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Phenolic Extract of Basil Prevents Lipid Oxidation in Sunflower Oil, Beef and Turkey Meat: A Comparison with Synthetic Antioxidant BHA

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Abstract: In this study we investigated the protective effect of phenolic extract from sweet basil against oxidation in sunflower oil and meat. The phenolic extract scavenges DPPH radical with an IC_{50}= 33.3±2.89 µg/mL which is less than that recorded by BHA (IC_{50}= 24.17±1.44 µg/mL, P<0.01). This extract inhibits oxidative bleaching of β-carotene (IC_{50}= 29.5±0.87 µg/mL) relatively less than BHA (IC_{50}= 21±1 µg/mL) (P<0.001). Moreover, the extract protects sunflower oil against oxidation induced by copper (IC_{50} = 17.78±0.04 µg/mL). This activity is lower than that exerted by BHA (IC_{50}= 16.53±0.14 µg/mL) (P<0.001). The extract also provides a significant prevention of sunflower oil peroxidation during storage at 37°C (-53%, P <0.001) comparing to BHA which decreases oil peroxidation by 61% (P<0.001). Against, the phenolic extract decreased the peroxidation of oil after heating at 370°C/5h by 49% (P <0.001) while the BHA acts by 57% (P<0.001). The basil phenolic extract significantly reduced malonaldehyde content in beef stored at 4°C (P<0.001) and in turkey meat (P <0.001). The extract contains 211.28±1.99 mg/g total phenol. The phytochemical characterization by HPLC showed that the extract contains four phenolic acids: caftaric acid (2.9%), chicoric acid (5.5%), caffeic (4.3%) acid and rosmarinic acid (87.3%) which represents the major compound. In conclusion, we suggest that the basil phenolic extract is a good source of natural antioxidants that might be exploited in food preservation.

Keywords: Sweet Basil, Phenolic Extract, Rosmarinic Acid, Antiradical Activity, Oil Peroxidation, Meat Lipid Oxidation

1. Introduction

Sweet basil (*Ocimum basilicum* L.) is a member of the Lamiaceae family. It is an aromatic annual herb classed as an important economic crop with wide level of cosmetic and pharmacological applications [1-3]. This plant grows in mountain regions, including Africa, Asia, and South America [1]. In tropical countries of Asia, sweet basil is a major essential-oil crop that is widely exploited in the food, perfume, pharmaceutical and aromatherapy industries [4, 5]. In the past, sweet basil was consumed for preventing cardiovascular related diseases, along with acting as an antispasmodic, carminative, digestive, stomachic, and tonic agent [1]. In east of Morocco, sweet basil is cultured as medicinal plant to treat hyperlipideamia and prevent cardiovascular diseases. Previous studies have shown that sweet basil extracts have various biological activities such as anticancer, antidiabetic, and antimicrobial [6, 7]. Phenolic compounds and especially rosmarinic acid are the major phytochemicals found in the plant [8].

Lipid oxidation is the major problem of the chemical deterioration of foods [9]. This phenomenon is triggered by the reaction of oxygen with the unsaturated fatty acids and progressed by intervention of free radicals in stored oils [9]. Indeed, among the direct factors that induce oil oxidation is the process of frying which is one of the most commonly used procedures for the preparation and production of foods around the world [10]. In fact, the oil is continuously heated

at high temperatures in the presence of moisture and atmospheric oxygen which promotes lipid oxidation; this is a problem that mainly affects organoleptic and nutritional qualities of industrial products [11].

On the other hand, oxidative stability of meat and meat products is dictated primarily by the species of animal as well as its fat and myoglobin/hemoglobin content [12]. Because of their high content of unsaturated fatty acids and their low antioxidant content, poultry meats are particularly more sensitive to oxidative phenomena [13]. Hence, the prevention of oxidation of foodstuffs during their technological transformations, storage and distribution is highly needed. Thus, among the effective technological treatments, the addition of antioxidants to oils and fat-rich foods such as meat and meat products [14]. However, synthetic antioxidants such as butylhydroxytoluene (BHT) and butylhydroxyanisole (BHA) are now recognized as having adverse effects on human health and are readily volatile at high temperatures [15]. So, the stabilization of vegetable oils and animal fat has been the subject of much research to replace synthetic antioxidants with natural ones with remarkable antioxidant power [16].

In this context, the extracts of several aromatic and condimental plants such as rosemary showed an interesting antioxidant activity in edible oil and meats [17, 18]. The observed beneficial effect is generally exerted by phenolic compounds; these phytochemicals are currently attracting much interest because of their high antioxidant power which can be exploited industrially in the prevention of the oxidation of dietary lipids [16].

In the present work, we characterized, qualitatively and quantitatively, a phenolic acid-rich extract of sweet basil cultivated in Morocco. Secondly, we investigated its preventive effect against the peroxidation of sunflower oil after storage and after heating at frying temperature, as well as against the oxidative process in stored beef and turkey meat.

2. Material and Methods

2.1. Preparation of Phenolic Acid-Rich Extract from Sweet Basil

O. basilicum was purchased from an herbalist in Oujda city (Morocco). The dried powder from aerial parts of the plant was defatted with n-hexane, in a Soxhlet extractor, to remove chlorophyll and liposoluble substances. The marc was air-dried and extracted with methanol (16 h). The methanol extract was then filtered and concentrated under a reduced pressure. The extract obtained was placed in a drying oven (40°C) to obtain the crud material. The yield of extraction, in terms of the starting dried plant material, was 11%. The crude extract was dissolved in distilled water and phenolic acids were extracted several times by *n*-butanol using liquid-liquid partition. Then, the *n*-butanol was evaporated under vacuum and the extract was desiccated in drying oven (40°C) to obtain fraction rich in phenolic acids. The extraction yielded 35%.

2.2. Determination of Total Phenol Content

Total phenol content of *O. basilicum* extract was determined by the Folin–Ciocalteu procedure as described by Dewanto et al. [19] with some modifications. 0.5 ml of each sample was mixed with 0.25 ml of Folin–Ciocalteu reagent and 0.5 ml of the sodium carbonate solution 20%. After stirring, the preparation was adjusted with distilled water to a final volume of 5 ml and mixed thoroughly. The coloring was allowed to grow for 30 min in the dark. The blue color was measured spectrophotometrically at 725 nm against a blank where sample was replaced by the same volume of methanol. The amount of total polyphenols was calculated from the calibration curve of rosmarinic acid standard solutions and expressed as mg rosmarinic acid/g dry extract. All measurements were done in triplicate.

2.3. HPLC Analysis of Phenolic Extract from Sweet Basil

HPLC analysis of the basil phenolic extract was carried out on an Agilent 1100 series chromatograph (Agilent, Palo Alto, CA) with a Diode Array Detector, using an Inerbil ODS II reverse phase (RP18) analytical column (250 × 4.6 mm, particle size 5 µm). The extract (20 µl, 1 mg/mL in methanol) was separated at 30°C at a flow rate of 1mL/min using the following gradient of aqueous orthophosphoric acid (pH3) (A) and acetonitrile (B): 0–5min: 0%B, 5–7min: 0–5%B, 7–10min: 5–7%B, 10–15min: 7–10%B, 15–20 min: 10–15%B, 20–25min: 15–18%B, 25–30min: 18–20%B, 30–33min: 20–25%B, 33–38min: 25–28%B, 38–42min: 28–34%B, 42–45min: 34–38%B, 45–65min: 38–45%B, 65–75min: 45–55%B, 75–85min: 55-0%B. The chromatogram was recorded at 280 nm. Compounds were identified by their retention times and UV–visible spectra using a database of analytical standards. Individual phenolic acid concentrations were quantified by comparison of integrated peak areas to calibration curves prepared with standards.

2.4. DPPH Radical-Scavenging Activity of Basil Phenolic Extract and BHA

The anti-radical effect of basil phenolic extract was determined by the DPPH (2.2-diphenyl-1-picryl-hydrazyl) test according to the method described by Blois [20]. 2495 µL of a methanol DPPH solution (0.1 mM) were added to 5 µL of the phenolic extract or buthylated hydroxyanisole (BHA) at final concentrations of 10, 25, 50, 100, 200, 400 µg/mL. After incubation for 30 min in the dark, the absorbance of the mixtures was measured at 517 nm against the blank containing 2495 µL of DPPH and 5 µL of methanol. The decrease in the absorbance indicates the presence of an anti-radical effect of tested substances. The radical scavenging activity (RSA) was calculated according to the following formula: RSA (%) = $(A_{blank} - A_{sample} / A_{blank})$ *100. The IC_{50} (concentration providing 50% inhibition) values were calculated from the plotted graph of scavenging

activity against concentrations of the samples. All tests were done in triplicate.

2.5. Inhibition of β-Carotene Oxidative Bleaching by the Basil Phenolic Extract and BHA

The determination of the effect of basil phenolic extract on β-Carotene oxidative bleaching was carried out according to the method described by Leouifoudi et al. [21] slightly modified. A stock solution of β-carotene–linoleic acid mixture was prepared as following: 2 mg of β-carotene dissolved in 1 mL of chloroform were mixed with 20 mg of linoleic acid and 200 mg of Tween 80; after evaporation of chloroform, 100 mL of distilled water were added with vigorous stirring to form the emulsion linoleate-β-carotene. 2495 µL of this emulsion were mixed with 5 µL of phenolic acid-rich extract at different concentrations (10, 25, 50, 100, 200, 400 µg/mL). Absorbance values were read before and after 24h incubation at 492 nm. The butylated hydroxyanisole was used as positive control in the same experimental conditions.

The inhibition of β-carotene oxidative bleaching was calculated according to the following formula: % inhibition = $100 - [((A_{blank} - A_{sample})/ A_{blank})*100]$ and IC_{50} values were calculated from the plotted graph of antioxidant activity against concentrations of the samples. The measurements were done in triplicate.

2.6. Inhibition of Copper-Induced Oxidation of Sunflower Oil by the Basil Phenol Extract and BHA

To determine the oil peroxidation, malondialdehydes (MDA) were quantified as thiobarbituric acid reactive substances (TBARS) according to the procedure described by Ramchoun et al. [22]. In the negative control, 40 µL of sunflower oil were incubated with dimethylsulfoxide (DMSO) only. In the positive control, 40 µL of sunflower oil were diluted in DMSO and incubated with 10 µL of copper sulphate ($CuSO_4$-$5H_2O$) solution (0.33 mg/mL). In the tested samples, the oil (40 µL) was diluted with DMSO and incubated with copper sulphate and phenolic extract or BHA at different concentrations (100, 200, 400, 600, 800, and 1000 µg/mL). The preparations were mixed vigorously and incubated 24h at 37°C. Then, 500 µL of 20% trichloroacetic acid (pH3.5) and 500 µL of 0.8% (w/v) thiobarbituric acid (TBA) were added to each sample before heating at 95°C for 30 min. After cooling, 2 mL of *n*-butanol were added and the solutions were centrifuged at 4500 rpm for 15 min. The absorbance of the colored layer was recorded at 532 nm. The amounts of thiobarbituric acid reactive substances were calculated from the calibration curve of standard solutions of MDA. The experiment was carried out in triplicates.

2.7. Inhibition of Sunflower Oil Peroxidation After Storage and After Frying by the Basil Phenol Extract and BHA

The investigation of the antioxidative preservation of phenolic extract and BHA on the sunflower oil during storage was carried out according to the following protocol:

1) Control: 5 g of sunflower oil stored at 37°C for 60 days.
2) Basil phenolic extract-treated oil: 5 g of sunflower oil was supplemented with the phenolic extract at a dose of 0.02% (w/w) and stored at 37°C for 60 days.
3) BHA-treated oil: 5 g of sunflower oil was supplemented with the BHA at a dose of 0.02% (W/W) and stored at 37°C for 60 days.

After 60 days incubation, the samples were dissolved in 15 ml of acetic acid-chloroform mixture (3:2 V/V). Then, 1 mL of a saturated potassium iodide (KI) solution was added to each sample and the reaction was allowed to proceed for 30 minutes with stirring. The peroxides in an acid medium and in the presence of KI produce the iodine which was assayed with a sodium thiosulfate solution (0.01N). The peroxide value is expressed as milliequivalent active oxygen per Kg of oil ($meqO_2$/Kg oil). All experiments were done in triplicate.

The determination of the antioxidant effect of basil phenolic extract and BHA on sunflower heated oil was carried out under the following experimental design:

1) Negative control: 5 g of unheated oil;
2) Positive control: 5 g of sunflower oil heated at 370°C for 5 h;
3) Basil phenolic extract-treated oil: 5 g of sunflower oil, treated with the phenolic extract at a dose of 0.02% (W/W) and heated at 370°C for 5 h;
4) BHA-treated oil: 5 g of sunflower oil treated with the BHA at a dose of 0.02% (W/W) and heated at 370°C for 5 h.

At the end of the experiment, heated oils were cooled and the peroxide values were determined as described above.

2.8. Effect of Basil Phenolic Extract and BHA on the Lipid Oxidation in Beef and Turkey Meat

The effect of basil phenolic extract and BHA on the oxidation of meat was evaluated by measuring the concentration of malondialdehydes (MDA) produced before and after incubation at 4°C as described by Kim et al. [23] with some modifications. The experiment was carried out according to the following procedure:

1) Beef control: 5 g of ground beef or ground turkey meat incubated for 7 days at 4°C;
2) Turkey meat control: 5 g of ground turkey meat incubated for 7 days at 4°C;
3) Basil phenolic extract-treated meats: 5 g of ground beef or ground turkey meat were mixed with the phenolic extract at a dose of 0.5% (W/W) and incubated at 4°C for 7 days;
4) BHA-treated meats: 5 g of ground beef or ground turkey meat were mixed with BHA at a dose of 0.5% (W/W) and incubated at 4°C for 7 days.

At the end of the experiment, each sample was homogenized in 15 mL of distilled water and 1 mL of 4N HCl and then centrifuged at 3500 rpm/10 min. The supernatants were used to MDA analysis as described above.

2.9. Statistical Analysis

Data obtained were analyzed using student t-test and one way ANOVA. P values less than 0.05 were considered as statistically significant. Our results are expressed as mean ± SD (n = 3).

3. Results and Discussion

3.1. Polyphenol Content and HPLC Analysis of the Basil Extract

The quantification of total polyphenol content showed that the basil extract is rich in phenolics, the amount was 211.28±1.99 mg rosmarinic acid equivalent/g dry extract. The figure 1 depicts the HPLC chromatogram of the extract. We identified four major phenolic acids:

1) peak 1; caftaric acid: 3.6±0.001 mg/g dry extract (2.9%);
2) peak 2; chicoric acid: 6.84±0.36 mg/g dry extract (5.5%);
3) peak 3; rosmarinic acid: 108.02±0.019 mg/g dry extract (87.3%);
4) peak 4; cafeic acid: 5.31±0.12 mg/g dry extract (4.3%).

The quantitative analysis shows that rosmarinic acid is the most abundant phenolic compound in the extract representing 87.3%. The finding agrees with previous study reporting the same phenolic acids profile in *O. basilicum* [24]. This result led as to suggest that rosmarinic acid might be the principal compound responsible of the preservation of oil and meat against oxidation.

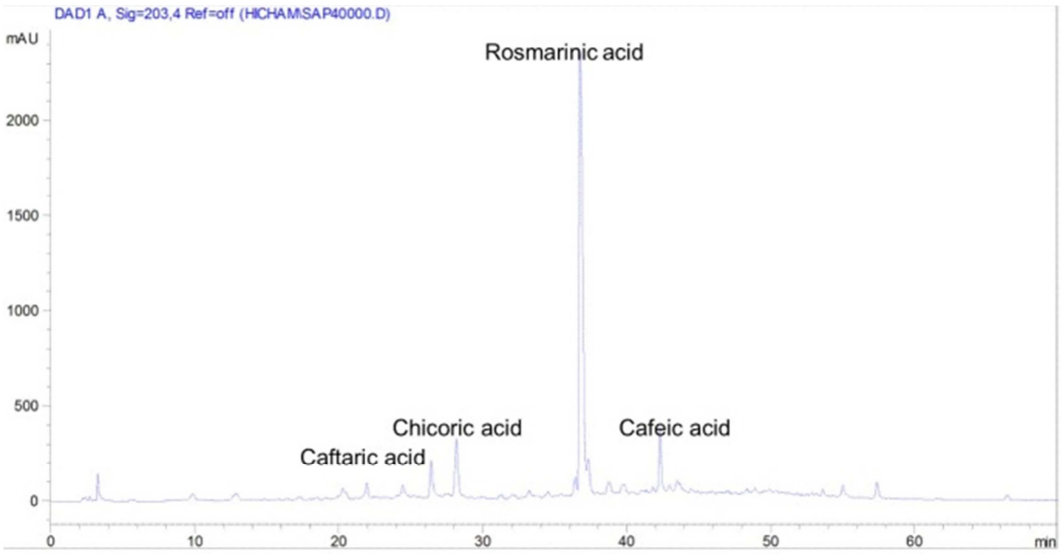


Figure 1. *HPLC analysis of the basil phenolic extract.*

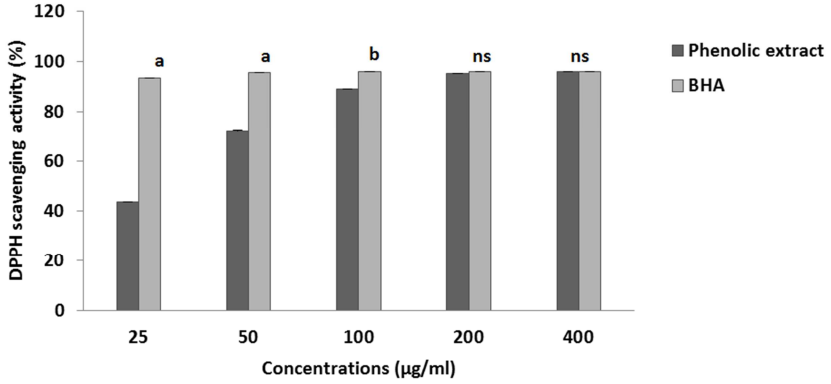
3.2. Free Radical Scavenging Activity of Basil Phenolic Extract and BHA

Free radical scavenging is one of the recognized mechanisms by which antioxidants inhibit lipid peroxidation [25]. The scavenging activity on DPPH radicals assay is generally used as a basic screening method for testing the anti-radical activity of natural and synthetic compounds [26]. In this work, we used such assay in order to evaluate the effectiveness of the phenolic extract of basil as an antioxidant.

The radical scavenging activity of the basil phenolic extract was summarized in figure 2. We note that the extract scavenge DPPH radical in a dose-dependent manner. The percentages of inhibition were 44%, 72%, 89%, 95% and 96% at doses of 25, 50, 100, 200 and 400 µg/mL, respectively. The BHA is more efficient than phenolic extract especially at law concentrations but since 100 µg/mL, the effects are relatively comparables. The percentages of inhibition exerted by BHA were between 93% and 96%, at the same concentrations described above. When comparing IC_{50} of the tested compounds, we concluded that the phenolic extract has a good anti-radical scavenging activity against the DPPH radical with an IC_{50}= 33.3±2.89 µg/mL compared to that recorded by BHA; IC_{50}= 24.17±1.44 µg/mL ($P<0.01$).

According to previous reports, polyphenolic compounds have been proven to be strong antioxidants against oxidation of biomolecules in food systems [27]. In general, the number and position of hydrogens donated by the aromatic ring of polyphenols directly determine their antioxidant capacities. The free radical scavenging activities of the basil extract might be due to the presence of phenolic acids with high capacity to donate protons and stabilize the radicals. Basically, the free radical DPPH reacts with polyphenols (ArOHs) through two different mechanisms: 1) direct abstraction of phenol H-atom by DPPH (HAT mechanism) and 2) electron transfer process from ArOH or its phenoxide anion (ArO−) to DPPH (ET mechanism) [28].

The sequential proton-electron transfer (SPLET) mechanism was also proposed for the reactions of DPPH radicals with phenolic acids. The SPLET mechanism comprises two consecutive steps: 1) the deprotonation of the antioxidant, and 2) an electron transfer from the deprotonated antioxidant to the free radical. The rate of SPLET depends on the ArOH ionization and the electron affinity of the radical [28].



Values are expressed as mean±SD (n=3). [a]P<0.001, [b]P<0.01, ns: not significant (basil phenolic extract *versus* BHA).

Figure 2. *DPPH scavenging activity of basil phenolic extract and BHA.*

3.3. Effect of basil phenolic extract and BHA on β-carotene oxidative bleaching

β-carotene bleaching test measures the potential of the samples to inhibit conjugated dienes and hydroperoxide formation from linoleic acid oxidation [29].

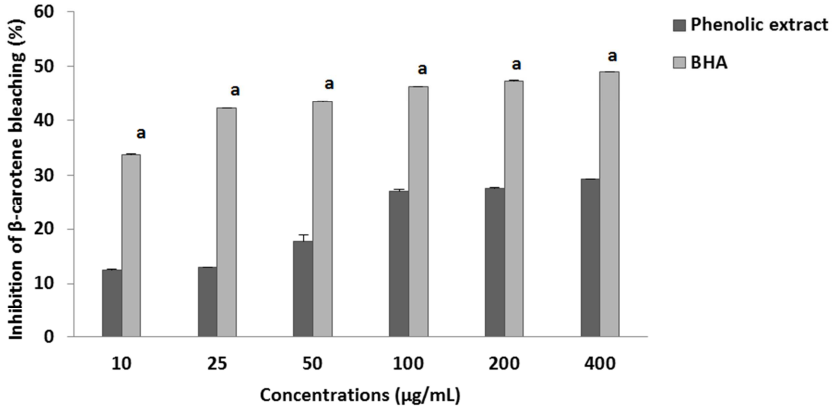
The results of the β-carotene discoloration test show that the basil phenolic extract inhibits oxidation of linoleic acid and consequently the discoloration of β-carotene. Figure 3 shows the antioxidant activity of the phenolic extract in comparison with BHA. As can be seen, the extract inhibits oxidation process by 12%, 13%, 18%, 27%, 28% and 29% at doses of 10, 25, 50, 100, 200 and 400 µg/mL, respectively. However, the BHT inhibits oxidation by 34%, 42%, 43%, 46%, 47% and 49% at the previous concentrations.

Comparing the effects of the tested compounds in inhibition of linoleic acid β-carotene-system oxidation, we note that the basil phenolic extract presents a considerable activity with an IC_{50}= 29.5±0.87 µg/mL. However, this activity is statistically lower than that of BHA having an IC_{50}= 21±1 µg/mL (P<0.001).

The property of the basil extract to slow down the β-carotene bleaching indicated its antioxidant activity and its ability to inhibit lipid peroxidation. This activity might be attributed mainly to rosmarinic acid and other phenolic acid as demonstrated by Taguchi et al. [30]. In fact, the phenolic compounds are able to scavenge the alkylperoxyl radical generated by autooxidation of linoleic acid and then the β-carotene bleaching was stopped.

The mechanism of the anti-β-carotene bleaching effect of phenolic compounds was based on hydrogen atom transfer (HAT). The HAT mechanism involves the abstraction of H-atom from

a phenolic hydroxyl group by peroxyl radicals, which is followed by rapid recombination of peroxyl and the resulting aryloxyl radicals to yield non-radical products [28].



Values are expressed as mean±SD (n=3). [a]P<0.001 (basil phenolic extract *versus* BHA).

Figure 3. *Inhibition of β-carotene oxidative bleaching by basil phenolic extract and BHA.*

3.4. Effect of Basil Phenolic Extract and BHA on the Copper-Induced Sunflower Oil Oxidation

The transition metals, such as copper, were strongly implicated in the generation of free radicals in food systems [31]. Transition metal ions may contribute to the chain initiation by a variety of mechanisms such as decomposition of hydroperoxides and hydrogen peroxide to give alkoxyl, peroxyl and hydroxyl radicals and also activation of oxygen [32]. It has been also pointed out that the higher oxidation

state of these metal ions plays an important role in the oxidation of polyunsaturated fatty acid-rich oils [32].

The development of lipid oxidation can be followed by the primary products of fatty acid oxidation, such as hydroperoxides and conjugated dienes, or secondary oxidation products, such as pentanal, hexanal and malonaldehyde which are usually determined colorimetrically as tiobarbituric reactive substances (TBARS) [33].

In this work, the oxidation of sunflower oil induced by copper, in the presence or absence of antioxidant compounds, was measured by assaying TBARS at 532 nm (Figure 4). We note that the copper induces a significant increase in oil oxidation at 37°C when compared to control ($P < 0.001$). However, treatment of copper-added oil with different concentrations of basil phenolic extract shows a significant and dose-dependent decrease of MDA (IC_{50} = 17.78 ± 0.04 µg/mL) (Figure 4). The inhibition of oil oxidation in the BHA-treated samples is relatively higher than the result showed by the phenolic extract (IC_{50} = 16.53 ± 0.14 µg/mL) ($P<0.001$).

The result led us to suggest that the basil phenolic extract might be acting as a potent copper chelator, mechanism by which natural polyphenols can inhibit lipid oxidation. In the fact, Chelation of Cu^{2+} is another mechanism involved in inhibition of free radical propagation and lipid peroxidation [28]. The metal chelator can affect in several different ways. It varies the redox-potential of the metal complex products and may also render steric effect which affects the rate or efficiency of coordination or binding to the target molecule or hydroperoxide [28]. Our result agrees with previous reports demonstrating that polyphenols can act as metal chelator and prevent lipid peroxidation [34]. On the other hand, as demonstrated above, the preservation of sunflower oil against oxidation can also be explained by the anti-radical effect of the phenolic acids present in the basil extract. In the fact, these compounds are able to exert a free radical neutralization resulting in the inhibition of polyunsaturated fatty acid peroxidation [28].

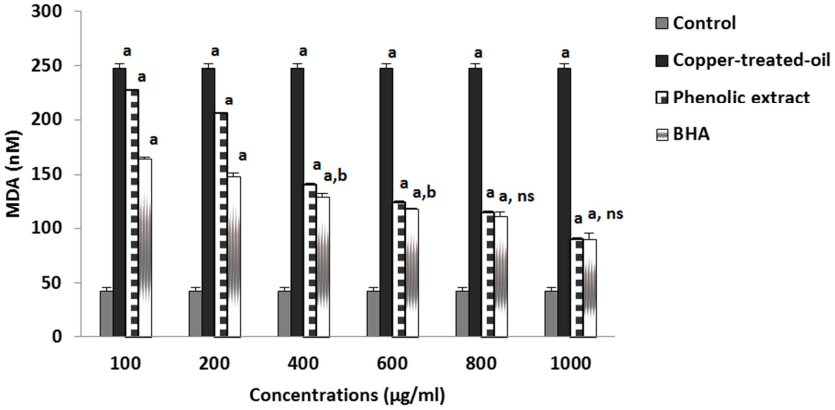


Figure 4. *Effect of basil phenolic extract and BHA on copper-induced sunflower oil oxidation.*

Values are expressed as mean±SD (n=3).
[a]$P<0.001$ (copper-treated oil *versus* control; phenolic extract and BHA *versus* copper-treated oil).
[b]$P<0.01$, ns: not significant (Phenolic extract *versus* BHA).

3.5. Effet of Basil Phenolic Extract and BHA on Sunflower Oil Peroxidation After Storage and After Heating

Autoxidation of edible oils during storage is a natural process that occurs between molecular oxygen and unsaturated fatty acids through a free-radical chain mechanism that involves the formation of fat free radicals, peroxide free radicals and hydroperoxides. The hydroperoxides are very unstable and decompose to secondary products including aldehydes, ketones, alcohols and acids, which cause off-odors and off-flavors [9]. The oils with higher contents of unsaturated fatty acids, especially polyunsaturated fatty acid, are more susceptible to oxidation. On the other hand, during the frying process, in the presence of air and moisture many chemical reactions occur, such as hydrolysis, polymerization and thermal oxidation, thereby producing a considerable number of harmful compounds such as polymer and ketones, which significantly change the quality of the frying oil [35]. In order to delay all these undesirable reactions, several antioxidants are added to edible oils in industrial processes. For this purpose, synthetic antioxidants such as BHA and BHT are widely used. Although these synthetic substances have the ability to increase the oxidative stability of oils, many researchers have stated that they also have undesirable side effects [15]. Because of the perceived detrimental effects of these compounds, researchers have focused on the use of natural antioxidants to stabilize oils [36]. In this context, we assayed the effect of basil phenolic extract, in comparison with BHA, on sunflower oil peroxidation after storage at 37°C and after heating at frying temperature.

Our results show that the storage of sunflower oil during 60 days at 37°C resulted in a significant increase in lipid peroxidation (+17 times) compared to control (the same oil before storage) ($P < 0.001$). For against, treatment with the basil phenolic extract, at a concentration of 0.02% (w/w), provides a statistically significant prevention of the oil peroxidation process during storage (-53%, $P < 0.001$). Moreover, the BHA decreases peroxide value of the stored

oil by 61% (P<0.001). So, we can concluded that the basil phenolic extract was relatively less efficient than the synthetic antioxidant BHA (P<0.001) (Figure 5)

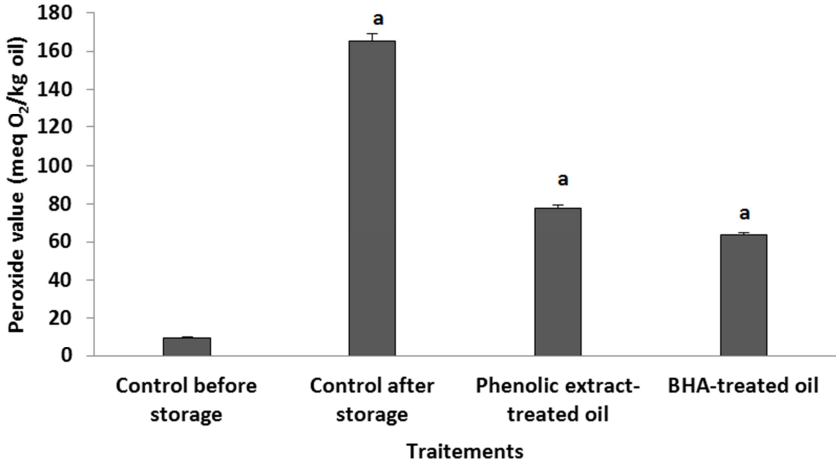


Figure 5. *Effect of basil phenolic extract and BHA on stabilization of stored sunflower oil.*

Values are expressed as mean±SD (n=3). [a]P<0.001, (Control after storage *versus* control before storage; phenolic extract and BHA-treated oils versus control after storage; basil phenolic extract *versus* BHA).

On the other hand, we demonstrated that heating the oil at 370°C for 5 h induces a significant increase in peroxide value compared to unheated oil (P<0.001) (Figure 6). However, the addition of basil extract or BHA at a concentration of 0.02% (w/w) exerts a significant preservation of oil during frying. In fact, the phenolic extract decreased the oil peroxide value by 49% (P <0.001) while the BHA acts by 57% (P<0.001) comparatively to control (oil fried without any additive). In term of comparison between the two assayed products, we note that the phenolic extract is always relatively less active than BHA (P<0.01).

Our results are in accordance with a number of previous reports studying the effect of natural products against the edible oil peroxidation. In the fact, Asnaashari et al. [37] demonstrated that the leaves extract from raspberry (*Rubus fruticosus*) showed higher effect on oxidative stability of sunflower oil. Another study data revealed the rosmary (*Rosmarinus officinalis* L.) extracts to be a potent antioxidant for stabilization of sunflower oil [38]. Baştürk and coworkers [39] studied the effect of natural antioxidants (*Salvia officinalis* L., *Mentha arvensis* L., *Rhus coriaria* L., *Thymus vulgaris*) on corn oil stability. The preservative effect of natural plant extracts against oil oxidation is mainly due to their high content of phenolic compounds [33]. These observations, led us to suggest that phenolic acids present in the basil extract are the major candidates acting as antioxidant agents on sunflower oil after long period storage or heating. On the other hand, according to the quantitative HPLC analysis of the extract, we can suggest that the beneficial effect is partly exerted by rosmarinic acid which is the major phenolic compound of the plant. Besides, this antioxidant effect can be the result of additive activities from each compound acting alone or in synergy with other phenolic compounds.

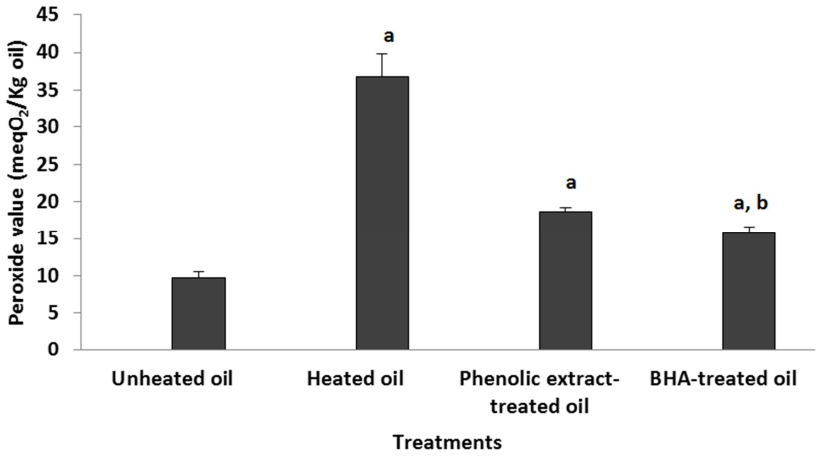


Figure 6. *Effect of basil phenolic extract and BHA on stabilization of heated sunflower oil.*

Values are expressed as mean±SD (n=3). [a]P<0.001 (Unheated oil *versus* heated oil; phenolic extract and BHA-treated oils *versus* heated oil), [b]P<0.01 (basil phenolic extract *versus* BHA).

3.6. Preventive Effect of Basil Phenolic Extract and BHA Against Lipid Oxidation in Beef and Turkey Meat

Meats are susceptible to quality deterioration due to their rich nutritional composition [40]. The most common form of meat chemical deterioration is the oxidation of lipids. This phenomenon depends on chemical composition of meat, light and oxygen access and storage temperature [41]. Lipid oxidation leads to the formation of several compounds which have negative effects on the quality of meat causing changes in sensory and nutritional qualities [42]. Lipid oxidation can be reduced or inhibited by the use of antioxidants in meat and thus the product quality and shelf-life can be improved.

The oxidation of beef lipids in the presence of basil phenolic extract or BHA was showed in figure 7. We demonstrated that the meat incubated at 4°C for 7 days had a higher and statistically significant oxidation rate than the reference values recorded before incubation (+129%, P<0.001). In contrast, treatment with basil phenolic extract at 0.5% significantly decreased the rate of oxidation by 46% (P<0.001). On the other hand, the BHA (0.5%) reduced meat oxidation by 44% (P<0.001) comparatively to control (untreated beef). The effect exerted by the phenolic extract was comparable to that of BHA under the same experimental conditions (P=0.26).

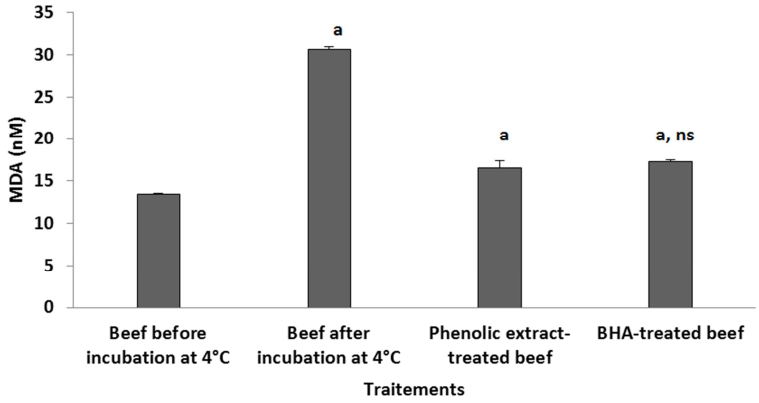


Figure 7. *Effect of basil phenolic extract and BHA on lipid oxidation in beef.*

Values are expressed as mean±SD (n=3). [a]P<0.001 (Beef after incubation *versus* beef before incubation; phenolic extract and BHA-treated beefs *versus* beef after incubation), ns: not sinificantant (phenolic extract-treated beef *versus* BHA-treated beef).

In the turkey meat, we observed that the oxidation was significantly induced after incubation for 7 days at 4°C (+121%, P<0.001). However, the addition of phenolic extract or BHA at a concentration of 0.5% significantly protects the oxidative process and reduces the MDA levels by 47% (P<0.001) and 46% (P<0.01), respectively. We note also that the difference between the effects of the phenolic extract and BHA is not statistically significant (P=0.27) (Figure 8). On the other hand, we observed that there is any significantly difference between beef and turkey meat in the induction of oxidation or its inhibition by antioxidants (basil extract and BHA).

Our findings agree with several works demonstrating that different plant products are effective as natural antioxidants to preserve and improve the overall quality of meat and meat products. These natural antioxidants from plants, in the form of extracts, have been obtained from different sources such as fruits [43], herbs and spices [44] and investigated to decrease lipid oxidation in meat.

The preventive action of basil extract against meat oxidation might be, most likely, attributed to its phenolic content as demonstrating with different studied plant extracts [43, 44]. In fact, phenolic antioxidants prevent formation of fatty free radicals and have metal ions chelation ability [28]. Thus, the basil phenolic extract can inhibit free radical formation and the propagation of lipid chain reactions by chelating metal ions, especially iron in meats. These properties were demonstrated above using DPPH radical, β-caroten-linoleate, and metal-induced lipid oxidation models.

The high rosmarinic acid content of basil extract seems to be responsible for antioxidant activity. However, as suggested above, a potential synergism between this compound and other phenolic acids might have enhanced its protective ability in beef and turkey meat.

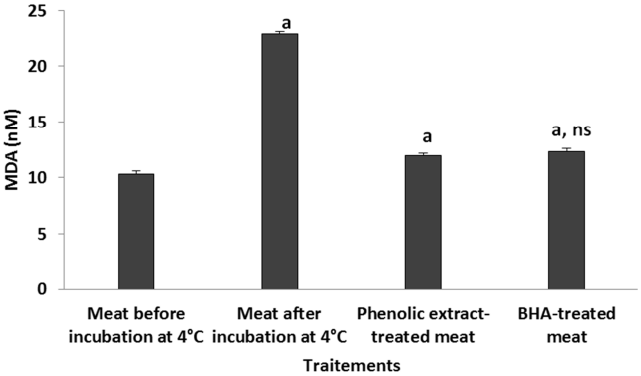


Figure 8. *Effect of basil phenolic extract and BHA on lipid oxidation in turkey meat.*

Values are expressed as mean±SD (n=3). [a]P<0.001 (Meat after incubation *versus* meat before incubation; phenolic extract and BHA-treated meats *versus* meat after incubation), ns: not significant (phenolic extract-treated meat *versus* BHA-treated meat).

4. Conclusion

From the present study, it is concluded that sweet basil phenolic extract can stabilize sunflower oil under longue period storage and during heating. The extract is also able to prevent lipid oxidation in beef and turkey meat. This preservative activity might be exerted by phenolic acids which are potent free radical scavengers and metal chalators. The basil phenolic extract not only is good source of natural antioxidants that can be used as alternative of synthetic substances, but also the high thermal stability of the extract shows an added advantage at high processing temperatures.

References

[1] Ahmad Ch M, Naz SB, Sharif A, Akram M, Saeed MA (2015). Biological Pharmacological Properties of the Sweet Basil (*Ocimum basilicum*) Br J Pharm Res 7: 330-339.

[2] Naji-Tabasi S, Razavi, SMA (2017). Functional properties and applications of basil seed gum: An overview. Food Hydrocol 73: 313-325.

[3] Złotek U (2018). Antioxidative, potentially anti-inflammatory, and antidiabetic properties, as well as oxidative stability and acceptability, of cakes supplemented with elicited basil. Food Chem 243: 168-174.

[4] Pirmoradi MR, Moghaddam M, Farhadi N (2013). Chemotaxonomic analysis of the aroma compounds in essential oils of two different *Ocimum basilicum* L. varieties from Iran. Chem Biodivers 10: 1361–1371.

[5] Radulovic NS, Blagojevic PD, Miltojevic AB (2013). Alpha-Linalool – amarker compound of forged/synthetic sweet basil (*Ocimum basilicum* L.) essential oils. J Sci Food Agric 93: 3292–3303.

[6] Elansary HO, Yessoufou K, Shokralla S, Mahmoud EA, Skalicka-Woźniak K (2016). Enhancing mint and basil oil composition and antibacterial activity using seaweed extracts. Ind Crops Prod 92: 50-56.

[7] Złotek U, Szychowski KA, Świeca M (2017). Potential in vitro antioxidant, anti-inflammatory, antidiabetic, and anticancer effect of arachidonic acid-elicited basil leaves. J Funct Foods 36: 290-299.

[8] Moghaddam M, Mehdizadeh L (2015). Variability of total phenolic, flavonoid and rosmarinic acid content among Iranian basil accessions. LWT - Food Sci Tech 63: 535-540.

[9] Sowicz EW, Gramza A, Hêoel M, Jeleñ HH, Korczak J, Matecka M, Mildner-Szkudlarz S, Rudziñska M, Samotyja U, Zawirska-Wojtasiak R (2004). Oxidation of lipids in food. Polish J Food Nutr Sci 13: 84-100.

[10] Karimi S, Wawire M, Mathooko FM (2017). Impact of frying practices and frying conditions on the quality and safety of frying oils used by street vendors and restaurants in Nairobi, Kenya. J Food Comp Anal 62: 239-244.

[11] Belkova B, Hradecky J, Hurkova K, Forstova V, Hajslova J (2018). Impact of vacuum frying on quality of potato crisps and frying oil. Food chem 241: 51-59.

[12] Shahidi F (2016). Chapter 10: *Oxidative* Stability and Shelf Life of *Meat* and *Meat* Products. *Oxidative* Stability and Shelf Life of Foods Containing Oils and Fats, Pages 373-389.

[13] Carvalho R, Shimokomaki M, Estévez M (2017). Chapter 6: Poultry Meat Color and Oxidation, Poultry Quality Evaluation. 133-157.

[14] Jiang J, Xiong YL (2016). Natural antioxidants as food and feed additives to promote health benefits and quality of meat products: A review. Meat Sci. 120: 107-117.

[15] Kahl R, Kappus H (1993). Toxicology of the synthetic antioxidants BHA and BHT in comparison with the natural antioxidant vitamin E. Z. Lebensm Unters Forsch 196: 329-338.

[16] Zamora R, Hidalgo FJ (2016). The triple defensive barrier of phenolic compounds against the lipid oxidation-induced damage in food products. Trends Food Sci Tech 54, 165-174.

[17] Muíño I, Díaz MT, Apeleo E, Pérez-Santaescolástica, C, De la Fuente J (2017). Valorisation of an extract from olive oil waste as a natural antioxidant for reducing meat waste resulting from oxidative processes. J Clean Prod 140: 924-932.

[18] Wang Y-Z, Fu S-G, Wang S-Y, Yang D-J, Chen, Y-C (2018). Effects of a natural antioxidant, polyphenol-rich rosemary (*Rosmarinus officinalis* L.) extract, on lipid stability of plant-derived omega-3 fatty-acid rich oil. LWT - Food Sci. Tech 89: 210-216.

[19] Dewanto V, Wu X, Adom KK, Liu RH (2002). Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. J Agric Food Chem 50: 3010-3014.

[20] Blois MS (1958). Antioxidant determinations by the use of stable free radical. Nature 181: 1119-1200.

[21] Leouifoudi I, Harnafi H, Zyad A (2015). Olive Mill Waste Extracts: Polyphenols Content, Antioxidant, and Antimicrobial Activities. Adv Pharmacol Sci. 1-11.

[22] Ramchoun M, Sellam K, Harnafi H, Alem C, Benlyas M, Khallouki F, Amrani S (2015). Investigation of antioxidant and antihemolytic properties of *Thymus satureioides* collected from Tafilalet Region, south-east of Morocco. Asian Pac J Trop Biomed 5: 93-100.

[23] Kim S-J, Cho AR, Han J (2013). Antioxidant and antimicrobial activities of leafy green vegetable extracts and their applications to meat product preservation. Food control 29: 112-120.

[24] Kwee EM, Niemeyer ED (2011). Variations in phenolic composition and antioxidant properties among 15 basil (*Ocimum basilicum* L.) cultivars. Food Chem 128: 1044–1050.

[25] Yang J, Xiong YL (2018). Comparative time-course of lipid and myofibrillar protein oxidation in different biphasic systems under hydroxyl radical stress. Food chem 243, 231-238.

[26] Geng D, Chia X, Donga Q, Hu F (2015). Antioxidants screening in Limonium aureum by optimized online HPLC–DPPH assay. Ind Crops Prod 67: 492-497.

[27] Cao Y, Ai N, Trued AD, Xiongd YL (2018). Effects of (−)-epigallocatechin-3-gallate incorporation on the physicochemical and oxidative stability of myofibrillar protein–soybean oil emulsions. Food chem 245: 439-445.

[28] Losada-Barreiro S, Bravo-Díaz C (2017). Free radicals and polyphenols: The redox chemistry of neurodegenerative diseases. Eur J Med Chem 133: 379-402.

[29] Yu S-H, Tsai M-L, Lin B-X, Lin C-W, MiTea F-L (2015). Catechins-cross-linked methylcellulose active films for inhibition of light irradiation and lipid peroxidation induced β-carotene degradation. Food Hydrocol 44: 491-505.

[30] Taguchi R, Hatayama K, Takahashi T, Hayashi T, Sato Y, Sato D, Ohta K, Nakano H, Seki C, Endo Y, Tokuraku K, Uwai K (2017). Structure–activity relations of rosmarinic acid derivatives for the amyloid β aggregation inhibition and antioxidant properties. Eur J Med Chem 138: 1066-1075.

[31] Asaduzzaman M, Biasioli F, Cosio MS, Schampicchio M (2017). Hexanal as biomarker for milk oxidative stress induced by copper ions. J Dairy Sci 100: 1-7.

[32] Carocho M, Morales P, Ferreira I (2018). Antioxidants: Reviewing the chemistry, food applications, legislation and role as preservatives. Trends Food Sci Tech 71: 107-120.

[33] Zhang Y-Y, Zhang F, Thakur K, Ci A-T, Wang H, Zhang J-G, Wei Z-J (2017). Effect of natural polyphenol on the oxidative stability of pecan oil, Food Chem. Toxicol. doi: 10.1016/j.fct.2017.10.001.

[34] Gulcin I, Bursal E, Sehitoglu MH, Bilsel M, Goren AC (2010). Polyphenol contents and antioxidant activity of lyophilized aqueous extract of propolis from Erzurum, Turkey. Food Chem Toxicol 48: 2227-2238.

[35] Gertz C (2000). Chemical and physical parameters as quality indicators of used frying fats. Eur. J. Lipid Sci Technol 102: 566–572.

[36] Horuz Tİ, Maskan M (2015). Effect of the phytochemicals curcumin, cinnamaldehyde, thymol and carvacrol on the oxidative stability of corn and palm oils at frying temperatures. J Food Sci Tech 52: 8041-8049.

[37] Asnaashari M, Tajik R, Haddad Khodaparast MH (2015). Antioxidant activity of raspberry (*Rubus fruticosus*) leaves extract and its effect on oxidative stability of sunflower oil. J Food Sci Technol 52: 5180–5187.

[38] Mezza GN, Borgarello AV, Grosso NR, Fernandez H, Pramparo MC, Gayol MF (2018). Antioxidant activity of rosemary essential oil fractions obtained by molecular distillation and their effect on oxidative stability of sunflower oil. Food chem 242: 9-15.

[39] Baştürk A, Ceylan M, Çavuş M, Boran G, Javidipour I (2018). Effects of some herbal extracts on oxidative stability of corn oil under accelerated oxidation conditions in comparison with some commonly used antioxidants. LWT Food Sci Tech 89: 358-364.

[40] Ahmed ST, Manirul Islam M, Rubayet Bostami ABM, Mun H-S, Kim Y-J, Yang C-J (2015). Meat composition, fatty acid profile and oxidative stability of meat from broilers supplemented with pomegranate (*Punica granatum* L.) by-products. Food chem 188: 481-488.

[41] Shah MA, Don Bosco SJ, Mir SA (2014). Plant extracts as natural antioxidants in meat and meat products. Meat Sci 98: 21-33.

[42] Karakaya M, Bayrak E, Ulusoy K (2011). Use of natural antioxidants in meat and meat products. J. Food Sci Eng 1: 1-10.

[43] Turgut SS, Işıkç F, Soyer A (2017). Antioxidant activity of pomegranate peel extract on lipid and protein oxidation in beef meatballs during frozen storage. Meat Sci 129: 111-119.

[44] Van Hecke T, Ho PL, Goethals S, De Smet S (2017). The potential of herbs and spices to reduce lipid oxidation during heating and gastrointestinal digestion of a beef product. Food Res Int 102: 785-792.