Relationship between Thermal Properties of Muscle Proteins and Pork Quality

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ABSTRACT : The purpose of this study was performed as model study using four animals to investigate the correction between the changes in Differential Scanning Calorimetry thermogram of muscle proteins during storage and meat freshness. *M. longissimus dorsi* of pork was obtained immediately after slaughter and chilled/stored at either -2°C or 25°C for up to 96 h for analyses. DSC thermograms were determined and compared with pH values, ATP-related compounds, K-values, volatile basic nitrogen (VBN) levels, bacterial counts and electrophoretic behavior. Changes in pH, bacterial counts, VBN and K-values were associated with increased storage temperature and time. The levels of pH values, bacterial counts, VBN and K-values of pork samples stored at 25°C were higher than those of the pork samples stored at -2°C. ATP concentration decreased faster in samples stored at 25°C. Only IMP increased in samples stored at -2°C, whereas the concentration of hypoxanthine and inosine increased in samples stored at 25°C. One exothermic peak and two endothermic peaks appeared on the thermograms of pork stored at either temperature. Lower transition temperature of myosin, sarcoplasmic protein and actin peaks were observed. The freshness parameters of K-value, VBN and hypoxanthine showed highly negative correlations (-0.742~-0.9980) to the changes in transition temperature. Therefore, the shift temperature on DSC thermogram can be used as an indicator of the freshness parameters of meat. ***(Asian-Aust. J. Anim. Sci. 2005. Vol 18, No. 3 : 427-432)***

Key Words : Association, Productive Traits, Polymorphism, Lipoprotein Lipase Gene, Pigs

INTRODUCTION

Differential scanning calorimetry (DSC) can detect the heat denaturation of a protein in complex protein systems as an endothermic peak in its thermogram (Judge et al., 1989). Moreover, the DSC technique has the advantage that it can be applied to observe thermal changes and denaturation of muscle proteins in meat (Stabursvik and Martens, 1980; Xiang and Brekke, 1990). Changes in exothermic and endothermic peaks on DSC thermograms of muscle during storage can be conducted to determine whether such changes could be uniquely associated with meat quality such as freshness, functionality and adulteration (Wright et al., 1977; Park and Lanier, 1988). The functional property of raw meat influences the quality of meat products and they are the major topic of research for meat scientists. Many factors influence meat quality and protein properties during postmortem storage (Hamm, 1981; Park and Lanier, 1988; Hwang and Thompson, 2002), and include genetics, nutrition, rate of chilling (Cannon et al., 1995), pH (Stabursvik and Martens, 1980; Samejima et al., 1983), and ion strength (Kijowski and Mast, 1988; Barbut and Findlay, 1991). The purpose of this study was performed as model study using four animals to investigate changes of DSC thermograms associated to freshness parameters including pH value, bacterial counts, ATP-related compounds, volatile basic nitrogen (VBN) levels and K-values. SDS-PAGE patterns were also evaluated.

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MATERIALS AND METHODS

Preparation of pork samples

The animals used in this study were 100 kg of body weight LYD pigs (n=4). Two kg *m. longissimus dorsi* between the 5th and the 11th pork rib was obtained within half hour after slaughtering from the slaughterhouse. Samples were package with polyethylene film and then chilled/stored at -2°C or 25°C (ambient temperature) for 0, 12, 24, 36, 48 or 96 h for analyses.

Freshness parameters analysis

The pH value was measured with an HI 8424 Microcomputer pH-meter (HANNA Instrument, Italy) by using insertion-type probe. Total bacterial count was measured by the FDA method (1975). Volatile basic nitrogen (VBN) content was determined by the modified AOAC (1984) method. The procedure of high performance liquid chromatography (HPLC) analysis of ATP-related compounds was modified according to the methods of Boyle et al. (1991) and Ryder (1985); HPLC analysis was performed on a model L-6200 (Hitachi Co., Japan). K-value was calculated as the ratio of inosine (HxR)+hypoxanthine (Hx) to the amount of ATP-related compounds using the data obtained from the result of HPLC analysis (Saito et al., 1959).

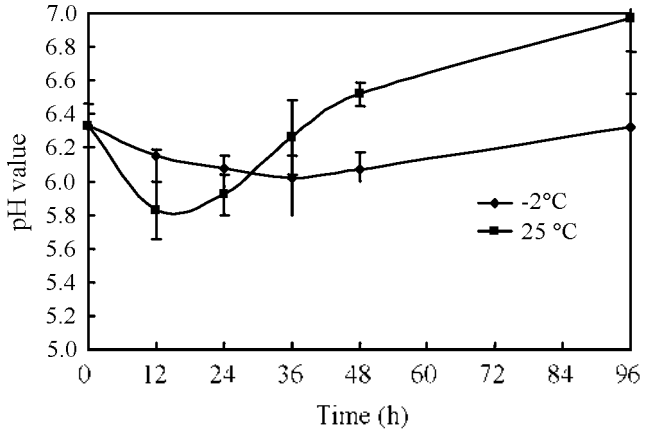


Figure 1. Changes of pH value of pork *m. longissimus dorsi* stored at -2°C and 25°C.

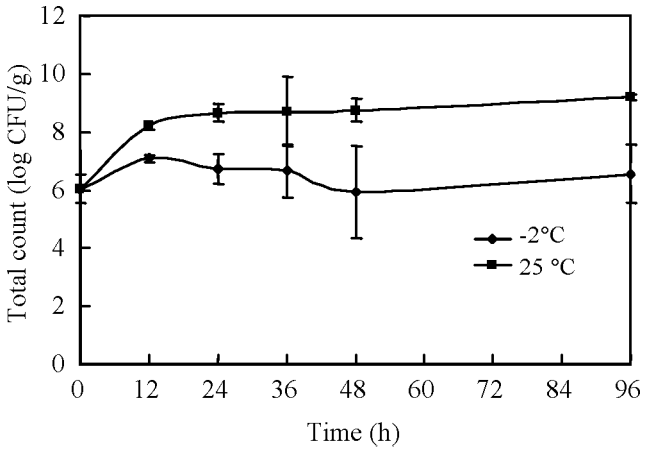


Figure 2. Changes of bacterial counts of pork *m. longissimus dorsi* stored at -2°C and 25°C.

DSC and SDS-PAGE analysis

DSC was performed on a UL Vac DSC-7000 (Sonku-Riko, Japan) equipped with a thermal analyzer. Triplicate samples (15-20 mg) were weighed in aluminum pans (No. 201-53090) and then sealed. The scanning temperature was 25-99°C at a heating rate of 10°C/min. A reference containing 12-13 mg of distilled water was used. The instrument temperature was calibrated using Indium. After DSC analysis, the sample pans were punctured and then dried at 105°C overnight for weight determination. The enthalpy of muscle proteins denaturation was also collected. Electrophoretic behavior of muscle proteins was carried out by the method of Laemmli (1970). One gram of *M. longissimus dorsi* was homogenized, centrifuged and washed three times using five volumes of washing buffer (pH=7.6). After final centrifuged, the myofibril pellet was then suspended in 5 ml of 0.05 M sodium phosphate solutions. A 20 µl of extracted proteins were loaded into the 7.5-20% gradient SDS-PAGE gels and run at 100 V electrophoretic tank (ATTO, Japan) for approximately 6 h at room temperature.

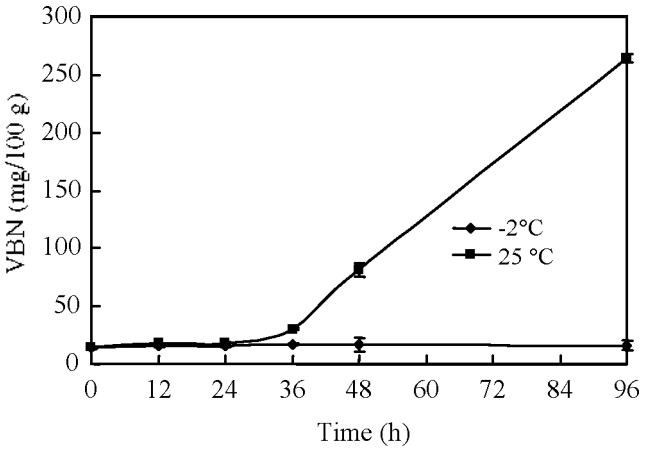


Figure 3. Changes of VBN of pork *m. longissimus dorsi* stored at -2°C and 25°C.

Statistical analysis

Data were analyzed using a statistics software package (SAS, 1995). The GLM system was used to calculate regress correlation. When significant ($p<0.05$) overall differences were found, differences between individual means were then assessed by Duncan's multiple range tests.

RESULTS AND DISCUSSION

The result of pH values and total bacterial count are shown in Figure 1 and 2 respectively. After being stored at 25°C for 24 h, the sample pH values decreased with storage time then increased. The pH values of pork stored at -2°C dropped more slowly than those of pork stored at 25°C. Initial numbers of bacterial count were 10^6 CFU/g, and then increased to 10^8 CFU/g of 25°C and 10^7 CFU/g of -2°C after 24 h. In early stage (12 h) bacterial counts of the sample stored at 25°C reached initial spoilage levels of 10^8 CFU/per gram. These results showed that when fresh pork is kept at high temperature, the sanitation of the pork samples obtained from a traditional meat market is insufficient.

The concentrations of VBN are shown in Figure 3, indicating the noticeable degradation in muscle proteins stored at higher temperature. Chen and Guo (1992) reported that accumulation of NH_3 related products might result in pH values above 8.0. However, the increased in pH value can be caused by enzyme or spoilage bacteria proteolytic activity (Hofmann, 1988). Furthermore, it may play an important role in affecting pork quality during storage.

The changes of ATP-related compounds for the pork stored at -2°C and 25°C are shown in Figure 4. ATP concentration depleted faster in the samples stored at 25°C than in those stored at -2°C. This result agrees with a report from Chen et al. (1992a) indicating that the level of ATP decreased from 7.18 m mole/kg meat to 1.0 m mole/kg

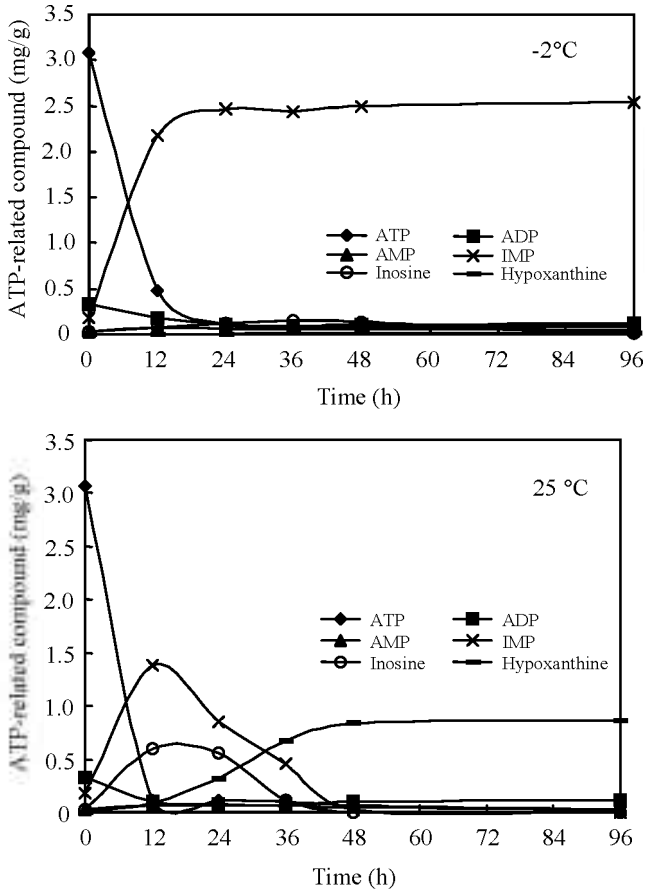
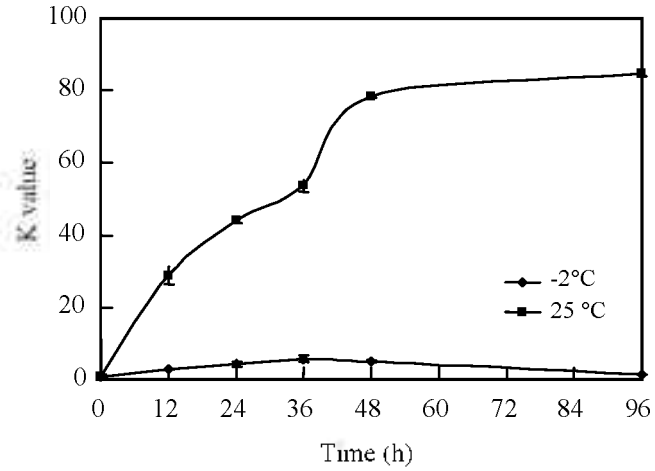


Figure 4. Changes of ATP-related compounds of pork *m. longissimus dorsi* stored at -2°C and 25°C.



K value = [(HxR+Hx)/(ATP+ADP+AMP+IMP+HxR+Hx)]×100%

Figure 5. Changes of K-value compounds of pork *m. longissimus dorsi* stored at -2°C and 25°C.

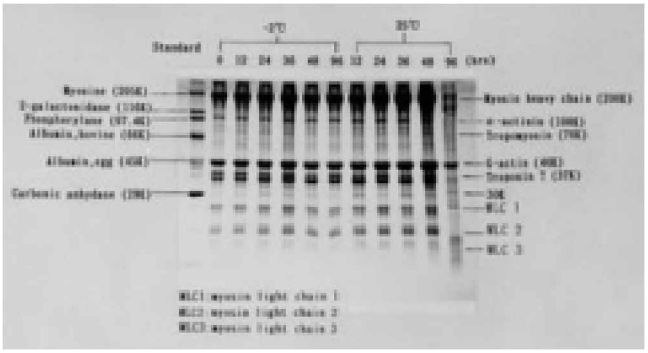


Figure 6. Changes with time for electrophoretograms of pork *m. longissimus dorsi* myofibrillar protein stored at -2°C and 25°C.

meat when stored at 25°C for 8 h. Normally, when ATP depletion occurs after slaughter, nucleotide-related compounds are ranked in the following order: ADP, AMP, IMP, inosine as well as hypoxanthine (Okuma and Watanabe, 2002). The results showed in the figure suggest that even stored at 25°C or -2°C the sample IMP level began increasing in the initial stage. However, after 12 h the IMP depletion rate was higher in the sample stored at 25°C. It was found that only little IMP degradation occurs at -2°C as well as inosine and hypoxanthine still remain at a lower level. At the same periods, the amounts of hypoxanthine and inosine only increased at the higher temperature. The results showed that the IMP levels of the samples increased in the early stage of the storage, regardless the treatments. However, the IMP level was found remarkable decrease at 12 h time point only in the sample stored at 25°C, but not in the -2°C group, suggesting that the lower storage temperature may limit IMP degradation.

K-value, an indicator of meat freshness was reported by Boyle et al. (1991). In general, K-value is the best indices to evaluate of fish freshness, this parameter correlate well with storage quality. This value coincides with the 60% limit set by Ehira (1976). We found that the K-value of pork stored at 25°C for 24 h rose to 44% and increased with time (Figure 5); this is close to spoilage stage, however the K-value of the pork stored at -2°C remained lower (0.7%) as described by Watanabe et al. (1992).

Figure 6 shows the changes in the electrophoretogram of myofibrillar proteins in the pork stored at -2°C and 25°C. The bands of myofibrillar proteins in the sample stored at -2°C remained stable during storage, and then 30 K Dalton component appeared on the electrophoretogram for all the pork samples and increased with postmortem time.

DSC thermal property analysis is shown in Figure 7 and 8. The exothermic peak and two endothermic peaks appeared on the thermogram of the pork stored at 25°C and -2°C for 1 h. However, the exothermic peak disappeared from the thermogram of the pork after 12 h of storage, and three endothermic peaks for myosin ($Tmax_1$), sarcoplasmic proteins ($Tmax_2$) and actin ($Tmax_3$) appeared on the thermogram. This agrees with the results obtained by Chen et al. (1992b), who indicated that an exothermic peak disappeared from the thermogram after 12 h. This result also agrees with Stabursvik et al. (1984), who reported that

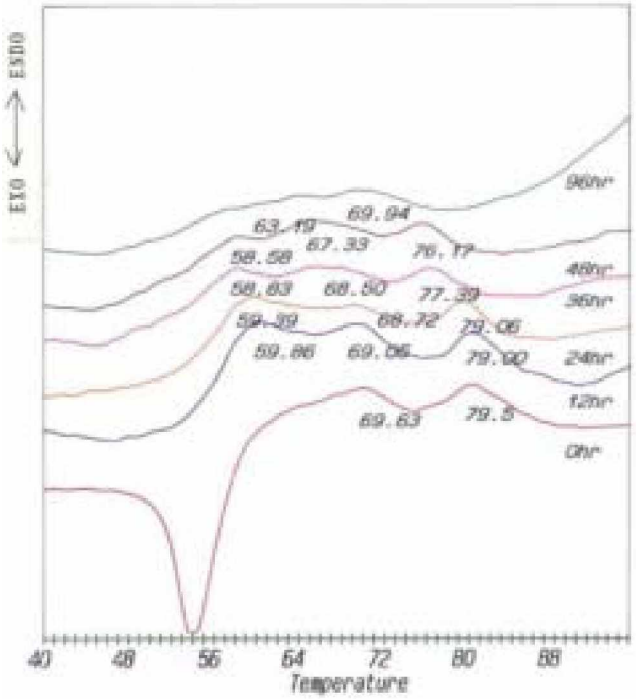


Figure 7. DSC thermogram of pork *m. longissimus dorsi* of stored at 25°C.

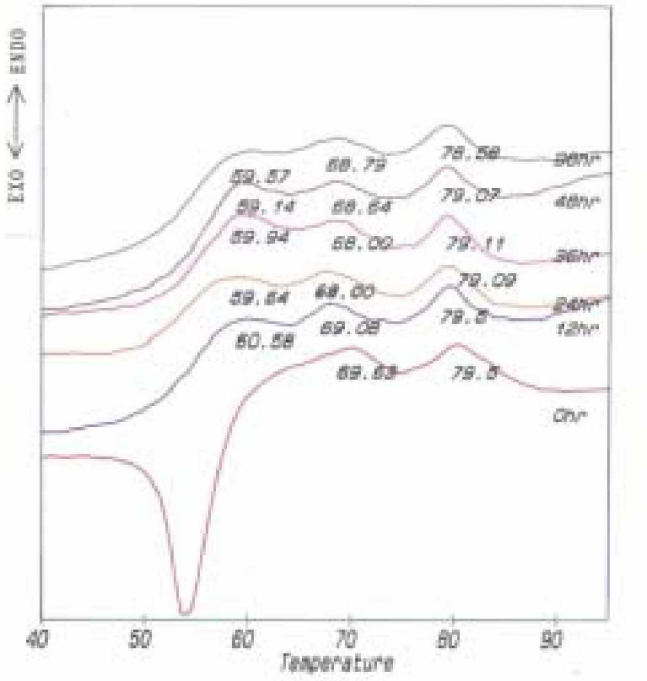


Figure 8. DSC thermogram of *m. longissimus dorsi* of pork stored at -2°C.

Table 1. Regression correlation coefficients among freshness parameters for pork compared with three proteins transition temperature of DSC thermogram during storage at 25°C within 96 h

Parameters	ΔH	Myosin	Sarcoplasmic protein	Actin
ΔH	1	0.822	-0.051	-0.021
Myosin	0.822	1	0.868	0.921
sarcoplasmic protein	-0.051	0.868	1	0.992
Actin	-0.021	0.921	0.992	1
K-value	0.487	-0.946	-0.795	-0.789
pH	-0.357	-0.963	-0.838	-0.876
Total plate count	0.806	-0.911	-0.602	-0.574
VBN	-0.063	-0.796	-0.911	-0.988
ATP	-0.927	0.767	0.415	0.383
Hypoxanthine	0.343	-0.998	-0.742	-0.765

N = 4.

the denaturation temperature of muscle protein was lower after postmortem. Wagner and Anon (1985) found that, with a meat pH comprised within 5.4-6.2, the thermal denaturation temperature of myosin decreased when the pH decreased. In contrast, an increased in actin denaturation temperature was observed. The pH value plays an important role in protein thermal denaturation, as reported by many authors (Goodno and Swenson, 1975; Wright et al., 1977; Samejima et al., 1983; Wagner and Anon, 1985).

It was noted that the transition temperature of myosin, sarcoplasmic proteins and actin gradually decreased with time. The most noticeable change was in the sample stored at 25°C for 24 h. We found that myosin and actin had decreased to 58.83°C and 77.39°C when compared with 59.94°C and 79.11°C for the sample stored at -2°C. After an extended storage time the three transition temperatures of the sample stored at 25°C shifted to 58.58°C, 67.39°C and 76.17°C. However, a recent study by Jensen and Jorgensen (2003) indicated that a shift of Tmax to a lower temperature was possibly related to a pH within 6.6-7.7, which originated from samples with a higher pH. It is apparent from Figure 1 in that the pH value of samples were above 6.2 when stored at 25°C. In addition, in the sample stored at the higher temperature, the loss of thermal stability of protein was clearly displayed on the DSC thermogram. This is the same as results obtained by Wright et al. (1997), who reported that a shift to lower temperature on the DSC thermogram of myosin occurred when the sample was stored at 25°C. Indeed, as mentioned above, after 36 h of storage, the hypoxanthine levels of the sample stored at 25°C nearly doubled to 0.67 mg/g (compared to 0.32 mg/g after 24 h), and that of the sample stored at -2°C nearly increased by a sixth to 0.11 mg/g (compared to 0.007 mg/g after 24 h). The same trend was also noted in K-value (53.6%), suggesting some of the DSC thermogram could be use as an indicator to evaluated pork freshness. Correlation coefficients data from Table 1 showed that three Tmax of myosin, sarcoplasmic protein and actin were linearly (R^2 above -0.74) related to most freshness parameters including K-value, VBN and hypoxanthine. The result from this study

Table 2. Change of transition heat (ΔH) of *M. longissimus dorsi* of pork stored at -2°C or 25°C (mcal/per mg of dry matter)

Time (h) post-mortem	-2°C	25°C
0	*	*
12	4.235±0.693^{ax}	5.287±0.939^{ax}
24	4.277±0.865^{ax}	4.825±1.031^{abx}
36	4.089±1.001^{ax}	4.964±1.096^{abx}
48	3.922±1.001^{ax}	4.340±1.044^{abx}
96	4.761±1.240^{ax}	3.825±1.288^{bx}

N = 4. Means±SD. Not detectable.
^{a,b} Means within the same column without the same superscript letters are significantly different ($p<0.05$).
^{x} Means within the same row without the same superscript letters are significantly different ($p<0.05$).

also suggest depend only ΔH data (Table 2) are difficult to express meat freshness after several days of storage. Regression analysis of the K value with Myosin transition temperature showed significant negative correlation ($Y_{Tmax}=0.0005X_{K\text{-}value}^{2}-0.076X_{K\text{-}value}+61.711$, $R^2=0.9633$).

In previous studies, several researchers have shown that DSC can detect the changes in physico-chemical properties of muscle postmortem. Wright et al. (1977) reported that the presence of an exothermal peak in the DSC thermogram of a pre-rigor rabbit at 54°C which disappeared with the onset of rigor mortis. We also found the same result in the porcine muscle within 4 h postmortem in our previous work (Chen et al., 1992b). Park and Lanier (1988) reported similar findings when a large exothermic peak near 50°C, observed soon after slaughter, was the most striking difference between the thermogram of pre-rigor muscle compared to that of post-rigor muscle.

In this study, we conclude that the variation in denatured peaks of DSC thermograms could be affected by higher pH values. Time course changes of certain freshness parameters have trends that the related to change in Tmax transition temperature values.

REFERENCES

AOAC. 1984. Official methods of Analysis, 14th ed., Association of Official American Chemists. Washington. DC. USA.

Barbut, S. and C. J. Findlay. 1991. Influence of sodium, potassium and magnesium chloride on thermal properties of beef muscles. J. Food Sci. 56:180-182.

Boyle, J. L., R. C. Lindsay and D. A. Stuiber. 1991. Adenosine nucleotide degradation in modified atmosphere chill-stored fresh fish. J. Food Sci. 56:1267-1270.

Cannon, J. E., J. B. Morgan, J. Heavner, F. K. McKeith, G. C. Smith and D. L. Meeker. 1995. Pork quality audit: A review of the factors influencing pork quality. J. Muscle foods. 6:369-402.

Chen, M. T. and S. L. Guo. 1992a. Studies on the microbial flora of Chinese-style sausage. 2. Action of selected organisms isolated from Chinese-style sausage on porcine muscle proteins. Fleischwirt. 72:1126-1128.

Chen, M. T., C. P. Lee and D. C. Liu. 1992b. Studies on 2, 3-Diphosphoglycerate, pyruvate kinase and mitochondrial ATPase activities in PSS pig. Proceeding of 38th International Cong. Meat Sci. Technol. 3:345-346.

Chen, M. T., C. P. Lee and D. C. Liu. 1992. DSC Studies on thermal properties of muscle proteins in normal and PSE pork. Proceedings of 38th International Cong. Meat Sci. Technol. 3:347-350. France.

Ehira, S. 1976. A biochemical study on the freshness of fish. Bull. Tokai. Reg. Fish Res. Labor. 88:1-132.

Food and Drug Administration. 1975. FDA Bacteriological Analytical Manual for Food. 4th ed., FDA, Washington, DC. USA.

Goodno, C. C. and C. A. Swenson. 1975. Thermal transitions of myosin and its helical fragments. II. Solvent-induced variations in conformational stability. Biochem. 14:873-878.

Hamm, R. 1981. Postmortem changes in muscle affecting the quality of comminuted meat products. In "Developments in Meat Sci. 2." (Ed. R. Lawrie) Appl. Sci. Pub., Inc., London.

Hofmann, K. 1988. pH a quality criterion for meat. Fleischwirt. 68:67-70.

Hwang, I. H. and J. M. Thompson. 2002. A technique to quantify the extent postmortem degradation of meat ultrastructure. Asian-Aust. J. Anim. Sci. 15:111-116.

Jensen, K. N. and B. M. Jorgensen. 2003. Effect of storage conditions on differential scanning calorimetry profiles from thawed cod muscle. Lebensm-Wiss. U. Technol. 36:807-812.

Judge, M. D., E. D. Aberle, J. C. Forrest, H. B. Hedrick and R. A. Merkel. 1989. Principles of meat science. 2nd edition Kendall. pp. 85-133. Hunt pub. Co.

Kijowski, J. M. and M. G. Mast. 1988. Thermal properties of proteins in chicken broiler tissues. J. Food Sci. 53:363-366.

Kimura, I. O., S. M. Sugimoto and T. O. Fujita. 1987. Measurement of fish flesh freshness by DSC. Netsu Sokutei. 14:37-38. Japan.

Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature. 277:680-685.

Okuma, H. and E. Watanabe. 2002. Flow system for fish freshness determination based on double multi-enzyme reactor electrodes. Biosensor and Bioelectronics 17:367-372.

Park, J. W. and T. C. Lanier. 1988. Calorimetric changes during development of rigor mortis. J. Food Sci. 53:1312-1314, 1372.

Ryder, J. M. 1985. Determination of ATP and its breakdown products in fish muscle by high-performance liquid chromatography. J. Agric. Food Chem. 33:678-680.

Saito, T. K. Ara and M. Matsuyoshi. 1959. A new method for estimating the freshness of fish. Bul. Japan. Soc. Sci. Fish 24:249-250.

Samejima, K., M. Ishioroshi and T. Yasui. 1983. Scanning calorimetric studies on the thermal denaturation of myosin and its subfragments. Agric. Biol. Chem. 47:2373-2379.

SAS. 1995. SAS system for window 95 (Release 6.111). SAS Inst. Inc., Cary, USA.

Stabursvik, E. and H. Martens. 1980. Thermal denaturation of proteins in post-rigor muscle tissue as studies by differential scanning calorimetry. J. Sci. Food Agric. 31:1034-1042.

Stabursvik, E., K. Fretheim and T. Frostein. 1984. Myosin denaturation in pale, soft and exudative (PSE) porcine muscle

tissue as studies by differential scanning calorimetry. J. Sci. Food Agric. 35:240-244.

Wagner, J. R. and M. C. Anon. 1985. Denaturation kinetics of myofibrillar proteins in bovine muscle. J. Food Sci. 50:1547-1550.

Watanable, A., H. Sato, E. Tsuneishi, M. Matsumoto and Y. Takimoto. 1992. Effect of slaughter methods on the post-mortem changes of pH and ATP-related compounds of beef muscles. Jpn. Anim. Sci. Technol. 63:935-941.

Wright, D. J., I. B. Leach and P. Wilding. 1977. DSCC studies of muscle and its constituent proteins. J. Sci. Food Agric. 28:577-564.

Xiang, Y. L. and C. J. Brekke. 1990. Thermal transitions of salt soluble proteins from pre- and post-rigor chicken muscles. J. Food Sci. 55:1540-1543.