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Effect of biologically active substances on antioxidant activity in rapeseed oil tested in accelerated oxidative process*

Wpływ dodatku wybranych substancji biologicznie czynnych na aktywność antyoksydacyjną oleju rzepakowego poddanego przyspieszonemu procesowi autooksydacji

Key words: tocopherols, free radical scavenging: DPPH, menadione K_3, ubiquinon Q_{10}, beta-carotene, quercetin

In the study rapeseed oil was enriched with such substances as: β-carotene, coenzyme Q_{10}, menadione K_3 and quercetin. Addition of these substances to rapeseed oil may increase its stability and exposed healthy properties.

The influence of mentioned above additives was analyzed in rapeseed oil which was to accelerated oxidation process in temperature 60°C. Process of autoxidation was monitored by determination of peroxide value. Decomposition of tocopherols and scavenging of free radicals $DPPH^{\bullet}$ were examined by evaluation of antiradical efficiency coefficient (AE).

Substances added to rapeseed oil improved antiradical efficiency (AE). Addition of biologically active substances restrained alpha-tocopherol decomposition. Similarly quercetin influenced gamma- and delta-tocopherols. Other additives restrained tocopherols decomposition after 24 hours of study. Only quercetin restrained rapeseed oil autoxidation process.

Słowa kluczowe: tokoferole, wygaszanie wolnych rodników DPPH, menadion K_3, koenzym Q_{10}, beta-karoten, kwercetyna

Zwiększona aktywność badawcza dotycząca substancji o właściwościach antyoksydacyjnych jest spowodowana ich funkcjami ochronnymi w organizmie ludzkim. Przeciwutleniacze wygaszają wolne rodniki, które przyczyniają się do rozwoju wielu chorób cywilizacyjnych, np. choroby niedokrwiennej serca. Do substancji o właściwościach przeciwutleniających obok tokochromanoli należą także β-karoten, koenzym Q_{10} i kwercetyna. Dlatego też w tej pracy podjęto badania nad wpływem tych substancji oraz menadionu K_3 na wygaszanie wolnych rodników $DPPH^{\bullet}$ przez natywne substancje zawarte w oleju rzepakowym w warunkach testu termostatowego (60°C).

Oznaczono zawartość tokoferoli, skład kwasów tłuszczowych oraz ogólną zawartość związków fenolowych analizowanego oleju. Zbadano zdolność wygaszania wolnych rodników $DPPH^{\bullet}$ oraz, dla obiektywnego wyznaczenia potencjału przeciwutleniającego, obliczono współczynnik aktywności antyrodnikowej (AE).

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Dodatek kwercetyny spowalniał proces autooksydacji oleju rzepakowego przechowywanego w 60°C. Pozostałe substancje przyspieszały utlenianie oleju. Wszystkie substancje z wyjątkiem menadionu spowalniały rozkład alfa-tokoferolu w oleju rzepakowym. W pierwszych 24 godzinach tylko dodatek kwercetyny spowalniał rozkład gamma-tokoferolu, w trzeciej i czwartej dobie doświadczenia także pozostałe substancje hamowały rozkład tego homologu. Po pierwszej dobie najwolniej ulegał rozkładowi delta-tokoferol w oleju rzepakowym z dodatkiem kwercetyny oraz menadionu K_3, w czwartej dobie zaobserwowano, że wszystkie substancje dodane do oleju rzepakowego spowalniały rozkład delta-tokoferolu.

Wszystkie substancje z wyjątkiem menadionu dodane do oleju rzepakowego zwiększały zdolność wygaszania rodników $DPPH^{\bullet}$. Współczynnik aktywności antyrodnikowej oleju rzepakowego zwiększał się po dodaniu substancji biologicznie czynnych.

Introduction

Vegetable oils are ones of the basic nutrients. They are a condensed source of energy and building material of cell membrane and a good source of unsaturated fatty acids and vitamins A, D, E (Ziemlański, Topolowska 1991). In Poland rapeseed oil is the most common consumption oil, which contains mostly α-, γ-tocopherols and small quantity of δ- and β-tocopherols (Nogala–Kałucka et al. 1985, 2000; Warner and Mounts 1990). Rapeseed oil contains poliphenolic compounds, mostly sinapic acid and its choline ester (Dąbrowski and Sosulski 1984, Shahidi et al. 1995).

These substances influence proper functioning of human organism. β-carotene is a precursor of vitamin A and it is an antioxidant which quenches singlet oxygen. Coenzyme Q_{10} takes part in electron transportation in respiration chain and its supplementation is recommended in older age. Quercetin is a polyphenolic compound which has strong antioxidant properties. Vitamin K_3 takes part in blood coagulation process and demonstrates antibacterial properties.

For many years different studies have been undertaken to examine the influence of oils enrichment with substances that extend their durability and healthy properties. The addition of each substance is strictly definite and restricted by numerous European law regulations No. 95/2/EC of 20 Feb. 1995.

Substances such as β-carotene, ubiquinon, menadione and quercetin are important for proper functioning of human organisms. β-carotene, precursor of vitamin A, is an antioxidant that quenches singlet oxygen (Yanishlieva-Maslarova 2001). Coenzyme Q_{10} take part in electron transportation in respiration chain and its supplementation is recommended in older age (Sinatra 1998). Quercetin is a polyphenolic compound, which has strong antioxidant properties (Oszmiański 1995, Trzeciak 2001). Vitamin K_3 takes part in blood coagulation process and demonstrates antibacterial properties (Ziemlański, Topolowska 1991). Addition of these substances to oil may increase its antiradical properties, extend its consumption duration and increase its health properties.

The aim of the study was to examine the influence of substances mentioned above on rapeseed oil properties tested in accelerated oxidative process at temperature 60°C. We determined the influence of additives on capacity of quenching of free radicals $DPPH^{\bullet}$ in rapeseed oil.

We studied the influence of additives on velocity of decomposition of tocopherols' homologues. The capacity of quenching free radical $DPPH^{\bullet}$ was examined and antiradical efficiency coefficient (AE) was evaluated.

Materials and methods

In the study we used "Kujawski" refined rapeseed oil. Samples of rapeseed oil with coenzyme Q_{10}, menadione, β-carotene, and quercetin in amount 0.001% were tested. We chose concentration of additives in amount of 0.001% because β-carotene in amount above that works prooxidative, furthermore other additives also have biological properties and their amount is strictly determined in a diet. Samples were stored in Petri dish, thickness of layer was 10 mm. Petri dishes were stored in darkness and incubated at the temperature 60±1°C. The process of autoxidation was monitored by changes of LOO which was determined periodically, according to Polish norm PN-ISO 390 of October 1996.

Determination of tocopherols' content

Separation and identification of tocopherols' homologues was performed using HPLC (Waters 600 Asc. Milford) systems consisting of the gradient pump Waters Model 600, fluorometric detector and Waters Millenium 32 data acquisition system. Samples dissolved in n-hexane were injected into the LiChrosorb Si 60 column (250 × 4.6 mm, 5 μm Merck), and the mixture of n-hexane and 1,4 diaxane (93:3 v/v) was used as a mobile phase. The flow rate was 1.5 ml/min. Fluorometric detector (Water 474) worked by excitation $\lambda = 290$ nm and emission $\lambda = 330$ nm. Quantitative identifications were calculated from calibration curves made for individual tocopherols.

Determination of total polyphenolic compounds content

Polyphenols compounds were extracted by threefold shacking of mixture of oil with methanol 1:1 v/v at room temperature by 1 hour. Next, mixture was centrifuged for 5 min at 3000 rpm in order to better separate methanol fraction (hydrophilic) (Espin et al. 2000). The level of polyphenolic compounds was determined using spectrophotometry with Folin-Ciocalteau reagent, using as an equivalent chlorogenic acid according to Swain and Hillis (1959).

Determination of antioxidant activity of investigated systems

In tested oils antioxidant activity was monitored by changes of absorption band of 1,1-diphenyl-2-picrylhydrazyl ($DPPH^{\bullet}$) under the influence of antioxidants contained in oils at wavelength 517 (Espin et al. 2000). Spectrophotometric measurement was conducted for 15 min from the moment the reagent was added, taking measurements at every 30 sec. In order to objectively compare antiradical activity in sampled mixtures parameter AE (antiradical effectivity) was denoted.

$$AE = 1/(T_{EC50} \times EC_{50})$$

EC_{50} — concentration needed to decrease initial content of $DPPH^{\bullet}$ by half.

T_{EC50} — time needed to reach steady state of $DPPH^{\bullet}$ at concentration of product corresponding to EC_{50}.

Determination of fatty acids

Fatty acids contained in rapeseed oil were determined as methyl esters (according to BN-80-50-05), using HEWLLET PACKARD 5890 II Plus chromatograph with flame-ionization detector. Chromatographic separations were made on HP-INNOWAX column at temperature 170–210°C. Helium was used as a gas carrier (1.56 cm^3/min) .

Statistical analysis

All experiments were repeated three times and the results were statistically analyzed using analysis of variance and Tuckey's post-hoc tests in order to determine homogenous groups. All tests were verified at the significance level $\alpha = 0,05$.

Results and discussion

Composition of fatty acids was typical of this type of oil, and it is confirmed in the literature (Wolff 1992, Szeliga 1997, Lampi et al. 1997). Rapeseed oil contains mainly gamma and alpha tocopherol homologues and small quantities of beta- and delta-tocopherol. Tocotrienols were not found; similar results were obtained by Nogala-Kałucka et al. (1985, 2000).

According to Dąbrowski and Sosulski (1984) the main phenolic compound contained in rapeseed is sinapic acid. Poliphenolic compounds are very sensitive to temperature and air by which they are easily decomposed. Refined rapeseed oil used in this study had general content of polyphenolic compounds of about 39.2±0.09 mg/kg.

The addition of coenzyme Q_{10}, menadione and β-carotene accelerated autoxidation process. Acceleration of autoxidation process in edible oils under the influence of menadione was observed by Kupczyk (2003). The biggest increase of

peroxide value was denoted in rapeseed oil with β-carotene, what may be caused by decomposition of β-carotene to compounds that accelerate oxidation. Similar results were received by Heinonen et al. (1997), Shibasaki-Kitawaka et al. (2004). During the time of storage only quercetin restrained this process (Fig. 1). Oszmiański (1995) also observed antioxidation properties of quercetin in relation to triacylglycerols.

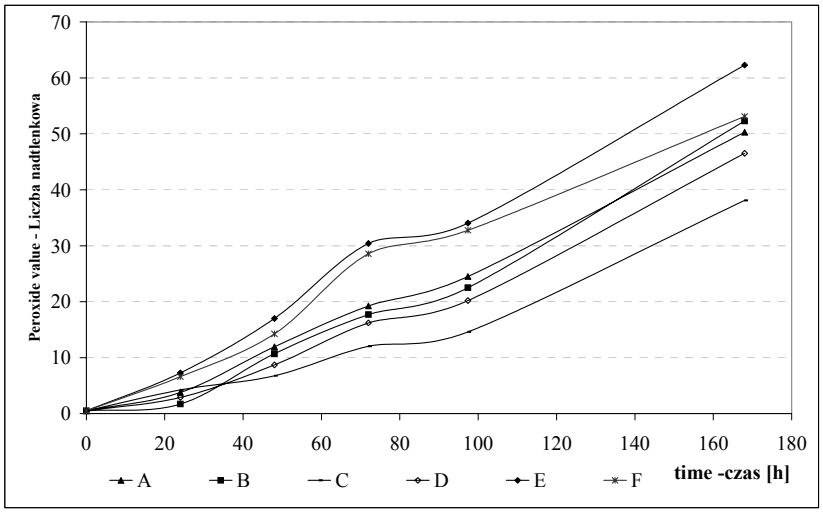
Changes in the content of particular homologues of tocopherol in rapeseed oil with additives during storage at the temperature 60°C are shown in figures 2–4. The greatest biological activity was revealed on α-T. These additives prevent lipid autoxidation process by reacting with hydroxide radical of propagation chain and therefore they are important to prevent α-T from decomposition (Aruoma 2003). During first 24 hours all substances except for menadione restrained α-T decomposition. The smallest decomposition, about 8.3% was denoted in the presence of quercetin, 13% with coenzyme Q_{10} and 19% with β-carotene. In pure rapeseed oil and in the presence of menadione the decrease of α-T was about 41%. Statistical analysis shows that the presence of coenzyme Q_{10} did not influence tocopherol decomposition after 4 days of study. Addition of menadione caused greater decomposition of tocopherol by 9%, but more α-T was left the presence of β-carotene about 9% and in presence of quercetin about 3%.

In rapeseed oil the content of beta-tocopherol was ver small and after 24 hours was completely decomposed in all mixtures.

Table 1

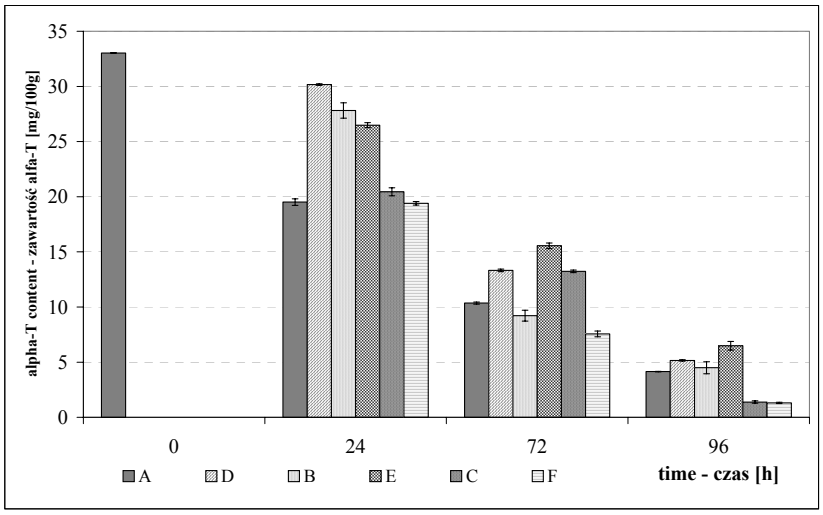
Fatty acids composition of Kujawski rapeseed oil
Skład kwasów tłuszczowych oleju rzepakowego Kujawski

Fatty acids — *Kwasy tłuszczowe*	Content — *Zawartość* [%]
$C_{16:0}$	4.49
$C_{16:1}$	0.20
$C_{17:0}$	0.13
$C_{18:0}$	1.79
$C_{18:1}$ generally	63.50
$C_{18:1}$ isomers trans	0.07
$C_{18:2}$ generally	17.38
$C_{18:3}$ generally	8.91
$C_{18:3}$ isomers trans	0.43
$C_{20:0}$	0.62
$C_{20:1}$	1.78
$C_{22:0}$	0.81



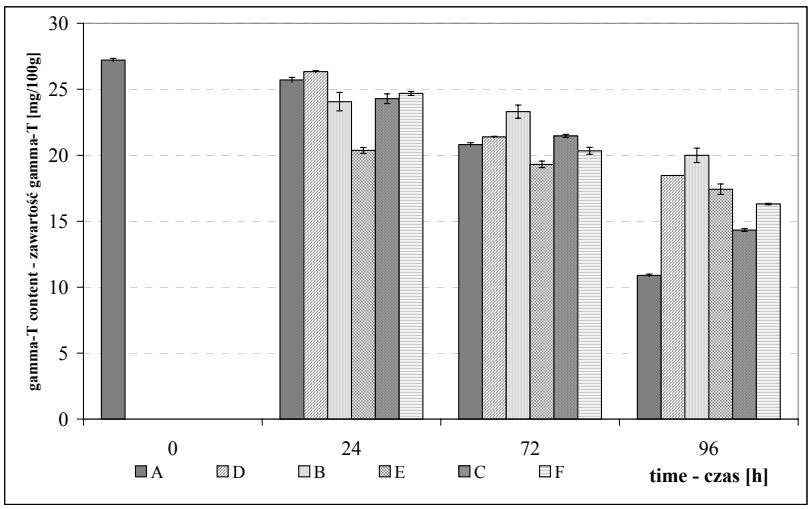
A — rapeseed oil — *olej rzepakowy*
B — oil + coenzyme Q_{10} — *olej + koenzym Q_{10}*
C — oil + p-benzoquinone — *olej + p-benzochinon*
D — oil + quercetin — *olej + kwercetyna*
E — oil + beta-carotene — *olej + beta-karoten*
F — oil + menadione — *olej + menadion*

Fig. 1. Changes of peroxidation value during the time of rapeseed oil storage at the temperature 60°C
Zmiany liczby nadtlenkowej oleju rzepakowego podczas przechowywania w temperaturze 60°C



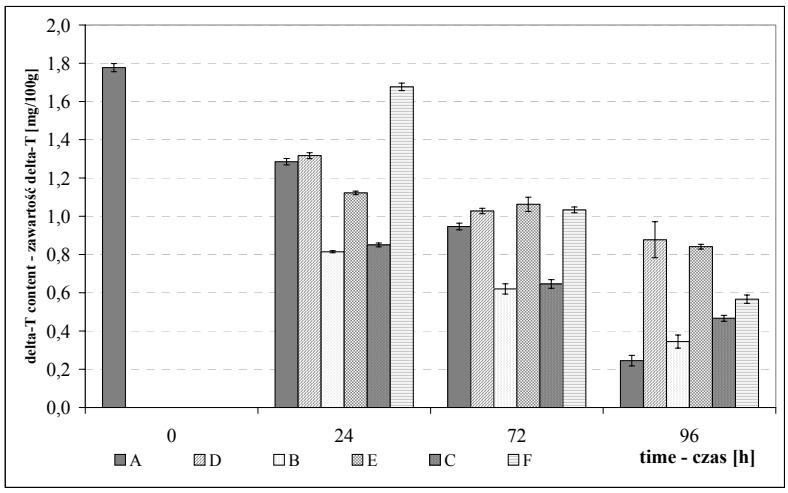
A — rapeseed oil — *olej rzepakowy*
B — oil + coenzyme Q_{10} — *olej + koenzym Q_{10}*
C — oil + p-benzoquinone — *olej + p-benzochinon*
D — oil + quercetin — *olej + kwercetyna*
E — oil + beta-carotene — *olej + beta-karoten*
F — oil + menadione — *olej + menadion*

Fig. 2. Changes of α-tocopherol content — *Zmiany zawartości alfa-tokoferolu*



A — rapeseed oil — *olej rzepakowy*
B — oil + coenzyme Q_{10} — *olej + koenzym Q_{10}*
C — oil + p-benzoquinone — *olej + p-benzochinon*
D — oil + quercetin — *olej + kwercetyna*
E — oil + beta-carotene — *olej + beta-karoten*
F — oil + menadione — *olej + menadion*

Fig. 3. Changes of γ-tocopherol content — *Zmiany zawartości gamma-tokoferolu*



A — rapeseed oil — *olej rzepakowy*
B — oil + coenzyme Q_{10} — *olej + koenzym Q_{10}*
C — oil + p-benzoquinone — *olej + p-benzochinon*
D — oil + quercetin — *olej + kwercetyna*
E — oil + beta-carotene — *olej + beta-karoten*
F — oil + menadione — *olej + menadion*

Fig. 4. Changes of δ-tocopherol content — *Zmiany zawartości delta-tokoferolu*

Table 2

Tocopherols and total phenolic compounds content in rapeseed
Zawartość tokoferoli i związków fenolowych ogółem w oleju rzepakowym

Tocopherol — *Tokoferol* [mg/100 g]				Total phenolic compounds *Związki fenolowe ogółem* [mg/kg]
α-T	β-T	γ-T	δ-T	
33.033 ± 1.443	0.131 ± 0.011	27.201 ± 1.323	1.777 ± 0.215	39.2 ± 0.09

mean ± standard deviation — *średnia ± odchylenie standardowe*

Only addition of quercetin restrained gamma-tocopherol decomposition during the first 24 hours. On the fourth day of storage all additives restrained decomposition. There was 33% of gamma-tocopherol more in the mixture with coenzyme Q_{10} than in pure rapeseed oil and in the mixtures with: quercetin — 27%; β-carotene — 24%; and menadione — 19%.

After 24 hours delta-tocopherol was decomposed most slowly in the presence of quercetin and menadione K_3. On the third day of storage, the mixture of rapeseed oil with coenzyme Q_{10} accelerated decomposition process by 16% comparing to pure rapeseed oil. After 96 hours of oils storage the decreases of delta tocopherol were in the mixture with quercetin 40%, with β-carotene 31%, with menadione 15%, with coenzyme Q_{10} 2% less than in pure rapeseed oil.

Kupczyk's (1995, 1997) observations confirm the influence of menadione on faster decomposition of tocopheroles. Sinatra (1998) reports that the presence of coenzyme Q_{10} in medium with tocopherols slows down their decomposition what is confirmed our research.

Capacity to scavenging the free radicals $DPPH^{\bullet}$ in rapeseed oil with addition of biologically active substances was examined. The reaction of free $DPPH^{\bullet}$ radicals proceeds in the following way (Brand-Williams et al. 1995):

$DPPH^{\bullet} + AH \rightarrow DPPH—H + A^{\bullet}$ AH – antioxidant

$R^{\bullet} + DPPH^{\bullet} \rightarrow DPPH—R$ $R^{\bullet}$ – radical

All substances added to rapeseed oil except for menadione increased capacity of scavenging of free $DPPH^{\bullet}$ radicals (Table 3). In the first 24 hours the best scavenging mixtures were rapeseed oil with coenzyme Q_{10} and with quercetin. During longer storage statistical analysis has shown no difference between scavenging of free radicals in pure oil and in mixture with quercetin. In mixtures with other additives deterioration of scavenging of free $DPPH^{\bullet}$radicals was observed.

According to Sanchez-Moreno et al. (1989) classification examined mixtures had average antiradical activity (Table 4).

The greatest antiradical activity was observed in the mixture with β-carotene. Antiradical activity of mixtures with quercetin and coenzyme Q_{10} was at the similar level. Addition of menadione into rapeseed oil increased parameter by 0.81.

Table 3

Scavenging of free radicals DPPH$^{\bullet}$ by rapeseed oil with additions different biologically active substances — *Zmiatanie wolnych rodników DPPH$^{\bullet}$ przez olej rzepakowy z różnymi biologicznie czynnymi dodatkami*

Samples *Próby*	Storage time of samples at temp. 60°C [hours] *Czas przechowywania prób w temp. 60°C [godz.]*			
	0	24	72	96
	DPPH$^{\bullet}$ [%]			
Rapeseed oil and quercetin *Olej rzepakowy z kwercetyną*	32.99 ± 0.266 b	33.98 ± 0.662 bc	52.13 ± 0.426 f	53.45 ± 2.537 f
Rapeseed oil and coenzyme Q_{10} *Olej rzepakowy z koenzymem Q_{10}*	31.155 ± 0.606 ab	31.32 ± 0.738 ab	63.27 ± 0.513 gh	65.91 ± 1.737 h
Rapeseed oil and β-carotene *Olej rzepakowy z β-karotenem*	28.17 ± 1.956 a	44.03 ± 0.148 e	61.06 ± 0.414 g	80.87 ± 1.417 j
Rapeseed oil and menadione *Olej rzepakowy z menadionem*	37.42 ± 1.463 cd	37.98 ± 0.978 cd	60.69 ± 1.00 g	70.21 ± 2.856 i
Rapeseed oil *Olej rzepakowy*	38.15 ± 0.271 d	43.97 ± 0.685 e	53.72 ± 0.514 f	55.90 ± 1.527 f

mean ± standard deviation — *średnia ± odchylenie standardowe*
values followed by the same letter are not significantly different at the $\alpha = 0.05$
wartości oznaczone tymi samymi literami nie różnią się statystycznie istotnie przy $\alpha = 0,05$

Table 4

Antiradical activity parameters of examioned oils
Parametry aktywności przeciwrodnikowej badanych olejów

Sample *Próba*	EC_{50} [g/kg DPPH$^{\bullet}$]	T_{EC50} [min]	AE [$\times 10^3$]	Class of activity *Klasa aktywności*
Rapeseed oil and quercetin *Olej rzepakowy z kwercetyną*	23.212	13.4	3.21	average— *średnia*
Rapeseed oil and coenzyme Q_{10} *Olej rzepakowy z koenzymem Q_{10}*	29.575	10.1	3.35	average— *średnia*
Rapeseed oil and β-carotene *Olej rzepakowy z β-karotenem*	31.751	6.6	4.77	average— *średnia*
Rapeseed oil and menadione *Olej rzepakowy z menadionem*	41.401	10.4	2.32	average— *średnia*
Rapeseed oil *Olej rzepakowy*	52.933	12.5	1.51	average— *średnia*

Conclusions

1. The addition of quercetin restrained autoxidation process in rapeseed oil stored at temperature 60°C. Other additives accelerated oxidation.
2. All substances except for menadione restrained decomposition of alpha-tocopherol in rapeseed oil.
3. On the first 24 hours only quercetin restrained decomposition of gamma-tocopherol. On the third and fourth day other substances started to restrain this homologue decomposition.
4. On the first day the slowest decomposition of delta-tocopherol was observed in samples with quercetin and menadione. On the fourth day all additives were observed to restrain delta-tocopherol decomposition.
5. All substances added into rapeseed oil except for menadione increased capacity of scavenging of free radicals $DPPH^{\bullet}$. Coefficient of antiradical activity of sampled oil increased after the addition of biologically active substances.

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