World Journal of Fish and Marine Sciences 4 (5): 462-466, 2012
ISSN 2078-4589

DOI: 10.5829/idosi.wjfms.2012.04.05.6439

Effect of Gelatin Coating on Fatty Acid Composition of Grass Carp (*Ctenopharyngodon idella*) During Refrigerated-Storage

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Abstract: The effect of gelatin coating on the fatty acid composition of refrigerated-stored slices of grass carp (*Ctenopharyngodon idella*) was investigated in this study. Monounsaturated fatty acids (MUFA) were the main class of fatty acids followed by polyunsaturated (PUFA) and saturated (SFA) fatty acids. C20:5 (EPA) ranged from 0.34-0.43% of total fatty acids (TFA) and C22:6 (DHA) ranged from 2.59-3.24% of TFA. In general coated slices (CS) had higher content of EPA and DHA when compared to non-coated slices (NCS). CS samples had lower n-6/n-3 (3.09) and higher polyene ratio (C20:5+C22:6/C16:0) (0.20) compare to control and NCS. Among all samples, NCS had the lowest PUFA/SFA ratio (1.10) while control and CS had the PUFA/SFA ratio of 1.14 and 1.16 respectively. The obtained results indicate the beneficial effect of gelatin as a natural and safe coating material.

Key words: Gelatin % Coating % Refrigerate-Storage % Fatty Acid Composition % Grass Carp

INTRODUCTION

For health concern, it is recommended to reduce dietary saturated fatty acids but increase dietary mono and polyunsaturated fatty acids. Seafood is rich source of n-3 polyunsaturated fatty acids such as eicosapentaenoic acid (C20: 5, EPA) and docosahexaenoic acid (C22: 6, DHA) [1], which play an important role in human nutrition, disease prevention and health promotion [2, 3]. It has been reported that a diet containing seafood and in particular omega 3 fatty acids, will reduce the occurrence of cardiovascular diseases and this is a reason that consumption of fish and fish products is encouraged [4]. Essential polyunsaturated fatty acids such as EPA and DHA are not synthesized in the human body, therefore their inclusion in diets is essential [5]. Due to the decline of wild fish stocks as a result of over-exploitation and serious habitat alternations, consumption of cultured fish is proposed as an alternative.

Lipid oxidation is a major cause of quality deterioration of refrigerated foods [6]. Lipid oxidation often leads to development of off-flavor and off-odors. It can reduce the nutritive value of muscle food as a result of oxidation of essential fatty acids [7]. In food industry in order to prevent lipid oxidation, antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), propyl gallate (PG) and tert-butylhydroquinone (TBHQ) are added to the foods, but there are concerns on the potential health risks of such synthetic antioxidants in humans [8].

Meat shelf-life can be extended using a surface coating. Surface coating provides a water and oxygen barrier. Collagen and gelatin coatings are used as a barrier on meat products to reduce purge, color deterioration, aroma deterioration and spoilage, improve sensory scores and act as an antioxidant [9]. Gelatin is a heterogeneous mixture of high molecular weight water-soluble proteins derived from collagen. In food industry, gelatin is used to improve elasticity, consistency and stability of foods [10]. Gelatin coating has reduced the formation of TBARS and improved colour stability in cooked ham and bacon pieces [11]. Bovine gelatin coating has increased the shelf-life of beef [12]. Antimicrobial coating from catfish skin gelatin has retarded the microbial growth and prolonged shelf life of fresh white shrimp [13]. Despite gelatin coating could preserve the quality of muscle foods during storage, there are little information about the effect of gelatin coating on the oxidative stability of essential long-chain (LC)

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unsaturated fatty acids. Therefore the objective of this study was to investigate how gelatin coating can preserve the long-chain (LC) essential polyunsaturated fatty acids of grass carp (*Ctenopharyngodon idella*) slices during refrigerated-storage.

MATERIAL AND METHODS

Fish Samples: Grass carp, *Ctenopharyngodon idella* (average weight: 2.5 kg) were purchased from a local market, placed in ice boxes (fish to ice ratio of 1:2 w/w) and transported to the laboratory where they were beheaded, gutted, washed and filleted. The obtained fillets were then cut into small slices of about 50 g which used for coating with gelatin solution.

Extraction of Fish Skin Gelatin: Frozen skin samples were thawed at 4°C for about 5 h and used for gelatin extraction. Skin was defatted using butyl alcohol (10% v/v) with skin to solution ratio of 1:10 (w/v) for 18 h and the solution was changed every 6 h. Defatted skin was washed with cold water for three times and then soaked in 0.1 M sodium hydroxide with skin/solution ratio of 1:10 (w/v) for 2 h at 4 °C to remove non collagenous proteins and pigments. The alkaline solution was changed every 40 min. Skin was then soaked in acetic acid (0.05 M) with skin/solution ration of 1:10 (w/v) at 4 °C for 3 h to swell the collagenous material in the skin matrix. Acid swollen skin was then washed with cold tap water until neutral pH of the washing water was achieved. Gelatin was extracted from the swollen skins at 45°C with sample/water ratio of 1:5 (w/v) for 4 h with continuous stirring. Following extraction, the mixture was centrifuged at 6,000 rpm for 15 min. to remove insoluble material. The supernatant was then collected and freeze-dried. The dry samples were blended to obtain fine powder. The obtained gelatin powder was used for coating the fish slices.

Coating Fish Slices with Gelatin Solution: The coating solution was prepared by dissolving 5% (w/v) of gelatin in distilled water. Gelatin was hydrated in distilled water at 50ºC for 60 min. in a water bath (DKZ, Shanghai Yiheng Technology Co., Ltd, Shanghai, China), Then stirred with a magnetic stirrer (IKA® C-MAG HS 7, Staufen, Germany) for 15 min until complete solubilization was achieved. Glycerol was added to gelatin solution as plasticizer at a concentration of 0.2 g/g gelatin, stirred magnetically for 10 min, then heated at 55 °C for 30 min. Previously prepared grass carp slices were dipped in the coating solution at a ratio of 1:2 (w/v) for 2 min. while gently stirred. Thereafter the slices were removed and allowed to drain for 20 min. in order to gelatin cool and set. Coated slices were packed in polyethylene bags and stored at 4-5°C for 10 days and analyzed.

Lipid Extraction: Lipid was extracted according to the method of Bligh and Dyer [14]. Twenty-five g of sample were homogenized in a blender for 2 min. with a mixture of 50 ml chloroform and 100 ml methanol. Then 50 ml of chloroform were added and further homogenized for 30 sec. Finally 50 ml of distilled water were added to the mixture and blended for 30 sec. The homogenate was centrifuged (Avanti® J-E, BECKMAN COULTER, Inc., USA) at 3000 rpm for 15 min at 4°C. Supernatant was then transferred into a separating flask and the lower phase (chloroform phase) was drained off into a 250 ml Erlenmeyer flask containing 4 g anhydrous sodium sulfate and shaken vigorously. The solution was then filtered through a Whatman No. 4 filter paper into a round-bottom flask. Rotary evaporator (Rotavapor R-114, BÜCHI, Flawil, Switzerland) was used for solvent evaporation at 25°C.

Fatty Acid Analysis: Fatty acid methyl ester was prepared as follows: Lipid samples (15-20 drops) were diluted with 2 ml of potassium hydroxide followed by the addition of 5 ml n-hexane in a sealed tube. The mixture was then shaken using a vortex for 1 min and left for about 20 min. until it was separated into two phases. From top layer, fatty acid methyl ester was then taken for analysis by using Trace GC (Thermo Finnigan, Italy). The GC conditions were as follows: capillary column (Bpx-70, 60 m, 0.32 mm, i.d. 0.25 µm); the split ratio of 90:1; injection port temperature of 250 °C; flame ionization detector temperature of 270 °C. Oven temperature was set at 195 ºC for 75 minutes. Flow rate of carrier gas (helium) was 1 ml min^{-1} and the makeup gas was N_2 (30 ml/min). The sample size injected for each analysis was 1 µl.

Statistical Analysis: Data were analyzed by one-way analysis of variance (ANOVA) followed by Duncan multiple range test. All data were expressed as mean ± SD. The significance of results was at 5%.

RESULTS AND DISCUSSION

The main fatty acids in grass carp raw flesh were C18:1 (39.68% of TFA), C16:0 (18.27% of TFA) and C18:2 (16.97% of TFA) representing about 75% of TFA (Table 1). Monounsaturated fatty acids constituted as the

Table 1: Fatty acid composition (% of TFA) of raw, coated and non-coated slices of refrigerated-stored grass carp

NCS	CS	R	Fatty acids
0.91	0.86	0.87	C14:0
18.57	18.38	18.27	C16:0
3.66	3.65	3.68	C16:1
3.56	3.65	3.47	C18:0
40.66	38.40	39.68	C18:1
17.19	16.80	16.97	C18:2
1.84	1.79	1.78	C18:3
0.15	0.11	0.17	C20:0
1.25	1.39	1.41	C20:1
3.59	4.35	3.96	C20:4
0.34	0.43	0.40	C20:5 (EPA)
2.59	3.24	2.87	C22:6 (DHA)

Table 2: Major class of fatty acid composition (% of TFA) of raw, coated and non-coated slices of refrigerated-stored grass carp

NCS	CS	R	Fatty acids
23.19	23.00	22.78	ESFA
45.57	39.79	44.77	EMUFA
25.55	26.61	25.98	EPUFA
4.77	5.46	5.05	EN-3
17.19	16.89	16.97	EN-6

Table 3: Some calculations in fatty acid composition (% of TFA) of raw, coated and non-coated slices of refrigerated-stored grass carp

NCS	CS	R	
3.60	3.09	3.36	N-6/n-3
0.15	0.20	0.17	C20:5+C22:6/C16:0
3.06	2.88	3.10	MUFA+PUFA/SFA
1.10	1.16	1.14	PUFA/SFA

most dominant class of fatty acids (44.77% of TFA) followed by polyunsaturated (25.98% of TFA) and saturated (22.78% of TFA) fatty acids, respectively (Table 2). Omega-6 fatty acids were higher than omega-3 fatty acids (16.97 versus 5.05% of TFA) and the omega-6/omega-3 ratio was 3.36 (Table 3). The result is in agreement with Ghomi *et al*. [15] who have reported higher amount of n-6 fatty acids in grass carp muscle than n-3 fatty acids with omega-6/omega-3 ratio of 2.36. The EPA and DHA contents of grass carp flesh were 0.4 and 2.87% of TFA. The EPA and DHA of grass carp flesh are lower that their contents in seabass (*Dicentrarchus labrax*) [16], perch (*Perca fluviatilis*) [17] and rohu (*Catla catla*) [18] with EPA and DHA in the ranges of 3.05-12.17 and 8.33-16.82% of TFA respectively. Fair *et al*. [19] have mentioned that in cultured fish species, body lipid content is influenced by the diet fish consumed.

The results of gelatin coating of grass carp slices during 10 days refrigerated-storage on fatty acid composition has been indicated in Tables 1. Slight difference in fatty acid composition was observed among samples. In gelatin coated slices (CS), the content of MUFA decreased and instead PUFA increased. There was no significant difference in the content of MUFA and PUFA between control and non-coated (NCS) samples (Table 2). The increase in PUFA in CS slices could be due to the higher DHA (3.24% of TFA) content compare to control (2.87% of TFA) and NCS samples (2.59% of TFA). The results indicated that gelatin coating has better preserved the long-chain (LC) n-3 fatty acids. Ecological studies have found a negative correlation between the risk of developing heart diseases and fish consumption because of their long chain omega-3 fatty acids [20]. In humans many chronic diseases such as cardiovascular, inflammatory and autoimmune diseases are associated with high intake of n-6 fatty acids and instead increased levels of omega-3 fatty acids such as EPA and DHA in diets and lower n-6/n-3 ratio have beneficial health effects [5]. The n-6/n-3 ratio of all samples were in the optimal ranges of 2:1-4:1 for human health as suggested by pepping [21]. However lower n-6/n-3 ratio was observed for CS samples which is due to the higher amount of n-3 fatty acids and particularly EPA and DHA in CS samples. Polyene ratio is an indirect measurement of lipid oxidation since it reflects the loss of omega-3 polyunsaturated fatty acids such as EPA and DHA as a consequence of oxidation [22]. Results indicated that CS exhibited higher polyene ratio (0.20) when compared to the control (0.17) and NCS (0.15) samples indicating lower oxidation in CS samples. The higher polyene ratio is CS samples indicated the protective effect of gelatin during refrigerated-storage. PUFA/SFA and PUFA+MUFA/SFA ratios are commonly used to indicate the nutritional quality of food lipid [23]. PUFA/SFA ratio in all samples in this study was in the ranges of 1.10-1.16 which is slightly higher than the recommended ratio of 0.4-1 [24]. CS samples exhibited slightly higher PUFA/SFA when compared to other samples.

CONCLUSION

Gelatin-coated slices (CS) had the higher content of EPA and DHA when compared to non-coated slices (NCS) and control samples. CS samples had lower n-6/n-3 (3.09) and higher polyene ratio (C20:5+C22:6/C16:0) (0.20). The results indicated the beneficial effect of gelatin as a natural and safe coating material.

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