

 Journal of Food Science and Technology (Iran)

Homepage:www.fsct.modares.ir

Scientific Research

Investigating the effect of nanoliposomes containing yarrow (*Achillea millefolium***) antioxidant extract on oxidative properties and fatty acid profile of sesame oil**

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ARTICLE INFO ABSTRACT

1. Introduction

Sesame seeds are considered one of the rich sources of plant-based protein and were among the first seeds used for oil extraction [1]. The oil content in sesame seeds varies from 28% to 59% [2]. This product has been cultivated since ancient times in Asia and some parts of Africa, especially in Sudan, Nigeria, and Ethiopia [3]. Sesame oil has a pleasant taste and aroma, and it can be used as a natural salad oil or in cooking. It is also used in the preparation of shortening, margarine, and some medicines [4, 5]. Due to the presence of polyunsaturated fatty acids and other compounds, sesame oil helps reduce blood pressure and cholesterol in humans [6]. The oxidation of fats in food significantly reduces their shelf life and results in the delivery of low-quality food to consumers. To prevent oil oxidation, several methods are employed, one of which is the addition of substances called antioxidants. Antioxidants are compounds that delay the onset of rancidity and off-flavors by extending the stability period. They work through various mechanisms, such as controlling oxidation substrates, controlling pro-oxidants, and deactivating free radicals to delay lipid oxidation [7]. On the other hand, the use of synthetic antioxidants like Butylated Hydroxytoluene (BHT), Butylated Hydroxyanisole (BHA), and Tertiary Butylhydroquinone (TBHQ) in food has become a concern due to their harmful effects on human health. In recent years, efforts to find new natural antioxidant sources have increased due to the adverse effects associated with synthetic antioxidants [8, 9]. Phenolic compounds are the most important category of natural antioxidants found in plant by-products. Studies have shown that phenolic compounds are effective antioxidants against fat oxidation in phospholipids and biological systems [10]. Yarrow, with the scientific name *Achillea Millefolium L.,* is a native plant of Iran containing various

substances such as tannins, choline, acetylcholine, flavonoids, valeric acid, amino acids, fatty acids, sterols, thiamine, ascorbic acid, calcium, and more [11]. Studies have shown that yarrow has oxytocic, anti-ulcer, and anti-inflammatory activities [12], and the methanolic extract of its flowers and leaves has antibacterial properties [13]. The most important compounds identified from the essential oil of this plant include flavonoids, piperitone, cineole, limonene, p-cymene, and camphor, which have high antioxidant properties [14, 15]. Farhadi and colleagues (2020) 15].Farhadi and colleagues investigated the changes in essential oil compounds, total phenol, flavonoids, and antioxidant capacity of the yarrow plant during different growth stages. They examined the quantity and quality of the antioxidant capacity of the yarrow plant during various stages of flowering and fruiting. The results showed that the amount of essential oils in the vegetative parts, flowers, and fruits of the plant was 0.17, 0.24, and 0.14 volume/weight, respectively. The amount of phenolic compounds and antioxidants decreased with plant growth, unlike flavonoids [16].Encapsulation is a technology that involves trapping solid, liquid, or gaseous substances in capsules that release their contents at controlled rates under specific conditions. It includes steps such as forming a wall around the bioactive compound, ensuring that these compounds do not leak out, and not encapsulating undesirable compounds [17]. One type of lipid carrier used for encapsulating bioactive compounds and food-drugs is liposomes. Liposomes are colloidal vesicles composed of polar lipids, especially phospholipids, which form spherical inlayer structures in the presence of water molecules. Due to their amphiphilic properties, these compounds can encapsulate a wide range of hydrophilic, lipophilic, and amphiphilic compounds [18].Although liposomes and nanoliposomes have the same structural, chemical, and thermodynamic properties, nanoliposomes provide a larger surface area due to their smaller particle size, increasing solubility, improving bioavailability, and providing controlled release and more precise delivery of encapsulated materials to target areas. They also have higher colloidal stability and create less turbidity [19]. In this context, Bozhmehrani and colleagues (2022) conducted a study to investigate the antioxidant effects of free grape pomace extract and nanoliposomes containing it on some oxidative parameters of soybean oil. The results of this study showed that using nanoliposomes containing grape pomace antioxidant extract is a suitable replacement for synthetic antioxidants available on the market [20]. Other studies in this field include microencapsulation of ascorbyl palmitate and synthetic antioxidants [21] and microencapsulation of hemp in the oxidative stability of soybean oil [22]. Therefore, this study aimed to investigate the effect of nanoliposomes containing antioxidant extract of yarrow on some oxidative parameters of sesame oil.

2- Materials and Methods

2-1- Materials

For this research, yarrow was obtained from reputable stores in Sabzevar. Refined sesame oil without antioxidants and lecithin used in this study were procured from the Khorasan Cotton and Oilseeds Company. Other chemicals used, such as gallic acid, were obtained from reputable companies.

2-2- Preparation of sesame oils containing nanoliposomes of yarrow extract

After obtaining the yarrow plant, its aerial parts were separated and dried in the shade. The samples were ground and subjected to a pulsed electric field process at the Food Industry Institute with an electric field intensity of 1.75 kV/cm and 50 pulses. They were then placed in an ultrasonic bath for 30

minutes at a frequency of 37 kHz. The resulting extract was immediately filtered and converted into powder using a rotary evaporator (Buchi, Switzerland) at 50°C under vacuum [23, 24]. To prepare nanoliposomes, the method of Bozhmehrani and colleagues (2022) was used, utilizing a 1 to 7 concentration of yarrow extract to lecithin. In this method, a certain amount of dried antioxidant extract powder was mixed in a water-ethanol solvent (70-30) and added to commercial lecithin derived from soybean oil. This mixture was placed in a rotary evaporator at 50°C under vacuum to ensure better mixing and to evaporate some of the solvent. The samples were then homogenized using a homogenizer at 12,000 rpm for 10 minutes at a temperature above the liposome phase transition. The liposome mixture was transferred to an ice water bath (to prevent excessive energy input into the solution and hydrolysis and oxidation of the lipid) and subjected to probe sonication (Hielscher, Germany) with 9 cycles of 20 seconds and 30 seconds rest between cycles. At this stage, single-layer nanoliposomes were produced. These samples were then dried using freeze drying (Christ α 1-4, Germany) for 48 hours [20]. After preparing nanoliposomes containing yarrow extract, 5 concentrations (50, 100, 200, 500, and 1000 ppm) were prepared and directly added to sesame oil without antioxidants. A sample without antioxidants was also prepared and these oils were kept at 63°C in a laboratory oven (Memert, Germany) for 7 days. After sampling at intervals of 0, 1, 3, 5, and 7 days, the following tests were conducted. It is worth mentioning that a sample containing 200 ppm BHT antioxidant and a sample containing free yarrow extract (equivalent to the optimal amount of nanoliposome containing extract) were also evaluated [20].

2-3- Acidity measurement

To determine the acidity, the AOCS Cd 3– 63 (1993) method was used, and the acid value was obtained using Equation 1 [25].

Eq (1)

$$
A = \frac{2.82 \times V}{W}
$$

In equation 1, V (ml) is the volume of sodium hydroxide used, W is the weight of the sample in grams, and A is the free fatty acids expressed as oleic acid per 100 grams of the sample.

2-4. Peroxide value determination

The peroxide value of the samples was determined according to the AOCS Cd 8– 53 (1993) method and was obtained using Equation 2 [25].

Eq (2) $P = \frac{S \times M \times 100}{W}$ W

In equation 2, S is the consumption of sodium thiosulfate in milliliters, M is the molarity of sodium thiosulfate, W is the oil weight in grams, and P is the oil peroxide in meq of O_2 per kg of oil.

2-5- Measurement of thiobarbituric acid index

To measure this index, 1 gram of the sample, 1 ml of 0.75% thiobarbituric acid solution, and 2 milliliters of 35% trichloroacetic acid solution were added to a 250-milliliter Erlenmeyer flask. The resulting mixture was placed in a boiling water bath for 20 minutes. Then, the mixture was centrifuged (Thermo, Japan) at 3000 rpm for 3 minutes. The aqueous phase was extracted with a syringe and transferred to a spectrophotometer cell. The absorbance of the sample was read at a wavelength of 532 nanometers using a spectrophotometer (Biochrom, UK), and thus, the absorbance of the sample at the mentioned wavelength was considered as the thiobarbituric acid index [20].

2-6- Determination of conjugated diene value

To measure the conjugated diene compounds, the oils were diluted at a ratio of 1:600 with hexane, and their absorbance was measured at a wavelength of 234 nanometers using a spectrophotometer [26].

2-7- Determination of oxidative stability To determine the oxidative stability of the oils, a Rancimat device (Metrohm, Switzerland) was used according to the AOCS Cd 12b-92 (1993) method, with a temperature of 110°C and an airflow rate of 20 liters per hour [25].

2-8- Fatty acid profile analysis

After identifying the best sample of sesame oil containing nanoliposomes with yarrow extract, the fatty acid profile of this sample and the antioxidant-free sample (control) was analyzed. First, the fatty acid methyl esters were prepared, and the analysis was conducted according to the AOCS Ce 2-66 (1993) method. A gas chromatography (GC) device equipped with a silica capillary column, 70 meters in length, 0.25 micrometers in diameter, and 0.25 micrometers in film thickness (Agilent, USA) was used for the analysis. The initial temperature was 80°C, which was increased by 15°C per minute to reach 200°C, and held at this temperature for 10 minutes. Then the temperature was raised to 220°C and held for 5 minutes. The injection port and detector temperatures were set at 210°C, and the carrier gas (helium) flow rate was 1 milliliter per minute. Finally, the chromatogram obtained from the device was compared with the standard curve to determine the type and amount of each fatty acid in the oil, expressed as a percentage [25].

2-9- Statistical analysis

To evaluate the effect of adding antioxidant (nanoliposomes containing yarrow extract) on the characteristics of sesame oil, the response surface methodology using MiniTab software was employed. Additionally, for comparing the optimal sample with other samples, a completely randomized design using SAS software was utilized. The Duncan test at a significance level of 5% was employed to compare the means.

3- Results and Discussion

3-1- Effect of Study Conditions on Oil Acidity

Acidity and acid value are crucial qualitative parameters of oils, reflecting their purity. Despite refined oils being nearly free of free fatty acids, significant amounts of these compounds exist in crude oils. Figure 1 illustrates that with increasing storage time, the acidity of oils consistently increased across all concentrations of yarrow extract-containing nanoliposomes used. However, with an increase in antioxidant extract concentration up to 500 ppm in the produced nanoliposomes, acidity initially decreased and then increased again. The equation predicting oil acidity (detailed below) demonstrated that the linear parameter of storage time had the greatest impact on oil acidity. The decrease in acidity with higher concentrations of yarrow extract-containing nanoliposomes can be attributed to the phenolic compounds' ability to interrupt the formation and release of free radicals and free fatty acids. Conversely, the increase in

acidity can be linked to impurities accompanying the extracted extract, acting as a pro-oxidant. The increase in acidity with prolonged storage time can be attributed to the high storage temperature and the production of free fatty acids from the breakdown of oil triglycerides. Yemani et al. (2022), in their study on the effects of storage time on some qualitative properties of virgin olive oil, noted an increase in oil acidity with prolonged storage time, consistent with the results of this section. Jalali-Zand and Goli (2021) also indicated that reducing the concentration of encapsulated selenium antioxidant and increasing storage time led to an increase in oil acidity in soybean oil. Li et al. (2021) compared two types of natural antioxidants, rosemary and senaze, during deep frying of soybean oil, demonstrating that prolonged frying time increased soybean oil acidity, albeit less intensively at higher concentrations of this extract.

Acidity = 0.06482 - 0.000098 extract content + 0.00462 time storage + 0.000310 time storage * time storage -0.000001 extract content *time storage

Figure 1- Effect of antioxidant concentration and storage time on acidity

3-2- Effect of study parameters on peroxide value

Peroxide value is the primary product of lipid oxidation and generally indicates the

degree of unsaturation of fats and oils. As unsaturated oils are more prone to oxidation, a higher peroxide value signifies a greater readiness for oxidation. When peroxide levels reach a certain threshold, various changes occur, resulting in the production of volatile aldehyde and ketone compounds that contribute to off-flavors and odors in fats and oils. Results indicated that with an increase in the concentration of antioxidant extract, the peroxide levels in samples initially decreased and then increased. Similarly, it was observed that with an increase in storage time up to 5 days, peroxide levels initially increased and then decreased (Figure 2). The decrease in peroxide levels with increased extract concentration can be attributed to the antioxidant properties of yarrow extractcontaining nanoliposomes, which donate hydrogen atoms to free radicals generated during oxidation processes, thereby interrupting chain reactions and slowing down the rate of auto-oxidation. However, adding excessive phenolic compounds to oils can diminish their antioxidant effect due to impurities accompanying the extract, which may act as pro-oxidants. The interaction of phenolic and flavonoid compounds present in the extract in enzymatic browning reactions can also lead to the loss of their antioxidant properties. Studies by Tepeh et al. (2005) highlighted that at high temperatures, peroxide values are unstable and readily convert to a mixture of aldehyde compounds, indicating that peroxide value alone cannot serve as a reliable measure of the actual progress of oxidation, which aligns with the findings of this section. Bozorgmehrani et al. (2022) demonstrated that with increased storage time up to 5 days, peroxide levels consistently increased in all soybean oil samples but decreased thereafter. It was also observed that with an increase in the concentration of grape pomace nanoliposomes containing antioxidants up to 500 ppm, the peroxide levels in oils exhibited less intensity in their increase, but at higher concentrations, peroxide levels increased more intensively.

Peroxide Value = 1.032- 0.00572 extract content $+1.200$ time storage $+0.000005$ extract content* extract content - 0.0892 time storage * time storage - 0.000199 extract content * time storage

Figure 2- Effect of antioxidant concentration and storage time on peroxide value

3-3- Effect of study parameters on thiobarbituric acid value

Thiobarbituric acid value (TBA) is a compound formed during the autooxidation of fatty acids, characterized by its absorption at 532-535 nanometers and indicating the presence of secondary oxidation products in the sample. Therefore, higher TBA values in oils indicate more extensive oxidation of the oil and consequently lower stability. Figure 3 showed that with an increase in storage time, the TBA value of samples increased, but with an increase in the concentration of yarrow extract-containing nanoliposomes up to 500 ppm, this indicator decreased and then increased. The decrease in TBA value with increased antioxidant extract concentration can be attributed to the increase in phenolic compounds present in the antioxidant extract, which effectively scavenges free radicals and secondary oxidation products, thus reducing the TBA value. The increase in TBA value is due to the oxidation of secondary oxidation products and the formation of carboxylic acids. Ahmadzadeh (2022) demonstrated that the TBA value of samples increased with time, but with an increase in extract

concentration up to 600 ppm, this indicator decreased, which is consistent with the results of this section. Akbari et al. (2013) showed that with increased storage time of fish fillet, the levels of secondary oxidation products, particularly aldehydes, increased due to the increased presence of free iron and other pro-oxidants, as well as the breakdown of hydroperoxides into secondary oxidation products.The equation predicting the TBA value of oils indicated that the parameter of storage time quadratic had the greatest impact on TBA values.

Thiobarbituric acid $= 0.1093 - 0.000519$ extract content $+0.0088$ time storage +0.00977 time storage * time storage - 0.000004 extract content * time storage

3-4- Impact of studied parameters on conjugated dienes

When polyunsaturated fatty acids containing three or more double bonds (such as linolenic acid) undergo oxidation, initially two double bonds form the structuere of conjugated dienes index, and subsequently, the third structure also undergoes positional changes, forming the triene double bond structure that absorbs at

268 nanometers [42]. Determining the indices of conjugated dienes and triene is quicker and simpler compared to determining peroxide and anisidine values and requires fewer samples and chemicals [43]. Findings indicated that with increased storage time, the DI values increased in all samples. However, with the increase in antioxidant concentration in the samples up to 500 ppm, this index initially decreased and then increased (Figure 4). Bojmehrani et al. (2022) also reported that increasing the concentration of antioxidants initially decreases and then increases this index in soybean oil. These researchers attributed the increase in this index to the oxidation of polyunsaturated fatty acids and stated that
with the increase in antioxidant the increase in antioxidant concentration, due to the presence of some peroxide compounds in the extract, the conjugated dienes index increased, which was consistent with the results of this section [20]. The formation rate of hydroperoxides is higher compared to their decomposition rate in the initial stages of oxidation. However, when the decomposition rate of hydroperoxides exceeds their formation rate, the peroxide number can decrease even if oxidation increases during storage [44]. When the decomposition rate of hydroperoxides or conjugated dienes exceeds their formation rate, their numbers can decrease even if oxidation increases during storage [45]. conjugated dienes is a method for measuring primary oxidation products that correlates highly with peroxide numbers during fat oxidation [46]. The equation predicting the conjugated dienes index of oils specified that the linear time parameter had the greatest effect on this index.

Conjugated dienes = 2.564 - 0.00696 extract content $+0.712$ time storage + 0.000007 extract content * extract content + 0.0044 time storage * time storage - 0.000175 extract content * time storage

Figure 4- Effect of antioxidant concentration and storage time on conjugated dienes

3-5- Optimization of Sesame Oil Formulation Containing Nanoliposomes with Boswellia Extract and Comparison with Samples Containing Free Boswellia Extract and BHT

According to the results of this study, the best sample of sesame oil in terms of oxidative stability was selected as the sample containing nanoliposomes with 500 ppm yarrow antioxidant extract. This sample was compared with samples containing the same amount of free yarrow extract and also with a sample containing 200 ppm BHT after 7 days of storage at 63

degrees Celsius. The results presented in Table 1 showed that the sample containing 500 ppm free yarrow extract had the highest acidity, peroxide value, thiobarbituric acid index, and conjugated dienes index. The sample containing nanoliposomes with 500 ppm yarrow antioxidant extract had the lowest acidity, peroxide value, and conjugated dienes, but its thiobarbituric acid did not significantly differ from the sample containing 200 ppm BHT. Ahmadi (2022) optimized the extraction of white tea antioxidant extract using ultrasound pretreatment, liposome encapsulation of the extract, and its use for stabilizing edible oils, stating that the sample containing nanoliposomes with white tea extract had the lowest peroxide value, thiobarbituric acid, and anisidine, while the highest values belonged to the sample lacking antioxidants $(p<0.05)$. The sample containing free white tea extract and its nanoliposome form also had better quality than the sample

containing BHT [40]. Shemsh et al. (2019) stated that the use of nanoliposomes in the application of natural and synthetic antioxidants in oils improves the antioxidant properties of these compounds, which was consistent with the results of this study [47].

The same letters in each row indicate lack of significance at the 5% level

3-6- The effect of antioxidant type on the oxidative stability of sesame oil

Oil oxidative stability index (OSI) is widely used to evaluate and predict the oxidative stability of oils by ransimet device. This test is performed based on the measurement of electrical conductivity of water while accumulating volatile compounds obtained from oil oxidation, especially carboxylic acids under accelerated oxidation conditions, and oil stability time is reported as an index of oxidation stability for an oil at a certain temperature [48]. Figure 5 showed that the highest oxidative stability (14.21 hours) was related to the oil with nanoliposome containing 500 ppm Yarrow extract, followed by the sample containing

200 ppm BHT (13.59 hours), free extract (12.42 hours) and the sample without antioxidants (11.62 hours). Bojemehrani et al. (2022) showed that the highest oxidative stability of soybean oil (7.6 hours) was related to the sample with 500 ppm nanoliposome containing antioxidant extract of grape pomace, followed by the sample containing BHT and the sample containing free antioxidant extract of grape pomace. which was in confirmation of the results of this study [20]. The studies of Lalas and Dourtoglou (2003) as well as Ramalho et al. (2008) in increasing the oxidative stability of oil by other plant extracts by Rancimet test were consistent with the results of the present research [49, 50].

Figure 5- Oxidative stability of sesame oils a) control, b) containing nanoliposomes, c) free extract and d) BHT

3-7- The effect of nanoliposome containing yarrow extract on the fatty acid profile of sesame oil

Table 2 showed that the predominant fatty acid in sesame oil containing nanoliposome and also the control was linoleic acid and the use of antioxidants did not significantly change the fatty acid profile of sesame oil. Hosseini et al. (2021) also showed that the use of antioxidants had no significant effect on the fatty acid profile of sesame oil. These researchers also stated in line with the results of this section that the dominant fatty acid in sesame oil is linoleic acid, after

which oleic acid was the second dominant fatty acid in sesame oil [51]. On the other hand, even with 85% unsaturated fatty acid, this oil shows good oxidation stability [52 and 53].

Table 2 - The effect of antioxidant addition on the fatty acid profile of sesame oil

Numbers with different letters in each column imply significant differences in the 5% level of probability.

4- Conclusion

The main aim of this study was to increase the oxidative stability of sesame oil by using the antioxidant compounds of yarrow plant. According to the findings of this research, it can be stated that the use of nanoliposome containing yarrow plant extract up to 500 ppm led to a decrease in acidity, peroxide, thiobarbituric acid index and conjugated dienes of sesame oil, and on the other hand, it was found that with the increase in storage time of these parameters

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increased and the sample with nanoliposomes containing 500 ppm of yarrow plant extract showed high resistance to oxidation, on the other hand, it was found that the use of nanoliposomes to increase oil stability did not significantly change the fatty acid profile of sesame oil. Finally, it can be stated that the use of nanoliposome containing Yarrow plant extract is a suitable alternative to synthetic antioxidants available in the market.

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مجله علوم و صنایع غذایی ایران

مقاله علمی_پژوهشی

بررسی تاثیر نانولیپوزوم های حاوی عصاره آنتیاکسیدانی گیاه بومادران)*millefolium Achillea* **)بر ویژگیهای اکسایشی و پروفایل اسیدهای چرب روغن کنجد**

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