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Original Research

Improving Oxidative Stability of Corn Oil by Curcumin and Beta-Carotene under Accelerated Oxidation Conditions

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Abstract

Curcumin (40, 80, and 120 µg/mL), β-carotene (5, 10, and 20 µg/mL), and TBHQ (200 µg/mL) were used to test how well they protected corn oil from oxidation under accelerated oxidation conditions. Linoleic acid has the highest amount (51.1 %), while linolenic acid has the lowest content (1.3%). Corn oil supplemented with 200 µg/mL TBHQ was shown to have the longest induction time (12.9 h) of the oils studied, followed by corn oil supplemented with 20 µg/mL β-carotene (11.78 h) and 40 µg/mL curcumin (11.53 h). Curcumin, and β-carotene showed greater antioxidant potential in all tested samples compared to the control in the accelerated storage experiment. Curcumin and β-carotene can potentially improve the shelf life of corn oil compared with TBHQ-200 µg/mL. It can be concluded that curcumin, and β-carotene were found to be effective antioxidants activity at 40, 80, and 120 µg/mL for curcumin and 5, 10, and 20 µg/mL for β-carotene, so TBHQ was not required.

Keywords: corn oil, conjugated diene and triene, oxidative stability, oven test, peroxide value

Introduction

Vegetable oils, such as corn oil, are highly recommended as a human cooking medium due to their high polyunsaturated fatty acid content and their many healthful impacts on the human body [1, 2]. Fried foods are well-liked across the world because of their distinctive flavor and texture. Takeouts and fast food, which are more popular than ever, both make use of frying and deep frying techniques on a regular basis [3]. When subjected to harsh environmental variables such as constant high temperature, air, and light, the chemical structure of fatty acids and their derivatives (triglycerides) become unstable. Due to their high-fat content, fatty acids and their derivatives are easily oxidized by chemicals, a process that leaves a rancid taste and reduces their shelf life [4]. When oils are improperly stored (chemically or ecologically), oxidation

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(rancidity) occurs, altering the oil's organoleptic qualities and so reducing its shelf life, nutritional value, and economic value [5]. As a result, the rancidity of oils greatly diminishes both their nutritional value and their market worth, which could lead to a financial loss [6]. Food spoilage during preparation and storage is often brought on by lipid peroxidation. Antioxidants are often added to foods, especially those high in fat, to extend their shelf life. Because of concerns that butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) and other synthetic antioxidants may cause cancer, their usage in food has been severely limited [7]. The synthetic antioxidant TBHQ, for example, was once widely regarded as the most effective in the world, but it is now banned in many developed nations [8]. As a result, natural antioxidants like curcumin are being considered as an alternative to synthetic antioxidants in order to avoid potentially harmful consequences [9] and β-carotene [10]. Vegetable oils are colored with oil-soluble pigments like beta-carotene, which can range in concentration from 2 to 8 parts per million (ppm) in margarine and buttery oils, 7 to 13 ppm in bakery shortening, 13 to 33 ppm in French fries or par-fry oil, and 66 to 132 ppm in popcorn oil [11]. Curcumin, a polyphenol extracted from the turmeric plant, has been linked to a variety of positive health effects [12]. Corn oil has a high nutritional value because it is rich in important fatty acids, especially linoleic acid (C18:2), with a concentration of more than 600 g kg^{-1}. Corn oil contains a tocopherol content of around 1 g kg^{-1}, despite its considerable unsaturation. Total tocopherol content in deodorized corn oil is typically between 0.8 and 1.2 g kg^{-1}, with γ-tocopherol accounting for 70-80%, α-tocopherol for 20-25%, and δ-tocopherol for 3-5% [13]. Corn oil fortified with antioxidants has been the subject of several recent investigations. To that end, we compared the effectiveness of the widely used synthetic antioxidant TBHQ (200 µg/mL) to that of lower concentrations of the natural antioxidant's curcumin (40, 80, and 120 µg/mL) and -carotene (5, 10, and 20 µg/mL) during the accelerated oxidation conditions of corn oils by measuring primary and secondary oxidation parameters.

Materials and Methods

Materials

Purified corn oil (free from antioxidant) was gained from Alfa Miser Company on the 10th of Ramadan, Egypt, and stored in the dark at 25ºC±2 ºC. Tert-butylhydroquinone (TBHQ) was supplied by Merck (Merck KGaA, Darmstadt, Germany), and the remaining reagents and solvents were of analytical degree. Curcumin and β-carotene were gained from Merck (Merck KGaA, Darmstadt, Germany), and the HPLC chromatogram is presented in Fig. 1(a, b).

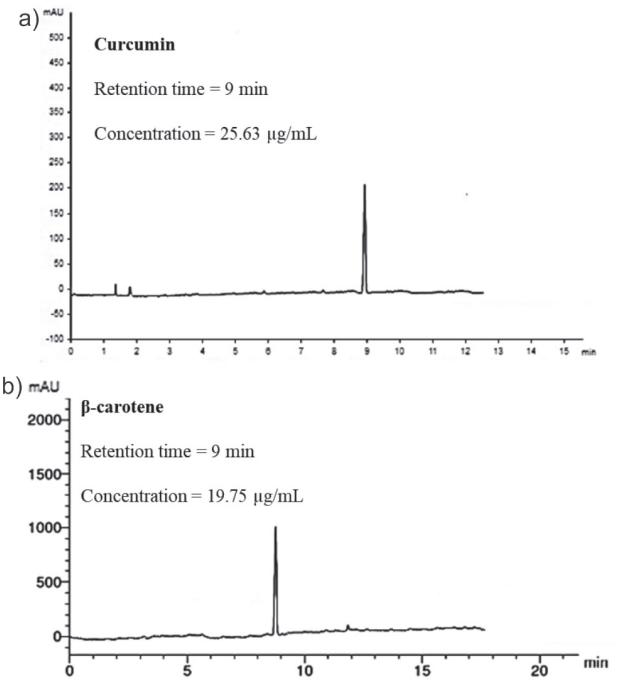

Fig. 1. HPLC chromatogram for curcumin a) and β-carotene b).

Fatty Acids Profile Estimation

Fatty acids profile of corn oil was estimated as described by [14]. The methyl esters were analyzed through gas chromatography (GC) in triplicate. This analysis was performed using an SP 2560 fused silica capillary column, precisely measuring 100 m in length, 0.25 mm in internal diameter, and with a film thickness of 0.2 m. The equipment utilized for detection was a flame ionization detector (FID) with model number 3800, manufactured by Varian in Walnut Creek, CA, USA. The samples were introduced into the system using an autosampler manufactured by Varian in Walnut Creek, CA, USA. The sensitivity of the gas chromatography (GC) equipment was adjusted to its maximum value of 12 using the Galaxie Chromatography Workstation software (version 1.9.3.2) to ensure adequate sensitivity. The FID parameters were configured as follows: the heater temperature was set to 250ºC, the sensitivity was set to 12, the flow rates of helium gas, hydrogen, and air were set to 30 mL/min, 31 mL/min, and 296 mL/min respectively, and the oven program duration was set to 111 minutes. Chromatograms of standard fatty acid methyl esters (Sigma, USA) were used to determine the identities of the peaks.

Corn Oil Samples Preparation

Curcumin (40, 80, and 120 µg/mL) and β-carotene (5, 10, and 20 µg/mL) as natural antioxidants were added and vigorously mixed with corn oil in comparison with the widespread synthetic antioxidant TBHQ (200 µg/mL) as mentioned in Table 1.

Table 1. Corn oil samples used in the present study.

Sample	Code
Corn oil	Control
Corn oil + TBHQ 200 µg/mL	TBHQ
Corn oil + β-carotene 5 µg/mL	BC-5
Corn oil + β-carotene 10 µg/mL	BC-10
Corn oil + β-carotene 20 µg/mL	BC-20
Corn oil + curcumin 40 µg/mL	C-40
Corn oil + curcumin 80 µg/mL	C-80
Corn oil + curcumin 120 µg/mL	C-120

Oxidative Stability (Rancimat Test)

According to ISO 6886:1997, oxidative stability was tested in 679 rancimat apparatus from Switzerland [15], using a 5 g±0.01 g sample. All samples were examined at 110ºC with a constant airflow rate of 20 L/h. Automatically and to within 0.005 seconds, the equipment software printed the induction times.

Oven Test

A series of 20 mL clear glass bottles containing corn oil and oil supplemented with curcumin, β-carotene, and TBHQ were prepared. Corn oil or oil supplemented with curcumin and β-carotene weighed 10 g each, and the bottles were kept open. For different periods (0, 2, 5, 8, and 12 days), the oxidation reaction was sped up in a forced-draft air oven T6 (Heraeus Instruments GmbH; Hanau, Germany) set at 70ºC±2ºC [1, 16]. The antioxidant activity (AA), conjugated dienes (CDs), and conjugated trienes (CTs) levels, as well as the peroxide value (PV) and acid value (AV) of these samples, were measured.

Peroxide Value Estimation

The PVs for corn oil and fortified oil with curcumin, β-carotene, and TBHQ were determined according to [17].

The PV was calculated from the following equation:

$$\text{PV (meq/1000 g sample)} = [(S - B) \text{ XMX1000}]/\text{Sample weight (g)}$$

where B is the volume of titrant, mL of blank, S is the volume of titrant, mL of test portion, and M is the molarity of sodium thiosulfate.

Acid Value Estimation

The AV of corn oil and fortified oil with curcumin, β-carotene, and TBHQ was determined according to AOCS [17]. A known weight of the test portion (56.4±0.20g) was added to an oil sample bottle or Erlenmeyer flask. 50 ml of hot neutralized alcohol and 2 ml of indicator were also added. Titrated with standard KOH, the mixture was shaken vigorously until the first permanent pink color appeared of the same intensity as that of the neutralized alcohol before adding the sample. The color must persist for 30 seconds.

The AV was calculated from the following equation:

$$\text{Acid value} = \text{Volume of KOH (ml)} \text{ X Normality of KOH/ Sample weight (g)}$$

Antioxidant Activity (DPPH-Assay)

For evaluation of the antioxidant activity of corn oil and fortified oil with curcumin, β-carotene, and TBHQ during the oven test, the DPPH• radicals dissolved in toluene (10^{-4} M) were used as described by Ramadan et al. [18] with some modification. For this experiment, 10 mg of each sample was combined with 10 mL of toluene solution, and then 1 mL of this mixture was combined with 3 mL of DPPH solution. The UV-260 visible recording of a Jenway 635001-6305 UV/Visible Spectrophotometer was used to detect the decrease in absorption at 515 nm in 1 cm quartz cells after 60 min of mixing, relative to a blank of toluene without DPPH. The antioxidant activity (% inhibition) was calculated from the following equation:

$$\%\ \text{Inhibition} = [(\text{Absorbance of control} - \text{Absorbance of sample})/ \text{Absorbance of control} \times 100]$$

Conjugated Diene (CDs) and Conjugated Triene (CTs) Estimation

Using a spectrophotometer (Jenway 635001-6305 UV/Visible), the CDs' and CTs' individual extinctions (given in units; U) at 232 nm and 270 nm were estimated. Isooctane was used to dilute oil samples in accordance with IUPAC method II. D.23 [1]. Polyunsaturated fatty acids' CD and CT were defined as their absorbance (U) at 232 and 270 nm, respectively. Oil oxidation was thought to be progressing when absorbance (U) values increased (at 232 nm and 270 nm).

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 Acids Profiles of Corn Oil

Table 2 shows the fatty acid profiles of corn oil; linoleic acid, oleic acid, palmitic acid, stearic acid, and linolenic acid are the most abundant fatty acids in corn oil. The highest percentage is linoleic acid (51.1%), followed by oleic acid (30.5%), palmitic acid (14.3%), and finally linolenic acid (1.3%). The fatty acid composition results for the corn oil are consistent with those found by other authors [13,19]. Table 2 statistics show higher levels of unsaturated fatty acids in the corn oil under study, consistent with information from the literature [20-22].

Oxidative Stability (Rancimat Test)

By carrying out the rancimat test, we can ascertain the induction time of the examined oils (Table 3). The longest induction times were determined for corn oil supplemented with TBHQ at 200 µg/mL (12.9 h), followed by corn oil supplemented with β-carotene at 20 µg/mL (11.78 h), then corn oil supplemented with curcumin at 40 µg/mL (11.53 h). The lowest induction times were recorded with corn oil supplemented with curcumin at 80 µg/mL (5.66 h). The induction time of corn oil increased with the addition of natural antioxidants [23-25] Redondo-Cuevas et al.[26] recorded the induction time of corn oil equal to 0.84 h.

Table 2. Fatty acids composition of corn oil.

Fatty acids	Common name	Percentage (%)
Palmitic acid	C16:0	14.3
Stearic acid	C18:0	2.8
Oleic acid	C18:1	30.5
Linoleic acid	C18:2	51.1
Linolenic acid	C18:3	1.3
Total saturated fatty acids		17.1
Total unsaturated fatty acids		82.9

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

Sample	Code	Induction time (h)
Corn oil	Control	10.28
Corn oil + TBHQ 200 µg/mL	TBHQ	12.9
Corn oil + β-carotene 5 µg/mL	BC-5	11.08
Corn oil + β-carotene 10 µg/mL	BC-10	9.23
Corn oil + β-carotene 20 µg/mL	BC-20	11.78
Corn oil + curcumin 40 µg/mL	C-40	11.53
Corn oil + curcumin 80 µg/mL	C-80	5.66
Corn oil + curcumin 120 µg/mL	C-120	11.31

Effect of Curcumin, β-Carotene, and TBHQ on Corn Oil Under Accelerated Oxidation Conditions

Corn oil was subjected to accelerated oxidation conditions (at 70ºC for 12 days) to evaluate the oxidation defense activity of curcumin at different concentrations (40, 80, and 120 µg/mL) and β-carotene (5, 10, and 20 µg/mL) as a natural antioxidant compared to the negative control and TBHQ-200 µg/mL. PV, AV, AA, CDs, and CTs were estimated.

Changes in Peroxide Value

Peroxide value (PV) is a measurement of the extent to which rancidity reactions have occurred during storage; it can be used to determine the quality and stability of lipids and oils [5]. In addition, the peroxide value of oil samples increased with storage time, temperature, and exposure to oxygen [27]. The peroxide value indicates how much rancidity the oil has undergone [28]. Except for the control, the initial rate of rise in PVs was relatively modest (Fig. 2). Peroxide values showed a statistically significant ($P<0.01$) difference between the control and all treatments containing curcumin, β-carotene, and TBHQ. Antioxidants (curcumin at 40, 80, and 120 µg/mL; β-carotene at 5, 10, and 20 µg/mL; and TBHQ at 200 µg/mL) significantly reduced PVs in corn oil when subjected to accelerated oxidation conditions (for 12 days) compared to the control. After 12 days of accelerated oxidation, the PV in corn oil control had increased significantly to 15.33 meq/kg. The PVs were significantly ($P<0.01$) reduced to 7.26, 7.1, and 6.43 meq/kg in corn oil when beta-carotene was used at different concentrations (5, 10, and 20 µg/mL, respectively). The PVs were significantly ($P<0.01$) reduced to 8.26, 7.53, and 6.56 meq/kg in corn oil when curcumin was used at different concentrations (40, 80, and 120 µg/mL, respectively). The PV was 5.33 meq/Kg in corn oil when TBHQ-200 µg/mL was used. In the early phases of oil oxidation, the PV is used to assess the formation of primary oxidation products (rancidity) like peroxides and hydroperoxides [29]. Oils' oxidative stability and storage life can be greatly improved by adding antioxidants (either natural or synthetic) to prevent autooxidation and prevent the accumulation of primary and secondary oxidation products. The antioxidant effect of natural plant extracts from various sources can effectively prevent the oxidation of corn oil [30, 31].

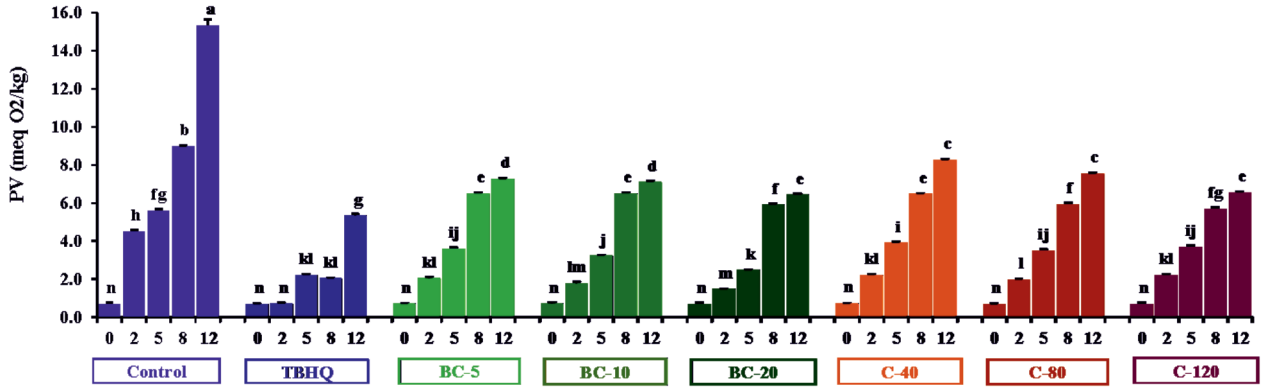

Fig. 2. Changes in peroxide value (meq O_2/Kg) of corn oil under accelerated oxidation conditions (at 70ºC for 12 days) using curcumin at different concentrations (40, 80, and 120 µg/mL) and β-carotene (5, 10, and 20 µg/mL) as a natural antioxidant in comparison with control (without any antioxidant) and positive control (using synthetic TBHQ-200 µg/mL). The bars on the top of the columns represent the SE, and different letters on the column differ significantly by Tukey's HSD LSD (*P<0.01*).

Changes in Acid Value

Corn oil quality and stability can be estimated based on AV changes measured over time during storage. Therefore, after subjecting fresh oil to rapid oxidation, basic chemical quality assessments were performed. The results obtained are shown in Fig. 3. All treatments recorded the same AV (0.7 mg KOH/kg) during storage for eight days. By the end of the 12-day accelerated oxidation trial, the AV had increased to 0.8 mg KOH/ kg across all treatments compared to the control.

Changes in Antioxidant Activity

In this study, the ability of corn oil under accelerated oxidation conditions (at 70°C for 12 days) using curcumin at different concentrations (40, 80, and 120 µg/mL) and β-carotene (5, 10, and 20 µg/mL) as a natural antioxidant compared with control (without any antioxidant) and positive control (using synthetic TBHQ-200 µg/mL) was screened using DPPH• radical. From the data presented in Fig. 4, adding natural (curcumin and beta-carotene) at different concentrations and synthetic antioxidants to corn oil increased the antiradical action of corn oil compared to the control (without antioxidants). The presence of TBHQ in corn oil increased the AA. On the other hand, the presence of curcumin and beta-carotene in corn oil increased the AA proportionally to the curcumin and β-carotene concentration. The method works for oil fractions with different polarities, like neutral lipids, glycolipids, and phospholipids [32]. In the current study, the presence of TBHQ in corn oil increased the AA. On the other hand, the presence of curcumin and beta-carotene in corn oil increased the AA proportionally to the curcumin and beta-carotene concentration. Lipid radicals, including alkoxyl (RO), peroxyl (ROO), and alkyl (R) radicals, are generated during autoxidation of polyunsaturated fatty acids [33]. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) can react with alkoxyl (RO), peroxyl (ROO), or alkyl (R)

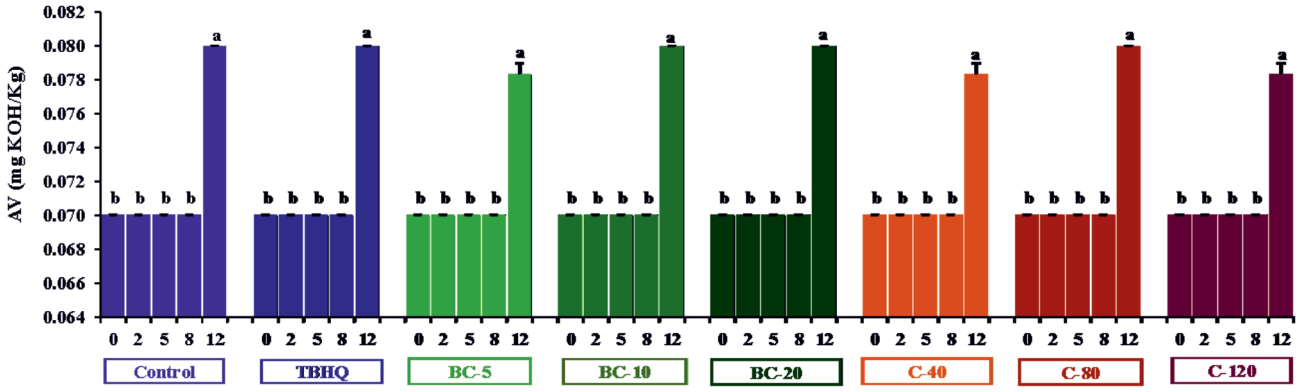

Fig. 3. Changes in acid value (mg KOH/Kg) of corn oil under accelerated oxidation conditions (at 70ºC for 12 days) using curcumin at different concentrations (40, 80, and 120 µg/mL) and β-carotene (5, 10, and 20 µg/mL) as a natural antioxidant in comparison with control (without any antioxidant) and positive control (using synthetic TBHQ-200 µg/mL). The bars on the top of the columns represent the SE, and different letters on the column differ significantly by Tukey's HSD LSD (*P<0.01*).

radicals of polyunsaturated fatty acids [34]. By factoring in the quantity of free radical scavenging chemicals at the outset and the amount of time needed for oxidation to consume these compounds, the DPPH method can make predictions about the oils' oxidative stability [35, 36].

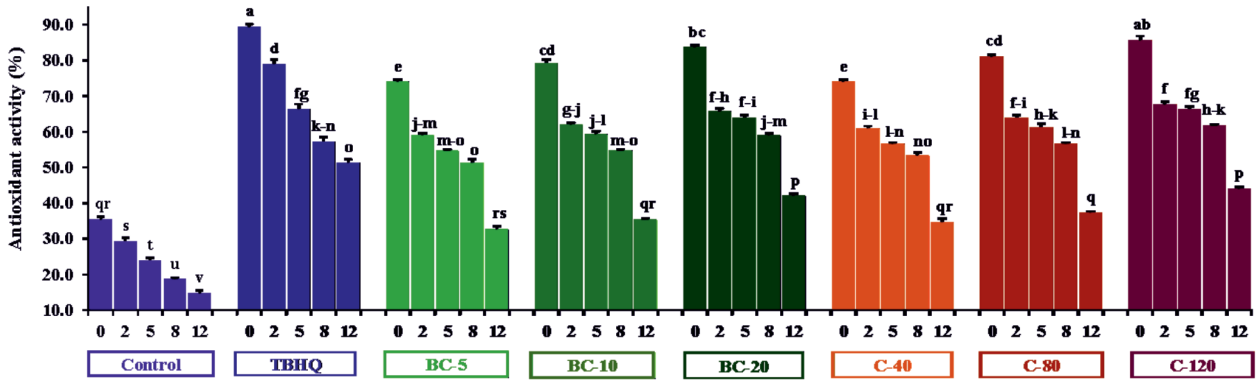

Fig. 4. Changes in antioxidant activity (%) of corn oil under accelerated oxidation conditions (at 70ºC for 12 days) using curcumin at different concentrations (40, 80, and 120 µg/mL) and β-carotene (5, 10, and 20 µg/mL) as a natural antioxidant in comparison with control (without any antioxidant) and positive control (using synthetic TBHQ-200 µg/mL). The bars on the top of the columns represent the SE, and different letters on the column differ significantly by Tukey's HSD LSD (*P<0.01*).

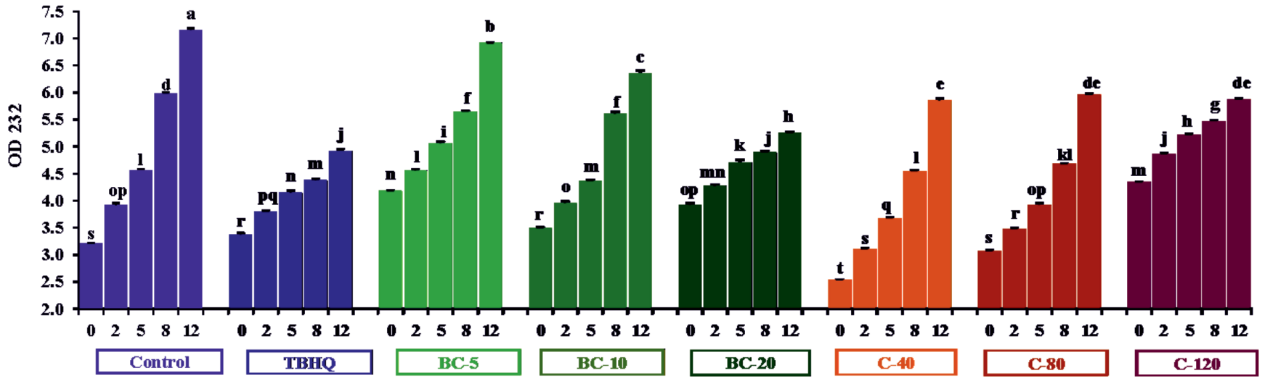

Fig. 5. Changes in conjugated dienes (OD232) of corn oil under accelerated oxidation conditions (at 70ºC for 12 days) using curcumin at different concentrations (40, 80, and 120 µg/mL) and β-carotene (5, 10, and 20 µg/mL) as a natural antioxidant in comparison with control (without any antioxidant) and positive control (using synthetic TBHQ-200 µg/mL). The bars on the top of the columns represent the SE, and different letters on the column differ significantly by Tukey's HSD LSD (*P<0.01*).

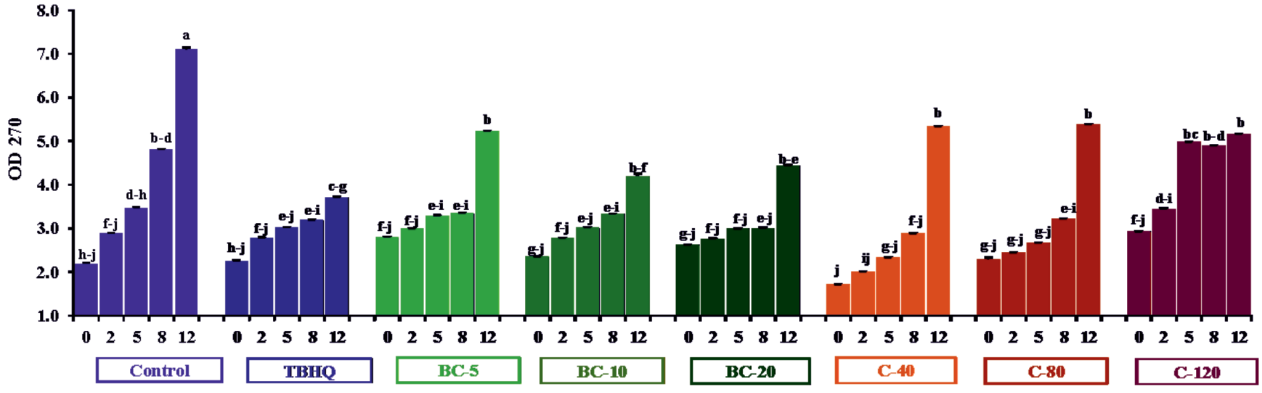

Fig. 6. Changes in conjugated trienes (OD270) of corn oil under accelerated oxidation conditions (at 70ºC for 12 days) using curcumin at different concentrations (40, 80, and 120 µg/mL) and β-carotene (5, 10, and 20 µg/mL) as a natural antioxidant in comparison with control (without any antioxidant) and positive control (using synthetic TBHQ-200 µg/mL). The bars on the top of the columns represent the SE, and different letters on the column differ significantly by Tukey's HSD LSD (*P<0.01*).

Changes in Conjugated Dienes and Conjugated Trienes

Absorptivity at 232 nm and 270 nm in corn oil under accelerated oxidation conditions (at 70ºC for 12 days) using curcumin at different concentrations (40, 80, and 120 µg/mL) and β-carotene (5, 10, and 20 µg/mL) as a natural antioxidant in comparison with control (without any antioxidant) and positive control (using synthetic TBHQ-200 µg/mL) are presented in Figs 5 and 6. Controls without antioxidants had the highest CDs (Fig. 5) and CTs (Fig. 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) and β-carotene (5, 10, and 20 µg/mL) as a natural antioxidant in comparison with control (without any antioxidant) and positive control (using synthetic TBHQ-200 µg/mL), significantly decreased CDs and CTs compared to control. At the end of the accelerated oxidation experiment (after 12 days), the CDs and triene developed rapidly and reached 7.17 and 7.09, respectively, in the controls of corn oil. The CDs and CTs were significantly ($P<0.01$) reduced to 4.9 and 3.7 in corn oil when TBHQ was used at 200 µg/mL. The CDs and CTs were significantly ($P<0.01$) reduced to 5.2 and 4.4 in corn oil when beta-carotene was used at 20 µg/mL and 5.8 and 5.1 for curcumin at 120 µg/mL. Changes in the concentration of CDs were an effective indicator for tracking lipid oxidation under accelerated oxidation settings against corn oil in the presence or absence of natural antioxidant extract or synthetic antioxidants [37-39]. A possible link between CDs synthesis and the abundance of polyunsaturated fatty acids in modern cooking oils has been suggested [16]. The relative rise in CDs, a measure of oxidative deterioration of oils, was used to find the most effective treatment that might be used as natural antioxidants in corn oil. CDs and CTs measurements are helpful in determining oils' oxidative stability. Absorbance at the UV spectrum indicates that the conjugation of double bonds in polyunsaturated fatty acids coincides with the formation of hydroperoxides [40]. During oxidation, the double bond position of lipids with polyenes or dienes is interrupted by methylene changes. CDs absorb strongly at 232 nm, and CTs absorbs strongly at 270 nm. The oxygen uptake is inversely correlated with the rise in CDs and CTs content. The oil's oxidative stability will be lower with higher CDs and CTs levels [1].

Conclusions

Since both curcumin and beta-carotene include AA, they may replace synthetic antioxidants in the oil business. At the end of the accelerated oxidation storage period, all the detected oxidative products in corn oil were stabilized and were significantly lower than the negative control (without antioxidant) and synthetic antioxidant (TBHQ-200 g µg/mL). Corn oil supplemented with natural and synthetic antioxidants showed low PV, CDs, and CTs content compared to the control (without antioxidants). This indicated that curcumin at different concentrations (40, 80, and 120 µg/mL) and β-carotene (5, 10, and 20 µg/mL) showed good AA, so there was no need to apply TBHQ.

Acknowledgments

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Conflict of Interest

The authors declare no conflict of interest.

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