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Stability of Grape Seed Oil and its Antioxidant Tocotrienols

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Abstract. For the experiment three different storage conditions were chosen: storage at room temperature of 22 °C in the light and in the dark and in the dark in a refrigerator at 4 °C. Parameters monitored were: peroxide value and changes in the content of α-, γ- and δ-tocotrienols and α - and γ-tocopherols during storage for 210 days (30 weeks). The peroxide value is an indicator of the content of primary oxidation products of oils. From analytical analyses results that the greatest destruction of grape oil occurs during storage at room temperature and access of light, where a peroxide value increased up to 484 meq. O_2/kg oil). The least intrusive method of storage was in terms of temperature refrigerator (4 °C) in the dark, when during 30 days of storage peroxide value had risen only to 71.9 meq. O_2/kg oil. Between these values were values stored at room temperature in the dark (after 30 weeks storage 196 meq. O_2/kg oil). From these parameters is clearly showed that to the stability of oil contribute significantly both factors - temperature and light conditions. The same trend was also found in tocotrienols. At room temperature and access of light was complete decomposition of α-tocotrienol in the 9th week of storage, γ-tocotrienol at 30 weeks of storage and δ-tocotrienol in the 18th week of storage. The most stable seems γ-tocotrienol > δ - tocotrienol > α-tocotrienol. When stored in the refrigerator in the dark, there was practically no decomposition of α-, γ- and δ-tocotrienols whose contents remained completely unchanged.

Introduction

Grapes are one of the major fruit crops and about 80% of the harvest is used by the winemaking industry, which leads to the generation of large quantities of seed by-product [1]. Grape seed oil has a large scale of application being used in various fields from cosmetics to cooking. Grape seed oil is gaining popularity as culinary oil, and has been studied as a possible source of specialty lipids. Grape seed oil is rich in biologically active compounds, between them in lipophilic antioxidants – tocotrienols and tocopherols. A high proportion of tocotrienols in the grape seed oil, which together with tocopherols are included in the group of substances with vitamin E activity, may cause that the grape oil is significantly different from other vegetable oils. The wide spectrum of different positive effects of tocotrienols on human health described in detail Aggarwal et al. [2].Of our knowledge, no study has been performed until now that focused on the potential use of seed oils of grape varieties grown in the Czech Republic. As a part of our ongoing efforts to develop value-added utilizations of fruit seeds, this study was conducted to determine grape seed oils stability and especially stability of tocotrienols fraction.

Materials and Methods

Plant material. In the experiment the André variety from the harvest in 2012 and the winery Lednice (Moravia) for determination of the stability of tocotrienols was used.

Storage method. For the experiment three different storage conditions were chosen: storage at room temperature of 22 °C in the light and in the dark and in the dark in a refrigerator at 4 °C (storage for 210 days, i.e. 30 weeks).

Determination of peroxide value. Peroxide value was determined by a slightly modified method based on the Czech technical standard ČSN EN ISO 27107. Principle of the method consists in the titration determining of the iodine released from potassium iodide hydroperoxides of unsaturated lipids in the acidic environment with 0.01 M sodium thiosulfate solution. End of the titration is indicated by the starch solution. Peroxide value represents the amount of substances in solution, which oxidize potassium iodide under given conditions and are characterized by degradation (oxidation) of unsaturated lipids present in the oil. The result is expressed in milliequivalents of active oxygen per 1 kg of oil.

Determination of the various forms of tocotrienols. 100 μl of the homogenized oil was pipetted into 10 ml volumetric flask and completed to volume with isopropanol. The sample was placed in an ultrasonic bath (Notus-Powersonic, Slovakia) and then mixed thoroughly. An aliquot was transferred though a nylon micro-filter (0.22 micron) in a dark vial. Individual tocotrienols were determined by HPLC with isocratic elution and fluorescence detection using the chromatographic system Ultimate 3000 (Dionex, USA) analytical column Develosil RPAQUEOUS 5u (250 x 4.6 mm) with the guard column Develosil 5u C30-UG 100A (10 × 4 mm) (Phenomenex, USA), which allows the separation of all forms of tocopherols and tocotrienols. The mobile phase consisted of a mixture of methanol and deionized water (97:3, v/v) at a flow rate of 1 ml/min. Column temperature was 30 °C and 10 μl sample injection. For detection of the samples the following wavelengths were used: Excitation - 292 nm and emission at 330 nm. Contents of tocotrienols were expressed in mg/kg oil.

Results and Discussion

In all samples of wine oil similar representation of tocotrienols was detected. Major components of vitamin E were γ- and α-forms. A small amount was represented by δ-tocotrienol (about 1%). Other tocotrienols did not exceed the limit of detection (LOD = 0.01-0.02 μg/ml, LOQ = 0.03-0.04 μg/ml). Dolde and Wang [3] reported that higher levels of some forms of vitamin E are one of the factors that may significantly affect the stability of the oil. Storage method, but also the variety of the vine had a significant impact on the total tocotrienol content and value of the peroxide value. From analytical analyses results that the greatest destruction of grape oil occurs during storage at room temperature and access of light, where a peroxide value increased up to 484 meq. O_2/kg oil (Fig. 1). The least intrusive method of storage was in terms of temperature refrigerator (4 °C) in the dark, when during 30 days of storage peroxide value had risen only to 71.9 meq. O_2/kg oil. Between these values were values stored at room temperature in the dark (after 30 weeks storage 196 meq. O_2/kg oil). From these parameters is clearly showed that to the stability of oil contribute significantly both factors - temperature and light conditions.

The same trend was also found in tocotrienols (Figs. 2-4). At room temperature and access of light was complete decomposition of α-tocotrienol in the 9th week of storage, γ-tocotrienol after 30 weeks of storage and δ-tocotrienol in the 18th week of storage. The most stable seems γ-tocotrienol > δ -tocotrienol > α-tocotrienol. When stored in the refrigerator in the dark, there was practically no decomposition of α-, γ- and δ-tocotrienols whose contents remained completely unchanged.

The increase in peroxide value of the values of different samples of wine oil at higher temperatures was also confirmed by Kim et al. [4]. The values of the peroxide value set in grape oil stored at 25 °C at the end of three months storage ranged from 50-100 meq. O_2 for 1 kg of oil corresponding to the values of the peroxide values provided in this study.

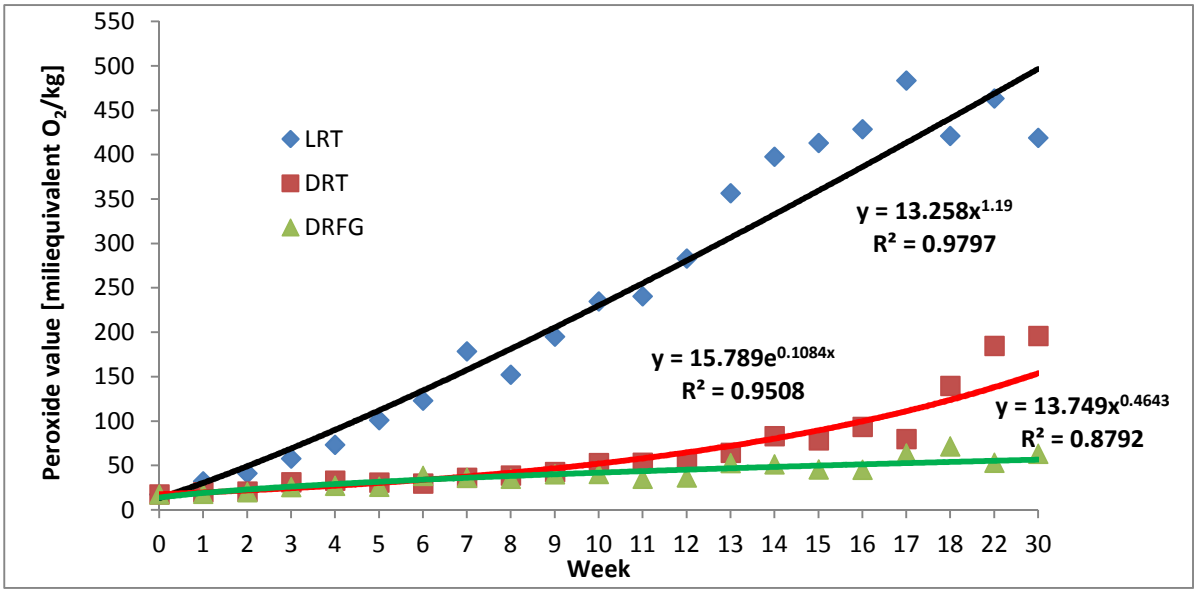

Fig. 1. Peroxide value of the oil samples during 30 weeks storage

LRT – light, room temperature, DRT – dark, room temperature, DRFG – dark, refrigerator

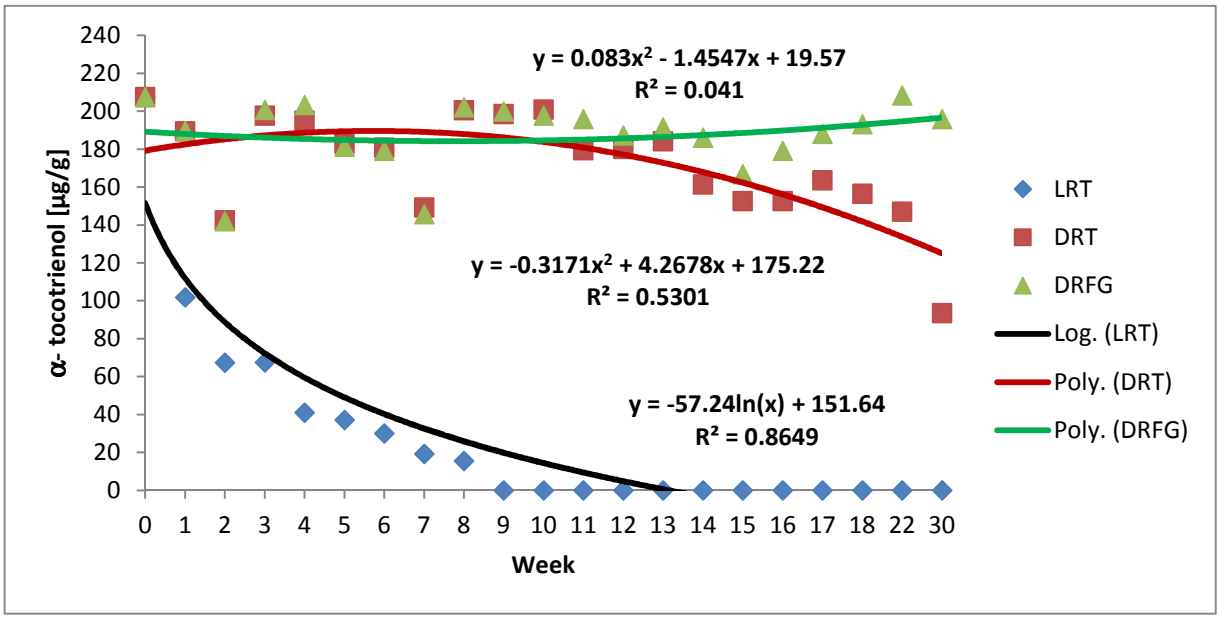

Fig. 2. Stability of α-tocotrienol

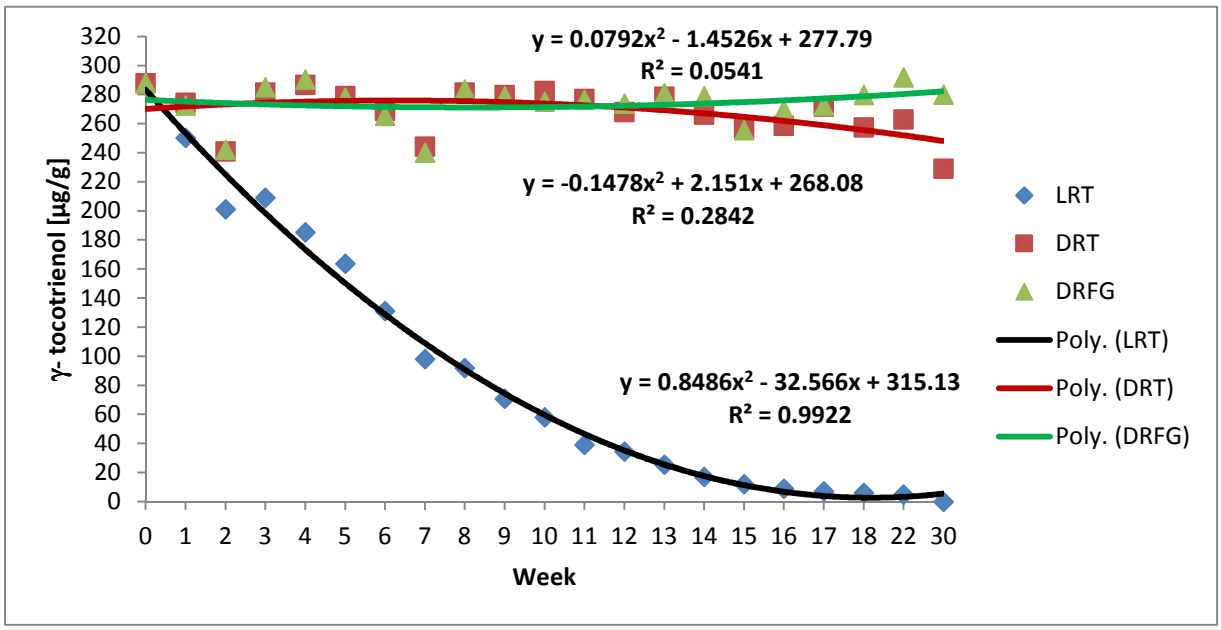

Fig. 3. Stability of γ-tocotrienol

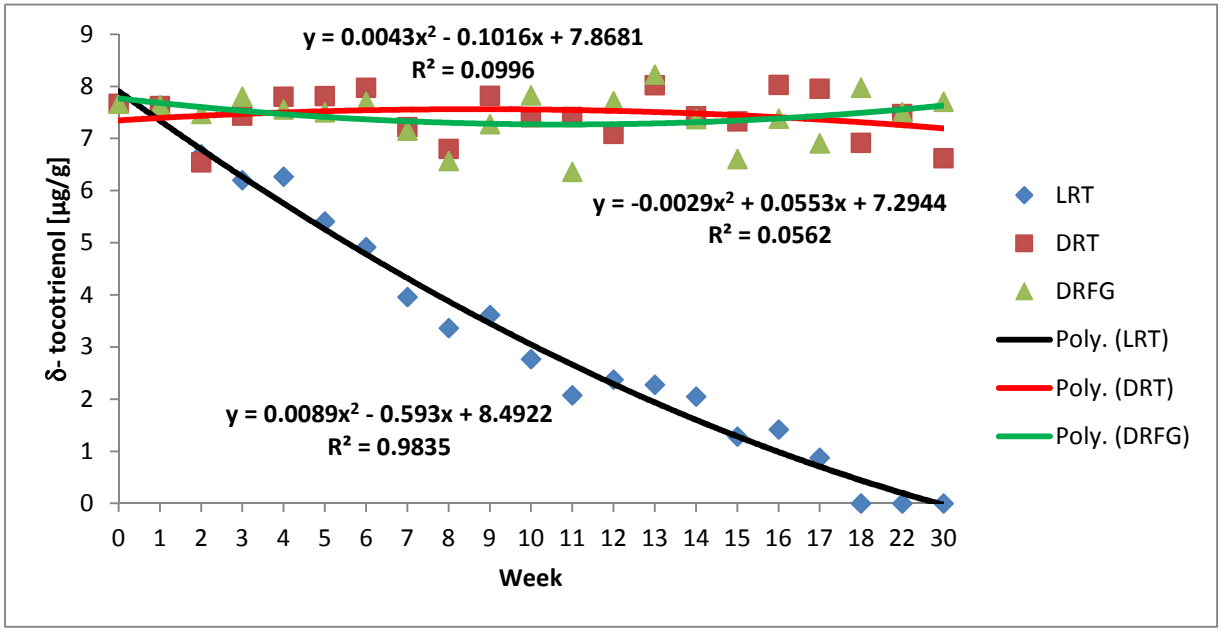

Fig. 4. Stability of δ-tocotrienol

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