INTRODUCTION

Evening primrose oil (*Oenothera biennis* L.) is a rich source of ω-6 series of polyunsaturated fatty acids. One of these fatty acids is gamma linolenic acid – GLA (cis-6,cis-9,cis-12-octadecatrienoic acid). The content of GLA is the single most important parameter to be determined [1,2].

The oil content of evening primrose seeds varies with such factors as the age of the seed, cultivar and growth conditions, and typically varies between 18 and 25%. The oil consists of about 98% triacylglycerols, with small amounts of other lipids (free acids, diacylglycerols, phospholipides) and about 1–2% unsaponifiable matter, of which sterols and tocopherols are some importance [1].

Evening primrose oil is used in increasing amount in nutritional and pharmaceutical preparations. Essential fatty acids are vital components of all membrane structures in the body and are also involved in the production of prostaglandins, which regulate the immune system. A prostaglandin deficiency can cause skin rashes, hair loss, lowered immunity [3,4]. One of the most important unsaturated fatty acids involved in beneficial prostaglandin production is gamma linolenic acid.

Gamma linolenic acid and evening primrose oil have been the focus of many medical studies. Some of these studies claim that evening primrose oil supplementation boost natural immunity, help lower cholesterol levels, reduce blood pressure, can help ease premenstrual syndrome, eczema, diabetes, osteoporosis [3,4].

MATERIALS AND METHODS

Samples

A total of eight evening primrose oils were tested. Samples were obtained from Aromatica, v.o.s. and the other producers. Common lipophilic compounds with stabilized properties were chosen as antioxidants: coenzyme Q₁₀, β-carotene, vitamin E and Origanox™ - extract of *Origanum Vulgare* containing flavonoids. Various ways of storage were chosen for assessment of stability of oils. Maximum time of storage was 183 days from opening the vial with sample.

Methanol esterification method

The fat sample (1.0 g) was saponified with 15 ml methanolic solution of potassium hydroxide (c = 0.5 mol·dm⁻³) for 30 minutes in distilling flask with condenser and was esterified after neutralization by sulphuric acid on methyl orange for 30 minutes again.

After cooling methyl esters were shaken with 10 ml of heptane three times. The extract was dried by anhydrous sodium sulphate and filtered to a 50 ml volumetric flask again. Both heptane portions were rinsed with 20 ml of water twice. The extract was dried by anhydrous
sodium sulphate and filtered to a 50 ml volumetric flask and filled up to the mark with heptane [5].

**GC analysis**

The GC method – gas chromatography was used for identification of the fatty acids. So prepared heptane methyl esters solutions were injected to gas chromatograph using autosampler. The compounds were identified according to available standards.

**GC conditions:** gas chromatograph TRACE GC (ThermoQuest Italia S. p. A., I) equipped with flame ionization detector, split/splitless injector and capillary column SPTM 2560 (100 m × 0.25 mm × 0.2 μm) with the temperature programme 60 °C held for 2 min, ramp 10 °C⋅min⁻¹ up to 220 °C, held for 20 min. The injector temperature was 250 °C and the detector temperature was 220 °C. The flow rate of the carrier gas N₂ was 1,2 ml⋅min⁻¹.

**RESULTS AND DISCUSSION**

Gamma linolenic acid (GLA) is found naturally to varying extents in the fatty acid fraction of some plant seed oils. The content of GLA is the one of the most important parameter for tested evening primrose oils. It is comprised of 18 carbon atoms and three double bonds. Double bonds can be cause of the reduce GLA and it leads to changes GLA during time of storage and used antioxidants.

**Virgin evening primrose oils**

Virgin evening primrose oils samples (Arnaud, Gustav Heess) were stored at 4 °C during time of storage. Evening primrose oil Arnaud was also stored under laboratory conditions. The percentage numbers GLA are shown in producer’s certificates (Arnaud – minimal 9 %, Gustav Heess - in range from 8 to 12 % GLA). The GLA amount changed significantly during storage. In the case of virgin evening primrose oil Arnaud the measure values GLA were lower for up to 1 %. In the case evening primrose oil sample Gustav Heess the content of GLA was about 8 % and it’s in the certificate range.

No-stabilized sample of evening primrose oil which was held on laboratory conditions recorded a considerable loss GLA (decrease of the GLA content – 2,9 %).

**Commercially produced oil samples**

Samples of evening primrose oils with antioxidants (vitamin E, vitamin E and coenzyme Q₁₀, vitamin E and β-carotene) obtained from Aromatica, v.o.s. The GLA content decreased during time of storage slightly. The loss GLA was observed independently of added kind, combination or amount antioxidants.

Dependent the content of GLA on time of storage three samples commercially produced evening primrose oils with the addition of antioxidants is shown in Fig. 1. In the case evening primrose oil with coenzyme Q₁₀ a vitamin E a decrease was 0,6 % GLA, the sample with β-carotene and vitamin E 1,3 % GLA and in the case evening primrose oil with vitamin E a loss was 1,0 % GLA. The decrease GLA related to the total content of fatty acids.
Laboratory prepared samples

Two samples (evening primrose oil with Origanox™ and evening primrose with mixture antioxidants coenzyme Q₁₀, β-carotene and vitamin E) were laboratory prepared. In the case both of samples the GLA content changed slightly over time of storage (Fig. 2).

Fig. 1: Variation over time of storage in gamma linolenic acid content of commercially produced evening primrose oils with antioxidants

Fig. 2: Variation over time of storage in gamma linolenic acid content of laboratory prepared evening primrose oils with antioxidants
Compare all tested evening primrose oils

The GLA content was similar for the all tested evening primrose oils and range of GLA was from 5 to 11 % of total fatty acids. The content of GLA decreased gradually during time of storage. Compare of the GLA content in various tested evening primrose oils at first and last (183) day of storage is shown in Table 1.

Maximum level GLA recorded virgin evening primrose oil Arnaud in 147 day of storage (10,40 ± 0,17 %). Minimal amount GLA was found in the sample evening primrose oil at 183 day of storage, which was stored under laboratory conditions (5,65 ± 0,06 %). Evening primrose oil with antioxidant Origanox™ changed minimally. Whereas, most losses recorded no-stabilized evening primrose oil which was hold on laboratory conditions.

<table>
<thead>
<tr>
<th>Evening primrose oils</th>
<th>Time of storage [days]</th>
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<tbody>
<tr>
<td></td>
<td>1</td>
<td>183</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GLA [%]</td>
<td>s_r [%]</td>
<td>GLA [%]</td>
</tr>
<tr>
<td>+ Q_{10} + vitamin E</td>
<td>8,00 ± 0,10</td>
<td>1,27</td>
<td>7,39 ± 0,03</td>
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<tr>
<td>+ β-carotene + vitamin E</td>
<td>8,47 ± 0,09</td>
<td>1,07</td>
<td>7,24 ± 0,10</td>
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<tr>
<td>+ vitamin E</td>
<td>8,77 ± 0,00</td>
<td>0,01</td>
<td>7,75 ± 0,04</td>
</tr>
<tr>
<td>+ Q_{10} + β-carotene + vitamin E</td>
<td>7,26 ± 0,18</td>
<td>2,44</td>
<td>7,00 ± 0,06</td>
</tr>
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<td>+ Origanox™</td>
<td>8,84 ± 0,04</td>
<td>0,43</td>
<td>8,72 ± 0,18</td>
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<td>virgin, Arnaud</td>
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<td>1,63</td>
<td>8,01 ± 0,13</td>
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<td>virgin, Gustav Heess</td>
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<td>0,68</td>
<td>7,63 ± 0,02</td>
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<tr>
<td>virgin, laboratory conditions</td>
<td>8,55 ± 0,00</td>
<td>0,04</td>
<td>5,65 ± 0,06</td>
</tr>
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</table>

CONCLUSION

Primrose is a pure natural vegetable oil processed from the seeds of the evening primrose plant. Evening primrose oil is higher in total essential fatty acids than any other vegetable oil. The oil contains 74 % linolenic acid (LA) and 8-10 % gamma linolenic acid (GLA).

The purpose of this work was to find out and compare the influences of used antioxidants and time of storage in respect of the content of fatty acids in evening primrose oil. Results of gas chromatography were statistically compared to determine the relationship between the stability of oils and the type of the antioxidant or the way of storage.

A total of eight evening primrose oils (virgin or with addition of antioxidants - coenzyme Q_{10}, β-carotene, vitamin E and Origanox™) were considered.

Various ways of storage were chosen for assessment of stability of oils. Methanol esterification method using potassium hydroxide catalysis was applied to oil for preparing fatty acids methyl esters. Gas chromatography (GC) was applied for the determination of fatty acids content (especially gamma linolenic acid - GLA). The compounds were identified by their retention times relative to authentic standards.

Gamma linolenic acid was present most often in concentration 7 – 9 % of total fatty acids in the samples of evening primrose oils. The content of GLA decreased gradually depending on increasing time of storage. Evening primrose oil, which was stored under laboratory conditions, showed the greatest losses GLA. Whereas, sample evening primrose oil containing Origanox™ had minimal changes. It was observed great dissimilarity GLA during another way of storage of oils with addition antioxidants. Virgin evening primrose oils have comparable levels of GLA.
Addition of antioxidants, their amounts or combination have no influence on changes in oils during the storage. However, the way of storage can influence on the content of GLA.

REFERENCES