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Effect of tunisian pomegranate peel extract on the oxidative stability of corn oil under heating conditions

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SUMMARY: The effect of pomegranate peel extract (PPE) on the oxidative stability of corn oil during heating was studied. Oxidation was followed by determining peroxide value (PV), *p*-anisidine value (*p*-AV), free fatty acid value (FFA), conjugated dienes (CD), conjugated trienes hydroperoxides (CT) and the calculated total oxidation value (TOTOX). Polyphenol (TPC) and *ortho*-diphenol (TOPC) contents as well as the antioxidant activity of each oil sample were evaluated before and after heating. PPE showed a significant inhibitory effect on lipid oxidation. Heating samples for 8 hours supplemented by PPE to a level of 1000 ppm resulted in the highest significant decreases in investigated indices compared to the control and BHT values. It was concluded that the antioxidant activity of PPE delayed oxidation and can be used in the food industry to prevent and reduce lipid deterioration in oil.

KEYWORDS: *Corn oil; Heating; Lipid oxidation; Oxidative stability; Pomegranate peel extract; Quality indexes.*

RESUMEN: ***Efecto del extracto de cáscara de granada tunecina sobre la estabilidad oxidativa del aceite de maíz en condiciones de calentamiento***. Se estudió el efecto del extracto de cáscara de granada (ECG) sobre la estabilidad oxidativa del aceite de maíz durante condiciones de calentamiento. La oxidación se siguió mediante la determinación del índice de peróxido (IP), el índice de *p*-anisidina (*p*-AV), el índice de acidez (IA), los dienos conjugados (DC), los hidroperóxidos de trienos conjugados (TC) y el valor calculado de la oxidación total (TOTOX). Se evaluó el contenido de polifenoles totales (PT) y de *orto*-difenoles (*o*-DF), así como la actividad antioxidante de cada muestra de aceite, antes y después del calentamiento. El ECG mostró un efecto inhibidor significativo sobre la oxidación de lípidos. El calentamiento de las muestras, durante 8 horas suplementadas con ECG a un nivel de 1000 ppm, dio como resultado una significativa disminución de los índices investigados en relación con los valores de control y con BHT. Se concluyó que la actividad antioxidante de los ECG retrasó la oxidación y que se puede utilizar en la industria alimentaria para prevenir y reducir el deterioro de los lípidos del aceite.

PALABRAS CLAVE: *Aceite de maíz; Calentamiento; Estabilidad oxidativa; Extracto de piel de granada; Índices de calidad; Oxidación de lípidos.*

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1. INTRODUCTION

Fried foods are very popular because of their desirable flavors, colors and textures. It is well known that frying oils used as a cooking medium at high temperatures in the presence of oxygen and water from the food to be fried are subjected to very complex reactions of oxidation, polymerization and hydrolysis (Aladedunye *et al.,* 2017; Jiménez *et al.,* 2017). These reactions produce undesirable constituents, such as polar compounds (Seo Yeong Gim *et al.,* 2017), *trans* fatty acids (Bhardwaj *et al.,* 2016) and diacylglycerols (Aniołowska and Kita, 2016). These oxidized lipids are responsible for undesirable aromas and decrease the nutritional quality of the fried product (Bhardwaj *et al.,* 2016; Esposto *et al.,* 2015) and their excessive ingestion induces a detrimental effect on health. This is why research on lipid oxidation, especially on PUFA oxidation, has been the subject of numerous studies. The addition of antioxidants is a practical strategy to control the rate of lipid oxidation in foods during processing, storage, and exhibition (Seo Yeong Gim *et al.,* 2017). Synthetic antioxidants such as butyl-hydroxy-anisole (BHA), butyl-hydroxy-toluene (BHT) and tertiary-butyl-hydro-quinone (TBHQ) are widely used in the food industry as potential inhibitors of the oxidation of lipids (Aladedunye *et al.,* 2017; Maskan and Horuz, 2017). The addition of these antioxidants to improve the oxidative stability of edible oils is discouraged because of their toxicity and carcinogenicity. As a result, there is a trend towards the use of plant-based natural antioxidants to replace these synthetic antioxidants (Aladedunye *et al.,* 2017; Esposto *et al.,* 2015; Kmiecik *et al.,* 2015).

In recent years, attention has been focused on industrial waste. Vegetable co-products are interesting because they contain important molecules such as phenolic compounds that include simple phenols, flavonoids and anthocyanins. Many co-products have been studied as a source of antioxidants and their use is encouraged regarding their high biological activities. Pomegranate skin (*Punica granatum* L.) is a co-product with high nutritional value. It has numerous preventive and curative effects against several diseases due to its richness in polyphenols (Amri *et al.,* 2017c) which show various biological activities such as the elimination of free radicals, inhibition of oxidation and decreased risk of cardiovascular diseases and certain cancers (Amri *et al.,* 2017a). The effectiveness of fruit extracts and/or fruit peels in preventing the oxidation of vegetable oils during heat treatment has been reported (Iqbal *et al.,* 2008).

Among vegetable oils, sunflower oil has been used as a model to investigate the ability of various plant extracts in preventing its peroxidation (Iqbal *et al.,* 2008; Poiana, 2012). However, no study has ever been conducted on the efficacy of pomegranate peel in preventing the lipid peroxidation of corn oil under frying conditions. Hence this work aims to study the evolution of the quality of corn oil during heat treatment in the presence of natural antioxidants from pomegranate skin. Oxidative changes were monitored by the peroxide value (PV), *p*-anisidine value (*p*-AV), free fatty acid value (FFA), conjugated dienes (CD), conjugated trienes hydroperoxides (CT) as well as the calculated total oxidation value (TOTOX). Subsequently, polyphenol (TPC) and *ortho*-diphenol (TOPC) content as well as the analysis of oil's antioxidant power during heating were characterized.

2. MATERIALS AND METHODS

2.1. Chemicals and reagents

All chemical standards including phenolic acids and reagents were purchased from Sigma-Aldrich Co. Ltd (St. Louis, MO. USA). Refined corn oil was recovered from the Agrimed group (Sfax, Tunisia).

2.2. Plant sample preparation

Fruits were harvested from Gabsi pomegranate trees from the Mahdia region in Tunisia. Fruits were washed and hand-peeled. Fruit peel was sundried and ground using a laboratory mill. The resulting powder was freeze-dried and protected from light under vacuum pack. 0.5 g of sample was extracted with 10 mL of methanol in the dark for 24 hours using an orbital shaker. Extractions were made in triplicate. The extracts were then filtered through a Whatman No.4 paper filter and concentrated under vacuum at 50 °C with a rotary evaporator. The crude extracts obtained where then freeze-dried, vacuum packed and stored under refrigeration until further analyses.

2.3. Phytochemical screening

The methanolic extracts of peels were evaluated for the content of: TPC, TOPC, anthocyanins (TAC), flavonoids (TFC), and total condensed tannins (TTC). All absorbance measurements were carried out using a UV-1601 Shimadzu spectrophotometer.

The TPC and TOPC of the methanolic fractions were determined according to the method of Montedoro *et al*. (1992) and expressed on dry weight (DW) basis as mg gallic acid equivalents (GAE)/g and mg hydroxytyrosol (HT)/g of sample. For TPC, PPE and diluted Folin–Ciocalteu reagent were mixed. Na_2CO_3 was added after 1 min and the mixture was incubated for 1 h. The wavelength used was 765 nm. For TOPC, 1 mL of HCl (0.5 N), 1 mL of a mixture of $NaNO_2$ and $NaMoO_4 \cdot 2H_2O$ in 100 mL·H_2O, and 1 mL of NaOH (1 N) were added to PPE. The absorbance was measured at 500 nm after 30 min.

TFC, expressed on a dry weight (DW) basis as mg catechin equivalents (CEQ)/g of sample, were evaluated according to the colorimetric assay developed by Zhishen *et al.* (1999). Diluted PPE was mixed with distilled water. At time zero, 5 min and 6 min, 0.3 mL of $NaNO_2$ (5% w/v), 0.3 mL of $AlCl_3$ (10% w/v) and 2 mL NaOH (1 M) were added, respectively. To made up the volume to 10 mL, 2.4 mL of distilled water were added. The absorbance was measured at 510 nm.

TAC were measured according to the method of Padmavati *et al*. (1997) and modified by Chung *et al.* (2005). They were expressed as mg cyanidin 3-glucoside equivalents per g of dry weight (mg CyE/g DW). PPE was mixed with acidified methanol (1% HCl/methanol) at room temperature, in the dark, for 2 h. After centrifugation for 15 min, at 1000g, the anthocyanin concentration in the supernatant was measured at 530 and 657 nm, respectively.

TTC was evaluated according to the procedure reported by Julkunen-Tiitto (1985) and expressed as mg tannic acid/g of dry weight (mg TA/g DW). PPE was mixed with 1.5 mL of vanillin (4%). Concentrated HCl was then added. The mixed solution was incubated for 20 min in the dark at ambient temperature. The absorbance was measured at 500 nm.

To evaluate the antioxidant activity of PPE, the DPPH test was used according to the method described by Braca *et al*. (2001). Different dilutions of the phenolic extract were prepared for each variety and then added to 1 mL DPPH (0.1 mM, in methanol). The reaction mixture was kept at room temperature. The optical density (OD) of the solution was measured at 517 nm. The optical densities of the samples in the absence of DPPH were subtracted from the corresponding OD with DPPH. The percentage reduction values were determined and compared to appropriate standards. The inhibition of the free radical DPPH, in percent (I_{DPPH} %) was calculated using the following equation:

$$I_{DPPH}\% = ((A\ blank - A\ sample)/A\ blank) \times 100$$

Where A blank is the absorbance of the control reaction (containing all reagents except the tested compound), and A sample is the absorbance of the tested compound.

2.4. Application of PPE to corn oil

Refined corn oil, free of synthetic antioxidants, was divided into five portions. Three of them were supplemented with 200, 500 and 1000 ppm PPE. The fourth one was mixed with synthetic antioxidant BHT at the permitted legal limit of 0.02% and was prepared for comparative purposes. The last portion, without additive, served as control. Before supplementation, PPE and BHT were mixed separately with a minimum amount of methanol to ensure dispersion in oil in an ultrasonic water bath. Then they were added to corn oil in brown glass bottles, and mixed for 30 min at 50 °C to get a diffusion of compounds from the PPE to the corn oil (Sultana *et al.,* 2007). All experiments were performed twice.

2.5. Heating processes

Samples with PPE, BHT and control samples were induced to oxidation by heating under simulated frying conditions using an incubator maintained at 200 °C. Samples were separately heated for 2, 4, 6, and 8 hours with temperature control using a calibrated chromel-alumel thermocouple (HI 935009, Hanna Instruments). The samples were taken out of the incubator and cooled after each heating time and stored at -20 °C until analysis.

2.6. Oxidative stability evaluation

The progress of lipid oxidation was evaluated by measuring standard chemical indices: FFA, PV, *p*-AV, CD, CT and TOTOX.

The FFA was determined by the titration of a solution of oil dissolved in ethanol/ether (1:1, vol/vol) with an ethanolic solution of potassium hydroxide (0.1M). The result was expressed as % of oleic acid.

The PV was determined using the standard titration method by the American Oil Chemists' Society (AOCS) (AOCS, 1994). This method determines all components, peroxides or other similar products of fatty acid oxidation. The results were expressed in milliequivalents of peroxide per kg of oil that oxidizes potassium iodide under test conditions ($mEqO_2/kg$).

The *p*-AV measures the carbonyl content in the oils or fats and was determined according to the AOCS official methods. In the presence of acetic acid, *p*-anisidine reacts with the aldehydiccarbonyl bond in oils, and leads to the formation of yellowish reaction products. The absorbance of the colored solution was measured at 350 nm (AOCS, 1994).

The CD and CT formed were determined using the standard method of AOCS (1994). The results were expressed as the specific extinction values K232 and K270.

TOTOX was used to estimate the oxidative deterioration of lipids. The TOTOX value was defined as the sum of both values (PV and *p*-AV) to total oxidation and was calculated according to the formula (Poulli *et al*., 2009):

$$\text{TOTOX value} = 2*\text{PV} + p\text{-AV}$$

2.7. Statistical analysis

All assays were run in triplicate. The results are reported as mean values of three analyses and standard deviation. Data was subjected to statistical analysis using the SPSS program, release 11.0 for Windows (SPSS, Chicago, IL, USA). The one-way analysis of variance (ANOVA) followed by Duncan's multiple range test were employed to study the differences between individual means and seemed to be significant at $p < 0.05$. Principal component analysis (PCA) was carried out using XLSTAT (2014) for Windows (Addinsoft, New York, USA).

3. RESULTS AND DISCUSSION

3.1. Phytochemical composition of PPE

The total analysis of the compound group defined by the total polyphenol (TPC), total flavonoid (TFC), total *ortho*-diphenol (TOPC), total tannin (TTC) and total anthocyanin (TAC) contents of PPE, were estimated (Figure 1). The results show that PPE is very rich in phenolic compounds which are known for their antioxidant power. In fact, the TPC of PPE reached a value of 171.57 mg GAE/g DW, lower than those reported by Amri *et al*. (2017c) (382 mg GAE/g DW) and by Harzallah *et al.* (2016) (215.54 mg GAE/g DW). The TOPC of PPE was 73.5 mg HT/g DW, which is much higher than those found by Mekni *et al*. (2013) where TOPC levels did not exceed 3 mg HT/g DW. The TFC of PPE was 95.12 mg QCE/g DW, similar to those found by Amri *et al*. (2017c). It is reported that tannins were the main phenolic compounds present in pomegranate. The PPE contained 2.28 mg TA/g DW of TTC. Concerning TAC, PPE contained moderate amounts (29.11 mg CyE/g DW) compared to those found by Amri *et al.* (2017c).

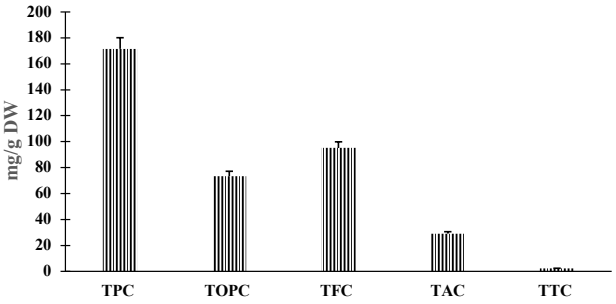

FIGURE 1. Phytochemical Composition of pomegranate peel extract. Results are expressed as means ± standard deviation (n = 3). TPC: total plolyphenols (mg GAE/g DW); TOPC: total ortho-diphenol (mg HTE/g D); TFC: total flavonoids (mg QCE/g DW); TTC: total tannins (mg TA/g DW); TAC: total anthocyanins (mg CyE/g DW).

3.2. Physicochemical properties of the frying/heating oils

3.2.1. Change in free fatty acid (FFA) contents

Hydrolysis is one of the most common reactions that causes frying oil degradation and therefore increased free fatty acid content. Therefore, free acidity is an important factor for oil quality (Amri, *et al.,* 2017b). The formation of free fatty acids might be an important measure of the rancidity of foods (Iqbal and Bhanger, 2007). The FFA value for fresh corn oil was 0.25%. This acidity is lower than that found in some edible oil such as olive oil, indicating that corn oil is suitable for edible

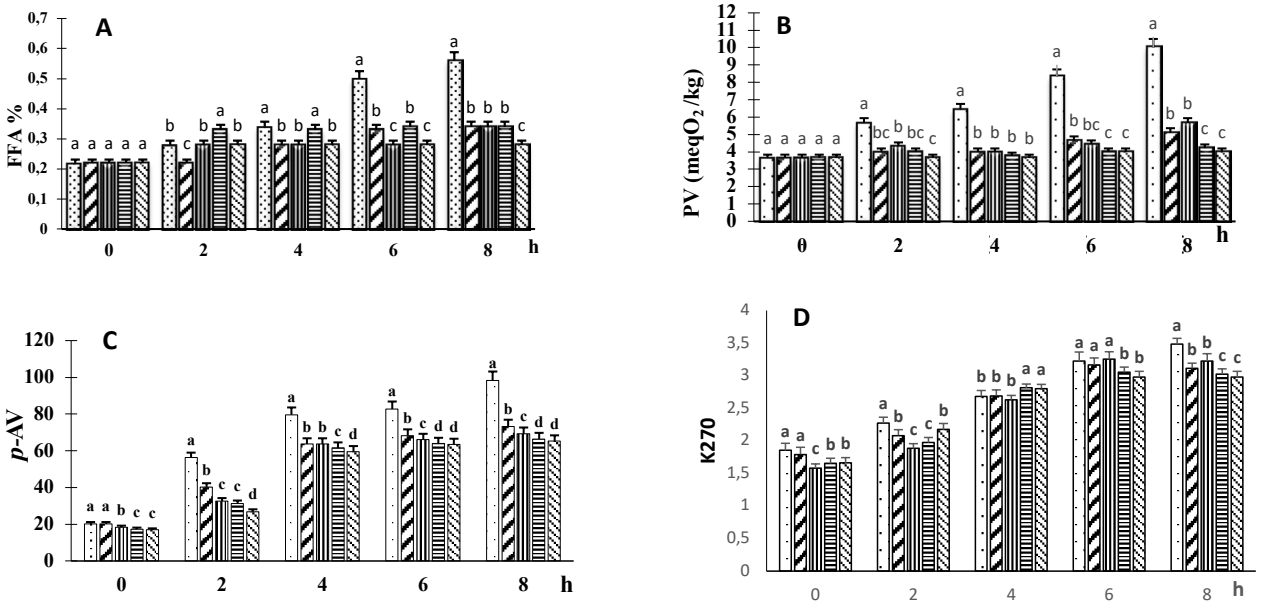

Figure 2: A: Changes in free fatty acid (FFA) contents in oils during heating. B: Changes in peroxide values (PV) in oils during heating. C: Changes in *p*-Anisidine values (*p*-AV) in oils during heating. D: Changes in conjugated triene values (CT) in oils during heating. Results are expressed as means ± standard deviation (n = 3). Bars (mean value ± SD) with different letters after each heating period are significantly different ($p < 0.05$) according to the one-way analysis of variance (ANOVA) followed by Duncan's multiple range test.

purposes. As shown in Figure 2.A, FFA performed remarkably well with the increase in the oil heating period for all the samples. Initially, there was no significant difference in FFA content between the different samples. After 4 hours, significant changes were detected between the control oil (CO) and oils with different supplements (by BHT (CO-BHT) or various doses of PPE: CO-200, CO-500 and CO-1000). The most relevant change in the free fatty acid content was observed in the CO sample. In fact, after 8 hours of heating, CO exhibited the highest FFA, while CO-1000 exhibited the lowest value (Figure 2.A). The FFA equal to BHT for CO-200 and CO-500. In addition, from 0 to 8 hours, the FFA of CO increased by 56% compared to 28% for CO-BHT, CO-200 and CO-500 and 16% for CO-1000. From the present results, it may be concluded that over long heating periods, PPE at 200 and 500 ppm had the same preventive effect as BHT but at 1000 ppm it was more effective. Thus, the increase in FFA content during heating would indicate oil degradation. This result agrees well with the previous investigations carried out by various researchers on corn oil (Sultana *et al.,* 2007). It was reported that FFAs were formed due to the hydrolysis of fats, especially polyunsaturated fatty acids, diglycerides and monoglycerides produced by thermal treatments (Iqbal and Bhanger, 2007; Maskan and Horuz, 2017).

Supplementation with PPE and BHT markedly reduced the FFA increase and inhibited the development of rancidity. BHT was often used traditionally as an antioxidant in oil products to retard oxidation. However, it is allowed for use within legal limits in the food industry due to its toxic and carcinogenic effects (Tan *et al.,* 2015). In addition, it is very effective during the storage and transport of oils and fats, but is less effective at frying temperatures due to its volatility (Nor *et al.,* 2008). In this case, natural extracts such as PPE are a viable source of natural antioxidants for the prevention of frying oil oxidation at high processing temperatures. The preventive effect of PPE against lipid oxidation was reported previously by Iqbal et *al.,* (2008) who demonstrated that the methanolic extracts of PP were more resistant than the conventional synthetic antioxidant, i.e. BHA. In fact, PPE was heated for different intervals at 180 °C and subjected to antioxidant activity evaluation in a linoleic acid system. After 80 min heating, PPE exhibited 66.23% antioxidant activity, i.e. inhibition of lipid peroxidation which is better than BHA.

3.2.2. Change in peroxide value

Peroxide value is an indicator of the extent of initial oxidation in oil and fats (Farhoosh and Moosavi, 2009). It was used to measure the primary oxidation products, the hydroperoxides which can be degraded to a complex range of secondary products (Poiana, 2012). A continuous and tremendous increase in PV with the increase in heating period was observed for CO (Figure 2.B). After 8 hours of heating, the PV of CO increased by 3 times, from 3 to 10. Such observations have been confirmed by Sultana *et al.* (2007) after microwave heating of corn oil for 21 min and by Karoui *et al.* (2011), who reported that PV increased from 0.46 to 9.37 meq O_2/kg after the heating of corn oil from 25 to 200 °C.

At any heating time, the PVs measured in oil samples containing PPE (natural antioxidant) and BHT (synthetic antioxidant) were significantly lower than that of the control. In addition, the PVs for these samples increased but this increase was very slow. Initially, PPE at various doses and BHT at 200 ppm were comparable up to 6 hours of heating, when the PVs for PPE at 500 ppm and 1000 ppm were significantly lower (4 and 4.5, respectively) than that of BHT (Figure 2.B). After 8 hours, the effectiveness compared to the control was 60, 55 and 50%, respectively, for PPE at 1000 ppm, PPE at 500 ppm and BHT at 200 ppm. This suggests that PPE was more stable and effective than BHT. The PVs found in corn oil stabilized by PPE are far lower than those of corn oil stabilized with corncob extract at 500 and 1000 ppm (Sultana *et al.,* 2007), sunflower oil stabilized by grape seed extract (Poiana, 2012) and palm oil by Za'atar essential oils (Maskan and Horuz, 2017) and similar to those of corn oil stabilized with thyme (Karoui *et al.,* 2011). The antioxidant efficacy of PPE has been also confirmed previously in the stabilization of sunflower oil (Iqbal *et al.,* 2008).

3.2.3. Change in p-anisidine value

p-AV is a reliable measurement of the amount of secondary oxidation products which were formed by the decomposition of primary oxidation products (hydrogen peroxides). Alcohols, carboxylic acids, aldehydes and ketones are the major secondary oxidation products (Nor *et al.,* 2008) and are generally responsible for the off-flavors and off-odors of edible oils (Poiana, 2012). The data on *p*-AV with time of heating and the effect of PPE and BHT antioxidants were presented in Figure 2.C. As can be seen, the *p*-AV of all the oils significantly increased as a function of heating time ($p < 0.05$). After heating, the *p*-AV of CO increased from 20 to 98 with the highest increment level. In addition, at any heating time, statistical analyses revealed that the *p*-AVs of CO were significantly higher than those of the supplemented oils. This rapid increase in *p*-AV indicates the lipid deterioration of corn oil by oven heating at 180 °C. The same effect was observed for corn oil after microwave heating for 21 min (Sultana *et al.,* 2007) and for other edible oils such as palm olein (Maskan and Horuz, 2017), sunflower oil (Poiana, 2012), soybean oil (Saoudi *et al.,* 2016). On the other hand, the addition of BHT and various levels of PPE resulted in a significant decrease in *p*-AV ($p < 0.05$) compared to the control sample. In fact, *p*-AV in CO-BHT, CO-200, CO-500 and CO-1000 increased marginally during heating compared to CO and the lowest level was ever recorded for CO-1000. The inhibition of *p*-AV rise by PPE was dose-dependent but at the end of heating, CO-500 and CO-1000 had statistically the same effect. They inhibited the *p*-AV rise by 33% compared to 28 and 23 for CO-200 and CO-BHT, respectively. PPE at 500 and 1000 ppm seemed to be more stable than the BHT. This data was in agreement with those reported by Poiana (2012), who found that grape seed extract was more stable than BHT against the *p*-AV rise in sunflower oil subjected to convection and microwave heating up to 240 min under simulated frying conditions. Other natural extracts such as rosemary and curcumin extracts (Ravi Kiran *et al.,* 2015) inhibited the secondary oxidation of oil by more than 30% compared to BHT. The protective effect of PPE against the secondary oxidation of oil can be attributed to its richness in polyphenolic compounds. In a recent study, gallic acid (GA), a potent antioxidant phenolic compound was grafted in chitosan (GA-g-CS) and its effect on the oxidative stability in bulk oil was tested at 60 and 140 °C. Results showed that GA-g-CS and GA acted as antioxidants at 140 °C by the inhibition of the conjugated dienoic acid value and *p*-AV increase (Seo Yeong Gim *et al.,* 2017).

3.2.4. Change in conjugated diene and triene contents

The measurements of CD and CT are indicators of the oxidative deterioration of oils. CD and CT

are generated by the oxidation of polyunsaturated fatty acids (PUFA). In fact, during heating PUFA are oxidized with the formation of hydroperoxides and their double bonds suffer a rearrangement and generate CD. CT are formed through the conjugation to include three or more double bonds. The resulting CD exhibit intense absorption at 232 nm and quantified by K232; similarly, CT absorb at 272 nm and quantified by K272. Thus, the higher the proportion of PUFA in the oil, the higher the levels of CD and CT formed during frying (Karoui *et al.,* 2011) the greater the levels of CD and CT the lower the oxidative stability of the oil will be.

Figure 2.D shows the relative increase in CT contents of corn oil under oven heating at 180 °C, as a function of heating time. Initially, the CD and CT values for fresh corn oil were 3.75 and 1.75, respectively, which were relatively higher than that found in another plant oils such as soybean oil (2.78 and 0.73) (Ravi Kiran *et al.,* 2015), and olive oil (2.52 and 0.2) (Saoudi *et al.,* 2016). This result confirms that corn oil is much more oxidized than these oils. There were no significant changes in CD values for all the tested oils (data not shown). Thus, it was clear that the thermal treatment of corn oil at 180 °C had no impact on CD content. These results are inconsistent with those reported by Karoui *et al.* (2011), who reported that corn oil supplemented by thyme extract heated from 25 to 200 °C showed an increase in absorption at 232 from 1.8 to 4.89. Furthermore, Yu *et al.* (2018) observed a linear increase in CD with frying time for some edible oils such as coconut oil, soybean oil and pure olive oil.

The CT contents increased with the increase in heating time at a greater rate for the control. After up to 6 hours of heating, CO-1000 showed the lowest level of CT followed by CO-500 followed by BHT. Based on these results, the antioxidant activity of pomegranate peel extract at 500 and 1000 ppm was better than BHT at its legal amounts. These findings are in accordance with those reported by Iqbal *et al.* (2008) sunflower oil supplemented by 1000 ppm of PPE appreciably resisted the increase in CT. The inhibitory effect of PPE against lipid oxidation can be explained by its richness in tannins, powerful antioxidant compounds (Amri *et al.,* 2017a; Amri *et al.*, 2017c). In a recent study, tannic acid (TA), tannyl stearate (TS), molecules synthesized from TA, BHA and BHT synthetic antioxidants were investigated for their power to retard and inhibit linoleic acid oxidation. The results revealed that TA exhibited the best antioxidant activity and its derivative (TS) was more stable than synthetic antioxidants against lipid oxidation as established by the Rancimat method and the addition of TS to frying oils offered good protection against oxidation (Soliman *et al.,* 2017).

3.2.5. Change in TOTOX value

TOTOX is useful for quantifying oxygen-directed oil degradation. The results in Table 1 show that the TOTOX value for oil samples subjected to thermal treatments at 180 °C markedly increased with increasing heating time. As was observed in the case of *p*-AV, PV and FFA, oils supplemented with antioxidants PPE and BHT were more stable to oxidative rancidity than the CO. In fact, after each heating period, the greatest TOTOX value was registered for CO, while the lowest value was recorded for CO-1000. At the end, after 8 hours of heating, the TOTOX value for CO increased by 4 times with a 76% increase compared to 68% for CO-200, 67% for

Table 1. Effect of PPE and BHT on TOTOX values during corn oil heating at 180 °C

h	CO	CO-BHT	CO-200	CO-500	CO-1000
0	27.51±0.13^{a}	27.51±0.38^{a}	25.65±0.24^{b}	24.63±0.29^{b}	24.24±0.32^{b}
2	67.73±0.21^{a}	48.33±0.70^{b}	41.28±0.62^{c}	39.35±0.008^{c}	34.12±0.04^{d}
4	92.5±0.49^{a}	71.63±0.39^{b}	71.63±0.20^{b}	69.04±1.02^{bc}	66.91±0.71^{c}
6	99.35±0.17^{a}	77.58±0.21^{b}	74.83±0.83^{c}	71.88±0.16^{d}	71.40±0.37^{d}
8	118.25±0.58^{a}	83.48±0.29 ^{b}	80.54±0.24^{c}	74.89±0.17^{d}	73.16±0.23^{e}

Results are expressed as means ± standard deviation (n = 3). For each condition, values in the same row 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. PPE: pomegranate peel extract; TOTOX: total oxidation value; CO: Corn oil; CO-BHT: oil supplemented with BHT; CO-200: oil supplemented with 200 ppm PPE; CO-500: oil supplemented with 500 ppm PPE; CO-1000: oil supplemented with 1000 ppm PPE.

CO-BHT and CO-500 and 66% for CO-1000. Thus, the addition of PPE to oil inhibited the TOTOX value increase by more than 10% compared to the control. The best inhibitory effect was attributed to the 1000 ppm of PPE. PPE at a dose of 500 ppm and BHT inhibited lipid oxidation in a similar manner. Poiana (2012) found the same results for the sunflower oil added with grape seed extract and they explained that natural antioxidants of grape seed prevented the lipid oxidation developed in the heating time by surrounding the interface of the lipid system.

3.2.6. Change in phenolic composition

Phenolic content is a primary parameter for vegetable quality evaluation and directly involved in the prevention of oxidation and oil preservation (Amri *et al.,* 2017b). Figure 3 provides the evolution of TPC (Figure 3.A) and TOPC (Figure 3.B) in the different oils during thermal oxidation. The initial phenol contents in the oils supplemented with various doses of PPE were significantly higher than that in the CO and CO-BHT, which had the same contents (27.75 mg GAE/kg oil). In fact, before heating, the TPC provided by CO-1000 (87.43 mg GAE/kg oil) was 3.5 times more than that found in CO and CO-BHT. This marked difference might be attributed to the diffusion of phenols from PPE into the oil, supplementing those already present (Karoui *et al.,* 2011). On exposure to 180 °C, the TPC of all oils decreased significantly as a function of heating time. After each thermal treatment, a significant difference was observed among the different oils, which are classified according to their phenolic content as follows: CO-1000 > CO-500 > CO-200 > CO-BHT > CO. The CO and CO-BHT had the lowest phenol contents and statistically no significant differences were detected between them. CO seemed to be resistant to phenolic degradation after 8 hours of thermal oxidation at 180 °C. However, Karoui *et al.* (2011) demonstrated that refined corn oil (76.74 mg GAE/ kg oil) exposed to 150 °C for just 30 min, lost its TPC. The difference in the remaining contents in phenols did not reflect resistance to phenol degradation. In fact, a decrease of 47, 39, 25, 48 and 49% compared to the initial concentration was verified at the end of the treatment for CO, CO-BHT, CO-200, CO-500 and CO-1000, respectively. So, CO-200 seemed to be the most stable system to TPC degradation followed by CO- BHT; whereas CO and CO-1000 had the same resistance degree. These results were not in agreement with those found by Esposto *et al.* (2015) who evaluated the TPCs evolution of refined oil supplemented with four doses of olive phenolic extract (OPE) after 30 minutes, 1, 2, 4, 6, 8, 10 and 12 hours of frying and compared them to EVOO (extra virgin olive oil, 1237.6 mg/kg TPC). The results demonstrated that oils mixed initially with high doses of OPE were more stable and resistant to phenolic degradation and at a concentration of at least 400 mg/kg of polyphenols. OPE was able to reduce oxidation. So, the thermal degradation of phenolic compounds, and consequently the formation of their oxidation products, was proportional to the initial concentration of OPE added to the oil.

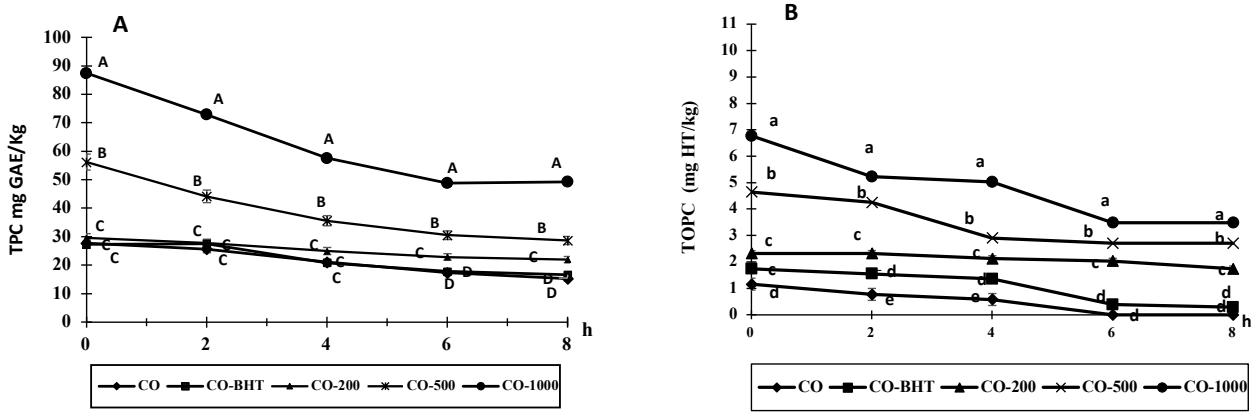

FIGURE 3. Heating impact on phenolic composition of corn oil supplemented with PPE and BHT. A: Total phenol contents (TPC), B: Total orthodiphenol contents (TOPC). Results are expressed as means ± standard deviation (n = 3). Data with different letters for the same heating time are significantly different ($p < 0.05$) according to the one-way analysis of variance (ANOVA) followed by Duncan's multiple range test.

The changes in the TOPC in the different oils during heat treatment are shown in Figure 3.B.

A significant difference ($p < 0.05$) between the TOPC of different oils was observed before and after each treatment period. The initial amount of TOPC in the CO (1.16 mg HT/kg oil) was significantly ($p < 0.05$) lower than that in the PPE supplemented oil. Thus, in CO-1000, it was six times more than the initial amount. TOPC declined as a function of heat treatment in all oils. After 6 h of heating, the TOPC disappeared completely in CO and CO-BHT and decreased by 50% in CO-1000 (3.48 mg HT/kg) and CO-500 (2.71 mg HT/kg) compared to initial values. These contents remained stable until 8 hours of heating. As observed in TPC, the thermal degradation of TOPC was proportional to the initial concentration of PPE added to the oil. CO-200 seemed to be the most stable system to TOPC degradation followed by CO-BHT. In CO-1000 and CO-500, the percent of TOPC loss was similar to that of CO. It can be concluded that, at a level of 200 ppm of PPE, the remaining phenols (TPC or TOPC) would be responsible for protecting the oil but higher than that, the pro-oxidant effect would be observed.

3.2.7. DPPH radical scavenging activities of heated oils

The DPPH radical scavenging activities of CO and supplemented oils significantly decreased as a function of the heating time (Figure 4). Initially, oils supplemented by PPE have high radical scavenging activities. Thus, PPE provided the oil with greater radical scavenging capacity in a dose-dependent manner. DPPH loss in CO decreased from 62 to 54%. Yu *et al.* (2018) reported that DPPH radical scavenging activities of refined coconut oil, refined soybean oil, pure olive oil and vegetable shortening significantly decreased after 80 cycles of repeated frying. It has been suggested that radical scavenging compounds such as a-tocopherol or phenols in edible oil are degraded during thermal oxidation in accordance with the decrease in the DPPH radical scavenging activity of the oil. As oxidation time increased to 8 h, DPPH loss in CO-BHT, CO-200, CO-500 and CO-1000 decreased respectively by 13, 16, 12 and 3% after 8 hours' treatment. This implies that the decrease in the DPPH radical scavenging activity observed in CO was inhibited by the added antioxidants. PPE was more stable than BHT at doses of 500 and 1000.

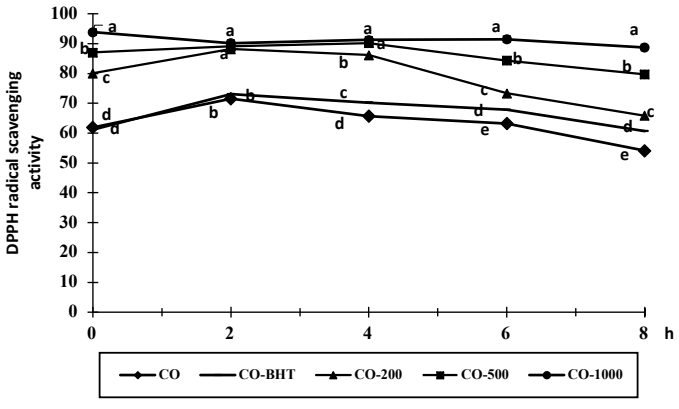

FIGURE 4. Heating impact on DPPH radical scavenging activity of corn oil supplemented with PPE and BHT. Data with different letters for the same heating time are significantly different ($p < 0.05$). Results are expressed as means ± standard deviation (n = 3) according to the one-way analysis of variance (ANOVA) followed by Duncan's multiple range test.

3.2.8. Principal components analysis

A multivariate statistical analysis of the data was performed using PCA to analyze the oxidative stability of different oil samples (CO, CO-BHT, CO-200, CO-500 and CO-1000) before and after 8 hours heating. Figure 5 was plotted according to the correlation among oxidative parameters (FFA, PV, CD, CT, *p*-AV, and TOTOX), phenolic compounds (TPC and TOPC) and DPPH antioxidant activities. PC1 accounted for 75.95% of the total variance (87.75%), and PC2 accounted for 11.80%. The position of each variable in the loading plot describes its relationship with the others. Variables that are close to each other had high correlations. Variables on the same side of the origin (0.0) were positively correlated and those on the opposite side of the origin were negatively correlated. Different corn oil samples could be discriminated on the PCA plane.

PC1 was positively related to TPC, TOPC and DPPH antioxidant activities. PC2 was related more closely to FFA, PV, *p*-AV, TC, DC and TOTOX. CO-1000, CO-500 and CO-200 were located on the positive side of PC1; whereas CO and CO-HBHT were located on the negative side.

This data revealed that the lowest levels for TPC and TOPC were observed in CO and CO-BHT, with the greatest extent of the oxidative deterioration, expressed by PV, *p*-AV, FFA, CT, CD and TOTOX. However, high levels were

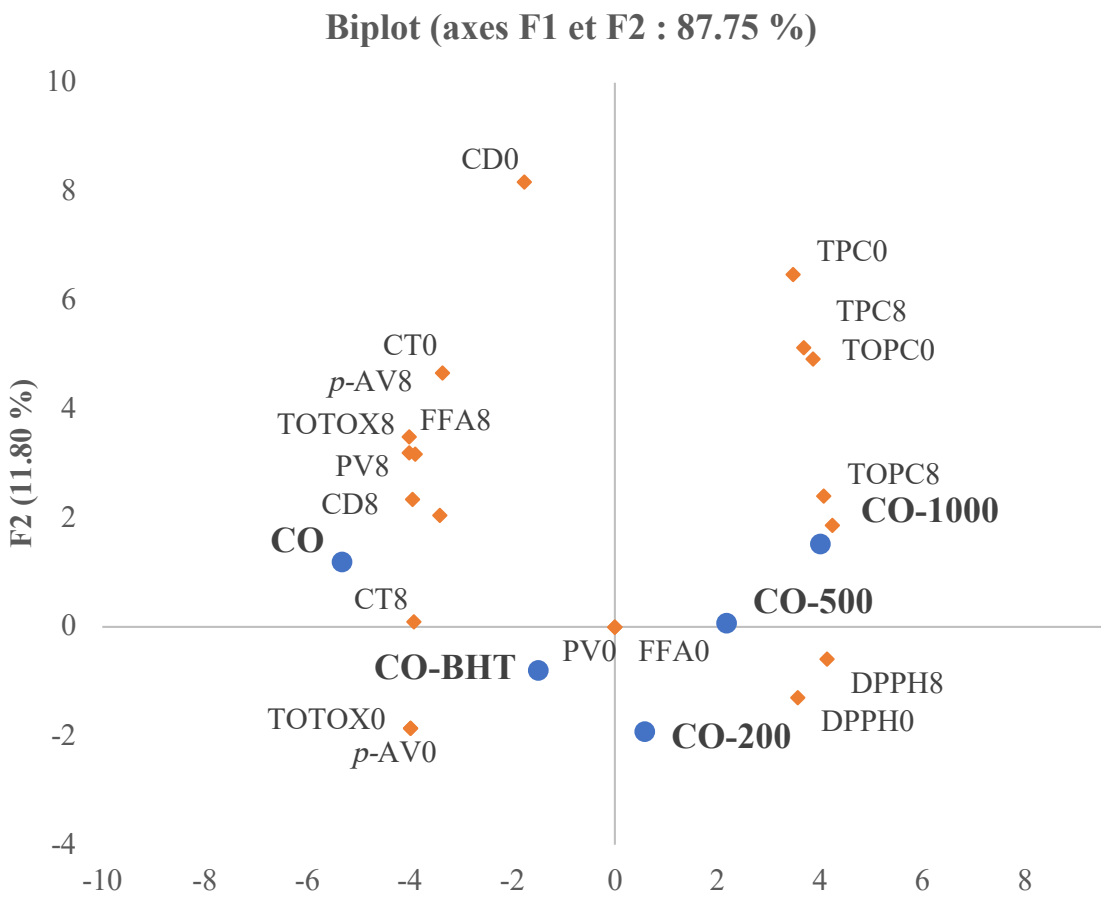

FIGURE 5. Principal component analysis (scores and loading plots, Biplot) applied to the data set for oxidative parameters, phytochemical contents (polyphenols and orthodiphenols) and DPPH antioxidant activities of different corn oil samples before and after 8 hours' heating. PV: peroxide value, *p*-AV: *p*-anisidine value, FFA: free fatty acid value, CD: conjugated dienes, CT: conjugated trienes, TOTOX: total oxidation value, TPC: polyphenol content, TOPC: ortho-diphenols.

registered for PPE-supplemented oils (200, 500 and 1000 ppm) with the lowest TOTOX values. This could be attributed to the protective action of TPC against thermo-oxidative degradation, explaining the negative correlation observed between TOTOX values and these phenolic compounds before and after 8 hours' heating. On the other hand, significant proportions of the primary and secondary oxidation products (PV, CD, CT and *p*-AV) were observed for CO, with negative correlations with CO-BHT and PPE-enriched oils (CO-200, CO-500 and CO-1000) respectively, according to PC2 and PC1.

The PCA results confirmed the deterioration effect on corn oil quality due to heating and the effectiveness of various PPE extracts used (200, 500 and 1000ppm) compared to BHT against the formation of primary and secondary oxidation products.

4. CONCLUSION

According to the results, it can be concluded that PPE exhibited significant potential to stabilize corn oil under heating conditions. They decreased the thermal deterioration of oil by enhancing its hydrolytic stability, inhibiting double bond conjugation and reducing the loss in polyunsaturated fatty acids. PPE at concentrations of 500 and 1000 ppm have potential stabilization efficiency compared to synthetic antioxidant (BHT). Therefore, PPE, a potential antioxidant source, can be recommended to extend the shelf-life of unsaturated vegetable oils.

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