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Further Insight into the Latex Metabolite Profile of *Ficus carica*

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Latex is a sticky emulsion that exudes upon damage from specialized canals from several plants. It contains several biologically active compounds, such as phytosterols, fatty acids, and amino acids. In plants, these compounds are involved in the interaction between plants, insects, and the environment. Despite its chemical, biological, and ecological importance, *Ficus carica* latex is still poorly studied. To improve the knowledge on the metabolite profile of this matrix, a targeted metabolite analysis was performed in a representative sample from *F. carica* latex. Seven phytosterols were determined by gas chromatography−ion trap mass spectrometry (GC−ITMS) and high-performance liquid chromatography coupled to diode array detection (HPLC−DAD), with β-sitosterol and lupeol being the compounds present in higher concentrations (ca. 54 and 14%, respectively). A total of 18 fatty acids were characterized by GC−ITMS, being essentially represented by saturated fatty acids (ca. 86.4% of total fatty acids). A total of 13 free amino acids were also identified by high-performance liquid chromatography coupled to ultraviolet−visible spectroscopy (HPLC/UV−vis), and cysteine and tyrosine were the major ones (ca. 38.7 and 31.4%, respectively). In humans, phytosterols and some polyunsaturated fatty acids, such as linoleic acid, are known for their anticarcinogenic properties. With regard to amino acids, some of them, such as glycine, are neurotransmitters. Our results reveal the presence of a wide diversity of compounds, from distinct classes, in *F. carica* latex, possessing various potential pharmacological activities; thus, its biological potential appears to be worth further exploring.

KEYWORDS: ***Ficus carica*** **latex; phytosterols; free and combined fatty acids; free amino acids**

INTRODUCTION

Ficus carica L., the common fig, is widely used in the Middle East as food and medicine (*1*). Its latex released upon picking the fruits has been traditionally used, via topical application, in several conditions, such as gout, ulcers, and warts (*1*), because of its viscosity and proteolytic and keratolytic activities (*2*). Latex is an aqueous suspension of a complex mixture of molecules, found in specialized secretory cells of plants, known as laticifers. These cells synthesize and store diverse secondary metabolites in appreciable amounts, namely, terpenoids, alkaloids, tannins, and sterols (*3*, *4*). Most of these compounds provide resistance to herbivores via toxicity or antinutritive effects, whereas others are involved in the stickiness that can trap different insects. In the context of humans, reduced amounts of latex are ingested only with the fresh fruits. However, latex is not ingested per se, and some precautions regarding the direct use are needed, because this material is known to have keratolytic and corrosive properties (*2*). Additionally, saponins are reported in latex (*5*), and these compounds are known to cause erythrocyte destruction (*6*). Some of the compounds present in latex are interesting regarding human health, even in small amounts (*4*); for example, some sterols show anti-inflammatory, antipyretic, and antidiabetic activities (*7*), and proteins, such as ficin, are known for their anti-helmintic activity (*8*).

Phytosterols are cholesterol-like molecules found in most plant foods, with the highest concentrations occurring in vegetable oils. They are absorbed only in trace amounts but inhibit the absorption of intestinal cholesterol, including recirculating endogenous biliary cholesterol, a key step in cholesterol elimination (*9*). Plant materials contain free and esterified sterols that can be acylated, with β-sitosterol, campesterol, and stigmasterol being the more abundant ones in nature. These compounds are involved in important cellular processes, such as the regulation of membrane fluidity, adaptation of membranes to temperature (*10*), and also participation in cellular differentiation and proliferation (*11*).

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Fatty acids constitute signal molecules, energy storage, and protect plants from environmental and biological stress (*12*). In the human body, fatty acids act like hormones or their precursors, help the digestion process, and are a source of metabolic energy (*13*). Polyunsaturated fatty acids (PUFAs) from the ω-6 family have intense biological properties and are the biosynthetic precursors of eicosanoids (i.e., prostaglandins), having effects on cardiovascular diseases, triglyceride levels, and arthritis (*14*).

Furthermore, some amino acids are essential to human life and have many functions in plant metabolism (*15*). These compounds are not only building blocks of proteins but also participate in many metabolic networks that control growth and adaptation to the environment. They are involved in secondary plant metabolism, namely, in the biosynthesis of phenolic compounds, glucosinolates, cyanogenic heterosides, and alkaloids (*15*, *16*). In humans, they are important in brain metabolism imbalances, as hormones, precursors of complex nitrogen-containing metabolites, neurotransmitters, metabolic intermediates, and alimentary supplements (*15*).

F. carica latex remains poorly studied. Its volatile and organic acid profiles have been recently described (*17*). The characterization of ficin, a cysteine proteinase (*18*), and a mixture of 6-*O*-acyl-β-D-glucosyl-β-sitosterol isoforms (*5*) were already reported. On the other hand, some biological activities, namely, antioxidant, acetylcholinesterase inhibition (*17*), antifungal, anti-helmintic (*19*, *20*), and anticarcinogenic (*1*, *5*, *21*), were previously found.

Thus, the aim of the present work was to improve the knowledge on its metabolite composition, to obtain more information on potential healthy constituents, namely, phytosterols, fatty acids, and free amino acids, for further possible exploitation. Analytical methods previously validated for other matrices were applied, and the chemical composition was compared to that of other *F. carica* materials. Nevertheless, some validation parameters for our matrix were also assessed. Following these purposes, the determination of phytosterol and fatty acid profiles was pursued, using high-performance liquid chromatography coupled to diode array detection (HPLC−DAD) and gas chromatography−ion trap mass spectrometry (GC−ITMS). In addition, the free amino acid composition was determined by high-performance liquid chromatography coupled to ultraviolet−visible spectroscopy (HPLC/UV−vis), after precolumn derivatization with dabsyl chloride.

MATERIALS AND METHODS

Standards and Reagents. All chemicals used were of analytical grade. The standard compounds were purchased from various suppliers. Myristic, pentadecanoic, palmitic, heptadecanoic, *cis*-10-heptadecenoic, stearic, oleic, elaidic, linoleic, arachidic, heneicosanoic, behenic, tricosanoic, and lignoceric acid methyl esters were from Supelco (Bellefonte, PA). All L-amino acids, β-sitosterol, lanosterol, lupeol, lupeol acetate, and betulin were from Sigma (St. Louis, MO). β- and α-amyrin were from Extrasynthése (Genay, France), and methyl jasmonate (internal standard) was from SAFC (St. Louis, MO). Methanol, dichloromethane, *n*-hexane, acetonitrile, ethanol, and phosphoric acid were obtained from Merck (Darmstadt, Germany). Anhydrous sodium sulfate, hydrochloric acid, and isooctane were from Panreac Quimica SA (Barcelona, Spain). Potassium hydroxide was obtained from Pronalab (Lisboa, Portugal). Boron trifluoride (BF_3) 10% methanolic solution was purchased from Supelco (Bellefonte, PA). Dabsyl chloride reagent, sodium hydrogen carbonate, sodium dihydrogenophosphate dimethylformamide, and triethylamine were obtained from Sigma (St. Louis, MO). Water was deionized using a Milli-Q water purification system (Millipore, Bedford, MA).

Latex Sample. To obtain a representative sample, latex was collected from 20 *F. carica* trees under the same environmental and soil characteristics, in the Mirandela region, Portugal. All samples were harvested manually at the same stage of maturation (June of 2009), without using steel knives, by incising the stalk of the green fruit from the main branch. The samples were obtained drop-by-drop without squeezing, mixed and homogenized, weighted, separated by aliquots, and kept at −20 °C until processed.

Phytosterol Analysis. *Solvent Extraction*. Two different solvents, dichloromethane and *n*-hexane, were tested. A total of 1.0 g of latex was extracted with 25 mL of each solvent for 4 h, in the dark, with magnetic stirring (300 rpm). The resulting extract was then filtered through a Buchner funnel, under vacuum. Afterward, the extract was dehydrated with 0.5 g of anhydrous sodium sulfate and concentrated under a stream of nitrogen to obtain a final volume of 0.3 mL. This procedure was performed in triplicate.

As Rubnov et al. (*5*) have described the presence of phytosterols conjugated with glucosyl groups in latex, acid and alkaline hydrolysis were performed to obtain the free phytosterol composition.

Acid Hydrolysis. The method described by Toivo et al. (*22*) was followed, with some modifications. Briefly, 1.0 g of latex was acidified by refluxing with 20 mL of 0.5 M ethanolic HCl for 1 h, under a constant temperature (80 °C). The mixture was then centrifuged, decanted, and transferred to a separation funnel, to extract the unsaponifiable fraction with *n*-hexane (3 × 10 mL). The organic phase was evaporated to dryness in a rotary evaporator (Büchi R-114), and the residue was dissolved in 1 mL of methanol and then filtered through a 0.22 μm polytetrafluoroethylene membrane (Millipore, Bedford, MA). This procedure was performed in triplicate.

Alkaline Hydrolysis. The conditions used for saponification and extraction were adapted from those proposed by Sánchez-Machado et al. (*23*), with some modifications. Briefly, 1.0 g of latex was saponified by refluxing with 20 mL of 1 M ethanolic KOH for 1 h, at constant temperature (80 °C). The mixture was then centrifuged, decanted, and transferred to a separation funnel, to extract the unsaponifiable fraction with *n*-hexane (3 × 10 mL). The organic phase was evaporated to dryness in a rotary evaporator (Büchi R-114), and the residue was dissolved in 1 mL of methanol and then filtered through a 0.22 μm polytetrafluoroethylene membrane (Millipore, Bedford, MA). This procedure was performed in triplicate.

GC−ITMS Conditions. The extracts obtained by hydrolysis and direct solvent extraction were analyzed on a Varian CP-3800 gas chromatographer (Walnut Creek, CA) equipped with a Varian Saturn 4000 mass selective detector (Walnut Creek, CA) and Saturn GC/MS workstation software, version 6.8 (Walnut Creek, CA). The column used for identification was a VF-5 ms, 30 m × 0.25 mm × 0.25 μm (FactorFour), from Varian. The injector port was heated to 250 °C. Solvent extracts were injected in split mode, with a 1:40 ratio. The carrier gas was helium C-60 (Gasin, Portugal), at 1 mL/min, constant flow. The oven temperature was 40 °C (for 1 min), then increasing 2 °C/min to 220 °C, and held for 30 min. All mass spectra were acquired in the electron impact (EI) positive mode. Ionization was maintained off during the first 3 min, to avoid solvent overloading. The ion trap detector was set as follows: the transfer line, manifold, and trap temperatures were 280, 50, and 180 °C, respectively. The mass range was m/z 50−1000, with a scan rate of 6 scan/s. The emission current was 50 μA, and the electron multiplier was set in relative mode to the auto-tune procedure. The maximum ionization time was 25 000 μs, with an ionization storage level of m/z 35. The injection volume was 1 μL, and the analysis was performed in full-scan mode. Compounds were identified by a comparison of retention times and MS fragmentation pattern to those of standards analyzed under the same conditions, and a mass spectra database search was performed using the National Institute of Standards and Technology (NIST) MS 05 spectral database.

HPLC−DAD Conditions. A total of 20 μL of extracts obtained by hydrolysis or direct solvent extraction were analyzed at room temperature in a HPLC unit (Gilson), using a reversed-phase Hypersil ODS (20 × 0.4 cm inner diameter × 5 μm particle size) column, according to Sánchez-Machado et al. (*23*). The mobile phase was methanol:acetonitrile (30:70), at a flow rate of 0.8 mL/min, in isocratic mode. Detection was achieved with Gilson DAD. Spectral data from all peaks were accumulated in the 190−400 nm range. The data were processed on Unipoint system software (Gilson Medical Electronics, Villiers le Bel, France). Peak purity was checked by the software contrast facilities. Phytosterols were identified by a comparison of retention times and UV absorption spectra to those obtained for authentic standards. Quantification was achieved by the

absorbance recorded in the chromatograms at 205 nm relative to external calibration standards.

Fatty Acid Analysis. *Derivatization*. The fatty acid derivatization was performed according to a described procedure (*24*), with some modifications. Briefly, 500 mg of *F. carica* latex was redissolved in 5 mL of methanol. The lipids in the extract were hydrolyzed with 1 mL of KOH methanolic solution (11 g/L), at 90 °C, for 10 min. The free fatty acids originally present and those resulting from the alkaline hydrolysis were derivatized to their methyl ester forms with 1 mL of BF_3 methanolic solution (10%), at 90 °C, for 10 min. The methyl ester derivatives were extracted with 2 × 6 mL of isooctane, and anhydrous sodium sulfate was added to ensure the total absence of water. The resulting extract was evaporated to dryness under a stream of nitrogen and redissolved in 400 μL of isooctane. All assays were performed in triplicate.

GC−ITMS Conditions. The conditions used were similar to those described for GC−ITMS phytosterol analysis, with some modifications. The oven temperature was set at 40 °C for 1 min, then increasing 5 °C/min to 250 °C, 3 °C/min to 300 °C, and held for 15 min. Ionization was maintained off during the first 4 min, to avoid solvent overloading. The mass ranged from m/z 50 to 600, with a scan rate of 6 scan/s. The amount of fatty acid methyl esters (FAMEs) present in the extract was achieved from the calibration curve of the respective FAME standards. The FAME values were then converted to the respective fatty acid contents.

Amino Acid Analysis. *Extraction*. A total of 3.0 g of latex was extracted with 10 mL of 0.1 M HCl, with magnetic stirring (300 rpm), for 1 h. The resulting extract was then filtered and kept at −20 °C until derivatization.

Derivatization. The dabsylation process was performed as reported by Silva et al. (*25*). Briefly, aliquots of 20 μL of standard solution and latex sample were diluted with 180 μL of the reaction buffer (0.15 M sodium hydrogen carbonate at pH 8.6, adjusted with 2 N NaOH). After thorough mixing on a vortex mixer, 200 μL of reagent (12.4 mM dabsyl chloride in acetone) was added and the solutions were agitated again. The resulting solutions were incubated at 70 °C in a water bath, for 15 min. The reaction was stopped by placing the vials in an ice bath for 5 min. A total of 400 μL of the dilution buffer [mixture of 50 mL of acetonitrile, 25 mL of ethanol, and 25 mL of 9 mM sodium dihydrogenophosphate; 4% dimethylformamide and 0.15% triethylamine (pH 6.55, adjusted with 85% phosphoric acid)] was added, followed by mixing and centrifugation (5 min, 5000 rpm), and 20 μL of the clear supernatants was directly set for injection.

HPLC/UV−vis Conditions. Dabsyl derivatives of free amino acids were separated on a Gilson HPLC unit, using a reversed-phase Spherisorb ODS2 (25.0 × 0.46 cm; 5 μm particle size) column, as before (*25*). The solvent system consisted of (A) 9 mM sodium dihydrogenophosphate, 4% dimethylformamide, and 0.15% triethylamine (pH 6.55, adjusted with 85% phosphoric acid) and (B) 80% acetonitrile in water. Elution was performed at a flow rate of 1 mL/min, starting with 20% B until 7 min and installing a gradient to obtain 35% B at 35 min, 50% B at 45 min, and 100% B at 66 min. Detection was achieved with a UV−vis detector set at 436 nm. Free amino acid quantification was accomplished by the absorbance recorded in the chromatograms relative to external calibration standards.

RESULTS AND DISCUSSION

As already referred, the chemical composition of *F. carica* latex is poorly studied. The few existing works concern the volatile and organic acid profiles (*17*), the characterization of a proteinase (*18*), and a mixture of 6-*O*-acyl-β-D-glucosyl-β-sitosterol isoforms (*5*). The presence of other compounds was checked to improve the knowledge on the metabolic profile of this matrix.

Phytosterol Profile. *Analytical Procedure*. To achieve the most suitable conditions for the analysis of phytosterols present in latex, several extracts were prepared. Thus, phytosterols in the extracts obtained using two organic solvents (dichloromethane and *n*-hexane) and in hydrolyzed (acid and alkaline) latex were identified by GC−ITMS and quantified by HPLC−DAD. The hydrolysis of latex was performed because phytosterols conjugated with glucosyl groups were previously described in this matrix (*5*). Therefore, the hydrolysis procedure allows for the separation of these compounds and their subsequent identification.

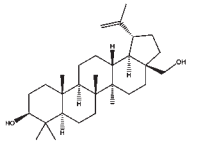
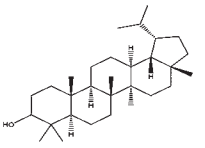
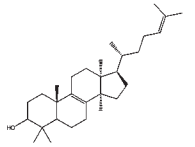
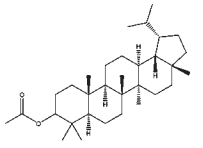
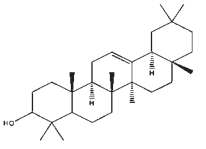
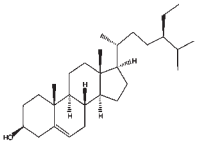
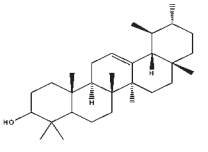
Using GC−ITMS, seven phytosterols were identified in the latex extracts (**Table 1**). The same extracts were also analyzed by HPLC−DAD, and the phytosterols were quantified by this technique because it revealed a better chromatographic separation, in a shorter analysis time (25 min), compared to GC−ITMS. Thus, HPLC−DAD was faster and cheaper, which renders it suitable for routine analysis, besides being more available than GC−ITMS. The analysis of both hydrolyzed extracts by HPLC−DAD allowed for the separation of different phytosterols, with the extract of alkaline hydrolysis being the one giving the best results (**Figure 1**).

Under the analyzed conditions (alkaline hydrolysis followed by HPLC−DAD analysis), seven compounds were identified in *F. carica* latex unsaponifiable fraction: betulol, lupeol, lanosterol, lupeol acetate, β-amyrin, β-sitosterol, and α-amyrin, with β-sitosterol being the compound present in highest quantities (ca. 54% of total determined phytosterols) and α-amyrin being the minor one (ca. 0.4%) (**Figure 1** and **Tables 1** and **2**). Calibration curves were obtained with standard solutions at four different concentrations, selected as representative of the range of concentrations of the compounds in *F. carica* latex. The calibration plots showed a good correlation, as indicated by correlation coefficient values higher than 0.98 for all phytosterols (**Table 2**). The limit of detection (LOD = $3S_0/b$) and limit of quantification (LOQ = $10S_0/b$) (where S_0 is the standard deviation of the signal-to-noise ratio of a low concentration standard and b is the slope of the calibration plot) (*26*) for the analyzed phytosterols are shown in **Table 2**. To study the phytosterol recovery, aliquots of lupeol standard solution were treated as the sample and quantified by HPLC−DAD, with a recovery of 91% (±1.5). Repeatability was checked by analyzing the same sample 5 times, by the same analyst, within the same day; the coefficients of variation were lower than 20% (**Table 2**), indicating that the repeatability of the procedure was good. Additionally, the interday precision was determined by analyzing the sample on 5 different days (5 injections/day). The coefficients of variation obtained were always lower than 15%, and the method presented a satisfactory interday precision (**Table 2**).

As far as we know, with the exception of β-sitosterol derivatives already reported (*5*), the other phytosterols are described for the first time in *F. carica* latex. β-Sitosterol was the main phytosterol identified in latex, which is in agreement with the results obtained before (*27*) with the unsaponifiable fraction of *F. carica* leaves and fresh and dried fruits. In comparison to other matrices known for their high level of phytosterols, such as nuts and seeds (0.95−4.13 g/kg) (*28*) and oilseed rape (1.41−15.57 g/kg) (*29*), *F. carica* latex is a richer source of this class of compounds (19.6 g/kg), which could be isolated or a purified extract could be prepared for further application in several industries.

In human nutrition, only small amounts of latex are ingested with fresh figs. Furthermore, the intake of phytosterols may cause a decrease in plasma cholesterol levels, which can be due to not only the inhibition of intestinal cholesterol absorption but also other effects on hepatic/intestinal cholesterol metabolism (*30*). Additionally, anticarcinogenic, anti-inflammatory, and antidiabetic activities are also verified for products given orally (*31*), being important health promoter components of the diet. In plants, these compounds are resultant from the isoprenoid biosynthetic pathway and derive from squalene (*11*). The differences between higher plants and yeast/mammals in the biosynthesis of sterols are generally accepted to begin at the cyclization step of 2,3-oxidosqualene, a common precursor. Phytosterols, such as

Table 1. Structures and Mass Spectrum Data of Identified Sterols

Phytosterol	Mass spectrum (m/z)[a]	Structure
Betulol	189(100); 95(78); 207(75); 203(71); 135(69); 411(64); 81(53); 121(51); 107(50); 109(48); 442 (M^+)	
Lupeol	43(100); 68(98); 55(87); 67(78); 81(76); 69(73); 95(72); 93(68); 41(67); 109(61); 426 (M^+)	
Lanosterol	69(100); 43(91); 55(64); 41(64); 411(54); 109(44); 95(42); 426(35); 81(33); 57(32); 426 (M^+)	
Lupeol acetate	43(100); 189(75); 95(75); 109(71); 69(70); 81(65); 121(61); 107(58); 93(56); 55(55); 468 (M^+)	
β-Amyrin	218(100); 203(26); 219(18); 189(10); 95(9); 69(8); 109(8); 135(7); 81(6); 207(6); 426 (M^+)	
β-Sitosterol	43(100); 55(36); 41(33); 57(31); 107(30); 81(28); 414(28); 95(27); 105(25); 69(24); 414 (M^+)	
α-Amyrin	218(100); 219(18); 203(16); 189(11); 135(10); 122(10); 95(10); 426(9); 207(9); 109(8); 426 (M^+)	

[a] Values represent fragmentation ions (relative abundance, %). GC−ITMS conditions: oven temperature at 40 °C (for 1 min), 2 °C/min to 220 °C, and held for 30 min; injector port heated to 250 °C, in split mode, with a ratio of 1:40; carrier gas, helium C-60 (Gasin, Portugal), at 1 mL/min, constant flow; chromatographic column, VF-5 ms, 30 m × 0.25 mm × 0.25 μm (FactorFour), from Varian.

campesterol, stigmasterol, and β-sitosterol, are biosynthesized via cycloartenol in higher plants. β-Sitosterol is the principal Δ^5-sterol in several plant materials, being one of the most efficient compounds acting in membranes to restrict the motion of fatty acyl chains, because of its stereochemistry, associated with the presence of a 24-ethyl group at C-24 (*11*).

Lupeol synthases cyclize 2,3-oxidosqualene, promote ring expansion and annulation to lupyl cation, and terminate by abstracting the C-29 proton to form lupeol (*32*). This compound has been described in *F. carica* fruits and exhibits several pharmacological properties, including beneficial activities against inflammation, cancer, diabetes, and renal and hepatic toxicities (*33*).

Lanosterol is synthesized from 2,3-oxidosqualene by lanosterol synthase (*32*). As far as we know, this compound is described for the first time in this species.

β- and α-amyrin synthases also form the lupyl cation but allow further ring expansion and some rearrangement before deprotonation to β- and α-amyrin, respectively (*32*), which have been known to possess anti-inflammatory, anti-ulcer, anti-hyperlipidemic, anti-tumor, and hepatoprotective activities (*34*). These compounds have been described in this species, namely, in dried fig fruits and branchs (*27*), and are known to be precursors of saponins (*32*).

Betulol [lup-20(29)-ene-3β,28-diol], also known as betulin, is an abundant naturally occurring triterpene, predominantly found in bushes and trees. This phytosterol can be easily converted to betulinic acid, which possesses a wide spectrum of biological and pharmacological activities, such as antimalarial and anti-inflammatory activities (*35*).

Considering its high phytosterol content, *F. carica* latex may be incorporated in products to be used orally, such as pharmaceutics (as therapeutic steroids) and functional foods (as anti-cholesterol additives), or topically, such as cosmetics (in creams and lipsticks) (*31*).

Fatty Acid Profile. The fatty acid profile of *F. carica* latex was determined by GC−ITMS and revealed to be composed of 14 major detectable fatty acids (**Table 3** and **Figure 2**). The linearity was obtained with standard solutions at five different concentrations, selected as representative of the range of concentrations of the compounds in *F. carica* latex, with a good correlation being

found (correlation coefficient values higher than 0.98 for all fatty acids) (**Table 3**). The LOD and LOQ for the analyzed compounds are indicated in **Table 3**. To evaluate the fatty acid recovery, aliquots of oleic acid standard solution were treated as the sample and quantified by GC−ITMS, with a recovery of 95% (±1.6) being obtained. Repeatability and interday precision were determined, and the coefficients of variation found were lower than 20 and 15%, respectively (**Table 3**), which indicate that the repeatability and interday precision of the procedure were good.

As far as we know, among the determined fatty acids, myristic, pentadecanoic, heptadecanoic, *cis*-10-heptadecenoic, linoleic, arachidic, heneicosanoic, behenic, tricosanoic, and lignoceric acids are reported for the first time in *F. carica* latex. Studies performed with latex produced from other species revealed that this material is characterized by low total lipid quantities (*36*), as with our sample. Nevertheless, Kitahara et al. (*37*) demonstrated that, even in small amounts, fatty acids show interesting biological activities, namely, as antimicrobial agents, for which they may be potential antimicrobial material and ointment base for infection control. Thus, the extraction and preconcentration of this matrix may provide an interesting material to be incorporated in pharmaceutical, cosmetic, and nutraceutical formulations for topical and oral use.

Palmitic, arachidic, and behenic acids were the major fatty acids (ca. 21.4, 44.1, and 13.1% of total fatty acid content, respectively) (**Table 3**). Latex is essentially constituted by saturated fatty acids (SFAs) (ca. 86.4% of total fatty acids), unlike what happens with dried and fresh fruits, which are predominantly composed of polyunsatured fatty acids (ca. 84 and 69% of total fatty acids, respectively) (*27*, *38*). According to other authors (*27*, *38*), the SFA profile of *F. carica* dried and fresh fruits mainly presents palmitic acid, which agrees with the high content of this compound in *F. carica* latex.

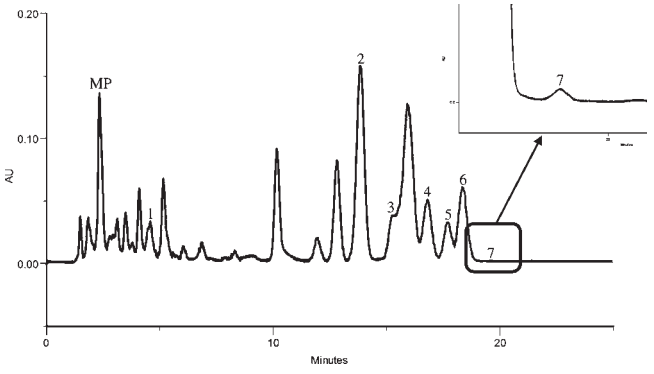


Figure 1. HPLC−DAD phytosterol chromatogram of *F. carica* latex hydrolyzed with 1 M KOH (detection at 205 nm). Peaks: (MP) mobile phase, (1) betulol, (2) lupeol, (3) lanosterol, (4) lupeol acetate, (5) β-amyrin, (6) β-sitosterol, and (7) α-amyrin.

With respect to monounsaturated fatty acids (MUFAs), oleic acid (C18:1*n*9) was the most abundant one (ca. 95% of MUFA total content), which is in agreement with data found for *F. carica* fruit (*27*, *38*) (**Table 3**). Oleic acid is included in the ω-9 family and is not essential for humans, because they possess all of the enzymes required for its synthesis. Under severe conditions of essential fatty acid deprivation, mammals elongate and desaturate oleic acid to produce mead acid (C20:3*n*9) (*39*). Oleic acid has been described in several materials, including fresh and dried fig fruits and is recognized for its effectiveness in reducing cholesterol levels and promoting the decrease of cardiovascular diseases and for its antidiabetic and anti-inflammatory properties (*27*, *38*, *40*).

With regard to polyunsaturated fatty acids (PUFAs), linoleic acid was the only compound identified (ca. 9.9% of total fatty acids) (**Table 3**). This compound has already been described in dried and fresh fig fruits (*27*, *38*). It is an essential fatty acid because it cannot be synthesized by humans, as a result of the lack of desaturase enzymes required for its production. It must be obtained from the diet and originates the ω-6 fatty acid series, which includes γ-linolenic and arachidonic acids (*39*). Linoleic acid can convert to hormone-like substances called eicosanoids, which affect physiological reactions ranging from blood clotting to immune response (*27*).

Amino Acid Profile. The characterization of the free amino acid profile of *F. carica* latex by HPLC/UV−vis revealed the presence of 13 compounds, comprising essential (not synthesized by humans) and non-essential amino acids (**Table 4** and **Figure 3**), which, as far as we know, are reported for the first time in this material. A linear relationship was obtained with standard solutions at five different concentrations, selected as representative of the range of concentrations of the compounds in *F. carica* latex; a good correlation, with coefficient values higher than 0.90 for all amino acids, was found (**Table 4**).

To determine the amino acid recovery, aliquots of cysteine standard solution were treated as the sample and quantified by HPLC/UV−vis, with a recovery of 90% (±1.3). Repeatability and interday precision were determined, with coefficients of variation lower than 20 and 15% being found, respectively (**Table 4**), thus indicating that the repeatability and interday precision of the procedure were good.

Despite *F. carica* latex exhibiting low levels of amino acids, when compared to *Hevea brasilliensis* latex, our matrix is characterized by higher total amounts of these compounds (*41*). Some of them, such as glycine and glutamine, have an important role in the treatment of brain metabolism imbalance and as neurotransmitters (*42*). Although *F. carica* latex presents reduced amino acid content, the extraction and preconcentration of this matrix may render an interesting material to be used topically or orally in formulations for health promotion. Cysteine and tyrosine were clearly the compounds present in higher quantities relatively to the other free amino acids (ca. 38.7 and 31.4% of total amino acid

Table 2. Linearity, Detection and Quantification Limits, Repeatability, Precision, and Phytosterol Composition of *F. carica* Latex[a]

phytosterol	regression equations	R^2	LOD (μg/mL)	LOQ (μg/mL)	repeatability (CV %)	interday precision (CV %)	mg/kg[b]
betulol	$y = 1 \times 10^8 x + 4 \times 10^6$	0.9915	1.4	4.7	6.1	9.0	326.6 ± 32.2
lupeol	$y = 3 \times 10^8 x + 5 \times 10^6$	0.9937	10.4	34.8	5.3	5.7	2827.4 ± 2.3
lanosterol	$y = 6 \times 10^8 x - 2 \times 10^7$	0.9897	117.9	392.9	5.6	10.5	2634.1 ± 275.6
lupeol acetate	$y = 2 \times 10^8 x + 7 \times 10^6$	0.9986	67.5	225.0	3.9	9.3	1988.9 ± 20.6
β-amyrin	$y = 3 \times 10^8 x + 2 \times 10^7$	0.9905	155.6	518.6	3.1	3.6	1197.0 ± 41.0
β-sitosterol	$y = 1 \times 10^8 x + 5 \times 10^6$	0.9884	62.8	209.4	4.0	4.9	10564.3 ± 251.8
α-amyrin	$y = 2 \times 10^8 x - 1 \times 10^7$	0.9959	194.2	647.3	12.5	13.5	76.2 ± 17.8
total							19614.5

[a] LOD, limit of detection; LOQ, limit of quantification; CV, coefficient of variation. [b] Values are expressed as mean ± standard deviation of three assays.

Table 3. Linearity, Detection and Quantification Limits, Repeatability, Precision, and Free Fatty Acid Composition of *F. carica* Latex

fatty acid[a]	mass spectrum (*m*/*z*)[b]	regression equations	R^2	LOD (μg/L)	LOQ (μg/L)	repeatability (CV %)	interday precision (CV %)	mg/kg[c]
C14:0	73 (100), 60 (95), 43 (86), 41 (73), 57 (73), 55 (57), 228 (44), 129 (44), 29 (42), 71 (38), 228 (M^+)	$y = 34968x - 407.1$	0.9998	18.96	63.20	1.07	2.04	0.56 ± 0.01
C15:0	43 (100), 73 (92), 60 (92), 41 (87), 55 (68), 57 (64), 29 (54), 69 (34), 71 (33), 242 (30), 242 (M^+)	$y = 41478x - 1075.9$	0.9947	91.27	304.23	7.74	11.9	1.35 ± 0.02
C16:0	43 (100), 73 (91), 60 (84), 41 (75), 57 (63), 55 (62), 29 (41), 69 (31), 71 (29), 61 (22), 256 (M^+)	$y = 69568x - 5994.2$	0.9880	266.5	888.37	2.13	9.99	28.94 ± 1.12
C17:0	73 (100), 80 (86), 57 (78), 43 (76), 270 (68), 129 (51), 71 (46), 41 (45), 55 (43), 69 (32), 270 (M^+)	$y = 28597x - 751.3$	0.9964	98.33	327.77	2.76	6.42	0.66 ± 0.03
C17:1	74 (100), 87 (66), 43 (55), 57 (42), 55 (41), 41 (34), 69 (28), 71 (23), 143 (23), 83 (22), 270 (M^+)	$y = 43962x - 1152.4$	0.9965	91.33	304.44	2.81	5.02	nq*
C18:0	43 (100), 73 (34), 60 (81), 57 (76), 41 (70), 55 (64), 29 (38), 71 (37), 69 (35), 129 (32), 284 (M^+)	$y = 49782x - 1094.5$	0.9933	77.17	257.22	0.99	2.89	8.62 ± 0.20
C18:1c	55 (100), 69 (76), 41 (75), 83 (59), 43 (55), 97 (44), 57 (44), 67 (35), 56 (35), 70 (32), 282 (M^+)	$y = 161434x - 5193.8$	0.9994	99.28	333.25	1.99	3.65	5.54 ± 0.73
C18:1t	55 (100), 69 (83), 41 (70), 83 (69), 264 (54), 43 (51), 97 (50), 57 (42), 70 (38), 56 (36), 282 (M^+)	$y = 56860x - 1589.6$	0.9864	93.68	312.28	4.45	8.48	0.35 ± 0.07
C18:2	67 (100), 81 (88), 82 (73), 95 (63), 68 (60), 55 (60), 96 (55), 41 (54), 54 (45), 69 (36), 280 (M^+)	$y = 315587x + 5906.3$	0.9974	54.29	181.27	2.25	7.35	14.59 ± 0.73
C20:0	43 (100), 57 (79), 73 (71), 41 (69), 55 (66), 60 (61), 71 (43), 69 (35), 29 (31), 85 (26), 312 (M^+)	$y = 43010x - 641.3$	0.9967	57.77	192.35	4.28	8.48	91.29 ± 1.12
C21:0	98 (100), 74 (79), 55 (73), 69 (49), 87 (46), 84 (41), 112 (39), 97 (29), 83 (29), 353 (25), 326 (M^+)	$y = 42367x - 1421.4$	0.9930	113.82	379.40	5.97	7.74	0.77 ± 0.08
C22:0	340 (100), 57 (51), 73 (41), 43 (39), 129 (38), 71 (35), 55 (29), 97 (29), 97 (29), 60 (28), 83 (26), 340 (M^+)	$y = 47023x - 2643.2$	0.9965	180.50	601.66	1.08	1.68	26.43 ± 0.49
C23:0	43 (100), 354 (75), 55 (71), 41 (69), 57 (68), 60 (63), 73 (62), 69 (38), 71 (37), 129 (35), 354 (M^+)	$y = 40762x - 1670.4$	0.9930	136.63	455.42	1.91	3.67	1.25 ± 0.01
C24:0	43 (100), 57 (100), 73 (80), 60 (61), 55 (58), 71 (56), 129 (51), 41 (51), 69 (42), 368 (M^+)	$y = 84071x - 3003.9$	0.9945	114.90	383.00	3.29	6.24	1.90 ± 0.17
total								182.25
SFA								161.77
MUFA								5.89
PUFA								14.59

[a] C14:0, myristic acid; C15:0, pentadecanoic acid; C16:0, palmitic acid; C17:0, heptadecanoic acid; C17:1, *cis*-10-heptadecenoic acid; C18:0, stearic acid; C18:1c, oleic acid; C18:1t, elaidic acid: C18:2, linoleic acid; C20:0, arachidic acid; C21:0, heneicosanoic acid; C22:0, behenic acid; C23:0, tricosanoic acid; C24:0, lignoceric acid; SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; LOD, limit of detection; LOQ, limit of quantification; CV, coefficient of variation. [b] Values represent fragmentation ions (relative abundance, %). Chromatographic conditions: oven temperature at 40 °C (for 1 min), 5 °C/min to 250 °C, 3 °C/min to 300 °C, and held for 15 min; injector port heated to 250 °C, in split mode, with a ratio of 1:40; carrier gas, helium C-60 (Gasin, Portugal), at 1 mL/min, constant flow; chromatographic column, VF-5 ms, 30 m × 0.25 mm × 0.25 μm (FactorFour), from Varian. [c] Results are expressed as mean ± standard deviation of three determinations. *Compound present in the matrix below the limit of quantification.

content, respectively), followed by tryptophan (ca. 22.2%) (**Table 4**). All of the determined amino acids were already described in dried figs (*43*).

In young plants, amino acid biosynthesis is regulated by a metabolic network that links nitrogen assimilation with carbon metabolism. This network is strongly controlled by the metabolism of four central amino acids, namely, glutamine, glutamate, aspartate, and asparagine, which are then converted into all other amino acids in various biochemical processes. They also serve as major transport molecules of nitrogen, including transport from vegetative to reproductive tissues. Its metabolism is subjected to a concerted regulation by physiological, developmental, and hormonal signals (*15*).

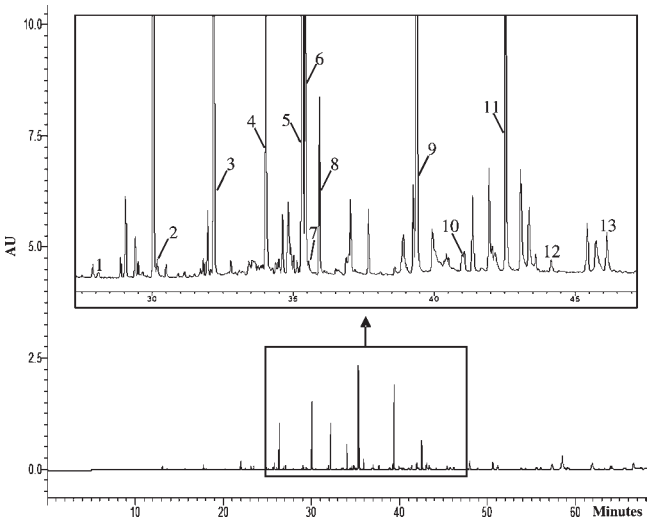


Figure 2. GC−ITMS profile of methyl esters of free fatty acids of *F. carica* latex. Peaks: (1) myristic acid (C14:0), (2) pentadecanoic acid (C15:0), (3) palmitic acid (C16:0), (4) heptadecanoic acid (C17:0), (5) linoleic acid (C18:2), (6) oleic acid (C18:1c), (7) elaidic acid (C18:1t), (8) stearic acid (C18:0), (9) arachidic acid (C20:0), (10) heneicosanoic acid (C21:0), (11) behenic acid (C22:0), (12) tricosanoic acid (C23:0), and (13) lignoceric acid (C24:0).

Cysteine, which is one of the major compounds identified, is formed from *O*-acetylserine and sulfide, catalyzed by *O*-acetylserine(thiol)-lyase (*44*). Its biosynthesis in plants constitutes an essential part of the sulfur cycle in nature (*45*). This amino acid is necessary for the detoxification of harmful toxins from the body, protecting the brain and liver from alcohol and drug damage (*45*, *46*).

Tyrosine, tryptophan, and phenylalanine are three aromatic amino acids, biosynthesized from the shikimate pathway, having chorismate as a precursor (*47*). These amino acids are precursors of a large variety of secondary metabolites, such as phenylpropanoids and alkaloids, which are important in plant metabolism (*47*). Tyrosine is a precursor of the neurotransmitters epinephrine and dopamine and is useful in the suppression of appetite (*46*). Tryptophan is a precursor of serotonin, being used in insomnia, anxiety, and depression conditions (*45*, *46*). When combined with UV radiation, phenylalanine can be used for vitiligo treatment (*46*).

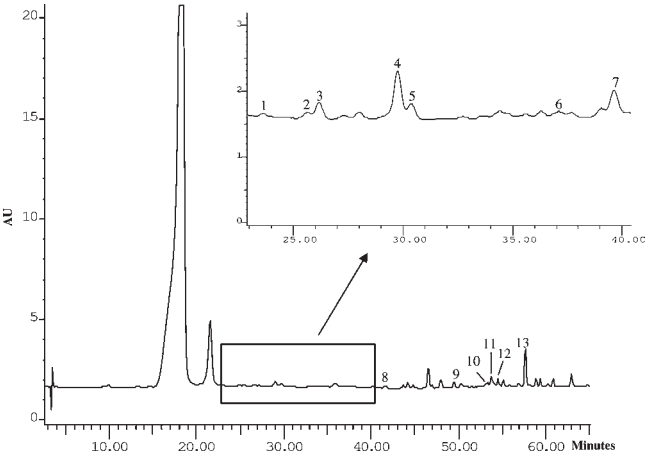


Figure 3. HPLC/UV−vis chromatogram of *F. carica* latex free amino acids (detection at 436 nm): (1) asparagine, (2) glutamine, (3) serine, (4) glycine, (5) alanine, (6) leucine, (7) tryptophan, (8) phenylalanine, (9) cysteine, (10) ornithine, (11) lysine, (12) histidine, and (13) tyrosine.

Table 4. Linearity, Detection and Quantification Limits, Repeatability, Precision, and Amino Acid Composition of *F. carica* Latex[a]

amino acid	regression equations	R^2	LOD (μg/mL)	LOQ (μg/mL)	repeatability (CV %)	interday precision (CV %)	mg/g[b]
			Essential Amino Acid				
leucine	$y = 1.0 \times 10^7 x + 2438.2$	0.9881	0.57	1.90	6.52	11.4	$0.036 \pm 1.8 \times 10^{-6}$
tryptophan	$y = 1.0 \times 10^7 x + 4065.5$	0.9829	1.20	4.01	9.61	13.4	$1.598 \pm 2.1 \times 10^{-3}$
phenylalanine	$y = 8.0 \times 10^7 x + 3584.1$	0.9834	0.13	0.43	13.4	13.8	$0.015 \pm 4.8 \times 10^{-6}$
lysine	$y = 1.0 \times 10^7 x + 3293.4$	0.9563	0.97	3.25	6.93	12.1	$0.010 \pm 2.5 \times 10^{-7}$
histidine	$y = 3.0 \times 10^6 x + 3384.4$	0.9455	3.33	11.10	5.25	14.3	$0.058 \pm 2.0 \times 10^{-4}$
Σ							1.717
			Non-essential Amino Acid				
asparagine	$y = 3.0 \times 10^6 x + 1432.9$	0.9603	7.79	25.98	8.98	10.8	$0.058 \pm 7.2 \times 10^{-5}$
alanine	$y = 1.0 \times 10^7 x + 5653.8$	0.9854	1.43	4.76	5.57	8.50	$0.008 \pm 3.8 \times 10^{-6}$
glutamine	$y = 3.0 \times 10^6 x + 762.39$	0.9910	0.60	2.00	11.3	11.5	$0.034 \pm 2.2 \times 10^{-5}$
serine	$y = 4.0 \times 10^6 x + 4344.5$	0.9832	3.04	10.14	8.70	10.5	$0.060 \pm 1.8 \times 10^{-7}$
glycine	$y = 1.0 \times 10^7 x + 20161$	0.9641	6.03	20.10	3.41	7.40	$0.004 \pm 4.2 \times 10^{-7}$
ornithine	$y = 1.0 \times 10^7 x + 4914.7$	0.9033	1.38	4.60	6.72	7.00	$0.270 \pm 3.4 \times 10^{-4}$
tyrosine	$y = 5.0 \times 10^6 x + 10991$	0.9197	6.34	21.10	10.2	13.2	$2.264 \pm 1.7 \times 10^{-3}$
cysteine	$y = 8.3 \times 10^5 x + 1434.5$	0.9162	4.74	15.80	7.65	10.0	$2.786 \pm 4.8 \times 10^{-4}$
Σ							5.484
total of all amino acids							7.201

[a] LOD, limit of detection; LOQ, limit of quantification; CV, coefficient of variation; Σ, sum of the determined amino acids. [b] Values are expressed as mean ± standard deviation of three assays.

In conclusion, this work provides quantitative and qualitative data of a large number of metabolites, namely, phytosterols, fatty acids, and amino acids, giving a broader view of the *F. carica* latex metabolic profile. A total of 6 phytosterols, 10 fatty acids, and 13 amino acids were described for the first time, improving the knowledge on the chemical composition of this matrix. All of these identified compounds develop determinant roles in defense, metabolism, and plant ecological interactions. Some of them also have important biological activities in both plants and humans. Attending to the compounds determined and to the biological activities attributed to them, further assays regarding the potential of this matrix as a source of bioactive compounds or even its direct use deserve to be developed.

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