



Investigating the Effect of Garlic Essential Oil Nanoemulsion Encapsulated with Arabic Gum on Antioxidant Activity, Shelf Life, and Sensory Properties of Flavored Olive Oil

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ABSTRACT

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The present study aims to shed light on the effects of encapsulation by the nanoemulsion (NE) method of garlic essential oil (GEO) on the oxidative stability of olive oil. To this end, the effect of different GEO percentages on NE droplet size, encapsulation efficiency, antioxidant properties, stability, and turbidity at concentrations of 200, 400, and 600 ppm was evaluated. The results showed that the droplet size of NEs ranged from 50.75 to 220.20 nm. The NEs were added to olive oil, and the oil's peroxide, thiobarbituric acid, iodine, acid values, antioxidant properties, and sensory properties were measured at 1, 30, 60, and 90 days of storage. According to the findings, from day 30th to 90th, the lowest amount of peroxide, thiobarbituric acid, and acid values, and also the highest antioxidant activity (lowest IC50), were detected in the sample with the highest concentration of NE (600 ppm of garlic essential oil nanoemulsion [GEON] encapsulated with Arabic gum).

1- Introduction

Cold pressing, without any refinement, is used to extract virgin olive oil; hence most of its bioactive compounds are conserved [1]. Olive oil is mainly fraught with triacylglycerols (99%). Its secondary components include free fatty acids, mono- and diacylglycerols, hydrocarbons (squalene), sterols, aliphatic alcohols, tocopherols, and pigments [2]. Virgin olive oil is very stable against oxidation thanks to its high content of monounsaturated fatty acids (especially oleic acid) and antioxidant compounds such as polyphenols and tocopherols (α -tocopherol); nonetheless, free fatty acids and photosensitizers, which are removed while refining, may result in oxidation [3]. Oxidation of lipids is closely tied to the attack of molecular oxygen on adjacent sites of double bonds. The formation of free radicals, in turn, generates hydroperoxides and, subsequently, small volatile compounds, including aldehydes, ketones, carboxylic acids, alkanes, and small alkenes [1]. Antioxidants like phenolic compounds found in enormous plants can prolong food's shelf life by diminishing food oxidation [4]. Garlic (*Allium sativum*) extracts and essential oils have many biological properties, i.e., antimicrobial, antioxidant, anti-cancer, immune system boosting, and prebiotic activities [5]. However, essential oils are unstable and susceptible to degradation when exposed to environmental factors such as oxygen, temperature, and light. For this reason, various colloidal systems have been developed to preserve them [6].

In the food and pharmaceutical industries, microencapsulation is used for numerous purposes. These include the protection of sensitive substances from light, heat, and moisture, the targeted release of bioactive compounds, and obviating of undesirable flavors and flavorings [7]. It is also utilized in texture modification, for example, in converting liquids into powders. Various techniques are used to produce microcapsules. The most common

methods are spray drying, freeze drying, fluidized bed coating, liposomal entrapment, and complex coacervation. The inner phase/core materials serve as active agents enclosed within the encapsulation [8].

The materials embedded around the active agents are coatings, wall/carrier materials, outer/external phases, and matrix materials, most of which are natural ingredients whose edible kinds can be utilized in food products or processes [9].

NE-based natural antioxidants encapsulation is a practical approach for enhancing antioxidant activity, dispersibility, stability, and solubility [10]. Some features, such as the controlled release of natural antioxidants and protected antioxidants in edible oils, improve their antioxidant activity [11]. NE is superior to conventional microencapsulation technologies in many ways [12].

For instance, its formation depends on a minimal quantity of surfactant; it is characterized by a higher surface area and small particle size, limiting the release of antioxidants and absorbing most of them. At the same time, compared with the microemulsion, it rarely displays instabilities such as coagulation, creaming, flocculation, and sedimentation regarding texture, appearance, and stability. In short, as a material delivery approach, NEs are small, highly available, bioavailable, and stable [10]. Many researchers have demonstrated the antioxidant activity of encapsulated natural phenolic compounds in edible oils [13-15]. Nano-encapsulated extracts indicate the slightest oxidation under accelerated conditions as antioxidants are released gradually during storage and better protected [16, 17]. According to Dehghan *et al.* (2020), encapsulated orange peel essential oil had superior antioxidant activity than free essential oil and TBHQ, resulting in better control of soybean oil oxidation [18]. In addition, Aboutalebzadeh *et al.* (2022)

proved that sweet basil essential oil nanocapsules are superior to the free essential oil in controlling Kilka fish oil's oxidative stability [19].

The present research evaluated the effect of GEON coated with Arabic gum on flavored olive oil's antioxidant activity, shelf life, and sensory properties.

2- Materials and methods

Fresh Garlic cloves (*Allium sativum L.*) were purchased from a local market in Tehran. Virgin olive oil was obtained by the Zeidar Oil Company (Rudbar, Iran). Arabic gum (spray-dried and up to 99% purity) and some chemicals were purchased from Merck Company (Darmstadt, Germany). The remaining chemicals necessary for the study were obtained from Sigma–Aldrich (St. Louis, MO, USA).

2-1- GEO Extraction:

Water vapor distillation was used to extract GEO by the Clevenger apparatus, consisting of a distillation flask connected to the refrigerant by connection pipes and ends in a graduated tube. First, fresh garlic was peeled, washed, milled in distilled water, poured into a balloon of the Clevenger apparatus, and heated for 3-4 hours. Subsequently, the resultant vapors were liquefied after passing through the apparatus's cooling pipes and accumulated in the receiver. Next, the collected essential oil was dehydrated with dry sodium sulfate and passed through a 0.45 μ m microfilter. After that, a centrifuge was used for sodium sulfate precipitation, and pure essential oil was obtained. Ultimately, the essential oil was placed into sterile, dark glass containers to protect it from light and kept in a refrigerator at 4°C [20].

2-2- Preparation of GEON encapsulated with Arabic gum:

In the first step, a magnetic stirrer combined GEO with Tween 80 as a

surfactant (Sigma Aldrich, Germany) in equal ratios. The aqueous phase (distilled water) was then mildly acidified by citric acid (0.3%). The oil phase and surfactant combination were gently mixed with the aqueous phase using a magnetic stirrer at 700 rpm. The emulsion pre-mix was then treated with ultrasonic waves for 20 minutes in an ultrasound water bath (100 watts power and frequency 40 kHz) for grinding the particles and producing NE [21]. To microencapsulate NEs, Arabic Gum was mixed with distilled water to form a solution with a solids content of 0.5% (w/w). The solution was stirred for 15 minutes at 20°C and then refrigerated for 24 hours. Next, Tween 80 at 2% (w/w) and GEON with concentrations of 200, 400, and 600 ppm were added to the mixture in a 1:5 ratio. Before homogenizing at 12000 rpm for 5 minutes, the mixture was stirred with a magnetic stirrer for 1.5 hours. Ultrasonic probes with six 30-second cycles were applied to minimize particle size. The NEs were placed at -20°C for 24 hours and dried by freeze-drying (0.017 mPa) at -57°C for 48 hours [22].

2-3- Evaluation of GEON

2-3-1- Particle size determination:

The mean volumetric diameter of the droplets was determined using dynamic light scattering (Malvern Instruments, Worcestershire, UK). All of the NEs diluted with distilled water with a ratio of 1:50 in order to avoid multiple particle dispersions. The experiments were carried out at a temperature of 25°C, and the refraction angle employed was 90° [23].

2-3-2- Turbidity and Stability Analysis:

The turbidity of NEs was evaluated using a turbidimeter model (HACH 2100 AN Turbidimeter) [24]. In addition, the stability and turbidity of NEs inside the tube were compared. Once it was produced and three months afterward, samples were

photographed and examined by inverting the test tubes to deposit particles [25].

2-3-3- Encapsulation Efficiency Analysis:

Using a syringe (about 5 cc), volatile substances from GEO were removed from the upper space of NE samples (filled into clean jars with closed tube lids) and injected into gas chromatography. The injection chamber temperature, the initial oven temperature, and the temperature gradient were 120°C, 40°C, and 5°C/min until reaching 90°C respectively. The apparatus was kept at this temperature for two minutes, and the experiment lasted 12 minutes. The column used was 50 meters long with an inner diameter of 0.32 mm and a layer thickness of 0.25 microns. The flow rate of nitrogen-carrying gas was 0.7mL/min, and the flame ionization detector with a temperature of 300°C was considered. Next, the total area under the peaks of volatile substances triggered by essential oils was measured to determine the percentage of odor concealment by the continuous phase in water-in-oil NEs. By comparing the total surface areas of sub-peaks obtained from GEON and GEO without encapsulation in the same concentrations of essential oil and under the same circumstances, the efficiency of encapsulating and coating was determined according to Equation 1 [23].

Encapsulation efficiency =

$$1 - \frac{\text{total area under the peak of NEs headspace}}{\text{total area under the peak of non-emulsion}} \times 100 \quad (1)$$

2-3-4- Investigation of antioxidant properties of GEON:

As per this method, 0.5 mL of the prepared NE combined with 3mL of 60µM free radical methanol solution of DPPH was stored for 30 minutes in the dark at room temperature. Finally, after centrifugation at 6000 for 5 minutes, the adsorption of the solution is measured at 517 nm using a spectrophotometer. Also, a

sample containing 0.5mL methanol with 3mL DPPH was considered as a control sample. The radical scavenging activity was determined according to Equation 2 [23].

$$\%inhibitor\ DPPH = \frac{ABS\ control - ABS\ sample}{ABS\ control} \times 100 \quad (2)$$

Abscontrol = DPPH solution light absorption without sample

ABSsample = Absorption of light with sample.

2-3-5- Evaluation of total phenol content (TPC) of GEON:

The TPC in GEON was assessed according to the Folin-Ciocalteu method. To this end, 0.1 ml of the NE solution (at a concentration of 1 mg/ml) was added to 0.5 mL of Folin-Ciocalteu (diluted with distilled water in a 1:10 ratio). Then, 0.4 ml sodium carbonate solution (at a concentration of 75 g/kg) was mixed. The test tube was incubated in a hot water bath at 37°C for 30 minutes. The spectrophotometer was used to measure sample absorption at 765nm. Results are expressed in terms of gallic acid equivalent (mg/g) as an indicator of phenolic compounds [26].

2-3-6- Addition of Essential Oil and NE to the Olive Samples

According to Table 1, GEO and GEON in different concentrations were added to the olive oil samples. Evaluations were conducted at 1, 30, 60, and 90 days at 25°C.

2-4- Evaluated Tests on the flavored Olive Oils Containing GEO and GEON

2-4-1- Measurement of Iodine value (IV):

Firstly, 0.2 grams of oil, 10 mL chloroform, and 10 mL Hanus solution were poured into the Erlenmeyer flask, stirred slowly, and placed in the dark for 30 minutes. Subsequently, 15 mL of unsaturated potassium iodide (15%) was added to Erlene and stirred. Afterward, 100 mL of distilled water was added. Then, the contents of the Erlenmeyer were titrated with 0.1 N sodium thiosulfate solution until it turned yellow. Subsequently, a few drops of starch adhesive reagent was added until the formation of a blue-to-brown color, and it was continued until the color disappeared. Finally, the IV was calculated according to Equation 3 [25].

$$\text{Iodin Index} = \frac{(B-S) \times N \times 12.66}{w} \quad (3)$$

B: Volume of control thiosulfate consumption

S: Volume of original sample thiosulfate

N: Normal consumption of thiosulfate

W: Oil sample weight (g)

2-4-2- Evaluation of Antioxidant Activity by Inhibition of Free Radicals DPPH:

Brand-Williams *et al.* (1995) method with some modifications was used to determine the DPPH radical inhibition activity of the oil samples. First, 0.5 mL of the oil sample was mixed with 2.5 mL of 0.5mM methanol DPPH solution; then, the mixture was homogenized entirely by a shaker and incubated for 30 minutes in the dark at room temperature. Light absorption at 517nm vs. control was measured using a UV-Vis spectrophotometer, and the results were expressed in terms of the percentage of DPPH radical inhibition according to Equation 2 [27].

2-4-3- Measurement of Acid Value (AV):

The oil acid value was determined by titration according to ISO standard 660 [28]. 20 mL of ethanol and 20 mL of diethyl ether in an Erlenmeyer flask were added, and five drops of phenolphthalein reagent were added and mixed. Then, 5 g oil sample was weighed in another 250mL Erlenmeyer flask, and the aforementioned solvent was added. The contents of Erlenmeyer were titrated with 0.1 N potassium hydroxide until a light pink color that lasted for 15 seconds. Control titration was conducted in the same manner through solvent titration (without an oil sample) until the appearance of light pink. The AV was calculated in terms of mg potassium hydroxide per gram of sample according to Equation 4.

$$AV = \frac{(A-B) \times N \times 56.1}{w} \quad (4)$$

A = Alkali volume used in sample titration (ml)

B= Alkali volume used in control titration (ml)

N = Normality of alkali

W = weight of consumed oil sample (g)

2-4-4- Measurement of Peroxide Value (PV):

The peroxide value was determined following the method outlined by Dehghan *et al.* (2020). In accordance with this procedure, a specified amount of oil sample (3 g) was combined with glacial acetic acid (30 mL) and chloroform (20 mL). Subsequently, 1mL of saturated potassium iodide solution was dissolved. The mixture was stored in a dark place for 1 min. Once 50mL of distilled water was added, titration was performed with (0.01 N) sodium thiosulfate in the presence of starch adhesive as a reagent. Finally, a control titration was performed, and the PV (meq of oxygen/ kg) was determined using Equation 5 [18].

$$\text{peroxide value} = \frac{(B-S) \times N \times 1000}{w} \quad (5)$$

S = Volume of sodium thiosulfate solution used in sample test (ml)

B = Volume of sodium thiosulfate solution used in the control experiment (ml)

N = Normality of sodium thiosulfate (ml)

W = weight of oil sample (g)

2-4-5- Measurement of Thiobarbituric acid Value (TBA):

The Thiobarbituric acid test of olive oil during storage was evaluated according to the method cited by Dehghan *et al.* (2020). Initially, a 200 mg oil sample was dissolved in 1-butanol (25 mL), after mixing 5 mL of this solution with 10 mL of TBA reagent (0.2%), heated in Ben Marie for 2 h at 95°C then cooled under water for about 10 minutes until room temperature was acquired. Finally, at 532 nm, absorbance was read versus a blank (all reagents without oil). The TBA value (mg of malondialdehyde per kg of oil) was determined using Equation 6 [18].

$$TBA = \frac{(A-B) \times 50}{w} \quad (6)$$

A: The test solution's absorbance

B: Reagent blank absorbance

W: weight of oil sample (mg)

2-5- Sensory Analysis

Seven trained panels assessed the olive oil samples in terms of taste, color, odor, and general acceptance characteristics in a 5-point hedonic test. In this test, the number 1 represents the most acceptability, and the number 5 represents the lowest acceptance.

2-6- Statistical Analysis

The experiment results were expressed as the mean \pm standard deviation by the measurements with three replications. Experimental data were compared with one-way ANOVA. In addition, statistically significant differences between the values of the means (in cases where the overall effect of the treatments was significant) were determined using Duncan's multiple range post hoc test. The results of statistical tests were performed using SPSS software version 26. A significance level of $P \leq 0.05$ was considered for all data comparisons.

3- Results and Discussion

3-1- GEON Results Analysis

3-1-1- Particle size of Nanocapsules and Encapsulation efficiency:

Particle size is a determinant of the stability and encapsulation efficiency of emulsions. In the present research, the particles' size and distribution indicate that the NEs have small and uniform particle sizes. The droplet size of NEs ranged from 50.75 to 220.20 nm. The smallest particles correspond to the 200 ppm of GEON with a diameter of 50.57 nm. In comparison, the largest particles are associated with 600 ppm of GEON, having a diameter of 220.20 nm. (Table 2, Supplementary Information). DLS analysis can be used as a physical technique to determine how particles are distributed throughout solutions and suspensions. According to this method, scattering and changes in light intensity are determined by their Brownian motion after the laser light interaction with particles, and the particle dimension distribution is assessed eventually. Hosseinialhashemi *et al.* (2021) found that NEs droplet sizes ranged from 310.34 to 354.19 nm [22].

In the present study, all NEs showed satisfactory microencapsulating efficiency. NEs coated the volatile compounds in the GEO efficiently. The encapsulation efficiency for 200, 400, and 600 ppm of GEON was 73.49 ± 2.08 , 79.75 ± 1.40 , and 85.82 ± 1.54 , respectively. Hassanzadeh *et al.* (2018) demonstrated encapsulation efficiency ranging from 91 to 77% for NEs containing 5–25% GEO [23].

3-1-2- Analyses of TPC and antioxidant activity:

The samples with a higher TPC demonstrated the most robust free radical scavenging effect (lower IC₅₀ values). Hence, the higher TPC in GEO results in greater antioxidant activity (lower IC₅₀) than in GEON. However, concentration growth enhanced the antioxidant activity of GEON (their IC₅₀ decreased) ($P \leq 0.05$). The maximum value of IC₅₀ was 241.21 ± 0.01 (mg/ml) found in 200ppm of GEON. Data are presented in Table 3. It is in line with Delazar *et al.* (2012), who showed that encapsulation did not increase antioxidant activity [29].

Phenolic compounds, as hydrogen donors, scavenge free radicals [30]. DPPH test is widely utilized to specify the activity of free radical scavenging of enormous plants and compounds. The IC₅₀ value is defined as the required sample concentration (here, GEON), resulting in 50% inhibition [31].

3-1-3- Turbidity of GEON:

According to the results, GEON turbidity increased significantly by raising the proportion of dispersed phases (from 200 to 600 ppm) and over time. The findings are presented in Table 4. The larger particle size changes light scattering in NEs, which in turn affects turbidity. Thus, these two parameters have a linear relationship ($P \leq 0.05$). Testing the stability of NEs at all concentrations and storage dates indicated no deposits on the walls of tubes containing samples.

Ziani *et al.* (2012) evaluated the effect of temperature on NE stability by assessing the turbidity of the NEs. Results showed that NEs with higher oil phase concentrations had higher turbidity [24].

3-2- The analysis results of changes of flavored olive oil samples containing GEO and GEON encapsulated with Arabic Gum over time

3-2-1- Peroxide value (PV) and Thiobarbituric acid value (TBA):

The PV denotes the primary products (hydroperoxides) of lipid oxidation (mainly unsaturated fatty acids) and is an index to measure oxidation progression [32]. The samples' PV did not differ statistically on day 1 ($P > 0.05$). The control sample and sample 5 accounted for the highest PV content on the other days. At the same time, sample 7 accounted for the lowest PV level ($P \leq 0.05$). PV elevated significantly over time ($P \leq 0.05$). PV was lower in GEON samples in comparison with control and GEO samples. The PV increased slightly from the day 60th to the 90th due to the slower release of GEON than essential oil at the same concentration. Upon increasing concentrations, PV decreased significantly ($P \leq 0.05$). Phenolic compounds and their high antioxidant activity are responsible for the lower PV of samples containing more GEO and GEON. The results are illustrated in Fig 1. Similarly, Nishad *et al.* (2021) reported that nanocapsules of grapefruit peel polyphenol (droplets dispersed in W/O emulsions) controlled peroxide values more efficiently than free peel extract applied in mustard oil [33]. During storage time, TBA was increased ($P \leq 0.05$), which can be attributed to the formation of secondary oxidation metabolites of fats (such as malondialdehyde, alcohol, acetone, and acids) obtained from the decomposition of Mono hydroperoxides; that reaction causes the faster lipid oxidization. Furthermore, it

directly affects the taste of food products. [32]. On the 30th day, the control sample and sample 5 did not statistically differ ($P > 0.05$) because of the slow release of phenolic compounds from the GEON. However, GEON samples had a lower TBA value on the 60th and 90th days than GEO samples at the same concentration ($P \leq 0.05$). The low level of TBA in samples containing high antioxidant content is probably due to the effect of antioxidants in reducing peroxide, as these compounds neutralize free radicals by giving hydrogen, reducing the progress of oxidation. The results are presented in Fig 2. Accordingly, Mohammadi *et al.* (2016) found that oil using nano-encapsulated extract had a lower TBA value than oil with the free extract [34].

3-2-2- Acid value (AV) and Iodine value (IV):

AV represents the amount of KOH (in mg) required to neutralize free fatty acids in 1 g oil. No statistically significant difference was found between the AV of all samples on day 1 ($P > 0.05$). However, as triglyceride groups were hydrolyzed and carbonyl groups were produced during oxidation, the AV increased in all samples [35]. This increasing trend was more observed in the control sample; nonetheless, the samples containing GEO and GEON rose slightly. On the 30th and 60th days of storage, the highest AV appeared in samples 1, 2, and 5, respectively ($P \leq 0.05$). On the 90th day of storage, the highest amount of AV belonged to the Control sample, followed by sample 2 ($P \leq 0.05$), and the lowest AV was observed in sample 7 ($P \leq 0.05$). Fig 3 illustrates a schematic of the results. These findings align with the results of Hosseinialhashemi *et al.* (2021), who found that sunflower oil's AV was enhanced during storage time. However, oil containing NE of *P. khinjuk* extract exhibited lower AV than oil containing TBHQ [22].

The IV of fat or oil is a determinant factor for its degree of unsaturation. Therefore, a decrease in the IV is attributed to the increase in lipid oxidation. The comparison of the average iodine value of the samples over 1, 30, 60, and 90 days showed no significant statistical difference in IV over time ($p > 0.05$), with an average value of 70.255. As Razavi Majd *et al.* (2021) pointed out, given the stabilizing effect of rosemary essential oil on sunflower and soybean oil, the IV of all samples was decreased at the end of the storage time at 70 °C. However, it did not differ significantly compared to the first day of storage [26].

3-2-3- Antioxidant Activity:

The intrinsic antioxidant properties of olive oil are attributed to tocopherols, phenolic compounds, and sterols. However, the effectiveness of natural antioxidants diminishes during storage [36]. Over time, the antioxidant activity of the samples decreased significantly (their IC50 rose) ($P \leq 0.05$). Overall, GEON samples exhibited higher antioxidant activity than the control and GEO samples. During the entire storage time, the lowest antioxidant activity (highest IC50) was found in the control and afterward in sample 2 ($P \leq 0.05$). On the other hand, sample 7 accounted for the highest antioxidant activity (lowest IC50) ($P \leq 0.05$).

Furthermore, from the 60th to the 90th day, the antioxidant activity decreased with a slower trend, which can be attributed to the slower release of GEON than GEO in the same concentration. Also, upon improving the concentration of GEO and its NE, the antioxidant activity of the samples enhanced significantly ($P \leq 0.05$), which is due to the presence of more phenolic compounds. Fig 4 illustrates the results. In agreement with our results, Ghosh *et al.* (2016) reported that the encapsulated SC-CO₂ polyherbal extract of spruce leaves, bo leaves, and cardamom

seeds had the highest antioxidant activity and controlled release after storing soybean oil for 30 days [37]. Mousavi *et al.* (2022) found that ordinary Virgin olive oil incorporated with 300 ppm Chavir ultrasound extract showed the highest antioxidant activity [36].

3-3- Sensory evaluation:

Based on the results, there was no significant differences in the samples' taste, color, odor, and general acceptance scores on the first day ($P \geq 0.05$). Similarly, color scores did not differ significantly on the 30th day ($P \geq 0.05$). However, on the 60th day of storage, samples 1, 2, 3, and 4 accounted for the lowest color scores ($P \leq 0.05$). On the 90th day of storage, sample 1 had the lowest color score, and samples 7 and 6 accounted for the highest color score, respectively ($P \leq 0.05$). From the 30th to the 90th day of storage, the lowest taste score was found in the Control sample, whereas samples 7 and 6 had the highest taste score ($P \leq 0.05$). Over time, the taste and color scores of the samples diminished substantially ($P \leq 0.05$). The results are depicted in Fig. 5a and 5b. On the 30th and 90th day of storage, the lowest odor and general acceptance scores were observed in sample 1 ($P \leq 0.05$). The highest odor scores were found in samples 7 and 6 ($P \leq 0.05$). On the 60th day of storage, the lowest odor and general acceptance score were observed in sample 1 ($P \leq 0.05$), and no significant difference was found in the odor score of other samples ($P > 0.05$). A significant decline in odor and general acceptance scores was observed over time ($P \leq 0.05$). The schematic of the results is illustrated in Fig. 5c and 5d.

As a whole, according to the data, all scores declined over time. GEON is superior to GEO and control samples thanks to the encapsulation method's ability to mask GEO's intense aroma and flavor during storage. Khoshtinat *et al.* (2022) reported that garlic oil in low-fat salad dressing accounted for the weakest sensory score. However, encapsulation of

garlic oil with β -cyclodextrin had no adverse impact on the sensory properties of the products [38].

4- CONCLUSIONS

This study established that Garlic Essential Oil (GEO) is rich in phenolic antioxidant compounds, and the encapsulation technique aids in preserving GEO throughout the storage period. Natural antioxidants are also known to enhance the shelf life of edible oils. Flavored olive oil samples with higher concentrations of GEO and Garlic Essential Oil Nanoemulsion (GEON) exhibited slower increases in peroxide, thiobarbituric, and acid values. The lowest rate of oxidation, highest antioxidant activity, and highest sensory scores were observed in olive oil containing 600 ppm of GEON encapsulated with Arabic gum. Therefore, this particular formulation emerged as the most effective and promising treatment.

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maceration extracts on the antioxidant activity and oxidative stability of ordinary virgin olive oil: identification of volatile organic compounds. Journal of Food Measurement and Characterization, 2022. **16**(5): p. 4236-4250. <https://doi.org/10.1007/s11694-022-01462-7>.

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TABLES

Table 1: Characteristics of the treatments

Samples	Characteristics
Code (1)	Control Sample
Code (2)	Olive oil contains 200 ppm GEO
Code (3)	Olive oil contains 400 ppm GEO
Code (4)	Olive oil contains 600 ppm GEO
Code (5)	Olive oil contains 200 ppm GEON encapsulated with Arabic gum
Code (6)	Olive oil contains 400 ppm GEON encapsulated with Arabic gum
Code (7)	Olive oil contains 600 ppm GEON encapsulated with Arabic gum

Table 2: results of particle size distribution by DLS

	200 ppm		400 ppm		600 ppm	
	Size of droplets(d.nm)	Percentage	Size of droplets(d.nm)	Percentage	Size of droplets(d.nm)	Percentage
peak(1)	64.01	88.10	105.70	61.90	190.10	72.60
peak(2)	78.82	8.80	91.28	28.50	164.20	23.20

peak(3)	58.77	7.20	78.82	6.40	220.20	3.10
peak(4)	91.28	3.50	122.40	1.90	148.1	1.10
peak(5)	50.75	0.40	141.80	0.60	-	-
Peak(6)	-	-	58.77	0.10	-	-

Table 3: Comparison and evaluation of antioxidant properties and TPC of GEO and GEON

	Phenol Content	Antioxidant Activity (IC50%)
	(mg/gr)	(mg/ml)
GEO	13.69±0.01 ^a	19.86±0.01 ^d
GEON (200 ppm)	0.84±0.01 ^d	241.21±0.01 ^a
GEON (400 ppm)	1.66±0.01 ^c	146.83±0.01 ^b
GEON (600 ppm)	2.69±0.01 ^b	81.56±0.01 ^c

The different lowercase letters indicate the significant difference in the column ($P \leq 0.05$)

Table 4: Turbidity changes of GEON over time

Test	Samples	Turbidity(NTU)			
		Day0	Day30	Day60	Day90
	GEON (200ppm)	1.90±0.08 ^{cD}	2.26±0.03 ^{cC}	2.63±0.06 ^{cB}	3.50±0.07 ^{cA}
	GEON (400ppm)	2.10±0.08 ^{bD}	2.50±0.03 ^{bC}	2.70±0.06 ^{bB}	3.70±0.07 ^{bA}
	GEON (600ppm)	2.36±0.08 ^{aD}	2.86±0.03 ^{aC}	2.83±0.06 ^{aB}	3.90±0.07 ^{aA}

Different lowercase letters indicate a significant difference in the column, and different uppercase letters indicate a significant difference in the row. ($P < 0.05$)

Figures

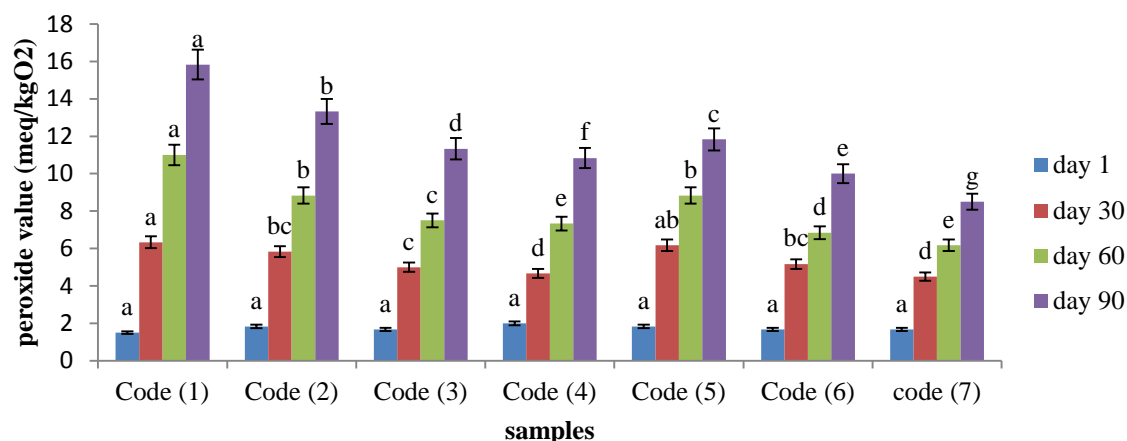


Fig 1: Changes in **peroxide value** of olive oil samples containing **GEO** and **GEON** encapsulated with Arabic Gum over time.

Code (1)- Control Sample: olive oil without **GEO** and **GEON**, **Code (2):** Olive oil contains 200 ppm **GEO**, **Code (3):** olive oil contains 400 ppm **GEO**, **Code (4):** olive oil contains 600 ppm **GEO**, **Code (5):** olive oil contains 200 ppm **GEON** encapsulated with Arabic gum, **Code (6):** olive oil contains 400 ppm **GEON** encapsulated with Arabic gum, **Code (7):** olive oil contains 600 ppm **GEON** encapsulated with Arabic gum.

Different letters indicate significant differences ($p < 0.05$).

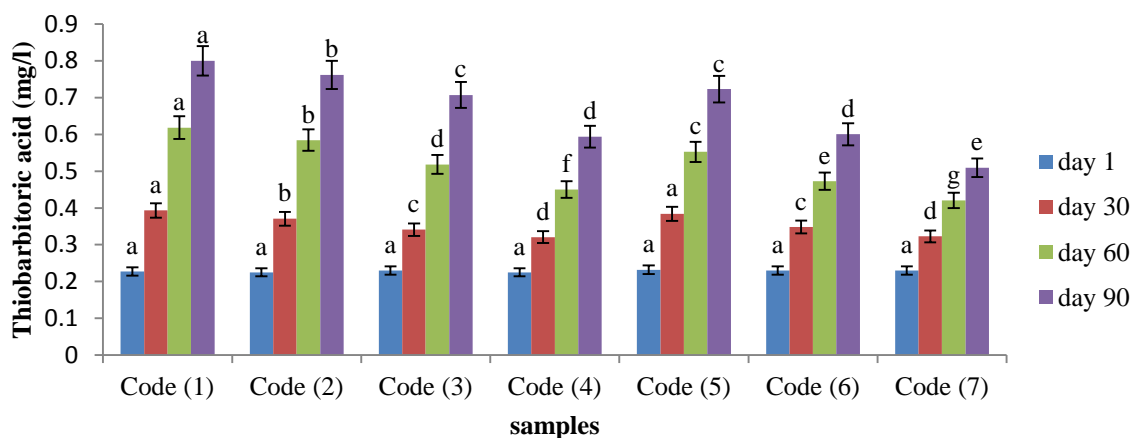


Fig 2: Changes in **Thiobarbituric acid value** of olive oil samples containing **GEO** and **GEON** encapsulated with Arabic Gum over time.

Code (1)- Control Sample: olive oil without **GEO** and **GEON**, **Code (2):** Olive oil contains 200 ppm **GEO**, **Code (3):** olive oil contains 400 ppm **GEO**, **Code (4):** olive oil contains 600 ppm **GEO**, **Code (5):** olive oil contains 200 ppm **GEON** encapsulated with Arabic gum, **Code (6):** olive oil contains 400 ppm **GEON** encapsulated with Arabic gum, **Code (7):** olive oil contains 600 ppm **GEON** encapsulated with Arabic gum.

Different letters indicate significant differences ($p < 0.05$).

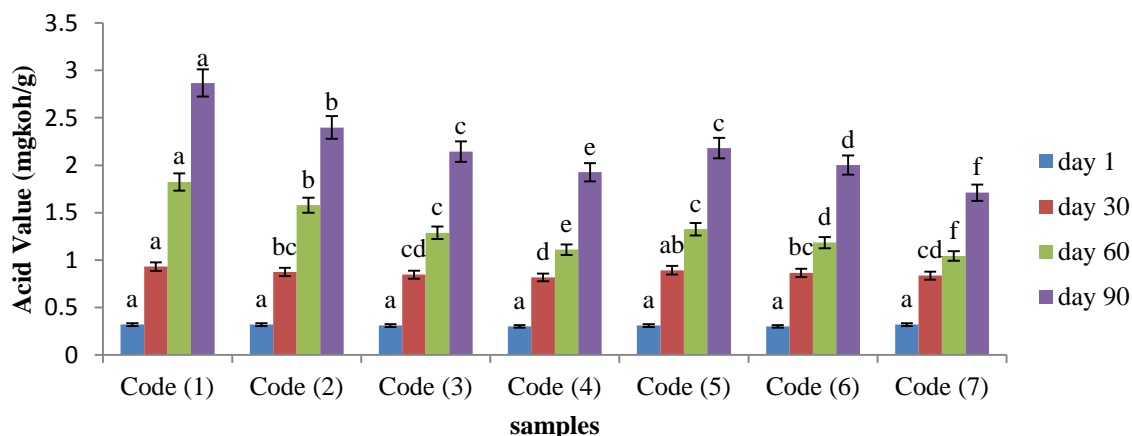


Fig 3: Changes in the acid value of olive oil samples containing **GEO** and **GEON** encapsulated with Arabic Gum over time.

Code (1)- Control Sample: olive oil without **GEO** and **GEON**, **Code (2):** Olive oil contains 200 ppm **GEO**, **Code (3):** olive oil contains 400 ppm **GEO**, **Code (4):** olive oil contains 600 ppm **GEO**, **Code (5):** olive oil contains 200 ppm **GEON** encapsulated with Arabic gum, **Code (6):** olive oil contains 400 ppm **GEON** encapsulated with Arabic gum, **Code (7):** olive oil contains 600 ppm **GEON** encapsulated with Arabic gum.

Different letters indicate significant differences ($p < 0.05$).

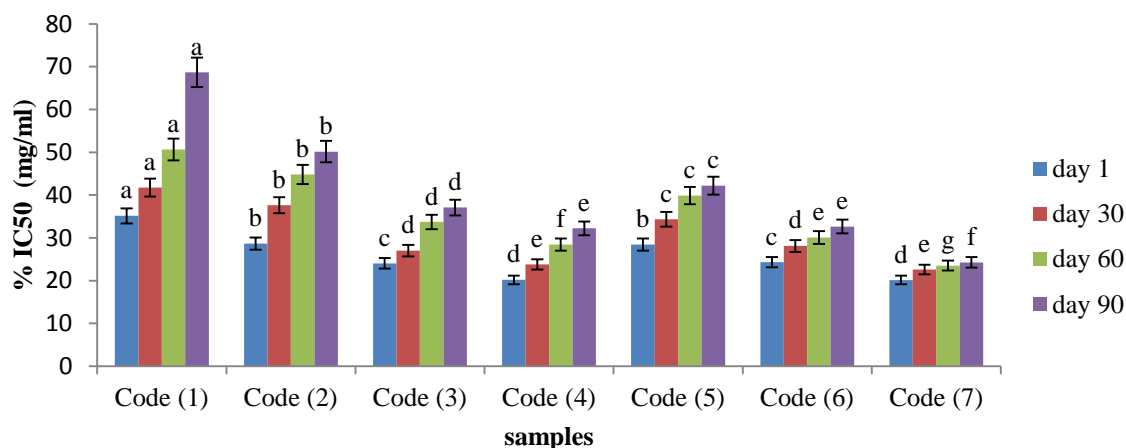


Fig4: Changes in antioxidant activity (IC50) of olive oil samples containing **GEO** and **GEON** encapsulated with Arabic Gum over time.

Code (1)- Control Sample: olive oil without **GEO** and **GEON**, **Code (2):** Olive oil contains 200 ppm **GEO**, **Code (3):** olive oil contains 400 ppm **GEO**, **Code (4):** olive oil contains 600 ppm **GEO**, **Code (5):** olive oil contains 200 ppm **GEON** encapsulated with Arabic gum, **Code (6):** olive oil contains 400

ppm **GEON** encapsulated with Arabic gum, **Code (7)**: olive oil contains 600 ppm **GEON** encapsulated with Arabic gum.

Different letters indicate significant differences ($p < 0.05$).

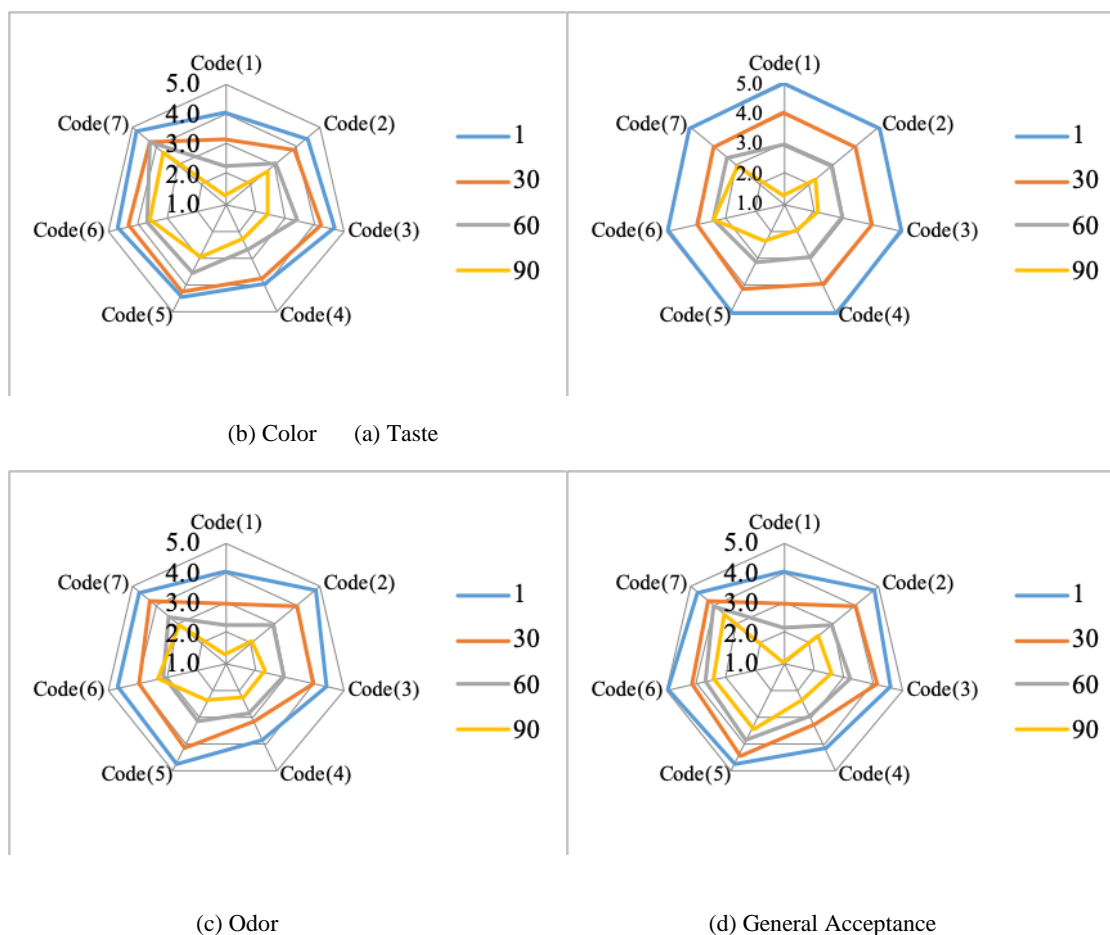


Fig. 5: The **sensory evaluation** results in olive oil samples containing **GEO** and **GEON** encapsulated with Arabic Gum over time.

Code (1)- Control Sample: olive oil without **GEO** and **GEON**, **Code (2):** Olive oil contains 200 ppm **GEO**,

Code (3): olive oil contains 400 ppm **GEO**, **Code (4):** olive oil contains 600 ppm **GEO**, **Code (5):** olive oil contains 200 ppm **GEON** encapsulated with Arabic gum, **Code (6):** olive oil contains 400 ppm **GEON** encapsulated with Arabic gum, **Code (7):** olive oil contains 600 ppm **GEON** encapsulated with Arabic gum.



تاثیر استفاده از نانوامولسیون اسانس سیر ریزپوشانی شده با صمغ عربی بر فعالیت آنتی اکسیدانی، ماندگاری و خواص حسی روغن زیتون طعم دار شده

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این مطالعه با هدف بررسی تاثیر کپسوله کردن اسانس سیر به روش

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از ۵۰،۷۵ تا ۲۲۰،۲۰ نانومتر متغیر بود. نانوامولسیون ها به روغن زیتون

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همچنین بیشترین میزان فعالیت آنتی اکسیدانی (کمترین IC50) در نمونه با

بالاترین غلظت نانوامولسیون اسانس (روغن زیتون محتوی ۶۰۰ ppm

نانوامولسیون اسانس سیر ریزپوشانی شده با صمغ عربی) مشاهده شد.

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