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A nanofiber application for thiamine stability and enhancement of bioaccessibility of raw, cooked salmon and red meat samples stored at 4 °C

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ABSTRACT

Nanofibers were fabricated by using the electrospinning technique. The diameter of gelatin nanofibers was measured as 41.511 nm. When thiamine was integrated into the nanofibers, it was increased to 100.156 nm. After raw red meat and salmon samples were coated with the nanofibers, the samples were stored at cold storage conditions. The thiamine levels of raw uncoated red meat (RM, 400 to 379 μg/100 g: $p < 0.05$) and salmon meat (SM, 68 to 62 μg/100 g: $p < 0.05$) were decreased. The coating increased thiamine contents in raw (519 to 563 μg/100 g) and cooked (416 to 485 μg/100 g) RM samples. Thiamine contents of raw (75 to 78 μg/100 g) and cooked (67 to 75 μg/100 g) SM samples were increased ($p < 0.05$). The changes in the bioaccessibility of uncoated and coated RM samples were in the range of 85–76%, and 87–79%, respectively while salmon samples were increased from 79 to 94% ($p < 0.05$).

1. Introduction

Nutrition is a vital issue for the sustainability of humankind. From vegetables to meat, there are so many kinds of foods having different nutritional values. Especially, in today's world, meat and meat products play a key role in a well-balanced diet. Red meat is defined as proteinaceous food having a high bioavailability as compared to alternative foods (Wyness, 2016). In contrast to red meat, fish meat has long-chain polyunsaturated fatty acids such as eicosapentaenoic acid, EPA (C20:5 $\omega - 3$), docosapentaenoic acid, DPA (C22:5 $\omega - 3$), and docosahexaenoic acid, DHA (C22:6 $\omega - 3$) which can limit serious diseases such as blood pressure, tumor, and brain function failure (Cetinkaya et al., 2021; Ahmmed et al., 2020; Zhang et al., 2019). Also, fish meat consists of water-soluble vitamins form of B_6 (Pyridoxal, Pyridoxamine, and Pyridoxine), thiamine (B_1), riboflavin (B_2), and niacin (B_3) (Çatak et al., 2020; Ceylan et al., 2018). As stated by Yaman (2019), vitamin B_1 playing a role in converting pyruvate to acetyl CosA in energy metabolism can be found in thiamine form in foods. Also, the daily need for vitamin B_1 in an adult is about 1.2 mg (Martel, Julianna L., Connor Kerndt., 2020). Despite its importance for the human diet, water-soluble vitamins such as B_1 are affected depending on the presence of the heating process, light, and pH of the environment. Therefore, significant losses in the amounts of water-soluble vitamins may be observed (Ceylan et al., 2018). However, B-complex vitamins not stored in the human body should be daily taken because of energy production and biosynthesis (Moore, 2012). Because of the insufficient intake of thiamine in the human diet, polyneuritis, beriberi, and inflammation, and degeneration of peripheral nerves could be observed (Li, 2017). Besides the importance of vitamin intake, their bioavailability and bioaccessibility take a great deal of attention for the well and balanced diet for humans. The bioaccessible amount of water-soluble vitamins in the gastrointestinal tract, including the mouth, stomach, and also small intestine, can vary depending on the different factors such as pH, temperature, bonds with polypeptides and polysaccharides, and the presence of metal ions and digestive enzymes inhibitors (Ball, 2005). The bioaccessibility of food can be determined using *in vitro* digestion methods, which simulate gastric and small intestinal conditions using Caco-2 cells, in the case of a cell culture laboratory is established (Courraud et al., 2013; Yaman et al., 2021). Because of the fact that vitamin B_1 is lost during processing, thiamine enrichment in foods is used in most countries (Ball, 2005; Yaman et al., 2021). For example, the thiamine bioaccessibility in wheat bread was found in the range of 69.1% and 91.2%. The particle size of dietary fibers in wheat bread and hydroxyl groups could reduce the thiamine bioaccessibility (Kurek et al., 2017). On the other hand, the

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bioaccessibility of thiamine was increased by decreasing particle size of durum wheat fractions (Zaupa et al., 2014). The bioaccessibility of thiamine in cereal-based baby foods in pH 1.5 and 4 was found as 81and 65% (Akça et al., 2019). As were seen from the studies given above, bioaccessibility value can vary depending on various factors from processing, pH, and the type of product.

Today, various food processing techniques from irradiation to food additives are used to prolong the shelf life and provide food safety. In food science and industry, recently, novel methods like nanotechnology take a great deal of attention. In this respect, nanoparticles (Ceylan, 2018), nanoemulsions (Durmuş et al., 2020), nanofibers (Ceylan, Meral, Cavidoglu, et al., 2018), and the studies based on nanoencapsulation of bioactive materials such as thyme, curcumin (Ceylan et al., 2018; Meral et al., 2019) have been used. A product fabricated using nanotechnology provides a larger contact area on the surface of the potentially used materials. So, besides the limitation of microbiological spoilage, more stable water-soluble vitamins in foods could be obtained.

The main aim of the present study was to successfully integrate the nanoencapsulated thiamine into the nanofibers by using the electrospinning technique. In this respect, the hypothesis of the study was to reveal how effectively thiamine nanofibers could be successfully used as an alternative material to provide thiamine stability and improve the bioaccessibility of red meat and salmon samples stored in refrigerated conditions.

2. Materials and methods

2.1. Materials

Food samples (salmon and red meat) were obtained from an international supermarket located in Istanbul. Thiamine, KH_2PO_4, HCl, $K_3Fe(CN)_6$, MeOH, acetonitrile, mucin, uric acid, urea, bile salts mixture, $NaHCO_3$, bovine serum albumin, NaCl, KCl, $CaCl_2{\cdot}2H_2O$, lipase (from porcine pancreas Type II, 100–500 units/mg protein), pepsin from porcine gastric mucosa lyophilized powder (≥250 units/mg solid), pancreatin (from porcine pancreas 8 × USP specifications), alpha-amylase from Aspergillus oryzae powder (1.5 U/mg), acid phosphatase, (potatoes, 0.5–3.0 U/mg) and taka diastase from Aspergillus oryzae (100 U/mg) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Fytronix ESP-900 Electrospinning unit (Elazığ-Turkey) consisting of a high voltage unit, syringe pump unit and flat collector was used to fabricate nanofibers.

2.2. Methods

2.2.1. Preparation of dope solution and electrospinning parameters

All process as graphical abstract is given in Fig. 3. A solution was prepared by dissolving gelatin (20 g) in 20 mL acetic acid and deionized water. Thiamin (0.75 g) was added to the solution (consisting of 10 mL (20%) gelatin and acidic acid (≥99%)) and mixed using a magnetic stirrer. At a range of between 23 °C and 25 °C, for electrospinning, supply high voltage was defined as 25 kV while flow rate was determined as 0.035 mL/min. Also, the distance between the Taylor cone and collector was arranged to 10 cm. For nanocoating of 50 g each food sample, 39.10^{-3} g thiamine was used. Fytronix ESP-900 electrospinning device was used in the production of nanofibers (Elazığ-Turkey).

2.2.2. Morphological characterization

Morphologies of thiamine electrospun nanofibers were analyzed under low vacuum in a field emission scanning electron microscope SEM using FEI, Quanta Feg 250, USA, at different magnifications with a working distance of 8 mm. An accelerating voltage of 5 kV was determined for obtaining secondary electron images. The average diameters of electrospun nanofibers were obtained by using different measurements as shown in Fig. 1 and described by (Meral et al., 2019).

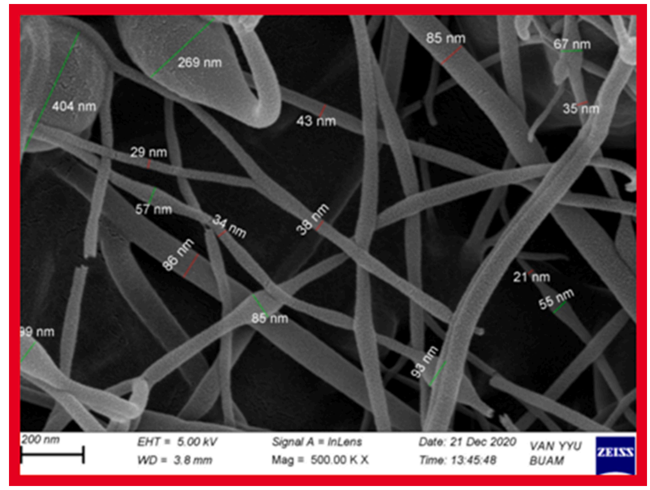

Fig. 1. Morphological images of thiamine loaded nanofibers.

2.2.3. Nano-treatment for foods

Salmon and red meat samples presented in Fig. 2 were separately coated with electrospun nanofibers. The food samples coated with nanofibers were coded as Sn and RMn and uncoated samples (UC) were stored at cold storage conditions (4 ± 1 °C). Following the nano-coating process of raw salmon and red meat, Sn and RMn samples and their control samples were cooked at 200 °C (kitchen oven) for 5 min, after cooling, all cooked samples were stored at 4 ± 1 °C for three days.

2.2.4. Extraction of vitamins B_1

Homogenized sample (5 g) was added into a 250 mL Erlenmeyer flask including 0.1 N 60 mL HCl and then autoclaved at 121 °C for 30 min. The obtained solution was cooled to room temperature and the pH of the solution was adjusted to 4.5 using 2.5 mM sodium acetate. For the enzymatic extraction, 10 mg acid phosphatase and 100 mg taka diastase were added to the samples and also incubated for 3 h at 37 °C using a shaking water bath. Acid phosphatase was utilized in the samples, collected *in vitro* digestion. Follow cooling; the samples were filtered using a filter paper. The method (HPLC and the extraction of vitamin B_1) described by Akça et al. (2019) was applied.

2.2.5. Derivatization of thiamine to trichrome

Potassium ferricyanide (1.5 mL) solution prepared with 0.25 g in 25 mL 15% NaOH solution was added to 20 mL filtrate used from the above solution and then arranged to pH 7.1 ± 1 with *ortho*-phosphoric acid. The derivative solution was filtered using a 0.45 μm cellulose acetate filter before injecting into the HPLC.

2.2.6. HPLC determination of vitamin B_1

A Shimadzu Nexera-i HPLC with a Shimadzu RF-20A fluorescence detector (Shimadzu Corporation, Kyoto, Japan) was used in order to separate thiamine. The mobile phase included a 75% buffer solution obtained from 0.033 M KH_2PO_4 and also methanol (25%). The pH was arranged to 7.1 ± 1 using *ortho*-phosphoric acid and then filtered with a 0.22 μm cellulose acetate filter under vacuum. The fluorescence detector excitation (290 nm) and emission (395 nm) wavelengths were defined. The separation was provided using an Eclipse X08-C18, 5 μm, 4.6 × 150 mm column (Agilent, USA) with a flow rate of 1 mL/min.

2.2.7. In vitro digestion procedure

The bioaccessibility of thiamine in the obtained samples was studied by using *in vitro* stimulated human digestive system. The stomach, mouth, and small intestinal medium were studied *in vitro* system (Yaman et al., 2019).

Saliva: 16 mL urea (2.5 g/100 mL), 3.4 mL NaCl (17.5 g/100 mL),30

Fig. 2. Food samples coated with thiamine loaded gelatin based nanofibers.

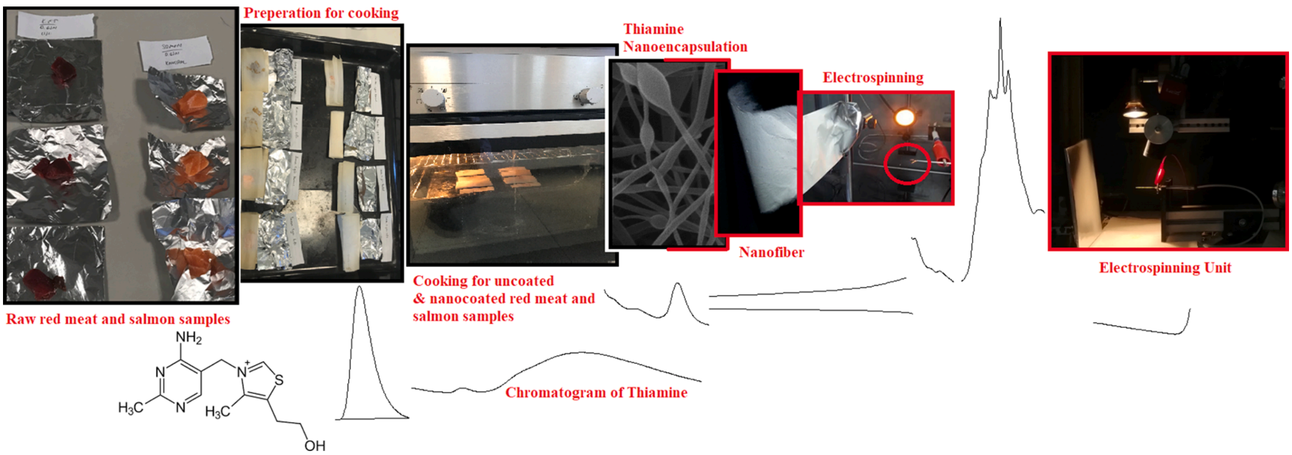

Fig. 3. Flow chart of the study.

mg uric acid, 50 mg mucin and finally 560 mg α-amylase were dissolved in 1 L volumetric flask using deionized water. The volume arranged at pH 6.8 was completed with deionized.

Gastric juice: 36 mL of $CaCl_2{\cdot}H_2O$ (2.22 g/100 mL), 13 mL of HCl (37 g/L), 2 g of bovine serum albumin, 5 g pepsin, and 6 g mucin were dissolved in 1 L volumetric flask using deionized water. The final volume was balanced at pH 1.5 using deionized water.

Duodenal juice: $CaCl_2{\cdot}2H_2O$ 18 mL (2.22 g/100 mL), KCl (9.0 g/ 100 mL) 12.6 mL, 4 g bovine serum albumin, 3 g lipase, and also 18 g pancreatin were dissolved in 1 L volumetric flask and then the volume was completed with deionized water. The final pH was 8.0.

Bile juice: $CaCl_2{\cdot}2H_2O$ (2.2 g /100 mL) 20 mL, $NaHCO_3$ 136.6 mL (17 g/200 mL), 3.6 g from bovine serum albumin, and 60 g bile were dissolved in 1 L volumetric flask with deionized water. The final volume was adjusted to pH 7.0.

The test sample (5 g) was mixed with 5 mL of saliva solution in a 100 mL beaker and incubated for 5 min at 37 °C in a shaking water bath. Following this, 12 mL of gastric juice was added to the fluid obtained from the mouth phase and incubated again for 1 h at 37 °C in a shaking water bath. 5 mL bile juice and 10 mL duodenal juice were added to the sample, which was obtained from the gastric phase. This mixture was incubated at 37 °C for 2 h in a shaking water bath. Finally, the volume was diluted using deionized water to 50 mL. Then, the samples were centrifuged at 8000 rpm and for 5 min and filtered through a filter paper. This prepared solution was utilized for the analysis of thiamine. Also, bioaccessibility was calculated by dividing the concentration of thiamine in the digesta by the thiamine concentration in the original non-digestible sample and expressed as a percentage.

2.2.8. Statistical evaluation

Measurements were repeated twice with three replications in the study. All obtained data were subjected to analysis of variance in order to reveal the raw, cooked, digestion and bioaccessibility ratio in red meat and salmon samples. Graphpad Prism software Version 5.00 (California Corporation, CA) was performed in order to determine significant differences, and also comparisons of all differences were evaluated using Tukey's Multiple Range Test ($p < 0.05$).

3. Result and discussion

3.1. Morphological properties of nanofibers

Scanning electron microscopy (SEM) images of thiamine nanoencapsulated gelatin-based nanofibers are presented in Fig. 1. SEM images for the gelatin clearly revealed smooth and ultrafine nanofibers. Depending on successful nanoencapsulation of thiamine into the gelatin nanofibers, the morphological structures were varied. As were seen from the images, the average diameter of gelatin nanofibers was found as 41.511 ± 18.641 nm calculated with the values having 90.260 nm maximum and 19.679 nm minimum diameters. Moreover, when thiamine nanoencapsulated gelatin-based nanofiber was examined, it was observed that the average diameter of the nanomaterials was defined to be 100.156 ± 97.735 nm. Nanoencapsulation of thiamine to the nanofibers was significantly increased the average diameters of the nanomaterials. Basically, flow rate, applied voltage, the distance between the Taylor cone and the collector, the sort of used materials play a key role to determine the average diameters of the nanomaterials (Ceylan, Meral, Karakaş, et al., 2018). On the other hand, nanoencapsulation process can remarkably affect the average diameter of the nanomaterials (Ceylan, Unal Sengor, & Yilmaz, 2018). Loading of different materials such as liquid smoke (115.3 nm), *L. reuteri* (381.83 nm), *L. rhamnosus* (583.1 nm), the combination of nisin and curcumin (172 nm), and fish oil (861 nm) to the carrier was successfully provided using electrospinning or electrospraying technique (Ceylan, Sağdıç, & Yilmaz, 2017; Ceylan et al., 2019; Zafer Ceylan, Meral, et al., 2018; Meral et al., 2019; Cetinkaya et al., 2021). As could be seen from previous studies given above, the average diameters of the obtained nanomaterials were in the range of 115.3 and 861 nm. In the present study, nanoencapsulation of thiamine into the gelatin nanofibers increased the average diameter up to 223%.

3.2. Thiamine content of cooked and raw salmon and red meat samples

The results with statistical evaluation of cooked and raw salmon and red meat samples (uncoated and coated with nanofibers) stored for three days are given in Tables 1 and 2. On the initial day of the experimental period, uncoated raw red meat (RM) and salmon (SM) samples had 400 and 68 μg/100 g thiamine levels. With the increase of the storage period, the thiamine contents of raw uncoated RM and SM samples began to decrease. For uncoated raw RM and SM samples, during three days, the declines as maximum percentage 5.25% and 8.82% were observed, respectively. Also, after the cooking process for uncoated samples, thiamine contents of RM and SM samples were determined as 395 and 49 μg/100 g. The initial thiamine content of RM was not significantly affected ($p > 0.05$), while that of SM was significantly decreased ($p < 0.05$), as compared to the raw materials. With the increase of storage period, thiamine content of cooked RM (395 to 372 μg/100 g, $p < 0.05$) samples was decreased. On the other hand, there was no statistical difference between the storage period of uncoated SM samples ($p > 0.05$). In this respect, during three days, raw salmon samples had a good resistance against the loss in thiamine.

For the samples coated with electrospun thiamine loaded gelatin-based nanofibers (RmN: red meat coated with nanofibers and SmN: salmon coated with nanofibers), it was observed for three days-storage that the nanocoating process increased thiamine content of raw RmN (change from 519 to 563 μg/100 g, $p < 0.05$) and raw SmN (change from 75 to 78 μg/100 g, $p > 0.05$) for the three-days storage period. Moreover, similar behavior between the storage days was observed in the cooked samples (increase in coated & cooked RmN and SmN: 16.58 and 11.9%, respectively) as could be seen from Table 1. This is because the release of thiamine from the nanomaterial with the increase in storage period could be associated with the continuous increase in thiamine content in cooked samples. After some food preservation methods or depending on storage conditions, cooking and processing types, thiamine losses can be observed, for example, irradiation process affected thiamine content about %34 (Liu et al., 1991; Rickman et al., 2007; Dionísio et al., 2009; Woodside, 2015; Ceylan and Ozogul, 2020). Another study showed that inactivation in thiamine content in pet meat samples depending on the heating process was observed (Moon et al., 2013). Thiamine content of African catfish was determined as 70 μg/100 g (Ersoy & Özeren, 2009). Besides thiamine content in fish meat, beef (40 μg/100 g), veal (60 μg/100 g), lamb (120 μg/100 g), and mutton (160 μg/100 g) are defined as the richest source of thiamin (Williams, 2007). As well known, thiamin defined as water-soluble can be lost in the cooking water. By the present nanofiber coating process, the release of thiamine within nanofibers and also barrier properties of nanomaterials protecting loss of water from salmon and red meat samples successfully might have been limited the loss of thiamine in the food samples.

Table 1
Thiamine levels of coated and uncoated red meat samples.

	Experimental Days	Uncoated (μg/100 g)	Coated with Nanofibers (μg/100 g)
Raw	0	400 ± 3^{Ba1}	519 ± 4^{Ac1}
	1	396 ± 4^{Ba1}	548 ± 4^{Ab1}
	3	379 ± 3^{Bb1}	563 ± 5^{Aa1}
Cooked	0	395 ± 5^{Ba1}	416 ± 2^{Ac2}
	1	391 ± 3^{Ba1}	456 ± 3^{Ab2}
	3	372 ± 1^{Bb2}	485 ± 4^{Aa2}

[A-B] Within each row, different superscript uppercase letters show differences between the two groups at the same experimental day in raw and cooked samples, separately ($p < 0.05$). [a-c]Within each column, different superscript lowercase letters show differences for each raw and cooked samples, separately ($p < 0.05$). Within each column, 1–2 indicate statistical differences between raw and cooked samples in uncoated and coated, respectively.

Table 2
Thiamine levels of coated and uncoated salmon samples.

	Experimental Days	Uncoated (μg/100 g)	Coated with Nanofibers (μg/100 g)
Raw	0	68 ± 1^{Ba1}	75 ± 2^{Aa1}
	1	66 ± 1^{Ba1}	77 ± 1^{Aa1}
	3	62 ± 2^{Bb1}	78 ± 2^{Ab1}
Cooked	0	49 ± 2^{Ba2}	67 ± 1^{Ab2}
	1	51 ± 2^{Ba2}	73 ± 2^{Aa1}
	3	52 ± 2^{Ba2}	75 ± 2^{Aa1}

[A-B] Within each row, different superscript uppercase letters show differences between the two groups at the same experimental day in raw and cooked samples, separately ($p < 0.05$). [a-c] Within each column, different superscript lowercase letters show differences for each raw and cooked samples, separately ($p < 0.05$). Within each column, 1–2 indicate statistical differences between raw and cooked samples in uncoated and coated, respectively.

3.3. Bioaccessibility of uncoated red meat and salmon samples

Table 3 shows bioaccessibility values of salmon & red meat samples. The initial bioaccessibility values of uncoated RM and SM samples were found to be 85% and 84%, respectively. On the first day of the cold storage, uncoated RM and SM samples almost had the same thiamine bioaccessibility. On the other hand, the bioaccessibility value of uncoated RM samples was sharply decreased (76%) depending on the increase in the storage day. Furthermore, in terms of thiamine bioaccessibility, uncoated salmon samples (84–85%) possessed higher stability against cold storage as compared to uncoated RM samples. At the end of three-day storage, it was clearly seen that the thiamine bioaccessibility of fish meat samples was found to be stable. The bioaccessibility values of foods can vary depending on the type of food and vitamin in food. For example, the bioaccessibility of folic acid in cereal-based baby foods was between 31 and 67% (Yaman et al., 2019). In another food, the bioavailability of the pyridoxal, pyridoxine, and pyridoxamine forms of vitamin B_6 were 38%, 67%, and 36%, respectively (Yaman & Mızrak, 2019). Revealing that the bioaccessibility of vitamins B_1 could be influenced by stability, temperature, pH of the gastrointestinal tract, dietary fiber content, and bonds with polysaccharides and

Table 3
Bioaccessibility levels of uncoated and coated red meat and salmon samples.

	Experimental Days	Uncoated (%)	Coated with Nanofibers (%)
Red Meat	0	85 ± 1^{Ba}	87 ± 1^{Aa}
	1	86 ± 1^{Aa}	81 ± 1^{Bb}
	3	76 ± 1^{Bb}	79 ± 1^{Ab}
Salmon	0	84 ± 1^{Aa}	79 ± 1^{Bb}
	1	85 ± 1^{Ba}	91 ± 1^{Aa}
	3	84 ± 1^{Ba}	94 ± 1^{Aa}

[A-B] Within each row, different superscript uppercase letters show differences between the two groups at the same experimental day in uncoated and coated samples with nanofibers, separately ($p < 0.05$). [a-c] Within each column, different superscript lowercase letters show differences for red meat and salmon samples, separately ($p < 0.05$).

polypeptides (Akça et al., 2019). By the present study, a decline in thiamine content of uncoated red meat samples was observed but the change in salmon samples was not significantly important ($p > 0.05$).

3.4. Bioaccessibility of red meat and salmon samples coated with nanofibers

Table 3 indicates the thiamine bioaccessibility of red meat and salmon samples coated with nanofibers. After the nanocoating process, the initial value of red meat samples was found as 87%. By the time, this value began to decrease, but this decline in RM samples coated with nanofibers was about a maximum of 9.19% for three days. On the other hand, initially, the thiamine bioaccessibility value of SM samples coated with nanofibers was 79%. On the contrary of RM samples, with time, the thiamine bioaccessibility continuously was increased (up to 94%) during the experimental period. Clearly seen that the nanocoating process improved the thiamine bioaccessibility in salmon samples (change: 18.9%: $p < 0.05$). In this respect, according to the study described by (Ceylan, Yaman, et al., 2018), thiamine loss in fish fillets was able be to be observed up to 37% for the cold storage period. The loss of thiamine can directly affect the bioaccessibility of food. The bioaccessibility value of some water-soluble vitamin forms can change depending on this gastric acidity (Ball, 2005). In the present study, for all food samples, gastric pH was 1.5. Akça et al., (2019) reported that thiamine was the most unstable in low acidic conditions and also the bioaccessibility level in gastric pH 4 was found as lower as compared to pH 1.5. Also, the potential presence of dietary fiber may reduce bioaccessibility of vitamins (Palafox-Carlos et al., 2011). Besides the mentioned issues and as could be seen in the results of the present study and two different meat samples were coated with nanofibers though, they had different bioaccessibility levels. In this sense, meat type and processing can affect the bioaccessibility as stated by (Afonso et al., 2015; Cardoso et al., 2015). The used nanofibers might more rapidly have been penetrated into the salmon meat samples because of the fact that salmon meat had a weak connective tissue as compared to the red meat samples. So, a higher bioaccessibility level might have been obtained in salmon meat samples during three days of storage.

4. Conclusion

The nano-coating process with thiamine nanofiber having 100.156 nm diameter was successfully applied for the enhancement of bioaccessibility and thiamine stability in raw and cooked red and salmon meat samples. Especially, in salmon samples, the nano-coating process showed more effectiveness in terms of bioaccessibility. Also, the nano-coating process provided a continuous increase in thiamine content of red meat and fish meat samples stored at cold storage conditions for three days. While maximum bioaccessibility of nanocoated red meat samples was found as 87%, the nano-coating process for salmon samples successfully increased the bioaccessibility up to 94%. In this regard, for further studies, this nanotechnology application can play a guiding role in food science with its present results.

CRediT authorship contribution statement

Mustafa Yaman: Resources, Investigation, Project administration, Writing – original draft, Writing – review & editing. **Melika Sar:** Formal analysis. **Zafer Ceylan:** Supervision, Writing – original draft, Conceptualization, Project administration, Methodology, Writing – review & editing, Validation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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