Journal of Food Processing and Preservation

WILEY

RESEARCH ARTICLE OPEN ACCESS

Investigating the Effect of Nanoemulsions Containing Yarrow (*Achillea millefolium*) Essential Oil on the Microbial and Oxidative Stability of Mayonnaise

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Received: 2 September 2025 | **Revised:** 2 January 2026 | **Accepted:** 7 January 2026

Academic Editor: Essam Hebishy

Keywords: encapsulation | essential oil | mayonnaise | nanoemulsion | oxidative stability | yarrow (*Achillea millefolium*)

ABSTRACT

This study investigated the effects of yarrow (*Achillea millefolium*) essential oil (YE) and its nanoemulsion (NEYE), at concentrations of 1% and 2%, on oxidative stability and microbial growth of mayonnaise stored for 45 days at 4°C. NEYEs were characterized with a particle size ranging from 75.5 to 152.65 nm, with a polydispersity index (PDI) of 0.7–0.91, and a zeta potential of −15.64 to −22.5 mV. The formation of NEYE was further confirmed using a scanning electron microscope (SEM). The results showed that both the free and nanoencapsulated essential oils significantly reduced the counts of *Staphylococcus aureus*, *Escherichia coli*, *Salmonella enteritidis*, mold, and yeast, as well as acidity, peroxide, anisidine, and Totox values of mayonnaise compared with the control sample ($p < 0.05$). However, the essential oil–based nanoemulsions demonstrated greater oxidative stability and more effective microbial growth control than the free essential oil ($p < 0.05$). The mayonnaise samples exhibited shear-thinning behavior, and those containing NEYE showed a higher apparent viscosity than both the YE-treated and control samples. The incorporation of NEYE had no adverse effect on the overall acceptability of mayonnaise, while the use of YE at the same concentrations altered color, odor, flavor, and overall acceptability. It has been revealed that essential oils can be successfully incorporated into a nanoemulsion delivery system, offering several advantages, including enhanced antioxidant and antimicrobial activity, as well as improved rheological and sensorial attributes.

1 | Introduction

Mayonnaise is a semisolid oil-in-water (O/W) emulsion composed of egg yolk, vegetable oil, acetic or citric acid, salt, spices, and various flavorings. Its oil content should not be less than 65% (w/w), and the final product must contain at least 2.5% acetic acid, although citric acid from lemon juice may be used as a substitute. Egg yolk functions as the primary emulsifier in mayonnaise, while its natural pigments contribute to the characteristic yellow color [1, 2].

Although mayonnaise is susceptible to microbial contamination due to the nature of its raw materials, such as eggs and the lack of heat treatment, the low pH restricts microbial spoilage primarily to yeasts, certain acid-tolerant bacteria, and molds [3]. Mayonnaise has been associated with outbreaks of salmonellosis

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due to the use of raw egg in its preparation. *Salmonella enteritidis* is an enteropathogenic bacterium, which is typically eliminated from food products through thermal treatment [4]. Mayonnaise is considered a microbiologically safe product when formulated with pasteurized eggs and when the acetic acid content is maintained at ≥1.4%. However, the growth of *Escherichia coli* has been reported in mayonnaise and salad dressings. Survival of *E. coli* in mayonnaise can be eliminated through the implementation of good manufacturing practices (GMPs) and the use of appropriate storage conditions [5]. A low pH (≤4.5) inhibits the growth of certain pathogens, such as *Staphylococcus aureus*, in mayonnaise. Enterotoxin production by *S. aureus* has been detected in homemade mayonnaise at pH ≥ 5.0, whereas no toxin has been found at pH 4.5 [5]. Yeasts like *Zygosaccharomyces baileyi* and *Saccharomyces cerevisiae*, as well as molds including *Aspergillus* and *Penicillium* species, are also capable of causing spoilage in mayonnaise [6].

On the other hand, mayonnaise is also susceptible to lipid oxidation, and the resulting oxidation products are directly associated with the quality of the final product. Lipid oxidation can negatively affect the appearance, flavor, texture, nutritional profile, and shelf life of food products. Oxidation in mayonnaise can occur in any of its three structural regions: the continuous phase, the dispersed oil phase, or the oil–water interfacial layer. The mechanism of lipid oxidation in food emulsions, such as mayonnaise, is complex and, unlike in bulk oils, is not yet fully understood. It has been reported that oxidation proceeds more rapidly in emulsions than in oils [7].

Because thermal treatment is not applied during mayonnaise production, chemical preservatives such as benzoic and sorbic acids are commonly used to inhibit microbial growth [3]. To achieve greater oxidative stability, the addition of antioxidants is also essential. Synthetic antioxidants are widely employed to prevent lipid oxidation. However, due to concerns regarding their potential side effects, natural antioxidants have been proposed as safer alternatives for improving the oxidative stability of food products. Natural antioxidants protect cells from oxidative damage, reduce the risk of certain diseases such as cancer and cardiovascular disorders, and delay rancidity in oils and fats [8]. The effectiveness of natural antioxidants in food emulsions is influenced by the complex physicochemical properties of the oil–water interface [7].

The phenolic compounds present in essential oils are among the most effective natural antimicrobials and antioxidants. Moreover, essential oils, as plant-derived secondary metabolites, are generally recognized as safe (GRAS) and are considered promising alternatives to synthetic additives [3]. The antibacterial activity of essential oils is attributed to their hydrophobic nature, which enables them to penetrate the phospholipid bilayer of bacterial cell membranes. This penetration increases membrane permeability, resulting in the release and leakage of intracellular ions and metabolites, ultimately leading to cell death [9].

One of the most widespread and commonly used medicinal plants worldwide is yarrow (*Achillea millefolium*), a member of the Asteraceae family native to Europe, North America, and Asia. In folk medicine, yarrow has been used to alleviate symptoms associated with gastrointestinal discomfort and oxidative stress. In addition, yarrow essential oil (YE) exhibits strong antibacterial and antioxidant activities under in vitro conditions [10] as well as in various food matrices [2, 3].

Ahmadi-Dastgerdi et al. identified 60 compounds in the GC–MS profile of YE, characterized by a phenolic and terpenoid composition, and confirmed its antioxidant and antimicrobial activity [10]. Both phenolic compounds and essential oils contribute to its bioactive properties, although the magnitude of their contribution varies depending on the specific antioxidant or antibacterial effect [2, 3, 10–12].

The major limitation of essential oils as antimicrobial and antioxidant agents is their low stability and high volatility, as well as uncontrolled release. Encapsulation of essential oils improves their solubility, stability, and bioavailability while also reducing the degradation of their flavor and aroma during processing and storage [13].

Lipid-based carrier systems, including nanoemulsions, represent a promising technology for essential oil delivery, offering high potential for therapeutic applications such as cancer treatment, as well as providing desirable antimicrobial effects [13]. Nanoemulsions with a droplet diameter of less than 100 nm are used in the food and pharmaceutical industries because they enhance the bioavailability of bioactive compounds. They are generally classified into two primary types: O/W and water-in-oil (W/O). The addition of surfactants is often necessary to obtain a kinetically stable emulsion. The reduction in droplet size enhances both the bioavailability and the physical stability of nanoemulsions [14]. They exhibit greater colloidal stability than conventional emulsions, protect oils from oxidation, and help mask off-flavors in food products [15].

Encapsulation of YE protects its active components from the degrading effects of sunlight. It also enhances its antibacterial activity against *S. aureus* and *E. coli* compared to free essential oil [16]. O/W nanoemulsions protect yarrow phenolics from degradation during gastrointestinal digestion and enhance their delivery to the colon, where these compounds can exert their proliferative activity [17].

Several studies have reported that the use of encapsulated essential oils, such as cumin essential oil nanoemulsion [18], garlic extract nanoemulsion [19], and microencapsulated mustard essential oil [20], enhances microbial and oxidative stability and improves the sensory properties of mayonnaise compared with the use of free essential oils.

Given that encapsulation can protect sensitive constituents and expand the potential applications of YE, this study encapsulated YE in O/W nanoemulsions (NEYE) to control microbial growth and delay the oxidation process in mayonnaise.

2 | Materials and Methods

2.1 | Materials

Tween 80, sodium benzoate, potassium sorbate, tert-butylhydroquinone (TBHQ), and all chemicals and microbial culture media were purchased from Merck Co. (Darmstadt, Germany).

Soybean oil, containing palmitic acid (10%–16%), stearic acid (3%–4%), oleic acid (18%–24%), linoleic acid (51%–55%), and linolenic acid (7%–13%), was obtained from Ladan Co. (Iran). Egg yolk, vinegar, salt, sugar, and mustard were purchased from a local market. The stabilizer and starch were obtained from Danisco Co. (Denmark).

2.2 | YE Extraction

Yarrow (*A. millefolium*) was collected from a medicinal plant center in Isfahan, Iran, and after taxonomic verification by the botanical herbarium, the plant material was dried and powdered. The essential oil was extracted by steam distillation using a Clevenger apparatus (Ashk Shishe model, Iran) for 4 h, yielding 0.86% [10].

2.3 | NEYE Preparation

NEYE was prepared using YE as the dispersed phase and deionized water as the continuous phase at ambient temperature ($25^{\circ}C \pm 1^{\circ}C$). First, Tween 80 (5% v/v) was added to deionized water and stirred at 800 rpm for 30 min at room temperature. Subsequently, YE (1% and 2% v/v) was incorporated into the mixture. The resulting coarse emulsion was homogenized at 12,000 rpm for 5 min using a laboratory stirrer (Behsan model C3, Iran). The emulsion was then subjected to ultrasonic processing using a probe ultrasonic device (Hielscher UP400S, Germany) for 10 min at 30% power output (230 W) and a frequency of 25 kHz [18].

2.4 | NEYE Properties

Dynamic light scattering (DLS) was used to determine the particle diameter and particle size distribution (polydispersity index [PDI]) using a particle size analyzer (Malvern Instruments, Malvern Instruments Ltd., Worcestershire, United Kingdom). The ζ-potential of the nanoemulsions was measured using a Zetasizer (Malvern Instruments, Malvern Instruments Ltd., Worcestershire, United Kingdom) [19].

2.5 | NEYE Morphology

The morphology of NEYE was examined using a scanning electron microscope (SEM) (QUANTA 200-EDAX, United States) operated at 25 kV. Prior to imaging, the NEYE samples were lyophilized and sputter-coated with a thin layer of gold to enhance conductivity [18].

2.6 | Mayonnaise Production

Mayonnaise samples were prepared according to a commercial low-fat mayonnaise formulation. The control mayonnaise contained 40% (w/w) soybean oil, 9% egg yolk, 9% vinegar, 1% salt, 5% sugar, 0.3% mustard, 0.15% stabilizer, 2% starch, and 42.55% water. The treated mayonnaise samples were produced by incorporating 1% and 2% YE or NEYE. Two sets of mayonnaise samples were separately prepared: one containing 750 ppm benzoate–sorbate (BS) and the other containing 200 ppm TBHQ. A control sample without additives was also prepared.

A standard blender was used for preparation. First, the starch and stabilizers were dissolved in a portion of the oil. YE or NEYE was then added to the oil phase. Next, the starch mixture and dry ingredients were blended, followed by the addition of the soluble stabilizers and approximately one-third of the water. A small amount of oil was added, and the mixture was blended for 5 min. Subsequently, one-third of the vinegar was incorporated. The remaining oil, water, and vinegar were then gradually added and mixed thoroughly using the blender. After preparation, the mayonnaise samples were filled into glass bottles and stored at 4°C for 45 days for further analyses [19].

2.7 | Microbial Assay of Mayonnaise

The enumeration of *S. aureus*, *S. enteritidis*, and *E. coli* was performed using the surface culture method on Baird–Parker agar, EC agar, and *Salmonella–Shigella* agar, respectively, followed by incubation at 37°C for 24 h. Mold and yeast counts were determined by surface plating on dichloran rose bengal chloramphenicol (DRBC) agar and incubating at 25°C for 72 h [3].

2.8 | Chemical Assay of Mayonnaise

The pH of the mayonnaise samples was measured at room temperature using a pH meter (Metrohm, Switzerland). For pH analysis, 5 g of the sample was mixed with 45 mL of deionized distilled water and homogenized for 1 min at 100 rpm.

To determine acidity, 5 g of the extracted mayonnaise oil was mixed with two to three drops of phenolphthalein and 96% ethanol. The mixture was then titrated with 0.1 N sodium hydroxide until the endpoint was reached [19].

2.9 | Oxidation Assay of Mayonnaise

For oil extraction, 1 g of mayonnaise was mixed with 3.75 mL of chloroform:methanol (1:2 v/v) and vortexed (IKA, Germany) to ensure thorough mixing. After the addition of 1.25 mL of distilled water, the mixture was centrifuged at 1000 rpm for 5 min to separate the oil layer from the aqueous phase and other components. The peroxide value (PV) of the extracted oil was determined by titration, while the anisidine value (AV) was measured spectrophotometrically (Hitachi, Japan). The Totox value was calculated using the formula: $\{2 \times PV + AV\}$, according to AOCS methods [2].

2.10 | Steady-State Shear Rheology of Mayonnaise

The apparent viscosity was measured at room temperature (25°C) using a controlled stress rheometer (Brookfield, United States) equipped with a temperature control unit. A cone–plate

geometry was used (cone diameter 35 mm, cone angle 4°, and gap size 0.143 mm). Measurements were performed over a shear rate range of 0.01–1000 s^{-1} with an applied torque of 10%–90%. Shear stress values were recorded and plotted as a function of shear rate over the range of 0–1000 s^{-1} [19].

2.11 | Sensory Assay of Mayonnaise

Sensory evaluation was conducted on Day 0, immediately after product preparation. Mayonnaise samples were coded to ensure objectivity and minimize bias. A panel of 20 trained evaluators (male and female) assessed the samples for flavor, odor, color, texture, and overall acceptability using a 5-point hedonic scale, in which panelists rated their degree of liking or disliking for each attribute, ranging from "*dislike extremely*" to "*like extremely*." All participants provided informed consent prior to evaluation [2].

2.12 | Statistical Analysis

All experiments were performed in triplicate. Analysis of variance (ANOVA) followed by Duncan's multiple range test was conducted using SPSS software (Ver. 22). Differences were considered statistically significant at a 95% confidence level ($p < 0.05$).

3 | Results and Discussion

3.1 | NEYE Characterization

The encapsulation efficiency was 86% ± 0.05%. Given that nanoemulsion stability, assessed through droplet size, PDI, and ζ-potential, plays a critical role in the oxidative and microbial stability of the final product, a stability evaluation was performed. DLS analysis of the O/W nanoemulsions showed that the particle size of NEYE increased significantly with increasing volume fraction of YE ($p < 0.05$). As the YE concentration increased from 1% to 2%, the particle size of NEYE rose from 75.5 to 152.65 nm, while the zeta potential decreased from −15.64 to −22.5 mV (Table 1).

This indicates that the particle size of NEYE is affected by volume fraction. As the volume fraction increased, the frequency of droplet collisions also increased, likely leading to coalescence within the emulsion. These results are consistent with the findings of Hassanzadeh et al. [19], who prepared W/O nanoemulsions containing garlic extract and reported that increasing the extract content from 5% to 25% led to an increase in particle size from 62 to 302 nm.

In similar studies, the average droplet size of cumin essential oil [18] and thyme essential oil [21] nanoemulsions was reported to be 106.1 and 125.5 nm, respectively.

Reducing the average size of essential oil droplets in the nanoemulsions, compared with their pure (bulk) form, enhances the availability of bioactive compounds and consequently the antioxidant and antimicrobial properties of the nanoemulsion [18].

TABLE 1 | DLS analysis of NEYE.

Type of nanoemulsion	Particle size (nm)	PDI	ζ-Potential (mV)
NEYE-1%	75.5 ± 1.11^{b}	0.7 ± 0.05^{b}	− 15.64 ± 0.7^{a}
NEYE-2%	152.65 ± 1.19^{a}	0.91 ± 0.06^{a}	− 22.5 ± 0.11^{b}

Note: Mean ± SD (standard deviation). Different lowercase letters indicate a significant difference in the columns ($p \leq 0.05$).
Abbreviations: NEYE-1%, nanoemulsions containing 1% yarrow essential oil; NEYE-2%, nanoemulsions containing 2% yarrow essential oil.

3.2 | NEYE Microstructure

Structural and morphological analysis of nanoemulsions is essential for understanding the droplet morphology, aggregation behavior, and their potential effects on oxidative stability. In the present study, the formation of NEYE was confirmed using SEM (Figure 1), which clearly revealed discrete dispersed structures and supported the droplet size trends observed in the DLS analysis. Similarly, Moradi et al. [18] confirmed the formation of cumin essential oil nanoemulsions using SEM.

3.3 | Microbial Assay of Mayonnaise

Figure 2 shows the growth of *S. aureus*, *S. enteritidis*, *E. coli*, mold, and yeast in mayonnaise during storage. Throughout the 45-day storage period, the highest microbial growth occurred in the control sample. In contrast, the sample containing BS, followed by NEYE and YE, exhibited the lowest microbial growth ($p < 0.05$).

The cross-contamination with raw materials, particularly contaminated eggs, containers, and equipment, can lead to the persistence of pathogenic bacteria in mayonnaise. In the control sample, *S. aureus* (9.11 ± 0.05 log cfu/g), *S. enteritidis* (26.75 ± 0.1 log cfu/g), and *E. coli* (22.95 ± 0.06 log cfu/g) survived throughout the 45-day storage period. Several factors, including pH, acidity, and temperature, presence of lysozyme, antimicrobial agents, and salt concentration, contribute to the rapid inactivation of bacteria in mayonnaise [3].

The pH of mayonnaise samples is approximately 4, which is close to the tolerance limit of most pathogens, including *S. aureus*, *S. enteritidis*, and *E. coli*. The synergistic effects of acetic acid and low pH play a role in preventing the growth of pathogens [3]. The effects of acidity on microorganisms include disruption of substrate transport and oxidative phosphorylation, cell membrane destruction, denaturation of proteins, and interference with DNA synthesis [22]. pH has an important effect on the death of Gram-negative bacteria. At low pH values, the acidophilic bacteria can survive in emulsion products such as mayonnaise, salad dressings, and ketchup [6]. The minimum pH required for the growth of *E. coli* and *Salmonella* has been estimated at approximately 4.4 and 4.5, respectively, while their maximum growth pH values are around 9 and 7.8. Therefore, acidic products limit the growth of *E. coli* and *Salmonella*. Because *E. coli* is generally more tolerant of acidic conditions than *Salmonella*, studies often report that *E. coli* can persist longer in mayonnaise despite

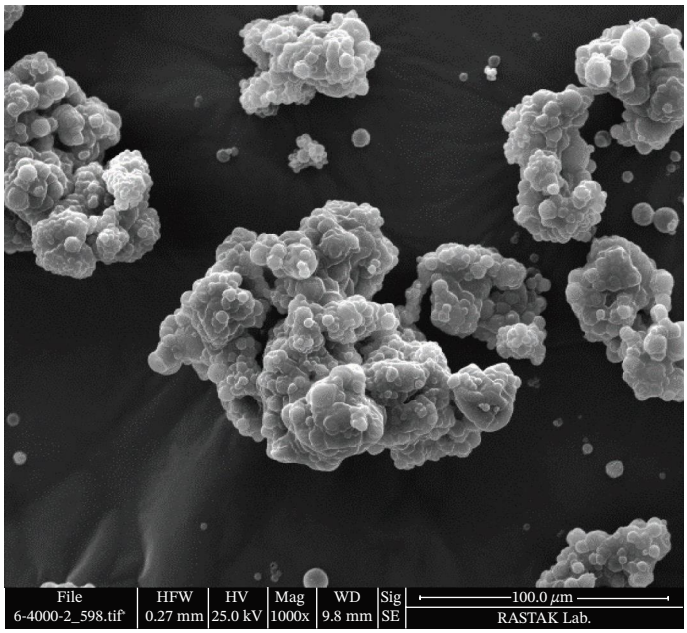


FIGURE 1 | Scanning electron microscope (SEM) of NEYE prepared at 1000 magnification.

its low pH. This suggests that certain protective factors within the mayonnaise matrix may reduce the effectiveness of acidity alone. Nevertheless, *E. coli* remains sensitive to low pH and high acidity, and under typical acidic conditions in mayonnaise, its survival is expected to decline over time [3].

Low temperatures protect *Salmonella* against the organic acids. Heo et al. [23] investigated the inactivation of *Salmonella* in mayonnaise at different storage temperatures and reported that the greatest reduction in viable cells occurred at 4°C compared to 10°C.

Pasteurization helps minimize the risk of *S. aureus* and *S. enteritidis* contamination. Products formulated with unpasteurized eggs are recommended to have a pH below 4.1 and an acetic acid content of approximately 1.4% to enhance microbial safety. In addition, the natural presence of lysozyme in eggs contributes to antimicrobial activity, particularly against Gram-positive bacteria [3].

The lower growth of *S. aureus* (7.41 ± 0.05 and 7.00 ± 0.04 log cfu/g), *S. enteritidis* (20.86 ± 0.03 and 17.44 ± 0.4 log cfu/g), *E. coli* (12.36 ± 0.06 and 10.87 ± 0.11 log cfi/g), and fungi (4.16 ± 0.02 and 3.1 ± 0.05 log cfu/g) for 45 days in YE-1% and YE-2% samples is likely associated with the presence of secondary metabolites in YE, including terpenes such as 1,8-cineol, camphor, eugenol, *p*-cymene, thymol, carvone, and terpinene, which are known for their antimicrobial properties. Terpenes can interact with and disrupt the lipid components of microbial cell membranes, leading to increased membrane permeability, protein dysfunction, and loss of cellular integrity, which ultimately results in cytoplasmic leakage and cell death. Thymol can compromise the outer membrane of Gram-negative bacteria and increase the permeability of the cytoplasmic membrane. *p*-Cymene, being highly hydrophobic, can interact with membrane lipids and cause membrane swelling. Other compounds such as caryophyllene oxide, limonene, pinene, and neomenthol also contribute to notable antimicrobial effects. Together, these substances likely account for the overall antimicrobial activity observed [10].

Recent advances in genomics and proteomics have enhanced our understanding of the antimicrobial mode of action of essential oils. Phenolic and other hydrophobic components of essential oils interact with the lipid constituents of microbial cell membranes, leading to metabolic disruption, altered membrane permeability, and ultimately cell death. These effects are largely attributed to the hydrophobic nature of phenolic compounds, which enables them to insert into the lipid bilayer and disturb membrane integrity. This disruption can cause leakage of cellular contents and interfere with essential membrane-associated functions [3].

Similar results have been reported regarding retardation of microbial growth in mayonnaise formulated with various natural antimicrobials, including yarrow [3]; cumin [18]; mustard [20]; basil and dill [24]; oregano, clove, and cinnamon [25] essential oils; and garlic extract [19].

The synergistic interactions between YE and other components of mayonnaise (e.g., egg yolk, acetic acid, and surfactants) contribute to controlling microbial growth [3]. Microbial sensitivity to the antimicrobial activity of essential oils generally increases as pH, temperature, and oxygen availability decrease. The storage temperature used in this study (4°C) likely enhanced the antimicrobial effectiveness of YE. In addition, low pH increases the apparent hydrophobicity of essential oils, promoting their association with microbial membranes and strengthening their antimicrobial action. Therefore, the naturally acidic environment of mayonnaise supports the ability of YE to interact with and compromise bacterial cell membranes [3, 6].

However, NEYE demonstrates enhanced antimicrobial effects, likely due to the slow and sustained release of volatile compounds [19]. At the end of storage, the population of *S. aureus* (4.14 ± 0.05 and 4.00 ± 0.04 log cfu/g), *S. enteritidis* (5.05 ± 0.03

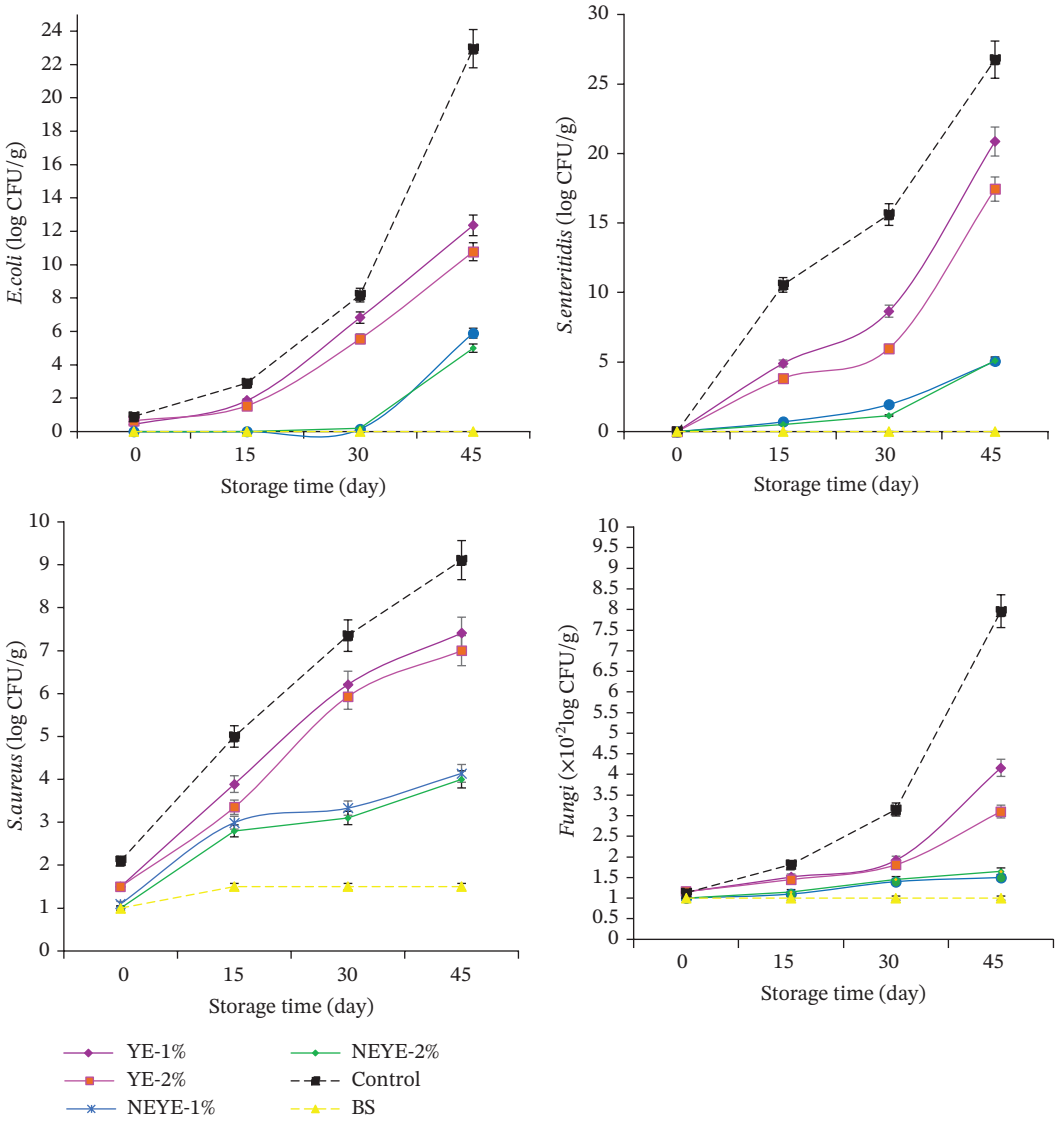


FIGURE 2 | Microbial properties of mayonnaise during storage at 4°C. YE-1%, mayonnaise containing 1% yarrow essential oil; YE-2%, mayonnaise containing 2% yarrow essential oil; NEYE-1%, mayonnaise containing 1% nanoemulsions of yarrow essential oil; NEYE-2%, mayonnaise containing 2% nanoemulsions of yarrow essential oil; BS, mayonnaise containing benzoate and sorbate.

and 5.11 ± 0.4 log cfu/g), *E. coli* (5.9 ± 0.06 and 5.00 ± 0.11 log cfi/g), and fungi (1.65 ± 0.02 and 1.5 ± 0.05 log cfu/g) was the lowest in NEYE-1% and NEYE-2%.

Encapsulation can modify both the efficacy and release kinetics of active compounds, thereby influencing the overall stability of the final product. In this study, the effective release of volatile antimicrobial constituents from NEYE, together with the large surface area associated with its fine droplets, resulted in a greater reduction in microbial populations compared with YE [18, 19]. Consequently, nanoemulsions with particle sizes below 100 nm are considered more suitable for maximizing the antimicrobial and antioxidant potential of essential oils [18].

The interaction of NEYE droplets with microbial cell membranes can compromise membrane integrity, leading to increased cytoplasmic leakage, altered membrane permeability, and overall structural destabilization, which ultimately results in cell death [18].

In agreement with our results, cumin essential oil and its nanoemulsion reduced the number of acid-resistant bacteria, fungi, and total microbial count in mayonnaise compared to the control sample. According to their results, pinenes, as one of the major components of cumin essential oil, inhibited the activity of esterase and phospholipase of microorganisms. Moreover, the nanoemulsified form of the essential oil provided more effective microbial growth control than the free essential oil, highlighting the enhanced antimicrobial potential of nanoemulsion systems [18]. Garlic extract–containing nanoemulsion, due to its organosulfur compounds such as allicin, was effective against *Staphylococcus aureus* and *Escherichia coli*, particularly after 4 months of mayonnaise storage [19]. Both free and encapsulated mustard essential oil could inhibit the growth of *E. coli* and *S. enteritidis* in mayonnaise for up to 40 days [20]. Mansouri et al. [26] evaluated the antibacterial properties of nanoemulsions containing *Thymus daenensis* L. essential oil in mayonnaise and reported that the nonencapsulated essential oil exhibited stronger antibacterial properties than the optimized nanoemulsion

against *Salmonella Typhimurium*, *Escherichia coli*, and *Listeria monocytogenes*. Gorjian et al. [27] reported that mayonnaise formulations containing nanoliposomes and nanoniosomes loaded with myrtle extract showed lower counts of *E. coli*, heterofermentative lactobacilli, mold, and yeast during storage compared with the control sample.

The control sample showed the highest level of fungal growth (7.96×10^2 cfu/g), likely resulting from exposure to air contaminants, equipment, processing environment, or personnel [3]. The antifungal activity of essential oils is commonly attributed to their terpene and terpenoid constituents, which, due to their high lipophilicity and low molecular weight, can interact with fungal cell membranes, compromise membrane integrity, and interfere with key physiological processes. These compounds may also inhibit sporulation and germination of spoilage fungi, thereby contributing to improved product stability [28].

According to the Iranian National Standard No. 2965 [29], *E. coli* and *Salmonella* must be absent in mayonnaise, and the maximum permissible count of mold and yeast is 10^2 cfu/g. Based on these criteria, only the BS treatment remained within the acceptable microbial limits throughout 45 days of storage. The NEYE treatments met the standard for up to 30 days, whereas in the other formulations, bacterial growth appeared after 15 days and fungal growth after 30 days of storage.

3.4 | Chemical Assay of Mayonnaise

Acidity and pH are very important chemical factors in mayonnaise. According to the Iranian National Standard (ISIRI, No. 2454), mayonnaise must have a pH of 4 or less and a total acidity of at least 0.6 g/100 g [30]. If the acidity is high, it will cause an undesirable taste in the final product, and if the acidity is too low, the product will spoil quickly. On the other hand, an increase in pH can lead to the growth of pathogenic bacteria, including *S. aureus*. Also, monitoring acidity and pH is one of the parameters of oxidation quality control [19].

As shown in Figure 3, at the beginning of the experiment (Day 0), no significant differences were observed in the pH or acidity of the samples ($p > 0.05$). However, during storage, the pH of all mayonnaise samples decreased while their acidity increased significantly ($p < 0.05$). Among the treatments, samples containing TBHQ and NEYE exhibited the lowest acidity throughout storage, whereas the control sample showed the highest acidity ($p < 0.05$).

The decrease in pH during storage may be attributed to the activity of lactic acid bacteria, which increases acidity [31].

Acidic pH enhances the stability of mayonnaise by restricting microbial growth and helping to maintain the emulsion structure. Maximum stability occurs when the pH approaches the isoelectric point of egg yolk phosvitin. pH also influences the distribution of volatile secondary oxidation products and can promote oxidation by disrupting the bonds between egg yolk proteins (e.g., low-density lipoproteins, lipovitellin, and phosvitin) and iron, thereby releasing iron ions that catalyze oxidative reactions. In addition, lower pH can activate lipase, leading to the hydrolysis of triglycerides in the oil phase, increased release of free fatty acids during storage, and a corresponding rise in acidity [31].

The initial acidity of the mayonnaise samples ranged from 0.04 to 0.05 mg KOH/g oil and increased gradually during storage. At the end of the storage period, the acidity values remained within the acceptable limits defined by the standard (≤ 2 mg KOH/g oil) [30], indicating that the mayonnaise maintained appropriate quality throughout storage.

The lower acidity in treatments containing antioxidants (synthetic and natural) indicates greater resistance to hydrolysis and

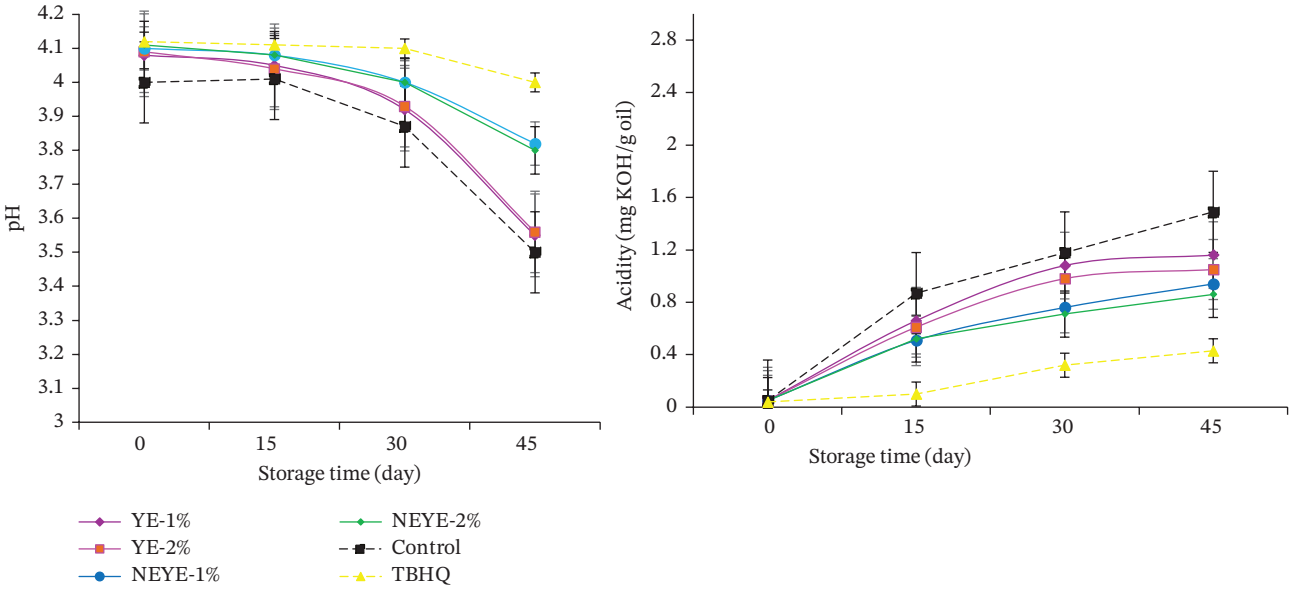


FIGURE 3 | pH and acidity of mayonnaise during storage at 4°C. YE-1%, mayonnaise containing 1% yarrow essential oil; YE-2%, mayonnaise containing 2% yarrow essential oil; NEYE-1%, mayonnaise containing 1% nanoemulsions of yarrow essential oil; NEYE-2%, mayonnaise containing 2% nanoemulsions of yarrow essential oil; TBHQ, mayonnaise containing TBHQ.

oxidative reactions during storage [31]. Similar findings have been reported in previous studies. Moradi et al. [18] demonstrated that cumin essential oil and its nanoemulsions reduced the acidity of mayonnaise compared to the control sample. Likewise, Gorjian et al. [27] reported that among treatments containing nanoliposomes and nanoniosomes loaded with myrtle extract, the highest pH value (4.2) was observed in the sample formulated with sodium benzoate.

3.5 | Oxidation Assay of Mayonnaise

Oxidative stability is a key indicator of mayonnaise quality and shelf life, as lipid oxidation produces low molecular weight compounds that can render the product unsuitable for consumption. Due to its high oil content and the unsaturated nature of the lipids used, mayonnaise is particularly susceptible to oxidation. This process leads to the formation of toxic oxidation products, off-flavors, discoloration, and ultimately a reduction in shelf life [32].

PV reflects the concentration of hydroperoxides formed during the initial stages of lipid oxidation [3, 22]. As shown in Figure 4a, the PV of the samples at Day 0 (0.79–0.89 meq O_2/kg) did not differ significantly ($p > 0.05$); however, PV increased significantly throughout storage ($p < 0.05$). At the end of storage, the treatments containing TBHQ and NEYE exhibited the lowest PVs (1.76 and 2.19–2.29 meq O_2/kg, respectively),

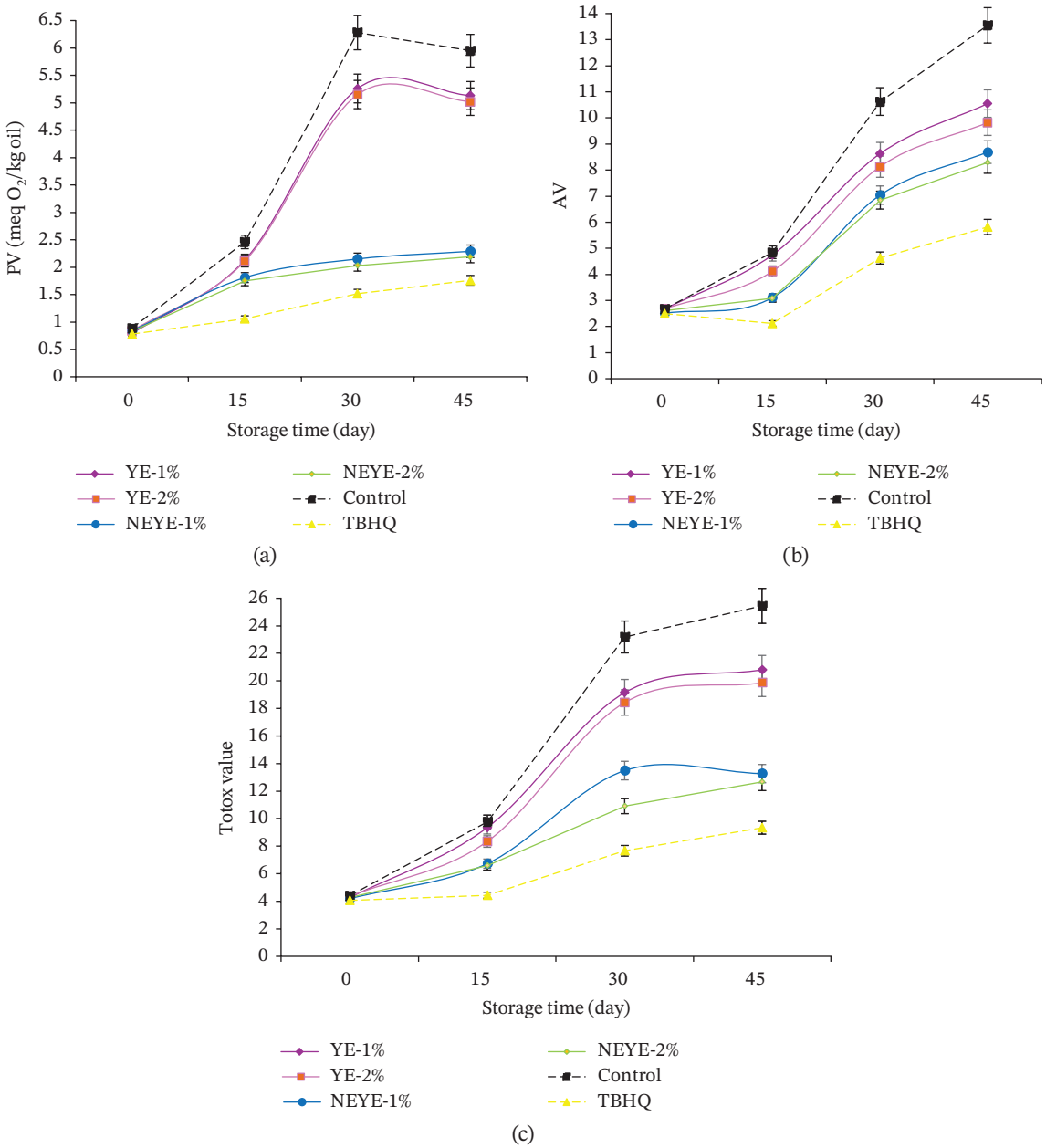


FIGURE 4 | (a–c) Oxidation properties of mayonnaise during storage at 4°C. YE-1%, mayonnaise containing 1% yarrow essential oil; YE-2%, mayonnaise containing 2% yarrow essential oil; NEYE-1%, mayonnaise containing 1% nanoemulsions of yarrow essential oil; NEYE-2%, mayonnaise containing 2% nanoemulsions of yarrow essential oil; TBHQ, mayonnaise containing TBHQ.

whereas the control sample showed the highest PV (5.95 meq O_2/kg) ($p < 0.05$).

Since mayonnaise is an O/W emulsion, its oil droplets are in direct contact with the aqueous phase, which contains dissolved oxygen that accelerates oxidative reactions [2, 22]. In addition, several intrinsic components of mayonnaise contribute to lipid oxidation. Egg yolk is a major source of iron, which catalyzes the decomposition of hydroperoxides into secondary oxidation products. Lemon juice, through its ascorbic acid content, can promote radical formation; ascorbic acid may act as a pro-oxidant by forming iron–ascorbate complexes that enhance hydroperoxide breakdown at the oil–water interface. Vinegar can also exhibit pro-oxidant effects by lowering pH and increasing the release of iron from egg-yolk lipoproteins. It has been reported that pasteurization of mayonnaise can improve oxidative stability by reducing microbial activity and modifying pro-oxidant components [33].

The mayonnaise samples were formulated with soybean oil, which contains a high proportion of linoleic acid and a lower amount of palmitic acid. Because soybean oil is rich in polyunsaturated fatty acids (PUFAs), it is particularly susceptible to oxidation and therefore expected to contribute to lipid deterioration in the formulation [2].

In the early stages of oxidation, peroxide formation proceeded slowly, but it increased rapidly as storage progressed. An increasing–decreasing pattern of PV was observed in the control sample and in treatments containing free essential oil. The subsequent decrease in PV was likely due to the decomposition of hydroperoxides into secondary oxidation products. In other words, the accumulation of hydroperoxides declined because their rate of formation became lower than their rate of degradation [2, 31].

The oil–water interface in emulsions plays a critical role in determining the rate of oxidation. Consequently, lipid oxidation can occur more rapidly and severely in emulsified systems than in bulk oils. Droplet size is an important factor influencing oxidation in O/W emulsions. Although emulsions with smaller droplets exhibit greater physical stability than those with larger droplets, their increased surface area exposes them more extensively to oxygen and pro-oxidant molecules. This heightened interfacial exposure can accelerate oxidative degradation despite improved physical stability [34].

Some studies have reported that droplet size does not significantly influence the rate of oxidation in O/W emulsions [35]. Instead, the oxidative stability of emulsions is largely determined by the effectiveness of antioxidants, which depends on their distribution within the system and their ability to scavenge free radicals, chelate pro-oxidant metals, or quench singlet oxygen. In emulsion systems, the “polarity paradox” has traditionally been used to explain the relative efficacy of antioxidants with different polarities. However, recent research indicates that the polarity paradox has notable limitations and cannot fully account for several observed behaviors in complex emulsion environments [34].

In multiphasic systems, the efficiency of antioxidants is influenced not only by their intrinsic radical-scavenging rates but also by their effective concentrations at the site of oxidation, which are determined by their partitioning among the oil, water, and interfacial regions of the emulsion. The antioxidant activity of phenolic compounds generally depends on their ability to donate hydrogen atoms from substituted phenols to peroxyl radicals. While antioxidant behavior in bulk oils has been extensively reviewed, their efficiency in emulsified systems remains less understood. For instance, increasing the alkyl chain length of an antioxidant alters its solubility and distribution within the emulsion phases and may also affect its redox potential. Therefore, identifying and applying the minimum effective concentration of antioxidants are essential for optimizing their performance in complex emulsion matrices [36].

Molecular diffusion in emulsions is also relevant for controlling chemical reactions that occur at liquid–liquid interfaces because emulsion droplets are constantly breaking and reorganizing, which includes oil, surfactants, and other emulsion components. The diffusion of reactive molecules into or between emulsion droplets depends not only on their molecular nature but also on the properties of the emulsion components (composition, polarity, and viscosity of the oil, aqueous phase components, nature of the emulsifier, etc.). The diffusion can be faster or slower than the ongoing chemical reaction. When the diffusion of reactants is rate-limiting, droplet dynamics must be considered to explain the fate of chemical reactions [37].

According to the Iranian National Standard No. 2454, the maximum permissible PV of mayonnaise is 2 meq O_2/kg [30]. Based on this criterion, the TBHQ-containing treatment remained acceptable for the whole 45-day storage period. The NEYE-1% and NEYE-2% treatments met the standard for up to 30 days. In contrast, the control sample and the YE-1% and YE-2% treatments remained within the acceptable PV limits for less than 15 days and approximately 15 days, respectively. These findings indicate that NEYE effectively extends the shelf life of mayonnaise by inhibiting lipid oxidation and thereby reducing spoilage during storage.

The decrease in PV observed in the YE-containing treatments can be attributed to the strong antioxidant activity of the essential oil and its constituents [2]. Phenolic compounds such as eugenol, thymol, and methyl chavicol present in YE play a key role in inhibiting lipid oxidation [10]. These polyphenols act through multiple mechanisms, including chain-breaking during free radical propagation, decomposition of hydroperoxides, and chelation of pro-oxidant metal ions. By scavenging free radicals, they interrupt the oxidation cycle; they can readily donate hydrogen atoms to reactive species, forming more stable radicals, or neutralize radicals via electron transfer. Additionally, phenolics inhibit enzymes involved in reactive oxygen species (ROS) generation and chelate transition metals, thereby reducing oxidative damage [2, 25]. According to the findings of El-Waseif et al. and Yi et al., flavonoids can increase the oxidative stability of lipids in O/W emulsions, which may help delay lipid oxidation in mayonnaise [31, 38].

The application of NEYE offers increased oxidative quality of mayonnaise. The controlled release of volatile antioxidant compounds from NEYE, combined with the large contact area of its droplets, enhances their antioxidant properties compared to YE [18, 19].

Nanoemulsions serve as an effective vehicle for protecting and delivering yarrow phenolics to the colon. Siles-Sánchez et al. investigated the behavior of yarrow phenolic compounds encapsulated in nanoemulsions during gastrointestinal digestion and found that most phenolics remained encapsulated and were protected from degradation. Although nanoencapsulation did not increase the bioavailability of yarrow phenolics, it significantly improved their delivery to the colon, where they may exert biological activity [17].

Reducing particle size to the nanometer scale improves stability, bioavailability, oil retention, and encapsulation efficiency. Lower levels of oxidation products have also been reported in soybean O/W emulsions with decreasing particle size [39]. In general, nanoemulsions with smaller droplet diameters and lower PDIs are more suitable for food shelf life because the essential oil can be dispersed more uniformly throughout the nanoemulsion [26]. The negative zeta potential observed for the nanoemulsions indicates desirable stability and good dispersion quality. This surface charge generates electrostatic repulsion between droplets, preventing aggregation and thereby improving the long-term stability of the nanoparticles [40].

Similarly, previous findings demonstrated that cumin essential oil and its nanoemulsion reduced the PV of mayonnaise to 3.21 and 1.91 meq O_2/kg oil, respectively, compared with 10.03 meq O_2/kg oil in the control sample. This indicates that the nanoemulsified essential oil provided superior oxidative stability compared to the free essential oil [18]. Garlic extract–containing nanoemulsion did not significantly affect the acidity of mayonnaise; however, it substantially reduced the PV to below 1 meq O_2/kg oil [19]. The oxidative stability of mayonnaise formulated with free and encapsulated mustard essential oil decreased during storage. The control sample without any preservatives showed the highest PV (3.59 meq O_2/kg oil), whereas the sample containing encapsulated mustard essential oil demonstrated improved oxidative stability (2 meq O_2/kg oil), following TBHQ (1.84 meq O_2/kg of oil) [20]. Similarly, the PV of mayonnaise was reduced in formulations incorporating nanoliposomes and nanoniosomes loaded with myrtle extract [27].

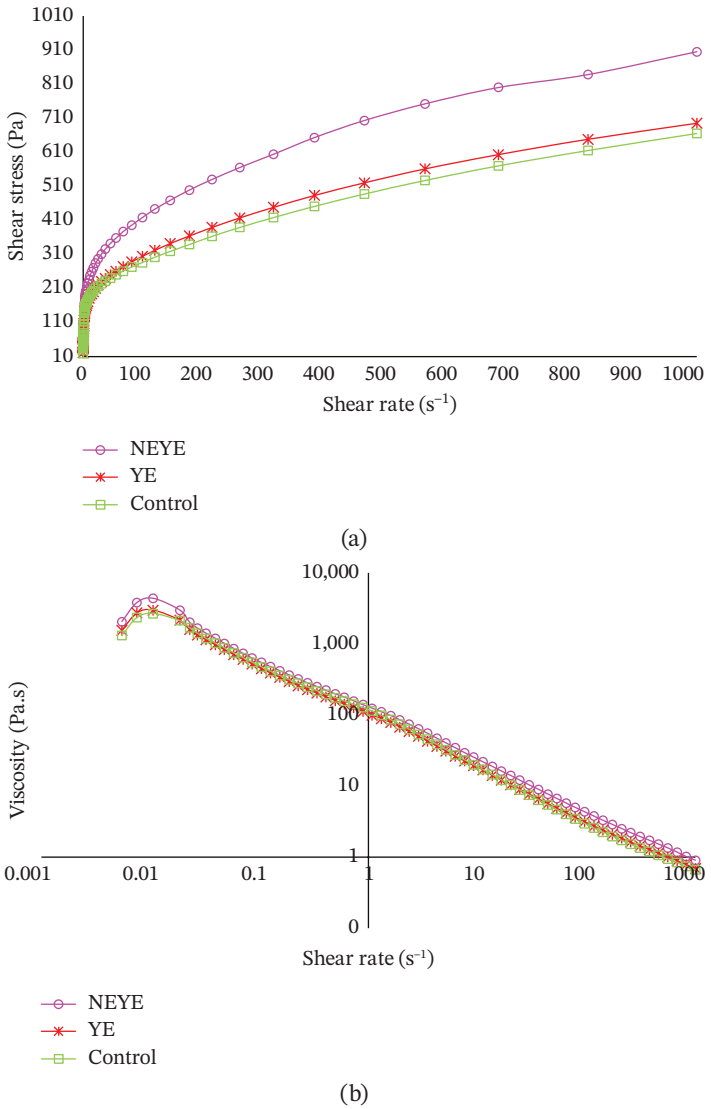


FIGURE 5 | (a) Shear stress versus shear rate and (b) apparent viscosity versus shear rate for the mayonnaise samples.

Hydroperoxides are unstable intermediates and therefore cannot serve as fully reliable indicators of lipid oxidation. In contrast, the AV reflects the concentration of secondary oxidation products, particularly aldehydes, which are more stable and provide a more accurate measure of oxidative deterioration [2].

As can be seen in Figure 3, the initial AV of the samples ranged from 2.5 to 2.6 with no significant differences ($p > 0.05$). However, AV increased significantly during storage ($p < 0.05$). Throughout the storage period, the treatments containing TBHQ and NEYE exhibited the lowest AV values, whereas the control sample had the highest ($p < 0.05$). These findings align with the PV results, suggesting that the decomposition of hydroperoxides intensified over time, leading to the formation of secondary oxidation products [31].

Totox value is an important indicator for monitoring the overall progression of oxidation in mayonnaise. The results showed that Totox values increased in all treatments throughout the storage period (Figure 3). By Day 45, the sample containing TBHQ exhibited the lowest Totox value (9.34), followed by the samples enriched with NEYE (12.67–13.27). In contrast, the control sample showed a substantial increase, reaching a Totox value of 25.45.

The antioxidant activity of YE, attributed to its phenolic compounds and monoterpene hydrocarbons, effectively reduced oxidation processes in mayonnaise. This may explain the observed decrease in Totox value with increasing concentrations of the essential oil nanoemulsion in our study.

Similarly, natural antioxidants such as essential oil of thyme [1]; yarrow [2]; cumin [18]; mustard [20]; oregano, clove, and cinnamon [25]; and extract of garlic [19], rosemary [41], and rose fruit [42] have been reported to reduce the Totox value in mayonnaise.

3.6 | Rheological Assay of Mayonnaise

Figure 5a shows the nonlinear relationship between shear stress and shear rate for the mayonnaise samples. Therefore, mayonnaise samples are classified as non-Newtonian fluids. On the other hand, due to the decrease in apparent viscosity with increasing shear rate, mayonnaise samples exhibit shear-thinning (pseudoplastic) behavior.

Figure 5b presents the relationship between apparent viscosity and shear rate for the mayonnaise samples. As the shear rate increases, the apparent viscosity decreases, confirming the shear-thinning behavior of the emulsions. When shear force is applied, the internal structure of the emulsion becomes disrupted, reducing its resistance to flow (apparent viscosity).

As shown in the results, the NEYE sample exhibited the highest apparent viscosity. The control sample had the lowest viscosity, while the viscosity of the YE sample was similar to that of the control. This can be explained by the nature of the essential oil, its interaction with the continuous phase of the mayonnaise, and its limited contribution to increasing the volume or structure of that phase. Consistent with these findings, a previous study reported that incorporating a W/O garlic extract nanoemulsion into mayonnaise formulations led to an increase in viscosity [18].

The rheological data are consistent with the microstructural observations, as reflected in the improved viscoelastic behavior and apparent stability of the mayonnaise system upon nanoemulsion incorporation. The microscopic interactions between droplets governed by size, volume fraction, interfacial properties, and flocculation behavior collectively determine the macroscopic flow behavior of the emulsion. These interactions give rise to the characteristic rheological features observed, including shear-thinning behavior, yield stress, and viscoelasticity.

3.7 | Sensory Assay of Mayonnaise

Figure 6 shows the effect of YE and NEYE on the sensory properties of mayonnaise. In terms of texture and color, no significant differences were observed among the different YE concentrations ($p > 0.05$). However, higher levels of YE had a negative impact on flavor and odor, resulting in reduced overall acceptability. The lowest sensory scores were typically associated with the 2% YE formulation, likely due to the strong taste and aroma of the oil. High doses of essential oil, although suitable for providing antimicrobial and antioxidant activity, may produce unacceptable taste and odor.

NEYE incorporation improved the flavor, odor, and overall acceptability of the mayonnaise, likely due to its ability to mask volatile off-notes or provide a more controlled release of aroma compounds [18, 19].

Overall, the samples containing YE exhibited the lowest organoleptic scores. Consistent with these findings, Moradi et al. [18], Hassanzadeh et al. [19], and Goli et al. [20] reported that incorporating essential oils in nanoemulsion form results in more favorable sensory properties in mayonnaise compared to using free essential oils.

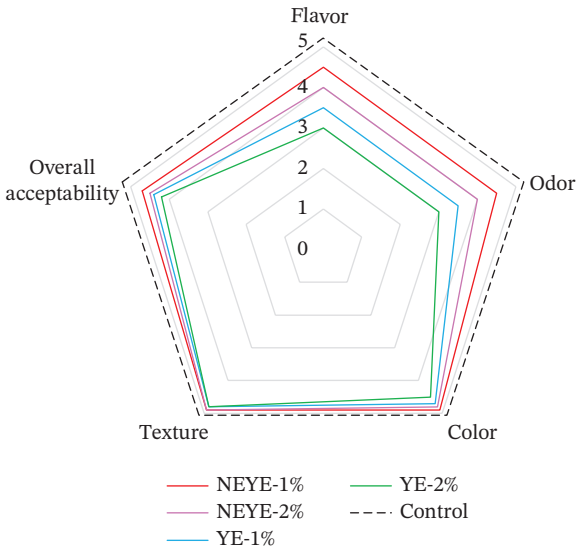


FIGURE 6 | Sensory properties of mayonnaise at Day 0, at 4°C. YE-1%, mayonnaise containing 1% yarrow essential oil; YE-2%, mayonnaise containing 2% yarrow essential oil; NEYE-1%, mayonnaise containing 1% nanoemulsions of yarrow essential oil; NEYE-2%, mayonnaise containing 2% nanoemulsions of yarrow essential oil.

Additionally, the potential phytotoxicity of essential oils represents a major challenge to their application, as certain components may exert harmful effects. In one study, YE rich in β-pinene and eucalyptol showed no phytotoxicity even at the highest tested concentrations [43]. In contrast, Czerniewicz et al. [44] reported that the highest concentration tested (0.8%) caused phytotoxic symptoms, a discrepancy likely attributable to anatomical differences in the leaf structures of the plant species examined. It has also been noted that yarrow oil contains a rare monoterpene alcohol, fragranol, and its esters, which exhibit greater toxicity in mice compared to oils containing thujone [45].

4 | Conclusion

Yarrow (*A. millefolium*) essential oil acted as a natural preservative and antioxidant in mayonnaise, significantly impacting its acidity and the levels of lipid oxidation products. The incorporation of YE, especially at certain concentrations, reduced the formation of primary (> 2 meq/kg) and secondary lipid oxidation products. In addition, NEYE demonstrated strong antimicrobial activity, especially after 30 days of storage. Overall, the estimated shelf-life of the mayonnaise (30–45 days) appears comparable to that of commercial products. Rheological analysis indicated that samples containing NEYE exhibited higher apparent viscosity. Sensory evaluation further revealed that NEYE-enhanced samples achieved superior scores in taste, odor, and overall acceptability compared to those containing YE. The limitations of the current work include scale-up potential, variability in essential oil composition, and long-term stability under real-world conditions. Despite the promising results, further research is needed to optimize the formulation of NEYE in mayonnaise to overcome some obstacles related to the sensory properties of the product. Although essential oils are GRAS, their toxicity testing at high concentrations should be performed. The particle size and ζ-potential monitoring over time are the important knowledge gaps of the present study and should be addressed in future investigations.

Nomenclature

YE	yarrow essential oil
NEYE	nanoemulsion of yarrow essential oil
PDI	polydispersity index
PV	peroxide value
AV	anisidine value
TBHQ	tert-butylhydroquinone

Author Contributions

Mahya Sheikhzadeh, Haniyeh Fateh, and Asiye Ahmadi-Dastgerdi designed the experiment and data collection. Shokufeh Najmi Khoei and Maryam Zokaei helped in the design and contributed to the compilation of the manuscript. Mehrdad Rabiei Nematabad performed SEM and rheology tests, analyzed their data, interpreted the results, and ensured the integrity and accuracy of the final manuscript with scientific editing, particularly the microbiology section. Asiye Ahmadi-Dastgerdi analyzed the data and revised and restructured the discussion for scientific clarity.

Funding

The researchers did not receive any specific grant from funding agencies from the public, commercial, or not-for-profit sectors.

Disclosure

Asiye Ahmadi-Dastgerdi read and approved the final version of the manuscript.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data used to support the findings of this study are available from the corresponding author upon request.

References

1. A. Ahmadi-Dastgerdi, N. Fallah, M. Zokaei, and M. Gholami-Ahangaran, "The Role of Thyme (*Zataria multiflora* Boiss) Essential Oil as Natural Antioxidant on the Lipid Oxidation in Mayonnaise," *Journal of Food Quality* 2022 (2022): 1527289, https://doi.org/10.1155/2022/1527289.

2. A. Ahmadi Dastgerdi, H. Ezzatpanah, S. Asgary, S. Dokhani, and E. Rahimi, "Oxidative Stability of Mayonnaise Supplemented With Essential Oil of *Achillea millefolium ssp millefolium* During Storage," *Food Science and Technology* 13, no. 1 (2019), https://doi.org/10.15673/fst.v13i1.1340.

3. A. Ahmadi Dastgerdi, H. Ezzatpanah, S. Asgary, S. Dokhani, and E. Rahimi, "Antibacterial and Antifungal Effect of *Achillea millefolium* Essential Oil During Shelf Life of Mayonnaise," *Food Science and Technology* 13, no. 4 (2019), https://doi.org/10.15673/fst.v13i4.1568.

4. L. Silva, and B. D. G. Melo Franco, "Application of Oregano Essential Oil Against *Salmonella enteritidis* in Mayonnaise Salad," *International Journal of Food Science and Nutrition Engineering* 2, no. 5 (2012): 70–75.

5. O. Sagdic, F. Tornuk, S. Karasu, M. Z. Durak, and M. Arici, "Chapter 26. Microbial Ecology of Mayonnaise, Margarine, and Sauces," *Quantitative Microbiology in Food Processing: Modeling the Microbial Ecology* (John Wiley & Sons, Ltd., 2017), https://doi.org/10.1002/9781118823071.ch26.

6. S. Manios, R. Lambert, and P. A. Skandamis, "A Generic Model for Spoilage of Acidic Emulsified Foods: Combining Physicochemical Data, Diversity and Levels of Specific Spoilage Organisms," *International Journal of Food Microbiology* 170 (2014): 1–11, https://doi.org/10.1016/j.ijfoodmicro.2013.10.021.

7. C. C. Berton-Carabin, M. H. Ropers, and C. Genot, "Lipid Oxidation in Oil-in-Water Emulsions: Involvement of the Interfacial Layer," *Comprehensive Reviews in Food Science and Food Safety* 13, no. 5 (2014): 945–977.

8. R. A. Holley, and D. Patel, "Improvement in Shelf-Life and Safety of Perishable Foods by Plant Essential Oils and Smoke Antimicrobials," *Food Microbiology* 22, no. 4 (2005): 273–292.

9. S. Burt, "Essential Oils: Their Antibacterial Properties and Potential Applications in Foods–A Review," *International Journal of Food Microbiology* 94, no. 3 (2004): 223–253.

10. A. Ahmadi-Dastgerdi, H. Ezzatpanah, S. Asgary, S. Dokhani, and E. Rahimi, "Phytochemical, Antioxidant and Antimicrobial Activity of the Essential Oil From Flowers and Leaves of *Achillea millefolium* subsp. *millefolium*," *Journal of Essential Oil Bearing Plants* 20, no. 2 (2017): 395–409.

11. M. Villalva, J. M. Silvan, T. Alarcón-Cavero, et al., "Antioxidant, Anti-Inflammatory, and Antibacterial Properties of an Achillea millefolium L. Extract and Its Fractions Obtained by Supercritical Anti-Solvent Fractionation Against Helicobacter pylori," *Antioxidants* 11, no. 10 (2022): 1849, https://doi.org/10.3390/antiox11101849.

12. F. Kiani Aliabadi, A. Ahmadi Dastgerdi, and N. J. Tabatabaeian, "The Oxidative Stability of Chia Seed Oil Enriched With Oregano

(*Origanum vulgare* L.) and Yarrow (*Achillea millefolium*) Extracts," *Journal of Food Quality* 2023, no. 1 (2023): 6263692, https://doi.org/10.1155/2023/6263692.

13. H. Emtiazi, A. Salari Sharif, M. Hemati, B. F. Haghiralsadat, and A. Pardakhti, "Comparative Study of Nano-Liposome and Nano-Niosome for Delivery of Achillea millefolium Essential Oils: Development, Optimization, Characterization and Their Cytotoxicity Effects on Cancer Cell Lines and Antibacterial Activity," *Chemistry & Biodiversity* 19, no. 10 (2022): e202200397, https://doi.org/10.1002/cbdv.202200397.

14. V. K. Venugopalan, L. R. Gopakumar, A. K. Kumaran, et al., "Encapsulation and Protection of Omega-3-Rich Fish Oils Using Food-Grade Delivery Systems," *Foods* 10 (2021): 1566.

15. X. Ren, Y. Liu, C. Fan, et al., "Production, Processing, and Protection of Microalgal N-3 Pufa-Rich Oil," *Food* 11, no. 9 (2022): 1215, https://doi.org/10.3390/foods11091215.

16. J. Rakmai, B. Cheirsilp, A. Torrado-Agrasar, J. Simal-Gándara, and J. C. Mejuto, "Encapsulation of Yarrow Essential Oil in Hydroxypropyl Beta-Cyclodextrin: Physiochemical Characterization and Evaluation of Bio-Efficacies," *CyTA–Journal of Food* 15, no. 3 (2017): 409–417, https://doi.org/10.1080/19476337.2017.1286523.

17. M. de las Nieves Siles-Sánchez, L. Jaime, M. Corredig, S. Santoyo, and E. Arranz, "Encapsulation of Yarrow Phenolic Compounds in Lupin Protein Nanoemulsions Increases Stability During Gastrointestinal Transit and Delivery in the Colon," *Food Hydrocolloids for Health* 6 (2024): 100186.

18. A. Moradi, N. Davati, and A. Emamifar, "Effects of *Cuminum cyminum* L. essential Oil and Its Nanoemulsion on Oxidative Stability and Microbial Growth in Mayonnaise During Storage," *Food Science & Nutrition* 11, no. 8 (2023): 4781–4793, https://doi.org/10.1002/fsn3.3457.

19. H. Hassanzadeh, M. Rahbari, Y. Galali, M. Hosseini, and B. Ghanbarzadeh, "The Garlic Extract-Loaded Nanoemulsion: Study of Physicochemical, Rheological, and Antimicrobial Properties and Its Application in Mayonnaise," *Food Science & Nutrition* 11, no. 7 (2023): 3799–3810, https://doi.org/10.1002/fsn3.3365.

20. S. A. H. Goli, S. Keramat, S. Soleimanian-Zad, and B. R. Ghasemi, "Antioxidant and Antimicrobial Efficacy of Microencapsulated Mustard Essential Oil Against *Escherichia coli* and *Salmonella enteritidis* in Mayonnaise," *International Journal of Food Microbiology* 410, no. 30 (2024): 110484, https://doi.org/10.1016/j.ijfoodmicro.2023.110484.

21. J. Xue, P. Michael Davidson, and Q. Zhong, "Antimicrobial Activity of Thyme Oil Co-Nanoemulsified With Sodium Caseinate and Lecithin," *International Journal of Food Microbiology* 210 (2015): 1–8, https://doi.org/10.1016/j.ijfoodmicro.2015.06.003.

22. K. Hunsakul, S. Siripongvutikorn, and W. Usawakesmanee, "Utilization of Tuna Roe and Using Inulin as Oil Replacer for Producing Value Added Omega-3 Mayonnaise Product," *Functional Food in Health and Disease* 6, no. 3 (2016): 158–172.

23. S. K. Heo, J. Y. Lee, S. H. Jo, D. H. Bae, and M. Kim-Chung, "Inactivation of *Salmonella enterica serovar Typhimurium* in Commercial Mayonnaise at Different Temperatures and pH," *Journal of the Korean Society for Applied Biological Chemistry* 53, no. 3 (2010): 351–355.

24. D. Teneva, Z. Denkova, R. Denkova-Kostova, et al., "Biological Preservation of Mayonnaise With *Lactobacillus plantarum* LBRZ12, Dill, and Basil Essential Oils," *Food Chemistry* 344 (2021): 128707.

25. R. Muñoz-González, C. Pino, H. Henríquez, et al., "Elucidation of Antimicrobial and Antioxidant Activities of Selected Plant-Based Mayonnaise-Derived Essential Oils Against Lactic Acid Bacteria," *Journal of Food Processing and Preservation* 46, no. 3 (2022): e16339, https://doi.org/10.1111/jfpp.16339.

26. S. Mansouri, M. Pajohi-Alamoti, N. Aghajani, B. Bazargani-Gilani, and A. Nourian, "Stability and Antibacterial Activity of *Thymus daenensis* L. Essential Oil Nanoemulsion in Mayonnaise," *Journal of the Science of Food and Agriculture* 101, no. 9 (2021): 3880–3888, https://doi.org/10.1002/jsfa.11026.

27. H. Gorjian, Z. Raftani Amiri, J. Mohammadzadeh Milania, and N. Ghaffari Khaligh, "Comparison of the Effect of Natural Preservatives (Nanoliposome and Nanoniosome Containing Myrtle Extract) and Sodium Benzoate on Physicochemical, Microbial, Sensorial and Properties of Mayonnaise Sauce," *Food Science and Technology* 18, no. 116 (2021): 313–325.

28. F. Nazzaro, F. Fratianni, R. Coppola, and V. De Feo, "Essential Oils and Antifungal Activity," *Pharmaceuticals* 10, no. 4 (2017): 86, https://doi.org/10.3390/ph10040086.

29. Iranian National Standardization Organization, *ISIRI. INSO 2965. Microbiology of Mayonnaise and Salad Sauce- Specifications and Test Methods* (2017).

30. Iranian National Standardization Organization, *ISIRI. INSO 2454. Mayonnaise and Cold Sauces- Specifications and Test Methods* (2023).

31. M. El-Waseif, B. Saed, H. Fahmy, et al., "Mayonnaise Enriched With Flaxseed Oil: Omega-3 Fatty Acids Content, Sensory Quality and Stability During the Storage," *Food* 11, no. 15 (2022): 2288, https://doi.org/10.3390/foods11152288.

32. M. A. Rahim, M. Imran, F. A. Khan, et al., "Omega-3-Enriched and Oxidative Stable Mayonnaise Formulated With Spray-Dried Microcapsules of Chia and Fish Oil Blends," *Omega* 9, no. 7 (2024): 8221–8228, https://doi.org/10.1021/acsomega.3c08807.

33. S. G. Gorji, H. E. Smyth, M. Sharma, and M. Fitzgerald, "Lipid Oxidation in Mayonnaise and the Role of Natural Antioxidants: A Review," *Trends in Food Science & Technology* 56 (2016): 88–102.

34. M. Zhang, L. Fan, Y. Liu, and J. Li, "Effects of Interface Generation, Droplet Size and Antioxidant Partitioning on the Oxidation Rate and Oxidative Stability of Water–In–Oil Emulsions: A Comparison of Coarse Emulsions and Nanoemulsions," *Food Hydrocolloids* 136 (2023): 108227, https://doi.org/10.1016/j.foodhyd.2022.108227.

35. M. Costa, J. Freiría-Gándara, S. Losada-Barreiro, F. Paiva-Martins, C. Aliaga, and C. Bravo-Díaz, "Interfacial Kinetics in Olive Oil–in–Water Nanoemulsions: Relationships Between Rates of Initiation of Lipid Peroxidation, Induction Times and Effective Interfacial Antioxidant Concentrations," *Journal of Colloid and Interface Science* 604 (2021): 248–259, https://doi.org/10.1016/j.jcis.2021.06.101.

36. M. Costa, S. Losada-Barreiro, F. Paiva-Martins, and C. Bravo-Díaz, "Polyphenolic Antioxidants in Lipid Emulsions: Partitioning Effects and Interfacial Phenomena," *Foods* 10, no. 3 (2021): 539, https://doi.org/10.3390/foods10030539.

37. M. Costa, F. Paiva-Martins, F. Losada-Barreiro, and C. Bravo-Díaz, "Modeling Chemical Reactivity at the Interfaces of Emulsions: Effects of Partitioning and Temperature," *Molecules* 26, no. 15 (2021): 4703, https://doi.org/10.3390/molecules26154703.

38. B. R. Yi, H. J. Ka, Y. J. Kwon, et al., "Oxidative Stability in Oil-in-Water Emulsions With Quercetin or Rutin Under Iron Catalysis or Riboflavin Photosensitization," *Journal of Food Science* 82, no. 4 (2017): 890–896, https://doi.org/10.1111/1750-3841.13681.

39. P. S. O'Dwyer, D. O'Beirne, D. N. Eidhin, and B. T. O'Kennedy, "Effects of Sodium Caseinate Concentration and Storage Conditions on the Oxidative Stability of Oil-in-Water Emulsions," *Food Chemistry* 138, no. 2-3 (2013): 1145–1152, https://doi.org/10.1016/j.foodchem.2012.09.138.

40. Z. Jafarifar, "Preparation and Characterization of Nanostructured Lipid Carrier (NLC) and Nanoemulsion Containing Vitamin D for Enrichment of Dairy Products," *Research and Reviews: Journal of Hospital and Clinical Pharmacy* 7, no. 2 (2021).

41. P. Savani, A. Puthiyedathb, R. Chandran, S. George, P. Prasadb, and U. Annapure, "Evaluation of the Sensory Properties and Antioxidant Activity of Clean Rosemary Extracts for an Effective Replacement of

EDTA in Mayonnaise," *Applied Food Research* 3, no. 1 (2023): 100302, https://doi.org/10.1016/j.afres.2023.100302.

42. O. Soltan, H. Gazwi, A. Ragab, et al., "Assessment of Bioactive Phytochemicals and Utilization of Rosa canina Fruit Extract as a Novel Natural Antioxidant for Mayonnaise," *Molecules* 28, no. 8 (2023): 3350, https://doi.org/10.3390/molecules28083350.

43. E. Górska-Drabik, K. Golan, C. Sempruch, G. Chrzanowski, M. P. Dybowski, and M. Poniewozik, "Chemical Composition and Toxicity of *Achillea millefolium* L. Essential Oil Against *Acrobasis advenella* (Lepidoptera, Pyralidae) Under Laboratory Conditions," *Molecules* 30, no. 9 (2025): 1927.

44. P. Czerniewicz, G. Chrzanowski, I. Sprawka, and H. Sytykiewicz, "Aphicidal Activity of Selected Asteraceae Essential Oils and Their Effect on Enzyme Activities of the Green Peach Aphid, *Myzus persicae* (Sulzer)," *Pesticide Biochemistry and Physiology* 145 (2018): 84–92, https://doi.org/10.1016/j.pestbp.2018.01.010.

45. N. Radulović, M. S. Dekić, P. J. Ranđelović, N. M. Stojanović, A. R. Zarubica, and Z. Z. Stojanović-Radić, "Toxic Essential Oils: Anxiolytic, Antinociceptive and Antimicrobial Properties of the Yarrow *Achillea umbellata* Sibth. Et Sm. (Asteraceae) Volatiles," *Food and Chemical Toxicology* 50, no. 6 (2012): 2016–2026, https://doi.org/10.1016/j.fct.2012.03.047.