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Original Articles

Lipid Peroxidation-derived Toxic Aldehyde, 4-Hydroxynonenal Contents in Roast Pork of Some Chinese Restaurants in Miyazaki

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Summary : The 4-hydroxynonenal (HNE), thiobarbituric acid reactive substances (TBARS) and total lipid (TL) contents of roast pork in some Chinese Restaurants in Miyazaki were analyzed.

HNE, TBARS and TL contents in roast pork samples were different from restaurant to restaurant. Weak negative correlation was observed between HNE and TBARS contents. No correlation was observed between HNE and TL or between TBARS and TL. Considering the toxicity, it is necessary to analyze the contents in many samples, because samples containing high HNE content were observed.

Key words : 4-Hydroxynonenal, Roast pork, Thiobarbituric acid reactive substances, Total lipid.

Introduction

Lipid peroxidation is one of major causes 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-alkenals are of particular interest because they have been reported to elicit a variety of powerful biological activities such as inhibition of enzymes, chemotactic activity toward neutrophils, and inhibition of protein synthesis (Benedetti *et al.* 1980 ; Benedetti & Comporti, 1987 ; Brembilla *et al.* 1986 ; Esterbauer *et al.* 1991 ; Poot *et al.* 1988 ; Tovar & Kaneda, 1977 ; Yoshioka & Kaneda, 1972). Among 4-hydroxy-2-alkenals, 4-hydroxy-2-nonenal (HNE) is the major aldehyde formed during peroxidation of n-6 polyunsaturated fatty acids (Esterbauer *et al.* 1991). Because there is a large amount of n-6 unsaturated fatty acids in foodstuffs, HNE could be a reliable marker for assessing the quality of food, particularly of meats. From the viewpoint of food hygiene, it is necessary to analyze the contents in many meats and meat products. However, HNE contents were analyzed only in beef and pork (Sakai *et al.* 1995 ; Sakai *et al.* 1998) and smoked meat products (Munasinghe *et al.* 2003). Therefore we analyzed the HNE contents in roast pork obtained from some Chinese restaurants in Miyazaki.

Materials and Methods

Materials

HNE was synthesized by the method of Sugamoto *et al.* (1997) and confirmed with the basis of the ^{1}H-NMR and ^{13}C-NMR spectra. Butyl hydroxy toluene (BHT) was obtained from Tokyo Kasei (Tokyo, Japan), 2, 4-dinitrophenylhydrazine (DPNH) from Wako Pure Chemicals (Tokyo, Japan), and 1, 3-diethyl-2-thiobarbituric acid (DETBA) from Aldrich Chemicals (Milwaukee, WI. USA). Other reagents were of analytical grade. Roast pork samples were obtained from 8 Chinese restaurants in Miyazaki and stored at -80°C until analysis.

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HNE analysis.

HNE contents in roast pork samples were determined by the method of Goldring *et al.* (1993). The meat samples were mixed with 20 ml of dichloromethane containing 0.5 % BHT for 30 min and then 20 ml of chloroform containing 2.5 mmol DNPH was added to the mixture. Then, the reaction mixture was filtered. The filtrate obtained was evaporated *in vacuo* to dryness and redissolved in 5 ml of chloroform. The sample was put on a disposable silica gel extraction column (Baker) which had been equilibrated with n-hexane/chloroform (2 : 1, vol/vol). The same solvent mixture was used to wash off the highly lipophilic DNPH derivatives in a discrete band. The remaining material was eluted using chloroform. The chloroform extract was evaporated in vacuo to dryness and redissolved in methanol. The DNPH-HNE derivatives were analyzed by HPLC by the method of Goldring *et al.* (1993). Analytical conditions were as follows : column, Ultrasphere C18 (25 cm × 4.6 mm i.d., Beckman) ; mobile phase, 30 mM sodium citrate /27.7 mM acetate buffer (pH 4.75) : methanol=1 : 3 ; flow rate, 1 ml/min ; column temperature, 40°C ; detection wavelength, 365 nm.

TBARS values analysis.

TBARS values in roast pork samples were measured by the distillation method of Yamauchi *et al.* (1980) and expressed as nmol of malonaldehyde (MA)/g tissue. To prevent lipid oxidation of the samples during distillation, BHT was added to the meat at a level of 0.3 % and mixed well in a mortar.

TL analysis.

Fatty acids were extracted by the method of Folch *et al.* (1957) and TL was determined by the gravimetric analysis.

Results and Discussion

All results were summarized in Table 1. HNE contents of store A and D were considerably high values, although those of other stores were less than 10 nmol/g. Especially, HNE contents of store A were very high values. Nishikawa *et al.* has been reported that the LD_{50} of HNE given orally in Fisher rats was 1000 mg/kg (Nishikawa *et al.* 1992). The oral toxicity seems to be relatively low. However, they also reported that acute tubular necrosis of kidney and diffuse liver cell necrosis were observed in rats given high dose of HNE (192 μmol/kg body) (Nishikawa *et al.* 1992). In addition, Siegel *et al.* (2007) reported that HNE exists at increased concentrations in Alzheimer's disease (AD) patients and is found in amyloid ß peptide (Aß) plaques associated with AD. From the viewpoint of food hygiene, it is noteworthy that considerable high contents of HNE were detected in some roast pork samples. Therefore it is necessary to analyze HNE contents in many samples. TBARS contents of roast pork of store B, C and E were higher than those of other stores although HNE contents of these stores were lower than those of other stores. As shown in Figure 1, weak negative correlation was observed between HNE and TBARS contents. This result was different from our previous results that positive correlation was observed between HNE and TBARS contents in pork stored at 0, -20 and -80°C (Sakai *et al.* 1998). TL contents were different from store to store. No correlation was observed between TL and HNE. Because we have been reported that positive correlation was observed between HNE and

Table 1. HNE, TBARS and TL contents of roast pork samples obtained from 8 Chinese Restaurants in Miyazaki.

Store	HNE (nmol/g)	TBARS (nmol/g)	TL (%)
A	52.97	9.3	33.35
B	3.67	76.1	26.90
C	4.35	82.8	17.43
D	23.18	2.4	5.60
E	6.79	67.1	23.40
F	4.18	30.6	34.12
G	4.18	5.1	26.17
H	9.80	11.1	26.87

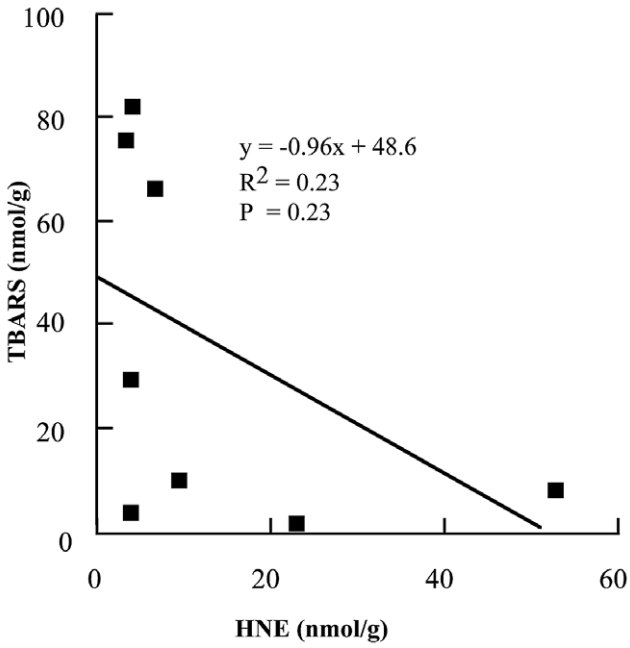

Fig. 1. Relationship between HNE and TBARS contents in roast pork samples.

n-6 polyunsaturated fatty acids (PUFA) contents (Sakai *et al.* 1995), it is necessary to analyze not only TL contents but also n-6 PUFA contents in many roast pork samples. We have been reported that processing methods and/or stored temperatures may affect the variation pattern of HNE contents in meat samples of roast pork and are thought to be different from store to store. Considering the facts that high dose of HNE (192 μmol/kg body) brings about sever damages to liver or kidney (Nishikawa *et al.* 1992) and that considerably high HNE contents was detected in some roast pork samples, further studies on changes in HNE contents in many meat products which are different processing and/or stored methods must be necessary.

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宮崎の中華店のチャーシュウ中の脂質過酸化由来有毒アルデヒド，4–ヒドロキシノネナール含量

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要　約

８店舗の中華店で入手したチャーシュウ中の4–ヒドロキシノネナール（HNE），チオバルビツール酸反応物質（TBARS）含量および脂質含量（TL）を測定した．

試料によりHNE，TBARSおよびTL含量に差が認められた．HNE含量とTBARS含量には有意ではないが負の相関関係が認められた．また，TLとHNEまたはTBARS含量とに相関関係は認められなかった．試料中には高濃度のHNEが検出された検体があり，その経口毒性を考慮すると，今後多くの試料について調査する必要がある．

キーワード：4–ヒドロキシノネナール，チャーシュウ，チオバルビツール酸反応物質，脂質含量