

Storage Stability of Seed Oils Extracted form Red Pepper (*Capsicum annuum* L.) Waste by a Green Approach

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Abstract

The present study is aimed to examine and compare the storage stability of seed kils extracted form red pepper (*Capsicum annuum* L.) waste by green techniques (cold pressing, ethanol solvent, and ultrasound-assisted ethanol solvent extraction). In accordance with this purpose, the oil samples were stored for a period of 6 weeks at 60°C under accelerated conditions and analyzed at weekly intervals for peroxide, conjugated diene-triene values tok follow their oxidation stability. It was observed that the peroxide, oxidative induction time, conjugated diene, and triene values of seed kils were significantly influenced by the extraction processes (p<0.05). The initial peroxide (9.83 meqO₂ kg⁻¹ oil), conjugated diene (8.63), and triene (3.36) values were higher in the oil extracted with ultrasound-assisted ethanol solvent extraction technique. At the end of accelerated oxidation conditions, the peroxide, conjugated diene, and triene values were ranked in the following order: ethanol solvent extraction (10 meqO₂ kg⁻¹, 7.31, and 3.76), ultrasound-assisted ethanol solvent extraction (12.50 meqO₂ kg⁻¹, 8.87, and 4.02), and cold press (28.50 meqO₂ kg⁻¹, 16.14, and 3.70). However, the seed oil exhibited a high value of oxidative induction time in the oil extracted with ethanol solvent technique (30.11 min). The results revealed that despite inherent anti-oxidative properties, red pepper seed kils can undergo some degree of auto-oxidation, which is dependent on the extraction method during storage. The best quality was preserved by the ethanol solvent extraction technique both in the first and the sixth week of storage.

Keywords: By-food product, green extraction, oil, red pepper seed, storage stability

Yeşil Bir Yaklaşımla Kırmızı Biber (Capsicum annuum L.) Atıklarından Elde Edilen Çekirdek Yağlarının Depolama Kararlılığı

Öz

Bu çalışmanın amacı, kırmızı biber (*Capsicum annuum L.*) atıklarından yeşil tekniklerle (soğuk presleme, etanolsolvent ve ultrases destekli solvent ekstraksiyonu) ekstrakte edilen çekirdek yağlarının depolama stabilitesini incelemek ve karşılaştırmaktır. Bu amaca uygun olarak, yağ numuneleri 6 hafta süreyle 60°C'de hızlandırılmış oksidasyon koşullarında depolanmış ve yağların oksidasyon kararlılıklarını takip etmek için peroksit, konjuge dien-trien değerleri haftalık aralıklarla analiz edilmiştir. Tohum yağlarının peroksit, oksidatif indüksiyon süresi, konjuge dien ve trien değerlerinin ekstraksiyon işlemlerinden önemli ölçüde etkilendiği gözlenmiştir (p<0.05). Başlangıç peroksit (9.83 meqO₂ kg-1 yağ), konjuge dien (8.63) ve trien (3.36) değerleri, ultrason destekli etanol solvent ekstraksiyon tekniği ile ekstrakte edilen yağda daha yüksek bulunmuştur. Hızlandırılmış oksidasyon koşullarının sonunda, peroksit, konjuge dien ve trien değerleri sırasıyla: etanol solvent ekstraksiyonu (10 meqO₂ kg-1, 7.31 ve 3.76), ultrason destekli etanol solvent ekstraksiyonu (12.50 meqO₂ kg -1, 8.87 ve 4.02) ve soğuk pres (28.50 meqO₂ kg-1, 16.14 ve 3.70) olarak belirlenmiştir. Bununla birlikte, tohum yağı, etanol- solvent tekniği ile ekstrakte edilen yağda (30.11 dakika) yüksek bir oksidatif indüksiyon süresi değeri sergilemiştir. Sonuçlar, doğal antioksidatif özelliklerine rağmen, kırmızı biber tohumu yağlarının, depolama sırasında ekstraksiyon yöntemine bağlı olarak bir dereceye kadar oto-oksidasyona maruz kalabileceğini ortaya koymuştur. En iyi kalite, depolamanın hem birinci hem de altıncı haftasında etanol solvent ekstraksiyon tekniği ile korunmuştur.

Anahtar Kelimeler: Atık ürün, depolama stabilitesi, kırmızıbiber çekirdeği, yağ, yeşil ekstraksiyon



INTRODUCTION

Oxidation of polyunsaturated fatty acids is the main reaction affecting oil quality and health during the storage period (Micić et al., 2015). In addition to the ratio of unsaturated fatty acids, the presence of oxygen, the number of minor components that have antioxidant and prooxidant effects, light, metal contamination, temperature, humidity, storage conditions, and oil extraction methods are the main factors that cause the initiation of lipid oxidation (Kayahan, 2003). As a result of the oxidation destruction mechanism that occurs in oils such as thermal oxidation, polymerization, and hydrolysis, the shelf life and nutritional value of the oils are reduced, and also, they gain toxic properties (Akçar, 2009). However, fat-soluble vitamins are destroyed, unsaturated fatty acids are reduced, so the biological, chemical, nutritional, color, texture, and sensory quality of the oil are adversely affected (Labuza, 1971). To overcome a major problem such as lipid oxidation, there is a need to develop new preservatives that will provide better oxidative stability of edible oils (Yang et al., 2010). At this point, antioxidants, which are defined as compounds that prevent or delay oxidative degradation in foods, are of great importance (Lee et al., 2010). Due to the undesirable effects of synthetic antioxidants on human health, interest in natural antioxidants is increasing (Cordeiro et al., 2012).

Vegetable oils, which are obtained from oilseeds, lead to many applications as a new, alternative and inexpensive source of natural antioxidants with their bioactive content (Silva et al., 2013). Red pepper seed oil produced from industrial by-products is of great importance in terms of one of these vegetable oils. In recent years, research and innovation projects under Horizon 2020 framework program include the main topics about plant products and by-products (Baenas et al., 2019). And at the same time, the storage stability and shelf life of edible oils has always been a topic of considerable interest to nutritionists, food manufacturers and consumers due to their impact on food quality and safety. For these reasons, red pepper seed oil remains among the popular topics of researches. Red pepper seed oil is rich in unsaturated fatty acids with 68-78% linoleic acid and 7-15% oleic acid content, and is mainly palmitic acid (11-14%), and stearic acid (3-4%), including saturated fatty acids (El-Adawy and Taha, 2001; Perez-Galvez et al., 1999). It has a strong antioxidant mechanism with alpha-tocopherol and capsaicin contents (Yang et al., 2010). Present studies have indicated that the red pepper seed oil is also rich in carotenoids especially capsanthin, lutein, and betacarotene (Konçsek et al., 2018). *Capsicum* compounds have an important role in reducing the oxidation of active substances by their antioxidant properties (Baenas et al., 2019).

Besides their own antioxidant content, the methods of oilseed extraction are also an important factor affecting the quality, quantity, and stability of the oil (Ozkan et al., 2010; Evren and Tekgüler, 2011). With appropriate extraction methods and applied conditions, seed oils with long shelf life can be obtained by ensuring that other bioactive components such as antioxidants and tocopherols in the seeds are transferred to the oil at the maximum rate. Solvent extraction that is most commonly used with hexane as a solvent is among the traditional extraction methods, but in recent years, effective methods such as cold pressing and ultrasoundassisted solvent extraction methods have been used to produce edible oils (Yetim and Kesmen, 2009). These pressing and ultrasonic applications stand out as a key technology in achieving the goal of sustainable "green extraction" (Dedebaş et al., 2021). "Green extraction" offers some potential to minimize or eliminate the toxic effects of organic solvents, and with a better quality to extract bioactive lipid-soluble compounds (Ramadan, 2020). Cold-pressed kils have an interest due tok their more polar phenolic compounds and natural antioxidants (Uluata, 2016). In addition, ultrasound-assisted extraction is one of the modern, green, economically, non-thermally effective techniques used to obtain bioactive oils and are preferred to modern techniques (Chemat et al., 2017). In recent years, consumers have preferred reliable, economical, and environmentally friendly products in terms of food safety, and for this reason, products are tried to be produced with a green approach (Farr and Proctor, 2014; Siger et al., 2015).

Although there are very few studies on red pepper seed oil so far, it is seen that solvent extraction (Soxhlet) and cold pressing methods, which are traditional, are mostly used. The results obtained from these studies contributed to the characterization of red pepper seed oil. In addition, in recent years, modern techniques such as pressure-assisted, supercritical fluid- CO_2 , microwave-assisted, and ultrasound-



assisted solvent extraction have been used to obtain red pepper seed oil, but these studies have been limited to the physicochemical, bioactive, and antioxidative properties of the oils (Chouaibi et al., 2019; Ma et al., 2019). No studies have been found on the production of red pepper seed oils by green extraction methods without using toxic organic solvents and the effectiveness of extraction methods on the oxidative stability of seed oils during storage.

The aim of the study was to investigate the storage stability of seed oils extracted form red pepper (*Capsicum annuum* L.) waste by cold-press, conventional solvent, and ultrasound-assisted solvent method with a green approach. The chemical quality of the oils was also determined by the peroxide, oxidative induction time, conjugated diene, and triene values.

MATERIAL AND METHODS

Materials

Red pepper (*Capsicum annum* L.) seeds as an industrial food waste were provided by MÜSAN Food Co. Ltd. (Kahramanmaraş, Turkey). The seeds were the post-production seeds of red pepper fruits that were harvested in the 2015 August-October harvest season for spicy red pepper products. The seeds were dried under the sun (moisture content \leq 6%) within that season. The seeds were ground into a powder with an average particle size of 500 µm by an electrical grinder (model Scm 2934; Sinbo, İstanbul, Turkey). All reagents were provided by Merck (Darmstadt, Almanya) and Sigma-Aldrich (St.Louis, ABD) and of the analytical grade.

Method

Ultrasound-assisted solvent extraction (UAE)

An ultrasound bath, Jeiotech UC-10 brand with 40 kHz ultrasound frequency and max.300 W ultrasound power (10 liter capacity ultrasonic tank, acoustic energy density of 0.026 W cm⁻³), was used for UAE. Dimensions of 10 L (40 kHz 300 W) volume capacity ultrasound tank were 295×240×160 mm. The seed powder was exposed to high intensity ultrasonic effect at a fixed sample: solvent ratio (1/10, weight/volume), at 40°C and for 40 min. The bath temperature was maintained constant at 40 °C (measured with a thermocouple) during treatment using cool-packes. Ethanol was selected as a green alternative solvent to hexane. The supernatants obtained after the treatment were filtered to obtain an

ethanol-containing extract phase. The supernatants were filtered and ethanol in the extract phase was volatilized by a rotary evaporator (Hei-VAP Value model, Heidolph, Germany) under vacuum at 40°C. Extraction conditions were determined by pre-extraction studies.

Solvent extraction (SE)

The solvent extraction was the non-ultrasoundassisted conventional solvent extraction. SE was performed with the same ultrasound bath Jeiotech UC-10 brand without using ultrasonic vibrations. The extraction conditions were carried out by the same method used in the UAE procedure.

Cold press (CP)

Red pepper seed oil was obtained using a coldpressing machine (model:6YL-68, Henan Double Elephants Machinery I/E Co., Ltd., China) (single head, 5.5 kW power, 50 kg seed/h capacity). It is ensured that the oil outlet temperature of the device does not exceed 40°C. After the cold-pressing process, the extracted seed oil was passed through a cloth filter and then the lipid fraction was centrifugated to purify from solid particles.

Storage in accelerated oxidation conditions

Oil samples produced by three different methods were stored in amber bottles at 60°C for 6 weeks in an oven (Samples were taken into different bottles for each storage period). In this process, samples were taken at the beginning of storage and for 6 weeks at 7-day intervals, and peroxide value (AOCS, 1984), conjugated diene, and triene determinations (AOCS, 1989) were performed.

Analyses

Oil yield (%)

Oil extraction yields were calculated according to Equation (1).

oil yield %= [(weight of oil (g))/(weight of seed (g))]×100
(1)

Peroxide value

The peroxide value expressed as milliequivalent gram oxygen (meq O_2 kg⁻¹) in 1 kg oil was determined using the Cd 8-53 method of AOCS (AOCS, 1984).

Conjugated diene and triene values



Conjugated diene and triene values were determined according to AOCS Ch5-91. Conjugated diene (K_{232}) and triene (K_{270}) values are determined by weighing certain amounts of oil samples (1%, w/v) and dissolving them in 10 ml hexane. By using UV-1800 Shimadzu UV / VIS spectrometer, the absorbance values at 232 nm and 270 nm are measured in 1 g oil at a concentration of 100 ml⁻¹ (AOCS, 1989).

Determination of oxidative induction time (T₀):

Oxidative stabilities of oil samples were determined by differential scanning calorimetry (DSC 4000, Perkin Elmer). Oil samples were weighed at 15 ± 1 mg and put into sample aluminum containers (Perkin Elmer; 40 µl). The reference container was used as empty. The heat flow and temperature calibration of the DSC device were made for the use of standard indium.

In order to determine the oxidative stability of the oil samples, analyzes were made using the isothermal method by DSC device. The isothermal temperature was programmed to 130°C and measurements were made by waiting until the end of the oxidation period in 20 mL min⁻¹ oxygen gas (99.9%) flow under atmospheric pressure. Oxidative induction time was determined using the method defined by Tan et al. (2002). The induction time of the oxidative reaction is closely related to the intersection of the exotherm's (leading) tangent line and the extrapolated base.

Statistical analysis

The results were given as the mean \pm standard error. Statistical significance was admitted at a level of p<0.05. The data were statistically analyzed by one-way analysis of variance (ANOVA). Duncan test was used to compare means (SPSS v.23, IBM, USA).

RESULTS AND DISCUSSION

Oil Yield (%)

The yields of seed kils extracted form red pepper (*Capsicum annuum* L.) waste by green techniques were ranked in the following order: ethanol solvent extraction (14.50%), ultrasound-assisted ethanol solvent extraction (16.80%), and cold press (11.32%). Higher oil yield was obtained with ultrasound application from red pepper seeds compared to other extraction procedures. Ultrasonic application mechanically breaks down the cell walls, which is the

main obstacle in oil extraction by pressing form oil seeds. This ensures easy transfer of oil out of the cell and supports the increase in oil yield (Atalay, 2019; Ergün et al., 2013).

Oxidative Induction Time (T₀) of Red Pepper (*Capsicum annum* L.) Seed Oils

The average oxidative induction time values of red pepper seed oils are presented in Figure 1.

The rate of lipid oxidation reactions increases after a specific phase, defined as the induction period that is an indicator of oxidative stability (Kayahan, 2003). Induction period has a relationship with oxidative stability. The oxidative stability and shelflife of the oil increase as the induction time increases (Kowalski et al., 2004). The oxidation induction times of the samples were calculated at a constant temperature of 130°C by DSC. DSC induction time measurements are significantly affected by different extraction techniques (p<0.05). At 130°C, the SE (30.11 min) method showed higher oxidative stability than the CP (24.71 min) and UAE (11.79 min) methods. The oil sample obtained by the UAE method exhibited the lowest induction time due to having the highest peroxide value at the beginning of the extraction. This shows that the oil obtained by the SE method is thermally more stable than the oils obtained by other methods. The high oxidative stability of the oil obtained by the SE method may be due to its higher amount of tocopherol and phenolic components and the low content of unsaturated fatty acids (Atalay and Inanc, 2023). Also, Gbogouri et al., (2013) reported that the differences in thermostability of oils may be due to their initial chemical characteristics (fatty acid composition, peroxide values, etc.).

When we compare the induction times of red pepper seed oils with the studies of Tan et al. (2002) investigating the induction times of different edible oils, it is seen that red pepper seed oils have good oxidative stability. Although the DSC (T_0) value of red pepper seed oil produced by SE (30.11 min) is higher than olive (21.99 min), safflower (18.19 min) and grape seed (17.60 min) oils; sunflower (33.34 min), and soybean oils (33.70 min) were found to be almost the same values. Micic et al. (2015) examined the oxidative stability of blackberry and raspberry seed oils, and it is seen that red pepper seed oils. There is no study on DSC-measured T_0 values for red pepper



seed oils. So, this study contributed to the literature with T_0 values of red pepper seed oils for comparison.

Storage under Accelerated Oxidation Conditions

The oxidative stability of red pepper seed oils extracted by different techniques was discussed by following the peroxide and conjugated diene-triene values in the storage period under accelerated oxidation conditions.

Change in peroxide values during storage

The influence of extraction methods during storage on PV in the red pepper seed oil samples is shown in Fig. 2.

Oxidation of vegetable oils is the main deterioration reaction that reduces shelf life and causes quality loss during storage. Peroxide value, one of the most widely used methods in determining oxidative rancidity in oils, is the measurement of the amount of the peroxides and hydroperoxides formed in the initial phase of oxidation (Zhang et al., 2010). The degree of oxidation of oil samples was determined by PV at the beginning and during storage at 60°C for 6 weeks. The initial peroxide value of seed oils renge form 6.67 tok 9.83 meq O₂ kg⁻¹oil. The peroxide value of UAE extracted oil was significantly higher than those of other oil tested (p < 0.05). It is thought that as the ultrasound intensity increases, the formation of primary oxidation products (peroxide and conjugated dienes) also increases and causes a rapid increase in the primary oxidation of oils.

When the data obtained as a result of storage were evaluated, it was seen that the peroxide values were found as CP, UAE, SE, respectively, starting from the highest. By comparison of seed oil groups extracted by green processes, statistically significant differences, as measured by peroxide value, were observed during accelerated storage for each week (p<0.05). In the comparison of weeks in the same method, the interaction was very significant (p<0.001).

It was observed that the peroxide value of the oil obtained by the cold press method increased linearly during storage and reached its highest value (28.50 meqO₂kg⁻¹) at the end of the 6th week of storage. Increases in peroxide value may occur due to the formation of primary oxidation products as a result of lipid oxidation and these causes to loss of quality of oil (Chong et al., 2015). During storage, peroxide values for the UAE and SE extraction

methods were lower than the CP method. The fact that the oil obtained by the CP method has higher peroxide values and lower antioxidant activity compared to other oils is attributed to be related to its initial low content of phenolic and tocopherol components (Atalay and Inanc, 2023). Also, the peroxide value may have reached high values due to uneven pressure application and temperature increase in cold pressing. In addition to these, it was observed that the peroxide values analyzed by the UAE method during the storage were partially higher than the peroxide values obtained in the SE method. This might be due to the cavitation effect, i.e. micromechanical shocks that influence structural and functional compounds up tok the point of lipid oxidation and deterioration during the ultrasound procedure (Chemat et al., 2004). Interestingly, it was observed that the analyzed peroxide values of oils produced by UAE and SE methods under hightemperature storage conditions were in accordance with the standards specified for edible vegetable oils and did not exceed 15 meq O2 kg-1 (Alimentarius, 2003).

Uluata (2016) followed the lipid oxidation of apricot kernel oils produced by cold press and solvent extraction methods and reported that peroxide values reached higher values than the current study during storage. The results of all oxidation tests similarly showed that apricot seed oils produced by the SE method are more oxidatively stable than the CP method. The fluctuations (increase-decrease) in peroxide values during thermal oxidation were in agreement with those obtained by Kıralan and Kıralan (2017). On the other hand, they reported that higher peroxide values for cold-pressed sunflower oil were analyzed. Halim and Thoo (2018) examined the effect of ultrasound application on the oxidative stability of sunflower and palm oil and reported that peroxide values of oils were lower without ultrasound application. The results were in agreement with those obtained by Halim ve Thoo (2018). There is no study on the storage stability of red pepper seed oils. So, this study contributed to the literature with peroxide, the conjugated diene, and triene values during storage of red pepper seed oils for comparison.

Change in conjugated diene-triene values during storage. The influence of extraction methods during storage on conjugated diene and triene values in the red pepper seed oil samples is shown in Fig. 3 and Fig. 4.



Similar to the peroxide value, the conjugated diene value is also an indicator of the primary oxidation products formed by lipid oxidation (Bouaziz et al., 2008). Hydroperoxides, which are the first degradation products, constitute the conjugated diene values at 232 nm, and the products formed as a result of the breakdown of hydroperoxides constitute the triene values of the oil at 270 nm (Karakuş, 2008). The two most commonly used methods to determine the shelf life of vegetable oils are the determination of peroxide value and conjugated diene-triene values.

The initial conjugated diene value of seed kils renge form 7.07 tok 8.63. The conjugated diene value of UAE extracted oil was significantly higher than those of other oil tested at the beginning of storage. Nevertheless, the value of the oil extracted with CP is higher when we consider the entire storage. When the conjugated diene values were evaluated for oils produced by different methods at every stage of the storage process, it was seen that the values were different from each other from the first week and the difference between the methods was important (p<0.05).

The initial conjugated triene value of seed oils renge form 1.92 tok 3.36. The conjugated triene value of UAE extracted oil was also significantly higher than those of other oil tested at the beginning and during of storage. When the variation of each

method according to the weeks was analyzed, the interaction was very important in both conjugated diene-triene values (p<0.001). Conjugated diene values (K₂₃₂) increased for all samples generally due to the formation of conjugated dienes and polyenes during storage. The values of the oils obtained with CP were higher than the others. During the storage period, a partial decrease was observed in the values for all oils in the first week's measurements. It is thought that this decrease may be due to the conversion of most of the dienes to polymer components with the increase occurring during storage. Similarly, fluctuations (increase-decrease) were observed in conjugated triene (K_{270}) values during storage. Although conjugated triene values were the highest in UDSE, it was observed that the values were close to each other in SE and SP methods. Increases and decreases in conjugated triene values of all oils were slower. For all methods, it was found that the data obtained for conjugated diene and triene at the end of storage had the highest values. It was observed that the conjugated diene values for all oil samples were higher than the triene values. Also, the attitude (increase-decrease) of conjugated diene and triene values was parallel to the peroxide values (Hosseini et al., 2015).



The values indicated with the same superscripts do not statistically differ (p>0.05). (Extraction methods 'a-c') Figure 1. The oxidative induction time of red pepper seed kils





CP: Cold pressing, UAE: Ultrasound-assisted solvent extraction and SE: Solvent extraction

There is no statistical difference between the values shown with the same letters (p>0.05), (Differences among the methods "a-c" series during each storage period, differences of methods according tok storage time "A-E" series)





CP: Cold pressing, UAE: Ultrasound-assisted solvent extraction and SE: Solvent extraction There is no statistical difference between the values shown with the same letters (p>0.05), (Differences among the methods "a-c" series during each storage period, differences of methods according tok storage time "A-E" series)

Figure 3. Changes in conjugated diene (K₂₃₂) values during storage









Low levels of conjugated diene and triene allow oils to show higher oxidative stability (Chatha et al., 2006). Tocopherols maintain the quality during storage because of their natural antioxidants role and thereby reduce the formation of bad odor-aroma and rancidity (Moradi et al., 2018). It is also known that its phenolic components have a protective effect against fat oxidation by inhibiting the formation of conjugated diene-triene and lipid hydroperoxides (Bouaziz ve ark., 2008). In the studies of Kıralan and Kıralan (2017), during the thermal oxidation of coldpressed sunflower oil and the photooxidation process under light, the attitude of K₂₃₂ values during storage was consistent with the result obtained in this study. However, it was seen that the values reached were higher than the values obtained in this study.

Since there is no storage study on red pepper seed oil under accelerated oxidation conditions, it is very difficult to compare the results with the studies made with other materials. Because of antioxidant activity; It may vary depending on factors such as different extraction techniques applied, extraction conditions, the presence of bioactive components that can show pro-oxidant or synergist activity, and the activities of these components in the system where they are located (Kamal-Eldin ve Appelqvist 1996). **CONCLUSION**

The results showed that there was a significant difference in quality and oxidative stability between the studied red pepper seed oils due to the different green extraction methods applied. Generally, the effect of the cold-pressing and ultrasound application exceeded that of the others tok influence the peroxide value and conjugated dienetriene values. Oils produced by solvent extractions under accelerated oxidation conditions preserved their quality in the best way and showed high storage stability compared to other oils. Consequently, it is necessary to evaluate the changes in the oxidative stability mechanisms and quality values of seed oils extracted by different techniques along their shelf life. The initial quality (chemical characteristics and natural antioxidants) of seed oils is also important for storage stability. However, if there is the formation of secondary oxidation products in storage conditions, it is necessary to analyze them and the change of antioxidant agent contents such as phenolic substances, tocopherol, capsaicin and carotenoid should also be monitored to have more enlightening information about oxidative stability of seed oils. Additionally, researches on the elimination of toxic solvents used and the usage of green technologies for oilseed extraction should be increased. Oils should be produced in reliable, economical, and environmentally friendly ways in terms of food



safety. Many analyzes were carried out for the first time for red pepper seeds and a contribution was made to literature research. It is recommended to continue studies on the storage of red pepper seed oil, to develop methods, and to apply them to the industry.

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CONFLICT OF INTEREST

The Authors report no conflict of interest relevant to this article

RESEARCH AND PUBLICATION ETHICS STATEMENT

The authors declares that this study complies with research and publication ethics.

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