Cis-trans isomerisation of unsaturated fatty acids in pork lipids by nitrite

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Abstract—**Many consumers are concerned about content of *trans* fatty acids (TFAs) in their diet. The effect of meat processing on TFA content in meat products is still unknown. Thus, the present study was conducted to elucidate the effect of curing followed by cooking on TFA content in pork lipids. First extracted pork fat and olive oil were reacted with 1.5 mmol of HNO_3 and 0.34 mmol of $NaNO_2$ at 65ºC for 30 min. As a result, a large amount of TFAs was formed in the extracted pork fat and the olive oil after the reaction. The major TFAs were elaidic acid (*trans*-9-octadecenoic acid) converted from oleic acid and linolelaidic acid (*trans*-9,12-octadecadienoic acid) from linoleic acid. On the other hand, no TFA was detected in the pork cured with a mixture of KNO_3 and $NaNO_2$ (5/1, wt/wt) and cooked at 70ºC for 50 min. However addition of nitric acid as a substitute for potassium nitrate resulted in the formation of a small amount of TFAs in the cured and cooked meat. The major TFA detected was elaidic acid that might derive from oleic acid. From the results of present study, there was no evidence that processing such as curing and cooking affects the TFA content in meat. Meanwhile, it was also suggested that unsaturated fatty acids in meats could be *cis-trans* isomerised by excess amount of nitrite in the presence of nitric acid.**

Keywords— ***trans* fatty acids, nitrite, isomerisation**

I. INTRODUCTION

Consumption of *trans* fatty acids (TFAs) increases the risk of coronary heart disease and possibly raises the risk of sudden cardiac death and diabetes [1]. Consequently, a strong concern for its consumption has been raised world-wide, and regulations on mandatory nutritional labeling of TFAs or limitation of TFA content in foods have been taken in Pan-American countries [2] and some Asian countries such as South Korea and Taiwan.

Major sources of TFAs are foods containing partially hydrogenated vegetable oils for use in margarines or frying oils. Naturally occurring TFAs are consumed in smaller amounts (about 0.5% of total energy intake) in meats and dairy products of ruminant animals [3]. These TFAs are produced by the action of rumen bacteria. Meanwhile, a small amount of TFAs is detected occasionally in meats of non-ruminant animals as pigs. These TFAs are believed to originate from feedstuffs for the animals.

On the other hand, there is little knowledge of production of TFAs in non-ruminant meats during its processing. Nitrite, an important ingredient of curing agents for meat processing, has been known as one of catalysts of *cis–trans* isomerisation since 1819 when Poutet [4] first reported the solidification of olive oil by oxides of nitrogen.

The objective of this study is to elucidate some effects of meat processing, especially curing with sodium nitrite followed by cooking, on content of TFAs in pork lipids.

II. MATERIALS AND METHODS

Pork meat (loin) and a commercial pure olive oil were purchased in a local food store. Extracted pork fat used in this study was prepared from back fat around loin meat with diethylether. Isomerisation reaction of these fat/oil was done by a following procedure: 3.5 g of fat/oil was vigorously shaken with 0.42 mL of an aqueous solution of nitric acids containing 1.5 mmol of HNO_3 and 0.34 mmol of $NaNO_2$ at 65ºC for 30 min. The lipids were extracted with chloroform, and the chloroform layer was washed with distilled water several times to remove the nitric acids.

To prepare experimental pork patties, 2% of NaCl, 10% of water and varying levels of a mixture of KNO_3 and $NaNO_2$ (5/1, wt/wt) were added to ground pork. The levels of the KNO_3/$NaNO_2$ mixture were 0, 100, 1000, and 10000 mg/kg of meat. After mixing these ingredients, the meat batters were stuffed into a casing with poly vinylidene chloride and stored at 5ºC for 24 hours. Then the cured meat was cooked in a steam

kettle at an internal temperature of 70ºC for 50 min. In the experiment using nitric acid in place of KNO_3, equivalent molar of HNO_3 to KNO_3 was added to meats, and other conditions were consistent. Although the actual weights of added HNO_3 differed from those of KNO_3, the same titles of treatments in Table 2 were used in Tables 3 and 4 for the sakes of simplicity and comparison.

Extraction of total lipid from the fat/oil samples and the patties were done according to the method of Folch et al. [5]. Fatty acid methyl esters (FAMEs) were prepared using a previously described method [6].

The FAMEs were applied to solid phase extraction (SPE) by use of a commercial Ag-ion column (Discovery Ag-ION, SUPELCO Inc., USA) to fractionate *trans*-octadecenoic acids and *cis*-ones. FAME (approximately 1 mg) dissolved in *n*-hexane was applied onto the SPE column equilibrated with dehydrated acetone and *n*-hexane. Then the first fraction containing saturated fatty acids and *trans*-monoenoic fatty acids was eluted with 9 mL of *n*-hexane/acetone (96/4, vol/vol). After that the second fraction containing *cis*-monoenoic acids and all polyunsaturated fatty acids was eluted with 10 mL of acetone/acetonitrile (80/20, vol/vol). Typical chromatograms of these fractions are shown in Fig. 1.

The resulting FAME fractions were analysed on a GC-2010 gas chromatograph (Shimadzu Corporation, Japan) equipped with a flame-ionization detector using a capillary column (SP-2560™, 100 m × 0.25 mm i.d., 0.25 µm thickness; SUPELCO Inc.). The operating conditions of the gas chromatograph were as follows: oven temperature was held at 170ºC for 30 min, increased to 220ºC at a rate of 2ºC/min and held at this temperature for 25 min. The temperatures of the injector and the detector were 240ºC. The carrier

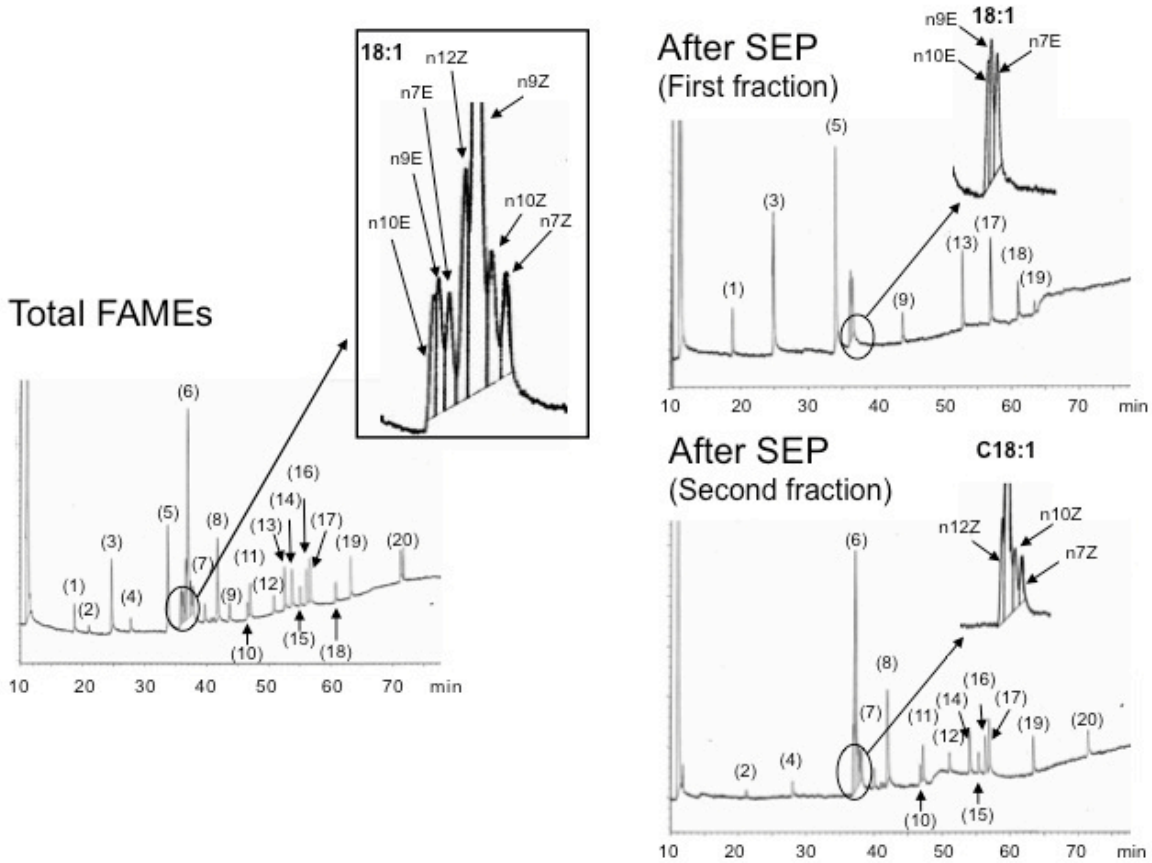

Fig.1 Chromatograms of fractionated FAMEs by a Discovery Ag-Ion column. The condition of GC analysis was as described in Materials and Methods. Peak identification: (1)14:0, (2)14:1n5E, (3)16:0, (4)16:1n7E, (5)18:0, (6)18:1n9Z, (7)18:2n6E, (8)18:2n6Z, (9)20:0, (10)20:1n9Z, (11)18:3n3Z, (12) 20:2n6Z, (13)22:0, (14)20:3n6Z, (15)20:4n6Z, (16)20:3n3Z, (17)23:0 (a syringe spike), (18)24:0, (19)24:1n9Z, (20)22:6n3Z

gas (Helium) was maintained at a flow rate of 20 cm/sec. The contents of TFAs were calculated by quantitative analysis using tricosanoic acid as an internal standard.

III. RESULTS AND DISCUSSION

Oleic and linoleic acids dramatically decreased after the reaction with nitric acids, and large amounts of elaidic (*trans*-9-octadecenoic) and linolelaidic (*trans*-9,12-octadecadienoic) acids were generated in both pork fat and olive oil (Table 1). The average amounts of elaidic acid were 355 mg/g of lipid (pork fat) and 563 mg/g of lipid (olive oil); meanwhile those of linolelaidic acid were 45.8 mg/g of lipid (pork fat) and 19.2 mg/g of lipid (olive oil), respectively. The isomerised ratios of oleic acid were approximately 80% for pork fat and 75% for olive oil, and those of linoleic acid were about 50% for pork fat and 40% for olive oil.

Table 1 *Cis-tarns* isomerisation of pork fat and olive oil by HNO_3 and $NaNO_2$

Fatty acid	Olive oil		Pork fat	
	Un-treated	Treated	Un-treated	Treated
14:0	1.0 (0.1)	1.1 (0.3)	1.1 (0.0)	1.35 (0.0)
16:0	10.9 (0.3)	11.8 (0.8)	25.2 (0.1)	26.8 (0.2)
16:1n7	0.5 (0.1)	0.3 (0.0)	1.5 (0.0)	1.5 (0.1)
18:0	2.3 (0.0)	2.6 (0.4)	12.5 (0.1)	13.5 (0.4)
18:1n9(*Z*)	78.1 (0.5)	20.1 (1.7)	46.7 (0.1)	7.1 (1.6)
18:1n9(*E*)	ND	59.1 (3.0)	0.0 (0.0)	40.4 (1.1)
18:2n6(*Z*)	6.5 (0.2)	2.4 (0.6)	11.6 (0.1)	3.6 (0.2)
18:2n6(*E*)	ND	2.0 (0.2)	ND	5.2 (0.1)
others	0.7 (0.2)	0.5 (0.1)	1.4 (0.0)	0.6 (0.1)

Data were presented as means (n = 3) of weight percent in total fatty acids. Values in parentheses were standard deviations. ND, Not detected.

Litchfield et al. [7] demonstrated that free oleic acid was *cis-trans* isomerised with nitrous acid and the converting ratio was above 60%. Our results are consistent with their report although those fatty acids were combined with glycerol in this study.

Even though excess amount of the nitrate/nitrite mixture was added to meats, significant production of TFA was not observed (Table 2). Use of nitrate and nitrite is regulated in many countries, and those allowances in finished meat products are often ~ 500 mg/kg for potassium nitrate and ~ 200 mg/kg for sodium nitrite, respectively [8]. Therefore, the present results suggested that addition of nitrite and nitrate within the limit of the regulations does not affect the TFA contents in meat products.

Table 2 Fatty acid composition (wt%) of cured pork with several levels of $KNO_3/NaNO_2$ mixture

Fatty acid	Added level of $KNO_3/NaNO_2$ mixture (mg/kg)			
	0	100	1,000	10,000
14:0	1.1 (0.1)	1.2 (0.1)	1.2 (0.1)	1.2 (0.0)
16:0	24.2 (0.9)	23.8 (1.7)	23.2 (0.4)	24.9 (0.3)
16:1n7	1.8 (0.3)	2.0 (0.4)	1.8 (0.2)	1.9 (0.2)
18:0	12.3 (0.6)	12.1 (0.9)	11.9 (0.4)	12.8 (0.2)
18:1n9(*Z*)	50.0 (2.9)	47.8 (2.3)	47.0 (0.8)	47.2 (0.7)
18:1n9(*E*)	ND	ND	ND	0.0 (0.0)
18:2n6(*Z*)	8.8 (1.6)	8.7 (1.7)	9.3 (0.2)	10.1 (0.1)
18:2n6(*E*)	ND	ND	ND	ND
others	1.7 (0.1)	1.9 (0.2)	1.8 (0.1)	1.8 (0.1)

Data were presented as means (n = 6) of weight percent in total fatty acids. Values in parentheses were standard deviations. ND, Not detected.

Table 3 Fatty acid composition (wt%) of cured pork with several levels of $HNO_3/NaNO_2$ mixture

Fatty acid	Added level of $HNO_3/NaNO_2$ mixture (mg/kg)			
	0	100	1,000	10,000
14:0	1.2 (0.1)	1.1 (0.1)	1.1 (0.2)	1.1 (0.0)
16:0	23.5 (0.4)	23.2 (0.3)	23.2 (0.2)	23.0 (0.2)
16:1n7	2.1 (0.2)	2.4 (0.1)	2.2 (0.1)	2.2 (0.1)
18:0	13.0 (0.2)	13.0 (0.0)	12.8 (0.2)	12.7 (0.3)
18:1n9(*Z*)	45.6 (0.2)	45.3 (0.1)	45.5 (0.3)	45.4 (0.3)
18:1n9(*E*)	0.00 (0.00)	0.01 (0.01)	0.01 (0.00)	0.18 (0.04)
18:1n7(*Z*)	3.1 (0.1)	3.4 (0.2)	3.4 (0.2)	3.5 (0.1)
18:2n6(*Z*)	9.6 (0.4)	9.5 (0.1)	9.6 (0.1)	9.2 (0.1)
18:2n6(*E*)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
others	1.9 (0.1)	2.2 (0.4)	2.2 (0.2)	2.4 (0.2)

Data were presented as means (n = 3) of weight percent in total FAMEs. Values in parentheses were standard deviations.

When potassium nitrate was replaced with equivalent molar of nitric acid, TFAs was slightly, but significantly, increased in the highest group as shown in Tables 3 and 4. Meanwhile there were no effects in the other groups. The major TFA produced in the

meats were elaidic acid that might be derived from oleic acid, and *trans* vaccenic acid (*trans*-11-octadecenoic acid) followed them. A small amount of *trans* dienoic acids such as linolelaidic acid were also detected in this study, however, any *trans* trienenoic acids not detected.

Table 4 *Trans* Fatty acid content (mg/100 g of food) of cured pork with several levels of $HNO_3/NaNO_2$ mixture

Fatty acid	Added level of $HNO_3/NaNO_2$ mixture (mg/kg)			
	0	100	1,000	10,000
Trans 18:1	0.330 (0.319)	2.48 (1.14)	1.78 (0.64)	25.7 (7.3)
Trans 18:2	0.208 (0.258)	0.175 (0.303)	1.60 (0.72)	1.85 (1.70)
Total TFAs*	0.538 (0.468)	2.65 (1.44)	3.38 (1.06)	27.6 (8.7)

Data were presented as means (n = 3) of weight percent in total fatty acids. Values in parentheses were standard deviations. *TFA; *trans* fatty acid.

A number of researches indicated that the oxides of nitrogen such as nitric acid catalyse *cis-trans* isomerisation of fatty acids [9, 10]. Chang and Miwa [9] demonstrated that nitrite anion (NO_2^-) derived from nitrous acid (HNO_2) could directly combine with *cis*-double bonds of unsaturated fatty acid and that the complex rotates to the opposite geometric configuration by a spin-orbital coupling process.

It is believed that the nitrogen is reduced by one electron from nitrite-nitrogen and nitric oxide (NO) could be formed. Nitric oxide forms very stable complexes with the transition metals in the heme pigments of meat, which generates a stable pink colour of cured meats. In addition, nitrous acid can react with free α-amino groups of amino acids in acidic condition and α-hydroxy acids can be librated, which is well known as the Van Slyke reaction [11]. These reaction leads to depletion of reactive species of nitrite for *cis-trans* isomerisation of fatty acids in meat. Thus excess amount of nitrite would be needed to produce a large quantity of TFAs in non-ruminant meat as pork.

IV. CONCLUSIONS

In the present study, there was no evidence that processing such as curing and cooking under a normal condition affects TFA content in meat. Meanwhile, it was suggested that oleic and linoleic acids in meat are *cis-trans* isomerised by a large amount of nitrite in the presence of nitric acid. Therefore, addition of excess amount of sodium nitrite to meats should be avoided.

ACKNOWLEDGMENT

We are grateful to Dr. S. Takenoyama with Faculty of Health and Nutrition, Minami Kyushu University, for his help and advice on printing of our poster.

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