Enhancing Oxidative Stability of *Nigella Sativa* Oil with Some Agro-Industrial Wastes Extracts and its Utilization in Mayonnaise

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ABSTRACT

The stability of lipid oxidation of fat-rich food products is one the most important factors to preserve the quality of food products. Therefore this study aimed to enhance the oxidative stability of *Nigella sativa* (NS) oil for 15 days at 50°C, by using different concentrations of extracts from olive leaves (OL), tomato by-product (TP), orange peel (OP) and water melon peel (WP) as naturally bioactive compounds, in addition to assess the quality of mayonnaise containing NS oil as a functional ingredient. The natural phenolic compounds of these agro- industrial wastes were extracted and identified using HPLC. The results showed that OL extract had the highest total phenolic content (121.09 mg GAE/g dw) which represented about 1.76, 3.91 and 13.34 times higher than the phenolics in the TP, OP and WP extracts, respectively. The results of the oxidative stability measurements indicated that the addition of all extracts to NS oil retarded the oxidative rancidity compared to the control as measured by the peroxide value, *p*-anisidine value, and thiobarbituric acid. Infrared analysis demonstrated the antioxidant power of the OL extract at 400 ppm and the TP at 600 ppm in preventing rancidity conditions, which were similarly effective as the synthetic antioxidant Tertiary-butyl hydroquinone (TBHQ) (200 ppm). This demonstrated the potential for using them as natural antioxidants to prevent rancidity in stored oil. The results of quality and sensory evaluation of NS oil mayonnaise showed an increase in viscosity and a change in colour parameters that were imperceptible to the human eye as the ΔE values were less than 3, and all mayonnaise products were "accepted" by the panelists.

Keywords: Lipid oxidation, Rancidity, Functional ingredients and Synthetic antioxidant.

INTRODUCTION

Nigella sativa (black cumin) oil is characterized by functionalities and biological therapeutic activities which make them suitable for utilization as a dietary supplement for health promotion or protection against some diseases (Hosni *et al*., 2023). Due to its antioxidant, antimicrobial, and nutritional effects, it is used for functional food (pickles, baked goods, dairy products confectionary, sauces, salads, and savory foods) and as additives for pharmaceuticals, and cosmeceuticals (Gholamnezhad *et al*., 2016 and Rashwan *et al*., 2023). The *Nigella sativa* seed has a fixed oil content of 26 – 34%, however, the seed oil contains 0.4%–2.5% essential oil (Yimer *et al*., 2019). The main component of the essential oil is thymoquinone that has a variety of therapeutic advantages (Isik *et al*., 2019). As reported by Cascella *et al*. (2018), *Nigella sativa* could be a useful compound for preventing and treating cerebral ischemic and neurodegenerative diseases and could induce effective neuroprotective activity. In addition, it is as functional oils due to its dietary characteristics is considered as a rich source of essential fatty acids like linoleic (54.0-70.0%) and oleic (15.0-24.0%) as well as palmitic acid (20.4%) and oil-soluble vitamins (Mamun and Absar, 2018). In recent years, *Nigella sativa* oil has drawn significant attention from researchers because of its potential uses in the development of novel functional food and pharmaceuticals (Mukhtar *et al*., 2019). The dairy products and ice cream were fortified with *Nigella sativa* oil using nanoemulsion to increase their nutritional value and boost consumer acceptance. However, one of the oldest and most popular sauces in the world is mayonnaise that is a product widely consumed by different age groups. Mayonnaise has high oil content (75%), making it particularly susceptible to oxidation and spoiling. Lipid oxidation is one of the major sources of deterioration in oils and lipid foods where it decreased its nutritional and functional qualities during storage and marketing. Oxidative changes of lipid have an impact on many interactions between food components, and can lead to rancidity, off flavours, and colour loss, reduction of shelf life, and may even produce toxic compounds that are bad for consumers' health (Ahmed *et al*., 2016). The most effective inhibitors of lipid oxidation are antioxidants. Therefore, natural phenolic compounds of plant origin have recently received more attention as antioxidants than synthetic products due to their functional and

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nutritional advantageous effects (Kabir *et al*., 2015 and Shalaby & Elhassaneen, 2021).

Recovery of antioxidants from agro-industrial wastes is interesting aspects that have biological properties as well as they are the most efficient method, economical and suitable approach. Therefore, these wastes are an excellent source of nutraceutical, bioactive, inherently functional and possess many components that are good for human health (Abdel-Razek *et al*., 2016). In Egypt, the agricultural processing industries produce around 67 million tons of wastes annually. The highest amount of wastes was recorded from processing industries of vegetables and fruits (peels, pomace, seed fractions, straws and other agro food wastes), which are a rich source of phytochemicals like phenolic compounds, polysaccharides, dietary fibers, flavour compounds (Elbasiouny *et al*., 2020). Among the most abundant phenolic compounds with antioxidant properties are anthocyanins, hydroxybenzoic, hydroxycinnamic acids, chlorophyll, *p*-cymene, limonene, carvone, thujene, thymoquinone, hesperidin, α-thujene and lycopene that can all be extracted at various concentrations from some wastes of tomato, orange, carrots, olive and water melon. Vitamins and carotenoids are some additional antioxidant substances that can be consumed. Phenolic compounds have antioxidants, prebiotic, antibacterial, antiviral, anti-inflammatory and anticancer effects (Leyva-López *et al*., 2020). Recently, there has been a focus on the antioxidative application of the active plant materials to control and reduce oxidation in food lipids as well as a trend towards replacing synthetic antioxidants with natural ones that may have health benefits (Uzombah, 2021 and Ashrafi *et al*., 2023). The addition of black seed to mayonnaise may reduce oxidation, enhance flavour, and extend shelf life. *Nigella sativa* oil can be added to food products as a nutritious source of healthy fats, as well as to enhance flavour and shelf life (Ozdemir *et al*., 2018 and Abdul Rahim *et al*., 2022).

Therefore, the objective of this study was to enhance the oxidative stability of *Nigella Sativa* oil at 50˚C using some agro- food processing industrial wastes extracts as natural bio-active compounds. This study also aimed to assess the quality of mayonnaise containing *Nigella Sativa* oil as a functional ingredients.

MATERIALS AND METHODS

Materials:

Agro-industrial wastes: Olive leaves were collected from the Horticultural Research Institute at the Agriculture Research Centre in Giza, Egypt. The fresh water melon peels were procured from the local juice shop of Alexandria, Egypt. Tomato by-product, and orange peel were obtained from Edfina Company, Alexandria, Egypt.

Olive leaves (OL), tomato by-product (TP), orange peel (OP) and water melon peels (WP) were cleaned under running water, spread on trays and dried at 40˚C for 12 h using air draft drying oven (WT-binder labortechnik GMBH). The dried waste materials were grinded in an electric grinder and passed through sieves to produce powders with a particle size of 0.5-0.85 mm. Then, the dehydrated powder was packaged and stored at 4˚C until used.

***Nigella sativa* oil**: The dry *Nigella sativa* seeds were purchased from a specialized herbal store located in Alexandria, Egypt and were mechanically pressed at room temperature using a screw press machine at this herbal store. The extracted crude oil was collected in dark glass bottles and stored at 4˚C until analyzed.

Refined sunflower oil: Refined sunflower oil was provided from Sila Company, at Fayoum, Egypt.

Ingredients for mayonnaise: Including egg yolk, vinegar, citric acid, lemon juice, salt, and mustard were purchased from the commercial markets in Alexandria, Egypt.

Chemicals: Analytical-grade chemicals and reagents were bought from Sigma Aldrich (St. Louis, USA).

Methods:

Preparation of agro-industrial wastes ethanolic extracts: Tested agro-industrial wastes (5g) were boiled under reflux in 70% ethanol (1:10, w/v) for 2 h at 60˚C to produce ethanolic extracts. The extraction was carried out 3 times. The extract was weighed to determine the extracted yield of each plant material, filtered through Whatman filter paper (No.2), evaporated to dryness using rotary evaporator at 40°C and then stored at 4°C before analysis.

Assessment of agro-industrial waste extracts on *Nigella sativa* oil stability: Different concentrations (200, 400, 600 and 800 ppm) of the olive leaves, tomato peel, orange peel, and water melon peel extracts were added separately to 50 g of the studied oil to select the best concentration of these waste extracts as antioxidants. At the same time Tertiary-butyl hydroquinone (TBHQ, food grade) as a synthetic antioxidant was added at 200 ppm. The Soniprep 150 was used to carefully mix the beakers using ultrasonic waves, and then the beakers were put into an oven (Model WT Binder) set at 50˚C for 15 days. The oxidation effect of oil containing no additives was measured for reference purposes.

Fatty acid composition determination of *Nigella sativa* seed oil: The fatty acid profile of *Nigella sativa* seed (NS) oils were determined by gas chromatography (GC). The fatty acid methyl esters were prepared

according to the procedure of Freedman *et al*. (1986) and analyzed using a Shimadzu 12 A GC equipped with FID detector and 3.2 m x 4 mm glass column packed with 10% SP 2330 on 100/120 Chromosorb; WAW (Supelco, USA) according to Neff *et al*. (1992).

Determination of total phenolic content: The phenolic content of the studied extracts as mg gallic acid equivalent (GAE) /g was measured spectrophotometrically using the Folin-Ciocalteu reagent according to Rafiee *et al*. (2011) method.

Identification of phenolic compounds and bioactive components of tested ethanolic extracts by HPLC: Ethanolic extracts were analysed according to Lin *et al*. (1998), using high-performance liquid chromatography (HPLC) with a Waters 600 E system controller. The phenolic compounds and the other bioactive components were identified based on a comparison of the retention times of unknown peaks to those of the authentic reference standards. The integrated datum offered by the Waters data module was used to estimate the concentration of each constituent in the tested agro-industrial waste extracts.

Measurement of oxidative rancidity in oil samples: Peroxide value (meq O_2 kg^{-1}), and *p*-anisidine value (*p*-AnV), were determined according to the AOCS Method (1992). Thiobarbituric acid (TBA) was determined as described by Allen and Hamilton (1989).

Identification of oxidative stability in treated *Nigella sativa* oil using infrared spectroscopy (IR):

A Perkin-Elmer 1420 infrared spectrophotometer was used to record the infrared spectra. The infrared scan took 3 min, and the data were collected over a wave number range of 4000 to 450 cm^{-1} (Vlachos *et al*., 2006). The pure oil and oil that had been treated with selected waste extracts or the addition of TBHQ (200 ppm) were placed between two sodium chloride plates.

Technological methods:

Mayonnaise product: According to Abd El-Razek *et al.* (2013), the following ingredients were used to make mayonnaise: 120 g of sunflower oil, 40 g of egg yolk, 1.34 g of sugar, 0.8 g of mustard, 0.27 g of citric acid, 0.22 g of lemon juice, 1.8 g of salt, and 2.26 g of vinegar. The sunflower oil (control) was substituted by NS oil containing 200 ppm TBHQ and NS oil containing selected waste extracts as mentioned by Ozdemir *et al*. (2018). Samples were made by mixing all of the ingredients excluding the oil at room temperature in a plastic beaker. Sunflower oil was gradually added, and the mixture was homogenized for two minutes by homogenizer running at10000 rpm. Other mayonnaise samples were prepared using the same technique. The mayonnaise samples were placed in glass beakers and kept at 4 ±1˚C.

Quality assessment of mayonnaise: The pH value of mayonnaise samples were recorded using a pH meter (pH MVx 100 Beckman. USA). A rotary viscometer (Brookfield DVIIPRO, USA) was used to measure the viscosity at 20 rpm and 25˚C (Nielsen, 2010). A Hunter Lab Ultra Scan VIS model colorimeter (USA) was used to assess the colour values of all samples. Sensory quality of the prepared mayonnaise samples were evaluated by hedonic test using 9-point hedonic scale as proposed by Khan *et al*. (2020). Twenty panelists from the Department of Food Science and Technology at Alexandria University have evaluated the appearance, colour, taste, flavour, texture, and overall acceptability.

Statistical analysis:

The results are presented as mean ± standard deviation and Co-Stat Software computer program (2004) was used to analyze the data.

RESULTS AND DISCUSSION

Extracted yield and total phenolic content in different samples:

Ethanolic extracted yield and total phenolic content in different studied materials is presented in Table (1). It is clear that the ethanolic extracted yield varied widely between samples and ranged from 5.20 to 12. 41 g /100 g dry weight. The highest level of yield was found in OL extract, while the lowest was obtained in OP extract. The OL extract also had a high content of phenolic content (121.09 mg gallic acid equivalent / g dw.) compared to the other industrial waste extracts TP (68.79 mg GAE/ g dw) and OP (30.95 mg of GAE/ g of dw). WP extract recorded the lowest of phenolic content (Table 1). TP extract produced the highest content of phenolics. The phenolic content for olive leaves was reported as 234 to 250 mg kg^{-1} as tannic acid equivalents (Farag *et al*., 2007). According to Lafka *et al*. (2011), the total phenol content of olive leaf extracts was 16.33 mg kg^{1}, which was lower than the obtained values in this study. The phenolic content of the orange peel extracts (35.6 mg GAE/g) was close to the results of Elkhatim *et al*. (2018). Moreover, the values of phenolic content ranging from 8.7 to 203.1 mg GAE /100g were found for extracts from water melon peels (Morais *et al*., 2017). Rolim *et al*. (2018) and Neglo *et al*. (2021), found lower values than obtained values in the present study. Higher phenolic content (7.626 mg GAE/ g) was found in hydrothermal extracts from water melon wastes obtained at 300˚C for 30 min by Kim *et al*. (2014). The polyphenolic compounds was 120.46 ± 12.7mg GAE/100g of tomato peels as reported by Gharbi *et al*. (2017), which were higher than the obtained values in the present study. Coelho *et al*. (2019), found that ethanolic extracts of tomato skins and seeds had phenolic content values ranging from 0.244 to

2.558 mg GAE/ g which were lower than the values found in the present study. Szabo *et al*. (2019), reported an average of phenolic content 76 ± 4 mg GAE /100 g tomato peels using 80% methanol. Nincevic-Grassino *et al*. (2020), found that the phenolic content of tomato peels was 2626 and 2866 mg GAE /100 g which were extracted for 6 h with 96% and 70% hot ethanol, respectively. Also, Esmer *et al*. (2022), reported that the total phenolic content of the tomato by product extract was 3819.32 mg GAE/100 g dry extract using extraction conditions at 100˚C for 5.26 h. These variations in total phenolic content may be caused by the plant variety, the environmental conditions, the harvest time fruit maturity and the method of analysis.

Table 1. Yield of ethanolic extracted and phenolic content of tested agro- industrial waste samples (on dry weight basis)

Agro-industrial wastes	Ethanolic extracted yield (%)	Phenolic content (mg GAE /g dw)
Olive leaves (OL)	12. 41± 2.05	121.09±1.33
Orange peel (OP)	5.20 ± 1.05	30.95 ±0.85
Water melon peel (WP)	7.81 ± 1.77	9.08 ±0.66
Tomato by-product (TP)	8.42 ±0.99	68.79 ±0.14

Identification of phenolic and other bioactive components: HPLC was used to separate and identify the ethanolic extracts. The results obtained are represented in Table (2), which showed the phenolic and the other bioactive compounds and their percentage of each ethanolic extract of the studied waste materials. OL extract contained seven phenolic compounds with oleuropein (7.2g/100g) having the highest concentration (78.01%), followed by protocatechuic acid, apigenin 7-glucoside, and hydroxytyrosol (Table 2). Rutin and caffeic acid were the minor phenolic compounds. These results are close to those reported by Ortega-García *et al*. (2008), who showed that the oleuropein was the predominant compound with a concentration ranging from 45.11 to 80.67 mg/g. These results are in agreement with Ghomari *et al*. (2019), who found that a large amount of oleuropein extracted from olive leaves with distilled water at 60˚C (pH = 3) for 4 h. At the same time, seven compounds were identified in OP extract where ascorbic acid, hesperidin, syringic acid, and naringin had the highest percent (36.06, 24.75, 18.51 and 10.34% respectively). The data of phenolic compounds for OP are nearly the same data obtained by Ahmed et al. (2019), who showed that hesperidin, naringenin, gallic and quercetin were the most abundant of orange peels . In contrast, Omoba *et al*. (2015), reported that the predominant phenolic compounds were quercitrin, rutin, and quercetin in orange peel. The most abundant compounds in bitter orange peel were hesperidin (0.066 to 66.095 mg/g d.b) followed by Naritutin (0.025 to 26.5 mg/g), whereas the major phenolic compounds are naringin (1.1 to 5.10 mg/g d.b) and neohesperidin (0.66 to 7.9 mg/g) as reported by Sawalha *et al*. (2009). In contrast, Omoba *et al*. (2015), reported that the predominant phenolic compounds were quercitrin, rutin, and quercetin in orange peel. In the WP extracts, chlorophyll was identified with the highest amount (42.21%) followed by catechins (23.66%) then diosmetin (12.55%). Rolim *et al*. (2018), identified that higher levels as the bioactive compounds were gallic acid, catechins, salicylic acid and eugenol in the water melon peel extract.

In the present data of HPLC analysis for TP extract, lycopene was the major components amounted to 50.51%, which is one of the most effective singlet oxygen quenchers of tomato by- product (Zhao *et al*., 1989). Quercetin and ascorbic acid were 2.89 and 1.27 g/ 100g dry extract of TP. Sánchez-Rodríguez *et al*. (2012), reported the presence of quercetin, and kaempferol, hesperetin, and hesperidin, have inhibitory effects against the enterovirus EV71. Kaempferol blocks the translation of viral proteins (Tsai *et al*., 2011). The *p*-coumaric acid was 429.99 ± 38.53 mg/100 g dry extract as the highest amount of acid in the tomato peel extract (Esmer *et al*., 2022). The presence of phenolic and the other bioactive compounds possibly explains the antioxidant potential found in all extracts. Among these, the phenolic compounds are predominant, which produce several bioactivities, including anticancer, anti-inflammatory, antiplatelet, antimicrobial, antimutagenic, anti-neurodegeneration, and cardioprotective characteristics.

Fatty acids composition of *Nigella sativa* (NS) oil:

The results of gas chromatogram showed the NS oil had a high content of linoleic acid (61.6%), followed by oleic acid (22.8%) then palmitic 12.4% and stearic acids (3.5%) with α Linolenic acid (C18:3) in minor amount (0.4%). Fatty acid composition affected by seed quality, environmental conditions, and oil-processing technique. Ashrafi *et al*. (2023), stated that it has a high concentration of linoleic acid as olive oil which is one of the three essential fatty acids for human nutrition and can reduce total cholesterol and the low density of lipoproteins. Therefore, antioxidants must be added to avoid and reduce their oxidation and loss during storage.

Table 2. Phenolic and other bioactive compounds (%) in ethanolic extract of various agro-industrial wastes as determined by HPLC

Agro-industrial wastes	Compound	Compound content (g /100 g dw)	Compound (%)
Olive leaves (OL)	Oleuropein	7.2	78.01
	Apigenin 7-glucoside	0.4	4.33
	Protocatechuic acid	0.97	10.51
	Rutin	0.02	0.22
	Vanillic acid	0.21	2.27
	Caffeic acid	0.1	1.08
	Hydroxytyrosel	0.33	3.57
Orange peel (OP)	*p*- Coumaric acid	0.12	2.88
	Ferulic acid	0.19	4.57
	Syringic acid	0.77	18.51
	Hesperidin	1.03	24.75
	Naritutin	0.12	2.88
	Naringin	0.43	10.34
	Ascorbic acid	1.5	36.06
Water melon peel (WP)	Chlorophyll	5.28	42.21
	Catechins	2.96	23.66
	Diosmetin	1.57	12.55
	Quercetin	0.20	1.59
	Pheophytin	1.27	10.15
	Salicylic acid	1.12	9.59
Tomato by-product (TP)	Lycopene	5.87	50.51
	Beta carotene	0.69	5.94
	Lutein	0.18	1.55
	Ascorbic acid	1.27	10.93
	Quercetin	2.89	24.87
	Kaempferol	0.72	6.19

Effect of agro-industrial waste extracts on oxidative stability of NS oil.

Different concentrations of each tested waste extracts (200, 400, 600, and 800 ppm) were combined with NS oil to choose the best concentrations for antioxidant activity of the wastes. Oil without additives and oil treated with 200 ppm TBHQ were used as controls. Peroxide value (PV), *p*-anisidine value (*p*-AnV), and thiobarbituric acid (TBA) were measured as indicative of the oxidative degradation according to the primary oxidative products and secondary changes.

Peroxide value (meq O_2/kg): Figure (1) illustrates the PV of NS oil with added the studied extracts during storage time at 50°C. PV values for the treated samples with extracts were lower than that of the control oil during the storage time. The OL extract had the highest antioxidant activity. The 400 ppm OL extract inhibited the formation of hydroperoxide in NS oil in similar way as that produced by the addition of 200 ppm TBHQ after 15 days of oil storage at 50°C. The 800 ppm concentration of OL extract revealed a reduction in PV of 80.39% when compared to the control sample. PV was reduced by 73.68% of oil treated with 800 ppm TP ethanolic extract, which had the same antioxidant effect of 200 ppm TBHQ throughout the entire storage time. Meanwhile, both OP and WP extracts at 800 ppm showed a reduction in PV of 64.74 and 45.26%, respectively when compared to the control oil sample. Moreover, the OP and WP extracts at 800 ppm of NS oil had higher PV than when 200 ppm of TBHQ was applied during the storage. This antioxidant activity increased with an increased concentration of these natural extracts added to *Nigella sativa* oil during storage time. The antioxidant activity can be summarized in the following order: OL > TP > OP > WP. The antioxidant activity of extracts is dependent on the type of plant used. This is in agreement with Zeyada

et al. (2008), who concluded that tomato peels, cucumber peels, water melon peels and potato peels had the lowest in PVs of vegetable oils and had antioxidant activity. Taghvaei and Seid (2015), stated that the addition of *Majorana syriaca* ethyl acetate extract (200 ppm) resulted in 28.9-43.2% protection against lipid oxidation because of their physiological effects, particularly their effectiveness in inhibiting the oxidation of lipids (Mnari *et al*., 2022). The extract lemon balm slowed down the rancidity of the heated corn oil at 70˚C and improved the stability of corn oil (Farahmandfar *et al*., 2019).

2. *p*- Anisidine value (*p*-AnV).

As can be seen in Figure (2), after 15 days of storage at 50˚C, the *p*-AnV. of the control NS oil (without any additions) has increased from 0.58 at zero time to 12.22. The *p*-AnV of NS oil with added extracts increased during storage, but the increasing rate is apparently lower than the rate of increase for the control sample without additives. The *p*-AnV of stored treated oil at 50˚C decreased when compared with the control sample. The treated oils with waste extracts at 800 ppm concentration showed the maximum reduction in *p*-AnV. The reduction of *p*-AnV was 50.9,51, 32.08 and 19.8% with the added OL, TP, OP and WP extracts, respectively when compared to the control samples. It was clear that the 400 ppm OL extract and 600 ppm TP extract are as effective as the synthetic antioxidants TBHQ used (200 ppm). It was evident that both OP and WP extracts had low antioxidant activity. Also, the *p*-AnV values decreased by increasing the concentration of the waste extracts. The results obtained in the present study are completely consistent with those of Zeyada *et al*. (2008), who discovered that the *p*-AnV determinations support the same trends as those found in the PV determination of oxidation. The *p*-anisidine values of stabilized soyabean oil at 60ºC with the rice bran extract showed a decline up to 24% when compared to the control (Chatha *et al*., 2006). Sultana *et al*. (2007), reported that the corncob methanolic extract at 1000 ppm concentration also decreased 41.8% in the *p*-ansidine values for stabilized corn oil samples as compared to the control. The extracts of agro wastes of different plant materials showed a reduction up to 59% in *p*-anisidine values of stabilizing corn oil. Tehseen *et al*. (2021), showed that spinach extract at 1800 ppm concentrations enhanced the oxidative stability of the stored corn oil at 25˚C and 60˚C.

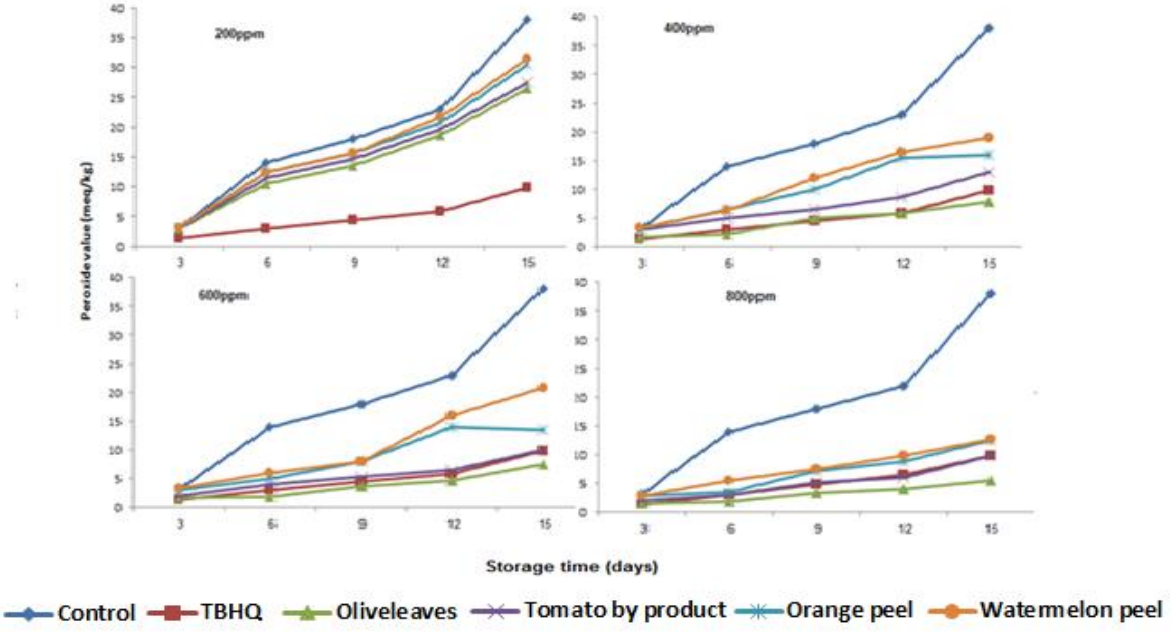

Fig. 1. Changes in peroxide value (meqO_2/ kg) of *Nigella sativa* oil treated with different concentrations of studied agro-industrial extracts compared to crude oil and oil treated with 200 ppm TBHQ during heating at 50˚C for 15 day

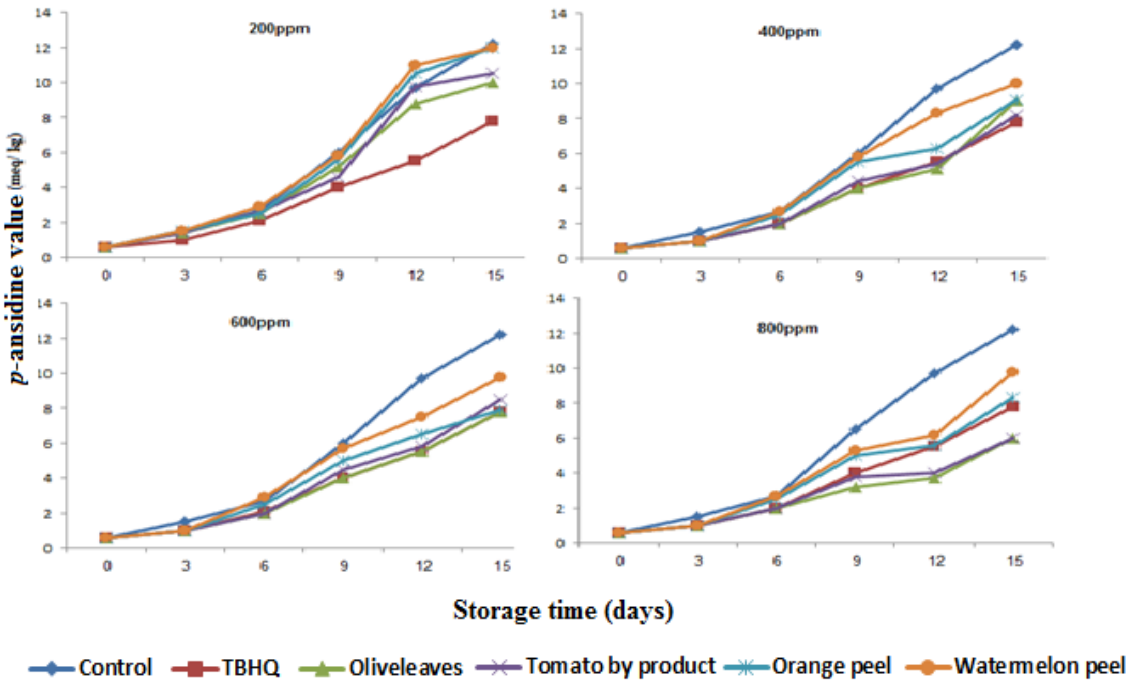

Fig. 2. Changes in *P*-anisidine value of *Nigella sativa* oil treated with different concentrations of studied agro-industrial extracts compared to crude oil and oil treated with 200 ppm TBHQ during heating at 50˚C for 15 day

3. Thiobarbituric Acid (TBA)

The TBA of oil containing antioxidants is shown in Figure (3). The TBA value significantly increased from 1 to 18.3 mg malonaldehyde / Kg oil without antioxidant, while oil containing 200 ppm TBHQ increased to 0.092 mg malonaldehyde / Kg oil after stored at 50˚C for 15 day. Supplementation with the tested ethanolic extracts showed a reduction in TBA values as compared to the control. The OL (400 ppm), TP (600 ppm), OP (800 ppm) and WP (800 ppm) extracts showed about 67.21, 66.94, 60.98%, and 57.38% reduction in TBA values as compared to the control. These concentrations of the tested ethanolic extracts affected TBA values quite close to the effect of 200 TBHQ (Figure 3). The data for TBA analysis generally indicated that remarkably faster formation of the di unsaturated aldehydes was observed in the control sample without any antioxidant addition. However, the TBA values for NS oil with different concentrations of each added OL, TP, OP and WP extracts showed similar trends to those produced by the peroxide value and *p*-anisidine value. These results are in accordance with that mentioned by Zeyada *et al*. (2008), Rafiee *et al*. (2011) and Mnari *et al*. (2022). Agro - extracts are rich in phenolic antioxidants and flavonoids that act with lipid or hydroxyl radicals to convert them into stable products and enhanced thermal stability (Amri *et al*., 2017). Seo *et al*. (2017), showed that gallic acid from chitosan acted as antioxidants at 140˚C by the inhibition of the conjugated dienoic acid value and the *p*-AnV increased. Pomegranate peel extract at 1000 ppm delayed oxidation and reduced lipid deterioration in corn oil during heating compared to BHT (Mnari *et al*., 2022).

Impact of selected plant extracted on the oxidation of *Nigella sativa* oil using infrared analysis. The oil samples that had been treated with TBHQ (200 ppm), OL (400 ppm), TP (600 ppm), OP (800 ppm), and TBHQ-free oil were all subjected to infrared analysis after being stored at 50˚C for 15 days. The plant extract concentrations were selected according to match the previous analysis for peroxide and *p*-anisidine values because they demonstrated the strongest antioxidant activity.

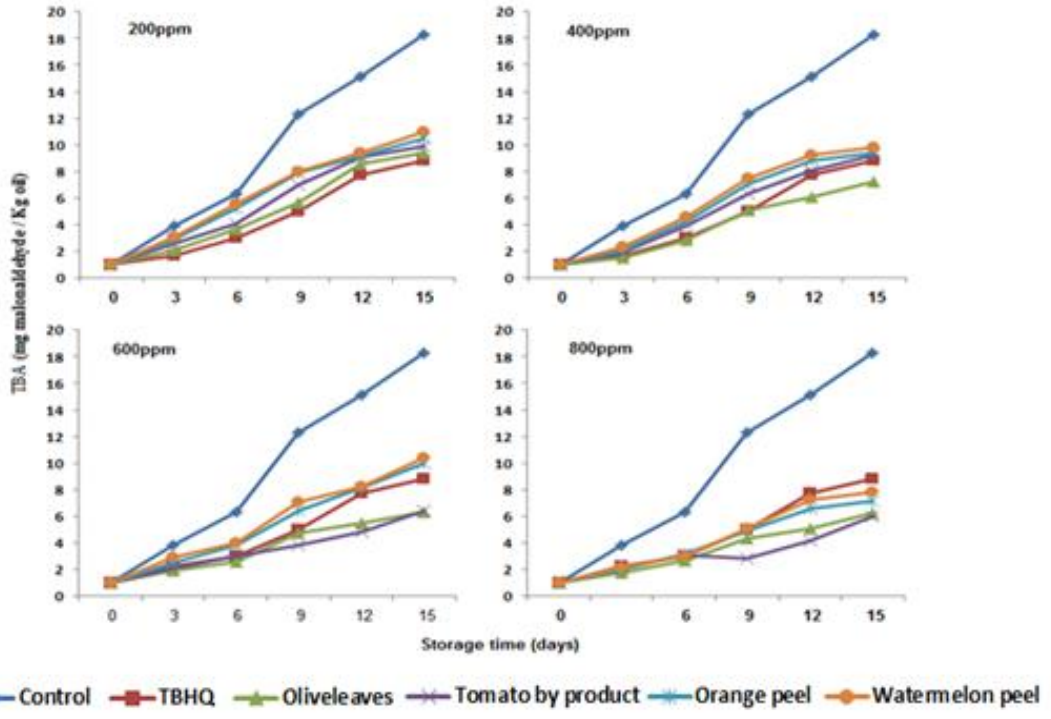

Fig. 3. Changes in thiobarbituric acid (TBA)value (mg malonaldehyde / kg) of *Nigella sativa* oil treated with different concentrations of studied agro-industrial extracts compared to crude oil and oil treated with 200 ppm TBHQ during heating at 50°C for 15 day

The functional groups of oil were discovered using the infrared spectrum, which can also be used to evaluate oil oxidative stability. Figure (4, a) depicts the IR spectrum of NS oil at zero time. A strong broad absorption bands were seen in the FTIR spectra as follows: at 3473 cm^{-1}, which are typical of hydroxyl groups involved in hydrogen bonds; at 2853-3009 cm^{-1}, which indicates the presence of the CH_3, CH_2 and CH resonances close to the saturated and olefinic backbone; the main region of interest between 1658-1744 cm^{-1}, which is attributed to carbonyl vibrations (C=O) and other group at 1464 (bending vibrations of the CH_2 and CH_3); 1379 (bending of CH cis-olefinic groups); 1377 (bending vibrations of CH_2 groups); 1236, 1165, and 1720 (C–O stretching vibration). It has been established that the presence of three additional bands at 1399 cm^{-1}, 1238 cm^{-1}, and 1163 cm^{-1} in oil correlates with the amount of saturated dacyl groups and CH cis-olefinic in the sample (Workman, 2001).

The changes of the frequency of most of the bands for all heated oil samples under the oxidative conditions of heating for 15 days were shown in Figures (3 b, c, d, e and f). The infrared spectra of the samples have shown some differences related to the width and intensity of some bands, as well as the presence or absence of others (Guill´en and Cabo, 1997). The wide background signal at 3400 - 3550 cm^{-1} is probably caused by a variety of oxidation products, including water and peroxides. The spectrum displayed variations in an absorbance at 1728 cm^{-1} that may be related to the appearance of saturated aldehyde groups or other secondary oxidation products. The bands of the carboxylic C= O band was lower in the oil sample heated at 50°C for 15 days without any antioxidant than the oil sample that was not heated at all. Figure (4) showed that the heated oil without any additives of antioxidants was oxidized as demonstrated by the intense absorption of the carbonyl band (Figure 4, b). The addition of OL and TP or OP extracts to the oil protected the oil from the secondary oxidation. The use of OL extracts (400 ppm) as potent antioxidants produced oil samples with the highest levels of oxidative stability that were comparable to the effects of synthetic TBHQ (200 ppm). This may be attributed to the presences of the amount and type of phenolic compounds in OL extracts which enhance the activity of the other antioxidants in the extracts or synthetic TBHQ effect. As shown in Figure (4), the tested oil samples, with the exception of oil treated with OP, showed no

change in shifting towards wavelength values in the band near 3009-2853 cm^{-1}, 1399 cm^{-1}, 1238, and 1163 cm^{-1}. These results agreed with Ciriminna *et al*. (2016) and confirm the conclusions reached previously using analytical chemical techniques. The addition of olive leaves and tomato extracts to NS oil have improved their oxidative stability and slowed the production of total oxidation products.

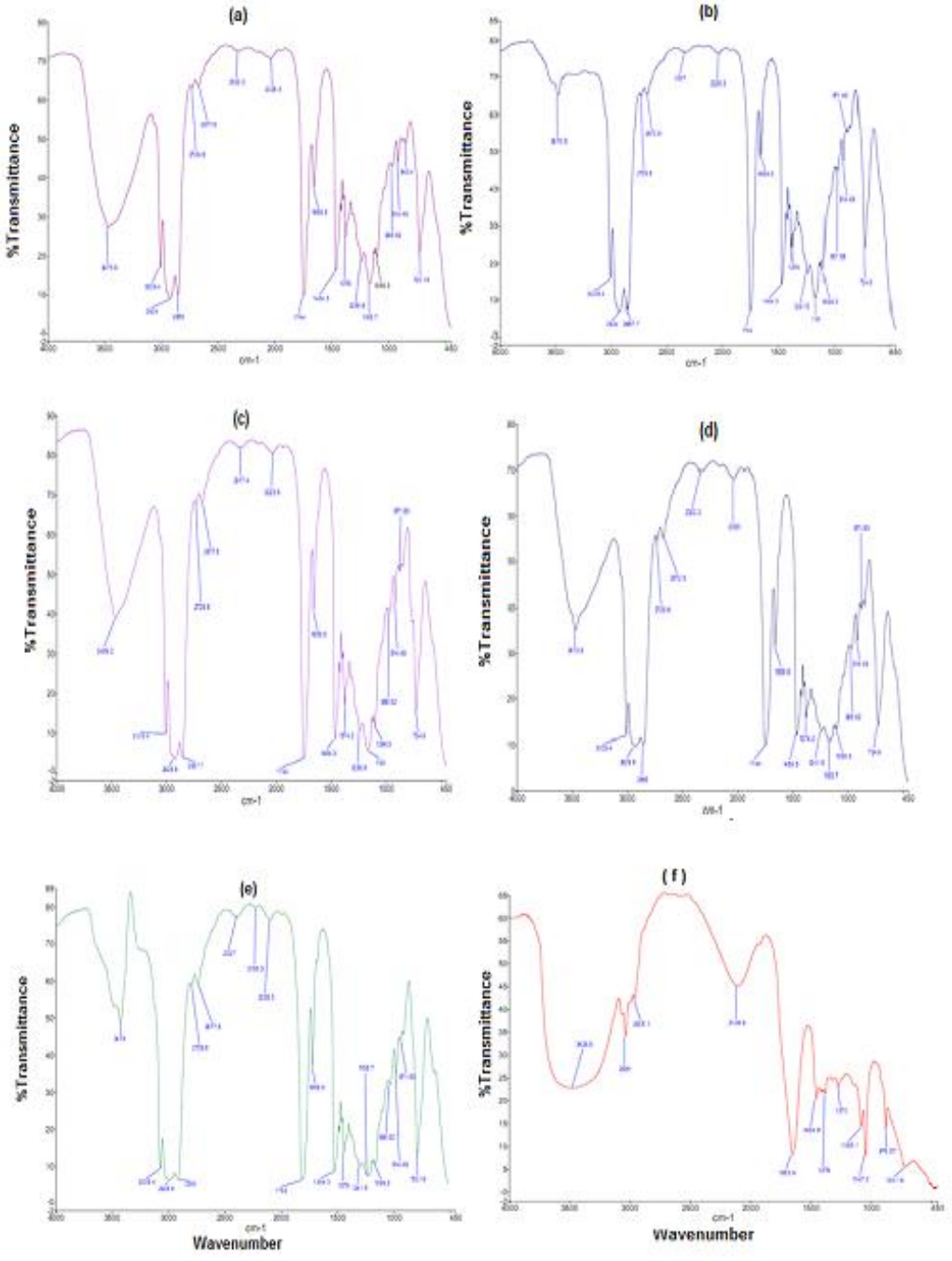

Fig. 4. IR spectra of *Nigella sativa* oil : (a) control (before heating); (b) without additives after 15 days at 50˚C; with 200 ppm TBHQ, (c); with 400 ppm olive leaf extract, (d); with 600 ppm tomato by-product extract (2) and 800 ppm orange peel extract (d)

Quality assessment of mayonnaise formulated with NS oil

Four mayonnaise formulations were prepared: with sunflower oil (control), NS oil containing 200 ppm TBHQ, NS oil containing 400 ppm OL extract, and NS oil containing 600 ppm TP extract (Figure 5). The changes in pH, viscosity, colour and sensorial attributes of the mayonnaise samples are shown in Table (3). The stability and structure of mayonnaise are significantly influenced by its pH (Depree and Savage, 2001). It can be clearly seen that the pH values varied from 4.14 to 4.20 at zero time. The samples NS oil containing extracts exhibited lower reduction in the pH compared to the control sample. The viscosity of mayonnaise samples prepared with NS oil was higher than the control sample. A decrease in the amount of linoleic and unsaturated fatty acids in oil may be the cause of the decrease in the viscosity value of mayonnaise samples (Ennouri *et al.*, 2005). Colour is the primary criterion consumers use to accept the product (Fernandesa and Mellado, 2018). The colour parameters of all mayonnaise were different by replacing oil with NS oil. As can be seen in Table (3) formulations of mayonnaise using NS oil had lower (L) lightness, higher a* values, and lower b* values than control mayonnaise. It was observed that the control mayonnaise had the highest L* values and a* values presented negative results that indicated a trend towards a green colour. There was no statistical difference between a* and b* values of the mayonnaises formulation with NS oil. On the other hand, the Chroma, which is an indicator of the intensity and saturation of the colour, also increased with oil replacement with NS oil, and it ranges from 19.01 to 19.35. The ΔE parameters were lower than 3 in formulations of mayonnaise using NS oil.

Sensorial attributes of mayonnaise

The sensory evaluation of mayonnaise was influenced by NS oil as shown in Table (3). The samples made with sunflower oil received higher appearance, and colour scores than the mayonnaise made with NS oil. This might be due to extracts colour that the lightness supported this result. However, the panellists gave in the same Table "like moderately" for colour of mayonnaise made with NS oil. The result was supported by Δ E values which indicated that the NS oil did not cause of consumers unpleasant colour properties. On the other hand, there were no statistical differences in texture, and overall acceptability scores. The lowest overall acceptance score was obtained to NS oil sample containing tomato extract. According to the obtained data, panelists accepted all the samples and scored "like very much" for acceptability. The sensory results of the present research are in agreement with Amin *et al*. (2014), who showed that sunflower and soybean oils were the most sensorial acceptable oils for low fat mayonnaise.

Table 3. pH, viscosity, colour parameters and sensory evaluation of mayonnaise samples with different formulations

Variables	Mayonnaise samples			
	Control (sunflower oil)	NS oil + TBHQ	NS oil + OL extract	NS oil + TP extract
pH	4.18±0.01	4.20±0.01	4.14±0.01	4.17±0.02
Viscosity (MPas)	2490	2500	2520	2510
Colour parameters				
L*	62.2^{a}	54.27^{b}	55.37^{b}	55.36^{b}
a*	$-.53^{b}$	2.05^{a}	1.9^{a}	2.11^{a}
b*	11.74^{b}	13.51^{a}	13.44^{a}	13.58^{a}
hue	92.25^{a}	82.84^{b}	83.27^{b}	82.63^{b}
Chroma	15.31^{b}	19.35^{a}	19.01^{a}	19.28^{a}
ΔE	---	2.58	2.43	2.64
Sensory evaluation				
Appearance	8.50^{a}±0.63	7.90^{b}±0.57	7.80^{b}±0.92	7.80^{b}±0.85
Colour	8.50^{a}±0.52	7.50^{b}±0.97	7.60^{b}±0.63	7.40^{b}±0.70
Odour	8.30^{a}±0.48	8.00^{b}±0.42	7.90^{b}±0.74	7.90^{b}±0.84
Taste	8.40^{a}±0.32	7.80^{b}±0.64	7.80^{b}±0.53	7.70^{b}±0.33
Texture	9.00^{a}±0.05	8.80^{a}±0.48	8.90^{a}±0.13	8.90^{a}±0.69
Overall acceptability	8.20^{a}±0.83	8.00^{a}±0.95	7.90^{a}±0.67	7.80^{a}±0.71

Values in each row followed by same letters are not significantly different (p≤0.05).NS: *Nigella sativa*, TBHQ: tertiary-butyl hydroquinone (200ppm), OL: olive leaf extract (400 ppm), TP: tomato by- product extract (600ppm).

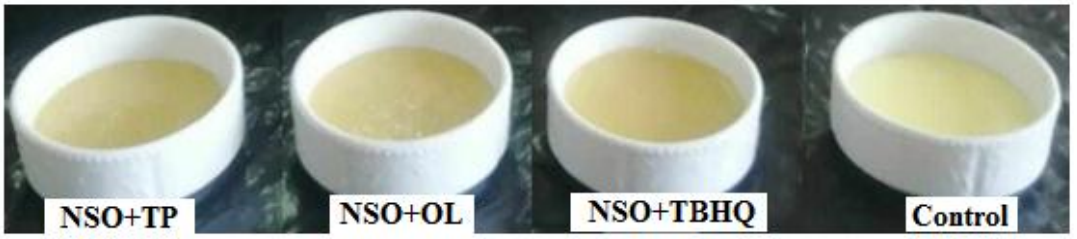

Fig. 5. Appearance of mayonnaise samples containing with sunflower oil as a control, *Nigella Sativa* oil (NSO) with 200 ppm Tertiary-butyl hydroquinone (TBHQ), 400ppm olive leaves (OL) and 600ppm tomato by-product (TP)

CONCLUSION

Extracts of olive leaves and tomato by-product or orange peels can be utilized as a natural promising BHQT substitute in the oxidative stability of *Nigella sativa* oil as well as the possibility of using NS oil in the production of functional products with preserving product stability.

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الملخص العربي

تحسين الثبات التأكسدى لزيت حبة البركة بإستخدام بعض مستخلصات المخلفات الزراعية والصناعية والإستفادة منه فى إنتاج المايونيز

نوال نشات زيادة [١]، منى ابراهيم مسعود[٢]، سعدية محمد هاشم [٣]

يعد تثبيط أكسدة الزيوت والدهون فى المنتجات الغذائية الغنية بالدهون من أهم العوامل التى تحافظ على جودة المنتجات الغذائية. لذلك هدفت الدراسة إلى تحسين وتقييم الثبات التأكسدى لزيت حبة البركة المخزن عند ٥٠ درجة مئوية لمدة ١٥ يوم بإستخدام تركيزات مختلفة من مستخلصات أوراق الزيتون (OL) والمخلفات الثانوية لتصنيع الطماطم (TP) وقشور البرتقال (OP) وقشور البطيخ (WP) كمصدر للمركبات الطبيعية النشطة حيويا. كما قيمت الدراسة جودة المايونيز المنتج من زيت حبة البركة (NS) كمكون وظيفى. تم الإستخلاص والتعرف على المركبات الفينولية الطبيعية من تلك المخلفات الزارعية والصناعية بإستخدام HPLC. أوضحت النتائج أن مستخلص أوراق الزيتون يتميز بإرتفاع محتواه من المركبات الفينولية الكلية (١٢١,٠٩ مجم حامض جاليك لكل جرام وزن جاف) و التى تزيد بنسبة ١,٧٦ و ٣,٩١ و ١٣,٣٤% عن الفينولات الكلية في مستخلصات مخلفات تصنيع الطماطم وقشور البرتقال وقشور البطيخ على التوالي. وقد أوضحت نتائج قياسات الثبات التأكسدى أن إضافة جميع المستخلصات إلى زيت حبة البركة قد أدى إلى تأخير حدوث التزنخ تحت ظروف التجربة مقارنة بالكنترول طبقا لقيم البيروكسيد و البارا–أنيزيدين وحامض الثيوباربتيوريك. كما أسفرت نتائج التحليل بطريقة الأشعة تحت الحمراء كفاءة قوة تضاد الأكسدة لكلا من مستخلص اوراق الزيتون عند تركيز ٤٠٠ جزء فى المليون ومخلفات تصنيع الطماطم بتركيز ٦٠٠ جزء فى المليون فى تثبيط التزنخ والتى تماثل تأثير مضاد الأكسدة الصناعى TBHQ بتركيز ٢٠٠ جزء فى المليون، مما يؤكد إمكانية إستخدامهما كمضاد أكسدة طبيعي في تثبيط التزنخ فى الزيت المخزن. وقد أظهرت نتائج إختبارات الجودة و التقييم الحسي للمايونيز زيادة اللزوجة وتغيير فى خواص اللون و الغير محسوس للعين البشرية حيث كانت قيم ΔE أقل من ٣ وكما حازت جميع منتجات المايونيز "قبولاً" من قبل المحكمين.