The dynamics of the bioactive compounds in oilseeds

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Abstract

Plant sterols have gained lot of interest in recent years. It is mainly because of their cholesterol-lowering effect in human blood serum, therefore they are used as active compounds in medicaments for cardiovascular diseases. Various other important applications, for example cure for some forms of cancer, are also known. Sterols are not only manufactured as medicaments but also as active ingredients in various functional foods. There's an ongoing search for rich sources of natural sterols. Sterols from wood industry's tall oil and soya oil deodorisation destillate have mostly been used so far. The obtained data could be used to acknowledge rapeseed not only as a good source of oil and animal feed but also for bioactive compounds.

The goal of our research was to determine the phytosterol and tocopherol level in locally grown rapeseed oil to evaluate its potential as a valuable source for bioactive compounds besides healthy food component. Moreover, the object of this study is to investigate the effect of various factors on the content and composition of said compounds.

Key words: rapeseed oil, fertilizers, sterol, tocopherol.

Introduction

Rape (*Brassica napus* L.) is now the third most important source of vegetable oil in the world. While the growing area of rapeseed in Estonia has increased tremendously in last two decades, yields have remained relatively low. Low yields are mainly due to misjudgements in agrotechnical principles (Kaarli, 2004).

Rapeseed is getting more popular among Estonian farmers besides linseed, hempseed and golden flax. Its agronomic and economic value is widely known. Besides two main products, oil and meal, rapeseed is also good source of bioactive compounds – phytosterols and tocopherols, containing about 0,5-1,2% of them in oil. It is well-known fact that the main sterol, sitosterol has cholesterol-lowering effect in blood serum and is therefore used as active compound in treatment of cardiovascular diseases. Numerous other medical and non-medical properties are also known (Moreau *et al.,* 2002; Quilez, 2003). Tocopherols are widely recognized as naturally occurring antioxidants. There's a everlasting search for rich sources of natural bioactive chemicals. Sterols from wood industry's tall oil and soya oil deodorisation destillate and tocopherols from various oilseeds have mostly been used so far.

Various scientists have analyzed the sterol composition of rapeseed oil but only few articles have been published on different factors affecting the sterol composition in plants. The obtained data could be used to acknowledge rapeseed not only as a good source of oil and animal feed but also various bioactive compounds. Nevertheless, lot of authors have mentioned that genetic backround, fertilization, weather parameters, growing medium as well as some oil refining steps could have possible effect on sterol content and composition. Therefore, information about the phytosterol and tocopherol content in local rapeseed oil is needed and due to emergence of many small-scale cold-pressing oil enterprises where the origin of the seeds could be detected, this information becomes more important.

The goal of our research was to determine the phytosterol and tocopherol level in locally grown rapeseed oil samples to evaluate its potential as a valuable source for bioactive compounds besides healthy food component. Moreover, the object of this study is to investigate the effect of various factors on the content and composition of said compounds.

Materials and Methods

In order to investigate the phytosterol and tocopherol content in oil of samples, cultivars were used from field trials at the Department of the Field Crop Husbandry at EMU and also National Testing Centers (3). Samples of other oilseeds were from trial fields of Jõgeva Plant Breeding Institute. For the the analysis of different fertilizers, the trial was carried out in 4 replications and the size of plots was 10 x 1 m. The soil type was pallescent soil LP (Kõlli & Lemetti, 1994), a glossisol by FAO classification and a Stagnic Luvisol by WRB classification (Deckers *et al.,* 1998). The trial soil was neutral – pH_{KCl} 6.2; humus 2.4%, available phosphorus 77.7 mg kg^{-1} (AL); mobile potassium 169.8 mg kg^{-1} (AL); calcium 5,648.0 mg kg^{-1}, sulphur 13.5 mg kg^{-1} of the soil. The field was fertilized with mineral complex granular combined fertiliser OptiCropNPK 21-08-12-S-Mg-B-Ca, calculating 120 kg of the active substance agent of nitrogen per hectare. In variants different microfertilizers were used.

Sterol analysis. Method for phytosterol analysis was worked out by using and optimising analytical steps known from literature. Total sterols and sterols in free form were determined. For quantification of sterols, internal standard method was used. Sterol content and composition was detected over a 3-year period (2001-2003) and 6 different microfertilizers were used as variants. Three major sterols (β-sitosterol, campesterol, brassicasterol) which take up about 90% of all the plant sterols in

rapeseed oil, were determined. As sterols occur in vegetable oils in free form or bound with fatty acids, the amount of both forms was calculated.

Sample treatment. For total sterol analysis, the dried seeds were cold-pressed and oil samples (each 500 mg) were taken in 3 replications. 200 µl of internal standard solution (500 mg of cholesterol in 100 ml ethanol) was added. After addition of 0.5 ml KOH solution (60% in water) and 4.5 ml ethanol, the oil samples were hydrolyzed for 45 minutes at 70 °C. Then 3 g of silica was added and the reaction mixture was evaporated to dryness. 1 g of Na_2SO_4, 1 g of silica and 3 g of silica with the sample were loaded onto glassfilter and eluated with 20 ml of ethyl acetate/diethyl ether mixture (1:1). The solvents were evaporated, 1 ml of dichloromethane was added. 1 µl of sample mixture was injected into gas chromatograph.

For analysis of tocopherols and sterols present in free form, oil samples of 250 mg were taken. 200 µl of cholesterol in ethanol and 3 ml of dichloromethane were added. 10 ml solid-phase extraction (SPE) cartridge, filled with 30 µm silica, was conditioned with 10 ml hexane/ethyl acetate mixture (80:1) and 3 ml of sample was loaded. Then, the cartridge was eluted with 20 ml hexane/ethyl acetate mixture (80:1), 30 ml (20:1) and finally the sterol fraction was collected with 20 ml hexane/ethyl acetate mixture (3:1). The solvents were evaporated, 1 ml of dichloromethane was added. 1 µl of sample mixture was injected into gas chromatograph.

Gas chromatography. GC analysis was performed on HP5890 instrument, using 25 m x 0.25 mm, i.d. 0.22 µm, BP-5 fused silica capillary column. Nitrogen was used as carrier gas. The temperature was 300 °C, total time 20 min. Injector 300 °C, flame-ionization detector 340 °C.

Results and discussion

Various rapeseed varieties from national testing centers and also from the trial fields of Estonian University of Life Sciences were used. Method containing oil sample clean-up and subsequent quantification by gas chromatography was worked out. Also the precision of sterol analysis was evaluated.

The limit of detection (LOD, S/N ratio > 3) and limit of quantification (S/N ratio > 10) were determined on the basis of sitosterol. LOD of the GC signal of sitosterol was 5 µg/ml and LOQ was fixed at 62 µg/ml. The average sitosterol content that was determined over a 4-month period twice a week, was 396.9 mg/100 g oil (relative standard deviation 1.09%), average campesterol content was 315 mg/100 g oil and 81.1 mg for brassicasterol, relative standard deviations were 1.15% and 1.17%, respectively.

Different seed samples were further characterized by their oil phytosterol content. In present research the effect of weather and fertilizers on plant sterol content and composition was evaluated. The total sterol content of oil samples varied between 5220 and 6550 mg kg^{-1}, the main sterol being β-sitosterol followed by campesterol and brassicasterol.

The amount of sterols present is decided by the intensity of their biosynthesis and their role in the plant cell. Free sterols are present in cell membrane bilayer where they regulate the fluidity of the membrane and therefore also the transport of different compounds through the cell. Steryl esters are probably the deponated forms of sterols inside the cell. It is also known that plants use phytosterols for adaptation to different temperatures (Piironen *et al.,* 2000).

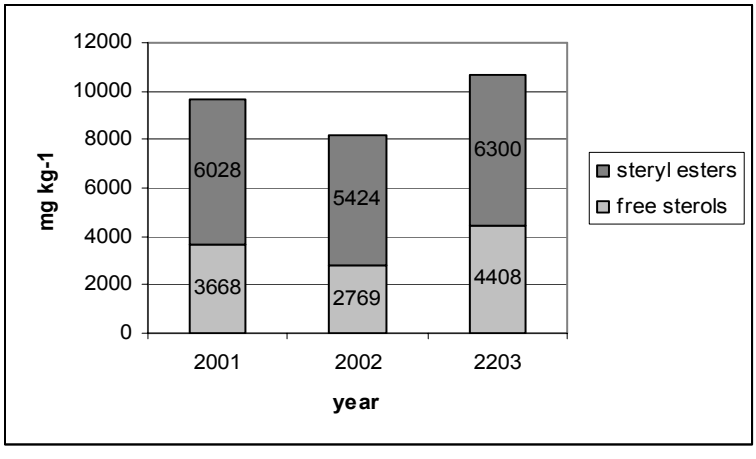

Fig. 1. The sterol content (as free sterols and steryl esters) of the oil from spring rapeseed variety's 'Mascot' in 2001-2003, mg kg^{-1}.

It was found that weather during the growing cycle of rapeseed had a clear impact on sterols. Summer in 2002 was extremely dry and hot, totally opposite to the vegetation period in 2003, while summer in 2001 had about average weather conditions. It was found that the total sterol content varied between 5220-5770 mg kg^{-1} in 2002 and 5910-6550 mg kg^{-1} in 2003 (Figure 1). It is rather difficult to assess the influence of different weather factors to the sterol content. It is known that the sterol levels could change drastically due to long drought period in some plants but linear interpolations could not be made for less extreme weather conditions.

There are couple of articles about the effect of temperature during growing period of soya on the total sterol content. Scientists have found a positive correlation of sterol and higher temperature values (Vlahakis *et al.,* 2000). Present research indicates rather the opposite. While in 2003 where average temperatures were lower than in two previous years, the total sterol content in oil samples was higher. This may be due to effect of other factors including precipitation, soil parameters,

fertilization, etc.

The ratio of free sterols to steryl esters is usually 1/3 to 2/3 according to Lampi *et al* (2004). The amount of different forms of sterols could change due to plants need to adapt to various conditions. Knowing the ratio is mainly necessary for manufacturing functional food products. Results obtained in present research fall into the category described in literature.

There are two important issues from the fertilization point of view. Does the fertilization which ensures good development of the rapeseed plant and high oil yield with good quality parameters also provide bigger amount of plant sterols? Does the fertilization with certain element(s) influence the sterol content and composition in plants? The effect of different microfertilizers on sterol levels was investigated during 3-year period. The dynamics of the main sterol (β-sitosterol) is given below (Table 1). Noticeable differences were observed but no certain influence could not be detected over research period. Correlations between oil yield and total sterol content was also not observed.

Table 1. Content of total β-sitosterol in different oil samples in 2001-2003, mg kg^{-1}.

Variant	β-sitosterol, mg kg^{-1}			Average
	2001	2002	2003	
0 (control)	-	2540	2970	2760
OptiCrop+Copper	2820	2630	2670	2700
OptiCrop+Boron	2690	2500	2760	2650
OptiCrop+Manganese	2690	2370	2890	2650
OptiCrop+Sulphur	-	2440	2890	2670
OptiCrop+Micro Rape	2430	2660	2730	2610
Average	2660	2520	2820	2670

No influence of various micronutrients on different forms of sterols and overall distribution of individual sterols was found.

Tocopherol content has been observed in 10 spring rapeseed cutivars so far. It has been found that only α- and γ-tocopherol have been found in rapeseed oil. Other isomers (β- and δ-tocopherol) were not found in determinable amounts. α- and γ-tocopherol contents varied, respectively 21-27 mg and 46-52 mg/100 g oil in samples of 2004.

It must be said that some of the findings are preliminary in nature and need to studied more deeply to be stated with more certainty. Nevertheless it was found that the weather of the trial years was the most influencing factor concerning the total amount of sterols. Also that spring and winter rapeseed varieties differ mostly in proportions of steryl esters and free sterols. Plant physiologs have suggested that plants tend to use its steryl esters as a defense mechanism against cold temperatures (Ferrari, 1997). Accordingly it was found that the oil of winter rapeseed varieties had significantly higher proportion of steryl esters. In general, the amount of different forms of sterols could change due to plants need to adapt to various conditions. Knowing the ratio of different forms of sterol conjugates is mainly necessary for manufacturing functional food products. Results obtained in present research for spring rapeseed variants fall into the category described in literature that ratio of free sterols to steryl esters is usually 1/3 to 2/3.

Influence of factors affecting sterol and tocopherol content and their content in other oilseeds in Estonia will be discussed in more detail.

Conclusions

There are several factors affecting the amount, proportions etc of said biochemical compounds that are of interest in light of making functional foods, biomedicines. Rapeseed is by far the best source of phytochemicals for industrial extraction in Estonia. It was found that mostly weather during growing period influenced the total content of sterols in rapeseed oil samples. The sterol content of oils from rapeseeds investigated where various fertilizers were used showed variation but the effect of different fertilizers on sterols could not been interpreted clearly. Various other preliminary findings were observed that need more studies.

References

Deckers, J. A. *et al* (Eds.) (1998) World Reference Base for Soil Resources. Introduction. ACCO Leuven, 165. p.

Ferrari, R. A. *et al* (1997) Alteration of Sterols and Steryl Esters in Vegetable Oils during Industrial Refining. Journal of Agricultural and Food Chemistry, 45, pp. 4753-4757.

Kaarli, K. (2004) Õlikultuuride kasvataja käsiraamat. Saku, OÜ Greif, 131 p. (in Estonian)

Kõlli, R. and Lemetti, I. (1994) Eesti muldade lühiiseloomustus I. - Normaalsed mineraalmullad, EPMÜ, Tartu: Trükokoda Tartumaa, 122 p. (in Estonian).

Lampi, A-M. *et al* (2004) Analysis of Phytosterols in Foods. In: Phytosterols as Functional Food Components and Nutraceuticals (Editor P. C. Dutta). New York, Marcel Dekker, Inc., pp. 33-70.

Moreau, R. A. *et al* (2002) Phytosterols, phytostanols and their conjugates in foods: strucutral diversity, quantitative analysis and health-promoting uses. Progress in Lipid Research, 41, pp. 457-500.

Piironen, V. *et al* (2000) Plant sterols: biosynthesis, biological function and their importance to human nutrition. Journal of Science of Food and Agriculture, 80, pp. 939-966.

Quilez, J. *et al* (2003) Potential uses and benefits of phytosterols in diet: present situation and future directions. Clinical Nutrition, 22 (4), pp. 343-351.

Vlahakis, C. and Hazebroek, J. (2000) Phytosterol Accumulation in Canola, Sunflower and Soybean Oils: Effects of Genetics, Planting Location and Temperature. Journal of the American Oil Chemists' Society, 77 (1), pp. 49-53.