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Oxidative stability of sunflower oil enriched with citrus flavonoids extracted using MWCNT-COOH during frying

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ABSTRACT

The purpose of this study was to compare the efficacy of citrus peel phenolic compounds extracted using carboxylated multi-walled carbon nanotubes (MWCNT–COOH) with common synthetic antioxidants [butylhydroxyanisole (BHA) and butylhydroxytoluene (BHT)] in stabilizing sunflower oil (SO) during frying (180 ± 5 °C, 24 h). To evaluate the antioxidant activity of these compounds, total phenolic, and flavonoid contents, reducing power, ABTS scavenging activity, β-carotene bleaching ability, and 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity were measured. Extracts were combined at various concentrations (100-1000 ppm) with SO, and both synthetic antioxidants were standardized at 200 ppm. Thermal-oxidative stability was investigated by assessing the change in peroxide value (PV), color, free fatty acid (FFA) content, conjugated diene value (CDV), and thiobarbituric acid (TBA). Bitter orange peel extract (BPE) displayed a higher content of phenolic compounds and antioxidant activity. At 1000 ppm, it showed a lower FFA content, PV, TBA, and CDV. It can be used as an alternative to synthetic antioxidants. As a result, citrus peel phenolic compounds extracted with MWCNT–COOH can be used as preservatives in frying oils.

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1-Introduction

Edible vegetable oils are required in the human diet and account for over 75% of edible oil consumption worldwide. Sunflower oil (SO) is one of the four main edible oils consumed worldwide. It is more susceptible than other edible oils to oxidative deterioration because it has high polyunsaturated fatty acids content (85-95%) [1] .People's diets have changed, but frying is still popular because it's fast. During frying, a wide range of changes occurs, including thermal-oxidative changes, loss of unsaturated fatty acids, degradation of color, flavor, nutritional quality, and the production of toxic components that have detrimental effects on human health [2] . Synthetic antioxidants that are both affordable and effective have been widely used for various years to prevent the oxidation of fats and oils.

However, the global concern over the toxicity of synthetic antioxidants has caused increased consumption of natural antioxidants [3] . The polyphenol content of plant extracts is the main reason for their antioxidant properties. Plants with high polyphenols have paramount importance to natural antioxidants [4] . Several reports have been published about citrus fruits' critical nutrition and health-promoting values [5] . Several studies have attributed the antioxidant properties of citrus peels to their many polyphenol substances, including flavonoids and phenolic acids [6] .The antioxidant capacity of grapefruit and bitter orange peels is positively associated with their flavonoid content. As a result, these peels have a strong radical scavenging ability [6-8] .Flavonoids have superior antioxidant activity in high-temperature oil systems by adsorbing at the air-oil interface [9].

The influence of citrus peel extracts on the thermal oxidation stability of vegetable oils during frying has been reported in several studies [6, 8, 10] . However, several factors are involved in oil thermal-oxidation stability, such as citrus species, extraction procedure, extract concentration, type of vegetable oil, heating condition, and method of oil oxidation. To create a cost-effective and efficient solution for absorption, citrus peel extract can be combined with nanomaterials that are easy to use in any situation [11] . Carbon nanotubes have been used as an effective adsorbent recently because of their large surface area, proper stability times, and π–π electrostatic interactions compared to other elements [12] . Thus, according to recent studies, the present research goal was to apply the carboxylated multi-walled carbon nanotubes (MWCNT–COOH) in the adsorption of bitter orange and grapefruit peels flavonoids and to consider evaluating the antioxidant impact of these compounds on the oxidation stability of sunflower oils during frying for 24 hours.

2- MATERIALS AND METHODS

2.1. Materials

The experimental fields of the Marzikola Citrus Garden Institute in Iran provided bitter oranges and pink grapefruits. Carboxylated multi-walled carbon nanotubes (95% purity, length: 0.5-2 μm, the content of COOH groups: 2 wt %, OD: 20-30 nm, Neutrino Co., Ltd) were used. Sunflower oil without antioxidants was gained from Ghoncheh Co. (Sari, Iran) and used as received. Most of the reagents were analytical and supplied by Merck Co. (Darmstadt, Germany)

2.2. Citrus peels preparation

Bitter oranges and grapefruit were washed with purified water and peeled manually. The gathered peels were dried in an oven at 40 °C for 48 h, and the peel powder was prepared using a hammer mill. The powders were maintained in sealed containers at 4 °C.

2.3. Extract preparation

Bitter orange/grapefruit peel powder (5 g) was mixed with 30 mL of ethanol-water (70:30, v/v) as solvent. After that, the mixtures were sonicated using an ultrasonic bath (to disperse) at room temperature (25 °C) for 30 min. The suspensions were then separated, and the residual powders were extracted twice more using 25 ml of the solvent. After filtration, the filtrates were diluted to 100 mL in a volumetric flask (100 mL) by the ethanol-water mixture (70:30, v/v) and stored at four °C for further use [12].

2.4. Adsorption-desorption of flavonoids by MWCNT-COOH

The extracted flavonoids were separated from the extract by adsorption-desorption with MWCNT-COOH. Adsorption was performed at 10 mL of the extract with 50 mg of MWCNT-

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COOH at 25 °C by agitating the mixture for 1 hour. After filtration, the MWCNT-flavonoids were immersed in 10 ml of ethanol-water (70:30, v/v) solution at pH=3. Afterward, the MWCNT-flavonoids were centrifuged from the solution and washed with water. Ultimately, the released flavonoids were dried at 40 °C in a vacuum [12] .

2.5. Evaluation of the antioxidant activity of extracts

2.5.1. Total phenolic content (TPC)

According to Delfanian, the Folin-Ciocalteu colorimetric procedure was slightly changed to determine TPC. 1 mL of the extract was combined with 2.5 mL of 10-fold diluted Folin-Ciocalteu reagent and 2 mL of saturated Na2CO3 solution (7.5 %). The mixture was kept at 45°C for 40 minutes. Then, a visible spectrophotometer (Model 7315) was used to measure its absorbance at 765 nm. The spectrophotometer was made by Jenway in the UK. A blank was prepared without the sample, and gallic acid was employed as the standard to draw the calibration curve. Finally, TPC was expressed as milligrams of gallic acid per gram of dry extract [13-15].

2.5.2. Total flavonoid content (TFC)

The TFC of the extracts was determined based on the method previously described by Singleton [16] ; 0.5 ml of the sample was blended with 0.5 ml of methanol, 50 µl of 10 % AlCl3, 50 µl of 1 M Potassium acetate, and 1.4 ml of water. Next, the mixture was incubated at 25 °C for 30 minutes, and its absorbance was measured at 415 nm. TFC was finally determined. The absorbance of the reaction mixture was determined at 415 nm with a UV-visible spectrophotometer. TFC was obtained from the calibration curve of quercetin.

2.5.3. DPPH radical scavenging activity

Radical scavenging activity was defined as bleaching the purple-colored solution of DPPH. According to Delfanian [13], 1 mL of the sample was mixed with 1 mL of 0.4 mM DPPH metabolic solution. The mixture was agitated quickly and incubated at 25 °C for 30 min in the dark. A UV-visible spectrophotometer (7310, Jenway, South Korea) was used to determine the absorbance of the solution at 517 nm. The following equation was used to calculate the percentage of DPPH radical scavenging activity:

$$I\% = \frac{A_0 - A_1}{A_0} \times 100 \quad (1)$$

Where A_0 is the absorbance of the control sample (without extract), and A_1 denotes the sample absorbance. BHT was employed as the positive control.

2.5.4. ABTS radical scavenging activity

According to Babbar, the ABTS analysis was conducted [17]. ABTS stock was prepared as seven mM ABTS, and 2.45 mM potassium persulfate suspensions were combined to react to darkness at 25 °C for 16 h. Subsequently, the ABTS suspension was diluted by ethanol (70 %) at a 1:25 ratio. Then, 20 µl of the extract was dissolved in 2 ml of the ABTS solution and mixed vigorously. The blank was created using the reaction solvent alone, with no sample. After 15 minutes, the absorbance was recorded at 734 nm. The following equation was applied to calculate the ABTS scavenging activity:

$$I\% = \frac{A_{blank} - A_{sample}}{A_{blank}} \times 100 \quad (2)$$

A sample and A blank represent the absorbance values of the extract and the control sample at 734 nm.

2.5.5. Reducing power

The reducing power of the extracts was performed based on the method reported by Oyaizu [18] . 1 mL of the different extracts (50-5000 μg/mL) was combined with 2.5 mL of 0.2 M sodium phosphate buffer (pH=6.6) and 2.5 mL of 1 % $K_3Fe(CN)_6$. The mixture was incubated at 50 °C for 20 min. Next, 2.5 mL of 10 % trichloroacetic acid was added, and the mixture was centrifuged at 3000 g for 10 min. The supernatant (2.5 mL) was distilled with 2.5 mL water and 0.5 mL of 0.1 % ferric chloride solution. The absorbance was determined at 700 nm, and ascorbic acid was employed as the positive control.

2.5.6. β-carotene bleaching assay

We used Gursoy's method [19] with minor modifications to check if the sample can

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prevent β-carotene from fading. The stock solution was prepared by mixing 0.5 mg of β-carotene, 1 mL of chloroform, 25 μL of linoleic acid, and 200 mg of Tween 40. Subsequently, the solvent evaporated into a rotary evaporator at 40 °C. Afterward, 100 mL of oxygenated distilled water was added, and the mixture was then agitated. Then, 2.5 ml of the emulsion was poured into the extract-containing tubes, and the mixtures were incubated in the water bath at 50 °C for 2 hours. The absorbance was measured at 490 nm at 15-min intervals. The antioxidant activity (percentage) of the extracts was evaluated according to the following equation:

$$AA\% = 100[1 - \frac{A0-At}{(C0-Ct)}] \quad (3)$$

Where A_t and C_t respectively show the absorbance values determined for the sample and the control after incubation for 120 min. C_t and C_0 respectively stand for the absorbance values of the model and the control determined at the beginning of incubation.

2.6. Evaluation of oxidative stability

2.6.1. Frying procedure and oil sampling

We tested extracts at 250, 500, and 1000 ppm in SO and compared their ability to prevent oxidation with synthetic antioxidants (BHA and BHT) at 200 ppm. For three days, the oils were heated in a domestic fryer (Moulinex, France) at 180 ± five °C for 8 hours per day to reach 24 hours. 20 mL of each batch was taken every two hours and kept at -18 °C until being analyzed. The fryer was turned off at the end of each day, and the oils were conditioned at refrigerator temperature.

2.6.2. Color development determination

The absorbance of the oil samples was quantified at 420 nm with a spectrophotometer (UV-Vis; Jenway, model: 7315) and used as an index of color development.

2.6.3. Peroxide value (PV)

The PV of the SO samples was evaluated using the AOAC method. Five grams of the extract, 30 ml of acetic acid–chloroform solution, and 0.5 ml of saturated potassium iodide were blended with shaking. After a minute, water was added, and the mixture was stirred and tested with sodium hyposulfite until the yellow color disappeared. Afterward, 0.5 ml of 1 % starch solution was incorporated into the mixture, and titration continued until the color disappeared. PV was calculated according to the following equation:

$$PV = \frac{S \times N \times 1000}{M} \quad (4)$$

Where S is the sodium hyposulfite consumed (ml), N shows the sodium hyposulfite solution normality, and M denotes the mass of the oil sample (g) [20].

2.6.4. Free fatty acids (FFA) content

The FFA content of the samples was evaluated through the titration procedure of AOAC [20] with slight modifications. 2.5 grams of the oil sample was mixed with 10 ml of ethanol-chloroform solution (50:50 v/v). Then, the mixture was stirred fast and titrated with potassium hydroxide (0.1 N) until a consistent deep pink color appeared and lasted for at least 1 minute. Phenolphthalein solution (1%) was applied as the indicator. The FFA content was computed according to the following equation:

$$\%FFA = \frac{(N \times V \times 28.2)}{M} \quad (5)$$

Where N and V are respectively, the normality and volume (ml) of NaOH, and M stands for the mass of the SO sample (g).

2.6.5. Conjugated diene value (CDV)

The CDV of the oil was determined by measuring its absorbance at 233 nm using a spectrophotometer.

2.6.6. Thio barbituric acid (TBA)

The content of TBA was evaluated according to AOAC by measuring the absorbance at 532 nm[20].

2.7. Statistical analyzes

Variance (ANOVA) analysis was employed to analyze the data obtained from triplicate measurements. SPSS (version 19) was used for statistical analysis, and mean comparison was conducted by Duncan's multiple range test ($P< 0.05$).

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3- RESULTS AND DISCUSSION

3.1. Antioxidant activity of citrus peel extract

Polyphenols include different bioactive compounds, with flavonoids being an influential group. The chemical structure of flavonoids allows them to play a critical role in neutralizing free radicals and decomposing peroxides. The TPC and TFC of the extracts are summarized in Table 1. TPC and TFC values for bitter orange peel extract were 2246.76 and 1967 µg/g respectively, and for grapefruit peel extract, they were 15.134 and 12.123 µg/g in the 30:70 v/v water-ethanol solution. Ghasemi declared that the TPC of BPE and GPE ranged from 164 to 222.3 µg/g, while their TFC varied between 7.7 and 23.2 µg/g [7] . Various studies [21] have found a significant relationship between TPC and the antioxidant activity of diverse fruits. As shown in Figure 1, the inhibition of BPE's β-carotene bleaching and radical scavenging activity were higher than those of GPE.

Flavonoids' antioxidant properties are linked to their ability to donate electrons or generate active hydrogen [21]. The extracts' scavenging activity based on ABTS radicals further assessed free radical scavenging ability. We picked this process for its ability to measure hydrophilic and lipophilic antioxidant activity, resist chromogen composition, and its simplicity, speed, and sensitivity. As with the DPPH results, increasing the extract concentration improved its ability to scavenge ABTS radicals. The reaction mechanisms can describe the difference between the DPPH and ABTS results.

By analyzing the structure and reaction of the molecules, the phenomenon can be explained. ABTS radicals show quicker electron transfer responses than DPPH radicals. According to Bendaoud et al., the discoloration rate is connected to the hydrogen-donating ability of compounds [22] . The β-carotene linoleate system measures a sample's ability to stop the production of conjugated dienes during the oxidation of linoleic acid. The results revealed that TFC was significantly correlated with inhibition ability. In addition, flavonoids could hinder lipid peroxidation by eliminating free radicals [23] .According to Table 2, the reducing power of the extracts was determined at the extract concentrations of 50-5000 ppm. The results of the antioxidant activities agreed with the formerly reported by Su, who examined the BPE antioxidant impact on reducing power [24]. Mutiah [25] reported that the extract of BPE had a high reducing power of 0.536 µg/ml. Amarowicz [26] cited that reducing power depended on antioxidant activity. Citrus peel extracts' antioxidant activity might be because of their ability to minimize anions, activate free radicals, and form metal ion complexes [25].

Table 1. Antioxidant properties of bitter orange extract (BPE) and grapefruit peel extract (GPE).

Antioxidant properties	BPE	GPE	Standard Curves	Correlation Coefficient (R^2)
Total phenolic content (µg/g)	2246.76^{c}	1967^{d}	(Gallic acid) y = 0.0003x + 0.0719	$R^2 = 0.963$
Total flavonoid content (µg/g)	15.134^{c}	12.123^{d}	(Quercetin) y = 0.0082x - 0.0331	$R^2 = 0.9756$

Table 2. Reducing power ability of BPE and GPE.

Extract(ppm)	50	100	250	500	1000	2500	5000
BPE	0.099^{b}	0.166^{b}	0.256^{b}	0.321^{b}	0.425^{b}	0.584^{b}	1.018^{b}
GPE	0.057^{c}	0.11^{c}	0.178^{c}	0.243^{c}	0.243^{c}	0.406^{c}	0.855^{c}

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Ascorbic acid	**0.983[a]**	**1.231[b]**	**1.376[a]**	**1.654[a]**	**1.887[a]**	**2.602[a]**	**3.774[a]**

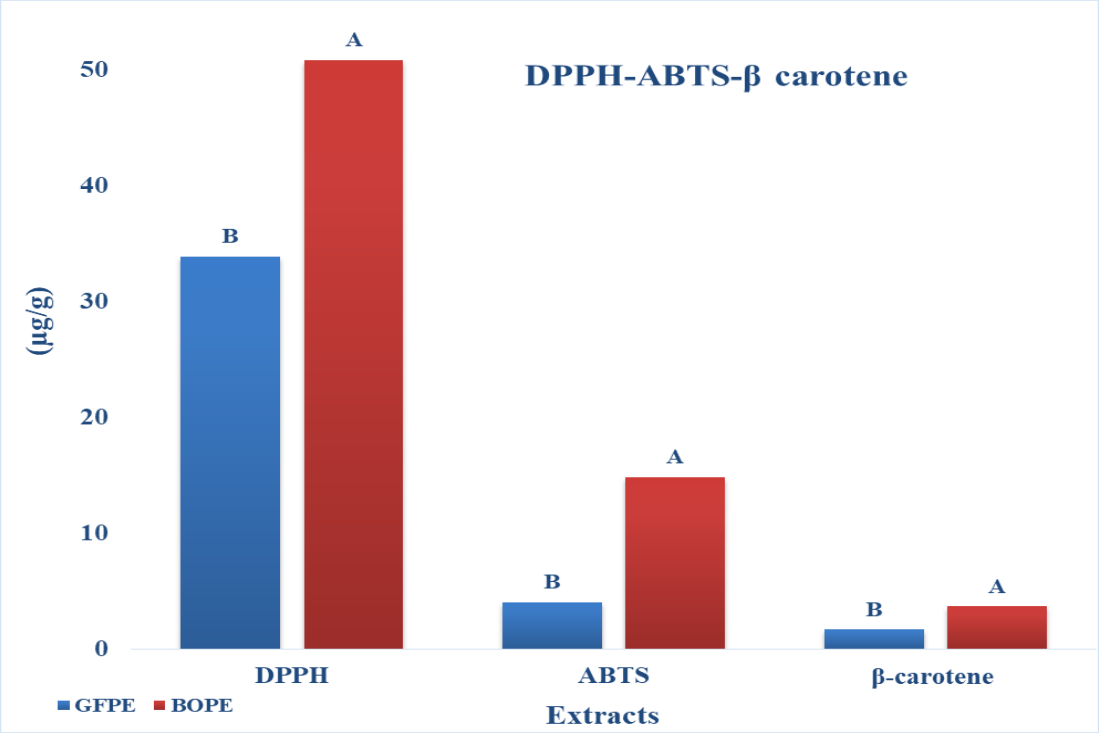

Figure1. Effects of BPE and GPE on DPPH, ABTS and β-carotene bleaching assays.

3. Oil oxidation stability

3.1. Color development

Figure 2 shows the color development of the SO samples. The TFC of the extracts had a significant effect ($p<0.05$) on the color development. The oil samples experienced a gradual and significant increase in color values. These findings were consistent with the results of Anwar [10].

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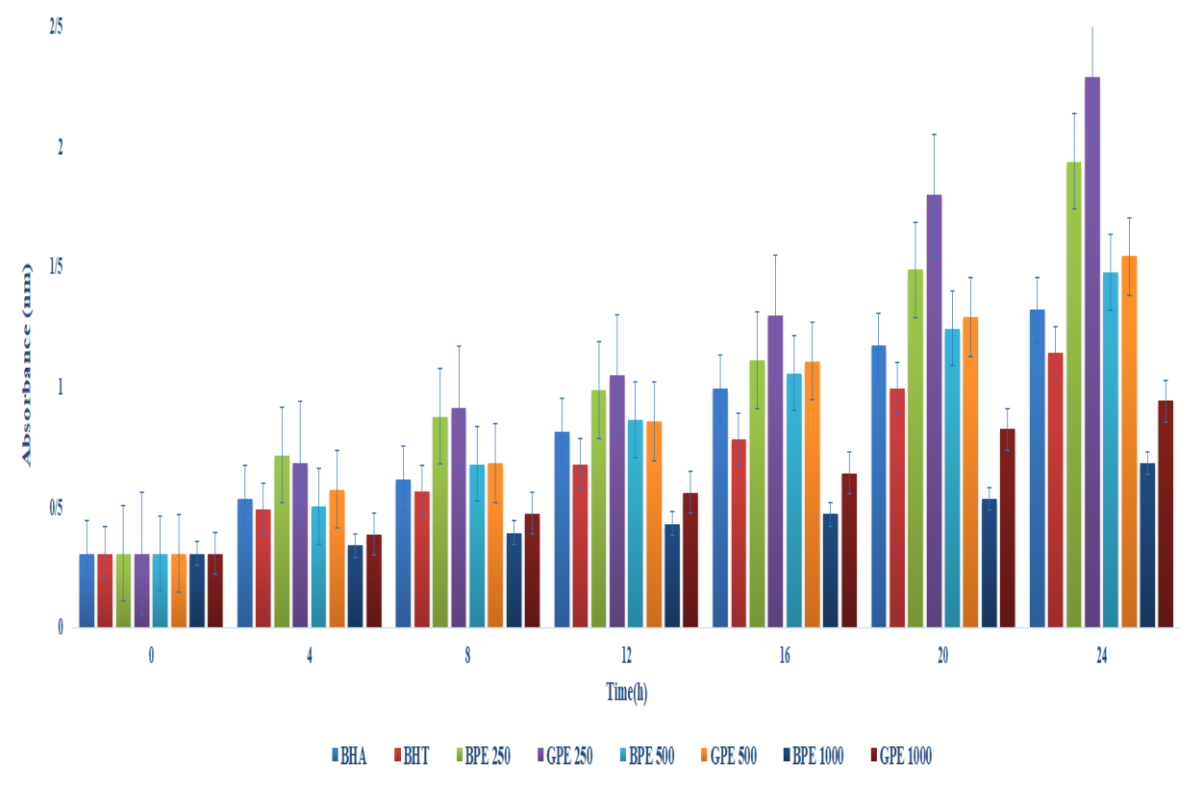

Figure 2. Effect of adding antioxidants on changes in color of sunflower oil during the frying process.

3.2. Free fatty acids (FFA) content

Figure 3 shows the FFA content of the samples throughout the frying process. Compared with the results declared by El-Badawi [8] , Nyam [27], and Lutfullah [28] , the FFA content was enhanced during frying. The FFA contents of the SO-BHA, SO-BHT, SO-BPE250, SO-GPE250, SO-BPE500, SO-GPE500, SO-BPE1000, and SO-GPE1000 were 1.41, 1.41, 0.99, 0.99, 0.85, 0.71, and 0.99 g $100g^{-1}$, respectively at the end of frying. Throughout the frying period, the low FFA content of the citrus peel extracts showed a great capacity to limit oil oxidation. The results also showed that the BPE had a better protective effect on the oil hydrolysis than the synthetic antioxidants. Lutfullah and the team got the same results as us on the effects of pomegranate and orange peel extracts on SO's antioxidants. Habib et al. found that SO-BPE1000 had lower FFA content than other samples while testing citrus peel extract's effects on SO's antioxidant properties.

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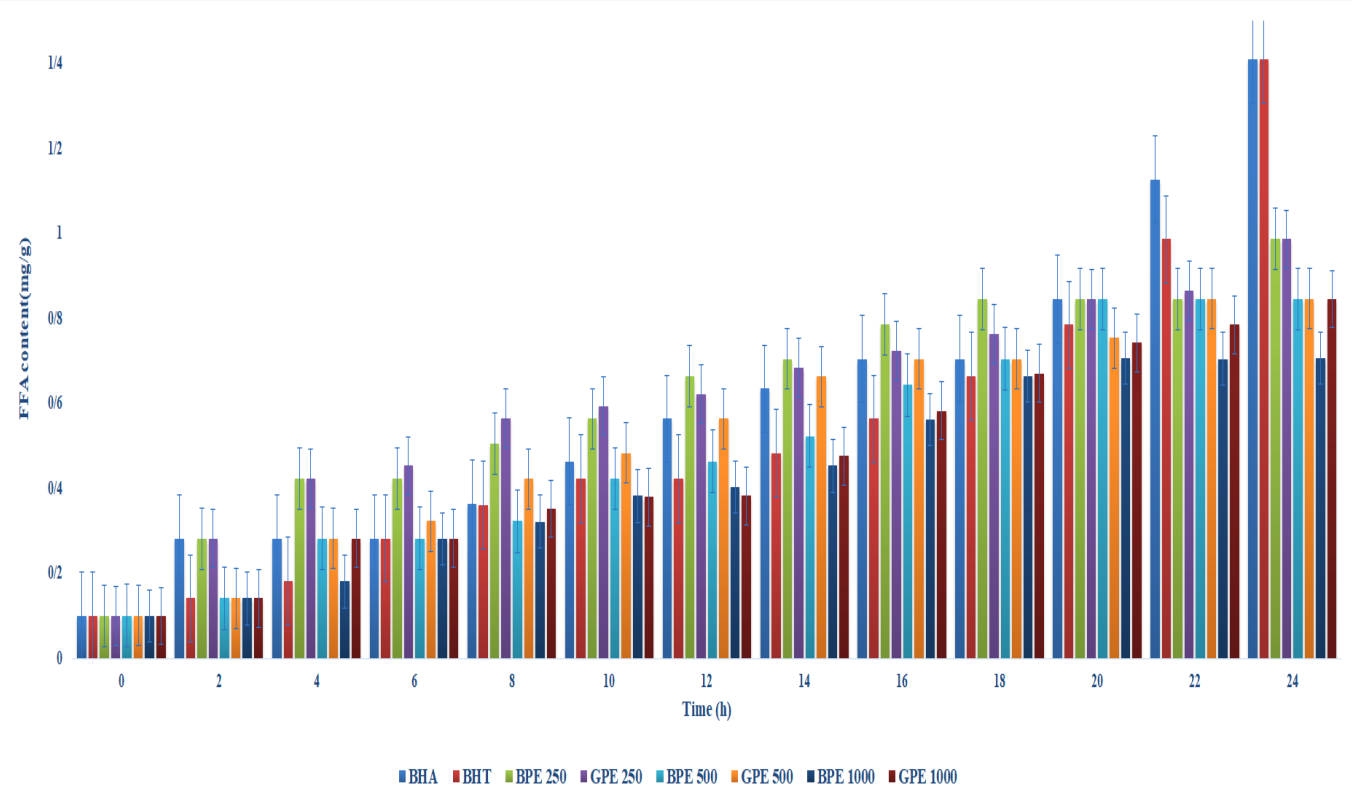

Figure 3. Effect of adding antioxidants on free fatty acid (FFA) content (b) of sunflower oil during the frying process.

3.3.3. Peroxide value (PV)

PV can show oil primary oxidation products [29] . Figure 4 shows the development of the sample PV during frying. The results show that the extracts significantly ($P<0.05$) decreased the PV of the SO samples relative to the control samples (SO-BHA, SO-BHT). The results indicated that PV increased as the frying time increased, conforming to El-Badawi [8]. Zia [30] compared the antioxidant effect of the citrus phenolic extract with that of BHA and BHT on corn oil. The results showed a dramatic decrease in the PV of the oil samples that contained the citrus extract at different concentrations. Zhang [29] evaluated the oxidative stability of SO-containing rosemary extract and denoted that the PV increased linearly.

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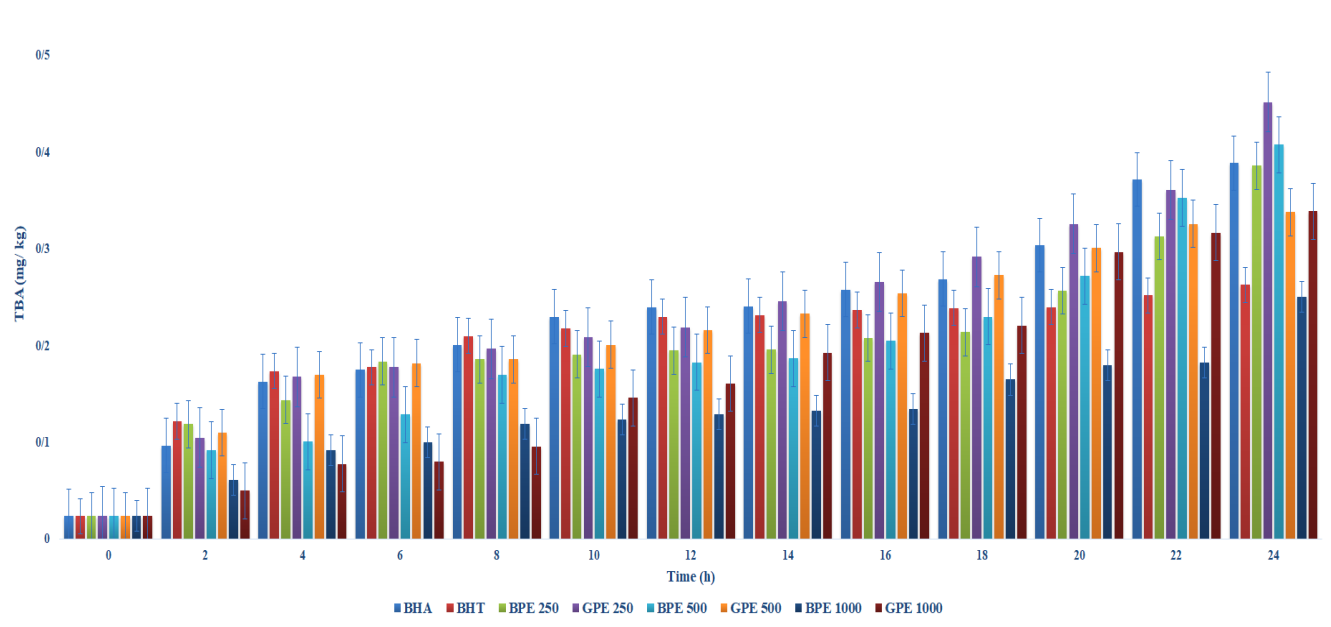

Figure 4. Effect of adding antioxidants on changes in peroxide values of sunflower oil during the frying process.

3.3.4. Thio barbituric acid (TBA)

TBA is the most routinely applied experiment for determining lipid oxidation in oils. Figure 5 illustrates the changes in the TBA value of all the SO samples during frying at 180 ± 5°C. The TBA value was elevated by increasing the frying time. BPE and GPE at 1000 ppm acted better in oil oxidation than the synthetic antioxidants. The study showed that soybean oil with almond peel extract at 60°C had a lower TBA value than BHA, which is consistent with Duh's findings [31] .Abd El-aal [32] reported that orange peel extract could reduce the TBA index by more than synthetic antioxidants in soybean oil during storage at 65 °C.

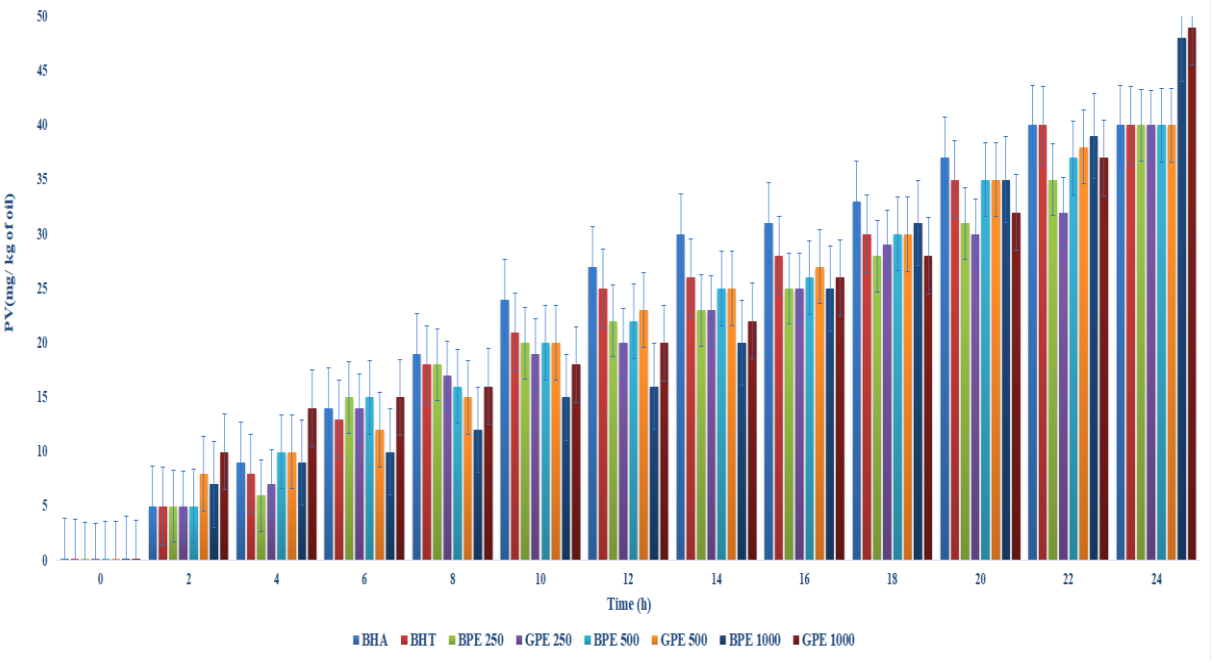

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Figure 5. Effect of adding antioxidants on changes in TBA values of sunflower oil during the frying process.

3.3.5. Conjugated diene value (CDV)

The CDV of oil throughout the frying process shows the production rate of primary oxidation products. Because of conjugated dienes' staying in fried oil, CDV is a better oxidation scale. According to Suleiman [33], the heating process rebuilds the position of the sunflower oil double bond. A fraction of non-conjugated dienes changes into conjugated ones. The production rate of conjugated dienes in the oil in Figure 6 is higher than their decomposition rate, leading to their accumulation. As time passed, CDV increased, which increased the primary oxidation products, as found in Bou's research [34].

The CDV of blended oils containing 1000 ppm of BPE and GPE had a considerable difference ($P<0.05$) from that of the other samples. These results followed prior research such as Abd Elaal [32] .They suggested natural extracts had a more protective effect on oil stability than BHT in the SO heated at 180 ± 5 °C. During the 24 hours of heating, the CDVs of the blended SO containing 1000 ppm of BPE, BHA, and BHT were 2.41, 2.43, and 2.49 mmol/L, respectively.

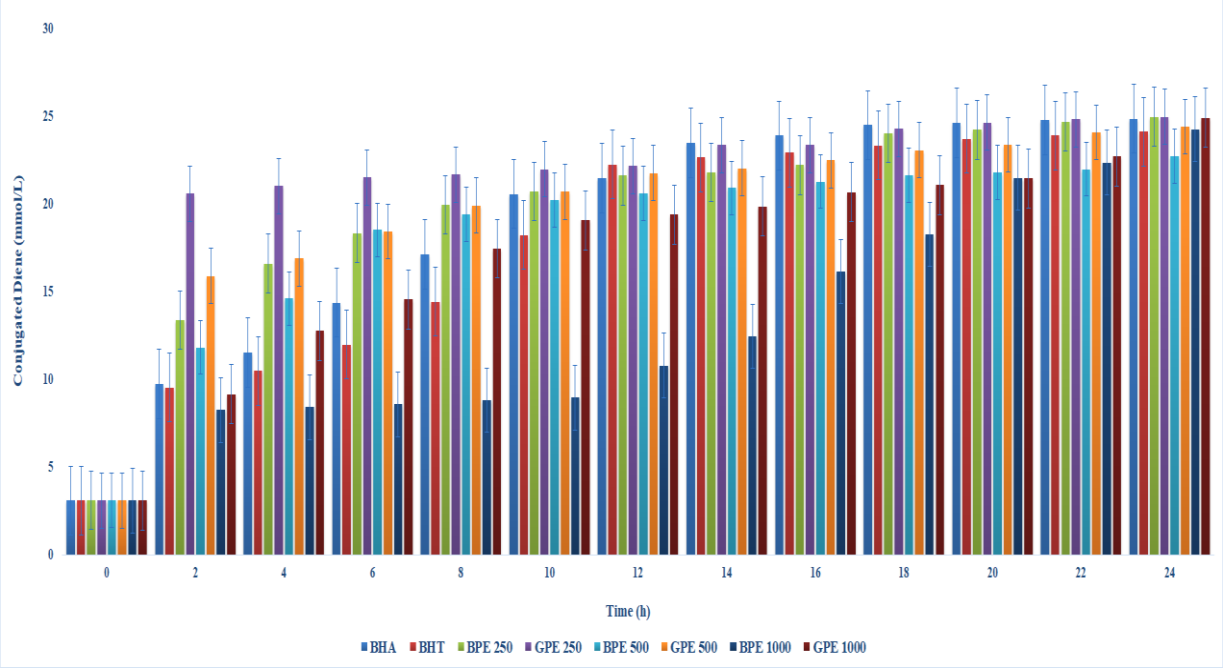

Figure 6. Effect of adding antioxidants on changes in conjugated diene (c) values of sunflower oil during the frying process.

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4- CONCLUSIONS

This study showed that the citrus peel extracts were acceptable sources of flavonoids due to DPPH and ABTS scavenging activity, reducing power, and β-carotene assays. The results corroborated that the protection presented by BPE at 1000 ppm was more pronounced than that of the broadly used BHA and BHT in terms of TBA, CDV, FFA, and PV. Bitter orange peel extract (BPE) displayed a higher content of phenolic compounds and antioxidant activity The antioxidant properties of citrus peel flavonoids can make them a useful substitute for synthetic antioxidants in SO.

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ETHICS STATEMENTS

This research did not include any human subjects or animal experiments.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

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پایداری اکسیداتیو روغن آفتابگردان غنی شده با فلاونوئیدهای مرکبات استخراج شده با استفاده از نانولوله های کربنی چند جداره در طی سرخ کردن

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نانولوله های کربنی چند جداره کربوکسیله،

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خواص آنتی اکسیدانی،

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چکیده

هدف از این مطالعه مقایسه کارایی ترکیبات فنلی پوست مرکبات استخراج شده با استفاده از نانولوله های کربنی چند جداره کربوکسیله (MWCNT-COOH) با آنتی اکسیدان های مصنوعی رایج [بوتیل هیدروکسی آنیزول (BHA) و بوتیل هیدروکسی تولوئن (BHT)] در تثبیت روغن آفتابگردان (SO) بود. در طول سرخ کردن (۵±۱۸۰ درجه سانتیگراد، ۲۴ ساعت). برای ارزیابی فعالیت آنتی اکسیدانی این ترکیبات، محتویات کل فنول و فلاونوئید، قدرت کاهش، فعالیت مهار ABTS، توانایی تثبت بتاکاروتن، و فعالیت مهار رادیکال ۱، ۱- دی فنیل-۲-پیکریل هیدرازیل (DPPH) اندازه گیری شد. عصاره ها در غلظت های مختلف (۱۰۰-۱۰۰۰ پی پی ام) با SO ترکیب شدند و هر دو آنتی اکسیدان مصنوعی در ۲۰۰ پی پی ام استاندارد شدند. پایداری حرارتی-اکسیداتیو با ارزیابی تغییر در مقدار پراکسید (PV)، رنگ، محتوای اسید چرب آزاد (FFA)، مقدار داین کونژوگه (CDV)، و اسید تیوباربیتوریک (TBA) مورد بررسی قرار گرفت. عصاره پوست پرتقال تلخ (BPE) محتوای بیشتری از ترکیبات فنلی و فعالیت آنتی اکسیدانی را نشان داد. در ۱۰۰۰ ppm، محتوای FFA، PV، TBA و CDV کمتری را نشان داد. می توان از آن به عنوان جایگزینی برای آنتی اکسیدان های مصنوعی استفاده کرد. در نتیجه، ترکیبات فنلی پوست مرکبات استخراج شده با MWCNT-COOH می توانند به عنوان نگهدارنده در روغن های سرخ کردنی استفاده شوند.

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