The Effects of Nitrite Treatment on the Lipid Composition, Fatty Acid Composition, and Susceptibility to Oxidation of Pork Biceps Femoris Muscle

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ABSTRACT : The purpose of this experiment was to investigate the antioxidant effect of nitrite on total, neutral, and polar lipids and fatty acid composition in laboratory-cooked ground pork. Muscle samples (*Biceps femoris*) were analyzed using Iatroscan, gas chromatography, phosphorus content, and TBARS value. The total and neutral lipid contents of muscle were higher in nitrite-untreated meat (0 ppm) than in nitrite-treated meat (100 ppm) but the reverse was observed for polar lipid contents. The results for neutral lipids showed a similar trend when compared with total lipids. Polyunsaturated fatty acids contents of total, neutral and polar lipid in 100 ppm treated meat were higher than that of 0 ppm. The phosphorus content was higher in 100 ppm meat than in 0 ppm but the reverse was observed for TBARS value. These results showed that the addition of 100 ppm nitrite to ground pork resulted in a remarkable antioxidant effect during refrigeration storage. *(Asian-Aus. J. Anim. Sci. 2000. Vol. 13, No. 12 : 1764-1769)*

Key Words : Antioxidant Effect, Lipid Components, Fatty Acid, Phosphorus Content, TBARS Value

INTRODUCTION

Nitrite is an important ingredient in cured meat. It produces the characteristic cured-meat color. Nitrite also acts as a potent antioxidant in meat system, and this probably plays a key role in production of the cured-meat flavor, and prevention of the warmed-over flavor on storage. In addition, it has an important antimicrobial effect (Shahidi et al., 1986).

Its effects include inhibition of iron-catalyzed oxidation by complexing metal ions or preventing iron release from myoglobin and haemoglobin, stabilization of lipids in the membrane, or formation of nitrite derivatives with antioxidant activity (Pearson and Gray, 1983; St. Angelo et al., 1987).

Pearson et al. (1983) also reported that overheating or retorting meat to produce antioxidative materials, or the addition of phosphates, nitrite, synthetic antioxidant, ascorbates, chelating agents, and the use of substances possessing natural antioxidant activity prevented the development of warmed-over flavor. Bailey and Swain (1973) postulated that nitrite may inhibit oxidation in meat products, which has since been confirmed by a number of investigators (Fooladi et al., 1979; Igene et al., 1979).

Therefore the purpose of this study was to investigate the effects of nitrite on the changes in fatty acid profile of cooked meat under storage conditions at 5℃, and to evaluate the beneficial effect of this preservative chemical on the basis of its role in imparting stability to polyunsaturated fatty acids in meat systems in storage conditions.

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MATERIALS AND METHODS

Preparation of samples

Fresh samples were obtained from the *Biceps femoris* muscles of pig and stored at 0℃ until the experiment procedures were carried out. After 3 days, the superfical fat part of the muscle was drained and ground, then treated with 2% NaCl and 0.01% ascorbic acid. The Japanese pig (Landrace) was purchased from a meat shop. The pooled meat samples were initially sliced into a 0.1×0.1 cm size, followed by fine mixing in a mortar for 30 min at 5℃. Meat from 3 carcasses, each 600 g were pooled and used as the source of samples. These were divided into two groups and one group was subjected to the nitrite treatment. The treated group (100 ppm) was prepared as follows: to 900 g of prepared meat sample 1 ml of 100 ppm (0.09 g/1 ml) $NaNO_2$ solution was added, and for the preparation of control group (0 ppm), distilled water (1 ml) was added instead of $NaNO_2$ solution. All samples were heated at 75℃ for one hour in a water bath and then kept in a refrigerator (5℃). Analyses were made on three random samples in each group during the refrigerator storage for 0, 5 and 14 days at 5℃.

Extraction and the estimation of the quantity of different lipid fractions

The extraction of lipids from the muscle was done according to the procedure described by Folch et al. (1957). The total lipid content was fractionated into neutral lipids and polarlipids by silicic acid column

chromatography according to the procedure described by Rouser et al. (1967). A glass column, 2.5×35 cm, was filled with 45 g of activated silicic acid and lipid fractions were eluted with 525 ml of chloroform to obtain neutral lipids, followed by 525 ml of methanol to obtain polarlipids. The solvents of neutral lipid fractions were evaporated at reduced pressure and the lipids were again made up to a known volume with 25 ml of chloroform. An aliquot of 10 ml extract was dried under nitrogen and weighed. The phosphorus content of polarlipid extract was estimated by the method described by Bartlett (1959).

Extraction of lipids and identification of lipid classes

The extraction of the lipids from the muscle samples was done according to the procedure described by Folch et al. (1957). Twenty grams of pork muscle samples were minced, blended twice with chloroform-methanol (2:1, v/v) as an extraction solvent and filtered in a separatory funnel. Then the methanol aqueous phase was discarded and the lipid fraction in the chloroform fraction was quantitatively collected into a 200 ml volumetric flask containing a small amount of anhydrous sodium sulfate. The chloroform extraction was then developed in an ascending chromatographic tank using hexane:chloroform:isopropylalcohol:formic acid=(80:14:1:0.1) as the first solvent system for the separation of triglyceride (TG), free fatty acid (FFA), cholesterol (CHO) and diglyceride (DG). Acetone (100%) was used as the second solvent system for the preparation of monoglyceride (MG). The third solvent system, chloroform:methanol:formic acid:water=(25:15:2:1) was used for the separation of phosphatidylethanol amine (PE), phosphatidylcholine (PC), sphingomyelin (SPM), lysophosphatidylcholine (LPC), and polar materials (PM). Quantification of the different lipid classes was done by method of Gunnlaugsodttir and Ackman al. (1993), using the Iatroscan (Iatron Co. Ltd, Tokyo, Japan).

Preparation of fatty acid methyl esters

A 30 mg quantity of lipid in chloroform lipid extract was transferred to reaction tubes where the chloroform was evaporated by passing a steady flow of nitrogen gas over the solution held in a 40℃ heating block. Derivatization of fatty acid methyl esters (FAME) was performed by a modified procedure of Jham et al. (1982). Tricosanoic acid (C23:0) was added to the lipid material as an internal standard prior to saponification. Lipid content was saponified to release free fatty acids by heating at 10 0℃ for 5 min, in the presence of 1 ml of 0.5 N KOH in MeOH. Methylesters of the fatty acids were prepared by the addition of 0.4 ml MeOH:HCl (4:4, v/v) to the reaction mixture and heating at 100℃ for 5 min. After 10 min, 2 ml distilled water was added to the reaction mixture and the fatty acid methyl esters (FAME) were extracted with 6 ml of hexane. The FAMEs in the hexane fractions were determined by gas chromatography with reference to known amounts of authentic standards which were positively identified by GC.

Gas chromatographic (GC) analysis

GC quantification of fatty acids was carried out by using a Shimadzu GC-14A instrument fitted with a flame ionization detector (FID) and a fused silica capillary column (30 m×0.32 mm ID×0.25 μm film thickness, SUPEL 15. - COWAX^{TM}-10). The sample solution (1 μl) in n-hexane was introduced into the injection port of the gas liquid chromatographic instrument, and chromatograms were recorded with a computing integrator Shimadzu C-R4A chromatopac. The flame gas was hydrogen and the carrier and make-up gas was helium. Carrier gas flow rate was 1.25 kg/cm^2. The column initial and final temperatures were 185 and 230℃, and were maintained for 8 min and 17 min, respectively, with a temperature ramping program at 3 ℃/min. The injector and detector temperatures were maintained at 240℃ and 250℃. The peaks were identified by comparing the retention times of the peaks of individual standard fatty acids. The percentage of each fatty acid component was calculated by dividing the area of each peak by the total area of peaks.

Measurement of lipid oxidation

The degree of lipid oxidation in the cooked meat systems was evaluated by the 2-thiobarbituric acid (TBA) test described by Yamauchi and Ando (1973). The TBARS values reported are the mean values of three replicates of the same samples from each batch ±standard deviation. First, to the all the samples, an antioxidant (butylated hydroxytoluene) was added. In the case of nitrite treated samples, Orange I reagent was added. Orange I (OI) reagent was prepared in a brown reagent bottle; acetic acid 13.8% (v/v in distilled water) was heated to 50℃ and sulfanilic acid powder, 1.0 g, and α-naphthol, 0.8 g, were added. The solution was left to cool to room temperature and the pH was adjusted to 4±0.05 by using 10% ammonia solution and made up to a final volume of 1,000 ml with distilled water. The solution was then filtered with 5A filter paper and stored at 5℃. An antifoam was added to all the samples just prior to distillation; 2.5 ml 4 N HCl was added for adjusting the pH to the 1.3 and the distillation was carried out for 20 minutes. Then 200 ml distillate was collected and filtered with filter paper. Out of 40 ml of filtrate, 5 ml was added to 5 ml of TBA reagent and kept at

37℃ for 15 hours to develop the red color of TBA-pigment. The absorbance was measured at 532 nm and the TBARS value was calculated by multiplying the value of absorbance by 18.7 (Ando and Yamauchi, 1968).

Statistical analysis

The data are expressed as mean±SE of three random samples of each group (0 ppm and 100 ppm nitrite) and the statistical analysis was done using student's t-test (Snedecor and Cochran, 1967) and the significance of the different of means was evaluated at the levels of $p<0.05$ and $p<0.01$.

RESULTS AND DISCUSSION

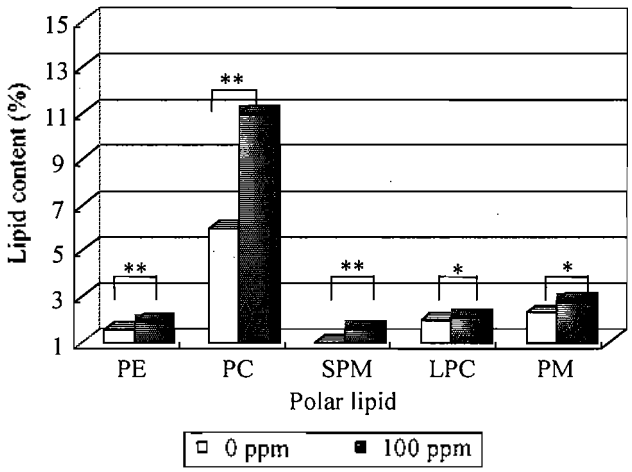
Lipid classes in muscle

Data presented in table 1 illustrate the effect of refrigeration storage on change of total, polar and neutral lipid, respectively. Total and neutral lipid contents of muscles showed slightly but non-significantly higher values in untreated (0 ppm) than treated meat (100 ppm). Polar lipid contents in all storage periods were higher with 100 ppm nitrite than with 0 ppm (table 1). In the case of composition of polar lipids in samples stored for 14 days, PE (phosphatidylethanolamine), PC (phosphatidylcholine), SPM (sphingomyelin), LPC (lysophosphatidylcholine), and PM (polar material) levels were higher in 100 ppm when compared with 0 ppm (figure 1). These results show that nitrite exerted some protective effects on individual phospholipids in meat to overcome its degradation at refrigeration.

Changes of lipid content

Fatty acid profiles of total lipid content of nitrite treated and untreated meat systems after different storage days at 5℃ are shown in the table 2. The C18:2 (ω6) and C20:4 (ω6) fatty acids, total polyunsaturated fatty acids (PUFA), and (ω6) PUFA levels in the fatty acid profile of 0 ppm nitrite meat declined with advancing storage period (table 2). In the 100 ppm group, the C18:2 (ω6) fatty acid contents showed a stable value although the C20:4 (ω6) fatty acid, total PUFA and (ω6) PUFA levels decreased slightly during storage (table 2). These values after storage for 14 days were significantly higher than those of the control group. These changes in the fatty acid profiles may have been caused by the oxidation of the unsaturated fatty acids present in the meat.

Tappel (1955) reported that the objectionable flavors that occur in uncured meats are caused by the oxidation of unsaturated fatty acids catalyzed by iron porphyrins and nonhaem iron. The stability of PUFA level in the nitrite treated meat, especially the (ω6) PUFA, may be mediated by the reported antioxidant effect of nitrite in meat in refrigeration storage conditions. Rubin (1977) reported that sodium nitrite is an important ingredient in the preparation of cured



PC=phosphatidylcholine SPM=sphingomyelin
LPC=lysophosphatidylcholine PM=polar material
PE=phosphatidylethanolamine
** $p<0.01$; *$p<0.05$.

Figure 1. Changes in lipid composition of polar lipids of nitrite untreated (0 ppm) and treated meat (100 ppm) after 14 days of storage

Table 1. Changes of lipid components in nitrite untreated (0 ppm) and treated (100 ppm) meat samples at different days of refrigeration

Lipid component (mg/100g muscle)	Day of refrigeration (at 5℃)								
	0 day			5th day			14th day		
	0 ppm	100 ppm	p value	0 ppm	100 ppm	p value	0 ppm	100 ppm	p value
Total lipid	5,653±12.3	5,642±10.2	0.67	5,626±11.7	5,625±12.6	ND	5,567±15.3	5,553±11.8	0.52
Neutral lipid	4,788±10.60	4,779± 9.40	0.79	4,725±10.3	4,717± 8.5	0.89	4,676± 8.4	4,663± 9.3	0.46
Polar lipid	791± 6.8	809± 5.9	0.39	772± 6.3	787± 5.4	<0.05	735± 5.5	766± 4.9	<0.01

The values are expressed as mean±SE of three random samples of each group (untreated=0 ppm & treated=100 ppm).
ND=not detectable.

Table 2. Composition of some selected fatty acids (mg/g lipid) of total lipids in nitrite untreated (0 ppm) and treated (100 ppm) meat samples

Fatty acids (mg/g lipid)	Storage (5℃)									
	0 day			5th day			14th day			
	0 ppm	100 ppm	p value	0 ppm	100 ppm	p value	0 ppm	100 ppm	p value	Pooled SE
C14:0	7.71[a]	10.08[a]	<0.05	8.27[a]	8.73[b]		7.73[a]	8.47[b]	<0.05	8.50±0.35
C16:0	179.19[b]	208.47[a]		183.23[a]	187.09[c]	<0.01	159.01[c]	197.18[b]	<0.01	185.70±6.86
C16:1 (ω7)	23.50[a]	26.18[a]		22.52[a]	21.60[a]		17.59[b]	22.44[a]	<0.05	22.30±1.14
C18:0	86.46[a]	95.13[a]		86.99[a]	88.28[a]		73.01[a]	97.55[a]	<0.05	87.90±3.51
C18:1 (ω9)	329.48[a]	359.27[a]		327.57[a]	330.14[b]	<0.05	267.50[b]	353.70[a]	<0.01	327.94±13.2
C18:1 (ω7)	44.84[a]	47.81[a]		45.34[a]	44.44[a]		31.04[b]	47.97[a]	<0.01	43.57±2.58
C18:2 (ω6)	55.23[a]	58.73[a]		51.97[b]	57.23[a]	<0.01	42.10[c]	56.32[b]	<0.01	53.60±2.47
C18:3 (ω6)	0.05	0.19		0.31	0.19	<0.05	0.00	0.13	<0.05	0.14±0.04
C18:3 (ω3)	1.49	1.74	<0.05	1.46	1.61		1.28	1.52	<0.05	1.52±0.06
CLA	0.36	0.36		0.34	0.36		0.25	0.36	<0.05	0.32±0.02
C20:0	1.19	1.30		1.13	1.20		1.03	1.28	<0.05	1.19±0.04
C20:1 (ω9)	5.31[a]	6.01[a]		5.60[a]	5.41[b]		4.83[b]	6.41[a]	<0.05	5.60±0.22
C20:2 (ω6)	2.06	2.13		1.88	1.99		1.66	2.12	<0.01	1.97±0.07
C20:3 (ω6)	0.79	1.05	<0.05	0.72	0.87	<0.05	0.61	0.83	<0.05	0.81±0.05
C20:4 (ω6)	3.59[a]	4.19[a]	<0.05	3.07[a]	4.08[a]	<0.01	2.19[b]	3.99[a]	<0.01	3.52±0.31
C20:5 (ω3)	0.22	0.07		0.18	0.19		0.20	0.21		0.18±0.02
C21:5 (ω3)	5.84[a]	5.19[a]		5.02[b]	5.09[a]		3.62[c]	5.35[a]	<0.05	5.02±0.3
C22:4 (ω6)	0.97	1.35	<0.05	1.00	1.15		0.68	0.98	<0.01	1.02±0.09
C22:5 (ω3)	0.65	0.74		0.61	0.68	<0.05	0.42	0.44		0.59±0.05
C22:6 (ω3)	0.28	0.34		0.28	0.32	<0.05	0.00	0.00		0.20±0.06
Total	751.75[a]	835.22[a]	<0.05	753.20[a]	812.99[b]	<0.05	619.50[b]	802.38[b]	<0.01	762.51±31.6
TFA	747.99[a]	830.67[a]		749.83[a]	809.19[b]	<0.05	616.85[b]	799.86[b]	<0.01	759.07±31.4
TSFA	275.65[a]	316.27[a]		280.79[a]	305.76[a]		241.90[b]	286.50[b]	<0.05	284.48±10.6
TUFA	472.34[a]	514.40[a]		469.04[a]	507.12[a]	<0.05	374.95[b]	503.43[a]	<0.05	473.55±21.1
TUSF/TSFA	1.72	1.63		1.67	1.66		1.55	1.65	<0.05	1.65±0.02
TPUFA	68.52[a]	76.17[a]	<0.05	66.86[a]	72.34[a]	<0.05	53.02[b]	70.69[a]	<0.01	67.93±3.25
TPUFA/TSFA	0.25	0.23		0.24	0.26		0.22	0.24		0.24±0
(ω6) PUFA	62.69[a]	67.63[a]	<0.05	58.97[a]	65.10[a]	<0.05	47.24[b]	63.90[a]	<0.01	60.92±2.97
(ω3) PUFA	5.48[b]	6.08[b]		7.56[a]	7.88[a]		5.52[b]	7.51[a]	<0.05	6.67±0.44
(ω6)/(ω3)	11.50[a]	11.15[a]		7.86[b]	8.26[b]	<0.05	8.55[b]	8.56[b]		9.31±0.64

The values were expressed as mean±SE of three random samples of each group (untreated=0 ppm and treated=100 ppm). TFA: total fatty acids; TSFA: total saturated fatty acid; TUFA: total unsaturated fatty acid; TPUFA: total polyunsaturated fatty acid.

meat because it confers stability against oxidation. Our results clearly indicate that nitrite treatment favourably protected the polyunsatutated fatty acids in the meat during storage at 5℃.

These results clearly indicate that meat storage without nitrite treatment resulted in a decline in (ω6) PUFA level with advancing storage. In contrast, the nitrite treatment has stabilized this component. These results are consistent with the fact that the most polyunsaturated fatty acids in meat are associated with phospholipids and these are very susceptible to oxidation.

Changes in phosphorus content

The phosphorus contents during different storage periods of 0 ppm and 100 ppm samples are shown in figure 2. The results clearly show that nitrite treatment resulted in remarkable inhibition in the decline of phosphorus content in polar lipids of meat during storage. Phosphorus content reflects the phospholipid level in meat and the contents in 100 ppm samples at 0, 5 and 14 days were higher than those in the 0 ppm samples.

Allen and Foegeding (1981) reported that lipid oxidation is associated with the polyunsaturated fatty acid moieties the of phospholipid component of the meat. The nitrite may have exerted some protective effect on the phospholipid component and therefore the phoshorus content in the nitrite-added meat did not decline sharply with storage time in comparison with

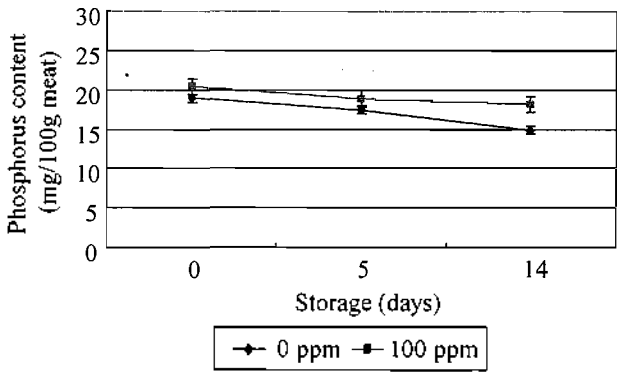


Figure 2. Changes in phosphorus content of nitrite untreated (0 ppm) and treated (100 ppm) meat samples

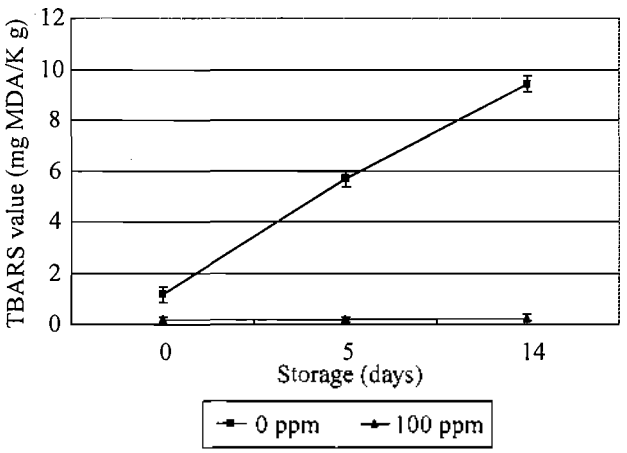


Figure 3. Changes in TBARS value of nitrite untreated (0 ppm) and treated (100 ppm) meat samples during 14 days of storage

0 ppm samples. This may have resulted from the antioxidative property of nitrite on polyunsaturated fatty acids in meat samples. This result is consistent with the previously reported antioxidative effect of nitrite in refrigeration-stored meat (Zubillaga and Maerker, 1987).

On the other hand nitrite play a key role in protecting the cured-meat lipids against oxidative degradation. Therefore nitrite can be regarded as a preservative that imparts a beneficial influence on the fatty acid profile of the meat under storage conditions and it can be regarded as an important chemical useful for the livestock industry.

TBARS value

The TBARS values during different storage periods of 100 ppm and 0 ppm samples are shown in figure 3. TBARS of the 0 ppm treatment at 14 days was increased about 8.1 times compared to the 0 day value. TBARS value of the 100 ppm sample after 14 days storage was increased about 1.4 times compared to 0 day storage. TBARS value of the 0 ppm was higher than the 100 ppm sample at all storage times. This result shows that the addition of 100 ppm nitrite has resulted in a remarkable antioxidation effect during the storage of the meat.

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