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ANTIOXIDATIVE EFFECT OF THYME (*THYMUS VULGARIS*) IN SUNFLOWER OIL

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ABSTRACT

Background. Lipid oxidation is a main problem during food processing, storage and consumption leading to losses of quality, stability, safety and nutritive value. Antioxidants have been used to prevent oxidation changes and off – flavor development in food products. Aim of the research was to evaluate antioxidative effect of thyme ethanol extract on sunflower oil during its storage in different temperature conditions. Oil samples were stored in darkness at 4°C, 18°C, 38°C.
Material and methods. Samples of thyme (thymus vulgaris) were purchased at a local pharmacy in Poznań, Poland and sunflower oil was acquired from a local supermarket. Thyme extract was characterized by total polyphenol content. Antioxidant activity was estimated with use of DPPH and ABTS radicals scavenging methods. Ethanol extract of thyme at 1% level was added to sunflower oil. Peroxide value (PV), anisidine value (AV), totox value (TxV) and fatty acids (FA) content were taken as parameters for evaluation of effectiveness of thyme extract in stabilization of sunflower oil.
Results. High polyphenol content, DPPH and ABTS radicals scavenging activity of ethanol thyme extract were evaluated. Results from different parameters were in agreement with other researchers, suggesting the antioxidant effect of thyme on antioxidant stability. Results show that thyme extract prolonged stability of sunflower oil and it may be a potent antioxidant for its stabilization.
Conclusions. Ethanol thyme extract may be used as a natural antioxidant to prolong stability of oils.

Key words: thyme extract, antioxidants, oxidation, sunflower oil

INTRODUCTION

Lipids belong to one of the most important food components. Oxidation of lipids that occurs in food during storage, technological processing and heat treatment is one of the basic process causing rancidity [Donelli and Robinson 1995, Bilska 2011]. The oxidation products of lipids decrease the nutritional value of food and may affect the organoleptic characteristics, including taste and aroma, making the final product unacceptable for consumption. Therefore, several investigations have been undertaken aimed at increasing the stability of oils and lipid-containing products. In recent years, there is a very popular to finding natural compounds, that could replace synthetic antioxidants such as BHT or BHA which are commonly used in food. Apart from using synthetic antioxidants which, as several studies show, are toxic to our bodies, most important method of prevention from the oxidation of lipids is the addition of natural antioxidants (spices,

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herbs and vegetable extracts) [Selmi and Sadok 2008, Jukić and Miloš 2005, Wójciak et al. 2011]. Antioxidants present in food are very important for human health since the reactive oxygen is recognized as aging and carcinogenesis factor. Plant components as antioxidants play an important role in food and living organisms because of the radicals scavenging ability and reducing cells degradation in human body [Ramarathnam et al. 1995, Hollman et al. 1996, Del Ré and Jorge 2011]. The antioxidant properties of herbs and spices are reported to be effective in delaying the progress of rancidity in oils and fats [Szabo et al. 2010, Hinneburg et al. 2006, Gramza-Michałowska and Stachowiak 2010, Kobus-Cisowska et al. 2010]. As a great scavengers of free radicals, hydroxyl radicals and superoxide anion radicals are polyphenols [Farag et al. 1989]. It is known that a lot of herbs, spices and natural extracts from selected herbs are stable to autooxidation due to presence of natural phenolic compounds [Ramarathnam et al. 1995]. One of such herbs is thyme which, as many other plants, has a remarkable antioxidative effect [Selmi and Sadok 2008]. Thyme belongs to the family of Labiatae and as an aromatic agent is widely used in many cooked dishes of European cuisine. Antioxidative effect of thyme is based essentially on polyphenolic compounds as flavonoids (Luteolin) [Hollman et al. 1996, Lacroix et al. 1997, Justesen and Knuthsen 2001]. It is also well known that essential oil of this plant is a rich source of thymol and carvacrol which has been reported to possess a high antioxidant activity [Farag et al. 1989]. Moreover thyme essential oil inhibits growth of Bacillus subtilis, Staphylococcus aureus (G+ bacteria), Escherichia coli and Enterobacter aerogenes (G– bacteria). It has a strong inhibition effect also on the growth of fungi and yeasts.

The objective of the present work was to study the effect of antioxidant activity of thyme ethanol extract on the shelf-life of sunflower oil during 29 days of storage in different temperature conditions.

MATERIAL AND METHODS

Chemicals. The following chemicals were used: 6-hydroxyl-2,5,7,8-tetramethylchroman-2-carboxylic acid – Trolox (Aldrich); 2,2-diphenyl-1-picryl-hydrazyl (DPPH) (Sigma-Aldrich); 2,2'-azinobis-(3-ethylbenzo-thiazoline-6-sulfonic acid) diammonium salt (ABTS) (Sigma); ethanol, methanol, n-hexane, heptane, acetic acid, starch, potassium iodine, chloroform, natrium chloride, sodium thiosulfate standard solution (POCH, Poland); potassium persulfate (Aldrich); Gallic acid (Sigma); Folin-Ciocalteu's phenol reagent (Sigma-Aldrich); Grain FAME mix 10 mg/ml (Supelco); sodium methoxide in methanol; p-Anisidine (Fluka).

All chemicals and reagents used were the highest analytical grade.

Plant material. Samples of thyme (thymus vulgaris) were purchased at a local pharmacy in Poznań, Poland and sunflower oil was acquired from local supermarket. Extract was prepared by 6 hours direct extraction of ground dried leaves (10 g) with 100 ml of 95% ethanol at boiling point of ethanol. Collected extract was filtered and centrifuged (4000 rpm, 10 min). Researches of antioxidant activity was conducted on the ethanol extract.

Determination of total phenolic content in extract was determined with Folin-Ciocalteu method [Singleton and Rossi 1965]. The determinations were carried out in duplicate. Results expressed as mg of gallic acid equivalent per gram of the extract (mg GAE/g).

Antiradical activity assays. Polyphenol antioxidative activity was estimated using DPPH [Chu et al. 2000, Nuutila et al. 2003] and ABTS [Re et al. 1999] radicals scavenging ability of the examined extract. The determinations were carried out in duplicate. The results were expressed as mg Trolox per 1 g of extract (mgT/g).

Peroxide value (PV). Oxidation rate was followed by periodic determination of the peroxide value. This was determined according to PN-EN ISO 3960:2005. The determinations were carried out in triplicate. PV was expressed as mmol O_2 per kg of oil.

Anisidine value (AV). AV was determined according to PN-EN-ISO 6885:2008 using a Philips UV-Vis spectrophotometer. The data were presented as mean ±standard deviation of duplicate determinations.

Totox values (TV). TV were calculated from the relationship TOTOX = 2PV + AV.

Fatty acids (FA). FA methyl esters were prepared by transmethylation 100 µl sample dissolved in 1 ml hexane using 1 ml 0.5N solution of sodium methoxide in methanol. After 10 minutes reaction at room

temperature, saturated solution of NaCl was added into the sample. Then 1 µl of hexane fraction was taken and analyzed by gas chromatography (HP 6890 series), using a capillary column of 25 m, 0.2I.D., 0.33 µm film (HP FFAP, Agilent Technologies, U.S.A); temperature was programmed from 90 to 240°C. Peaks were identified using FAME mix reference compounds.

Storage conditions. Oil samples were put into glass bottles. The bottles were closed and exposed to different storage conditions: 1 – at 4°C in darkness, 2 – at 18°C in darkness, 3 – at 38°C in darkness. For each storage conditions two bottles were used, one with sunflower oil and without thyme extract, the second with oil and added thyme extract. The thyme extract was added to oil in the 1% concentration. Once a week from each bottle a sample of oil was taken for determination of the analysed discriminants.

Statistical analysis. All analyses were performed in duplicate. The recorded results were subjected to statistical analysis using the STATISTICA 9 and Excel 2007 software. The results were interpreted at the significance level $\alpha = 0.05$.

RESULTS AND DISCUSSION

A big part of natural antioxidants constitute poliphenols found in every part of the plant including fruits and leaves [Selmi and Sadok 2008]. The major constituent of thyme (*Thymus vulgaris*) which posses the antioxidant properties is luteolin [Justesen and Knuthsen 2001]. The thymol and carvacrol, the main constituents of thyme essential oil has antibacterial effect and also a considerable antioxidant effect on the oxidation of lipids [Babović et al. 2010, Grosso et al. 2010]. The antioxidant activity may occur various mechanisms such as the inhibitory effect on lipid peroxidation and by scavenging the radicals.

Evaluation of total polyphenols content with Folin-Ciocalteu method showed that thyme extract contained 77.6 mg GAE/ml. Plant polyphenols are well characterized with its radicals scavenging potential. The most popular methods for evaluations of antiradical activity of the plants, herbs and their extracts are DPPH and ABTS methods. Researches showed that thyme extract received by using hot extraction possess high DPPH and ABTS radicals scavenging activity namely 54.5 mgT/ml and 77.6 mgT/ml. Selmi and Sadok [2008] also have reported that the ethanol thyme extract exhibit good radical scavenging activities.

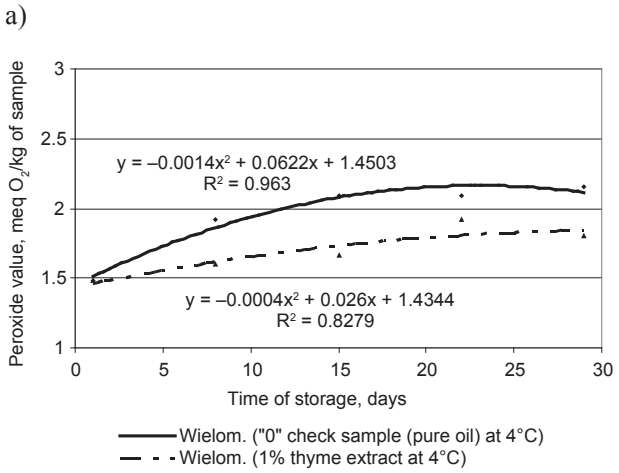
The effect of thyme extract on the formation of the oxidation products expressed as peroxide value in sunflower oil stored in darkness at 4°C, 18°C, 38°C versus time of storage is shown in Figure 1 and Table 1.

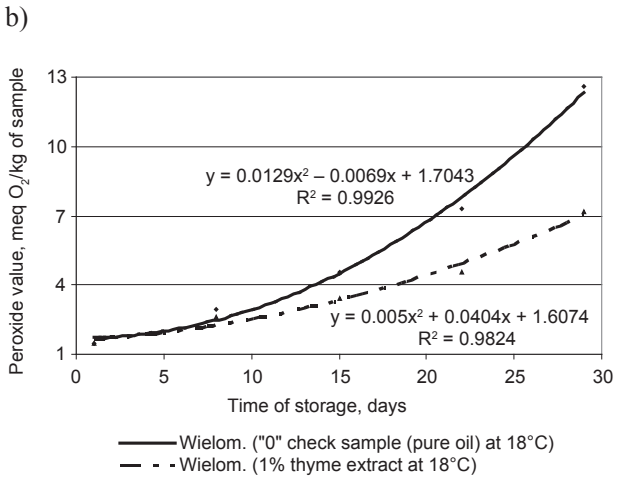
Figure 1 a, b, c illustrates that independently of the storage temperature the addition of 1% thyme extract effectively delayed increase of peroxide values in oil samples.

Table 1. Effect of addition of thyme extract on the peroxide value (PV) of sunflower oil during 29 days storage in darkness at 4°C, 18°C, 38°C, meq O_2/kg

Sample	Storage temperature	Peroxide value				
		1 day	8 day	15 day	22 day	29 day
"0" sample (pure oil)	4°C	$1.48^{a} \pm 0.01$	$1.92^{a} \pm 0.01$	$2.09^{a} \pm 0.01$	$2.09^{a} \pm 0.01$	$2.15^{a} \pm 0.01$
1% thyme extract		$1.48^{a} \pm 0.01$	$1.60^{a} \pm 0.01$	$1.66^{a} \pm 0.01$	$1.92^{a} \pm 0.01$	$1.80^{a} \pm 0.01$
"0" sample (pure oil)	18°C	$1.48^{a} \pm 0.01$	$2.92^{a,b} \pm 0.02$	$4.55^{a,b} \pm 0.01$	$7.29^{b,c} \pm 0.01$	$12.60^{c} \pm 0.01$
1% thyme extract		$1.48^{a} \pm 0.01$	$2.58^{a,b} \pm 0.01$	$3.40^{a,b} \pm 0.01$	$4.53^{a,b} \pm 0.01$	$7.20^{b} \pm 0.01$
"0" sample (pure oil)	38°C	$1.48^{a} \pm 0.01$	$6.94^{a} \pm 0.02$	$24.77^{b} \pm 0.02$	$43.0^{c} \pm 0.06$	$63.80^{d} \pm 0.1$
1% thyme extract		$1.48^{a} \pm 0.01$	$3.06^{a} \pm 0.01$	$9.52^{b} \pm 0.01$	$16.62^{c} \pm 0.02$	$25.82^{d} \pm 0.04$

a, b, c, d – means within rows with different letters are significantly different $p \leq 0.05$.





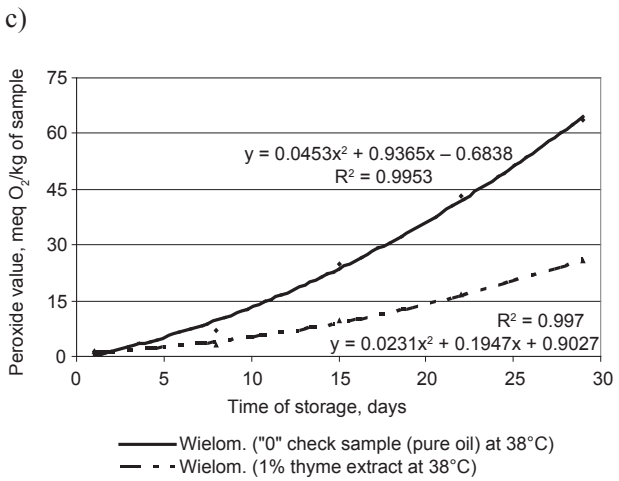
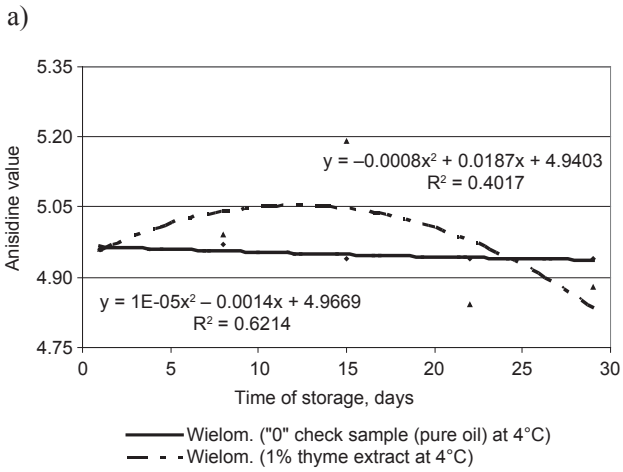
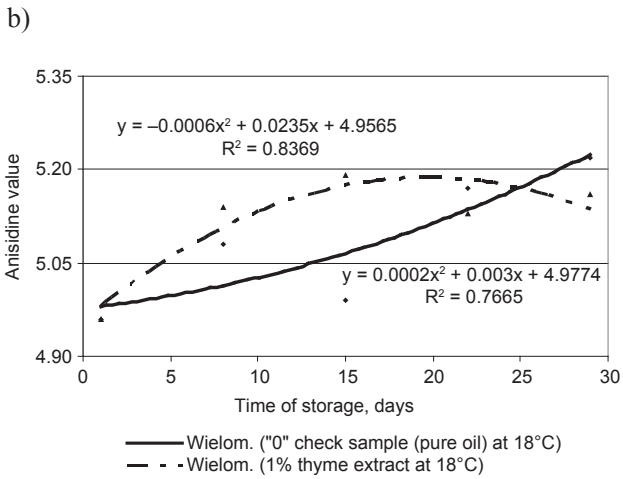


Fig. 1. Effect of addition of thyme extract on the peroxide value of sunflower oil during storage in darkness at 4°C, 18°C, 38°C





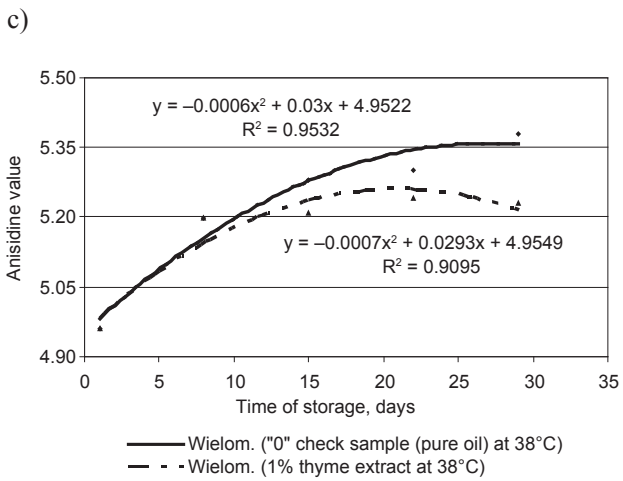


Fig. 2. Effect of addition of thyme extract on the anisidine value of sunflower oil during storage in darkness at 4°C, 18°C, 38°C

Samples of sunflower oil stored at 4°C did not show statistically important changes during storage but in oil sample with thyme extract addition, the value was lower than in pure oil. The same situation took place in oil samples stored at 18°C. PV of the pure oil increased significantly from 22 day of storage and in oil sample with antioxidant on added the last day. The peroxide value of the check sample (pure oil) stored at 38°C started to increase markedly from 15 day. During the next days the oxidation process occurred more progressively and the end of the experiment the PV value of oil reached 63.80 meq O_2/kg. The sample with thyme extract also statistically increased from 15 day and the last day of storage exceeded the value of 25.82 meq O_2/kg. After that time the peroxide value in oil containing thyme extract was more than 100% lower than PV in the oil with no extract. Regardless of storage temperature the significant influence of thyme extract on PV was observed by all the storage time. The antioxidative effects of thyme are confirmed by Takacsova et al. [1995] in rapeseed oil during storage at 60°C. Also Nguyen et al. [2000] reported that the peroxide value of rapeseed oil samples with the addition of thyme increased later than check sample. Bensmira et al. [2007] analysed changes in PV of sunflower oil during heat treatment. In their studies, results showed a significant difference between PV of sunflower oil with thyme and PV of oil without thyme. The amount of peroxides found in original sunflower oil (pure oil) was higher than in sunflower oil with thyme. They found that the addition of thyme into sunflower oil helped to inhibit oxidation to a certain extent, thus reducing the amount of peroxides formed during its exposure to simulated frying temperatures.

The susceptibility of sunflower oil to oxidation was also measured by anisidine value (AV). In Table 2 we can also see that the susceptibility to oxidation is affected by the storage conditions. The higher was the temperature of storage the higher was AV of samples. In all storage conditions the control samples possess statistically higher AV values ($p \leq 0.05$) than samples with thyme extract. In oil samples storage in 38°C from 8 day to the end of storage period, AV for the control oil increased significantly. In oil sample containing thyme extract AV increased significantly from 8 day but after this did not change. The same situation took place in samples stored at 18°C.

Anisidine value is often used in conjunction with PV to calculate the so-called total oxidation or Totox value given as: TOTOX = 2 × PV + p – AV. The results of this analysis are shown in Table 3 and Figure 3. In oil samples stored at 4°C the Totox values did not changes significantly ($p \leq 0.05$). The thyme extract addition to oil sample stored at 18°C caused its stability in comparison to check sample in which Totox value increased significantly from 22 day of storage. In oil samples stored at 38°C Totox value increases very rapidly during storage. In cases of both samples the Totox increase

Table 2. Effect of addition of thyme extract on the anisidine value (AV) of sunflower oil during 29 days storage in darkness at 4°C, 18°C, 38°C

Sample	Storage temperature	Anisidine value				
		1 day	8 day	15 day	22 day	29 day
"0" sample (pure oil)	4°C	4.96^{a} ±0.02	4.97^{a} ±0.02	4.94^{a} ±0.02	4.94^{a} ±0.02	4.94^{a} ±0.02
1% thyme extract		4.96^{a} ±0.02	4.99^{a} ±0.02	5.19^{b} ±0.02	4.84^{c} ±0.03	4.88^{d} ±0.02
"0" sample (pure oil)	18°C	4.96^{a} ±0.02	5.08^{b} ±0.04	4.99^{a} ±0.02	5.17^{c} ±0.01	5.22^{d} ±0.02
1% thyme extract		4.96 ±0.02	5.14^{b} ±0.02	5.19^{b} ±0.03	5.13^{b} ±0.02	5.16^{b} ±0.03
"0" sample (pure oil)	38°C	4.96^{a} ±0.02	5.20^{b} ±0.03	5.28^{c} ±0.01	5.30^{c} ±0.01	5.38^{d} ±0.02
1% thyme extract		4.96^{a} ±0.02	5.20^{b} ±0.02	5.21^{b} ±0.02	5.24^{b} ±0.02	5.23^{b} ±0.02

Explanations – as in Table 1.

Table 3. Effect of addition of thyme extract on the Totox value of sunflower oil during 29 days storage in darkness at 4°C, 18°C, 38°C

Sample	Storage temperature	Totox value				
		1 day	8 day	15 day	22 day	29 day
"0" sample (pure oil)	4°C	7.92[a]	8.81[a]	9.08[a]	9.12[a]	9.24[a]
1% thyme extract		7.92[a]	8.19[a]	8.51[a]	8.68[a]	8.48[a]
"0" sample (pure oil)	18°C	7.92[a]	10.92[a]	14.09[a,b]	19.75[b]	30.42[c]
1% thyme extract		7.92[a]	10.30[a]	11.99[a]	14.19[a]	19.56[a]
"0" sample (pure oil)	38°C	7.92[a]	19.08[a]	54.82[b]	91.30[c]	132.98[d]
1% thyme extract		7.92[a]	11.32[a]	24.25[b]	38.48[c]	56.87[d]

Explanations – as in Table 1.

Table 4. Fatty acids content in sunflower oil with and without thyme extract content before and after 29 days storage (temp. 4°C, 18°C, 38°C, in darkness)

Fatty acids	Fresh sunflower oil	Oil samples after 29 days storage					
		in 4°C		in 18°C		in 38°C	
		sunflower oil	sunflower oil with 1% thyme extract addition	sunflower oil	sunflower oil with 1% thyme extract addition	sunflower oil	sunflower oil with 1% thyme extract addition
C 14 :0	0.07 ±0.01	0.06 ±0.01	0.06 ±0.01	0.06 ±0.01	0.06 ±0.01	0.05 ±0.01	0.06 ±0.01
C 16:0	6.44 ±0.03	5.99 ±0.04	6.23 ±0.07	5.85 ±0.02	6.33 ±0.03	6.47 ±0.03	6.24 ±0.01
C 16: 1	0.10 ±0.01	0.26 ±0.01	0.62 ±0.01	0.35 ±0.01	0.52 ±0.02	0.68 ±0.05	0.37 ±0.01
C 18: 0	3.40 ±0.01	3.49 ±0.03	3.39 ±0.04	3.34 ±0.05	3.40 ±0.02	3.61 ±0.01	3.36 ±0.01
C 18: 1	28.52 ±0.33	28.77 ±0.38	28.48 ±0.22	27.63 ±0.11	28.77 ±0.16	28.89 ±0.09	28.97 ±0.20
C 18: 2	59.97 ±0.50	60.04 ±0.57	59.63 ±0.52	57.59 ±0.48	59.97 ±0.66	59.32 ±0.37	59.71 ±0.48
C 18: 3	0.09 ±0.02	0.09 ±0.01	0.12 ±0.01	0.11 ±0.01	0.10 ±0.01	0.18 ±0.03	0.14 ±0.24
C 20: 0	0.23 ±0.06	0.27 ±0.02	0.28 ±0.02	0.30 ±0.04	0.22 ±0.03	0.38 ±0.04	0.22 ±0.02
C 20: 1	0.21 ±0.01	0.25 ±0.01	0.34 ±0.01	0.25 ±0.02	0.22 ±0.01	0.40 ±0.02	0.20 ±0.01
C 22: 0	0.60 ±0.01	0.69 ±0.08	0.68 ±0.06	0.75 ±0.04	0.69 ±0.06	0.77 ±0.03	0.68 ±0.01
ΣSFA	10.74	10.5	10.64	10.3	10.70	11.28	10.61
SMUFA	28.83	29.28	29.44	28.23	29.51	29.97	29.54
ΣPUFA	60.06	60.13	59.75	57.7	60.07	59.50	59.85

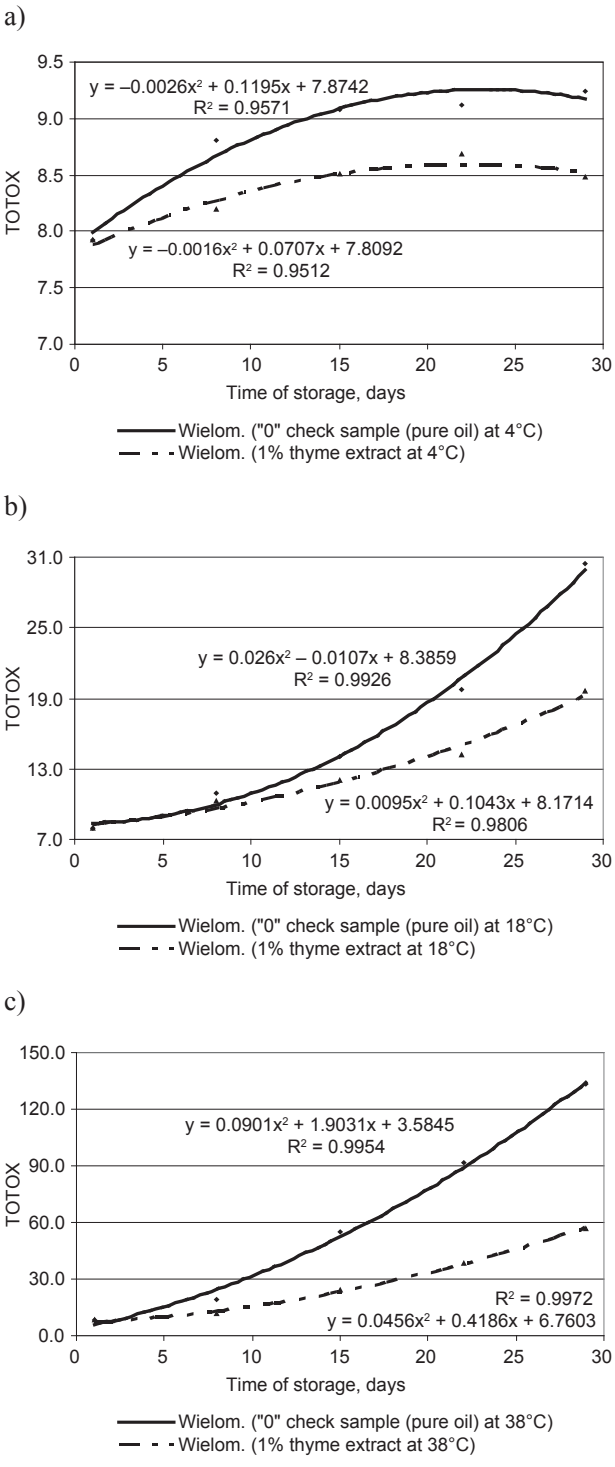


Fig. 3. Effect of addition of thyme extract on the Totox value of sunflower oil during storage in darkness at 4°C, 18°C, 38°C

was statistically significant from 15 day of storage. In pure oil sample the initial Totox value increased for the all storage time nearly twenty times. In comparison to check sample the Totox values on the last day of storage of sample with thyme extract was two times lower.

Sunflower oil is among the healthiest vegetable oils available. It is an excellent source of the essential fatty acids required by the human body. One of the main fatty acids in sunflower oil is the linoleic acid. Fatty acids composition of fresh oil and samples with and without extract addition after storage are presented in Table 4.

The major fatty acids were linoleic acid (C18:2) which constituted about 60% all measured acid, oleic acid (C 18:1) – nearly 30% and palmitic acid (C16:0) about 6%. Polyunsaturated fatty acids (PUFAs) constitute the majority of the fatty acids pool (~60%), followed by monounsaturated fatty acids (MUFAs) (~28%) and saturated (SFAs) (~11%). Bensmira et al. [2007] have found that sunflower oil with thyme exhibited relatively reduced FFA contents.

CONCLUSIONS

1. Thyme ethanol extract possess high polyphenol content, DPPH and ABTS radicals scavenging activity.

2. Thyme extract addition at the level of 1% to sunflower oil inhibited oxidation processes during its storage (up to 29 days) in different temperature conditions.

3. Thyme extract may be used as a natural antioxidant.

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PRZECIWUTLENIAJĄCY WPŁYW TYMIANKU (*THYMUS VULGARIS*) NA OLEJ SŁONECZNIKOWY

STRESZCZENIE

Wstęp. Utlenianie lipidów jest jednym z najważniejszych procesów zachodzących podczas wytwarzania i przechowywania żywności, który prowadzi do obniżenia jej jakości, trwałości, bezpieczeństwa i wartości żywieniowej. W celu zabezpieczenia żywności przed zmianami oksydacyjnymi oraz powstawania niepożądanego zapachu stosuje się przeciwutleniacze. Celem badań było zbadanie wpływu dodatku etanolowego ekstraktu tymianku na procesy utleniania oleju słonecznikowego w czasie jego przechowywania w różnych warunkach temperaturowych. Próbki oleju były przechowywane w ciemności w 4°C, 18°C, 38°C.
Materiał i metody. Próby tymianku użyte do badań zakupiono w sklepie zielarskim na terenie Poznania, natomiast olej słonecznikowy – w lokalnym markecie. W gotowym ekstrakcie oznaczono ogólną zawartość związków fenolowych metodą Folina-Ciocalteu'a oraz zdolność wychwytywania wolnych rodników za pomocą metody DPPH i ABTS. Etanolowy ekstrakt tymianku dodawano do prób oleju w ilości 1%. W celu zbadania wpływu ekstraktu na stabilność oksydatywną oleju słonecznikowego oznaczono liczbę nadtlenkową, liczbę anizydynową, wskaźnik Totox oraz oznaczono skład kwasów tłuszczowych.
Wyniki. Uzyskany ekstrakt tymianku charakteryzował się dużą zawartością związków fenolowych oraz wykazywał duże zdolności wychwytywania wolnych rodników (test DPPH i ABTS). Dodatek ekstraktu tymianku skutecznie spowalniał procesy oksydacyjne zachodzące w trakcie przechowywania prób oleju słonecznikowego, co potwierdzają także badania innych naukowców.
Wnioski. Etanolowy ekstrakt tymianku może być stosowany jako naturalny przeciwutleniacz przedłużający trwałość olejów.

Słowa kluczowe: ekstrakt tymianku, antyoksydanty, utlenianie, olej słonecznikowy

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