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# STEROL COMPOSITION OF RAPESEED VARIETIES INTRODUCED IN BULGARIA

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## Abstract

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The composition of glyceride oils isolated from the seeds of 6 rapeseed varieties introduced in Bulgaria was investigated focusing on the biologically active substances: sterols and sterol esters. Sterols in the glyceride oils were found to be totally 3 $g.kg^{-1}$ including 2 $g.kg^{-1}$ free sterols and 1 $g.kg^{-1}$ esterified sterols. β-Sitosterol predominated in both free and esterified sterols, being respectively 658–790 $g.kg^{-1}$ and 471–700 $g.kg^{-1}$, followed by campesterol and stigmasterol. Oleic acid (609–768 $g.kg^{-1}$) was the main component among the fatty acids of the sterol esters, followed by palmitic acid (72–144 $g.kg^{-1}$).

*Key words*: rapeseed oil, sterols, biologically active substances, fatty acids

## Introduction

Rapeseed oil is one of the most important vegetable oils concerning both its industrial application and nutritional consumption. The high nutritional value of the rapeseed oil is due to the high levels of unsaturated fatty acids including the biologically active linoleic (*cis* 9, *cis* 12-18:2) and linolenic (*cis* 9, *cis* 12, *cis* 15-18:3) acids, and the low level of saturated fatty acids. The oil is rich in sterols and tocopherols which are natural antioxidants and synergists preventing to some extent the autoxidation of lipids at ambient temperature. The sterol composition is an important indicator for the quality of the oil. However, knowledge about the sterols in the rapeseed oils of new varieties is still fragmentary and when it is available it concerns only the total sterols. No data is available about the content and composition of the two sterol fractions – free sterols and sterol esters. The objective of the present research is to investigate the oil of 6 rapeseed varieties introduced in Bulgaria about the content and composition of the sterols in both fractions – free sterols and sterol esters, as well as to determine the fatty acid composition of the sterol esters.

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## Materials and Methods

The seeds of the investigated rapeseed varieties were provided from the Plovdiv region in South Bulgaria, crop 2007. The investigations were carried out on air dried seeds in technical ripeness.

The oil was extracted in Soxhlet apparatus with hexane for 8 h. After that the solvent was removed by a rotary evaporator and the residue was weighed to determine the oil content of each rapeseed variety.

***Sterols.*** The total oil sample (sample size of 100 mg, precisely measured) was applied on a 20 cm x 20 cm glass plate with ca. 1 mm thick silica gel 60 G layer (*Merck*, Darmstadt, Germany) and developed with hexane-acetone, 100:8 (by volume) to a front of 19 cm. Free ($R_f$=0.4) and esterified ($R_f$=0.8) sterols were detected under UV light by spraying the edges of the plate with 2',7'-dichlorofluorescein and then they were scraped, transferred to small glass columns and eluted with diethyl ether. The solvent was evaporated under a stream of nitrogen, the respective residues were weighed in small glass containers to a constant weight and 2 % solutions in hexane were prepared. Free sterols were subjected to gas chromatography (GC) without derivatization. Sterol esters were hydrolyzed with ethanolic KOH (Ivanov et al., 1972) and then sterols were extracted with light petroleum ether and purified by TLC under the conditions given above prior to the GC analysis. Sterol composition was determined on HP 5890 gas chromatograph (Hewlett Packard GmbH, Austria) equipped with 25 m x 0.25 mm HP5 capillary column (Agilent Technologies, Santa Clara CA, USA) and flame ionization detector (FID). Temperature gradient from 90°C (held for 2 min) to 290°C at 15°C/min then to 310°C at 4°C/min and held at this temperature for 10 min; the injector temperature was 300°C and the detector temperature was 320°C. Nitrogen was the carrier gas at a flow rate of 0.8cm$^3$/min; split 100:1. Identification was performed by comparison of the retention times with those of a standard mixture of sterols (ISO 12228, 1999).

***Fatty acids.*** The fatty acid composition of sterol esters was determined by GC after transmethylation of the respective sample with 2N methanolic KOH at 50°C according to Metcalfe and Wang (1981). Fatty acid methyl esters (FAME) were purified by silica gel TLC on 20 cm x 20 cm plates covered with ca. 1 mm silica gel 60 G layer (Merck, Darmstadt, Germany) with mobile phase n-hexane-acetone, 100:8 (by volume). GC was performed on a HP 5890 (Hewlett Packard GmbH, Austria) gas chromatograph equipped with a 30 m x 0.25 mm capillary InnoWax column (cross-linked PEG, Hewlett Packard GmbH, Austria) and a FID. The column temperature was programmed from 165°C to 240°C at 4°C/min and held at this temperature for 10 min; injector and detector temperatures were 260°C. Nitrogen was the carrier gas at a flow rate of 0.8cm$^3$/min; split was 100:1. Identification was performed by comparison of the retention times with those of a standard mixture of fatty acid methyl esters subjected to GC under identical experimental conditions (ISO 5008, 2000).

## Results and Discussion

Data about oil and sterol contents of the investigated rapeseed varieties are presented in Table 1. As can be seen the seeds have high glyceride oil content (381-458 g.kg$^{-1}$) like the widely cultivated rapeseed varieties in the world (ca. 400 g.kg$^{-1}$). The content of total sterols in the investigated oils was found to be 3g.kg$^{-1}$. This value is close to that for sterols in sunflower oils (CODEX STAN 210, 2003, 2005). Among total sterols in the six rapeseed varieties the free sterols are twice more than the esterified sterols (Table 1).

The qualitative and quantitative compositions of the free and esterified sterols are shown in Table 2. β-Sitosterol predominates in both sterol fractions being 473-522 g.kg$^{-1}$ in free sterols and 522-565 g.kg$^{-1}$ in sterol esters. High levels of campesterol and brasicasterol have been determined as well, respectively 297-371 g.kg$^{-1}$ and 111-190 g.kg$^{-1}$ in free sterols, and 350-417 g.kg$^{-1}$ and 55-81 g.kg$^{-1}$ in sterol esters. These amounts are close to that reported in CODEX STAN 210 (1999). The cholesterol content was measured to be 2-5 g.kg$^{-1}$ (Table 2), whereas $\Delta^5$–avenasterol

**Table 1**

**Content of oil and sterols in rapeseed varieties***

| Rapeseed variety | Oil content, $g.kg^{-1}$ | Total sterols, $g.kg^{-1}$ | Free sterols, $g.kg^{-1}$ | Esterified sterols, $g.kg^{-1}$ |
|---|---|---|---|---|
| *Expres* | 381.00 | 3.00 | 2.00 | 1.00 |
| *Baldur* | 383.00 | 3.00 | 2.00 | 1.00 |
| *Elite* | 392.00 | 3.00 | 2.00 | 1.00 |
| *Trabant* | 432.00 | 3.00 | 2.00 | 1.00 |
| *Taurus* | 458.00 | 3.00 | 2.00 | 1.00 |
| *Rasmus* | 417.00 | 3.00 | 2.00 | 1.00 |

*Mean of three determinations

**Table 2**

**Sterol composition of free and esterified sterols, $g.kg^{-1}$***

| Sterols, g.kg-1 | Variety | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *Expres* | | *Baldur* | | *Elite* | | *Trabant* | | *Taurus* | | *Rasmus* | |
| | free | esterified | free | esterified | free | esterified | free | esterified | free | esterified | free | esterified |
| Cholesterol | 4 | 3 | 3 | 4 | 3 | 4 | 3 | 5 | 2 | 5 | 4 | 4 |
| Brasicasterol | 190 | 81 | 178 | 80 | 136 | 61 | 111 | 55 | 128 | 65 | 148 | 71 |
| Campesterol | 322 | 378 | 297 | 350 | 333 | 383 | 357 | 417 | 371 | 407 | 326 | 368 |
| Stigmasterol | 11 | ** | 4 | 1 | 6 | 1 | 7 | 1 | 1 | ** | 7 | ** |
| ß - Sitosterol | 473 | 538 | 518 | 565 | 522 | 551 | 522 | 522 | 498 | 523 | 515 | 557 |

*Mean of three determinations, ** Not detected

was found in traces only (not given in Table 2).

Great difference in sterol amounts in the free and esterified sterol fractions was established only for brasicasterol and stigmasterol (Table 2). The corresponding values for free sterols were above twice higher than that for esterified sterols. On the other hand, b-sitosterol and campesterol as free sterols were slightly less then as sterol esters. Total cholesterol content was low - about 7-8 $g.kg^{-1}$ and the proportion between the free and esterified cholesterol depended on the rapeseed variety.

The fatty acid composition of sterol esters is presented in Table 3.

Oleic and palmitic acids predominated in the sterol esters, being respectively 609-768 $g.kg^{-1}$ and 72-144 $g.kg^{-1}$, followed by linoleic acid (58-119 $g.kg^{-1}$). This composition differed from the fatty acid composition of triacylglycerols. The quantities of saturated fatty acids in sterol esters, mainly palmitic and stearic acids, were found to be 102-203 $g.kg^{-1}$ (Table 3) whereas these amounts in the corresponding glyceride oils were markedly lower (73-89$g.kg^{-1}$). These observations are in agreement with the data reported earlier about the fatty acid composition of sterol esters in other seed oils (Zlatanov et al., 1997; Kiosseouglou and Boskou, 1989). According to Munshi et al. (1982) the differences between fatty acid composition of triacylglycerols and sterol esters may be due to different biosynthetic phases of these compounds and the stages of biosynthesis and accumulation of fatty acids. Thus, firstly sterol esters have been synthesized, and then triacylglycerols have been accumulated. The first stage is also characterized by a high concentration of saturated fatty acids, especially palmitic and stearic acids, which are accumulated in sterol esters.

**Table 3**

**Fatty acid composition of sterol esters and triacylglycerols, g.kg$^{-1}$***

| Fatty acids | Variety | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *Expres* | | *Baldur* | | *Elite* | | *Trabant* | | *Taurus* | | *Rasmus* | |
| | SE | TAG | SE | TAG | SE | TAG | SE | TAG | SE | TAG | SE | TAG |
| $C_{12:0}$ | 10 | ** | 7 | ** | 3 | ** | 2 | ** | 14 | ** | 3 | ** |
| $C_{12:1}$ | 5 | ** | ** | ** | ** | ** | 3 | ** | 3 | ** | 3 | ** |
| $C_{14:0}$ | 19 | ** | 23 | ** | 4 | ** | 4 | ** | 13 | ** | 5 | ** |
| $C_{14:1}$ | 7 | ** | 29 | ** | 5 | ** | 6 | ** | 3 | ** | 4 | ** |
| $C_{16:0}$ | 144 | 51 | 137 | 46 | 97 | 46 | 72 | 46 | 130 | 51 | 73 | 43 |
| $C_{16:1}$ | 7 | 3 | 4 | 2 | 4 | 2 | 2 | 2 | 2 | 4 | 2 | 2 |
| $C_{17:0}$ | 3 | ** | 11 | ** | 2 | ** | 1 | ** | 3 | ** | 3 | ** |
| $C_{18:0}$ | 48 | 19 | 49 | 23 | 39 | 19 | 30 | 18 | 59 | 17 | 42 | 19 |
| $C_{18:1}$ | 609 | 663 | 628 | 663 | 726 | 677 | 768 | 643 | 675 | 639 | 746 | 653 |
| $C_{18:2}$ | 119 | 175 | 87 | 170 | 73 | 164 | 75 | 179 | 58 | 189 | 77 | 176 |
| $C_{18:3}$ | 3 | 71 | 3 | 73 | 4 | 68 | 4 | 74 | 4 | 76 | 4 | 77 |
| $C_{20:0}$ | 6 | 7 | 9 | 10 | 12 | 10 | 10 | 13 | 12 | 9 | 13 | 11 |
| $C_{20:1}$ | 10 | 12 | 11 | 14 | 19 | 14 | 15 | 25 | 16 | 18 | 18 | 19 |
| $C_{22:0}$ | 8 | ** | 1 | ** | 6 | ** | 7 | ** | 8 | ** | 7 | ** |
| $C_{22:1}$ | 3 | ** | 1 | ** | 6 | ** | 2 | ** | ** | ** | 1 | ** |

*Mean of three determinations, ** Not detected

SE – sterol esters, TAG - triacylglycerols

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