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ANTIOXIDANT ACTIVITY OF CURCUMIN AS A NATURAL ANTIOXIDANT ON THE OXIDATIVE STABILITY OF SOYBEAN OIL UNDER THERMOXIDATION

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ABSTRACT: The aim of the present study was to examine and compare oxidative stability of soybean oil under accelerated oxidation conditions (at 70°C for 12 days) using curcumin at different concentrations (40, 80, and 120 µg/mL) as a natural antioxidant in comparison with control (without any antioxidant) and positive control (using synthetic TBHQ-200 µg/mL). The major fatty acids in soybean oil are linoleic acid, oleic acid, palmitic acid, linolenic acid, and stearic acid. Linoleic acid recorded the highest ratio (53.3 %), followed by oleic acid (23.7 %), then palmitic acid (11.4%), while stearic acid had the lowest level (4.1%). In rancimate test, the longest induction times were determined for soybean oil supplemented with curcumin at 80 µg/mL (6.39 h), followed by soybean oil supplemented with TBHQ at 200 µg/mL (5.83 h). The results from the accelerated storage experiment indicated that curcumin (40, 80, and 120 µg/mL) exhibited stronger antioxidant capability in all tested samples rather than control (without antioxidants). Under accelerated oxidation conditions, curcumin has a potential capability to improve the shelf life of soybean oil in comparison with TBHQ-200 µg/mL. It can be concluded that soybean oil supplemented with curcumin and TBHQ showed low peroxide value, conjugated diene, and conjugated triene content compared to control (without antioxidant). This indicated that curcumin at different concentrations (40, 80, and 120 µg/mL) has a good antioxidant activity. So, it replaces TBHQ in different applications.

Key words: Soybean oil, oxidative stability, thermoxidation, peroxide value, conjugated diene and triene.

INTRODUCTION

Lipid oxidation is one of the major causes of food deterioration and shelf-life reduction in the food industry. The oxidation process results in rancidity and decrements in the nutritional quality, color, flavor, texture, and safety of food (**Adams *et al.*, 2011**). Moreover, lipid oxidation can generate free radicals, which contribute to diseases, like cancer and cardiovascular disease (**Fransen *et al.*, 2012**). One of the simplest and most effective approaches to decrease lipid oxidation is to incorporate antioxidants. In this context, natural antioxidants are preferred due to the potential toxic health effects of synthetic Additives (**Fransen *et al.*, 2012**). Consequently, there is great interest in the use of natural antioxidants from different sources (**Blasi and Cossignani, 2020**). Fatty acids and their derivatives, or triglycerides, have an unstable chemical structure, especially when they are exposed to extreme environmental factors like constant high temperatures, light, and air. As a result, fatty acids and their derivatives are susceptible to chemical oxidation, which shortens their shelf life and results in an unpleasant flavor (**Roszkowska *et al.*, 2015**). Oils undergo additional oxidation (rancidity) because of improper chemical or environmental storage, modifying their organoleptic properties, reducing their shelf life, and affecting their nutritional and economic characteristics. These rancid oils lose a lot of their nutritional value and economic value as a result, which could result in a financial loss (**Condori *et al.* 2020**).

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One of the primary causes of food deterioration during processing and storage is lipid peroxidation. One strategy for extending the shelf life of foods containing lipids is the addition of antioxidants. Being probably carcinogenic, synthetic antioxidants like butylated hydroxy anisole (BHA) and butylated hydroxytoluene (BHT) are not allowed in food **(Zhou *et al.*, 2017)**. For a number of years, TBHQ was widely regarded as the most potent synthetic antioxidant; However, it has recently been outlawed in a number of developed nations, including Canada, Japan, and Europe **(Shahidi and Zhong, 2010)**. Therefore, the idea of eliminating undesirable side effects and substituting natural antioxidants like curcumin for synthetic antioxidants **(Kharat *et al.*, 2020)** Curcumin is a natural polyphenol that comes from the turmeric plant. It has many promising health benefits, like antioxidants **(Hewlings and Kalman, 2017)**. Soybean oil is rich in polyunsaturated fatty acids such as linoleic and linolenic acids, which are the main responsible of its oxidative instability (**Su *et al.*, 2003**), due to the low dissociation energy of their double bonds. In this respect, oxygen plays a leading role in the free radical chain reaction of lipid oxidation mechanism that is explained through the stages of initiation, propagation, and termination. The addition of antioxidants as free radical scavengers before the propagation phase, is considered a strategy to counteract the lipid autoxidation and to enhance the lag time until the production of the volatile rancidity markers (**Johnson and Decker, 2015**). By measuring primary and secondary oxidation parameters, the current study compared curcumin's 40, 80, and 120 µg/mL levels as a natural substitute of the widely used synthetic antioxidant TBHQ (200 µg/mL) under accelerated oxidation conditions of soybean oils.

MATERIALS AND METHODS

Materials

Refined soybean oil was obtained from Alfa Miser Company on the 10th of Ramadan, Egypt and stored at room temperature in the dark. The soybean oil sample was free from antioxidants. The synthetic antioxidant TBHQ (*tert*-butylhydroquinone) was supplied by Merck (Merck KGaA, Darmstadt, Germany), and the remaining reagents and solvents, of analytical degree. Curcumin was obtained from Merck (Merck KGaA, Darmstadt, Germany).

Determination of the fatty acid profiles of the soybean oil

Fatty acids composition was carried out according to **Ramadan *et al.* (2006)** using a Hewlett Packard HP 6890 gas chromatograph, operated under the following conditions: Detector, flame ionization (FID); column, capillary, 30.0 m x 530 µ m, 1.0 µ m thickness, polyethylene glycol phase (INNO Wax); N2 with flow rate, 15 mL/min with average velocity 89 cm/s (8.2 psi); H_2 flow rate, 30 mL/min; air flow rate, 300 mL/min; split ratio, 8:1, split flow, 120 mL/min; gas saver, 20 mL/min. Detector temperature, 280ºC; column temperature, 240ºC; injection temperature, 280ºC. Programmed temperature starting from 100ºC to reach a maximum of 240ºC was used for eluting the fatty acid methyl esters. The identification of the peaks was made as compared with chromatograms of standard fatty acids methyl esters (Sigma, USA).

Preparation of soybean oil samples

The curcumin (40, 80, and 120 µg/mL) was added and mixed vigorously with soybean, oil in comparison with the widespread synthetic antioxidant TBHQ dissolved in propylene glycol (200 µg/mL). Table 1 shows the codes used for the samples of soybean oil, with and without additives.

Oxidative stability test

The oxidative stability was determined in 679 Rancimat apparatus from Switzerland according to ISO 6886:1997 **(Ciemniewska-Żytkiewicz *et al.*, 2014)**. Utilizing a sample of 5 ± 0.01 g. All samples were studied at 110ºC), under a constant airflow (20 L h^{-1}). The induction times were printed automatically by apparatus software with an accuracy of 0.005.

Oven test

Soybean oil, and fortified oil with curcumin, or TBHQ were placed in a series of transparent glass bottles having a volume 20 mL each. The bottles were filled with 10 g of Soybean oil, and/ or fortified oil with curcumin and left open. The oxidation reaction was accelerated in a forced-draft air oven T6 (Heraeus Instruments GmbH; Hanau, Germany) set at 70°C±2 **(Konsoula, 2016; El-Hadary and Taha, 2020)** for different periods (0, 2, 5, 8 and 12 days). These samples were analyzed for peroxide value (PV) (**AOCS, 1998**), acid value (AV) **(AOCS, 1998),** antioxidant

Table 1. Soybean oil samples used in the present study

Sample	Code
Soybean oil	Control
Soybean oil + TBHQ 200 µg/mL	TBHQ
Soybean oil + curcumin 40 µg/mL	C-40
Soybean oil + curcumin 80 µg/mL	C-80
Soybean oil + curcumin 120 µg/mL	C-120

activity (AA), conjugated diene (CD), and conjugated triene (CT) (**El-Hadary and Taha, 2020)**.

Antioxidant activity (DPPH-assay)

The antioxidant activity of soybean oil before and after fortification with curcumin, and TBHQ during oven test was assayed with DPPH• radicals dissolved in toluene according to **Ramadan *et al.* (2006).** Radical scavenging activity (RSA) and presence of hydrogen donors in tested oils during accelerated oxidation test were examined by reduction of DPPH$^{\bullet}$ in toluene. Toluene solution of DPPH$^{\bullet}$ radicals was freshly prepared at a concentration of 10^{-4} M. The radical in the absence of antioxidant compounds was stable for more than 2 h of normal kinetic assay. For evaluation, 10 mg of each sample during oven test (in 100 µL toluene at room temperature) were mixed with 390 µL toluene solution of DPPH$^{\bullet}$ radicals and the mixture was vortexed for 20 s at ambient temperature. Against a blank of toluene without DPPH$^{\bullet}$, the decrease in absorption at 515 nm was measured in 1 cm quartz cells after 60 min of mixing using UV-260 visible recording a model Jenway 635001 - 6305 UV/Visible Spectrophotometer. RSA toward DPPH$^{\bullet}$ was estimated from the differences in the absorbance of toluene DPPH$^{\bullet}$ solution with or without sample (control) and the inhibition percent was calculated from the following equation:

$$\% \text{ Inhibition} = [\text{Ac} - \text{As/Ac}]\text{x } 100$$

Where: Ac is absorbance of control and as is absorbance of test sample

Conjugated diene, and conjugated triene estimation

Specific extinctions (presented in units; U) at λ 232 nm and 270 nm were determined for the conjugated dienes and conjugated trienes, respectively, using a spectrophotometer (Jenway 635001 - 6305 UV/Visible). Oil samples were diluted with isooctane following the standard method of IUPAC method II. D.23 **(El-Hadary and Taha 2020)**. The absorbance (U) measured at λ 232 and 270 nm was referred to as the conjugated diene (CD) and conjugated triene (CT) of polyunsaturated fatty acids (PUFAs), respectively. Increasing absorption (U) values (at λ 232 nm and 270 nm) were considered as an indication of proceeding oil oxidation.

Statistical Analysis

The data were subjected to factorial completely randomized analysis using R statistical software version 4.1.1. The differences among the studied factors were separated using the protected Tukey's HSD test at a significance level of $p \leq 0.01$.

RESULTS AND DISCUSSION

Fatty Acid Profiles of the Soybean Oil

Fatty acids composition of soybean oil is presented in Table 2. The major fatty acids in soybean oil are linoleic acid, oleic acid, palmitic acid, linolenic acid, and stearic acid. Linoleic acid has the highest ratio (53.3%), followed by oleic acid (23.7%), followed by palmitic acid (11.4%), while stearic acid has the lowest level (4.1%). The results for the fatty acids composition of the selected refined edible oils are like those obtained by other authors (**Panthee *et al.*, 2006; Chowdhury *et al.*, 2007; Clemente and Cahoon, 2009)**. Table 2 statistics show that there were higher levels of unsaturated fatty acids in the soybean oil under study, which is consistent with information from the literature (**Kostik *et al.* 2013**).

Table 2. Fatty acids composition of soybean oil

Fatty acids	Common name	Percentage (%)
Palmitic acid	C16:0	11.4
Stearic acid	C18:0	4.1
Oleic acid	C18:1	23.7
Linoleic acid	C18:2	53.3
Linolenic acid	C18:3	7.5
Total saturated fatty acids		15.5
Total unsaturated fatty acids		84.5

Oxidative stability (Rancimat test)

Conducting the rancimat test allows us to determine the time of induction of the tested oils (Table 3). Among the tested oils, the longest induction times were determined for soybean oil supplemented with curcumin at 80 µg/mL (6.39 h), followed by soybean oil supplemented TBHQ at 200 µg/mL (5.83 h). The lowest induction times were recorded with soybean oil without any additives (4.46 h). The induction time of soybean oil increased by addition natural antioxidants such as *Thymbra spicata* oil **(Yagcı *et al.* 2012)**, herbs and spices **(Redondo-Cuevas *et al.*, 2017),** and vitamin A **(Halbaut *et al.*, 1997)**.

Effect of Curcumin, and TBHQ on Soybean Oil under Thermoxidation

Changes in peroxide value and acid value

The peroxide value is commonly used to measure the development of primary oxidative products (rancidity), such as peroxides and hydro-peroxides, in the early stages of oil oxidation (**Marmesat *et al.*, 2009**). The initial increase rate in peroxide values was very slow except for the control (Table 4). A significant difference ($P<0.01$) in peroxide values were observed between the control and all treatments containing curcumin, or TBHQ. Under accelerated oxidation conditions (for 12 days), the effect of different antioxidants (curcumin at 40, 80, and 120 µg/mL, and TBHQ-200µg/mL) was significant in reducing peroxide values in soybean oil when compared to the control. At the end of the accelerated oxidation experiment (after 12 days), the peroxide value developed rapidly and reached 15.33 meq/kg in the control of soybean oil. The peroxide values were significantly ($P<0.01$) reduced to 8.83, 8.43, and 7.93 meq/kg in soybean oil when curcumin was used at different concentrations (40, 80, and 120 µg/mL, respectively). The peroxide value was 5.33 meq/ Kg in soybean oil when TBHQ-200 µg/mL was used. It is frequently suggested that natural or synthetic antioxidants can not only delay the auto-oxidation of oils but also the accumulation of primary and secondary oxidation products, significantly increasing their oxidative stability and shelf life.

Soybean oil's stability and quality can be estimated based on the changes in its acid value that are measured during storage. As a result, fresh oil was subjected to conditions of accelerated oxidation and basic chemical quality tests were conducted. The results obtained are shown in Table 4. In all treatments, the same acid value (0.07 mg KOH/kg) was recorded during storage for 8 days for control and TBHQ treatment, while in curcumin treatment reached 0.05 mg KOH/kg.

At the end of the accelerated oxidation experiment (after 12 days), the acid value developed rapidly and reached 0.08 mg KOH/ kg in control and TBHQ treatment compared to the curcumin (80 µg/mL) treatment (0.052 mg KOH/ kg).

Changes in antioxidant activity

In this study, the ability of soybean oil under accelerated oxidation conditions (at 70°C for 12 days) using curcumin at different concentrations (40, 80, and 120 µg/mL) in comparison with control (without any antioxidant) and positive control (using synthetic TBHQ-200 µg/mL) was screened using DPPH• radical. From the data presented in Table 5, when compared to the control (without antioxidants), the antiradical activity of soybean oil increased when natural (curcumin) and synthetic antioxidants were added to it at various concentrations. The presence of TBHQ in soybean oil increased the antioxidant activity. Polyunsaturated fatty acid alkoxyl (RO), peroxyl (ROO), and alkyl radicals (R) are the lipid radicals produced during autoxidation **(Lee *et al.*, 2007)**.

Table 3. Induction time (h) of the tested soybean oil

Sample	Code	Induction time (h)
Soybean oil	Control	4.46
Soybean oil + TBHQ 200 µg/mL	TBHQ	5.83
Soybean oil + curcumin 40 µg/mL	C-40	5.59
Soybean oil + curcumin 80 µg/mL	C-80	6.39
Soybean oil + curcumin 120 µg/mL	C-120	5.64

Table 4. Changes in peroxide value (meq/Kg) and acid value of soybean oil under accelerated oxidation conditions

Treatment	Storage time (days)	PV (meq O2/kg)	AV (mg KOH/Kg)
Control	0	0.697±0.053 n	0.07±0.0 b
	2	4.5±0.058 h	0.07±0.0 b
	5	5.6±0.1 fg	0.07±0.0 b
	8	8.967±0.033 b	0.07±0.0 b
	12	15.333±0.333 a	0.08±0.0 a
TBHQ	0	0.693±0.033 n	0.07±0.0 b
	2	0.7±0.058 n	0.07±0.0 b
	5	2.2±0.058 kl	0.07±0.0 b
	8	2.033±0.033 kl	0.07±0.0 b
	12	5.333±0.088 g	0.08±0.0 a
C-40	0	0.717±0.017 n	0.07±0 b
	2	2.2±0.058 kl	0.07±0 b
	5	3.9±0.058 i	0.07±0 b
	8	6.467±0.033 e	0.07±0 b
	12	8.267±0.033 c	0.078±0.001a
C-80	0	0.683±0.017 n	0.07±0 b
	2	1.967±0.033 l	0.07±0 b
	5	3.5±0.058 ij	0.07±0 b
	8	5.933±0.067 f	0.07±0 b
	12	7.533±0.033 c	0.08±0 a
C-120	0	0.693±0.067 n	0.07±0 b
	2	2.2±0.058 kl	0.07±0 b
	5	3.7±0.058 ij	0.07±0 b
	8	5.7±0.058 fg	0.07±0 b
	12	6.567±0.033 e	0.078±0.001a

The different letters on the column differ significantly by Tukey's HSD LSD ($P<0.01$).

Changes in conjugated diene and conjugated triene

Changes in the content of conjugated dienes and triene were a useful indicator for lipid oxidation that occurred after the accelerated oxidation of soybean oil in the presence or absence of natural antioxidant extract or synthetic antioxidant (**Marmesat *et al.*, 2009**). It has been reported that the production of conjugated dienes may be related to the high polyunsaturated fatty acid content of edible oils **(Konsoula, 2016)**.

A useful parameter for determining oils' oxidative stability is the measurement of conjugated diene and conjugated triene. The UV absorbance indicates that the conjugation of double bonds in polyunsaturated fatty acids and the formation of hydroperoxides occur simultaneously **(Mohdaly *et al.*, 2010)**. During oxidation, the double bond position of lipids with polyenes or dienes is interrupted by methylene changes. Conjugated diene (CD) absorbs strongly at 232 nm, and conjugated triene (CT) absorbs strongly at 270 nm. The oxygen uptake is inversely correlated with the rise in conjugated diene and conjugated triene content. The oil's oxidative stability will be lower the higher the levels of CD and CT **(El-Hadary and Taha, 2020)**. Absorptivity at 232 nm and 270 nm in soybean oil under accelerated oxidation conditions (at 70°C for 12 days) using curcumin at different concentrations (40, 80, and 120 µg/mL) in comparison with control (without any antioxidant) and positive control (using synthetic TBHQ-200 µg/mL) are presented in Table 6. Controls without antioxidants had the highest conjugated dienes and conjugated triene (Table 6), content, indicating a higher intensity of oxidation during accelerated storage. All treatments such as curcumin at different concentrations (40, 80, and 120 µg/mL) as a natural antioxidant in comparison with control (without any antioxidant) and positive control (using synthetic TBHQ-200 µg/mL) significantly decreased conjugated diene and conjugated triene compared to control.

Table 5. Changes in antioxidant activity (%) of soybean oil under accelerated oxidation conditions.

Treatment	Storage time (days)	Antioxidant activity (%)
Control	0	35.333±0.882 qr
	2	29.333±0.882 s
	5	24±0.577 t
	8	18.667±0.333 u
	12	14.667±0.882 v
TBHQ	0	89.333±0.882 a
	2	79±1.155 d
	5	66.333±1.453 fg
	8	57.333±1.202 k-n
	12	51.333±0.882 o
C-40	0	74±0.577 e
	2	61±0.577 i-l
	5	56.667±0.333 l-n
	8	53.333±0.882 no
	12	34.667±0.882 qr
C-80	0	81±0.577 cd
	2	64±0.577 f-i
	5	61.333±0.882 h-k
	8	56.667±0.333 l-n
	12	37.333±0.333 q
C-120	0	85.667±1.202 ab
	2	67.667±0.882f
	5	66.333±0.667 fg
	8	61.667±0.333 h-k
	12	44±0.577 p

The different letters on the column differ significantly by Tukey's HSD LSD ($P<0.01$).

Table 6. Changes in conjugated diene (OD 232) and conjugated triene (OD 270) of soybean oil under accelerated oxidation conditions

Treatment	Storage time (days)	Conjugated diene (OD 232)	Conjugated triene (OD 270)
Control	0	3.202±0.01 s	2.184±0.018 h-j
	2	3.915±0.033 op	2.889±0.003 f-j
	5	4.57±0.014 l	3.469±0.009 d-h
	8	5.989±0.004 d	4.804±0.014 b-d
	12	7.151±0.04 a	7.095±0.05 a
TBHQ	0	3.375±0.023 r	2.259±0.003 h-j
	2	3.799±0.017 pq	2.778±0.013 f-j
	5	4.149±0.031 n	3.025±0.002 e-j
	8	4.38±0.021 m	3.189±0.005 e-i
	12	4.921±0.033 j	3.713±0.008 c-g
C-40	0	2.543±0.001 t	1.711±0.001 j
	2	3.114±0.002 s	2.013±0.001 ij
	5	3.681±0.009 q	2.333±0.001 g-j
	8	4.552±0.009 l	2.881±0.004 f-j
	12	5.851±0.031 e	5.333±0.001 b
C-80	0	3.077±0.001 s	2.29±0.052 g-j
	2	3.487±0.002 r	2.444±0.001 g-j
	5	3.923±0.03 op	2.674±0.001 g-j
	8	4.681±0.008 kl	3.225±0.001 e-i
	12	5.955±0.017 de	5.387±0.001 b
C-120	0	4.35±0.001 m	2.926±0.001 f-j
	2	4.86±0.018 j	3.451±0.001 d-i
	5	5.222±0.007 h	4.981±0.001 bc
	8	5.473±0.015 g	4.897±0.001 b-d
	12	5.871±0.017 de	5.166±0.001 b

The different letters on the column differ significantly by Tukey's HSD LSD ($P<0.01$).

Conclusions

Soybean oil supplemented with curcumin and TBHQ is characterized by low peroxide value, conjugated diene, and conjugated triene content compared to control (without antioxidant). Accordingly, curcumin enriched soybean oil at different concentrations (40, 80, and 120 µg/mL) that can have a good substitute for TBHQ substitution soybean oil.

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نشـــاط الكـــوركمين كمضـــاد طبيعـــى للأكســـدة علـــى الثبـــات التـــأكســـدى لـــزيـــت فـــول الصـــويـــا تحت ظـــروف الأكســـدة الحـــراريـــة

ياسر محمد حسين - رجب عبدالفتاح المصري – على محمد الجابى – على عثمان

قسم الكيمياء الحيوية - كلية الزراعة - جامعة الزقازيق - الزقازيق - مصر

تهدف الدراسة الحاليه الى فحص الثبات التأكسدى لزيت فول الصويا تحت ظروف الاكسدة الحرارية (70 درجه مئوية لمدة 12 يوم) وتم استخدام الكركمين كمضاد أكسدة طبيعى بتركيزات مختلفة (40 ، 80 ، 120 ميكروجرام/مل) بالمقارنه مع مضاد أكسدة صناعى (TBHQ) بتركيز 200 ميكروجرام/ملل ومقارنتهم بزيت الصويا بدون أيه اضافات (كنترول). تم بدراسة محتوى زيت الصويا من الاحماض الدهنية وأظهرت النتائج أنه يحتوي على حمض اللينوليك بأعلى نسبة (53.3%) ، يليه حمض الأوليك (23.7%) ، يليه حمض البالمتيك (11.4%). أشارت نتائج تجربة التخزين تحت ظروف الأكسدة الحرارية إلى أن الكركمين بتركيز (40 و 80 و 120 ميكروجرام/مل) أظهر قدرة عاليه كمضاد للأكسدة مقارنه بمضاد الأكسدة الصناعى ومجموعه الكنترول (بدون أي مضادات أكسدة) كما يمتلك الكركمين القدرة على زيادة العمر الافتراضي لزيت فول الصويا مقارنةً بمضاد الأكسدة الصناعى وظهر ذلك من انخفاض قيم رقم البيروكسيد ورقم الحامض والروابط الزوجيه المتبادله الثنائية والروابط الزوجيه المتبادله الثلاثية مقارنه بالكنترول. وبالتالى يمكن استخدام الكركمين بديلا لمضادات الاكسدة الصناعية لمنع أكسدة وفساد زيت فول الصويا

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