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Physicochemical properties evaluation of Sandalwood (*Santalum album linn)* essential oil nanoemulsion

Amiri, E. [1]**, Hosseini, S. E.** [2*]**, Sharifi, A.** [3*]

1. Masters. graduated, Department of Food Science and Technology, Islamic Azad University, Science and Research Branch, Tehran, Iran.
2. Associate Professor, Department of Food Science and Technology, Science and Research Branch, Islamic Azad University, Tehran, Iran
3. AssociateProfessor, Department of Food Science and Technology, Qazvin Branch, Islamic Azad University, Qazvin, Iran.

ABSTRACT

The use of nanotechnology in the food industry provides facilities such as encapsulation, targeted transfer of plant compounds and extracts without unfavourable sensitive effects. In this study, the essential oil (EO) of *Santalum album (Santalum album* linn.*)* was used to prepare a nanoemulsion. The nanoemulsion characteristics were evaluated and the data was optimized. To conduct the research, Design-Expert 12, statistical method of response surface, Central composite designwith variables such as ultrasound frequency (25-31 kHz), ultrasound duration (10-20 sec.), and *Santalum albu*m EO (1%-10%) were used. After extraction of EO and identification of its effective compounds; percentage of antioxidant activity, viscosity, color indexes and the particle size of nanoemulsions tests were done. After optimization, stability test was performed on the optimized sample. The optimization results showed that with 5.5% *Santalum album* EO, ultrasound frequency of 28 KHZ, ultrasound duration of 15 seconds a nanoemulsion having optimum characteristics can be produced. After repeating the experiments for the obtained point, resulted in production of a nanoemulstion having particle size of 230 nm, the viscosity of 3.10 mPa.s, the brightness index of 78, the whiteness index of 58, and the antioxidant activity of 70%. It is believed these nanoemulsions have the potential to be used in the cosmetics, pharmaceutical and food industries.

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*Corresponding Author E-Mail:
ebhoseini@yahoo.com
asharifi81@gmail.com

1. Introduction

Undoubtedly, food is considered one of the most important human needs and providing healthy food is related to the health of society. One of the methods of controlling pathogenic microorganisms is the use of man-made chemical preservatives in food. However, today it is emphasized to reduce the use of these methods, because on the one hand, food consumers demand natural foods with a long shelf life, with the least change in their structure, and on the other hand, the carcinogenicity and toxicity of some chemical preservatives for humans. It has been proven. Therefore, the pressure on the food industry to quickly replace chemical preservatives and use natural preservatives is one of the new approaches to improve the microbial health of foods and then increase the general health level of societies. Among the natural compounds that can be used as preservatives in food are the essential oils of medicinal plants[1]. Essential oils are one of the natural compounds that are widely used as natural preservatives in food [2].

White sandal (sandal wood)*Sandalwood white linn*.) a fragrant tree from the Santalase family[1][3]. According to the tests, more than 100 constituents have been identified in sandalwood oil, the main composition of which is Santalol.[2] Is. Sandalwood oil consists almost exclusively of sesquiterpenoids[3] Composed. According to research, sandalwood oil is used as a flavoring component in many food products, including alcoholic and non-alcoholic beverages, frozen dairy desserts, candy, gelatin, and puddings [4]. Sandalwood is a small shrub that symbiotically takes its food from the roots of various plants with its sucking spines. Its leaves are opposite, full and pointed, its flowers are small, clusters and the color of the flowers is shiny red. Its fruit is egg-shaped and juicy, which contains a kernel and is enclosed between the flowers. The color of the outer surface of the shrub wood is white or yellow. The core of the tree is lemon-colored and in some cases light brown, fragrant, pleasant and has a smell similar to nutmeg and rose, which is also used in traditional medicine in addition to various uses [5]. The main components of white sandalwood essential oil alpha santalol[4], say Santalol[5], Cedrol[6], esters[7], aldehydes[8]and phytosterols[9] Usually, 50-70% of the total essential oil is made up of santaloles. Its most important fatty acids include palmitic acid[10] and oleic acid[11]are[6].

Since the stability of flavor-generating compounds is low in different environmental conditions and is lost during processing, packaging and storage through physical and chemical interactions; Using a method that can preserve these compounds against environmental factors and also realize its release in a certain time and completely; It is of special importance. Micro-coating is one of the most important processes that can achieve these goals. Micro-coating of effective compounds is used in food and pharmaceutical industries to cover dyes, perfumes and other effective substances [7].

The use of essential oils in food formulations is generally associated with difficulties due to low solubility in water, high vapor pressure, and physical and chemical instability [8]. In addition, essential oils create a smell and taste in products, which is not pleasant for consumers, so today efforts are made to reduce the adverse effects of essential oils. Nanodispersion[12], are a promising method to overcome the problems related to low solubility and bioavailability of these compounds, as well as protecting them from interactions with other compounds and increasing antimicrobial properties through increasing cellular uptake, and they are used to design and produce lipid carrier systems [9].

The use of new technologies in this sector is a new approach that can be considered. One of the interesting technologies in this field is nano technology. Nanotechnology is the science of studying and researching phenomena and

1 .Santalaceae

2 .Santalol

3 .Sesquiterpene

4 .a-santalol

5 . β-santalol

6 .Cedrol

7 .Ester

8 .Aldehyde

9 .Phytosterols

10 .Palmitic acid

11 . Oleic acid

12 .Nanoemulsion

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functions at the atomic, molecular, and nanomolecular levels. Nano technology is widely used in various fields, which has received more attention in the food industry. Nanotechnology has the potential to create fundamental changes in the food and beverage industries with new tools for detoxification, improving taste by passing specific molecules based on the specific shape of materials instead of their size, packaging, improving the plant's ability to absorb nutrients, etc. [10]. Basically, nanoemulsions, due to their specific size, have a transparent or semi-transparent appearance, and in terms of droplet size distribution, they have high kinetic stability, low viscosity, and high stability against precipitation, creaming, joining together, and coagulation phenomena, and for these reasons, they are used for many industrial applications today. they have been noticed. Often, in scientific sources, emulsions with droplet sizes around nanometers (typically, in the range of 20 to 200 nm) are referred to as miniemulsions, nanoemulsions, ultrafine emulsions, submicron emulsions, etc. The unique structure and characteristics of nanoemulsions compared to normal emulsions have created advantages for their use in many industries, including food industries. One of the applications of nanoemulsion systems in food industries is their role in microcoating (encapsulation).[13] and controlling the release of beneficial compounds such as various colors, essential oils, vitamins, etc. [11].

In recent years, extensive research has been done on the formulation of nanoemulsions. In a research, the nanoemulsion formulation of sage essential oil and its physicochemical and microbial properties have been investigated [1]. In another article, the effect of the microcoating process using a freeze dryer on the physicochemical and antioxidant properties of angelica essential oil was studied [12]. Researchers also investigated the physicochemical properties of lime essence alginate oil nanoemulsions [13] and in another article, the antibacterial and anticancer effects of nanoemulsions containing clove plant were investigated [14].

In this research, after extracting and identifying the effective compounds of white sandalwood essential oil, this essential oil was prepared in the form of nanoemulsion and its properties were evaluated and optimized.

2- Materials and methods

White sandal plant (S*long list*linn.) was prepared from the Research Institute of Medicinal Plants of Tehran province and chemicals were also purchased from reputable companies.

2-1- Extraction of white sandal plant essential oil

First, white sandalwood was milled and then essential oil was extracted from it by steam distillation using a Clonger machine. The duration of essential oil extraction lasted about 2 hours. Dehydration of essential oil was also done by adding a little sodium sulfate. Essential oil without water was stored in a dark container in a refrigerator at a temperature of 4 degrees Celsius [15].

2-2- Analysis and identification of constituent compounds of white sandal plant essential oil

Shimadzu model 8050-TQ gas chromatograph (GC-MS) connected to mass spectrometer was used for essential oil analysis. The column used was HP-5 type (length 30 meters, inner diameter 0.25 μm, thickness of stationary phase layer 0.25 mm). Thermal programming was 330-40 degrees Celsius and scanning time was 0.5 seconds and mass range from 35 to 300, ionization energy was equal to 70 electron volts and using helium gas with a flow rate of 1 ml/min. Analyzing the essential oil and identifying the constituent compounds after injecting the essential oils into the gas chromatography GC device and finding the most suitable thermal planning of the column, in order to achieve the best separation, the resulting essential oils were diluted with normal hexane and injected into the gas chromatography device coupled with a GC/MS mass spectrometer and spectra The corresponding mass and chromatograms were obtained. Identifying the resulting spectra by plotting the chromatogram of a fraction of normal paraffins (C_5-C_{30}) was performed under the same conditions with the

[13]. Encapsulation

injection of essential oils and according to the inhibition time of these compounds, the Quatz index was calculated for each component in the essential oil chromatogram. These values were compared with the values of the quartz index in the standard tables, and the compounds in the white sandalwood essential oil were identified based on these data and the information available in the GC-MS library [16].

2-2-1- Preparation of nano emulsions containing sandalwood essential oil

In high-energy methods for nanoemulsion production, mechanical equipment capable of producing strong tearing forces is used. To produce the desired nanoemulsion, an ultrasonic homogenizer of the Scientists-IID model was used, and the frequency of this device can be changed from 25 to 31 kHz. The mechanism of nanoemulsion production with this method is based on cavitation. In order to prepare azotoin nanoemulsion[14]80 Vaspin[15] 83 and ultrasound method was used. The nanoemulsion contained 2% by weight of surfactant, 95% by weight of water and 3% by weight of essential oil. For the synthesis of nanoemulsions, an oil-in-water emulsion was used to create oily nanoemulsions. The intended emulsion solution was mixed with white sandal plant essential oil with different amounts of essential oil (1-10%), Tween 80 and Span 83 non-ionic surfactants and distilled water. In the next step, the emulsion solution was homogenized using an ultrasonic probe device at frequencies (25-31 kHz), wave propagation times (10-20 seconds) and input power of 400 watts for different periods of time [17].

2-3- Evaluation of the physicochemical properties of the produced nanoemulsion

2-3-1- Determining the size of nanoemulsion particles

To measure the nanoemulsion droplets, a Zetasizer device (Malvern, England, Zetasizer Nano ZS) was used, which is based on the method of dynamic light scattering or dynamic light scattering, which is so-called[16]DLS is said to be done. DLS measures the Brownian motion of nanosized droplets and relates this motion to the nanometer-equivalent hydrodynamic diameter. For particle size measurement, each sample was diluted 20 times with distilled water. The average particle size was measured based on the average volume diameter [18].

2-3-2- Viscosity of nanoemulsion

In the next experiment, the synthesized nanoemulsion formulations were investigated through viscosity measurement (Brookfield Viscometer, made in America, model RV-DV III Ultra). Experiments were performed by placing device probes inside the nanoemulsion solution. Nanoemulsion solutions have low viscosity, which is one of the main characteristics of nanoemulsions [17].

2-3-3- Color indicators

Indicators*L** (brightness index) and WI (whiteness index) at laboratory temperature (25 degrees Celsius) using a chromameter (modelCR400, Konica Minolta, made in Japan) was determined. To perform the test, the angle of the device was set to 10 and calibrated with a standard white screen [13].

2-3-4- Determination of antioxidant activity

The ability to lose hydrogen atom by antioxidant compounds was measured by the amount of discoloration of 2 and 2-diphenyl-1-picryl-hydrazyl (DPPH) violet solution in methanol. In this test, DPPH was used as a stable radical compound. The antioxidant activity of essential oil was evaluated before and after nanoemulsion production. First, 0.008% DPPH solution was prepared with methanol. Different dilutions of nanoemulsion and essential oil were prepared with methanol, and finally, the optical absorption of the samples was read by a spectrophotometer at a wavelength of 517 nm after 3 minutes in a greenhouse at room temperature [1]. The reduction percentage of DPPH free radicals was calculated by equation (1):

$$\text{Inhibition percentage} = 100\times\frac{\text{Sample absorption - sample absorption control}}{\text{Sample absorption control}}$$

2-3-5- Stability of nanoemulsions

After optimizing the samples, the long-term

14. Tween

15. Span

16. Dynamic Light Scattering

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stability of the produced nanoemulsions was checked by measuring the changes in the size of the particles as well as their appearance during storage at the laboratory temperature (25 degrees Celsius) for 60 days. The stability of nanoemulsions by the polydispersity index of the optimal nanoemulsion produced on days 1, 30 and 60 using an active optical scattering device (Nanotrac Wave),modelMicrotrac (made in USA) was measured [14].

2-4- Designing experiments

response surface approach[17]It is a combination of statistical techniques and is among the multiple test design techniques[18]It is a method in which the effects of several variables on the response are studied simultaneously, and it has important applications in the design, development and formulation of new products and processes, as well as the improvement of existing product designs and processes. In this research, a central composite design was used with 20 treatments and 4 replications in the central points. The variables of percentage of sandalwood essential oil were selected from 1 to 10 percent by volume/volume, ultrasound frequency from 25 to 31 kHz, and ultrasound duration from 10 seconds to 20 seconds, and twenty experiments were designed by Design-Expert 12 software. The coded and actual values of the independent variables used are shown in Table 1.

Table 1 Coded values and levels of independent variables for production optimization of Sandalwood essential oil nanoemulsion

-1	0	+1	Independent variables
10	5.5	1	Sandalwood essential oil (%)
31	28	25	Ultrasound frequency (kHz)
20	15	10	Ultrasound time (S)

After conducting experiments, research using the analysis of variance method[19] The significance of the model and the effects of each of the parameters alone as well as their mutual effects were examined in each of the experiments and the relationship between the variables was reported in the form of the following regression equation:

relationship (2)

$$Y = b_0+b_1A + b_2B + b_3C + b_{12}AB + b_{13} \text{ AND} + b_{23} BC + b_{11}A^2 + b_{22}B^2 + b_{33}C^2$$

The above-mentioned fair skin expresses the desired answers, including the determination of particle size, viscosity, color indicators, antioxidant activity, etc.$_n$ It indicates the regression coefficients. The values of A, B and C respectively represent the independent factors of the process, including sandalwood essential oil (percentage), ultrasonic frequency (kHz) and ultrasonic time (seconds). After performing the tests and recording the answers and performing statistical analysis, the statistical results were obtained in the form of the mutual effect of the variables (sandal essence, ultrasound frequency and ultrasound time) and the relationship between the variables was reported as a regression equation.

2-4-1- Model fitting

The appropriate model considering the significance of the F test and the non-significance of the index of inconsistency with the test findings[20] as well as R values^{2}and R^2 Adjusted and coefficient of variation was selected. According to the analysis of variance tables, it can be seen that the F test of all the investigated parameters is completely significant. In order to check the influencing parameters in the study according to the analysis of variance table, the sentences with significant differences were retained in the model [19].

3. Results and Discussion

After performing the tests and recording the answers and performing statistical analysis, the model is suitable according to the significance of the F test ($p \geq 0.01$), the non-significance of the lack of fit ($p>0.05$) and the highest R values.^{2} and R^2 Adjusted and coefficient of variation was selected. The results of the tests on the parameters related to the process, including particle size, viscosity, whiteness index, brightness index and strength

17. Response Surface Methodology (RSM)
18. Multivariate
19. ANOVA
20. Lack of Fit

Antioxidants are shown in Table 2, which includes the observed responses and the values predicted by the model.

Table 2 Observed responses and values predicted by the model for production optimization of Sandalwood essential oil nanoemulsion

A	B	C	Observed responses					predicted responses				
Essential **(%)**	Ultrasound frequency (kHz)	Ultrasound time (S)	particle size (nm)	viscosity (mPa.s)	brightnessindex	white index	Antioxidant activity (%)	particle size (nm)	viscosity (mPa.s)	brightnessindex	white index	Antioxidant activity (%)
5.5	31	15	263.36	3.77	86.78	68.61	95.26	219.68	2.81	78.80	56.49	60.73
1	25	10	289.47	3.25	89.77	77.21	97.74	236.67	2.09	80.12	62.57	55.99
5.5	31	10	273.16	4.02	81.58	65.61	94.99	226.29	2.99	73.01	52.62	57.94
10	31	15	343.45	4.24	87.05	70.57	107.25	296.78	3.22	78.53	57.62	70.36
5.5	28	15	273.31	4.04	86.70	70.30	104.43	230.43	3.10	78.86	58.41	70.52
10	25	20	364.61	4.51	82.23	67.66	94.39	312.92	3.37	72.78	53.33	53.53
1	28	10	269.22	3.44	86.02	71.53	83.90	220.96	2.37	77.20	58.14	45.75
5.5	28	15	273.31	4.04	86.70	70.30	104.43	230.43	3.10	78.86	58.41	70.52
5.5	28	10	280.46	4.15	83.33	66.15	108.26	236.78	3.19	75.35	54.03	73.73
1	31	20	233.11	3.17	94.44	82.61	55.63	180.30	2.01	84.79	67.96	13.89
5.5	28	15	273.31	4.04	86.70	70.30	104.43	230.43	3.10	78.86	58.41	70.52
10	28	15	346.16	4.57	84.34	67.14	108.65	302.52	3.61	76.36	55.04	74.15
1	31	10	258.27	3.45	82.29	66.87	65.70	205.47	2.29	72.64	52.23	23.96
10	31	10	347.58	4.64	82.71	71.58	104.43	295.88	3.51	73.26	57.25	63.57
10	25	10	358.78	4.89	81.28	63.93	112.47	307.08	3.75	71.84	49.59	71.60
1	25	20	265.31	3.31	92.81	94.95	63.67	212.50	2.15	83.16	80.31	21.92
5.5	28	15	273.31	4.04	86.70	70.30	104.43	230.43	3.10	78.86	58.41	70.52
10	31	20	352.41	3.91	92.75	73.32	110.36	300.72	2.77	83.31	58.99	69.49
5.5	28	15	285.06	3.97	85.26	70.95	103.3	241.39	3.01	77.28	58.84	68.77
1	28	15	252.82	3.40	89.59	78.70	74.68	207.10	2.40	81.24	66.02	38.54

1-3- The results of identifying the components of white sandal plant essential oil

The highest amount of compounds in alpha-santhalol essential oil and the lowest amount of identified compounds in white sandal plant essential oil is beta-almane.[21] Is. Other compounds identified in white sandal plant essential oil are listed in Table 3. According to studies, alpha-santalol has the most anti-cancer and antioxidant properties and is a non-toxic compound. Also, the main factor responsible for the pleasant scent of sandalwood is alpha-santalol. According to the evidence of the sandalwood plant, due to the high percentage of alpha-santhalol, it has antimicrobial properties against Staphylococcus aureus bacteria.[22], Ashershia Clay[23] as well as Pseudomonas[24] has[20].

[21] 1. b-Elements

[22] 2. *Staphylococcus aureus*

[23] 3. *Escherichia coli*

[24] 4. *Pseudomonas*

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Table 3 Chemical components of white sandalwood essential oil analyzed with gas chromatography mass spectrometry (GC-MS)

Percentage	RI	components	Order
28.75	1675	α-santalol	**1**
9.42	1720	β-santalol	**2**
7.7	1123	Pyrazine	**3**
6.92	1465	The β-santales	**4**
5.17	1454	g-Neoclovene	**5**
3.76	1640	Cyclotetradecane	**6**
3.58	1975	octadecanoic acid	**7**
3.35	1600	n-Hexadecane	**8**
2.94	1107	Longifolene	**9**
2.35	1800	n-Octadecane	**10**
1.93	1399	n-Tetradecane	**11**
1.90	1310	Teresantalol	**12**
0.83	1503	Germacrene A	**13**
0.5	1299	Azulene	**14**
0.36	1069	5-Methyl-2-furfural	**15**
0.33	1052	Butanedioic acid	**16**
0.32	1244	Benzoic acid	**17**
0.28	1153	Benzyl alcohol	**18**
0.24	873	2-Methylbutanoic acid	**19**
0.13	1393	b-Elements	**20**

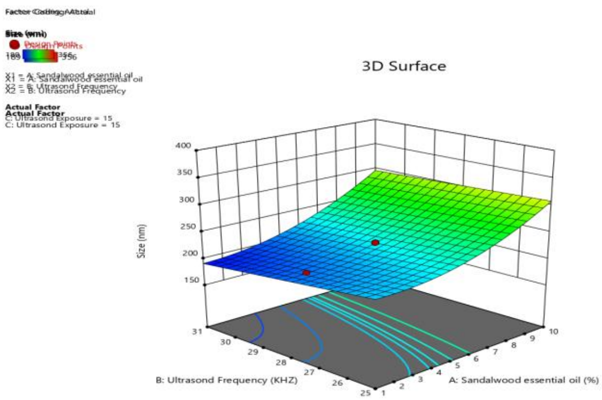
2-3- The results of nanoemulsion particle size evaluation

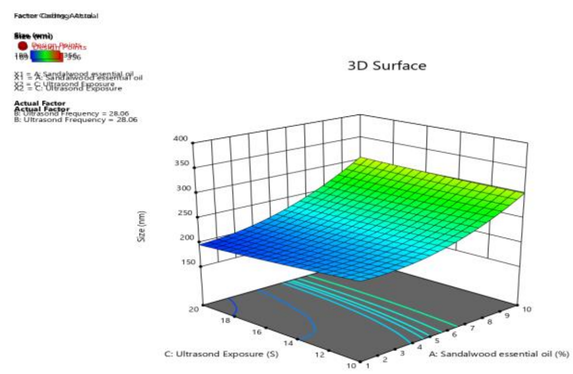
White sandal essential oil nanoemulsions have a homogeneous morphological structure and are spherical in shape without agglomeration. The spherical shape of nanoemulsions is also due to the electrostatic balance of the carrier and sandalwood essential oil, which also has the ability to form an egg network, and by acting as a nucleus, it can be used to entrap sandalwood essential oil and cause the entrapment of sandalwood essential oil. As can be seen in the results of Figure (1), with the increase in the use of sandalwood essential oil in the formulation of nanoemulsions, the size of nanoemulsions increased significantly, which is related to the molecular weight of nanoparticles, in other words, the increase in the molecular weight of compounds increases the size of the resulting nanoemulsions. became The fitted equation for this answer is coded as follows:

Y=71/47 +43/230+A-85/10B-83/۴C00/5 +AB+50/7AC39/24 +A^2+1093/0$B^2$52/1+C^2relationship (3)

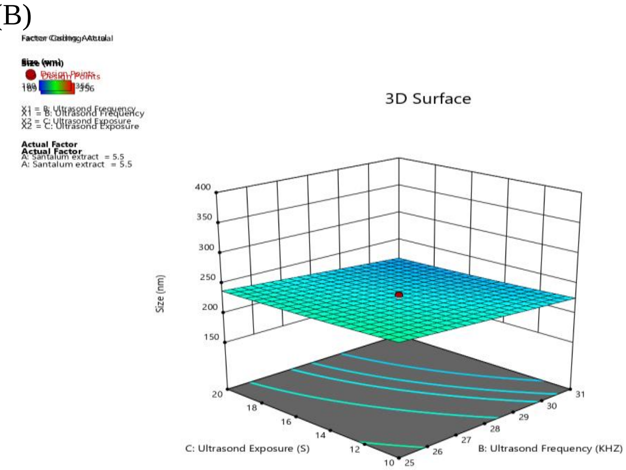
According to the results of Figure (1), it was observed that the size of the nanoemulsion particles decreased with the increase in the frequency and time of ultrasonication. The reason for this effect is the cavitation process, in this process, repeated tensions and bursting of bubbles cause rapid movement of the liquid flow towards the formed nanoemulsions, and their resistance to the liquid flow leads to damage to the walls of the nanoemulsion particles, and as a result of these severe damages, local stresses and It creates ephemerality on the surface of the produced nanoemulsion particles, which leads to a smaller size of the nanoemulsion particles [21]. In a similar study investigating the effect of ultrasonic waves on the size of cocoa milk particles, similar results were obtained that increasing the frequency leads to a smaller size of cocoa milk particles, which was in agreement with the findings of the present research [22]. According to the results, sandalwood essential oil had a positive and significant effect on the particle size, but the particle size decreased with the increase of the ultrasonic frequency and time variable.

Coefficient of explanation (R^2) the predicted model for the above answer is 0.9305; Therefore, the model has been able to explain 93.05% of the total changes in the scope of the examined variables. The lack of fit index was not significant ($p>0.05$), which indicates the appropriateness of the model in predicting the range of tested variables.





(B) (A)



(C)

Fig 1 Interaction of sandalwood essential oil (%),time (S) and ultrasound frequency (kHz) on particle size (nm). A) The effect of sandalwood essential oil and ultrasound frequency on particle size B) The effect of sandalwood essential oil and ultrasound time on particle size C) The effect of ultrasound frequency and time on particle size

3-3- Viscosity of nanoemulsion

According to the obtained results, it was observed that the viscosity of nanoemulsion treatments increases significantly with the increase in the percentage of sandalwood essential oil, which is due to the increase in the percentage of essential oil entrapment up to 5.5% of sandalwood essential oil, which increases the molecular weight of sandalwood essential oil nanoemulsions. The viscosity also increases. The fitted equation for this answer is coded as follows:

Y=6088/0+10/3+A-985/0B-1667/0C-1125/0AB1125/-0AC875/0-BC-0941/0A^{2}1871/・-B^{2}785/0-C^2 Relationship (4)

According to the results of Figure (2), increasing the duration of ultrasound up to 15 seconds can create a homogenous texture in the emulsions due to preventing the nanoemulsions from sticking together and their agglomeration. Creation leads to mono and tri connections and the creation of dimeric structures instead of nanoemulsions, which can increase the viscosity of nanoemulsion to some extent by increasing the amount of sandalwood essential oil. Ultrasound frequency has similar effects as ultrasound time.

Similar results were obtained in a research that investigated the effects of the ultrasonic process

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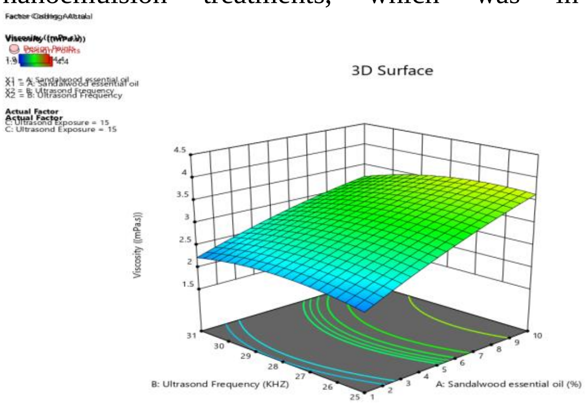
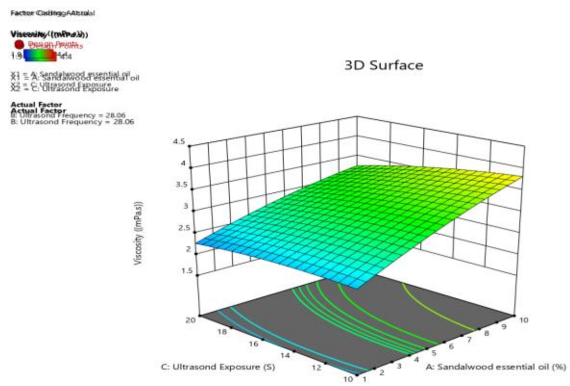
and duration on the production of nanoemulsion. They found that increasing the duration and frequency of ultrasound in very high amounts by creating entanglement in the structure of the primary nuclei of emulsions and creating tension in the environment leads to the creation of irregular gel structures that increase the viscosity, which is in agreement with the findings of the current research [23]. Also, in the investigation of the effects of optimization of nanoemulsions of seed oil, they found that the use of high ultrasonic powers can increase the viscosity of nanoemulsion treatments, which was in accordance with the findings of the present research [24]. According to the results of Figure (2), sandalwood essential oil had the most positive and significant effect, which significantly increased the viscosity.

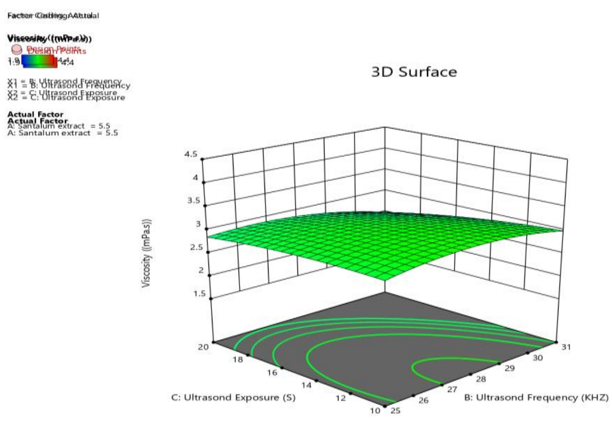
Coefficient of explanation (R^2) the predicted model for the above answer is 0.7764; Therefore, the model has been able to explain 77.64% of the total changes in the scope of the studied variables. The misfit index was not significant ($p > 0.05$), which indicates the appropriateness of the model in predicting the range of tested variables.



(B)



(A)



(C)

Fig 2 Interaction of sandalwood essential oil (%), time (S) and ultrasound frequency (kHz) on viscosity (mPa.s) A) The effect of sandalwood essential oil and ultrasound frequency on viscosity B) The effect of sandalwood essential oil and ultrasound time on viscosity C) The effect of ultrasound frequency and time on viscosity

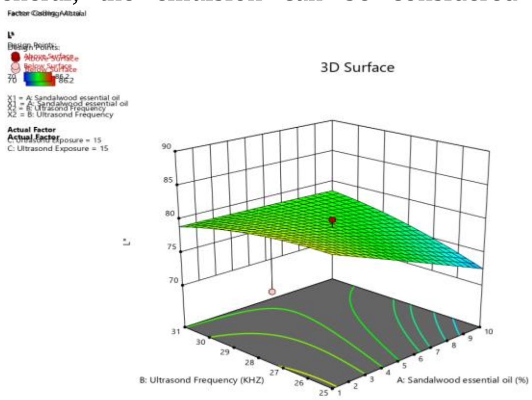
4-3- Color indicators

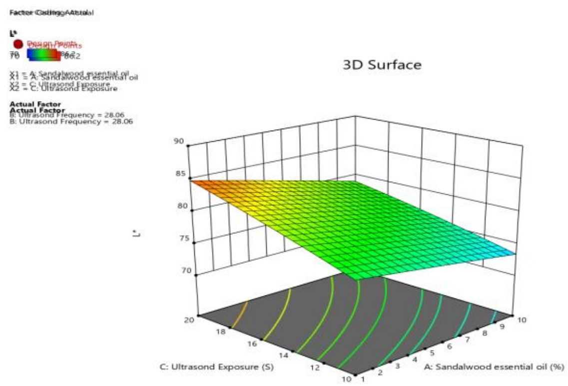
According to the results of figure (3) evaluation of the brightness index ($^*L$) showed that increasing the percentage of essential oil in the formulation of nanoemulsion treatments reduces the brightness index because nanoemulsions have a limited and specific entrapment capacity and in higher amounts of essential oil, with an increase in the amount of essential oil, it is not trapped in the structure of nanoemulsions resulting in high turbidity It is caused by the accumulation of sandalwood essential oil in the environment that this increase in turbidity, brightness index ($^*L$) reduces. The fitted equation for this answer is coded as follows:

relationship (5)

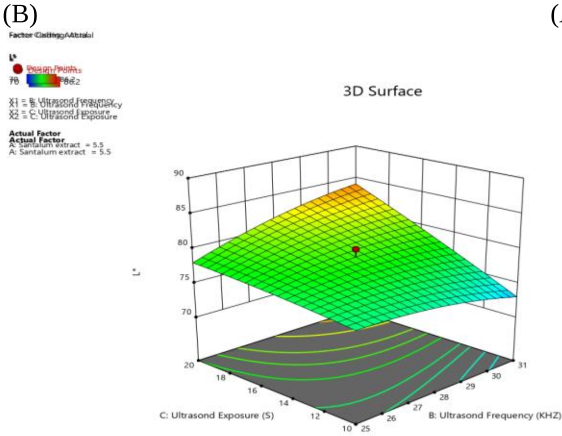
$$Y=44/2-87/78+A+7632/0B+27/3C+23/2AB+5250/0AC-28/2BC-0605/0A^2-8227/0B^2-2394/0\ C^2$$

In general, the emulsion can be considered transparent if its turbidity is less than 0.05 NTU and the average diameter of its droplets is less than 80 nm, and it will be suitable for use in clear drinks. In the investigation of the nano coating of garlic essential oil using the water-in-oil emulsion method, they also found that increasing the amount of essential oil in the nano emulsion formulation with increasing the opacity of the brightness index ($^*L$) which was in agreement with the findings of the present research [25].





(B) (A)



(C)

Fig 3 Interaction of sandalwood essential oil (%), time (S) and ultrasound frequency (kHz) on brightnessindex A) The effect of sandalwood essential oil and ultrasound frequency on brightness index B) The effect of sandalwood essential oil and ultrasound time on brightness index C) The effect of ultrasound frequency and time on brightness index

Increasing the amount of ultrasound time and frequency due to disturbances in the environment of nanoemulsions in high amounts can break the structural arrangement of nanoemulsions and create an environment full of irregular structures and untrapped sandalwood essential oil particles in the center of nanoemulsions, which leads to an increase in turbidity and a decrease brightness index ($^*L$) treatments. There was a similar research in this regard. According to the results of Figure (3), the variable of ultrasound time and sandalwood essential oil had a positive and

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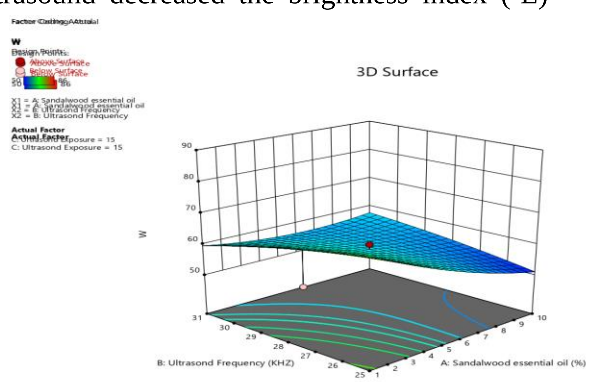
significant effect on the colorimetric components. Coefficient of explanation (R^2) predicted model for the above answer is 0.7448. Therefore, the model has been able to explain 74.48% of the total changes in the scope of the investigated variables. The misfit index was not significant ($p > 0.05$), which indicates the appropriateness of the model in predicting the range of tested variables.

As the results of the brightness index ($^*L$) showed that increasing the amount of sandalwood essential oil as well as the frequency and time of ultrasound decreased the brightness index ($^*L$) and since there is a positive and significant relationship between whiteness index and brightness ($^*L$) is present, so according to Figure (4), the whiteness index of nanoemulsion treatments also experienced a significant decrease, which was consistent with the article on the properties of garlic essential oil nanoemulsions [25]. The fitted equation for this answer is coded as follows:

relationship (6)

$Y=49/5-42/58+A-17/1B+87/4C-50/4AB-50/3AC-0005/0BC+12/2A^2-7453/0B^2-4921/0C^2$



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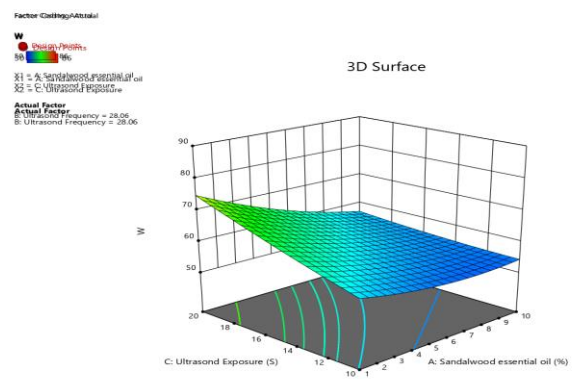
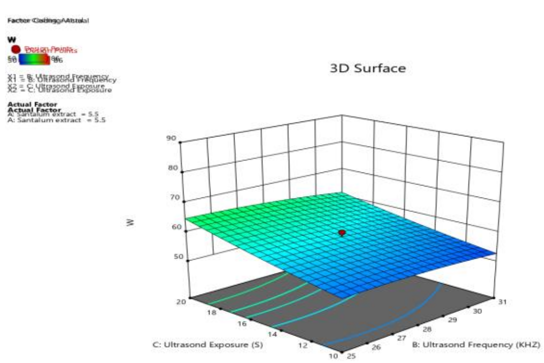


(A)



(C)

Fig 4 Interaction of sandalwood essential oil (%), time (S) and ultrasound frequency (kHz) on white index A) The effect of sandalwood essential oil and ultrasound frequency on white index B) The effect of sandalwood essential oil and ultrasound time on white index C) The effect of ultrasound frequency and time on white index

Coefficient of explanation (R^2) the predicted model for the above answer is 0.7869; Therefore, the model has been able to explain 78.69% of the total changes in the scope of the investigated variables. The misfit index was not significant ($p > 0.05$), which indicates the appropriateness of the model in predicting the range of tested variables.

5-3- The results of antioxidant activity evaluation

Antioxidants in low concentrations are able to prevent or delay oxidation caused by reactive oxygen species. Nowadays, the use of synthetic antioxidants is very popular in medicine, agriculture and pharmaceutical industries. But several studies indicate the toxicity of these antioxidants. For this reason, finding natural antioxidants, especially from

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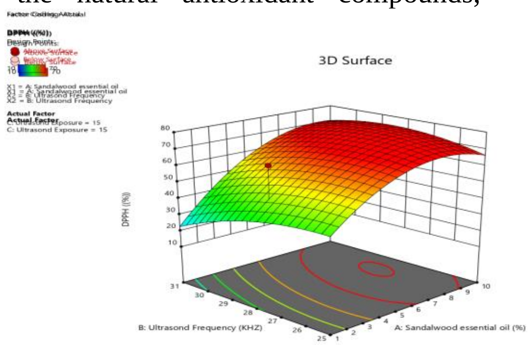
Plant sources and their use in medicine, agriculture and pharmaceutical industries are very desirable to reduce the possibility of side effects and poisoning with them, especially in controlled doses. In recent years, extensive studies have been conducted on plants and their essential oils to find antioxidant compounds. Among the natural antioxidant compounds, polyphenols, flavonoids and phenolic compounds that are widely distributed in nature are of great interest [26]. The fitted equation for this answer is coded as follows:

relationship (7)

$$AND=80/17+52/70+A-02/4B-04/7C+00/6AB-00/4AC+00/6BC-18/14A^2-77/5B^2-83/3\,C^2$$



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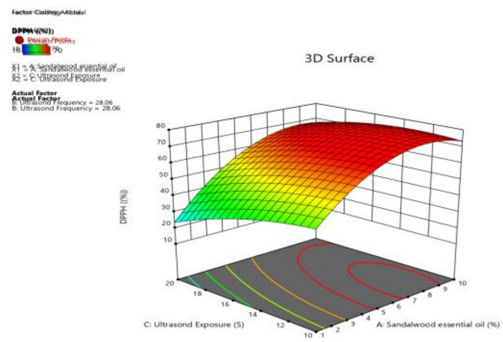
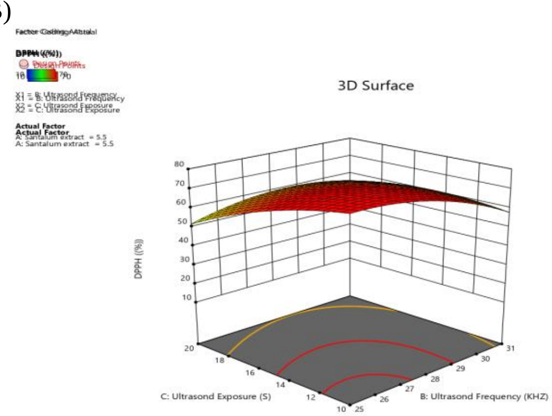


(A)



(C)

Fig 5 Interaction of sandalwood essential oil (%), time (S) and ultrasound frequency (kHz) on Antioxidant activity (%) A) The effect of sandalwood essential oil and ultrasound frequency on Antioxidant activity B) The effect of sandalwood essential oil and ultrasound time on Antioxidant activity C) The effect of ultrasound frequency and time on Antioxidant activity

Considering that the concentration and number of phenolic compounds present in plant essential oils determine the percentage of antioxidant activity, therefore according to Figure (5) naturally, with the increase in the percentage of sandalwood essential oil, the amount of phenolic rings to receive the H+ group in the environment increases, which is a problem It leads to an increase in the antioxidant capacity of the produced nanoemulsions; But in addition to the amount of essential oil used in the formulation, factors such as the percentage of entrapment and release of essential oil are among the determining factors in the amount of antioxidant capacity, which increases with the frequency and time of ultrasound due to the environmental stresses that were discussed in the previous sections, with the reduction of the amount of entrapment and Also, the release of sandalwood essential oil can significantly reduce this capacity. In an article that examined the effects of sandal essential oil microcapsules in detergent, similar results were obtained [27]. According to the results, sandalwood essential oil had the most positive and significant effect, which significantly increased the amount of antioxidants.

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Coefficient of explanation (R^2) the predicted model for the above answer is 0.7546; Therefore, the model has been able to explain 75.46% of the total changes in the scope of the investigated variables. The misfit index was not significant ($p > 0.05$), which indicates the appropriateness of the model in predicting the range of tested variables.

6-3- Optimization

In the process of investigating the physicochemical properties of the nanoemulsion of white sandal plant, the optimal operating conditions were carried out using the numerical optimization technique. For this purpose, optimization goals, response levels and independent variables were set at first. The point predicted by the software was sandalwood essential oil equal to 5.5%, ultrasonic frequency equal to 28 kHz and ultrasonic duration equal to 15 seconds, after repeating the experiments for the obtained point, the following answers were obtained, the particle size was 230 nm, the viscosity was equal to 3.10 milliliters. Pascal seconds, the brightness index was equal to 78, the whiteness index was equal to 58 and the antioxidant activity was equal to 70%. The obtained results show that the amount predicted by the model is consistent with the value obtained experimentally. These conditions indicate that the model can properly show the effect of the variables of ultrasound time, ultrasound frequency and the amount of sandalwood essential oil on the research responses (dependent variables).

7-3- Results of the stability of nanoemulsions

Table 4 shows the results of evaluating the stability of optimal nanoemulsion during sixty days of storage, that the amount of polydispersity index has increased from 0.12 to 0.45, which indicates a decrease in stability on the 60th day; But 80% of the stability of the nanoemulsion has been preserved on the 60th day compared to the first day.

Table 4 Results of stability evaluation of nanoemulsions

60th day	30th day	1st day	Storage time
0.45	0.32	0.12	polydispersity index (PDI)
575	314	235	particle size (nm)
80	93	100	Nanoemulsion stability (%)

Nanoemulsions, like conventional emulsions, are semi-stable systems and break over time due to various physicochemical processes, including gravitational separation, agglomeration, integration, and alcohol drying treatment. Compared to conventional emulsions, nanoemulsions are more stable to the phenomena of gravity separation and droplet accumulation. But due to the small size of their particles, they are highly susceptible to Stowald treatment. In this study, the stability of nanoemulsions decreased with increasing storage time.

4 - Conclusion

In this research, white sandalwood essential oil was prepared in the form of nanoemulsion and its properties were evaluated and optimized. After preparing and identifying the effective compounds of sandalwood essential oil, the percentage of antioxidant activity, viscosity, colorimetric indices and particle size of the nanoemulsion were evaluated and after optimization, the stability test was performed. It was done on the optimized sample. In relation to determining the size of the particles, with the increase in the amount of essential oil, its value increased, and with the increase in the frequency and time of ultrasound, the size of the particles decreased due to the cavitation phenomenon. In relation to viscosity, with increasing the amount of sandalwood essential oil, the viscosity increased, and with increasing the frequency and time, the viscosity was somewhat constant, and from the 20th second onwards, the viscosity increased. Regarding the colorimetric indices, the brightness and whiteness indices decreased with increasing the amount of sandalwood essential oil from 1 to 10%. Also, with the increase of ultrasound time from 10 to 20 seconds, the brightness and whiteness index increased significantly, and with the frequency increase from 25 to 31 kHz, the whiteness and brightness index decreased slightly. In relation to the antioxidant activity, with increasing the amount of sandalwood essential oil, the antioxidant activity increased significantly, but the ultrasonic frequency did not have a great effect on the antioxidant activity, and in relation to the stability of the nanoemulsion, it had 80% stability at the end of 60 days. The use of these nanoemulsions with antimicrobial properties in the cosmetic, pharmaceutical and food industry and as packaging coatings in the meat and fishery industries and food preservation, in the perfume industry to increase the stability of the perfume and in microbiology and the production of preservatives can be effective and practical.

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مقاله علمی_پژوهشی

بررسی خصوصیات فیزیکوشیمیایی نانوامولسیون اسانس گیاه صندل سفید (*Santalum album linn*)

عرفان امیری[۱]، ابراهیم حسینی[۲]*، اکرم شریفی[۳]*

۱- دانش آموخته ارشد، گروه علوم و صنایع غذایی، واحد علوم و تحقیقات، دانشگاه آزاد اسلامی، تهران، ایران.

۲- دانشیار، گروه علوم و صنایع غذایی، واحد علوم و تحقیقات، دانشگاه آزاد اسلامی، تهران، ایران.

۳- دانشیار، گروه مهندسی علوم و صنایع غذایی، واحد قزوین، دانشگاه آزاد اسلامی، قزوین، ایران.

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* مسئول مکاتبات:

ebhoseini@yahoo.com
asharifi81@gmail.com

چکیده

استفاده از نانوتکنولوژی در صنایع غذایی امکاناتی مانند درون‌پوشانی و انتقال هدفمند ترکیبات و اسانس‌های گیاهی بدون تأثیرات حسی نامطلوب را فراهم می‌سازد. در این تحقیق اسانس گیاه صندل سفید (*Santalum album* linn.) برای تهیه نانو امولسیون استفاده شد.خصوصیات نانوامولسیون ارزیابی و بهینه‌یابی شد. برای انجام تحقیق از نرم‌افزار آماری Design-Expert 12 و روش آماری سطح پاسخ و طرح مرکبمرکزی با متغیرهای آماری، فرکانسفراصوت (۳۱-۲۵ کیلوهرتز)، مدت‌زمان فراصوت (۲۰-۱۰ ثانیه) و میزان اسانس صندل (۱-۱۰ درصد) استفاده شد. پس از استخراج و شناسایی ترکیبات مؤثره اسانس آزمون‌های درصد فعالیت آنتی‌اکسیدانی، ویسکوزیته، شاخص‌های رنگی واندازه ذرات نانو امولسیون انجام شدوپس از بهینه‌یابی آزمون پایداری بر روی نمونه بهینه انجام گرفت. نتایج بهینه‌یابی نشان داد با شرایط اسانس صندل به میزان ۵/۵ درصد، فرکانس فراصوت به میزان ۲۸ کیلوهرتز، مدت‌زمان فراصوت به میزان ۱۵ ثانیه، نانوامولسیونی با خصوصیات بهینه می‌توان تولید نمود. پس از تکرار آزمایش‌ها برای نقطه به‌دست‌آمدهپاسخ‌های ذیل به دست آمد، میزان اندازه ذرات ۲۳۰ نانومتر، ویسکوزیته معادل ۳/۱۰ میلی‌پاسکال ثانیه، شاخص روشنایی معادل ۷۸، شاخص سفیدی ۵۸ و فعالیت آنتی‌اکسیدانی معادل ۷۰ درصد بود. انتظار می رود این نانوامولسیون‌ها پتانسیل کاربرد در در صنایع آرایشی، دارویی و غذایی را دارا باشند.