

# Comparative Efficacy of Pomegranate Juice, Peel and Seed Extract in the Stabilization of Corn Oil under Accelerated Conditions

Zoi Konsoula

**Abstract**—Antioxidant-rich extracts were prepared from pomegranate peels, seeds and juice using methanol and ethanol and their antioxidant activity was evaluated by the 1,1-diphenyl-2-picrylhydrazine (DPPH) radical scavenging and Ferric Reducing Antioxidant Power (FRAP) method. Both analytical methods indicated a higher antioxidant activity in extracts prepared from peels, which was comparable to that of butylated hydroxytoluene (BHT). Furthermore, the antioxidant activity was correlated to the phenolic and flavonoid content of the various extracts. The antioxidant effectiveness of the extracts was also assessed using corn oil as the oxidation substrate. More specifically, preheated corn oil samples stabilized with extracts at a concentration of 250 ppm, 500 ppm or 1,000 ppm were subjected to accelerated aging (100 °C, 10 days) and the extent of oxidative alteration was followed by the measurement of the peroxide, conjugated dienes and trienes, as well as p-aniside value. BHT at its legal limit (200 ppm) served as standard besides the control sample. Results from the different parameters were in agreement with each other suggesting that pomegranate extracts can stabilize corn oil effectively under accelerated conditions, at all concentrations tested. However, the magnitude of oil stabilization depended strongly on the amount of extract added and this was positively correlated with their phenolic content. Pomegranate peel extracts, which exhibited the highest not only phenolic and flavonoid content but also antioxidant activity, were more potent in inhibiting oxidative deterioration. Both methanolic and ethanolic peel extracts at a concentration of 500 ppm exerted a stabilizing effect comparable to that of BHT, while at a concentration of 1000 ppm they exhibited higher stabilization efficiency in comparison to BHT. Finally, heating oil samples resulted in a time dependent decrease in their antioxidant capacity. Samples containing peel extracts appeared to retain their antioxidant capacity for a longer period, indicating that these extracts contained active compounds that offered superior antioxidant protection to corn oil.

**Keywords**—Antioxidant activity, corn oil, oxidative deterioration, pomegranate.

## I. INTRODUCTION

THE oxidative deterioration of fats and oils in food products is responsible for rancidity and off flavors, which are coupled with the decrease in nutritional quality and safety [1]. Edible oils with higher contents of unsaturated fatty acids, especially polyunsaturated fatty acids, are more susceptible to oxidation [2]. The addition of antioxidants has become popular as means of increasing the shelf-life of food products and improving the stability of lipids and lipid-containing foods

[3]. Synthetic antioxidants, such as butylated hydroxyanisole (BHA), BHT, tert-butyl hydroquinone (TBHQ) have been used as food additives. However, recent reports reveal that these compounds may be implicated in many health risks, including carcinogenesis [2], [4]–[6]. As a result, the most powerful synthetic antioxidant (TBHQ) is not allowed for food application in Japan, Canada and Europe. Similarly, BHA has also been removed from the generally recognized as safe (GRAS) list of compounds [2], [7].

Due to these safety concerns, attention is now increasingly paid to the development and utilization of effective and non-toxic antioxidants from natural sources [1]. Extracts of many plants have been shown to have various degrees of antioxidant activity in different fats and oils [3]. Reference [8] utilized different sesame extracts as alternative natural antioxidants for the stabilization of various edible oils, while [2] employed garlic extract for the stabilization of sunflower oil under accelerated storage conditions. Furthermore, natural antioxidants have been explored from agricultural wastes such as old tea leaves, rice bran, wheat bran and peanut hulls [6]. The antioxidant activity of these plants and their extracts can be attributed to the presence of various bioactive compounds including phenolic acids, flavonoids and tocopherols [3], [5].

Pomegranate products have been used for centuries for medicinal purposes [1]. However, the past 15 years the fruit's popularity has risen due to its high content in polyphenols [9]. Pomegranate fruits come from a shrub or a small tree (*Punica granatum L.*) belonging to the family of Punicaceae. Although the particular plant is a native to the tropical and subtropical regions of the Middle East and India, the cultivation of pomegranate is nowadays widely distributed to the temperate regions of the Mediterranean. The fruit comprises of four parts, the non-edible exocarp and mesocarp (commonly known as the peel) and also the edible endocarp which contains the juice sacs (commonly known as arils) and the seeds [10]. All of these parts are considered rich sources of bioactive compounds including phenolic acids, tannins, flavonols and anthocyanins, which promote health by reducing the risk of atherosclerosis, cancer, diabetes and neurodegenerative disorders [9]. The continuously increasing scientific evidence linking its consumption to better health has resulted in a concomitant rise in pomegranate's commercial importance in food and health industries [11].

Taking into consideration the enormous interest that the bioactive components isolated from pomegranate have raised in the scientific community, the present study aimed at the

Zoi Konsoula is with the Department of Life and Health Sciences, University of Nicosia, 1700 Nicosia, Cyprus (e-mail: konsoula.z@unic.ac.cy).

preparation of antioxidant-rich extracts from pomegranate peels, seeds and juice and the evaluation of their stabilizing efficiency against the oxidative deterioration of corn oil by measuring both primary (hydroperoxides) and secondary oxidation products. Additional objectives of this study were the investigation of the relationship between the prepared extracts' antioxidant activity, as well as efficacy in retarding the oxidative deterioration of corn oil and their total phenolic content.

## II. MATERIAL AND METHODS

### A. Materials

*Punica granatum* L. fruits and commercial corn oil were obtained from the local market in Thessaloniki, Greece. All chemicals used were of analytical grade and they were obtained from Sigma Chemie (Steinheim, Germany), and Fluka Chemie GmbH (Buchs, Switzerland).

### B. Preparation of Pomegranate Extracts

The different parts of pomegranates were separated and freeze dried for preparation of the peel, juice, and seed extract. The seeds were washed with excess water for the removal of sugars and adhering materials prior to freeze drying. Dried peels and seeds were ground to fine powder in an electric coffee grinder (Black and Decker, Canada Inc., Brockville, ON). To obtain ethanol extracts and methanol extracts, 100 g of samples were mixed with 400 mL of solvent and extracted in an ultrasonic bath (ULTRASONS-H, J.P. SELECTA s.a., Barcelona, Spain) for 2 h at room temperature. The samples were stirred every 30 min to ensure a well-mixed extraction. Extracts were filtered by Whatman No. 4 filter, and the residue was re-extracted twice using fresh solvent. After filtration, the combined filtrates were desolvated under vacuum at 40 °C using a rotary evaporator.

### C. Assessment of the DPPH Scavenging Activity

The ability of plant extracts to scavenge the stable DPPH radical was assessed according to the method described by [12]. Also, the heated and unheated oil samples were appropriately diluted in ethyl acetate prior to the determination of their DPPH radical scavenging power [12]. The EC<sub>50</sub> value was defined as the effective concentration which was required to decrease the initial DPPH concentration by 50%. The residual DPPH scavenging capacity after the heat treatment of oils was determined according to [8].

### D. Assessment of the Reducing Capacity

The capacity of plant extracts to reduce ferric ions was assessed by the method described by [13].

### E. Assessment of Oil Thermal Stability

Samples (100 mL) of corn oil with or without additives were placed in open and transparent beakers, which enabled direct contact between oil surface and atmospheric air, and the oil samples were heated at 100 °C in an oven (Hower, FIRLABO, IATRIN, Thessaloniki, Greece). Aliquots of the oil samples were removed at predetermined time intervals in

order to assess their oxidative stability and antioxidant activity. Oxidation was assessed by the measurement of peroxide value, p-anisidine value, conjugated dienes and trienes. Ethanolic and methanolic extracts of pomegranate peel, seed or juice, as well as BHT, were used as additives in the present study and they were added at different concentrations in the corn oil.

### F. Analytical Methods

Official AOCS Methods [14] were used for the determination of peroxide value (PV, Method Cd 8-53), while the p-anisidine value (p-AV, Method 2.504) and conjugated dienes (CD, Method 2.505) were determined by [15], [16], respectively. The concentration of phenolic compounds was determined using the Folin-Ciocalteau reagent. The results were expressed as gallic acid equivalents [17]. The aluminum chloride colorimetric technique was used for the total flavonoid content estimation. The total flavonoid content was expressed as rutin equivalents (RE) [18].

### G. Statistical Analysis

All chemical and instrumental measurements were performed in triplicate and the data presented in this work are the mean value of the three different experiments. Statistical analyses were performed using Student's *t*-test and a probability value lower than 0.05 was considered significant.

## III. RESULTS AND DISCUSSION

### A. Determination of the Pomegranate Extracts Yield and Quantification of their Total Phenolic and Flavonoid Content

The yields of extracts obtained from pomegranate peel, juice and seeds using various solvents are shown in Table I. In all cases the highest yield of extracts was obtained by extraction with methanol.

TABLE I  
YIELD, PHENOLIC AND FLAVONOID CONTENT OF POMEGRANATE PEEL, SEED  
AND JUICE EXTRACTS

	EXTRACT		
	PEEL	SEED	JUICE
Yield (% w/w)			
METHANOL	28.9 ± 0.5	19.7 ± 0.4	14.3 ± 0.5
ETHANOL	21.9 ± 0.6	15.1 ± 0.4	11.2 ± 0.6
<b>Total phenolic compounds (mg GAE/g)</b>			
METHANOL	190 ± 5.2	69.8 ± 2.2	34.2 ± 0.8
ETHANOL	146.5 ± 4.8	54.3 ± 2.1	26.8 ± 0.9
<b>Total flavonoid compounds (mg RE/g)</b>			
METHANOL	20.5 ± 0.6	8.8 ± 0.4	4.3 ± 0.3
ETHANOL	16.2 ± 0.7	7 ± 0.4	3.4 ± 0.4

Data are mean (*n* = 3) ± standard deviation (*n* = 3), (*p* < 0.05)

More specifically, pomegranate peel extracted with methanol gave maximum yield, reaching approximately 29% (w/w), while the yield of the juice extract was considerably lower since it did not exceed 15% (w/w). These findings were supported by previous results of [6], who reported that methanol is usually recommended for the extraction of the antioxidant compounds contained in pomegranate.

Phenolic compounds, which are recorded among the most occurring phytochemicals in plants, are secondary metabolites. In addition to their contribution to color and sensory characteristics of fruits, phenolic compounds also play a significant role in the protection against *in vivo* and *in vitro* oxidation [1]. Moreover, according to [1], phenolics are polar constituents and, therefore, polar solvents are more efficient for the extraction of active antioxidants from pomegranate. In the present study, the phenolic content of the methanolic extracts was higher in comparison to the ethanolic extracts (Table I). Furthermore, in line with previous data reported by [10], [19], [20], the results of the present study indicated a higher total phenolic content in the peel extract. More specifically, statistical mean values showed that the peel extracts contained at least 2.7-fold and 4.7-fold higher amount of phenolic compounds in comparison to seed and juice extracts, respectively. These findings designated pomegranate peel as an enriched source of phenolic compounds.

According to the data presented in Table I, the flavonoid fraction represented almost 11% to 13% of the total phenolic content in all analyzed extracts. These findings are considerably lower than the observations reported by [10], [21], according to whom the flavonoid fraction reached up to 24-30% of the total polyphenols present in the extracts. In the present study, the extracts of pomegranate peel were richer in total flavonoid compounds, followed by the seed extracts. Moreover, the total flavonoid content correlated with the total phenolic content ( $r^2 \geq 0.98$ ) in all pomegranate extracts. These data are comparable to those reported by [20].

#### B. Antioxidant Activity of the Pomegranate Extracts

The DPPH free radical scavenging method has been widely used to evaluate the antioxidant activity of plant extracts due to the simple, rapid, sensitive and reproducible procedure. The DPPH radical is a stable organic free radical, which loses its absorption maxima at 517 nm when accepting an electron or a free radical species [22]. The particular method was employed for the evaluation of the free radical scavenging potential of pomegranate extracts at different concentrations.

The EC<sub>50</sub> value is defined as the effective concentration which is required to decrease the initial DPPH concentration by 50% and a lower EC<sub>50</sub> value reflects better protective action [20], [22]. The EC<sub>50</sub> values of all extracts were calculated by using the results of DPPH scavenging activity between 50 µg/mL and 1,000 µg/mL extract concentrations. As it can be seen in Fig. 1 (a), the EC<sub>50</sub> value of DPPH scavenging activity varied within 0.06–0.08 mg/mL in the peel, 1.3–1.7 mg/mL in the juice, and 0.48–0.62 mg/mL in the seed extract. In general, the DPPH scavenging activity was shown significantly to be higher in the peel extract when compared with juice and seeds. According to EC<sub>50</sub> mean values, while DPPH scavenging activity in the peel extract was found approximately 21-fold and 8-fold higher than the juice and the seed extract, respectively. These results show similarity with [20], [21] who demonstrated that pomegranate peel extract had markedly higher antioxidant capacity than the pulp extract. Finally, the EC<sub>50</sub> value of BHT was recorded

equal to 0.10 mg/mL, slightly higher than the EC<sub>50</sub> values of the peel extracts (Fig. 1 (a)). This finding indicated the pomegranate peel extracts prepared in the present study exhibited antioxidant capacity comparable to the synthetic antioxidant, BHT.

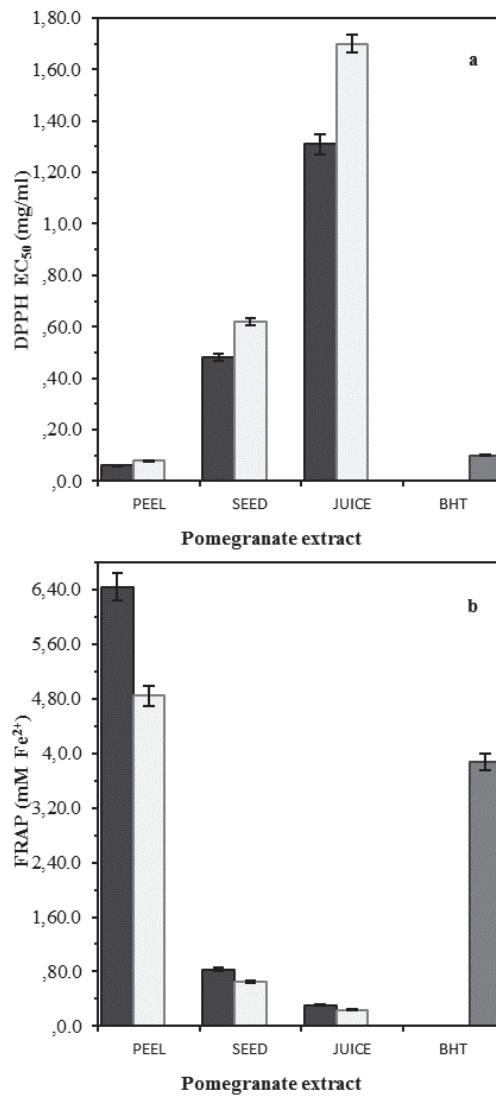


Fig. 1 Antioxidant activity of methanolic (■) and ethanolic (□) pomegranate extracts in comparison to BHT (■) determined by the DPPH (a) and FRAP (b) method. Data are mean ( $n = 3$ )  $\pm$  standard deviation ( $n = 3$ ), ( $p < 0.05$ )

The FRAP assay is also commonly used to study the antioxidant capacity of plant materials. The antioxidant capacity of the extracts is determined by the ability of the antioxidants in these extracts to reduce ferric iron to ferrous in the FRAP reagent, which consists of 2,4,6-tris(1-pyridyl)-5-triazine (TPTZ) prepared in sodium acetate buffer, pH 3.6. The reduction of ferric iron in the FRAP reagent will result in the formation of a blue product (Ferrous-TPTZ complex) whose absorbance can be read at 593 nm [13]. According to

this method the antioxidant activity values ranged from 4.8 mM Fe<sup>2+</sup> to 6.4 mM Fe<sup>2+</sup> for peel extracts, from 0.65 mM Fe<sup>2+</sup> to 0.83 mM Fe<sup>2+</sup> for seed extracts and from 0.24 mM Fe<sup>2+</sup> to 0.31 mM Fe<sup>2+</sup> for juice extracts (Fig. 1 (b)). In accordance with the results obtained for the DPPH radical scavenging activity, the reducing capacity of the pomegranate peel extracts was significantly higher than the seed and juice extracts. Furthermore, according to the FRAP assay, the reducing capacity of the peel extracts appeared greater than that of BHT (Fig. 1 (b)).

#### C. Correlation of the Antioxidant Activity to the Total Phenolic and Flavonoid Content of the Pomegranate Extracts

References [10], [19], [20] claimed that the antioxidant activity may be correlated to the phenolic content of the peel extracts. As inferable from the data on the correlation analysis shown in Fig. 2, the antioxidant activity measured by both methods followed the differences in the content of total phenolic compounds.

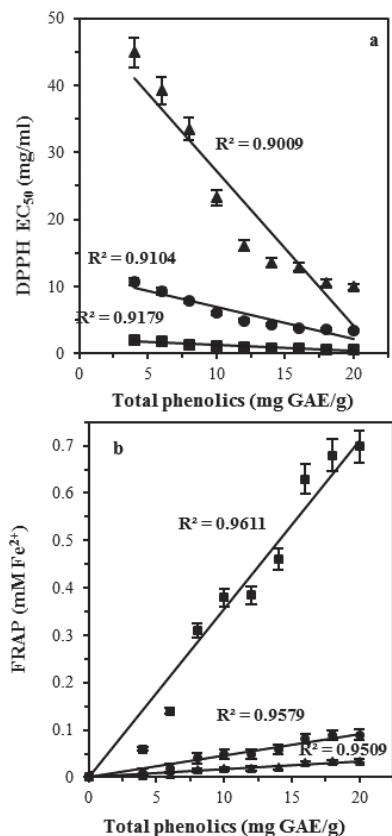


Fig. 2 Correlation between the antioxidant activity of peel (■), seed (●) and juice (▲) extract determined by the DPPH (a) and FRAP (b) method. Data are mean ( $n = 3$ )  $\pm$  standard deviation ( $n = 3$ ), ( $p < 0.05$ )

In the peel extract, there were strong correlations between total phenolic content and the DPPH scavenging activity ( $r^2 = 0.92$ ) or the reducing capacity ( $r^2 = 0.96$ ). The hierarchy for the correlation between antioxidant activity and phenolic

content was: peel > seed > juice extract (Fig. 2). Similar results were obtained in the case of the total flavonoid content (data not shown).

#### D. Heat Stability of Corn Oil Containing Various Pomegranate Extracts

The primary products of lipid oxidation are hydroperoxides, which are generally referred to as peroxides. Therefore, the determination of the peroxide value can be used as an oxidative index of lipid oxidation. The formation of peroxides is also accompanied by an increase in UV absorbance with a maximum at about 232 nm and 270 nm, which is characteristic of CD and triene systems, respectively. However, peroxides are unstable on heating and transform rapidly to secondary oxidation products. The degree of secondary oxidation is usually reflected by the changes in p-anisidine value. It is apparent, therefore, that the estimation of the absolute oxidative state of oil requires the measurement of four different oxidation parameters, such as peroxide value, CD and trienes, as well as p-anisidine value [2], [8], [23].

Various concentrations of pomegranate extracts (250, 500 and 1,000 ppm) were incorporated in corn oil in order to delay the accumulation of oxidation products and thus to improve its oxidative stability. The synthetic antioxidant BHT at its legal limit served as standard besides the control. As it can be seen in Fig. 3 (a), corn oil containing the different methanolic extracts of pomegranate resisted oxidation, since a significant difference ( $p < 0.05$ ) in the peroxide value was observed between the control and corn oil samples containing the extracts, which indicated a retarded rate of peroxide formation. However, each extract inhibited the oxidative deterioration of corn oil to a different extent. The peroxide value of corn oil containing 1,000 ppm of methanolic pomegranate peel extract, seed extract, juice extract, and BHT were found to be approximately 64.9, 103.24, 188.81 and 79.94 meq/kg, respectively, while the peroxide value of the control sample reached up to 345.62 meq/kg, after 10 d of treatment. It is apparent, therefore, that the methanolic pomegranate extracts prepared from the peel were more effective in preventing the generation of oxidation products. Also, these data suggested the superiority of antioxidant activity of pomegranate peels over the synthetic antioxidant.

Similar findings were obtained when different ethanolic extracts were added in corn oil (data not shown). Nevertheless, the stabilizing effect of the ethanolic extracts appeared to be slightly inferior in comparison to the methanolic extracts. More specifically, the peroxide value of corn oil containing 1000 ppm of ethanolic pomegranate peel extract, seed extract and juice extract were found to be approximately 71.55, 113.24 and 204.92 meq/kg, respectively (data not shown).

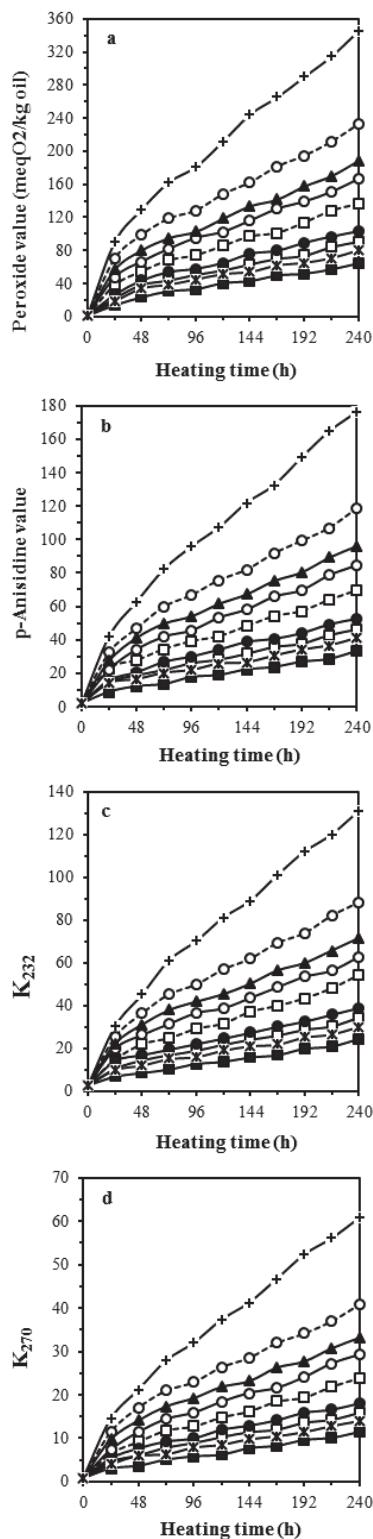


Fig. 3 Changes in the peroxide (a), p-anisidine (b), CD (c) and CT (d) value during heat treatment of corn oil (+) containing 250 ppm (---), 500 and 1000 ppm (—) peel extract (□, ■); seed extract (●, ○); juice extract (▲) or BHT (\*) at its legal limit. Data are mean ( $n = 3$ )  $\pm$  standard deviation ( $n = 3$ ), ( $p < 0.05$ )

Oil stabilization depended strongly on the amount of extract added to corn oil. When corn oil was supplemented with methanolic extracts prepared from pomegranate peel or seeds, the formation of oxidation products decreased almost 2-fold and 2.3-fold, respectively, upon increasing the amount of extract from 250 ppm to 1,000 ppm (Fig. 3 (a)). On the contrary, the stabilizing effect of juice extract depended far more pronouncedly on its concentration, indicating once again the inferior protective effect of pomegranate extracts prepared from juice (data not shown). It should be also underlined that the peroxide value achieved by the methanolic juice extract at 1,000 ppm was 2.9-fold and 1.8-fold higher in comparison to that attained in case of peel and seed extract, respectively (Fig. 3 (a)).

The determination of the p-anisidine value confirmed further the oxidative stabilization of corn oil by the addition of the different pomegranate extracts (Fig. 3 (b)). The p-anisidine value of oil samples supplemented with 1,000 ppm peel, seed and juice extract were 5.3, 3.3 and 1.83-fold lower in comparison to control. Moreover, the incorporation of 200 ppm BHT in corn oil resulted in the reduction of the p-anisidine value by 4.3-fold. The results presented in Fig. 3 (b) indicated that the methanolic extract prepared from pomegranate peel exhibited higher antioxidant activity than BHT at a concentration of 1,000 ppm, while its stabilizing efficacy was comparable to BHT in case the concentration was reduced to 500 ppm. Finally, the antioxidant activity of pomegranate peel extracts was followed by seed extracts (Fig. 3 (b)), while juice extracts possessed significantly lower stabilizing efficacy at all concentrations tested (data not shown).

The assessment of CD and trienes (CT) is a good parameter for the measurement of oxidative deterioration of oils, hence indicates the effectiveness of antioxidants in oils [2], [8]. [23]. The increase in CD and CT contents is proportional to the uptake of oxygen, therefore, the greater the levels of CD and CT the lower the oxidative stability of the oils. Formation of high contents of CD may be related to the presence of higher contents of polyunsaturated fatty acids in corn oil, while conjugated trienes may be produced by dehydration of CD hydroperoxides [2], [23]. Figs. 3 (c) and (d) show the relative increase in CD and CT contents of corn oil samples under accelerated conditions as function of treatment time, respectively. Highest contents were observed for control, indicating greater intensity of oxidation, followed by pomegranate juice, seed and peel extracts in all concentrations tested. In line with the results presented previously on the peroxide and p-anisidine value, lower CD and CT contents were recorded when the concentration of the pomegranate extracts in corn oil reached up to 1000 ppm. Also at the particular concentration the stabilizing efficacy of the peel extract was superior to that of BHT.

#### E. Antioxidant Activity of Corn Oil Containing Various Pomegranate Extracts

Antioxidants in foods play a major role in maintaining the quality of oils by retarding the oxidative breakdown of lipids.

It is, therefore, necessary to evaluate the effect of heating on the antioxidant activity of oils in order to gain a complete perspective on the oxidative state as well as the nutritional value of oils [8]. Heating oil samples resulted in a time dependent decrease in their DPPH radical scavenging capacity (Fig. 4). Although corn oil lost its radical scavenging capacity after 96 h of heating under cooking conditions, the samples containing pomegranate extracts appeared to retain their radical scavenging capacity for a longer period, indicating that these extracts offered antioxidant protection to corn oil. Based on the data presented in Fig. 4, pomegranate extracts prepared from peel were the most effective in preserving the radical scavenging capacity of corn oil. More specifically, the time required to reduce DPPH radical scavenging capacity by 50% was decreased from 48 h to 36 h or 24 h when 1,000 ppm of peel, seed or juice extract was added to corn oil, respectively (Fig. 4). Similar results were observed in the case of the ferric reducing capacity of pomegranate extracts (data not shown). Therefore, the relative order of antioxidant activity of oil samples containing pomegranate extracts prepared from different fruit parts was as follows: peel > seed > juice. Finally, these findings were in agreement with the results obtained previously regarding the oxidative stability induced by the different pomegranate extracts (Fig. 3).

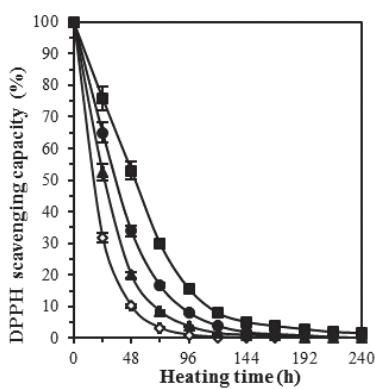


Fig. 4 Effect of heat treatment on the residual DPPH radical scavenging activity of corn oil ( $\diamond$ ) containing 1000 ppm peel ( $\blacksquare$ ), seed ( $\bullet$ ) or juice extract ( $\blacktriangle$ ). Data are mean ( $n = 3$ )  $\pm$  standard deviation ( $n = 3$ ), ( $p < 0.05$ )

The antioxidative activity of corn oil supplemented with pomegranate extracts was concentration dependent. Increase of the content of pomegranate extracts prepared from peel, from 250 ppm to 1,000 ppm was followed by a significant rise in the DPPH radical scavenging activity. More specifically, the time required to reduce DPPH radical scavenging capacity by 50% was increased from approximately 28 h to 36 h or 52 h, when the concentration of the pomegranate peel extract added to corn oil was raised from 250 ppm to 500 ppm or 1,000 ppm, respectively (data not shown). Similar results were obtained in case of seed and juice extracts (data not shown). These findings suggested that pomegranate peel extracts could be utilized in order to formulate oil products of unique antioxidative potential.

#### F. Phenolic Compounds Content of Corn Oil Supplemented with Various Pomegranate Extracts

The results of the present study suggested that pomegranate extracts contained antioxidative compounds which contributed to the stabilization of corn oil. For this reason, the changes in the total phenolic content of corn oil treated with the different pomegranate extracts was monitored parallel to the changes in the four different oxidation parameters. The phenolic compounds profile of heated oil is presented in Fig. 5 (a).

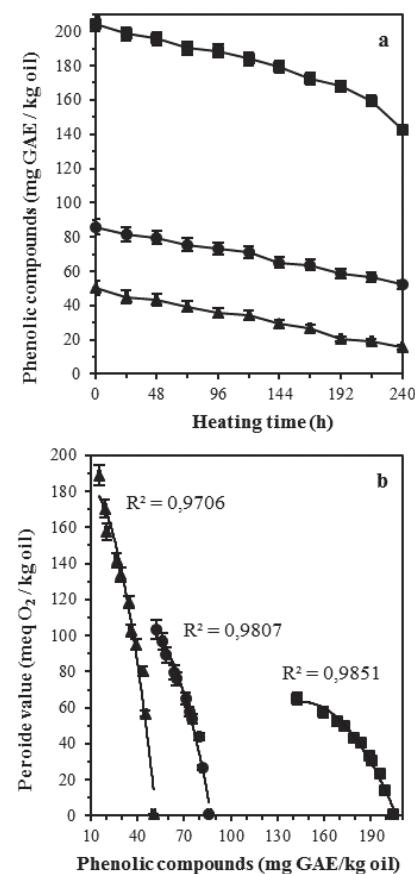


Fig. 5 Effect of heat treatment on the amount of phenolic compounds (a) contained in corn oil supplemented with 1000 ppm peel ( $\blacksquare$ ), seed ( $\bullet$ ) or juice extract ( $\blacktriangle$ ) and its correlation with the peroxide value changes (b). Data are mean ( $n = 3$ )  $\pm$  standard deviation ( $n = 3$ ), ( $p < 0.05$ )

In all cases, the plot of phenolic compounds concentration in corn oil samples against heating time gave a straight line; however, the reduction in the phenolic constituents' content proceeded in significantly different rate. More specifically, the decomposition rate of phenolic compounds in oil samples increased in the following order: peel < seed < juice, indicating the superior heat stability of the phenolic constituents of pomegranate peel. As far as oil samples treated with pomegranate peel extracts are concerned, it is noteworthy that they contained considerably higher amounts of phenolic compounds during the whole period of heat treatment (Fig. 5

(a)). Finally, after taking into account that each phenolic compound exhibits different thermal stability, it was apparent that the composition of phenolic compounds presents in the various pomegranate extracts determined their profile during heating.

The reduction of the phenolic compounds concentration in oil samples treated with pomegranate extracts induced their oxidative deterioration (Fig. 5 (b)). More specifically, a second order equation fitted the relationship between the peroxide value and phenolic compounds concentration of corn oil supplemented with peel or seed extract ( $r^2 \geq 0.98$ ) and juice ( $r^2 \geq 0.97$ ). These findings not only indicated a direct correlation between the phenolic content of corn oil and its oxidative deterioration rate, but it also suggested that the composition of phenolic compounds present in pomegranate extracts was the most effective in retarding oil oxidation. Furthermore, the phenolic compounds, which were present in pomegranate peel and were subsequently transferred to the respective extract, contributed more significantly to the inhibition of oxidative rancidity, since the changes in their concentration induced less pronounced changes in the peroxide value of corn oil samples (Fig. 5 (b)).

#### IV. CONCLUSION

The results of the present study demonstrated that pomegranate extracts could be used as alternative antioxidants for the protection of corn oil against oxidative deterioration. The protection offered by pomegranate peel extract at concentration of 500 ppm was comparable to that of the commonly employed synthetic antioxidant BHT at its legal limit, while at concentration of 1,000 ppm the peel extract appeared to be the more effective in retarding oxidative deterioration of corn oil. This is in accordance with results of [24] who reported that in soybean oil carnosic acid was more active than BHT and BHA, but less active than TBHQ, as well as with [23], who reported that potato peel extract at concentration of 100 ppm and 200 ppm and sugar beet pulp extract at 200 ppm had stabilization efficiency comparable to BHT and BHA at their legal limit, but less effective than TBHQ.

Taking into account the constituents of the various pomegranate extracts, it was suggested that the phenolic compounds, which were present in the different pomegranate parts and were subsequently transferred to the extracts, acted as significant oxidation inhibitors. Furthermore, a direct correlation between the phenolic content of oil samples and their oxidative deterioration rate was reported in this work for the first time. More specifically, it was found that the decomposition of phenolic compounds presents in oil samples during heat treatment induced a significant increase in the formation of oxidation products. It is apparent that supplementing vegetable oils with pomegranate extracts, and specifically extracts prepared from peel, may lead to the formulation of oil samples with unique antioxidation potential, high oxidative stability and significant nutritional value.

#### ACKNOWLEDGMENT

Z. Konsoula would like to express her gratitude to Professor M. Liakopoulou-Kyriakides, who is with the Aristotle University of Thessaloniki, for providing access to the university's facilities, as well as for the peer interactive communication and contribution to the implementation of the present study.

#### REFERENCES

- [1] M. S. Shiban, M. M. Al-Otaibi, and N. S. Al-Zoreky, "Antioxidant Activity of Pomegranate (*Punica granatum L.*) Fruit Peels," *Food and Nutrition Sciences*, vol. 3, pp. 991-996, 2012.
- [2] S. Iqbal, and M. I. Bhanger, "Stabilization of sunflower oil by garlic extract during accelerated storage," *Food Chemistry*, vol. 100, pp. 246-254, 2007.
- [3] A. Padmaja, and N. B. L. Prasad, "Pomegranate (*Punica granatum L.*) Peel Extract as a Source of Natural Antioxidant," *Journal of Food Science and Engineering*, vol. 1, pp. 171-182, 2011.
- [4] M. I. Ibrahim, "Efficiency of Pomegranate Peel Extract as Antimicrobial, Antioxidant and Protective Agents," *World Journal of Agricultural Sciences*, vol. 6, pp. 338-344, 2010.
- [5] B. Sultana, F. Anwar, M. R. Asi, and S. A. S. Chatha, "Antioxidant potential of extracts from different agro wastes: Stabilization of corn oil," *GRASAS YACEITES*, vol. 59, pp. 205-217, 2008.
- [6] S. Iqbal, S. Haleem, M. Akhtar, M. Zia-ul-Haq, and Jamshed Akbar, "Efficiency of pomegranate peel extracts in stabilization of sunflower oil under accelerated conditions," *Food Research International*, vol. 41, pp. 194-200, 2008.
- [7] R. S. Farag, A. Z. M. A. Badei, and G. S. A. El Baroty, "Influence of thyme and clove essential oils on cottonseed oil oxidation," *Journal of American Oil Chemists Society*, vol. 66, pp. 800-804, 1989.
- [8] Z. Konsoula, and M. Liakopoulou-Kyriakides, "Effect of endogenous antioxidants of sesame seeds and sesame oil to the thermal stability of edible vegetable oils," *LWT-Food Science and Technology*, vol. 43, pp. 1379-1386, 2010.
- [9] A. Masci, A. Coccia, E. Lendaro, L. Mosca, P. Paolicelli, and S. Cesa, "Evaluation of different extraction methods from pomegranate whole fruit or peels and the antioxidant and antiproliferative activity of the polyphenolic fraction," *Food Chemistry*, vol. 202, pp. 59-69, 2016.
- [10] R. R. Mpahlele, O. A. Fawole, L. M. Mokwena, and U. L. Opara, "Effect of extraction method on chemical, volatile composition and antioxidant properties of pomegranate juice," *South African Journal of Botany*, vol. 103, pp. 135-144, 2016.
- [11] S. A. Zaki, S. H. Abdelatif, N. R. Abdelmohsen, and F. A. Ismail, "Phenolic Compounds and Antioxidant Activities of Pomegranate Peels," *International Journal of Food Engineering*, vol. 1, pp. 73-76, 2015.
- [12] G. Kalantzakis, G. Blekas, K. Pegklidou, and D. Boskou, "Stability and radical-scavenging activity of heated olive oil and other vegetable oils," *European Journal of Lipid Science and Technology*, vol. 108, pp. 329-335, 2006.
- [13] I. F. F. Benzie, and J. Strain, "The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay," *Anal. Biochem.*, vol. 239, pp. 70-76, 1996.
- [14] AOCS (American Oil Chemists' Society), "Method Cd 8-53. Peroxide value acetic acid-chloroform method" in *Official Methods and Recommended Practices of the American Oil Chemists' Society*, 4<sup>th</sup> ed., F. Gunstone Ed. Champaign, IL: AOCS Press, 1993.
- [15] IUPAC (International Union of Pure and Applied Chemistry), "Method number 2.504. Determination of the p-anisidine value (P-AV)," in *Standard Methods for the Analysis of Oils, Fats and Derivates*, 7<sup>th</sup> ed., C. Paquet and A. Hautefenne, Ed. Oxford, U.K.: Blackwell Scientific Publications, 1987, pp. 143-144.
- [16] IUPAC (International Union of Pure and Applied Chemistry), "Method number 2.505. Evidence of purity and deterioration from ultraviolet spectrometry," in *Standard Methods for the Analysis of Oils, Fats and Derivates*, 7<sup>th</sup> ed., C. Paquet and A. Hautefenne, Ed. Oxford, U.K.: Blackwell Scientific Publications, 1987, pp. 144-145.
- [17] T. Gutfinger, "Polyphenols in olive virgin oils," *Journal of American Oil Chemists' Society*, vol. 58, pp. 966-968, 1981.

- [18] J. L. Lamaison, and A. Carnat, "Teneurs en principaux flavonoids des fleurs de Crataegeus monogyna Jacq et de Crataegeus laevigata (Poiret D. C) en fonction de la vegetation," *Pharm. Acta Helv.*, vol. 65, pp. 315–320, 1990.
- [19] R. P. Singh, K. N. Chidambara Murthy, and G. K. Jayaprakash, "Studies on the Antioxidant Activity of Pomegranate (*Punica granatum*) Peel and Seed Extracts Using *in vitro* Models," *J. Agric. Food Chem.*, vol. 50, 81–86, 2001.
- [20] H. H. Orak, H. Yagar, and S. S. Isbilir, "Comparison of Antioxidant Activities of Juice, Peel, and Seed of Pomegranate (*Punica granatum* L.) and Inter-relationships with Total Phenolic, Tannin, Anthocyanin, and Flavonoid Contents," *Food Sci. Biotechnol.*, vol. 21, pp. 373–387, 2012.
- [21] Y. F. Li, C. J. Guo, J. J. Yang, J. Y. Wei, J. Xu, and S. Cheng, "Evaluation of antioxidant properties of pomegranate peel extract in comparison with pomegranate pulp extract," *Food Chem.*, vol. 96, pp. 254–260, 2006.
- [22] M. J. Kaneria, and S. V. Chanda, "The Effect of Sequential Fractionation Technique on the Various Efficacies of Pomegranate (*Punica granatum* L.)," *Food Anal. Methods*, vol. 6, pp. 164–175, 2013.
- [23] A. A. A. Mohdaly, M. A. Sarhan, A. Mahmoud, M. F. Ramadan, and I. Smetanska, "Antioxidant efficacy of potato peels and sugar beet pulp extracts in vegetable oils protection," *Food Chemistry*, vol. 123, pp. 1019–1026, 2010.
- [24] O. I. Aruoma, B. Halliwell, R. Aeschbach, and J. Loliger, "Antioxidant and prooxidant properties of active rosemary constituents: Carnosol and carnosic acid," *Xenobiotica*, vol. 22, pp. 257–268, 1992.