Cholesterol Oxidation in Meat Products and Its Regulation by Supplementation of Sodium Nitrite and Apple Polyphenol before Processing

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The levels of cholesterol oxidation derivatives (OxChol) in eight commercial species of meat products were examined. These products contained more than 1 mg/100 g of OxChol, and 7β-hydroxycholesterol + 5β-epoxycholesterol (111−1092 μg/100 g), 5α-epoxycholesterol (80−712 μg/100 g), cholestanetriol (0−368 μg/100 g), and 7-ketocholesterol (708−1204 μg/100 g) were detected. To know the interaction of sodium nitrite supplementation against cholesterol oxidation in meat products, sausage was produced with or without varying levels of sodium nitrite and stored in the refrigerator for 15 days. As a result, cholesterol oxidation in sausage was inhibited by addition of sodium nitrite in a dose-dependent manner. This observation may be associated with inactivation of O_2^- radical and stabilization of polyunsaturated fatty acids (PUFAs). In fact, the levels of OxChol in sausage increased, accompanying the decrease of coexisting linoleic acid when sodium nitrite was not added to sausage meat. Thus, cholesterol oxidation in meat products seems to be considarably promoted by the oxidation of coexisting PUFAs. On the other hand, additive apple polyphenol also inhibited linoleic acid oxidation in sausage and then suppressed cholesterol oxidation through its radical scavenging effects. Therefore, apple polyphenol, having a large amount of an oligomer of catechin, may interfere with cholesterol oxidation in meat processing or storage of meat products through its antioxidative action and be useful as a new antioxitant for meat products when it is added to the original meat before processing.

Keywords: *Meat; cholesterol; oxidation; sodium nitrite; apple polyphenol; antioxidation*

INTRODUCTION

A number of pieces of evidence suggested that some cholesterol oxidation derivatives are inhibitors of sterol biosynthesis (Brown et al., 1974) and cytotoxic (Guyton et al., 1990; Hwang et al., 1992), atherogenic (Morin and Peng, 1989), apoptosis inductive (Nishio and Watanabe, 1996), and immunosuppressive agents (Küçük et al., 1994) in various biological assays. In addition, we observed that dietary cholesterol oxidation derivatives showed perturbative action on lipid metabolism in rats (Osada et al., 1994a, 1995, 1998, 1999). In fact, mixtures of cholesterol oxidation derivatives were absorbed into lymph via the small intestine at the rate of approximately 30% in rats (Osada et al., 1994b), although the absorption rate of individual cholesterol oxidation products differed considerably depending on their structure. Contrary to this observation, Nakatsugawa and Kaneda (1983) observed that the absorption rate of fatty acid oxidation products into lymph was surprisingly low, below 0.6%. Thus, dietary cholesterol oxidation derivatives seem to be absorbed more readily and may disturb homeostasis as compared with oxidized fatty acids. Therefore, the presence of cholesterol oxidation derivatives in processed foods raises a lot of questions concerning the safety of the consumption of them. Recently, Paniamgvait et al. (1995) summarized levels of various types of cholesterol oxidation derivatives in foods of animal origin. Seven species of cholesterol oxidation derivatives were identified in processed meats and meat products, although fresh meat had none or only trace amounts of them. For example, Park and Addis (1987) reported that 3-year-old freeze-dried pork contained cholesterol oxidation derivatives at approximately 460 ppm. Csallany et al. (1989) also found that freeze-dried pork muscle had 177 ppm cholesterol oxidation derivatives. Thus, processed meats seem to contain cholesterol oxidation derivatives at the unnegligible level, although their levels per weight in meat products were not always high compared to those in other foods such as egg, dairy, and marine products (Paniangvait et al., 1995). However, we generally consume a lot of cooked meats or processed meat products in the daily diet. Despite these conditions, the mechanisms of cholesterol oxidation during meat processing or storage and its regulation are in chaos and have not been completely elucidated until now. Therefore, we report a possible mechanism of cholesterol oxidation in meat processing through the production and storage of sausage. On the other hand, the regulation of cholesterol oxidation in processed meat was also examined by supplementation of either sodium nitrite or apple polyphenol before processing because plant polyphenol such as tea catechins, as well as α-tocopherol or other chemicals (Nanjo et al., 1996; Frankel et al., 1997), has potent antioxidative activity against lipid oxidation in various reaction systems.

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Table 1. TBARS Values in Eight Commercial Spices of Meat Products

meat product	TBARS value[a] (nmol of MDA/g of wet weight)	meat product	TBARS value[a] (nmol of MDA/g of wet weight)	meat product	TBARS value[a] (nmol of MDA/g of wet weight)
retort hamburger steak	60.8 ± 17.8	roast pork	91.5 ± 3.0	bacon	9.91 ± 0.53
uncooked hamburger steak	31.5 ± 1.1	roast ham	9.07 ± 0.51	salami sausage	57.1 ± 4.9
sausage	109 ± 21	raw ham	74.8 ± 7.4		

[a] Data are the mean ± SE of triplicate analyses. TBARS, thiobarbituric acid reactive substrate; MDA, malondialdehyde.

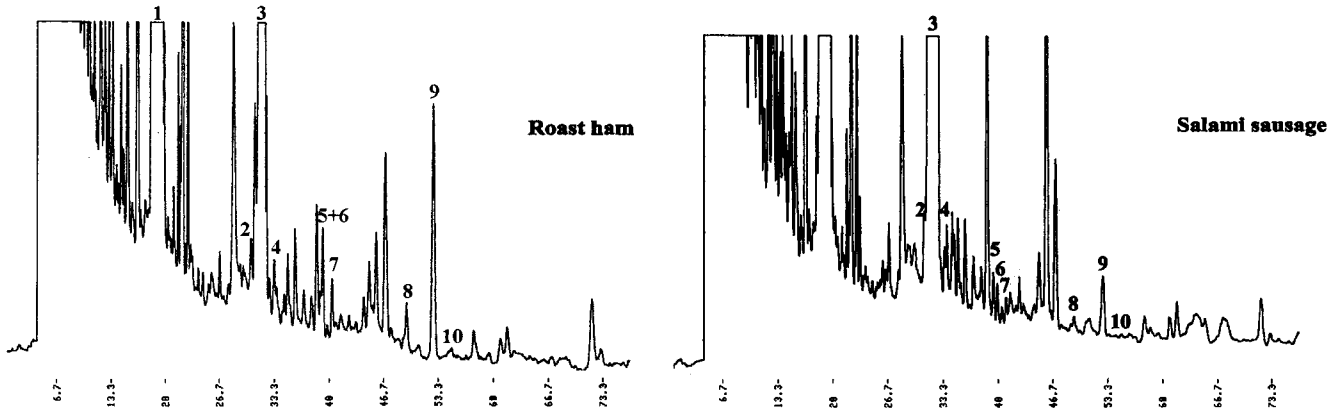

Figure 1. GLC chromatographic pattern of cholesterol oxidation derivatives of roast ham and salami sausage. Peaks: 1, 5α-cholestane (internal standard); 2, 7α-hydroxycholesterol; 3, cholesterol; 4, 3,5-cholestadien-7-one; 5, 7β-hydroxycholesterol; 6, 5β-epoxycholesterol; 7, 5α-epoxycholesterol; 8, cholestanetriol; 9, 7-ketocholesterol; 10, 25-hydroxycholesterol. The *m/e* values of trimethylsilyl esters of each major oxidized cholesterol were as follows: 7α-, 7β-, and 25-hydroxycholesterols, 546, 456, 441, 366, and 351; 5α- and 5β-epoxycholesterols, 474, 456, 384, and 366; cholestanetriol, 546, 456, 403, 367, and 321; 3,5-cholestadien-7-one, 382, 367, and 340; 7-ketocholesterol, 472, 457, 382, and 367.

Uniquely, apple polyphenol has a large amount of condensed tannin consisting of a polymer of catechin (Ohnishi-Kameyama et al., 1997). We also show the possibility of apple polyphenol as a new antioxidant against meat lipids, especially cholesterol, in this study.

MATERIALS AND METHODS

Materials. Eight commercial species of meat products including retort hamburger (beef), nonheated hamburger (beef), sausage (pork), roast ham (pork), raw ham (pork), bacon (pork), and salami sausage (beef) and pork loin meat as the original of sausage meat were purchased from the local market. The casing was a gift from the Aomori Agricultural Products Processing Center (Aomori, Japan). Pure apple polyphenol derived from unripe apple was kindly donated by the Institute for Production Research and Development, Nikka Whisky Co. Ltd. (Tokyo, Japan). Cholest-5-en-3β-ol (cholesterol), cholest-5-ene-3β,7α-diol (7α-hydroxycholesterol), cholest-5-ene-3β,7β-diol (7β-hydroxycholesterol), 5,6α-epoxy-5α-cholestan-3β-ol (5α-epoxycholesterol), 5,6β-epoxy-5β-cholestan-3β-ol (5β-epoxycholesterol), 5α-cholestane-3β,5,6β-triol (cholestanetriol), 3β-hydroxycholest-5-en-7-one (7-ketocholesterol), 3,5-cholestadien-7-one, and cholest-5-ene-3β,25-diol (25-hydroxycholesterol) were purchased from Sigma Chemical Co. (St. Louis, MO) as the available sterol standards. Other solvents and chemicals were of reagent grade or better quality and purchased from a local supplier.

Analyses. Each meat product was homogenized after mincing and addition of 1.15% KCl. Then thiobarbituric acid reactive substance (TBARS) values were measured as an index of peroxidation degree using each homogenate solution according to the method of Ohkawa et al. (Ohkawa et al., 1979) to know the levels of oxidized lipids. Lipids in meat products were extracted from each material by the method of Folch et al. (1957) and saponified by 4 N KOH−ethanol containing 0.001% 2,6-di-*tert*-butyl-4-methylphenol (BHT) at 4 °C for 12 h to avoid more oxidation. Then the unsaponifiable fraction was derivatized to trimethylsilyl ester, and it was analyzed by gas−liquid chromatography (GLC; Shimadzu GC-14B) with a flame ionization detector (FID) using an ULBON HR-1 column (0.25 mm × 50 m, 0.25 μm, Shinwa Chemical Industries, Kyoto, Japan) using 5α-cholestane (Sigma Chemical, St. Louis, MO) as internal standard (Osada et al., 1993a) to measure the concentration of cholesterol oxidation derivatives. Analysis of GLC−mass spectrometry was also performed to identify the major available cholesterol oxidation derivatives using Shimadzu GCMS-QP2000GF with GC-14A and ULBON HR-1 columns. Both the injector and interface temperatures were 300 °C. Mass spectra were measured within a mass range of *m/e* 50−600. The scan speed was 1 scan/s. The ionization energy was 70 eV.

Additive Effects of Sodium Nitrite or Apple Polyphenol on Cholesterol Oxidation in Sausage. To know the effect of sodium nitrite supplementation on cholesterol oxidation in meat products, sausage meat was prepared using commercial pork loin meat and casing after addition of 0.1% Na_2HPO_4 as binding agent. Sodium nitrite was added to sausage meat before processing at the level between 0.005% and 0.15%, although it was not added to the control sample. Moreover, 0.1% apple polyphenol was also added to sausage meat with or without sodium nitrite (0.05%) to examine the antioxidative effect of apple polyphenol. Our used apple polyphenol consisted of (+)-catechin, (−)-epicatechin, chlorogenic acid, caffeic acid, *p*-cumaric acid, phloridzin, quercetin, phloretin, procyanidin B1, procyanidin B2, and procyanidin C1 as relatively low molecular fractions (HPLC data were not shown). After the meat was placed in the casing, each sausage meat was boiled at 70 °C for 40 min. Prepared sausages were stored in the refrigerator at 4 °C for 15 days after being packed into a sterilized freezer bag. TBARS values and levels of both linoleic acid and cholesterol oxidation derivatives in each sausages were measured at intervals of 3 or 5 days. The linoleic acid level was analyzed by GLC (Shimadzu GC-8A) with an FID using a Silar 10C column (2 mm × 3 m) after addition of pentadecanoic acid as internal standard and transmethylation by BF_3−methanol (Ikeda et al., 1989).

RESULTS

TBARS Values in Commercial Meat Products. TBARS values of uncooked hamburger steak, roast ham,

Table 2. Concentrations of Five Major Species of Cholesterol Oxidation Derivatives in Commercial Meat Products[a]

meat product	7β-HOC + 5β-EOC	5α-EOC (μg/100 g)	Ctriol (μg/100 g)	7KetoC (μg/100 g)
retort hamburger steak	1092 ± 96	526.8 ± 117.1	341.5 ± 97.9	909.8 ± 419.5
uncooked hamburger steak	805.9 ± 142.4	712.1 ± 172.7	262.9 ± 193.0	973.2 ± 275.6
sausage	474.1 ± 175.6	383.4 ± 64.4	368.4 ± 241.3	729.3 ± 48.8
roast pork	634.1 ± 189.2	352.7 ± 168.3	ND	814.6 ± 97.6
roast ham	243.9 ± 60.5	83.17 ± 29.27	262.9 ± 91.1	1204 ± 61
raw ham	156.1 ± 68.3	80.85 ± 26.34	51.22 ± 48.94	1049 ± 390
bacon	111.1 ± 29.3	86.10 ± 26.34	67.15 ± 20.49	708.3 ± 136.6
salami sausage	327.8 ± 39.0	144.9 ± 13.2	227.6 ± 93.3	890.2 ± 131.7

[a] Data are the mean ± SE of triplicate analyses. 7β-HOC = 7β-hydroxycholesterol; 5β-EOC = 5β-epoxycholesterol; 5α-EOC = 5α-epoxycholesterol; Ctriol = cholestanetriol; 7KetoC = 7-ketocholesterol. ND = not detected.

Table 3. Effects of Sodium Nitrite Supplementation on TBARS Formation, Linoleic Acid Remaining, and Cholesterol Oxidation in Sausage Stored at 4 °C[a]

added level of sodium nitrite (%)	storage day	TBARS value (nmol/g of wet weight)	level of linoleic acid (wt %)	cholesterol oxidation derivatives			
				7β-HOC + 5β-EOC	5α-EOC (μg/100 g)	Ctrio (μg/100 g)l	7KetoC (μg/100 g)
0	0	146 ± 21	9.68 ± 0.14	286 ± 85	138 ± 42	146 ± 18	458 ± 86
	1	NM	9.51 ± 0.28	476 ± 102	96 ± 12	90.0 ± 21.4	608 ± 56
	3	271 ± 52	9.23 ± 0.61	467 ± 95	120 ± 28	111 ± 36	1119 ± 122
	6	174 ± 65	9.12 ± 0.15	571 ± 38	171 ± 26	68.9 ± 24.2	1031 ± 106
	9	220 ± 47	9.03 ± 0.15	500 ± 68	122 ± 42	75.6 ± 18.2	962 ± 115
	12	175 ± 61	9.15 ± 0.41	548 ± 95	110 ± 36	122 ± 32	1165 ± 185
	15	160 ± 49	8.88 ± 0.21	1391 ± 482	140 ± 46	456 ± 102	1692 ± 146
0.005	0	129 ± 38	9.76 ± 0.18	524 ± 49	91 ± 24	323 ± 104	500 ± 104
	1	NM	9.56 ± 0.14	343 ± 65	221 ± 56	158 ± 34	692 ± 74
	3	195 ± 58	9.53 ± 0.42	429 ± 102	229 ± 44	139 ± 16	239 ± 54
	6	137 ± 18	9.56 ± 0.55	276 ± 53	108 ± 18	222 ± 18	223 ± 28
	9	149 ± 64	9.39 ± 0.68	310 ± 25	132 ± 38	76 ± 21	462 ± 66
	12	191 ± 54	9.35 ± 0.22	309 ± 74	126 ± 46	87 ± 18	415 ± 52
	15	122 ± 25	9.29 ± 0.29	333 ± 69	144 ± 62	167 ± 26	419 ± 80
0.015	0	116 ± 15	9.39 ± 0.48	191 ± 18	211 ± 68	144 ± 28	531 ± 78
	1	NM	9.42 ± 0.78	157 ± 85	152 ± 36	137 ± 14	612 ± 64
	3	196 ± 28	9.41 ± 0.12	324 ± 39	172 ± 48	147 ± 36	927 ± 101
	6	145 ± 24	9.50 ± 0.25	191 ± 28	150 ± 36	128 ± 34	819 ± 95
	9	114 ± 51	9.46 ± 0.23	152 ± 45	102 ± 44	91.1 ± 20.7	765 ± 106
	12	120 ± 28	9.43 ± 0.62	229 ± 34	109 ± 16	57.8 ± 14.3	723 ± 74
	15	54.3 ± 28.4	9.13 ± 0.33	190 ± 52	136 ± 34	111 ± 12.6	750 ± 82
0.05	0	60.1 ± 12.5	9.48 ± 0.58	157 ± 36	200 ± 86	206 ± 38	350 ± 68
	1	NM	9.35 ± 0.42	229 ± 52	76 ± 36	110 ± 42	569 ± 111
	3	105 ± 15	9.50 ± 0.54	276 ± 42	156 ± 24	68.8 ± 26.6	254 ± 56
	6	44.2 ± 10.5	9.62 ± 0.24	338 ± 98	68 ± 12	124 ± 22.4	223 ± 48
	9	45.4 ± 11.2	9.55 ± 0.11	228 ± 75	158 ± 18	217 ± 56	465 ± 86
	12	30.9 ± 8.5	9.50 ± 0.15	171 ± 65	69 ± 26	108 ± 20	419 ± 42
	15	19.7 ± 6.7	9.69 ± 0.10	100 ± 28	120 ± 42	64.4 ± 10.2	423 ± 48
0.15	0	60.0 ± 12.4	9.71 ± 0.78	248 ± 78	164 ± 34	252 ± 56	627 ± 82
	1	NM	9.63 ± 0.42	195 ± 38	179 ± 18	226 ± 38	539 ± 122
	3	22.9 ± 5.8	9.86 ± 0.48	105 ± 18	83 ± 38	167 ± 14	300 ± 68
	6	20.6 ± 8.5	9.70 ± 0.25	186 ± 56	56 ± 12	142 ± 42	362 ± 48
	9	12.0 ± 5.3	9.56 ± 0.23	152 ± 42	99 ± 32	108 ± 28	512 ± 82
	12	8.86 ± 3.54	9.62 ± 0.47	276 ± 32	176 ± 52	142 ± 22	615 ± 96
	15	9.14 ± 3.12	9.71 ± 0.29	105 ± 22	144 ± 28	88.9 ± 15.6	423 ± 54

[a] Data are the mean ± SE of triplicate analyses. NM = not measured.

and bacon were below 40 nmol of malondialdehyde (MDA)/g, whereas their levels of retort hamburger steak, sausage, roast pork, raw ham, and salami sausage were more than 50 nmol of MDA/g; especially, its level in sausage showed the highest value among the eight examined species of meat products (Table 1).

Cholesterol Oxidation Derivatives in Meat Products. Figure 1 shows the GLC chromatographs of the trimethylsilyl ester of cholesterol oxidation derivatives in roast ham and salami sausage as the representatives of measured meat products. Eight species of major cholesterol oxidation derivatives including 7α-, 7β, and 25-hydroxycholesterols, 5α- and 5β-epoxycholesterols, 3,5-cholestadien-7-one, cholestanetriol, and 7-ketocholesterol were identified in the examined meat products. More unknown cholesterol oxidation derivatives were also found in these meat products. 7β-Hydroxycholesterol (peak 5) and 5β-epoxycholesterol (peak 6) were not adequately separated in roast ham, although they were separated in salami sausage. This observation may be caused by a too high level of 5β-epoxycholesterol compared to 7β-hydroxycholesterol as shown by previous data (Osada et al., 1999).

Table 2 summarizes the levels of five major species of cholesterol oxidation derivatives in each products. Retort hamburger steak had 7β-hydroxycholesterol + 5β-epoxycholesterol at the highest level. The concentration of 5α-epoxycholesterol was the highest in uncooked hamburger steak. The level of cholestanetriol was higher in retort hamburger steak and sausage than those in six other meat products, although the cholestanetriol level in each product was below 370 μg/100 g and significant differences were not observed except in raw ham and bacon. Interestingly, cholestanetriol was not detected in roast pork. Contrary to these observations, each product had 7-ketocholesterol at the level

above 700 μg/100 g; particularly, its level in roast ham was the highest among the identified cholesterol oxidation derivatives. The concentrations of total cholesterol oxidation products in each measured product were above 1 mg/100 g (10 ppm).

Effects of Sodium Nitrite on Cholesterol Oxidation in Sausage. The effect of the additive sodium nitrite on cholesterol oxidation was examined in sausage of our own making because our measured commercial sausage showed the highest TBARS value among eight samples and it had cholesterol oxidation derivatives at above 1900 μg/100 g. In our measurement, additive sodium nitrite significantly inhibited lipid peroxidation in sausage in the dose-dependent manner as summarized in Table 3. TBARS values showed a maximum at 3 days of storage, and their levels in each sausage tended to decrease in a time-dependent manner after that, even when sodium nitrite was not present in the products. The concentration of linoleic acid was lower in sausages without sodium nitrite than in those supplemented with it. Similarly, each level of cholesterol oxidation products such as 7β-hydroxycholesterol, 5β-epoxycholesterol, 5α-epoxycholesterol, cholestanetriol, and 7-ketocholesterol in sausages without sodium nitrite was the highest among examined products after 15 days, although significant differences were not observed in sausages with sodium nitrite.

Additive Effects of Apple Polyphenol on Cholesterol Oxidation in Sausage. The increase of TBARS values in sausages was inhibited by the addition of apple polyphenol even when sodium nitrite was not supplemented in sausage meats (Figure 2A). Moreover, reduction of linoleic acid accompanying oxidation was also inhibited by supplementation of apple polyphenol in sausages with or without sodium nitrite in meats (Figure 2B). Reflecting on these observations, cholesterol oxidation derivatives were inhibited even when polyphenol was not added to sausage meats, although their production was highly lowered in sausage with sodium nitrite (Figure 2C).

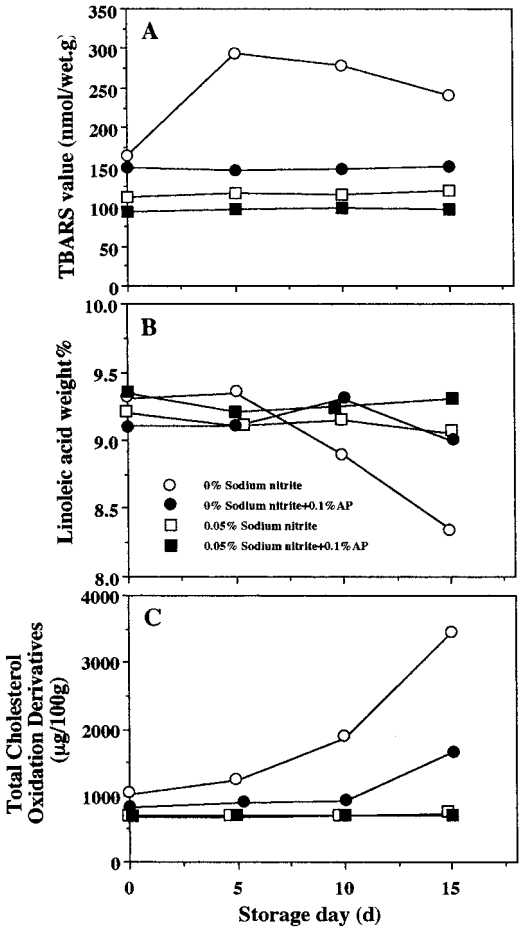

Figure 2. Additive effect of apple polyphenol on TBARS formation (A), linoleic acid remaining (B), and production of cholesterol oxidation derivatives (C) in sausage with or without sodium nitrite. Each sausage was stored at 4 °C. Data are the mean of triplicate analyses. AP = apple polyphenol.

DISCUSSION

TBARS values in available commercial meat products were measured. As a result, five species of meat products except for uncooked hamburger steak, roast ham, and bacon among our examined samples had more than 50 nmol of MDA/g of TBARS value. Pearson and Grey (1983) suggested that nonheme iron may accelerate the propagation step of the free radical chain reaction. The level of nonheme iron is generally higher in beef meat than in pork meat (Hazell, 1982). However, TBARS values were varied in our examined meat products independent of their original. The storage conditions of the products contribute to this discrepancy. Moreover, various factors including diet conditions in the original animal may affect lipid oxidation of meat as α-tocopherol or β-carotene supplementation in the animal diet lowered oxidative degradation in both beef and pork meat (Engeseth et al., 1993; Maraschiello et al., 1998).

The total levels of cholesterol oxidation derivatives in each meat product were not consistent with the TBARS values. The major cause of these discrepancies is not known. This observation may be caused by the effect of an additive component such as sodium nitrite as described and a variety of lipid components including the level of polyunsaturated fatty acids in the original meats.

Our detected cholesterol oxidation derivatives in meat products were found in other reports (De Vone, 1988; Nourooz-Zadeh and Appelqvist, 1989; Sander et al., 1989; Pie et al., 1991). However, previous data showed higher levels of cholesterol oxidation derivatives in meat products as compared with our results, especially in beef tallow or lard (Bascoul et al., 1986; Yan and White, 1990). Moreover, Highly et al. (1986) showed that meat products including cooked bratwurst, raw hamburger, cooked lean bacon, and beef franks contained an unusual amount of cholesterol oxidation derivatives at a level above 4800 ppm, although these data were a little questionable. However, we found a lot of unknown peaks as shown in Figure 2. These peaks may be cholesterol oxidation products, although we must identify these products. Therefore, our common consumed meat products usually appear to have cholesterol oxidation products at above 10 ppm.

Concerning the mechanism of cholesterol oxidation in processed foods, Li et al. (1994) observed that cholesterol oxidation was promoted depending on the degree of unsaturation of coexisting triacylglycerols. Kim and Nawer (1993) also suggested that coexisting triacylglycerol accelerated the decomposition of cholesterol at high temperature. We also observed that cholesterol was unstable in the presence of unsaturated fats; however, it was relatively stable at 100 °C (Osada et al., 1993b). Thus, first, hydroperoxy or hydroxy radicals derived from unsaturated fatty acid oxidation may attack

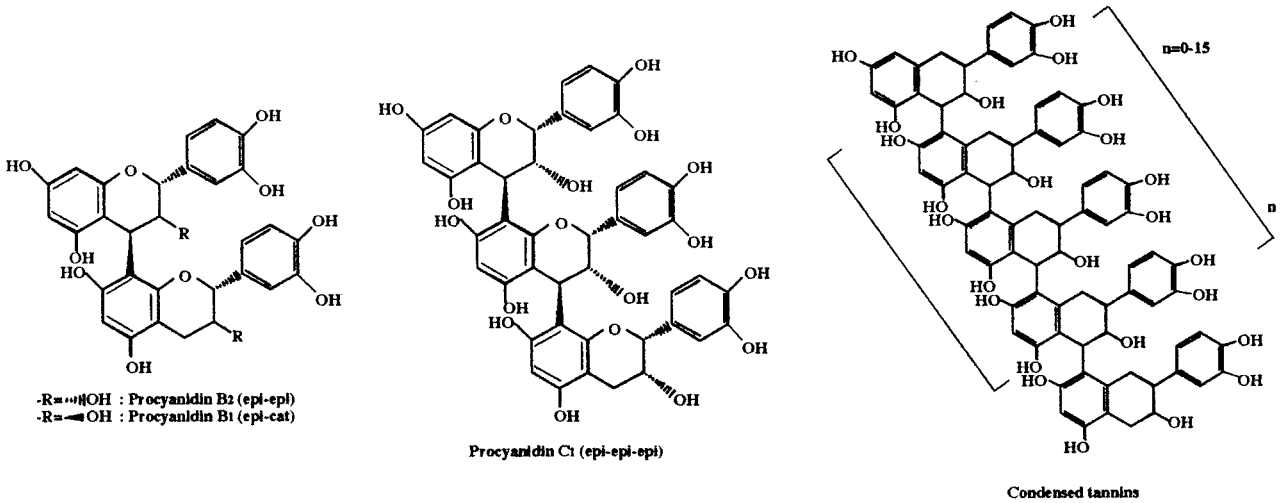

Figure 3. Structure of condensed tannins (high molecular fraction) consisting of catechin in apple polyphenol.

cholesterol and start the chain reaction of cholesterol oxidation. In fact, Ansari and Smith (1978) showed the oxidation of cholesterol by hydroxyl radical using the aqueous cholesterol dispersion system. Moreover, the levels of cholesterol oxidation derivatives increased accompanying linoleic acid reduction by oxidation in our sausage model.

Nitrite is an important and widely used food additive, particularly in the preservation of meats, and it produces a characteristic flavor and pink color in treated meats. Yamauchi and Ando (1973) showed that nitrite in meats interferes with the formation of TBARS. Our data show additive sodium nitrite inhibited both reduction of linoleic acid and production of cholesterol oxidation derivatives in a dose-dependent manner. The opposite was observed when sodium nitrite was not added to sausage. In addition, other reports (Miller et al., 1985; Igene et al., 1985; Shahidi et al., 1987) also suggested that additive sodium nitrite inhibited lipid peroxidation and increased the TBARS value or TBA number. Nakamura and Nakamura (1996) proved that formation of NO myoglobin (Mb) in meat-curing by addition of nitrite is as follows.

$$\text{MetMb} + H_2O_2 \rightarrow \text{FerrylMb} \quad (1)$$

$$\text{FerrylMb} + \text{NADH} \rightarrow \text{MetMb} + \text{NAD}^{\bullet} \quad (2)$$

$$\text{NAD}^{\bullet} + O_2 \rightarrow \text{NAD}^{+} + O_2^{\bullet -}(O_2H^{\bullet}) \quad (3)$$

$$O_2H^{\bullet} + \text{NADH} \rightarrow \text{NAD}^{\bullet} + H_2O_2 \quad (4)$$

$$\text{NADH} + NO_2^{-} \rightarrow \text{NAD}^{\bullet} + \text{NO} + OH^{-} \quad (5)$$

$$\text{NAD}^{\bullet} + \text{MetMb} \rightarrow \text{Mb} + \text{NAD}^{+} \quad (6)$$

$$\text{Mb} + \text{NO} \rightarrow \text{MbNO} \quad (7)$$

Thus, superoxide is produced in this chain reaction; however, it is an intermediate in MbNO formation when nitrite is present in meat; that is, the generated O_2^{-} radical may be actively used as the intermediate substrate of MbNO formation. The generated superoxide may not serve as an oxidative agent of meat lipids in the presence of nitrite. Nitrite seems to bind to polyunsaturated fatty acid related to the degree of unsaturation (Goutefongea et al., 1977). Therefore, nitrite may associate with the stability of polyunsaturated fatty acids against various oxidative factors. This observation was also shown by Zubillaga and Maerker (1987). However, our added level of sodium nitrite was very high, although the permitted level of sodium nitrite as a food additive is different in each country. Therefore, more detailed examination using other meat processing methods will be needed in the case of the addition of sodium nitrite at a low level.

A lot of polyphenolic compounds consisting of various structures have been identified and isolated from a large number of plants used for foods. They have a variety of biological activities; especially their antioxidative activity has been studied in diverse fields. In our trial, additive apple polyphenol inhibited an increase of the TBARS value, linoleic acid reduction, and cholesterol oxidation even when sodium nitrite was not present in sausage. In addition, peroxidation of fish oil was considerably suppressed by addition of apple polyphenol (data not shown). Moreover, Britt et al. (1998) observed cholesterol oxidation in ground beef patties was reduced by addition of two species of tart cherries. They suggested indirectly that a phenolic compound in cherry tissue may inhibit cholesterol oxidation in their discussion. Their results are similar to our data, although phenolic compounds may be different from apple polyphenol. Thus, apple polyphenol may show potent antioxidative action when it is supplemented before processing. However, more comparative studies will be required between apple polyphenol and natural antioxidants including α-tocopherol and various flavonoids to confirm the potency of this antioxidative action.

Some reports (Engeseth et al., 1993; Maraschiello et al., 1998) suggested that dietary treatment such as α-tocopherol supplementation to the original animal inhibited lipid oxidation including cholesterol in meats. Dietary polyphenol may also reduce the formation of cholesterol oxidation products in muscle foods since other reports showed that dietary polyphenol such as tea catechins inhibited lipid peroxidation in tissues (Nanjo et al., 1993; Piskula and Terao, 1998).

Recently, Ohnishi-Kameyama et al. (1997) identified the existence of catechin oligomers ranging from dimers to pentadecamers in apple polyphenol using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry, and they suggested apple polyphenol had a large amount of these oligomers. In fact, apple polyphenol had a high molecular fraction consisting of catechin oligomers at a level above 45% in our measure-

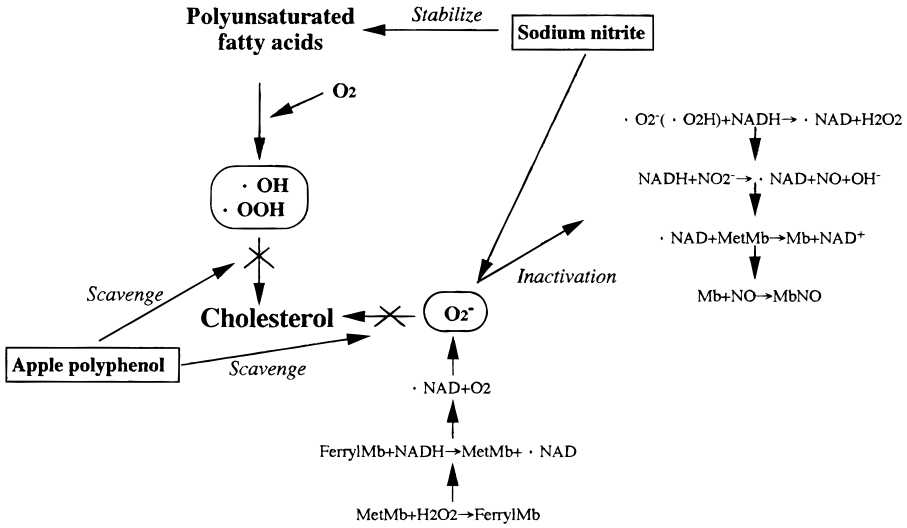

Figure 4. Possible mechanism of antioxidation of additive sodium nitrite and apple polyphenol against cholesterol oxidation in meat products.

ment using LH-20 column separation (data not shown). This level was dependent on its original. These oligomers had many diphenolic compounds including (+)-catechin or (−)-epicatechin as compared with the monomers as shown in Figure 3. Therefore, apple polyphenol may show a potent antioxidative action equal to the activity of (−)-epicatechin gallate having a triphenol structure.

In conclusion, cholesterol oxidation in meat was inhibited through stabilization of coexisting polyunsaturated fatty acids and radical scavenging by addition of sodium nitrite and apple polyphenol as summarized in Figure 4. Thus, apple polyphenol, as well as sodium nitrite, may be useful as a new antioxidant against lipid oxidation containing cholesterol during food processing. More studies are needed to elucidate concerning the application of apple polyphenol as lipid antioxidant in meat products because it may affect the organoleptic properties of processed meats.

ABBREVIATIONS USED

BHT, 2,6-di-*tert*-butyl-4-methylphenol; cholesterol, cholest-5-en-3β-ol; 7α-hydroxycholesterol, cholest-5-ene-3β,7α-diol; 7β-hydroxycholesterol, cholest-5-ene-3β,7β-diol; DPPH, EDTA, disodium ethylenediamine tetraacetate; 5α-epoxycholesterol, 5,6α-epoxy-5α-cholestan-3β-ol; 5β-epoxycholesterol, 5,6β-epoxy-5β-cholestan-3β-ol; cholestanetriol, 5α-cholestane-3β,5,6β-triol; FID, flame ionization detector; GLC, gas−liquid chromatography; 7-ketocholesterol, 3β-hydroxycholest-5-en-7-one; 25-hydroxycholesterol, cholest-5-ene-3β,25-diol; MDA, malondialdehyde; Mb, myoglobin; OxChol, cholesterol oxidation products; TBARSs, thiobarbituric reactive substances.

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