S2P04.WP

BIOCHEMICAL CHARACTERISTICS OF MUSCLE OF DFD PORK CAUSED BY TYING ANTEMORTEM STRESS

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Please refer to Folio 6.

INTRODUCTION

Some butchers doubt to get PSE pork, so they force drive hogs and tie the hogs' four limbs with a cord giving the animals a severe stress pre-slaughter and result in a DFD pork to deceive the consumers as normal pork in Tainan area of Taiwan. The butchers do not know the problem with DFD pork having a shorter shelf-life and undesirable quality. There are very few papers dealing with normal pigs being stressed and resulting DFD pork as a result of PSS hog stressed pre-slaughter in Taiwan. This study was to investigate biochemical and metabolites changes of DFD porcine muscle post-mortem from the tied-limbs hogs as compared to the normal muscle.

MATERIALS AND METHODS

Muscle samples were obtained from the meat market slaughterhouse of Tainan County. Fifty-nine hogs (100kg of body weight, LYD three crossbred) were forced driven and the limbs were tied in the pen pre-slaughter (see Photo 1). pH_1 and pH_{24} of ham and loin were measured for determination of DFD and PSE muscles. The identification was done by the method of Briskey *et al.* (1964). There were 34 hogs being judged as DFD pork and 25 hogs as the normal pork from the 59 hogs.

One kg of *m.semimembranosus* was taken and cut into 50-100g and wrapped with aluminum foil and then placed in liquid nitrogen. The rest of meat samples were placed in an ice box and then transported to laboratory and stored at 2 to 4°C.

The pH value and colour of muscle were measured at one and four hours post-mortem, at the same time, approximately 100g of muscle sample was taken at 1, 6 and 12 hours after slaughter and wrapped in aluminum foil and stored in liquid nitrogen for glycogen, lactic acid and NAD^+ determinations.

Samples for NMR studies: Pieces of about 50g of muscle were cut out from porcine *m.semimembranosus* 15 minutes after slaughter and then wrapped in aluminum foil and placed in liquid nitrogen and transported to laboratory for NMR study. Samples were taken out from liquid nitrogen container and cut into pieces of 20g and trimmed of fat and connective tissue, and then dipped in liquid nitrogen to be frozen and crumbled in mortar bowl. The crumbled meat was added with 60ml cold $1MHC10_3$ and homogenized at 6000rpm, 30 sec. The suspension was centrifuged at 2°C (15000xg, 30 minutes). The pH of homogenate was adjusted to 7.0 with $5MK_2CO_3$ and the supernatant was then recentrifuged (15000xg, 10 minutes). The supernatant was frozen and kept at -18°C until the NMR experiment could be performed.

DSC

Whole muscle fibres were removed from the chilled meat (4°C) and cut into 2cm long for DSC samples, or the whole

muscle fibres were dipped in liquid nitrogen for 12 hours and then crumbled for another DSC analysis.

Analyses

The pH value was measured with an HI 8424 Microcomputer pH-meter (HANNA Instrument, Italy) Meat color was measured with Hunter Colorimeter (Model TC-III Tokyo, Denshoky Co., LTD., Japan). The glycogen level of meat was determined according to the method described by Nuss and Wolf (1980). NAD^+ content was determined according to the End-point UV-methods of Martin Klingenberg (in Methods of Enzymatic Analysis, 3rd ed.). Carbon-13 NMR:7.05 Tesla NMR measured electrical magnet waves were transformed into frequency signals. The samples were placed in a 0.5mm NMR tube to which isotonic D_2O solution had been added, methanol (CH_3OH) was used as reference for chemical shift scale.

DSC was performed on a ULVac DSC-7000 (Sinku-Riko, Japan) equipped with a thermal analyzer. Samples (15-20mg) were weighed in aluminum pans (No.201-53090) and then sealed. The scanning temperature was 25°C-99°C at a heating rate of 10°C/min. Triplicate samples were analyzed. A reference containing 12 to 13mg distilled water was used. The instrument was temperature calibrated using indium. Each DSC analysis was repeated three times. After DSC analysis, the sample pans were punctured and the dry weight of the samples determined after drying at 105°C overnight. The enthalpy of denaturation of muscle proteins was also collected.

RESULTS AND DISCUSSION

Muscle samples were classified as normal muscle or DFD muscle according to the method described by Briskey (1964). If the pH of porcine muscle which was maintained above 6.2, 24 hours post-mortem was defined as DFD pork, while below 6.0 was defined as the normal pork.

Color changes of DFD and normal pork were shown in Table 1. There was no significant difference in L-value between 30 minutes and 24 hours postmortem for both DFD and normal pork. However, there was a significant difference ($P<0.05$) in L-value between DFD and the normal meat. The L-value of DFD pork was darker red than the normal pork. Redness (a-value) for DFD and the normal pork 24 hours post-mortem was higher than that for both samples 30 minutes post-mortem. The a-value for both DFD and the normal pork were not significantly different for the sample obtained at the same post-mortem time. However, color of DFD pork showed in dark red, and the normal pork appeared bright red visually. Yellowness (b-value) for both the normal and DFD pork was not significantly different. Gariepy *et al.* (1989) reported that L-value and b-value and water holding capacity could be used to evaluate meat quality and differentiate the normal pork, DFD and PSE pork. The L-values obtained from this study were similar to the data of their study.

Changes in glycogen level of DFD and normal muscle are presented in Figure 1. Glycogen content of the normal pork 30 minutes post-mortem was significantly higher ($P<0.05$) than DFD pork. Maieda *et al.* (1986) reported that the glycogen content of normal porcine muscle was 1.5 times DFD muscle 90 minutes after slaughter while approximately double in concentration in this study. The level of glycogen in muscle of live animal before slaughter plays an important role on meat quality because that glycogen level in muscle is associated with the ultimate pH of meat (Fernadez *et al.*, 1991; 1992).

Glycogen content of DFD muscle 30 minutes post-mortem might be inferred more highly than the normal muscle before slaughter. In this experiment the animals in lairage were suffered with tying stress and resulting in difference quality of meat after slaughter because of the individual difference in glycogen content or capability of resistance to stress or variation of energy levels. DFD pork incidence postmortem increased and energy levels of muscle would affect meat quality after slaughter (Barton, 1974; Nielsen, 1981).

Glycogen content of both normal and DFD pork decreased with time post-mortem. However, glycogen content of the normal pork was significantly higher ($P<0.01$) than DFD pork for 30 min, 1, 6, and 12 hours post-mortem. Glycogen

content of DFD pork 12 hours post-mortem was not detected or remained only in trace amounts.

Changes in the lactate content of the normal and DFD pork post-mortem is presented in Figure 2. The result indicated there was no significant difference in lactate levels between the normal and DFD pork 30 minutes post-mortem. This result was in agreement with the report of Maieda *et al.* (1986). However, the lactate level in the normal muscle 1, 6 and 12 hours post-mortem were significantly higher ($P<0.05$) than DFD pork at the same time post-mortem. These changes were in accordance with the changes in glycogen levels.

pH changes of muscle 30 minutes post-mortem were associated with the lactate content of meat (Fischer and Augustine, 1977; Fernandez and Gueblez, 1992). According to the above mentioned results, the ultimate pH value of muscle post-mortem was related to the glycogen concentration in muscle, in addition, the lactate level increased rapidly because of anaerobic glycolysis of glycogen. The lactate level of the normal pork increased markedly (Figure 2) and its glycogen level dropped obviously in this study (Figure 1).

DFD pork may be caused by a decrease in the concentration of NAD^+. Since NAD^+ was reduced into NADH, myoglobin therefore converted into metmyoglobin and the oxygen concentration reduced. Thus, these conditions caused pork to become DFD under anaerobic circumstances. NAD^+ concentrations normal and DFD meats 30 minutes post-mortem were not significantly different. However, the NAD^+ concentration of the normal muscle was significantly higher ($P<0.05$) than the DFD muscle six and 12 hours after slaughter. The NAD^+ level of both the normal and DFD muscles decreased with increasing post-mortem time (Figure 3).

Carbon 13-NMR spectra were measured according to Lundberg and Vogel (1986). Table 2 presents result on the peaks of compounds appearing on the carbon 13-NMR spectrum obtained from porcine muscle post-mortem. Figure 5-A presented carbon 13-NMR-spectrum for the muscle 30 minutes post-mortem obtained from the animals stressed before slaughter. The numbers taken from the Figure 5-A correspond to the numbers listed in Table 2. Figures 5-B, 5-C and 5-D present carbon-13 NMR spectra for the muscle 6, 12 and 24 hours post-mortem respectively. Obviously, there are many well separated resonances that have been assigned to specific carbon atoms of metabolites by comparison with the known compositions of pork (see Table 2) and by using the earlier reported assignments of Lundberg and Vogel (1986).

The main changes that could be detected in carbon-13 NMR spectra during post-mortem metabolism were the increasing lactate peaks at 20, 68 and 182ppm as the numbers of 1, 6 and 20 assigned for the resonances in Table 2.

Figures 4-A and 4-B present carbon-13NMR spectra for the normal muscle post-mortem. It was found that the peak composition of metabolites in the normal muscle was similar to the pork obtained from the animal stressed before slaughter as compared to Table 2. Lactate content of the normal muscle post-mortem was higher than the sample obtained from the animal stressed before slaughter. These results indicates that the energy level in the normal hogs was higher than the stressed hogs before slaughter. The reason that the level of lactate of DFD muscle was lower than the normal muscle might be caused by driving force and tying and ATP and subsequent disappearance at muscle glycogen. Figures 5-A, 5-B, 5-C and 5-D show that the peaks on carbon-13NMR spectra appeared at the position of chemical shift-32, 36, 53, 117, 133, 172ppm for the metabolites of the stressed pigs before slaughter.

The numbers taken from these figures corresponded to the numbers of 2, 3, 5, 11, 12, 16 and 19 presented in Table 2. These compounds might be assigned as carnosine.

Carnosine which is one of peptide presenting in muscle of bird and mammal is water soluble and related to meat flavour (Chen, 1987). It was observed that carnosine concentration was high in DFD muscle 30 minutes post-mortem, and tended to be stable in the muscle 6, 12 and 24 hours post-mortem. Carnosine concentration of the normal muscle 30 minutes and six hours post-mortem apparently did not change, but was slightly higher than DFD muscle six hours post-mortem.

The peaks appeared at 37, 53, 157 and 174ppm on carbon-13 NMR spectrum for Figures 5-A, 5-B, 5-C and 5-D corresponded to the numbers of 4, 5, 14 and 18 listed in Table 2 which were assigned as creatine or creatine phosphate

(CrP). It is difficult to differentiate creatine from creatine phosphate (phosphocreatine+ ADP=ATP+creatine) appearing on NMR spectrum because creatine and creatine phosphate has the same chemical shifts. Yang (1990) reported that CrP and ATP of the normal muscle disappeared from the 31-PNMR spectrum resulting from rigor mortis and preceding metabolism. Miri *et al.* (1992) reported that CrP level of the normal pork on 31-PNMR spectrum was higher than DFD pork. The CrP level of the normal pork decreased with increasing post-mortem time (Lundberg and Vogel, 1986). The CrP level of the sample obtained from the muscle 30 minutes post-mortem was higher than creatine content, but the creatine increased in the muscle six hours after slaughter.

The peaks at 71, 74 and 76ppm appear in the carbon-13 NMR spectrum presented in Fig. 5-A, 5-B, 5-C and 5-D and correspond to the numbers of 7, 8 and 9 in Table 2 which were assigned as inositol. Peaks on the carbon-13NMR spectrum of the normal pork 30 minutes and six hours post-mortem appeared at the same position of chemical shift as the DFD pork. Inositol concentration of muscle 30 minutes post-mortem for the stressed hog was higher, and decreased six hours after slaughter, then tended to be stable. However, inositol content of the normal muscle apparently did not change. There were two peaks at 83 and 152ppm on carbon-13NMR spectrum for DFD and normal pork 30 minutes post-mortem (Figures 5-A and 4-A). These two peaks nearly disappeared from the normal muscle and the muscle six hours post-mortem obtained from the stressed hogs pre-slaughter (see Figures 5 and 7). The compounds have not been identified and the cause for the change of these compounds requires more work in the future. In conclusion, lactate, carnosine, inositol, creatine and phosphocreatine could be detected on carbon-13NMR spectra of the normal and DFD pork from hogs stressed pre-slaughter from the muscle 30 minutes, 6, 12 and 24 hours post-mortem. From the lactate content changes in muscle post-mortem, it was noted that energy level in the normal muscle was higher than muscle of hogs being stressed pre-slaughter.

DSC thermal property analysis is shown in Figure 6. It was found that the exothermic peak appeared on thermogram of the normal muscle stored at 4°C and 25°C, and remained for at least 8.5 hours and three hours after slaughter respectively. There was no exothermic peak detected on the thermogram of DFD muscle both of muscles at 4°C and 25°C. It was found that a different change in the Tmax of transitions for normal and DFD muscles. It seemed that myosin, sarcoplasmic proteins and actin had a gradually shift to lower temperature with increasing post-mortem time. The changes in apparent enthalpies of denaturation of the muscle proteins in the normal and DFD pork were also studied.

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Table 1. Changes in Hunter Lab values of normal and DFD pork post-mortem.

Hunter lab values	DFD		Normal(?)	
	1 hour	24 hours	1 hour	24 hours
L value	29.87[b]	31.27[b]	34.84[a]	38.27[a]
a value	5.00[b]	6.76[a]	5.34[b]	7.85[a]
b value	2.87	3.5	1.70	3.61

[a,b] Different superscripts within a row indicates significant differences between normal and DFD.

Table 2. Carbon-13 NMR spectra of pork post-mortem.

Peak	Compound	Carbon	Chemical shift (ppm)
1	lactic acid	C_3	20
2	carnosine	C_4	32
3	carnosine	$-CH_3NH_3-$	36
4	aspartate, creatine, CrP	C_3rep-CH_2N	37
5	CrP, creatine, aspartate	$-CH_3N$-rep $(CH_3)_3N$	53
6	lactic acid	C_2	68
7	inositol	C_2+C_6	71
8	inositol	C_1+C_3	74
9	inositol	C_5	76
10	ni	-	83
11	carnosine	C_4	117
12	carnosine	C_2	133
13	ni	-	152
14	creatine	$N-C(NH_2)_2$	157
15	ni	-	160
16	creatine	C_1	172
17	aspartate	C_1	173
18	creatine	-COO	174
19	carnosine, aspartate	C_4	177
20	lactic acid	C_1	182

ni = not identified.