

## Comparison of the Concentrations of Long-Chain Alcohols (Policosanols) in Three Tunisian Peanut Varieties (*Arachis hypogaea* L.)

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Policosanols (PC) is a mixture of high molecular weight aliphatic primary alcohols. Literature about the contents and compositions of PC derived from peanut varieties is scarce. Total PC composition and content in whole peanut grain samples from three varieties of peanut (two cultivars, AraC and AraT, and a wild one, AraA) were identified using a gas chromatograph system coupled with a mass spectrophotometer. The results show that, qualitatively, 21 components of peanut aliphatic alcohols were identified (C<sub>14</sub>–C<sub>30</sub>). Besides (C<sub>18=</sub>), the results exhibited a previously unreported mixture of PC compositions in the peanuts: the unsaturated PC (UPC), which are (C<sub>20=</sub>), (C<sub>21=</sub>), (C<sub>22=</sub>), and (C<sub>24=</sub>). The main components of total PC in Tunisian peanut kernels are docosanol (C<sub>22</sub>), (Z)-octadec-9-en-1-ol (C<sub>18=</sub>), hexadecanol (C<sub>16</sub>), and octadecanol (C<sub>18</sub>). Quantitatively, the total PC content of the whole peanut samples varied from 11.18 to 54.19 mg/100 g of oil and was higher than those of beeswax and whole sugar cane, which are sources of dietary supplements containing policosanols.

**KEYWORDS:** Wild cultivars; peanuts; policosanols; GC-MS; development

### INTRODUCTION

Peanut (*Arachis hypogaea* L.), an economically important crop throughout the world, is also known to be a valuable source of phenolic antioxidant compounds (1). However, scientific literature about aliphatic alcohols, including the policosanols (PC) group in peanut varieties, is scarce. PC is the trivial name of a mixture of high molecular weight (HMW) aliphatic primary alcohols (C<sub>20</sub>–C<sub>36</sub>), originally isolated from sugar cane (*Saccharum officinarum* L.) (2). It is also extracted from a diversity of other natural sources such as beeswax, rice bran, and wheat germ (3). These plants represent good sources of PC with its health-enhancing components. In fact, PC is present in the fruits, leaves, and surfaces of plants and whole seeds (4, 5). The major components of the PC mixture are octacosanol (C<sub>28</sub>, 60–70%, w/w), triacosanol (C<sub>23</sub>, 10–15%, w/w), and hexacosanol (C<sub>26</sub>, 4–10%, w/w). A PC supplement has been approved as a cholesterol-lowering drug in over 25 countries (6). Several studies showed that PC supplements are potent antioxidants that promote proper arterial endothelial cell function and inhibit platelet aggregation and thrombosis (6). They serve also as an effective treatment for intermittent claudication (7). However, Francini et al. (8) have reported that the mechanisms by which PC improves plasma lipid profile are unclear, and there is a continuing

debate about the exact effect of PC. In fact, some of the previous works indicated that PC is unhelpful in reducing blood cholesterol (9–12). Nevertheless, PC was successful worldwide, and it is sold as a lipid-lowering supplement in more than 40 countries (8). Finally, a consensus is built that PC is a health-enhancing compound, and nowadays the industry has focused its attention on plant matrices rich in PC for functional foods and nutraceuticals applications.

The objective of this study is to investigate biologically active and health-beneficial peanut PC components and the effect of Tunisian peanut varieties in terms of compositions and contents on these PC components.

### MATERIALS AND METHODS

**Plant Materials.** Three varieties of local peanuts were collected: one wild variety, Arbi (AraA), and two cultivated varieties, Trabelsia (AraT) and Chounfakhi (AraC). They were grown on a private farm at Dar-Allouch in northeastern Tunisia, in the same eco-environmental conditions. Kernels were hand-picked starting mid-May until the end of October 2008. Samples were collected at different intervals after the date of podding (DAP) until maturity.

**Reagents and Standard.** Methanol and *n*-hexane solvents for HPLC grade have been purchased from Panreac Quimica SA (Barcelona, Spain). Chloroform and petroleum ethers are from Fisher Scientific SA (Loughborough, U.K.). Ethanol was purchased from Scientific Limited (Northampton, U.K.). Aliphatic alcohol internal standard was acquired

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from Sigma-Aldrich (Madrid, Spain). TLC silica plates (silica gel 60G F254, 20 × 20 cm, and 0.25 mm thickness), potassium hydroxide pellets (KOH), and anhydrous sodium sulfate (Na<sub>2</sub>SO<sub>4</sub>) were obtained from Merck (Darmstadt, Germany).

**Lipid Extraction.** Plant oil was extracted from dry material of peanuts with petroleum ether using a Soxhlet apparatus. This extraction took 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 was determined by measuring the relative weight of extracted oil from the dried peanuts (13).

**Saponification of the Lipids (ISO 11294, 1994).** Unsaponifiable lipids were determined by saponifying 5 g of oil mixed with both 200 μL of 5α-cholesterol 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<sub>2</sub>O 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<sub>2</sub>O (1:1) (v/v).

The unsaponifiable fraction was collected from the ether fraction and dried under anhydrous Na<sub>2</sub>SO<sub>4</sub> to eliminate residual water. Na<sub>2</sub>SO<sub>4</sub> 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 thin-layer chromatography (TLC) analysis.

**Aliphatic Alcohol Fractionation by Preparative TLC.** The unsaponifiable matter was separated into subfractions on preparative silica gel thin-layer plates, using one-dimensional TLC with hexane/Et<sub>2</sub>O (65:35 by volume) as developing solvent. The unsaponifiable fraction (5% in CHCl<sub>3</sub>) was applied on the silica gel plates in 3 cm bands. To correctly identify the aliphatic alcohols bands, a reference sample of 1-icosanol was applied to the left side of the TLC plates. After development, the plates were sprayed with 2',7'-dichlorofluorescein and viewed under UV light. On the basis of the reference spots, the band corresponding to aliphatic alcohols was scraped off separately and extracted three times with CHCl<sub>3</sub>/Et<sub>2</sub>O (1:1), filtered to remove the residual silica, dried in a rotary evaporator, and stored at -20 °C for GC-FID and then GC-MS analysis.

**Silylation of Sterol Fraction.** An amount of 2 mg of aliphatic alcohols residue was mixed with 80 μL of *N,O*-bis(trimethylsilyl) trifluoroacetamide (BSTFA; Acrös Organics BVBA98%) 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. One microliter of the solution was then directly injected to the gas chromatograph.

**GC-FID.** We employed GC-FID method (14) to determine the policosanol composition. The aliphatic alcohol fraction was silylated to form the trimethylsilyl ether and injected into a GC (Hewlett-Packard 5890 A series II GC), which was equipped with a MEGASE 54 column (Precis HB-sterol; 30 m × 0.22 mm i.d. and 0.22 μm film thickness).

The initial column temperature was set to 150 °C, programmed to increase at the rate of 8 °C/min until 310 °C, and then held constant for 10 min. The injector and detector temperatures were set to 320 °C. Helium was used as carrier gas given a column flow of 1 mL/min.

The level of each aliphatic alcohol was calculated using the formula

$$\text{amount (mg/100 g of oil)} = \frac{P_{A.I.S.} m_{I.S.}}{P_{A.I.S.} m_S} \times 100$$

where  $P_{A.S}$  = the aliphatic alcohol peak area,  $P_{A.I.S.}$  = the internal standard peak area,  $m_{I.S.}$  = the weight (mg) of the internal standard, and  $m_S$  = the weight (mg) of oil taken for analysis (15–17).

**GC-MS Analysis (14).** We employed the GC-MS method to determine policosanol contents as described by Irmak (3). The aliphatic alcohol fraction was silylated to form trimethylsilyl ether, which was injected into the GC (Hewlett-Packard 5890-A series II) 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 under the same conditions reported for the GC-FID analyses. Manual injection of 1 μL of aliphatic alcohols solution was performed in splitless mode.

**Chemicals.** With extreme care and informed judgment, the PC compositions of the samples were identified by direct comparison of their chromatographic retention indices and mass spectra with those of the

reference compounds from the NIST 05 Chemstation Library, which is available from ref 18.

**Statistical Analysis.** The differences between means and medians of alcohol composition and its components were tested using Student (*t*) and Wilcoxon tests. All of the statistical analysis was performed with R software for statistical computing version 2.10 (19). Tests were deemed to be significant when  $p < 0.05$ .

## RESULTS AND DISCUSSION

**PC Content and Composition.** The total ion chromatogram of the aliphatic alcohols fraction is shown in **Figure 1**. Twenty-one aliphatic alcohols were identified in the peanut kernels, and they ranged from C<sub>14</sub> to C<sub>30</sub> (**Table 1**). In total, 5 unsaturated PCs (UPC) and 16 saturated PC (SPC) were identified. The target (M) and [M - 15]<sup>+</sup> used for the extraction of individual PC peaks are also shown in **Table 1**. Trimethylsilyl (TMS) ethers had characteristic mass spectra with fragment ions observed between [M - 15]<sup>+</sup> and  $m/z$  103 [(CH<sub>3</sub>)<sub>3</sub>SiOCH<sub>2</sub>]<sup>+</sup>.

The mass fractionation pattern of the PC displayed unusual forms of unsaturated policosanol (UPC) with their elementary components, which are eicosen-1-ol (C<sub>20=</sub>), heneicosen-1-ol (C<sub>21=</sub>), docosen-1-ol (C<sub>22=</sub>), and tetracosen-1-ol (C<sub>24=</sub>), in addition to (*Z*)-octadec-9-en-1-ol (C<sub>18=</sub>) (**Table 1**).

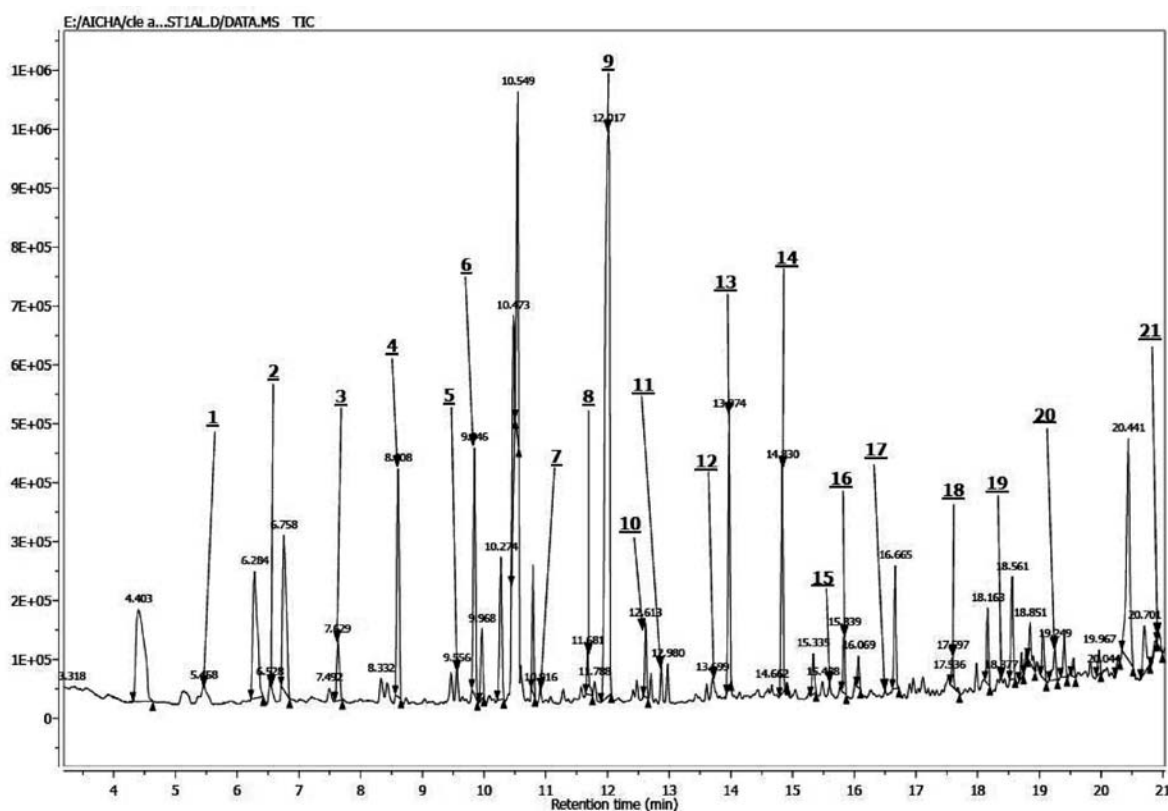
In fact, we have quantified only 13 unsaturated and saturated aliphatic alcohols. The remaining 8 components were too scant to be evaluated during all stages. The main constituents of the aliphatic alcohol fractions were the PC family members (C<sub>20</sub>–C<sub>36</sub>). The PC group accounts for >90% of the total of aliphatic alcohol content. Other aliphatic compounds, such as tetradecanol (C<sub>14</sub>), pentadecanol (C<sub>15</sub>), and hexadecanol (C<sub>16</sub>), represent <5% of the total aliphatic alcohol content.

In comparison to major sources of PC production cited in the literature such as sugar cane, in which the PC level has been found to be 27 mg/100 g of oil (3), wheat germ, in which it was found to be about 16.4 mg/100 g of oil (3), corn kernels, in which it was found to be about 1.52 mg/100 g of oil (20), and olive fruits, in which the total content of aliphatic and triterpenes alcohols does not exceed 1.33 mg/100 g total alcohols (21), total PC contents in peanuts are markedly higher (54.2 mg/100 g of oil in AraA) than those previously mentioned.

**Distribution of PC Component among Cultivars and Wild Species.** PC composition and content for the three varieties of peanut are given in **Table 2**.

The PC content and composition in peanut kernels vary significantly among the three varieties and through time (DAP). The highest level of PC reached was about 54.2 mg/100 g of oil in AraA at 68 DAP, which was higher than those of cultivars AraC (38.85 mg/100 g of oil) at 48 DAP and AraT (11.18 mg/100 g of oil) at 12 DAP (**Table 2**). These differences in the accumulation of PC content may be related to genetic differences among the three varieties (22–24). In addition, these results indicate that AraA peanut contains an interesting quantity of policosanol and represents a better source of PC than the cultivated ones. Thus, these three varieties of peanut can be considered as potential sources of health-enhancing compounds for functional food and nutraceutical applications comparable to the widely known natural sources of PC.

In AraA, (*Z*)-octadec-9-en-1-ol was the major aliphatic alcohol of UPC and reached its maximum (45.64 mg/100 g of oil) at 68 DAP. However, it is in lower amount and does not exceed 1.83 mg/100 g of oil in AraT and 9.11 mg/100 g of oil in AraC (**Table 2**). In fact, it was shown that there is a significant difference ( $p = 0.04772$ ) between AraA and AraC in term of (*Z*)-octadec-9-en-1-ol content. A similar result was obtained for AraA and AraT with a  $p = 0.04796$ . In contrast, between the two cultivars,



**Figure 1.** GC-MS total ion chromatogram of the aliphatic alcohol fraction of AraA (Arbi). (For peak assignments, see **Table 1**.)

**Table 1.** Retention Indices and Mass Spectrometric Data for TMS Derivatives of Aliphatic Alcohols Identified by GC-MS in Wild Variety at 12 DAP

peak <sup>a</sup>	retention index (min)	M <sup>b</sup>	[M - 15] <sup>+</sup> (m/z)	alcohol <sup>c</sup>
1	5.46	286	271	tetradecan-1-ol
2	6.56	300	285	pentadecan-1-ol
3	3.63	314	299	hexadecan-1-ol
4	8.61	328	313	heptadecan-1-ol
5	9.56	340	325	(Z)-octadec-9-en-1-ol
6	9.85	342	327	octadecan-1-ol
7	10.91	356	341	nonadecan-1-ol
8	11.68	368	353	eicosen-1-ol
9	12.02	370	355	eicosan-1-ol
10	12.61	382	367	heneicosen-1-ol
11	12.87	384	369	heneicosan-1-ol
12	13.70	396	381	docosen-1-ol
13	13.96	398	383	docosan-1-ol
14	14.83	412	397	tricosan-1-ol
15	15.61	424	409	tetracosen-1-ol
16	15.84	426	411	tetracosan-1-ol
17	16.89	440	425	pentacosan-1-ol
18	17.60	454	439	hexacosan-1-ol
19	18.36	468	453	heptacosan-1-ol
20	19.25	482	467	octacosan-1-ol
21	20.94	510	495	triacontan-1-ol

<sup>a</sup> See **Figure 1**. <sup>b</sup> Mass of the trimethylsilylated alcohol. In all cases the mass spectrum exhibited a major peak due to CH<sub>3</sub> loss, [M - 15], and a peak characteristic of the trimethylsilyl group on a terminal ether site, m/z 103 [(CH<sub>3</sub>)<sub>3</sub>SiOCH<sub>2</sub>]<sup>+</sup>. <sup>c</sup> Compounds tentatively identified.

AraC and AraT, there was no significant difference. Weber (25) has reported that under aerobic conditions, large proportions of the alcohol (Z)-octadec-9-en-1-ol (C<sub>18=</sub>) are oxidized to oleic acid and incorporated predominantly into phospholipids. Indeed, AraA was richer in (Z)-octadec-9-en-1-ol (C<sub>18=</sub>) than AraC and AraT during maturation.

**Table 2.** Changes in Total Aliphatic Alcohols Contents Expressed in Milligrams per 100 g of Oil during Development of Tunisian *Arachis hypogaea* L. Species<sup>a</sup>

AraA	IS (12 DAP)	MIS (68 DAP)	MS (96 DAP)
AA	7.54	56.15 <sup>s</sup>	43.84 <sup>s</sup>
PC	6.70	54.19 <sup>s</sup>	36.35 <sup>s</sup>
SPC	5.68	3.80	9.89 <sup>s</sup>
UPC	1.02	46.59 <sup>s</sup>	26.46 <sup>s</sup>
AraC	IS (12 DAP)	MIS (48 DAP)	MS (96 DAP)
AA	9.17 <sup>s</sup>	39.53 <sup>s</sup>	12.49 <sup>s</sup>
PC	7.27	38.85 <sup>s</sup>	11.76 <sup>s</sup>
SPC	6.90	6.68 <sup>s</sup>	8.85 <sup>s</sup>
UPC	0.37	32.17 <sup>s</sup>	2.91
AraT	IS (12 DAP)	MIS (68 DAP)	MS (96 DAP)
AA	14.64 <sup>s</sup>	6.01	8.55
PC	11.18 <sup>s</sup>	4.94	7.35
SPC	9.89 <sup>s</sup>	3.30	3.07
UPC	1.29 <sup>s</sup>	1.64	4.28 <sup>s</sup>

<sup>a</sup> Abbreviations: AraA, Arbi; AraC, Chounfakhi; AraT, Trabelsia; DAP, days after podding; AA, aliphatic alcohols; PC, policosanol; SPC, saturated policosanol; UPC, unsaturated policosanol; IS, immature stage; MIS, middle stage; M, maturity. Within a column, <sup>s</sup> denotes a significant difference among peanut kernels, at  $p = 0.05$ .

Interestingly, cultivars AraC and AraT showed a high specificity for the synthesis of docosanol (C<sub>22</sub>). AraC presented the highest amount of docosanol (C<sub>22</sub>) (6.67 mg/100 g of oil). This may be due to the difference previously mentioned in the genetic background between the cultivated peanut (*A. hypogaea* L.) and those of wild species of *Arachis* (22–24). In addition, differences in the activities of oil-synthesizing enzymes have been shown among the three varieties. This enzymatic complex in AraA is actually more active than those in cultivated species (24).

**Table 3.** Content and Composition of Aliphatic Alcohols (Milligrams per 100 g of Oil) in Peanut Kernels<sup>a</sup>

AA	IS (12 DAP)			MIS			M (96 DAP)		
	AraA	AraC	AraT	AraA	AraC	AraT	AraA	AraC	AraT
C16	0.84	1.90	3.46 <sup>s</sup>	5.76 <sup>s</sup>	0.68	1.07	7.49 <sup>s</sup>	0.73	1.20
C17	0.03 <sup>s</sup>	nd	nd	nd	nd	nd	nd	nd	nd
C18=	0.23 <sup>s</sup>	nd	1.04 <sup>s</sup>	45.64 <sup>s</sup>	0.08	0.41 <sup>s</sup>	23.70 <sup>s</sup>	2.40	1.83 <sup>s</sup>
C18	2.46 <sup>s</sup>	3.17	3.90	1.10 <sup>s</sup>	1.01	0.81	2.60 <sup>s</sup>	1.40	1.23
C20=	0.50 <sup>s</sup>	0.16	0.16	0.35	14.30 <sup>s</sup>	1.11	1.27	0.23 <sup>s</sup>	1.90
C21	0.26 <sup>s</sup>	0.09	0.12	0.13	0.12	0.10	0.09	0.11	0.09
C22=	0.23 <sup>s</sup>	0.11	nd	0.36	14.73 <sup>s</sup>	nd	1.20 <sup>s</sup>	0.14	0.35
C22	1.16	3.35	5.50 <sup>s</sup>	2.50	5.34 <sup>s</sup>	1.76	6.70	6.70	1.60 <sup>s</sup>
C23	0.11 <sup>s</sup>	nd	nd	nd	nd	nd	0.35 <sup>s</sup>	nd	nd
C24=	0.06 <sup>s</sup>	0.10	0.09	0.24	3.06 <sup>s</sup>	0.12	0.29	0.14 <sup>s</sup>	0.20
C24	0.56 <sup>s</sup>	0.20	0.23	0.07	0.16 <sup>s</sup>	nd	0.15 <sup>s</sup>	0.07	0.04
C26	0.50 <sup>s</sup>	nd	nd	nd	nd	0.60 <sup>s</sup>	nd	0.37 <sup>s</sup>	0.11
C28	0.20	0.09 <sup>s</sup>	0.14	nd	0.05 <sup>s</sup>	nd	nd	0.20 <sup>s</sup>	nd
C30	0.40 <sup>s</sup>	nd	nd	nd	nd	0.03 <sup>s</sup>	nd	nd	nd

<sup>a</sup> Abbreviations: AraA, Arbi; AraC, Chounfakhi; AraT, Trabelsia; DAP, days after podding; IS, immature stage (12 DAP); MIS, middle stage (AraA and AraT, 68 DAP; AraC, 48 DAP); M, maturity at 96 DAP; =, unsaturation; nd, not detected. Within a column, <sup>s</sup> denotes a significant difference among peanut kernels, at  $p = 0.05$ .

The PC's component (C<sub>16</sub> and C<sub>22</sub>) in AraA, C<sub>20=</sub> and C<sub>22=</sub> in AraC, and C<sub>22=</sub> in AraT were the other PC components present in significant quantities in peanut kernels. Their levels varied among species, with AraA containing the highest amount of C<sub>16</sub> (23%) and the lowest amount of C<sub>22=</sub> (9.1%), whereas AraC contained the highest level of C<sub>22=</sub> (37.26%) and AraT the lowest level of C<sub>20=</sub> (22.21%). These results are in agreement with a previous study reporting that wheat varieties grown under identical conditions and management differ significantly in PC content and composition (26). For C<sub>23</sub>–C<sub>30</sub>, the levels of PC components were nearly identical for the three varieties of peanut and < 5% of the total content of the aliphatic alcohols. Generally, even-number-indexed PC components such as docosanol (C<sub>22</sub>), (Z)-octadec-9-en-1-ol (C<sub>18=</sub>), hexadecanol (C<sub>16</sub>), and octadecanol (C<sub>18</sub>) are present in higher amount than the odd-number-indexed ones in our peanut kernels, which contributes to the abundant beneficial effect of peanut product (27).

A comparison of our results to major sources of PC is important for the evaluation of the peanut potential as an alternative PC source. Our PC content and composition are markedly different from those of sugar cane, which contains mostly octacosanol (81%), followed by hexacosanol (28) and beeswax, in which the major PC components are triacontanol (36.9%), dotriacontanol (20.8%), octacosanol (18.3%), and hexacosanol (13.9%). In wheat germ, the major PCs were tetracosanol, followed by hexacosanol and octacosanol (3), and in Adlay grain, octacosanol is the main component, followed by hexacosanol and docosanol (C<sub>22</sub>) (29). Also, corn kernels contained dotriacontanol (C<sub>32</sub>) as the main component in all samples (30–35%), followed by triacontanol (C<sub>30</sub>) and tetracosanol (C<sub>24</sub>) (20). In fact, several studies have reported the beneficial health effects of octacosanol, and it was reported that it increases the lipid catabolism to generate more energy for endurance enhancement (30). The UPC components identified are little known, and no information is available in the literature about their potential therapeutic effect.

**Changes in PC Content and Composition during the Development of Peanut Kernels.** The distributions of PC compounds in the three varieties among the different stages of maturity are markedly dissimilar (Table 3). Gülz (31) reported that the level of primary aliphatic alcohols changes during leaf development. Figure 2A and Table 2 show that in the early stages of development, the PC content increases to 6.70 mg/100 g of oil in AraA and increases to 7.27 mg/100 g of oil in AraC, starting from their lowest levels. In immature AraT, the PC content started from its

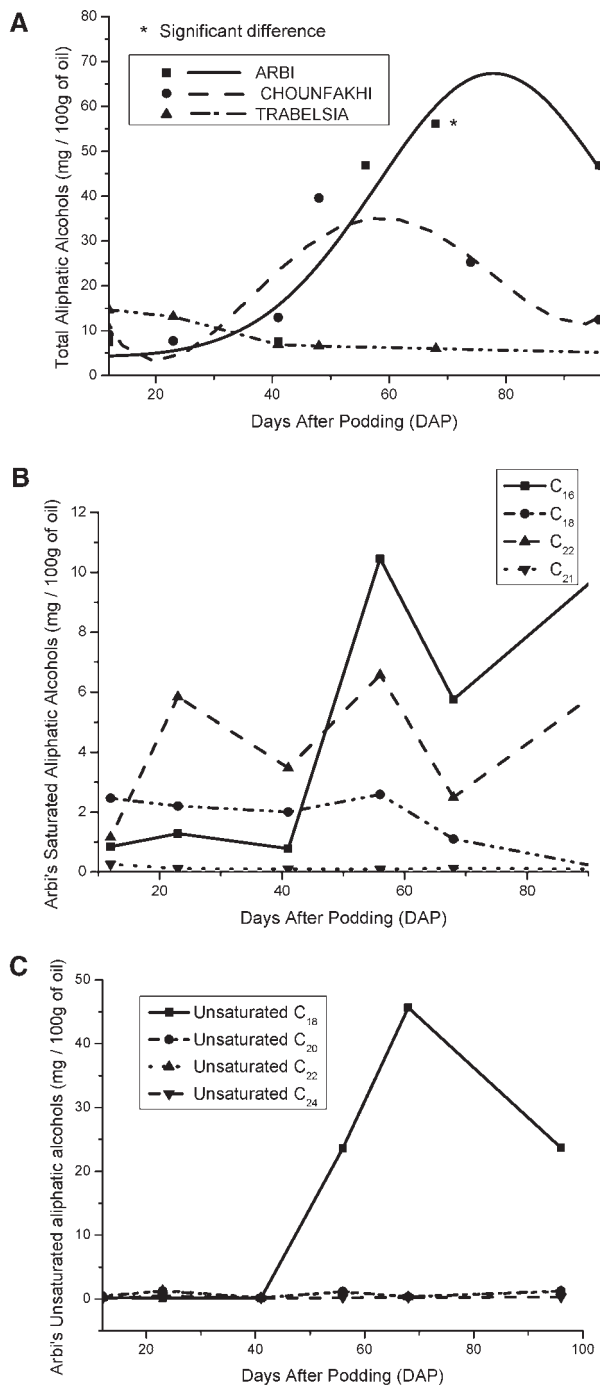
maximum level (11.18 mg/100 g of oil) and decreased slowly during the first period of maturity. The low level of PC content noted in immature kernels of AraA and AraC may be explained by the fact that AraA had the highest oil content (29.50 ± 1.50%) compared to AraC (20.50 ± 1.50%) and AraT (20.10 ± 1.00%) (25). In this immature stage, oil content might have caused a slight dilution of the PC in the wild AraA compared with AraC than AraT (3, 24).

The biggest change of PC content occurred at 41 DAP for AraC and at 56 DAP for AraA. From this time point, there was a dramatic increase in the total amount of PC until 48 DAP for AraC (38.85 mg/100 g of oil) and in the case of AraA (54.19 mg/100 g of oil) at 68 DAP. These date points are important indicators of the best time to exploit maximally these beneficial health components. Perhaps, in this date points, there is a drop in the synthesis of oil in the three varieties, especially AraA and AraT. Thus, the production of PC is favorably activated. From 68 DAP in AraA and from 74 DAP for AraC until maturity, there is a decrease in the content of PC. At maturity, AraA (36.35 mg/100 g of oil) had a significantly higher amount ( $p < 0.05$ ) of PC than AraC (11.76 mg/100 g of oil) and AraT (7.35 mg/100 g of oil). In maturity, the drop of PC content may be also due to their conversion to others metabolites such as fatty acids (30). Weber (32) reported that the percentage of oleic acid increased in matured corn kernels.

The pattern of accumulation of different SPC and UPC components in peanut species varied significantly during maturation (Figure 2B,C). The overall mean production of total SPC through the developmental stages is not significantly different among the three varieties.

In the case of UPC, we noted that AraA produced through the development stage a significantly higher ( $p = 0.04375$ ) amount compared to the cultivars ones. In fact, from 12 to 41 DAP, docosanol and octadecanol were the most abundant components of UPCs in AraA, whereas from 56 DAP until maturity (Z)-octadec-9-en-1-ol and hexadecanol were the main ones, followed by docosanol and octadecanol.

From 12 to 68 DAP, docosanol and octadecanol were the main components of PC in AraC and AraT and showed similar metabolic profiles. In contrast, from 68 DAP until maturity stage, AraC had docosanol and (Z)-octadec-9-en-1-ol (C<sub>18=</sub>) as the major PC components, whereas AraT had octacosanol and hexacosanol as the most predominant PCs. The highest level of docosanol was detected at 56 DAP for AraA (6.67 mg/100 g of oil), whereas in AraC the highest level (9.96 mg/100 g of oil) was



**Figure 2.** (A) Change in total aliphatic alcohols (mg/100 g of oil) during maturation of three varieties of peanut: Arbi (●); Trabelsia (▲); Chounfakhi (■). (B) Change in saturated policosanol compounds (SPC) content (mg/100 g of oil) during peanut kernel maturation (Arbi variety): C<sub>16</sub> (■); C<sub>18</sub> (◆); C<sub>22</sub> (▲); C<sub>21</sub> (▼). (C) Change in unsaturated policosanol compounds (UPC) content (mg/100 g of oil) during peanut kernel maturation (Arbi variety): C<sub>18</sub>= (▲), C<sub>20</sub>= (●); C<sub>22</sub>= (▲); C<sub>24</sub>= (▼).

detected at 41 DAP and in AraT it was reached at 23 DAP (5.87 mg/100 g of oil). Marcelletti (33) reported that docosanol inhibits replication of herpes viruses in vitro and in vivo. This component was generally the most abundant PC component during the maturity of peanut kernels. Among the different stages of peanut kernel developmental, octadecanol level varied significantly between AraA and AraC ( $p = 0.03524$ ) and between the two cultivars AraC and AraT ( $p = 0.0462$ ). The quantities of the other individual PC components such as octacosanol in peanut

kernel were minor and become unquantifiable at maturity. This can be explained by the fact that they contribute to the biosynthesis of fatty acids. Actually, it has been reported that octacosanol might be oxidized and degraded to fatty acids via  $\beta$ -oxidation in mammals (34).

This study is the first step that provides information on health-beneficial phytochemicals PC content and composition in Tunisian peanut kernels. The presence of UPC was not reported previously. Even-number-indexed policosanols, docosanol (C<sub>22</sub>), (Z)-octadec-9-en-1-ol (C<sub>18</sub>=), hexadecanol (C<sub>16</sub>), and octadecanol (C<sub>18</sub>), are present in higher amount than the odd-number-indexed ones, which increases the beneficial effect of peanut product (27). Wild Arbi presents a significantly higher amount of total PC than the cultivated species. The PC content in Tunisian peanut is markedly higher than in beeswax and whole sugar cane and can represent a valuable source of these health-enhancing compounds. The time points that indicated the maximum accumulation of PC in the three varieties represent the best times to take advantage of these health-beneficial components. This information could be successfully used in peanut breeding situations to provide a good source of natural bioactive compounds that can be incorporated into functional foods and nutraceuticals.

#### ABBREVIATIONS USED

AA, aliphatic alcohols; AraA, *Arachis* Arbi; AraC, *Arachis* Chounfakhi; AraT, *Arachis* Trabelsia; DAP, days after podding; GC-FID, gas chromatography–flame ionization detector; GC-MS, gas chromatography–mass spectrometry; HMW, high molecular weight; IS, immature stage; M, maturity; MIS, middle stage; MW, molecular weight; PC, policosanol; SPC, saturated policosanol; TMS, trimethylsilyl; UPC, unsaturated policosanol.

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