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Effect of electrospun thymol-loaded nanofiber coating on vitamin B profile of gilthead sea bream fillets (*Sparus aurata*)

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ABSTRACT

The effect of electrospun chitosan-based nanofibers (CH) and thymol-loaded electrospun chitosan-based nanofibers (TCH) were investigated in terms of stability of unstable B vitamin complex during cold storage period. Scanning Electron Microscopy revealed that the average fiber diameters were determined to range between 98.12 nm and 135.94 nm. CH and TCH were both effective against loss and rapid changes of unstable B vitamin complex. 7% average change in thiamin value was observed for samples coated with TCH, but it increased up to 39% in uncoated samples. In terms of thiamin content, the positive effect of nanofiber coating for the samples coated with TCH was observed on the 11th day of storage. Average change in riboflavin for CH and TCH was found to be lower. Particularly, sharply changes in nicotinamide acid, pyridoxal, pyridoxine and pyridoxamine contents were determined for uncoated samples, as compared to slightly changes in CH and TCH. The objective of this work was to obtain nanofibers. In this sense, potential effect of nanofibers on vitamin B profiles of fish fillets was to investigate. The results revealed that coating of the fish fillets with the nanofibers could be a promising technique to keep the stability of unstable B vitamin complex.

1. Introduction

Food is considered to be unique source of protein, fat, vitamin and mineral for well-balanced human diet. Cheese, egg yolk contain smaller amounts of vitamin as compared to meet. Furthermore, fish or different sort of seafood includes much more important nutrients such as omega-3 fatty acids (EPA; Eicosapentaenoic Acid, DHA; Docosahexaenoic Acid), vitamins, which are so important for human health (Stark, Van, Higgins, Weatherford, & Salem, 2016). While vitamin D_3 (cholecalciferol) is found in fatty fish species (salmon, tuna fish, swordfish) (USDA, 2011), vitamin B_1 (thiamine), B_2 (riboflavin), niacin, B_5 (pantothenic acid), B_6 (pridoxine, pridoxamine and pyridoxal), B_{12} (cynocobalamin) are important some water-soluble vitamins (Boyaci et al., 2012; Esroy & Özeren, 2009; Lebiedzińska, Marsza, Kuta, & Szefer, 2007), which can be easily lost during cooking/heating due to the activity of enzymes (Puthesseri, Divya, Lokesh, & Neelwarne, 2013) or food processing. Especially, B-complex vitamins that are not stored in the body, but should be taken into body daily play a key role for biosynthesis, production of energy (Bellows & Moore, 2012). Also, riboflavin converts the amino acids into niacin, which is also responsible for energy production, neural and enzymatic functions in the body. In addition to niacin, vitamin B_6has an important functionality on amino acid metabolism, glycogen and different kinds of enzymatic reactions (Schellack, 2015). Because of all mentioned above reasons, directly or indirectly of preservation or stability of vitamins in food is critical subject for food makers and also especially for the all consumers.

In this respect, different kinds of preservation methods such as irradiation technique, food preservatives, packaging techniques, processing methods have been utilized for a long time in scientific researches. Different studies on conventional preservation reveal that there are different disadvantages of using this kind of preservation method. For example, food irradiation gives rise to increase in malondialdehyde (MDA) level and vitamin losses (Dionisio, Gomes, & Oetterer, 2009; Ceylan, 2014, p. 104), the use of chemical originated food additives is not desired by most of consumers because of their potential toxic effects as well.

Novel approaches on food preservation have been recently taking a great deal attention in scientific area. In this respect, recent studies

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have revealed that microbial spoilage (growth in total viable bacteria count, growth in psychrophilic bacteria and total yeast and molds) of fish fillets can be delayed by using nano-scale material (Ceylan, Şengör, Sagdıç, & Yılmaz, 2017a). Also, rapid chemical deterioration indices such as total volatile basic nitrogen, oxidative stability and trimethylamine in fish fillets can be easily delayed by using nanofiber or nanoencapsulated material (Ceylan, Sengor, & Yilmaz, 2017b). The use of more than one core material in nanostructure can fortify the nanofibers, so the use of this kind of coating material enriches the antimicrobial effectiveness as compared to the single coating core material (Ceylan, Şengör, & Yılmaz, 2018). Except for mentioned studies, nanotechnological applications such as nanoparticle (Abdou, Osheba, & Sorour, 2012) and nanofibers are being used to delay rapid deterioration, maintain color and aroma of fish (Ceylan, 2017, p. 159; de Faria, Perreault, Shaulsky, Arias, & Elimelech, 2015). Besides nanofibers used to extend stability of food, directly nanoencapsulation of vitamins has been presented as a promising approach in recent studies (Almouazen, Bourgeois, Jordheim, Fessi, & Briançon, 2013; Katouzian & Jafari, 2016).

Type of used material to obtain nanostructure should be of very critical, in this respect, the use of GRAS material is highly important. Chitosan and thymol can be already regarded as GRAS by U.S. Food and Drug Administration (FDA; 21 CFR 182.10) (2009). Because of the fact that these materials can be converted into nanoscale material, potential minimum adverse effect on human health of these materials is decreased and also the use cost of food additive can be decreased. Furthermore, contact area of material can be increased by nanofiber (Ceylan & Vicente, 2017). Thus, chitosan as biopolymer based nanocarrier and thymol as nanoencapsulated bioactive material may play a key role for delaying rapid deterioration, loss and changes in vitamin content of fish fillets.

The first aim of study was to successfully obtain bioactive material-loaded biopolymer-based nanocarrier by using electrospining technique and coat the fish fillets with obtained nanofibers. The final target of the study was to reveal the effect of the nanocoating process on unstable vitamin B profile of fish fillets during cold storage conditions.

2. Material and methods

2.1. Vitamin analysis

2.1.1. Materials

The vitamin standards (nicotinic acid, nicotinamide, riboflavin, thiamine, pridoxine hydrochloride, pridoxamine hydrochloride and pyridoxal hydrochloride), enzymes (taka diastase, aspergillusoryzae, 100 U/mg and acid phosphatase, potato, 0.5–3.0 U/mg) and teflon tube "tubing" (length: 20 m diameter: 0.5 mm), potassium ferricyanide, potassiumdihydrogen phosphate, and 1-heptanesulfonicwere obtained from Sigma-Aldrich (St. Louis, MO, U.S.A). In this study, all other chemicals were used in high purity.

2.1.2. Standard preparation

Standard stock solution of each vitamin was prepared in 0.1 N hydrochloric acid solution. Each standard is freshly prepared daily. Working standards in three levels are prepared from stock solution.

2.1.3. Extraction of thiamin, riboflavin, niacin and vitamin B_6in fish fillets

Extraction methods described Ndaw, Bergaentzlé, Aoudé-Werner, and Hasselmann (2000; 2002) was used with some modifications. The fish fillets was homogenized and 2 g of this sample was put in a 100 ml erlenmeyer flask, then 60 ml 0.1 N hydrochloric acid solution was added and it was kept in autoclaved at 121 °C for 30 min. After this stage, enzymatic treatment was performed to liberate the phosphorylated forms of thiamine (TMP, TDP and TTP) riboflavin (FAD and FMN), and vitamin B6(PLP, PNP and PMP). There is no need for enzymatic extraction for niacin. For this reason, the sample is cooled and directly filtered and injected into the HPLC. Extraction procedure for thiamine, riboflavin and vitamin B6 as follows: after cooling to room temperature, pH adjusted to 4.5 using sodium acetate (2.5 mM) solution. 100 mg taka-diastase and 5 mg acid phosphatase enzyme have been added to the sample and incubated for 18 h for vitamin B_6 and 3 h for thiamine and riboflavin at 37 °C in shaking water-bath. Then, the samples were cooled to room temperature and the volume was completed to 100 ml with 0.1 N hydrochloric acid solution, afterwards it was filtered with 0.45 μm filter and injected into the HPLC.

2.1.4. HPLC determination of B_6Vitamers

The level of B_6vitamers (PL, PN and PM) in fish fillets were determined by a reversed-phase HPLC method. HPLC conditions described by Kall (2003) was used with some modifications. HPLC system was a Shimadzu LC 20AT pump with a Shimadzu RF-10AXL fluorescence detector (Shimadzu Corporation, Kyoto, Japan). The Mobile phase was prepared as follows: the buffer solution was prepared by dissolving 11 g of KH_2PO_4 and 0.5 g of 1-heptanesulfonic acid in 940 ml deionize water. Then, 60 ml of methanol was added and pH adjusted to 2.4 with ortho-phosphoric acid. The fluorescence detector excitation and emission wavelengths were set at 290 and 395 nm, respectively. B_6 vitamers were separated with Eclipse X08-C18, 5 μm, 4x6x150 mm column (Agilent, USA) with a flow rate of 0.8 mL/min. Column oven temperature was set to 30 °C.

2.1.5. HPLC determination of riboflavin

The content of riboflavin an in fish fillets was determined by HPLC, consisting of Shimadzu LC 20AT pump with a Shimadzu RF-10AXL fluorescence detector (FLD) (Shimadzu Corporation, Kyoto, Japan) according to procedure described by Eroglu, Senem, Mustafa, Baris and Sibel (2016) et al. The Mobile phase was prepared by mixing 750 ml of water and 250 ml of methanol. The mixture was filtered through with 0.45 μm filter. Excitation and emission wavelengths 445 and 525 nm for riboflavin, respectively. An Agilent eclipse X08-C18 column (5 μm, 4.6 × 150 mm, Agilant Technologies) was used and flow rate of 1 mL/min.

2.1.6. HPLC determination of thiamine

Due to its structure, thiamine can not be detected in the fluorescence detector alone. Therefore, pre-column derivatization is required to be detected by the fluorescence detector. Detection methods described Finglas et al. (1984)Finglas & Faulks, 1984 was used with some modifications. 25 ml of the filtered sample is taken up in 50 ml polyethylene tubes, 1.5 ml of potassium ferricyanide solution (0.25 g of potassium ferricyanide dissolved in 25 ml of 15% sodium hydroxide solution) is added, adjusted to pH 7.0–7.1 with ortho-phosphoric acid and injected into HPLC by filtration through 0.45 μm filter. The Mobile phase was prepared by mixing 750 ml of water and 250 ml of methanol. The mixture was filtered through with 0.45 μm filter. Excitation and emission wavelengths 366 and 445 nm for thiamine, respectively. An Agilent eclipse X08-C18 column (5 μm, 4.6 × 150 mm, Agilant Technologies) was used and flow rate of 0.8 mL/min. Column oven temperature was set to 25 °C.

2.1.7. HPLC determination of niacin

Similarly, as in thiamine, post-column derivatization is required to determine niacin. For post-column derivatization, a photo-chemical derivatization system was established in the laboratory. Detection methods described S. Lahely, Bergaentzle, and Hasselmann (1999) was used with some modifications. The derivatization system was made by wrapping a teflon tubing with a length of 20 m and a diameter of 0.5 mm on a UV-A lamp 60 cm long. The system is connected between the analytical column and the fluorescence detector. The Mobile phase was prepared by mixing 750 ml of water and 250 ml of methanol. The mobile phase should be prepared daily and protected from light. The mobile phase was prepared by mixing 1 L of 0.07 M potassium