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Phytostanols and phytosterols distributions in corn kernel

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ABSTRACT

Recently, industry has focused attention on plant matrices rich in phytosterols and phytostanols for their ability to reduce serum cholesterol levels. Therefore, the objective of this study was to examine the phytosterols and phytostanols contents of different fractions (endosperm, pericarp, germ) of corn kernel. The germ fraction contained more oil (24.2–30.7%) than endosperm and pericarp fractions (0.4–1.2%). Endosperm oil had the highest levels of phytostanols and 4,4-dimethylsterols, while pericarp oil had the greatest amounts of 4-desmethylsterols and 4-monomethylsterols. In the oil extracted from three corn kernel fractions sitostanol was the predominant phytostanols (77–87%), followed by campestanol (13–23%). The high percentages of 24–methylencycloartanol and cycloartenol were detected in the endosperm and pericarp parts, respectively. Citrostadienol was detected in corn germ oil as the main component of the 4-monomethylsterols fraction. In different parts of corn kernel, β-sitosterol (62–69%) was the major 4-desmethylsterol, followed by campesterol (11–18%) and stigmasterol (5–13%).

1. Introduction

Plant sterols, called phytosterols, were found to be minor constituents of vegetable oils. They are also characteristic of the genuineness of vegetables oils (Crane, Aurore, Joseph, Mouloungui, & Bourgeois, 2005). According to the number of methyl groups at the C-4 position, phytosterols have been classified into three groups: the 4-desmethylsterols or simply sterols, the 4, 4-dimethylsterols and the 4-monomethylsterols (Grunwald, 1975). They have been recognized as cancer preventive biologically active substances together with other secondary plant product (Cañabate-Díaz et al., 2007). Phytostanols, a fully saturated subgroup of phytosterols, are less abundant in nature than plant sterols (Nair, Kanfer, & Hoogmartens, 2006).

Most studies comparing the activity of phytostanols and phytosterols conclude that their ability to reduce serum cholesterol levels is equal, although some consider that plant stanols are more efficient (Santos et al., 2007). This better effectiveness is related to the fact that phytostanols, which have practically no absorption, remain for longer period in the intestinal lumen where they interfere continually and in a more efficient way with the absorption of cholesterol (Hicks & Moreau, 2001; Nguyen, 1999). Diminishing of plasmatic cholesterol levels is vital for the prevention of cardiovascular diseases, which are the main cause of death in Europe (Santos et al., 2007). Therefore, the development of food technology has created some foods enriched with phytostanols and phytosterols. At present, several functional food product types such as yoghurts and milk with added plant sterols and stanols are available on the market (Lagarda, García-Llatas, & Farré, 2006). Furthermore, one of the main objectives of industry is to identify plant matrices rich in those compounds.

The oil extracted from whole corn kernel is very rich in phytosterols (Verleyen et al., 2002; Ferrari, Schulte, Esteves, Brühl, & Mukherjee, 1996). However, commercial corn oil is extracted from corn germ and could thus more accurately be called "corn germ oil" (Moreau, Singh, Nuñez, & Hicks, 2000). We have previously reported that corn oil extracted from whole kernel contained phytostanols, 4-desmethylsterols, 4-monomethylsterols and 4,4-dimethylsterols (Harrabi et al., 2007). The purpose of this study was to determine the distribution of those four groups of sterols among corn kernel fractions (endosperm, germ and pericarp). The goal is to evaluate seed parts for its potential use as source of those high value-added compounds.

2. Experimental

2.1. Reagents and standard

Methanol and *n*-hexane, solvents of HPLC grade, were purchased from Panreac Quimica SA. (Barcelona, Spain). Chloroform and petroleum ether were from Fisher Scientific SA (Loughborough, Spain). Ethanol was purchased from Sientific Limited (Northampton, UK). Sterol standards were acquired from Sigma Aldrich

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(Madrid, Spain). TLC silica plates (silica gel 60G F254, 20 × 20 cm, 0.25 mm thickness), Potassium hydroxide pellets and anhydrous sodium sulphate were obtained from Merck (Darmstadt, Germany).

2.2. Plant material

Astro (dent maize) was obtained from Spain, while *Bonus* and *GH2547* (sweet maize) were obtained from USA. The three varieties of maize (*Zea mays* L.), were grown in restricted zones (15 m × 3 m) on the Agronomy farm of the INRAT (Institut National Recherche Agronomie Tunis, North of Tunisia) from the middle of April until the end of August 2005. Each sample was collected at maturity. Corn kernels were steeped for 3 h in 0.1% sodium metabisulfite solution at 50 °C before dissection.

2.3. Lipid extraction

The total lipids were extracted by the method of Folch, Lees, and Sloane Stanley (1957) modified by Bligh and Dyer (1959). Seeds (2.5 g) were washed with boiling water for 5 min to denature the phospholipases (Douce, 1964) and then crushed in a mortar with a mixture of $CHCl_3$–MeOH (2:1, V/V). The water of fixation was added and the homogenate was centrifuged at 3000*g* for 15 min. The lower chloroformic phase containing the total lipids was kept and dried in a rotary evaporator at 40 °C.

2.4. Saponification

Unsaponifiable lipids were determined by saponifying 5 g of lipid extracts with 50 mL ethanolic KOH 12% (w/v) and heating at 60 °C for 1.30 h. After cooling, 50 mL of H_2O was added and the unsaponifiable matter was extracted four times with 50 mL petroleum ether. The combined ether extract was washed with 50 mL of EtOH–H_2O (1:1). The ether extracted was dried over anhydrous Na_2SO_4 and evaporated. The dry residues were dissolved in $CHCl_3$ for TLC analysis.

2.5. Thin-layer chromatography

TLC of unsaponifiable fraction: the unsaponifiable matter was separated into subfractions on preparative silica gel thin-layer plates, using 1-dimensional TLC with hexane–Et_2O (9:1 by volume) as the developing solvent. The unsaponifiable (4 mg in 100 μl $CHCl_3$) containing 1% (w/w) each of 5-α-cholestanol and lanosterol as the internal standard for 4-desmethyl sterols and dimethylsterols, respectively, was applied on the silica gel plates in 3-cm bands. To correctly identify the sterols bands, a reference sample of purified sterol (5-α-cholestanol and lanosterol) were applied on the left and the right sides of the TLC plates. After development the plate was sprayed with 2′,7′-dichlorofluorescein and viewed under UV light. On the basis of the reference spots, the sterols bands were identified. The bands corresponding to 4-desmethylsterols and triterpene alcohol (4,4-dimethylsterols) were scraped off separately and each fraction was extracted three times with $CHCl_3$–Et_2O (1:1), filtered to remove the residual silica, dried in a rotary evaporator and stored at −10 °C for further analysis.

2.6. GC–MS analysis

Each sterols fraction was silylated with 200 μl of BSA (bis N,O-trimethylsilyl acetamide) agent in dry pyridine at 40 °C for 20 min. The derivatized sterols fractions were immediately injected separately into a GC (Hewlett Packard 6890) coupled to a HP5973 mass selective detector (Agilent technologies), set to scan from 50 to 550 *m*/*z*. The system was fitted with a capillary HP-5 column (5% phenyl methyl siloxane, 30 m × 0.25 mm, 0.25 μm film thickness) and Helium was used as the carrier gas at 1 mL min^{-1}. GC–MS operating temperatures were as follows: injector 260 °C, detector 310 °C and oven temperature was programmed from 150 °C to 320 °C at 10 °C min^{-1}. The ionization energy was 70 eV. Manual injection of 1 μl of the solution of sterol was performed in the split mode at a 1:50 split ratio.

The sterols were identified by comparing the relative retention times (to a campesterol standard) and mass spectra with those previously published (Harrabi et al., 2007). Likewise, identification of stanols was done by comparing retention times with a stigmastanol standard and their reported mass spectra. The peaks were also confirmed with Wiley 275.L Mass Spectral Library. Compounds were quantified by directly comparing their total ion chromatogram peak areas with that of an internal standard. For 4-desmethylsterols and phytostanols the internal standard was 5-α-cholestanol while for triterpene alcohols and 4-monomethylsterols it was lanesterol. The GC–MS response factor of sterols, calculated by using 5-α-cholestanol, was 0.94 ± 0.05. A typical GC–MS chromatograms of the trimethylsilyl ether derivatives of 4-desmethylsterols, phytostanols, 4,4-dimethylsterols and 4-monomethylsterols of germ fraction are shown in Fig. 1.

2.7. Statistical analysis

Statistical analysis was performed by using the Proc ANOVA in SAS (Software version 8).

Duncan's Multiple Range Test was used. For each sample three determinations have been done.

3. Results and discussion

3.1. Total lipids in the corn kernel fractions

The weights of the dissected corn kernel fractions show that dent corn (*Astro*) had significantly ($p < 0.05$) higher endosperm and lower germs than sweet corn (*Bonus* and *GH2547*) (Table 1). This difference could be linked to the characteristics of the seeds. In fact, dent seeds which contain soft starch differ from sweet seeds by a single recessive gene which prevents some of the sugars being converted to starch (Bland, 1971). This latter, was mainly accumulated in endosperm fraction. A comparison between three corn kernels parts demonstrated that there was a statistical difference ($p < 0.05$) in their contents of lipids. The level of oil from the germ fraction was very high (24.2–30.7%), while the amounts of oil from the endosperm and pericarp fractions were very low (0.4–1.2%). This result might be related to the differences in the activities of the oil-synthesizing enzymes and the availability of their respective substrates among corn kernel fractions. Corn germ fraction had more lipids than wheat germ fraction which contained 11.8% (Jiang, & Wang, 2005).

3.2. Phytostanols distribution

The average dietary consumption of phytosterols and phytostanols is approximately 250 and 25 mg/day, respectively (Nair et al., 2006). Mean values of phytostanols were significantly ($p < 0.05$) lower in germ fraction of three varieties compared to the endosperm and the pericarp fractions. Corn germ oil contained the least amount of phytostanols (270.2–460.0 mg kg^{-1} of oil) (Table 2). This result is in contrast with a result of Moreau et al. (2000) which indicated that the corn germ oil contained phytosterols but not phytostanols. Differences in variety were likely the reason for the observed results. In fact, the sterol composition and content of vegetable oils is affected by geographical growing area, difference in

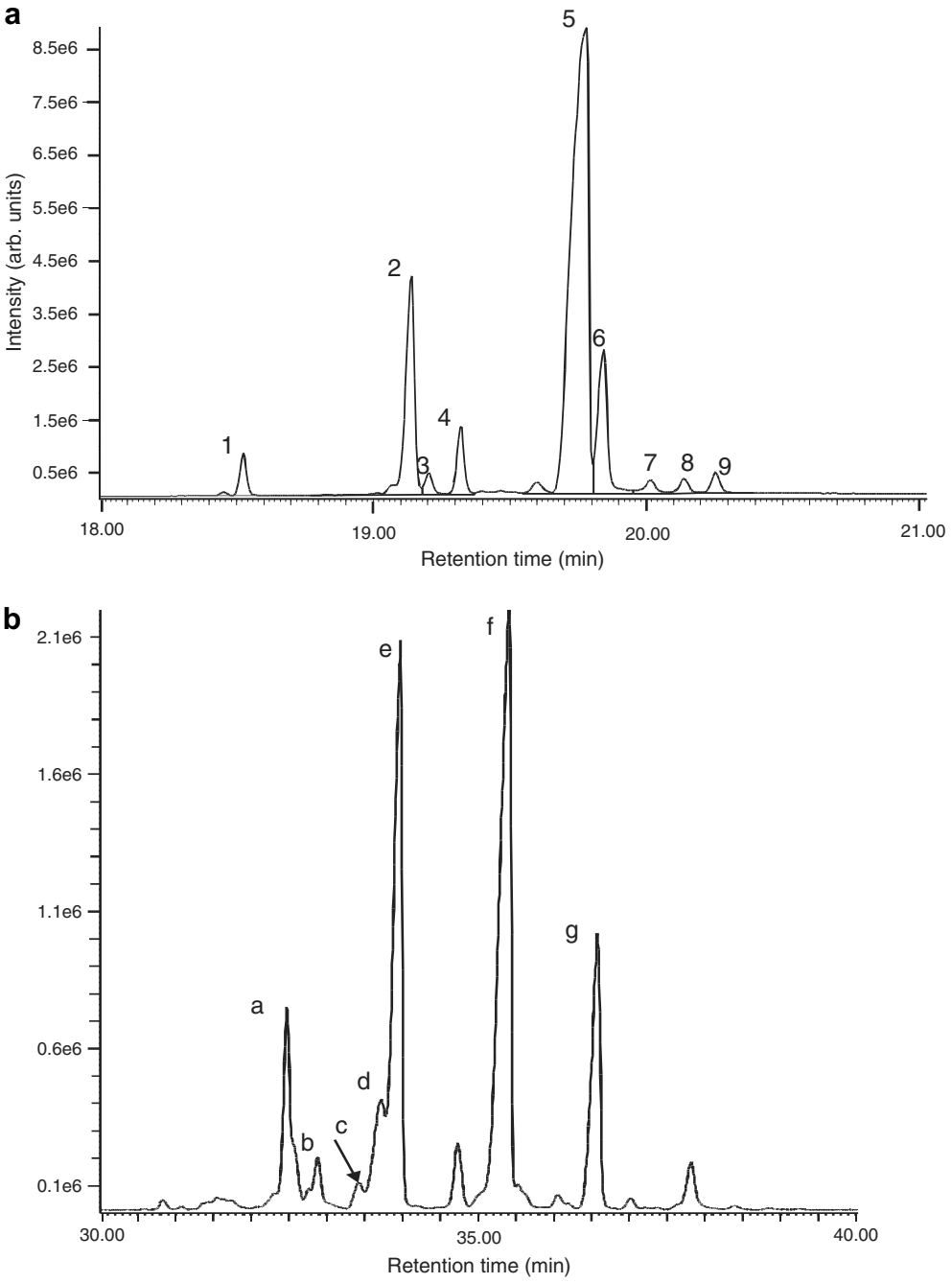

Fig. 1. (a) The GC–MS total ion chromatogram of the trimethyl silyl derivatives of 4-desmethylsterols and stanols and (b) 4-monomethylsterols and 4,4-dimethylsterols of the germ fraction. 1. 5-α-cholestanol (S.I.), 2. campesterol, 3. campestanol, 4. stigmasterol, 5. siosterol, 6. stigmastanol, 7. Δ-5 avenasterol, 8. Δ-7 avenasterol, 9. Δ-7 stigmastenol, a. obtusifoliol, b. lannesterol (S.I.), c. β-amyrine, d. gramisterol, e. cycloartenol, f. 24-methylencycloartenol and g. citrostadienol.

Table 1
Total lipid content (% of dry weight) of dissected corn kernel

Sample	Astro		GH2547		Bonus	
	% of kernel fraction[A]	Lipid content[A]	% of kernel fraction[A]	Lipid content[A]	% of kernel fraction[A]	Lipid content[A]
Pericarp	$^{*}6.5 \pm 0.3^{c}$	$^{*}0.8 \pm 0.1^{a}$	$^{*}5.9 \pm 0.3^{c}$	$^{*}1.2 \pm 0.1^{a}$	$^{*}5.4 \pm 0.2^{d}$	$^{*}0.4 \pm 0.1^{b}$
Germ	$^{*}13.9 \pm 0.6^{c}$	$^{*}24.2 \pm 0.5^{a}$	$^{*}19.2 \pm 0.4^{d}$	$^{*}29.0 \pm 1.0^{b}$	$^{*}18.9 \pm 0.3^{d}$	$^{*}30.7 \pm 0.4^{b}$
Endosperm	$^{*}79.6 \pm 0.5^{c}$	$^{*}0.4 \pm 0.1^{a}$	$^{*}74.8 \pm 0.3^{d}$	$^{*}1.0 \pm 0.1^{b}$	$^{*}75.7 \pm 0.2^{d}$	$^{*}1.2 \pm 0.1^{b}$

Different letters within a row denote a significant difference between varieties (a,b for lipid content, c,d for % of kernel fraction).
Asterisk (∗) within a column denotes a significant difference among corn kernel parts, at $p \leqslant 0.05$.
[A] Each value is a mean ± standard deviation (SD) of three determinations.

Table 2
Phytostanols and phytosterols content (mg kg^{-1} of oil ± SD) of corn kernel parts

Sample		Phytostanols[A]	Dimethylsterols[A]	Monomethylsterols[A]	Desmethylsterols[A]
Astro	G	*460.0 ± 30.0^a	*1270.2 ± 36.0^a	*56.0 ± 5.2^a	*5870.0 ± 548.3^a
	P	*3800.0 ± 132.2^b	*2500.0 ± 47.6^b	*152.0 ± 3.4^b	*36700.0 ± 1539.4^b
	E	*6793.3 ± 138.9^c	*2900.0 ± 200.0^c	*72.0 ± 2.6^c	*10500.0 ± 1000.0^c
GH2547	G	*270.2 ± 26.4^a	*1890.0 ± 127.6^a	*85.0 ± 2.0^a	*3740.0 ± 515.0^a
	P	*2300.0 ± 130.1^b	*3400.0 ± 180.2^b	*304.0 ± 12.1^b	*28000.0 ± 3605.5^b
	E	*7720.2 ± 72.1^c	*4000.0 ± 435.8^c	*63.0 ± 2.0^c	*4083.3 ± 629.1^c
Bonus	G	*320.0 ± 10.0^a	*917.0 ± 38.3^a	*73.0 ± 2.0^a	*4770.0 ± 278.7^a
	P	*1700.0 ± 55.6^b	*2260.0 ± 144.2^b	*232.0 ± 4.0^b	*21500.0 ± 2883.1^b
	E	*7150.3 ± 99.8^c	*3560.0 ± 115.3^c	*46.0 ± 2.0^c	*6700.0 ± 608.2c

G, germ, P, pericarp, E, endosperm.
Mean values with * within a column for the same part of different varieties are significantly different, at $p \leqslant 0.05$.
Mean values with different letters within a column for different parts of corn kernel are significantly different, at $p \leqslant 0.05$.
[A] Each value is a mean ± standard deviation (SD) of a triplicate analysis performed on different samples.

species or processing, and the ripening stage of the fruit or the seed (Rivera del Alamo, Fregapane, Aranda, Gómez-Alonsa, & Salvador, 2004; Phillips, Ruggio, & Ashraf-Khorassani, 2005). This study shows that corn germ oil had lower levels of phytostanols than wheat germ oil which had 150 mg/100 g of oil (Jiang, & Wang, 2005). Among the three corn kernel fractions, endosperm oil had the most amounts of phytostanols (6793.3–7720.2 mg $kg^{-1}$of oil). This result suggested that cells in the endosperm part may require more saturated phytosterols than the cells in germ part. Thus, corn endosperm may be potential source of these health-enhancing compounds for functional foods and nutraceutical applications. In the oil obtained from three corn kernel fractions, sitostanol was the predominant stanols (77–87%), followed by campestanol (13–23%) (Table 3). This contrasts with the composition of wheat germ oil where sitostanol and campestanol amounted 55.3 and 44.7% of total phytostanols, respectively (Jiang, & Wang, 2005). Abundance of those compounds might be considered as characteristic of the chemotype analyzed or might be linked to the abundance of their precursors. The pericarp of *Astro* had significantly higher ($p < 0.05$) amount of sitostanol and campestanol than those of pericarp fractions of *Bonus* and *GH2547* (Table 3), presumably because the highest levels of β-sitosterol and campesterol, which are the precursors of sitostanol and campestanol, respectively, were detected in the pericarp of *Astro*.

3.3. 4-Desmethylsterols distribution

The total lipids extracted from corn pericarp contained significantly higher ($p < 0.05$) amount of 4-desmethylsterols than had endosperm and germ fraction (Table 2). Considering the fact that Δ-5 sterols were mainly accumulated in the plasma membrane (Grandmougin, Bouvier-Navé, Ullman, Benveniste, & Hartmann, 1989), we suggested that cells in the pericarp fraction may require more 4-desmethylsterols than the cells in the germ and endosperm fractions to regulate membrane fluidity and permeability. Jiang, & Wang (2005) reported that usually the more unsaturated a lipid molecule is, the more fluidity it will provide to the membrane. Plant sterols reside predominantly in the plasma membrane, but are synthesized in the membranes of the endoplasmic reticulum (Hartmann, Perret, Carde, Cassagne, & Moreau, 2002). Therefore, we suggested that biosynthesis of sterols in endoplasmic reticulum in cells of the pericarp fraction was most important. Comparing 4-desmethylsterols compositions between three kernels parts for each variety, it was found that significant ($p < 0.05$) differences between three fraction occurred for several sterols (Table 3). For all corn fractions, β-sitosterol (62–69%) was the major 4-desmethylsterols, followed by campesterol (11–18%) and stigmasterol (5–13%). The other 4-desmethylsterols (Δ-5-avenasterol, Δ-7-avenasterol and Δ-7-stigmastenol), were present in low amounts. Yoshida and Niki (2003) reported that β-sitosterol, campesterol and stigmasterol exerted antioxidant effects on the oxidation of methyl linoleate oil solution. β-sitosterol was reported to be the main sterol in seeds parts of wheat (Jiang, & Wang, 2005), adlay (Wu, Charles, Huang, 2007) and sterculiaceae (*Theobroma subincanum*) (Bruni et al., 2002). These observations suggested that this sterol is an essential component of membrane of cells in different parts of seed. Among the various plant sterols, β-sitosterol had been most intensively investigated with respect to its beneficial and physiological effects on health (Yang, Karlsson, Oksman, & Kallio, 2001). The endosperm fraction of *GH2547* was distinguished by the lowest levels of β-sitosterol, campesterol and stigmasterol, while the endosperm fraction of *Astro* showed the highest levels of those three compounds. The germ fraction of *Bonus* appeared to contain the highest levels of Δ-5-avenasterol, Δ-7-avenasterol and Δ-7-stigmastenol, however, the pericarp fraction of *Bonus* was characterized by the lowest levels of β-sitosterol, campesterol and stigmasterol. This difference could be linked to the differences in relative's activities and abundances of the complex of enzymes responsible for sterols synthesis and catalysis.

3.4. 4,4-Dimethylsterols and 4-monomethylsterols distributions

Moreau et al. (2000) reported the distribution of 4-desmethylsterols and phytostanols in corn kernel, but not the distribution of 4,4-dimethylsterols and 4-monomethylsterols. A comparison between three corn kernels parts demonstrated that there was a significant ($p < 0.05$) differences in their contents of various sterol fractions.

This study shows that endosperm oil had the most levels of 4,4-dimethylsterols (2900–4000 mg kg^{-1} of oil), while pericarp oil had the most amount of 4-monomethylsterols (152−304 mg kg^{-1} of oil) (Table 2). Compared with phytostanols, 4,4-dimethylsterols and 4-desmethylsterols, 4-monomethylsterols fraction had the lowest levels in all corn kernel parts and this might be linked to its role as an intermediate in biosynthetic sterol pathways. Mean values of each 4,4-dimethylsterol were significantly ($p < 0.05$) different among three corn kernels fractions (Table 3). β-amyrine was detected in the germ while it was absent in the endosperm and in the pericarp parts. This result could be linked to the abundance and activity of β-amyrine synthase, enzyme which converted 2,3-oxidosqualene to β-amyrine (Volkman, 2005). The highest level of 24-methylenecycloartanol (77%) was detected in endosperm oil while the highest level of cycloartenol (62%) was detected in pericarp oil. Considering the fat that sterol C-24 methyltransferase (SMT1) catalyses the conversion of cycloartenol to 24-methylenecycloartenol (Nes et al., 2003), we suggested that this enzyme was more abundant and active in endosperm than in

Table 3
Content of phytostanols and phytosterols (mg kg^{-1} ± SD)[A] in corn kernel parts

Peaks	Sterols	Samples	Endosperm	Germ	Pericarp
2	Campesterol	Astro	*1884.7 ± 179.5^{a}	*1172.8 ± 54.0^{b}	*4000.3 ± 843.2^{c}
		Bonus	*1152.4 ± 104.6^{a}	*849.0 ± 49.6^{b}	*2345.6 ± 314.2^{c}
		GH2547	*746.4 ± 115.0^{a}	*552.2 ± 76.7^{b}	*3847.2 ± 475.9^{c}
3	Campesterol	Astro	*1494.6 ± 30.4^{a}	*101.2 ± 6.6^{b}	*881.6 ± 21.1^{c}
		Bonus	*1529.8 ± 132.6^{a}	*42.6 ± 5.8^{b}	*394.1 ± 3.2^{c}
		GH2547	*1775.6 ± 16.5^{a}	*24.8 ± 2.4^{b}	*575.0 ± 33.0^{c}
4	Stigmasterol	Astro	*615.3 ± 58.6^{a}	*609.8 ± 28.1^{a}	*4881.4 ± 234.0^{b}
		Bonus	*381.9 ± 34.6^{a}	238.5 ± 13.9^{b}	*2859.5 ± 383.4^{c}
		GH2547	*232.3 ± 35.7^{a}	274.8 ± 37.8^{b}	*4032.0 ± 519.1^{c}
5	Sitosterol	Astro	*6709.5 ± 639.0^{a}	*3891.6 ± 178.5^{b}	*24,405.5 ± 1053.0^{c}
		Bonus	*4147.3 ± 376.5^{a}	*3291.3 ± 192.3^{b}	*14,491.0 ± 1954.7^{c}
		GH2547	*2436.5 ± 372.3^{a}	*2741.0 ± 377.4^{b}	*18,244.0 ± 2372.4^{c}
6	Sitostanol	Astro	*5298.6 ± 108.5^{a}	*358.2 ± 23.4^{b}	*2918.4 ± 111.1^{c}
		Bonus	*5620.1 ± 170.1^{a}	*277.3 ± 4.2^{b}	*1305.9 ± 57.9^{c}
		GH2547	*5944.3 ± 55.6^{a}	*245.1 ± 24.0^{b}	*1725.0 ± 99.2^{c}
7	Δ-5-Avenasterol	Astro	*178.5 ± 17.0^{a}	7.61 ± 1.6^{b}	*1541.4 ± 33.8^{c}
		Bonus	*207.6 ± 18.8^{a}	*119.2 ± 6.9^{b}	*918.0 ± 121.0^{c}
		GH2547	*104.9 ± 16.1^{a}	11.2 ± 1.5^{b}	*851.2 ± 109.6^{c}
8	Δ-5-Avenasterol	Astro	*689.8 ± 65.7^{a}	95.0 ± 4.3^{b}	*1321.2 ± 36.9$_{c}$
		Bonus	*442.2 ± 40.1^{a}	100.1 ± 5.8^{b}	*780.5 ± 103.7^{c}
		GH2547	*323.4 ± 49.8^{a}	*77.4 ± 10.6^{b}	*705.6 ± 90.8^{c}
9	Δ-5-Stigmastenol	Astro	*424.2 ± 40.4^{a}	82.7 ± 3.7^{b}	*550.5 ± 4.6^{c}
		Bonus	*361.8 ± 32.8^{a}	*166.9 ± 9.7^{b}	105.5 ± 43.2^{c}
		GH2547	*239.2 ± 36.8^{a}	83.1 ± 11.2^{b}	140.0 ± 18.0^{c}
a	Obtusifoliol	Astro	21.5 ± 0.7^{a}	*16.4 ± 1.8^{a}	*46.2 ± 1.0^{b}
		Bonus	19.5 ± 0.8^{a}	*23.5 ± 0.6^{a}	*126.4 ± 2.1^{b}
		GH2547	*28.3 ± 0.9^{a}	*28.1 ± 0.7^{a}	*90.5 ± 3.6^{b}
b	Amyrine	Astro	–	*36.3 ± 1.0	–
		Bonus	–	*27.0 ± 8.6	–
		GH2547	–	*42.3 ± 2.8	–
c	Gramisterol	Astro	15.9 ± 0.5^{a}	15.7 ± 1.4^{a}	*35.7 ± 0.8^{b}
		Bonus	*11.1 ± 0.4^{a}	16.4 ± 0.4^{b}	*26.9 ± 0.4^{c}
		GH2547	14.3 ± 0.4^{a}	*20.3 ± 0.4^{b}	*3.5 ± 2.9^{c}
d	Cycloartenol	Astro	*696.0 ± 48.0^{a}	*427.2 ± 12.1^{b}	*1625.0 ± 30.9^{c}
		Bonus	808.3 ± 4.0^{a}	*368.5 ± 18.4^{b}	*1411.5 ± 131.2^{c}
		GH2547	848.1 ± 92.3^{a}	*759.9 ± 51.3^{b}	*2040.0 ± 108.1^{c}
e	24-Methylen cycloartenol	Astro	*2204.0 ± 152.0^{a}	*806.43 ± 22.83^{b}	*874.96 ± 16.72^{c}
		Bonus	*2751.7 ± 115.6^{a}	*521.46 ± 20.12^{b}	*848.43 ± 52.95^{c}
		GH2547	*3151.8 ± 343.5^{a}	*1087.5 ± 73.49^{b}	*1360.0 ± 72.11^{c}
f	Citrostadienol	Astro	*34.5 ± 1.2^{a}	*23.7 ± 1.9^{b}	70.0 ± 1.5^{c}
		Bonus	15.2 ± 0.6^{a}	32.9 ± 0.9^{b}	79.5 ± 1.3^{c}
		GH2547	20.2 ± 0.6^{a}	36.5 ± 0.7^{b}	*139.8 ± .5^{c}

Mean values with different letters within a row for different parts of same corn kernel are significantly different, at $p \leqslant 0.05$.
Mean values with * within a column for the same part of different varieties are significantly different, at $p \leqslant 0.05$.
[A] Each value is a mean ± standard deviation (SD) of a triplicate analysis performed on different samples.

pericarp parts. In seeds of sterculiaceae (*Theobroma subincanum*), the high percentage of cycloartenol was detected in the endosperm and 24-methylenecycloartenol has been detected only in the endosperm (Bruni et al., 2002). Additionally, rice bran oil contained very high levels of cycloartenol and 24-methylenecycloartenol, which made up over 40% of total phytosterols (Jiang & Wang, 2005). The abundance of these compounds in seed parts might be considered as characteristic of the genotype analysed. *GH2547* germ had significantly higher ($p < 0.05$) cycloartenol (2040.0 ± 108.1 mg kg^{-1} of oil) and 24-methylenecycloartenol (1360.0 ± 72.1 mg kg^{-1} of oil) than had *Astro* germ and *Bonus* germ. These two sterols are mainly converted to 4-desmethylsterols in the sterol biosynthetic pathway. This result could explain the detection of the lowest level of 4-desmethylsterols in germ fraction of *GH2547*.

In three corn kernel fractions, 4-monomethylsterols were a mixture of obtusifoliol, gramisterol and citrostadienol (Table 3). This later was the most abundant compound (45%) of 4-monomethylsterols fraction in corn germ parts. Also, citrostadienol was detected in wheat germ oil as the main component of the 4-monomethylsterols fraction (Sims, Fioriti, & Kanuk, 1972). Małecka (2002) has reported that citrostadienol may exhibit antioxidant activity. The highest level of citrostadienol (139.8 ± 5.5 mg kg^{-1} of oil) was detected in *GH2547* pericarp fraction, presumably because this later had the highest level of gramisterol (73.5 ± 2.9 mg kg^{-1} of oil), which is the precursor of citrostadienol in the sterol biosynthetic pathway. Among pericarp fractions, *Bonus* showed the highest amount of obtusifoliol (126.4 ± 2.1 mg kg^{-1} of oil) and the lowest value of gramisterol (26.9 ± 0.4 mg kg^{-1} of oil), most probably because the conversion of obtusifoliol to gramisterol was less important in the pericarp part of *Bonus*.

4. Conclusions

This study revealed that each corn kernel part had a characteristic composition in phytosterols and phytostanols fractions. Phytostanols are localized mainly, but not exclusively, in the endosperm fraction. The high phytosterols and phytostanols content in

the pericarp and endosperm fractions, respectively, suggest the exploitation of these fractions as low-cost sources of high value-added compounds.

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