

## Investigation of the Effect of Olive Leaf and Clove Extracts Mixture on the Stability of Sunflower Oil During Repeated Deep Frying of Potatoes

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### Abstract

In this study, in order to increase the stability of the sunflower oil; hazelnut oil, sesamol, olive oil, basil oil, black seed oil, olive leaf extract, clove extract, rosemary extract and thyme oleoresin were investigated. The blend of olive leaf extract and clove extract in hazelnut oil (M28) was chosen as the sample showing the highest antioxidant capacity during deep frying of potatoes. According to  $\beta$ -Carotene-Linoleic Acid assay system, the antioxidant capacities of M28 were measured as  $61.52 \pm 3.28\%$  and  $54.35 \pm 1.19\%$ , after 120 and 960 minutes, respectively. Similarly, the antioxidant capacities of hazelnut oil and BHA mixtures were detected as  $67.64 \pm 2.64\%$  and  $49.09 \pm 1.55\%$  for the same time intervals. Peroxide values showed that, after deep frying of potatoes in  $190^\circ\text{C}$ , oxidation time of the sunflower oil was enhanced by 20% after the donation of 15% of M28.

**Keywords:** Deep frying;  $\beta$ -carotene-linoleic acid assay; Olive leaf extract; Clove extract; Peroxide value

### Introduction

Due to the increase in the working population and the change in people's life standards, worldwide eating habits were varied and fast foods gained interest. The main components of the fast food are mainly fats, oils and carbohydrates. When these components in foods meet oxygen in the air during frying at  $150^\circ\text{C}$ - $250^\circ\text{C}$ , they get into autoxidation which brings out peroxides and hydroperoxides [1]. These peroxides turn into aldehydes, ketones, epoxides, dimers and polymers [2].

Releasing of the peroxides and harmful components not only leads to undesired food taste and odour, but also damages the quality of the food and causes health hazards in tissues and cardiovascular system [3]. In order to prevent these undesired affects and to cease the lipid oxidation reactions, various compounds can be added into the frying oils as thermally stable synthetic antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and tertiary butylated hydroquinone (TBHQ) [4]. However, commercially used synthetic antioxidants have been strictly controlled therefore people are more aware against the use of these food additives and they are interested in the use of natural antioxidants.

Studies have proved that, natural extracts of herbs and spices show antioxidant activities and stabilizing properties in fats and oils. Gamel and Kiritsakis proved the antioxidant capacity of the extracts of dry rosemary leaves and olive vegetable water filtrate combined with BHA on olive oil and sunflower oil during frying process [5]. The antioxidant capacity of oleoresin rosemary and sage extract combination was studied widely and it was introduced that oleoresin rosemary showed higher antioxidant capacity than BHA while sage showed higher activity than BHT [3]; furthermore the combination lowered the rate of oxidation of oil during deep frying and improved the sensory properties of the potato chips [6]. Bitar showed that p-cymene 2,3-diol extracted from thyme could be a potential replacement for synthetic antioxidants [7] while summer savory (*Satureja hortensis* L.) extract was introduced to be a stabilizer for the unsaturated lipids in sunflower oil against autoxidation [4]. Poiana, on the other hand, observed grape seed extract as an antioxidant in oils by inhibiting the lipid oxidation in high temperatures [8].

Besides, there is an increasing interest in the effects of olive leaf

extract and clove extract on the stability of sunflower oil. The olive tree, *Olea europaea* L., is naturally grown in Mediterranean region and is a kind of Oleaceae family. Oleuropein is a major bioactive component of olive leaf extract while it also contains a wide variety of phenolic compounds like oleuropein, hydroxytyrosol, tyrosol, cumaric acid, ferulic acid and caffeic acid. Olive leaf extract was examined and showed antioxidant activity in sunflower oil at  $98^\circ\text{C}$  [9]. Clove (*Syzygium aromaticum* L.), on the other hand, is a kind of Myrtaceae family and widely used in medicinal applications especially in dental care. Clove contains a wide variety of bioactive compounds like sesquiterpenes and triterpenoids [10]. On the other hand, rosemary extract (E392) and sesamol have long been used as antioxidants. In recent years, basil essential oil additionally showed antioxidant effect when added into frying oils. Therefore, in this study; hazelnut oil, sesamol, olive oil, basil oil, black seed oil, olive leaf extract, clove extract, rosemary extract and thyme oleoresin were studied since there are limited studies on this subject. They were observed to improve the stability of the sunflower oil during deep frying of potatoes.

### Materials and Methods

#### Materials

The industrial type sunflower oil was supplied from a commercial company. Household type sunflower oil, hazelnut oil, olive oil and frozen potatoes were purchased from a local market. BHA, BHT and alpha tocopherol were purchased from Sigma-Aldrich. Deep fryer was purchased from a market whose brand is Arzum and natural extracts and essential oils were supplied from Aromsa A.S. Analytical grade reagents and solvents were supplied from Merck.

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Code	Basil Oil	Olive Leaf Extract	Sage Extract	Clove Extract	Rosemary Extract	BHA	BHT	α-Tocopherol
M1 <sup>a</sup>	50	50	-	-	-	-	-	-
M2 <sup>b</sup>	50	50	-	-	-	-	-	-
M3 <sup>a</sup>	-	-	50	50	-	-	-	-
M4 <sup>b</sup>	-	-	50	50	-	-	-	-
M5 <sup>a</sup>	50	-	50	-	-	-	-	-
M6 <sup>b</sup>	50	-	50	-	-	-	-	-
M7 <sup>a</sup>	-	-	-	-	-	100	-	-
M8 <sup>b</sup>	-	-	-	-	-	100	-	-
M9 <sup>a</sup>	-	-	-	-	-	-	100	-
M10 <sup>b</sup>	-	-	-	-	-	-	100	-
M11 <sup>a</sup>	-	-	-	-	-	-	-	100
M12 <sup>b</sup>	-	-	-	-	-	-	-	100
M13 <sup>a</sup>	-	40	20	-	40	-	-	-
M14 <sup>b</sup>	-	40	20	-	40	-	-	-
M15 <sup>a</sup>	30	70	-	-	-	-	-	-
M16 <sup>a</sup>	-	50	50	-	-	-	-	-
M17 <sup>a</sup>	25	50	25	-	-	-	-	-
M18 <sup>b</sup>	-	70	30	-	-	-	-	-
M19 <sup>a</sup>	30	70	-	-	-	-	-	-
M20 <sup>a</sup>	-	50	50	-	-	-	-	-
M21 <sup>a</sup>	25	50	25	-	-	-	-	-
M22 <sup>a</sup>	-	100	-	-	-	-	-	-
M23 <sup>a</sup>	-	-	100	-	-	-	-	-
M24 <sup>a</sup>	100	-	-	-	-	-	-	-
M25 <sup>a</sup>	-	20	80	-	-	-	-	-
M26 <sup>a</sup>	-	80	20	-	-	-	-	-
M27 <sup>a</sup>	-	30	70	-	-	-	-	-
M28 <sup>a</sup>	-	70	30	-	-	-	-	-

<sup>a</sup>The extracts were diluted by 50 g of hazelnut oil.

<sup>b</sup>The extracts were diluted by 50 g of olive oil.

**Table 1:** Amounts of extracts (mg) in the carrier oils.

## Sample preparation

The mixtures were prepared by dissolving different concentrations of each extract in the carrier oils (hazelnut oil and olive oil) and they were coded as M1, M2, M3,... M28 (Table 1). All the samples were stored at 0-4°C until being used.

## Methods

**β-Carotene-Linoleic acid system model:** Antioxidant capacity of the extracts can also be measured by β-carotene-linoleic acid system model [11]. Firstly the β-carotene solution was prepared. For this, 2.5 mg of β-carotene was dissolved in 5 mL of chlorophorm then 125 μL of linoleic acid and 1 g of Tween40 were mixed with the solution in a flask. Chlorophorm was evaporated at 40°C under vacuum and the residue was diluted to 500 mL by oxygenated water.

2000 ppm of samples of extracts, oils and synthetic antioxidant standards were prepared in ethanol. 700 μL were taken from each sample and 5 mL of β-carotene-linoleic acid solution was added. The samples were then diluted to 6 mL by ethanol and they were stored in a 50°C water bath (Nuve BM402 Model, Turkey) for 120 min before immediately measuring the zero time absorbance (T<sub>0</sub>) at 470 nm. After 2 and 16 hours of storage, absorbance values (T<sub>120</sub> and T<sub>960</sub>, respectively) of the extracts were similarly detected in 470 nm. A mixture prepared as above without β-carotene served as control sample (T<sup>0</sup>). Antioxidant capacity was determined as:

$$\text{Antioxidant Capacity (\%)} = \left[ 1 - \frac{T_0 - T_{120}}{T_0 - T_{120}^0} \right] \times 100$$

**Frying process:** Different concentrations of extracts, oils and synthetic antioxidants were prepared in the sunflower oil (100-2000 ppm). 400 ± 20 g of frozen potatoes were added into 2 ± 0.2 L of sunflower oil for the frying process at 190°C ± 5°C for 12 min in stainless steel home type deep fryer (Arzum, Cipsco). Before starting each frying cycle, the necessary amount of fresh oil with antioxidant was provided to the same oil level of the fryer.

**Peroxide value (PV):** The rancidity of the sunflower oil was defined by the amount of peroxides and the concentration of the peroxides were detected based on the AOAC official method for the fats and oils with the equation [12]:

$$\text{PV} \left( \frac{\text{mEq peroxide}}{\text{kg sample}} \right) = S * N * \frac{1000}{\text{g Sample}}$$

Where,

S: mL Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> (blank correlated) and N: Normality of Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> solution.

PV was expressed as miliequivalent (meq) O<sub>2</sub> per Kg oil. The determinations were carried out in triplicate. Peroxide values of sunflower oil were measured before the frying process (t<sub>0</sub>) and at the end of each frying process (t<sub>1</sub>, t<sub>2</sub>, t<sub>3</sub>,... etc.).

## Statistical analysis

All the data of all antioxidant capacity and peroxide value tests are mean values of triplicate analyses. The data were recorded as mean ± standard deviation. Analysis of variance was performed by ANOVA

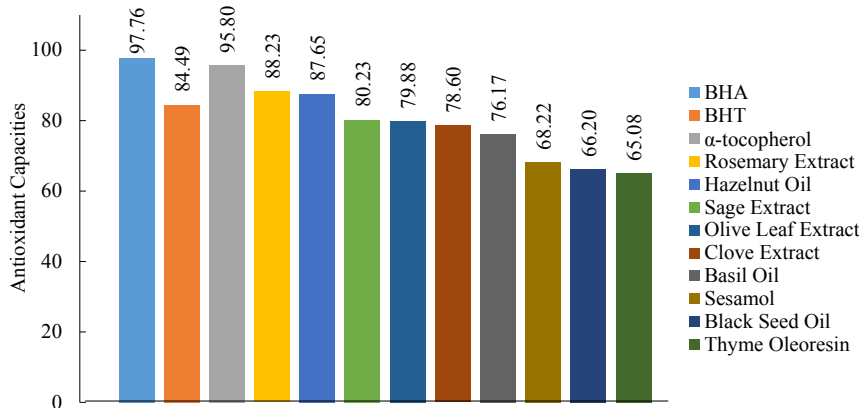


Figure 1: Antioxidant capacities measured after 120 min (T<sub>120</sub>).

Code	T <sub>120</sub> AA%	T <sub>960</sub> AA%
M1 <sup>a</sup>	52.96 ± 0.67	38.34 ± 1.11
M2 <sup>b</sup>	35.56 ± 0.90	34.09 ± 1.71
M3 <sup>a</sup>	55.07 ± 2.01	26.87 ± 2.26
M4 <sup>b</sup>	66.19 ± 1.47	36.45 ± 1.73
M5 <sup>a</sup>	43.16 ± 1.67	na
M6 <sup>b</sup>	43.32 ± 1.65	na
M7 <sup>a</sup>	67.54 ± 2.35	48.38 ± 1.15
M8 <sup>b</sup>	50.77 ± 1.23	na
M9 <sup>a</sup>	57.25 ± 0.75	34.96 ± 0.67
M10 <sup>b</sup>	61.88 ± 2.13	32.98 ± 0.98
M11 <sup>a</sup>	43.05 ± 2.06	14.63 ± 0.87
M12 <sup>b</sup>	51.77 ± 1.17	34.33 ± 1.02
M13 <sup>a</sup>	62.78 ± 1.71	na
M14 <sup>b</sup>	69.21 ± 1.65	na

T<sub>120</sub>: Analysis after 120 min.

T<sub>960</sub>: Analysis after 960 min.

na: Antioxidant capacity was not detected.

AA: Antioxidant activity.

<sup>a</sup>The extracts were diluted by 50 g of hazelnut oil.

<sup>b</sup>The extracts were diluted by 50 g of olive oil.

\*Values expressed are means ± SD of three parallel measurements (p<0.05).

Table 2: β-carotene-linolenic acid assay results of M1-M14.

Code	T <sub>120</sub> AA%	T <sub>960</sub> AA%
M15 <sup>a</sup>	51.33 ± 2.86	26.85 ± 0.26
M16 <sup>a</sup>	37.76 ± 1.26	28.85 ± 0.80
M17 <sup>a</sup>	69.59 ± 4.80	39.64 ± 0.38
M18 <sup>b</sup>	39.90 ± 1.68	29.35 ± 1.53
M7 <sup>a</sup>	68.11 ± 1.67	45.28 ± 1.14
M9 <sup>a</sup>	56.95 ± 0.87	33.99 ± 0.71
M10 <sup>b</sup>	61.86 ± 2.18	33.12 ± 1.01
M12 <sup>b</sup>	49.85 ± 1.18	32.83 ± 0.97

T<sub>120</sub>: Analysis after 120 min

T<sub>960</sub>: Analysis after 960 min

AA: Antioxidant activity

<sup>a</sup>The extracts were diluted by 50 g of hazelnut oil.

<sup>b</sup>The extracts were diluted by 50 g of olive oil.

\*Values expressed are means ± SD of three parallel measurements (p < 0.05).

Table 3: β-carotene-linolenic acid assay for M7, M9, M10, M12 and M15-M18.

procedures. Significant differences between means were determined by One-way ANOVA (Tukey Test), p values smaller than 0.05 (p < 0.05) were regarded as significant.

## Results and Discussion

Diraman and Hisil indicated that spices; especially rosemary and

peppermint in olive oil, rosemary and thyme in refined hazelnut oil and thyme in mixed vegetable oil, significantly increased the oxidative stability [13]. Additionally, clove extract, additionally showed antioxidant capacity in lipid and protein oxidation [14,15]. In this study, antioxidant capacities of sage extract, hazelnut oil, sesamol, olive oil, olive leaf extract, thyme oleoresin, basil oil, clove extract, black seed oil and rosemary extract with BHA, BHT and α-tocopherol were detected by β-carotene-linolenic acid assay. The results were plotted as in Figure 1. There are small differences between the antioxidant capacities of the samples, carrier oils and the references (BHT, BHA and α-tocopherol). The antioxidant capacity results of basil oil, olive leaf extract, sage extract, clove extract and rosemary extract are close to the results of the synthetic antioxidants and higher than the other samples (sesamol, thyme oleoresin and black seed oil) except carrier oils.

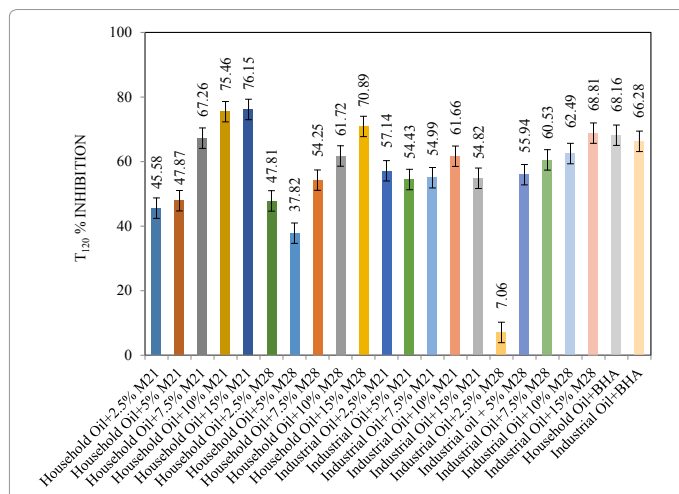
Since organoleptic problems were observed when the extracts were individually added into the sunflower oil, the extracts were decided to be mixed with hazelnut oil and olive oil. Antioxidant capacities of oils and extracts were proved to show a synergistic effect by β-carotene-linolenic acid assay as given in Table 2. As it can be seen, after 120 min, the mixtures of all the samples showed antioxidant capacity; however, after 960 min, no antioxidant capacity was performed for the samples M5 (basil oil and sage extract in hazelnut oil), M6 (basil oil and sage extract in olive oil), M8 (BHA in olive oil), M13 (olive leaf extract, sage extract and rosemary extract in hazelnut oil) and M14 (olive leaf extract, sage extract and rosemary extract in olive oil). According to Table 2, M1 and M2 performed higher antioxidant capacities after 960 min (38.34 ± 1.11 and 34.09 ± 1.71%, respectively) than the synthetic antioxidants. Therefore M1, M2 and new mixtures coded from M15 to M18 were analysed for β-carotene-linolenic acid assay tests and the results are given in Table 3. Table 3 introduces that M17 (basil oil, clove extract and olive leaf extract) performed improved antioxidant capacity after 960 min (39.64 ± 0.38%) compared with M1, M2 and oil mixtures with synthetic antioxidants. M1, M2 and M17 are the mixtures of basil oil and olive leaf extract and M17 includes clove extract. Therefore, new mixtures, coded from M19 to M28 were prepared including basil oil, olive leaf extract and clove extract. The antioxidant capacity results of this study are presented in Table 4.

In Table 4, it is clearly seen that M21 and M28 performed the highest activities after 960 min (41.64 ± 0.38 and 54.35 ± 1.19%, respectively), therefore they were doped into household and industrial oils in different concentrations. BHA is permitted to be used at 200 ppm in the frying oils by EU Regulation on Food Additives [16] so BHA added frying oils and the mixtures were analysed for the β-carotene-linoleic

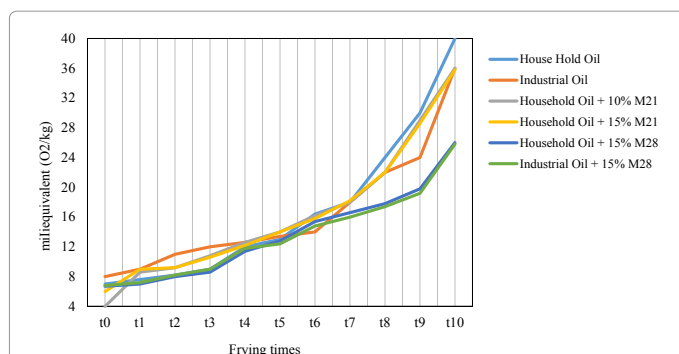
Code	T <sub>120</sub> AA%	T <sub>960</sub> AA%
M7 <sup>a</sup>	67.64 ± 2.64	49.09 ± 1.55
M9 <sup>a</sup>	57.07 ± 0.95	34.53 ± 0.73
M10 <sup>b</sup>	62.34 ± 2.36	33.08 ± 1.03
M12 <sup>b</sup>	50.73 ± 1.23	33.13 ± 1.02
M18 <sup>b</sup>	39.90 ± 1.68	29.35 ± 1.53
M19 <sup>a</sup>	51.33 ± 2.86	26.85 ± 0.26
M20 <sup>a</sup>	37.76 ± 1.26	35.85 ± 0.80
M21 <sup>a</sup>	69.59 ± 4.80	41.64 ± 0.38
M22 <sup>a</sup>	67.42 ± 3.73	40.55 ± 0.27
M23 <sup>a</sup>	61.88 ± 2.76	37.72 ± 0.34
M24 <sup>a</sup>	52.24 ± 1.77	31.68 ± 0.29
M25 <sup>a</sup>	41.33 ± 1.97	25.38 ± 1.24
M26 <sup>a</sup>	56.38 ± 3.21	36.72 ± 2.31
M27 <sup>a</sup>	45.52 ± 0.73	32.57 ± 0.43
M28 <sup>a</sup>	61.52 ± 3.28	54.35 ± 1.19

T<sub>120</sub>: Analysis after 120 min.  
 T<sub>960</sub>: Analysis after 960 min.  
 AA: Antioxidant activity.  
<sup>a</sup>The extracts were diluted by 50 g of hazelnut oil.  
<sup>b</sup>The extracts were diluted by 50 g of olive oil.  
<sup>\*</sup>Values expressed are means ± SD of three parallel measurements (p<0.05).

**Table 4:** β-carotene-linoleic acid assay results of M7, M9, M10, M12 and M18-M28.



**Figure 2:** β-carotene-linoleic acid assay test results of the extract and oil combinations.



**Figure 3:** Comparison of peroxide values during deep frying of potatoes.

acid assay test results (Figure 2). The average β-carotene-linoleic acid assay result of industrial and household type oils doped with 200 ppm BHA was 67.22% inhibition after 120 min. According to Figure 2, the

samples showing higher activity than 65% inhibition after 120 min were selected to be tested for the peroxide value analysis after each deep frying of potatoes. The potatoes were fried for 10 times with each sample. Peroxide values are plotted in Figure 3. Peroxide value results presented that, when extract-oil mixtures were added into household and industrial oils, the acceleration of the frying oil rancidity was decreased. It is clear from Figure 3 that the acceleration of the frying oil degradation, when 15% of M28 was added, started after 9 times of frying while it started after 7 times of frying for the other samples. It can be concluded that the addition of olive leaf extract and clove extract in hazelnut oil mixture improved the performance of sunflower oil during repeated frying of potatoes which was supported by the β-carotene-linoleic acid assay results. The organoleptic results of the fried potatoes introduced no taste and odour change.

## Conclusion

This study was performed in order to improve the stability of the sunflower oil during deep frying of potatoes by the addition of several mixtures of hazelnut oil, sesamol, olive oil, basil oil, black seed oil, olive leaf extract, clove extract, rosemary extract and thyme oleoresin in hazelnut oil and olive oil compared with the synthetic antioxidants. β-carotene-linolenic acid assay results proved that basil oil, olive leaf extract, sage extract, clove extract and rosemary extract in hazelnut oil were close to the results of the synthetic antioxidants and higher than the other samples. Among the different concentrations of these extracts and oils; basil oil, clove extract and olive leaf extract in hazelnut oil (M21) and clove extract and olive leaf extract in hazelnut oil (M28) performed the highest activities after 960 min (41.64 ± 0.38 and 54.35 ± 1.19%, respectively) according to the β-carotene-linolenic acid assay. M21 and M28 were added into sunflower oil for deep frying of potatoes. The results of β-carotene-linolenic acid assay results before frying and the peroxide value results after frying showed that, the rancidity of the sunflower oil was enhanced by the addition of M28 (30 mg clove extract + 70 mg olive leaf extract in 50 g of hazelnut oil).

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