



Production of Rosmarinic Acid Nanoparticles, and Investigation of Anti-Oxidation Effects on Salmon Fish Meat

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Abstract: In this study, an anti-oxidant nanoformulation to prevent the oxidation of salmon was developed using rosmarinic acid (RA). Minced salmon samples (MSs) were treated with 8.10^{-3} g (RAT1) and 16.10^{-3} g (RAT2) RA-loaded nanoparticles for 100 g MS rosmarinic acid, separately. The thiobarbituric acid (TBA) values of control (C), RAT1, and RAT2 samples stored at 4 °C were found as 2.995, 1.350, and 0.994 mg MDA/kg; respectively, after 9 days. While the initial free fatty acid (FFA) value of C samples was 2.011%, RAT1 and RAT2 were found as 2.765% and 2.494%, respectively. The 2,2-diphenylpicrylhydrazyl (DPPH) values of MSs treated with RAT1 and RAT2 were observed to be higher than that of C samples. C samples were evaluated as unfit for human consumption on the 5th day of the storage, it was revealed that but the sensory scores of MSs treated with RAT1 and RAT2 were still acceptable for human consumption.

Keywords: Nanotechnology application, nanoparticle, oxidation, rosmarinic acid, salmon quality.

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INTRODUCTION

Well-balanced nutrition is a very crucial issue for consumers. In this respect, fish is a unique essential nutrient source for human (1). However, as soon as fish samples are caught, they must be protected by using appropriate food preservation methods. Therefore, different conventional food preservation methods are already used to delay the rapid deterioration in fish meat after harvesting. Ionizing irradiation treatment, packaging treatments, food additives, chilling, and freezing technology are widely used to limit the rapid deterioration in fish all over the world (2-4). Yet, food additives are applied to processed, lightly processed, or raw fish samples.

Furthermore, most of the food additives used in the fish processing industry are based on chemical origin. Since natural or organic-based food additives are highly expensive, they are not preferred by the fish processing industry. Also, some of the micro or macro natural-based food additives may have a lower antioxidant or bactericidal effect on fish fillets/samples. In this regard, cost-effective food preservation methods having stronger antimicrobial and antioxidant properties are currently being tried, particularly in the scientific area. For example, in the last decade, there has been a significant increase in microencapsulation methods. Moreover, food nanotechnology applications such as nanoencapsulation, nanocoating, nanoemulsions,

nanoparticles, nanofibers recently take a great deal of attention in the food industry and scientific area. Ceylan, Sengor (5) revealed that nanofibers could be used as a nanocoating material for delaying the chemical deterioration in fish fillets. Özogul, Durmus (6) noted that nanoemulsions could be utilized to limit the rapid deterioration of fish samples. Osheba, Sorour (7) revealed that chitosan nanoparticle application successfully limited the rapid increase in chemical deteriorations parameters such as total volatile nitrogen, trimethylamine, and thiobarbituric acid in fish fingers. Ceylan, Sengor (8) reported that nano-thymol and the liquid smoke application delayed the rapid microbial spoilage in fish fillets during cold storage conditions. Also, the encapsulation of bio-based materials and functional ingredients are important and promising approaches for food nanotechnology applications. In this respect, encapsulations of nisin (9), rosehip seed oil (10), α -tocopherol (11), and zinc oxide (12) were successfully carried out, as can be seen from previous literature studies, as well. Additionally, nanomats that integrated with curcumin and nisin (13) or pomegranate seed oil (14), grape seed oil-loaded nanofibers (15) and zein nanofibers (16) effectively improved the acceptability of fish meats during cold storage.

In this study, rosmarinic acid (RA), which is one of the polyphenolic substances contained in culinary herbs such as perilla, rosemary, sage, mint, and basil, was used. Therefore, there are many studies in which rosmarinic acid is provided with effective antioxidants and other quality properties. To the best of our knowledge, the number of antioxidant and lipid oxidation studies about RA-loaded nanoparticles of the salmon fish is limited. In this sense, the initial aim of the study was to encapsulate RA and characterize RA nanoparticles, such as average particle size and in vitro release profile. Also, the main aim of the present study was to investigate the potential antioxidative effect of RA-loaded nanoparticles on the salmon fish mince stored under 4 °C, and its effects on its shelf life.

MATERIALS AND METHOD

Materials

Salmon fish samples were obtained from an international supermarket in Konya, Turkey. The fish samples were immediately transferred to the food processing laboratory. RA (96%, Mw=360.31 g/mol), polycaprolactone (PCL) (Mn=80,000), and polyvinyl alcohol (PVA) (Mw = 31,000–50,000, 87–89%) were purchased from Sigma-Aldrich (USA).

Treatment with Nanoparticles of Fish Samples

The samples were grouped as control (C), RAT1 (8.10⁻³ g RA-loaded nanoparticle/ 100 g minced fish), and RAT2 (16.10⁻³ g RA-loaded nanoparticle/ 100 g of minced fish), as compared to RAT1). 100 g of fish samples were separated as C, RAT1, and

RAT2, respectively. Following the nano treatment, the minced fish samples were placed into the locked plastic pouch and then stored at 4 °C for 9 days.

Calibration Process

The calibration curve of RA was obtained using UV-Vis Spectrophotometer (Shimadzu, Japan). Standard solutions of RA were prepared to give final concentrations of 1.5625, 3.125, 6.25, 12.5, and 25 μ g/ mL. Then the absorbance values of these samples were measured by UV-Vis Spectrophotometer at 324 nm, and the calibration curve was plotted.

Preparation of RA-Loaded PCL-Based Nanoparticles

The double-emission precipitation method was used to prepare the RA-loaded PCL nanoparticles. 10 mg of RA was dissolved in 1 mL of distilled water, 30 mg of PCL was dissolved in dichloromethane, and then the PCL solution was added to the RA solution, respectively. All solutions were mixed and sonicated for 10 minutes using an ultrasonicator at 55 W. This solution was placed into a syringe and then added dropwise into 4 mL of 5% PVA solution under continuous stirring. Following these first step procedures, the solution was sonicated again for ten minutes. After that, the mixture was left overnight under continuous stirring in the sonicator. Finally, the obtained RA-loaded nanoparticles were isolated by centrifugation (at 10,000 rpm for 30 min). The samples were washed three times to remove the organic solvent and freeze-dried for further characterization analysis and food nanotechnology applications.

Zeta Size (ZS) of Nanoparticles

The average size distribution of RA-loaded nanoparticles was obtained using a Zeta-Sizer Nano ZS (Malvern Instruments, Malvern, UK) instrument equipped with a 4.0 mV He-Ne laser.

Encapsulation Efficiency

The loading capacity (LC) and encapsulation efficiency (EE) of RA-loaded PCL nanoparticles were measured by separating the nanoparticles from the aqueous nanoparticle suspension via centrifugation. In this respect, the concentration of free RA in the supernatant was determined using a UV-visible Spectrophotometer via the RA standard curve. The loading capacity and encapsulation efficiency of RA-loaded PCL nanoparticles were respectively calculated by the formulas given below. Encapsulated RA amount was calculated by UV-vis absorbance value of supernatant which was obtained after centrifugation of the RA loaded PCL nanoparticles. The UV-vis absorbance value of supernatant put the formula which was obtained from the calibration curve.

$$EE = ((\text{Total RA Amount} - \text{Free RA Amount}) / \text{Total RA Amount}) \times 100 \quad (\text{Eq. 1})$$

$$LC = \left(\frac{\text{Encapsulated RA}}{\text{Total Nanoparticles Weight}} \right) \times 100 \quad (\text{Eq. 2})$$

The Amount of Released RA

To determine the in vitro release profile, the release study experiment was conducted at the optimum storage temperature (+4 °C) for meat products. 1 mg of RA-loaded PCL nanoparticles was dissolved in 2 mL distilled water and placed in a dialysis capsule. 100 mL PBS media at pH = 7.2 phosphate buffer solution was used as release medium. At designated time intervals, 1 mL of the release medium was taken and replaced by an equal volume of fresh pre-warmed release medium. The amount of released RA in samples was analyzed by UV-Vis spectrometer at 324 nm wavelength. The amount of RA released at the end of 144 h was evaluated from Equation 3, and the % amount released over time is plotted.

$$\text{Release Amount (\%)} = \left(\frac{\text{Released Amount of RA}}{\text{Total Amount of RA}} \right) \times 100 \quad (\text{Eq. 3})$$

Thermogravimetric Analysis (TGA) of Nano-Scale Material

Thermal behaviors of PCL nanoparticles and RA-loaded PCL nanoparticles were determined using Thermogravimetric Analyzer, TGA (SDT Q600, TA Instruments, and Newcastle, DE USA). As stated by Ceylan, Meral (17) continuous nitrogen flow at a rate of 20 mL/min in the temperature range of 25 °C to 800 °C at a rate of 10 °C min⁻¹ was applied.

Thiobarbituric Acid (TBA) Analysis

10 g of minced salmon fish samples, 4 N HCl (2.5 mL), and 97.5 mL of distilled water were added into the flask and then heated for distillation. After the distillation, 5 mL from the distillate sample was taken and added to the test tubes. All tubes were heated in hot water at 85°C for 30 min. Absorbance values of C, RAT1, and RAT2 were measured at 530 nm using a spectrophotometer (Shimadzu-UV mini 1240, Kyoto-Japan). Finally, TBA results of the samples were calculated as mg malondialdehyde per kg (mg MDA kg⁻¹) according to Tarladgis, Watts (18).

Antioxidant Activity Measurement by DPPH

The antioxidant capacity of the minced salmon samples was determined using a modified method as described by Brand-Williams, Cuvelier (19). Salmon fish samples, 5 g per sample, were mixed with 25 ml of methanol in ice Polytron homogenizer, centrifuged at 7000 × g for 10 min, and filtered by using Whatman No. 1. The supernatant was mixed with methanol to a volume of 25 mL, and then 50 µL was added to 2950 µL of 100 µM DPPH in methanol solution in a test tube. Methanol, 50 µL, and 2950 µL DPPH solution were used to be a blank.

The tubes were covered with parafilm, vortexed, and kept in the dark at room temperature. The absorbance value of the solution was obtained at 517 nm. The standard curve was developed with ascorbic acid and DPPH.

Free Fatty Acid (FFA)

FFA value as an index of fish fat hydrolyzes was determined according to Egan, Kirk (20). The samples were extracted and then 25 mL of the extract was mixed with 25 mL of ethyl alcohol (95%). The mixture was titrated against 0.1 N NaOH using an indicator (phenolphthalein). The percentages of FFAs in C, RAT1, and RAT2 samples were calculated as oleic acid by using the formula below.

$$\text{FFA} = \left(\frac{V \times N \times 2.82}{W} \right) \quad (\text{Eq. 4})$$

V: Volume of NaOH

N: Normality of NaOH

W: Weight of Lipid in Extractions

Sensory Evaluation

Sensory deteriorations in C, RAT1, and RAT2 groups were evaluated by trained sensory panelists as stated by Fan, Sun (21) (n=10). The samples were presented to the trained panelists in a room under well-ventilated and lighted conditions every analysis day. A 10-point hedonic scale was used to evaluate the samples in terms of the overall sensory score (SOVS; obtaining from odor, texture, and color parameters). Score 5.0 was considered as the borderline of all minced fish samples (C, RAT1, and RAT2) acceptability.

Statistical Analysis

All measurements were repeated twice with three replications. Collected data were subjected to analysis of variance (ANOVA) to evaluate the TBA, DPPH, FFA, and SOVS in all groups. Graphpad Prism software Version 5.00 (California Corporation, CA) was used to reveal significant differences between C, RAT1, and RAT2, and also comparisons of all differences among them were evaluated by the Tukey's Multiple Range Test (p<0.05).

RESULTS AND DISCUSSION

Particle Distribution of Nanoparticles

RA solutions were prepared with different concentrations (1.5625, 3.125, 6.25, 12.5, and 25 µg/mL). Then the absorbance values of these samples were measured by UV-Vis Spectrophotometer at 324 nm, and the calibration curve was plotted (Figure 1).

Zeta Size values of PCL and RAT-loaded PCL nanoparticles were determined to be 213.9±3.52 and 235.8±2.98 nm, respectively Figure 2A and 2B (Table 1).

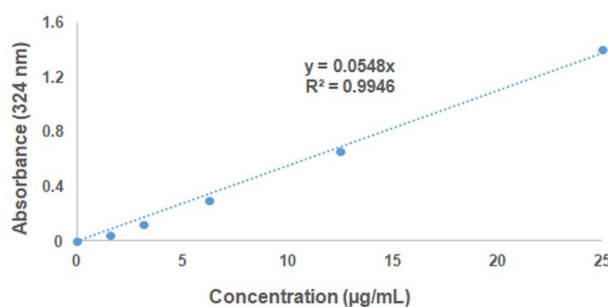


Figure 1: The calibration curve.

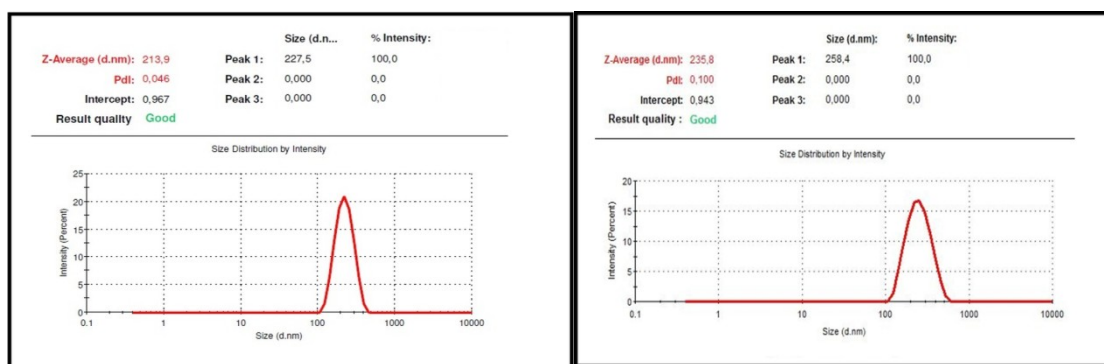


Figure 2: Zeta Size result of (A) blank and (B) RA PCL nanoparticles.

Table 1: Average particle size of nanoparticles.

Samples	Average Particle Size (nm)
PCL NP	213.9±3.52
RAT NP	235.8±2.98

PCL and RAT NPs define poly(ε-caprolactone) and rosmarinic acid-loaded poly(ε-caprolactone), respectively.

The in vitro release study was performed and the release profile graph was drawn based on time and cumulative RA release (%) (Figure 3) by using calibration curve data of RA. It was determined that RA was released 58.46% in the first 24 h. Moreover,

93.85% of RA was released from the RA-loaded PCL nanoparticles end of the 144 th hour. According to the results of in vitro release study, we concluded that the active ingredient (RA) has a controlled manner and slow-release rate over time.

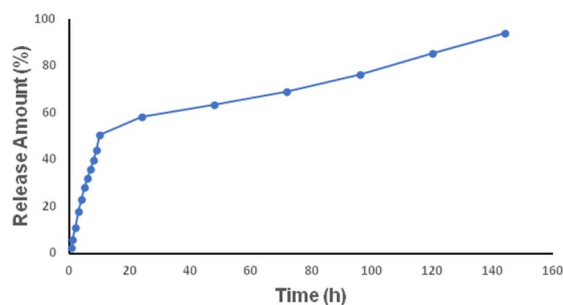


Figure 3: The cumulative RA release.

Loading of RA into PCL increased the average diameter of the RA-loaded nanoparticle according to the results of zeta size. Also, the biggest RA-

loaded nanoparticle detected by TEM analysis was given in Figure 4.

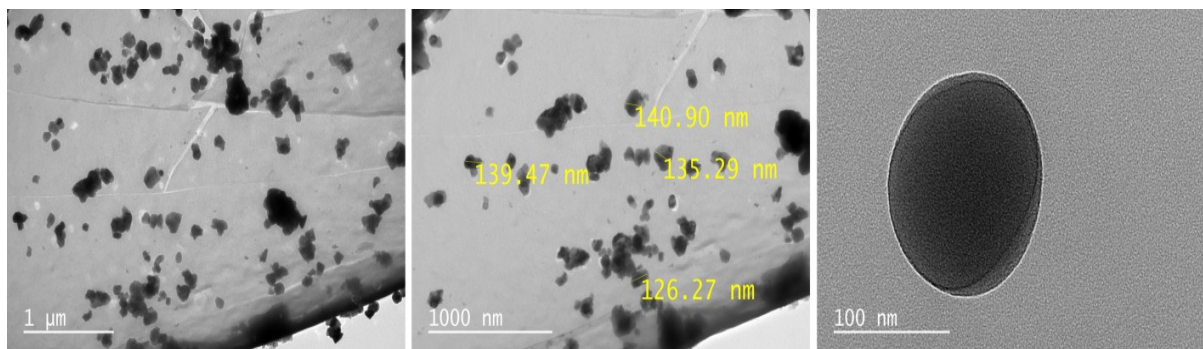


Figure 4: TEM image of RA-loaded nanoparticle.

PCL was used as a nanocarrier in the present study, the antioxidant activity of RA-loaded PCL nanoparticles on the minced salmon samples was revealed. In any nanotechnology application, the diameter of the nanomaterial can play a key role in food applications. Loading of RA into PCL provided an antioxidant effect on the minced salmon samples for 9 days, although RA did not significantly increase the diameter of the nanoparticle ($p < 0.05$). On the other hand, as can be seen from the previous study (8), loading of thymol and liquid smoke into chitosan nanofiber increased the average diameter of the chitosan-based nanofiber. Also, according to Merrell, McLaughlin (22), the diameter of PCL nanofibers fabricated by the electrospinning technique was found to be between 300 and 400 nm. da Silva, Ferreira (23) reported that RA encapsulated chitosan nanoparticles could have a diameter ranging from 200 to 300 nm. In this respect, the loading of RA possessing 235.8 ± 2.98 nm diameter provided a larger surface area to prevent the rapid oxidation of the minced salmon for 9 days.

Loading Efficiency and Encapsulation of RA-loaded PCL Nanoparticles

The encapsulation efficiency of the RA was found to be $99.0 \pm 0.35\%$. This ratio revealed that the RA was highly successfully encapsulated and also effective RA-loaded PCL nanoparticles were obtained. The loading efficiency of the RA-loaded PCL nanoparticles for each 10 mg was found as 9.2 mg of RA. For this study, loading and encapsulation efficiency are important to reveal the effectiveness of RA nanoparticles. Also, the results revealed that the high loading and encapsulation efficiency of RA known as an antioxidant may affect the stability of TBA, DPPH, FFA, and SOVS parameters obtained from the minced salmon samples. In the previous study, Snehalatha, Venugopal (24) reported that encapsulation and loading efficiency of etoposide (into PCL) were found to be 80.15% and 28.8%, respectively. In another study, the loading capacity

of RA in chitosan nanoparticles was defined to be lower as compared to our study results (25). As can be also seen from the previous study results, the nanocarrier and loading material play an important role to obtain effective productivity from the nanomaterials.

Thermal Decomposition of the Nanoparticles

The loss mass of the RA-loaded PCL nanoparticles and PCL nanoparticles was detected by the TGA analysis, as can be seen from Figures 5 and 6. Cold storage, especially at about 4 °C, is important in the fish processing industry. Therefore, revealing the nanoparticle's thermal decomposition in all mentioned temperature ranges (e.g. 60 °C - 80 °C, 121 °C) is much more important for further food nanotechnology studies as well. In this respect, there was no remarkable mass loss for PCL and RA nanoparticle samples in the range of cold storage temperatures. However, a mass reduction < 0.1328 mg (5.662%) and < 0.04301 mg (2.970%) was recorded respectively for PCL and RA nanoparticle samples at about in the range of 65 and 80 °C. As compared to 80°C, there was no remarkable mass reduction for both nanoparticle samples at 121 °C.

Thermal decomposition started at 250 °C for PCL nanoparticles with above 15%, but at the same temperature, loss in mass was found about 8.5% for the RA nanoparticles. Previous study results indicated that the type of the used material (probiotic bacteria or polymer etc.) play an important role to determine the thermal behavior of the nanoparticles. Ceylan, Meral (17) defined that the lost weight in nanofibers obtained from *L. rhamnosus* was found to be $\geq 5\%$ up to 200 °C degrees. However, Zhao, Li (26) reported that thermal decomposition of chitosan-based nanofibers started at 240°C and was completed at 650 °C. Therefore, the thermal behavior of obtained nanomaterials should be revealed for further studies related to food nanotechnology.

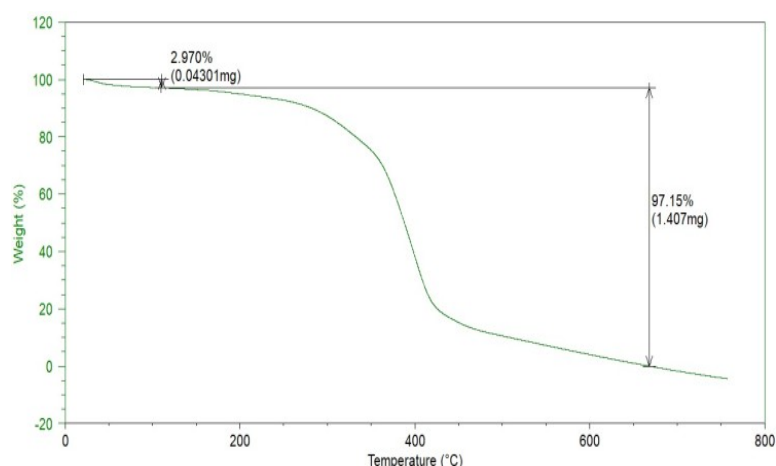


Figure 5: Thermal decomposition of rosmarinic acid-loaded PCL-based nanoparticles.

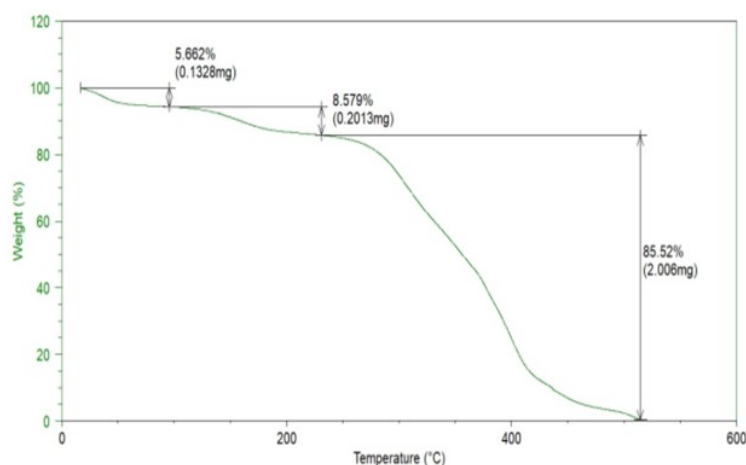


Figure 6: Thermal decomposition of PCL nanoparticles.

The Role of RA Nanoparticles in Lipid Oxidation and Antioxidant Activity (TBA)

The TBA results of processed and untreated minced salmon samples are given in Table 2. The TBA values of untreated minced salmon were higher than those of RA nanoparticles with different ratios ($p < 0.05$). While the TBA value of untreated samples was measured to be $0.51 \text{ mg MDA kg}^{-1}$ fish meat on the first day of the cold storage period, TBA values of RAT1 and RAT2 were found to be 0.18 and $0.155 \text{ mg MDA kg}^{-1}$, respectively. In another word, as compared to the control group samples, the increase in TBA of RAT1 and RAT2 samples was limited by 64.7 and 69.6% , respectively. As stated by Khalafalla, Ali (27), TBA value is evaluated to be an indicator of secondary lipid oxidation products. Therefore, in terms of revealing the lipid oxidation in fish products, it could play a key role. TBA value in fish samples, depending on the increase in storage period, can increase. With the increase of the storage period, the TBA level in C samples rapidly has increased as compared to the samples treated with nanomaterials (RAT1 and RAT2). In this

respect, on the 5th day of the cold storage, TBA values of C, RAT1, and RAT2 samples were defined as 1.12 , 0.4 (64.28% decrease), and $0.175 \text{ mg MDA kg}^{-1}$ (84.37% decrease), respectively ($p < 0.05$). At the end of the cold storage period, the TBA value of C samples reached $2.995 \text{ mg MDA kg}^{-1}$ when the TBA values of RAT1 and RAT2 samples could reach 1.35 and $0.994 \text{ mg MDA kg}^{-1}$. In addition, RA nanoparticles treatments have provided the limitation in the range of 54.92% and 84.37% for 9 days ($p < 0.05$). Roomiani, Ghaeni (28) reported that rosemary essential oils at 0.2% and 0.4% were effective in controlling lipids of fish fillets stored at -18°C for 6 months. Li, Mei (29) reported that the treatment of 1% chitosan coating incorporated with 20 , 30 , or 40 mg/L RA to delay the increase in TBA value of rainbow trout samples was successfully applied. When compared to the previous literature studies, it is seen that nanotechnology applications with 8.10^{-3} g and 16.10^{-3} g provided a big advantage to limit the rapid increase in the TBA value of minced fish products. Because nanomaterials could provide a larger contact area as compared to

microsized materials (5) and they can more successfully limit the penetration of oxygen that may cause the oxidation in fatty fish.

There is a linear correlation between the total phenolic substance amount and total antioxidant, which can be defined by the DPPH method (30). DPPH is one of the organic nitrogen radicals, also RA has phenolic compounds and fish meat has nitrogen, therefore some phenolic compounds could react with DPPH radicals. The amino acid composition, the abundance of free amino acids, peptide size, and solubility may have a key role in defining the DPPH radical scavenging capacity (31-34). The results of the DPPH (%) analysis are presented in Table 2. According to these results, the DPPH values of control group samples ranged from 1.184% to 1.669%. On the other hand, the DPPH values of the minced fish treated with RA nanoparticles rapidly increased as compared to those of the control group samples. On the 1st day of the cold storage, the DPPH value of RAT1 samples (2.843%) was found to be lower than those of the minced salmon samples treated with RAT2 samples (3.757%) ($p < 0.05$). Depending on the increase of the storage period, DPPH values of RAT1 and RAT2 samples highly increased because the release profile of RA may

begin to increase as can be seen from Figure 2. During the storage period, the DPPH value in C samples can increase because of nitrogen compounds, peptide structure in fish meat. However, DPPH values of RAT1 and RAT2 samples can more rapidly increase because they may have an interaction between the phenolic compound and nitrogen compounds in fish meat. Ceylan, Uslu (35) reported that the DPPH activity of the rainbow trout fillets treated with nanoencapsulated *L. reuteri* significantly was increased (up to 100%). Similarly, Hu, Wang (36), and Badawy, Lotfy (37) indicated that chitosan nanoparticles showed an excellent antioxidant effect for the meat samples during refrigerated storage. They also reported that the antioxidant activity significantly reduced with the storage time increase. Morsy, Mekawi (38) claimed that lyophilized pomegranate peel nanoparticles had a high phenolic content and antioxidant capacity. Meral and Köse (39) revealed that there is a good relationship between the total phenolic compounds and antioxidant activity in foods. Moreover, Meral (40) noted that antioxidant activity may be affected by different factors such as food composition, the amount of the food components, the interaction of food components, and technological processes.

Table 2: Oxidation parameters, antioxidant activity, and sensory analysis results of the minced salmon samples treated with nanoparticles and untreated samples.

Storage days	Parameters	Groups		
		C	RAT1	RAT2
1	TBA	0.510±0.000 ^A	0.180±0.000 ^B	0.155±0.000 ^C
	DPPH	1.184±0.335 ^C	2.843±0.191 ^B	3.757±0.430 ^A
	FFA	2.011±0.020 ^A	1.399±0.014 ^B	0.964±0.023 ^C
	SOVS	9.666±0.516 ^A	9.165±0.752 ^A	8.650±1.032 ^A
5	TBA	1.120±0.009 ^A	0.400±0.019 ^B	0.175±0.000 ^C
	DPPH	1.202±0.680 ^C	2.646±0.113 ^B	3.568±0.170 ^A
	FFA	2.507±0.205 ^A	2.029±0.405 ^A	2.101±0.072 ^A
	SOVS	4.660±0.816 ^C	7.500±0.547 ^B	7.600±0.547 ^A
9	TBA	2.995±0.064 ^A	1.350±0.044 ^B	0.994±0.004 ^C
	DPPH	1.669±1.331 ^C	5.282±0.073 ^B	7.112±0.443 ^A
	FFA	4.338±0.390 ^A	2.765±0.076 ^B	2.494±0.153 ^C
	SOVS	1.600±1.032 ^A	3.500±1.366 ^A	2.833±0.410 ^A

A-C Within each row, different superscript lowercase letters show differences between treatment groups for the same analysis group ($p < 0.05$). C: Minced salmon fish samples untreated, RAT1 and RAT2: Salmon fish samples treated with 8.10^{-3} g and 16.10^{-3} g RA nanoparticles, respectively. TBA: mg MDA kg^{-1} , DPPH: %, FFA: %

The Effect of RA Nanoparticles in Free Fatty Acid Levels (FFA)

The results of the FFA analysis are indicated in Table 2. The FFA values in the minced salmon samples (C, RAT1, and RAT2) increased with storage time. The FFA values of C group samples were found to be

higher when compared with those of RAT1 and RAT2 samples. Also, for 9 days, the FFA value of the minced salmon fish samples treated with RAT2 nanoparticles was determined to be lower than that of RAT1 samples. During 9 days, there were also statistical differences among all groups ($p < 0.05$).

Moreover, the initial FFA value of C samples was 2.011%, but at the end of the cold storage, this value reached 4.338% while FFA values of RAT1 and RAT2 samples were found to be 2.765% and 2.494%, respectively. A high-level FFA value can be defined by an undesirable aroma. Fish oil contains a great deal of PUFAs that can easily lead to the initiation of oxidation reactions and the formation of hydroperoxides of fatty acids. Also, bacterial activity plays a key role in the increase of FFA value in fish meat (41, 42). In addition, FFA values are used as a quality indicator of fish oils (43). Bimbo (44) reported that the FFA values of food-grade fish oil were found to be higher at the end of nine days of storage. The recommended FFA value is determined in the range of 1 and 7%. In this respect, as can be seen from the results of the study, nano-application in the present study provided a higher quality for minced salmon samples at 4 °C. In this respect, rosmarinic nanoparticle applications (8.10^{-3} and 16.10^{-3} g) with a larger contact area property successfully limited the rapid increase in the FFA value of fish meat.

The Role of RA Nanoparticles in Sensory Quality

Overall sensory scores of the minced salmon fish samples treated with RA nanoparticles, and without RA nanoparticles samples are shown in Table 2. Once the sensory score of all samples reached the point of minimum acceptability (5) grades, the samples were evaluated to be unfit for human consumption. All sensory attributes of every sample showed a declining trend for 9 days. After 5 days of refrigerated storage, putrid and fishy odor in untreated (C) samples was determined. On the 5th day of the refrigerated storage period, the FFA value of C samples reached 2.507, but, on the 9th day of the refrigerated storage, RAT2 samples could be measured as 2.494. The sensory quality of the samples treated with 8.10^{-3} and 16.10^{-3} g RA nanoparticles delayed the oxidation by four days when compared to the control samples. Tsai, Su (45) defined that the shelf life of salmon loins that were dipped into chitosan solution (1%) could be prolonged by four days as compared to control group samples stored at 4 °C. When compared to the amount of the used substance, in the present study better quality was provided with highly less nanomaterial.

CONCLUSION

The RA-loaded PCL nanoparticles were obtained with 235.8 ± 2.98 nm average particle size. The oxidation in the minced salmon fish samples was effectively limited using RA-loaded PCL nanoparticles for 9 days under cold storage conditions. The rapid changes in TBA, FFA values, and overall sensory score were limited although DPPH values of the minced salmon samples increased with time. Per 100 g minced salmon fish sample, especially, the

use of 8.10^{-3} and 16.10^{-3} g nanoform of RA provided well preservation for the limitation of the rapid increase in the tested oxidation parameters. It was revealed that the high loading and encapsulation efficiency of RA, in this respect especially RAT2 application, had an important effect on the oxidative stability of minced salmon samples. The nano-application presented a larger contact area on the surface of the minced fish. In this sense, the present study revealed that the use of RA in a nanotechnology application could be a promising approach, especially for fatty and minced food products.

CONFLICT OF INTEREST

Authors have no conflict of interest.

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