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DETERMINATION OF TOCOPHEROLS CONTENT IN SUNFLOWER OIL DURING OXIDATION USING FLUORESCENCE TECHNIQUE

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Abstract. The fluorescence intensity of tocopherols originated from cold-pressed sunflower oil at different degree of oxidation in presence of 1,2-dipalmitylo-sn-glicero-3--fosfatidylocholine lipid (DPPC) membrane have been measured. It has been shown that fluorescence of tocopherol depends on amount of oil as well as on membrane concentration in the sample. Addition of oil with increasing peroxide value PV decreased the tocopherol fluorescence due to its disappearance in the sample. At constant membrane concentration chromatographically determined the amount of tocopherol in oil sample correlated with peroxide value and fluorescence intensity. Such results allowed us construct calibration curve which may be used for fast and accurate peroxide value determination using fluorescence technique.

Key words: sunflower oil, tocopherols, peroxide value, DPPC membrane, fluorescence

INTRODUCTION

Edible oils and plant fats obtained from oily plant seeds are becoming a growing component of our diet. However, this growing business increases the responsibility of the producers for the high quality of the offered products [O'Brien 2004, Flaczyk and Korczak 2002]. To assure high oil quality, it is necessary to perform many types of analysis e.g. the determination degree of hydrolysis and amount of the peroxides [Kania et al. 2002]. Evaluation of the composition of unsaturated fatty acids, especially its transisomers and tocopherol content, is of major importance due to the significant role they play in our body. The presence of those components influences the quality and nutritional value of the oils [Eitenmiller and Lee 2004].

In oil vitamin E, a mixture of four tocopherol homologues, acts as a natural antioxidant. Its specific function played in vivo is protection of the membrane against free radi-

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cals and fatty acids peroxides. Oxidation of membrane phospholipids introduces its disfunction which influences its rigidity, permeability and morphology, leading finally to the development of degenerative diseases or causing aging of the cells [Mawatari and Murakami 1998, Gal et al. 2003]. In lipid tissue tocopherol locates itself at the interface of hydrophyllic and hydrophobic phases of the membrane where it efficiently prevents oxidation of unsaturated fatty acids [Gal et al. 2003, Paiva-Martins et al. 2003]. Its specific location depends also on the fatty acid composition of the membrane [Wang and Quinn 2002].

Tocopherol stabilizes the membrane structures through the formation of complexes between vitamin E and products of membrane lipid hydrolysis such as lysophospholipids and free fatty acids [Wang and Quinn 1999, 2000]. The presence of tocopherol in liquid crystalline phase of the lipid bilayer decreases the acyl chains mobility whereas in the gel phase the mobility increases [Massey 2001, Aalast et al. 2004]. The functions of vitamin E are not restricted to membrane protective role but a variety of actions of the vitamin have been recognized.

The distribution of vitamin E in the body differs from one tissue to another. Its highest concentration was found in adipose, liver and muscle tissue [Wang and Quinn 2002]. It has been shown that higher vitamin E concentration was measured in tissue which is exposed to UV radiation [Shapiro and Saliou 2001].

The composition and properties of cellular membranes depend not only on the age but its condition depends on the presence of the fatty acids in its structure. The presence of oleic acid in the diet, as in the Mediterranean diet, is connected with its increasing presence in human cells [Prades et al. 2003]. Nutritional, pharmaceutical and epidemiological data indicate that oxidative destruction of the cells, especially the fatty acids from ω-3 and ω-6 family, may be repaired by intake of those fatty acids in food by proper selection of plant and fish fatty acids. Among schizophrenia patients using food rich in ω-3 fatty acids the improvement in physical behaviour was observed, whereas in the control group fed with food with animal fatty acids such an effect was not observed [Mahadik et al. 2001].

The key factor to understand how vitamin E acts in the membrane is how and where it is localized and how it influences the membrane structure. Due to the partition of tocopherols between water-oil emulsion and DPPC membrane and the fact that intrinsic fluorescence of monomeric tocopherol molecule arises only from hydrophobic environment, it was possible to quantitatively determine the amount of intact tocopherol molecules in the membrane. In this work the studies of α-Toc fluorescence originated from cold-pressed sunflower oil at different degrees of oxidation in the presence of DPPC membrane were carried out.

MATERIAL AND METHODS

Materials

Cold-pressed sunflower oil was purchased from the local market. Thermal autooxidation of the oil was carried out in the dark, at 60º C in the presence of air on the flat Petri dish. Membrane 1,2-dipalmitylo-sn-glicero-3-fosfatydylocholine (DPPC), purity > 99%, from SIGMA. The standards of tocopherols: alpha-, beta-, gamma-, delta-tocopherol, purity ≥ 95%, from CALBIOCHEM.

Method of investigations

Peroxide value. Peroxide value was marked according with methodology PN-ISO 3960 [1996].

Tests content of tocopherols by HPLC. In order to determine the tocopherol content, samples of sunflower oil were saponified using 60% KOH. After saponification the unsaponifiable substances were extracted three times using peroxide free diethyl ether. Then the ether was distilled off and the residue was dissolved in n-hexane. The qualitative identification and quantitative determination of homologous tocopherols were carried out by HPLC [Nogala-Kałucka et al. 2003]. The tocopherols were analysed on the Waters HPLC system equipment with LiChrosorb Si 60 column (250 × 4.6 mm; 5 μm) and precolumn LiChrospher Si 60. The mobile phase was n-hexane and 1,4-dioxane. The tocopherols were monitored by a fluorimetric detector (295, 330 nm). Contents of individual tocopherol homologoues were calculated on the basis of calibration curves for pure forms of these compounds. Standards of alpha-, beta-, gamma- and delta--tocopherol (α-T, γ-T, β-T, δ-T; 99%) were purchased from Merck (Darmstadt, Germany). Two oil samples were used and all analyses were repeated three times.

Investigation in model arrangements membrane-vegetable oil. Membrane DPPC was dissolved in chloroform, and sunflower oil in hexane. They were mixed and vaporized in temperature 30°C during 5 min on evaporator vacuums ROTAVAPOR-EL from BUCHI. Samples were kept 24 h in exsiccator with silica gel under vacuum. After hydration 10 ml of distilled water was added and stirred by 20 min on magnetic stirrer MR 3001K from Heidolph, at 51°C, temperature above the gel-liquid crystalline transition. Then the sample was cooled down to 22°C. Fluorescence spectra were measured by Shimadzu 1501 PC fluorimeter in 1 cm cuvette at room temperature, 22º C, at excitation wavelength λ = 295 nm and emission λ = 305-450 nm.

RESULTS AND DISCUSSION

Synthetic membranes are structurally close to cell membranes and therefore are often used for the investigation of the structure in the presence of the other membrane cell components like protein or antioxidants [Castelli et al. 1997].

In order to estimate the tocopherol concentration in the membrane its endogenous monomer fluorescence was used. The fluorescence intensity changes with increasing oil content at the presence of 3.35 mg/ml DPPC membrane are presented in Figure 1. Nonlinear character of that dependence reflects very probably the formation of different sizes of DPPC membranes including multilamellar vesicles. The inclusion of α-Toc into membrane occurs fast at low concentration. The self quenching and location in the membrane in internal vesicles are the processes responsible for the dependence observed at higher tocopherol concentrations.

In order to estimate the intake of the α-Toc from the oil samples by the membrane the fluorescence intensity changes during increasing DPPC membrane concentration at constant oil concentration (50 mg/ml) were carried out. The results are presented in Figure 2. The observed linear dependence suggests that increasing membrane concentration offers more locations where tocopherol molecule may be included.

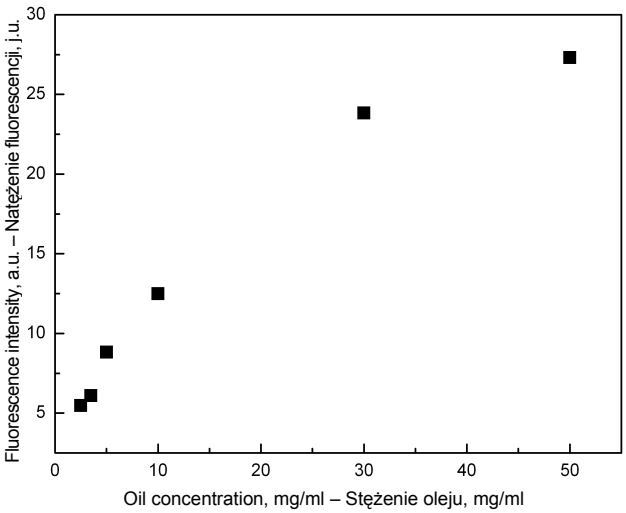

Fig. 1. Fluorescence intensity changes with increasing sunflower oil content at constant DPPC membrane concentration (3.35 mg/ml)
Rys. 1. Zmiany intensywności fluorescencji wraz ze wzrostem zawartości oleju słonecznikowego przy stałej koncentracji membrany DPPC (3,35 mg/ml)

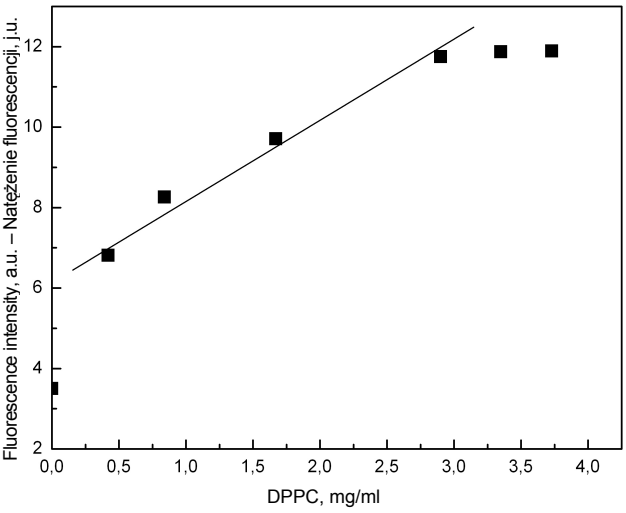

Fig. 2. Fluorescence intensity changes depending on the DPPC membrane concentration at constant sunflower oil concentration (50 mg/ml)
Rys. 2. Zmiany intensywności fluorescencji w zależności od stężenia membrany DPPC przy stałym stężeniu oleju słonecznikowego (50 mg/ml)

Due to their hydrophobic nature tocopherols are insoluble in water but soluble in organic solvents and in the hydrophobic interior of the lipid bilayer. In emulsion tocopherol fluorescence is very weak, whereas in the presence of added DPPC membrane α-Toc fluorescence increases significantly. The results suggest that α-Toc present in the oil in aqueous solution emulsion should be transferred into DPPC membrane where its fluorescence increases as the oil is added to the sample.

Native antioxidants including tocopherols present in the oil during oxidation undergo chemical processes decreasing its activity and concentration. To estimate how tocopherol content in oil sample changes during oxidation the investigation of fluorescence intensity changes with oil samples with different peroxide value in the presence of DPPC was carried out. The results presented in Figure 3 indicate that with increasing peroxide value of the added oil sample the tocopherol fluorescence decreases. This suggests that oil with higher peroxide value contains lower amount of tocopherol monomers which may be embedded into DPPC membrane.

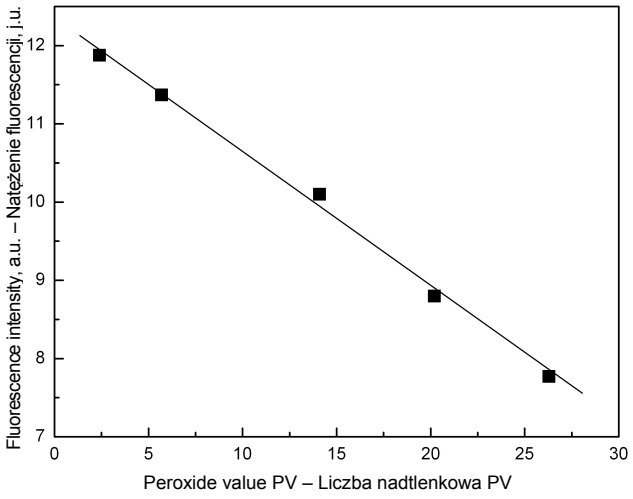

Fig. 3. Fluorescence intensity changes depending on the peroxide value of sunflower oil in the presence of DPPC membrane at concentration (3.35 mg/ml)
Rys. 3. Zmiany intensywności fluorescencji w zależności od wartości liczby nadtlenkowej próbek oleju słonecznikowego (3,35 mg/ml)

The HPLC method was used to determine the amount of tocopherols present in sunflower oil at different degrees of its oxidation. The results were correlated with the tocopherol fluorescence observed in model DPPC membrane-oil system and are presented in Figure 4. This relation in the whole studied region is nonlinear however, in the range up to PV = 20 linear dependence with high correlation coefficient between tocopherol fluorescence arising from the DPPC and tocopherol content in the oil sample is obtained.

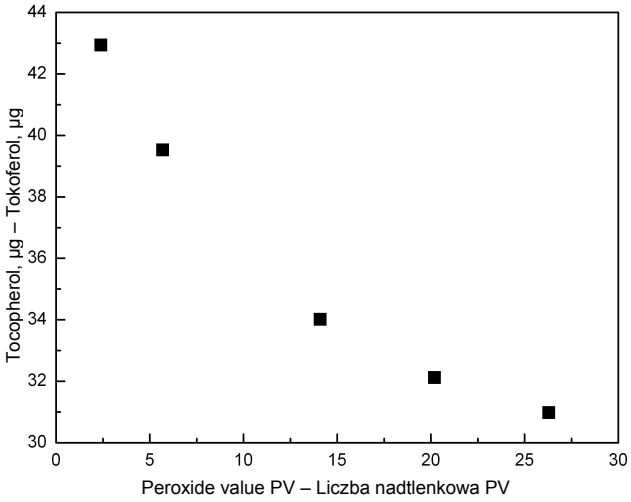

Fig. 4. Amount of tocopherol obtained by HPLC method from the oil samples at different peroxide value PV
Rys. 4. Zawartość tokoferoli w próbkach olejów o różnej liczbie nadtlenkowej oznaczonych metodą HPLC

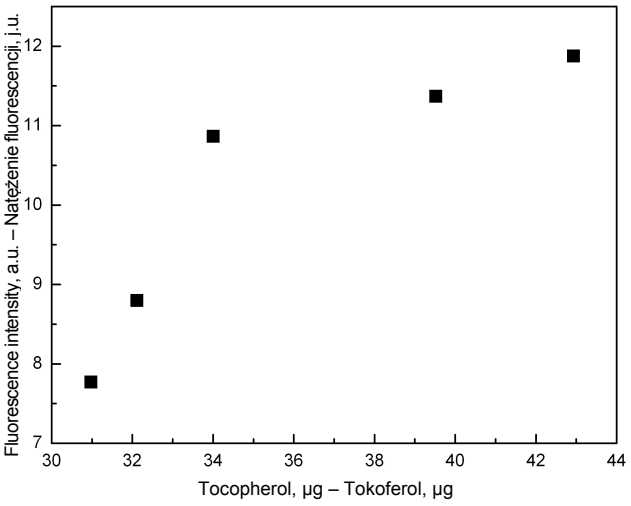

Fig. 5. Fluorescence intensity changes versus amount of tocopherol present in the sample. Calibration curve obtained from the data presented in Figure 3 and Figure 4
Rys. 5. Zmiany intensywności fluorescencji w stosunku do zawartości tokoferolu w próbce. Krzywa kalibracyjna otrzymana na podstawie danych przedstawionych na rysunku 3 i 4

CONCLUSIONS

The obtained data allowed to prepare a calibration curve which shows the dependence between a fluorescent, native tocopherol molecule and the degree of oil oxidation described by its peroxide value. Such a calibration curve is presented in Figure 5. The observed dependence between fluorescence and the amount of tocopherols in oil is nonlinear. This nonlinearity arises very probably from the fact that the cold pressed sunflower oil includes the other antioxidants and components which may screen the tocopherol fluorescence or introduce undefined errors to the presented results. However, for the specific product obtained calibration curve may be used for fast and accurate determination of the peroxide value as well as for the accurate tocopherols content during oil oxidation.

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OKREŚLENIE ZAWARTOŚCI TOKOFEROLI PODCZAS UTLENIANIA OLEJU SŁONECZNIKOWEGO METODAMI FLUORESCENCJI

Streszczenie. Przeprowadzono badania intensywności fluorescencji tokoferoli pochodzących z oleju słonecznikowego o różnej liczbie nadtlenkowej w obecności membrany lipidowej DPPC. Stwierdzono, że ilość oleju roślinnego i stężenie membrany w układzie mają wpływ na intensywność fluorescencji tokoferoli. Dodatek oleju słonecznikowego o wzrastającej liczbie nadtlenkowej wpływał na zmniejszenie fluorescencji, związane ze spadkiem zawartości tokoferoli w układzie. Pokazano, że przy stałym stężeniu membrany natężenie fluorescencji tokoferolu, chromatograficznie zmierzona zawartość tokoferolu oraz liczba nadtlenkowa oleju są skorelowane ze sobą. Pozwoliło to na przygotowanie krzywej kalibracyjnej do szybkiego oznaczania liczby nadtlenkowej określonej porcji oleju metodą fluorescencyjną.

Słowa kluczowe: olej słonecznikowy, tokoferol, liczba nadtlenkowa, membrana DPPC, fluorescencja

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