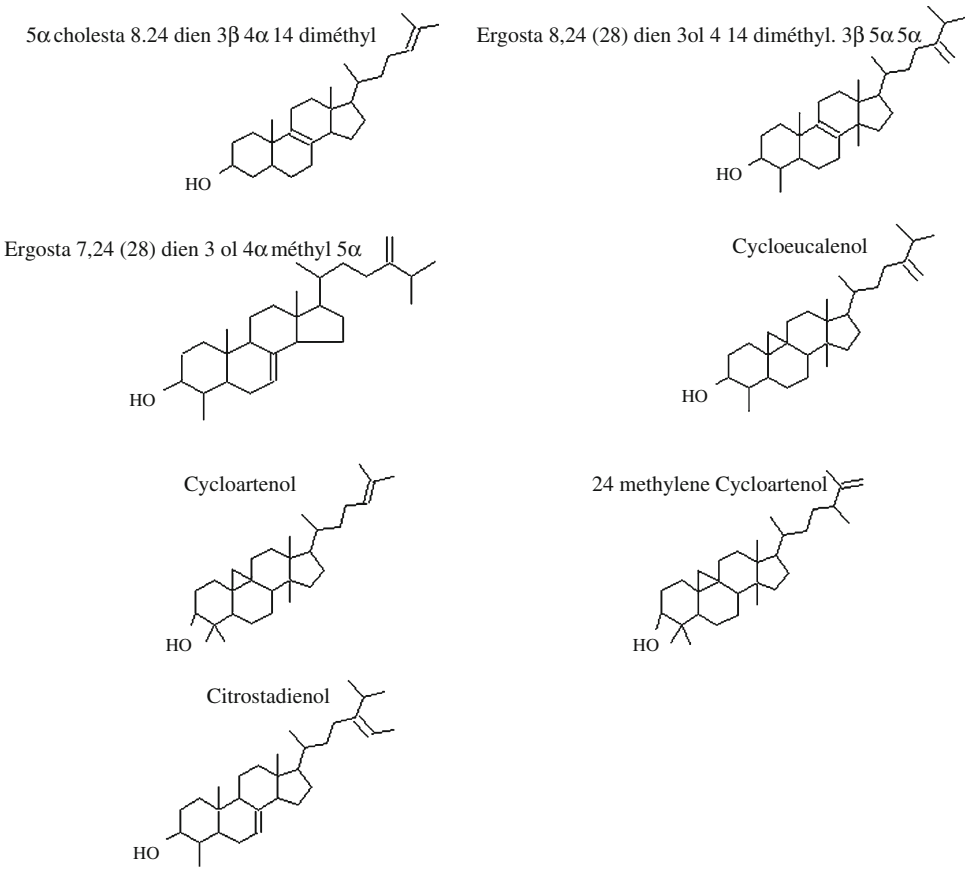
Triterpenic Alcohol and Methyl Sterol Composition

Triterpenic alcohols and methyl sterols were analyzed and quantified by GC-MS (Table 4; Figs. 5, 6). They were found to be present in the neutral lipid fraction of the total extractible matter, although in limited amounts (less than 1%). Among triterpenic alcohols, cycloeucalenol was the major one (39.7%). This molecule is known to be a precursor for the biosynthesis of sterols [33, 34]. Cycloartenol was found in 16.6%, 24 methylene cycloartenol in 12.9% and citrostadienol in 13.5%. Concerning methyl sterols, ergosta 8,24 (28) dien 3 ol 4 14 dimethyl 3*β* 5α 5α was the most important (8.9%), followed by an unknown one that we were unable to identify (4.8%). Finally, ergosta 7,24 (28) dien 3 ol 4α methyl 5α was 3.6%, while only traces of 5α cholesta 8.24 dien 3*β* 4α 14 dimethyl were detected.

Conclusion

The neutral lipid characterization of the non-hydrosoluble fraction of CPL showed a particular profile, especially

Fig. 6 Structures of triterpenic alcohols and methyl sterols



regarding its fatty acid composition for which a significant amount of odd-numbered long-chain fatty acids was detected (around 22%), the main ones being the C25:0 and C27:0 fatty acids. To the best of our knowledge, these results are the first to describe the presence of such fatty acids in CPL non-water-soluble fractions. The presence of such rare fatty acids, which are of potential interest to the lipochemistry industry, could then represent a possible way to valorize this waste material from papain production.

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