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Lipophilic compounds from maize fiber and rice husk residues – An abundant and inexpensive source of valuable phytochemicals

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ABSTRACT

Maize (*Zea mays* L.) fibers and rice (*Oryza sativa* L.) husks are abundant and low cost by-products generated during grain milling. In this work, the composition of the lipophilic compounds present in these materials, which accounted for 4.1 % in maize fibers and 2.2 % in rice husks, was thoroughly studied by GC–MS. The most abundant lipophilic compounds identified in these residues were *n*-fatty acids and acylglycerols (mono-, di-, and triglycerides), that altogether accounts for up to 88 % of all extractives in maize fibers and up to 95 % in rice husks. Steroid compounds, including free and esterified sterols, hydrocarbons, and ketones were identified in both samples, being present in significant amounts in maize fibers. Tocopherols were also present in both cereal by-products, albeit in lower amounts, with α-tocopherol being the most abundant one. Minor amounts of *n*-fatty alcohols and high molecular esters were also found in rice husks. Maize fibers and rice husks can, therefore, be potential feedstocks to obtain valuable phytochemicals of diverse industrial interest.

1. Introduction

Maize (*Zea mays* L.) and rice (*Oryza sativa* L.) are the two major staple food crops used for human nutrition worldwide. Maize is the crop with the highest global annual grain yield, reaching an all-time high in 2018/2019 with 1076 million tons, while rice production amounted up to 488 million tons in the same period (International Grains Council, 2019). Processing and refining of these cereal grains generate large amounts of by-products, as is the case of the so-called maize fibers and rice husks. Maize fiber is the by-product of the grain wet-milling operation, and consists mostly of the kernel outer seed coat (Rose et al., 2010; Gáspár et al., 2007). Maize fiber is typically used as cattle feed. Rice husks are the hard protective coating of rice grains that are separated from the grains during milling. Rice husk are usually dumped and/or burned for co-generation of heat and power.

Maize fibers and rice husks are lignocellulosic materials, mostly composed of carbohydrat and lignin. Maize fiber is predominantly composed of hemicellulose (35 %), cellulose (18 %), and starch (20 %), with lower amounts of lignin (6 %) (Gáspár et al., 2007; del Río et al., 2018). Rice husks consist mainly of lignin (22 %), hemicellulose (18 %), and cellulose (38 %), but also contain important amounts of silica (20 %) that limits some of its industrial uses (Salanti et al., 2010). However, these cereal by-products also contain some amounts of lipophilic compounds (~2-4 %) that can also be valorized as a source for obtaining high-value compounds (Moreau et al., 1996, 2009; Singh et al., 2000; Friedman, 2013). Plant lipophilic compounds (phytochemicals) comprise a wide range of chemical compounds (e.g. hydrocarbons, fatty acids, acylglycerols, terpenoids, sterols) with widespread applications in the pharmaceutical, nutraceutical, cosmetic, food, or chemical industries (Hernandez, 2005; Metzger and Bornscheuer, 2006; Tao, 2007). The high abundance, availability and low cost of maize fibers and rice husks indicate that these cereal by-products might be excellent candidates for the selective extraction of "green" phytochemicals with potential industrial applications (Carciochi et al., 2017).

Cereal by-products, in particular cereal bran oils, have already been shown to be a rich source of phytochemicals (Jiang and Wang, 2005; Friedman, 2013; Prinsen et al., 2014; Górnas et al., 2016). Cereal bran oils are known to be rich in health-promoting compounds, as is the case of the sterols and sterol esters that are known to lower plasma cholesterol levels, among other health benefits (Nicolosi et al., 1993; Gerhardt and Gallo, 1998; Moreau et al., 2002; Patel and Naik, 2004; Awika, 2011), although some studies have indicated that the lipids in maize fibers present even greater health benefits than those from the respective maize bran oil (Wilson et al., 2000). However, despite their high significance, studies aimed at elucidating the composition of the lipids from other cereal by-products, such as maize fibers and cereal husks, have been insufficient, and additional studies are therefore

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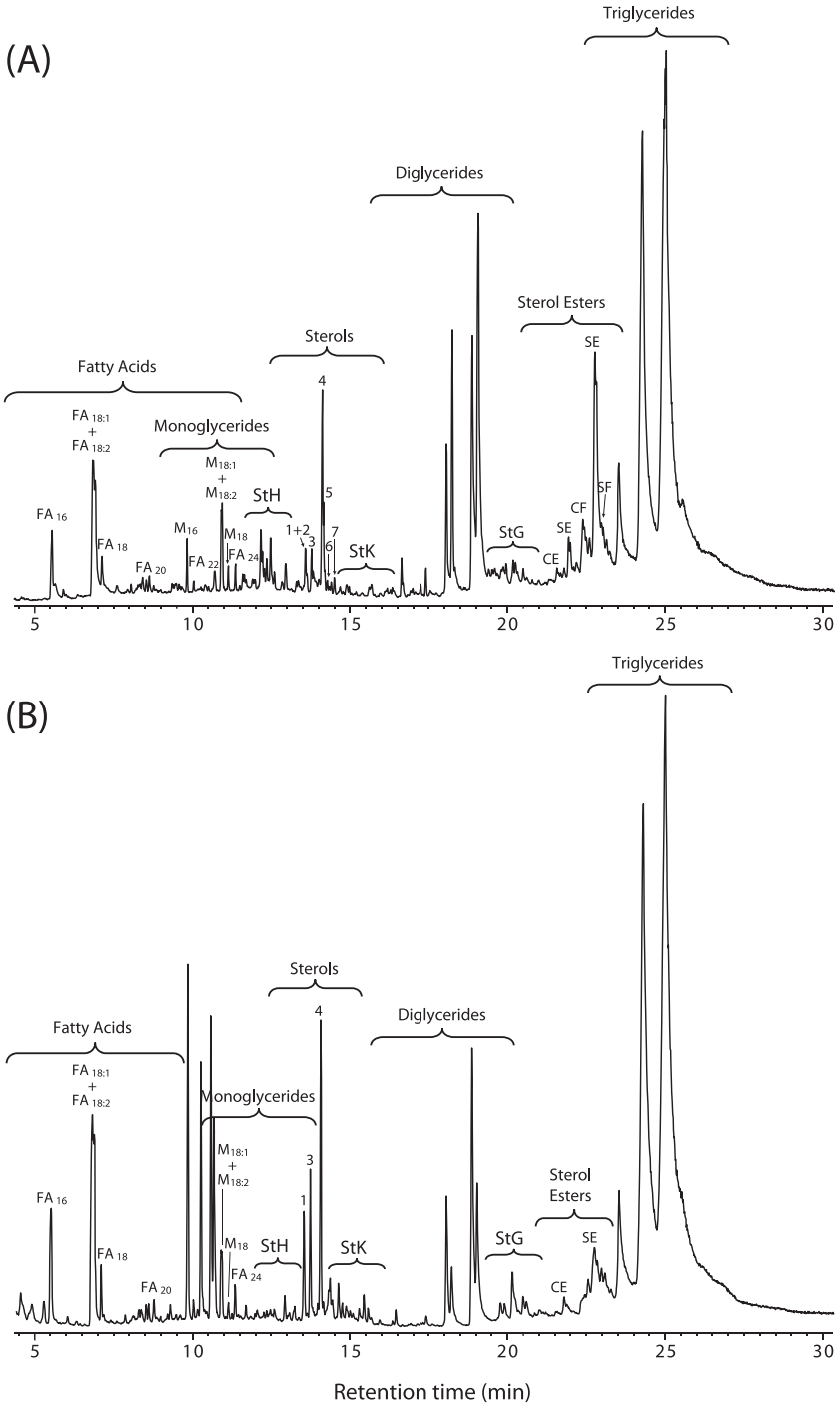

Fig. 1. GC–MS chromatograms of the lipid extracts (as TMS-ether derivatives) from (A) maize fibers, and (B) rice husks. FA(n), *n*-fatty acid series; M(n), monoglycerides; StH, steroid hydrocarbons; StK, steroid ketones; StG, sterol glucosides; CE, campesterol esters; SE, sitosterol esters; CF, campestanyl ferulate; SF, stigmastanyl ferulate. Labels for sterols are: 1, campesterol; 2, campestanol; 3, stigmasterol; 4, sitosterol; 5, stigmastanol; 6, Δ^5-avenasterol; 7, Δ^7-stigmastenol.

needed to know the precise composition of the lipophilic compounds in these materials. Previous works have only reported the identification of a limited range of lipophilic compounds in maize fibers (Moreau et al., 1996, 2009; Singh et al., 2000; Jiang and Wang, 2005) and rice husks (Hartman and Lago, 1976; Kim et al., 2012). As a result, a comprehensive description of the composition and content of the lipophilic fraction in these cereal by-products is still lacking.

In the present work, we perform a thorough study of the lipophilic compounds present in maize fibers and rice husks by acetone extraction and subsequent compositional analysis by gas chromatography–mass spectrometry (GC–MS). The results presented here will significantly improve our knowledge of the lipophilic compounds in these cereal by-products that can be used as potential sources of highly valuable phytochemicals of diverse industrial interest.

2. Material and methods

2.1. Samples

Maize (*Zea mays* L.) fibers were provided by Cargill, Inc. (Brazil), and were the by-product of the wet-milling process during the production of maize starch. Rice (*Oryza sativa* L.) husks were obtained from

Table 1
Composition and abundance (mg/kg) of the compounds identified in the maize fiber and rice husk residues.

Compound	Maize fibers	Rice husks	Compound	Maize fibers	Rice husks
Fatty acids	**7590**	**2770**	**High molecular weight esters**	**n.d.**	**60**
cis-9-hexadecenoic acid	50	100	hexadecanoic acid, octacosyl ester	n.d.	5
n-hexadecanoic acid (I)	1470	510	docosanoic acid, tetracosyl ester	n.d.	10
n-heptadecanoic acid	30	10	tetracosanoic acid, tetracosyl ester (IX)	n.d.	25
cis,cis-9,12-octadecadienoic acid (II)	3530	930	tetracosanoic acid, hexacosyl ester	n.d.	10
cis-9-octadecenoic acid (III)	1210	870	tetracosanoic acid, octacosyl ester	n.d.	10
n-octadecanoic acid	330	110			
n-nonadecanoic acid	20	10	**Tocopherols**	**20**	**40**
n-eicosanoic acid	200	40	α-tocoferol (X)	20	20
n-heneicosanoic acid	20	10	β-tocopherol (XI)	n.d.	5
n-docosanoic acid	120	40	γ-tocoferol(XII)	tr.	10
n-tricosanoic acid	80	10	δ-tocopherol (XIII)	n.d.	5
n-tetracosanoic acid	270	80			
n-pentacosanoic acid	70	10	**Sterols**	**700**	**250**
n-hexacosanoic acid	120	20	campesterol (XIV)	90	40
n-heptacosanoic acid	20	tr.	campestanol (= ergostanol) (XV)	10	tr.
n-octacosanoic acid	40	10	stigmasterol (XVI)	60	60
n-triacontanoic acid	10	10	sitosterol (XVII)	380	110
			sitostanol (= stigmastanol) (XVIII)	60	tr.
Monoglycerides	**330**	**80**	Δ^5-avenasterol (XIX)	40	5
1-monopalmitin	70	15	Δ^7-stigmastenol (XX)	20	5
1-monolinolein (IV)	125	25	Δ^7-avenasterol (XXI)	30	10
1-monoolein	105	35	cycloartenol (XXII)	0	5
1-monostearin	30	5	24-methylenecycloartanol (XXIII)	0	10
			7-oxo-sitosterol (XXIV)	10	5
Diglycerides	**2090**	**400**			
1,2-dipalmitin	10	10	**Sterol glycosides**	**30**	**70**
1,3-dipalmitin	40	5	campesteryl 3β-D-glucopyranoside	5	10
1,2-pamitoyllinolein	120	30	stigmasteryl 3β-D-glucopyranoside	5	10
1,2-palmitoylolein	110	40	sitosteryl 3β-D-glucopyranoside (XXV)	20	50
1,3-palmitoyllinolein	200	10			
1,3-palmitoylolein	190	15	**Sterol esters**	**1540**	**70**
1,2-dilinolein	130	50	campesterol palmitate	20	tr.
1,2-linoleoylolein (V)	270	70	campesterol linoleate/oleate	190	10
1,2-diolein	120	70	campestanol palmitate	10	n.d.
1,3-dilinolein	150	20	campestanol linoleate/oleate	90	n.d.
1,3-linoleoylolein (VI)	300	30	stigmasterol palmitate	10	tr.
1,3-dilinolein	200	50	stigmasterol linoleate/oleate	40	20
1,3-oleylstearin	250	tr.	sitosterol palmitate	110	10
			sitosterol linoleate/oleate (XXVI)	760	30
Triglycerides	**8400**	**10400**	stigmastanol palmitate	50	n.d.
dipalmitoylolein	280	280	stigmastanol linoleate/oleate	260	n.d.
dipalmitoyllinolein	250	270			
dioleoylpalmitin	1430	2110	**Sterol ferulates**	**370**	**n.d.**
lineoyloleoylpalmitin	960	1380	campestanyl ferulate (XXVII)	120	n.d.
dilinoleoylpalmitin	500	630	stigmastanyl ferulate (XXVIII)	250	n.d.
triolein (VII)	1890	2750			
dioleoyllinolein	1410	1710	**Steroid ketones**	**70**	**30**
dilinoleoylolein	1010	910	stigmasta-3,5-dien-7-one (XXIX)	25	5
trilinoolein	670	360	stigmast-4-en-3-one (XXX)	25	15
			stigmastane-3,6-dione (XXXI)	20	10
Fatty alcohols	**n.d.**	**340**			
n-tetracosanol	n.d.	10	**Steroid hydrocarbons**	**180**	**30**
n-hexaconsanol	n.d.	20	ergost-3-ene	20	tr.
n-octacosanol	n.d.	40	ergosta-3,5-diene	10	tr.
n-triacontanol (VIII)	n.d.	120	stigmast-3-ene	65	tr.
n- dotriacontanol	n.d.	80	stigmasta-3,5,22-triene	10	15
n-tetratriacontanol	n.d.	50	stigmasta-3,5-diene (XXXII)	75	15
n-hexatriacontanol	n.d.	20			

n.d. not detected; **tr.** detected in trace amounts; Roman numbers refer to the structures in Figs. 2 and 3.

a local paddy field located in Isla Mayor (Spain). Both residues were air-dried, knife milled, and Soxhlet extracted with acetone for 8 h (Gutiérrez et al., 1998). The extracts were determined gravimetrically after evaporating the acetone in a rotary evaporator, and accounted for 4.1 % ± 0.2 (dry basis) in maize fibers, and to 2.2 % ± 0.2 (dry basis) in rice husks. The determination was achieved in triplicate.

2.2. GC–MS analysis of lipids in the maize fiber and rice husk residues

Around 1 mg of dried extracts was dissolved in 0.100 mL pyridine and silylated with 0.200 mL bis(trimethylsilyl)trifluoroacetamide (BSTFA) for 2 h at 80 °C. The silylated extracts were analyzed by GC–MS as previously described (del Río et al., 2016). Identification of individual compounds was achieved by comparison of their electron-impact mass spectra with those present in the NIST library, by comparison with literature and by comparison with authentic standards. Quantification was achieved by using response factors and appropriate external calibration curves using a mixture of authentic standards that included palmitic acid, sitosterol, cholesteryl linoleate, sitosteryl 3β-D-glucopyranoside, monopalmitin, 1,3-dipalmitin, tripalmitin and

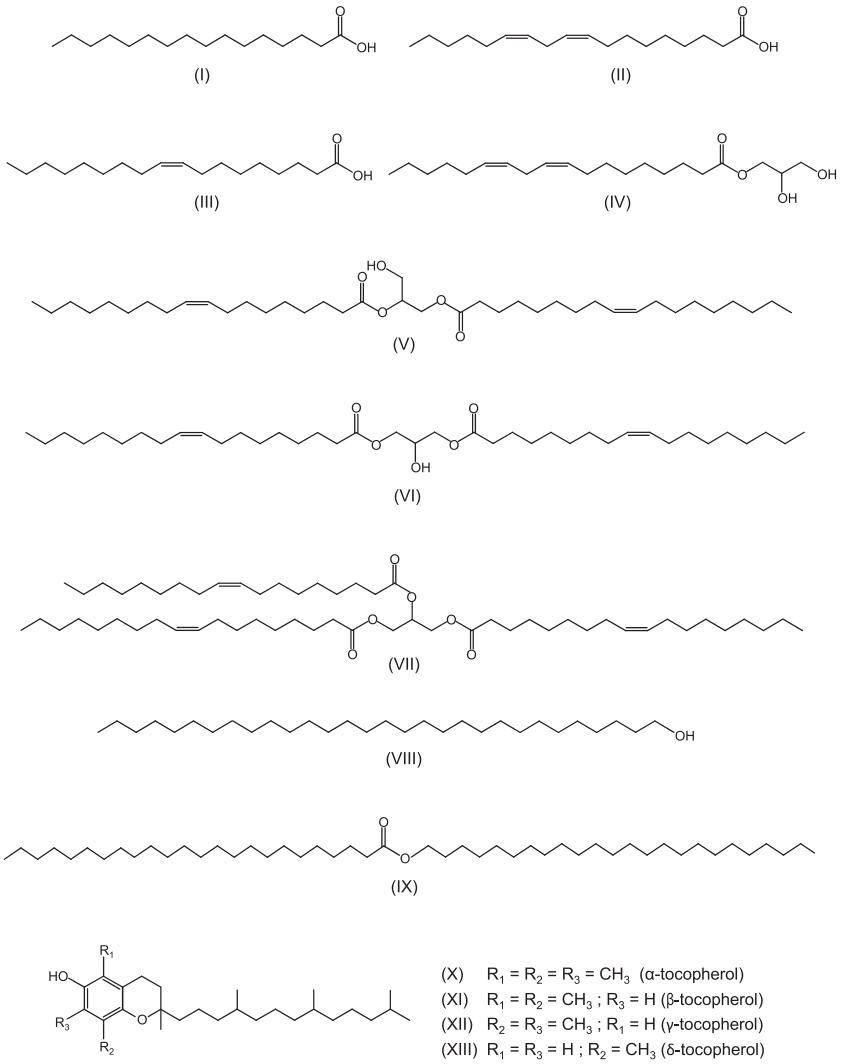

Fig. 2. Structures representative of the main aliphatic lipophilic compounds identified in the acetone extracts of maize fibers and rice husks and referred in the text. I: palmitic acid; II: linoleic acid; III: oleic acid; IV: monolinolein; V: 1,2-linoleoylolein; VI: 1,3-linoleoylolein; VII: triolein; VIII: triacontanol; IX: tetracosanoic acid, tetracosyl ester; X: α-tocopherol; XI: β-tocopherol; XII: γ-tocopherol; XIII: δ-tocopherol.

tetracosane. Quantification was given as the mean of three replicates. In all cases, the standard deviations from the three replicates were below 10 % of the mean values.

3. Results and discussion

3.1. Lipid composition of maize fiber and rice husks

The lipophilic compounds present in maize fibers and rice husks were analyzed by GC–MS (after derivatization to their trimethylsilyl-ether derivatives) using medium-length (12 m) high-temperature capillary columns, with thin films, that allowed extending the range of compounds identified up to higher molecular weight lipids, as is the case of sterol glycosides, sterol esters, and triglycerides that do not usually elute in more standard GC conditions (Gutiérrez et al., 1998).

The chromatograms of the lipophilic compounds (as their TMS-ether derivatives) present in maize fiber and rice husk residues are shown in Fig. 1. The compounds identified, together with their abundances (as mg/kg, dry basis), are reported in Table 1. Structures representatives of the main compounds identified are depicted in Fig. 2 (for aliphatic compounds) and Fig. 3 (for steroid compounds).

A series of peaks were detected around 10 min retention time in the chromatogram of the lipophilic extractives of rice husks, that were not present in the chromatogram of maize fiber extracts. According to their mass spectra, these peaks appear to correspond to glycolipids. However, their identities could not be definitively confirmed because their mass spectra only show fragments arising from the carbohydrate moiety (*m/z* 147, 217, 361), without the occurrence of any other diagnostic fragment and, therefore, they still remain unknown to us and have not been included in Table 1. Several classes of glycolipids, including mono- and digalactosyl monoacylglycerols and mono- and digalatosyl diacylglycerols, are known to occur in some cereals, including rice bran oils (Moazzami et al., 2011), but their occurrence in rice husks could not be unambiguously confirmed.

The analysis revealed that the predominant lipophilic compounds present in maize fibers and rice husks were *n*-fatty acids and acylglycerols (mono-, di- and triglycerides), along with minor amounts of steroid compounds (including free sterols, sterol esters, sterol ferulates, sterol glycosides, hydrocarbons, and ketones). For convenience, Fig. 4 shows the relative abundances of the main classes of lipids identified in maize fiber and rice husk extracts. Maize fibers were dominated by *n*-fatty acids (36 % of all identified compounds) and triglycerides (39 %), with minor amounts of diglycerides (10 %), free sterols (3 %) and sterol esters (7 %). Rice husks were dominated by triglycerides (72 %), and *n*-fatty acids (19 %), together with lower amounts of steroid compounds, mostly free sterols (2 %). It is interesting to note that, altogether, fatty

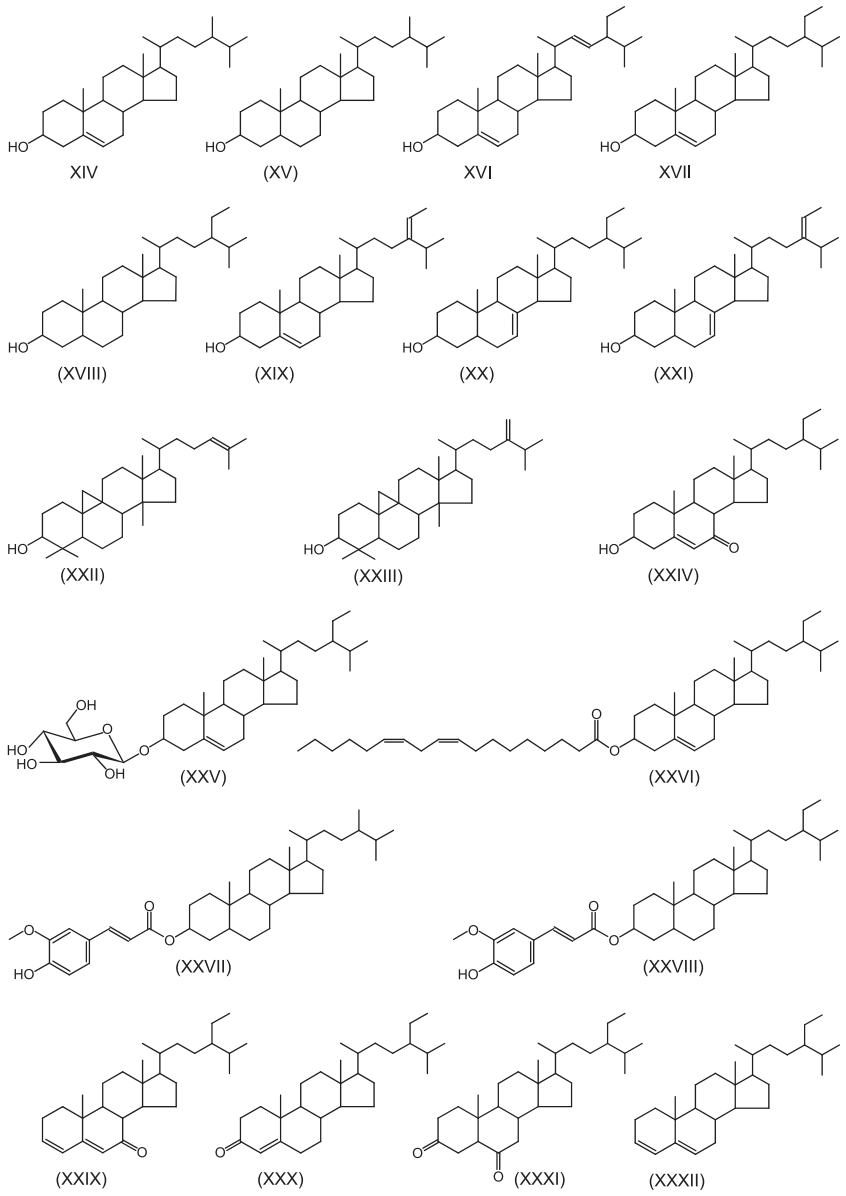

Fig. 3. Structures of the main steroid compounds identified in the acetone extracts of maize fibers and rice husks and referred in the text. XIV: campesterol; XV: campestanol; XVI: stigmasterol; XVII: sitosterol; XVIII: sitostanol; XIX: Δ^5-avenasterol; XX: Δ^7-stigmastanol; XXI: Δ^7-avenasterol; XXII: cycloartenol; XXIII: 24-methylenecycloartanol; XXIV: 7-oxositosterol; XXV: sitosteryl β-D-glucopyranoside; XXVI: sitosteryl linoleate; XXVII: campestanyl ferulate; XXVIII: stigmastanyl ferulate; XXIX: stigmasta-3,5-dien-7-one; XXX: stigmast-4-en-3-one; XXXI: stigmastane-3,6-dione; XXXII: stigmast-3,5-diene.

acids and acylglycerols accounts for up to 87 % of the acetone extracts in maize fibers and up to 95 % in rice husks.

3.1.1. Aliphatic series

The main aliphatic compounds present in maize fibers and rice husks were series of *n*-fatty acids, in free and esterified form (as mono-, di- and triglycerides). In addition, the extractives of rice husks also showed minor amounts of *n*-fatty alcohols and high molecular weight esters that were absent among the extractives of maize fibers.

The series of *n*-fatty acids were present in important amounts in both cereal by-products, accounting for 7590 mg/kg in maize fibers and 2770 mg/kg in rice husks. The series presented a similar distribution in both residues, ranging from *n*-hexadecanoic acid (C_{16}, palmitic acid, **I**) to *n*-triacontanoic acid (C_{30}), with the former being the major saturated fatty acid in both materials, accounting for 1470 mg/kg in maize fibers and 510 mg/kg in rice husks. Large amounts of the unsaturated fatty acids *cis*,*cis*-9,12-octadecadienoic acid (linoleic acid, **II**) and *cis*-9-octadecenoic acid (oleic acid, **III**) occurred in both samples, with linoleic acid being the major fatty acid in both cases, accounting for 3530 mg/kg in maize fibers and 930 mg/kg in rice husks, whereas oleic acid accounted for 1210 mg/kg in maize fibers and 870 mg/kg in rice husks.

Acylglycerols including mono-, di-, and triglycerides were the most predominant lipophilic compounds identified in these residues and were present in similar amounts, accounting for a total of 10,820 mg/kg in maize fibers and 10,880 mg/kg in rice husks. In both residues, triglycerides were the most abundant class of compounds accounting for 8400 mg/kg in maize fibers, and 10,400 mg/kg in rice husks, followed by diglycerides 2090 mg/kg in maize fibers, and 400 mg/kg in rice husks and monoglycerides in lower amounts (330 mg/kg in maize fibers, and 80 mg/kg in rice husks). The monoglycerides corresponded to the unsaturated monolinolein ($M_{18:2}$; **IV**) and monoolein ($M_{18:1}$), together with lower amounts of the saturated monopalmitin (M_{16}) and monostearin (M_{18}). Diglycerides occurred as a complex mixture of different compounds that resulted from the combination of palmitic, linoleic, oleic and stearic acids in different positions of the glycerol molecule. Individual diglycerides could be discerned by their

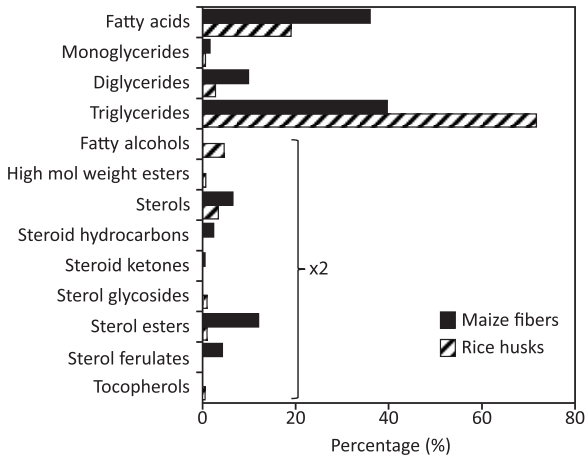

Fig. 4. Percentage of the main lipid classes identified in the acetone extracts of maize fibers and rice husks.

characteristic mass spectra (Curstedt, 1974), and those identified are listed in Table 1. The most abundant diglycerides in maize fibers were 1,2-linoleoylolein (**V**) and 1,3-linoleoylolein (**VI**), with important amounts of the 1,2- and 1,3-palmitoyllinolein, 1,2- and 1,3-palmitoylolein, 1,2- and 1,3-dilinolein, and 1,2- and 1,3-diolein. In all cases, the 1,3-isomers predominated over the respective 1,2-isomers. In rice husks, the most prominent diglycerides identified were 1,2- and 1,3-dilinolein, 1,2- and 1,3-linoleoylolein, and 1,2- and 1,3-dilinolein, and in contrast to what occurred in maize fibers, the 1,2-isomers predominated over the 1,3-isomers. The unsaturated oleic and linoleic acids were the most important fatty acids present as diglycerides in both cereal residues. Triglycerides also resulted to be a complex mixture of different compounds formed by the combination of palmitic, linoleic and oleic acids. The identification of individual triglycerides was also achieved by GC–MS based on the individual mass spectrometric patterns (Lauer et al., 1970; del Río et al., 2016) and the list of triglycerides identified in both residues are shown in Table 1. The most abundant triglycerides in both samples were triolein (**VII**), dioleoylpalmitin and dioleoyllinolein, among others. Oleic and linoleic acids were also the most important fatty acids forming triglycerides in both materials.

The series of fatty alcohols could only be detected in the extractives of rice husks, accounting for 340 mg/kg of residue. This series appeared in the range from *n*-tetracosanol (C_{24}) to *n*-hexatriacontanol (C_{36}), with the exclusive occurrence of the even carbon atom number analogs, and with *n*-triacontanol (C_{30}, **VIII**) as the most prominent compound (120 mg/kg).

A series of high molecular weight esters (waxes) also occurred among the extractives of rice husks, although in low amounts (60 mg/kg rice husks), being absent in maize fibers. The esters identified were formed by long chain fatty acids (from C_{16} to C_{24}), esterified with long chain fatty alcohols (from C_{24} to C_{28}), and the identities of the different individual esters were determined according to their mass spectra, as already reported (del Río et al., 2009, 2013). This series occurred in the range from C_{44} (hexadecanoic acid, octacosyl ester) to C_{52} (tetracosanoic acid, octacosyl ester), with C_{48} (tetracosanoic acid, tetracosyl ester, **IX**) being the predominant ester.

Finally, low amounts of tocopherols were also identified among the acetone extractives in both residues, being more predominant in rice husks (40 mg/kg) than in maize fibers (20 mg/kg). The identification of individual tocopherols was accomplished by comparison of their mass spectra with those already published (Snyder et al., 1993; del Río et al., 2015) and revealed that the major tocopherol present in both residues was α-tocopherol (**X**), which is the most active form of vitamin E, with minor amounts of β-tocopherol (**XI**), γ-tocopherol (**XII**) and δ-tocopherol (**XIII**). The results obtained in the present work contrast with those reported in a previous work that indicated that γ-tocopherol was the most abundant tocopherol in maize fibers (Moreau et al., 1996). In the case of rice husks, the distribution of tocopherols was similar to that reported for rice hulls after germination (Kim et al., 2012).

3.1.2. Steroid compounds

Different families of steroid compounds were identified among the extractives of maize fibers and rice husks, and included free sterols, sterol glucosides, sterol esters, sterol ferulates, steroid ketones, and steroid hydrocarbons, with a higher abundance of all of them in maize fiber extracts (Table 1, Fig. 4). Free sterols were identified in significant amounts in maize fibers (700 mg/kg) and in lower amounts in rice husks (250 mg/kg). Sitosterol (**XVII**) was the most abundant sterol identified in both residues, accounting for 380 mg/kg in maize fibers and 110 mg/kg in rice husks. Other sterols identified in these samples were campesterol (**XIV**), campestanol (=ergostanol, **XV**), stigmasterol (**XVI**), sitostanol (=stigmastanol, **XVIII**), Δ^5-avenasterol (**XIX**), Δ^7-stigmastenol (**XX**), Δ^7-avenasterol (**XXI**) and 7-oxositosterol (**XXIV**); minor amounts of cycloartenol (**XXII**) and 24-methylenecycloartanol (**XXIII**) were only identified in rice husks. The composition of sterols identified in maize fibers was similar to that previously reported (Moreau et al., 2009).

Sterol glucosides were also identified, although in relatively low amounts, among the extractives of maize fibers (30 mg/kg) and rice husks (70 mg/kg). Their identification was achieved by comparison with the retention times and mass spectra of authentic standards, as already published (Gutiérrez and del Río, 2001). Sitosteryl β-D-glucopyranoside (**XXV**) was the most abundant sterol glucoside in both residues, with campesteryl and stigmasteryl β-D-glucopyranosides also being present, albeit in lower amounts. This is the first report of the occurrence of sterol glucosides in these cereal by-products as they were biased in previous studies, probably due to GC conditions used.

Sterol esters were also found in important amounts in maize fibers (1540 mg/kg) but occurred in lower amounts in rice husks (70 mg/kg). The identification of the individual sterol esters was obtained from their mass spectra that show characteristic fragments arising from the sterol moiety (Evershed et al., 1989; Lusby et al., 1984). Hence, by monitoring in the GC–MS the specific fragment ions characteristic of the different sterol moieties, it was possible to resolve and identify the different sterol esters series involving different sterols, and that resulted to be a complex mixture of different compounds. Fig. 5 shows the distributions of the different sterol ester series present in maize fibers by monitoring the characteristic fragments for each sterol moiety, that is, *m/z* 382 for campesterol esters, *m/z* 384 for campestanol esters, *m/z* 394 for stigmasterol esters, *m/z* 396 for sitosterol esters, and *m/z* 398 for stigmastanol esters; in addition, the fragment at *m/z* 266, which is the characteristic base peak in the mass spectra of sterol ferulates (Prinsen et al., 2014), was also monitored. Hence, the sterol ester series present in maize fibers and rice husks corresponded to campesterol, campestanol, stigmasterol, sitosterol and stigmastanol esterified with different fatty acids. The single ion chromatograms of the different sterol ester series presented two major peaks that corresponded to the esterification with C_{16} and C_{18} fatty acids, including the unsaturated oleic ($C_{18:1}$) and linoleic ($C_{18:2}$) acids; the most abundant sterol ester in both residues being sitosterol linoleate (**XXVI**), that was particularly abundant in maize fibers where it accounted for 760 mg/kg. Sterol ferulates were also present in important amounts among the lipids of maize fibers, accounting for 370 mg/kg, but were absent in rice husks. Sterol ferulates were identified by comparison with the mass spectra previously published (Prinsen et al., 2014), and consisted of the saturated campestanyl ferulate (**XXVII**) and stigmastanyl ferulate (**XXVIII**). Sterol ferulates are characteristics components of cereal bran oils (Norton, 1994; Iwatsuki et al., 2003; Patel and Naik, 2004; Friedman, 2013; Prinsen et al., 2014) and have collectively been termed as γ-oryzanol. These compounds have been implicated in lowering blood lipid levels, among other health benefits (Nicolosi et al., 1993; Gerhardt and Gallo,

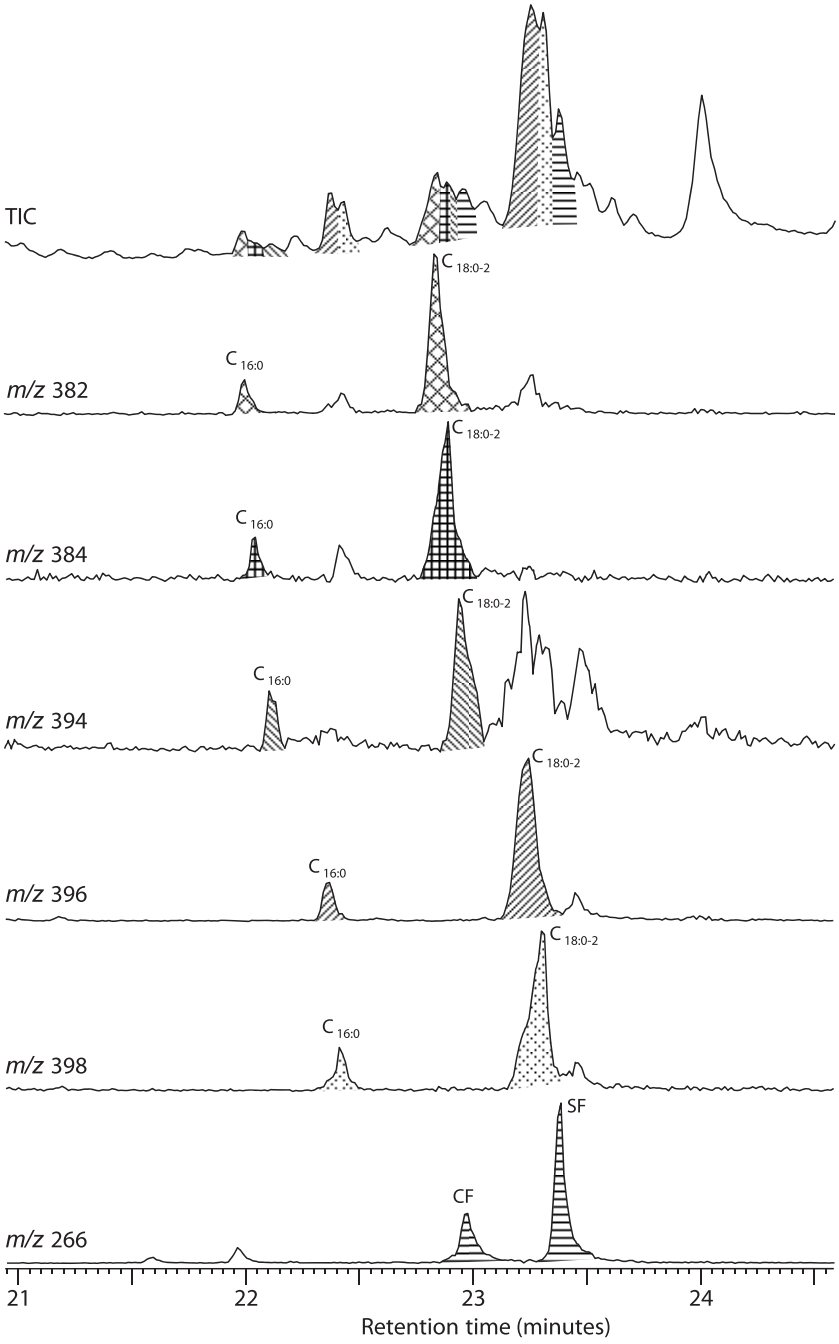

Fig. 5. Total ion chromatogram (TIC) and individual-ion chromatograms showing the distribution of the different sterol esters in the acetone extracts of maize fibers. *m/z* 382, campesterol ester series, *m/z* 384, campestanol ester series, *m/z* 394, stigmasterol ester series, *m/z* 396, sitosterol ester series, *m/z* 398, stigmastanol ester series; *m/z* 266, ferulate ester series. CF: campestanyl ferulate: SF: stigmastanyl ferulate.

1998; Patel and Naik, 2004).

Minor amounts of steroid ketones were also identified among the extractives of maize fibers (70 mg/kg) and rice husks (30 mg/kg). The main steroid ketones identified were stigmasta-3,5-dien-7-one (**XXIX**), stigmast-4-en-3-one (**XXX**), and stigmastane-3,6-dione (**XXXI**). Finally, steroid hydrocarbons were also found in maize fibers (180 mg/kg) and rice husks (30 mg/kg), and included ergost-3-ene, ergosta-3,5-diene, stigmast-3-ene, stigmasta-3,5,22-triene and stigmast-3,5-diene (**XXXII**); these compounds are, most probably, degradation products arising from free and conjugated sterols.

From our analysis, the high abundance of steroid compounds in maize fibers, particularly free and esterified sterols, makes this cereal by-product an interesting potential feedstock for obtaining valuable phytosterols that are known for their nutraceutical and health promoting benefits (Hicks, 1998; Moreau et al., 2002; Wilson et al., 2000).

4. Conclusions

The detailed composition of the lipophilic compounds present in maize fibers and rice husks has been reported. The analyses revealed that both cereal by-products predominantly contains fatty acids and acylglycerols, together with lower amounts of free sterols, sterols esters, sterol ferulates, and tocopherols, that were particularly prominent in maize fibers. Although the content and composition of the lipophilic compounds may slightly vary depending on environmental and cultivation conditions (e.g. cultivar, location, type of soil, age of harvesting),

the information reported here suggests new opportunities for the valorization of these residues that cannot be restricted solely to the valorization of the carbohydrates and lignin. Due to the large amounts of maize fibers and rice husks produced annually, their wide availability, and their low cost, these cereal by-products can also be regarded as a promising source to obtain high-value phytochemicals for use in the cosmetic, pharmaceutical, and food industries. Maize fibers are particularly rich in free and conjugated sterols, which are known to have beneficial effects on human health, as lowering plasma cholesterol levels (Wilson et al., 2000), making this cereal residue an appropriate feedstock for their extraction. Due to their nutraceutical properties, these valuable sterols could be used as food supplements. On the other hand, the high content of free fatty acids and acylglycerols in these residues could also make them interesting feedstocks for obtaining oils for different purposes, including their use in cosmetics. The keystone for the valorization of lipophilic compounds from these cereal residues would be their extraction at industrial-scale using economic feasible and green extraction processes that can be integrated into a Biorefinery. Several extraction techniques, including microwave, ultrasound, instantaneous pressure drop, pressurized-liquid extraction, supercritical-fluid extraction, or pressing techniques (Rombaut et al., 2014; Carciochi et al., 2017), have been developed that could be easily implemented into a Biorefinery facility for the green extraction of plant functional compounds from these cereal by-products.

CRediT authorship contribution statement

Gisela Marques: Conceptualization, Methodology, Investigation, Writing - review & editing. **Jorge Rencoret:** Methodology, Investigation. **Ana Gutiérrez:** Supervision, Resources. **José C. del Río:** Conceptualization, Methodology, Writing - review & editing, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Awika, J.M., 2011. Health promoting effects of cereal and cereal products. In: Tokusoglu, Ö., Hall, C.A. (Eds.), Fruit and Cereal Bioactives – Sources, Chemistry, and Applications. CRC Press, Boca Raton, FL, pp. 9–17.

Carciochi, R.A., D'Alessandro, L.G., Vauchel, P., Rodriguez, M.M., Nolasco, S.M., Dimitrov, K., 2017. Valorization of agrifood by-products by extracting valuable bioactive compounds using green processes. In: Grumezescu, A.M., Holban, A.M. (Eds.), Ingredients Extraction by Physicochemical Methods in Food – Handbook of Food Bioengineering. Academic Press, London, UK, pp. 191–228.

Curstedt, T., 1974. Mass spectra of trimethylsilyl ethers of ^{2}H-labelled mono- and diglycerides. Biochim. Biophys. Acta 360, 12–23.

del Río, J.C., Marques, G., Rodríguez, I.M., Gutiérrez, A., 2009. Chemical composition of lipophilic extractives from jute (*Corchorus capsularis*) fibers used for manufacturing of high-quality paper pulps. Ind. Crops Prod. 30, 241–249.

del Río, J.C., Prinsen, P., Gutiérrez, A., 2013. A comprehensive characterization of lipids in wheat straw. J. Agric. Food Chem. 61, 1904–1913.

del Río, J.C., Marques, G., Lino, A.G., Lima, C.F., Colodette, J.L., Gutiérrez, A., 2015. Lipophilic phytochemicals from sugarcane bagasse and straw. Ind. Crops Prod. 77, 992–1000.

del Río, J.C., Evaristo, A.B., Marques, G., Martín-Ramos, P., Martín-Gil, J., Gutiérrez, A., 2016. Chemical composition and thermal behavior of the pulp and kernel oils from macauba palm (*Acrocomia aculeata*) fruit. Ind. Crops Prod. 84, 294–304.

del Río, J.C., Rencoret, J., Gutiérrez, A., Kim, H., Ralph, J., 2018. Structural characterization of lignin from maize (*Zea mays* L.) fibers: evidences for diferuloylputrescine incorporated into the lignin polymer in maize kernels. J. Agric. Food Chem. 66, 4402–4413.

Evershed, R.P., Prescott, M.C., Spooner, N., Goad, L.J., 1989. Negative ion ammonia chemical ionization and electron impact ionization mass spectrometric analysis of steryl fatty acyl esters. Steroids 53, 285–309.

Friedman, M., 2013. Rice brans, rice bran oils, and rice hulls: composition, food and industrial uses, and bioactivities in humans, animals, and cells. J. Agric. Food Chem. 61, 10626–10641.

Gáspár, M., Kálmán, G., Réczey, K., 2007. Corn fiber as a raw material for hemicellulose and ethanol production. Process Biochem. 42, 1135–1139.

Gerhardt, A.L., Gallo, N.B., 1998. Full fat rice bran and oat bran similarly reduce hypercholesterolemia in humans. J. Nutr. 128, 865–869.

Górnas, P., Rudzinska, M., Raczyk, M., 2016. Lipophilic bioactive compounds in the oils recovered from cereal by-products. J. Sci. Food Agric. 96, 3256–3265.

Gutiérrez, A., del Río, J.C., 2001. Gas chromatography/mass spectrometry demonstration of steryl glycosides in eucalypt wood, kraft pulp and process liquids. Rapid Commun. Mass Spectrom. 15, 2515–2520.

Gutiérrez, A., del Río, J.C., González-Vila, F.J., Martín, F., 1998. Analysis of lipophilic extractives from wood and pitch deposits by solid-phase extraction and gas chromatography. J. Chromatogr. A 823, 449–455.

Hartman, L., Lago, R.C.A., 1976. The composition of lipids from rice hulls and from the surface of rice caryopsis. J. Sci. Food Agric. 27, 939–942.

Hernandez, E., 2005. Pharmaceutical and cosmetic use of lipids. In: Shahidi, F. (Ed.), Bailey´s Industrial Oil and Fat Products. John Wiley & Sons, New York, pp. 391–411.

Hicks, K.B., 1998. Cholesterol-lowering plant oil products. Genetic Eng. News 18, 14.

International Grains Council, 2019. Five-year Baseline Projections of Supply and Demand for Wheat, Maize (corn), Rice and Soyabeans to 2023/24. (Accessed 09 September 2019). https://www.igc.int/en/markets/marketinfo-forecasts.aspx.

Iwatsuki, K., Akihisa, T., Tokuda, H., Ukiya, M., Higashihara, H., Mukainaka, T., Iizuka, M., Hayashi, Y., Kimura, Y., Nishino, H., 2003. Sterol ferulates, sterols, and 5-alk(en)ylresorcinols from wheat, rye, and corn bran oils and their inhibitory effects on Epstein-Barr virus activation. J. Agric. Food Chem. 51, 6683–6688.

Jiang, Y., Wang, T., 2005. Phytosterols in cereal by-products. J. Am. Oil Chem. Soc. 82, 439–444.

Kim, H.Y., Hwang, I.G., Kim, T.M., Woo, K.S., Park, D.S., Kim, J.H., Kim, D.J., Lee, J., Lee, J.R., Jeong, H.S., 2012. Chemical and functional components in different parts of rough rice (*Oryza sativa* L.) before and after germination. Food Chem. 134, 288–293.

Lauer, W.M., Aasen, A.J., Graff, G., Holman, R.T., 1970. Mass spectrometry of triglycerides: I. Structural effects. Lipids 5, 861–868.

Lusby, W.R., Thompson, M.J., Kochansky, J., 1984. Analysis of sterol esters by capillary gas chromatography electron impact and chemical ionization-mass spectrometry. Lipids 19, 888–901.

Metzger, J.O., Bornscheuer, U., 2006. Lipids as renewable resources: current state of chemical and biotechnological conversion and diversification. Appl. Microbiol. Biotechnol. 71, 13–22.

Moazzami, A.A., Lampi, A.-M., Kamal-Eldin, A., 2011. Bioactive lipids in cereals and cereal products. In: Tokusoglu, Ö., Hall, C.A. (Eds.), Fruit and Cereal Bioactives – Sources, Chemistry, and Applications. CRC Press, Boca Raton, FL, pp. 229–249.

Moreau, R.A., Powell, M.J., Hicks, K.B., 1996. Extraction and quantitative analysis of oil from commercial corn fiber. J. Agric. Food Chem. 44, 2149–2154.

Moreau, R.A., Whitaker, B.D., Hicks, K.B., 2002. Phytosterols, phytostanols, and their conjugates in foods: structural diversity, quantitative analysis, and health-promiting uses. Progr. Lipid Res. 41, 457–500.

Moreau, R.A., Lampi, A.-M., Hicks, K.B., 2009. Fatty acid, phytosterols, and polyamine conjugate profiles of edible oils extracted from corn germs, corn fiber, and corn kernels. J. Am. Oil Chem. Soc. 86, 1209–1214.

Nicolosi, R.J., Rogers, E.J., Ausman, L.M., Orthoefer, F.T., 1993. Rice bran oil and its health benefits. In: Marshall, W.E., Wadsworth, J.I. (Eds.), Rice Science and Technology. Marcel Dekker, New York, pp. 421–437.

Norton, R.A., 1994. Isolation and identification of steryl cinnamic acid derivatives from corn bran. Cereal Chem. 71, 111–117.

Patel, M., Naik, S.N., 2004. Gamma-oryzanol from rice bran oil – a review. J. Sci. Ind. Res. 63, 569–578.

Prinsen, P., Gutiérrez, A., Faulds, C.B., del Río, J.C., 2014. Comprehensive study of valuable lipophilic phytochemicals in wheat bran. J. Agric. Food Chem. 62, 1664–1673.

Rombaut, N., Tixier, A.-S., Bily, A., Chemat, F., 2014. Green extraction processes of natural products as tools for biorefinery. Biofuels, Bioprod. Bioref. 8, 530–544.

Rose, D.J., Inglett, G.E., Liu, S.X., 2010. Utilisation of corn (*Zea mays*) bran and corn fiber in the production of food components. J. Sci. Food Agric. 90, 915–924.

Salanti, A., Zoia, L., Orlandi, M., Zanini, F., Elegir, G., 2010. Structural characterization and antioxidant activity evaluation of lignins from rice husk. J. Agric. Food Chem. 58, 10049–10055.

Singh, V., Moreau, R.A., Haken, A.E., Eckhoff, S.R., Hicks, K.B., 2000. Hybrid variability end effect of growth location on corn fiber yields and corn fiber oil composition. Cereal Chem. 77, 692–695.

Snyder, J.-M., Taylor, S.C., King, J.W., 1993. Analysis of tocopherols by capillary supercritical fluid chromatography and mass spectrometry. J. Am. Oil Chem. Soc. 70, 349–354.

Tao, B.Y., 2007. Industrial applications for plant oils and lipids. In: Yang, S.-T. (Ed.), Bioprocessing for Value-Added Products from Renewable Resources – New Technologies and Applications. Elsevier, Amsterdam, pp. 611–627.

Wilson, T.A., DeSimone, A.P., Romano, C.A., Nicolosi, R.J., 2000. Corn fiber oil lower plasma cholesterol levels and increases cholesterol excretion greater than corn oil and similar to diets containing soy sterols and soy stanols in hamsters. J. Nutr. Biochem. 11, 443–449.