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Potential Application of Olive Mill Waste Extracts as Natural Antioxidants for Improving the Oxidative Stability of Fatty Foods

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ABSTRACT

In this study, polyphenols were extracted from olive mill wastes (OMWs) i.e. olive leaves, traditional waste, 3-phase waste, 2-phase waste and olive mill wastewater to valorize these vegetable biomass by evaluation of their capacity as natural antioxidants. Each of these extracts were added at concentration of 1000 ppm to sunflower oil and refrigerated minced meat to evaluate the anti-oxidative stability of these samples compared to those with BHT at 200 ppm and the control one. The sunflower oil stability was measured by accelerated oxidation at 60 °C/8 days. However, the refrigerated minced meat stability was measured at 5 °C/8 days. The change in the oxidation state was followed by measuring the refractive index, peroxide value, *p*-anisidine value and totox value for all samples. In addition, the thiobarbituric acid reactive substances (TBARS) were measured for refrigerated minced meat. From the results, it was found that sunflower oil samples and refrigerated minced meat treated with the polyphenol extracts of OMWs had undergone oxidative stability much more pronounced than that of the control one, and closed to that of reference sample with BHT.

Key words: Olive wastes – Polyphenols – Natural antioxidants – Oxidative stability – Fatty foods

Introduction

Recently two modern processes methods are used for the extraction of olive oil: the three-phase and the two-phase systems. Olive mill waste produced by the latter is composed of two main byproducts, an aqueous liquid known as olive mill wastewater (OMWW) and a solid waste (pomace). The newer two-phase system produces only a semisolid waste known as "*alperujo"* (Lesage-Meessen *et al.* 2001 and Fernandez-Bolanos *et al.* 2005).

Olive mill and olive processing residues are attractive sources of natural antioxidants. An important part of these residues is olive tree leaves (usually 5%, but possibly reaching up to 10% of the total olives' weight depending on practices applied). In addition, during olive tree cultivation, the pruning step generates a considerable volume of olive leaves, which are usually used as animal feed, and which could also be used for antioxidant or olive-leaf extract production (Fki *et al.* 2005; Guinda 2006; De Leonardis *et al.* 2008; Lafka *et al.* 2011; Taamalli *et al.* 2012 and Zahran *et al.* 2015).

Vegetable edible oils and fats are recognized as important components of our diet. They provide linoleic and linolenic essential fatty acids, which are precursors of important hormones, such as prostaglandins and control many physiological factors such as blood pressure, cholesterol level and the reproductive system (Walisiewicz-Niekbalska *et al.* 1997). Lipid peroxidation is responsible for the quality deterioration of oils and fats (Kazuhisa 2001). On the other hand, Che-Man and Tan (1999) reported that the consumers do not accept oxidized products and industries suffer from economic loss. The soft oils (as sunflower oil and soybean oil) are more susceptible to oxidation.

Lipid oxidation plays an important role in the development of undesirable flavors commonly known as ‘‘warmed-over-flavor" and in the formation of toxic carcinogenic compounds in cooked meat products. For many years, meat processors used synthetic antioxidants like butyl hydroxyanisol (BHA) or butyl hydroxytoluene (BHT) to prevent, or reduce, flavor deterioration. However, concerns about safety and consumer's preference for more natural foods have resulted in a high demand for ‘‘natural" additives, which can extend the shelf life of both processed and unprocessed meat products (Sharon and Maria 2009).

In addition, lipid oxidation can have negative effects on the quality of meat and meat products causing changes in sensory attributes (color, texture, odor, and flavor) and nutritional quality (Decker and Mei 2009; Rababah *et al.* 2004). One method to reduce lipid oxidation is the application of antioxidants. Natural antioxidants are widely used in meat products to reduce or prevent oxidation and have an ability to counteract

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damaging effects of free radicals in tissues and thus are believed to protect against cancer, arteriosclerosis, heart disease and several other diseases (Barlow 1990).

The objective of the present study is to shed light on the effect of natural antioxidants extracted from olive mill wastes on oxidative stability and shelf life of edible oils and refrigerated minced meat.

Materials and Methods:

Materials:

Refined, bleached and deodorized (RBD) sunflower oil free from additives was kindly supplied from Cairo Company for Oils and Soap, Al-Ayat, Giza, Egypt. The oil was stored at 5°C until laboratory investigation. Fresh beef meat and tallow were obtained from local market, Cairo, Egypt. Solid wastes, wastewater and olive leaves were obtained during 2013 harvest season from the Quality Standard Company, El-Sadat City, Menofiya, Egypt. All chemicals and reagents used in the analytical methods were analytical grade and obtained from Merck Darmstadt, Germany.

Methods:

Preparation of olive mill wastes extracts:

Preparation of olive mill waste extracts were carried out according to Theodora-Ioanna *et al.* (2011), with some modifications using water/ethanol at ratio of 1:1 for 30 min with ultrasound-assisted extraction method.

Oxidative stability of sunflower oil (oven test):

One hundred grams of RBD sunflower oil were individually treated with each of olive leave extract, 3-phase wastes extract, 2-phase wastes extract, traditional wastes extract and OMWW extract at concentration of 1000 ppm. (The OMWs extracts were dissolved in polypropylene glycol before addition to the oil). The oil treated samples as well as a reference sample with synthetic antioxidant (BHT) at concentration 200 ppm were incubated at 60 °C for 8 days according to the modified Schaale oven test and control sample without antioxidants. PV and *p*-AV were measured every two days.

Oxidative stability of refrigerated minced meat:

Beef semi membranous muscle (top round) purchased locally was trimmed of all visible extra muscular fat, then ground via a 4.5 m/m head on a model Ts-285 grinder (Ta-sin Ltd., Taichung City, Taiwan) with 25% of fresh tallow. One hundred grams of minced meat were treated with a concentration of 1000 ppm of each of olive leave extract, 3-phase wastes extract, 2-phase wastes extract, traditional wastes extract and OMWW extract. The reference sample treated with synthetic antioxidant (BHT) at concentration 200 ppm, and the control sample without antioxidants were also conducted. Each additive was added to the minced meat and mixed for 1 min in a *Moulenix* mixer. Minced meat samples were individually packed in polyethylene bags and stored in refrigerator at 5 °C. Lipid oxidation measurement was carried out every 24 h for 8 days.

Physico-chemical quality analysis:

The refractive indexes (RI) and peroxide value (PV) of oil samples were determined according to the methods described by AOAC (2005). *p*-Anisidine value (*p*-AV) was determined spectrophotometrically using the standard method 2504 IUPAC (IUPAC 1987) using an Genesys 10 UV-Vis Spectrophotometer. The Totox value was calculated according to the equation, $totox = p\text{-}AV + 2PV$. Thiobarbituric acid (TBA) was determined according to the method of Pearson (1976).

Statistical analysis:

Results are representing the average and statistical significance of the differences between mean values was assessed by ANOVA; ($p \leq 0.05$) was considered statistically significant. The data obtained were exposed to proper statistical analysis according to Statistical Package for the Social Sciences (SPSS) Ver. 20.0 (2011).

Results and Discussion

The effect of OMWs extracts on the oxidative stability of sunflower oil (oven test):

As shown in Table (1), the refractive index of all oil samples, increased during incubation period through the eight days at 60 ˚C of incubation. It was noticed that the refractive indices of the reference oil sample treated with BHT, as well as sunflower oil samples treated with OMWs extracts were lower than the values of control sample (sunflower oil without any addition).

From the results, it was clear that the differences in the values of refractive index between the treatments and control sample may be referred to the existence of a secondary oxidation product in the control sample. It was noticed that in oil samples treated with olive extracts, the refractive indices were exceeded that of the reference sample treatment with BHT, while in the manners of the oil treated with 2-phase extract the RI

values were nearly similar to those of the reference samples. It was observed that the olive waste extracts had an antioxidant effect. These results were in agreement with obtained by Amro *et al.* (2002).

Table 1: Changes in refractive index of sunflower oil treated with different OMWs extracts during incubation at 60 ˚C/8 days

Oil samples	Refractive index at *(20 ˚C)*				
	Period per day				
	0	2	4	6	8
RBD sunflower oil (Control)	1.46606	1.46612	1.46625	1.46641	1.46652
Oil + BHT (200 ppm)	1.46601	1.46609	1.46617	1.46628	1.46634
Oil + leave extract (1000 ppm)	1.46602	1.46608	1.46618	1.46629	1.46635
Oil + traditional waste ext. (1000 ppm)	1.46603	1.46611	1.46618	1.46632	1.46640
Oil + 3-phase waste ext. (1000 ppm)	1.46602	1.46612	1.46623	1.46630	1.46643
Oil + 2-phase waste ext. (1000 ppm)	1.46603	1.46610	1.46625	1.46636	1.46651
Oil + OMWW ext. (1000 ppm)	1.46604	1.46609	1.46621	1.46636	1.46650

The evaluation of oxidative stability of sunflower oil treated with OMWs extracts by determination of peroxide value was presented in Table (2). The presence of unsaturated fatty acids together with low concentrations of antioxidants in sunflower oil promoted the susceptibility of oils to oxidation (Quiles *et al.* 2002). The increase of incubation time resulted to increase peroxide values in all samples. The peroxide values rate of treated sunflower oil samples with OMWs extracts were markedly lesser than those of the control oils until 8 days. It was clear that, the synthetic BHT exhibited the highest antiradical activity, followed by OMWs extracts, whereas it showed statistically similar ($P \leq 0.05$) oxidative stability in sunflower oil till the fourth day. The formation of peroxides after 6 days of incubation, were reduced in treated oil samples compared to control and ranged from 57.87 to 63.05 %. These data are in accordance with those reported by De Leonardis *et al.* (2007).

Table 2: Changes in peroxide value of sunflower oil treated with OMWs extracts during incubation at 60 ˚C/8 days

Oil samples	Peroxide value *(meq./kg)**				
	Period per day				
	0	2	4	6	8
RBD sunflower oil (control)	0.13^{a}	3.73^{c}	23.46^{b}	49.06^{d}	103.80^{c}
Oil + BHT (200 ppm)	0.13^{a}	2.03^{a}	8.79^{a}	17.38^{a}	70.21^{a}
Oil + leave extract (1000 ppm)	0.13^{a}	2.63^{b}	9.51^{a}	18.13^{ab}	79.12^{b}
Oil + traditional waste ext. (1000 ppm)	0.13^{a}	2.68^{b}	10.40^{a}	18.89^{b}	78.97^{b}
Oil + 3-phase waste ext. (1000 ppm)	0.13^{a}	2.76^{b}	9.53^{a}	20.36^{c}	81.16^{b}
Oil + 2-phase waste ext. (1000 ppm)	0.13^{a}	2.11^{a}	9.75^{a}	19.17^{b}	81.10^{b}
Oil + OMWW ext. (1000 ppm)	0.13^{a}	2.51^{b}	9.19^{a}	20.67^{c}	81.09^{b}

** Means with the same letter in the same column are not significant at ($p \leq 0.05$)*

As a measure of secondary oxidation products (hydroperoxide degradation), *p*-anisidine value of oil samples after 2 and 4 days went more or less in the same trend. Where, there was no significant ($p \leq 0.05$) difference between OMWs extracts among themselves and synthetic antioxidants (BHT), while significant ($p \leq 0.05$) difference was found between the treatments and the control sample. After 8 days there were no significant ($p \leq 0.05$) differences between the OMWs extracts and reference antioxidant (BHT) except samples treated with olive leaves extract. While, significant ($p \leq 0.05$) decreases were observed in *p*-anisidine values of oils treated with OMWs extracts at every incubation period compared to control sample as shown in Table (3). In this respect, Zahran *et al.* (2015) concluded that olive leaves extract at concentration of 1000 ppm was effectively stabilized sunflower, soybean oils and their blend as compared with BHT at 200 ppm during the deep-frying at 180 ± 5 °C. Also, they recommended that the olive leave extract can be use as a potent source of antioxidants for the stabilization of oil food systems, especially soft edible oils.

Table 3: Changes in *p*-anisidine value of sunflower oil treated with OMWs extracts during incubation at 60 ˚C/8 days

Oil samples	*p*-Anisidine value*				
	Period per day				
	0	2	4	6	8
RBD sunflower oil (Control)	6.79^{a}	7.24^{a}	8.22^{b}	9.81^{d}	11.12^{c}
Oil + BHT (200 ppm)	6.66^{a}	6.73^{a}	6.86^{a}	6.56^{a}	7.12^{a}
Oil + leave extract (1000 ppm)	6.43^{a}	6.57^{a}	6.83^{a}	7.02^{b}	8.88^{b}
Oil + traditional waste ext. (1000 ppm)	6.55^{a}	6.37^{a}	7.01^{a}	7.01^{b}	7.35^{a}
Oil + 3-phase waste ext. (1000 ppm)	6.64^{a}	6.52^{a}	6.58^{a}	7.20^{bc}	7.59^{a}
Oil + 2-phase waste ext. (1000 ppm)	6.49^{a}	6.40^{a}	6.85^{a}	7.14^{bc}	7.49^{a}
Oil + OMWW ext. (1000 ppm)	6.54^{a}	6.41^{a}	6.73^{a}	7.47^{c}	7.14^{a}

** Means with the same letter in the same column are not significant at ($p \leq 0.05$)*

The totox value measured the total oxidation state of the oil, so it reflects the sum of PV and *p*-AV (i.e. primary and secondary oxidation products). The obtained results in Table (4) revealed that totox values after 8

days of incubation were in the decreasing order: control > 3-ph > 2-ph > OMWW > traditional > olive leaves extract > BHT and that there were significant ($p \leq 0.05$) differences between the totox values of oils treated with OMWs extracts and the reference antioxidant being lower in the later, while, there was no significant ($p \leq 0.05$) difference between totox values of oils treated with OMWs extracts among themselves. In the manner of reference sample, there were also no significant ($p \leq 0.05$) differences between its totox values and of those of treated oils with OMWs extracts after four days of incubation. However, significant ($p \leq 0.05$) lesser values were observed in totox values of oils treated with OMWs extracts compared to control sample.

Table 4: Changes in totox value of sunflower oil treated with OMWs extracts during incubation at 60˚C/8 days

Oil samples	Totox value*				
	Period per day				
	0	2	4	6	8
RBD sunflower oil (Control)	7.05[a]	14.70[b]	55.13[b]	107.92[d]	218.72[c]
Oil + BHT (200 ppm)	6.92[a]	10.80[a]	24.44[a]	41.31[a]	147.55[a]
Oil + leave extract (1000 ppm)	6.69[a]	11.84[a]	25.84[a]	43.27[ab]	167.11[b]
Oil + traditional waste ext. (1000 ppm)	6.80[a]	11.73[a]	27.81[a]	44.79[b]	165.29[b]
Oil + 3-phase waste ext. (1000 ppm)	6.90[a]	12.04[a]	25.63[a]	47.91[c]	169.92[b]
Oil + 2-phase waste ext. (1000 ppm)	6.75[a]	10.62[a]	26.35[a]	45.47[b]	169.70[b]
Oil + OMWW ext. (1000 ppm)	6.80[a]	11.43[a]	25.11[a]	48.80[c]	169.32[b]

[*] *Means with the same letter in the same column are not significant at ($p \leq 0.05$)*

Effect of OMWs extracts on lipid peroxidation of minced meat during storage at 5 °C/8 days

Minced meat was treated with OMWs extracts at a concentration of 1000 ppm as natural antioxidants compared with minced meat treated by synthetic antioxidant (BHT). The oxidative stability of treated minced meat during refrigerated storage up to 8 days at 5 ºC was evaluated using thiobarbituric acid (TBA) assay.

The evaluation of the oxidative stability of minced meat treated with OMWs extracts by determination of peroxide value is presented in Table (5). The increase of storage period resulted in increasing peroxide values in all the samples. It was noticed that the peroxide values of minced meat lipids treated with BHT and OMWs extracts decreased after 6 days, and then continued to increase after 8 days. The synthetic BHT exhibited the highest oxidative stability compared to OMWs extracts. Regarding to control sample, the formation of peroxides, after 2, 4, 6 and 8 days of storage, were reduced by 29.0-37.5, 31.1-34.0, 30.6-42.7 and 30.5-34.4% in the minced meat samples, respectively, when added of OMWs extracts at concentration of 1000 ppm.

This results may be attributed to the amount of hydroxyl groups within the phenolic structures of constituents present in crude olive mill extracts mainly hydroxytyrosol and oleuropein. It is assumed that inhibition of lipid oxidation and hydrogen donor ability is enhanced with the increasing amount of hydroxyl groups (McDonald *et al.* 2001).

Table 5: Changes in peroxide value of minced meat treated with OMWs extracts during storage at 5 °C/8 days

Meat samples	Peroxide value *(meq./kg)**				
	Period per day				
	0	2	4	6	8
Minced meat (M.M.) (Control)	2.42[a]	6.99[c]	8.00[c]	8.05[c]	9.21[c]
M.M. + BHT (200 ppm)	2.39[a]	4.17[a]	4.39[a]	3.85[a]	5.49[a]
M.M. + leave extract (1000 ppm)	2.46[a]	4.39[a]	5.28[b]	4.61[a]	6.40[b]
M.M. + traditional waste ext. (1000 ppm)	2.42[a]	4.37*a*	5.38[b]	5.56[b]	6.08[b]
M.M. + 3-phase waste ext. (1000 ppm)	2.47[a]	4.94[b]	5.29[b]	4.88[a]	6.04[b]
M.M. + 2-phase waste ext. (1000 ppm)	2.48[a]	4.48[a]	5.42[b]	5.40[b]	6.05[b]
M.M. + OMWW ext. (1000 ppm)	2.41[a]	4.96[b]	5.51[b]	5.59[b]	6.28[b]

[*] *Means with the same letter in the same column are not significant at ($p \leq 0.05$)*

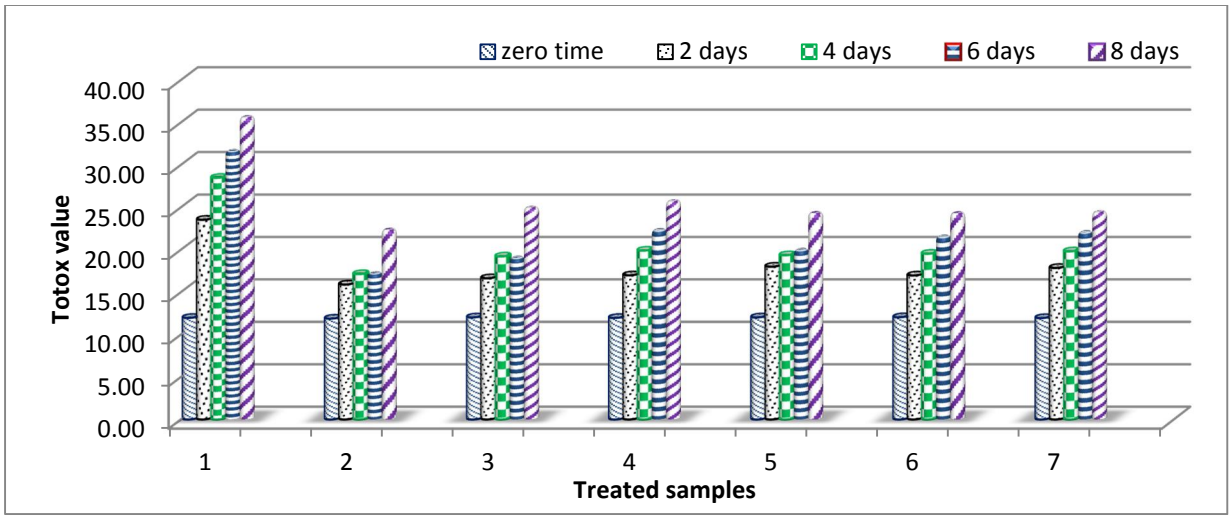
Table (6) shows the changes occurred in the *p*-AV of the control and antioxidant treated minced meat samples during storage up to 8 days at 5 °C. The results indicated that the *p*-AV of all samples increased with increasing the storage period. The control sample exhibited the highest values. Also, it was cleared that the *p*-AV of samples containing OMWs natural extracts were closed to those of reference sample containing synthetic antioxidant, while significant ($p \leq 0.05$) lower values were observed between *p*-AV of samples containing OMWs extracts compared to control sample. Also there were significant differences in OMWs extracts among themselves. These data are in accordance with those reported by Dejong and Lanari (2009).

Peroxide value and *p*-anisidine value may be combined to form total oxidation or "Totox" value. The lower the totox value, the better the quality of oil (Carol and Edward 1999). The changes occurred in the totox value of the control and antioxidant treated minced meat samples during storage are shown in Fig (1). It could be noticed that the totox value of all samples increased with increasing the storage period. The results took the same trend of peroxide value and p-anisidine value of control and treated samples.

Table 6: Changes in *p*-anisidine value of minced meat treated with OMWs extracts during storage at 5 °C/8 days

Meat samples	*p*-Anisidine value*				
	Period per day				
	0	2	4	6	8
Minced meat (M.M.) (Control)	7.12^{a}	9.55^{c}	12.47^{c}	15.46^{d}	17.11^{c}
M.M. + BHT (200 ppm)	7.09^{a}	7.56^{a}	8.34^{a}	9.46^{a}	11.28^{a}
M.M. + leave extract (1000 ppm)	7.11^{a}	7.85^{a}	8.64^{a}	9.77^{a}	12.08^{ab}
M.M. + traditional waste ext. (1000 ppm)	7.13^{a}	8.24^{b}	9.14^{b}	11.10^{c}	13.46^{b}
M.M. + 3-phase waste ext. (1000 ppm)	7.08^{a}	8.12^{b}	8.76^{a}	10.14^{bc}	12.15^{ab}
M.M. + 2-phase waste ext. (1000 ppm)	7.09^{a}	8.01^{b}	8.69^{a}	10.66^{bc}	12.11^{ab}
M.M. + OMWW ext. (1000 ppm)	7.12^{a}	7.94^{ab}	8.78^{a}	10.82^{bc}	11.79^{ab}

** Means with the same letter in the same column are not significant at ($p \leq 0.05$)*



Where: 1= RBD sunflower oil (control), 2= Oil + BHT (reference), 3= Oil + leave extract, 4= Oil + traditional waste ext., 5= Oil + 3-phase waste ext., 6= Oil + 2-phase waste ext. and 7= Oil + OMWW ext.

Fig. 1: Totox value of refrigerated minced meat treated with different OMWs extracts during storage at 5 °C/8 days.

Table (7) shows the TBARS (thiobarbituric acid reactive substances) values of the treated minced meat samples. From the obtained results it could be noticed that, the TBARS values of all treated meat samples and the control were gradually increased during storage period. Samples treated with BHT and OMWs extracts showed significant ($p \leq 0.05$) lower values than of the control sample. It was clearly noticed that there were significant ($p \leq 0.05$) differences between TBARS values of treated meat with OMWs extracts and with reference synthetic antioxidant being lesser in the later treated with BHT**.** Also there were significant differences between TBARS values of treated meat with OMWs extracts among themselves. The obtained results are in agreement with those reported by Dejong and Lanari (2009).

Table 7: Changes in TBARS content of minced meat treated with OMWs extracts during storage at 5 °C/8 days

Meat samples	TBARS *(mg MAD/kg)**				
	Period per day				
	0	2	4	6	8
Minced meat (M.M.) (Control)	0.527^{a}	0.909^{c}	1.466^{c}	2.991^{c}	3.631^{d}
M.M. + BHT (200 ppm)	0.538^{a}	0.667^{a}	0.975^{a}	1.615^{a}	1.817^{a}
M.M. + leave extract (1000 ppm)	0.538^{a}	0.776^{b}	1.076^{a}	1.962^{b}	2.145^{b}
M.M. + traditional waste ext. (1000 ppm)	0.542^{a}	0.800^{b}	1.108^{a}	1.985^{b}	2.153^{b}
M.M. + 3-phase waste ext. (1000 ppm)	0.538^{a}	0.803^{b}	1.053^{a}	1.981^{b}	2.473^{c}
M.M. + 2-phase waste ext. (1000 ppm)	0.538^{a}	0.878^{c}	1.119^{b}	1.884^{b}	2.527^{c}
M.M. + OMWW ext. (1000 ppm)	0.546^{a}	0.788^{b}	1.080^{a}	1.950^{b}	2.402^{c}

** Means with the same letter in the same column are not significant at ($p \leq 0.05$)*

Conclusion

From the obtained results, it could be concluded that OMWs are a valuable resources of bio-active compounds as a natural antioxidants. The olive waste extracts at 1000 ppm can effectively stabilize sunflower oil as compared with BHT at 200 ppm, which were superior in protecting sunflower oil during the incubation at 60 °C for 8 days, since it improved resistance of sunflower oil against thermal deteriorative changes. In addition, the polyphenol extract from the OMWs significantly inhibited lipid oxidation in beef minced meat. Therefore,

OMWs extracts can be recommended as a potent source of antioxidants for the stabilization of fatty food systems, especially soft vegetable oils.

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