Efficiency of dietary sodium alginate coating incorporated with lycopene in preserving rainbow trout

Ali Ehsani¹ · Marzieh Paktarmani² · Mohammad Yousefi³

Received: 27 August 2016 / Revised: 29 November 2016 / Accepted: 7 February 2017 / Published online: 29 May 2017
© The Korean Society of Food Science and Technology and Springer Science+Business Media Dordrecht 2017

Abstract The main aim of this study was to investigate the effect of alginate coating without or with lycopene at levels of 0.5, 1, 1.5, and 3% on the quality of rainbow trout stored under refrigerated conditions for 16 days. Samples coated with alginate with or without lycopene showed significantly lower free fatty acid (FFA) and total volatile basic nitrogen (TVB-N) values compared to uncoated (control) filets, particularly after day 4. Moreover, significant differences ($p < 0.05$) were observed between the coated samples containing lycopene and samples not containing lycopene on the last day. In relation with pH, none of the treatments resulted in significant differences. In regard to oxidation parameters, the effect of alginate-lycopene coatings was only seen on day 16 of storage. In general, although alginate-lycopene coating had no significant effect on the reduction of thiobarbituric acid and peroxide values, it succeeded in decreasing the TVB-N and FFA values.

Keywords Rainbow trout · Lycopene · Alginate · Coating

Introduction

Food materials, owing to their nature, are at the risk of physical, chemical, and biological contamination during storage. Sustainability and quality of food depend on chemical composition (proteins, lipids, carbohydrates, and water) and environmental and technological conditions. Fish is a perishable food product. Fat oxidation in fishes, particularly in fatty fishes such as salmon, can be considered as one of the main reasons for quality diminution [1] and can be one of the biggest concerns regarding marine products [2]. Fish oil is very sensitive and vulnerable because it contains a significant amount of polyunsaturated fatty acids [3]. This can result in bad odor and taste, color changes, changes in texture, reduction in water retention capacity and nutritional value, and production of compounds that may be toxic [1]. Given the high nutritional value of fish, preventive strategies should be considered to increase the shelf life and prevent the oxidative corruption of fish.

In this regard, food packaging and edible coatings can maintain the quality of foods for their preventive and inhibitory properties [4, 5].

Food coatings are often made of natural substances such as proteins, polysaccharides, and fats. They are used alone or in combination with other materials as a thin layer on the surface of foods. The use of such coatings, besides preserving the quality, minimizes the risks of chemical additives in packaging and pollution caused by packaging waste [6].

Sodium alginate is a linear, water-soluble polysaccharide and a derivative of alginic acid extracted from brown algae. Alginate, owing to its properties of biodegradability, eco-friendliness, sustainability, and production of elastic gels in the presence of polyvalent cations, has been focused on in the food industry [7].

✉ Mohammad Yousefi
yousefi.sbu@gmail.com

¹ Department of Food Science and Technology, Faculty of Nutrition and Food Sciences, Tabriz University of Medical Sciences, Tabriz, Iran

² Department of Health and Food Safety, Faculty of Health, Qazvin University of Medical Sciences, Qazvin, Iran

³ Talented Students Center, Student Research Committee, Department of Food Science and Technology, Faculty of Nutrition and Food Sciences, Tabriz University of Medical Sciences, Tabriz, Iran

Because of the high ability of alginate for forming films, it can be suitable for use as a food coating. Moreover, the presence of antibacterial and antioxidant materials can improve the preservative properties. Antioxidants are added to foods as coating to inhibit the oxidation of lipids [8]. In this study, we incorporated lycopene as an antioxidant agent into an alginate solution to produce a protective coating against chemical changes.

Materials and methods

Fish preparation

Rainbow trout with an average weight of 300 g were purchased from fish farms located in the Center of Artemia and the Aquatic Animals Research Institute, University of Urmia, and immediately transferred to the chemical analysis laboratory. The fish were beheaded and fins were removed. Then the fish were fileted after processing. The filets were divided into six groups. The groups included the control (no cover), treatment with alginate coating without lycopene, treatment with alginate coating containing 0.5% lycopene, treatment with alginate coating containing 1% lycopene, treatment with alginate coating containing 1.5% lycopene, and treatment with alginate coating containing 3% lycopene.

Preparation of coating solution

To prepare the coating solution, 4 g of sodium alginate powder, 0.2 g of calcium chloride (Sigma-Aldrich Co., LLC, St Louis, MO, USA), and 200 mL of distilled water were mixed into a sterile breaker at a temperature range of 55–60 °C and stirred for 10–15 min until a favorable transparency was achieved (the alginate solution volume was equal to 209 mL) [7]. In a separate sterile Erlenmeyer flasks, lycopene (Sigma-Aldrich Co., LLC., St Louis MO USA) was dissolved in the amounts of 1.11, 2.22, 3.31, and 6.63 g in the same solutions, including 10 mL of 20% ethanol and 2.0 mL of Tween 80 emulsifier (Scharlau, Sentmenat, Barcelona, Spain), and stirred vigorously [8]. Afterward, the lycopene solutions were added to four alginate solutions to produce 0.5, 1, 1.5, and 3% (w/v) final solutions. Finally, glycerol (Merck, Germany) was mixed with the final solutions at the ratio of 2% at 45 °C for 15 min to make the appropriate mixtures [7].

Addition of clay nanoparticles to the alginate coating

Different levels of clay nanoparticles (1, 3, and 5% w/w) as supporting agents in reducing the penetration of oxygen were mixed with distilled water for the preparation of an alginate solution and stirred for 24 h [9].

Filet treatment

After preparing the coating solution, fish were immersed in the prepared solution for 30 s. This operation was performed three times with an interval of 2 min and 30 s to allow the excess water to be dripped. After packing in polyethylene plastic bags, the filets were refrigerated for 16 days [10] and considered for chemical analyses.

Measuring fat oxidation parameters

The oil extraction step is necessary to measure free fatty acid (FFA), peroxide value (PV), and thiobarbituric acid (TBA). To extract the fish oil, the Bligh and Dyer method was employed [11]. One hundred grams of fish samples were cut and placed into the mixer along with 200 mL of chloroform and 400 mL of methanol and mixed well. Then, 200 mL of distilled water and 200 mL of chloroform were added in two steps and mixed again. The mixture was filtered using Whatman filter paper and separated via a separatory funnel. The permeates were then transferred to a rotary flask to evaporate the solvents and distilled water from the extracted oil.

Measuring free fatty acids

This test was performed as described by Egan et al. [12]. First, 50 mL of the solution (equal mixture of 96% ethanol with diethyl ether in terms of volume) was added to the sample, and then 1–2 drops of phenolphthalein were added to the mixture. Afterward, the solution was titrated with 0.1 N NaOH. FFAs were calculated using the following formula:

$$\text{Acid value} = (56.1 \times N \times V) / W \rightarrow \text{FFA (\%)} \\ = \text{Acid value} \times 1/2,$$

where V is the consumed volume of NaOH, W is the weight of the oil, and N is the normality of NaOH.

Determination of peroxide value

PV was measured according to the method of Egan et al. [12]. First, 25 mL of the solvent (a mixture of chloroform and acetic acid in a ratio of 2:3) was added to the sample. Then, 1 mL saturated solution of potassium iodide was added to it and placed in the dark for 10 min. Next, 20 mL of distilled water and 1 mL of a solution of 1.5% starch were added. The mixture was titrated with 0.01 N sodium thiosulfate. A test was also performed without the oil and PV was calculated using the following equation:

$$\text{PV} = ((V_1 - V_2) \times 100 \times N) / W,$$

where PV is the milli-equivalent g of free iodine per one kg of fat, V_1 is the consumed volume of thiosulfate, V_2 is the consumed volume of thiosulfate for the test without fat, W is the weight of the fat, and N is the normality of the sodium thiosulfate solution.

Measurement of total volatile basic nitrogen

The total volatile basic nitrogen (TVB-N) was measured according to the method of Douglas et al. [13]. Ten grams of minced fish meat along with 2 g of magnesium oxide and 300 mL of distilled water were heated in the kjeldahl flask. The vapor was condensed into a flask containing 25 mL of boric acid and a few drops of 2% methyl red. The collected solution was then titrated by 0.1 N sulfuric acid. TVB-N was calculated according to the following Eq. (1):

$$\text{TVB-N} = V \times 14, \quad (1)$$

where V is the consumed volume of sulfuric acid.

Measurement of thiobarbituric acid

TBA was measured according to Kirk et al. [15] and colorimetric methods. Two hundred milligrams of fish oil were transferred to a 25 mL flask and then brought to volume with butanol-1. Next, 5 mL of the above mixture was entered to a dry, tightly closed pipe and 5 mL of TBA reagent was added. The tightly closed pipes were placed in a water bath (Ecotecs/c08v) with a temperature of 95 °C for 2 h and then cooled to room temperature. Then, the absorbance value was read by a spectrophotometer (As) at 530 nm against distilled water (Ab). The TBA (mg malondialdehyde per kg fish oil) was calculated using the following equation:

$$\text{TBA value} = ((As - Ab) \times 50) / 200$$

pH measurement

Twenty grams of fish filet were mixed with 100 mL of distilled water in a mixer. The obtained mixture was filtered through the filter paper to remove the excess parts of fish flesh. Then, the pH was measured using a pH meter (crison GLP 22+), which was previously calibrated by 4 and 7 standard buffers [14].

Statistical analysis

Data analyses were performed using SPSS (version 16.0 for Window, SPSS, Inc., Chicago, IL, USA). All data were subjected to one-way analysis of variance (ANOVA) to evaluate the effects of the experimental conditions. The

normality of data was tested using the Kolmogorov–Smirnov test. Data were then subjected to Duncan's post hoc test. Differences were considered statistically significant at $p < 0.05$.

Results and discussion

The amount of free fatty acids

Our results showed that the amount of FFA increased with the increasing duration of fish preservation in the refrigerator. Figure 1 shows the results of this parameter. The highest and lowest amounts of FFA were related to the control group (uncoated samples) and treated group coated with alginate containing 3% lycopene, respectively. The figure shows significant differences ($p < 0.05$) between coated samples containing lycopene and other groups on days 8, 12, and 16. The same condition was established between the control and coated samples without lycopene on days 12 and 16. Moreover, differences among the samples containing lycopene were less and probably insignificant.

Although FFA production is not the only index of reduction in the nutritional value of food, its evaluation is of importance in the study of fish spoilage [16, 17]. This is related to the peroxidant effect of FFA. Besides, FFA compared to larger fat molecules (i.e., triglyceride and phospholipid) are smaller in size and have higher oxidation rates [16, 17]. The formation of FFA occurs because of the catalytic activity of enzymes on the lipids (primarily lipase and phospholipase) [16].

Based on these results, FFA increases with time in all treatments. This increase was intense in the control group, particularly on days 12 and 16. Therefore, the results

Fig. 1 Changes in FFA values in six batches of fish filets during 16 days of storage at 4 °C (values are mean $\pm$ SD)

demonstrated that the alginate coating successfully reduced the production rate of FFA in the fish filets.

Our observations about the effect of sodium alginate were compatible with those of Li et al. They demonstrated that the release rate of FFA from lipid droplets coated with alginate and chitosan was significantly lower than that from control (uncoated lipid droplets) samples [18]. In another study, a 12–100% decrease in the release rate of FFA from lipid droplets encapsulated within alginate beads compared to uncapsulated lipid droplets was shown [19]. In addition, our results showed the positive effect of lycopene in the reduction of FFA; however, different percentages of lycopene showed similar results.

Peroxide measurement

Figure 2 represents the results of peroxide measurement. The results showed that the peroxide amount of all samples increased during storage. At the end of the storage period, although the lowest value was related to the treatment group containing sodium alginate coating with 3% lycopene, there were no significant differences ($p > 0.05$) between the samples during the cold storage days (except for control samples on the last day).

In the complex process of fat oxidation, unsaturated fatty acids act with molecular oxygen to produce free radicals. Fat peroxy radical (ROO°) is combined with other molecules to form fatty acid composition, free radicals such as hydroperoxide, and other free radicals. Repetition of this action may ultimately lead to the accumulation of hydroperoxides [20].

Hydroperoxide is the primary product of lipid oxidation. As peroxides are compounds with no taste and odor, they cannot be noticed by consumers. However, these

compounds cause the formation of secondary compounds such as aldehydes and ketones [21].

Our observations showed no differences between all samples during storage (except for control samples on the last day). In general, this coating (lycopene) was not successful in the reduction of PV. Our observations were not in agreement with the results of Hamzeh et al. [22] considering that the PV results for the trout filets coated with sodium alginate and kept for 20 days under refrigeration. In their results, significant differences were observed between the coated rainbow trout with sodium alginate and control samples. However, the results of the present study corroborated the results of Ojagh et al. [23] such that no significant differences were found between coated (with chitosan) samples and control trout samples during cold storage.

Total volatile basic nitrogen

TVB-N chart results (Fig. 3) indicated that the index increased with the increase of storage time in the refrigerator. Moreover, after day 8, a significant difference was observed ($p < 0.05$) between the control and other samples. The rainbow trout coated with alginate without lycopene displayed a significantly higher ($p < 0.05$) TVB-N value compared to the coated samples containing lycopene. The filets coated with alginate lycopene were close to each other in terms of TVB-N values, and the coated samples containing 3% lycopene had the lowest TVB-N value.

TVB-N is an indicator to detect corruption in the meat and contains many volatile compounds such as ammonia, methylamine, dimethylamine, trimethylamine, and other similar compounds that are produced by microbial and enzymatic activities [24].

Our findings were compatible with the findings of Song et al. [10] and Lu et al. [25] who showed a significant

Fig. 2 Changes in PV values in six batches of fish filets during 16 days of storage at 4 °C (values are mean $\pm$ SD)

Fig. 3 Changes in TVB-N values in six batches of fish filets during 16 days of storage at 4 °C (values are mean $\pm$ SD)

difference between control and fishes coated with sodium alginate in TVB-N values. These observations, likewise, were consistent with the findings of Hamzeh et al. [22] on the similarity of TVB-N in the trout filets coated with sodium alginate. Furthermore, the results of this research were in agreement with the results of Ojagh et al. [23] and Arashisar et al. [26].

TBA value

The effect of different treatments on the TBA value is represented in Fig. 4. According to this figure, increasing the storage time heightened the TBA value. The maximum index at the end of the storage period was related to the control group and the treatment group with alginate coating without lycopene, which had a significantly higher TBA value ($p < 0.05$) compared to other groups. In general, concerning the coated treatments containing lycopene on days 0, 4, 8, and 12, no significant differences were observed or the differences very too low to be considered significant.

One of the indicators of secondary fat oxidation is TBA. The TBA test investigates the effect of lipid oxidation on foods by determining malondialdehyde (MDA) content. MDA is produced as a secondary product through the degradation of lipid hydroperoxides. However, according to Auburg et al. [27], the MDA produced by oxidation can react with other compounds such as nucleic acids, phospholipids, nucleotides, proteins, amino acids, and aldehyde compounds; therefore, it is removed. In this case, TBA cannot be a precise indicator to measure the secondary oxidation of fat.

The increase in the TBA value of the treatments during storage can be attributed to the increase in the oxidation of unsaturated fatty acids, production of volatile metabolites

Fig. 4 Changes in TBA values in six batches of fish filets during 16 days of storage at 4 °C (values are mean $\pm$ SD)

in the presence of oxygen, and water loss in a part of the filet [28].

Our results did not introduce the alginate-lycopene coating as an effective solution against MDA production. The same results were seen for PV; therefore, the prevention of lipid oxidation was not significant by such a coating.

Our findings were opposite to the results of Hamzeh et al. [22] and Lu et al. [29] in the trout and snakehead fish filets, respectively, coated with sodium alginate solution.

pH measurement

Changes in the pH value calculated during storage in the refrigerator are shown in Fig. 5. According to the results, at the beginning of the storage period, the pH value decreased and at the end of the period, it increased. At the end of the refrigeration storage period, the lowest pH was related to the treatment with sodium alginate coating containing 1.5% lycopene. As it is obvious from Fig. 5, the changes in the pH values for all treatments were small. The results showed that there were no significant differences ($p > 0.05$) between all treatments, although control samples showed higher pH value compared to the other samples on day 8.

Changes in pH depend on seasons, species, and other factors. After death, when the blood circulation is cut off and the subsequent oxygen supply disrupted, the carbohydrates are stored as glycogens for the anaerobic decomposition and production of lactic acid in fish; therefore, pH of tissues reduces. However, many researchers attribute the reduction of pH to the dissolution of CO₂ in the filet [24, 30]. On the other hand, increase in pH is related to enzymatic and microbial activities followed by the decomposition of amine compounds (a TMA to TMA) [31, 32]. Our designed coatings could reduce the pH values of filets; however, the reductions were not significant in comparison to uncoated treatments. The same results were

Fig. 5 Changes in pH values in six batches of fish filets during 16 days of storage at 4 °C (values are mean $\pm$ SD)

obtained by Song et al. in 2011. The difference in the pH of breams (*Megalobrama amblycephala*) coated with alginate was insignificant compared to control samples in a period of 16-day storage in the refrigerator [10].

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

References

- Mielnik MB, Herstad O, Lea P, Nordal J, Nilsson A. Sensory quality of marinated frozen stored chicken thighs as affected by dietary fish fat and vitamin E. *Int. J. Food Sci. Tech.* 37: 29–39 (2002)
- Serdaroğlu M, Yıldız-Turp G, Korczak J, Heś M, Gramza A, Jędrusek-Golińska A, Lisiewska Z, Słupski J, Kmiecik W, Gębczyński P. The effects of ascorbic acid, rosemary extract and α -tocopherol/ascorbic acid on some quality characteristics of frozen chicken patties. *Food Sci. Tech.* 7(1): 1–5 (2004)
- Losada V, Barros-Velázquez J, Gallardo JM, Aubourg SP. Effect of advanced chilling methods on lipid damage during sardine (*Sardina pilchardus*) storage. *Eur. J. Lipid Sci. Tech.* 106: 844–850 (2004)
- Suppakul P, Miltz J, Sonneveld K, Bigger SW. Active packaging technologies with an emphasis on antimicrobial packaging and its applications. *J. Food Sci.* 68: 408–420 (2003)
- Robertson GL. *Food packaging: Principles and practice*. CRC press, Boca Raton, FL, USA. pp. 50–55 (2016)
- Ryu S, Rhim J, Roh H, Kim S. Preparation and physical properties of zein-coated high-amylose corn starch film. *LWT-Food Sci. Tech.* 35: 680–686 (2002)
- Oussalah M, Caillet S, Saucier L, Lacroix M. Antimicrobial effects of alginate-based film containing essential oils for the preservation of whole beef muscle. *J. Food Protect.* 69: 2364–2369 (2006)
- Pokorny J, Yanishlieva N, Gordon MH. *Antioxidants in food: Practical applications*. CRC press. pp. 128–129 (2001)
- Abdollahi M, Rezaei M, Farzi G. A novel active bionanocomposite film incorporating rosemary essential oil and nanoclay into chitosan. *J. Food Eng.* 111: 343–350 (2012)
- Song Y, Liu L, Shen H, You J, Luo Y. Effect of sodium alginate-based edible coating containing different anti-oxidants on quality and shelf life of refrigerated bream (*Megalobrama amblycephala*). *Food Control.* 22: 608–615 (2011)
- Bligh EG, Dyer WJ. A rapid method of total lipid extraction and purification. *Can. J. Biochem. Phys.* 37: 911–917 (1959)
- Egan H, Kirk R, Sawyer R. *Pearson's chemical analysis of foods*. Churchill livingstone, New York. pp. 507–546 (1981)
- Goulas AE, Kontominas MG. Effect of salting and smoking-method on the keeping quality of chub mackerel (*Scomber japonicus*): biochemical and sensory attributes. *Food Chem.* 93: 511–520 (2005)
- Kirk S, Sawyer R. *Pearson's composition and analysis of foods*. Longman group Ltd. pp. 609–634 (1991)
- Sallam KI, Samejima K. Microbiological and chemical quality of ground beef treated with sodium lactate and sodium chloride during refrigerated storage. *LWT-Food Sci. Tech.* 37: 865–871 (2004)
- Losada V, Barros-Velázquez J, Aubourg SP. Rancidity development in frozen pelagic fish: Influence of slurry ice as preliminary chilling treatment. *LWT-Food Sci. Tech.* 40: 991–999 (2007)
- Lugasi A, Losada V, Hóvári J, Lebovics V, Jakoczi I, Aubourg S. Effect of presoaking whole pelagic fish in a plant extract on sensory and biochemical changes during subsequent frozen storage. *LWT-Food Sci. Tech.* 40: 930–936 (2007)
- Li Y, McClements DJ. Controlling lipid digestion by encapsulation of protein-stabilized lipid droplets within alginate–chitosan complex coacervates. *Food Hydrocolloid.* 25: 1025–1033 (2011)
- Li Y, Hu M, Du Y, Xiao H, McClements DJ. Control of lipase digestibility of emulsified lipids by encapsulation within calcium alginate beads. *Food Hydrocolloid.* 25: 122–130 (2011)
- Simic MG, Taylor KA. Free radical mechanisms of oxidation reactions. pp. 69–72. In: *Warmed-over flavor of meat*. A. J. St. Angelo & M. E. Bailey, (Eds.). Academic Press. New York (1987)
- Özyurt G, Polat A, Tokur B. Chemical and sensory changes in frozen (-18°C) wild sea bass (*Dicentrarchus labrax*) captured at different fishing seasons. In: *J. Food Sci. Tech.* 42: 887–893 (2007)
- Hamzeh A, Rezaei M. The effects of sodium alginate on quality of rainbow trout (*Oncorhynchus mykiss*) filets stored at $4 \pm 2^{\circ}\text{C}$. *J. Aquat. Food Prod. Tech.* 21: 14–21 (2012)
- Ojagh SM, Rezaei M, Razavi SH, Hosseini SMH. Effect of chitosan coatings enriched with cinnamon oil on the quality of refrigerated rainbow trout. *Food Chem.* 120: 193–198 (2010)
- Fan W, Chi Y, Zhang S. The use of a tea polyphenol dip to extend the shelf life of silver carp (*Hypophthalmichthys molitrix*) during storage in ice. *Food Chem.* 108: 148–153 (2008)
- Lu F, Ding Y, Ye X, Liu D. Cinnamon and nisin in alginate–calcium coating maintain quality of fresh northern snakehead fish filets. *LWT-Food Sci. Tech.* 43: 1331–1335 (2010)
- Arashisar Ş, Hisar O, Kaya M, Yanik T. Effects of modified atmosphere and vacuum packaging on microbiological and chemical properties of rainbow trout (*Oncorhynchus mykiss*) filets. *Int. J. Food Microbiol.* 97: 209–214 (2004)
- Aubourg SP. Interaction of malondialdehyde with biological molecules—new trends about reactivity and significance. *Int. J. Food Sci. Tech.* 28: 323–335 (1993)
- Keshri R, Sanyal M. Effect of sodium alginate coating with preservatives on the quality of meat patties during refrigerated ($4 \pm 1^{\circ}\text{C}$) storage. *J. Muscle Foods.* 20: 275–292 (2009)
- Lu F, Liu D, Ye X, Wei Y, Liu F. Alginate–calcium coating incorporating nisin and EDTA maintains the quality of fresh northern snakehead (*Channa argus*) filets stored at 4°C . *J. Sci. Food. Agric.* 89: 848–854 (2009)
- Manju S, Gopal TS, Jose L, Ravishankar C, Kumar KA. Nucleotide degradation of sodium acetate and potassium sorbate dip treated and vacuum packed Black Pomfret (*Parastromateus niger*) and Pearlscale (*Etroplus suratensis*) during chill storage. *Food Chem.* 102: 699–706 (2007)
- Church N. MAP fish and crustaceans sensory enhancement. *Food Sci. Tech. Today* 12: 73–83 (1998)
- Hernández M, López M, Alvarez A, Ferrandini E, García BG, Garrido M. Sensory, physical, chemical and microbiological changes in aquacultured meagre (*Argyrosomus regius*) filets during ice storage. *Food Chem.* 114: 237–245 (2009)