OXIDATIVE STABILITY OF MAYONNAISE SUPPLEMENTED WITH ESSENTIAL OIL OF *ACHILLEA MILLEFOLIUM SSP MILLEFOLIUM* DURING STORAGE

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Abstract. Lipid oxidation is the main chemical process affecting mayonnaise deterioration. Today, essential oils from aromatic plants have been qualified as natural antioxidants and proposed as potential substitutes of synthetic antioxidants in food products. In this research, antioxidant activity of *Achillea millefolium* essential oil was determined based on oxidative stability of treated mayonnaise (homogenized) during 6 months of storage at 4°C. The following analysis were performed: peroxide value (PV), anisidine value (AV), Totox value and thiobarbituric acid (TBA) to assess the extent of oil deterioration. Mayonnaise samples were divided into three experimental treatments, namely: E.O (essential oil in concentrations: 3.83, 5.85 and 7.2 mg/ml), T (TBHQ in concentration: 0.12 mg/ml) and C (control: no antioxidant). Subsequently, the samples from each treatment were stored at 4 °C. The results showed that the treatments containing essential oil and TBHQ significantly reduced the oxidation ($p < 0.05$), while the control sample was oxidized faster. Among the essential oils, concentrations of 5.85 and 7.2 mg/ml showed the best antioxidant activity. PV, TBA, AV and totox values increased during the storage time for all treatments. Our results suggest that essential oil of *Achillea millefolium* has potential source of natural antioxidant for the application in food industries to prevent lipid oxidation particularly lipid-containing foods such as mayonnaise, due to its reaction with oxidative free radicals and therefore can increase food shelf life.

Key words: *Achillea millefolium*, auto-oxidation, essential oil, food emulsion.

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Introduction. Formulation of the problem

Microbial spoilage and lipid oxidation lead to the development of off-flavours and off-odours which result in a shorter shelf life of fresh and processed foods containing lipids [1-4]. Although the impact of lipid oxidation products on human health has not been confirmed yet, it is recommended to keep to a rich antioxidant diet, as lipid oxidation products have some chronic effect on a consumer’s health [1,2,5-10].

Analysis of recent research and publications

Lipid oxidation generates free radicals which are responsible for carcinogenesis, mutagenesis, inflammation, and cardiovascular diseases [2,7,8,10]. Depending on the physical form of the oil, e. g., bulk oil and oil-in-water emulsions (O/W), factors affecting the oxidative stability of oils are different [1,11]. Many lipid oxidation reactions occur at air-oil and water-oil interfaces [1]. The characteristics of interfacial membranes of dispersed particles in O/W emulsions can have a crucial effect on the rates of lipid oxidation [1]. The mechanism of lipid oxidation and flavour deterioration in food emulsions, such as mayonnaise, is complex and not fully understood as most studies of oxidation have been performed on bulk oil or in simple model systems [11,12]. Oil-in-water emulsions like mayonnaise contain at least three phases, namely the aqueous phase, the oil phase, and the oil-water interface. In emulsions, oxidation may be initiated in any of these three phases [1,12]. It has been reported that the rate of lipid oxidation in emulsified oils is higher than that in bulk oils [1]. Nowadays, mayonnaise is one of the most popular and widely used sauces in the world, and it is produced by emulsifying oil with other components like eggs, vinegar, and mustard. Lipid oxidation in mayonnaise is one of the reasons for quality deterioration during storage, due to a high oil content and the nature of the raw materials, e. g., the unsaturated character of the oil. This process is responsible for the development of rancid flavours and discolouration of mayonnaise [4,13,10,12]. Therefore, slowing down lipid oxidation can contribute significantly to the extension of the shelf life of mayonnaise. Traditionally, chemically synthesized antioxidants, such as BHA, BHT, and TBHQ, are widely used in oil products [9,13-15]. It has been reported that food additives such as synthetic antioxidants have detrimental effects on human health, as Sanchez-Alonson *et al*., reveal that BHT causes liver expansion [16]. Besides, the lower incidence of cardiovascular and chronic diseases and cancers was due to natural antioxidants. Nowadays, there is an increasing interest among some consumers of food in replacing synthetic antioxidants with those of natural origin [1,10]. So, the search for useful natural antioxidants is becoming more and more important. Natural antioxidants are constituents of plants and spices notable for their anti-carcinogenic potential and other health promoting effects [10]. The antioxidant properties of many medicinal plants are reported to be effective in retarding rancidity in oils and fatty foods [14]. Medicinal plants and their products such as essential oils have been used for many centuries for human health [9]. It is known that a number of natural essential oils obtained from herbs are resistant to autoxidation due to some natural components, such as phenolic compounds, and improve oxidative stability of foods [18]. The antioxidant activity of essential oils depends on the type and polarity of the extraction solvent, the isolation methods, purity and identity of antioxidant active components of the raw materials [4,14]. Moreover, the activity of natural antioxidants in different food emulsions is affected by their complex interfacial affinities between oil-water interfaces [1,10]. The addition of essential oils into food emulsions improves their consumer acceptance and flavour characteristics. There are many articles considering the oxidative changes in bulk oils and oil-in-water emulsions [1]. The antioxidant effects of natural plant materials, such as essential oils and extracts from berries [19], green tea [20], raisins [21], olives [22], and grape seeds [23], in a variety of O/W emulsions and a purple maize extract [10], and in mayonnaise, have been considered previously. There are many plants, valuable to different extents, that are not explored yet nor used in foods. Plants contain many novel compounds with medicinal properties that need scientific investigation. Among them, *Achillea millefolium* (yarrow) is less known for its food and pharmaceutical applications. *A. millefolium* essential oil has a potent antioxidant activity and can be used in food industry. The antioxidant activity of the essential oil of *A. millefolium* has been investigated in different *in vitro* tests [24-28], but not many references have been found concerning the effect of the essential oil of this plant on the oxidative stability of oil-in-water emulsions such as mayonnaises.

The purpose of this study was to compare the antioxidant effectiveness of TBHQ and that of the essential oil of *Achillea millefolium* subsp. *millefolium* during storage of mayonnaise, and to evaluate the formation of primary and secondary oxidation products when mayonnaise is oxidized at 4°C.

Research objectives:

1. To study and analyse antioxidant effectiveness of TBHQ and essential oil of *Achillea millefolium* during storage of mayonnaise.
2. To study and evaluate the formation of primary and secondary oxidation products in mayonnaise.
3. To study the effect of TBHQ and essential oil on sensory properties of mayonnaise.

Research materials and methods

Chemical materials. Refined soy bean oil with the composition of unsaturated fatty acids (oleic acid – 23.34%, linoleic acid – 52.59%, linolenic acid – 7.23%) and without added synthetic antioxidants was obtained from Naz Company, Isfahan, Iran. Chloroform, acetic acid, potassium iodide, starch reagent, thiobarbituric acid, *p*-anisidine, isooctane, potassium sorbate, and sodium benzoate were obtained from Merck (Germany). TBHQ was purchased from Sigma-Aldrich (USA). All chemicals and reagents used were analytical grade. Eggs, sugar, salt, and other ingredients for the mayonnaise formulation were supplied by the local grocery store. Xanthan and CMC (Carboxy Methyl Cellulose) were purchased from Oleon (Oleon Company, Belgium). The *Achillea millefolium* subsp. *millefolium* was collected from Research Shahid Fozve Institute (Isfahan, Iran) [24].

Introduction of antioxidants into the oil phase of mayonnaise. According to the calculated amounts of the antioxidant activity of essential oil in a DPPH assay [24], the best concentrations of the essential oil (3.83, 5.85, and 7.2 mg/ml) and the synthetic antioxidant TBHQ, in the concentration 0.12 mg/ml (T), were added to the oil phase, as the above mentioned concentrations were provided in the final mayonnaise samples. TBHQ was used as a reference substance for comparison. The additives were mixed in the oil phase with a magnetic stirrer for 10 min at the ambient temperature.

Mayonnaise production. In this experiment, essential oil extracted from flowers of *Achillea millefolium* subsp. *millefolium* as a natural condiment was selected to study its antioxidant and sensory properties [24]. An attempt was made to develop a useful formula of mayonnaise free from chemical preservatives and to produce finally a product with desirable sensory properties. First, mayonnaise was produced in batches of 5 kg with a high speed homogenizer (Arkan felez, Iran), in a continuous process in the pilot plant of a local factory (Paknam Sepehr Sepahan Company, Naji Mayonnaise, Isfahan, Iran). The mayonnaise was formulated and manufactured with the oil phase (soy bean: 65% w/w), water, vinegar, egg, NaCl, sugar (sucrose), citric acid, mustard powder, potassium sorbate, and sodium benzoate. Then the mayonnaise was divided into three experimental samples, namely: EO (essential oil), T (TBHQ), and C (control) (Table 1). The prepared mayonnaises were transferred to glass jars and kept at 4°C. Samples were taken every 1 month to determine the level of oxidation deterioration during 6 months of storage.

Lipid extraction. Oil was extracted from the mayonnaise samples by the Bligh & Dyer method [29]. It was based on the solvent extraction by CHCl3:MeOH (v/v).

Determination of oxidative stability in mayonnaise. The peroxide value (PV), thiobarbituric acid index (TBA) and anisidine value of the samples were determined according to AOCS [30-32]. The (mg malonaldehyde/Kg oil) was determined according to AOCS-Cd 19-90. The test was carried out according to AOCS-Cd 18-90. The total oxidation (TOTOX) values of the samples were determined using the following equation [33]: TOTOX = 2 × PV + AV

Sensory analysis. After the preparation of the mayonnaise and its storage at 4°C, its sensory characteristics (flavour, odour, colour, texture, and overall acceptability) were evaluated by a panel of 10 experts on a 5-point hedonic scale, with 5 being the highest and 1 the lowest. The experts were recruited from the Paknam Sepehr Sepahan Company, Isfahan, Iran (Naji brand). A quantitative descriptive analysis was carried out [18]. All sensory work was carried out according to the international standards [34,35]. Each panellist evaluated 3 replicates of all sample groups. The sensory attributes were measured as follows: flavour (from conventional flavour to unexpected flavour), colour (from extremely light to extremely dark), odour (from conventional odour to unexpected odour), texture (from extremely soft to extremely tough), and appearance (from conventional appearance to unexpected appearance). The experts agreed that the flavour and odour were conventional. At the end of the test, the panellists were asked to grade the quality of the product 1 to 5. The sensory grades were only used to approximate the organoleptic qualities of the products and not to determine precisely their shelf life [16,36]. The reference product was mayonnaise without added E.O.

Statistical analysis. The statistical analysis of the experimental results was performed with the SPSS statistical package (version 20, SPSS Inc., Woking, Surrey, UK). ANOVA was performed to determine the statistical significance of the samples ($p < 0.05$). The software Design Expert 8.0 (Stat-Ease, Inc., Minneapolis, MN, USA) was only used with the concentrations in the ranges indicated in Table 1. The initial experimental area for the essential oils was 0.45 to 7.2 mg/ml [24].

Table 1 – Addition of antioxidants to the experimental mayonnaises

Antioxidant	Antioxidant addition (mg/ml)
EO.1	3.83
EO.2	5.85
EO.3	7.2
T	0.12
C	0

EO: Essential oil. T: TBHQ. C: Control (no antioxidant).

Results of the research and their discussion

Peroxide value. Primary oxidation products (hydroperoxides) were determined by peroxide value measurements. The peroxide values of mayonnaise (PV) with and without antioxidants at 4°C are presented in Table 2. The results have shown that the essential oil of *Achillea* retarded the hydroperoxide formation significantly ($p<0.05$). In all the samples, the PV was increasing from the beginning of the storage period up to the end of the experiment. For instance, in month 6, the PV of EO.2 was almost eight times as great as the PV in month 0.

A higher PV of the mayonnaise in EO.1 and EO.2, compared with the PV in EO.3 (Table 2), revealed that the higher lipid oxidation in EO.1 and EO.2 took place due to the concentration-dependent effect.

Table 2 – Peroxide value (meq peroxides/kg oil) in the experimental mayonnaises during storage for 6 months

Sample	Storage Time (month)					
	1	2	3	4	5	6
EO.1	1.35 ± 0.045^{Bf}	1.79 ± 0.16^{De}	2.98 ± 0.12^{Bd}	3.91 ± 0.10^{Cc}	4.5 ± 0.10^{Bb}	6.18 ± 0.17^{Ba}
EO.2	0.8 ± 0.10^{Cd}	2.22 ± 0.22^{Bc}	3.08 ± 0.08^{Bd}	3.18 ± 0.17^{Db}	3.8 ± 0.10^{Cb}	6.10 ± 0.10^{Ba}
EO.3	1.14 ± 0.04^{Be}	1.39 ± 0.11^{Cf}	2 ± 0.20^{Dd}	2.97 ± 0.13^{Dc}	3.35 ± 0.05^{Db}	3.95 ± 0.05^{Ca}
T	0.8 ± 0.05^{Cf}	1.19 ± 0.10^{Ce}	2.47 ± 0.17^{Cd}	6.37 ± 0.17^{Ba}	4.2 ± 0.10^{BCb}	3.87 ± 0.07^{Cc}
C	2.97 ± 0.17^{Af}	3.82 ± 0.12^{Ae}	5.10 ± 0.10^{Ad}	7.23 ± 0.23^{Ac}	8.51 ± 0.30^{Ab}	10.45 ± 0.15^{Aa}

For the description of the samples, see Table 1. The peroxide value of all samples for storage time 0 was 0.79 meq peroxides/kg oil. The mean ± SD (standard deviation) within columns with different capital letters differs significantly ($p<0.05$). The mean ± SD (standard deviation) within rows with different small letters differs significantly ($p<0.05$).

By comparing the PV of all samples, it was found that the control sample contained a higher PV than the samples with antioxidants added throughout the storage ($p<0.05$). In other words, the essential oil lowered the peroxide value compared to the control sample. It is known that lipid autoxidation is a chain reaction which can proceed faster when more lipid hydroperoxides are available for the reaction with O_2 [4,37]. As can be seen in Table 2, mayonnaise without additional antioxidants showed the highest PV during storage (10.45 mequiv peroxides/kg oil), whereas a PV higher than 5 mequiv peroxides/kg oil is not acceptable [38,39]. It has been hypothesized that a high content of unsaturated fatty acids in soybean oil and the absence of added antioxidant in the control sample can make the control sample highly susceptible to oxidation.

It seems that the essential oil with the concentration 7.2 mg/ml was more effective in lowering the PV than the TBHQ after 3 months of storage. The TBHQ showed a slight increase in the PV in the 4^{th} month of storage (6.37 mequiv peroxides/kg oil). After 5 months of storage at 4°C, the peroxide value of the sample with added TBHQ began decreasing (3.87 mequiv peroxides/kg oil). It is due to the reduced accumulation of hydroperoxides and to hydroperoxide formation slower than hydroperoxide breakdown (Table 2). The results have indicated the efficient antioxidative activity of essential oil in mayonnaise and reduced lipid oxidation. Some compounds, e. g. thymol, camphor, pinene, borneol, terpinen-4-ol, α-terpineol, and 1.8 cineole have been found to have a strong inhibiting effect on the peroxide value [24]. The antioxidant activities of phenolic compounds can result from different mechanisms, such as chain-breaking antioxidants, hydroperoxide decomposition, and binding metal ions [10,40]. Similar results were reported for mayonnaise with other natural plant materials such as extracts and powders for the purple maize extract [10], and the black glutinous rice extract [40]. Li *et al*. noticed the increased oxidation stability of mayonnaise during storage with an increase in the amount of the maize extract, and mayonnaises with the extract exhibited stronger oxidative stability than those with synthesized antioxidants (BHT). They reported the PV of mayonnaise with the purple maize extract to be significantly lower than those of the control. The purple maize extract exhibited a significant antioxidant effect against primary oxidation in the mayonnaise, due to the phenolic content, and a high concentration of the extract showed a great antioxidant activity [10].

TBA value. The oxidation reaction has been estimated by a TBA test which is based on the reaction between TBA and MDA (malondialdehyde) and other carbonyl compounds arising from lipid oxidation [15].

The results of the secondary lipid oxidation products as shown by the TBA for all mayonnaises are presented in Table 3. There are significant differences between samples with and without added antioxidants (essential oil and TBHQ) during storage.

Table 3 – TBA value (mg MDA/kg) in the experimental mayonnaises during storage for 6 months

Sample	Storage Time (month)					
	1	2	3	4	5	6
EO.1	16.41±0.10^{Bf}	27.25±0.25^{Be}	31.64±0.62^{Bd}	37.29±2.50^{Bc}	44.06±2.00^{Bb}	52.71±2.50^{Ba}
EO.2	8.90±0.40^{Be}	12.82±0.45^{Dd}	17.82±0.80^{Dc}	21.63±1.50^{Cb}	25.5±1.50^{Ca}	26.75±1.75^{Ca}
EO.3	13.84±0.04^{Cc}	14.39±0.49^{Cc}	15.09±1.00^{Cbc}	17.75±2.50^{Cab}	18.77±0.80^{Dab}	19.21±2.20^{Ca}
T	12.40±0.40^{Df}	14.5±0.50^{Ce}	18.5±0.50^{Cd}	20.11±3.00^{Cc}	23.94±1.00^{Db}	26.35±1.35^{Ca}
C	22.79±0.30^{Af}	30.21±1.00^{Ae}	41.12±0.8^{Ad}	54.31±2.30^{Ac}	59.01±4.00^{Ab}	67.25±7.50^{Aa}

For the description of the samples, see Table 1. The TBA value of all samples for storage time 0 was 7.63 mg/kg. The mean ± SD (standard deviation) within columns with different capital letters differs significantly (p<0.05). The mean ± SD (standard deviation) within rows with different small letters differs significantly (p<0.05).

The TBA in all antioxidant-containing samples was considerably lower (p<0.05) than in the control, thus indicating the resistance of mayonnaise against lipid oxidation. The strongest protective effect of added antioxidants was observed for the essential oil at 7.2 mg/ml, especially after 3 months of storage. It seems that the efficacy of the essential oil was highly dependent on the concentration. Although a low concentration of essential oil has a better inhibition effect than that in the control sample, a higher concentration of essential oil has proved to be far more effective. Obviously, a rapid increase in the TBA values was observed in the control samples. The maximum value of TBA in the control samples was obtained in the 6^{th} month of storage (67.25 mg/kg). By comparing the TBA values of the samples, it could be concluded, that the mayonnaises with added antioxidants had less of secondary oxidation products than the control. The same results have been reported by Tananuwong and Tewaruth [40], who established that the addition of a crude extract of black glutinous rice could retard an increase in TBA.

The TBA values of samples with essential oil were lower than those of the controls in the storage period. The same effect was in the case of TBHQ. Besides, it was established that storage time had a significant effect on mayonnaise oxidation, where an increasing level of TBA was observed in all samples from the first month to the last month of storage (Table 3). The TBA value of freshly prepared mayonnaise was 7.63 mg/kg, which indicates carbonyl arising from lipid oxidation and the MDA of oils and other mayonnaise ingredients (Table 3). However, most food products naturally contain some level of MDA as a secondary product of lipid oxidation – especially fried foods, cheese, cooked meats, and dehydrated foods [15]. The increase in the TBA values after 5 month of storage in the sample containing TBHQ coincided with a decrease in the PV. A decrease in the level of primary oxidation products can have happened because of hydroperoxide degradation and the formation of secondary lipid peroxidation products (Table 3).

The results have shown that essential oil can protect the mayonnaise from oxidation (compared to the control), probably because of the terpenes and phenolic compounds of the essential oil such as thymol, camphor, pinene, borneol, terpinen-4-ol, and 1.8 cineole [24].

Anisidine value. The formation of secondary oxidation products was also measured by the anisidine value (AV). Peroxide compounds are known to be unstable, and they decompose with the formation of secondary products of oxidation (aldehydes, ketones, and their derivatives). Besides, malonaldehyde and other short-chain carbon products of lipid oxidation are not stable and decompose into organic alcohols and acids that are not determined by the TBA test [3,41]. Anisidine values of mayonnaises stored at 4 °C are shown in Table 4.

Table 4 – Anisidine value (mMol Aldehyde/Kg) in the experimental mayonnaises during storage for 6 months

Sample	**Storage Time (month)**					
	1	**2**	**3**	**4**	**5**	**6**
EO.1	4.605±0.20^{Bf}	5.91±0.05^{Be}	7.915±0.17^{Bd}	8.87±0.25^{Bc}	9.514±0.50^{Bb}	10.32±0.80^{Ba}
EO.2	1.896±0.10^{Be}	3.355±0.20^{Dd}	4.235±0.14^{Cc}	4.735±0.75^{Cc}	5.207±1.00^{Cb}	5.971±1.00^{Ca}
EO.3	4.049±0.20^{Cf}	4.91±0.11^{Ce}	7.31±0.17^{Bd}	8.49±0.50^{Bc}	8.901±0.40^{Bb}	9.405±0.30^{BCa}
T	2.605±0.25^{Dc}	2.895±0.11^{Db}	4.07±0.17^{Ca}	0.85±0.15^{Df}	1.015±0.40^{De}	2.414±0.20^{Dd}
C	5.79±0.20^{Ae}	9.775±0.14^{Ad}	10.61±0.34^{Ad}	13.25±0.75^{Ac}	15.82±2.50^{Ab}	22.84±2.50^{Aa}

For the description of the samples, see Table 1. The anisidine value of all samples for storage time 0 was 1.29 mMol/Kg. The mean ± SD (standard deviation) within columns with different capital letters differs significantly

The results of anisidine value measurements were similar to the results of peroxide values. Among them, EO.2 and EO.3 exhibited the best antioxidative activity. Essential oil in the concentration 5.85 mg/ml was more effective in preventing the formation of secondary oxidation products, than the concentration 7.2 mg/ml, perhaps because of the peroxidation effect of some phenolic constituents at high concentrations [40,42]. Therefore, the concentration 5.85 mg/ml of essential oil can be recommended as a potent source of antioxidants to stabilize food systems containing unsaturated oils such as mayonnaise. The mayonnaise, with essential oil added, had a significantly lower anisidine value compared to the control sample throughout the storage period. The anisidine value of the control sample reached 22 at the end of storage, while the maximum anisidine value accepted for oils is 10 [38,39]. The rate of oxidation was reduced by the two tested antioxidants (EO and TBHQ). Among all tested antioxidants, the highest stabilization factor was observed in the sample with added TBHQ, and the lowest in the sample with added essential oil in the concentration 3.83 mg/ml (p<0.05). Generally, there were increases in the anisidine value for all the samples during storage. There are too few similar studies in the scientific literature to compare their results with those obtained in this study. Li *et al*., by primary and secondary oxidation measurements, showed that purple maize extracts had a similar protection effect against lipid deterioration in mayonnaise, and that the high anisidine value indicated that the oil was oxidized and deteriorated [10].

TOTOX value. The TOTOX value measures both hydroperoxides and their breakdown products and provides a better estimation of the progressive oxidative deterioration. The TOTOX values of all the samples increased over the storage time, and the antioxidant activity of EO.2 was similar to that of the synthetic antioxidant (Table 5).

Table 5 – TOTOX value in the experimental mayonnaises during storage for 6 months

Treatment	**Storage Time (month)**					
	1	**2**	**3**	**4**	**5**	**6**
EO.1	7.305±0.17^{Bf}	9.49±0.80^{Be}	13.875±0.80^{Bd}	16.69±0.57^{Bc}	18.514±3.00^{Bb}	32.68±2.50^{Ba}
EO.2	3.496±0.11^{Cf}	7.795±0.80^{Be}	10.395±0.40^{Dd}	11.095±0.31^{Cc}	12.807±1.51^{Cb}	18.171±1.00^{Ca}
EO.3	6.329±0.17^{Bf}	7.69±0.70^{Be}	11.31±0.70^{Cd}	14.43±0.86^{Bc}	15.601±1.05^{Bb}	17.305±2.00^{Ca}
T	4.205±0.40^{Ce}	5.275±0.20^{Bd}	9.01±0.50^{DCc}	13.59±0.40^{Ba}	9.415±0.40^{Dc}	10.154±2.00^{Db}
C	11.464±0.86^{Af}	17.415±2.50^{Ae}	20.81±1.80^{Ad}	27.71±0.44^{Ac}	32.84±2.50^{Ab}	43.74±3.75^{Aa}

For the description of the samples, see Table 1. The TOTOX value of all samples for storage time 0 was 2.87. The mean ± SD (standard deviation) within columns with different capital letters differs significantly (p<0.05). The mean ± SD (standard deviation) within rows with different small letters differs significantly (p<0.05).

The mayonnaise samples with essential oil prevented the formation of primary and secondary oxidation products, especially at concentrations of 5.85 and 7.2 mg/ml. According to the obtained results, it can be concluded that essential oil has a preventing effect on oil oxidation in mayonnaise. The ability of essential oil to inhibit lipid oxidation totally must be due to the fact that this is the ability of its constituents. A high amount of borneol (16.51%) and camphor (8.37%) in essential oil may be a possible reason of the powerful antioxidant effects of *Achillea* [24]. Also, a mixture of mono- and sesqiuterpene hydrocarbons (e. g., α-pinene, β-pinene, 1.8 cineole), as well as camphor and some of the monoterpene alcohols and borneol, were, probably, the most active scavengers in these essential oils [24]. The antioxidant activity of the main components of essential oil camphor, pinene, borneol, terpinen-4-ol, thymol, α-terpineol, and 1.8 cineole was previously reported [25-27,43]. These results mean that the main components in these essential oils can act synergistically upon each other or upon other components in mayonnaise. Previous papers have shown that essential oil of *A. millefolium* has a rich qualitative and quantitative phenolic profile, and our results agree with those of other studies in the point that essential oils of *Achillea* have antioxidant properties [24-28,43,]. However, it is difficult to evaluate natural antioxidants in oil-in-water emulsions due to the complex air-oil and oil-water interfaces. Frankel *et al*. demonstrated that the relative effectiveness of lipophilic and hydrophilic antioxidants was dependent on the lipid substrate, physical state (bulk oil, emulsion), antioxidant concentration, time, temperature, and the analytical method used to determine oxidation [44,45]. The location of antioxidants determines the prevention of lipid oxidation in O/W emulsions [1]. It has been reported that hydrophilic antioxidants become dilute in the water phase and cannot adequately protect the oil in the oil-water interface. Unlike polar, non-polar antioxidants are generally more efficient in O/W emulsions, perhaps because they are more easily located at the oil-water interface [1]. In this study, the essential oil of *A. millefolium* and its main components had a lipophilic nature, thus were more effective at the oil-water interface and prevented oxidation in mayonnaise. For example, camphor is highly lipophilic and is quickly absorbed and transported to the oil phase. Borneol is another lipophilic compound, and it is freely soluble in the oil phase. As for monoterpenes, pinene also has relatively lipophilic activity, and thymol is lipophilic, able to interact with the oil-water interface [46].

Sensory properties. In Table 6, the results of a sensory analysis of the mayonnaise samples containing different concentrations of the essential oil of *Achillea millefolium*are are shown for the storage period at 4°C. ANOVA has proved that essential oil has a significant effect on the odour, flavour, and overall acceptability of different samples. Also, there was no statistical difference in the texture and colour of the samples, and the experts did not detect any significant differences among the represented samples ($p < 0.05$).

Table 6 – Grades of the sensory evaluation of the mayonnaise samples (n=3)

Formula name	EO (mg/ml)	Colour	Odour	Flavour	Texture	Overall acceptability
C	0.00	4.00 ± 0.26^{a}	4.03 ± 0.18^{c}	4.03 ± 0.18^{c}	4.03 ± 0.32^{a}	4.10 ± 0.40^{c}
EO.1	3.85	4.03 ± 0.18^{a}	4.20 ± 0.41^{bc}	4.33 ± 0.48^{b}	4.10 ± 0.30^{a}	4.40 ± 0.56^{b}
EO.2	5.83	4.07 ± 0.25^{a}	4.33 ± 0.48^{b}	4.67 ± 0.48^{a}	4.13 ± 0.35^{a}	4.80 ± 0.41^{a}
EO.3	7.2	4.07 ± 0.45^{a}	4.60 ± 0.50^{a}	4.03 ± 0.41^{c}	4.17 ± 0.46^{a}	4.17 ± 0.53^{bc}

The values are expressed as means ± standard deviation. The mean ± SD (standard deviation) within columns with a different letter differs significantly ($p<0.05$).

For the flavour, the lowest grade (4.02) was given to the control sample. Along with the increase in the essential oil content from 3.83 to 5.85 mg/ml, the flavour grade improved considerably to 4.65. This confirms the advantage of essential oil of *Achillea millefolium* as the main flavouring component of mayonnaise. According to the results, with an increase in the concentration of essential oil from 5.85 mg/ml to 7.2 mg/ml, the flavour and the overall acceptability grades of the mayonnaise samples decreased significantly ($p<0.05$) while the grade of the odour went up. This may have resulted from an increase in the content of the chemical components of the added essential oil such as thymol and camphor [46]. The formulations EO.1 and EO.2 showed the highest values for the flavour, while the formulation EO.3 was the worst.

Regarding the overall acceptability grades, the highest grade (4.82) was given to the 5.85 mg/ml concentration of essential oil in mayonnaise, which agreed with the obtained anisidine (Table 4) and TOTOX values (Table 5). Although, at these concentrations, essential oil manifested evident antioxidant activity, their addition to the mayonnaise had various effects on the flavour of the mayonnaise. It is practical to use essential oil for mayonnaise preservation since, besides extending the shelf life of mayonnaise, it makes its odour pleasant to consumers.

Conclusion

The application of synthetic antioxidants is restricted because of their potentially toxic and carcinogenic decomposition products. Studies on natural antioxidant alternatives to synthetic antioxidants are of topical importance. The negative effects of synthetic antioxidants borne in mind, the results of the present research suggest that the essential oil of *A. millefolium* has a desirable protective effect on mayonnaise oxidation and can preserve the product during six months of storage. So it seems that the essential oil of *A. millefolium* is a good substitute for TBHQ or any other synthetic antioxidant in food emulsions such as mayonnaise. To the best of our knowledge, it is the first time the antioxidant activity of *A. millefolium* essential oil in the mayonnaise has been assessed. Although the antioxidant activity of essential oil in mayonnaise is evident, its addition had notable effects on the flavour and taste of the mayonnaise at concentrations ≥7.2 mg/ml. The best solutions to the above problem may consist in using these additives at lower concentrations (e. g. at a concentration of 5.85 mg/ml) and in combinations with different food preservation systems, or other natural preservatives, or methods that would expose the advantages of each of them. For instance, adding spices and keeping mayonnaise cold can play a key role. By using this method, a stable and safe mayonnaise can be produced without loss in the sensory quality. Therefore, more work on the acceptability of these ingredients will be necessary to make their commercial use possible, namely work on the fluctuations of the sensory attributes during refrigeration and on the effect of other ingredients used in combination with the essential oil. Our findings suggest that there are still many herbs in Iran that are not traditionally used but are of medicinal value. So, scientific studies should focus on such plants, too.

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