宮 崎 大 学 農 学 部 研 究 報 告 56 (111－114) (2010)
Bulletin of the Faculty of Agriculture, University of Miyazaki 56 (111－114) (2010)

Original Article

Effect of NaCl on 4-Hydroxy-2-alkenal Formation of Frozen Pork and Yellowtail Meat

Tadashi SAKAI, Saori KIRIAKE, Shusaku OHTUBO, Yukiko SHIMIZU

Department of Biochemistry and Applied Biosciences, Faculty of Agriculture, University of Miyazaki.

(Accepted on November 26, 2009)

Summary : 4-Hydroxy-2-nonenal contents in pork and 4-hydroxy-2-hexenal contents in yellowtail meat both containing 0, 0.3, 0.6, and 0.9 M NaCl stored at -20 °C were analyzed for 20 weeks. HNE content decreased in all pork samples during storage period. After 20 weeks of storage, HNE was not detected in all samples. HHE contents increased in all yellowtail samples. After 16 weeks of storage, HHE contents of NaCl added sample were significantly higher than those of control.

Key words : 4-Hydroxy-2-alkenal, Pork, Yellowtail meat

Introduction

Lipid oxidation is major cause of quality deterioration in meat and meat products (Pearson *et al.* 1983). A variety of secondary reactions occurs and many aldehydes are formed during the lipid peroxidation process. Among these aldehydes, 4-hydroxy-2-nonenal (HNE) which can be formed through peroxidation of n-6 polyunsaturated fatty acids (n-6 PUFAs) (Esterbauer *et al.* 1991) and 4-hydroxy-2-hexenal (HHE) which can be formed through peroxidation of n-3 PUFAs (Esterbauer, *et al.*, 1991) are highly reactive and may be considered as second toxic messengers which disseminate and augment initial free-radical events (Benedetti & Comporti 1987 ; Brembilla *et al.* 1986 ; Esterbauer, *et al.*, 1991 ; Poot *et al.* 1988 ; Sakai *et al.* 2000). We have reported that HNE exists in pork and beef (Sakai *et al.* 1995) and HHE exist some fish meats (Sakai *et al.* 1997). We have also reported that HNE decreased in the pork containing sugi wood vinegar (Munasinghe *et al.* 2003a) but increased in the meats containing NaCl stored at 0 °C (Sakai *et al.* 2004). In contrast, HHE increased in yellowtail meats containing sugi wood vinegar stored at 0 °C (Munasinghe *et al.* 2003b), but decreased in yellowtail meats containing NaCl stored at 0 °C (Munasinghe *et al.* 2006). Changes of HNE contents in pork are clearly different form those of HHE contents in yellowtail meats although each experimental condition used was same.

Sodium chloride (NaCl) is added to muscle foods for a variety of purposes, including flavor and the inhibition of microorganisms. NaCl has been shown to have an accelerating effect on lipid oxidation of meats.(Kanner *et al.* 1991) We have recently reported the effects of NaCl on HNE or HHE formation in pork and beef (Sakai *et al.* 2004) or yellowtail meat (Munasinghe *et al.* 2006). As mentioned above, the changes are different from each other. However, the changes of both aldehydes in the meats stored at -20 °C is uncertain. Therefore it is necessary to study the effect of NaCl addition on HNE or HHE formation in pork or fish meat. In this background, variation of HNE or HHE were investigated in pork or yellowtail meat containing NaCl stored at -20 °C for 20 weeks.

Materials and Method

HHE and HNE were synthesized by the method of Sugamoto *et al.* (1997), and identified on the basis of the ^{1}H-NMR and ^{13}C-NMR spectra. Butyl hydroxy toluene (BHT) was obtained from Tokyo Kasei

責任著者：境 正
宮崎大学農学部生物機能科学講座
〒889-2192 宮崎市学園木花台西1-1

Corresponding author : Tadashi Sakai
Division of Biotechnology and Biochemistry,
Faculty of Agriculture, University of Miyazaki
1-1 Gakuen Kibana-dai Nishi, Miyazaki 889-2192, Japan

(Tokyo, Japan). Other reagents were of analytical grade. Yellowtail (*Seriola quinqueradiata*) samples and pork were obtained from commercial markets. Both muscles were ground, mixed with revelant amount of NaCl and stored at -20 °C. In all experiments, 3 samples from each group were analyzed. MA and HHE or HNE contents were analyzed after 0, 4, 8, 12, 16, and 20 weeks of storage. 2,4-Dinitrophenylhydrazine (DNPH) was obtained from Wako Pure Chemicals (Tokyo). The chemical reaction of HNE or HHE essentially followed the procedure of Goldring *et al* (1993). The HNE-DNPH derivative was analyzed by the HPLC method reported by Goldring *et al* (1993) and the HHE-DNPH derivative was analyzed by a slight modification of the HPLC method reported by Sakai *et al* (1997). Analytical conditions are as follows : column, Ultrasphere C18 (250 × 4.6 mm i.d., Beckman) ; mobile phase, 30 mmol/L sodium citrate/ 27.7 mmol/L acetate buffer (pH 4.75) : methanol=25 : 75 (HNE) or=35 : 65 (HHE) ; flow rate, 1 mL/min ; detection wavelength, 365 nm. All data were expressed as the mean ± SE. Significant differences among means were determine by the Duncan multiple range tests (Duncan 1955).

Results and Discussion

Table 1 shows the changes in HNE contents in pork containing 0, 0.3, 0.6 and 0.9 M NaCl. HNE was detected in all control samples at 0 week. After storage of 4 and 12 weeks, HNE contents in the control samples were significantly higher than those containing NaCl. HNE was not detected in all samples at 20 weeks of storage. Addition of NaCl may suppress HNE formation in frozen pork. This result was different from those of refrigerated pork containing NaCl (Sakai *et al.* 2004). In the refrigerated pork, addition of NaCl accelerated HNE formation.

HHE contents in all samples fluctuated during storage. HHE contents in the samples containing 0.3 and 0.6 M of NaCl significantly increased after 20 weeks of storage but those in control samples did not increase during 20 weeks of storage (Table 2). Those containing 0.9 M of NaCl increased after 20 weeks of storage, although the difference was not significant (Table 2). We have previously reported that the HHE content was significantly higher in the control than in the samples containing NaCl in the yellowtail meats stored at 0 °C (Munasinghe *et al.* 2006). Effects of NaCl on HHE formation in frozen yellowtail meats may be different from those in the refrigerated meats.

Results obtained present study also showed that HNE formation mechanism in pork was different form HHE formation mechanism in yellowtail meats although each experimental condition used was same. It is uncertain why these differences occurred. Further studies must be necessary to elucidate why these difference occur. Studies along these lines are currently in progress in our laboratory.

Table 1. The variation of HNE contents (nmol/g tissue) in the control and the pork that contained 0.3, 0.6 and 0.9 M NaCl during 20 weeks at -20 °C storage

Sample	Weeks					
	0	4	8	12	16	20
Control	$0.10\pm0.03^{a,x}$	$0.07\pm0.03^{ab,x}$	$0.10\pm0.02^{a,x}$	$0.07\pm0.03^{ab,x}$	$0.03\pm0.03^{ab,x}$	$ND^{b,x}$
0.3M NaCl	$0.18^{1}\ 0.07^{a,x}$	$ND^{a,y}$	$0.10\pm0.02^{a,x}$	$0.01\pm0.00^{a,y}$	$0.00\pm0.00^{a,x}$	$ND^{a,x}$
0.6M NaCl	$0.12\pm0.06^{a,x}$	$ND^{bc,y}$	$0.10\pm0.04^{ac,x}$	$ND^{bc,y}$	$0.01\pm0.01^{b,x}$	$ND^{bc,x}$
0.9M NaCl	$0.76\pm0.25^{a,y}$	$ND^{b,y}$	$0.12\pm0.04^{b,x}$	$0.01\pm0.01^{b,y}$	$0.02\pm0.02^{b,x}$	$ND^{b,x}$

a-c Means (n=3) ± standard error within the same row with no common superscript differs significantly ($P < 0.05$)
x-y Means (n=3) ± standard error within the same column with no common superscript differs significantly ($P < 0.05$).
ND : not detected (< 0.1 nmol/kg)

Table 2. The variation of HHE contents (nmol/g tissue) in the control and the yellowtail meat that contained 0.3, 0.6 and 0.9 M NaCl during 20 weeks at -20 °C storage

Sample	Weeks					
	0	4	8	12	16	20
Control	$0.62\pm0.27^{a,x}$	$0.10\pm0.04^{b,x}$	$0.02\pm0.01^{b,x}$	$0.16\pm0.00^{b,x}$	$0.11\pm0.02^{b,x}$	$0.17\pm0.05^{b,x}$
0.3M NaCl	$0.14\pm0.06^{a,xy}$	$0.03\pm0.01^{a,x}$	$0.03\pm0.00^{a,x}$	$0.17\pm0.05^{a,x}$	$0.52\pm0.07^{b,y}$	$0.59\pm0.08^{b,x}$
0.6M NaCl	$0.12\pm0.06^{a,y}$	$0.02\pm0.01^{a,x}$	$0.05\pm0.01^{a,xy}$	$0.15\pm0.00^{a,x}$	$0.49\pm0.02^{ab,y}$	$0.80\pm0.45^{b,x}$
0.9M NaCl	$0.17\pm0.03^{a,xy}$	$0.06\pm0.03^{a,x}$	$0.12\pm0.04^{a,y}$	$0.15\pm0.05^{a,x}$	$0.56\pm0.20^{a,y}$	$0.51\pm0.37^{a,x}$

a-b Means (n=3) ± standard error within the same row with no common superscript differs significantly ($P < 0.05$)
x-y Means (n=3) ± standard error within the same column with no common superscript differs significantly ($P < 0.05$).

Acknowledgments

This work was supported in part by Grants-in-Aid for Scientific Research (No. 16580168 and No. 19580239) from the Ministry of Education, Science, and Culture, Japan and by The Salt Science Research Foundation (No. 0256 and No. 0650).

References

Benedetti, H., M. Comporti (1987). *Bioelectrochem. Bioenerg.* **18**, 187-202.

Brembilla, G., L. Sciaba, P. Faggin, A. Maura, U. M. Marinari, M. Ferro, H. Esterbauer (1986) *Mutat. Res.* **171**, 169-176.

Buckley, D. J., J. I. Gray, A. Asghar, J. F. Price, R. L. Krackel, A. M. Booren, A. M. Pearson, E. F. Miller (1989) *J. Food Sci.* **54**, 1193-1197.

Cuppett, S. L., J. I. Gray, A. M. Booren, J. F. Price, M. A. Stachiw (1989) *J. Food Sci.* **54**, 52-54.

Duncan, D. B. (1955) *Biometrics* **11**, 1-42.

Esterbauer, H., R. J. Schaur, H. Zollner (1991) *Free Radic. Bio. Med.* **11**, 81-128.

Goldring, C., A. F. Casini, E. Maellaro, B. Del Bello, M. Comport (1993) *Lipids* **28**, 141-145.

Kanner, J., S. Harel, R. Jaffe (1991) *J. Agric. Food Chem.* **39**, 1017-1021.

Munasinghe, D. M. S., K. Ichimaru, t. Matsui, K. Sugamoto, t. Sakai (2003a) *Meat Sci.* **63**, 377-380.

Munasinghe, D. M. S., K. Ichimaru, M. Ryuno, N. Ueki, T. Matsui, K. Sugamoto, S. Kawahara, T. Sakai (2003b) *Fish. Sci.* **69**, 189-194.

Munasinghe, D. M. S., S. Kawahara, T. Sakai (2006) *Biosci. Biotechnol. Biochem.* **70**, 3036-3038.

Osinchak, J., H. O. Hultin, O. T. Zajicek, S. D. Kelleher, G. Huang (1992) *Free Radic. Bio. Med.* **12**, 35-41.

Pearson, A. M., J. I. Gray, A. M. Wolzak, N. A. Horenstein (1983) *Food Technol.* **37**, 121-129.

Poot, M., H. Esterbauer, P. S, H. Hoehn (1988) *J. Cell. Physiol.* **137**, 421-429.

Rhee, K., G. C. Smith, R. N. Terrell (1983) *J. Food Protect.* **46**, 578-581.

Sakai, T., S. Kawazuru, K. Yamauchi, K. Uchida (1995) *Biosci. Biotechnol. Biochem.* **59**, 1379-1380.

Sakai, T., Y. Matsushita, K. Sugamoto, K. Uchida (1997) *Biosci. Biotechno. Biochem.* **61**, 1399-1400.

Sakai, T., D. M. Munasinghe, M. Kashimura, K. Sugamoto, S. Kawahara (2004) *Meat Sci.* **66**, 789-792.

Sakai, T., K. Sugamoto, N. Eto (2000) *J. Food Hygien. Soc. Japan* **41**, 368-370.

Sugamoto, K., Y. Matsushita, T. Matsui (1997) *Lipids* **32**, 903-905.

Takiguchi, A. (1989) *Nippon Suisan Gakkaishi* **55**, 1649-1654.

NaCl添加が凍結ブタおよびブリ肉中の4-ヒドロキシ-2-アルケナール生成に及ぼす影響

境　正・桐明沙織・大坪周策・清水佑希子

宮崎大学農学部応用生物科学科生物機能科学講座

要　約

NaCl添加ブタおよびブリ肉を−20℃にて20週間貯蔵し，その4−ヒドロキシノネナール (HNE) および4−ヒドロキシヘキセナール (HHE) 含量を測定した.

ブタ肉中のHNEは貯蔵20週目で，検出できなかった。貯蔵16週間後，NaClを加えたブリ肉中のHHE含量は対照区に比べ有意に高かった.

キーワード：4−ヒドロキシ−2−アルケナール，ブタ肉，ブリ肉