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Adding Tunisian grape seed extract to corn oil during heating: Impact on stability and fatty acid composition

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SUMMARY: A study was conducted to examine the impact of grape seed extract (GSE) on the oxidative stability of corn oil when heated. After oxidation, the peroxide (PV), p-anisidine (P-a), acidity (AV), conjugated trienes (CT), and total oxidation (TOTOX) values were determined. Every oil sample was assessed both before and after heating for its polyphenol content (TP) and fatty acid composition. GSE demonstrated a significant reduction in lipid oxidation. The samples supplemented with 1000 ppm GSE followed an 8-hour heating period presented the greatest significant decreases in the investigated indices when compared to the control and BHT values. As a result, it can be said that GSE's antioxidant activity delayed oxidation and prevented the deterioration of oil lipids.

KEYWORDS: *Assurance tests; Corn oil; Fatty acids composition; Grape seed extract; Heating and quality; Polyphenol content.*

RESUMEN: ***Adición de extracto de semilla de uva tunecina al aceite de maíz durante el calentamiento: Impacto en la estabilidad y la composición de ácidos grasos***. Se realizó un estudio para examinar el impacto del extracto de semilla de uva (GSE) en la estabilidad oxidativa del aceite de maíz al calentarse. Tras la oxidación, se determinaron los valores de peróxido (PV), p-anisidina (P-a), acidez (AV), trienos conjugados (CT) y oxidación total (TOTOX). Cada muestra de aceite se evaluó antes y después del calentamiento para determinar su contenido de polifenoles (TP) y composición de ácidos grasos. El GSE demostró una reducción significativa de la oxidación lipídica. Las muestras suplementadas con 1000 ppm de GSE se sometieron a un período de calentamiento de 8 a 15 horas, lo que produjo disminuciones significativas en los índices investigados en comparación con los valores de control y BHT. Como resultado, se puede afirmar que la actividad antioxidante del GSE retrasó la oxidación y previno el deterioro lipídico del aceite.

PALABRAS CLAVE: *Aceite de maíz; Composición de ácidos grasos; Contenido de polifenoles; Control de calidad; Extracto de semilla de uva; Pruebas de calentamiento.*

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1. INTRODUCTION

Grape (*Vitis vinifera*) seeds are a valuable processing by-product and are used in a variety of food products. Ten to twenty percent of grape seeds are made of oil, of which the majority is unsaturated fatty acid (Apaydin *et al.,* 2017). Because of its abundance in polyphenols, and phytosterols which have been shown to exhibit antioxidant properties, grape seeds have considerable potential for curative and preventive effects against a number of diseases i.e. hypertension, inflammation, cardiovascular disease, cancer, diabetes, microbial infections, peptic ulcer, etc. (Apaydin *et al.,* 2017; Gupta *et al.,* 2020; Gómez-Mejía *et al.,* 2021; Bonassi and Lavelli, 2024). They have shown a wide range of pharmacological characteristics, including the ability to prevent thrombosis, inhibit cardiovascular diseases, protect against the oxidation of low-density lipoproteins (LDL), dilate blood vessels and lower cholesterol (Apaydin *et al.,* 2017; Gupta *et al.,* 2020; Gómez-Mejía *et al.,* 2021). Because of their appealing flavors and textures, fried foods are highly popular among foodies. Frying oils undergo extremely complicated reactions when used at high temperatures (Wu *et al.,* 2019). A number of degraded compounds including diacylglycerols, trans fatty acids, and polar molecules (Wu *et al.,* 2019; Erickson *et al.,* 2023), compromise the shelf-life of frying oils. Hence, oxidized lipids give out offensive odors, lower the fried food's nutritional value and have negative health effects such as inflammation and cardiovascular diseases (Erickson *et al.,* 2023). Nevertheless, by giving free radicals the hydrogen they need to initiate and spread, primary antioxidants stop the oxidation of fats and oils (Barrera-Arellano *et al.,* 2019; Erickson *et al.,* 2023).

Regarding his high concentration of polyunsaturated fatty acids (PUFA), primarily linoleic acid (18:2), corn oil is regarded as nutritious (Barrera-Arellano *et al.,* 2019). On the other hand, it is even more sensitive to oxidative degradation, which can result in discoloration, rancidity, and off flavors. Therefore, in industrial processes, edible oils are treated with a number of synthetic antioxidants to delay all these degradation reactions (Jiménez *et al.,* 2017; Wu *et al.,* 2019; Xu *et al.,* 2021). However, many researchers have reported that these synthetic substances have carcinogenic side effects in addition to their ability to increase the oxidative stability of oils (Jiménez *et al.,* 2017; Wu *et al.,* 2019; Xu *et al.,* 2021). Consequently, there's a movement to substitute these artificial antioxidants with naturally occurring antioxidants derived from plants and spices (Zhang *et al.,* 2018; Wu *et al.,* 2019; Drinić *et al.,* 2020). Owing to their strong biological activity, a number of co-products have been investigated as sources of antioxidants (Drinić *et al.,* 2020; Mnari Bhouri *et al.,* 2022).

Sunflower oil is frequently used as a model to test various plant extracts' ability to prevent peroxidation (Kehili *et al.,* 2018; Mezza *et al.,* 2018; Cherif and Slama, 2022; Lužaić *et al.,* 2022). However, the effectiveness of grape seeds in preventing the lipid peroxidation of fried corn oil has never been studied. Thus, the goal of this study is to investigate how the natural antioxidants found in grape seed extract (*Vitis vinefera.)* affect the quality of corn oil upon heating.

2. MATERIALS AND METHODS

2.1. Chemicals and reagents

All chemical, standards and reagents used were purchased from Sigma-Aldrich Co. Ltd (St. Louis, MO. USA).

2.2. Plant sample preparation

Grapes were harvested from the Nabeul region in Tunisia. The fruits were washed and hand-peeled. The grape seeds were sundried and then ground into a powder. The resulting powder was vacuum-sealed, freeze-dried, and kept out of the light. Using an orbital shaker, 0.5 g of sample were extracted with 10 ml of methanol for 48 hours in the dark. Three separate extractions were carried out. The extracts were then concentrated using a rotary evaporator operating under vacuum at 50 °C after being filtered through

Whatman No. 4 filter paper. After that, the crude extracts were vacuum-packed, freeze-dried, and kept cold until an additional analysis could be done.

2.3. Phytochemical screening

Total phenols, ortho-diphenols, flavonoids and total condensed tannins were assessed in the methanolic grape seed extracts (GSE). The total phenolic and O-diphenol contents of the methanolic fractions were calculated using the method of Montedoro *et al.* (1992), and the results were expressed as mg gallic acid equivalents (GAE)/g of sample on a dry weight (DW) basis. The GSE total flavonoid contents were assessed using a colorimetric assay created by Zhishen *et al.* (1999). The results were expressed on a dry weight (DW) basis as mg catechin equivalents (CEQ)/g of sample.

The method of Julkunen-Tiitto (1985) was used to measure total condensed tannin content.

Tannic acid concentration was given as milligrams (mg TA/g DW) of dry weight. Using the protocol outlined by Braca *et al.* (2001), the DPPH test was used to assess the antioxidant activity of GSE.

2.4. Application of GSE to corn oil

Five samples of refined corn oil were used. Three samples had 200, 500, and 1000 ppm of GSE added to them (C200; C500 and C1000). For comparison, the fourth one was prepared with a synthetic antioxidant, BHT at the allowed legal limit of 0.02% (CBHT). The final sample acted as a control and contained no additives (CO). GSE and BHT were combined individually prior to supplementation, with a small quantity of methanol added to guarantee oil dispersion in an ultrasonic water bath. In order to achieve a diffusion of compounds from GSE to oil, they were then added to corn oil in brown glass bottles and mixed for 30 minutes at 50 °C (Mnari Bhouri *et al.,* 2022). Every experiment was run three times.

2.5. Heating processes

Using an incubator kept at 180 °C, samples containing GSE, BHT, and control samples were heated to simulate frying conditions and cause oxidation. The samples underwent 2, 4, 6, and 8 hours of independent heating. A calibrated chromel-alumel thermocouple (HI 935009, Hanna Instruments, France) was inserted into the oil samples to control their temperature. Following each heating cycle, the samples were removed from the incubator and quickly cooled before being kept at -20 °C until analysis.

2.6. Oxidative stability evaluation

Standard chemical indices, such as conjugated trienes (CT), p-anisidine value (P-a), acidityvalue (AV), and peroxide value (PV), were measured to assess the degree of lipid oxidation. Furthermore, the oxidative degradation of lipids was estimated using the total oxidation value (TOTOX). The titration of an oil solution dissolved in ethanol/ether (1:1, vol/vol) with an ethanolic potassium hydroxide solution (0.1M) was used to measure the free acidity. The outcome was given as an oleic acid percentage (AOCS, 1994). The American Oil Chemists' Society (AOCS) (1994) standard titration method was used to calculate the peroxide value (PV). This technique finds all of the fatty acid oxidation's constituent parts, peroxides, and other related products. The findings were reported as milliequivalents (mEqO2/kg) of peroxide per kilogram of oil used in the test to oxidize potassium iodide. Anisidine value (P-a), which gauges the amount of carbonyl present in oils or fats, was calculated using official AOCS procedures (AOCS, 1994). P-anisidine reacts with the aldehydic carbonyl bond in oils in the presence of acetic acid, producing reaction products which are yellow in color. At 350 nm, the colored solution's absorbance was measured (AOCS, 1994). Using the accepted procedure of AOCS, the conjugated triene hydroperoxides (CT) formed were ascertained. K 270 is the specific extinction value that represents the results. To calculate the oxidative degradation of lipids, the total oxidation value (TOTOX) was employed. The TOTOX value, which was determined using the following formula (Mnari Bhouri *et al.,* 2022), is the total of the PV and P-a values to total oxidation.

Value of TOTOX = 2*PV + P-a

2.7. Fatty acids composition

For the analysis of fatty acids, esterification was carried out using methanolic potassium hydroxide (KOH) and hexane (Mekni *et al.,* 2014). Then Fatty Acid Methyl Esters (FAMEs) obtained were injected onto a Hewlett-Packard gas chromatograph (Hewlett-Packard, Palo Alto, CA) for total fatty acid analysis (Mekni *et al.,* 2014). The gas chromatograph (Hewlett-Packard, Palo Alto, CA) was equipped with a split-splitless injector and a flame ionization detector, set at 270 ºC, in accordance with the European Union Commission's modified Regulation EEC 2568/91. A fused silica Agilent DB23 capillary column (60 m length, 0.32 mm inner diameter, and 0.25 µm film thicknesses) was used for elution, and the carrier gas was nitrogen (1 ml/min). The initial column temperature was 130 ºC. Step 1 was 6.5 ºC/min to 170 ºC. Step 2 was 2.8 ºC/min to 215 ºC and maintained for 12 min. Step 3 was 40 ºC/min to 230 ºC and maintained for 20 min. The conditions were as follows: injector temperature, 270 ºC; flame ionization detector, 280 ºC; injector split ratio, 1:50. Relative and absolute retention times were compared to those of authentic cis-fatty acid standards, which were analyzed under the same conditions in order to identify FAMEs.

2.8. Statistical analysis

Every assay was conducted three times. The standard deviation and mean values from the three analyses are presented as the results. The SPSS software, version 11.0 for Windows, was used to statistically analyze the data (SPSS, Chicago, IL, USA). The differences between individual means were examined using the one-way analysis of variance (ANOVA) and the Duncan multiple range test, which were found to be significant at $p < 0.05$. XLSTAT (2014) for Windows was used to perform principal component analysis (PCA) (Addinsoft, New York, USA).

3. RESULTS AND DISCUSSION

3.1. Phytochemical composition of GSE

Total polyphenol (TP), total flavonoid (TF), total ortho-diphenol (TOP) and total tannin (TT) contents of GSE were used to estimate the phenolic composition (Table 1). The results indicate that phenolic compounds are abundant in GSE. The TP reached a value of 173.8 mg GAE/g DW. Farhadi *et al.* (2016) have reported that the total phenolic compounds in grapes were 5 times more concentrated in the seeds than in the skin. These polyphenols in grape seeds are mainly flavonoids, including gallic acid, flavan-3-ols monomer catechin, epicatechin, allocatequin, epigallocatechin and 3-O-gallate Epicatechine, but also dimers and trimers of more highly polymerized procyanidins (Farhadi *et al.,* 2016; Gupta *et al.,* 2020; Gómez-Mejía *et al.,* 2021). The TOP of GSE was 21.3 mg HTE/g D. The TF of GSE was 25.5 mg QCE/g DW. The GSE contained 46 mg tannic acid/g DW of condensed tannins. For grapes, the accumulation of condensed tannins was observed at the seed level followed by the stems, skin and pulp (Farhadi *et al.,* 2016; Gupta *et al.,* 2020; Gómez-Mejía *et al.,* 2021). In general, grape seed extracts are very rich in tannins and flavonoids such as catechin andepicatechin and also have good antioxidant activities. The flavonoids found in grape seed extract have an anti-radical activity which is 50 times greater than that of vitamin E and 20 times that of vitamin C (Gupta *et al.,* 2020).

TABLE 1. Phytochemical composition of grape seed extract.

	TP (mg GAE/g DW)	TOP (mg HTE/g DW)	TT (mgTAE/g DW)	TF (mg QCE /g DW)
GSE	113.8±1.33	21.3±0.74	46±0.77	25.5±0.1

TP: Total polyphenol content; TOP: Total orthodiphenol content; TT: Total Tannin Content; TF: Total flavonoid content. Values are expressed as means ± standard deviation (n = 3).

3.2. Acidity value (AV)

One of the most frequent processes that degrades frying oil and raises the amount of free fatty acids in it is hydrolysis (Baştürk, 2019; Mnari Bhouri *et al.,* 2022). Figure 1 illustrates how, for every oil sample, the heating time increased AV in a very noticeable way. Fresh corn oil has an FFA value of 0.22%. Since this acidity is less than that of some edible oils, like olive oil, CO is safe for consumption. It was found that during the two first hours of heating, in the CO containing 200–1000 ppm GSE and 200 ppm BHT, the FFA of the oil samples increased only very slightly.

The FFA of the oil samples increased gradually and steadily over the duration of the last eight hours of heating at 180 °C. Additionally, the differences in the FFA trend between CO with additives (BHT and GSE) and CO without additives were found to be statistically different for each heating period ($p < 0.05$). -The control oil showed the most significant shift in the amount of free fatty acids. In fact, the control showed the highest FFA after 8 hours of heating, while C1000 showed the lowest (Figure 3). The FFA content in C200 is equivalent to BHT.

Based on the current findings, it can be said that GSE at 200 ppm has the same preventive effect as BHT over a longer heating period, but GSE at 1000 ppm is more effective. As a result, an increase in the FFA content during heating would suggest deteriorated oil. This finding is in good agreement with earlier studies on corn oil conducted by different researchers (Baştürk, 2019; Saeed and Naz, 2019). In fact, FFAs are produced by the hydrolysis of fats, particularly the diglycerides, monoglycerides, and polyunsaturated fatty acids that are boosted by heat treatments (Iqbal and Bhanger, 2007; Maskan and Horuz, 2017).

GSE and BHT supplementation significantly decreased the rise in FFA and prevented rancidity from developing. When it comes to preventing frying oil at high processing temperatures from degredation, natural extracts like GSE are a good source of antioxidants. GSE has been shown to enhance oxidative stability of sunflower oil during convective and microwave heating (Baştürk, 2019).

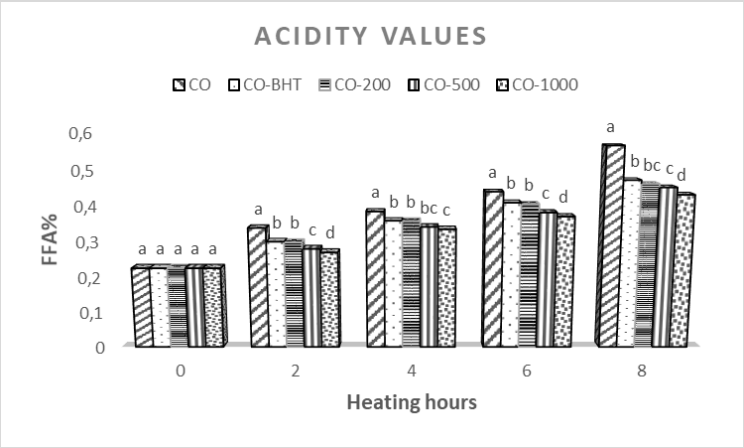

FIGURE 1. Change in acidity value of oils during heating. Results are expressed as means ± standard deviation (n = 3). Bars (means value ± SD) with different letters after each heating period are significantly different ($p < 0.05$).

3.3. Peroxide value and oxidation inhibition (IO)

The peroxide value was used to indicate oils primary oxidation in the presence of multiple antioxidants, which will eventually result in the formation of colorless and odorless hydroperoxides (Maskan and Horuz, 2017). The peroxide values for CO with additives and CO without additives under heating were measured and are displayed in Figure 2 (A).

For CO without additives, a steady and enormous increase in PV was noted as the heating time increased. Following an 8-hour heating period, the control oil's PV increased by three times, from 3.6 to 10. The observed findings are similar to those mentioned by Baştürk (2019) and Saeed and Naz (2019) for corn oil heated at 200 °C.

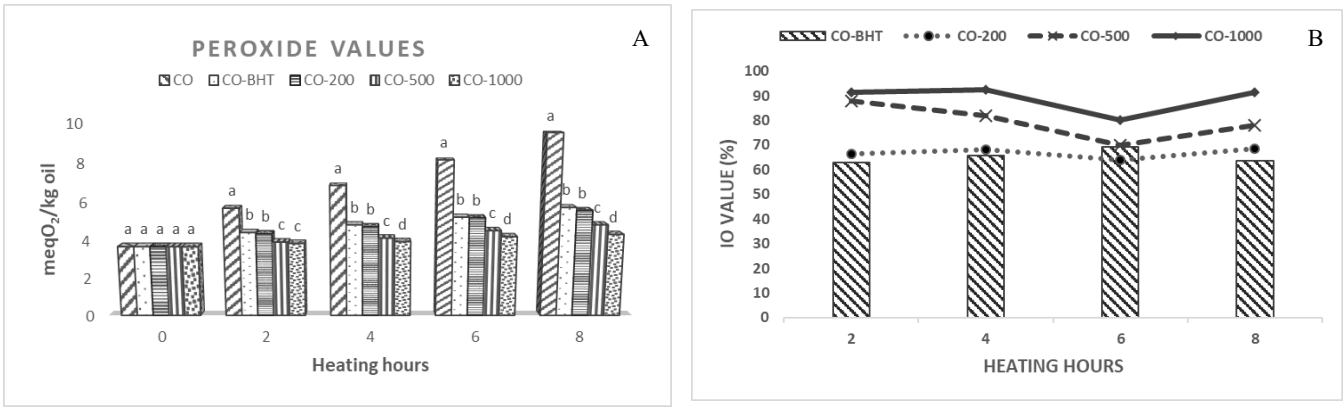

FIGURE 2. Change in peroxide values (PV) (A) and GSE and BHT's inhibitory effects on primary lipid oxidation (B) during oil heating. Results are expressed as means ± standard deviation (n = 3). Bars (means value ± SD) with different letters after each heating period are significantly different ($p < 0.05$).

The PVs measured in oil samples containing BHT and GSE were significantly lower than that of CO at any heating time. Furthermore, although the increase was extremely gradual, the PVs for these samples increased. After six hours of heating, the PVs for GSE at 500 and 1000 ppm were significantly lower (respectively 4 and 4.4) than that of BHT (5) ($p < 0.05$). Initially, GSE and BHT were comparable. After eight hours, GSE at 1000 ppm, GSE at 500 ppm, and BHT showed 60, 55, and 43% efficacy in comparison to the control, respectively. This implies that GSE outperformed BHT in terms of stability and effectiveness. The PVs in corn oil stabilized by GSE are comparable to those in corn oil supplemented with thyme and far lower than those in sunflower oil supplemented by grape seed extract, and palm oil supplemented by Za'atar (Maskan and Horuz, 2017; Baştürk, 2019).

The information about GSE and BHT's ability to inhibit primary lipid oxidation during heating is shown in Figure 2 (B). The statistical test resulted in the conclusion that increasing the GSE level during heating treatments led to a significant increase in IO ($p < 0.05$). The results also showed that GSE at a concentration of 200 ppm had the same inhibition power compared to BHT. However, GSE at 1000 ppm demonstrated an inhibition power greater than BHT. During heating at 180 °C, GSE did not exhibit a pro-oxidative effect for 8 hours. When additives were added and heating was prolonged, the amount of oxidized products increased, demonstrating the pro-oxidative effect (Drinić *et al.,* 2020).

3.4. P-anisidine value

The amount of secondary oxidation products: alcohols, carboxylic acids, aldehydes, and ketones (produced in the presence of primary oxidation products, such as hydrogen peroxides) can be accurately measured using the P anisidine value (P-a) (Baştürk, 2019). Figure 3 (A) displays the data for P-a with heating time as well as the impact of GSE and BHT antioxidants. It is evident that all of the oils' P-a substantially increased with heating time ($p < 0.05$). The P-a of the control oil increased from 20 to 98 following heating. Furthermore, statistical analyses showed that the P-a of CO is significantly higher than the P-a of the supplemented oils at all heating times ($p < 0.05$). This sharp rise in P-a suggests that heating corn oil at 180 °C degrades its lipids. The same result was noted for corn oil following a microwave heating period (Wu *et al.,* 2019) or oven heating (Baştürk, 2019; Mnari Bhouri *et al.,* 2022), as well as for other edible oils like soybean oil (Saoudi *et al.,* 2016), palm oil (Maskan and Horuz, 2017), and sunflower oil (Kehili *et al.,* 2018; Baştürk, 2019). However, the addition of BHT and different GSE levels led to a significant decrease in P-a ($p < 0.05$). As compared to CO, P-a in CBHT, C200, C500, and C1000 actually increased slightly during heating, with C1000 recording the lowest level ever. GSE inhibited the rise in P-a in a dose-dependent manner, but C1000 showed the greatest effect statistically after heating for 8 hours. In comparison to 31; 25 and 23% for C500; C200 and CBHT, respectively, C1000 reduced the rise in P-a by 35%.

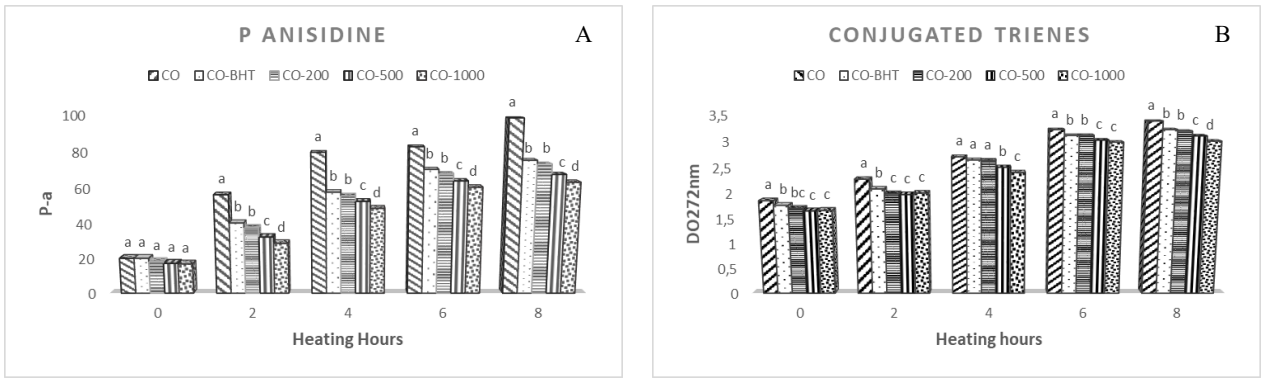

FIGURE 3. Change in p-Anisidine value (P-a) (A) and conjugated Trienes value (CT) (B) of oils during heating. Results are expressed as means ± standard deviation (n = 3). Bars (means value ± SD) with different letters after each heating period are significantly different ($p < 0.05$).

GSE appeared to be more stable than BHT at 500 and 1000 ppm. These results are consistent with those published by (Mnari Bhouri *et al.,* 2022), who discovered that when corn oil was heated in the oven for 8 hours, pomegranate peel extract exhibited greater stability against the rise in P-a. According to (Di Lorenzo *et al.,* 2016), other natural extracts like curcumin and rosemary extracts prevented secondary oxidation in oil by more than 30% when compared to BHT. The abundance of polyphenolic compounds in GSE was responsible for its shielding effect against secondary oil oxidation (Piona, 2012; Di Lorenzo *et al.,* 2016; Baştürk, 2019).

3.5. Conjugated trienes contents

A precise estimate of the oxidative degradation of oils is the evaluation of conjugated dienes (CD) and (trienes) CT. The more polyunsaturated fatty acids an oil contains, the more CD and CT that are produced during frying. The oxidative stability of the oil decreases as CD and CT levels increase (Kehili *et al.,* 2018; Saeed and Naz, 2019). The relative rise in conjugated trienes (CT) in corn oil heated to 180 °C is displayed in Figure 3 (B), along with the heating time. The initial CT value for CO is 1.85. This level is comparatively higher than those of other oils, such as olive oil (0.2) and soybean oil (0.73) (Kehili *et al.,* 2018; Saeed and Naz, 2019). With an elevated level for control, the CT contents increased in tandem with the heating time. GSE had a dose-dependent inhibitory effect on the formation of CT. At every heating stage, the oil samples that received the highest dose of GSE supplementation exhibited the lowest levels of CT. After heating for up to eight hours, C1000 displayed the lowest amount of CT, followed by C500 ppm; C200 ppm and CBHT. The differences observed were statistically significant ($p < 0.05$). These findings showed that at 1000 ppm, GSE had greater antioxidant activity than BHT at allowed dosages. These findings demonstrate the effectiveness of naturally occurring antioxidants derived from grape seeds in reducing lipid degradation and enhancing the oil's oxidative stability, even in the presence of elevated temperatures. GSE at 200 ppm revealed the same efficacy as BHT in preventing the formation of CT. These results are consistent with those of (Poiana, 2012), who found that, compared to the control, CT accumulation in sunflower oil after convective heating decreased by 41% when GSE was added at a 1000 ppm level. Mnari Bhouri *et al.* (2022) have also reported that corn oil supplemented with 1000 ppm pomegranate peel extract significantly resisted an increase in CT.

3.6. TOTOX value

TOTOX is a helpful tool for measuring oil degradation caused by oxygen. The TOTOX value for the oil samples heated at 180 °C increased considerably with increasing heating time, according to the results in Table 2. An extensive picture of the oxidation process in oils can be obtained by integrating PV and P-a. The TOTOX value was correlated with the degree of oil deterioration and was used as a marker of overall

oxidative stability (Mnari Bhouri *et al.,* 2022). The oils enriched with GSE and BHT were more stable to oxidative rancidity than the control oil, as was seen in the cases of P-a, PV, and AV. In fact, the oil mixed with 1000 ppm of GSE had the lowest TOTOX value after each heating period, while the oil under control had the highest value. Following eight hours of heating, the control oil's TOTOX value increased by four times, reaching a 77 % increase at the end, as opposed to 68% for CBHT and C500, 70% for C200, and 66% for C1000.

Consequently, the TOTOX value increase was inhibited by the addition of GSE to oil by 11% when compared to the control. C1000 was found to have the strongest inhibitory effect. BHT and C500 both prevented lipid oxidation in a comparable way. Several studies have reported similar outcomes for oils supplemented with grape seed extract, and mentioned that the grape seed's essential antioxidants surround the lipid system's interface, preventing lipid oxidation that develops during heating (Poiana, 2012; Lachman *et al.,* 2014).

TABLE 2. Effect of GSE and BHT on TOTOX value during corn oil heating at 180 °C

h	CO	CBHT	C200	C500	C1000
0	27.51±0.8a	27.51±0.19a	25.05±0.25b	24.63±0.3b	24.24±0.11b
2	67.51±1.3a	49.13±0.22b	46.29±0.31c	40.15±0.23d	36.46±0.14e
4	93.3±0.35a	67.23±0.36b	65.03±0.52c	60.7±1.3d	56.43±0.41e
6	98.95±0.6a	80.66±0.51b	78.29±0.29c	72.88±0.25d	68.76±0.35e
8	117.25±1.6a	86.63±0.4b	84.32±0.91c	77.05±1.5d	71.76±0.43e

Results are expressed as means ± standard deviation (n = 3). For each heating time, values in the same line with different letters are significantly different at ($p< 0.05$) according to the one-way analysis of variance (ANOVA) followed by Duncan's multiple range test. GSE: grape seed extract; TOTOX: total oxidation value; CO: Corn oil; CBHT: oil with BHT; C200: oil with 200 ppm GSE; C500: oil with 500 ppm GSE; C1000: oil with 1000 ppm GSE.

3.7. Phenolic composition

As the primary indicator for assessing the quality of vegetables, phenol content plays a direct role in preventing oxidation and preserving oil (Zhang *et al.,* 2018; Mnari Bhouri *et al.,* 2022). The evolution of the total phenol content (TPC) in the various oils during thermal oxidation is shown in Figure 4. The starting phenol contents in the oils enriched with different GSE levels were substantially greater than those in the CBHT and CO, which both had the same content (~28 mg GAE/kg oil). Actually, TPC from C1000 ppm of GSE (~63 mg GAE /kg oil) was 3 times higher than TPC from CBHT and CO prior to heating. The observed significant variation could potentially be explained by the introduction of additional phenols into the oil through the diffusion of extract phenols (Mnari Bhouri *et al.,* 2022). The TPC in all oils considerably decreased as a consequence of heating time upon exposure to 180 °C. Statistically significant differences were found among the various oils which can be classified as follows based on their phenolic content, following each heat treatment: C1000 > C500 > C200 > CBHT > CO ($p<0.05$). The phenol contents in CO and CBHT were lower ($p > 0.05$). After eight hours of thermal oxidation at 180 °C. The corn oil used in this study without any supplements appeared to be resistant to phenolic degradation.

The variation in the amount of remaining phenols does not indicate a resistance to the degradation of phenols. In fact, at the end of treatment, it was confirmed that the concentrations of control, CBHT, C200, C500, and C1000 had decreased by 45, 41, 37, 35, and 36%, respectively, in comparison to the initial

concentration. Therefore, C500 and C1000 appeared to be the most stable systems to TPC degradation. The results showed that oils enriched with high levels of GSE were more stable and resistant to phenolic degradation; additionally, GSE was able to reduce oxidation at concentrations of at least 200 mg/kg of polyphenols. Consequently, the formation of their oxidation products and the thermal degradation of phenolic compounds are directly correlated with the amount of GSE added to the oil.

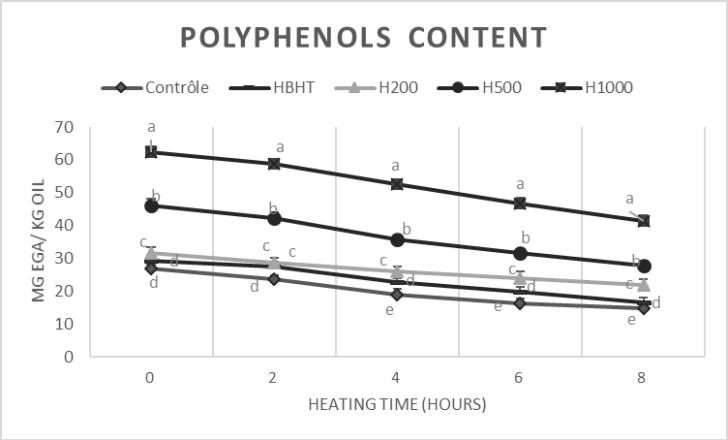

FIGURE 4. Heating impact on phenolic content in corn oil enriched with GSE and BHT. Results are expressed as means ± standard deviation (n = 3). Data with different letters for the same heating time are significantly different ($p < 0.05$).

3.8. Fatty acid composition

Analyzing the composition of fatty acids (FA) is a useful method for assessing the oxidative changes that occur in oils when they are heated (Dhibi *et al.,* 2014). Table 3 shows the difference in the amount of fatty acids in each oil before and after heating. The amount of polyunsaturated fatty acids (PUFA), monounsaturated fatty acids (MUFA), and saturated fatty acids (SFA) was determined based on the composition of FA. The results indicated that PUFA was significantly more affected during frying operations than MUFA and SFA (Table 3).

TABLE 3. Alterations in the fatty acid composition of the different samples heated for eight hours at 180 °C.

	CO		CBHT		C200		C500		C1000	
	0h	8h	0h	8h	0h	8h	0h	8h	0h	8h
C16:0	11,31±0,14b	15,82±0,06a	10,87±0,19b	12,60±0,21a	11,08±0,12b	11,46±0,05a	11,35±0,12b	11,64±0,02a	11,55±0,08b	11,79±0,04a
C18:1	35,48±0,18b	39,33±0,12a	34,49±0,03b	36,61±0,11a	34,50±0,05b	36,25±0,14a	35,55±0,11b	36,25±0,08a	35,62±0,03b	36,09±0,07a
C18:2	46,34±0,21a	42,81±0,16b	46,09±0,04a	44,28±0,01b	46,97±0,11a	45,82±0,08b	46,73±0,06a	45,97±0,12b	46,53±0,04a	45,92±0,05b
SFA	14,39±0,14b	19,51±0,11a	14,06±0,05b	15,77±0,12a	13,81±0,12b	14,36±0,07a	14,28±0,16a	14,54±0,11a	14,52±0,08a	14,66±0,15a
MUFA	35,97±0,09b	39,30±0,3a	36,09±0,2a	37,55±0,16b	35,37±0,17b	36,65±0,13a	36,01±0,11b	36,56±0,05a	36,06±0,13b	36,68±0,09a
PUFA	49,62±0,21a	43,18±0,13b	49,83±0,22a	46,77±0,04b	49,81±0,08a	47,38±0,15b	49,61±0,2a	47,98±0,11b	49,41±0,07a	47,75±0,12b

SFA: Saturated Fatty acids, MUFA: Mono-unsaturated Fatty acids, PUFA: poly-unsaturated Fatty acids. Values are expressed as means ± standard deviation (n = 3). ∗For each oil sample, means within the same row were significantly different at the level of $p < 0.05$ according to the one-way analysis of variance (ANOVA) followed by Duncan's multiple range test.

For CO heated for 8 hours, the percentage of SFA and MUFA significantly increased, although the proportion of PUFA decreased significantly ($p < 0.05$). The oil supplemented with GSE did not exhibit any alterations in its composition of fatty acids, indicating its resistance to oxidation. After 8 hours of heating, there was a significant difference ($p < 0.05$) in the SFA composition between the oils. After heating, the evolution of this composition is estimated to be 35.58, 12, 16, 3.98, 1.82 and 0.96%, respectively for CO, CBHT, C200, C500 and C1000.

The MUFA amounts were raised by 9.25, 4.04, 3.61, 1.71 and 1.52%, respectively for CO, CBHT, C200, C1000 and C500. Meanwhile, the PUFA amounts were lowered by 12.31, 4.31, 2.95, 1.35 and 1.28%,

respectively for CO, CBHT, C200, C1000 and C500. Unsaturated fatty chains are extremely susceptible to oxidation reactions and will eventually transform into trans or SFA. Thus, adding GSE to the CO before heating helps to effectively delay the PUFA's thermal oxidation and lower the rise in SFA. This can be attributed to the phenolic composition of GSE with high antioxidant activity, responsible for the inhibition of the oxidation of unsaturated fatty acids (Farhadi *et al.,* 2016; Gupta *et al.,* 2020). Edible oils containing natural extracts have been shown to exhibit a protective effect of phenolic compounds on the lipid profile when heated (Dhibi *et al.,* 2022).

3.9. Principal components analysis

In order to examine the oxidative stability of various oil samples (CO, CBHT, C200, C500, and C1000) both before and after 8 hours of heating, a multivariate statistical analysis of the data was carried out using PCA. The correlation between phenolic compounds (P), fatty acid composition (SFA, MUFA and PUFA) and oxidative parameters (AV, PV, CT, P-a, and TOTOX) was plotted in Figure 5. Of the total variance (89.78%), PC1 accounted for 66.47%, and PC2 for 23.31%. Every variable's placement in the loading plot reveals how it relates to the others. There is a strong correlation between variables that are near to one another. There was a positive correlation between variables on the same side of the origin (0.0) and a negative correlation between those on the opposite side of the origin. On the PCA plane, various corn oil samples could be distinguished from one another.

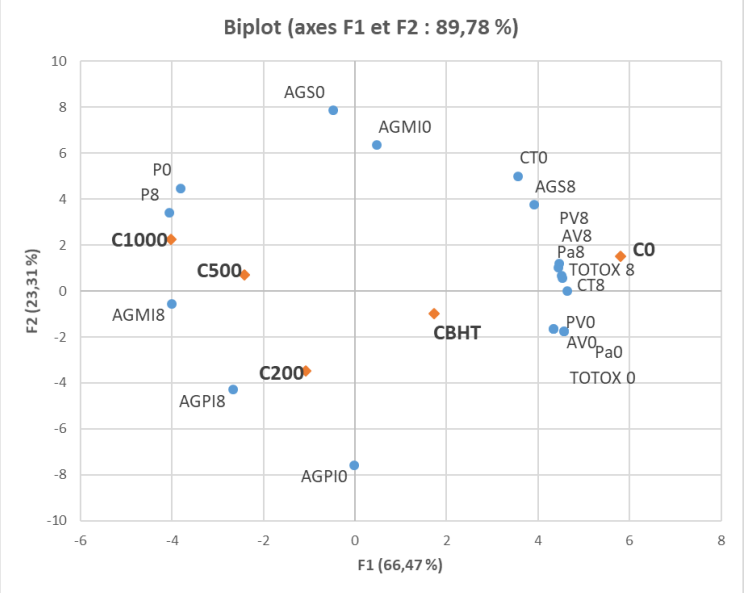

FIGURE 5. Principal component analysis (scores and loading plots, Biplot) applied to the dataset of oxidative parameters, polyphenol content and fatty acid composition of different corn oils samples before and after 8 hours heating. PV: peroxide value, Pa: p-anisidine value, AV: free fatty acid value, CT:conjugated trienes, TOTOX: total oxidation value, P: polyphenols content, AGS: saturated fatty acids AGMI: monounsaturated fatty acids AGPI:polyunsaturated fatty acids.

AV, PV, P-a, CT and TOTOX were positively correlated with PC1. PC2 exhibited a stronger correlation with polyphenol contents and fatty acid composition (PUFA, MUFA and SFA). On the positive side of PC1, CO and CBHT were found, while C200; C500 and C1000 were on the negative side. These data showed that the highest degree of oxidative deterioration, as indicated by PV, P-a, AV, CT and TOTOX, was found in CO and CO-BHT, where the lowest levels of PC were found. High levels were registered for GSE-supplemented oils (200, 500 and 1000 ppm) with the lowest TOTOX values. This could explain the negative correlation between TOTOX values and these phenolic compounds before and after 8 hours of heating, which is linked to protective action against thermo-oxidative degradation. Conversely, according to PC2 and PC1, substantial amounts of primary and secondary oxidation products (PV, CT, and P-a) were found for CO,

and these products had negative correlations with GSE-enriched oils (C200, C500, and C1000, respectively). The PCA results verified that heating had a detrimental effect on the quality of corn oil and that different GSE amounts (200, 500, and 1000 ppm) were more effective than BHT at preventing the production of primary and secondary oxidation products.

4. CONCLUSIONS

The examination of corn oil heated at 180 °C with the addition of different Tunisian GSE concentrations according to standard chemical indices seems to be a useful method for determining how effectively this natural extract prevents lipid oxidation. The oil samples showed significant changes in quality due to the production of hydroperoxides and secondary oxidation products. Corn oil's oxidative stability was greatly increased by adding GSE and BHT before heating. The effectiveness of GSE in improving the oxidative stability of corn oil in thermal applications was found to increase as the concentration of antioxidants in the studied range (200–1000 ppm) increased. Similar to BHT, oil supplementation with GSE up to a concentration of 200 ppm inhibited lipid oxidation; however, GSE is more effective than BHT at limiting the thermo-oxidative degradation of corn oil. The notion that total antioxidant capacity is inversely related to the degree of lipid oxidation in the heating time range is strengthened by the results, which also show that the polyphenol content in samples may be connected to oxidative deterioration. These findings demonstrate that Tunisian GSE is a highly effective lipid oxidation inhibitor in applications with oil to be heated to high temperatures, and the edible oil industry may find use for Tunisian GSE as a potential natural antioxidant.

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AUTHOR CONTRIBUTIONS

A. Mnari Bhouri: Conceptualization, methodology, software, validation, formal analysis, investigation, resources, data curation, writing—original draft preparation, writing—review and editing, visualization, project administration, funding acquisition.

Z. Amri: Validation, formal analysis, data curation, writing—original draft preparation, writing—review and editing, project administration.

M. Dhibi: Writing—original draft preparation, project administration.

N. Koubaa: Visualization project administration.

M. Hammami and S. Hammami: Supervision

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The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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