冷凍タラ肉中におけるタンパク質酸化におよぼす食塩添加の影響

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Original Article

Effect of NaCl on Protein Oxidation in Frozen Cod Meat

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Summary : Protein carbonyls (PC) and malonaldehyde (MA) content in cod meat with or without 2 % NaCl stored at -20 °C were analyzed for 24 weeks. The increase in the PC contents that was observed in the control as well as the NaCl-treated samples indicates the progression of protein oxidation even during frozen storage. Significant differences were not observed between the PC and MA contents of the control and NaCl-treated samples.

Key words : Cod, Malon aldehyde, Protein carbonyl.

Introduction

Proteins from animal tissues are targets for oxygen radical attack in vivo and in foods. (Mercier *et al.* 2004 ; Mercier *et al.* 1998 ; Stadtman, 1992) Protein oxidation by oxygen radicals is responsible for protein fragmentation or aggregation and affects the quality of meat and meat products. Although appearance of carbonyl groups is certainly not specific for oxidative modification, protein carnonyl (PC) measurement is the most common and important method for detecting and quantifying protein oxidation (Levine *et al.* 1990.). There have been several studies on the effects of lipid peroxidation on proteins in fish meat (Kawasaki *et al.* 1992 ; Kawasaki *et al.* 1991 ; Takama *et al.* 1972 ; Takiguchi, 1996). However, protein carnonyl (PC) content was not measured in these studies. To our knowledge, few quantitative studies (Munasinghe *et al.* 2005 ; Sakai *et al.* 1998 ; Srinivasan & Hultin, 1995) have been conducted on the oxidative modification of proteins in fish meat during storage.

Sodium chloride (NaCl) is added to muscle foods for a variety of purposes, including flavor and the inhibition of microorganisms. NaCl, nevertheless, has been shown to have an accelerating effect on lipid peroxidation in a variety of meats, including beef, pork, chicken, and fish (Buckley *et al.* 1989 ; Cuppett *et al.* 1989 ; Kanner *et al.* 1991 ; Osinchak *et al.* 1992 ; Rhee *et al.* 1983 ; Takiguchi, 1989). However, it is uncertain why protein oxidation caused by lipid peroxidation progresses or suppresses in fish meats containing NaCl. In this background, variation of PC and malon aldehyde (MA), a index of lipid peroxidation, contents in relation to protein oxidation caused by lipid peroxidation were investigated in minced cod meat containing NaCl stored at -20 °C for 24 weeks.

Materials and Method

A filet of fresh cod *Gadus morhua* was bought from a local market and brought to the laboratory under refrigeration. The skin was removed and ordinary muscle thus obtained was cut into small pieces. Once, ordinary muscle was minced for 30 seconds, the minced muscle was divided into two portions ; one

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was treated with NaCl, whereas the other was untreated (control). Each portion was then blended for 30 s using a food processor and was further divided into seven equal portions to prepare samples. Six out of seven samples were wrapped with wrapping papers, followed by aluminum foils and stored at - 20 °C. For the purpose of analysis one of six sample was removed in the 4th week. The remaining five samples were analyzed subsequently in 8th, 12th, 16th, 20th, and 24th week. The seventh sample was used on day 0, which was the day on which the seven samples were prepared from a single portion.

PC contents were determined by the 2,4-dinitrophenylhydrazine (DNPH) (Wako Pure Chemicals, Tokyo, Japan) method of Nakamura and Goto. (Nakamura & Goto, 1996). One g of muscle was homogenized in 10 to 20 volumes of 50 mM Tris-HCl buffer (pH 7.4) containing 1 mM EDTA in a Polytron homogenizer. The homogenates were centrifuged at 15,000 xg for 10 min at 4 °C. Proteins in two equal portions of the supernatant were precipitated with 10 % trichloroacetic acid. The precipitates were treated with either 2 N HCl alone (control) or 2 N HCl containing 10 mM DNPH at 15 °C for 1 h. After the reaction, the mixture was centrifuged, the precipitates were washed with an ethanol-ethyl acetate (1 : 1) mixture three times and the final precipitates were dissolved in 8 M urea. The absorbancy was measured at 360 nm and the carbonyl content was obtained as nmole per mg protein using a molar extinction coefficient of $22{,}000^{-1}$. Samples processed similarly but without DNPH treatment were used as controls. Con centrations of protein were measured with a Bio-Rad assay kit (Bio-Rad Laboratories Inc. Hercules, California, USA) using bovine serum albumin as a standard. 1,3-Diethyl-2-thiobarbituric acid (DETBA) was obtained from Aldrich Chemicals, Milwaukee, Wisconsin, USA. The chemical reaction between DETBA and MA was conducted accordance to the method of Sakai *et al* (1999). The DETBA-MA adduct was applied to the HPLC system under the following conditions : column, Inertsil ODS (particle size of 5 μm, 250 × 4.6 mm i.d. (GL Sciences, Tokyo, Japan) ; mobile phase, acetonitrile : 0.1 M sodium chloride (75 : 25, v/v) ; flow rate, 1.0 ml/min. ; detection, excitation and emission at 515 nm and 555 nm, respectively. Total lipid was extracted from cod meat following the method developed by Folch et al (1957). The n-3 fatty acids contents were measured following the gas chromatographic method developed by Takenoyama *et al.* (1999) with tricosanoic acid as internal standard. All data were analyzed by the Duncan multiple range tests (Duncan, 1955).

Results and Discussion

The PC content of both the control and the NaCl treated samples showed a more or less similar pattern of variation during the 24th week of the storage period (Table 1). A significant increase was observed in the PC content both the control and the NaCl treated samples on the 8th week relative to their respective initial values. Furthermore, the addition of NaCl did not show any significant effect on the PC content of cod mince, except on the 8th week in which a significantly high level of PC was observed in samples with NaCl in comparison with that in the control. The MA contents of both control and NaCl treated samples showed a similar trend of MA variation during six months storage period (Table 1). The MA contents showed an initial significant declined followed by an increased. This sudden decline of MA content may be due to rapid reaction of already formed MA with exposed proteins at low temperature (Buttkus, 1967) while lipid peroxidation was progressing slowly. Furthermore, Ohsima *et al.* (1984) also observed a drastic decline followed by an increase of Thiobarbituric acid number (TBA number) in minced and washed cod meat stored at -16 °C. The addition of NaCl did not

Table 1. The protein carbonyl (PC) content (nmol/mg protein) and the malonaldehyde (MA) content (μmol/g tissue) of minced cod meat stored at -20 °C for 24 weeks.

Storage period (Weeks)	0	4	8	12	16	20	24
PC							
Control	$0.78\pm0.05^{a,x}$	$1.58\pm0.13^{ac,x}$	$2.51\pm0.08^{bc,x}$	$2.65\pm0.31^{b,x}$	$2.34\pm0.66^{bc,x}$	$3.33\pm0.37^{b,x}$	$2.88\pm0.12^{b,x}$
2 % NaCl	$0.85\pm0.11^{a,x}$	$1.55\pm0.07^{a,x}$	$2.93\pm0.10^{b,y}$	$3.34\pm0.10^{b,x}$	$3.07\pm0.69^{b,x}$	$2.78\pm0.12^{b,x}$	$2.89\pm0.25^{b,x}$
MA							
Control	$1.41\pm0.17^{a,x}$	$0.78\pm0.07^{b,x}$	$1.75\pm0.32^{a,x}$	$1.71\pm0.14^{a,x}$	$0.65\pm0.05^{b,x}$	$0.83\pm0.04^{b,x}$	$0.81\pm0.07^{b,x}$
2 % NaCl	$1.18\pm0.05^{a,x}$	$0.84\pm0.08^{b,x}$	$1.88\pm0.08^{c,x}$	$1.65\pm0.04^{d,x}$	$0.78\pm0.03^{bc,x}$	$0.61\pm0.10^{c,x}$	$0.67\pm0.02^{bc,x}$

$^{a-g}$Values (means ± SE, n = 4) within the same row with no common superscripts differed significantly.
$^{x-y}$Values (means ± SE, n = 4) within the same column with no common superscripts differed significantly.

Table 2. Total lipids (%) and fatty acid contents (mg/g lipid) of minced cod meat stored at -20 °C for 24 weeks

	0 day		12 weeks		24 weeks	
	Control	2 % NaCl	Control	2 % NaCl	Control	2 % NaCl
Total lipids (%)	0.67	0.61	0.62	0.68	0.71	0.78
Fatty acids (mg/g lipid)						
Eicosapentaenoic acid (n-3)	109.6	109.9	110.3	108.5	108.3	101.4
Docosahexaenoic acid (n-3)	189.8	195.4	201.8	178.6	195.8	186.5

(n=2)

make any significant effect on lipid peroxidation in cod mince. Eicosapentaenoic acid and docosahexaenoic acid content of NaCl-containing samples were slightly lower than those of the controls after the 12 th and 24 th week (Table 2). This result might suggest that oxidation of these lipids induced the decrease.

No significant correlation was observed between the PC and the MA content during the 24 th week of the storage period ($r = 0.01$, $p = 0.98$). Sakai *et al.* reported that a linear correlation was observed between PC and MA content in the red (Sakai, *et al.*, 1998) and ordinary muscle (Munasinghe, *et al.*, 2005) of yellowtail *Seriola quinqueradiata* when stored at 0 °C. Protein oxidation was reported to link to lipid oxidation in turkey (Mercier, *et al.*, 1998) and beef (Mercier *et al.* 1995) meats. Srinivasan and Hultin found a relathionship between PC and thiobarbituric acid reactive substances values in cod and mackerel fillets when exposed to free radicals generating system. (Srinivasan & Hultin, 1995) In contrast to these studies, protein oxidation may not link to lipid peroxidation in frozen cod meat. However, there are few studies of relationship between protein and lipid oxidation in fish meat products (Munasinghe, *et al.*, 2005; Sakai, *et al.*, 1998; Srinivasan & Hultin, 1995). In addition, linear correlation was also observed between PC and MA contents in the yellowtail ordinary muscle stored at -20 °C. (Shimizu *et al.* 2009) Further studies are necessary to elucidate the relationship between protein oxidation and lipid peroxidation in fish meat products. The addition of NaCl did not make any significant effect on protein oxidation and lipid peroxidation in cod mince. This result is different from the finding that addition of NaCl accelerated protein oxidation and lipid peroxidation in yellowtail ordinary muscle stored at -20 °C (Shimizu, *et al.*, 2009). The difference of lipid contents in the muscle may be one of the causes. Further studies are also necessary to elucidate the effects of NaCl on protein oxidation and lipid peroxidation in fish meat products.

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冷凍タラ肉中におけるタンパク質酸化におよぼす食塩添加の影響

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要　約

0および2％NaClを添加したタラ肉を-20℃にて24週間貯蔵し，カルボニル修飾タンパク質（PC）およびマロンアルデヒド（MA）含量の変動を測定した．

PC含量は貯蔵期間中に増加し，タンパク質酸化が-20℃でも進行することを示していた。貯蔵期間中，NaCl添加区と対照区でPCおよびMA含量には有意な差は認められなかった．また，PC含量とMA含量には相関関係は認められなかった．

キーワード：タラ，マロンアルデヒド，カルボニル修飾蛋白質