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Gas Chromatography−Mass Spectrometry Screening for Phytochemical 4-Desmethylsterols Accumulated during Development of Tunisian Peanut Kernels (*Arachis hypogaea* L.)

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4-Desmethylsterols, the main component of the phytosterol fraction, have been analyzed during the development of Tunisian peanut kernels (*Arachis hypogaea* L.), Trabelsia (AraT) and Chounfakhi (AraC), which are monocultivar species, and Arbi (AraA), which is a wild species, by gas chromatography−mass spectrometry. Immature wild peanut (AraA) showed the highest contents of β-sitosterol (554.8 mg/100 g of oil), campesterol (228.6 mg/100 g of oil), and Δ^5-avenasterol (39.0 mg/100 g of oil) followed by peanut cultivar AraC with β-sitosterol, campesterol, and Δ^5-avenasterol averages of 267.7, 92.1, and 28.6 mg/100 g of oil, respectively, and similarly for AraT 309.1, 108.4, and 27.4 mg/100 g of oil, respectively, were found. These results suggest that, in immature stages, phytosterol contents can be important regulator factors for the functional quality of peanut oil for the agro-industry chain from plant to nutraceuticals.

KEYWORDS: Wild peanut; cultivars peanut; peanut in development; phytochemical 4-desmethylsterols; GC-MS

INTRODUCTION

At present, 70 wild species of *Arachis* have been found and collected in South America. The probable centers of origin of *Arachis* species and *Arachis hypogaea* are in Brazil and Bolivia (*1*). Substantial evidence suggests that the genetic bases of the cultivated peanut (*A. hypogaea* L.) are different from those of wild species of *Arachis*, which contain new sources of germplasm to defend against pathogen factors (*2*, *3*). Peanut seeds make an important contribution to diet in many countries, especially Africa and parts of Asia, because they are a potential source of food-grade protein, lipids, and fatty acids. As a result, they are readily cultivated commercially and have attained widespread acceptability (*4*). Besides having a favorable fatty acid profile, peanuts contain many micronutrients and bioactive constituents as "phytochemicals", which are responsible for their nutritional and physicochemical properties (*5*). There is sufficient evidence to recommend a diet high in food sources rich in bioactive compounds such as phytosterols, which are enriched in lipid content plant foods, such as nuts and legumes including peanuts and seeds such as sesame seeds (*6*). Recently, plant sterols (phytosterols) have been added to margarine and vegetable oils as examples of successful functional foods (*7*). Phytosterols are generally dominated by the chemically defined group 4-desmethylsterols, which have the same structural base as cholesterol but with one or two extra carbon atoms in the side chain. Whereas about 250 types of phytosterols are actually reported in the literature, nutrition research has focused mostly upon the unsaturated β-sitosterol, campesterol, and stigmasterol. Phytostanols, a fully saturated subgroup of phytosterols, are less abundant in nature than phytosterols and are not found in peanut kernels (*8*).

Phytosterols exist in all foods of plant origin and are known to have several bioactive properties with possible benefits for human health (*9*). Phytosterols in particular, are important agricultural products used in the health and nutrition industries. They are useful emulsifiers for cosmetic manufacturers and supply the majority of steroidal intermediates and precursors for the production of hormone pharmaceuticals (*10*). The most well-known property is the serum cholesterol-lowering effect (*9*). In fact, cholesterol-lowering effects by phytosterol are suggested by many authors (*11*). Although the mechanism of action of phytosterol is not yet fully understood, it is likely that phytosterols compete with cholesterol in adsorption into mixed bile salts and acid micelles. A great part of cholesterol is therefore not available for absorption and excreted with the feces (*11*). Naturally occurring phytosterols even in the habitual dietary intake range from 150 to 450 mg/day and are negatively correlated with cholesterol absorption (*12*). Phytosterols might also prevent the development of colon cancer and benign prostatic hyperplasia, a subject of considerable interest in research and commercialization of

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phytosterols (*13*). The oil extracted from whole peanut kernel is rich in phytosterols, as is olive oil, and contains 900−3000 ppm total phytosterols (*14*, *15*).

In Tunisia, some of the commercially important *Arachis* cultivars are Chounfakhi (AraC) and Trabelsia (AraT), which are used for the production of peanut oil, but the wild species Arbi (AraA) is less known and is slowly disappearing. However, chemical studies of peanuts originating from Tunisia have not been undertaken. Although phytosterol composition has been studied at full maturity stage (*1*), to our knowledge, no data are available about the maturity stage and the developmental stages of Arbi.

The aim of this investigation is to differentiate the three varieties of peanut oils in terms of phytosterols during development and maturity. This can provide an innovative contribution toward the production of more functional peanut oils.

MATERIALS AND METHODS

Plant Material. Three varieties of local peanuts were collected: a wild variety, Arbi (AraA), and cultivar varieties Trabelsia (AraT) and Chounfakhi (AraC). They were grown on a private farm in Dar Allouch in northeastern Tunisia. Kernels were hand-picked starting mid-May until the end of October 2008. Samples have been collected at different intervals after the date of flowering (DAF) until maturity.

Lipid Extraction. Plant oil was extracted from dry material of peanut with petroleum ether using a Soxhlet apparatus. This extraction takes 4 h at 42 °C and was repeated three times for each sample. The extract was dried in a rotary evaporator at 32 °C. Oil was weighed and stored at −10 °C. The oil percentage is determined by measuring the relative weight of extracted oil from dried peanuts (*16*).

Saponification of the Lipids (Iso 11294, 1994). Unsaponifiable lipids were determined by saponifying 5 g of oil mixed with both 200 μL of 5-α-cholestanol solution (internal standard; 0.2% (w/v)) and 50 mL of ethanolic KOH 12% (w/v) solution; the mixture was heated at 60 °C for 1.30 h. After cooling, 50 mL of H_2O was added to the mixture. The unsaponifiable matter was extracted four times with 4 × 50 mL of petroleum ether. The combined ether was washed with 50 mL of EtOH/H_2O (1:1) (v/v).

The unsaponifiable fraction was collected from the ether fraction and dried under anhydrous Na_2SO_4 to eliminate residual water. Na_2SO_4 was removed by filtration, and organic solvent was evaporated to dryness using first a rotary evaporator and then dry nitrogen. Finally, the dry residues were dissolved in chloroform for TLC analysis.

Sterol Fractionation by Preparative Thin-Layer Chromatography (TLC). The unsaponifiable matter was separated into subfractions on preparative silica gel thin-layer plates, using one-dimensional TLC with hexane/Et_2O (9:1 by volume) as the developing solvent. It was dissolved in 5% of chloroform ($CHCl_3$) containing 1% of 5-α-cholestanol (following the internal standard for 4-desmethylsterols). One hundred and fifty microliters from this solution was deposited as a streak of 3 cm on the silica gel plates. To correctly identify the sterol bands, a reference sample of purified sterol (5-α-cholestanol) was applied on the left side of the TLC plates. After development, the plat was sprayed with 2′,7′-dichlorofluorescein and viewed under UV light. On the basis of the reference spots, the sterol band was identified. The band corresponding to 4-desmethylsterols was scraped separately, extracted three times with $CHCl_3/Et_2O$ (1:1), and filtered to remove the residual silica. It was finally dried in a rotary evaporator and stored at −20 °C for further analysis. Sterol levels were estimated on the basis of peak areas of known standard concentrations.

Silylation of Sterol Fraction. An amount of 2 mg of sterol residue was mixed with 80 μL of *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA; Acrôs Organics BVBA 98%) and 20 μL of chlorotrimethylsilane (TMCS; Sigma-Aldrich; MW = 108.64 g/mol). The mixture was vortexed (homogenized by vortex agitator) and heated at 60 °C for 30 min. After silylation reaction, 100 μL of hexane was added to the mixture, and 1 μL of the solution was directly injected to the gas chromatograph.

Gas Chromatography−Flame Ionization Detection (GC-FID) (*17*). Quantification of phytosterols was performed using a GC-FID apparatus. The GC system used was a thermo GC (Thermo Quest Trace GC). The initial column temperature was set to 170 °C and programmed to increase at a rate of 10 °C/min to 310 °C and then held constant for 5 min. The injector and detector temperatures were set to 320 °C. Helium was used as carrier gas, giving a column flow of 1 mL/min. Quantification of sterols was achieved by the addition of 5-α-cholestanol as an internal standard.

The level of each sterol was calculated using the formula

$$\text{amount (mg/100 g (oil))} = \frac{P_{As} m_{is}}{P_{Ais} m_s} \times 100$$

where P_{As} = sterol peak area, P_{Ais} = internal standard peak area, m_{is} = weight (mg) of the internal standard, and m_s = weight (mg) of oil taken for analysis (*18*−*20*).

GC-MS Analysis (*17*). GC-MS analysis was performed on a Hewlett-Packard 5890 A series II GC equipped with a MEGASE 54 column (Precix HB-sterol; 30 m × 0.22 mm i.d. and 0.22 μm film thickness) fitted to a 5989 II series mass spectrometer with a Mass lab data system. Helium was used as carrier gas at 1 mL/min. The injector temperature was set to 320 °C, and the samples were injected at the same conditions reported for the GC-FID analyses. The initial oven temperature was set to 170 °C, programmed to increase at a rate of 10 °C/min to 310 °C, and then held constant for 15 min. The injector and the detector temperatures were set to 320 °C. Manual injection of 1 μL of sterol solution was performed in splitless mode. Qualitative analysis was based on comparisons between the retention times (see **Table 1**), the mass spectra, and the corresponding data in the literature. All sterols were identified by referring to the data in the literature and the spectra of pure standards and with entries in NIST 05 HP Chemstation Library.

Table 1. Retention Time and Mass Spectrometric Data for Trimethylsilyl Derivatives of Each Sterol Identified by GC-MS[a]

peak	RRT	compound	MW	main fragmentation ions (*m*/*z*) and others
1	19.14	campesterol	472	382, 343, 255, 129
2	19.49	stigmasterol	484	394, 255, 175, 129, 83
3	20.59	β-sitosterol	486	396, 381, 357, 255, 129, 73
4	20.70	Δ^5-avenasterol	484	469, 386, 379, 355, 296, 129, 55
5	21.04	fucosterol	484	469, 386, 296, 129, 55
6	21.30	Δ^7-sitosterol	486	303, 255, 229, 213
7	21.56	Δ^7-avenasterol	486	386, 343, 255, 213

[a] RRT, relative retention time (min); MW, molecuarl weight (g/mol).

Statistical Analysis. Using a linear mixed-effects model for longitudinal data (*21*) but allowing for nested random effects, and where the within-group (variety) errors are permitted to be correlated and/or have unequal variances, we tested for difference in trends (variety effect) as well as time−variety interaction, between curves illustrating the variation of the total moisture, total lipids, and total unsaponifiable through time for each variety of peanuts. In addition, for specific time point analysis Wilcoxon (*22*) and Student *t* tests were used to test for median and mean difference of these variables between varieties. All of the statistical analysis was performed with the R software for statistical computing (version 2.10) (*23*). The differences between individual means was deemed to be significant at $p < 0.05$.

RESULTS AND DISCUSSION

Changes in Total Moisture Content. During peanut development, the total moisture content followed a similar pattern for the three varieties (**Figure 1**). **Table 2** shows that in the early stage (12 DAF), the moisture content reached an average of 89% of seed weight for AraA and an average of 94% for AraT and AraC. This initial percentage of moisture underscores the essential role of water as an inductive factor in the synthesis of reserves such as starch, protein, and lipids and in the embryonic morphogenesis in the oilseeds. However, the initial percentage of moisture difference between wild and cultivar species may be explained by the fact that in the immature stage, there are more differences in the activities of oil-synthesizing enzymes, which in the case of the wild species are more activated and start earlier than in the cultivars.

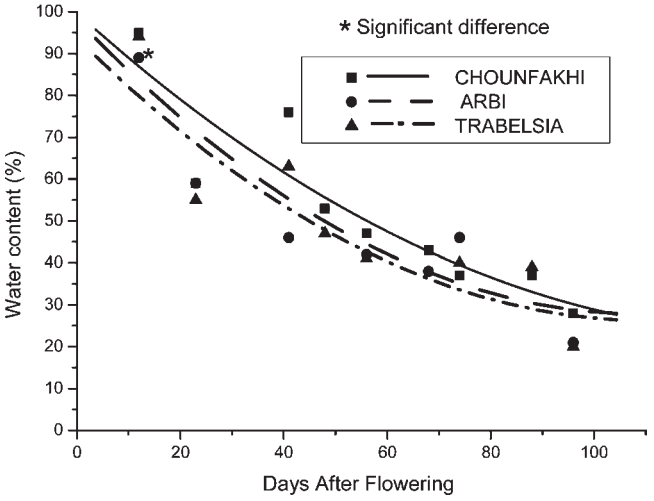

Figure 1. Distribution of moisture among the three peanut varieties during maturation.

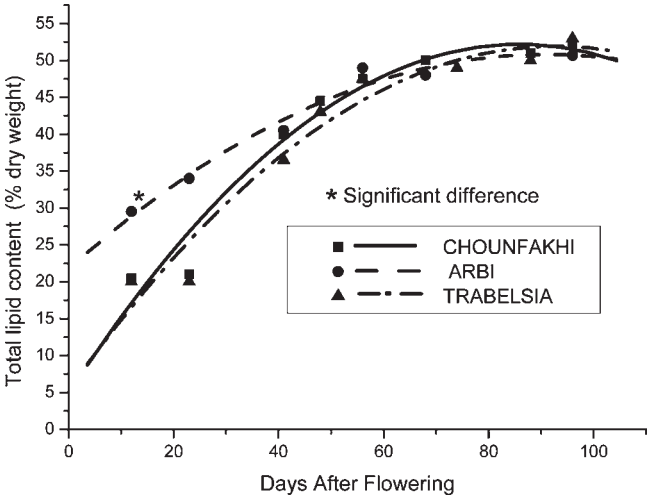

Figure 2. Distribution of total lipid content among the three peanut varieties during maturation.

Table 2. Total Moisture Content (Percent by Weight of Dry Weight) of Dissected Peanut Kernel[a]

DAF	maturity stage	sample	% moisture content
12	immature	AraA	88.66 ± 0.58[b]
		AraC	94.50 ± 0.50
		AraT	94.16 ± 1.04
56	intermediate	AraA	42.50 ± 0.50
		AraC	46.66 ± 0.57
		AraT	44.33 ± 0.57
96	mature	AraA	21.17 ± 1.04
		AraC	28.50 ± 1.32
		AraT	16.50 ± 3.96[b]

[a] Each value is the mean ± standard deviation of a triplicate analysis performed on different samples. DAF, days after flowering; AraA, Arbi; AraC, Chounfakhi; AraT, Trabelsia. [b] Mean value significantly different at $p < 0.05$.

Table 3. Total Lipid Content (Percent by Weight of Dry Weight) of Dissected Peanut Kernel[a]

DAF	maturity stage	sample	% total lipid content
12	immature	AraA	29.50 ± 1.50[b]
		AraC	20.50 ± 1.50
		AraT	20.10 ± 1.00
56	intermediate	AraA	48.00 ± 1.00
		AraC	47.50 ± 3.50
		AraT	47.50 ± 4.50
96	mature	AraA	50.66 ± 2.51
		AraC	52.16 ± 0.28
		AraT	53.16 ± 0.76

[a] Each value is the mean ± standard deviation of a triplicate analysis performed on different samples. DAF, days after flowering; AraA, Arbi; AraC, Chounfakhi; AraT, Trabelsia. [b] Mean value significantly different at $p < 0.05$.

This is may be due to differences in their genotypes (*24*). During maturation, moisture contents decreased gradually and reached an average of 30%. This reduction is closely related to the increase of the dry weight (*25*), as the seed completes its maturity by a dehydration, during which it enters dormancy. In the mature stage, all levels of moisture are similar (**Table 2**).

The statistical analysis of the overall trend for the three varieties using a linear-mixed model showed a large significant difference between AraA, AraC, and AraT in the total moisture content through time ($p = 0.0001327$). **Table 2** shows that in the immature stage (12 DAF), AraA (mean = 88.66% ± sd = 0.58) has significantly ($p < 0.05$) smaller moisture content compared to AraC (94.5% ± 0.50) and AraT (94.16% ± 1.04).

However, in the mature stage (96 DAF), no significant difference ($p = 0.172$) was observed between AraA (21.17% ± 1.04) and AraT (16.50% ± 3.96). In addition, significant differences were observed between AraC (28.50% ± 1.32) and AraT (16.50% ± 3.96) and also between AraC and AraA with $p = 0.025$ and $p = 0.00204$), respectively.

Changes in Total Lipid Content. During peanut development, oil accumulation follows a similar pattern for the three varieties with a significant difference ($p < 0.05$) for AraA in the early stage (12 DAF) (**Figure 2**). The latter shows a very high level of oil (expressed as %percent of dry weight) and reached 24.7−30.0%, whereas the amounts of oil from AraT and AraC were very low (7−8%). This result may be related to the low level of moisture shown previously (*26*). From 5 to 28 DAF, the lipid biosynthesis was much slower. This result can be explained by the fact that during this period, the lipids synthesized by immature peanut are used for the development of new seed tissues.

The most active period of oil accumulation occurred in the early development stage (12 DAF). Then, the total amount of lipids increased (intensively for the AraA) and reached a maximum at 90 DAF (about 48% for AraA variety and about 50% for AraC and AraT) and decreased from that point slightly to 47% at complete maturity of kernels. The increase in lipid content may be explained by the fact that, in this period, the weight of the seeds increases in favor of the formation of lipid reserve. No significant differences were detected in the oil content for the three varieties at maturity. In fact, in the last maturity stages, oil biosynthesis ceases, which explains the regression of the total lipid at complete maturity of seed. Therefore, this study suggests that AraA is an early variety, and a large amount of lipid was observed at 90 DAF for all varieties. Immature seeds of AraA are rare and richer in oil than those of AraT and AraC at 12 DAF. These results indicate approximately the best time for harvesting these varieties of peanuts.

Accordingly, **Table 3** shows that AraA (29.50%) at immature stage (12 DAF) has significantly higher lipid content ($p < 0.05$) than AraC (20.50%) and AraT (20.10%). However, at mature stage (96 DAF), no significant difference is observed among the

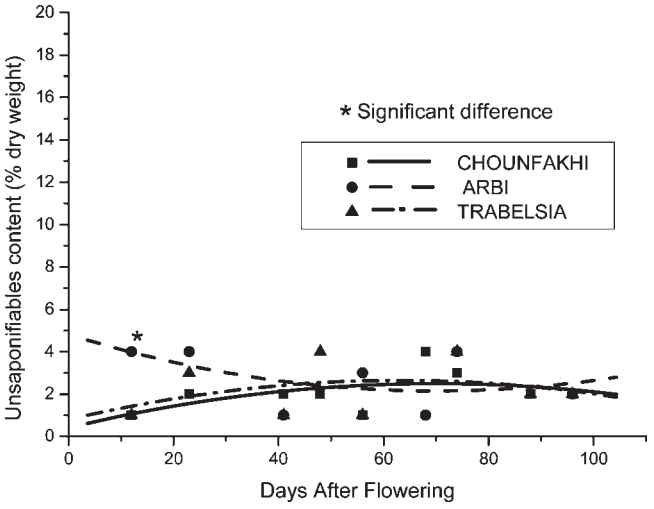

Figure 3. Distribution of total unsaponifiable content among the three peanut varieties during maturation.

Table 4. Total Unsaponifiable Content (Percent of Dry Weight) of Dissected Peanut Kernel[a]

DAF	maturity stage	sample	% unsaponifiable content
12	immature	AraA	4.00 ± 0.0[b]
		AraC	1.00 ± 0.0
		AraT	1.00 ± 0.0
56	intermediate	AraA	1.00 ± 0.0
		AraC	1.50 ± 0.5
		AraT	1.00 ± 0.0
96	mature	AraA	3.00 ± 0.0[b]
		AraC	1.50 ± 0.1
		AraT	1.50 ± 0.1

[a] Each value is the mean ± standard deviation of a triplicate analysis performed on different samples. DAF, days after flowering; AraA, Arbi; AraC, Chounfakhi; AraT, Trabelsia. [b] Mean value significantly different at $p < 0.05$.

three varieties. These results are in agreement with a previous work (*3*).

Dynamic Distribution of Total Unsaponifiable among the Three Varieties of Peanut. The profile of the unsaponifiable fraction is utilized as a fingerprint for the identification of oilseeds. With few exceptions, vegetable oils contain on average 0.2−1.5% of unsaponifiable compounds (*1*).

During peanut seed development, the three varieties presented different profiles with respect to the distribution of the unsaponifiable fraction (**Figure 3**). A relatively high amount of unsaponifiable (4% of total lipid) was recovered from AraA in the early stage of development (12 DAF) because it contained a high amount of oil as mentioned previously. AraC and AraT presented lower amounts (0.7−1.0% of total lipid). The total unsaponifiable content decreased in AraA, but increased in AraT and AraC, and reached the same level (2%) at 60 DAF. At maturity (96 DAF), the level of AraA unsaponifiable content increased slightly (3%) and remained higher than that of AraT and AraC (1.5%). This result can be related to differences in the activities of oil-synthesizing enzymes mentioned previously, among the three varieties, which in the case of AraA are very important at early stage (*26*).

Table 4 shows also a significant difference at early stage (12 DAF) among the three varieties, although no significant difference was detected during maturity.

Content of Total 4-Desmethylsterols during Peanut Kernel Development. Phytosterols and other nonsaponifiable compounds in

Table 5. Total Desmethylsterol Content of Peanut Kernels[a]

DAF	maturity stage	sample	content of DEMS (mg/100 g of oil)
12	immature	AraA	881.07[b]
		AraC	416.12
		AraT	476.58
56	intermediate	AraA	197.07
		AraC	90.75[b]
		AraT	196.92
96	mature	AraA	192.35
		AraC	198.60
		AraT	196.92

[a] DAF, days after flowering; AraA, Arbi; AraC, Chounfakhi; AraT, Trabelsia; DEMS, desmethylsterols. [b] Mean value significantly different at $p < 0.05$.

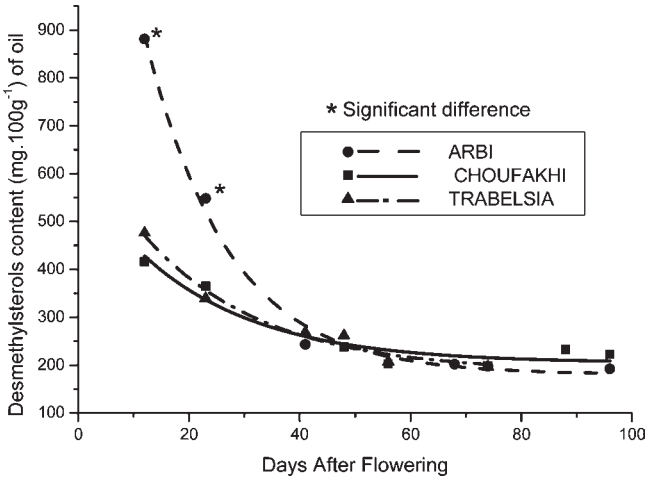

Figure 4. Distribution of total phytosterol content among the three peanut varieties during maturation.

oils are often used as markers for the assessment of adulterated oils (*3*, *10*, *27*). Plant oils and products made from them are generally regarded as important food sources of phytosterols, because in general they contain relatively higher phytosterols than fruits and vegetables (*28*−*30*). The analysis of free sterols (ST) provides important information about the quality and identity of lipids investigated (*7*, *31*).

According to Lagarda (*32*), the sensitivity and precision of currently available methods for sterol analysis can be classified as follows: GC > HPLC (high-performance liquid chromatography) > SFC (supercritical fluid chromatography). GC-FID (or GC-MS, when peak identity confirmation is needed) can be considered the method of choice for the determination of phytosterols in foods and diets and appears to provide a better selectivity than HPLC for analyzing certain isomers (*10*). In this work we opted for GC-MS and GC-FID to identify and quantify phytosterol content in peanut species.

In this study, we found that total 4-desmethylsterol content changes during the development of the plant, which is in agreement with ref *33*. In immature seeds (12 DAF), high levels of 4-desmethylsterol in the three varieties were observed and can be explained by intense cell division in the immature seeds (*13*). Indeed, this process requires biosynthesis of molecules essential for the construction of membranes such as phytosterols (*9*, *25*). In addition, 4-desmethylsterol content was significantly higher ($p < 0.05$) in AraA (881.07 mg/100 g of oil) compared to AraC (416.12 mg/100 g of oil) and AraT (476.58 mg/100 g of oil) (see **Table 5**). This result may be related to the differences shown previously in the activity level of oil-synthesizing enzymes, among the three

Table 6. Content and Composition of Desmethylsterols (Milligrams per 100 g of Oil) in Peanut Kernels[a]

		immature stage (12 DAF)			intermediate stage (56 DAF)			maturation stage (96 DAF)		
peak	sterol	AraA	AraC	AraT	AraA	AraC	AraT	AraA	AraC	AraT
2	campesterol	228.57[b]	92.13	108.36	23.30	24.62	24.35	22.74	25.76	24.35
3	stigmasterol	39.21[b]	19.16	23.00	15.32	14.32	13.64	16.86	16.22	13.64
4	β-sitosterol	554.79[b]	267.67	309.07	122.00	132.40	131.88	130.48	152.50	131.88
5	Δ^5-avenasterol	39.01[b]	28.65	27.36	22.23	25.00	25.11	20.35	25.53	25.10
6	fucosterol	3.67[b]	2.43	1.89	1.12	0.90	0.86	1.01	1.14	0.86
7	Δ^7-sitosterol	3.92[b]	1.63	2.42	0.30	0.23	0.15	0.30	0.30	0.15
8	Δ^7-avenasterol	11.90[b]	4.43	4.60	0.83	1.11	0.94	0.52	0.89	0.94

[a] DAF, days after flowering; AraA, Arbi; AraC, Chounfakhi; AraT, Trabelsia. [b] Mean value significantly different at $p < 0.05$.

varieties, which in AraA is more intense at 12 DAF. Moreover, substantial evidence suggests that the genetic base of the cultivated peanut (*A. hypogaea* L.) is different from that of the wild species of *Arachis* (*2*, *3*). Total 4-desmethylsterol, then, decreased during maturation to achieve the harvest values for AraA (192.35 mg/100 g of oil), AraC (196.60 mg/100 g of oil), and AraT (198.60 mg/100 g of oil) (**Figure 4**). The decrease in 4-desmethylsterol can be explained by their conversion into other lipid compounds (steroidal hormones and vitamins) that regulate the growth and development of immature tissues (*26*). During the development, the sterols become diluted and more oils were produced. At mature stage (96 DAF), there was no significant difference ($p > 0.05$) between the amounts of 4-desmethylsterols among the three varieties.

Content and Composition of 4-Desmethylsterols during Maturation. Most 4-desmethylsterols have a double bond between carbons 5 and 6 of the ring system and are thus called Δ^5 phytosterols and are mainly accumulated in the plasma membrane, where they are believed to regulate membrane fluidity (*34*).

However, another group of common 4-desmethylsterols that are abundant in certain plants have a double bond between carbons 7 and 8 instead of carbons 5 and 6 and are hence referred to as Δ^7 phytosterols.

Seven compounds were identified (**Table 4**); the first peak of interest corresponds to campesterol, which is followed by stigmasterol, β-sitosterol, Δ^5-avenasterol, and fucosterol (isomer of Δ^5-avenasterol). These five compounds or Δ^5-sterols are the major 4-desmethylsterols in the developing peanut kernels and account for 99% (AraC and AraA) and 69% (AraT) at maturity.

β-Sitosterol was the predominant sterol, followed by campesterol, Δ^5-avenasterol, stigmasterol, and fucosterol. These compounds are already reported to occur in peanut in similar amounts (*1*, *3*). Among different plant sterols, β-sitosterol has been most intensively investigated with respect to its beneficial effects in human (*7*, *35*, *36*). β-Sitosterol has been shown to exhibit anti-inflammatory, antineoplastic, antipyretic, and immunomodulating activities (*37*). Also, β-sitosterol, campesterol, and stigmasterol exert antioxidant effects on the oxidation of methyl linoleate oil solution. Indeed, the percentage of Δ^5-avenasterol is important among peanut varieties and has been associated with many important antioxidant effects (*26*). In fact, it was found that Δ^5-avenasterol has an essential antipolymerization effect, which could protect oils from oxidation during prolonged heating at high temperatures (*38*).

AraC was distinguished by the lowest levels of β-sitosterol, campesterol, Δ^5-avenasterol, and stigmasterol, followed by AraT, whereas AraA showed the highest amounts of those four compounds, especially at 12 DAF (881.1 mg/100 g of oil) (**Table 5**). In general, β-sitosterol and Δ^5-avenasterol are strongly and positively correlated (*15*). AraA showed the highest values in Δ^5-avenasterol in immature kernels, but AraC showed the highest one in mature kernels.

Samples were also checked for cholesterol and phytostanols, but neither was detected. This result is at odds with those of Baiyi et al. (*8*) and Grosso et al. (*1*).

Other phytosterols (Δ^7-sterols) are represented in **Table 6**. They presented lower amounts than Δ^5-sterols. Δ^7-Avenasterol was the main component of the Δ^7-sterols, which is in contrast with Gaydou (*39*). AraA contained the highest levels of the Δ^7-sterol 4-desmethylsterols relative to the other varieties. The distribution of Δ^7-sterols presents a pattern similar to the profile of Δ^5-sterol. In general, however, our results are similar to those reported in the literature (*3*).

During development, peanut moderates the biosynthesis of Δ^5-sterols and Δ^7-sterols to serve the peanut need for these sterols in order to regulate the growth and development of new tissues (*40*). During the last DAFs, the levels of Δ^5-sterols and Δ^7-sterols decreased, which indicates the end of their biosynthesis. In fact, during the end of peanut maturity, the phytosterols were not de novo synthesized but, instead, they are the result of the conversion of existing phytosterols to other sterol forms (stanols and steryl esters) (*41*).

To sum up, the highest amount of total 4-desmethylsterols was observed at 12 DAF and exclusively in wild immature varieties (AraA). As a result, immature peanut kernels could become naturally enriched functional foods for the industry and a novel ingredient to reduce the levels of "bad" serum low-density lipoprotein cholesterol for consumers. Thus, a functional peanut oil could be best produced from the appropriate selection of wild peanut oil such as AraA. Therefore, crop improvement is possible by using these wild species further. Finally, this chemical composition data of peanut can also contribute to the characterization of germplasm bank materials.

ABBREVIATIONS USED

AraA, *Arachis* Arbi; AraC, *Arachis* Chounfakhi; AraT, *Arachis* Trabelsia; DAF, days after flowering; DEMS, 4-desmethylsterols; GC-FID, gas chromatography−flame ionization detection; GC-MS, gas chromatography−mass spectrometry; MW, molecular weight; ppm, parts per million; TLC, thin-layer chromatography.

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