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Policosanol distribution and accumulation in developing corn kernels

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ABSTRACT

Policosanol is a mixture of long-chain primary aliphatic alcohols. The policosanol composition of whole corn kernel was determined in three varieties of corn (Astro, GH2547, Local). The total policosanol content of GH2547 (20.5 mg/kg of dry weight) was higher than those of Local (16.6 mg/kg) and Astro (15.2 mg/kg). The major policosanol components of whole corn kernel were dotriacontanol, triacontanol and tetracosanol. The distribution of policosanol in the germ, endosperm and pericarp of corn kernels was also determined. Corn pericarp had higher contents of policosanols (72.7–110.9 mg/kg) than the endosperm (4.0–16.2 mg/kg) and germ (19.3–37.1 mg/kg) fractions. Corn pericarp policosanol was mainly triacontanol (33.63–46.29 mg/kg), dotriacontanol (22.31–39.46 mg/kg) and octacosanol (8.13–14.0 mg/kg). In contrast, the corn germ fraction contained mostly dotriacontanol (more than 50%) and no triacontanol. The main components of corn endosperm policosanol were triacontanol and hexacosanol. The level of tetracosanol was highest in the germ fraction and lowest in the endosperm fraction. The greatest change in policosanol content (expressed as mg/100 g of oil) occurred during the early stages of corn kernel development. Although the structures of the alcohol constituents of policosanol are similar, their patterns of accumulation were different. The highest levels of octacosanol and dotriacontanol were detected at 20 days after pollination (DAP) followed by a rapid decrease between 20 and 30 DAP. Triacontanol levels decreased rapidly between 10 and 40 DAP, and then remained nearly constant.

1. Introduction

There has been significant recent interest in long-chain aliphatic alcohols as dietary supplements or nutraceuticals in the treatment of various chronic diseases including diabetes and hypercholesterolemia (Jackson & Eller, 2006).

Policosanol is the common name that refers to a mixture of long-chain (C20 to C36) aliphatic primary alcohols containing mainly docosanol, tetracosanol, hexacosanol, octacosanol and triacontanol (Irmak, Dunford, & Milligan, 2006). It is relevance human health stems from a demonstrated effectiveness in the prevention and treatment of cardiovascular disease (Varady, Wang, & Jones, 2003). Policosanol can reduce cholesterol levels by inhibiting cholesterol biosynthesis and enhancing low-density lipoprotein decatabolism (Menéndez et al., 2001). Also, it has been shown to decrease endothelial damage, platelet aggregation and the development of foam cells (Arruzazabala, Carbajal, Garcia, & Fraga, 1993; Carbajal, Arruzazabala, Valdes, & Mas, 1998).

Several studies have reported the beneficial health effects of octacosanol which is one of the policosanol family members (Taylor, Rapport, & Lockwood, 2003). Dietary octacosanol was thought to increase lipid catabolism to generate more energy for enhancement of motor endurance (Xu, Fitz, Riediger, & Moghadasian, 2007). The contribution of triacontanol in changing the chemical composition and physical status of membrane lipids has also been studied (Shripathi & Swamy, 1994; Shripathi, Swamy, & Chandrasekhar, 1997). An anti-inflammatory effect of triacontanol has been demonstrated in animals and it has been shown that this compound reduces thymus weight, number of thymus cells, number of splenocytes and amount of interleukin-1 (Warren, Burger, Sidwell, & Clark, 1992). Currently, a number of dietary supplements containing policosanol are commercially available in the US market (Irmak et al., 2006).

Policosanol was originally isolated from sugar cane wax and is also found in a number of other natural substances such as beeswax, rice bran, and wheat germ (Lin et al., 2004). These long-chain primary alcohols are present in fruit, leaves and surfaces of plants, and whole seeds (Irmak et al., 2006). Growth promoting effects of triacontanol have been observed in various plants and it has been shown that triacontanol increases dry weight, carbon dioxide fixation, reducing sugars, soluble proteins and free amino acids leading to the enhancement of plant growth and crop yield (Ramanarayan, Bhat, Shripathi, Swamy, & Rao, 2000).

Although the policosanol composition of wheat has been studied extensively (Irmak & Dunford, 2005), information on policosanol content of maize is limited, particularly concerning their metabolism (Avato, Bianchi, & Murelli, 1990). The objective of this

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study was to examine the policosanol composition of maturing grains of three varieties of corn and the distribution of those compounds among kernel fractions (endosperm, pericarp and germ). Information from this study could serve for the understanding of policosanol metabolism in plants.

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 Scientific Limited (Northampton, UK). Sterol standards were acquired from Sigma Aldrich (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) and *GH2547* (sweet maize) were obtained from Spain and U.S.A., respectively; while *Local* (Flint maize) was from I.N.R.A.T., Tunisia. The three varieties of maize (Zea mays L.), were grown in restricted zones (15 × 3 m) on the Agronomy farm of the INRAT (Institute National Recherche Agronomie Tunis, North of Tunisia) from the middle of April until the end of August 2005. Samples were collected at intervals after the date of hand-pollination. At maturity, corn kernels were steeped for 3 h in 0.1% sodium metabisulfite solution at 50 °C before dissection.

The seeds of dent maize (*Zea mays* indentata) contain soft starch grains which shrink when ripe and are dried to give the characteristic "dent" in the crown. Sweet maize *(Zea mays sacchrata)* differs from dent maize by a single recessive gene which prevents some of the sugars being converted to starch. The seeds of flint maize *(Zea mays indurata)* are small, smooth and hard, containing little soft starch.

2.3. Oil extraction

Oil was extracted by the method of Folch, Lees, and Sloane (1957) modified by Bligh and Dyer (1959). Seeds 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 oil extract with 50 mL of 12% ethanolic KOH (w/v) and heating at 60 °C for 1.30 h. After cooling, 50 mL of water was added and the unsaponifiable matter was extracted four times with 50 mL of petroleum ether. The combined ether extract was washed with 50 mL of EtOH-H_2O (1:1). The extracted ether was dried over anhydrous Na_2SO_4 and evaporated to dryness using N_2. The dry residues were dissolved in $CHCl_3$ for TLC analysis.

2.5. Thin-layer chromatography

TLC of the unsaponifiable fraction: unsaponifiable matter was separated into subfractions on preparative silica gel thin-layer plates (silica gel 60G F254) using one-dimensional TLC with hexane-ethyl ether (65/35 by volume) as the developing solvent. The unsaponifiable fraction (5% in $CHCl_3$) was applied on the silica gel plates in 3-cm bands. To correctly identify the aliphatic alcohol bands, a reference sample of 1-eicosanol was applied to 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 band corresponding to aliphatic alcohols was scraped off separately and 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

We employed a GC–MS method to determine policosanol content similar to those described previously by others (Irmak et al., 2006). The aliphatic alcohol fraction was silylated to form the trimethylsilylethers and injected into a GC (Hewlett Packard 6890) coupled to a mass selective detector set to scan from m/z 50 to m/z 550. The system was fitted with a capillary HP-5 column (5% Phenyl Methyl Siloxane; 30 × 0.25 mm, 0.25 μm film thickness) and helium was used as the carrier gas at 1 mL/min. The oven temperature was programmed from 150 °C to 320 °C at 10 °C/min. Manual injection of 1 μl of the solution of alcohols was performed in the split mode at a 1:50 split ratio.

Hexacosanol was identified by retention time (using a hexacosanol standard) and from its mass spectrum. The other compounds were identified by comparing to reference mass spectra. Terminal TMS ethers have characteristic mass spectra with few or no fragment ions observed between $[M\text{-}15]^+$ and m/z 103, $[(CH_3)_3SiOCH_2]^+$. Secondary alcohols form TMS derivatives that fragment at the ether site. Compounds were quantified by directly comparing their respective total ion chromatogram peak areas with that of the internal standard 1-eicosanol (17.3 min in Fig. 1), which was absent in all oil samples. The GC–MS response factor of policosanol, calculated by using eicosanol, was 0.91 ± 0.03.

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. Policosanol content of whole kernels

The analysis of individual policosanol components required mass spectrometric for tentative identification of the chromatographic peaks (Irmak et al., 2006). The total ion chromatogram of the aliphatic alcohols fraction of a corn sample is shown in Fig. 1. The elution of the compounds from the capillary column was molecular weight dependent. The chromatogram for the sample displayed numerous peaks. Their mass spectra show a single intense peak corresponding to the ion fragment (M-15), formed by CH_3 loss from the trimethylsilyl group (Fig. 2). Nine aliphatic alcohols were detected in corn kernels and they range from C16 to C32. Table 1 lists the identified compounds, their retention times and characteristic ion fragments. The main constituents of the aliphatic alcohol fractions were the policosanol family members (C20-C36), accounting for over 90% of the total aliphatic alcohol content. Hexadecanol, Z-9-octadecenol and octadecanol amounted to less than 8% of the total aliphatic alcohol amount.

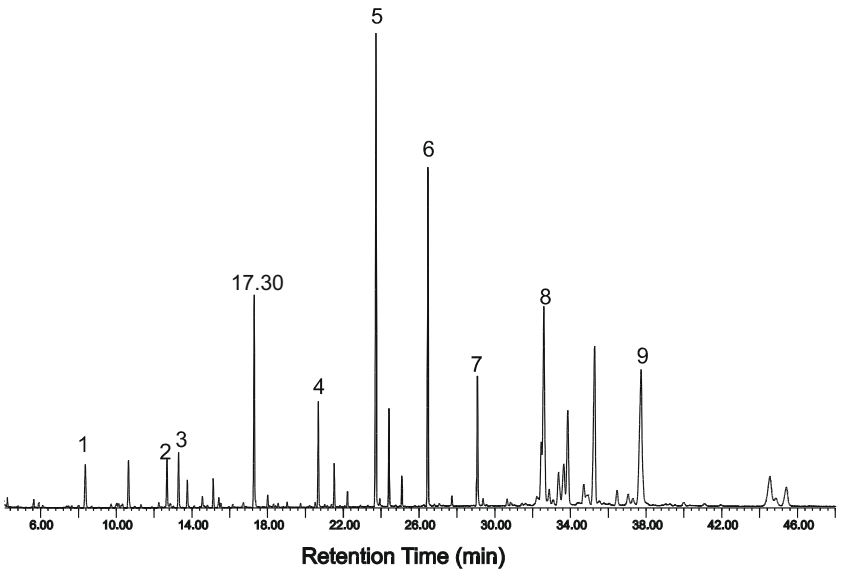

Fig. 1. The GC–MS total ion chromatogram of the aliphatic alcohol fraction of GH2547. For peak assignments, see Table 1. The peak at 17.30 min is for the 1-eicosanol internal standard.

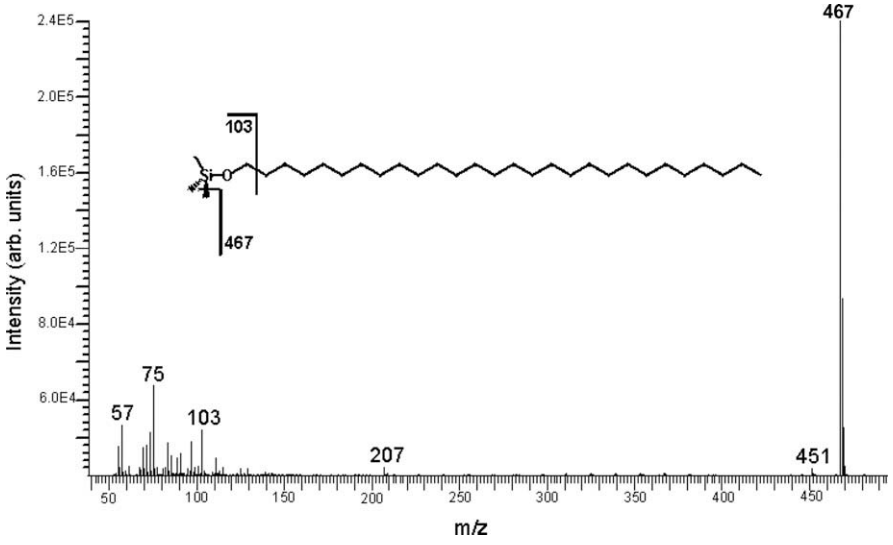

Fig. 2. EI mass spectrum (70 eV) of octacosanol (peak 7 in Fig. 1).

Table 1
m/z ratios for aliphatic alcohols identified in corn kernel.

Peak[a] N	Retention Time (min)	M[b]	$[M-15]^+$ (m/z)	Chemical formula	Alcohol
1	8.36	314	299	$C_{19}H_{42}OSi$	Hexadecan-1-ol
2	12.68	340	325	$C_{21}H_{44}OSi$	Z-9-octadecen-1-ol
3	13.30	342	327	$C_{21}H_{46}OSi$	Octadecan-1-ol
4	20.69	398	383	$C_{25}H_{54}OSi$	Docosan-1-ol
5	23.73	426	411	$C_{27}H_{58}OSi$	Tetracosan-1-ol
6	26.47	454	439	$C_{29}H_{62}OSi$	Hexacosan-1-ol
7	29.10	482	467	$C_{31}H_{66}OSi$	Octacosan-1-ol
8	32.62	510	495	$C_{33}H_{70}OSi$	Triacontan-1-ol
9	37.77	538	523	$C_{35}H_{74}OSi$	Dotriacontan-1-ol

[a] See Fig. 1.
[b] Mass in amu of the trimethylsilated alcohol. In all cases the mass spectrum exhibited a major peak due to CH_3 loss, [M-15], and a peak characteristic of the trimethylsilyl group on a terminal ether site, m/z 103 $[(CH_3)_3SiOCH_2]^+$.

Sugar cane is the major source for the production of commercial policosanol-enriched products. The policosanol levels of sugar cane peel, leaves and whole sugar cane have been found to be 270 mg/kg, 181 mg/kg and 17.4 mg/kg, respectively (Irmak et al., 2006). The content and composition of policosanol extracted from the three corn samples are listed in Table 2. Their total policosanol

content is similar to that of whole sugar cane, with that for *GH2547* (20.5 ± 1.2 mg/kg) being higher than that of *Local* (16.6 ± 1.4 mg/kg) and *Astro* (15.2 ± 1.2 mg/kg). This is an indication that kernel corn should be a good source of the long-chain alcohols.

Dotriacontanol was the most abundant policosanol present in whole corn kernels (30.1–35.5% of total policosanol). Corn seeds show high a specificity for the synthesis of dotriacontanol, though. Avato et al. (1990) assumed that dotriacontanol may be translocated to seeds during plant development.

Triacontanol and tetracosanol are the two other policosanols present in large quantities in the corn kernels. Their levels varied among samples with Astro having the highest and the lowest amounts of triacontanol (24.8%) and tetracosanol (15.2%), respectively. Results for Local were the opposite, with this variety exhibiting the highest level of tetracosanol (25.7%) and lowest level of triacontanol (17.7%) of the three corns. This result is in agreement with a previous study reporting that wheat varieties grown under identical conditions and management differ significantly in policosanol content and composition (Irmak & Dunford, 2005). Other data have indicated the importance of triacontanol as chemiotaxonomic markers for panicoid grasses (Avato et al., 1990). The levels of hexacosanol (11.3–12.7%) and octacosanol (6.7–7.6%) were nearly the same for the three varieties of corn.

The policosanol content and composition of corn kernel is markedly different from those of adlay grain, which contained mainly octacosanol, hexacosanol, tetracosanol and docosanol (Wu, Charles, & Huang, 2007) and sugar cane wax, which were mostly octacosanol (60–70%), followed by hexacosanol, triacontanol and dotriacontanol (Laguna et al., 1997). In wheat grain the most significant components of the policosanol fraction are tetracosanol, hexacosanol and octacosanol (Irmak & Dunford, 2005). The main components of beeswax policosanol are triacontanol (36.9%), dotriacontanol (20.8%), octacosanol (18.3%), hexacosanol (13.9%), and tetracosanol (9%) (Jackson & Eller, 2006).

3.2. Distribution of policosanol in the germ, endosperm and periacarp of corn kernels

The distribution of policosanol compounds in the principal parts of the caryopsis was also investigated. Table 3 shows the total policosanol content of the pericarp, endosperm and germ from dissected kernels. The highest policosanol levels are found in the pericarp (72.7 ± 2.6–110.9 ± 4.7 mg/kg), in agreement with literature data indicating that policosanol is a part of the protective and water proofing surface layer which acts as an interface between plant tissue and the growth environment (Irmak et al., 2006). The lowest level of policosanol (4.0 ± 0.7 mg/kg) was detected in Local corn endosperm. This study shows that corn germ contains more policosanol (19.3 ± 1.2–37.1 ± 3.4 mg/kg) than the wheat germ (10 mg/kg, (Irmak et al., 2006).

Triacontanol (33.63 ± 1.16–46.29.1 ± 1.1 mg/kg) was the most abundant policosanol present in corn pericarp, followed by dotriacontanol (22.31 ± 0.79–39.46 ± 1.72 mg/kg), and octacosanol (Table 3). In contrast, the corn germ fraction contained mostly dotriacontanol (more than 50%), and its composition is entirely different from wheat germ which contained mainly octacosanol, docosanol and triacontanol (Irmak et al., 2006). The main components of corn endosperm are triacontanol, dotriacontanol and hexacosanol. The level of tetracosanol is highest in the germ fraction (2.31 ± 0.26–2.56 ± 0.15 mg/kg) and lowest in the endosperm fraction (0.29 ± 0.05–1.36 ± 0.24 mg/kg). Docosanol was only detected in the germ and endosperm fractions, which likely explains the low levels detected in the whole corn kernel.

3.3. Changes in policosanol composition during maturation

Gülz, Müller, and Prasad (1991) reported that the amount of primary alcohols change during leaf development. The change in policosanol composition during corn kernel development has

Table 2
Policosanol[a] contents[b] (mg/kg of dry weight) of three samples of corn kernels.

Sample	C22	C24	C26	C28	C30	C32	Total PC (mg/kg)
Astro	[1]0.84 ± 0.06a	[1]2.32 ± 0.17b	[1]1.78 ± 0.13b	[1]1.16 ± 0.08a	[1]3.82 ± 0.29c	[1]5.91 ± 0.41d	15.2 ± 1.2[1]
GH2547	[1]0.79 ± 0.04a	[2]4.11 ± 0.27b	[1]2.31 ± 0.13c	[1]1.37 ± 0.08a	[2]4.67 ± 0.24b	[2]7.17 ± 0.42c	20.5 ± 1.2[2]
Local	[1]1.01 ± 0.08a	[2]4.59 ± 0.36b	[1]2.1 ± 0.2c	[1]1.14 ± 0.09a	[3]2.60 ± 0.25c	[3]4.99 ± 0.42b	16.6 ± 1.4[1]

Mean values with different letters within a row are significantly different, at $p < 0.05$.
Mean values with different numbers in the same column are significantly different, at $p < 0.05$.
[a] Docosanol (C22), tetracosanol (C24), hexacosanol (C26), octacosanol (C28), triacontanol (C30), dotriacontanol (C32) and policosanol (PC).
[b] Each value is a mean ± standard deviation (SD) of a triplicate analysis performed on different samples.

Table 3
Policosanol[a] contents in dissected corn kernels (mg/kg of dry weight)[b].

Sample	C22	C24	C26	C28	C30	C32	Total PC
P	nd	[1]1.52 ± 0.05a	[1]3.77 ± 0.13b	[1]11.48 ± 0.41c	[1]33.63 ± 1.16d	[1]22.31 ± 0.79e	72.7 ± 2.6[1]
Astro G	[1]2.06 ± 0.13a	[2]2.56 ± 0.15a	[2]1.32 ± 0.08b	[2]2.15 ± 0.13a	nd	[2]11.21 ± 0.61c	19.3 ± 1.2[2]
E	[2]0.57 ± 0.41a	[1]1.36 ± 0.24b	[3]1.97 ± 0.35b	[3]0.84 ± 0.15a	[2]2.73 ± 0.42c	[3]1.46 ± 0.26b	8.9 ± 1.6[3]
P	nd	[1]2.15 ± 0.11a	[1]5.18 ± 0.26b	[1]8.13 ± 0.41c	[1]37.0 ± 1.9d	[1]27.3 ± 1.4e	79.8 ± 4.1[1]
Local G	[1]1.53 ± 0.17a	[1]2.31 ± 0.26b	[2]0.76 ± 0.08c	[2]1.66 ± 0.18a	[2]0.55 ± 0.06c	[2]18.83 ± 2.12d	25.7 ± 2.9[2]
E	[2]0.19 ± 0.03a	[2]0.29 ± 0.05a	[2]0.95 ± 0.16b	[3]0.21 ± 0.03a	[3]1.29 ± 0.23b	[3]1.01 ± 0.17a	4.0 ± 0.7[3]
P	nd	[1]2.5 ± 0.1a	[1]8.53 ± 0.36b	[1]14.1 ± 0.6c	[1]46.3 ± 1.1d	[1]39.46 ± 1 .72e	110.9 ± 4.7[1]
GH2547 G	[1]2.80 ± 0.26a	[1]2.97 ± 0.27a	[2]1.63 ± 0.15b	[2]3.48 ± 0.32a	nd	[2]26.2 ± 2.4c	37.1 ± 3.4[2]
E	[2]0.82 ± 0.12a	[2]1.09 ± 0.16a	[3]2.81 ± 0.41b	[3]0.84 ± 0.12a	[2]3.88 ± 0.57b	[3]6.70 ± 0.99c	16.2 ± 2.4[3]

nd, not detected.
Mean values with different letters within a row are significantly different, at $p < 0.05$.
Mean values with different numbers in the same column for different parts of same corn kernel are significantly different, at $p < 0.05$.
[a] Docosanol (C22), tetracosanol (C24), hexacosanol (C26), octacosanol (C28), triacontanol (C30), dotriacontanol (C32) and policosanol (PC).
[b] Each value is a mean ± standard deviation (SD) of a triplicate analysis performed on different samples.

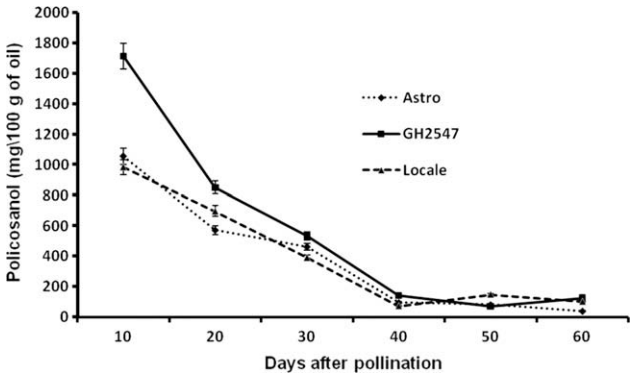

Fig. 3. Change in total policosanol content (mg 100 g^{-1} oil) during maturation of three varieties. Variety Local is represented by (●), Astro is represented by (■) and GH2547 is represented by (▲).

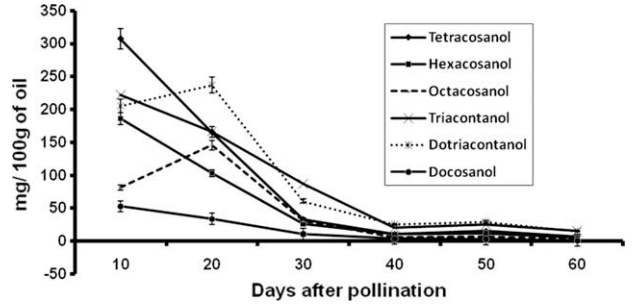

Fig. 4. Changes in the policosanol compounds content (mg 100 g^{-1} oil) during corn kernel maturation (variety *Astro*). Tetracosanol (■), dotriacontanol (♦), triacontanol (×), hexacosanol (∗), octacosanol (●), docosanol (▲).

never previously been studied. Fig. 3 shows that the greatest change in policosanol content (expressed as mg/100 g of oil) occurred during the early stages of corn kernel development. From10 to 40 DAP there is a dramatic decrease in the total amount of policosanol, but for the remainder of the period it was relatively constant. The decline of policosanol may be due to their conversion to others metabolites such as fatty acids. Menéndez et al. (2005) demonstrated that shortened saturated (myristic, palmitic and stearic) and unsaturated (oleic, palmitoleic) fatty acids are formed after oral dosing of monkey with policosanol. In addition, Weber (1970) reported that the percentage of oleic acid increased as the corn grain matured.

Differences in the concentrations of policosanol compounds over the course of corn kernel (Astro) are evident in Fig. 4. Although, alcohol constituents of policosanol are structurally similar, their patterns of accumulation were different. At 10 DAP tetracosanol was the major policosanol, while from 20 DAP until maturity dotriacontanol and triacontanol were the most predominant policosanol. The level of triacontanol decreased rapidly from 10 to 40 DAP, and then plateaued to maturity. This trend could be linked to its role as a potent plant growth promoting substance (Ramanarayan et al., 2000). It has been shown that triacontanol increases dry weight (Ries, 1991) and corn kernels rapidly accumulate dry weight from 10 to 40 DAP (Davis & Poneleit, 1975; Harrabi et al., 2007). Octacosanol and dotriacontanol showed similar trends, suggesting a similar metabolic profile. In contrast to the other compounds, the highest levels of octacosanol and dotriacontanol were detected at 20 DAP, followed by a steep decline between 20 and 30 DAP, suggesting they contribute to the biosynthesis of fatty acids. In fact, it has been assumed that octacosanol might be oxidised and degraded to fatty acids via β-oxidation in mammals (Menéndez et al., 2005). Additionally, Weber (1969) reported that if the weights of the individual fatty acids per 100 kernels are calculated, the largest increase in fatty acid synthesis is found between 15 and 30 DAP. Docosanol, which inhibits replication of herpesviruses in vitro and in vivo (Marcelletti, 2002), was the minor policosanol during corn kernel development.

4. Conclusion

This study shows that corn can be a significant dietary source of policosanols, exhibiting concentrations similar to whole sugar cane wax. In addition, the policosanol appears to be highly localised in the pericarp which exhibits higher policosanol content than does the endosperm and germ. Thus, corn pericarp may be potential source for functional foods and nutraceutical applications. Triacontanol was the most abundant policosanol present in corn pericarp, while dotriacontanol was the major components in whole corn kernel. During corn kernel maturation, the octacosanol profile suggests it plays a role in the biosynthesis of fatty acids. The observed trend for triacontanol seems to be influenced by its role in the increase of dry weight.

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