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Development of lipid damage of pumpkin seed oil stabilized with different antioxidants during long-term storage

Zh.Y. Petkova*, G.A. Antova, M.Y. Angelova-Romova

University of Plovdiv 'Paisii Hilendarski', Department of Chemical Technology, 24 Tzar Asen Street, Plovdiv 4000, Bulgaria

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Oxidative stability of pumpkin seed oil during long-term storage at different conditions was investigated. The examinations were carried out with pumpkin seed oil (as a control sample) and stabilized oil with different natural antioxidants such as caffeic acid, ethyl gallate and combination of both. Long-term storage for all samples was led for 6 months at room temperature (25°C) and in a dark place at 10°C. Indicators that define oxidative stability of the oils (acid and peroxide value, fatty acid composition and total tocopherol content) were monitored during the long-term storage. Significant increase in the peroxide value was detected in all samples during the whole period, especially in those that were kept at room temperature (25°C), while in acid value and fatty acid composition the deterioration was minor. Same tendency was observed in total tocopherol content where the latter decreased insignificantly in samples, stored at 10°C in a dark place. Generally, it could be considered that pumpkin seed oil was more stable when was stabilized with ethyl gallate and stored at 10°C.

Keywords: Pumpkin seed oil, Antioxidants, Long-term storage, Fatty acids, Tocopherols

INTRODUCTION

The oxidation of lipids is an issue with a great importance for the food industry. It is related with deterioration of fats and oils which can lead to lowering of the nutritive value of the food [1]. This provokes searching of different ways to minimize the process of oxidation and improve oxidative stability of lipid products [2]. Therefore, adding antioxidants to the food play an important role in food processing and storage because they have the ability to inhibit the oxidation [1].

Pumpkins are annual plants which belong to Cucurbitaceae family. Their seeds are by-products by food industry but they exhibit a high oil content (37.8-50.0%) which make them a rich source of lipids. The main fatty acid in the lipid fraction is linoleic acid (42.0-68.5%) followed by oleic (20.0-38.0%) and the content of palmitic and stearic acid is much lower (13.0 and 6.0%, respectively). The amount of unsaturated fatty acids is about 80.0% but the quantity of saturated fatty acids is relatively lower (about 19.0%) [3–9]. This is the reason pumpkin seed oil to be susceptible to oxidation process. The quality of vegetable oils is mainly based on their fatty acid composition, the content of natural antioxidants, as well as their ability to be stable during long-term storage. Linoleic acid, which is predominant in pumpkin seed oil, is easily susceptible to oxidation processes under the influence of light and oxygen, which may cause deterioration of the oils. It is established that during the refining process some of the tocopherols which are the major antioxidants in vegetable oils are also removed [10]. Irrespectively of the type of the oil, this leads to significantly decrease of its stability. Pumpkin seed oil that is available on the market is cold pressed, so it has higher stability. According to Vidrih *et al*. (2010) [11] the induction period of the oil at 110°C is 12.8-25.7 h which is higher than those of olive oil (9.1-23.2 h), sunflower oil-high oleic type (11.5-15.0 h), rape seed oil (7.4-10.5 h), soybean oil (6.2-8.8 h) and sunflower oil (5.3-7.8 h). Poiana *et al*. (2009) [12] examined the influence of the storage conditions on the oxidative stability and antioxidant properties of sunflower and pumpkin oil and established that during the period of 120 days the pumpkin seed oil showed higher oxidative stability than sunflower oil. They also proved that the oxidation processes were induced at a greater extent by the impact of the daylight than by the temperature.

Tocmo (2012) [13] examined the effect of packaging, light and storage temperature on the oxidative stability of pumpkin oil obtained by cold pressing of seeds of the species *Cucurbita pepo* and *Cucurbita moschata*. The oils were found to be stable for at least 20 weeks when stored in brown bottles at temperatures below 30°C, whereas from the accelerated deterioration of the oxidative stability of both oils the suggested shelf life was 15 months.

There is no information about the development of lipid damage of pumpkin seed oil produced in

* To whom all correspondence should be sent:
E-mail: zhanapetkova@uni-plovdiv.net

Bulgaria. Therefore, the aim of the present study was to monitor the changes of peroxide, acid value, tocopherol content and fatty acid composition of non-stabilized and stabilized pumpkin seed oil with different antioxidants during long-term storage.

MATERIALS AND METHODS

The examinations were carried out with pumpkin seed oil (as a control sample) and stabilized oil with different antioxidants such as caffeic acid, ethyl gallate and combination of both. Antioxidants were diluted in ethanol; after that they were added to the oil at a concentration of 0.05% if only one was used and 0.025% with more than one antioxidant used. Long-term storage for all samples was led for 6 months at 25°C and at 10°C. Indicators that defined oxidative stability of the oils (acid and peroxide value, total tocopherol content and fatty acid composition) were monitored during the long-term storage – on the 30th, 60th, 90th, 120th, 150th and 180th day of storage.

Peroxide and acid value

Peroxide and acid values were determined titrimetrically by procedures of ISO [14, 15].

Fatty acids

The fatty acid composition was determined by gas chromatography (GC) after transmethylation of the sample with 2% H_2SO_4 in absolute CH_3OH [16]. Determination of fatty acid methyl esters was performed on an HP 5890 series II (Hewlett Packard GesmbH, Vienna, Austria) gas chromatograph equipped with a 75 m × 0.18 mm (I.D.) × 25 μm (film thickness) capillary column Supelco and a flame ionization detector. The column temperature was programmed from 140°C (5 min), at 4°C/min to 240°C (3 min); injector and detector temperatures were kept at 250°C. Hydrogen was the carrier gas at a flow rate of 0.8 mL/min. Fatty acids methyl esters were identified and quantified relative to the Supelco 37 component FAME mix (Supelco, USA) which was subjected to GC under identical experimental conditions [17].

Tocopherols

Tocopherols were determined directly in the oil by high performance liquid chromatography (HPLC) on a Merck-Hitachi (Merck, Darmstadt, Germany) instrument equipped with 250 mm × 4 mm Nucleosil Si 50-5 column (Merck, Darmstadt, Germany) and fluorescent detector Merck-Hitachi F 1000. The operating conditions were as follows: mobile phase n-hexane:dioxan, 96:4 (v/v), flow rate 1.0 mL/min, excitation 295 nm, emission 330 nm. 20 μL 2% solution of oil were injected. Tocopherols were identified by comparing the retention times with those of authentic individual tocopherols. The tocopherol content was calculated on the base of tocopherol peak areas in the sample *vs*. tocopherol peak area of standard tocopherol solution [18]. α-, γ- and δ-tocopherols were identified in the examined pumpkin seed oils. γ-Tocopherol predominated in all samples (over 90.0%).

RESULTS AND DISCUSSION

Data about changes of acid and peroxide value of control sample and stabilized pumpkin seed oil with different antioxidants stored at 10°C and 25°C during the whole period is shown in Figures 1 and 2.

There were insignificant changes of acid value in all samples during the storage, which varied between 8.1-9.6 mg KOH/g. Overall, there was no significant hydrolysis process that occurred in the oil during the storage at both conditions. This could be explained by the lack of moisture in the oils which caused hydrolysis.

The peroxide value of pumpkin seed oil considerably increased during the storage at 25°C. The highest value was observed in the control sample on the 120th day (81.4 meq active oxygen/kg). The best results were noticed in the stabilized oils with ethyl gallate (up to 70.2 meq active oxygen/kg on the 120th day) and combination of ethyl gallate and caffeic acid (up to 60.2 meq active oxygen/kg). The peroxide value of the stabilized pumpkin seed oil with caffeic acid, ethyl gallate and a combination of the two antioxidants met the standards for unrefined vegetable oils (PV ≤ 20 meq active oxygen/kg) [19] until the 30th day of storage at room temperature. Though it was relatively lower in the first month, this indicator was 25 times higher on the 60th day of storage in comparison with the value at the starting point of the examination and finally doubled on the 90th day. After that the peroxide value slightly increased on the 120th day.

The peroxide value of the oil that was kept at 10°C was almost the same on the 30th day (1.5-2.5 meq active oxygen/kg), but slightly increased up to 4.6-6.1 meq active oxygen/kg on the 60th day. The change was minor in the oil that was stabilized with caffeic acid (up to 4.6 meq active oxygen/kg in the second month). Then, the peroxide value doubled on the 90th day of storage (11.0-13.7 meq active oxygen/kg) and reached the value of 20.5-27.9 meq active oxygen/kg on the 120th day.

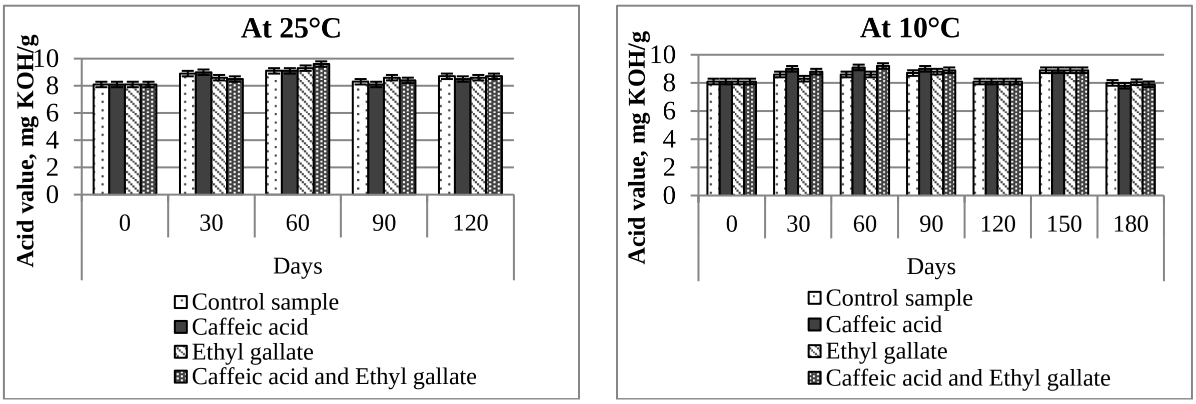

Fig. 1. Changes of acid value of pumpkin seed oil during long-term storage at different conditions

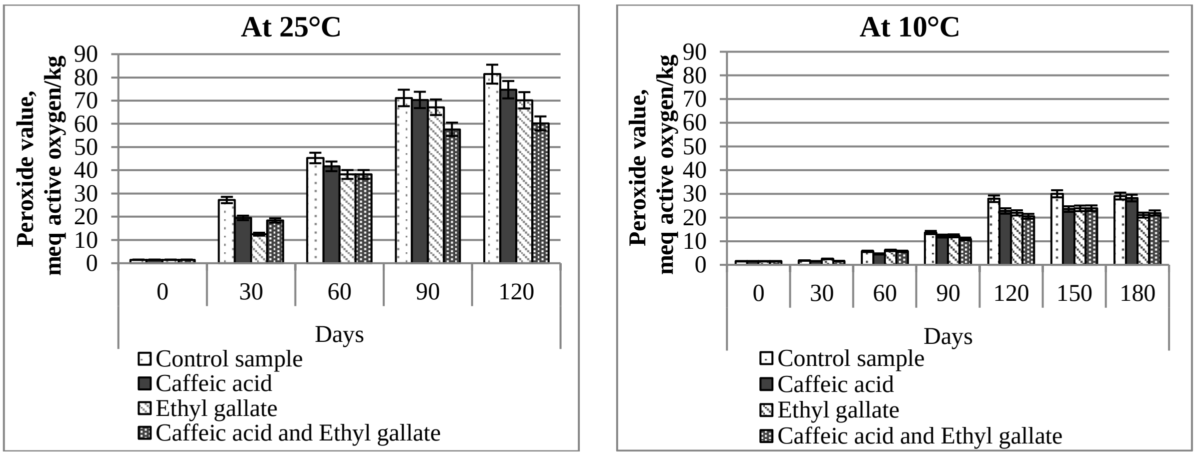

Fig. 2. Changes of peroxide value of pumpkin seed oil during long-term storage at different conditions

After that this indicator slightly increased up to 21.0-29.1 meq active oxygen/kg on the 180th day. Generally, the control sample of pumpkin seed oil that was kept at 10°C had the highest peroxide value at the final stage of examination, while those of the oil stabilized with ethyl gallate was relatively lower (21.0 meq active oxygen/kg). Therefore, ethyl gallate was the most efficient antioxidant of pumpkin seed oil stored at lower temperature.

According to the results it could be considered that pumpkin seed oil, kept at these conditions, was appropriate for human consumption no more than 120 days after its production.

Changes of total tocopherol content of non-stabilized and stabilized pumpkin seed oils with different antioxidants during long-term storage were also monitored. The results are presented in Figure 3.

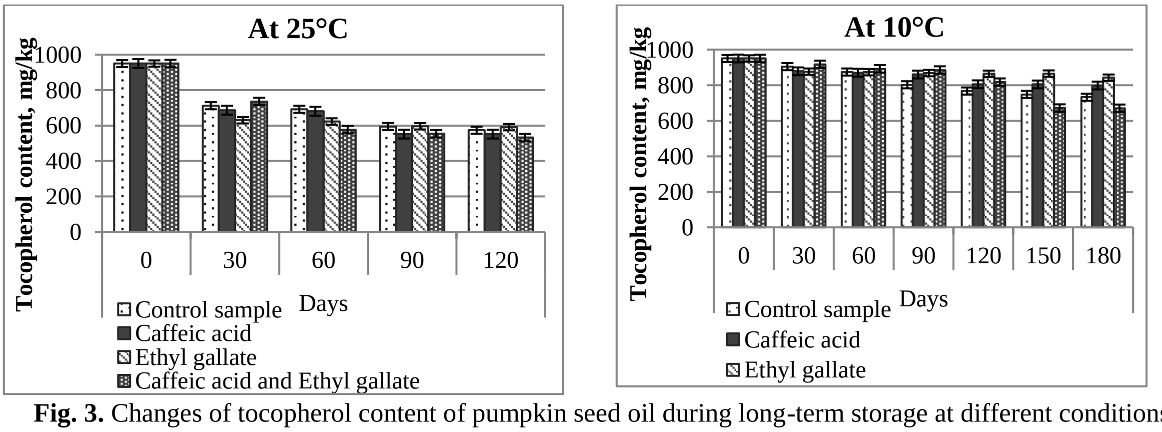

Fig. 3. Changes of tocopherol content of pumpkin seed oil during long-term storage at different conditions

Total tocopherol content of the control sample kept at room temperature (25°C) gradually decreased from 950 to 712 mg/kg on the 30th day and after that continued to decrease up to 574 mg/kg on the 120th day. Total tocopherols in the sample that was stored at 10°C slightly decreased from 904 mg/kg (30th day) to 732 mg/kg (180th day).

It was observed that tocopherol content of oil stabilized with caffeic acid and stored at 25°C decreased drastically in the final days of examination (552 mg/kg) and its amount was lower than those of non-stabilized oil. On the other hand, its quantity decreases slightly in the pumpkin seed oil stabilized with the same antioxidant and stored at 10°C – it was 878 mg/kg on the 30th day and reached the value of 798 mg/kg on the 180th day.

The tocopherol content of the oil stabilized with ethyl gallate and stored at 25°C decreased sharply from 950 to 630 mg/kg on the 30th day and then remained almost constant (591 mg/kg on the 120th day). When this oil was stored at 10°C, tocopherols were reduced smoothly with slight differences in their amount during long-term storage (876 mg/kg on the 30th day and 842 mg/kg on the 180th day).

The content of tocopherols decreased drastically in pumpkin seed oil stabilized with caffeic acid and ethyl gallate and stored at 25°C (from 950 to 577 mg/kg on the 60th day), but after that they reduced gradually to 532 mg/kg on the 120th day. Their amount slightly decreased from 950 to 817 mg/kg on the 120th day in the oil kept at 10°C and reached up to 671 mg/kg on the 150th day. After that, total tocopherol content remained almost the same in the final period of examination.

It could be concluded that the most significant change in tocopherol content was observed in the control sample and in the oil stabilized with both caffeic acid and ethyl gallate and the reducing of the tocopherols was noticed to a lesser extent in the oil stabilized with ethyl gallate. Long-term storage of pumpkin seed oil at a lower temperature (10°C) did not affect significantly the total tocopherol content, whereas its amount decreased to a greater degree at room temperature and when the oil was exposed to daylight. The two factors (higher temperature and light) initiated oxidation of the oil which could lead to reduction of the amount of tocopherols because of their binding to the free radicals.

Table 1. Changes of fatty acid composition of pumpkin seed oil during long-term storage at 25°C

At 25°C						
Stabilized with:	Fatty acids, %	Days				
		0	30	60	90	120
Control sample	$C_{16:0}$ Palmitic	21.6±0.4	17.3±0.3	20.3±0.5	23.6±0.6	21.1±0.1
	$C_{18:0}$ Stearic	7.4±0.2	6.9±0.5	2.5±0.1	4.7±0.4	3.5±0.2
	$C_{18:1}$ Oleic	47.2±0.6	40.8±0.5	46.8±0.7	42.6±0.2	41.3±0.3
	$C_{18:2}$ Linoleic	22.2±0.2	33.1±0.1	28.0±0.4	27.4±0.5	33.4±0.3
Caffeic acid	$C_{16:0}$ Palmitic	21.6±0.6	20.0±0.4	17.8±0.2	17.5±0.5	17.1±0.1
	$C_{18:0}$ Stearic	7.4±0.2	7.6±0.4	5.1±0.3	0.9±0.1	0.9±0.1
	$C_{18:1}$ Oleic	47.2±0.4	41.1±0.2	45.2±0.4	40.9±0.5	39.1±0.2
	$C_{18:2}$ Linoleic	22.2±0.3	29.2±0.5	29.4±0.4	39.8±0.5	42.4±0.4
Ethyl gallate	$C_{16:0}$ Palmitic	21.6±0.2	18.0±0.4	17.2±0.3	21.6±0.5	21.7±0.2
	$C_{18:0}$ Stearic	7.4±0.1	7.0±0.2	4.9±0.2	4.3±0.1	3.5±0.1
	$C_{18:1}$ Oleic	47.2±0.5	41.4±0.6	41.4±0.5	44.8±0.4	41.3±0.3
	$C_{18:2}$ Linoleic	22.2±0.2	31.8±0.5	34.8±0.3	27.6±0.4	32.9±0.4
Caffeic acid and Ethyl gallate	$C_{16:0}$ Palmitic	21.6±0.2	18.3±0.3	19.1±0.1	19.8±0.4	17.1±0.2
	$C_{18:0}$ Stearic	7.4±0.3	7.0±0.1	2.1±0.2	1.0±0.1	1.0±0.1
	$C_{18:1}$ Oleic	47.2±0.3	40.7±0.5	46.6±0.4	41.5±0.5	37.8±0.2
	$C_{18:2}$ Linoleic	22.2±0.3	32.2±0.2	30.3±0.5	36.4±0.4	43.3±0.3

The results about changes of fatty acid composition of the examined oils are shown in Tables 1 and 2. The amount of oleic acid decreased during the first month of examination of non-stabilized oil and oil stabilized with caffeic acid, and combination of caffeic acid and ethyl gallate stored at 25°C, after that slightly increase of its quantity in the second month was observed. Then, the content of the same acid decreased again up to the 120th day of storage.

Table 2. Changes of fatty acid composition of pumpkin seed oil during long-term storage at 10°C

At 10°C								
Stabilized with	Fatty acids, %	Days						
		0	30	60	90	120	150	180
Control sample	$C_{16:0}$ Palmitic	21.6± 0.1	22.1±0.4	16.3±0.3	16.9±0.5	18.4±0.4	15.7±0.7	23.7±0.5
	$C_{18:0}$ Stearic	7.4±0.2	6.8±0.5	5.3±0.3	6.3±0.2	1.6±0.1	1.6±0.1	6.4±0.4
	$C_{18:1}$ Oleic	47.2±0.5	44.2±0.4	43.1±0.4	38.7±0.3	37.7±0.5	38.2±0.4	49.6±0.6
	$C_{18:2}$ Linoleic	22.2±0.2	25.0±0.2	33.5±0.4	36.2±0.5	40.8±0.6	43.6±0.5	19.4±0.2
Caffeic acid	$C_{16:0}$ Palmitic	21.6±0.3	21.5±0.5	20.3±0.5	15.1±0.4	18.9±0.2	17.0±0.2	18.7±0.3
	$C_{18:0}$ Stearic	7.4±0.1	6.7±0.2	4.6±0.2	5.8±0.2	1.1±0.1	2.4±0.1	4.6±0.3
	$C_{18:1}$ Oleic	47.2±0.5	44.3±0.5	43.4±0.4	37.4±0.4	37.6±0.3	39.9±0.2	41.5±0.5
	$C_{18:2}$ Linoleic	22.2±0.3	25.8±0.4	30.1±0.4	40.1±0.4	40.8±0.3	38.6±0.3	34.5±0.4
Ethyl gallate	$C_{16:0}$ Palmitic	21.6±0.2	21.2±0.3	16.8±0.2	15.9±0.2	17.7±0.4	17.4±0.3	21.9±0.5
	$C_{18:0}$ Stearic	7.4±0.1	6.1±0.2	1.9±0.1	5.9±0.2	1.5±0.1	2.3±0.1	5.4±0.1
	$C_{18:1}$ Oleic	47.2±0.4	44.4±0.4	40.6±0.5	37.6±0.3	37.5±0.2	40.2±0.5	46.9±0.5
	$C_{18:2}$ Linoleic	22.2±0.2	26.2±0.3	39.3±0.4	38.8±0.5	41.9±0.4	38.8±0.6	24.8±0.4
Caffeic acid and Ethyl gallate	$C_{16:0}$ Palmitic	21.6±0.3	20.8±0.3	18.3±0.2	17.8±0.3	18.8±0.3	16.3±0.3	19.3±0.4
	$C_{18:0}$ Stearic	7.4±0.2	2.1±0.1	5.0±0.2	6.7±0.3	1.6±0.1	1.8±0.1	4.4±0.2
	$C_{18:1}$ Oleic	47.2±0.5	45.1±0.4	41.8±0.5	39.9±0.3	39.3±0.4	39.3±0.4	40.7±0.5
	$C_{18:2}$ Linoleic	22.2±0.3	30.2±0.3	33.3±0.4	33.7±0.3	39.0±0.2	41.6±0.5	34.7±0.4

On the other hand, it was observed inconsiderably reducing of the amount of oleic acid in the oil stabilized with ethyl gallate which was almost the same until the 60th day. After that its quantity gradually increased on the 90th day and decreased up to the 120th day. The amount of linoleic acid increased during long-term storage of the oil stabilized with caffeic acid and the mixture of the antioxidants. On the other hand, the results about the same acid differed in the stabilized oil with ethyl gallate. The content of linoleic acid slightly increased during the second month of storage, after that decreased on the 90th day and finally gradually increased up to the 120th day. Its quantity increased in non-stabilized pumpkin seed oil during the first month and again decreased in the second month of storage. The amount of linoleic acid remained almost the same during the third month and finally increased after the fourth month. Changes of fatty acid composition of the examined pumpkin seed oils at 10°C were also established and the content of oleic acid gradually decreased on the 90th day in non-stabilized and stabilized oil with both antioxidants. Then its amount remained almost the same up to the 150th day. After that the quantity of the same fatty acid increased sharply in the control sample and gradually in the stabilized oil. The content of oleic acid in the rest of the samples decreased on the 90th day and its amount remained the same up to the 120th day. The quantity of the latter acid increased gradually in the oil stabilized with caffeic acid, but the increase was sharp in the oil with addition of ethyl gallate.

The amount of linoleic acid increased gradually in the control sample and in the oil stabilized with both antioxidants during five months of storage, but after that it decreased. The content of this fatty acid in the stabilized pumpkin seed oil with caffeic acid increased on the 90th day and remained almost the same up to the 120th day and finally decreased gradually on the 180th day. The amount of linoleic acid in the sample stabilized with ethyl gallate smoothly increased up to the second month, remained the same up to the 150th day and sharply decreased on the 180th day.

Finally, changes of fatty acids of the oils stored at 10°C were less than those kept at 25°C and exposed to a day light.

CONCLUSION

According to the obtained results pumpkin seed oil was more stable when was stored at 10°C in a dark place. On the other hand, even stabilized oils deteriorated for 120 days at 25°C. Peroxide value increased to a higher level in the control sample and in stabilized oils kept at 25°C. The changes in acid value and fatty acid composition were minor but the content of tocopherols decreased significantly during storage. Overall, ethyl gallate could be considered as the most appropriate antioxidant for preserving pumpkin seed oil during long-term storage.

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ПРОМЕНИ ПРИ ОКИСЛЕНИЕ НА ТИКВЕНО МАСЛО, СТАБИЛИЗИРАНО С РАЗЛИЧНИ АНТИОКСИДАНТИ ПРИ ДЪЛГОСРОЧНО СЪХРАНЕНИЕ

Ж. Ю. Петкова*, Г. А. Антова, М. Й. Ангелова-Ромова

ПУ „Паисий Хилендарски”, Катедра „Химична технология”, ул. „Цар Асен” 24, 4000 Пловдив, България,

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(Резюме)

Проследени са промените, протичащи при окислението на масло от семена на тиква при дългосрочно съхранение и различни условия. Изследванията са проведени с тиквено масло и такова, стабилизирано с различни антиоксиданти (кафеена киселина, етилгалат и комбинация от двата антиоксиданта). Дългосрочното съхранение е проведено за период от шест месеца при стайна температура (25°C) и на тъмно при 10°C. На определени интервали от време са проследени важни индикатори, които определят оксидантната стабилност на маслата, като киселинно и пероксидно число, мастнокиселинен състав и общо съдържание на токофероли. Значителни промени са установени в стойностите на пероксидното число при всички проби за целия период на изследването, като по-големи са в тези, съхранявани при стайна температура. Промените в стойностите на киселинното число и мастнокиселинния състав са незначителни. Общото съдържание на токофероли намалява при всички изследвани масла, като промяната е изразена в по-малка степен при тези, съхранявани при по-ниска температура (10°C). Установено е, че най-стабилно е тиквено масло с добавка от етил-галат и съхранявано при 10°C.