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Physico-Chemical Properties, Fractionation and Antioxidant Activity of Some Essential Oils Utilized in Mayonnaise Preparation and Storage

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Abstract

Two types of essential oils (thyme and Stachys) were used in this Estimation. The chemical composition, antioxidant activity (by DPPH assay) and the rancimat method were used to assess the stability effect of these essential oils. The tested oils were added to mayonnaise to evaluate its capability for inhibition of secondary oxidation products. By DPPH assay, antioxidant activity showed that thyme essential oil was the best antioxidant agent as radical scavenging agents at 450 and 500 ppm concentrations, even better than BHT at the 200 ppm concentration. As a typical antioxidant, butylated hydroxytoluene was used for comparison. The mayonnaise samples prepared by adding thyme, stachys essential oils and TBHQ (control +) at concentrations of 200,400, 600 and 200 ppm into the sunflower oil free from antioxidant. At the amounts used in this study, thyme and stachys oils were significantly successful in reducing the oxidation of sunflower oil, especially, at 500 ppm which showed more efficient than BHT. Specifically, in the presence of thyme oil, the induction period of sunflower oil was considerably elongated. However, butylated hydroxytoluene (BHT) was more efficient than thyme and Stachys oils at the 200-ppm concentration against oxidation of oils. all concentrations of thyme and stachys essential oils had an antioxidant activity similar to synthetic antioxidant of TBHQ at a concentration of 200 ppm for inhibition of secondary oxidation products in mayonnaise in comparison with the control sample. As a conveniently available source of natural antioxidants, thyme and stachys essential oils can be used for use in fats, oils and food containing fats.

Keywords: Rancimat, Antioxidant, Stachys, Thyme, Essential oil, Mayonnaise.

1 Introduction

One of the main factors causing degradation is lipid oxidation through storage and producing oils, fat-containing products and edible fats. It alters fat and oils' main quality control parameters, such as colour, flavor, aroma, and nutritional value, affecting consumption suitability (Nogala-Kalucka et al 2005). Oils chemical structure makes them more vulnerable to oxidation because of the higher content of polyunsaturated fatty acids (PUFA). To address the fat and oil stability problem, synthetic antioxidants such as BHA and BHT are integrated with fats and oil (Choe and Min 2009).

Consumers are today becoming more aware of their food and food additives throughout the world's nutritional value and safety. Natural food and food additives are preferred around the same time that is considered safer, cheaper and less toxic than artificial ones (Zugravu et al 2017). However, the decrease in the usage of synthetic antioxidants in food products was attributed to their instability and their supposed position as promoters for carcinogenesis. As a result of these safety concerns, food scientists are increasingly replacing synthetic with natural antioxidants, which are usually expected to be safer (Yanishlieva and Marinova 2001). It is understood that adding specific aromatic spices or herbs to lipid-containing materials has retarded the oxidation development for some time (Choe and Min 2009). Many medical herbs and spices have been declared to be more effective in retarding the lipid oxidation development in oils and fats with antioxidant properties (Yanishlieva and Marinova 2001, Nogala-Kalucka et al 2005, Choe and Min 2009, Roy et al 2010).

Based on complex toxicity studies, in foods for safety and approval at low concentrations, synthetic antioxidants were tested. Although in most countries synthetic antioxidants are commonly used, confusion remains concerning their safety (Shahidi 2005). Because of their potential toxic effects during long-term usage, the safety of synthetic antioxidants is still a controversial problem. If synthetic antioxidants are unlikely to be harmful, it seems fair to attempt to substitute them with natural antioxidants that are more compatible with human existence (Taghvaei and Jafari 2015).

Mayonnaise is an emulsion of oil-in-water. The emulsion comes from a slow blend of oil and pre-mixed egg yolk, vinegar and mustard as a water-in-oil emulsion is created by immediate addition of the oil and aqueous phase. It is one of the oldest and most used sauces in the world today (Depree and Savage 2001). One of the main causes of mayonnaise chemical spoilage is oxidation, leading to rancidity and/or degradation of nutritional quality, colour, taste, texture and food safety. In lipid-bearing foods, oxidation of lipids is a major problem, particularly in food products that have highly polyunsaturated fatty acids (PUFA). Unless careful steps are taken, lipid oxidation happens nearly immediately in the food containing these lipids. When highly unsaturated oils are emulsified in different food systems, specific problems arise (Frankel et al 2002). A growing interest in aromatic herbs, in both the industry and in science study due to their antimicrobial and antioxidant properties (Vahidyan et al 2012).

Essential oils are volatile plant components that cause plant aromas (Ali et al 2015). Mankind has been utilized in food, cosmetics and traditional healing medicines since ancient times. Throughout the food and medicine industry, these aroma components of plant oils are used as flavor enhancers and fragrances industry, etc. (Shrivastava et al 2010). Thyme is an evergreen shrub aromatic from the *Lamiaceae* family, also known in Arabic as Zaatar is a widespread species planted in the Arab world (Basch et al 2004). Several lab experimental studies have also shown that essential thyme oil has good antimicrobial and antioxidant properties (Boskabady et al 2006, Rota et al 2008, Grigore et al 2010). Thyme oil, throughout its aroma and commercial value is used in different types of nutrients and drinks. It is also used in the food industry as a natural food preservative (Bhavaniramya et al 2019).

Stachys herbal tea is used for the treatment of gastrointestinal and respiratory disorders in southern Anatolia. The hydrodistillation derivatives of stachys essential oil were also studied, by both of GC-FID and GC/MS techniques. There have been 37 known compounds made up of 98.3% of the essential oil. The main components of stachys oil are β-Phellandrene, α-pinene and germacrene-D. (27, 18.5 and 13%, respectively). Antioxidant properties showed a surprisingly radical scavenging behavior (Iscan et al 2012).

This study will open the way for thyme and stachys essential oils to classify the main volatile constituents and their antioxidant capabilities as natural antioxidants agents in mayonnaise stored at room temperature (25^{O}C) which can be used in the food industry.

2 Materials and Methods

2.1 Materials

Thyme "*Thymus vulgaris L.*" leaves were purchase from the local market (haraz store, Cairo, Egypt), Stachys "*Stachys aegyptiaca* " was collected from Bani Sweif governorate, Egypt. The leaves were air-dried at 40^{o}C overnight until it is completely dry after detached from the branches and stored in a polyethylene bag at 4^{o}C. Free antioxidant sunflower oil was kindly supplied from ARMA (10th of Ramadan city, Egypt). Butylated hydroxytoluene (BHT) was obtained from Merck (Darmstadt, Germany). Sugar, mustard flour, white pepper, eggs, lemon and salt were purchased from a local market, Giza, Egypt.

2.2 Extraction of essential oils

In Clevenger's apparatus, 3 hours hydrodistilled extraction process for the isolation of the essential oils from air-dried thyme and stachys were carried out. Anhydrous sodium sulphate was used for drying the essential oil then the resulted oil was stored in dark glass bottles before analysis and the extraction yield was measured (Yağci et al 2012).

2.3 Preparation of mayonnaise

The following ingredients were included in the Mayonnaise control sample (w/w): sunflower oil 70%, egg yolk 22.17%, vinegar 0.63%, sugar 0.63%, salt 1.26%, mustard powder 0.31%, white pepper 0.31 and lemon juice 2.20%. Thyme, Stachys essential oils and TBHQ (control+) were individually used for the preparation of mayonnaise samples in the antioxidant-free oil of sunflower at concentrations of 200.400, 600 and 200 ppm for each. Egg yolk and other powdered materials were initially mixed for 30s with water 2.49%. In the aqueous process sunflower oil and vinegar have then been added, and the emulsion is then mixed for 1 minute. The mayonnaises were labelled after preparation and covered with screw caps in glass jars. These samples have been kept for 5 weeks at 25^{o}C **(Nadir et al 2003).**

2.4 Physicochemical measurements of thyme and Stachys essential oils

2.4.1 Specific gravity (SP.gr): using a pycnometer (10 mL capacity) at 20°C, the specific gravity of thyme and stachys essential oils was determined according to the reporting procedure by Parthiban et al (2011).

2.4.2 Refractive index (RI): The refractive index (RI) of thyme and Stachys essential oils was determined by using an Abbe Refractometer at 20°C, by the method defined by Parthiban et al (2011).

2.4.3 Acid value (AV): Acid value (AV) of thyme and stachys essential oils as mg KOH/gm oil was determined according to the methods outlined by Parthiban et al (2011).

2.5 Identification of thyme and Stachys essential oil constituents by GC/MS technique

The essential oils were analysed using the capillary column VF-5, MS (30mX 0.25mm ID and 0.25µm thickness film) and interfaced with the selective mass selective detector – GC/MS Model (Varian240-MS). A helium flow rate at 1ml/min as the carrier gas. Direct injection (1µL) into the divided injector has analysed the essential oils of thyme and Stachys. The temperature of the injector and detector was 250°C. The column oven was programmed to temperatures between 45 and 240°C at 45°C/10 and 6°C/15min at a rate of 240°C. A range of mass-spectrums from 20-425 amu was acquired, with 0.5 scans per second. Thyme and Stachy's essential oils were identified in a comparison between the retention times and the authentic standards that

were injected in the devices under the same conditions and their mass spectra were compared to those of the Wiley library. The quantitative measurement was based on the integrations of the peak area **(Shrestha et al 2013).**

2.6 Radical scavenging activity using DPPH assay

DPPH experiments with a 517 nm spectrophotometer have determined antioxidant activity. The potential to donate thyme and Stachys essential oils hydrogen atoms or electrons was calculated by bleaching DPPH solution to light-yellow (Goze et al 2009). Fifty microliters of various concentrations (50, 100, 200, 250, 300, 350, 400, 450 and 500 µg/mL: "ppm") of the essential oil in methanol as well as BHT, as a standard antioxidant (50, 100, 150 and 200 µg/mL) were put into appropriate tubes, and 4 mL of 0.004% methanolic solution of DPPH was added to each tube and vigorously shaken (Gülçin 2006). The tubes should stand for 30 minutes at room temperature. Control without any sample was prepared simultaneously. For the preparation samples, absorption variations have been measured at 517 nm. Radical scavenging activity was calculated by using the following formula as the % inhibition:

$$\% \text{ Inhibition} = \frac{AB-AA}{AB} \text{ X100.}$$

Where: AB: absorption of blank sample (t=0 min), AA: absorption of sample solution (t=30 min).

2.7 Measurement of oxidative stability of sunflower oil

Rancimat test was used to determine the antioxidant activity of thyme and stachys essential oil added at levels of 200 and 500 ppm which were mixed well with sunflower oil using magnetic stirrer, in comparison to BHT (200 ppm), and pure sunflower oil (without any addition, as a control).

Sunflower oil oxidative stability with and without the addition of antioxidant was determined under accelerated conditions (100ºC, Oxygen flow at 20L/hr.) using Rancimat 743 (Metrohm, Switzerland), the induction period (IP) was conducted with Rancimat (Anwar et al 2003).

The antioxidant activity (AA) and increasing index % were calculated from the measured induction times according to Holasova et al (2006), as follows:

$$\text{Antioxidant activity (AA)} = \frac{\text{Ind. time of oil with antioxidant}}{\text{Ind. time of control}}$$

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$$\text{Increasing Index\%} = \frac{\text{IP with antioxidant} - \text{IP of the control}}{\text{IP of the control}} \text{ X100.}$$

2.8 Measurement of acid value (AV) of mayonnaise

The acid value (AV) of mayonnaise samples were performed according to AOCS (2005).

2.9 Thiobarbituric acid value (TBA) of mayonnaise

Thiobarbituric acid value (TBA) of mayonnaise were performed according to AOCS (2005).

2.10 Statistical Analysis

Statistical analyses were conducted by SPSS V.15.0 program. Data on chemical, oxidation and microbiological evaluations were carried out by ANOVA (Benjakul et al 2003).

3 Results and Discussion

3.1 Yield percentage and physicochemical properties of thyme and stachys essential oils

In this research, two essential oils (thyme and Stachys) were tested as antioxidant agents, their physicochemical being defined first.

The percentage of thyme and stachys essential oils yield was determined and the obtained results are presented in **Table 1**. The present findings showed that the average yield of thyme and stachys essential oils obtained from the three-hour hydrodistillation of shade-dried thyme and stachys leaves was 0.92 and 1.5% (v/w), respectively, implying that, from an economic point of view, dried thyme and stachys leaves were good sources of highly valuable essential oils. Results were consistent with Gedikoğlu et al (2019).

To get closer to the consistency and purity of essential oils, physicochemical properties such as specific gravity, refractive index and acidity are very useful. These criteria are critical for the production of quality essential oil that will help to improve the community as well as the nation's economic condition (Boukhatem et al 2014). The physicochemical properties of thyme and stachys essential oils have been assessed and the obtained findings are presented in **Table 1**. Results in **Table 1** revealed that specific gravity of thyme and stachys essential oils at 20°C was 0.912 and 0.9782 gm/cm^3, respectively, which indicates high quality and purity of the volatile oil. In this concept, it should be mentioned that the specific gravity value for thyme and stachys essential oils is less than 1gm/cm^3 (Guenther, 1961). The refractive index of thyme and stachys essential oils as shown in **Table 1** was 1.4680 and 1.4125, respectively. The refractive index of pure oil depends on the molecular weight of the compounds within the volatile oil. Results are in agreement with those obtained by Constantin et al (2014), Shaheen et al (2017). Thyme and stachys essential oils obtained were yellow-brown and Colorless, respectively Results are in agreement with those obtained by Constantin et al (2014), Shaheen et al (2017). Data demonstrated in **Table 1** cleared that the acid value of thyme and stachys essential oils was 2.52 and 3.18 mg KOH/gm oil, respectively. The acid value is an important criterion of the freshness, quality and purity of the essential oil, most essential oils contain only a small amount of acid values (Kumar, 2014).

Table 1. Physio-chemical properties of thyme and Stachys essential oils

Characteristic	Thyme	Stachys
Appearance	transprent fluid	transprent fluid
Colour	yellow-brown	Colorless
Odor	thyme	menthol
Relative density, g/ cm^3	0.912	0.9782
Refractive index (20 °C)	1.4860	1.4125
Acid value, mg KOH/g	2.52	3.18
Yield%	0.92	1.5

3.2 Identification of the chemical constituents of thyme and Stachys essential oils

Using gas chromatography coupled with mass spectrometry (GC/MS) technique, thyme *'Thymus vulgaris L.'* and stachys *'Stachys aegyptiaca'* essential oils obtained by hydro-distillation were fractionated and classified. The chemical components were calculated based on retention time and compared with the standard compound mass spectral database and their relative proportions (area%) are shown in **Table 2**.

It was evident from the results of **Table 2** that thyme had 15 components, representing 99.99% of the total essential oil, while unknown components represented 0.01% of the total oil. Stachys oil, however, has 33 components that represent 99.22%, while unknown components represent 0.78%. GC/MS analysis **(Table 2)** indicated that the major components in thyme essential oil were thymol (62.28%) and in stachys essential oil were β-elemenone and Isojasmone (33.63 and 31.57%, respectively). These results were consistent within the ranges of thyme and stachys essential oils published by Shaheen et al (2017), Alsaraf et al (2020).

Due to their antioxidant activity, phenolic compounds such as thymol in essential thyme oil plays an important role (Braga 2005). Similar classification groups were observed for both thyme and stachys essential oils with small variations in component concentrations

Table 2. Fractionation of thymus & stachys essential oils using GC- MS

R_t	Compound Name	Area%	
		Stachys	thyme
3.19	p-cymeme	N.D.	6.28
3.29	Eucalyptol	3.13	N.D.
4.59	α- linalool	N.D.	6.83
4.93	3-octanol-acetate	0.45	N.D.
5.16	Imidazole	0.15	N.D.
5.52	(1)-isomenthone	0.42	N.D.
5.79	p-menthan-3-one	3.12	N.D.
6.01	Cis-iso pulegone	1.1	N.D.
7.7	β-elemenone	33.63	N.D.
7.92	Ascaridole	11.02	N.D.
7.99	Cis-carvone oxide	0.34	N.D.
8.11	D-carvone	0.16	N.D.
8.4	Piperitenone oxide	0.15	N.D.
8.62	Delta-elemene	0.42	N.D.
8.69	4-hydroxypiperitone	0.11	N.D.
8.85	n-nonanylacetate	0.4	N.D.
9.03	Thymol	0.36	62.28
9.39	Ascaridole epoxide	N.D.	0.39
9.45	Verbenone	1.14	N.D.
10.37	Isojasmone	31.57	N.D.
10.77	6-allyl-o-cresol	N.D.	4.16
10.79	Cinerolon	1.23	N.D.
11.07	Caryophyllene	5.23	1.81
11.79	1,4-dimethoxy-2,5 dimethylbenzene	N.D.	8.07
11.88	β-cedren	0.13	N.D.
11.99	Linalyl isobutyrate	0.21	N.D.
12.24	Germacrene D	0.46	N.D.
12.55	C- elemene	0.32	N.D.
12.63	p- tert- butylcatechol	N.D.	1.32
12.81	β-bisabolen	0.64	N.D.
12.89	Bicyclo(3,3,1)nonane-2,4-dione,3-(2,2-dimethyl propylielene)-methyl	0.8	N.D.
13.7	O-isopropyl phenetole	N.D.	0.81
14.18	Caryophyllene oxide	0.37	0.64
14.44	Carotol	0.22	N.D.
14.8	Cubenol	0.11	N.D.
15.3	e-cadinol	1.11	N.D.
15.59	Neryl butyrate	0.28	N.D.
15.65	Neryl acetate	0.13	N.D.
18.22	Cis-z-α-bisabolene epoxide	N.D.	1.91
18.35	2,6-di-tert-butylhydroquinone	N.D.	1.91
18.85	Hexahydro farnesylacetone	0.16	N.D.
21.12	Manoyloxide	0.15	N.D.
24.47	M-cymen-4-ol	N.D.	0.79
26.57	7-oxoAbieta-8,11,13-triene	N.D.	1.46
30.96	Cirsimaritin	N.D.	1.35

*N.D. = not detected

(Imelouane et al 2009, Mostafavi et al 2013). The variations in the content and compositional pattern of thyme and Stachys essential oils, however, may be due to several factors, including plants, growth stages, herb origin, environment and drying conditions (Sellami et al 2009, Baatour et al 2012).

3.3 DPPH radical scavenging assay

Using the DPPH radical scavenging assay, essential oils under investigation were subjected to antioxidant activity and compared with synthetic antioxidant (BHT) at 50, 100, 150 and 200 ppm, as well as, the findings are presented in **Fig 1**. It was revealed that activity was high at the level of 400 ppm as the synthetic antioxidant BHT at 200 ppm (77.29%). Whilst thyme essential oil exceeds the synthetic antioxidant BHT activity at levels 450 and 500 ppm. Stachys oil concentrations showed lower activity than synthetic antioxidant (BHT) up to 200 ppm concentration. These findings indicate that the antioxidant activity increases with the increasing level of essential oil. These results confirmed the results obtained by Işcan et al (2012), Alsaraf et al (2020).

3.4 Oxidative stability of sunflower oil and essential oils by Rancimat

To evaluate substances that slow oxidation processes, fat and oil stability Rancimat measurement was used. Rancimat determines the induction period by measuring the rising volatile acidic by-products released at 100°C from oxidizing oil (Choe and Min 2009).

In the present work, the Rancimat test was used to assess the antioxidant activity of thyme and stachys essential oils applied to sunflower oil at different concentrations (200 and 500 ppm) compared to BHT (200 ppm) and refined sunflower oil (without antioxidants) as mentioned in **Fig 2.**

As shown in Fig (2, the induction period for control sunflower oil (without additives) was 12.2 hour (hr.), which was increased to 13.84 hr. in the same oil by adding 200 ppm BHT. While the levels of thyme and stachys oils (200 ppm) gave the lower induction periods (as compared with the synthetic one but the induction period increased to 13.97 hr. in stachys oil and 14.2 hr. in thyme oil at 500 ppm.

Results are shown in **Fig 2** suggested that BHT exhibits the highest antioxidant activity (1.13) compared to the sunflower oil samples under investigation at 200 ppm but at 500 ppm both thyme and stachys essential oils have higher antioxidant activity than BHT at 200 ppm. At the level of 500 ppm, Thyme and stachys essential oils deserve special attention because they were more effective than BHT at the level of 200 ppm in delaying oxidation, and oxidative stability was directly proportional to the increase in the concentration of thyme and stachys essential oils. These results are in agreement with Hailemariam and Emire (2013).

3.4.1 Acid value (AV) of mayonnaise

As shown in **Table 3**, AVs of the control mayonnaise - samples changed from 0.274 in week 0 to 0.713 in week 5 during the storage period at 25°C. A similar increasing trend versus storage period was also reported by Kishk and Elsheshetawy (2013). The rising pattern for AVs over time is attributable to mayonnaise low pH causing the iron bridges between iron and phosvitin to be broken and released. Hydrolytic and oxidative enzymes of an egg can be activated by Iron (Kishk and Elsheshetawy 2013, Honold et al 2016). Mayonnaise with additional essential stachys oil had the lowest AV at the end of the storage period (0.485), nevertheless, the discrepancy between test samples and samples of added antioxidants was not important. This shows that the addition of antioxidant in the spectrum of application does not affect AVs of mayonnaises.

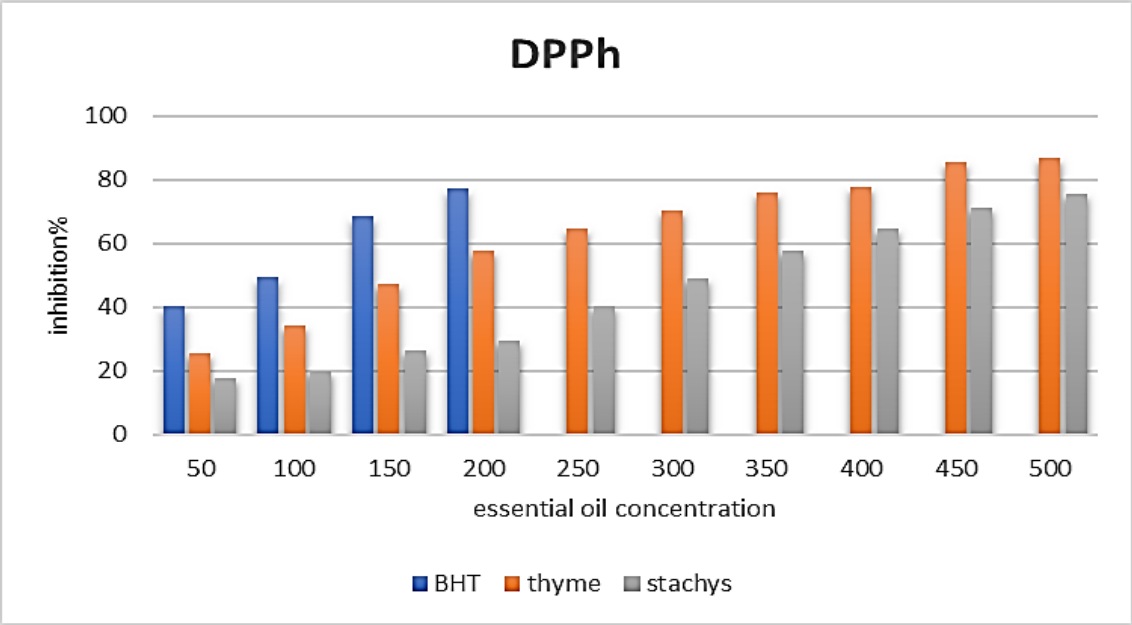

Fig 1 Antioxidant activity of Essential oil extracted by hydrodistillation using DPPH method (inhibition ratio %)

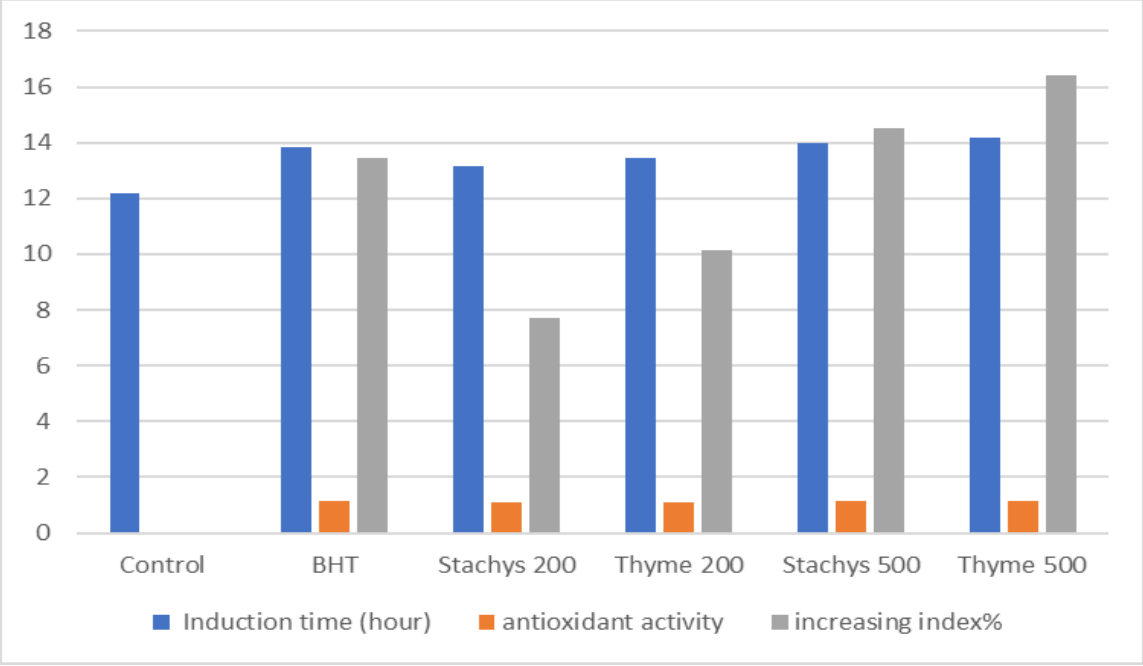

Fig 2. Effect of some essential oils on the oxidative stability of sunflower oil by Rancimat.

Table 3. The acid value of mayonnaise as affected by different levels of essential oils extracted by hydro-distillation during storage period at 25±2 °C

	Storage period (weeks)					
	0	**1**	**2**	**3**	**4**	**5**
Control -	0.274^{Af}	0.331^{Ae}	0.408^{Ad}	0.493^{Ac}	0.557^{Ab}	0.713^{Aa}
Control +	0.268^{Ae}	0.268^{Be}	0.329^{Bd}	0.369^{Bc}	0.398^{Bb}	0.494^{Ba}
Thyme 200	0.26^{Af}	0.274^{Be}	0.337^{Bd}	0.377^{Bc}	0.407^{Bb}	0.505^{Ba}
Thyme 400	0.272^{Ae}	0.279^{Be}	0.343^{Bd}	0.384^{Bc}	0.415^{Bb}	0.515^{Ba}
Thyme 600	0.277^{Ae}	0.277^{Be}	0.34^{Bd}	0.381^{Bc}	0.412^{Bb}	0.51^{Ba}
Staychs 200	0.277^{Ae}	0.28^{Be}	0.344^{Bd}	0.386^{Bc}	0.417^{Bb}	0.517^{Ba}
Staychs 400	0.283^{Af}	0.275^{Be}	0.339^{Bd}	0.379^{Bc}	0.41^{Bb}	0.508^{Ba}
Staychs 600	0.277^{Af}	0.263^{Be}	0.323^{Bd}	0.362^{Bc}	0.391^{Bb}	0.485^{Ba}

*Means with the same capital superscripts at the same column are not significant at ($p<0.05$)
* Means with the same small superscripts at the same row are not significant at ($p<0.05$)

Table 4. Thiobarbituric acid of mayonnaise as affected with different levels of essential oils extracted by hydrodistillation during storage period at 25±2 °C

	Storage period (weeks)					
	0	**1**	**2**	**3**	**4**	**5**
Control -	0.152^{Af}	0.213^{Ae}	0.383^{Ad}	0.575^{Ac}	0.711^{Ab}	0.895^{Aa}
Control +	0.149^{Ae}	0.162^{Be}	0.178^{Bd}	0.267^{Bc}	0.33^{Bb}	0.416^{Ba}
Thyme 200	0.148^{Af}	0.176^{Be}	0.191^{Bd}	0.287^{Bc}	0.354^{Bb}	0.446^{Ba}
Thyme 400	0.151^{Af}	0.171^{Be}	0.187^{Bd}	0.281^{Bc}	0.347^{Bb}	0.437^{Ba}
Thyme 600	0.154^{Ae}	0.165^{Be}	0.183^{Bd}	0.275^{Bc}	0.339^{Bb}	0.427^{Ba}
Staychs 200	0.154^{Ae}	0.18^{Be}	0.203^{Bd}	0.305^{Bc}	0.377^{Bb}	0.475^{Ba}
Staychs 400	0.157^{Af}	0.177^{Be}	0.197^{Bd}	0.296^{Bc}	0.366^{Bb}	0.46^{Ba}
Staychs 600	0.154^{Af}	0.169^{Be}	0.186^{Bd}	0.279^{Bc}	0.345^{Bb}	0.434^{Ba}

*Means with the same capital superscripts at the same column are not significant at ($p<0.05$)
* Means with the same small superscripts at the same row are not significant at ($p<0.05$)

3.4.2 Thiobarbituric acid value (TBA) of mayonnaise

Results showed the effect of various concentrations of thyme and stachys essential oil in comparison with the control sample (without any antioxidant). In the last week of antioxidant activity, the concentration of 600 ppm for both essential oils was the best treatment and had a significant difference in comparison with synthetic antioxidant of 200 ppm TBHQ **Table 4**. In short, all concentrations of thyme and stachys essential oils had an antioxidant activity similar to synthetic antioxidant of TBHQ at a concentration of 200 ppm for inhibition of secondary products. These results are in agreement with Vahidyan et al (2012).

4 Conclusion

It is generally accepted that, unlike synthetic antioxidants, essential oils from thyme and stachys can be added in larger quantities to obtain optimal effects below their threshold level (addition of synthetic antioxidants is limited under food laws and regulations). As a result, with the addition of essential oil, which has antioxidant activity, the stability of refined oils has been increased. The essential oils of Thyme and Stachys could therefore be used in the food industry as a safe, effective and easily accessible source of the natural preservative ingredient.

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