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Effect of Lemon Oil Nanoemulsion and Heat Treatment on Physical Properties and Lipid Quality Parameters of Fish Meat During Storage

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ABSTRACT

In this study, raw and steamed fish (SF) (*Oncorhynchus mykiss*) samples treated with lemon oil nanoemulsion (LON) were examined. Four groups were designated: raw fish (RF), raw fish treated with LON (RFL), SF, and steamed fish treated with LON (SFL). Changes in the physical properties and lipid quality parameters of fish samples were monitored over 30 days of storage at 4°C ± 2°C following vacuum packing. The DL-limonene compound, a major component of lemon oil, was detected at a concentration of 75.49%. The LON exhibited a zeta size (ZS) of 197.067 nm, a polydispersity index (PDI) of 0.217, and a zeta potential (ZP) of −3.56 mV. Regarding color characteristics, L^* whiteness value, $-a$ greenishness, and $+b$ yellowness values of the nanoemulsion showed a decrease by the end of storage. In all sample groups, except for the L^* value in the SFL group, a general trend was observed in which L^* increased, a^* decreased, and b^* increased compared to Day 0 at the end of storage. During storage, thiobarbituric acid reactive substance (TBARS) and free fatty acid (FFA) values remained within acceptable limits for consumption across all groups throughout storage. In conclusion, the application of LON without heat treatment had a positive effect on pH levels. Conversely, when applied to heat-treated fish meat, the nanoemulsion improved the L^* value, TBARS, FFA, and overall lipid quality parameters, with the exception of polyene index (PI).

Practical applications: Essential lemon oil possesses sensory, antioxidant, and antimicrobial properties. From a sensory perspective, the practical use of freshly squeezed lemon juice on fish meat is widely preferred by consumers. Inspired by this culinary practice, the effects of lemon oil nanoemulsion at a nanoscale on certain quality characteristics of both raw and thermally treated fish meat were investigated. In thermally treated fish meat, the lemon oil nanoemulsion positively influenced color parameters, notably enhancing the brightness (L^*) value, as well as markedly improving overall lipid quality indicators. Conversely, the thermal treatment itself adversely affected the pH values of fish meat. Considering lipid quality preservation throughout storage, it is recommended that thermal treatment be applied prior to the incorporation of lemon oil nanoemulsion, ensuring optimal results in both shelf-life and product appeal.

[Correction added on 19 February 2026, after first online publication: The copyright line was changed.]

1 | Introduction

The high water and nutritional contents of meat and fish products make them prone to spoilage. Thus, one of the most critical challenges the meat industry faces is extending the shelf life of meat and fish products [1]. As seafood products contain high levels of polyunsaturated fatty acids (PUFAs), they are highly vulnerable to spoilage through lipid oxidation. Lipid oxidation causes loss of flavor and nutritional value and rancidity in fish and fish products, decreasing the shelf life and quality of these products. Such deterioration may cause economic losses in the aquaculture sector by reducing consumer acceptability [2–4]. Key factors that accelerate lipid oxidation include heat, light, moisture, and the surface with which the oil interacts [5].

Nanoemulsions are transparent or translucent heterogeneous emulsions in which at least two insoluble liquids are dispersed as droplets within each other. The preparation of nanoemulsion requires at least three components: the oil phase, the water phase, and the surfactant, as well as the use of mechanical tools [6, 7]. In literature, nanoemulsion droplet sizes for water-in-oil dispersions are reported to vary; some sources indicate a range of 10–100 nm [8, 9], whereas others report a broader range of 20–200 nm [10, 11].

Nanoemulsion applications in aquaculture products have gained momentum in recent years. Studies show that farmed fish species such as sea bream and sea bass, and especially trout, are commonly used in these applications. In order to improve the quality and shelf life of seafood products, various oils, surfactants, and water are used in the production of nanoemulsion. These applications have been shown to positively affect sensory parameters such as color, odor, taste, texture, and overall acceptability of seafood products, as well as the sensory quality of the products. In addition, nanoemulsions have been observed to delay the increase of chemical parameters in general and play an influential role in preventing lipid oxidation [12]. Nanoemulsions can suppress fish spoilage via reducing microbial flora amount and delaying the oxidation of lipids and proteins, thereby maintaining the quality attributes of fish [13]. When the studies conducted with different oils and fish species in literature are examined, nanoemulsions are suggested to be effective in limiting lipid oxidation in fish meat [14–17].

People have been processing lemon fruit into juice and lemon oil for centuries. The essential oils extracted from lemons have long been used for perfumery and flavor applications due to their fresh and delicious aroma. Citral is almost synonymous with lemon flavor. However, as with other citrus oils, the dominant component (60%–70%) in lemon essential oil is D-limonene. It is followed by β-pinene, γ-terpinene, α-pinene, sabinene, myrcene, α-thujene, and other components. Monoterpenes account for 85%–95% of lemon oil, whereas citral, the most active aroma, accounts for only 1.8%–2.5% of lemon oil. Other active flavor compounds include neryl acetate, geranyl acetate, and citronellal. Different types of lemon can provide different flavor features [18]. The main components of lemon oil were reported as valencene, limonene, and ocimene by other authors [19, 20]. In addition, lemon essential oil nanoemulsion is reported to have attractive organoleptic properties and antioxidant and antimicrobial properties [20]. It is reported that the quality of muscle foods nanocapsulated with various bioactive components is improved, lipid oxidation is inhibited, physicochemical changes during storage are reduced, microbial growth is limited, and shelf life is increased [21]. A few researchers have investigated the effects of lemon oil nanoemulsion (LON) on fish meat quality [17, 15, 22]. However, there is no study comparing the effects of LON on raw and cooked fish meat. In literature, studies investigating the effects of nanoemulsions on the physical, chemical, and microbiological properties of fish meat were generally carried out on raw fish (RF) [12, 14, 15, 17, 23, 24]. However, Ceylan et al. [16] examined the fat stability of raw and cooked mackerel fillets during storage, and they observed that phenolic compounds and tocopherol in nanoemulsions containing wheat germ oil delayed the oxidation of cooked fish samples.

Türkiye is a developing country in aquaculture and exports, with trout (*Oncorhynchus mykiss* Walbaum, 1792) weighing 1 kg and above being among the most exported species [25]. Due to their accessibility and ease of processing, large-sized trout were used in this study. The aim of the present study was to investigate the physical properties (pH, water activity, color, and qualitative component analysis by solid phase microextraction (SPME)–GC–MS, as well as nanoemulsion characterization analysis) and lipid quality parameters (thiobarbituric acid reactive substance [TBARS]; free fatty acids [FFA]; atherogenic index [AI]; thrombogenicity index [TI]; hypocholesterolemic/hypercholesterolaemic index [H/H]; polyene index [PI]; flesh lipid quality [FLQ]) of raw and cooked fish meat treated with LON during a 30-day storage period at 4°C ± 2°C.

2 | Materials and Methods

In the present study, 25 kg of rainbow trout (*O. mykiss*, Walbaum 1792) with an average length of 47.95 ± 0.77 cm and an average weight of 1659.05 ± 70.87 g were used. The fish were purchased from a fisherman in Türkiye and transported to the laboratory within 2 h of harvest. The heads, fins, skin, bones, and all internal organs were removed, and the fish were cut into cubes. The dimensions of the fish meat cubes were $2.73 \pm 0.08 \times 2.31 \pm 0.07 \times 1.73 \pm 0.09$ cm^3 with an average cube weight of 13.47 ± 0.57 g.

2.1 | Methods

2.1.1 | Nanoemulsion Production and Formation of Experimental Groups

An oil-in-water nanoemulsion of lemon oil was prepared according to the method of Hamouda et al. [26]. The nanoemulsion consists of fabricated lemon oil (14%) (Ugurluoglu, Konya, Türkiye), ethanol (3%) (Supelco 1.00983.2511), and surfactant (Tween 80, 3%; GRAS = generally recognized as safe) (ISOLAB, 960.161.1000). This mixture was left at 86°C for 1 h and then cooled to room temperature. Later, 80% distilled water was added and homogenized in an ultrasound device (Bandelin, Sonopuls HD

2200) for 15 min. Prepared nanoemulsions were exposed to an ultraviolet light (Bil-Ser, Türkiye) for 20 min.

Fish samples were assigned to four groups represented by their initials: RF, raw fish treated with LON, LON (RFL), steamed fish (SF), and steamed fish treated with LON (SFL). Raw trout fish samples in group RF were vacuum-packed without any treatment. Fish samples were immersed in the prepared nanoemulsion solution in the RFL group, left for 3 min, filtered, and then vacuum-packed. In the SF group, fish samples were cooked for 12 min in a steamer (Fakir Tolero, Stuttgart, Germany). After cooling to room temperature, they were vacuum-packed. In the SFL group, fish samples were cooked in a steamer (Fakir Tolero, Stuttgart, Germany) for 12 min. Then they were immersed in the prepared nanoemulsion solution and kept for 3 min, filtered out, and then vacuum-packed. Finally, each group is represented by 200 g fish in the vacuumed package (Abant Group, MG42, Hellas, Sydney, Australia). A total of 11 fish samples were collected, and the study was carried out in two replicates. After vacuum packaging, all group samples were stored at 4°C ± 2°C for 30 days.

2.1.2 | Physical Properties of Nanoemulsion

Characterization analysis of the LON was carried out using a dynamic light scattering (DLS) at 25°C (Malvern Instruments, Worcestershire, UK). An average of 30 measurements were taken for each sample. Zeta size (ZS), polydispersity index (PDI), and zeta potential (ZP) values of the samples were determined accordingly.

2.1.3 | pH and Water Activity (a_w) Analysis

The pH was measured using a WTW pH meter (Model 3110, 2AA112, Germany). The pH of the nanoemulsion was measured by immersing the pH meter probe in 20 mL of nanoemulsion solution. The nanoemulsion was stored at 4°C ± 2°C during the measurements. The same procedure was applied to 5 g of fish meat to determine the pH of fish meat ($n = 6$). The a_w values were detected using an a_w measurement device (Novasina Lab Swift) at 25°C. For the water activity measurement of nanoemulsion and fish meat, 5 mL of nanoemulsion solution and 5 g of fish sample were used, respectively ($n = 6$).

2.1.4 | Color Measurement of Nanoemulsion and Fish Samples

Color differences of nanoemulsion and fish samples were measured via a colorimeter (Konica Minolta (CR-A 33a) Osaka, Japan) [27] ($n = 6$).

2.1.5 | SPME–GC–MS Analysis of Lemon Oil

Component analysis of lemon oil was analyzed using SPME–GC–MS (Thermo Trace GC ultra Milan, Italy/Trace DSQ/USA) equipped with an autosampler. The column used was HP INNOWAX (30 m–0.25 mm–0.25 μm). The temperature was initially held at 60°C for 1 min and then increased to 250°C at a rate of 3°C/min. The injection mode and carrier flow were splitless and 1 mL/min, respectively. The front inlet temperature was set to 175°C, and the MS transfer line and ion source temperatures were 200°C. The mass range was m/z 40–450. A volume of 75 μm Carboxen/PDMS was used as the SPME tip. The incubation time was set to 30 min, and the incubation temperature was set to 60°C.

2.1.6 | TBARS Analysis

The TBARS analysis was carried out according to the method reported by Weilmeier and Regenstein [28] and Khan et al. [29] with slight modifications. About 5 g of fish meat was homogenized with 7.5% trichloroacetic acid (TCA) and 100 μL of butylated hydroxytoluene (BHT) for about 2 min. The mixture was filtered through filter paper (Whatman: No. 1). The filtrate samples were transferred to the respective test tubes and kept in a boiling water bath for 30 min. After cooling, absorbance readings were taken against the blind in a spectrophotometer. The malondialdehyde (MDA) concentration in the samples was then calculated using the standard curve.

2.1.7 | FFA Analysis

FFA analysis was performed according to AOAC [30]. A sample of 5 g was weighed into an Erlenmeyer. It was then dissolved in 50 mL ethyl alcohol-diethyl ether mixture. Three to four drops of phenolphthalein were added. It was titrated with 0.5 N ethyl alcohol KOH in a burette until the color turned pink (this color should remain for 30 s). The amount of KOH consumed was then read from the burette, and the result was recorded. FFA% was calculated according to the following formula:

$$\text{FFA}\,(\%) = \left(\frac{\text{KOH consumed}}{\text{sample weight}}\right) \times 2.82 \quad (1)$$

2.1.8 | Lipid Extraction and Fatty Acid Compositions

The lipid extraction of fish samples was performed according to Bligh and Dyer [31]. A GC–MS instrument (Thermo Scientific ISQ LT) with a specific column (Trace Gold TG-WaxMS) was used for fatty acid analysis of the samples. The temperature was kept constant at 100°C for 3 min and then gradually increased (4°C/min) to 240°C. The mass spectroscopy transfer line temperature was 250°C (ionization mode, 70 eV). A fatty acid methyl esters stock solution (Supelco, 37, Bellefonte, Pennsylvania, USA) was used to supply standards for comparison.

2.1.9 | Lipid Quality Indices (LQI)

AI, TI, PI, H/H index, and flesh lipid quality (FLQ) were calculated by using the following equations described by Ulbritcth and Southgate [32], Lubis and Buckle [33], Santos-Silva et al. [34], and

Abrami et al. [35]:

$$AI = \frac{C12 + (4 \times C14) + C16}{\Sigma n6 + \Sigma n3 + \Sigma MUFA} \quad (2)$$

$$TI = \frac{C14 + C16 + C18}{(0.5 \times \Sigma MUFA) + (0.5 \times \Sigma n - 6) + (3 \times \Sigma n - 3) + (n - 3)/(n - 6)} \quad (3)$$

$$PI = \frac{(C20 : 5 + C22 : 6)}{C16} \quad (4)$$

$$HH = \frac{(C18 : 1n - 9 + C18 : 2n - 6 + C18 : 3n - 3 + C20 : 4n - 6 + C20 : 5n - 3 + C22 : 5n - 3 + C22 : 6n - 3)}{(C14 : 0 + C16 : 0)} \quad (5)$$

$$FLQ = \frac{(EPA\% + DHA\%)}{\text{Total fatty acids}\%} \times 100 \quad (6)$$

2.1.10 | Statistical Analysis

All data obtained were analyzed using the Microsoft Office Excel 2020 (means and standard errors) and performed with one-way analysis of variance (ANOVA) and Tukey test using Minitab 17 (Minitab, State College, PA, USA) ($p < 0.05$).

3 | Results and Discussion

3.1 | Physical Properties of Nanoemulsion

The ZS, PDI, and ZP values of LON are shown in Figure 1. Ucar [17] described LON's droplet size (ZS) as 47.40 nm. However, other researchers reported the droplet size of LON as 181.5, 380, and 486.7 nm [22, 36, 37]. It is seen that the droplet size values in the literature are larger or smaller than the 197.1 nm value in our study. These differences in droplet size are believed to be related to the lemon oil, surfactants, and other chemicals used in preparing nanoemulsions and the methods applied.

The small droplet size of nanoparticles or nanoemulsions is critical, as it provides a larger surface area and enhanced reactivity, which are advantageous for various applications, including packaging [38]. In the context of citrus essential oils, nanomaterials ranging in size from 20 to 200 nm provide larger surface area by increasing the solubility of the aqueous phase and protect citrus essential oil compounds from oxidation [39]. In our study, the LON exhibited a particle size value of 197.1 nm, which falls within this range and was intentionally targeted to remain below 200 nm. The results indicated positive outcomes in terms of improved surface area effects and enhanced oxidative stability of the oils.

In our study, the ZP value of the LON was measured at -3.56 ± 0.61 mV. A high ZP value indicates high stability, whereas lower values indicate low stability [40]. It is generally accepted that a ZP of +30 and −30 mV is sufficient to ensure the physical stability of nanoemulsions [41]. In this sense, the LON we used in our study may be considered stable. Kaur et al. [42] and Chen et al. [43] reported negative ZP values of −14.9 and −27.83 mV, respectively. Our findings are consistent with the published data.

The PDI in nanoemulsions is defined as the ratio of the standard deviation to the average droplet size, serving as an indicator of droplet size homogeneity [44]. A PDI value below 0.2 is considered ideal, where droplet sizes are similar without any adhesion or agglomeration [45]. In our study, the PDI value was calculated as 0.217, which is very close to the ideal threshold, and no agglomeration was observed in the nanoemulsion. Comparable values have been reported by other researchers as 0.238 [43], 0.125 [42], 0.413 [17], and 0.114 [22] for LONs.

3.2 | Determination of Volatile Constitutes of Lemon Oil

The components of lemon oil identified by SPME–GC–MS are shown in Figure 2. DL-Limonene is an important constituent in several citrus essential oils (lemon, orange, grapefruit, and tangerine). It is classified as "generally recognized as safe" (GRAS) by the US Food and Drug Administration (FDA) and is widely used as a seasoning and food preservative [46]. According to the results of the qualitative component analysis of lemon oil, DL-limonene (cyclohexene, 1-methyl-4-(1-methylethenyl) was detected at the highest concentration with 75.49%, followed by 2-ȧ-pinene (11.29%), γ-terpinene (7.55%), and ȧ-pinene (2.46%), respectively. Ucar [17] and Yazgan et al. [22] also reported higher DL-limonene content in lemon oil than in other components. However, unlike our study, those researchers identified *p*-cymene and β-pinene as the compounds found at the highest rate in lemon oil after DL-limonene.

Similarly, in another study, limonene was identified as the most dominant compound in lemon oil with 40.93%, followed by α-pinene and linalyl acetate [42]. Zhao et al. [47] also reported D-limonene as the major active component of lemon oil with a composition of 47.48% D-limonene, 14.69% β-pinene, 9.61% gamma terpinene, 4.89% β-myrcene, and 4.28% α-pinene. In a separate study, the lemon oil was found to contain 48.54% limonene, 30.9% α-pinene, and 3.65% β-citral [48]. These findings support the results of our study, in which D-limonene was identified as the major compound, indicating consistency with literature.

3.3 | pH and Water Activity (a_w)

The pH values of LON and RF, RFL, SF, and SFL groups are shown in Figure 3. In our study, the pH values of LON varied

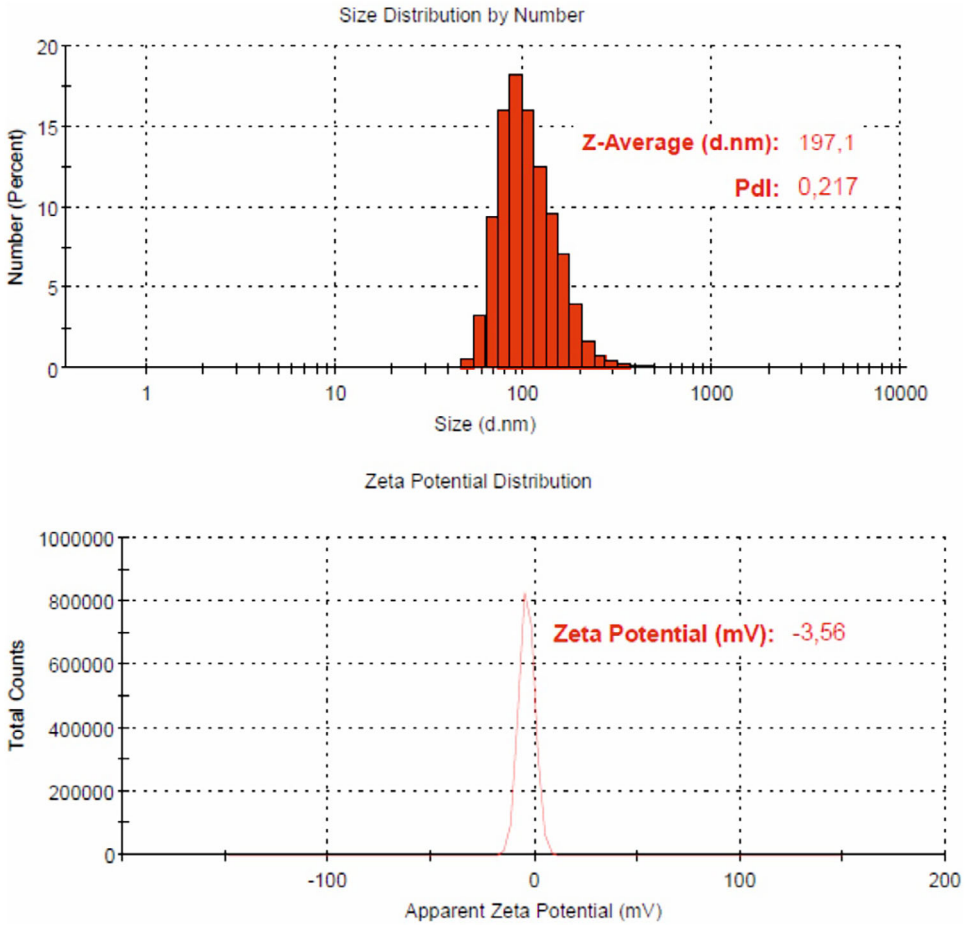

FIGURE 1 | The particle size distribution and zeta potential of lemon oil nanoemulsion (LON). PDI, polydispersity index.

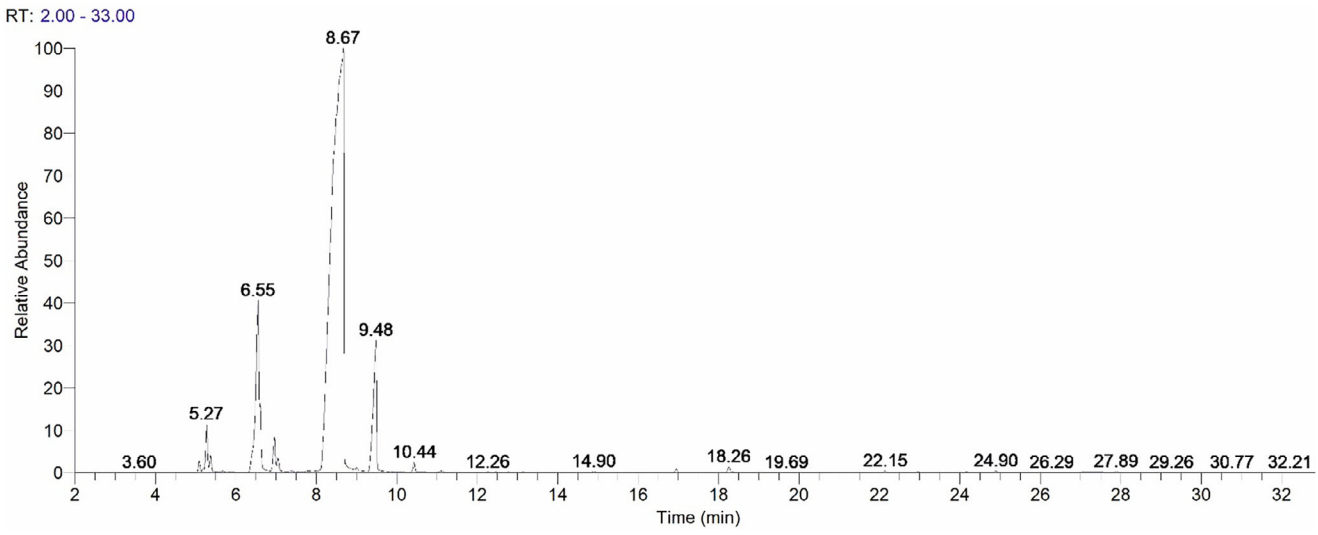

FIGURE 2 | Major volatile component of lemon essential oils identified by SPME–GC–MS.

between 4.18 and 5.51 throughout 30 days of storage. Despite the fluctuations observed during storage, the pH value of the LON stored at 4°C ± 2°C showed a general increase. However, the first 15 days showed more stable values compared to the next 15 days. In previous studies on the preservation of fish products using nanoemulsions obtained with various oils, the properties of nanoemulsions such as pH, a_w, FFA, and color have not been investigated [12, 23, 49]. However, a study reported the pH values of the oil-in-water nanoemulsion of lemon and fish by-product oils as 4.27 on Day 0 of storage at 25°C and 4.22 on Day 90 of storage, whereas these values were reported to remain stable at 4°C as 4.27 on both Days 0 and 90 [50]. Kaur et al. [42] reported

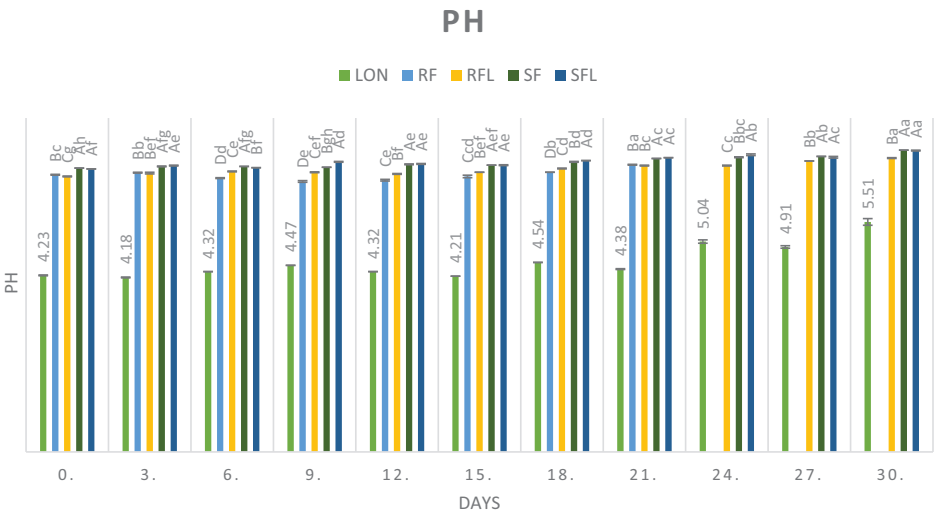

FIGURE 3 | pH values of lemon oil nanoemulsion (LON) and experimental groups. RF, raw fish; RFL, raw fish treated with LON; SF, steamed fish; SFL, steamed fish treated with LON. *A, B, C, … →: There is a difference between different letters between groups ($p < .05$) *a, b, c, … ↓: There is a difference between different letters between days ($p < .05$).

the pH value of lemon oil as 3.5 and the pH value of LON as 5.3.

The pH values of RF, RFL, SF, and SFL groups were measured as 6.64, 6.60, 6.80, and 6.78, on the first day of storage, respectively. Compared to the initial values, pH values increased in all groups throughout the storage period. Durmus [15] reported the Day 0 pH value of the control group of raw trout as 6.31 and the corresponding value for LON-treated raw trout as 6.38. Until Day 12 of storage, the pH values fluctuated compared to the control group, but from Day 12 to the end of storage (Day 16), they were lower than the control. In our study, raw trout treated with LON exhibited lower pH values than the control during the first 3 days of storage; however, these values increased thereafter. Although the pH value of the treated group on Day 21 was lower than that of the control, the difference was not statistically significant ($p > 0.05$). In another study, Ozogul et al. [23] applied the nanoemulsions prepared from sunflower, canola, and corn oils to raw sea bass. The fish were wrapped with stretch film and stored at 2°C ± 2°C for 12 days to examine quality changes. It was reported that the pH values of raw sea bass treated with nanoemulsions containing sunflower, canola, corn, hazelnut, soybean, and olive oil were lower compared to those of the control group.

It is known that chemical and enzymatic changes occurring in fish meat can increase the pH value [51]. This may be due to the breakdown of hydrogen bonding and electrostatic interactions [52]. However, the reason why the pH value of the trout treated with LON increased after Day 3 may be attributed to the incline in the pH value of the LON, which exhibited an increase after Day 6. The RFL (7.04) group was found to be lower than the SF (7.23) and SFL (7.22) groups at the end of storage ($p < 0.05$). In the present study, the pH value of the cooked fish samples was higher than that of the raw samples, which is believed to have link to the chemical and/or enzymatic changes that occur in the fish meat during the process of cooking. Kılıç et al. [53] attributed the increase in pH to microbial growth, autolysis as well as protein degradation. It is known that heat treatment causes denaturation of proteins and also increases the pH value [54].

The water activity (a_w) values for LON and RF, RFL, SF, and SFL groups are presented in Figure 4. The a_w values for LON remained stable between 0.98 and 0.99 throughout the storage period. Water activity values of raw trout (RF and RFL groups) were measured consistently as 0.98 during storage. Similarly, a previous study indicated that the a_w values for raw sea bass (*Dicentrarchus labrax*) did not show significant differences over the storage period, remaining within the range of 0.97–0.98. In the same study, nanoemulsions containing 25% and 50% rosehip oil were applied to raw sea bass fish. The a_w values on Day 0 of storage were reported as 0.979 and 0.980 and noted to be 0.975 and 0.977 on the final day of storage (Day 9), respectively [24]. The lowest water activity value on the first day of storage was found in the SFL group ($p < 0.05$). During the storage period, the water activity values between SF and SFL groups showed statistically significant differences on specific storage days (0, 3, 6, 12, 15, 24). However, no statistical difference was observed between the steamed groups (SF and SFL) at the end of the storage period ($p > 0.05$). This suggest that the water activity values of steamed trout are partially affected by the LON and/or the duration of storage. However, further comprehensive studies on this topic are necessary.

3.4 | Color Measurement of Nanoemulsion and Fish Samples

The L^*, a^*, and b^* values of LON and experimental groups are shown in Table 1. The L^*, a^*, and b^* values of the LON showed a fluctuating trend during storage. However, compared to the values at the beginning of storage, the L^*, a^*, and b^* values at the end of storage were lower. This is thought to be related to the decrease in the stability of the nanoemulsion during storage or the loss of volatile lemon oil components. According to the end of storage data, the highest L^* value was measured in the SFL group ($p < 0.05$), and the lowest L^* value was measured in the RFL group. When SF and SFL groups were compared, L^* values of SFL group were higher than the SF group. Accordingly, the cooking process increased the L^* value, but treatment with

TABLE 1 | Color (L^*, a^*, b^*) values of lemon oil nanoemulsion (LON) and experimental groups.

Storage days	LON			RF			RFL			SF			SFL		
	L^*	a^*	b^*	L^*	a^*	b^*	L^*	a^*	b^*	L^*	a^*	b^*	L^*	a^*	b^*
0	102.94 ± 0.04	−1.30 ± 0.01	2.02 ± 0.04	60.47 ± 0.37^{De}	16.51 ± 0.31^{Bde}	25.11 ± 0.70^{Be}	65.82 ± 0.85^{Ccd}	17.74 ± 0.31^{Aa}	27.99 ± 0.43^{Acd}	79.67 ± 0.23^{Bg}	12.85 ± 0.09^{Ca}	23.67 ± 0.11^{Bd}	81.87 ± 0.56^{Acd}	11.97 ± 0.38^{Ca}	23.63 ± 0.37^{Bd}
3	103.11 ± 0.03	−1.24 ± 0.01	2.16 ± 0.02	66.73 ± 0.62^{Ccd}	17.73 ± 0.51^{Abc}	29.47 ± 0.58^{Ac}	67.58 ± 0.35^{Cab}	16.26 ± 0.64^{Ab}	28.48 ± 0.95^{Acd}	80.32 ± 0.29^{Bef}	12.77 ± 0.11^{Ba}	23.16 ± 0.25^{Bef}	82.19 ± 0.24^{Abc}	10.62 ± 0.03^{Cb}	22.39 ± 0.05^{Bef}
6	94.64 ± 0.98	−1.22 ± 0.02	−1.48 ± 0.04	64.96 ± 0.91^{Bd}	18.50 ± 0.21^{Aa}	29.75 ± 0.67^{Abc}	61.70 ± 0.79^{Cf}	17.96 ± 0.12^{Aa}	28.74 ± 0.42^{Ac}	81.28 ± 0.19^{Acd}	12.12 ± 0.08^{Bb}	22.83 ± 0.10^{Bfg}	82.29 ± 0.29^{Abc}	10.65 ± 0.10^{Cb}	22.74 ± 0.04^{Be}
9	101.50 ± 0.17	−1.31 ± 0.01	−0.16 ± 0.05	62.20 ± 0.95^{Be}	16.21 ± 0.24^{Ae}	27.60 ± 0.18^{Ad}	55.71 ± 0.33^{Ch}	12.97 ± 0.31^{Bcd}	21.07 ± 0.42^{De}	81.22 ± 0.11^{Acd}	12.84 ± 0.17^{Ba}	23.36 ± 0.14^{Bde}	82.49 ± 0.12^{Abc}	10.55 ± 0.11^{Cb}	22.09 ± 0.16^{Cf}
12	104.56 ± 0.05	−1.34 ± 0.01	1.79 ± 0.04	69.01 ± 0.14^{Bab}	17.86 ± 0.14^{Aab}	28.77 ± 0.22^{Acd}	64.15 ± 1.04^{Cde}	18.24 ± 0.65^{Aa}	28.25 ± 0.55^{Acd}	81.55 ± 0.13^{Abc}	13.03 ± 0.22^{Ba}	23.19 ± 0.21^{Bef}	83.40 ± 0.38^{Aa}	10.54 ± 0.13^{Cb}	22.22 ± 0.07^{Bf}
15	103.80 ± 0.10	−1.56 ± 0.01	0.52 ± 0.04	70.59 ± 0.55^{Ca}	17.03 ± 0.06^{Acd}	29.08 ± 0.53^{Ac}	62.76 ± 0.32^{Def}	16.20 ± 0.25^{Bb}	27.12 ± 0.28^{Bd}	80.70 ± 0.04^{Bde}	11.85 ± 0.20^{Cb}	22.74 ± 0.12^{Cg}	82.34 ± 0.06^{Abc}	10.37 ± 0.06^{Db}	22.45 ± 0.10^{Cef}
18	97.01 ± 0.42	−6.61 ± 0.04	3.68 ± 0.13	67.09 ± 1.09^{Bbc}	13.69 ± 0.09^{Af}	31.55 ± 0.31^{Aa}	58.92 ± 0.69^{Cg}	13.28 ± 0.33^{Ac}	28.95 ± 0.36^{Bc}	81.99 ± 0.36^{Aab}	8.32 ± 0.05^{Bc}	27.47 ± 0.10^{Ca}	83.45 ± 0.09^{Aa}	6.79 ± 0.25^{Ccd}	26.94 ± 0.23^{Cab}
21	100.65 ± 0.77	−6.76 ± 0.03	5.26 ± 0.50	70.02 ± 0.26^{Ca}	12.72 ± 0.13^{Ag}	31.00 ± 0.14^{Aab}	65.46 ± 0.43^{Dcd}	11.93 ± 0.32^{Bd}	30.56 ± 0.34^{Ab}	80.34 ± 0.06^{Bef}	8.42 ± 0.11^{Cc}	27.16 ± 0.05^{Bab}	81.74 ± 0.08^{Acd}	6.41 ± 0.09^{Dde}	26.67 ± 0.10^{Bb}
24	102.15 ± 0.42	−7.10 ± 0.08	6.62 ± 0.30	—	—	—	66.07 ± 0.68^{Bbc}	12.93 ± 0.32^{Acd}	31.53 ± 0.96^{Aab}	81.16 ± 0.35^{Acd}	8.44 ± 0.06^{Bc}	27.27 ± 0.09^{Ba}	82.17 ± 0.27^{Abc}	7.06 ± 0.03^{Cc}	27.18 ± 0.16^{Ba}
27	100.46 ± 0.93	−7.18 ± 0.08	5.67 ± 0.56	—	—	—	68.85 ± 0.35^{Ba}	12.95 ± 0.14^{Acd}	32.19 ± 0.33^{Aa}	82.28 ± 0.09^{Aa}	7.92 ± 0.04^{Bd}	26.76 ± 0.05^{Bb}	82.70 ± 0.39^{Aab}	6.85 ± 0.24^{Ccd}	27.24 ± 0.23^{Ba}
30	92.11 ± 1.43	−7.18 ± 0.18	1.76 ± 0.24	—	—	—	66.87 ± 0.19^{Cbc}	13.74 ± 0.21^{Ac}	32.50 ± 0.28^{Aa}	79.78 ± 0.07^{Bfg}	7.08 ± 0.18^{Be}	25.77 ± 0.20^{Bc}	81.22 ± 0.06^{Ad}	5.94 ± 0.11^{Ce}	25.99 ± 0.07^{Bc}

Note: →: There is a difference among different letters (A–C) between groups ($p < 0.05$). ↓: There is a difference among different letters (a–c) between days ($p < 0.05$).
Abbreviations: LON, lemon oil nanoemulsion; RF, raw fish; RFL, raw fish treated with LON; SF, steamed fish; SFL, steamed fish treated with LON.

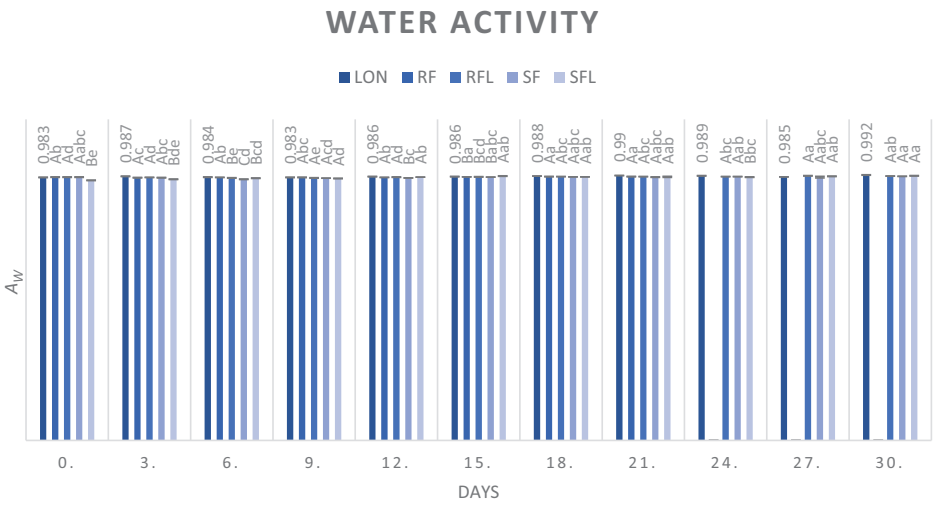

FIGURE 4 | a_w values of lemon oil nanoemulsion (LON) and experimental groups. RF, raw fish; RFL, raw fish treated with LON; SF, steamed fish; SFL, steamed fish treated with LON.*A, B, C, … →: There is a difference between different letters between groups ($p < .05$) *a, b, c, … ↓: There is a difference between different letters between days ($p < .05$).

nanoemulsion had an additional contribution. Similar results were also reported by Ceylan et al. [16]. The highest a^* values were observed during storage in RF and RFL groups. This value was lower in the cooked groups (SF and SFL). The flesh color of farmed fish fed with salmon or carotenoid-containing feeds starts to turn white 10 min after heat treatment (cooking) [55]. In the present study, the primary reason for the lower redness ($+a$) values compared to the raw groups is the cooking effect. However, it was also observed that lemon oil nanoemulsion and the cooking process also decreased the redness value to some extent. The highest b^* value was measured in the RFL group, and the lowest b^* value was measured in the SF group at the end of the storage period. The b^* values of LON ranged between −1.48 and 6.62 on different days throughout the storage. The differences between the values in terms of $+b^*$ and $-b^*$ values are believed to be related to the inability of the nanoemulsion to maintain daily stability changes. In both raw and steamed groups, $+b$ values generally increased during storage. However, the $+b$ values of the steamed groups were found to be lower. The $+b$ values of both steaming and nanoemulsion-treated group (SFL) were lower than the other groups, except for Day 30 of storage ($p > 0.05$). This may suggest that both steaming and treating with LON processes are effective on the $+b$ values. The increase in the $+b$ value can be attributed to the formation of yellowish-colored compounds due to lipid oxidation in fresh fish meat depending on the storage time [24]. In the present study, it can be concluded that steamed trout is more resistant to lipid oxidation than raw trout. The LON also contributed to this.

3.5 | TBARS Values

Lipid oxidation occurs when atmospheric oxygen reacts with lipids to produce peroxides. The formation of peroxides is a chain reaction, and the product formed is a hydroperoxide. The decomposition of hydroperoxides results in the formation of alkoxy and hydroxy free radicals. These radicals are responsible for the formation of aldehydes and ketones, which are responsible for undesirable taste and odor changes in lipids [56].

The TBARS values of experimental groups are shown in Figure 5. On Day 0 of storage, TBARS values of the RF, RFL, SF, and SFL groups were 0.22, 0.24, 1.15, and 0.65 μg MDA/g, respectively. At the end of storage (Day 21 for RF and Day 30 for RFL, SF, and SFL), the TBARS values were 0.23 μg MDA/g for RF, 0.26 μg MDA/g for RFL, 0.92 μg MDA/g for SF, and 0.74 μg MDA/g for SFL. In the present study, TBARS values in the RF groups (RF and RFL) were significantly lower than those in the SF groups ($p < 0.05$). Moreover, TBARS values of the LON-treated group after steaming were significantly lower during storage ($p < 0.05$). Although TBARS values fluctuated in all groups throughout the storage period, none of the groups exceeded the TBARS value of 7–8 mg MA/kg, which is the consumption limit value for fish meat [57].

Ozogul et al. [14] reported the TBARS value of raw trout as 0.50 μg MDA/g on Day 0 of storage and 1.77 μg MDA/g at the end of storage (Day 24). These values were higher than values observed for raw trout in our study on both Day 0 and the end of storage (Day 21). In their research, nanoemulsions of rosemary, laurel, thyme, and sage essential oils were applied to raw trout, and TBARS values were monitored over 24 days of storage. TBARS values fluctuated in all groups, and it was reported that the nanoemulsion-treated groups had lower TBARS values compared to the control group. In another study, Yang et al. [58] investigated the effect of oregano/*Litsea cubeba* (6:4, wt%/wt%) nanoemulsion on grass carp fillets. The researchers found that lipid oxidation was lower in the nanoemulsion-treated group compared to the control group. Durmus [15] reported the Day 0 TBARS values of raw trout treated with LON and plain raw trout (control) as 0.32 and 0.41 μg MDA/g, respectively. By the end of 16 days of storage, these values increased to 1.72 and 2.75 μg MDA/g, respectively. These results are higher than the corresponding values observed for both RFL (raw trout treated with LON) and RF (plain raw trout) groups in our study. Additionally, the statistical difference between RF and RFL groups in our study was found to be insignificant ($p > 0.05$). This outcome is likely to depend on several factors such as the characteristics of the fish used in the study (e.g., size, processing, and packaging), as

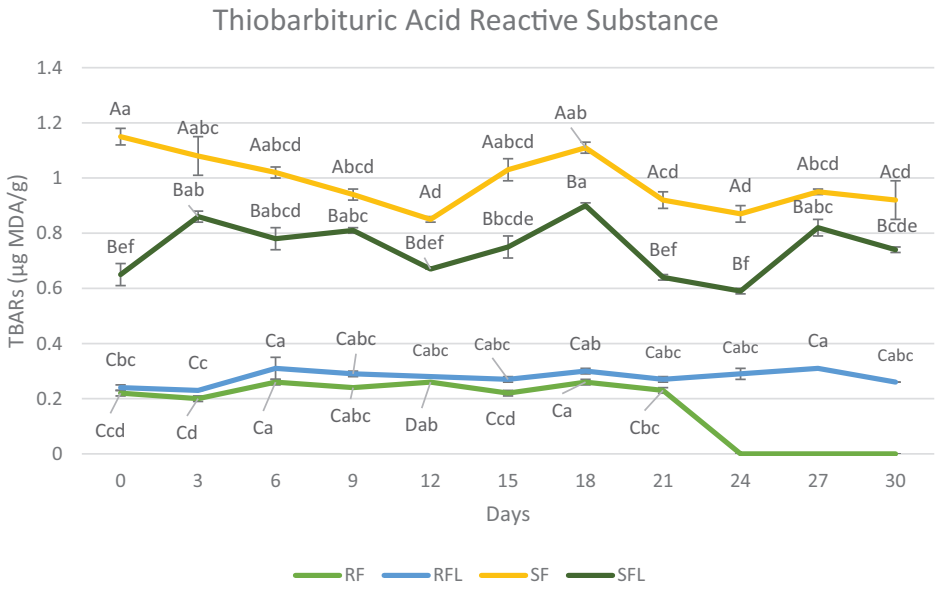

FIGURE 5 | TBARs values of experimental groups. RF, raw fish; RFL, raw fish treated with LON; SF, steamed fish; SFL, steamed fish treated with LON.*A, B, C, … →: There is a difference between different letters between groups ($p < .05$) *a, b, c, … ↓: There is a difference between different letters between days ($p < .05$).

well as the nanoemulsion preparation method, composition, and characterization properties.

It is well-known that cooking or heat treatment increases TBARS values by causing lipid oxidation and hydrolysis in fish meat. As a result of higher TBARS levels, undesirable odor and taste formation and quality loss occur in fish meat [16, 59]. In our study, although cooking is considered a disadvantage in terms of TBARS values, LON was found to be more effective in inhibiting lipid oxidation in SF (SFL) compared to RF (RFL). Cinnamon, clove, and thyme nanoemulsions have been reported to reduce lipid oxidation in steamed chicken fingers compared to the control group during storage [60]. Similar results were observed between SF and SFL groups in our study. In the steamed samples, the LON-treated group exhibited significantly lower lipid oxidation ($p < 0.05$). In another study, Ceylan et al. [16] reported the TBARS values of mackerel fish cooked in a conventional oven as 4.28, 12.72, and 12.67 µg MDA/g on Days 1, 6, and 11, respectively. For conventional oven-cooked fish treated with wheat germ oil nanoemulsion, the values were 3.59, 8.13, and 9.02 µg MDA/g, respectively. The study concluded that wheat germ oil nanoemulsion effectively limited lipid oxidation in cooked fish. Unlike Ceylan et al. [16], the nanoemulsion application in our study was performed after cooking. These findings suggest nanoemulsion application can be effective both before and after cooking in preventing lipid oxidation. However, further comprehensive studies are needed to better understand the efficacy of applications.

3.6 | FFA Values

FFAs are not bound as triglycerides and are formed as a result of hydrolysis of liquid and/or solid fats. They are affected by factors such as time, temperature, and humidity [61]. The FFA values of LON and experimental groups are shown in Figure 6. Although the FFA value of the LON was 0.18 on Day 0 of storage, it almost doubled to 0.35 on the final day of storage. To date, there are no studies in literature specifically investigating the changes in FFA levels of nanoemulsions during any storage period. However, several studies have examined FFA changes in fish and shrimp meat following the application of nanoemulsions [15, 62, 63]. In our study, the FFA values of LON stored at 4°C ± 2°C remained stable only on certain days up to Day 24, with an increase observed on Day 27. The observed increase in FFA values of LON over time is a significant finding, particularly for studies investigating the effects of different nanoemulsions on meat, fish, chicken, pork, and other products. This result suggests that nanoemulsions may not maintain full stability depending on the storage time, which is consistent with the pH and color instability of LON observed in the present study.

FFAs are recommended to be between 1% and 7% for food-grade fish oils [64]. On Day 0 of storage, the FFA values of RF, RFL, SF, and SFL groups were determined as 1.33%, 1.38%, 2.19%, and 1.48%, respectively. Although the FFA value of the RF group was 2.75% on the last day of storage (Day 21), the end of storage values of RFL, SF, and SFL groups (Day 30) increased to 3.39%, 2.50%, and 2.26%, respectively. At the end of storage, the FFA value of RFL group was found to be significantly different from SF and SFL groups ($p < 0.05$). In addition, the FFA values of the SF group were higher than those of the other groups until Day 24. Although a general increase was observed in all groups during storage, the highest value at the end of storage was found in the RFL group with 3.39%, whereas the lowest was in the SF group with 2.26% ($p < 0.05$). Similar to the TBARS results, FFA values increased after cooking. However, applying nanoemulsion to fish meat after cooking positively affected the quality of the fish meat by decreasing FFA values.

Ozogul et al. [14] reported the FFA value of raw trout as 4.92% on Day 0 and 12.54% at the end of storage (Day 24). Although fluctuations in FFA values were observed in the groups

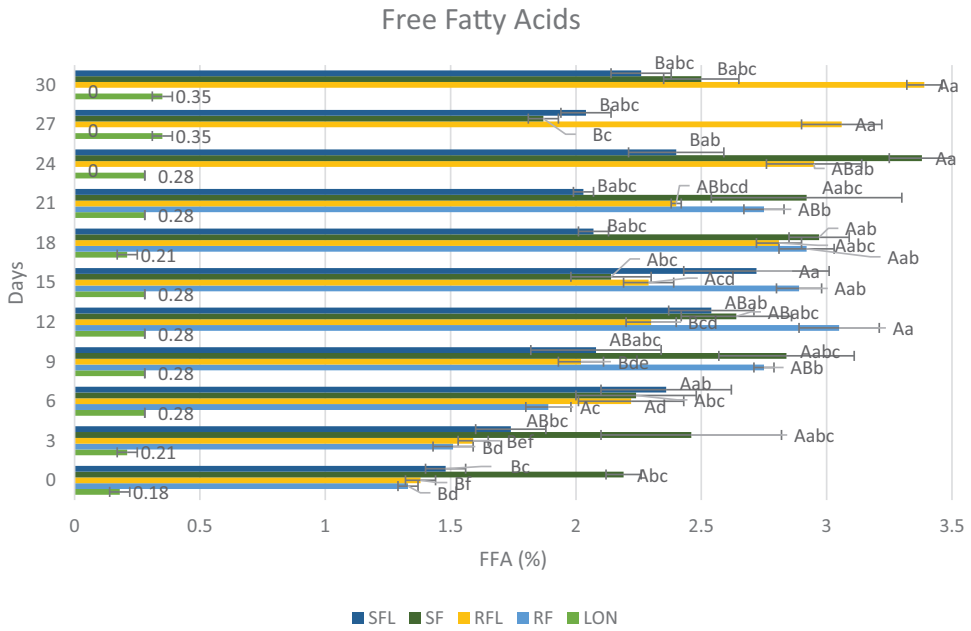

FIGURE 6 | FFA values of lemon oil nanoemulsion (LON) and experimental groups. RF, raw fish; RFL, raw fish treated with LON; SF, steamed fish; SFL, steamed fish treated with LON.*A, B, C, … →: There is a difference between different letters between groups ($p < .05$) *a, b, c, … ↓: There is a difference between different letters between days ($p < .05$).

treated with thyme, rosemary, laurel, and sage nanoemulsions, it was reported that nanoemulsions slowed down lipid oxidation compared to the control group. In another study, the FFA value of raw trout was reported as 2.38%, which decreased to 2.21% in the group treated with LON on Day 0 of storage. Over a 16-day storage period, FFA values in the LON-treated group showed fluctuations similar to those observed in our study, with a final FFA value of 2.31% reported at the end of storage [15]. Hashemi et al. [1] applied nanoemulsions containing different concentrations of *Zataria multiflora* essential oil to raw trout and investigated FFA changes during storage at 4°C ± 1°C. On Day 0, the FFA values for raw trout treated with 0.25%, 0.5%, and 1% nanoemulsions were 4.21%, 4.20%, and 4.20%, respectively. By Day 16, these values had increased to 6.88%, 5.61%, and 5.61%, respectively. The lowest FFA level at the end of storage was observed in the group treated with 1% nanoemulsion. In our study, although there were no significant differences between RF and RFL groups, except on Days 9 and 12, when SF and SFL groups were compared, the FFA values, which increased with the cooking process, decreased with the application of LON. This suggests that LON had a positive effect on reducing the FFA values in cooked fish meat. In addition, it can be stated that none of the groups exceeded the consumability threshold (1%–7%) during the storage period, indicating that the samples remained at good quality.

3.7 | Total Fatty Acids and LQI Values

The total fatty acids and lipid quality index results of experimental groups are shown in Table 2. The ΣSFA, ΣMUFA, and ΣPUFA values were similar in RFL, SF and SFL groups on the first day of storage ($p > 0.05$). When Day 0 and the final day of storage (Day 30) were compared, it was observed that the ΣSFA value decreased in the RFL and the SF groups, whereas it increased in the SFL group ($p < 0.05$). A slight increase in ΣMUFA values was observed in RFL group, whereas ΣMUFA values decreased in SF and SFL groups ($p < 0.05$). At the end of storage, ΣPUFA values increased in all three groups—RFL, SF, and SFL ($p < 0.05$).

Durmus and Ozogul [65] applied nanoemulsions prepared with sunflower, canola, corn, soybean, hazelnut, and olive oils to sea bass fillets and examined fatty acid profiles at 2°C ± 2°C. After 12 days, they reported an increase in SFA levels and decrease in MUFA and PUFA levels in both control and nanoemulsion treatment groups. Similarly, Ucar [17] reported the ΣSFA, ΣMUFA, and ΣPUFA values of trout treated with lemon oil as 17.18%–17.18%, 45.26%–45.25%, and 28.98%–28.01% on Days 0 and 16, respectively. Accordingly, it was reported that total SFA and MUFA values remained stable, whereas PUFA values decreased. These values are lower than total SFA and PUFA values and higher than total MUFA values of raw trout treated with LON in our study. In another study, raw trout were treated with nanoemulsions containing different concentrations of *Z. multiflora* essential oil (0.25%, 0.5%, 1%). At the end of 16 days (end of storage), the lowest total SFA value (21.62%) was found in the groups treated with 0.5% and 1% nanoemulsions. However, ΣSFA values increased in all nanoemulsion-treated groups by the end of storage. It was reported that total MUFA values increased, whereas PUFA values decreased in all nanoemulsion groups at the end of storage compared to Day 0 [1]. The differences in SFA, MUFA, and PUFA values of raw trout between our study and other studies may be attributed to various factors such as fish species, storage method and duration, and the type and preparation method of the nanoemulsion. Incorporation of essential oils (thyme) into nanoemulsions has previously been reported to prevent lipid oxidation in fish burgers [66]. In our study, the application of LON to raw trout resulted in a decrease in ΣSFA values and increases in ΣMUFA and ΣPUFA values at the end of storage. However, these differences were not statistically significant ($p > 0.05$). Unusan [67] reported that SFA and PUFA levels decreased,

TABLE 2 | Total fatty acids and lipid quality index results of experimental groups.

	0				15			30		
Days	**RF**	**RFL**	**SF**	**SFL**	**RFL**	**SF**	**SFL**	**RFL**	**SF**	**SFL**
ΣSFA	24.16 ± 0.19	26.74 ± 0.26^{BC}	26.62 ± 0.15^{BCD}	27.02 ± 0.08^{B}	26.97 ± 0.18^{B}	25.71 ± 0.30^{D}	27.22 ± 0.27^{B}	25.83 ± 0.14^{CD}	24.39 ± 0.08^{E}	28.17 ± 0.02^{A}
ΣMUFA	31.02 ± 0.15	32.15 ± 0.07^{ABC}	32.35 ± 0.27^{AB}	31.80 ± 0.01^{BC}	32.47 ± 0.10^{A}	31.84 ± 0.11^{BC}	30.64 ± 0.11^{D}	32.73 ± 0.05^{A}	31.64 ± 0.14^{C}	30.22 ± 0.05^{D}
ΣPUFA	44.73 ± 0.12	40.78 ± 0.10^{EF}	41.03 ± 0.19^{DEF}	41.15 ± 0.08^{DEF}	40.55 ± 0.07^{F}	42.42 ± 0.19^{B}	42.11 ± 0.22^{BC}	41.43 ± 0.10^{DE}	43.96 ± 0.05^{A}	41.57 ± 0.04^{CD}
Σ*n*-3	20.86 ± 0.09	19.19 ± 0.06^{DE}	19.53 ± 0.08^{CD}	19.58 ± 0.06^{CD}	18.96 ± 0.04^{EF}	19.77 ± 0.10^{BC}	19.98 ± 0.16^{B}	18.72 ± 0.05^{F}	20.88 ± 0.03^{A}	19.73 ± 0.05^{BC}
Σ*n*-6	20.41 ± 0.08	18.30 ± 0.03^{DE}	18.15 ± 0.25^{E}	18.49 ± 0.04^{DE}	18.42 ± 0.03^{DE}	19.43 ± 0.07^{AB}	18.98 ± 0.09^{BC}	19.47 ± 0.05^{A}	19.88 ± 0.03^{A}	18.75 ± 0.05^{CD}
Total	99.91 ± 0.46^{A}	99.67 ± 0.32^{A}	99.99 ± 0.00^{A}	99.97 ± 0.01^{A}	99.98 ± 0.02^{A}	99.96 ± 0.01^{A}	99.97 ± 0.01^{A}	99.99 ± 0.01^{A}	99.99 ± 0.01^{A}	99.96 ± 0.01^{A}
Unidentified	0.09 ± 0.07^{A}	0.33 ± 0.32^{A}	0.01 ± 0.00^{A}	0.03 ± 0.01^{A}	0.02 ± 0.02^{A}	0.04 ± 0.01^{A}	0.03 ± 0.01^{A}	0.01 ± 0.01^{A}	0.01 ± 0.01^{A}	0.04 ± 0.01^{A}
n-3/*n*-6	1.02 ± 0.00	1.05 ± 0.00^{ABC}	1.08 ± 0.02^{A}	1.06 ± 0.00^{AB}	1.03 ± 0.00^{BC}	1.02 ± 0.00^{C}	1.05 ± 0.01^{ABC}	0.96 ± 0.00^{D}	1.05 ± 0.00^{ABC}	1.05 ± 0.00^{ABC}
AI	0.29 ± 0.00	0.30 ± 0.00^{AB}	0.31 ± 0.00^{A}	0.27 ± 0.00^{D}	0.30 ± 0.00^{ABC}	0.31 ± 0.01^{AB}	0.29 ± 0.00^{C}	0.29 ± 0.00^{ABC}	0.29 ± 0.00^{BC}	0.30 ± 0.00^{ABC}
TI	0.23 ± 0.00	0.25 ± 0.00^{ABC}	0.26 ± 0.00^{A}	0.22 ± 0.00^{E}	0.25 ± 0.00^{BCD}	0.25 ± 0.01^{AB}	0.23 ± 0.00^{DE}	0.25 ± 0.00^{ABC}	0.24 ± 0.00^{DE}	0.24 ± 0.00^{CD}
H/H	4.66 ± 0.03	4.56 ± 0.04^{BCD}	4.48 ± 0.03^{CD}	5.01 ± 0.01^{A}	4.61 ± 0.05^{BCD}	4.44 ± 0.09^{D}	4.70 ± 0.02^{B}	4.67 ± 0.04^{BC}	4.64 ± 0.02^{BCD}	4.58 ± 0.01^{BCD}
PI	1.34 ± 0.01	1.22 ± 0.01^{C}	1.20 ± 0.01^{C}	1.43 ± 0.00^{A}	1.21 ± 0.01^{C}	1.20 ± 0.03^{C}	1.34 ± 0.01^{B}	1.19 ± 0.01^{C}	1.35 ± 0.01^{B}	1.33 ± 0.00^{B}
FLQ	14.53 ± 0.06	13.01 ± 0.01^{EF}	13.13 ± 0.07^{E}	14.44 ± 0.07^{B}	12.79 ± 0.02^{F}	13.59 ± 0.10^{D}	14.13 ± 0.11^{C}	12.94 ± 0.05^{EF}	15.13 ± 0.02^{A}	14.32 ± 0.04^{BC}
EPA/DHA	0.44 ± 0.00	0.46 ± 0.00^{AB}	0.45 ± 0.00^{B}	0.42 ± 0.00^{C}	0.45 ± 0.00^{B}	0.43 ± 0.00^{C}	0.43 ± 0.00^{C}	0.46 ± 0.00^{A}	0.43 ± 0.00^{C}	0.43 ± 0.00^{C}

Note: → There is a difference among different letters (A–C) between groups on the same day ($p < 0.05$).

Abbreviations: AI, atherogenic index; H/H, hypocholesterolemic/hypercholesterolaemic; LON, lemon oil nanoemulsion; PI, polyene index; PUFA, polyunsaturated fatty acid; RF, raw fish; RFL, raw fish treated with LON; SF, steamed fish; SFL, steamed fish treated with LON; TI, thrombogenicity index.

whereas MUFA levels increased in raw trout after oven cooking. In contrast, in our study, SFA and MUFA values increased and PUFA values decreased following the cooking process. However, by the end of storage, ΣSFA and ΣMUFA values of cooked trout decreased, whereas ΣPUFA values increased ($p < 0.05$). Additionally, at the end of storage, ΣSFA ($p < 0.05$) and ΣPUFA ($p > 0.05$) values of trout treated with LON after cooking were found to have increased, whereas the ΣMUFA ($p < 0.05$) value decreased. Ceylan et al. [16] reported that an increase in SFA values due to PUFA loss after cold storage in fish meat is an expected outcome. However, this effect was not observed in the cooked trout samples in our study. On the contrary, SFA and MUFA values decreased, whereas PUFA values increased at the end of storage. In addition, both SFA and PUFA values increased and MUFA values decreased at the end of storage in the group treated with LON after cooking (SFL). This situation is believed to be linked to a series of events beginning with the decrease in lipid oxidation due to decreased microbiological and enzymatic activity following the cooking process and then continuing with factors such as the protective effect of LON, vacuum packaging, and other storage conditions. However, further research is required to understand these findings.

AI and TI values were similar in the RFL and SF groups but were significantly lower in the SFL group ($p < 0.05$). It has been reported that AI and TI values should be below 1 to prevent heart diseases [32, 68]. In our study, AI and TI values remained within the recommended range in all groups on Days 0, 15, and 30. Additionally, it can be concluded that the SFL group is the best group from a health perspective. Kocatepe et al. [25] reported the AI and TI values of raw trout as 0.42 and 0.83, respectively, which are higher than the values found in our study. In our study, AI and TI values of raw trout were 0.29 and 0.23, respectively, whereas Ucar [17] reported these values as 0.26 and 0.25. For raw trout treated with LON, the researcher reported AI and TI values of 0.25 and 0.24, respectively. In comparison, our study found these values to be 0.30 and 0.25. It can be concluded that these values are comparable to those reported by Ucar [17].

H/H, PI, and FLQ values were higher in the SFL group compared to other groups ($p < 0.05$). A high H/H value is desirable for healthy nutrition. Although the H/H value of raw trout was 4.66, this value was 4.56 in the group treated with LON. Ucar [17] reported the H/H value of raw trout as 4.54 at the beginning of storage. Following the LON treatment, the H/H value was reported as 4.76 at the beginning of storage and 4.71 at the end of storage, showing values comparable to those found in our study. Additionally, in our study, the H/H value of cooked trout increased from 4.48 at the beginning of storage to 4.64 at the end. In the cooked group treated with nanoemulsion, these values were 5.01 at the beginning and 4.58 at the end of storage. This trend is believed to be related to the positive effect of cooking and nanoemulsion application on H/H values and their decrease with storage time.

The PUFA/SFA ratio, also known as PI, is usually considered a measure of oil polyunsaturation and its sensitivity toward oxidation [69]. PI was found to be the lowest in the RFL group and significantly different from the other groups ($p < 0.05$). Although the PI value of raw trout was reported as 0.69, it was 0.71 in the group treated with LON [17]. In our study, however, the PI values of raw trout and raw trout treated with lemon oil were higher (1.34 and 1.22, respectively) according to Ucar [17]. This is likely due to the different biochemical contents of the trout which may be influenced by factors such as age, size, diet, and freshness.

On Days 0 and 15 of storage, the PI value was significantly higher in the SFL group compared to the SF group ($p < 0.05$). However, the difference between SFL and SF groups was not significant at the end of storage ($p > 0.05$). Similarly, Ceylan et al. [16] reported an increase in the PI value of cooked fish (*Scomber scombrus*) during storage in the group treated with wheat germ oil nanoemulsion, compared to the control group. This may suggest that post-cooking nanoemulsion application to fish meat makes PUFAs sensitive to spoilage. However, further research is needed to better understand this phenomenon.

Flesh lipid quality (FLQ) is calculated by multiplying the ratio of total EPA + DHA to total fatty acids by 100, and it represents the flesh lipid quality. In our study, FLQ value of raw trout was found to be 14.53%. Kocatepe et al. [25] reported an FLQ value of 7.42 in trout. The highest FLQ values on Days 0 and 15 of storage were found in the SFL groups. However, by the end of storage, the highest FLQ value was found in the SF group, followed by the SFL group ($p < 0.05$). These results suggest that both the cooking process and the combination of cooking process and nanoemulsion application positively affected the FLQ values of trout.

4 | Conclusion

The application of nanoemulsion to fish meat without heat treatment positively affected the pH. In parallel with the TBARS values, the FFA values of the RFL group, which was not subjected to heat treatment, were lower, except on Day 15 and during the last 2 days of storage. However, the combination of heat treatment and nanoemulsion generally showed a positive effect on FFA values during the final days of storage. Water activity values did not differ between the groups. Color, pH, and FFA values of LON showed changes over the storage period, indicating that the stability of these parameters depends on storage duration. Additionally, the combined application of nanoemulsion and heat treatment positively affected the whiteness (L^*) value of fish meat. At the beginning of storage, the combined application of heat treatment and nanoemulsion to fish meat showed better results in AI, TI, H/H, and FLQ values. However, by the end of storage, the best FLQ value was observed in the heat-treated group only. As a result, although nanoemulsion application without heat treatment had a positive effect on pH, its application to heat-treated fish meat had a positive effect on L^* value, TBARS, FFA, and lipid quality parameters (except PI value).

Author Contributions

Bengunur Corapci: conceptualization, methodology, validation, writing – original draft, writing – review and editing. **Hulya Turan**: validation. **Can Okan Altan**: investigation, validation, writing – review and editing. **Bayram Kostekli**: investigation. **Zafer Ceylan**: validation. **Demet Kocatepe**: investigation, validation.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Data will be made available on request.

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