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CHEMICAL COMPOSITION OF LIPOPHILIC COMPOUNDS FROM 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. 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 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.

I. INTRODUCTION

Plant biomass is the main source of renewable materials in 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 [1]. 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 [2]. Wheat straw also contains significant amounts of lipids (*ca.* 1-2% by weight) that can be extracted to produce high-value waxes [3].

Studies concerning the composition of lipids in wheat straw have been relatively scarce, although some papers have been published in this regard [3,4]. In the present work, a thorough and comprehensive characterization of the lipophilic extractives in wheat straw has been performed by gas chromatography-mass spectrometry (GC-MS) using medium-length high temperature capillary columns 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 [5]. The knowledge of the precise composition of the lipophilic extractives in wheat straw will help to maximize the exploitation of this important agricultural waste.

II. EXPERIMENTAL

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, 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.

GC-MS analyses

The GC-MS analysis were performed on a Varian Star 3400 gas chromatograph 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). Helium was used as carrier gas at a rate of 2 mL/min. The samples were injected directly onto the column using a SPI (septum-equipped programmable injector) system. The temperature of the injector during the injection was 60 ºC, and 0.1 min after injection was programmed to 380 ºC at a rate of 200 ºC min-1 and held for 10 min. The oven was heated from 120 ºC (1 min) to 380 ºC (5 min) at 10 ºC min-1. The temperature of the transfer line was set at 300 ºC. Bis(trimethylsilyl)trifluoroacetamide (BSTFA) silylation were used to form the TMS-derivatives. 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. Peaks were quantified by area, and a mixture of standards (octadecane, palmitic acid, sitosterol, cholesteryl oleate, and sitosteryl 3β-D-glucopyranoside) with a concentration range between 0.1 and 1 mg/mL, was used to elaborate calibration curves.

III. RESULTS AND DISCUSSION

The total acetone extractives of wheat straw accounts for 2.7% of 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. This content is similar to that reported in other grasses and nonwoody materials [6,7].

The lipophilic extracts from wheat straw were analyzed (as TMS-ether derivatives) by GC-MS using medium-length high-temperature capillary columns with thin films, according to the method previously described [5]. The GC-MS chromatogram of the TMS-ether derivatives of the lipid extracts from wheat straw is shown in **Figure 1**.The identities and abundances of the main lipid compounds identified are detailed in **Table 1**.

The 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 (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.

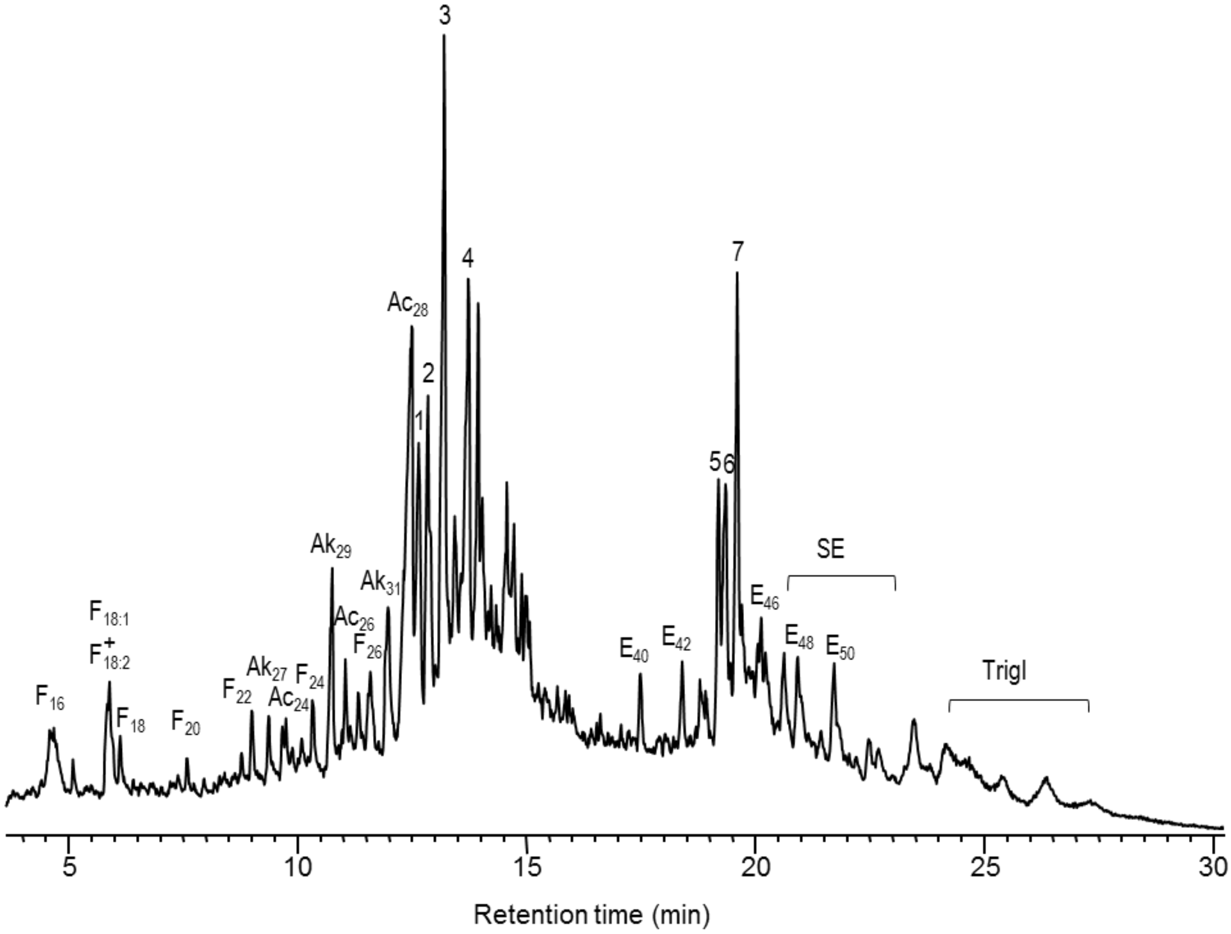

Figure 1. GC-MS chromatograms of the lipid extracts from wheat straw, as TMS-ether derivatives. F(n): *n*-fatty acid series; Ak(n): *n*-alkane series; Ac(n): *n*-fatty alcohol series; Ad(n): *n*-aldehyde series; E(*n*): high molecular weight ester series; n denotes the total carbon atom number. SE: sterol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

Aliphatic series

Free fatty acids were the most predominant series, 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 palmitic acid being the most predominant. The unsaturated oleic and linoleic acids were also found in important amounts. Free fatty alcohols were the second most abundant class of aliphatic series in wheat straw, accounting for 1615 mg/Kg. 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 being the most predominant homolog in the series. 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 being the predominant homolog, 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 being the major compound. The distribution of aldehydes parallels that of free alcohols, as usually occurs in the plant kingdom and observed in other plants [6], suggesting that aldehydes are intermediates in the biosynthesis of alcohols from fatty acids [8].

The series of high molecular weight esters occurred in important amounts (915 mg/Kg). This series was 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. 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 based on the mass spectra of the peaks [6]. Quantitation of individual esters was accomplished by integrating the areas in the chromatographic profiles of the ions characteristic for the acidic moiety. 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}). 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.

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 carbon atom number predominance, and with 1-monopalmitin 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 and 1,3-dipalmitin. Finally, triglycerides were also identified and accounted for 198 mg/Kg, dioleoylpalmitin 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. The molecular ion at *m/z* 464 indicates that this is a hentriacontanedienone, and the fragments at *m/z* 250 and *m/z* 278 that arise from the McLafferty rearrangement at both sides of the diketone group followed by loss of water [9] clearly indicate that the structure of this β-diketone is 14,16-hentriacontanedione. 14,16-hentriacontanedione was the second most abundant single compound among the lipophilic extractives in wheat straw. 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 [7,10].

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 was the most important sterol, together with campesterol and stigmasterol. Minor amounts of sterols were found esterified forming sterol esters (70 mg/Kg), sitosteryl palmitate being the most important one. Sterol glycosides were also identified in important amounts (680 mg/Kg). Sitosteryl 3β-D-glucopyranoside 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 [11]. Steroid ketones were observed in low amounts (88 mg/Kg) and consisted mainly of stigmasta-4,22-dien-3-one, stigmasta-3,5-dien-7-one, ergost-4-ene-3,6-dione, stigmast-4-ene-3,6-dione, ergostane-3,6-dione, stigmastane-3,6-dione, stigmasta-4,22-diene-3,6-dione, and stigmast-22-ene-3,6-dione. Finally, minor amounts of steroid hydrocarbons (16 mg/Kg) were also identified, stigmasta-3,5-diene being the most important one, and 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.

Table 2. Composition and Abundance (mg/Kg fiber, d.a.f.) of Main Lipids Identified in the Extracts of Wheat Straw

Compound	Abundance
n-Fatty acids	2080
n-Fatty alcohols	1615
n-Alkanes	371
n-Aldehydes	99
High molecular weight esters	915
Monoglycerides	127
Diglicerides	85
Triglycerides	198
β-Diketones	883
Steroid hydrocarbons	16
Steroid ketones	88
Sterols	1121
Sterol glycosides	680
Sterol esters	70

IV. CONCLUSIONS

The present paper provides a detailed description of the lipophilic compounds in wheat straw, which is a highly valuable information for a more complete industrial utilization of this lignocellulosic material.

V. ACKNOWLEDGEMENT

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