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Araştırma
(Research)

Oxidation Stability of Hazelnut Oil Supplemented with β-Carotene During Light Exposure Using Xenon Test Instrument

Sümeyye ŞAHİN[1*]

[1]Ordu University Faculty of Agriculture, Department of Food Engineering, Ordu/TÜRKİYE

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Sorumlu yazar: Sümeyye ŞAHİN, e-posta: gmsumeyyesahin@gmail.com

Abstract

Objective: The inhibition of lipid oxidation is important for the application of edible fats and oils in food processing. High oleic oils exhibit high stability during oxidation. Hazelnut oil, naturally rich in oleic acid, has recently become widely used in the food industry due to its high oxidation stability. Antioxidants are added to oils to make them more resistant to oxidation. β-carotene is a natural antioxidant and leads to increased resistance to photooxidation. The aim of this work was to investigate the effect of a natural antioxidant β-carotene on light-induced lipid stability of hazelnut oils.

Materials and Methods: The hazelnut oil was supplemented with β-carotene in 25 ppm. Not-supplemented hazelnut oil (HO) and the beta-carotene added hazelnut oil (HO with β-car.) were irradiated in a xenon test instrument by different light intensities at 275 and 765 W/m^2 for 2, 4, 6, 8, 10, 12, 18, 24, 30, 36, 42 and 48 hours. Fatty acids from the hazelnut oil samples were determined as fatty acid methyl esters (FAMEs) using GC-FID method. To investigate the degree of oxidation of the hazelnut oil samples, the induction time with an accelerated aging test using a Rancimat, peroxide value, volatile lipid oxidation compounds using SPME-GC-FID and antioxidative capacity using DPPH-radical scavenging assay were measured.

Results: The results indicated that the change in fatty acid composition with irradiation intensity and duration of irradiation is not so strong. Among the fatty acids, a significant change was detected only in the linoleic acid content between the HO and the HO with β-car. after light exposure. The amounts of hexanal, heptenal, E-2,4-heptadienel, nonenal, and nonanoic acid increased after photooxidation. The antioxidant capacity of the hazelnut oil samples decreased during light exposure. After light exposure, HO with β-car. showed a higher induction time and lower peroxide value compared to HO.

Conclusion: These results confirm that the β-carotene supplemented hazelnut oil showed higher oxidative stability than hazelnut oil without additional β-carotene. In conclusion, the results suggest that β-carotene could slow down the photooxidation of hazelnut oil.

Keywords: Hazelnut oil, β-carotene, oxidative stability, rancimat, light exposure

Xenon Test Cihazıyla Işığa Maruz Bırakılan β-Karoten Takviyeli Fındık Yağının Oksidasyon Kararlılığı

Öz

Amaç: Lipid oksidasyonunun önlenmesi, yenilebilir katı ve sıvı yağların işlenmesinde gıda sanayi için önemlidir. Yüksek oleik yağlar oksidasyon sırasında yüksek stabilite göstermektedirler. Doğal olarak oleik asit açısından zengin olan fındık yağı, yüksek oksidasyon stabilitesi nedeniyle son zamanlarda gıda endüstrisinde yaygın olarak kullanılmaya başlanmıştır. Yağlara oksidasyona karşı daha dayanıklı olmaları için antioksidan ilavesi yapılmaktadır. β-karoten doğal bir antioksidandır ve fotooksidasyona karşı direncin artmasına neden olur. Bu çalışmanın amacı, doğal bir antioksidan olan β-karoten ilavesinin, ışık maruziyeti altında fındık yağlarının lipid stabilitesi üzerindeki etkisini araştırmaktır.

Materyal ve Yöntem: Fındık yağına 25 ppm oranında β-karoten ilave edildi. Katkısız fındık yağı ve beta-karoten ilaveli fındık yağı, bir ksenon test cihazında

275 ve 765 W/m^2'lik iki farklı ışık yoğunluğunda 2, 4, 6, 8, 10, 12, 18, 24, 30, 36, 42 ve 48 saat süresince ışığa maruz bırakıldı. Fındık yağı örneklerinden elde edilen yağ asitleri, GC-FID yöntemi kullanılarak yağ asidi metil esterleri (FAME'ler) olarak belirlendi. Fındık yağı örneklerinin oksidasyon derecesini araştırmak için, Ransimat cihazında hızlandırılmış yaşlandırma testi ile indüksiyon süresi, peroksit değeri, SPME-GC-FID kullanılarak uçucu lipit oksidasyon bileşikleri ve DPPH-radikal temizleme testi kullanılarak antioksidatif kapasite ölçüldü.

Araştırma Bulguları: Sonuçlar, ışınlama yoğunluğu ve ışınlama süresi ile yağ asidi bileşimindeki değişimin çok güçlü olmadığını gösterdi. Işık maruziyeti sonrası linoleik asit içeriği açısından β-karoten ilaveli ve ilavesiz fındık yağları arasında önemli farklılıklar tespit edildi. Hekzanal, heptenal, E-2,4-heptadienel, nonenal ve nonanyonik asit miktarları fotooksidasyonla artmıştır. Fındık yağı örneklerinin antioksidan kapasitesi ışığa maruz kalma süresince azaldı. Işık maruziyeti sonrasında β-karoten ilaveli fındık yağının, antioksidan katkısız fındık yağına kıyasla daha yüksek bir indüksiyon süresi ve daha düşük peroksit değeri gösterdiği bulundu.

Sonuç: Bu sonuçlar, β-karoten katkılı fındık yağının, β-karoten içermeyen fındık yağına göre daha yüksek oksidatif stabilite gösterdiğini doğrulamaktadır. Sonuç olarak, elde edilen bulgular β-karotenin fındık yağının fotooksidasyonunu yavaşlatabileceğini göstermektedir.

Anahtar kelimeler: Fındık yağı, β-karoten, oksidatif stabilite, ransimat, ışığa maruz kalma

Introduction

Physical, chemical, and biological causes lead to lipid oxidation (Frede, 2010). Oxidation of lipids leads to the formation of undesirable rancid taste and odor substances (Ternes et al., 2005). In addition, the safety of the product is reduced by the formation of secondary oxidation products (Frankel, 2005). The rate of autoxidation depends on some factors including the fatty acid composition, the concentration and effectiveness of pro- and antioxidants, oxygen partial pressure, the surface in contact with oxygen and storage conditions such as temperature, light and water content Among these factors, the light accelerates the process of oxidation of lipids (Belitz et al., 2008). Saturated fatty acids are relatively stable against oxidation compared to unsaturated (mono- and polyunsaturated) fatty acids (Krist et al., 2008). However, for health reasons, it is recommended to consume unsaturated fatty acids instead of saturated fatty acids (WHO, 2023). Polyunsaturated fatty acids are more exposed to autooxidation than monounsaturated fatty acids (Krist et al., 2008). For this reason, oils rich in monounsaturated fatty acids are more suitable for consumption due to their oxidation resistance and positive effects on health.

Hazelnut oil is naturally high in oleic acid which is a monounsaturated fatty acid (Crews et al., 2005; Köksal et al., 2006; Şahin et al., 2022). Due to its high oleic content, it lowers high cholesterol levels (Krist et al., 2008). In addition to high oleic acids, the hazelnut oil contains lipid-soluble vitamins, especially vitamin E which has health benefits due to its high antioxidant capacity (Alasalvar et al., 2006; Crews et al., 2005). The hazelnut oil is obtained from hazelnut kernels by cold pressing or solvent extraction techniques. Cold pressed or refined hazelnut oil is used as edible oil in cooking, frying, and salad dressings (Alasalvar and Shahidi, 2009; Krist et al., 2008). Besides the food industry, hazelnut oil is also used as massage oil in cosmetics for skin care and as base oil in pharmacy for the treatment of varicose veins (Krist et al., 2008).

Carotenoids, as an antioxidant, protect fatty foods from photooxidation (Belitz et al., 2008). β-Carotene, which is the most abundant carotenoid in fruits and vegetables (Sergio et al., 1999), is the most important inhibitor against type 2 photooxidation (Frankel, 2005). It has been reported that β-Carotene exhibited antioxidant effects in several vegetable oils such as soybean oil (Jung and Min, 1991), sunflower oil (Yanishlieva et al., 2001), conventional and high-oleic rapeseed oils (Sahin et al. 2011). However, there is no data in the literature concerning the role of β-carotene in hazelnut oil oxidation. Therefore, the aim of this study was to investigate the antioxidant effect of β-Carotene on lipid stability of the hazelnut oil during light exposure.

Materials and Methods

Refined hazelnut oil (HO) was supplied from Çotanak (Ordu, Türkiye). Trolox, β-Carotin (pure 97,0%[UV]), DPPH, and potassium methoxide were purchased from Sigma-Aldrich (St. Louis, USA). Hexane, 25% sulfuric acid, methanol, sodium hydrogen sulphate and n-butanol were obtained from Merck (Darmstadt, Germany). Supelco 37 Component FAME (fatty acid

methyl ester) standard mixture was purchased from Sigma-Aldrich (Taufkirchen,Germany).

Storage under accelerated light conditions

β-Carotene was added as an antioxidant at a concentration of 25 ppm in the hazelnut oil. The amount of β-Carotene to be added was set at 25 ppm, because Codex Standard 19-1981 set the highest amount of β-carotene as a coloring agent at 25 ppm in the oil (Codex,1981). Not-supplemented hazelnut oil (HO) and the beta-carotene added hazelnut oil (HO with β-car.) were stored under defined light conditions in a SUNTEST CPS+, which is a xenon testing device (ATLAS Material Testing Solutions, Linsengericht / Altenhaßlau, Germany). The xenon lamp with an appropriate filter provides the complete sunlight spectrum in the UV/VIS range necessary for the vast majority of weathering tests. Using the xenon lamp technology, natural daylight can be simulated. The SUNTEST CPS+ emits light at 275 and 765 W/m^2 to simulate natural daylight. A light intensity of 275 W/m^2 simulates sunlight when it is cloudy, while a light intensity of 750 W/m^2 simulates sunlight when it is completely sunny with no clouds. Therefore, in this study, two different light intensities of 275 and 765 W/m^2 were used to simulate normal daylight. 200 ml of the oil samples were irradiated in an open glass bottle for 2, 4, 6, 10, 12, 18, 24, 30, 36, 42 and 48 hours at 275 W/m^2 and 765 W/m^2 in SUNTEST CPS+ (Figure 1).

Figure 1. SUNTEST CPS+, a xenon test instrument

Analysis of total antioxidative capacity

Total antioxidant capacity of not-supplemented hazelnut oils and the beta-carotene supplemented hazelnut oils were assayed by the DPPH radical scavenging method described by Şahin et al. (2022). Briefly, 40 µL of oil were mixed with 1500 µL DPPH (600 µM in n-butanol). After incubation at room temperature for 30 min, the absorbance of the mixture was recorded at 515 nm with a UV/Vis spectrophotometer from Analytik Jena (Jena, Germany). Trolox was chosen as the standard. Total antioxidant capacity was calculated as millimoles of trolox equivalents per liter (mmol TE/L).

Determination of fatty acid composition

The fatty acid composition of hazelnut oils was analyzed by a gas chromatograph with a flame ionization detector (GC-FID, Agilent, USA) of FAME according to literature (Petersen et al. 2012). To produce the fatty acid methyl esters, the samples (60 mg) at room temperature are dissolved in hexane (4mL) and then transesterified with potassium methoxide solution (25% in methanol). To prevent saponification of the methyl esters, the mixture was neutralized with sulfuric acid. After filtration of organic layer with sodium hydrogen sulfate, FAMEs were injected (1 µL) into a CP-Sil 88 column (100 m x 0.25 mm I.D., 0.2 µm film thickness; Varian Inc., Lake Forest, USA). A CONCEPT autosampler (PAS Technology, Magdala, Germany) was used for injection under the following chromatographic conditions: carrier gas hydrogen, flow rate 1.3 mL/min, split ratio 10:1, injector temperature 240°C, the oven temperature program 140°C (5 min) then 4°C/ min to 240°C (15 min).

Determination of volatile compounds

To determine the volatile component, the HS-SPME (headspace-solid phase microextraction) method with GC was used according to literature described by Petersen et al. (2012). 5 grams of oil were weighed into the 20 mL glass headspace vial. Then the vials were placed in CONCEPT autosampler of PAS Technologie (Magdala, Germany). After extraction at 40°C by exposing the SPME fiber divinylbenzene/carboxen/polydimethylsiloxane (DVB/PDMS/CAR from SUPELCO, Bellefonte, USA) in the headspace above the sample for 90 minutes, the analytes were thermally desorbed in a Hewlett-

Packard (HP) 6890 GC injection port at 270 °C. GC conditions were as follows: carrier gas helium, flow rate 0.6 mL/min, split ratio 5:1, , the oven temperature program 40°C (6 min) then 5°C/ min to 100°C and 30°C/ min to 300°C (2 min). A HP-5 MS column (30 m x 0.25 mm x 0.25 mm; Agilent, Waldbronn, Germany) was used separation of volatile compounds. The volatile compounds were detected using a flame ionization detector (FID).

Rancimat analysis

To determine the oxidative stabilities of hazelnut oil samples, a Rancimat 617 (Metrohm AG, Herisau, Switzerland) was used. Induction time was detected by the Rancimat method described by Petersen et al. (2012). The principle of the method was based on the artificial aging of the oil at high temperatures and the simultaneous continuous measurement of its volatile degradation product. Briefly, the oil sample (3 g) in the reaction vessel was heated to 120°C with an air flow of 20 L per hour. The volatile degradation products were thrown into a receiver filled distilled water and conductivity of the distilled water was measured. The rancimat analysis result was given as induction time.

Measurement of peroxide value

To determine the peroxide numbers of oil samples, a rapid test was performed using FOODLAB fat device (cdRsrl, Florence/Italy). The procedure is carried out using the test kit instructions. Briefly, a sample amount of 2.5 µl is pipetted into the test cell. 10 µl of reagent is then immediately pipetted in. Then the reagent and the sample were mixed by rotating and pivoting. After the 3-minute incubations, the peroxide number is measured in milliequivalent of oxygen per kg (meq O_2 / kg) (FOODLAB, 2007).

Data Analysis

The statistical evaluation of the results was carried out by comparing two mean values using a two-sided t-test at the significance level α=5%.The t-test is intended to test whether the difference between the two means is solely due to random error, that is, whether both means come from populations with the same mean (Doerffel, 1987).

Results and Discussion

Fatty acid composition

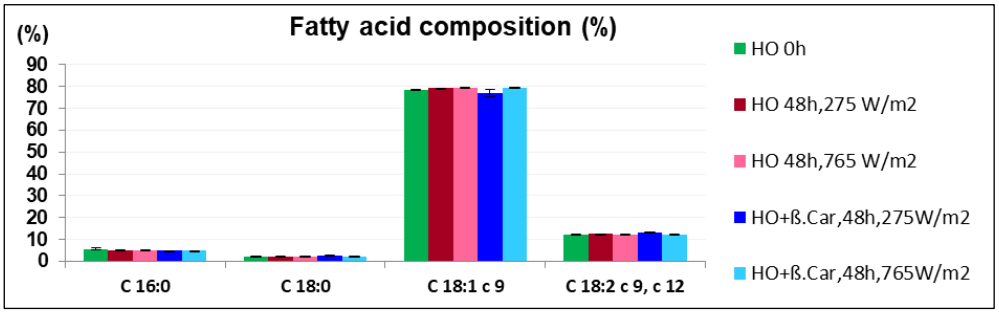

Figure 2. Changes in the fatty acid contents [%] of HO and HO with β-Car. during light exposure

Hazelnut oil, which is rich in unsaturated fatty acids, contains mostly monounsaturated oleic acid (C 18:1 c9). This is followed by polyunsaturated linoleic acid (C 18:2 c9, c12), saturated palmitic acid (C 16:0) and saturated stearic acid (C 18:0) (Alasalvar et al. 2003, Köksal et al. 2006). In this study, the fatty acid compositions of not-supplemented hazelnut oils (HO) and the beta-carotene supplemented hazelnut oils (HO+β.Car.) were examined and the change of the most abundant fatty acids (oleic, linoleic, palmitic and stearic acids) during light exposure (275 W/m² and 765 W/m²) was evaluated (Figure 2). As shown in Fig. 2, HO had 78.44 % oleic acid, 12.42 % linoleic acid, 5.71 % palmitic acid, and 2.26 % stearic acid in its fatty acid composition. Similar results for these four fatty acids of hazelnut oils have been shown before by Köksal et al. (2006) and Crews et al. (2005). It was found that there was a significant difference between HO and HO with β-Car. only in the content of linoleic acid among fatty acids (Fig. 2). Linoleic acid content decreased after light exposure. Since linoleic acid contains more allyl groups in its structure than other fatty acids here, a change in its amount during photooxidation is an expected result. It is known that if there are more alkyl groups in the fatty acid molecule, the induction period is shorter and oxidation occurs faster (Belitz et al., 2008). Here, the fact that the linoleic acid amount of hazelnut oil with beta carotene added did not decrease after photooxidation shows that the addition of beta carotene protects linoleic acid against oxidation.

Peroxide Number

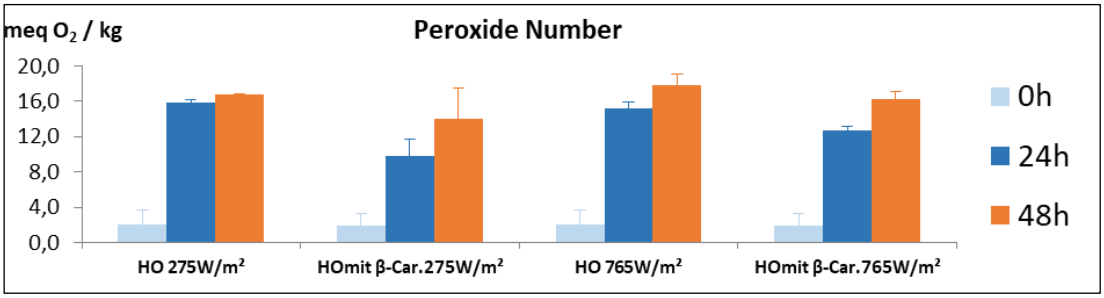

Figure 3. Peroxide numbers [meq O_2 / kg] of HO and HO with β-Car. during light exposure

Figure 3 shows the peroxide number of HO and HO with β-Car. after 24 and 48 h at 275 W/m² and 765 W/m². As expected, the peroxide number increased with light exposure time and light intensity. The addition of β-carotene significantly reduced the peroxide number of hazelnut oil after irradiation for 24 h at 275 W/m² and 765 W/m². A similar decrease in peroxide number due to the addition of β-carotene was also observed in hazelnut oil after irradiation for 48 h at 275 W/m² and 765 W/m², but this decrease was not significant ($p>0.05$). After 24 hours of light exposure, the peroxide numbers are in the order: HO, 275 W/m² > HO, 765 W/m² > HO with β-Car., 765W/m² > HO with β-Car., 275W/m². After 48 hours of light exposure, the peroxide numbers are in the order: HO, 765 W/m² > HO, 275W/m² > HO with β-Car., 765W/m² > HO with β-Car., 275W/m². Similar to this work, Goulson and Wartheseen (1999) examined the antioxidant activity of β-carotene at various concentrations in refined conventional rapeseed oil and high-oleic rapeseed oil. For this purpose, they added 15, 30, 60 and 120 mg/kg β-carotene (22% β-carotene suspension) to both oils, then exposed these oils to light at 8000 lux for 21 days. They found that β-carotene prevented peroxide formation in both oils. Junk and Min (1991) evaluated the effect of some carotenoids including β -carotene on the photooxidation of soybean oil. They reported that the addition of carotenoids decreased the peroxide values of soybean oil.

Induction time

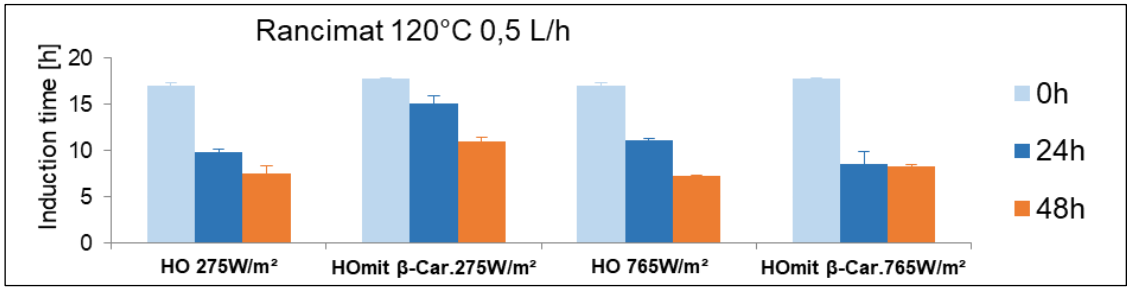

Figure 4. Induction time (Rancimat, 120°C 0.5 L/h) of HO and HO with β-Car. during light exposure

Figure 4 shows the induction times for HO and HO with β-Car. during 24 h and 48 h of light exposure at 275 W/m² and 765 W/m² light intensities. Before light exposure, HO with β-Car. exhibited a significantly longer induction time than HO. After 24h and 48h of light exposure at 275 W/m² light intensity, the induction times of HO with β-Car. were significantly longer than HO. Similarly, it was observed that the addition of β carotene significantly increased the induction time of hazelnut oil after 48h of light exposure at 765 W/m² light intensity. After 24 h of light exposure, the induction times are: HO with β-Car, 275 W/m² > HO, 765 W/m² > HO, 275 W/m² > HO with β-Car., 765W/m². After 48 hours of light exposure, induction times are: HO with β-Car., 275 W/m² > HO with β-Car., 765 W/m²> HO, 275 W/m²> HO, 765 W/m².

Şahin (2011) evaluated the antioxidant effect of ß-carotene supplementation (5, 8, 15, 22, and 25 mg/kg) to refined conventional and high-oleic rapeseed oils under accelerated storage (75 h of light exposure at 275, 347, 520, 693 and 765 W/m² light intensities) and reported that the induction times of the rapeseed oils increased significantly by the addition of ß-carotene.

Antioxidant capacity

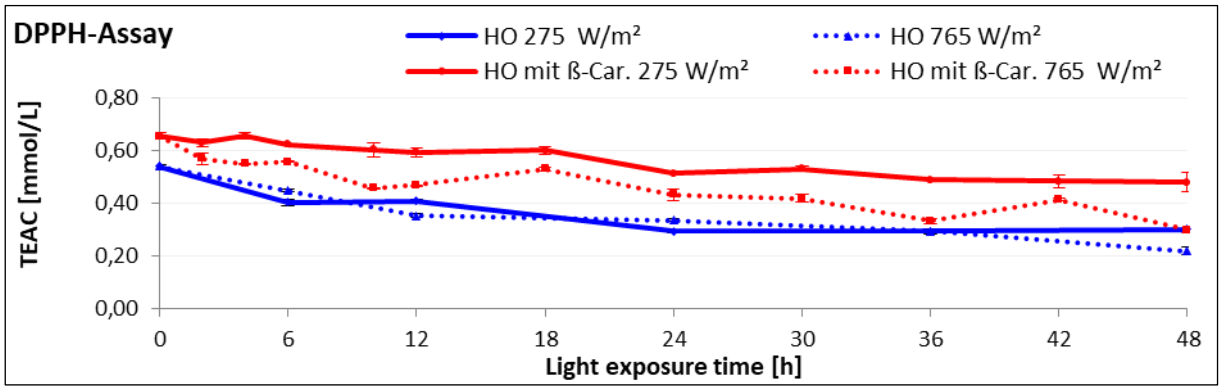

Figure 5. Total antioxidant capacity [mmol TE/L] of HO and HO with β-Car. during light exposure

Total antioxidant capacities of HO and HO with β-Car. after 2, 4, 6, 10, 12, 18, 24, 30, 36, 42 and 48 hours at 275 W/m² and 765 W/m² are shown in Figure 5. As expected, the addition of β-carotene increased the antioxidant capacity of the hazelnut oil. After irradiation for 24 h and 48 h at 275 W/m² and 765 W/m², HO with β-Car. had significantly higher antioxidant capacity than HO. After 24 hours of light exposure, total antioxidant capacities are in the order: HO with β-Car., 275 W/m² > HO with β-Car., 765 W/m² > HO, 765 W/m² > HO, 275 W/m². After 48 hours of light exposure, total antioxidant capacities are in the order: HO with β-Car., 275 W/m² > HO, 275 W/m² = HO with β-Car., 765 W/m² > HO, 765 W/m².

Similarly, Şahin (2011) determined that the refined conventional and high-oleic rapeseed oils supplemented with 5, 8, 15, 22, and 25 mg/kg ß-carotene showed higher antioxidant capacity after 75 h of light exposure at 275, 347, 520, 693 and 765 W/m² light intensities than the refined conventional and high-oleic rapeseed oils containing no ß-carotene supplementation.

Volatile Lipid Oxidation Compounds

Table 1. Concentration [µg/g] of volatile lipid oxidation compounds [µg/g] of HO and HO with β-Car. during light exposure

Volatile compounds	HO 0h	HO 48h, 275 w/m²	HO 48h, 765 w/m²	HO with β.Car. 48h, 275 w/m²	HO with β.Car. 48h, 765 w/m²
Propanol	1,51	0,39	0,47	0,00	0,00
Hexanal	0,02	1,12	1,49	1,15	0,96
Heptanal	0,03	0,15	0,69	0,21	0,60
E, E-2,4-hexadienal	0,00	0,00	0,09	0,00	0,05
E-2 heptenal	0,00	0,00	0,12	0,00	0,12
1-octen-3-ol	0,00	0,08	0,20	0,05	0,19
3-octanone	0,03	0,05	0,26	0,02	0,07
Octanol	0,00	0,00	0,27	0,00	0,07
E, E-2,4-heptadienel	0,00	0,69	3,24	1,04	2,24
E-2-octenal	0,19	0,16	0,33	0,07	0,09
Nonenal	0,01	0,45	1,87	0,25	0,84
Decanal	0,11	0,12	0,13	0,12	0,12
E, E-2,4 nonadienal	0,21	0,24	0,00	0,23	0,26
E-2 decenal	0,00	0,01	0,05	0,01	0,06
Nonanoic acid	0,00	0,00	2,58	1,09	1,58
E, E-2,4-decadienal	4,11	4,93	0,00	4,57	5,02
E, Z-2,4 decadienal	4,42	4,98	5,23	4,63	6,16

Table 1 shows volatile compounds [µg/g] in HO and HO with β-Car during light exposure. A total of 17 volatile compounds were detected in HO and HO with β-Car. Among these volatile compounds, E, E-2,4-hexadienal, E-2 heptenal, 1-octen-3-ol, octanol, E, E-2,4-heptadienel, E-2 decenal, and nonanoic acid were formed 48 hours of light exposure at 275 W/m² and 765 W/m² light intensities. The amounts of hexanal, heptenal, E-2,4-heptadienel, nonenal, and nonanoic acid increased after light photooxidation.

E-2 heptenal and octanol were formed only at high-intensity light exposure (765 W/m^2).

Conclusion

In the present work, the influence of β-carotene (25 mg/kg) on photo-oxidative changes in hazelnut oil was examined under accelerated storage conditions (2, 4, 6, 10, 12, 18, 24, 30, 36, 42 and 48 hours of light exposure at 275 W/m^2 and 765 W/m^2 light intensities). After light exposure, hazelnut oil supplemented with β-carotene had a longer induction time, less peroxide values, and higher antioxidant capacity compared to hazelnut oil containing no β-carotene supplement. In conclusion, the results suggest that β-carotene shows an antioxidant effect in hazelnut oil and protects it against lipid oxidation during photooxidation.

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