The Sterol Composition of Freshly Harvested Compared to Stored Seeds of Rape, Sunflower and Poppy

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ABSTRACT

The composition of the free sterols and the sterol esters of freshly harvested seeds of rape, sunflower and poppy was compared to that of stored seeds. The sterol composition of rapeseed was not changed during storage, whereas in sunflower seed the free sterols had less of Δ5-avenasterol and Δ7-stigmastenol in ten-month-old seeds compared to fresh seeds. The greatest relative changes were observed for esterified sterols in poppy seed, with a drop in the percentage of Δ5-avenasterol from 25.3% in freshly harvested to 16.9% in seeds stored for 10 months.

INTRODUCTION

A previous study has shown a very small variation in the composition of the sterols and sterol esters of *Brassica napus* with cultivar and location of growing within Sweden (1). In the Swedish samples, no Δ7-sterols were detected, whereas Seher and Homberg (2) found Δ7-sterols in freshly harvested seeds of *B. napus*, but not in stored seeds.

On the other hand, the sterols of sunflower seeds have rather high proportions of Δ7-sterols, and in poppyseed sterol esters are rich in Δ5-avenasterol (3), a sterol which has been shown to minimize the oxidative polymerization of oils during heating (4).

In view of these literature reports and the great overall variation found in compilations of sterol composition of seeds and oils (1, 3), it was deemed important in our studies on variability to analyze the sterols and sterol esters of rapeseed, sunflowerseed and poppy seed immediately after harvest and after some time of storage.

MATERIALS AND METHODS

Seed Material

Rapeseed (*Brassica napus L.*), cultivar WW Olga, was obtained from the Weibullsholms Plant Breeding Institute, Landskrona, Sweden. The seed was harvested on September 2, 1977, and was of high quality (viability 94%). Sunflower seed (*Helianthuus annuus*), harvested on September 27, 1977 (viability 64%), and poppy seed (*Papaver somniferum*), cultivar Soma, harvested on September 27, 1977 (viability 89%), were both obtained from the Swedish Seed Association, Svalöv, Sweden. All the seed samples were kept in plastic bags at 4 C until used for analysis.

Reagents and Techniques

The seeds were extracted with hexane/ethanol (3:1 v/v), and the solvent was removed in vacuo as described in detail in a previous communication (1). The crude lipids were separated by preparative thin layer chromatography, and the sterol ester and sterol bands were recovered. The sterol esters were saponified and the sterols and the fatty acids were separated by preparative thin layer chromatography. The sterols were silylated and the silyl ethers were analyzed by gas chromatography on an OV-17 column. The fatty acids were converted to methyl esters by BF_3 treatment and analyzed on an EGA column. The analyses were performed in a Varian Aerograph 2100, and a digital integrator, Varian 480, was used for the calculation of peak areas. Reference compounds were used to identify the sterols and fatty acids. The details of the techniques used have been given in previous publications (1,3).

RESULTS AND DISCUSSION

The quantitative composition of the free sterols and the sterols of the sterol esters of rapeseed, cv WW Olga, analyzed immediately (9 days) after harvest (marked "storage time 0 months" in Table I) was rather similar to that previously found for the same cultivar harvested in 1976 (cf. ref. 1, Table I). No changes in sterol compositions were observed after 6 or after 10 months of storage. Some previous studies have reported variable levels of Δ7-sterols (2, and Appelqvist, L.-Å, A. Johansson, and J. Wennerholm, unpublished results); whereas other investigators found no such sterols in rapeseed (1,5).

The composition of the fatty acids of the sterol esters of freshly harvested rapeseed (WW Olga) was similar to that previously reported (cf. ref. 1, Table II) and did not undergo marked changes upon 10 months' storage of seeds.

The quantitative composition of the free and the esterified sterols of sunflower are obviously different after 10 months of seed storage compared to that of freshly harvested seeds (Table I). In the free sterol fraction, a drop in relative concentration of Δ5-avenasterol, Δ7-stigmastenol and "unknown" sterol II is balanced by the sitosterol percentage. In view of the extremely low metabolic activity prevailing in such very dry seeds (6), we assume that the losses of Δ5-avenasterol and of Δ7-sterols are due to chemical oxidation rather than enzymatic conversion. The changes in sterol composition in the esterified sterol fraction of sunflower are in most cases not beyond experimental error. In view of the many steps involved in the acquisition of the fatty acid pattern of the sterol esters (cf. ref. 1), the differences noted between 0 and 10 months' storage (Table II) are supposed to be within the experimental errors.

In poppy seeds, there appears to be changes in the sterol esters but not in free sterols. There was a remarkable drop during seed storage in the relative content of Δ5-avenasterol an important component of the esterified sterols of poppy seed (Table I). A relative decrease in Δ5-avenasterol has also been reported, very recently, in the oil of olives which were stored for various time periods (7). The fatty acid composition of the sterol esters after seed storage for 10 months indicated losses, probably by oxidation, since the linolenic acid content dropped from 51% to 36% (Table II). It should be noted that sterol esters are present in relatively small amounts in poppy seed (0.05% of the oil) compared to the levels in oils of rapeseed (0.71%) and of sunflower seed (0.28%). The content of free sterols are similar, ca. 0.35%, in these three oils (1,3).

The results of these studies should be of concern to all who analyze dry seed material for content of natural constituents. The seed age is regarded of limited interest for major components, such as fatty acids of the triacylglycerols, but obviously not for minor components which can be easily oxidized. Analyses of the content of lipid peroxides in the laboratory-extracted seed oils from *Brassica napus* and *Sinapis alba* (8) demonstrated that

TABLE I

Free and Esterified Sterols in Freshly Harvested and in Stored Rapeseed, Sunflower Seed and Poppy Seed

Species and cultivar	Lipid class	Time of storage, months	Composition of sterols, %									
			Brassica sterol	I	II	Campesterol[a]	Stigmasterol	III	Sitosterol	Δ5-Avenasterol	Δ7-Stigmastenol	Δ7-Avenasterol
Rapeseed WW Olga	Free sterols	0	16.1			31.2		tr	52.7	tr		tr
		6	15.0			31.7		---	53.3	tr		---
		10	15.4			31.4		---	53.2	---		---
	Esterified sterols	0	5.4			40.9		---	53.7	tr		tr
		6	5.1			42.2		---	52.7	tr		---
		10	6.8			41.9		---	51.2	tr		---
Sunflower seed Sv. Sunbred	Free sterols[b]	0		0.8	2.9	7.3	15.3	---	55.1	8.5	5.5	3.5
		6		0.8	1.3	6.9	14.2	---	67.1	4.8	3.0	1.1
		10		0.6	1.0	6.5	15.2	---	65.0	5.5	2.8	2.4
	Esterified sterols	0	0.3	0.4		8.6	2.8	4.9	54.3	6.8	14.1	7.9
		6	---	tr		8.8	1.9	1.8	53.8	6.7	18.3	8.8
		10	---	---		8.6	2.0	3.0	58.9	5.3	13.6	8.6
Poppy seed Sv. Soma	Free sterols	0				18.9	3.3		71.3	6.5		tr
		6				20.1	5.6		69.7	4.6		---
		10				19.5	3.0		71.5	6.1		---
	Esterified sterols	0				40.0	3.3		31.5	25.3		tr
		6				43.1	3.6		32.6	20.8		tr
		10				44.9	4.0		34.2	16.9		---

[a]Campesterol refers to a 24-methylcholesterol with a configuration (α) at C-24 which is not verified in sunflower and poppy seeds. However, this sterol is generally reported as campesterol in the literature.

[b]Also ca. 1% of cholesterol.

TABLE II

The Fatty Acids of Sterol Esters in Freshly Harvested and in Stored Rapeseed, Sunflower Seed and Poppy Seed

Species and cultivar	Time of storage, months	Fatty acids of sterol esters, %								
		16:0	16:1	18:0	18:1	18:2	18:3	20:0	22:0	24:0
Rapeseed	0	7	1	2	32	51	6			
WW Olga	10	11	1	6	30	44	8			
Sunflower seed	0	6	1	2	10	35	1	12	22	9[a]
Sv. Sunbred	10	11	--	4	11	36	tr	12	18	7[b]
Poppy seed	0	18	7	5	19	51				
Sv. Soma	10	26	3	15	20	36				

[a]Also 1% of 20:1 and 1% of an unidentified fatty acid.

[b]Also 1% of an unidentified fatty acid.

stored viable seeds had no detectable levels of lipid peroxides, provided many precautions were taken during laboratory extraction, but that nonviable seeds after 7-8 months in storage had low but significant levels of lipid peroxides.

The variable appearance of the less stable Δ5-avenasterol and Δ7-sterols in rapeseed, the loss of such sterols in the free sterol fraction of sunflower, and the loss of Δ5-avenasterol in the sterol esters of poppy seed certainly limit the usefulness of such data in chemotaxonomy (cf. ref. 9) but may be of significance to the edible oil industry (4).

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