Changes in Tocopherol and Plastochromanol-8 Contents in Seeds and Oil of Oilseed Rape (*Brassica napus* L.) during Storage As Influenced by Temperature and Air Oxygen

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The changes in tocopherol and plastochromanol-8 contents in seeds and oil of oilseed rape (*Brassica napus* L.) were studied during a storage period of 24 weeks at different incubation temperatures and exposure to air oxygen (open and closed flasks). In the extracted oil, total tocopherol content remained unaltered at 5 and 20 °C throughout the 24 weeks of storage. At 40 °C, a beginning degradation was observed already after 4 weeks in both open and closed flasks; the α-tocopherol content was affected most, followed by γ-tocopherol and plastochromanol-8. After 16 weeks at 40 °C, the total tocopherol content in the oil was reduced by more than 90%. In intact seeds, no tocopherol degradation was observed; only the seeds incubated at 40 °C and in open flasks showed slightly lower tocopherol contents. However, the analysis of the tocopherol composition in the stored seeds showed a decrease in the α-tocopherol content and an increase in the γ-tocopherol content, which resulted in a decreasing α-/γ-tocopherol ratio. This trend was most apparent at 40 °C and after 24 weeks of storage. A reduction of plastochromanol-8 occurred only at 40 °C and was more pronounced in open flasks. At 40 °C and in closed flasks a gradual increase in the content of α-tocotrienol was observed, a compound normally not accumulated in rapeseed.

Keywords: *Tocopherol degradation; plastochromanol-8; α-tocotrienol; Brassica napus*

INTRODUCTION

The deterioration of the quality of lipids due to oxidative processes is of great economic and nutritional importance for the food industry, because of the loss of essential fatty acids and lipid-soluble vitamins. In addition, lipid peroxidation produces free radicals, which are purportedly associated with carcinogenesis, mutagenesis, and aging (Tagi, 1987). Although antioxidants can neither avoid the autoxidation of lipids nor reverse the formation of peroxides, they delay the process by eliminating the free-fatty acid radicals.

The most important fat-soluble antioxidants in cell membranes and vegetable oils are the tocopherols. They are not only effective in vivo but also in vitro. There are four forms of tocopherols: α-, β-, γ-, and δ-tocopherol, the α-derivative being known as vitamin E.

Several studies reported the effect of tocopherols on the oxidative stability of oils and different lipid systems (Huang et al., 1994; Blekas et al., 1995; Frankel et al., 1994; Martínez de la Cuesta et al., 1995). They revealed the effectiveness of tocopherols to inhibit peroxide formation, but without reporting the reduction of tocopherol contents during oxidation. Coors and Montag (1988) studied the decomposition of tocopherols during a 12 week storage time in several refined and cold pressed oils, including rapeseed oil. The reduction of tocopherols was strongly influenced by temperature, light, and air oxygen. Chemical changes of lipids, including total tocopherol contents, were also monitored in ground seeds (Gopalakrishnan et al., 1996) during storage. But no investigation was carried out comparing tocopherol degradation in both extracted oils and intact seeds.

Rapeseed oil contains about 64% γ-tocopherol, 35% α-tocopherol, a very low percentage (<1%) of δ-tocopherol, and plastochromanol-8, a chromanol-6 derivative related to tocopherols with antioxidant properties (Olejnik et al., 1997). The present study was aimed at investigating the loss of tocopherols and plastochromanol-8 during 6 months of storage in both intact seeds and extracted oil as influenced by different temperature and air oxygen treatments in rapeseed.

MATERIALS AND METHODS

Plant Material. Three different winter rapeseed cultivars, "Bristol", "Falcon", and "Lirajet", with similar fatty acid composition and oil content but different tocopherol content and profile were cultivated under field conditions in 1997/98 in Göttingen, Germany (data are presented in Tables 1 and 2). After seed maturity, the plants were immediately harvested and seed samples were taken. A part of the harvested seeds were used for the oil extraction.

Reagents. Isooctane and *tert*-butyl methyl ether were of HPLC grade (Merck, Darmstadt, Germany). Petroleum ether (40 °C) was of analytical grade (>98%) (Merck, Darmstadt, Germany).

Soxhlet Extraction. From each cultivar 500 g of seeds was ground and then extracted with petroleum ether in a Soxhlet apparatus for 18 h. After extraction, the samples were ground again, but more finely, and extracted for 6 h (second extraction). Petroleum ether was evaporated under reduced pressure using a rotorvapor. Oil content is expressed as wt %/wt.

GC Analysis of Fatty Acids. Fatty acid composition of seed oils was determined by gas−liquid chromatography of

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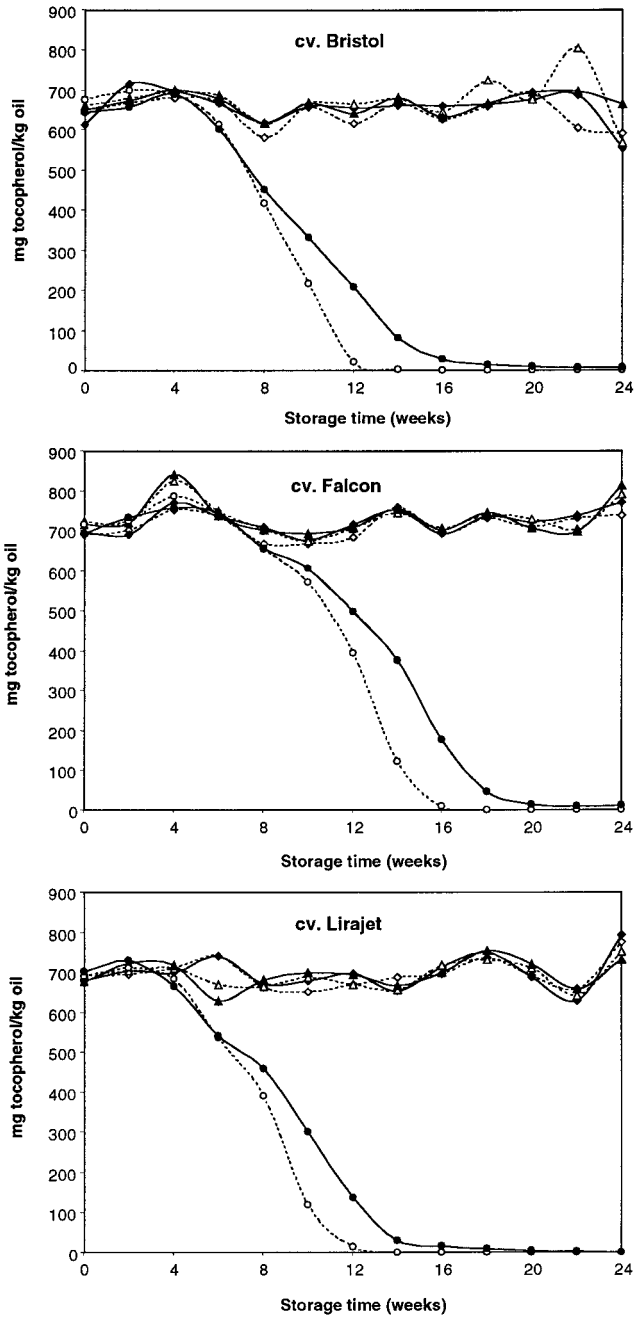

Figure 1. Changes of total tocopherol contents (mg tocopherol/kg oil) in extracted oil of three different rapeseed cultivars during storage at 5 °C, open flasks (--◇--); 5 °C, closed flasks (--◆--); 20 °C, open flasks (--△--); 20 °C, closed flasks (--▲--); 40 °C, open flasks (--○--); 40 °C, closed flasks (--●--).

fatty acid methyl esters (Thies, 1971) on a Perkin-Elmer gas chromatograph model 8600 (Perkin-Elmer Corp., Norwalk, CT) equipped with a fused silica capillary column FFAP, 25 m × 0.25 mm × 0.25 μm film thickness (Macherey & Nagel GmbH + Co KG, Düren, Germany). The oven, detector, and injector temperatures were 200, 250, and 250 °C, respectively. The carrier gas was hydrogen, at a pressure of 100 kpa. Two microliters of sample was injected, at a split rate of 1:70.

Treatments. Intact seed and crude oil samples were placed in 200 mL plastic flasks and stored in the dark at three different temperatures (5, 20, and 40 °C) and subjected to two air conditions (closed and open flasks) for 24 weeks. Subsamples were taken at 2 week intervals and stored at −80 °C. Two replications were performed for each treatment. The samples were then analyzed for tocopherol and plastochromanol-8 contents.

Table 1. Oil Content and Fatty Acid Composition of the Three Studied Rapeseed Cultivars

		fatty acid composition[b]						
cultivar	oil[a] (%)	C16:x[c]	C18:0	C18:1	C18:2	C18:3	C20:x[d]	C22:x[e]
Bristol	51.3	5.3	1.6	61.5	19.3	10.3	1.7	0.3
Falcon	50.7	5.7	1.3	54.4	23.2	13.1	1.6	0.7
Lirajet	45.7	4.7	1.4	60.8	19.8	11.4	1.6	0.3

[a] Expressed as wt %/wt. [b] Expressed as percent of total fatty acids. [c] Sum of C16:0, C16:1, and C16:2. [d] Sum of C20:0 and C20:1. [e] Sum of C22:0 and C22:1.

Table 2. Total and Individual Tocopherol and Plastochromanol-8 Contents, Expressed as mg kg^{-1} Oil, and the α-/γ-Tocopherol Ratio in the Three Studied Rapeseed Cultivars

			tocopherols[b]				
cultivar	n	total-T[a]	α-T	γ-T	δ-T	α-/γ-T ratio[c]	Plasto-8[d]
Bristol	6	650 b	236 c	408 b	6 b	0.58 c	68 b
Falcon	6	707 a	291 a	404 b	12 a	0.72 a	79 a
Lirajet	6	690 a	256 b	425 a	9 b	0.60 b	54 c

[a] Total-T = total tocopherol content. [b] Tocopherols: α-T = α-tocopherol, γ-T = γ-tocopherol, δ-T = delta-tocopherol. [c] α-/γ-tocopherol ratio. [d] Plasto-8 = plastochromanol-8. Values followed by the same letter are not significantly different ($p < 0.01$).

Tocopherol, Tocotrienol, and Plastochromanol-8 Analyses. Tocopherols and plastochromanol-8 were determined by normal-phase HPLC as described by Thies (1997). Tocotrienols were determined according to Balz et al. (1992).

(a) Oil Samples. Twenty milligrams of extracted oil was dissolved in 2 mL of isooctane, and 5 μL of the solution was directly injected in the HPLC without further purification.

(*b) Intact Seed Samples.* About 200 mg of seeds was ground and placed in a 5 mL volumetric test tube. The samples were extracted with 2 mL of an isooctane−ethanol (2:1) solution for 24 h at room temperature in the dark (batch extraction method). After extraction, the tubes were centrifuged and the over layer passed through a lipophilic filter. The samples were extracted twice. The collected filtrates were evaporated under reduced pressure. Analyses of the extracted crude oils were carried out as for the oil samples.

RESULTS AND DISCUSSION

Degradation of Tocopherol and Plastochromanol-8 Contents in Oils. Figure 1 shows the changes of total tocopherol contents in the three studied rapeseed lines during 24 weeks storage. Total tocopherol contents remained unaltered at 5 and 20 °C and by both air treatments throughout 24 weeks of storage. At 40 °C and under both air treatments, tocopherols were completely degraded, following a sigmoidal pattern of decomposition. After an initial period of induction of 4 weeks in "Bristol" and "Lirajet" and 8 weeks in "Falcon", tocopherols were linearly decomposed with similar degradation rates in all studied cultivars. The 4 week delay observed in "Falcon" for the induction time cannot be explained by higher initial total tocopherol content or by differences in the fatty acid composition of this line (see Tables 1 and 2). The higher α-tocopherol content of "Falcon" could not cause this delay either, since this tocopherol derivative decomposed faster than γ-tocopherol (see Figure 2). Other minor compounds with antioxidant effect present in rapeseed oil could play an important role in protecting tocopherols from decomposition. Prior et al. (1991) observed variation in the oxidative stability of rapeseed press oils with relatively uniform tocopherol contents and found a positive correlation between oil stability and P content, suggesting a synergism between tocopherols and phospholipids. An

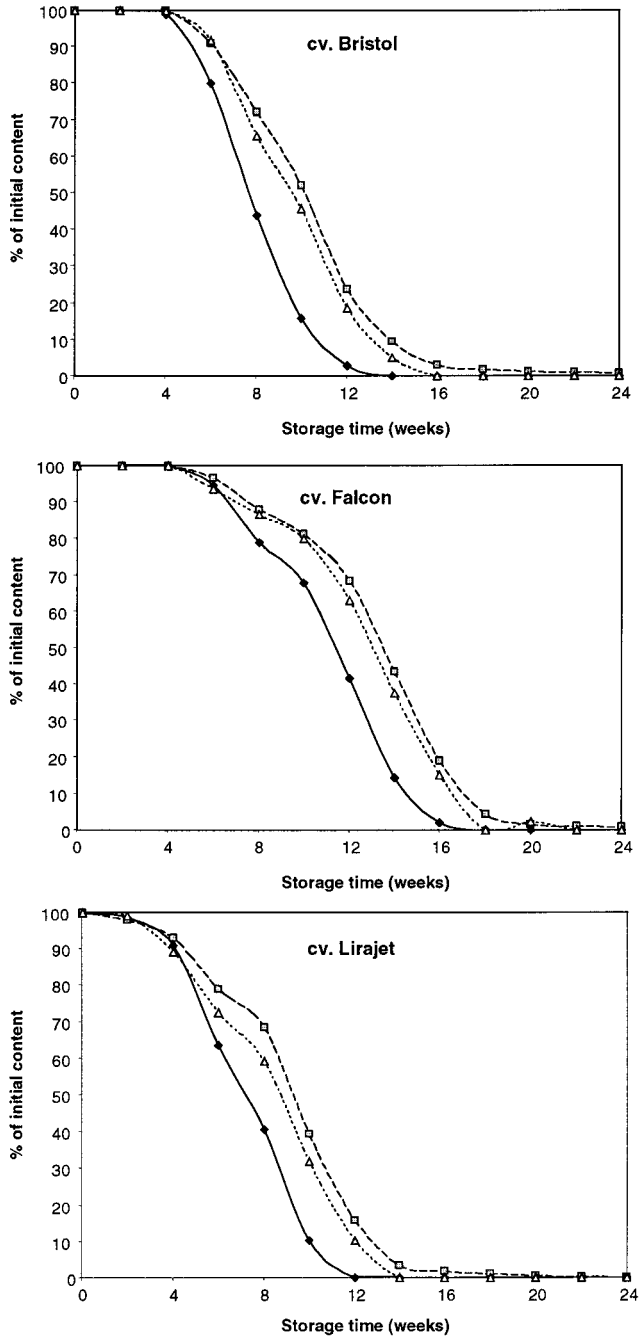

Figure 2. Reduction of α-tocopherol (--◆--), γ-tocopherol (--□--), and plastochromanol-8 (--△--) contents in extracted oil of three different rapeseed cultivars, expressed as percent of their initial content (=100%) at 40 °C (mean values of both air treatments).

antioxidant synergy between α-tocopherol and phopholipids was reported in sardine oil (Barranda et al., 1999). Another group of fat-soluble compounds present in rapeseed oil are the carotenoids. They have been widely recognized for their ability to prevent oxidative damage (Larson, 1988). In addition, hydrophilic antioxidants (vitamin C and phenolic acids) may also be protective against lipid oxidation. Frankel et al. (1994) found in corn oil that vitamin C acts as antioxidant by being oriented in the air−oil interface. The observation that vitamin C is particularly active as an antioxidant in bulk oils is not new (Uri, 1961). Further detailed work using a higher number of lines containing different

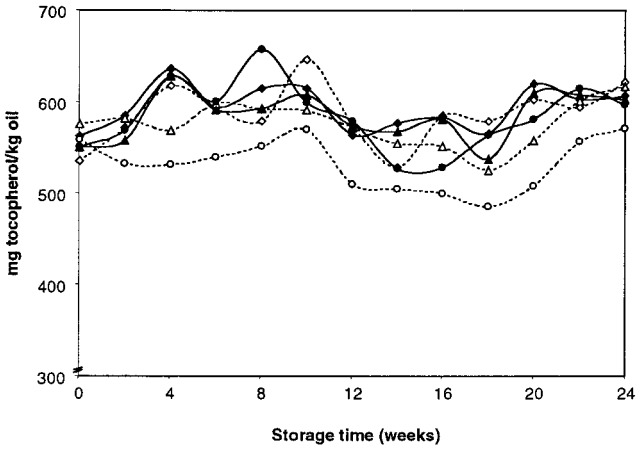

Figure 3. Changes of total tocopherol contents (mg tocopherol/kg oil) in intact seeds of three different rapeseed cultivars (mean value of all studied lines) during storage at 5 °C: open flasks (--◇--); 5 °C, closed flasks (--◆--); 20 °C, open flasks (--△--); 20 °C, closed flasks (--▲--); 40 °C, open flasks (--○--); 40 °C, closed flasks (--●--).

tocopherol contents and profiles and analyzing other antioxidant compounds present in rapeseed is required to explain the differences in the induction time. On the other hand, the decrease of total tocopherol content in closed flasks at 40 °C occurred at a lower rate than in open flasks, indicating that air oxygen accelerates tocopherol decomposition at this temperature.

Individual tocopherols and plastochromanol-8 showed no degradation at 5 and 20 °C in all studied lines (data not shown). However, at 40 °C, degradation was already observed after 4−6 weeks (Figure 2). α-Tocopherol was decomposed faster than γ-tocopherol, and plastochromanol-8 showed an intermediate behavior between both tocopherol-derivatives, more closely to that of γ-tocopherol. The same order of decrease (α-tocopherol > γ-tocopherol) was found in oxidized fatty acids from soybean oil mixed with tocopherols, stored at room temperature (Kajimoto et al., 1989). Coors and Montag (1988) found in refined rapeseed oil after 10 weeks of storage at 5 °C a 10% reduction of γ-tocopherol.

Degradation of Tocopherol and Plastochromanol-8 Contents in Intact Seeds. The total tocopherol contents were ca. 15% lower in seeds than in the extracted oil because Soxhlet extraction is more efficient in extracting tocopherols than the batch extraction method used for the intact seed samples. Decomposition of tocopherol and plastochromanol-8 was very similar in all studied cultivars. Therefore, they are displayed as the mean values of all cultivars included in this study. For total tocopherol contents, no significant changes were detected in intact seeds at 5, 20, and 40 °C (closed flasks). At 40 °C and open flasks, total tocopherol contents were significantly lower compared to the other treatments throughout the storage time (Figure 3). For this reason, high temperatures combined with high availability of air oxygen appears to conduce to a decomposition of tocopherols in seeds. Gopalakrishnan et al. (1996) reported a 50% reduction of total tocopherol contents in ground canola seeds already after 10 days of storage at room temperature (20 °C) and also after 30 days of storage at 5 °C. We have not found a reduction neither in extracted oils nor in intact seeds at these temperatures during 6 months of storage. The larger interfacial contact between lipids and air oxygen in ground seeds compared to the extracted oil and intact

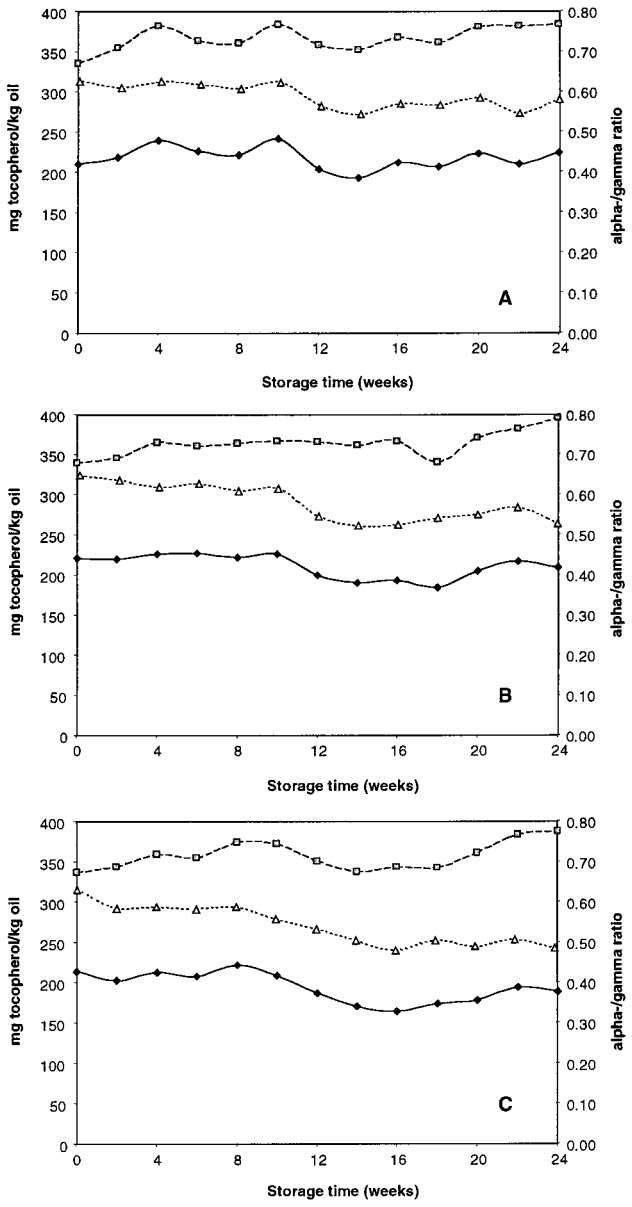

Figure 4. Changes of α-tocopherol (--◆--) and γ-tocopherol (--□--) contents and the α-/γ-tocopherol ratio (--△--) in intact seeds of three different rapeseed cultivars (mean of all studied lines and both air treatments) during storage at **(**A**)** 5 °C; **(**B**)** 20 °C, and **(**C**)** 40 °C.

seeds could be the cause of the higher decomposition of tocopherols in the meal.

Individual tocopherols were differently influenced during storage (Figure 4). α-Tocopherol remained largely constant at all temperatures, whereas γ-tocopherol contents increased by 20% at the end of the storage time. The constant reduction of the α-/γ-tocopherol ratio during storage by both temperature treatments confirms this. A modification of the contents of individual tocopherols and tocopherol composition takes place during storage in intact seeds of oilseed rape.

Plastochromanol-8 was strongly affected during storage at 40 °C (Figure 5). In open flasks, degradation of plastochromanol-8 was considerable during the first 4−6 weeks; after this period, degradation proceeded somewhat slower. In closed flasks, plastochromanol-8 decomposed only slightly throughout the storage time. The decrease in plastochromanol-8 content was stronger in open flasks (ca. 45%) compared with closed flasks (ca.

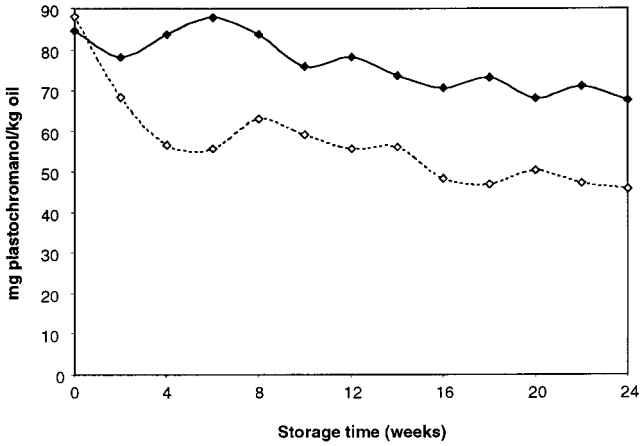

Figure 5. Reduction of plastochromanol-8 contents (mg plastochromanol/kg oil) in intact seeds of three different rapeseed cultivars (mean values of all studied lines) during storage: 40 °C, open flasks (--◇--); 40 °C, closed flask (--◆--).

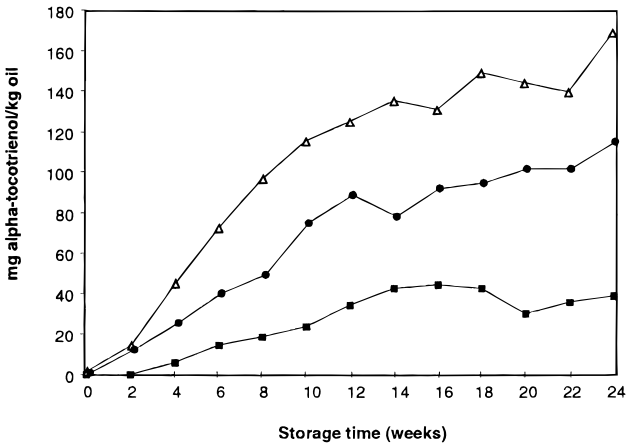

Figure 6. Increase of α-tocopherol content (mg/kg oil) in intact seeds of three different rapeseed cultivars: (--■--) cv. Bristol; (--△--) cv. Falcon; (--●--) cv. Lirajet during storage at 40 °C (closed flasks).

20%). At 5 and 20 °C, it was not degraded (data not shown). The significance of this compound as antioxidant in vivo is unknown. Nevertheless, due to the high consumption of plastochromanol-8 at 40 °C compared to α-tocopherol, it could be possible that this chromanol derivative exhibits higher antioxidant activity in seeds than α-tocopherol. In lard oil, plastochromanol-8 appeared to be a better inhibitor of autoxidation than α-tocopherol (Olejnik et al., 1997).

Synthesis of α-Tocotrienol in Intact Seeds. At 40 °C (closed flasks), α-tocotrienol, an antioxidant component closely related to α-tocopherol, is surprisingly produced in seeds during storage (Figure 6). The synthesis of α-tocotrienol in nontocotrienol-containing seeds such as rapeseed has to our knowledge not been reported before. Normally, this compound is not synthesized in rapeseed as end product. In all other treatments, no α-tocotrienol was detected throughout the storage time. It can be speculated that the high temperature (40 °C) resulted in a high consumption of air oxygen and possibly generated high amounts of carbon dioxide in the closed flasks, which could be responsible for the production of this compound. The consequences of the appearance of α-tocotrienol and its explanation require further investigations.

CONCLUSIONS

Great differences in the contents and composition of tocopherols and plastochromanol-8 may occur in dependence of the storage conditions. This has to be considered when seed samples of unknown origin or storage condition are analyzed. Seed and oil samples obtained, e.g., from screening germoplasm for genetic variability, can be stored under appropriate conditions for at least 6 months without the danger of degradation of tocopherols and plastochromanol-8.

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