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HAEMATOCOCCUS PLUVIALIS DERIVED ASTAXANTHIN – A POWERFUL BIOACTIVE COMPOUND FOR VEGETABLE OILS

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Keywords: *Astaxanthin, Haematococcus pluvialis, bioactive compound, antioxidant, vegetable oil, conjugated dienes.*

Introduction. *The beneficial effects on health result from the protective action of astaxanthin, (AXT) a powerful antioxidant capable of scavenging free radicals and protecting cells from oxidative stress. The study aimed to evaluate the protective role of astaxanthin derived from Haematococcus pluvialis in reducing the thermal oxidation of fatty acids in vegetable oils.*

Material and methods. *Astaxanthin, obtained by extraction from the biomass of Haematococcus pluvialis, at a concentration of 0.26-0.29 mg/mL, was added to olive, sunflower, almond, walnut, sesame, and poppy seed oils. The progression of oxidation was monitored based on the formation of conjugated dienes. The formation of conjugated dienes was monitored spectrophotometrically.*

Results. *Astaxanthin reduced the content of conjugated dienes in sesame, almond, and walnut oils by 30-34%. A strong antioxidant effect of AXT was noted in the case of poppy seed oil, for which the formation of conjugated dienes was decreased by 42% and the oxidation was delayed by 60 min when exposed to high temperatures compared to native oil. For sunflower oil, which exhibited a high degree of thermal oxidation, addition of AXT reduced the formation of conjugated dienes by 22% during the experiment.*

Conclusions. *Astaxanthin from Haematococcus pluvialis significantly reduced the formation of conjugated dienes, indicating that it does not act as a prooxidant in various vegetable oils.*

Cuvinte-cheie: *Astaxantina, Haematococcus pluvialis, compus bioactiv, antioxidant, ulei vegetal, diene conjugate.*

ASTAXANTINA DERIVATĂ DIN *HAEMATOCOCCUS PLUVIALIS* – UN PUTERNIC COMPUȘ BIOACTIV PENTRU ULEIURILE VEGETALE

Introducere. *Beneficiile astaxantinei (AXT) pentru sănătate rezultă din acțiunea sa protectoare. Acest antioxidant puternic neutralizează radicalii liberi și protejează celulele împotriva stresului oxidativ. Studiul a avut ca scop evaluarea rolului protector al astaxantinei, obținute din Haematococcus pluvialis, în reducerea efectelor oxidării termice asupra acizilor grași din uleiurile vegetale.*

Material și metode*. Astaxantina, obținută prin extragere din biomasa de Haematococcus pluvialis, a fost adăugată la uleiurile: de măsline, floarea-soarelui, migdale, nuci, susan și de mac în concentrația de 0,26-0,29 mg/ml. Evoluția procesului oxidativ a fost monitorizată în baza formării dienelor conjugate. Formarea dienelor conjugate a fost înregistrată spectrofotometric.*

Rezultate*. Astaxantina a redus cu 30-34% conținutul dienelor conjugate în uleiurile de susan, migdale și de nuci. Astaxantina a demonstrat un efect antioxidant semnificativ pentru uleiul de mac, expus temperaturilor înalte, diminuând formarea dienelor conjugate cu 42% și amânând oxidarea cu 60 de minute, în comparație cu uleiul netratat. Pentru uleiul de floarea-soarelui, care a prezentat un grad ridicat de oxidare termică, adăugarea de AXT a redus formarea dienelor conjugate cu 22%.*

Concluzii*. Astaxantina derivată din Haematococcus pluvialis a redus semnificativ formarea dienelor conjugate în diverse uleiuri vegetale, indicând lipsa efectului prooxidant.*

INTRODUCTION

Astaxanthin (AXT) is a xanthophyll carotenoid with a unique molecular structure. This distinctive structure imparts strong antioxidant properties to AXT, allowing it to function both inside and outside the cell membrane (1). It has been widely studied for its health benefits, including alleviating diabetes mellitus, neurodegenerative and cardiovascular diseases, hepatic disorders, and providing protection against various cancers. These effects stem from its ability to scavenge free radicals and shield cells from oxidative stress (2, 3).

One of the most recognized unconventional sources of astaxanthin is the unicellular green alga *Haematococcus pluvialis*, which can accumulate 1.5-3% pigment on a dry cell weight basis (4). Astaxanthin, produced by *H. pluvialis*, is the main natural source for human consumption. Due to its high price and limited sources, AXT is not well-known to consumers and is undervalued by food manufacturers. The antioxidant potential of AXT allows food technologists to offer a wide range of functional foods (5, 6). Incorporating astaxanthin into oils is a promising alternative to using this pigment (7).

Haematococcus pluvialis, a unicellular green alga, is a major natural source of astaxanthin, primarily used for human consumption (4). Despite its high price and limited sources, astaxanthin's antioxidant potential makes it valuable in developing functional foods (5, 6). Incorporating astaxanthin into oils enhances oxidative stability, offering a promising alternative for health-promoting food products (7).

Vegetable oils have long been regarded as functional foods and nutraceuticals, offering a variety of beneficial effects on human health (8). For example, olive oil is an essential component of the Mediterranean diet and plays a crucial role in reducing the incidence of cardiovascular diseases, including myocardial infarction and stroke. Oleic acid is the most abundant monounsaturated fatty acid in olive oil, with its concentration ranging from 56% to 84% of the total fatty acid content. Tocopherols, hydrophilic and lipophilic phenols, and other minor constituents account for 1-2%. All these components help boost heart health (9). Walnut oil is widely used in traditional medicine and has become a popular dietary supplement in many countries. Walnut-rich nutrition is viable to prevent declining cholinergic function in the brain and reduce oxidative stress in neurons by activating antioxidant enzymes like superoxide dismutase and glutathione peroxidase (10). Research is being conducted to identify the mechanism of action of the oil as a valuable supplement in treatment of multiple sclerosis (11). In addition to its antioxidant activity, walnut oil significantly decreases serum tumor necrosis factor-α, interleukin-6, and IL-1β levels, improving the anti-inflammatory ability and generating anti-inflammatory compounds by restoring bacterial balance (12). The protective effects of sesame oil are manifested through the reduction of proinflammatory cytokines (11). Poppy seed oil can improve the plasma lipid profile and the antioxidant status of hepatocytes (13). Based on its fatty acid profile, almond oil is also a nutraceutical product with notable antiatherosclerotic, antihepatotoxic, and regenerative effects in humans (14, 15). Functional foods are defined as foods consumed as part of a normal diet that contain biologically active components (whether added or naturally present) with the potential to improve health and/or reduce the risk of disease (16). Vegetable oils are an excellent source of bioactive compounds that can be utilized in the nutraceuticals and functional foods field (17).

Incorporating natural bioactive into traditional foods to create new functional foods (such as oils, beverages, baked goods, and dairy products) is a rapidly growing global market (18). In the commercial segment of functional foods, the category of vegetable oils is experiencing the fastest growth. Consequently, a recent study has focused on the potential of astaxanthin as a natural additive of health-promoting compounds, useful for production of functional foods. Thus, the combination of linseed oil and astaxanthin can mitigate the effects of oxidative stress and reduce inflammatory processes, positioning itself as a functional food to prevent cardiovascular diseases (19).

Vegetable oils have been studied as solvents and stabilizers for astaxanthin obtained from natural sources. Vegetable oil may be one of the factors responsible for the increased bioavailability of astaxanthin, thus defining the application areas of the final product (20).

Incorporating natural astaxanthin into vegetable oil leverages astaxanthin's potent antioxidant properties, without pro-oxidant effects. This combination can substantially inhibit the oxidation of oils. Considering astaxanthin's potential to improve the oxidative stability of vegetable oils, *this study aimed* to evaluate its antioxidant effect when added to various vegetable oils.

MATERIAL AND METHODS

The biomass of unicellular green alga *Haematococcus pluvialis* CNMN-AV-05 strain, deposited in the National Collection of Nonpathogenic Microorganisms (Technical University of Moldova, Institute of Microbiology and Biotechnology, Chisinau, Moldova) served as a source of astaxanthin. The microalga was cultured in a mineral medium with the following composition (in g/L): $NaNO_3$ – 0.3; KH_2PO_4 – 0.02; K_2HPO_4 – 0.08; NaCl – 0.02; $CaCl_2$ – 0.05; $MgSO_4\cdot 7H_2O$ – 0.01; $ZnSO_4\cdot 7H_2O$ – 0.0001; $MnSO_4\cdot 5H_2O$ – 0.0015; $CuSO_4\cdot 5H_2O$ – 0.00008; H_3BO_3 – 0.0003; $(NH_4)6Mo_7O_{24}\cdot 4H_2O$ – 0.0003; $FeCl_3\cdot 6H_2O$ – 0.0175; $Co(NO_3)2\cdot 6H_2O$ – 0.0002; EDTA – 0.0075; at a temperature of 26°C, under constant illumination of 28 µmol m-2 s-1 with periodic stirring for the first ten days of cultivation. Astaxanthin production was induced by excessive illumination at 56 µmol $m^{-2}s^{-1}$ for 72 hours.

H. pluvialis biomass consisting of aplanospores (red cysts) was separated from the culture liquid by centrifugation for 5 min at 1500 g, and the cell pellet was disrupted by microwave treatment at 450 W for 120 seconds. To extract astaxanthin, 10 mg of the prepared biomass was mixed with 5 mL of 96% ethanol. The extraction process involved continuous stirring at room temperature for 30 minutes.

The astaxanthin content was determined spectrophotometrically at 478 nm. A calibration curve was prepared using synthetic astaxanthin with 98% purity (Merck KGaA, Darmstadt, Germany) over a concentration range of 0.5 to 4.0 µg/mL (n=7). The linearity of the calibration curve was confirmed with an r^2 value of 0.999.

Vegetable oils from sunflower, almond, poppy seeds, and walnut kernels were manufactured by "INDUSTRY INVESTMENT" SRL, Chisinau, Republic of Moldova. Sesame oil was produced by "Condiprod-Com" SRL, Ukraine, and olive oil-by "Helcom," Portugal.

To solubilize astaxanthin in oils, 100 mL of an oil suspension with 1.0 g of MW-treated biomass was prepared and stirred at room temperature for 180 minutes. The oils were then separated from the remaining biomass by decantation. The amount of astaxanthin in oils was determined spectrophotometrically and recalculated according to the calibration curve.

The presence of astaxanthin in oils was confirmed by recording absorption spectra with the determination of the specific maximum of astaxanthin at a wavelength of 482-484 nm.

In this study, lipid oxidation was induced by maintaining the samples at a temperature of 60°C in the dark. The formation of conjugated dienes was monitored using spectrophotometry. Vegetable oils were diluted with hexane at a ratio of 1:600 (v/v). The absorbance of these diluted oil samples was measured at 234 nm. Changes in absorbance values were recorded as indicators of diene formation (17).

The investigations were conducted in three independent experiments. The results were statistically analyzed by calculating the arithmetic mean, standard error, and confidence interval, using the parametric t-test with a significance level of $p<0.05$.

RESULTS

Astaxanthin obtained from *H. pluvialis* biomass was supplemented with vegetable oils in concentrations of 0.26-0.29 mg/mL. The temperature of 60℃, chosen as the inductor of the oxidation process, caused a slow process of fatty acid oxidation when exposed to an oxidizing factor.

Figure 1 shows the spectra of oils before and after solubilization of natural astaxanthin in them.

Vegetable oils with AXT exhibited absorption peaks at 482-484 nm, indicating the solubilization of astaxanthin in oils. These recorded absorption peaks are characteristic of the pigment. The content of astaxanthin in oils ranged from 0.26 mg/mL in the case of sesame oil and olive oil (fig. 1D, 1B), 0.72 mg/ml in sunflower oil and almond oil (fig. 1A, 1F) to 0.287-0.29 mg/mL in poppy seed oil and wal nut oil (fig. 1E, 1C). In most cases, the solubilization of astaxanthin in oils did not depend on the degree of unsaturation.

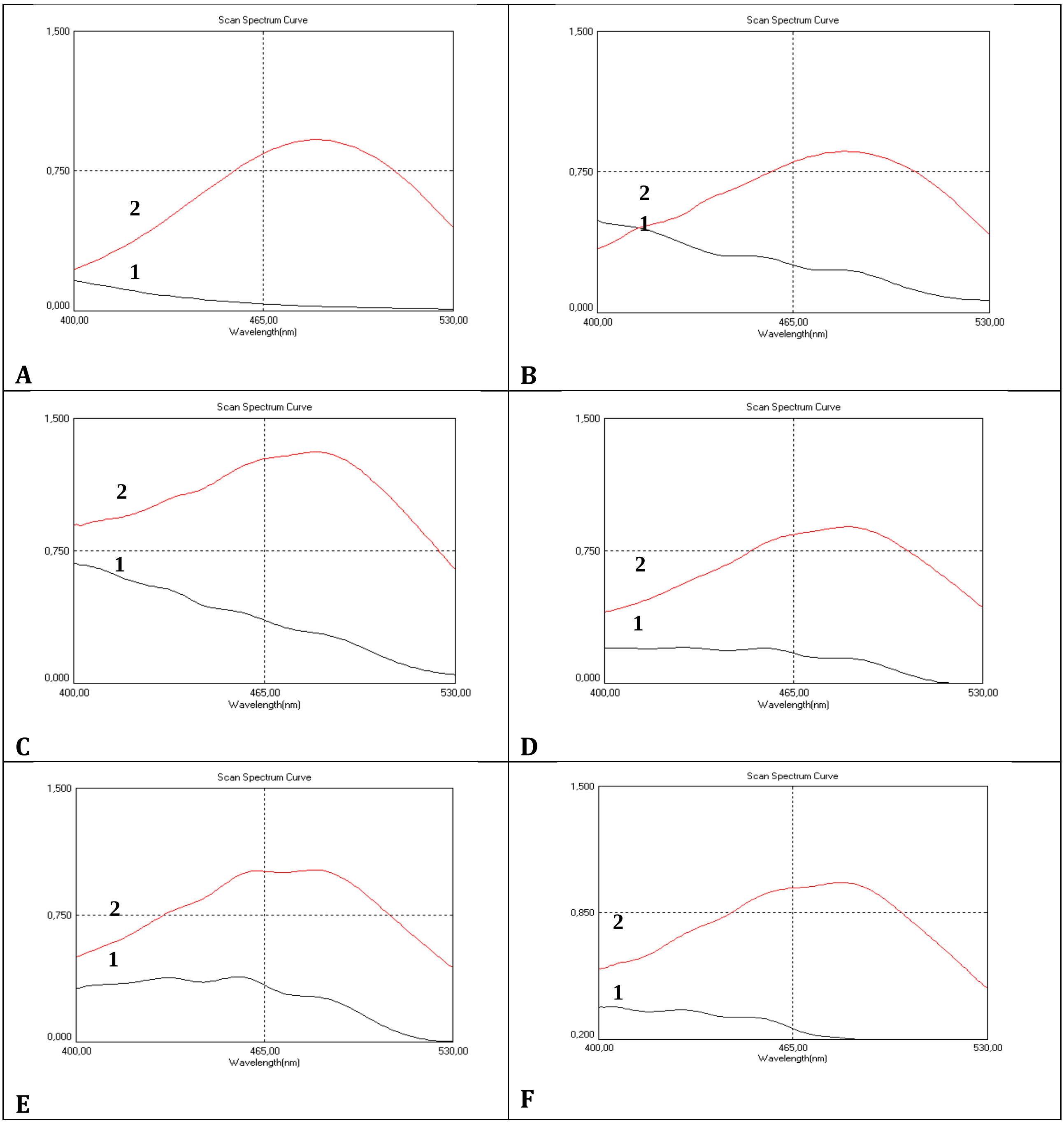
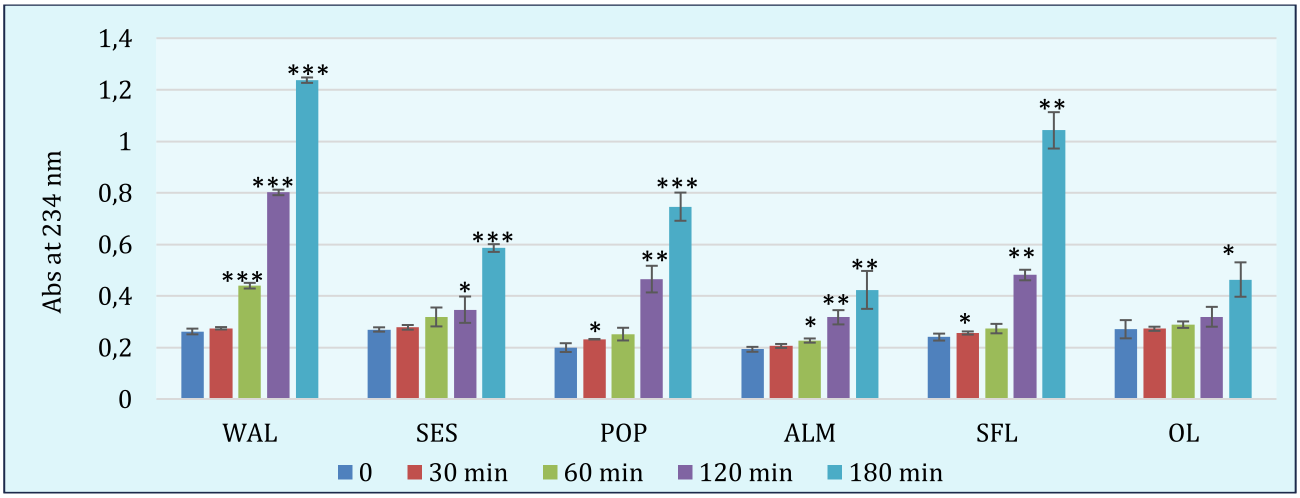


Figure 1. Spectra of oils used to solubilize natural AXT from *H. pluvialis*: A – sunflower oil; B – olive oil; C – walnut oil; D – sesame oil; E – poppy seed oil; F – almond oil; 1 – spectrum of native vegetable oil; 2 – spectrum of oil with solubilized natural astaxanthin.

Native vegetable oils and oils with solubilized natural astaxanthin were subjected to lipid oxidation. Vegetable oils and their mixtures with AXT that have not passed the oxidation test are called the zero variant. The change in the absorbance at 234 nm of oils in their native form was analyzed. Within 30 min, the absorbance at 234 nm for the oils under study did not change significantly. In poppy seed oil, the content of conjugated dienes increased by 16% ($p<0.05$). After 60 min, the stability of the olive oil was assessed. The content of conjugated dienes in sesame and almond oils increased by 18%, and in sunflower oil – by 14%. The absorption value of poppy seed oil increased by 26% at 234 nm. In the case of walnut oil, the absorption increased by 68% ($p<0.001$).



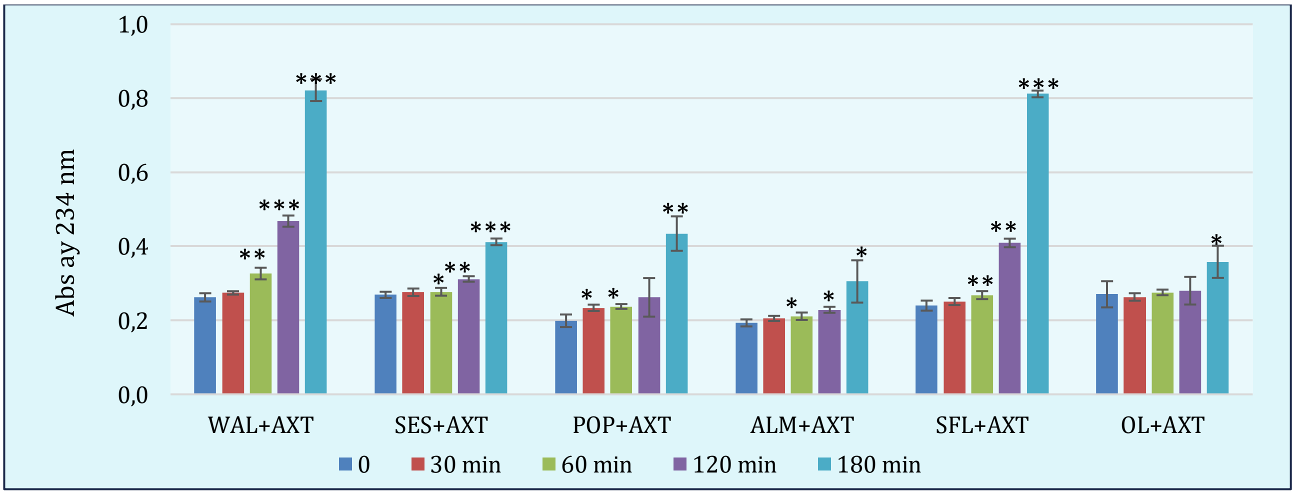
Note: n=3, "0"- native oils, * $p<0.05$; ** $p<0.01$; *** $p<0.001$

Figure 2. Changes in absorption data at 234 nm of vegetable oils subjected to a temperature of 60°C.

After 120 min of oil incubation at 60°C, the olive oil remained the most stable, with an 18% increase in absorbance at 234 nm. In sesame and almond oils, the absorption value increased by 28% and 64%, respectively. In the case of sunflower and poppy seed oils, the absorbance at 234 nm was doubled. A tripling of the content of conjugated dienes was recorded for walnut oil.

The duration of 180 min heat stress further induced lipid oxidation, which in the case of walnut and olive oils increased by another 45-54% compared to the content of dienes determined after an oxidation time of 120 min. The absorption value of sesame and poppy seed oils increased by 60-70%. The content of conjugated dienes in sunflower oil increased by more than 100%. Almond oil slowed the oxidation process, increasing the absorbance at 234 nm by 33%.

Compared to the zero variant, vegetable oils subjected to moderate heat stress accumulated conjugated dienes differently. Thus, the content of conjugated dienes in walnut and sunflower oils increased more than four times. In poppy seed oil, the content of conjugated dienes increased by 3.7 times ($p<0.001$). In sesame and almond oils, absorbance at 234 nm increased by 2.17 ($p<0.001$) – 2.19 ($p<0.01$) times. Olive oil turned out to be the least susceptible to the oxidation process caused by a temperature of 60°C, the increase in the absorbance value at 234 nm was 1.7 times ($p<0.05$).



Note: n=3, "0"- native oils, * $p<0.05$; **$p<0.01$; *** $p<0.001$

Figure 3. Changes in absorption data at 234 nm of vegetable oils with natural astaxanthin subjected to a temperature of 60°C.

Table 1. Fatty acid content (average values) in selected oils (8).

The type of oil	**Unsaturated fatty acids, % total fatty acids**				**Saturated fatty acids, % total fatty acids**	
	18:1n9	18:2n6	18:3n3	n3 + n6	18:0	16:0
Sunflower	28	62	-	62	3	6
Olive	66	16	2	18	2	16
Walnut	11	66	10	76	3	7
Sesame seeds	42	41	-	41	6	10
Poppy seeds	12	74	-	74	2	10
Almond	67	23	-	23	3	7

Astaxanthin, derived from microalgae *Haematococcus pluvialis*, has been added to vegetable oils to monitor the pigment's ability to stop fatty acid oxidation. During a 30-minute exposure at 60°C, the absorbance at 234 nm for oils with the addition of AXT changed similarly to native vegetable oils. The content of conjugated dienes in poppy seed oil increased by 17% ($p<0.05$). Heat stress exposure of oils with AXT for 60 min resulted in absorption stability at 234 nm for sesame, olive, and almond oils and a 24% ($p<0.01$) increase in walnut oil. Sunflower and poppy seed oils, supplemented with AXT, slightly changed their absorbance at 234 nm. After 120 min incubation at 60°C, the excess of conjugated dienes in sunflower and walnut oils was determined, while the absorption value increased by 70% and 80%, respectively.

In oils with AXT, for which the oxidation process started at a temperature regime of 60°C, the accumulation of conjugated dienes occurred slowly. The absorption value of poppy seed oil increased by 31% ($p<0.01$) at 234 nm. In the presence of astaxanthin, no doubling of conjugated dienes content was observed during the 120-minute heat stress accumulation period. Keeping vegetable oils with the addition of AXT for 180 min at a temperature of 60°C led to the oxidation of fatty acids with an increase in the absorbance value at 234 nm by 53% ($p<0.001$) in sesame oil and by 58% ($p<0.05$) in almond oil. In walnut and sunflower oils, the absorption value increased by more than threefold. Olive oil was stable, content of conjugated dienes was higher by 32% ($p<0.05$).

DISCUSSIONS

Vegetable oils examined as lipid carriers for astaxanthin can be utilized to produce nutraceuticals or AXT-based functional foods (21). The varied content of fatty acids in oils diversifies and enhances the nutraceutical component of oily astaxanthin preparations, defining their application areas. Among the identified fatty acids in walnut oil (about 77%), α-linoleic acid (ω-3) predominated at 66% (tab. 1).

Poppy seed oil is characterized by having more than 70% linoleic acid, and sunflower oil has over 62%. On the contrary, both olive and almond oils are dominated by oleic acid (66-67% of the total fatty acids). Sesame oil contains equal parts of oleic acid and linolenic acid. The content of polyene acids in vegetable oils is one factor that largely determines the oxidation process with the formation of conjugated dienes (22, 23). Among the vegetable oils subjected to a long-term test (63°C), rapeseed oil, containing up to 60% monounsaturated fatty acids, was the least susceptible to oxidation. Among the oils selected for the study, the most resistant to thermal oxidation were olive and almond oils with an oleic acid content of more than 65%, and sesame seed oil with over 40% oleic acid (tab. 1).

In another study of the thermal stability of vegetable oils, depending on their content of polyenoic fatty acids, the thermal stability of native sunflower oil was compared with that of sunflower oil with added oleic acid. The oils were exposed to 180-190°C for 8 hours over three days. The oil enriched with oleic acid showed better thermal stability than native sunflower oil (24).

When comparing oils that are rich in polyunsaturated omega-6 and omega-3 fatty acids, such as sunflower, walnut, and poppy seed oils, the oxide tion of these oils occurred at different rates. Walnut oil was the most prone to degradation processes, likely due to the presence of 10% linolenic acid. In our case, walnut oil had the highest values of formed dienes. Some studies have established that a determining factor in the intensity of oxidative processes in this oil is the palmitic acid content (25). Therefore, the technological conditions affecting the fatty acid composition of this oil, are important (26).

Native sunflower and poppy seed oils similarly accumulated oxidative degradation products of fatty acids. In the oxidation process of vegetable oils at 180°C, sunflower oil exhibited the highest levels of oxidative degradation products (27). Poppy seed oil is considered stable against thermal-oxidative stress (28). The inherent antioxidants of the oils, those added for product preservation, and the extraction method applied were not considered (29). This assumption is supported by results showing the addition of astaxanthin, which significantly reduced the oxidation process in walnut, sunflower, and poppy seed oils, with the latter showing the most favorable result: a reduction in conjugated diene content by 42% ($p<0.01$).

The lowest values of diene content in native vegetable oils subjected to oxidation were found in olive, sesame, and almond oils (fig. 2). Olive oil contains the lowest levels of polyunsaturated fatty acids, followed by almond and sesame oils (tab. 1). Olive oil is considered one of the most resistant vegetable oils to high temperatures. These properties are due to its low polyunsaturated acid content (30) as well as the presence of antioxidant components, the content of which depends on the extraction techniques applied (31). Almond oil has also proven to be resistant to hyperthermic conditions. Studies have shown that this property depends on the origin of the almonds (32). In the experiment, sesame oil demonstrated stability against thermal oxidation, a known characteristic of the oil (33). Adding astaxanthin to olive oil, almond, and sesame oils did not alter their antioxidant properties, as they continued to exhibit low levels of conjugated dienes (fig. 3).

Astaxanthin in the studied oils delayed the oxidation of fatty acids. In native oils, the accumulation of conjugated dienes begins after 60 minutes of hyperthermia (fig. 2), whereas in oils with added astaxanthin, this process starts after 120 minutes (fig. 3).

Adding pigment extracts from *H. pluvialis* to sunflower oil, significantly increased ($p<0.05$) its oxidation stability during short-term exposure of 180°C (34). Astaxanthin donates a hydrogen atom to peroxyl radicals due to the presence of dihydroxyl groups in the β-ionone rings (35). In several edible oils, including sunflower, olive, and mustard oils, subjected to thermal oxidation at 50-70°C, astaxanthin remains an active component. However, a temperature of 180°C leads to the degradation of the antioxidant (20).

Products of microalgae origin are being studied as vegetable oil protectors. Microalga *Nannochloropsis oculata* in powder form added to soybean oil inhibited its oxidation (36). A positive dependence of the oxidative process in oils on the concentration of the added microalgae product has been demonstrated. Thus, 5.0% microalgal powder increased more than twice the stability index of soybean oil subjected to oxidation at temperatures of 120 and 130°C. Microalga *N. oculata* has been proposed as an antioxidant additive to vegetable oils to prevent the generation of free radicals during processing at high temperatures. Microalga *Chlorella vulgaris*, added to olive oil in concentrations of 0.5, 1.0, and 1.5%, significantly delayed the formation of oxidative degradation products by 20-33% compared to industrial antioxidants. The antioxidant effect of microalgae was even higher than that of β-carotene used as a control stabilizer (37). Microalgae-derived antioxidants are starting to gain popularity in the processing of functional and organic foods. Astaxanthin is included in the list of these antioxidants (9, 38).

CONCLUSIONS

1. Astaxanthin from *Haematococcus pluvialis* significantly reduced the formation of conjugated dienes, indicating that it does not act as a prooxidant in various vegetable oils. The addition of astaxanthin notably enhanced the thermal stability of oils, particularly those high in oleic acid. For example, the formation of conjugated dienes decreased by 42% in poppy seed oil and by 30-34% in sesame, almond, and walnut oils.
2. Olive oil showed the smallest increase in conjugated dienes when supplemented with astaxanthin, even under high-temperature conditions, demonstrating that astaxanthin can modify the functional properties of oils without acting as a pro-oxidant. Future studies should examine the long-term stability of enriched oils under various conditions and assess their sensory and nutritional impacts. Exploring the combination of astaxanthin with other antioxidants could provide insights into developing more effective antioxidant systems for food applications.

CONFLICT OF INTERESTS

The authors of the article deny the existence of any conflict of interest in the publication of this material.

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