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A Comprehensive Characterization of Lipids in Wheat Straw

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ABSTRACT: The chemical composition of the lipids in wheat straw was studied in detail by gas chromatography and mass spectrometry. Important discrepancies with the data reported in previous papers were found. The predominant lipids identified were series of long-chain free fatty acids (25% of total extract), followed by series of free fatty alcohols (ca. 20%). High molecular weight esters of long chain fatty acids esterified to long chain fatty alcohols were also found (11%), together with lower amounts of other aliphatic series, such as *n*-alkanes, *n*-aldehydes, and glycerides (mono-, di-, and triglycerides). Relatively high amounts of β-diketones (10%), particularly 14,16-hentriacontanedione, which is the second most abundant single compound among the lipids in wheat straw, were also identified. Finally, steroid compounds (steroid hydrocarbons, steroid ketones, free sterols, sterol esters, and sterol glycosides) were also found, with sterols accounting for nearly 14% of all identified compounds.

KEYWORDS: *Wheat straw, lipids, fatty acids, fatty alcohols, sterols, β-diketones*

INTRODUCTION

There is a growing need to consider alternative agricultural strategies that move an agricultural industry focused on food production to one that also supplies the needs of other industrial sectors, such as paper, textiles, biofuels, or added-value chemicals, in the context of the so-called lignocellulose biorefinery. The term "biorefinery" has been established to describe future processing mills that will use renewable raw materials to produce energy together with a wide range of everyday commodities in an economic and sustainable manner.[1−3] Plant biomass is the main source of renewable materials on Earth and represents a potential source of renewable energy and biobased products. Biomass is available in high amounts at very low cost (as forest, agricultural or industrial lignocellulosic wastes and cultures) and could be a widely available and inexpensive source for biofuels and bioproducts in the near future.

The high abundance, wide availability, and very low-cost of some agricultural wastes, as cereal straws, makes them excellent raw materials for future biorefineries. Among them, wheat straw has the greatest potential of all agricultural residues because of its wide availability and low cost.[4,5] Wheat straw is an abundant byproduct from wheat production in many countries. The average yield of wheat straw is 1.3−1.4 kg/kg of wheat grain, with a world production of wheat estimated to be around 680 million tons in 2011. Wheat straw contains 35−45% cellulose, 20−30% hemicelluloses, and around 15% lignin, which makes it an attractive feedstock to be converted to ethanol and other value-added products.[6]

Wheat straw also contains significant amounts of lipids (ca. 1−2% by weight) that can be extracted to produce high-value waxes.[7] Natural waxes have a wide range of industrial applications in cosmetics, personal care products, polishes, and coatings. On the other hand, these lipids, even when present in low amounts in the raw material, may play an important role during the industrial processing, as in pulp and paper production, since they are at the origin of the so-called pitch deposits.[8] Lipids include different classes of compounds (i.e., alkanes, fatty alcohols, fatty acids, free and conjugated sterols, terpenoids, and triglycerides), which have different behavior during pulping and bleaching.[8−12] Pitch deposition is a serious problem in the pulp and paper industry, being responsible for reduced production levels, higher equipment maintenance costs, higher operating costs, and an increased incidence of defects in the finished products, which reduces quality and benefits.[8]

Studies concerning the composition of lipids in wheat straw have been relatively scarce, although some papers have been published in this regard.[7,13−15] However, most of these studies are somewhat limited and controversial. Thus, some papers have reported the occurrence of resin acids (i.e., abietic acid), which are exclusively restricted to conifers, among the lipophilic extractives in wheat straw.[13−15] Moreover, high amounts of ergosterol, a sterol that only occurs in fungi and that is absent in plants, were also reported in those studies.[13−15] Therefore, the presence of these compounds clearly indicates cross-contamination from other lignocellulosic materials, as well as fungal degradation of the studied wheat straw sample, which certainly impairs obtaining accurate information about the authentic composition of the lipids present in wheat straw. In the present work, a thorough and comprehensive characterization of the lipophilic extractives in wheat straw has been performed, and important discrepancies with the data reported in previous papers have been found. In this paper, the composition of the lipophilic compounds was carried out by gas chromatography (GC) and gas chromatography−mass spectrometry (GC−MS) using short- and medium-length high temperature capillary columns, respectively, with thin films, which enables the elution and analysis of a wide range of compounds from fatty acids to intact high molecular weight lipids such as sterol esters, sterol glycosides or triglycerides.[16] The knowledge of the precise composition of the lipophilic extractives in wheat straw will help to maximize the exploitation of this important agricultural waste.

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■ MATERIALS AND METHODS

Samples. Wheat straw (*Triticum durum* var. Carioca) was harvested from an experimental field in Seville (South Spain) in June 2009. Wheat straw was air-dried, and the dried samples were milled using a knife mill (Janke and Kunkel, Analysenmühle), and subsequently extracted with acetone in a Soxhlet apparatus for 8 h. The acetone extracts were evaporated to dryness and resuspended in chloroform for chromatographic analysis of the lipophilic fraction. Two replicates were used for each sample.

GC and GC−MS Analyses. An HP 5890 gas chromatograph (Hewlett-Packard, Hoofddorp, Netherlands) equipped with a split−splitless injector and a flame ionization detector (FID) was used for GC analyses. The injector and the detector temperatures were set at 300 and 350 °C, respectively. Samples were injected in the splitless mode. Helium was used as the carrier gas. The capillary column used was a high temperature, polyimide-coated fused silica tubing DB5-HT (5 m x 0.25 mm i.d., 0.1 μm film thickness; J&W Scientific). The oven was temperature-programmed from 100 °C (1 min) to 350 °C (3 min) at 15 °C min^{-1}. Peaks were quantified by area, and a mixture of standards (octadecane, palmitic acid, sitosterol, cholesteryl oleate, and sitosteryl 3*β*-D-glucopyranoside) with a concentration range between 0.1 and 1 mg/mL was used to elaborate calibration curves. The correlation coefficient was higher than 0.99 in all the cases. The data from the two replicates were averaged. In all cases, the standard deviations from replicates were below 10% of the mean values.

The GC−MS analyses were performed on a Varian Star 3400 gas chromatograph (Varian, Walnut Creek, CA) coupled with an ion-trap detector (Varian Saturn) equipped with a high-temperature capillary column (DB-5HT, 15 m × 0.25 mm i.d., 0.1 μm film thickness; J&W Scientific). Helium was used as carrier gas at a rate of 2 mL/min. The samples were injected with an autoinjector (Varian 8200) directly onto the column using a SPI (septum-equipped programmable injector) system. The temperature of the injector during the injection was 60 °C and 0.1 min after injection was programmed to 380 °C at a rate of 200 °C min^{-1} and held for 10 min. The oven was heated from 120 °C (1 min) to 380 °C (5 min) at 10 °C min^{-1}. The temperature of the transfer line was set at 300 °C. Bis(trimethylsilyl)trifluoroacetamide (BSTFA) silylation was used when required. Compounds were identified by comparing their mass spectra with mass spectra in the Wiley and NIST libraries, by mass fragmentography, and, when possible, by comparison with authentic standards.

■ RESULTS AND DISCUSSION

The abundance of the main constituents of wheat straw (water-solubles, acetone extractives, Klason lignin, acid-soluble lignin, holocellulose, α-cellulose, and ash) is shown in Table 1. The total acetone extractives of wheat straw accounts for 2.7% of the dry material. However, the lipohilic content, estimated as the chloroform solubles, is lower and accounts for 2% while the rest (0.7%) correspond to polar compounds extracted in acetone. This content is similar to that reported for wheat straw in previous papers[13−15] and also similar to that found in other nonwoody materials such as flax,[9,10] hemp,[17] kenaf,[18] sisal,[19] abaca,[20] jute,[21] giant reed,[22] or *Miscanthus*.[23]

Table 1. Abundance of the Main Constituents (% dry weight) of Wheat Straw

water-solubles	9.6
total acetone extractives	2.7
lipophilics	2.0
polars	0.7
Klason lignin[a]	16.2
acid-soluble lignin	1.5
holocellulose	67.2
cellulose	36.5
hemicelluloses	30.7
ash	6.6

[a] Corrected for proteins and ash.

The underivatized and TMS-ether derivatives of the lipophilic extracts from wheat straw were analyzed by GC and GC−MS using short- and medium-length high-temperature capillary columns, respectively, with thin films, according to the method previously described.[16] The GC−MS chromatograms of the underivatized and TMS-ether derivatives of the lipid extracts from wheat straw are shown in Figure 1. The identities and abundances of the main lipid compounds identified are detailed in Table 2.

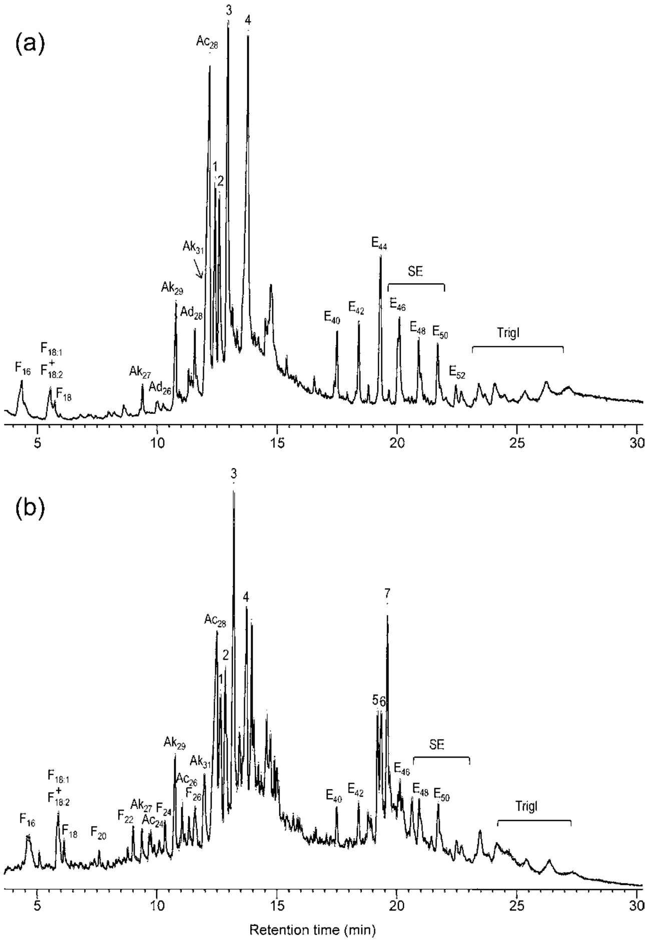

Figure 1. GC−MS chromatograms of the lipid extracts from wheat straw (a) underivatized, (b) as TMS-ether derivatives. F_n, *n*-fatty acid series; Ak_n, *n*-alkane series; Ac_n, *n*-fatty alcohol series; Ad_n, *n*-aldehyde series; E_n, high molecular weight ester series; *n* denotes the total carbon atom number. SE, sterol esters; Trigl, triglycerides. Other compounds reflected are 1, campesterol; 2, stigmasterol; 3, sitosterol; 4, 14,16-hentriacontanedione; 5, campesteryl 3*β*-D-glucopyranoside; 6, stigmasteryl 3*β*-D-glucopyranoside; 7, sitosteryl 3*β*-D-glucopyranoside.

The most predominant lipids present in wheat straw were series of fatty acids that accounted for 25% of all identified compounds, followed by series of free fatty alcohols (ca. 20%). High molecular weight esters of long-chain fatty acids esterified to long-chain fatty alcohols were also found in significant amounts (11%). Additionally, lower amounts of other aliphatic series such as *n*-alkanes, *n*-aldehydes, and glycerides (mono-, di-, and triglycerides) were also observed. Important amounts of β-diketones (10% of all identified compounds) were also found in the extracts of wheat straw. Steroid compounds

Table 2. Composition and Abundance (mg/kg fiber, daf) of Main Lipids Identified in the Extracts of Wheat Straw

compd	abund
n-fatty acids	2080
n-tetradecanoic acid	24
n-pentadecanoic acid	8
n-hexadecanoic acid	400
n-heptadecanoic acid	9
octadeca-9,12-dienoic acid	164
octadec-9-enoic acid	228
n-octadecanoic acid	112
n-nonadecanoic acid	5
n-eicosanoic acid	53
n-heneicosanoic acid	20
n-docosanoic acid	122
n-tricosanoic acid	66
n-tetracosanoic acid	114
n-pentacosanoic acid	32
n-hexacosanoic acid	104
n-heptacosanoic acid	13
n-octacosanoic acid	213
n-nonacosanoic acid	9
n-triacontanoic acid	104
n-hentriacontanoic acid	5
n-dotriacontanoic acid	69
n-tritriacontanoic acid	2
n-tetratriacontanoic acid	13
n-fatty alcohols	1615
n-docosanol	14
n-tricosanol	1
n-tetracosanol	49
n-pentacosanol	7
n-hexacosanol	94
n-heptacosanol	30
n-octacosanol	1392
n-nonacosanol	12
n-triacontanol	16
n-alkanes	371
n-tricosane	1
n-tetracosane	1
n-pentacosane	6
n-hexacosane	3
n-heptacosane	34
n-octacosane	7
n-nonacosane	157
n-triacontane	7
n-hentriacontane	128
n-dotriacontane	0
n-tritriacontane	27
n-aldehydes	99
n-eicosanal	2
n-heneicosanal	0
n-docosanal	2
n-tricosanal	0
n-tetracosanal	3
n-pentacosanal	0
n-hexacosanal	10
n-heptacosanal	4
n-octacosanal	69
n-nonacosanal	1
n-triacosanal	6
n-dotriacosanal	2
high molecular weight esters[a]	915
esters C_{38}	17
esters C_{39}	3
esters C_{40}	102
esters C_{41}	10
esters C_{42}	113
esters C_{43}	17
esters C_{44}	273
esters C_{45}	10
esters C_{46}	189
esters C_{47}	8
esters C_{48}	85
esters C_{49}	7
esters C_{50}	90
esters C_{51}	3
esters C_{52}	42
esters C_{54}	5
monoglycerides	127
2,3-dihydroxypropyl tetradecanoate	1
2,3-dihydroxypropyl hexadecanoate	26
2,3-dihydroxypropyl octadecadienoate	8
2,3-dihydroxypropyl octadecenoate	10
2,3-dihydroxypropyl octadecanoate	9
2,3-dihydroxypropyl eicosanoate	2
2,3-dihydroxypropyl docosanoate	5
2,3-dihydroxypropyl tricosanoate	1
2,3-dihydroxypropyl tetracosanoate	5
2,3-dihydroxypropyl pentacosanoate	1
2,3-dihydroxypropyl hexacosanoate	6
2,3-dihydroxypropyl heptacosanoate	1
2,3-dihydroxypropyl octacosanoate	28
2,3-dihydroxypropyl nonacosanoate	2
2,3-dihydroxypropyl triacontanoate	22
diglycerides	85
1,2-dipalmitin	13
1,3-dipalmitin	14
1,2-palmitoyllinolein	5
1,2-palmitoylolein	5
1,2-palmitoylstearin	2
1,3-palmitoyllinolein	7
1,3-palmitoylolein	10
1,3-palmitoylstearin	12
1,2-diolein	3
1,3-diolein	4
1,2-distearin	1
1,3-distearin	9
triglycerides	198
dipalmitoylolein	46
dioleoylpalmitin	91
triolein	61
β-diketones	883
14,16-hentriacontanedione	875
12,14-tritriacontanedione	7
steroid hydrocarbons	16
ergosta-3,5-diene	3
stigmasta-3,5,22-triene	4
stigmasta-4,22-diene	1
stigmasta-3,5,7-triene	2
stigmasta-3,5-diene	6
steroid ketones	88
stigmasta-4,22-dien-3-one	6
stigmasta-3,5-dien-7-one	23
ergost-4-ene-3,6-dione	4

Table 2. continued

compd	abund
ergostane-3,6-dione	6
stigmasta-4,22-diene-3,6-dione	1
stigmast-22-ene-3,6-dione	3
stigmast-4-ene-3,6-dione	21
stigmastane-3,6-dione	24
sterols	1121
campesterol	300
stigmasterol	240
sitosterol	581

compd	abund
sterol glycosides	680
campesteryl *β*-D-glucopyranoside	164
stigmasteryl *β*-D-glucopyranoside	191
sitosteryl *β*-D-glucopyranoside	325
sterol esters	70
campesterol esters	12
stigmasterol esters	6
sitosterol esters	53

[a] See Table 3 for the detailed description of the individual esters.

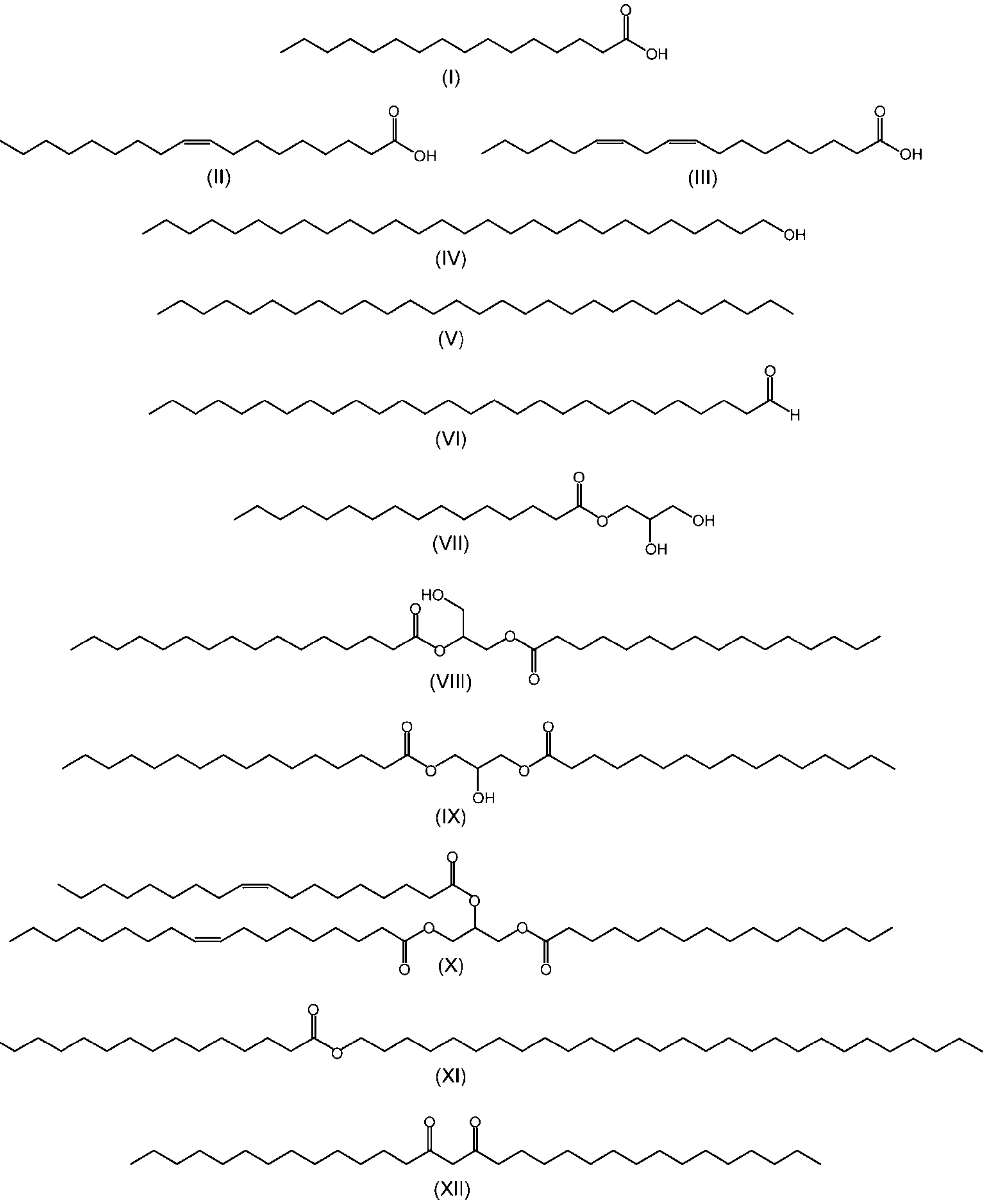

Figure 2. Structures representative of the main aliphatic lipophilic compounds identified in wheat straw and referred in the text: (**I**) hexadecanoic (palmitic) acid, (**II**) 9-octadecenoic (oleic) acid, (**III**) 9,12-octadecadienoic (linoleic) acid, (**IV**) *n*-octacosanol, (**V**) *n*-nonacosane, (**VI**) *n*-octacosanal, (**VII**) 2,3-dihydroxypropyl hexadecanoate (1-monopalmitin), (**VIII**) 1,2-dipalmitin, (**IX**) 1,3-dipalmitin, (**X**) dioloylpalmitin, (**XI**) hexadecanoic acid octacosyl ester, and (**XII**) 14,16-hentriacontanedione.

(hydrocarbons, ketones, free sterols, sterol esters, and sterol glycosides) were also present among the lipophilic extracts of wheat straw in important amounts, with sterols accounting for nearly 14% of all identified compounds. The structures of the main lipophilic compounds present in wheat straw are depicted in Figures 2 and 3. The distributions of the main aliphatic series

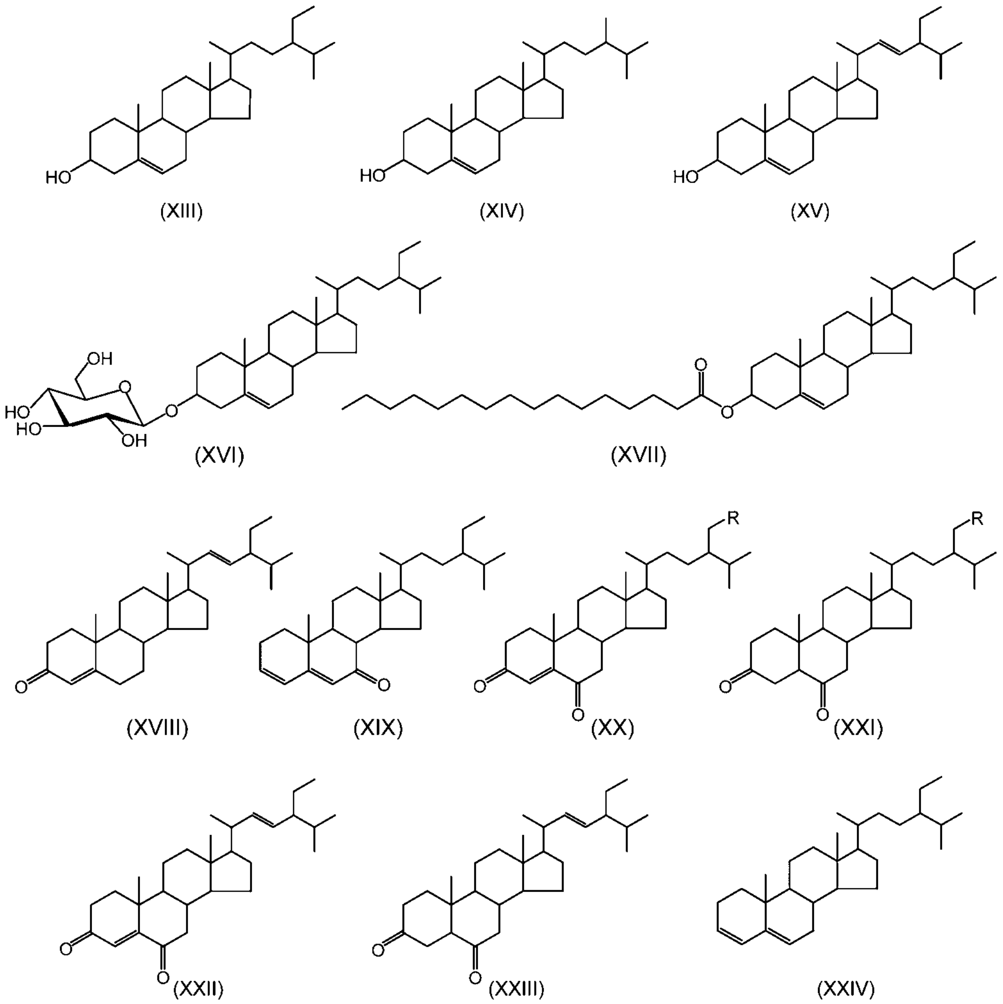

Figure 3. Structures of the main steroid compounds identified in wheat straw and referred in the text: (**XIII**) sitosterol, (**XIV**) campesterol, (**XV**) stigmasterol, (**XVI**) sitosteryl 3*β*-D-glucopyranoside, (**XVII**) sitosteryl palmitate, (**XVIII**) stigmasta-4,22-dien-3-one, (**XIX**) stigmasta-3,5-dien-7-one, (**XX**, R = H) ergost-4-ene-3,6-dione, (**XX**, R = CH_3) stigmast-4-ene-3,6-dione, (**XXI**, R = H) ergostane-3,6-dione, (**XXI**, R = CH_3) stigmastane-3,6-dione, (**XXII**) stigmasta-4,22-diene-3,6-dione, (**XXIII**) stigmast-22-ene-3,6-dione, and (**XXIV**) stigmasta-3,5-diene.

are represented in the histograms of Figure 4. It is important to note that significant differences were observed with the composition reported in previous papers.[13−15] Previous papers also indicated a predominance of free fatty acids in wheat straw. However, they failed to report the occurrence of fatty alcohols, which are the second most abundant class of aliphatic compounds in wheat straw, as well as the presence of series of alkanes and aldehydes. In addition, they did not report the presence of the important amounts of *β*-diketones that were observed in our work. Finally, previously published papers reported the presence of important amounts of free and esterified sterols,[13−15] but they to identify other important steroids such as sterol glycosides, steroid ketones, and steroid hydrocarbons.

Aliphatic Series. Free fatty acids were the most predominant series in the extracts of wheat straw, accounting for 2080 mg/kg. The series ranges from tetradecanoic acid (C_{14}) to tetratriacontanoic acid (C_{34}), with a strong even-over-odd carbon atom number predominance and hexadecanoic (palmitic) acid (**I**) being the most predominant. The unsaturated 9-octadecenoic (oleic, **II**) and 9,12-octadecadienoic (linoleic, **III**) acids were also found in important amounts, as already seen.[13] However, previous papers have reported the occurrence of important amounts of abietic acid,[13−15] a compound that is restricted only to conifers and should not be present among the lipids in wheat straw. Its occurrence could suggest cross-contamination of the lipids from other lignocellulosic sources. Free fatty alcohols were the second most abundant class of aliphatic series in the extracts of wheat straw, accounting for 1615 mg/kg, although their occurrence was not reported before.[13−15] Free fatty alcohols were found in the range from *n*-docosanol (C_{22}) to *n*-triacontanol (C_{30}), with a strong even-over-odd carbon atom number predominance and *n*-octacosanol (**IV**) being the most predominant homologue in the series. In fact, *n*-octacosanol was the most important single compound among the lipids of wheat straw. The series of *n*-alkanes was present in lower amounts (371 mg/kg) and ranged from *n*-tricosane (C_{23}) to *n*-tritriacontane (C_{33}), with a strong odd-over-even atom carbon number predominance and nonacosane (**V**) being the predominant homologue in the series, followed by hentriacontane. Finally, minor amounts of *n*-aldehydes (99 mg/kg) were identified from *n*-eicosanal (C_{20}) to *n*-dotriacosanal (C_{32}), with a strong even-over-odd atom carbon atom predominance and *n*-octacosanal (**VI**) being the major compound in the series. The distribution of the aldehyde series parallels that of free alcohols, as usually occurs in the plant kingdom and observed in other plants,[9,10] suggesting that aldehydes are intermediates in the biosynthesis of

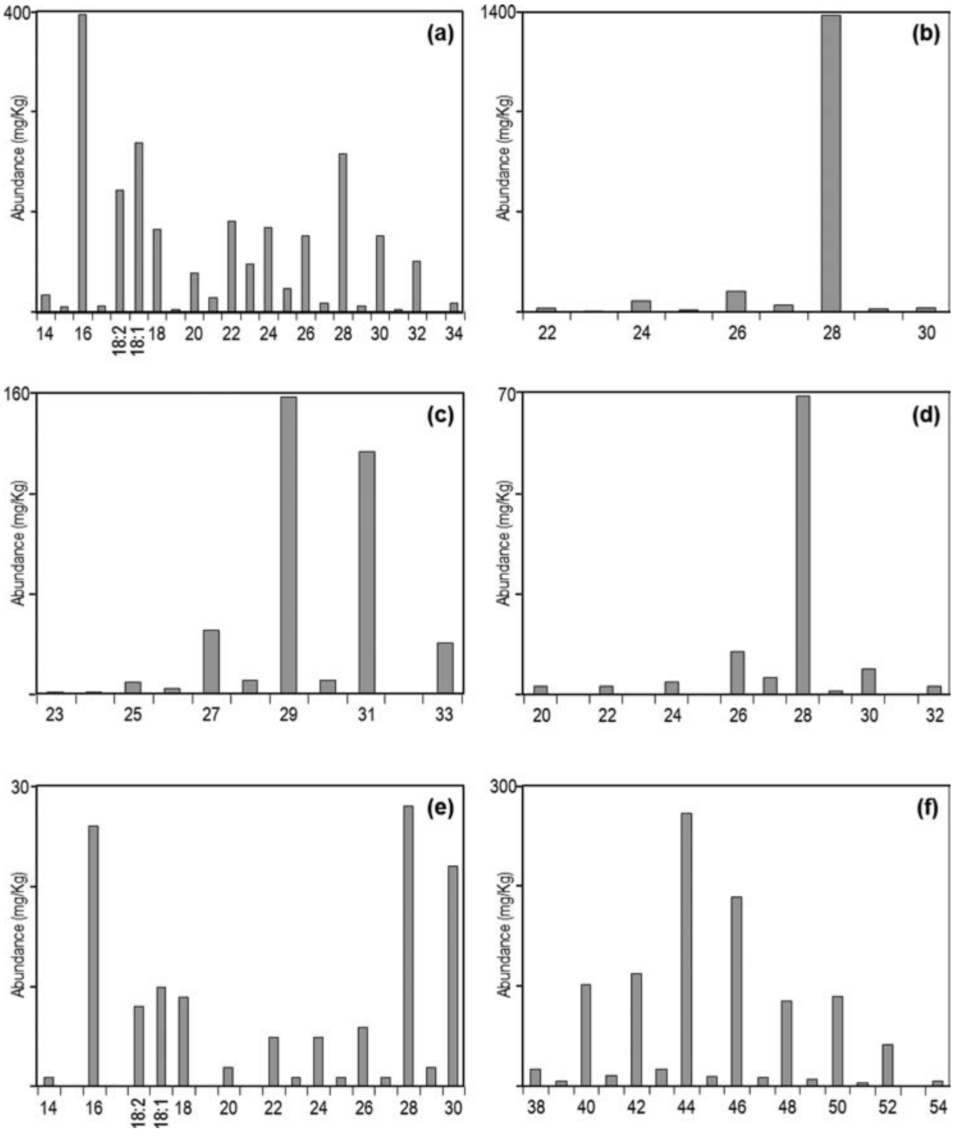

Figure 4. Distribution of the main aliphatic series identified in the extracts of wheat straw: (a) *n*-fatty acid, (b) *n*-fatty alcohols, (c) *n*-alkanes, (d) *n*-aldehydes, (e) monoglycerides, and (f) high molecular weight esters. The histograms are scaled up to the abundance of the major compound in the series.

alcohols from fatty acids.[24,25] Fatty alcohols, alkanes, and aldehydes were not detected in previous papers,[13−15] although alkanes and aldehydes were already reported in wheat straw by Deswarte et al.[7]

A series of high molecular weight esters also occurred in wheat straw extracts in important amounts (915 mg/kg). High molecular weight esters were found in the range from C_{38} to C_{48}, with a strong predominance of the even atom carbon number homologues and the C_{44} and C_{46} analogs being the most abundant ones. Our results completely differ from those of previous papers that only reported the presence of high molecular weight esters C_{32} and C_{34},[13−15] which were not detected in our study, and failed to detect the important presence of esters of higher molecular weight. A close examination of each chromatographic peak indicated that they consisted of a mixture of esters of different long-chain fatty acids esterified to different long-chain fatty alcohols. The identification and quantitation of the individual long-chain esters in each chromatographic peak was resolved on the basis of the mass spectra of the peaks. Figure 5 shows the mass spectra of the chromatographic peaks corresponding to the high molecular weight esters C_{44}, C_{46}, C_{48}, and C_{50}. The mass spectra of long-chain esters are characterized by a base peak produced by a rearrangement process involving the transfer of 2H atoms from the alcohol chain to the acid chain, giving a protonated acid ion.[26] The fragments at m/z 257, 285, 313, and 341 therefore correspond to the protonated hexadecanoic, octadecanoic, eicosanoic, and docosanoic acids, respectively. Hence, the base peak gives information about the number of carbon atoms in the acid moiety, while the molecular ion provides information about the total number of carbon atoms in the ester. It is possible then to determine the contribution of individual esters in every chromatographic peak by mass spectrometric determination of the molecular ion and the base peak. Quantitation of individual esters was accomplished by integrating the areas in the chromatographic profiles of the ions characteristic for the acidic moiety. The detailed structural composition of the different high molecular weight ester waxes identified in wheat straw is shown in Table 3. The esterified fatty acids ranged from dodecanoic acid (C_{12}) to octacosanoic acid (C_{28}) and the esterified fatty alcohols from octadecanol (C_{18}) to triacontanol (C_{30}). The acyl moiety of the high molecular

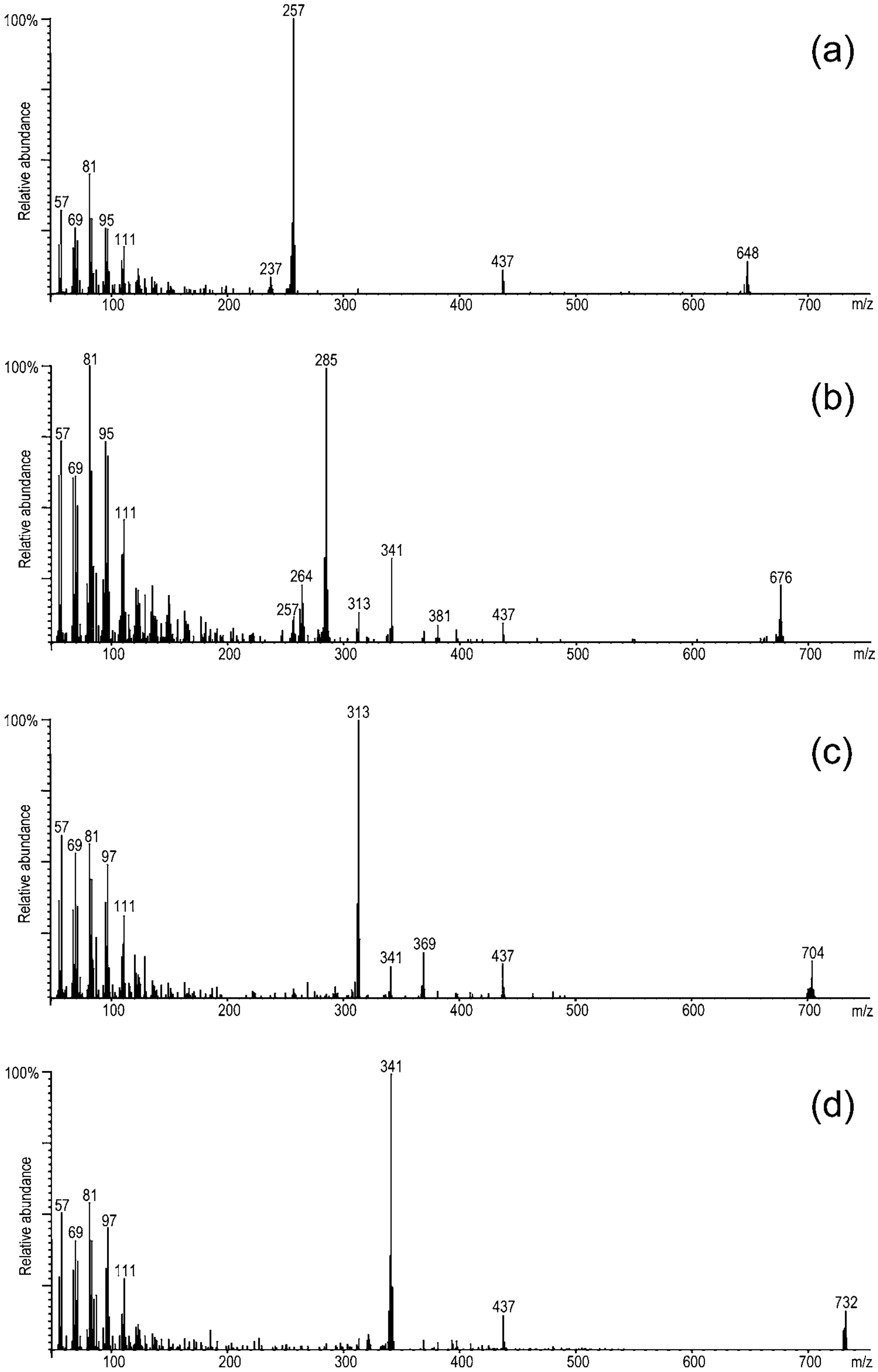

Figure 5. Mass spectra of the chromatographic peaks corresponding to the high molecular weight esters (a) C_{44}, (b) C_{46}, (c) C_{48}, and (d) C_{50}. Mass fragments at *m*/*z* 257, 285, 313, and 341 correspond to the protonated fatty acid moieties (hexadecanoic, octadecanoic, eicosanoic, and docosanoic acids, respectively).

weight ester waxes was mostly constituted by saturated fatty acids with even carbon atom number, although high molecular weight esters with unsaturated fatty acids (oleic and linoleic acids) could also be detected. In addition, even atom carbon number esters were also identified, and they mostly corresponded to odd carbon atom number fatty alcohols. According to our analyses, the predominant high molecular weight ester in wheat straw was C_{44}, which was mostly constituted by hexadecanoic acid, octacosyl ester (**XI**).

Finally, glycerides (mono-, di-, and triglycerides) were also found among the lipophilic extractives in wheat straw, although in lower amounts. monoglycerides accounted for 127 mg/kg and ranged from 2,3-dihydroxypropyl tetradecanoate to 2,3-dihydroxypropyl triacontanoate, with a strong even-over-odd

Table 3. Composition and Abundance (mg/kg, daf) of the Different Individual Esters Identified among the Waxes Identified in the Extracts of Wheat Straw

compd	fatty acid:fatty alcohol	abund
esters C_{38}		17
tetradecanoic acid, tetracosyl ester	C_{14}:C_{24}	7
hexadecanoic acid, docosyl ester	C_{16}:C_{22}	8
octadecanoic acid, eicosyl ester	C_{18}:C_{20}	1
eicosanoic acid, octadecyl ester	C_{20}:C_{18}	1
esters C_{39}		3
hexadecanoic acid, tricosyl ester	C_{16}:C_{23}	3
esters C_{40}		102
dodecanoic acid, octacosyl ester	C_{12}:C_{28}	3
tetradecanoic acid, hexacosyl ester	C_{14}:C_{26}	6
hexadecanoic acid, tetracosyl ester	C_{16}:C_{24}	81
octadeca-9,12-dienoic acid, docosyl ester	$C_{18:2}$:C_{22}	2
octadec-9-enoic acid, docosyl ester	$C_{18:1}$:C_{22}	2
octadecanoic acid, docosyl ester	C_{18}:C_{22}	2
eicosanoic acid, eicosyl ester	C_{20}:C_{20}	4
docosanoic acid, octadecyl ester	C_{22}:C_{18}	2
esters C_{41}		10
hexadecanoic acid, pentacosyl ester	C_{16}:C_{25}	10
esters C_{42}		113
tetradecanoic acid, octacosyl ester	C_{14}:C_{28}	46
hexadecanoic acid, hexacosyl ester	C_{16}:C_{26}	43
octadeca-9,12-dienoic acid, tetracosyl ester	$C_{18:2}$:C_{24}	5
octadec-9-enoic acid, tetracosyl ester	$C_{18:1}$:C_{24}	1
octadecanoic acid, tetracosyl ester	C_{18}:C_{24}	10
eicosanoic acid, docosyl ester	C_{20}:C_{22}	6
docosanoic acid, eicosyl ester	C_{22}:C_{20}	2
esters C_{43}		17
hexadecanoic acid, heptacosyl ester	C_{16}:C_{27}	11
octadecanoic acid, pentacosyl ester	C_{18}:C_{25}	1
eicosanoic acid, tricosyl ester	C_{20}:C_{23}	1
docosanoic acid, heneicosyl ester	C_{22}:C_{21}	4
esters C_{44}		273
hexadecanoic acid, octacosyl ester	C_{16}:C_{28}	253
octadeca-9,12-dienoic acid, hexacosyl ester	$C_{18:2}$:C_{26}	4
octadec-9-enoic acid, hexacosyl ester	$C_{18:1}$:C_{26}	1
octadecanoic acid, hexacosyl ester	C_{18}:C_{26}	3
eicosanoic acid, tetracosyl ester	C_{20}:C_{24}	7
docosanoic acid, docosyl ester	C_{22}:C_{22}	5
esters C_{45}		10
hexadecanoic acid, nonacosyl ester	C_{16}:C_{29}	6
octadecanoic acid, heptacosyl ester	C_{18}:C_{27}	1
eicosanoic acid, pentacosyl ester	C_{20}:C_{25}	1
docosanoic acid, tricosyl ester	C_{22}:C_{23}	2
esters C_{46}		189
hexadecanoic acid, triacontyl ester	C_{16}:C_{30}	22
octadeca-9,12-dienoic acid, octacosyl ester	$C_{18:2}$:C_{28}	52
octadec-9-enoic acid, octacosyl ester	$C_{18:1}$:C_{28}	19
octadecanoic acid, octacosyl ester	C_{18}:C_{28}	74
eicosanoic acid, hexacosyl ester	C_{20}:C_{26}	9
docosanoic acid, tetracosyl ester	C_{22}:C_{24}	27
tetracosanoic acid, docosyl ester	C_{24}:C_{22}	4
esters C_{47}		8
docosanoic acid, pentacosyl ester	C_{22}:C_{25}	8
esters C_{48}		85
hexadecanoic acid, dotriacontyl ester	C_{16}:C_{32}	4
octadecanoic acid, triacontyl ester	C_{18}:C_{30}	1
eicosanoic acid, octacosyl ester	C_{20}:C_{28}	62
docosanoic acid, hexacosyl ester	C_{22}:C_{26}	7
tetracosanoic acid, tetracosyl ester	C_{24}:C_{24}	10
esters C_{49}		7
docosanoic acid, heptacosyl ester	C_{22}:C_{27}	7
esters C_{50}		90
eicosanoic acid, triacontyl ester	C_{20}:C_{30}	2
docosanoic acid, octacosyl ester	C_{22}:C_{28}	81
tetracosanoic acid, hexacosyl ester	C_{24}:C_{26}	2
hexacosanoic acid, tetracosyl ester	C_{26}:C_{24}	3
octacosanoic acid, docosyl ester	C_{28}:C_{22}	2
esters C_{51}		3
docosanoic acid, nonacosyl ester	C_{22}:C_{29}	3
esters C_{52}		42
docosanoic acid, triacontyl ester	C_{22}:C_{30}	1
tetracosanoic acid, octacosyl ester	C_{24}:C_{28}	27
hexacosanoic acid, hexacosyl ester	C_{26}:C_{26}	2
octacosanoic acid, tetracosyl ester	C_{28}:C_{24}	12
esters C_{54}		2
hexacosanoic acid, octacosyl ester	C_{26}:C_{28}	1
octacosanoic acid, hexacosyl ester	C_{28}:C_{26}	1

carbon atom number predominance and with 2,3-dihydroxypropyl hexadecanoate (1-monopalmitin, **VII**) being the most abundant. The unsaturated monoglycerides 1-monoolein and 1-monolinolein were also present in minor amounts. Diglycerides were also found in low amounts (85 mg/kg), the most abundant being 1,2-dipalmitin (**VIII**) and 1,3-dipalmitin (**IX**). Finally, triglycerides were also identified among the lipophilic extractives of wheat straw and accounted for 198 mg/kg, dioleoylpalmitin (**X**) being the most abundant.

β-Diketones. The analysis of the lipophilic extractives of wheat straw revealed the presence of important amounts (883 mg/kg) of a compound with a β-diketone structure. The identification of this compound was achieved based on its mass spectrum (Figure 6). The molecular ion at m/z 464 indicates that this is a hentriacontanedienone, and the fragments at m/z 250 and 278 that arise from the McLafferty rearrangement at both sides of the diketone group followed by loss of water[27] clearly indicate that the structure of this β-diketone is 14,16-hentriacontanedione (**XII**). Despite 14,16-hentriacontanedione being the second most abundant single compound among the lipophilic extractives in wheat straw, its occurrence was not reported in previous papers.[13−15] Minor amounts of 12,14-tritriacontanedione were also present among the lipophilic compounds of wheat straw. β-Diketones are relatively common constituents of plant waxes and have been identified in the leafs of different grasses, including wheat straw.[28−35]

Steroid Compounds. Different classes of steroid compounds were present in the extracts of wheat straw, namely, steroid hydrocarbons, steroid ketones, sterols, sterol glycosides, and sterol esters. Free sterols were the most abundant steroid compounds, accounting for 1135 mg/kg. Sitosterol (**XIII**) was the most important sterol in wheat straw, together with campesterol (**XIV**) and stigmasterol (**XV**). Surprisingly, previous papers reported the occurrence of ergosterol in wheat straw.[13−15] However, ergosterol is a characteristic sterol in fungi and does not occur in plant cells; therefore, its occurrence may be attributable to fungal

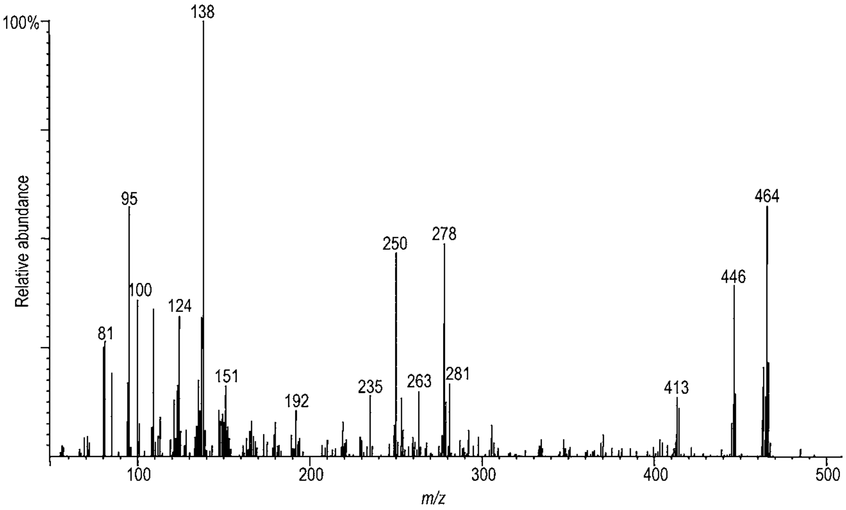

Figure 6. Mass spectrum of the *β*-diketone (14,16-hentriacontanedione, **XII**) identified among the lipophilic extractives of wheat straw.

presence in the wheat straw sample analyzed in those papers and its probable degradation. Minor amounts of sterols were found esterified to form sterol esters (70 mg/kg), sitosteryl palmitate (**XVII**) being the most important one. Sterol glycosides were also identified among the lipophilic extractives of wheat straw in important amounts (680 mg/kg). Sitosteryl 3*β*-D-glucopyranoside (**XVI**) was the most predominant, with lower amounts of campesteryl and stigmasteryl *β*-D-glucopyranosides. The identification of sterol glycosides was accomplished (after BSTFA derivatization of the lipid extract) by comparison with the mass spectra and relative retention times of authentic standards.[36] Sterol glycosides were not reported previously among the lipophilic compounds in wheat straw, despite their high abundance.[13−15]

Steroid ketones were observed in low amounts (88 mg/kg) and consisted mainly of stigmasta-4,22-dien-3-one (**XVIII**), stigmasta-3,5-dien-7-one (**XIX**), ergost-4-ene-3,6-dione (**XX**, R = H), stigmast-4-ene-3,6-dione (**XX**, R = CH_3), ergostane-3,6-dione (**XXI**, R = H), stigmastane-3,6-dione (**XXI**, R = CH_3), stigmasta-4,22-diene-3,6-dione (**XXII**), and stigmast-22-ene-3,6-dione (**XXIII**). Finally, minor amounts of steroid hydrocarbons (16 mg/kg) were also identified, stigmasta-3,5-diene (**XXIV**) being the most important one, with lower amounts of ergosta-3,5-diene, stigmasta-3,5,22-triene, stigmasta-4,22-diene, and stigmasta-3,5,7-triene. Most probably, these steroid hydrocarbons might arise from degradation of free and conjugated sterols, either within the plant or during the lipids isolation and/or analysis.

In conclusion, the present paper provides for the first time a detailed and comprehensive description of the lipophilic compounds in wheat straw, which is highly valuable information for a more complete industrial utilization of this lignocellulosic material that is regarded as waste.

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Notes

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