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The effects of electrical stimulation and aging times on the physical, biochemical and sensory parameters of Chinese dry-cured and aged ham

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The Ohio State University, 1994
THE EFFECTS OF ELECTRICAL STIMULATION AND AGING TIMES ON THE PHYSICAL, BIOCHEMICAL AND SENSORY PARAMETERS OF CHINESE DRY-CURED AND AGED HAM

DISSERTATION

Presented in Partial Fulfillment of the Requirements for the Degree Doctor of Philosophy in the Graduate School of The Ohio State University

By

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***

The Ohio State University
1994

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DEDICATION

Special appreciation goes to my parents, Mr. and Mrs. Yipoung Chow, for their moral support and continuous encouragement during the period of the advanced education. Moreover, sincere gratitude is extended to my wife, Shu-Fang, my daughter, Yu-Tzu, and my son, Ke-Chun for their love and understanding to allow me in finishing graduate study.
ACKNOWLEDGMENTS

It would be greatly appreciated that Dr. Michael E. Mangino, my adviser, for his valuable advice, guidance and suggestions in supervising this study and author's Ph.D. program. His patient and close cooperation are gratefully acknowledged. I would also like to express my deepest thanks for Dr. Ned A. Parrett, my co-adviser, for his help in the development of this research and his timely counsel in the preparation, organization, and writing of this dissertation.

I wish to express my sincere appreciation to Dr. Vern R. Cahill, Dr. Charles V. Morr, and Dr. Herbert W. Ockerman for their suggestions and tremendous help in doing this research, and for their contributions to my final manuscript. In addition, a special thanks go to Dr. S. Y. Chen, Director of Animal Industry Research Institute, Taiwan Sugar Corporation (TSC), for his generous support and assistance give me this chance to complete advanced study. My thanks also go to Dr. Isabelle Laye, for her assistance in using the technique of Dynamic Headspace Analysis in Haas Chair Laboratory. The author gratefully acknowledges the financial support of the TSC and the National Science Council of Republic of China during my graduate study.
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PUBLICATIONS


FIELD OF STUDY

Major Field: Food Science and Nutrition
# TABLE OF CONTENTS

DEDICATION .................................................................................................. ii  
ACKNOWLEDGMENTS ................................................................................ iii  
VITA .................................................................................................................... iv  
TABLE OF CONTENTS ............................................................................... viii  
LIST OF TABLES .......................................................................................... xii  
LIST OF FIGURES ......................................................................................... xiv  

## CHAPTER

I. INTRODUCTION .................................................................................... 1  
II. LITERATURE REVIEW ...................................................................... 7  

2.1. Structural Changes of Myofibrils during Postmortem Aging .................. 7  
2.2. Endogenous Proteases ..................................................................... 8  
2.3. Calcium-Dependent Proteases Involved in Postmortem Aging ............ 9  
2.4. Electrical Stimulation Related to Postmortem
2.5. Sodium Dodecyl Sulfate Polyacrylamide Gel
Electrophoresis (SDS-PAGE).............................. 15
2.6. Myofibril Fragmentation Index (MFI).............. 16
2.7. Biochemical Changes in Muscle Postmortem....... 17
2.8. Curing Ingredients and Their Role in Cured
Meat Flavor...................................................... 20
2.9. Chemical Compounds Related to Flavor in
Dry-cured and Aged Hams.................................. 23
2.10. Volatile Compounds Related to Flavor in
Dry-cured and Aged hams................................. 26

III. METHODS AND MATERIALS........................................... 29

3.1. Objectives.......................................................... 29
3.2. Experimental Procedures................................. 31
3.3. Preparation of Ham Samples for Analyzing......... 37
3.4. Determination of pH Value................................. 39
3.5. Determination of Calcium-Dependent Protease
Activities.......................................................... 39
3.6. Determination of Protein Concentration by
Biuret Method.................................................... 43
3.7. Determination of Myofibril Fragmentation index
(MFI)................................................................. 46
3.8. Sodium Dodecyl Sulfate Polyacrylamide Gel
Electrophoresis.................................................. 48
3.9. Determination of Proximate Analysis and Sodium Chloride
3.10. Determination of Non-protein Nitrogen
3.11. Automated Analysis of Free Amino Acids
3.12. Determination of Total Nucleotides and Nucleosides
3.13. Determination of Total Free Fatty Acids
3.14. Determination of Carbonyl Compounds
3.15. Determination of T.B.A. Value and Sodium Nitrite Residues
3.16. Determination of Volatile Compounds by Using Dynamic Headspace Analysis (DHA)
3.17. Sensory Panel Evaluation
3.18. Statistical Analysis

IV. RESULTS AND DISCUSSION

4.1. Changes in pH Value Affected by Electrical Stimulation
4.2. Relative Activity of Low-Calcium-Requirements Calcium-Dependent Protease (CDP-I) during the Aging Period
4.3. Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis
4.4. Myofibril Fragmentation Index (MFI)
4.5. Protein Concentration ........................................................... 73
4.6. Changes of Proximate Analysis and Salt
   Content during the Aging Periods........................................ 78
4.7. Changes of Non-protein Nitrogen during the
   Aging Periods............................................................................. 80
4.8. Changes of Free Amino Acids during the Aging
   Periods.................................................................................................. 80
4.9. Changes of Total Nucleotides and Nucleosides
   during the Aging Periods............................................................. 85
4.10. Changes of Total Free Fatty Acids and Carbonyl
   Compounds during the Aging Periods................................. 87
4.11. Changes of T.B.A. Value and Sodium Nitrite
   Residues during the Aging Periods......................................... 90
4.12. Changes of Volatile Compounds during the Aging
   Periods by Using Dynamic Headspace Analysis.... 93
4.13. Sensory Evaluation of Chinese-style Dry-cured
   and Aged Hams............................................................................. 99

V. SUMMARY AND CONCLUSIONS....................................................... 109

BIBLIOGRAPHY......................................................................................... 115
LIST OF TABLES

<table>
<thead>
<tr>
<th>TABLES</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. The effect of electrical stimulation and aging times on</td>
<td>74</td>
</tr>
<tr>
<td>the myofibril fragmentation index of Chinese style dry-cured and aged hams</td>
<td></td>
</tr>
<tr>
<td>2. The effect of electrical stimulation and aging times on</td>
<td>77</td>
</tr>
<tr>
<td>the protein concentration (mg/ml) of Chinese style dry-cured and aged hams</td>
<td></td>
</tr>
<tr>
<td>3. The effects of electrical stimulation and aging times on</td>
<td>79</td>
</tr>
<tr>
<td>the mean proximate analysis and salt content (standard error) of fresh ham and Chinese dry-cured and aged hams</td>
<td></td>
</tr>
<tr>
<td>4. The effect of electrical stimulation and aging times on</td>
<td>81</td>
</tr>
<tr>
<td>the non-protein nitrogen (mg/g of freeze-dry lean) of Chinese style dry-cured and aged hams</td>
<td></td>
</tr>
<tr>
<td>5. The effect of electrical stimulation and aging times on</td>
<td>82</td>
</tr>
<tr>
<td>the free amino acids (µ mole/g DM) of fresh ham and Chinese style dry-cured and aged hams</td>
<td></td>
</tr>
<tr>
<td>6. The effect of electrical stimulation and aging times on</td>
<td></td>
</tr>
<tr>
<td>the total nucleotides and nucleosides (mg/g of freeze-</td>
<td></td>
</tr>
<tr>
<td>xii</td>
<td></td>
</tr>
</tbody>
</table>
7. The effect of electrical stimulation and aging times on the total free fatty acids (g/100g freeze-dry fat) of Chinese style dry-cured and aged hams................................. 88
8. The effect of electrical stimulation and aging times on the carbonyl compounds (μ mole/g of freeze-dry fat) of Chinese style dry-cured and aged hams............................... 89
9. The effects of electrical stimulation and aging times (0, 6, 9, and 12 months) on the volatile flavor compounds in Chinese style aged hams and fresh ham......................... 94
10. The two way ANOVA of individual volatile compounds affected by aging times, electrical stimulation and the interaction between these two treatments............................. 96
11. The volatile flavor compounds related to flavor characteristics of Chinese style dry-cured and aged ham.................................................................................. 98
12. The effects of electrical stimulation and aging times on the mean sensory parameters (standard error) of fresh ham and Chinese dry-cured and aged hams.................. 100
13. The correlation coefficients between ham flavor intensity and biochemical measurements related to flavor characteristics of Chinese ham........................................ 102
14. The correlation coefficients of individual volatile compounds between ham flavor intensity and overall xiii
15. The correlation coefficients between tenderness, texture and relative activity of CDP-I and myofibril fragmentation index.
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>FIGURES</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. The schematic diagram of the experimental design for proteolytic activity...</td>
<td>3</td>
</tr>
<tr>
<td>2. The schematic diagram of the experimental design for examining biochemical changes and sensory parameters related to flavor characteristics of Chinese style dry-cured and aged hams...</td>
<td>6</td>
</tr>
<tr>
<td>3. The effect of electrical stimulation on postmortem pH decline of the meat sample taken from the <em>semimembranosus</em> muscle...</td>
<td>6</td>
</tr>
<tr>
<td>4. The effects of electrical stimulation and aging times on the changes of pH values of the Chinese style dry-cured and aged hams...</td>
<td>6</td>
</tr>
<tr>
<td>5. The effects of electrical stimulation and aging times on relative activity of CDP-I of the Chinese style dry-cured and aged hams...</td>
<td>7</td>
</tr>
<tr>
<td>6. The 5 - 15% SDS electrophotograms of myofibrillar...</td>
<td></td>
</tr>
</tbody>
</table>
protein of the Chinese style dry-cured and aged hams affected by electrical stimulation and aging period........ 70

7. The 17-27% SDS electrophotograms for myofibrillar protein of the Chinese style dry-cured and aged hams affected by electrical stimulation and aging period........ 71

8. The protein standard curve calibrated by using crude porcine protein.......................................................... 76

9. The effects of electrical stimulation and aging times on the T.B.A. values (mg/kg) of Chinese style dry-cured and aged hams................................................................................... 91

10. The effects of electrical stimulation and aging times on the sodium nitrite residue (ppm) of Chinese style dry-cured and aged hams................................................................. 92
CHAPTER I

INTRODUCTION

Chinese style dry-cured and aged ham has an excellent texture and a unique flavor produced through the processing techniques of dry curing and long postmortem aging. It is often used as a garnish or as a main ingredient in Honey Dew Ham, King-Hua Chicken with ham, and stuffed Winter Melon with ham. The most famous Chinese hams are produced in "King-Hua", a city in the midsouth of China (Ockerman and Chow, 1984).

Chinese-style dry-cured and aged hams require a long postmortem aging process (six months to one year) for the production of high quality product. A clear understanding is needed of the biochemical changes which occur, due to the proteolysis of muscle proteins, maillard reaction and lipid oxidation, which contribute to the formation of ham flavor during the aging process. This information will assist industry in optimising its production. In this study consists two major experiments to investigate (1) the proteolytic
enzyme activity, and (2) the biochemical changes related to the formation of ham flavor compounds.

The first experiment focuses on the muscle proteases. Muscle proteinases are important to the development of tenderness, flavor and eating quality in red meat during the postmortem aging (Penny, 1980; Etherington, 1984). Pearson et al. (1983) indicated that muscle myofibrils undergo proteolysis during the postmortem aging process. They were able to detect extra protein bands in sodium dodecyl sulfate polycrylamide gel electrophoresis (SDS-PAGE) patterns. The proteolytic disintegration of myofibrillar proteins is an indicator of meat tenderization.

Recent studies have attempted to show how individual enzymes may contribute to weakening myofibrillar and connective tissue structures (Goll, et al. 1983). Calcium-dependent proteases (CDP) may be involved in the carcass postmortem aging (Arakawa et al., 1976; Penny and Ferguson-Pryce, 1979).

The primary investigations of electrical stimulation have been focused on the improvement of quality and palatability characteristics from electrical stimulated meat animal carcasses, including improvements in tenderness (Davey, et
al. 1976.; George, et al. 1980; Seideman, et. al. 1979; Smith et al. 1977), enhancement of lean color (Bendall, 1980; Tang and Henrickson, 1980), improving lean firmness and texture (Salm et al. 1981), increasing "set-up" of marbling (Savell, et al. 1979; Savell, et al. 1978) and prevention of "heat-ring" development (Stiffler, et al. 1986). Moreover, Dutson et al. (1980) indicated that postmortem electrical stimulation of carcasses speeds up the tenderization process in meat during the aging period. They suggested that electrical stimulation may promote the release of calcium from the sarcoplasmic reticulum and hydrolysis of ATP. The combination of these two factors may increase the Ca ++ ion concentration in the cell, which may activate the low Ca^{2+}-requiring calcium-dependent protease (CDP-I) to cause partial degradation of muscle myofibrils (Dayton et al., 1975).

Therefore, the changes in the activity of such endopeptidases as low calcium activated protease in electrical stimulated carcasses may be responsible for textural changes during the aging process of Chinese ham. However, there is very little information regarding the effect of electrical stimulation on the muscle proteinases during the long-term required for the aging process. In the present study we have examined the levels of calcium dependent proteases in fresh pork and dry-cured hams in order to
determine their activity during the aging period. In addition, this experiment was designed to study the effects of electrical stimulation and aging treatment on the alterations in myofibrillar structures which include determination of protein concentration by using the Biuret method, the myofibril fragmentation index, and SDS-PAGE.

The second experiment focuses on biochemical changes that occur, due to proteolysis of muscle proteins, which contribute to the formation of ham flavor during the aging process. Recently research on meat flavor of cured and aged ham has focused on (1) identification of volatile flavor compounds released from cured hams and the carbonyl compounds which progressively increased during aging time and their relationship which significantly contributed to ham flavor (Gray and Pearson, 1984; Minor et al., 1965; and Ockerman et al., 1964); (2) investigation of the precursors of meaty flavor, glycoprotein and inosinic acid, which are water soluble and are converted to flavor compounds during cooking of meat (Batzer et al., 1962; Wood et al., 1957). Characteristic differences in flavor were attributed to the components derived from lipids (Hornstein and Crowe, 1960; Hornstein et al., 1961); (3) the degradation products of protein and fat produced during aging of hams. These
free amino acids, non-protein nitrogen, water soluble nitrogen, free fatty acids, nucleotides and nucleosides were recognized as important in the formation of cured ham flavor either as flavor compounds or flavor precursors (Fu, et al., 1980; Lillard and Ayres, 1969); (4) the composition of amino acids, especially the sulfur-containing amino acids, tyrosine, histidine and perhaps other amino acids such as aspartic acid, arginine and proline, have also been reported to contribute to the meaty aroma during heating of a model meat system (Hsieh et al., 1980); (5) the identification of volatile compounds by dynamic headspace analysis (DHA), direct sampling by gas chromatography and mass spectrometric techniques (Issenberg and Teranishi, 1970; Hsieh et al. 1980); (6) the possible flavor precursors of isolated muscle fractions are determined by sensory evaluation (Shahidi et al., 1986; Cross et al., 1978).

In the second experiment, chemical constituents of Chinese-style dry-cured and aged hams that contributed to flavor of the product were investigated. The chemical entities included are proximate analysis, salt content, non-protein nitrogen, free amino acids, total nucleotides, total nucleosides, total free fatty acids, carbonyl compounds, sodium nitrite residue, and T.B.A. values. The volatile compounds were determined by using dynamic headspace
analysis. In addition, sensory panel evaluation was obtained. These flavor characteristics may be affected by electrical stimulation of the carcass which may hasten the aging process of Chinese-style ham.
CHAPTER II

LITERATURE REVIEW

2.1. Structural Changes of Myofibrils during Postmortem Aging

Consumers consider tenderness as the predominant quality factor of meat products and probably the most important organoleptic characteristic. With adequate tenderness, consumers appreciate the more subtle characteristics of flavor and juiciness (Chrystal and Hagyard, 1976). Lehmann (1907) reported that there was a 30% increase in meat tenderness during 8 days postmortem aging. This postmortem tenderization results from the action of indigenous muscle proteases to cause alterations in ultrastructure of myofilaments and/or changes in other proteins of the cytoskeleton. Penny (1980) and Goll, et al. (1983) investigated the structural changes of myofibrils during postmortem aging at refrigerated temperature. They found that Z-disks were weakened;
troponin-T gradually was degraded, a 30,000 dalton component simultaneously appeared; and the two major contractile proteins, myosin and actin, were not degraded. These subtle but important structural changes appeared to be a major contributor to the tenderization process during postmortem aging.

2.2. Endogenous Proteases

Two proteolytic enzyme systems which could be related to postmortem tenderization consist of catheptic enzymes (Moeller et al., 1977; Dutson, 1983) and Ca$^{2+}$-dependent proteases (Goll et al. 1983; Koohmarae et al, 1988a). Some have suggested that a cooperative action of these two proteolytic systems occur (Penny and Ferguson-pryce, 1979; Asghar and Bhatti, 1987).

Thirteen lysosomal proteases have been identified so far, but only seven (cathepsin A, B1, C, D, H, L and lysosomal carboxypeptidase B) have been found to exist in skeletal muscle cells. All of these lysosomal enzymes are small proteins (20,000 - 40,000 daltons). They are quite stable and show optimal activity at acidic pH values (Goll et al., 1983).
In addition, both the low-calcium-requiring calcium-dependent protease (CDP-I) and the high-calcium-requiring calcium-dependent protease (CDP-II) are found in skeletal muscles. CDP-I and CDP-II have the same optimum temperature and optimum pH. But CDP-I requires a very low concentration of calcium for 50% activation, when compared to CDP-II (Goll et al., 1985).

2.3. Calcium-Dependent Proteases Involved in Postmortem Aging

There is considerable experimental evidence (Goll et al. 1983; Penny, 1980) indicating that both the Ca\(^{2+}\) dependent proteases and certain capthesins found in skeletal muscle are capable of degrading myofibrillar proteins. CDPs, however, play a more important role than lysosomal proteases in postmortem aging (Koohmaraie et al. 1988 a, b, c, d).

1) **CDP-I, not CDP-II, may be involved in postmortem aging.**

Goll, et al. (1983) indicated that from the aspects of the optimum temperature, optimum pH and Ca\(^{2+}\) concentration requirement, CDP-II may not be involved in postmortem tenderization: (a) the intracellular pH of muscle cells is
decreased from pH 7.0 immediately after death to pH 5.5 during postmortem storage. CDP-II is maximally active at pH 7.5 to pH 8.0. Therefore, it would have very little activity at post rigor pH conditions. (b) CDP-II is maximally active at 23° to 26 °C, whereas postmortem aging occurs at 2° to 4°C. (c) The intracellular free Ca\(^{2+}\) concentration is approximately 50 μM during postmortem storage. The Ca\(^{2+}\) concentration requirement for CDP-II activation (approximately 3 mM) exceeds the Ca\(^{2+}\) level in postmortem muscle (Goll, et al. 1983).

Koohmaraie et al., (1986) used SDS-PAGE, phase microscopy and electron microscopy to investigate the effect of CDP-I on myofibrils during postmortem storage. CDP-I and myofibrils were purified independently and then incubated together at different temperature (5°C and 25°C), and different pHs (5.5, 5.8, 6.2 and 7.5). Results indicated that CDP-I retained 24% to 28% of its activity at pH 5.5 to 5.8 and 5°C. This level of activity was sufficient to cause the myofibrillar changes related to postmortem aging.

Koohmaraie et al. (1987) further confirmed that CDP-I, not CDP-II, may be involved in postmortem aging. They observed that both CDPs undergo autolysis in in-vitro assays where sufficient Ca\(^{2+}\) is available for their activation. Therefore, the rapid loss of CDP-I during postmortem storage may be a good
indicator that CDP-I, not CDP-II, is activated under the conditions existing in postmortem muscle.

2) CDP and lysosomal enzyme activities in different muscles

Koohmaraie et al. (1988b) studied the aging responses (decrease in shear force value) that are associated with protease activities during postmortem aging by utilizing three different muscles Psoas major (PM), Biceps femoris (BF) and Longissimus dorsi (LD) within the same beef carcass. Results indicated that the activities of cathepsins (B, H, and L) were the same for all three muscles. However, the CDP activities followed the same pattern as the aging response; LD had the highest aging response, also had the highest CDP-I activity. In turn, PM, showed the least aging response, and had the lowest CDP-I activity. They suggested that the initial levels of CDP-I activity may determine the aging response of a given muscle.

3) Postmortem changes are Ca²⁺ mediated

Koohmaraie et al. (1988c) indicated that postmortem tenderization events were Ca²⁺ mediated and Ca²⁺-dependent proteases were responsible for postmortem tenderization. They conducted their experiments by incubating the muscle slices with a buffer containing CaCl₂ or with a buffer containing EDTA (chelator of divalent ions) or EGTA (chelator of Ca²⁺ ions). They reported that (1) the slices which were incubated with a
solution containing CaCl₂ increased the myofibril fragmentation index (MFI) and that such fragmentation did not change when slices were incubated with solutions containing Ca²⁺ chelators. (2) The appearance of the 30,000 dalton component and disappearance of desmin occurred within 24 hr of incubation in the presence of CaCl₂, but these changes were inhibited in the presence of Ca²⁺-chelators. In this experiment, based on the incubation of slices in the presence of CaCl₂, which activated Ca²⁺-dependent proteases, postmortem changes were accelerated. It was concluded that CDPs play an important role in the myofibrillar changes of meat. However, the incubation of slices in EDTA and EGTA had no effect on the activities of cathepsins B, H, L, and postmortem changes did not take place. Thus, it is concluded that lysosomal enzymes play minor roles and/or have no detectable effects on postmortem aging of meat.

4) Acceleration of postmortem tenderization process

Koohmarai et al. (1988d) investigated low-frequency electrical stimulation (LFES) and infusion of water containing 0.3 M CaCl₂ on the lysosomal enzymes and CDPs. The ovine carcasses, after treatment of LFES and prior to evisceration, were infused with water or water containing 0.3 M CaCl₂ through the carotid artery by using a ham pumping device. The activities of cathepsin B, H, L, CDP-I, CDP-II, and CDP-inhibitor
were determined at 24 hr postmortem. The results showed:
(1) No treatment effect was observed on the activities of Cathepsin B, H, and L. (2) The control, LFES and LFES + H2O treatments had similar CDP-I, CDP-II and CDP-inhibitor activities. (3) Infusion of lamb carcasses with CaCl2 caused a decrease in CDP-I, CDP-II and CDP-inhibitor activities. Because the postmortem tenderization is Ca2+ - mediated, the carcasses infused with CaCl2 had an autolysis effect on CDP-I, CDP-II and the CDP-inhibitor. Hence, it was concluded that the activation of Ca2+ -dependent proteases was responsible for the postmortem proteolysis and tenderization.

2.4. Electrical Stimulation Related to Postmortem Aging

Ducastaing, et al. (1985) reported that electrical stimulation (ES) enhances the aging process of meat during postmortem storage. Several mechanisms may be involved to explain this effect. Cross, (1979) indicated that high voltage (500 V) ES applied to beef and lamb carcasses resulted in a rapid drop in pH while the carcass temperature was still high and the cellular disruption caused violent contractions. These effects caused the rupture of the lysosomes and the release of lysosomal enzymes
which may break the interlaced proteins and tenderize the muscle. One theory proposed for improved tenderness by using ES is that ES may increase the release of calcium from the sarcoplasmic reticulum and hydrolysis of ATP. The combination of these two factors may increase the intracellular Ca++ ion concentration in the muscle cell. The calcium ion in the cell could activate the CDP-II which is thought to cause partial degradation of myofibrils by removing a actinin from the Z-line with the concomitant hydrolysis of troponin-T, troponin-I, C-protein, tropomyosin, connectin and desmin. The activity of CDP-I was also affected by the increase of calcium ions (Dutson and Pearson, 1984).

Ducastaing et al. (1985) studied the effects of ES on postmortem changes in the activities of CDP-I, CDP-II and their inhibitors in beef Longissimus dorsi muscles. The research results indicated (1) After ES treatment, the activities of CDP-I and inhibitors were greatly decreased, while the CDP-II was only slightly changed. (2) The lower level of intracellular calcium in the muscle cell would suggest that CDP-I is the most possible cause of postmortem changes in myofibrillar proteins. (3) The effect of most of the CDP-I actually occurred at the prerigor stage, because its activity was tremendously decreased during the rigor mortis process.
2.5. Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE)

The technique of polyacrylamide gel electrophoresis in sodium dodecyl sulfate (SDS) was utilized to study the changes of myofibrillar proteins as a means of analyzing the molecular weight, and the composition and purity of these proteins during postmortem storage (Porzio and Pearson, 1977). It appeared that specific alterations in myofibrillar proteins were closely related to postmortem tenderization of meat. Proteolysis in postmortem muscle has been considered to be one of the possible mechanisms by which postmortem tenderization occurs (Penny, 1980).

Pearson et al. (1983) studied proteolysis in postmortem muscle following storage of rabbit muscle myofibrils at 4°C for 48 hr before solubilization in SDS. They found extra protein bands, when myofibrils were solubilized in SDS. The appearance of degradation products was due to proteolysis by indigenous muscle proteases.

Ishiura et al. (1979) reported that the various purified myofibrilar proteins such as myosin, troponin-I, tropomysin, α-actinin, and C-protein can be digested by CDPs. The CDP-II may be active immediately postmortem when the pH is
approximate 7.0 to 7.2 and carcass temperature is high. As the pH drops to 5.4-5.6, due to glycogen being metabolized to lactic acid through glycolysis, the CDP-I would become active and continue to digest the myofibrils during postmortem tenderization.

2.6. Myofibril Fragmentation Index (MFI)

Myofibril fragmentation index (MFI) has been used as a method of determining the tenderness of meat. Due to weakening of the Z-disks, the myofibrils of aged meat break into shorter segments upon homogenization and allows more fragments to pass through the cheese cloth when filtered than unaged meat. MFI is measured by a spectrophotometric method. The light absorbance of a myofibril suspension which reflects the extent of myofibril fragmentation and indicates the degree of fragmentation (Moeller et al. 1973; Parrish et al. 1973; and Olson et al. 1976).

MFI correlates significantly with shear force values of aged meat. Parrish et al., (1973) studied that the effect of postmortem conditions on chemical, morphological and organoleptic properties of bovine muscle. They reported that the sarcomere length was longer for meat aged at 16°C for 3
days than those aged at 2°C for 3 days. The length of the sarcomere was highly correlated with the results obtained from the Warner-Bratzler shear. Moller et al. (1973) used MFI as an index of tenderness in bovine *Longissimus dorsi*. They reported that the correlation coefficient between the absorbance value of a myofibrillar suspension and beef tenderness utilizing the Warner-Bratzler shear was 0.78. Moreover, Olson et al. (1976) investigated the correlation between MFI and shear resistance of three bovine muscles during postmortem storage. They concluded that the increase in myofibrillar fragmentation index corresponded to a decrease in Warner-Bratzler shear force values.

2.7. Biochemical Changes in Muscle Postmortem

2.7.1. ATP depletion and glycolysis

Glycogen is stored in animal tissue and has a major influence on the behavior of postmortem tissue. In postmortem muscle there is no blood circulation to supply oxygen to the tissue or to remove waste products from the muscle. Early postmortem, a small amount of ATP is regenerated by the conversion of creatine phosphate to creatine and the transfer of its phosphate to ADP. After the creatine phosphate has been used, anaerobic glycolysis continues to regenerate small
quantities of ATP. During glycolysis, glycogen is converted to available energy by the Emden-Meyerhoff pathway through a series of phosphorylated six-carbon and three-carbon intermediates to pyruvate, which is then reduced to lactate. Finally, when glycogen is exhausted, and ATP is depleted, lactic acid is produced which lowers the tissue pH in 24 hr postmortem or less. In addition, in the living muscle cells, ATP and ADP serve as plasticizers for actin and myosin to prevent interaction of these two proteins. When ATP and ADP are depleted in postmortem tissue, actin and myosin interact and the muscle passes into "rigor mortis." (Hultin, 1985).

2.7.2. Increase in tenderness on postmortem aging

In the rigor mortis stage, muscle tissue becomes tough due to the interaction of actin and myosin to form actomyosin. Conditioning could alleviate the toughness if the carcasses were held at an appropriate temperature of approximately 15°C for 16 to 24 hr., until the development of rigor mortis was near completion. There is noted during postmortem aging an increase in tenderness and flavor, which results from a breakdown of proteins to peptides and amino acids (Hultin, 1985). This postmortem tenderization process caused structural alterations to occur in the myofibril: (1) the degradation of troponin and tropomyosin; and (2)
disintegration of the Z-disk. Two proteolytic systems of Ca\(^{2+}\)-dependent proteases and catheptic enzymes are related to postmortem tenderization and are described section 2.1., 2.2., 2.3., and 2.4.

2.7.3. ATP breakdown to hypoxanthine

In muscle postmortem, ATP breaks down and is converted to hypoxanthine. Many 5'-mononucleotides, intermediates in the production of hypoxanthine, are also important enhancers in meat products. For example, IMP (inosinic acid) has flavor-enhancing properties, the ribose component must be phosphorylated at position 5 and the purine component must contain a hydroxyl group at position 6. ATP is first converted to ADP and then to AMP by the disproportionation reaction. AMP is then deaminated to IMP, a flavor enhancer. IMP, however, can degrade to inosine and eventually to hypoxanthine. Hypoxanthine has been reported to be related to meat flavor (Hultin, 1985).

2.7.4. Changes in lipid postmortem

Important change occurring in the lipid components of muscle tissue postmortem were mediated by lipid oxidation. The fatty acids of meat lipids contain highly unsaturated fatty acids which would be expected to undergo oxidation following
hydrolysis with lipases and phospholipases. These oxidation products are associated with the development of aged ham flavor as well as off-odors (Hultin, 1985).

2.8. Curing Ingredients and Their Role in Cured Meat Flavor

Kramlich et al. (1973) reported that most country-style hams use the dry curing method which is applied without any added water. The cure is solubilized by the original moisture present in the meat products. The curing ingredients consist of salt, and nitrite, or nitrate, or may be a mixture of nitrite and nitrate. Sugar or other sweetening agents may also be used in the cure.

2.8.1. Role of sodium chloride

Sodium chloride is the most important constituent in the curing mixture. The major reasons salt is applied to meat products are (1) as a preservative agent due to the increase in osmotic pressure of the meat and lowering of the water activity which inhibits certain microorganisms that cause putrefaction; (2) contributes to a desirable flavor due to the interaction of salt with the lean and/or fatty tissues to produce desirable flavor components (Bard and Townsend, 1978).
Blumer (1954), Craig et al. (1964), and Kelly (1965) indicated that development of aged flavor in country-style hams results from a number of factors including increase in salt concentration and levels of free amino acids, free fatty acids, non-protein nitrogen, and water-soluble organic acids. However, salt also promotes the development of rancidity of fat and produces an undesirable dark-colored lean resulting in oxidation of the pigment myoglobin that is objectionable to some consumers (Greene and Price, 1975).

2.8.2. Role of sugar and/or other sweetening agents.

Sugar and/or corn syrup solids are added to some meat products. The primary functions of sugar are to impart sweet flavor to the cured meat, to counteract the harsh hardening effects of salt by preventing excessive removal of moisture and by directly moderating the flavor (Kramlich et al., 1973). In the production of fermented sausages, sugars provide nutrients for desirable flavor-producing bacteria. For example, small amounts of dextrose may be added to fermented sausages to accelerate the fermentation process (Pearson and Tauber, 1984). Sugar also contributes to the browning reaction. The browning products are developed during smoking and cooking and impart flavor to cured meats. Sugar can improve the color of cured meats by establishing reducing conditions.
reducing conditions aid in converting the nitrite to nitric oxide, which reacts with the myoglobin, to produce the bright pink pigment, a characteristic of cured meat color (Ockerman, 1983).

2.8.3. Role of nitrite and/or nitrate

Cahill et al. (1980) summarized the important functions of nitrite as follows: (1) to stabilize color, (2) to contribute to the characteristic flavor of cured meat, (3) to inhibit the growth of a number of food poisoning and spoilage bacteria, especially of *Clostridium botulinum*, and (4) to retard development of rancidity. Nitrite is widely used in meat products, because it reacts quicker and less time is required for color fixation and stabilization than when nitrate is used. Nitrite is reduced to nitric oxide which actively combines with myoglobin to form nitric oxide myoglobin. During heating and/or smoking, a stable and typical cured pink pigment, nitrosomyochrome, is formed. According to USDA meat inspection regulations, nitrate is allowed in dry-cured and long-term aged hams. Tarr (1944) indicated that nitrites at pH values below 7.0 can effectively retard the growth of *C. botulinum* and *C. sporogenes* and a number of other kinds of bacteria associated with meat spoilage. Nitrite retards the rate of phospholipid oxidation by chelating trace metals which may serve as catalysts for lipid oxidation. Moreover, Eakes et al. (1975) indicated that nitrite
imparted greater firmness to country-style cured hams.

2.9. Chemical Compounds Related to Flavor in Dry-cured and Aged Hams

Meat flavor is a complex stimulus involving such characteristics as odor, taste, texture, and temperature (Lawrie, 1979). Aging is an essential step in the production of country-style hams. There is an increase in degradation products of protein and fat including free fatty acids, free amino acids, and carbonyls. Amino acids and fatty acids are known to contribute to flavor of country cured hams either directly or as flavor precursors (Blumer, 1954; Ockerman et al., 1964; Eakes et al., 1975). The flavors produced during long-term aging are quite different than those of other cured products such as hams and bacons. They are discussed in the following sections:

2.9.1. Free amino acids

Fu et al. (1980) reported that the quantity of free amino acids increased with the increase of aging time. Among free amino acids lysine, glutamate, alanine, valine and leucine showed the greatest amount of increase. No remarkable changes in the pattern of essential amino acids were observed
during aging.

Hsieh et al. (1980) in their model system compared an autoclaved mixture of simple sugars, amino acids, 5'-nucleotidases, glucoprotein, monosodium glutamate, and salt with fat as an optional component. Results demonstrated that the sulfur-containing amino acids and simple sugars interacted during heating to produce meaty aromas and/or flavors, whereas, the other components either mask the harsh sulfur-like taste or enhanced the meat flavor. They concluded that sulfur-containing amino acids, tyrosine, histidine and perhaps other amino acids contributed to the meaty aroma of the heated mixture. Schrodter and Wolm (1980) have observed that glutamic acid, aspartic acid, arginine and proline contributed to optimization of the meaty aroma during the heating of a model meat system. One source of aroma in sugar-amino acid reactions is the production of aldehydes through Strecker degradations. Amino acid breakdown products also contribute to the formation of meaty flavors.

2.9.2. Free fatty acids

Moody (1983) found the basic meaty flavor residues in the water-soluble fraction and showed that it was essentially the same for all species, whereas the characteristics species flavor
and aroma appeared to arise from the lipids. He also concluded that fats can influence meat flavor in two ways; (1) by providing a substrate for oxidation, with the formation of carbonyl compounds in organoleptically significant amounts and (2) by serving as a depot of fat-soluble compounds that volatilize upon heating and strongly affect flavor.

Fu, et al. (1980) indicated that free fatty acids increased while triglyceride decreased during the aging period. Diglyceride and monoglyceride increased first, then decreased. All the free fatty acids except linoleic acid increased with the increased aging.

2.9.3. Total Nucleotides and Total Nucleosides

Cantoni et al., (1967) stated that the major nucleotide present initially in sausage is inosinic acid (IMP) which is formed by deamination of adenylic acid, soon after rigor mortis. During ripening, phosphomonoesterase and nucleosidase activity produce inosine nucleoside and hypoxanthine, respectively, from inosinic acid. Batzer et al. (1962) found that meat flavors are water soluble and converted to flavor compounds during cooking of the meat. In addition to glucose and inosinic acid (IMP) was necessary for development of meaty flavor in pork. Nucleotides and
nucleosides are also potential precursors of free ribose and ribose phosphate, which have been implicated in the Maillard browning reactions.

2.10. Volatile Compounds Related to Flavor in Dry-cured and Aged Hams

A critical step to understand what contributes to flavor volatiles in country cured hams is to examine the chemical nature of the volatile that produce characteristic flavor responses in these products. The flavor compounds of ham are measured at parts per million (ppm) to parts per billion (ppb) levels. Techniques of analysis of flavor components include sample preparation, isolation, concentration, separation, and identification of the volatile compounds.

Ockerman et al. (1964) employed vacuum distillation (at 60°C under a vacuum of 200 µ for 24 hr) to isolate the volatile compounds from the dry cured hams. After distillation, the volatiles were concentrated and collected in a series of cold traps (ice-salt, dry ice-methanol and liquid nitrogen). In addition, the carbonyl compounds were isolated by 2,4-dinitrophenyldrazones reaction column, followed by separation by gas chromatography.
Infrared spectroscopy is one of the best means of identifying functional groups as well as for fingerprinting the molecule. Ockerman et al. (1964) tentatively identified by gas chromatography retention times and further verification of the compounds made by infrared spectroscopy. These volatiles were found in country ham as follows: formaldehyde, acetaldehyde, propionaldehyde, isobutyraldehyde, n-valeraldehyde, iosvaleraldehyde, acetone, diacetyl, methyl ethyl ketone, formic acid, acetic acid, propionic acid, butyric acid, and isocaproic acid.

Recently dynamic head space analysis (DHA) method, by using gas chromatography (GC) and mass spectrometry (MS), have been used extensively for monitoring changes in aroma volatiles during meat processing and storage. The DHA technique includes (1) sweeping the volatiles over the meat products with a stream of purified carrier gas such as helium; (2) condensing the volatiles in a suitable liquid nitrogen-cooled trap; (3) volatilizing the compounds by heat into a gas chromatograph (GC) column; (4) after separation of the flavor volatiles by GC-MS, the spectra are fed into a forward spectra-search computer program containing the spectra of a large number of volatile compounds; (5) the computer matches the spectral pattern for the unknown compounds with that of the cataloged compounds by using relative correlations for each
Chang and Peterson (1977) used GC-MS techniques and identified a large number of volatile compounds in meat products. These compounds consisted of lactones, acyclic sulfur-containing compounds (mercaptans and sulfides), nonaromatic heterocyclic compounds containing sulfur, nitrogen, and oxygen (e.g., hydrofuranoids), and aromatic heterocyclic compounds containing sulfur, nitrogen, and oxygen (pyrazines and thiophenes) which are probably the main contributors to meat flavor.
CHAPTER III

METHODS AND MATERIALS

3.1. Objectives

The first experiment was designed to investigate the activity of calcium-dependent proteases in Chinese ham. The ham was subjected to electrical stimulation and aged for six months. Enzyme activities were examined using a constant level of curing ingredients and electrical stimulation (electrical stimulation vs non-electrical stimulation), and different aging times (0, 2, 4, and 6 months). In addition, the fresh ham which is not subjected to the dry-curing and aging process, was used as a control treatment.

The objective of the first experiment was to examine the effect of electrical stimulation and different aging periods on:

(1) the enzyme activity of the low-calcium-requiring calcium-dependent protease (CDP-I);
(2) the separation of proteins by using the sodium dodecyl
sulfate polyacrylamide gel electrophoresis (SDS-PAGE); (3) the concentration of protein by utilizing the Biuret method; (4) the determination of myofibril fragmentation index by using a spectrophotometer.

Moreover, the second experiment was designed to investigate the biochemical changes which contribute to the flavor characteristics of Chinese style dry-cured and aged ham. Hams received the same treatments described in previous section, and were subjected to electrical stimulation and aging for 6 months. Variables examined included electrical stimulation and aging time (0, 2, 4, and 6 months). Fresh ham not subjected to the dry curing process was used as the control.

The second experiment focuses on the investigation of biochemical changes which relate to flavor characteristics of Chinese hams. The treatments of electrical stimulation and aging periods are the same as those in the first experiment.

The objectives of the second experiment were to evaluate
the influence of electrical stimulation and different aging time on:

(1) the changes in proximate analysis which consists of the moisture content, crude fat, crude protein, crude ash and sodium chloride;

(2) the quantitative determination of non-protein nitrogen, free amino acids, total nucleotide and total nucleosides, total free fatty acids, carbonyl compounds, and sodium nitrite residue;

(3) the oxidation of fatty acid by examining the T.B.A. value;

(4) analysis of volatile compounds using a dynamic headspace procedure;

(5) sensory characteristics including color uniformity, cohesiveness texture, ham flavor intensity, tenderness and overall acceptability.

3.2. Experimental Procedures

3.2.1. Slaughter and electrical stimulation treatment

For the first experiment, a total of five 95±3 kg crossbred
(Yorkshire, Duroc, Hampshire) pigs of similar genetic and feeding background were utilized. They were conventionally slaughtered at The Ohio State University Meat Laboratory. The investigation of Chinese ham was carried out by studying the effect of electrical stimulation on enzyme activity during the aging period. The schematic diagram of the experimental design is shown in Figure 1. The experiment was replicated five times.

The right sides and left sides of the pork carcasses, were randomly selected within 30 min post-mortem, to receive the electrically stimulated (ES) and non-electrically stimulated (NES) treatments. Each electrically stimulated side received 15 electrical impulses of 90 V alternating current of 1.5 sec duration followed by 1.5 sec of no current with a low voltage JASEC Electrical Stimulator. The opposite side of the carcass was used for a control and was non-electrically stimulated (NES). The hams of both sides of the carcasses were removed immediately after stimulation for the stimulated side and at the same time period for the non-stimulated side.

The ham, based on the length of femur bone, was marked around the skin into five nearly equal parts. Before dry curing, the first part of the ham, was cross cut through the femur bone, was assigned as a control treatment of fresh
Examining items (after completion of each aging period):

1. enzyme activity of CDP-I;
2. separation of proteins by utilizing SDS-PAGE gel electrophoresis;
3. determination of protein concentration by using Biuret method;
4. determination of myofibril fragmentation index by utilizing a spectrophotometer.

Figure 1. The schematic diagram of the experimental design for proteolytic activity.
ham. The fresh ham was dipped in liquid nitrogen, vacuum packaged and kept frozen (-70 °C). The remaining four parts of the ham were dry-cured to manufacture the Chinese ham and assigned to different aging periods (0, 2, 4, and 6 months).

3.2.2. Manufacture of Chinese dry-cured and aged hams

Specific processing procedures for producing Chinese-style ham were followed according to the procedures of Haung (1985). The hams, after electrical stimulation treatment, were chilled in the cooler (2±1°C) for 24 hr until the internal temperature of the ham reached approximately 3°C and the pH of ham was approximately 5.5 - 5.8. The hams were then rubbed with the curing mixture at three intervals (the first time was immediately after cutting from the carcass, the second time was on the seventh day and the third time was on the seventeenth day. The curing mixture contained 2.5% salt, 0.3% sugar, 0.02% sodium nitrate, and 0.01% sodium nitrite based on the weight of the ham. The hams were placed on a shelf in a cooler for 40 days of curing at a temperature of 2.5 -4.5 °C and a relative humidity of 70-90%. After curing, the hams were rinsed in cold water to wash off the excess cure and put into an equalization room (temperature 5 - 10°C
and relative humidity 65-75%) for 20 days to allow for salt equalization. During the 20 days, the salt become more uniformly distributed throughout the ham. Most Chinese-style cured hams are not smoked. After cure and salt equalization, hams were hung in the aging room (temperature 18-21°C and relative humidity 50-60%). At specific aging time (0, 2, 4, and 6 months), each designated part was cross cut through the femur bone and vacuum packaged and held at -20°C until biochemical, physical and sensory analyses were performed. The frozen parts were tempered at -2 °C for 12 hr prior to analysis. Each part of the ham was separated individually into lean meat and fat and trimmed free of connective tissues.

Furthermore, a schematic diagram of the second experimental design is shown in Figure 2. A total of five, 95±3 kg, crossbred pigs were conventionally slaughtered at The Ohio State University Meat Laboratory. Within 30 min. postmortem, the pork carcasses received electrical stimulation (ES) and non-electrical stimulation (NES) treatments which were described in previous section (3.2.1). The ham, based on the length of femur bone, were divided into five nearly equal parts and assigned as fresh ham, ham
Examining items (after completion of each aging period):
(1) changes of proximate analysis and sodium chloride;
(2) determinations of non-protein nitrogen, free amino acids, total nucleotide and nucleosides, total free fatty acids, carbonyl compounds and sodium nitrite residue.
(3) oxidation of fatty acid by examining T.B.A. value.
(4) volatile compounds analyzed by using dynamic head space; and sensory panel evaluation.

Figure 2. The schematic diagram of the experimental design for examining biochemical changes and sensory parameters related to flavor characteristics of Chinese hams
aged 0, 2, 4, and 6 months. The fresh ham served as a control treatment and was removed immediately after electrical stimulation and dipped in liquid nitrogen, vacuum packaged and kept frozen (-70°C).

The remaining hams were chilled at 2±1 for 24 hr then processed onto Chinese dry-cured and aged hams. The processing procedures were described in previous section (3.2.2). At specific aging times (0, 2, 4, and 6 months), sections were cross cut through the femur bone, vacuum packaged and held at -20°C until biochemical and physical analyses were performed and sensory parameters were evaluated. Prior to analysis, hams were removed from the freezer and tempered (-2°C) for 12 hr. Ham sections were separated individually into lean meat and fat and trimmed free of connective tissue.

3.3. Preparation of Ham Samples for Analyzing
1. For considering the differences in moisture content of fresh hams and Chinese-style hams with different treatments at aging times (0, 2, 4, and 6 months) and with electrical stimulation (or non-electrical stimulation), the meat sample moisture content must be adjusted to an equal dry matter.
2. Triplicate 10-g of 1.27-cm cores were randomly taken from
the ham's four major muscles (rectus femoris, biceps femoris, semimembranosus, semitendinosus) of fresh and Chinese style hams.

3. The ham cores were finely knife-minced, visible amounts of fat and connective tissue were removed and then freeze-dried (temperature at -40°C and vacuum at 50 microns) for two days.

4. After freeze-drying the meat samples, the dry matter of the hams was calculated: the fresh ham, and hams aged 0 month, 2 months, 4 months and 6 months as 2.35-g, 1.63-g, 1.38-g, 1.12-g and 1.00-g, respectively. There was no difference between the treatment of electrical stimulation (ES) and non-electrical stimulation (NES).

5. Based on the dry matter of hams, 100-g of fresh ham, and 69.36-g, 58.72-g, 47.66-g, and 42.55-g of ham samples which were aged 0, 2, 4, and 6 months, respectively, were used for assaying the activities of calcium-dependent proteases.

6. In addition, 11.75-g of fresh ham, and 8.15-g, 6.90-g, 5.60-g, and 5.00-g of ham samples which were aged 0, 2, 4, and 6 months, respectively were used for determining the protein concentration, sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), and myofibril fragmentation index.
3.4. Determination of pH Value

1. For determining the pH of pork carcass as affected by electrical stimulation, samples were taken from the semimembranosus muscle at a 4-6 cm distance from the end of aitch bone at 0, 0.5, 4, 8, 12, 16, 20, and 24 hr post-mortem.
2. The pH was determined by using a Expandomatic pH meter (Beckman Expandomatic Model SS-2). The meter was standardized before each measurement using a pH 5 and pH 7 buffer solution.
3. A ten gram sample was blended with 100 ml of distilled water for two min for the pH determination.
4. The pH determination of aged hams (0, 2, 4, and 6 months) samples was randomly taken from the ham's four major muscles (rectus femoris, biceps femoris, semimembranosus, semitendinosus).
5. The ham cores were finely knife-minced and mixed well.
6. A ten gram sample was used for pH measurement following the previous procedures.

3.5. Determination of Calcium Dependent Protease Activities

3.5.1. Preparation of calcium-dependent proteases
1. Low-calcium-requiring calcium-dependent protease (CDP-I) was isolated from 100-g fresh ham and Chinese style hams (69.36-g for aged 0 month 58.72-g for aged 2 months, 47.66-g for aged 4 months, and 42.55-g for aged 6 months; based on the same freeze-dried matter) which was treated with ES or NES.
2. The muscles were taken to a cold (2-4°C) room, trimmed of fat and connective tissue, and ground immediately in a prechilled meat grinder.
3. The ground muscle was suspended in 2.5 volumes (vol/weight) of extracting buffer, consisting of 100 mM Tris-base, 10 mM 2-mercaptoethanol, 5 mM EDTA and the pH was adjusted to 8.3 with 1M HCl.
4. The suspended muscles were then homogenized three times for 30 sec each in a Waring Blendor at low speed setting (3,000 rpm) and once at a high speed setting (7,500 rpm) for 30 sec with a 30-sec cooling period interposed between each blending.
5. After one hr's extraction with gentle shaking (five times back and forth per min; Dubnoff Metabolic Shanking Incubator, Precison Scientific Co. Chicago IL), the homogenate was centrifugated at 30,000Gmax for 50 min. The supernatant was filtered through one sandwich (cheese cloth / glass wool / cheese cloth) and its pH was adjusted to 7.5
with 1 M HCl followed by centrifugation at $48,000 G_{\text{max}}$ for 90 min.

6. The supernatant of the $48,000 G_{\text{max}}$ was filtered through glass wool and was passed through a PB-10 desalting column (Bio-Rad Laboratories, Hercules, CA) to remove salt from the supernatant.

7. The supernatant was applied to a DEAE-Sephacel column (Phamacia Fine Chemicals AB, Uppsala, Sweden) that had been equilibrated with elution buffer consisting of 20 mM Tris-Base, 5 mM EDTA, 10 mM 2-mercaptoethanol, and the pH adjusted to 7.5 with 1M HCl.

8. The column was washed with the same buffer to remove unbound protein until absorbance of the outflow at 278 nm was below 0.2.

9. The bound proteins were then eluted with an increasing gradient of elution buffer (20 mM Tris-Base, 5 mM EDTA, 10 mM 2-mercaptoethanol, and the pH adjusted to 7.5 with 1M HCl) consisting of 25 mM NaCl added to the same elution buffer consisting of 350 mM NaCl (250 ml of each).

10. The flow rate of the elution was 30 ml/hr controlled by a Masterflex Easy-Load Pump (Cole-Parmer Instrument Co., Chicago, IL) and 140 fractions (3.0 ml) were collected from the column.
3.5.2. Assay of CDP-I activity

1. After dissociation by the DEAE Sephadex column, the proteolytic activities of the CDP-I were measured.
2. The CDP-I was assayed by taking a 1-ml aliquot from 56th to 86th fractions. Then 1 ml of Ca\(^{2+}\) assay media was added which consisted of 7 mg/ml Casein-Hammersten (United State Biochemical Corp. Cleveland OH) as a substrate in 100 mM Tris-Base, 1 mM NaN\(_3\), 100 \(\mu\)M CaCl\(_2\), pH adjusted to 7.5 with 1 M acetic acid. Then, 100 \(\mu\)l of 100 mM CaCl\(_2\) was added and the mixture incubated at 25°C for 60 min.
3. A control sample contained 1.0 ml of sample and 1.0 ml of EDTA assay media which consisted of 7 mg/ml Casein-Hammersten, 100 mM Tris-Base, 1 mM NaN\(_3\), 10 mM EDTA, pH adjusted to 7.5 with 1 M acetic acid, was incubated at 25°C for 60 min.
4. The reaction was subsequently stopped by adding 2 ml of 5% of trichloroacetic acid.
5. A sample blank contained 1.0 ml of sample and 1.0 ml of Ca\(^{2+}\) assay media, but 2 ml of 5% of trichloroacetic acid was added before the enzyme was incubated at 25°C for 60 min.
6. After centrifugation at 35,000G\(_{max}\) for 10 min, the supernatant was read at 278 nm wavelength (A\(_{278}\)) using a spectrophotometer.
7. The activity of CDP-I was calculated according to the
following formula:

$$\text{CDP-I activity} = [A_{278} \text{ in Ca}^{2+} \text{ assay}] - [A_{278} \text{ in EDTA assay}].$$

8. The total activity was determined as total absorbance units at 278 nm per 100-g of muscle.

3.6. **Determination of Protein Concentration by Biuret Method**

Protein concentrations were determined by using the biuret method (Gornall et al., 1949). This method was modified by using crude porcine protein instead of using bovine serum albumin to plot the protein standard curve.

3.6.1. **Preparation of crude porcine protein**

1. Ten g of fresh ham was taken from the pork carcass immediately after slaughter. All preparation procedures of crude porcine protein were conducted at 4°C.
2. Myofibrils were isolated from meat samples by homogenizing with 40 ml of chilled (2°C) isolating medium containing 100 mM KCl, 50 mM Tris-HCl and 5 mM EDTA which was then adjusted to a pH of 7.6 with 1.0 M NaOH.
3. The suspended muscles were then homogenized in a polytron homogenizer (Brinkman Instruments, Rexdale, Ont.).
Canada) using two bursts of homogenization, once for 30 sec at the low speed (2,500 rpm) and once for 30 sec at the high speed with (6,000 rpm) a 30 sec cooling period interposed between the bursts.

4. The homogenate was centrifuged (used rotor type SS-34, Sorvall Super-speed RC2-B, IVAN SORVALL Inc., Newrown, Connecticut) at 2,000 G\text{max} for 20 min and the supernatant decanted.

5. The sedimented myofibrils were washed by suspending in 40 ml of 100 mM NaCl and centrifuged at 2,000 G\text{max} for 20 min.

6. The sediment was resuspended in 40 ml of the washing solution and again centrifuged at the same speed and for the same time. Additional washing procedures were repeated twice.

7. Finally, the sedimented myofibrils were dissolved by adding 4 ml of dissolving solution which contained 8 M urea, 2.5% SDS, 5 mM EDTA, 5 mM dithothreitol, 100 mM Tris/glycine (pH 8.8) and 10 mM 2-mercaptoethanol.

8. After adding 10 ml of distilled water, the suspension was well mixed and centrifuged at 48,000 G\text{max} for 20 min.

9. The supernatant was obtained and called crude porcine protein.
3.6.2. **Plotting protein standard curve**

1. The crude porcine protein is used for preparing a standard curve by pipetting 1 ml of various levels of the protein standard containing 0.0, 2.5, 5.0, 7.5, and 10 mg of crude porcine protein / ml distilled water in 10 ml test tube.
2. Adjust the total volume of liquid in each tube to 1.0 ml by adding an appropriate amount of distilled water.
3. Add 4 ml of the biuret reagent (Laboratory store of The Ohio State University) to each tube and vortex the mixture for 2-3 sec and then incubate all tubes for 20 min at 37°C.
4. Adjust wavelength to 540 nm and standardize Hitachi Perkin-Elmer Spectrophotometer (UV-VIS Model 139) with a blank containing 1 ml of distilled water / dissolving solution (2.5/1 v/v) and 4 ml of the Biuret reagent.
5. The absorbance value of each sample with known concentration of crude porcine protein was determined at a wavelength of 540 nm.
6. The standard curve was plotted by using absorbance value on the Y-axis and protein content on the X-axis.

3.6.3. **Determination of protein concentration of meat samples**

1. The preparation of meat samples was accomplished according to the procedures previously described (section 3.3.)
preparation of meat samples).

2. The crude porcine protein of meat samples were extracted by following the previous procedures (section 3.6.2. from procedure 2 to procedure 9) and assayed by using the Biuret method.

3. The protein concentration in an unknown sample can be determined by comparing absorbance reading with the protein standard curve.

3.7. Determination of Myofibril Fragmentation Index (MFI)

MFI was determined by a modification of the procedure of Culler et al. (1978).

1. The preparation of meat samples was accomplished according to the previously described procedure (section 3.3. preparation of meat samples).

2. Myofibrils were isolated from muscle by homogenizing the meat samples in a Waring Blender (at high speed position approximately 7,500 rpm) for 30 sec in 40 ml of 2°C isolating solution consisting of 100 mM KCl, 20 mM KH$_2$PO$_4$, and 1mM EDTA.

3. The homogenate was centrifuged (rotor type SS-34, Sorvall Superspeed RC2-B, IVAN SORVALL Inc., Newtown, Connecticut) at 1,500 G$_{\text{max}}$ for 20 min and then the
supernatant was decanted.
4. The sediment was resuspended in 30 ml of isolating solution and passed through a polyethylene strainer (18 mesh) to remove connective tissue and debris.
5. An additional 20 ml of isolating solution were used to facilitate passage of myofibrils through the strainer. Again, the suspension was sedimented at 1,500 Gmax for 20 min and the supernatant decanted.
6. The sediments were washed three more times by suspending in 40 ml of the isolating solution and centrifuging at 1,500 Gmax for 20 min.
7. Finally, the sedimented myofibrils were resuspended in 40 ml of the isolating solution and the protein concentration determined by the biuret procedure of Gornall et al. (1949).
8. An aliquot of the myofibril suspension was diluted with isolating solution to a protein concentration of 0.5±0.05 mg/ml.
9. The diluted myofibril suspension was stirred and poured into a cuvette; absorbance of this suspension was measured immediately by a Hitachi Perkin-Elmer Spectrophotometer (UV-VIS Model 139) at a wavelength of 540 nm.
10. Myofibril fragmentation index was calculated as absorbance units per 0.5 mg/ml myofibril protein concentration multiplied by 200.
3.8. Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis

Myofibrils were isolated from meat samples according to the procedure described by Goll et al. (1974). Isolated myofibrils were then analyzed by the SDS-PAGE procedure described by Porzio and Pearson (1977).

1. Preparation of meat samples was accomplished according to the previously described procedure (section 3.3. preparation of meat sample).

2. The crude porcine protein of meat samples were extracted by following the previous procedures (section 3.6.2. from procedure 2 to procedure 9) and assayed by using the Biuret method and their protein contents were determined by comparing with the protein standard curve.

3. Isolated myofibrils were then analyzed by using 5 - 15% and 17 - 27% Daiichi gels. Each gel was loaded with 60 μg of protein / 15 μl.

4. SDS-PAGE Molecular Weight Standards (Bio-Rad Laboratories) containing myosin, β-galactoside, phospholase B, bovine serum albumin, ovalubin, carbonic anhydrase, soybean trypsin inhibitor and lysozyme were used.

5. A constant current (40-50 mA) was utilized for running the gel electrophoresis.

6. After electrophoresis, the gel staining procedures described by Neuhoff and Stamm (1990) Integrated Separation Systems
(ISS) was utilized which included the gel being fixed in 12\% trichloroacetic acid for one hr with gentle shaking (five times of back and forward per min), and incubated in a pretreatment solution (consisting of water, phosphoric acid, and ammonium sulfate) for one hr with gentle shaking (five times back and forward per min).

7. The gel was incubated in staining solution (consisting of water and coomassie blue) for more than 12 hr, and rinsed with distilled water.

3.9. Determination of Proximate Analysis and Sodium Chloride

1. Two hundred g of ham was trimmed of skin and cut from the femur bone.

2. The meat mass was ground three times in a prechilled meat grinder, passing through plates of 10, 6 and 3 mm.

3. Moisture was determined by the A.O.A.C. method 950.46B (1990). Ten g was used for analysis. The percentage of moisture was calculated from the weight of moisture lost during heating (100 °C for 18 hr) divided by the weight of the unheated sample.

4. The percentage of crude fat was determined by using the ether extraction method (A.O.A.C. section 960.39, 1990). Dried
ham samples, following moisture analysis, were placed in a thimble on a fat extractor for approximately 8 hr.

5. The percentage of crude protein was determined by the Kjeldahl method (A.O.A.C., section 981.10, 1990).

6. The percentage of sodium chloride in ham sample was determined by the A.O.A.C. method described in section 935.47 (1990).

3.10. Determination of Non-protein Nitrogen

Non-protein nitrogen compounds were determined by a modification of the procedures of Dierick et al. (1974).

1. Six g freeze-dried ham samples were combined with 30 ml of trichloroacetic acid (10 % w/v) and homogenized by using a Waring Blender at high speed (6,000 rpm) for 2 min.

2. The homogenate was vacuum-filtered by using 2 layers of Whatman No. 1 filter paper.

3. The residues were washed with distilled water.

4. A 20 ml sample of filtrate was used to determine non-protein nitrogen using the Kjeldahl method (A.O.A.C. Section 981.10, 1990).
3.11. Automated Analysis of Free Amino Acids

Free amino acids were determined by a modification of the procedures of Stein and Moore (1954).

1. One g of freeze-dried sample was homogenized with 25 ml of 1% picric acid by using a Waring Blender at high speed (6,000 rpm) for 2 min.
2. The homogenate was vacuum filtered through Whatman No. 1 filter paper and the residue washed with 5 ml of distilled water.
3. Filtrates were pooled and passed through Dowex 2-x8 resin. The resin was prepared by washing with HCl and then rinsed with water.
4. The wall of a chromatograph tube and the resin bed were washed with five 3 ml aliquots of 0.02 N HCl solution. The clear, colorless effluent and washings were adjusted to a volume of 50 ml.
5. Five ml of effluent was dried in a freeze-dryer (temperature at -40°C and vacuum at 50 micron), then, dissolved in 0.5 ml 0.2 N sodium citrate buffer (pH 2.2), and injected into an Amino Acid Auto Analyzer (Waters Picotag System; precolumn derivatization with phenyl isochorocyanate, analysis by reverse phase LC (C18), acetate buffer / acetonitrile mobile phase).
6. The amount of free amino acids were determined according to the Picotag Method Manual (Cohen et al., 1989).

3.12. Determination of Total Nucleotides and Nucleosides

Total nucleotides and nucleosides are determined by ion-exchange chromatography. The method was as described by Macy, et al (1970).

1. A thirty g ham sample was homogenized with 50 ml of 0.6N perchloric acid in a Waring Blender at high speed (6,000 rpm) for 2 min and filtered through Whatman No. 1 filter paper.

2. The residue was reextracted with 50 ml of 0.6N perchloric acid, filtered and washed with two 10 ml aliquots of distilled water.

3. The filtrate was neutralized with 10 ml of 30% (w/w) potassium hydroxide. Precipitated perchlorate was removed by filtration (Whatman No. 1 filter paper) and the filtrate diluted to 150 ml with distilled water.

4. One ml of ham extract was mixed with 1 g of Amberlite IRA 400 (Sigma Co.) resin for at least 15 min with intermittent stirring.
5. The mixture of extract and resin were washed into a funnel plugged with a small amount (approximately 5 g) of glass wool. The resin was washed with distilled water until a volume of 25 ml was collected.

6. The absorbency of the effluent was read at 248 and 260 nm wavelength with a Hitachi Perkin-Elmer Spectrophotometer (UV-VIS Model 139).

7. Total purine nucleosides and bases were calculated from the absorbency at 260 nm of the effluent from the resin using the molar absorptivity for hypoxanthine (7.35 X 10^3) (Jones and Murray, 1964).

8. Total nucleotides absorbed onto the resin were determined by the difference between the absorbencies at 248 nm of the resin treated samples and similar non-resin treated samples. The molar absorptivity of inosinic acid (12.25 X 10^3) (Jones and Murray, 1964) was used to calculate total nucleotides.

3.13. Determination of Total Free Fatty Acids

1. Fat cut from fresh and aged hams was finely knife-minced and then freeze-dried.

2. A 10-g sample was homogenized with 20 ml of chloroform/
methanol mixture (2/1 v/v) in a Waring blender at high speed (6,000 rpm) for 1 min.
3. The homogenate was filtered through Whatman No. 1 filter paper and the residue with filter paper was again homogenized with 20 ml of the same mixture for 1 min. and filtered through Whatman No. 1 filter paper.
4. The filtrate was pooled and dried over anhydrous Na2SO4.
5. This lipid extract was used for the determination of total fatty acids by the titration method described by Ockerman (1981).

3.14. Determination of Carbonyl Compounds

Carbonyl compounds were determined by a modification of the procedure of Boyd et al. (1965)
1. One g of fat was mixed in a mortar with 3 g of celite 545 to the consistency of a dry powdery paste and extracted with 150 ml of carbonyl-free hexane with five strokes of reciprocating shaking per min in a Dubnoff Metabolic Shaking Incubator (Precision Scientific Co., Chicago, IL) for 24 hr.
2. The mixture was filtered onto a 2,4-dinitrophenyl hydrazine (DNPH) celite column. The DNPH celite column was made by the followed procedures: 0.5 g DNPH and 6 ml 85%
H\textsubscript{3}PO\textsubscript{4} were put into a mortar and ground until the DNPH was completely dissolved. Then, 4 ml of distilled water was added, the precipitated DNPH (orange pigment) was visible, and grinding was continued until the precipitated DNPH had disappeared. Ten g of dried celite 545 was added and mixed well. The mixture of DNPH and celite 545 was poured into a chromatography tube that was plugged with a small amount (approximately 5 g) of glass wool and then 30 ml of hexane were added.

3. Then, (2 -3 ml/min.) hexane was added to wash the DNPH celite column and the effluent (500 ml) was collected. At this time, the carbonyl compounds reacted with the DNPH and became yellow pigment of 2,4-dinitrophenyl hydrazones.

4. The same volume of effluent was collected from a blank column without sample.

5. The effluent, collected from the procedure 3, was measured at 340 nm wavelength by using a Hitachi Perkin-Elmer Spectrophotometer (UV-VIS Model 139).

6. The concentration of carbonyl compounds was calculated, using a mean molar absorbency of 22,500 (Jones et al., 1956).

3.15. Determination of T.B.A. Value and Sodium Nitrite Residue
The determinations of T.B.A. and sodium nitrite residue followed the procedures described by Ockerman (1981).

3.16. Determination of Volatile Compounds by Using Dynamic Headspace Analysis (DHA)

3.16.1. Preparation of Meat Samples

1. Triplicate 10-g 1.27-cm cores were randomly taken from the ham's four major muscles (rectus femoris, biceps femoris, semimembranosus, seditendinosus) of fresh ham and Chinese style hams aged 6, 9, and 12 months.
2. The ham cores were finely knife-minced, and visible amounts of fat and connective tissue were removed.
3. The ham samples were wrapped in aluminum foil, and a thermocouple was inserted into the geometric center of the wrapped ham samples. The ham samples and thermocouple were placed in a broiler pan and placed in the oven for broiling. The heat radiated from above the meat, so that the meat must be turned once at 40°C during cooking. The meat samples were removed when they reached an internal temperature of 75°C.
4. After processing, 0.5 g of ham was weighed into 30 ml
serum bottles and sealed air-tight with Teflon-coated rubber septa and aluminum caps (Supelco Inc., Bellefonte, PA). Samples were kept in a -18°C freezer until analyzed. They were removed from the freezer and equilibrated 1 hr in a 0-5°C refrigerator before subjecting them to DHA.

3.16.2 Dynamic headspace analysis of volatile compounds

Dynamic headspace analysis (DHA) of volatile flavor compounds was done according to the procedure previously described by Laye et al. (1993).

1. The bottle containing the ham was connected to the Model LSC 2000 Purge-and-Trap Concentrator (Tekmar, Cincinnati, OH).

2. Headspace volatiles were purged 10 min. with ultra pure helium at a flow rate of 40 ml/min.

3. Volatiles were sequentially adsorbed on a Tenax TA trap (Tekmar), desorbed by flash heating, cryogenicly focused at the liquid nitrogen cooled capillary interface, and then automatically injected into the GC/MS by heating 1 min. at 180°C.

3.16.3 Gas chromatography/mass spectrometry

1. Volatile compounds were then fractionated with a gas
chromatography unit (Hewlett Packard 5890 series II) equipped with a Mass selective detector (MSD 5971A, Hewlett Packard, Palo Alto, CA).

2. Volatiles were separated with a fused silica capillary column (DB-WAX; 30 m x 0.25 mm i.d. x 0.25 mm film thickness, J&W Scientific Inc., Folsom, CA).

3. Ultrahigh purity helium was used as carrier gas at a flow rate of 1 ml/min.

4. The oven temperature was increased from 32°C to 80°C at 2°C/min., to 160°C at 5°C/min., to 220°C at 10°C/min., and maintained at 220°C for 2 min.

5. The MS ion source was maintained at 180°C throughout the analysis. Mass spectra were acquired with an ionization energy of 70 eV and within the mass range of m/z 19-300.

6. Compounds were tentatively identified by computer-matching of full or partial mass spectral data in the Hewlett Packard chemstation NIST/EPA/MSDC 49K mass Spectral Database and by matching retention index (RI) values.

7. The reproducibility for recovery of headspace volatile compounds was calculated by the % relative standard deviation (% RSD) method (Sensel & Griffiths, 1990; Ha et al., 1992).
3.17. Sensory Panel Evaluation

3.17.1. Preparation of Meat Samples

1. Two hundred g of ham samples, aged 0, 2, 4, and 6 months, were trimmed of all bone, subcutaneous fat, and epimysial connective tissue.
2. Hams were sliced within muscle cross-sections and cut into pieces (7.0 cm long, 3.0 wide and 0.5 cm thick).
3. The sliced samples were wrapped in aluminum foil, and a thermocouple was inserted into the geometric center of the wrapped meat samples.
4. The meat samples and thermocouple were placed in a broiler pan and placed in the oven for broiling. The broiling procedures are described as section 3.16.1.
5. The ham broths were prepared for triangle tests. Two hundred g of ham was trimmed as previous procedures (Section 3.16.1) and soaked in 1,000 ml cold water overnight.
6. The hams were homogenized with 500 ml of distilled water in a Waring blender at high speed (6,000 rpm) for 2 min.
7. Then, the homogenate was poured into a pot for boiling 10 min. and filtered by passing through a stainless steel strainer (18 mesh), ten drops of red food color (Kroger Co.) was added to the filtrate to mask the color differences.
3.17.2 Testing Environment

1. Sensory evaluation was conducted in the Sensory Evaluation Laboratory of Howlett Hall, The Ohio State University.
2. Panelists were served in individual booths to avoid interruption, distractions and communication during testing.
3. The environmental factors included adequate lighting, comfortable setting, comfortable room temperature, and air-conditioning.
4. The panelists were instructed to rinse their mouth with water between samples.

3.17.3 Selection of a Sensory Panelist by Using Triangle test

1. Twenty graduate students, from the Food Science and Technology and Animal Science Departments of The Ohio State University, were screened by using triangle tests.
2. In the first set, the broth of ham aged two months were alike and the different sample was represented by broth of a ham aged six months.
3. In the second set, two slices (7.0 cm long, 3.0 cm wide, and 0.5 cm thick) of ham aged two months were alike and the different sample was a slice (the same size as previous
samples; section 3.17.1) of ham aged six months.

4. For each set, sensory panelists were asked to determine which of the three samples was different.

3.17.4. Instruction of a Descriptive Panel

1. After the triangle tests, twelve taste panelists were selected and instructed according to procedures outlined by AMSA (AMSA, 1978).
2. Panelists had a clear understanding of the test procedures, the score sheets, the terminology used, and were able to precisely and consistently discriminate the sensory characteristics of cured aged hams.

3.17.5. Performance of Descriptive Evaluation

1. Ham samples (7.0 cm long, 3.0 wide and 0.5 cm thick) were prepared for sensory evaluation. The sample serving procedure was of a completely randomized design.
2. Scoring was accomplished by using a 9-point Hedonic scale (9 = extremely dark red in color, extremely intensive ham flavor, tender, fine texture, and overall acceptable; 1 = extremely light red in color, extremely bland flavor, tough, coarse texture, and overall unacceptable).
3.18. Statistical Analysis

Statistical analysis of the data was performed using two way analysis of variance (ANOVA) (SYSTAT 5.2, 1992). Mean separations were performed using t-test and correlation coefficients were determined by using Spearman correlation (Steel, and Torrie, 1984).
CHAPTER IV

RESULTS AND DISCUSSION

4.1. Changes in pH Value Affected by Electrical Stimulation

Smith et al., (1980) and Crenwelge et al., (1984) found that electrical stimulation (ES) of pork carcasses results in a paler color, less firmness and increased muscle separations. Therefore, low frequency of ES on swine carcasses was recommended (Lawlis et al. 1992).

The effect of electrical stimulation on carcasses did accelerate glycolysis. The glycogen was converted to available energy for muscle contraction. Lactic acid was produced and increased the rate of tissue pH decline. There was an approximately a 0.2 unit of pH difference (Figure 3) between the stimulated carcass and the control at 30 min postmortem.
Figure 3. The effect of electrical stimulation on postmortem pH decline of the meat sample taken from the *semimembranosus* muscle.

Footnote: ES and NES represent electrical stimulation and non-electrical stimulation, respectively.
The pH value of both treatments were the same after 20 hr postmortem. However, Ducastaing et al. (1985) indicated that a pH drop of 0.6 unit at 30 min postmortem during electrical stimulation means this treatment was efficient.

4.2. Relative Activity of Low-calcium-requiring Calcium-dependent Protease (CDP-I) during the Aging Periods

The pH value (Figure 4) of Chinese style dry-cured and aged hams increased (p<0.01) during the postmortem aging times. Demeyer and Vandekerckhove (1979) indicated that the pH increase in the late stages of curing is related to a decrease in electrolyte dissociation and/or an increase in the concentration of buffering proteins as well as proteolytic activity with the formation of ammonia. In addition, in the current study, there was significant difference (p<0.01) between the treatments of electrical stimulation and non-electrical stimulation.

Figure 5 shows that the relative activity of low-calcium-requiring calcium-dependent proteases (CDP-I) was significantly (p<0.01) affected by the ES and aging times. CDP-I
Figure 4. The effect of electrical stimulation and aging times on the changes of pH values of the Chinese style dry-cured and aged hams.

Footnote: The pH values of the Chinese style dry-cured and aged hams are significant (p<0.01) for both electrical stimulation and aging times.
Figure 5. The effect of electrical stimulation and aging times on relative activity of CDP-I of the Chinese style dry-cured and aged hams.

Footnote: The relative activities of CDP-I are significant (p<0.01) for both electrical stimulation and aging times.
could be involved in the postmortem aging process in Chinese style dry-cured and aged hams. Ducastain et al. (1985) reported that the activities of both CDP-I and CDP-II (high-calcium-requiring calcium-dependent protease) were mediated by Ca$^{2+}$ concentrations. However, considering the fact that the Ca$^{2+}$ concentration required to activate CDP-II far exceeds the low level of intracellular free Ca$^{2+}$ level. In this experiment, it seems that the CDP-I is more likely to cause most of the postmortem changes in the myofibrillar proteins. Results also indicated that the high correlation coefficient ($r = 0.95$) between relative activity of CDP-I and aged hams' pH. The higher the pH values (Figure 4), it was more likely the relative activity of CDP-I (Figure 5) was greater. The activity and relative contributions of CDP-I to the aging process may be determined by the ultimate postmortem pH value of the muscle system (Arakawa et al., 1976; Penny and Ferguson-Pryce, 1979).

Koohmaraie et al. (1986) investigated the effect of CDP-I on myofibrils during postmortem storage. They found that CDP-I retained 24% to 28% of its activity and this was sufficient to cause the myofibrillar changes related to postmortem aging at pH 5.5 to 5.8 and 5°C. Results (Figure 5) showed that CDP-I retained 27% to 40% of its activity during
ham aging (pH 6.0 to 6.15 and temperature of 18°C to 21°C), which would be a sufficient level of activity to produce the myofibrillar changes observed during aging. In addition, the presence of the curing salts in the tissue may stabilize the muscle proteolytic activities against autolysis during the dry-curing and aging processes (Toldra and Etherington, 1988).

Furthermore, Dutson and Pearson (1984) reported that during electrical stimulation (ES), the massive contractions cause the release of calcium from the sarcoplasmic reticulum. The increased intracellular Ca^{2+} ion concentration in the muscle cell may activate the CDP-I. CDP-I could degrade the myofibrillar proteins which is thought to cause postmortem tenderization. Ducastaing et al. (1985) also reported that most of the effect of ES on CDP-I could occur at the very beginning of the postmortem changes.

4.3. Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE)

SDS-polyacrylamide gel electrophoresis (SDS-PAGE) is a very sensitive method to monitor the proteolytic degradation of myofibrils isolated from skeletal muscle during postmortem aging. In this study, SDS-PAGE employed the 5 - 15% (Figure
Figure 6. The 5 - 15% SDS electrophotograms of myofibrillar protein of the Chinese style dry-cured and aged hams affected by electrical stimulation and aging periods.

Footnotes: A = BIO-RAD protein standard; B = Fresh ham, ES; C = Fresh ham, NES; D = Aged 0 mon, ES; E = Aged 0 mon, NES; F = Aged 2 mon, ES; G = Aged 2 mon, NES; H = Aged 4 mon, ES; I = Aged 4 mon, NES; J = Aged 6 mon, ES; and K = Aged 6 mon, NES.
Figure 7. The 17 - 27% SDS electrophotograms of myofibrillar protein of the Chinese style dry-cured and aged hams affected by electrical stimulation and aging periods.

Footnotes: A = Fresh ham, ES; B = Fresh ham, NES; C = BIO-RAD protein standard; D = Aged 0 mon, ES; E = Aged 0 mon, NES; F = Aged 2 mon, ES; G = Aged 2 mon, NES; H = Aged 4 mon, ES I = Aged 4 mon, NES; J = Aged 6 mon, ES; and K = Aged 6 mon, NES.
Figure 6, the electrophotogram of myofibril showed that the myosin heavy chain, C-protein, α-actinin, and tropomyosin were completely degraded during six months of postmortem aging. The degradation of these myofibrillar proteins resulted in the formation of polypeptides with molecular weight of 150,000-, 110,000-, 95,000-, 82,000-, 75,000- and 65,000-daltons. Troponin T (37,000-dalton) was also degraded and polypeptides with molecular weights of 34,000-, 30,000- and 25,000-dalton appeared. Figure 7 also showed that troponin-I and myosin light chain were completely degraded. But the actin was not affected.

Penny and Dransfield (1979) reported that degradation of myosin to polypeptides with molecular weights of 150,000-dalton and 82,000-dalton has been observed when bovine muscle is stored at 35°C. Koohmaraie et al. (1984) reported that degradation products of 110,000-, 95,000- and 30,000-dalton components gradually appeared during postmortem storage. Penny and Dransfield (1979) demonstrated that the reduction in toughness during conditioning was quantitatively related to the loss of troponin-T by measuring the intensity of the troponin T-band in gels by densitometry. There was also a good correlation ($r = 0.78$) between the extents of loss of troponin-T and toughness.
4.4. Myofibril Fragmentation Index (MFI)

Table 1. shows that myofibril fragmentation index (MFI) increased with aging time (p<0.01). The MFI has been used as a technique to determine the extent of postmortem tenderization (Parrish, et al., 1973). Because the weakening of the myofibrillar structure as a result of the Z-disk degradation of aged meat produced a higher proportion of smaller fragment on homogenization than unaged meat. MFI correlates significantly with the increase tenderness of meat cooked after aging. Thus, the MFI is an important indicator related to the changes in myofibrillar proteins and to the reduction in toughness during aging. Olson and Parrish (1977) indicated that the loss of troponin-T and the appearance of the 30,000 component in gels of myofibrils related to the reduction in toughness and increase in the MFI during the aging of beef. Sonaiya (1982) also reported that electrical stimulated meat had higher MFI than untreated meat. However, current research indicated that there was no a significant difference (p>0.05) in MFI by utilizing ES.

4.5. Protein Concentration

The biuret method is one of the most popular colorimetric assay methods for the experimental determination of protein
Table 1. The effect of electrical stimulation\(^1\) and aging times\(^2\) on the myofibril fragmentation index of Chinese style dry-cured and aged hams.

<table>
<thead>
<tr>
<th>Myofibril fragmentation index</th>
<th>0 mon</th>
<th>2 mon</th>
<th>4 mon</th>
<th>6 mon</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hams dry-cured and aged</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Electrical stimulation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Means</td>
<td>35.3(^a)</td>
<td>52.4(^b)</td>
<td>74.6(^c)</td>
<td>100.0(^d)</td>
</tr>
<tr>
<td>(Standard error)</td>
<td>(2.5)</td>
<td>(3.0)</td>
<td>(3.5)</td>
<td>(5.9)</td>
</tr>
<tr>
<td><strong>Non-electrical stimulation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Means</td>
<td>36.2(^a)</td>
<td>50.7(^b)</td>
<td>72.5(^c)</td>
<td>95.1(^d)</td>
</tr>
<tr>
<td>(Standard error)</td>
<td>(3.3)</td>
<td>(3.2)</td>
<td>(3.9)</td>
<td>(5.2)</td>
</tr>
</tbody>
</table>

Footnotes: (1) There is no difference (p>0.05) between electrical stimulation and non-electrical stimulation.

(2) Means within row with different superscript letter (a,b,c,d) are significantly different (p<0.01) aging times.
concentrations. According to Gornall et al. (1949), the reddish-violet colored complex developed involving the complexation of copper in alkaline solution with peptide bonds and tyrosine side chains. This color can be measured by using a spectrophotometer at 540 nm. The higher absorbance value represents more protein content in the sample. Bovine serum albumin (BSA) is commonly used as a calibration curve with known protein concentration to allow the protein content of the unknown samples to be determined. However, the choice of BSA as a standard protein may lead to inaccuracies, since the use of two different standard proteins (for example: BSA vs crude porcine protein) with quite different tyrosine content yields different calibration curves (Bell, 1988). Therefore, in this experiment, the more accurate result of protein concentration can be obtained by using crude porcine protein for the preparation of a standard protein curve (Figure 8). There was a correlation coefficient of 0.94 between the standard protein curve of crude porcine protein and BSA.

Table 2 indicated that the protein concentration was significantly different (p<0.01) between fresh ham and hams aged 0, 2, 4, and 6 months. From the results of the SDS-PAGE (Figures 6 and 7), it appeared that the decrease of protein concentration was due to the degradation of the myofibrillar proteins by indigenous proteases with the formation of
Figure 8. The protein standard curve calibrated by using crude porcine protein.
Table 2. The effect of electrical stimulation\(^1\) and aging times\(^2\) on the protein concentration (mg/ml) of Chinese style dry-cured and aged hams.

<table>
<thead>
<tr>
<th>Protein concentration (mg/ml)</th>
<th>Fresh ham</th>
<th>Hams dry-cured and aged</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 mon</td>
<td>2 mon</td>
</tr>
<tr>
<td><strong>Electrical stimulation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Means</td>
<td>19.4(^a)</td>
<td>16.2(^b)</td>
</tr>
<tr>
<td>(Standard error)</td>
<td>(0.9)</td>
<td>(0.8)</td>
</tr>
<tr>
<td><strong>Non-electrical stimulation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Means</td>
<td>19.4(^a)</td>
<td>16.2(^b)</td>
</tr>
<tr>
<td>(Standard error)</td>
<td>(1.1)</td>
<td>(0.7)</td>
</tr>
</tbody>
</table>

Footnotes: (1) There is no difference (p>0.05) between electrical stimulation and non-electrical stimulation.

(2) Means within row with different superscript letters (a,b,c,d,e) are significantly different (p<0.01) with aging times.
non-protein nitrogen components, such as peptides, free amino acids and ammonia.

4.6. Changes of Proximate Analysis and Salt Content during Aging Periods

Changes in proximate analysis and salt content during aging periods are presented in Table 3. Moisture content decreased (p<0.05) quickly during dry-curing, salt equalization and the first two months of aging. After aging 4 months, the moisture content remained constant. This result agreed with Fu et al. (1980), who reported that the moisture content of Chinese-style ham dropped from 52% to 34% during a 12-month aging period. An increasing quantity (p<0.05) of crude protein and crude fat was observed due to the loss of water during the six months aging. In addition, there was a difference (p<0.05) between fresh ham and aged hams for crude ash and salt content, because salt was added during the dry-curing process. However, the electrical stimulation treatment resulted in no difference (p>0.05) in proximate analysis and salt.
### Table 3. The effects of electrical stimulation\(^1\) and aging times\(^2\) on the mean proximate analysis and salt content (standard error) of fresh ham and Chinese dry-cured and aged hams

<table>
<thead>
<tr>
<th>Proximate analysis</th>
<th>Fresh ham</th>
<th>Ham dry-cured and aged</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 mon</td>
<td>2 mon</td>
</tr>
<tr>
<td><strong>Moisture (%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ES</td>
<td>71.2(^a)</td>
<td>58.7(^b)</td>
</tr>
<tr>
<td></td>
<td>(3.3)</td>
<td>(2.5)</td>
</tr>
<tr>
<td>NES</td>
<td>70.8(^a)</td>
<td>59.1(^b)</td>
</tr>
<tr>
<td></td>
<td>(3.1)</td>
<td>(2.7)</td>
</tr>
<tr>
<td><strong>Crude protein (%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ES</td>
<td>17.8(^a)</td>
<td>23.6(^b)</td>
</tr>
<tr>
<td></td>
<td>(0.8)</td>
<td>(0.7)</td>
</tr>
<tr>
<td>NES</td>
<td>17.9(^a)</td>
<td>23.9(^b)</td>
</tr>
<tr>
<td></td>
<td>(0.5)</td>
<td>(1.0)</td>
</tr>
<tr>
<td><strong>Crude fat (%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ES</td>
<td>9.6(^a)</td>
<td>10.3(^a)</td>
</tr>
<tr>
<td></td>
<td>(0.3)</td>
<td>(0.7)</td>
</tr>
<tr>
<td>NES</td>
<td>9.8(^a)</td>
<td>9.5(^a)</td>
</tr>
<tr>
<td></td>
<td>(0.5)</td>
<td>(0.8)</td>
</tr>
<tr>
<td><strong>Crude ash (%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ES</td>
<td>1.4(^a)</td>
<td>7.4(^b)</td>
</tr>
<tr>
<td></td>
<td>(0.1)</td>
<td>(0.1)</td>
</tr>
<tr>
<td>NES</td>
<td>1.5(^a)</td>
<td>7.5(^b)</td>
</tr>
<tr>
<td></td>
<td>(0.1)</td>
<td>(0.1)</td>
</tr>
<tr>
<td><strong>Salt content (%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ES</td>
<td>0.05(^a)</td>
<td>4.2(^b)</td>
</tr>
<tr>
<td></td>
<td>(0.0)</td>
<td>(0.1)</td>
</tr>
<tr>
<td>NES</td>
<td>0.06(^a)</td>
<td>4.3(^b)</td>
</tr>
<tr>
<td></td>
<td>(0.0)</td>
<td>(0.1)</td>
</tr>
</tbody>
</table>

1. There is no difference (p>0.05) between treatments of electrical stimulation (ES) and non-electrical stimulation (NES).

2. a,b,c,d - Means within row with different superscript letters are significantly different (p<0.05) with aging times.
4.7. Changes of Non-protein Nitrogen during Aging Periods

Table 4 demonstrated that the non-protein nitrogen increased (p<0.05) during aging. The progressive increase in the non-protein nitrogen may be due to the continuous action of muscle proteases, peptidases, nucleosidase system and deaminases on myofibrillar proteins (McCain et al., 1968). Lillard and Ayres (1969) reported that degradation products of myofibrillar proteins which are associated with ham flavor increased in American country-cured ham with aging.

4.8. Changes of Free Amino Acids during the Aging Periods

Table 5 indicates the changes of 18 free amino acids among fresh ham and aged hams (0, 2, 4, and 6 months) as affected by ES and aging times. Analysis of individual amino acids, data showed that for every amino acids was significant increase (p<0.01) with aging times. Neither ES nor the interaction between ES and aging times was significant (p>0.05) for any of the amino acids. A correlation was run between CDP-I activity and total amino acid content. They both increased with aging times. The correlation was significant with an $r = 0.722$ and $p = 0.008$. 
Table 4. The effect of electrical stimulation\(^1\) and aging times\(^2\) on the non-protein nitrogen (mg/g of freeze-dry lean) of Chinese style dry-cured and aged hams.

<table>
<thead>
<tr>
<th>Hams dry-cured and aged</th>
<th>Non-protein nitrogen (mg/g freeze-dry lean)</th>
<th>Fresh ham</th>
<th>0 mon</th>
<th>2 mon</th>
<th>4 mon</th>
<th>6 mon</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Electrical stimulation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Means</td>
<td></td>
<td>3.65(^a)</td>
<td>8.33(^b)</td>
<td>9.48(^b)</td>
<td>10.71(^b,c)</td>
<td>11.58(^c)</td>
</tr>
<tr>
<td>(Standard error)</td>
<td></td>
<td>(0.08)</td>
<td>(0.32)</td>
<td>(0.30)</td>
<td>(0.23)</td>
<td>(0.17)</td>
</tr>
<tr>
<td>Non-electrical stimulation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Means</td>
<td></td>
<td>3.28(^a)</td>
<td>9.36(^b)</td>
<td>9.29(^b)</td>
<td>10.51(^b,c)</td>
<td>11.97(^c)</td>
</tr>
<tr>
<td>(Standard error)</td>
<td></td>
<td>(0.05)</td>
<td>(0.31)</td>
<td>(0.26)</td>
<td>(0.30)</td>
<td>(0.39)</td>
</tr>
</tbody>
</table>

1. There is no difference (p>0.05) between electrical stimulation and non-electrical stimulation.

2. \(^a,b,c\) - means within row with different superscript letters are significantly different (p<0.05) with aging times.
Table 5. The effects of electrical stimulation\textsuperscript{1} and aging times\textsuperscript{2} on the free amino acids (μ mole/g DM) of fresh ham and Chinese dry-cured and aged hams.

<table>
<thead>
<tr>
<th>Free amino acid (μ mole/g DM)</th>
<th>Retention Time</th>
<th>Fresh ham</th>
<th>0 mon</th>
<th>2 mon</th>
<th>4 mon</th>
<th>6 mon</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspartic acid</td>
<td>1.92</td>
<td>0.37</td>
<td>0.51</td>
<td>4.46</td>
<td>10.75</td>
<td>10.72</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>2.14</td>
<td>0.49</td>
<td>1.63</td>
<td>13.96</td>
<td>23.95</td>
<td>24.68</td>
</tr>
<tr>
<td>Serine</td>
<td>3.82</td>
<td>0.42</td>
<td>1.55</td>
<td>10.72</td>
<td>13.98</td>
<td>13.60</td>
</tr>
<tr>
<td>Glycine</td>
<td>4.23</td>
<td>1.30</td>
<td>1.89</td>
<td>11.47</td>
<td>17.84</td>
<td>17.36</td>
</tr>
<tr>
<td>Histidine</td>
<td>4.88</td>
<td>0.70</td>
<td>0.38</td>
<td>4.72</td>
<td>6.98</td>
<td>7.02</td>
</tr>
<tr>
<td>Arginine</td>
<td>5.33</td>
<td>21.17</td>
<td>19.77</td>
<td>22.52</td>
<td>27.25</td>
<td>22.34</td>
</tr>
<tr>
<td>Threonine</td>
<td>5.65</td>
<td>1.19</td>
<td>2.15</td>
<td>8.58</td>
<td>13.07</td>
<td>12.52</td>
</tr>
<tr>
<td>Alanine</td>
<td>5.73</td>
<td>1.85</td>
<td>3.46</td>
<td>16.82</td>
<td>26.83</td>
<td>25.79</td>
</tr>
<tr>
<td>Proline</td>
<td>5.94</td>
<td>1.56</td>
<td>1.13</td>
<td>7.46</td>
<td>11.84</td>
<td>12.68</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>7.20</td>
<td>0.09</td>
<td>0.54</td>
<td>3.51</td>
<td>4.60</td>
<td>4.31</td>
</tr>
<tr>
<td>Valine</td>
<td>7.68</td>
<td>0.23</td>
<td>1.18</td>
<td>9.14</td>
<td>15.21</td>
<td>15.02</td>
</tr>
<tr>
<td>Methionine</td>
<td>8.10</td>
<td>0.14</td>
<td>0.45</td>
<td>2.57</td>
<td>3.92</td>
<td>3.97</td>
</tr>
<tr>
<td>Cysteine</td>
<td>8.33</td>
<td>0.16</td>
<td>0.15</td>
<td>1.09</td>
<td>1.28</td>
<td>1.54</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>9.07</td>
<td>0.16</td>
<td>0.96</td>
<td>6.14</td>
<td>9.21</td>
<td>9.35</td>
</tr>
<tr>
<td>Leucine</td>
<td>9.22</td>
<td>0.24</td>
<td>1.62</td>
<td>10.56</td>
<td>15.58</td>
<td>16.81</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>10.00</td>
<td>0.09</td>
<td>0.64</td>
<td>4.72</td>
<td>7.15</td>
<td>7.23</td>
</tr>
<tr>
<td>Trptophan</td>
<td>10.48</td>
<td>0.07</td>
<td>0.02</td>
<td>0.96</td>
<td>1.63</td>
<td>2.29</td>
</tr>
<tr>
<td>Lysine</td>
<td>11.41</td>
<td>0.31</td>
<td>1.38</td>
<td>14.30</td>
<td>23.22</td>
<td>21.65</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Ham aged</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>0 mon</td>
<td>30.5\textsuperscript{a}</td>
<td>39.4\textsuperscript{a}</td>
<td>153.7\textsuperscript{b}</td>
<td>234.3\textsuperscript{c}</td>
<td>228.9\textsuperscript{c}</td>
<td></td>
</tr>
<tr>
<td>2 mon</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 mon</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 mon</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1. There was no difference (p>0.05) between electrical stimulation (ES) and non-electrical stimulation (NES).
2. Every individual amino acids was significant (p<0.01) with aging times.
Table 5. The effects of electrical stimulation\(^1\) and aging times\(^2\) on the free amino acids (\(\mu\) mole/g DM) of fresh ham and Chinese dry-cured and aged hams.

<table>
<thead>
<tr>
<th>Free amino acid ((\mu) mole/g DM)</th>
<th>Retention Time</th>
<th>Fresh ham</th>
<th>0 mon</th>
<th>2 mon</th>
<th>4 mon</th>
<th>6 mon</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspartic acid</td>
<td>1.92</td>
<td>0.31</td>
<td>0.54</td>
<td>2.64</td>
<td>8.59</td>
<td>10.38</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>2.14</td>
<td>0.35</td>
<td>1.77</td>
<td>12.21</td>
<td>26.64</td>
<td>21.84</td>
</tr>
<tr>
<td>Serine</td>
<td>3.82</td>
<td>0.40</td>
<td>1.18</td>
<td>7.10</td>
<td>12.64</td>
<td>13.89</td>
</tr>
<tr>
<td>Glycine</td>
<td>4.23</td>
<td>1.71</td>
<td>1.78</td>
<td>9.31</td>
<td>15.55</td>
<td>17.76</td>
</tr>
<tr>
<td>Histidine</td>
<td>4.88</td>
<td>0.81</td>
<td>0.58</td>
<td>2.96</td>
<td>6.04</td>
<td>6.97</td>
</tr>
<tr>
<td>Arginine</td>
<td>5.33</td>
<td>22.92</td>
<td>19.11</td>
<td>20.54</td>
<td>20.65</td>
<td>22.56</td>
</tr>
<tr>
<td>Threonine</td>
<td>5.65</td>
<td>1.34</td>
<td>1.93</td>
<td>7.44</td>
<td>12.69</td>
<td>12.79</td>
</tr>
<tr>
<td>Alanine</td>
<td>5.73</td>
<td>2.17</td>
<td>3.27</td>
<td>15.48</td>
<td>24.23</td>
<td>26.50</td>
</tr>
<tr>
<td>Proline</td>
<td>5.94</td>
<td>1.06</td>
<td>1.07</td>
<td>6.34</td>
<td>10.61</td>
<td>12.58</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>7.20</td>
<td>0.12</td>
<td>0.52</td>
<td>3.14</td>
<td>5.41</td>
<td>4.17</td>
</tr>
<tr>
<td>Valine</td>
<td>7.68</td>
<td>0.26</td>
<td>1.02</td>
<td>8.20</td>
<td>14.04</td>
<td>15.12</td>
</tr>
<tr>
<td>Methionine</td>
<td>8.10</td>
<td>0.10</td>
<td>0.43</td>
<td>2.69</td>
<td>4.01</td>
<td>3.94</td>
</tr>
<tr>
<td>Cysteine</td>
<td>8.33</td>
<td>0.09</td>
<td>0.20</td>
<td>1.26</td>
<td>1.58</td>
<td>1.58</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>9.07</td>
<td>0.18</td>
<td>1.02</td>
<td>5.45</td>
<td>9.27</td>
<td>9.18</td>
</tr>
<tr>
<td>Leucine</td>
<td>9.22</td>
<td>0.23</td>
<td>1.44</td>
<td>9.25</td>
<td>16.23</td>
<td>16.24</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>10.00</td>
<td>0.14</td>
<td>0.70</td>
<td>4.40</td>
<td>7.09</td>
<td>7.04</td>
</tr>
<tr>
<td>Trptophan</td>
<td>10.48</td>
<td>0.12</td>
<td>0.17</td>
<td>1.15</td>
<td>1.97</td>
<td>2.14</td>
</tr>
<tr>
<td>Lysine</td>
<td>11.41</td>
<td>0.25</td>
<td>0.99</td>
<td>13.03</td>
<td>21.19</td>
<td>21.79</td>
</tr>
</tbody>
</table>

Total                                           32.5\(^a\) 37.7\(^a\) 132.6\(^b\) 218.4\(^c\) 226.5\(^c\)

1. There was no difference (p>0.05) between electrical stimulation (ES) and non-electrical stimulation (NES)
2. Every individual amino acids was significant (p<0.01) with aging times.
Hsieh et al. (1980) in their investigation of synthetic meaty flavor have indicated that both cysteine and methionine, sulfur-containing amino acids, are important compounds which contribute to meaty flavor during the cooking of muscle tissue. Other amino acids from myofibrillar proteins such as alanine, leucine, isoleucine and histidine have been found to also contribute to meat flavor (Hsieh et al. 1980), whereas, Schroedter and Wolm (1980) have concluded that glutamic acid, aspartic acids, arginine and proline contribute to meat flavor and/or aroma. Lysine has also been demonstrated to be involved in the development of browning products on heating of muscle proteins and may be involved in development of meat flavor (Miller et al. 1965; Skurray and Cumming 1974).

Pearson et al. (1983) examined amino acid composition and they reported that myofibrillar proteins contain all of the amino acid residues which were found. Especially, myosin contained 60% of the total myofibrillar protein. The principal sources of cysteine, methionine, alanine, leucine, isoleucine, histidine, lysine, arginine, aspartic and glutamic acids appears to be myosin and the other myofibrillar proteins contribute lesser quantities.
Figure 6 and 7 showed the SDS electrophotogram of myofibrillar protein of Chinese-style dry-cured and aged hams and indicates that the myosin heavy chain, C-protein, α-actinin, troponin-T, troponin-I and tropomyosin were degraded, while the non-protein nitrogen and free amino acids were increased during six months' aging. Therefore, it can be concluded that the degradation products of myofibrillar proteins were the major sources to cause the increase of non-protein nitrogen and free amino acids which significantly contributed to the development of ham flavor during postmortem aging.

4.9. Changes of Total Nucleotides and Nucleosides during the Aging Period

Table 6. indicates that the total nucleotides decreased (p<0.05), while the total nucleosides increased (p<0.05) in Chinese-style hams during aging. Jones and Murray (1964) demonstrated that the increase in flavor of meat during aging may be related to the progressive nucleotide breakdown. For example, the ADP and AMP are respectively dephosphorylated and deaminated to inosinic acid (IMP), and latter dephosphorylated to inosine. Macy, Naumann and Bailey (1970) suggest that inosine has been implicated in
Table 6. The effect of electrical stimulation\(^1\) and aging times\(^2\) on the total nucleotides and nucleosides (mg/g of freeze-dry lean) of Chinese-style dry-cured and aged hams.

<table>
<thead>
<tr>
<th>Hams dry-cured and aged</th>
<th>Fresh ham</th>
<th>0 mon</th>
<th>2 mon</th>
<th>4 mon</th>
<th>6 mon</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total nucleotides</strong> (mg/g of freeze-dry lean)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Electrical stimulation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Means</td>
<td>0.67(^a)</td>
<td>0.53(^a)</td>
<td>0.55(^a)</td>
<td>0.42(^{a,b})</td>
<td>0.31(^b)</td>
</tr>
<tr>
<td>(Standard error)</td>
<td>(0.05)</td>
<td>(0.03)</td>
<td>(0.04)</td>
<td>(0.01)</td>
<td>(0.02)</td>
</tr>
<tr>
<td>Non-electrical stimulation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Means</td>
<td>0.66(^a)</td>
<td>0.54(^a)</td>
<td>0.49(^{a,b})</td>
<td>0.43(^{a,b})</td>
<td>0.34(^b)</td>
</tr>
<tr>
<td>(Standard error)</td>
<td>(0.03)</td>
<td>(0.03)</td>
<td>(0.02)</td>
<td>(0.01)</td>
<td>(0.02)</td>
</tr>
<tr>
<td><strong>Total nucleosides</strong> (mg/g of freeze-dry lean)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Electrical stimulation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Means</td>
<td>0.20(^a)</td>
<td>0.24(^a)</td>
<td>0.44(^b)</td>
<td>0.41(^b)</td>
<td>0.50(^b)</td>
</tr>
<tr>
<td>(Standard error)</td>
<td>(0.01)</td>
<td>(0.02)</td>
<td>(0.03)</td>
<td>(0.03)</td>
<td>(0.04)</td>
</tr>
<tr>
<td>Non-electrical stimulation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Means</td>
<td>0.18(^a)</td>
<td>0.23(^a)</td>
<td>0.42(^b)</td>
<td>0.44(^b)</td>
<td>0.55(^c)</td>
</tr>
<tr>
<td>(Standard error)</td>
<td>(0.01)</td>
<td>(0.03)</td>
<td>(0.02)</td>
<td>(0.03)</td>
<td>(0.02)</td>
</tr>
</tbody>
</table>

1. There is no difference (p>0.05) between electrical stimulation and non-electrical stimulation.

2. a,b,c - Means within row with different superscript letters are significantly different (p<0.05) with aging times.
meat flavor and browning because of its further split to ribose and hypoxanthine. The increase in concentration of hypoxanthine parallels the organoleptic changes occurring during conditioning. Morton, Arkroyd and May (1960) reported that a ribose heated with cysteine in excess water can artificially produce the odor and taste of various meats.

4.10. Changes of Total Free Fatty Acids and Carbonyl Compounds during Aging Periods

Table 7 and Table 8, respectively show that the total free fatty acids and carbonyl compounds increased during aging. Changes in free fatty acids and carbonyl compounds during aging no doubt contribute to the flavor alterations observed. Hydrolytic and oxidative changes occurred in the lipid fraction during the aging process. Hydrolytic changes involved liberation of the glycerol ester bound fatty acids. Demeyer et al., (1974) reported that free fatty acids increased while triglyceride decreased during the aging period. Furthermore, the oxidative changes in unsaturated fatty acids resulted in the production of lipid peroxides and carbonyl compounds. Ockerman (1961) indicated that the unsaturated fatty acids, such as palmitoleic, oleic and linoleic acids decreased during the aging period. Saturated fatty acids, such as myristic,
Table 7. The effect of electrical stimulation\textsuperscript{1} and aging times\textsuperscript{2} on the total free fatty acid (g/100g freeze-dry fat) of Chinese style dry-cured and aged hams.

<table>
<thead>
<tr>
<th>Hams dry-cured and aged</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total free fatty acid (g/100g freeze-dry fat) ham</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>-------------------------</td>
</tr>
<tr>
<td>Electrical stimulation</td>
</tr>
<tr>
<td>Means</td>
</tr>
<tr>
<td>(Standard error)</td>
</tr>
<tr>
<td>Non-electrical stimulation</td>
</tr>
<tr>
<td>Means</td>
</tr>
<tr>
<td>(Standard error)</td>
</tr>
</tbody>
</table>

1. There is no difference (p>0.05) between electrical stimulation and non-electrical stimulation.

2. a,b,c - Means within row with different superscript letters are significantly different (p<0.05) with aging times.
Table 8. The effect of electrical stimulation\(^1\) and aging times\(^2\) on the carbonyl compounds (\(\mu\) mole/g of freeze-dry fat) of Chinese style dry-cured and aged hams.

<table>
<thead>
<tr>
<th>Hams dry-cured and aged</th>
<th>Fresh ham</th>
<th>0 mon</th>
<th>2 mon</th>
<th>4 mon</th>
<th>6 mon</th>
</tr>
</thead>
</table>

Carbonyl compounds  
(\(\mu\) mole/g of freeze-dry fat)

**Electrical stimulation**

<table>
<thead>
<tr>
<th>Means</th>
<th>1.07(^{a})</th>
<th>1.38(^{a})</th>
<th>2.79(^{b})</th>
<th>3.23(^{b})</th>
<th>4.54(^{c})</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Standard error)</td>
<td>(0.09)</td>
<td>(0.08)</td>
<td>(0.17)</td>
<td>(0.05)</td>
<td>(0.23)</td>
</tr>
</tbody>
</table>

**Non-electrical stimulation**

<table>
<thead>
<tr>
<th>Means</th>
<th>0.96(^{a})</th>
<th>1.28(^{a})</th>
<th>2.78(^{b})</th>
<th>3.28(^{b})</th>
<th>4.62(^{c})</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Standard error)</td>
<td>(0.05)</td>
<td>(0.03)</td>
<td>(0.26)</td>
<td>(0.17)</td>
<td>(0.30)</td>
</tr>
</tbody>
</table>

1. There is no difference (p>0.05) between electrical stimulation and non-electrical stimulation.

2. \(a,b,c\) - Means within row with different superscript letters are significantly different (p<0.05) with aging times.
palmitic and stearic acids showed less decrease. Total carbonyl compounds were shown to increase during the aging of hams between two months and fifteen months.

4.11. Changes of T.B.A. Value and Sodium Nitrite Residues during Aging Periods

The thiobarturic acid (T.B.A.) test was used for evaluating the extent of lipid oxidation. Figure 9 showed that T.B.A. value of Chinese ham increase (p<0.05) between aging times of 0 month and 4 month. After 4 months, the TBA values slightly decline due to further degradation of malonaldehyde.

Changes in sodium nitrite content are presented in Figure 10. The sodium nitrite residue rapidly decrease (p<0.05) between aging time 0 month and 2 months. After two months of aging only a limited amount of nitrite remained.

Fooladi et al. (1979) reported that nitrite retards the rate of oxidative rancidity, because nitrite protects against oxidation of phospholipids. MacDonald et al., (1980) also indicated that nitrite served as a metal chelator for trace metals in meat. In this study the nitrite level quickly disappeared and could not effectively retard lipid oxidation.
Figure 9. The effects of electrical stimulation\(^1\) and aging times\(^2\) on the T.B.A. value (mg / Kg) of Chinese dry-cured and aged hams.

1. There is no difference (p>0.05) between electrical stimulation and non-electrical stimulation.

2. There is significant differences (p<0.05) among aging times.
Figure 10. The effects of electrical stimulation\(^1\) and aging times\(^2\) on the sodium nitrite residue (ppm) of Chinese dry-cured and aged hams.

1. There is no difference (p>0.05) between electrical stimulation and non-electrical stimulation.

2. There is significant differences (p<0.05) among aging times.
4.12. Changes of Volatile Compounds during Aging Time by Using Dynamic Headspace Analysis

Table 9 shows the effects of electrical and non-electrical stimulation and aging times (0, 6, 9 and 12 months) on the volatile flavor compounds in Chinese style hams. Results indicated the volatile flavor compounds increased from 0 month to 6 months and this may be due to the presence of muscle proteases breaking down myofibrillar proteins and the formation of volatile compounds during the dry-curing and aging of hams (McCain et al., 1968). However, the volatiles decreased from 9 to 12 months. Because the aged hams were only 3 cm thick, the longer aging times resulted in a drier product and the more volatile compounds escaped. In addition, the cooking treatment can increase (p<0.05) the quantity of volatile compounds. The heat serves several functions including (1) releasing flavor precursors from fat; (2) allowing intimate mixing of fat and water-soluble compounds; (3) accelerating the browning reaction; and (4) combining volatile compounds produced during the cooking of meat (Herz and Chang, 1970). Moreover, Table 10 indicates that the individual volatile compounds affected by ES and aging times and the interaction between these two treatments analyzed by using two way ANOVA. Heptane, octane,
Table 9. Effects of electrical stimulation and aging times (0, 6, 9, and 12 months) on the volatile flavor compounds in the Chinese style hams and fresh ham as a control.

<table>
<thead>
<tr>
<th>Peak No.</th>
<th>Compounds</th>
<th>Fresh ham (x10^6)</th>
<th>0 mon</th>
<th>6 mon</th>
<th>9 mon</th>
<th>12 mon</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Pentane</td>
<td>(cooked) 9.4</td>
<td>46.2</td>
<td>109.4</td>
<td>14.5</td>
<td>1.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(uncooked) --</td>
<td>4.8</td>
<td>6.6</td>
<td>15.4</td>
<td>2.6</td>
</tr>
<tr>
<td>2.</td>
<td>Heptane</td>
<td>(cooked) 2.6</td>
<td>2.9</td>
<td>98.8</td>
<td>7.2</td>
<td>0.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(uncooked) --</td>
<td>--</td>
<td>--</td>
<td>2.9</td>
<td>0.9</td>
</tr>
<tr>
<td>3.</td>
<td>Octane</td>
<td>(cooked) 3.3</td>
<td>4.0</td>
<td>100.8</td>
<td>10.4</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(uncooked) --</td>
<td>--</td>
<td>0.8</td>
<td>4.1</td>
<td>1.1</td>
</tr>
<tr>
<td>4.</td>
<td>2-Propanone</td>
<td>(cooked) 20.9</td>
<td>56.6</td>
<td>55.1</td>
<td>93.5</td>
<td>46.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(uncooked) 4.0</td>
<td>61.6</td>
<td>20.9</td>
<td>95.2</td>
<td>28.2</td>
</tr>
<tr>
<td>5.</td>
<td>2-Butanone</td>
<td>(cooked) 4.2</td>
<td>3.3</td>
<td>8.8</td>
<td>9.6</td>
<td>4.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(uncooked) --</td>
<td>0.9</td>
<td>6.4</td>
<td>6.3</td>
<td>2.5</td>
</tr>
<tr>
<td>6.</td>
<td>2-Ethyl furan</td>
<td>(cooked) --</td>
<td>0.7</td>
<td>0.7</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(uncooked) --</td>
<td>--</td>
<td>0.6</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>7.</td>
<td>2-Pentanone</td>
<td>(cooked) 7.7</td>
<td>32.2</td>
<td>26.8</td>
<td>31.3</td>
<td>5.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(uncooked) --</td>
<td>29.7</td>
<td>28.4</td>
<td>27.0</td>
<td>5.2</td>
</tr>
<tr>
<td>8.</td>
<td>Methylbenzene</td>
<td>(cooked) 0.2</td>
<td>0.8</td>
<td>1.0</td>
<td>1.5</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(uncooked) --</td>
<td>--</td>
<td>--</td>
<td>1.5</td>
<td>--</td>
</tr>
<tr>
<td>9.</td>
<td>Dimethyl-disulfide</td>
<td>(cooked) 2.3</td>
<td>2.0</td>
<td>5.4</td>
<td>23.1</td>
<td>6.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(uncooked) --</td>
<td>0.9</td>
<td>2.9</td>
<td>3.7</td>
<td>2.4</td>
</tr>
<tr>
<td>10.</td>
<td>Hexanal</td>
<td>(cooked) 47.8</td>
<td>206.3</td>
<td>15.2</td>
<td>186.7</td>
<td>18.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(uncooked) 2.8</td>
<td>316.5</td>
<td>294.6</td>
<td>797.9</td>
<td>58.5</td>
</tr>
<tr>
<td>11.</td>
<td>2-Methyl-thiophene</td>
<td>(cooked) --</td>
<td>--</td>
<td>1.5</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(uncooked) --</td>
<td>--</td>
<td>1.4</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>12.</td>
<td>2-Heptanone</td>
<td>(cooked) 2.5</td>
<td>6.1</td>
<td>3.7</td>
<td>1.3</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(uncooked) --</td>
<td>--</td>
<td>--</td>
<td>1.7</td>
<td>--</td>
</tr>
<tr>
<td>13.</td>
<td>2-Pentyl furan</td>
<td>(cooked) --</td>
<td>--</td>
<td>4.7</td>
<td>5.3</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(uncooked) --</td>
<td>--</td>
<td>1.7</td>
<td>--</td>
<td>--</td>
</tr>
</tbody>
</table>
Table 9. Effects of electrical stimulation and aging times (0, 6, 9, and 12 months) on the volatile flavor compounds in the Chinese style hams and fresh ham as a control.

<table>
<thead>
<tr>
<th>Peak No.</th>
<th>Compounds</th>
<th>Non-electrical stimulation</th>
<th>Fresh ham</th>
<th>0 mon</th>
<th>6 mon</th>
<th>9 mon</th>
<th>12 mon</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td>Pentane</td>
<td>(cooked)</td>
<td>3.7</td>
<td>17.3</td>
<td>31.4</td>
<td>12.3</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(uncooked)</td>
<td>--</td>
<td>12.9</td>
<td>1.8</td>
<td>12.4</td>
<td>2.6</td>
</tr>
<tr>
<td>2.</td>
<td>Heptane</td>
<td>(cooked)</td>
<td>2.7</td>
<td>2.9</td>
<td>29.7</td>
<td>7.6</td>
<td>1.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(uncooked)</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>1.2</td>
<td>0.9</td>
</tr>
<tr>
<td>3.</td>
<td>Octane</td>
<td>(cooked)</td>
<td>3.5</td>
<td>4.0</td>
<td>28.4</td>
<td>9.9</td>
<td>4.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(uncooked)</td>
<td>--</td>
<td>0.8</td>
<td>0.5</td>
<td>1.9</td>
<td>0.7</td>
</tr>
<tr>
<td>4.</td>
<td>2-Propanone</td>
<td>(cooked)</td>
<td>22.4</td>
<td>44.5</td>
<td>72.3</td>
<td>119.5</td>
<td>49.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(uncooked)</td>
<td>8.4</td>
<td>39.5</td>
<td>30.9</td>
<td>108.6</td>
<td>41.7</td>
</tr>
<tr>
<td>5.</td>
<td>2-Butanone</td>
<td>(cooked)</td>
<td>3.7</td>
<td>3.3</td>
<td>8.8</td>
<td>12.4</td>
<td>4.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(uncooked)</td>
<td>--</td>
<td>0.9</td>
<td>3.7</td>
<td>6.4</td>
<td>2.0</td>
</tr>
<tr>
<td>6.</td>
<td>2-Ethyl furan</td>
<td>(cooked)</td>
<td>--</td>
<td>0.6</td>
<td>0.7</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(uncooked)</td>
<td>--</td>
<td>0.5</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>7.</td>
<td>2-Pentanone</td>
<td>(cooked)</td>
<td>3.3</td>
<td>69.2</td>
<td>16.8</td>
<td>32.2</td>
<td>4.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(uncooked)</td>
<td>--</td>
<td>58.7</td>
<td>16.9</td>
<td>27.0</td>
<td>5.2</td>
</tr>
<tr>
<td>8.</td>
<td>Methyl-benzene</td>
<td>(cooked)</td>
<td>--</td>
<td>--</td>
<td>1.0</td>
<td>1.5</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(uncooked)</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>1.5</td>
<td>--</td>
</tr>
<tr>
<td>9.</td>
<td>Dimethyl-disulfide</td>
<td>(cooked)</td>
<td>4.2</td>
<td>2.5</td>
<td>5.4</td>
<td>3.6</td>
<td>4.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(uncooked)</td>
<td>--</td>
<td>--</td>
<td>1.0</td>
<td>3.1</td>
<td>2.0</td>
</tr>
<tr>
<td>10.</td>
<td>Hexanal</td>
<td>(cooked)</td>
<td>5.6</td>
<td>123.8</td>
<td>15.2</td>
<td>118.1</td>
<td>19.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(uncooked)</td>
<td>2.8</td>
<td>516.5</td>
<td>78.8</td>
<td>640.5</td>
<td>171.2</td>
</tr>
<tr>
<td>11.</td>
<td>2-Methyl-thiophene</td>
<td>(cooked)</td>
<td>--</td>
<td>--</td>
<td>1.8</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(uncooked)</td>
<td>--</td>
<td>1.7</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>12.</td>
<td>2-Heptanone</td>
<td>(cooked)</td>
<td>--</td>
<td>--</td>
<td>2.1</td>
<td>1.7</td>
<td>1.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(uncooked)</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>1.5</td>
</tr>
<tr>
<td>13.</td>
<td>2-Pentyl furan</td>
<td>(cooked)</td>
<td>--</td>
<td>--</td>
<td>1.8</td>
<td>3.9</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(uncooked)</td>
<td>--</td>
<td>--</td>
<td>1.7</td>
<td>0.5</td>
<td>--</td>
</tr>
</tbody>
</table>
Table 10  The two way ANOVA of individual volatile compounds affected by aging times, electrical stimulation and the interaction between these two treatments.

<table>
<thead>
<tr>
<th>Volatile compounds</th>
<th>Probability Between</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Aging time</td>
<td>Electrical stimulation</td>
<td>Interaction</td>
</tr>
<tr>
<td>Pentane</td>
<td>0.021</td>
<td>0.063</td>
<td>0.172</td>
</tr>
<tr>
<td>Heptane</td>
<td>0.001</td>
<td>0.041</td>
<td>0.018</td>
</tr>
<tr>
<td>Octane</td>
<td>&lt;0.001</td>
<td>0.022</td>
<td>0.006</td>
</tr>
<tr>
<td>2-propanone</td>
<td>0.075</td>
<td>0.579</td>
<td>0.813</td>
</tr>
<tr>
<td>2-butanone</td>
<td>0.002</td>
<td>0.207</td>
<td>0.648</td>
</tr>
<tr>
<td>2-pentanone</td>
<td>0.006</td>
<td>0.206</td>
<td>0.114</td>
</tr>
<tr>
<td>Dimethyl disulfide</td>
<td>0.487</td>
<td>0.201</td>
<td>0.482</td>
</tr>
<tr>
<td>Hexanal</td>
<td>0.001</td>
<td>0.185</td>
<td>0.624</td>
</tr>
<tr>
<td>2-methyl thiophene</td>
<td>0.874</td>
<td>0.043</td>
<td>0.976</td>
</tr>
<tr>
<td>2-Heptanone</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
2-methyl thiophene, and 2-heptanone were affected (p<0.05) by ES. Pentane, heptane, octane, 2-butanone, 2-pentanone, hexanal, and 2-heptanone were significantly (p<0.05) affected by aging times.

There were 13 volatile compounds identified by utilizing the method of dynamic headspace analysis (DHA), and contributed to flavor characteristics of Chinese style aged ham (Table 11). Hydrocarbons are derived from the thermal oxidative decomposition of lipids, a reaction catalyzed by heme compounds such as hemoglobin and myoglobin. They do not contribute significantly to the flavor of meat (Ben-Aziz et al. 1970). Aldehydes are ubiquitous in nature and are contributors to the odor and flavor of meat. They also can react further with other components to produce flavor chemicals (Ohloff and Flament, 1978). Hexanal is the result of oxidation, usually of C18 polyunsaturated fatty acids such as linoleic and linolenic and the C20 arachidonic acid (Cross and Ziegler, 1965). In this study, the amount of hexanal showed great variation, possible due to the different content of intramuscular fat in the muscle tissue. The ketones are produced from the thermal nonoxidative reaction and/or from oxidative reactions of saturated fatty acids (Nawar, 1985). It is believe that most of the ketones are not primary
Table 11. The volatile flavor compounds related to flavor characteristics of Chinese style dry-cured and aged ham

<table>
<thead>
<tr>
<th>Volatile compounds</th>
<th>Related to flavor characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hydrocarbons:</strong></td>
<td></td>
</tr>
<tr>
<td>Pentane</td>
<td>Burnt, green</td>
</tr>
<tr>
<td>Heptane</td>
<td>Cooked meat</td>
</tr>
<tr>
<td>Octane</td>
<td>Meaty</td>
</tr>
<tr>
<td>Benzene methyl</td>
<td>Strong, fruity, becoming dank, bitter</td>
</tr>
<tr>
<td><strong>Aldehyde:</strong></td>
<td></td>
</tr>
<tr>
<td>Hexanal</td>
<td>Strong, rancid, unpleasant, pungent</td>
</tr>
<tr>
<td><strong>Ketones:</strong></td>
<td></td>
</tr>
<tr>
<td>2-Propanone</td>
<td>Dull, meat-broth</td>
</tr>
<tr>
<td>2-Butanone</td>
<td>Sickly</td>
</tr>
<tr>
<td>2-Pentanone</td>
<td>Buttery, sweet, sickly, meaty</td>
</tr>
<tr>
<td>2-Heptanone</td>
<td>Green, pungent, sickly</td>
</tr>
<tr>
<td><strong>Furans:</strong></td>
<td></td>
</tr>
<tr>
<td>Furan 2-ethyl</td>
<td>Acid, sour, whey butter-like</td>
</tr>
<tr>
<td>Furan 2-pentyl</td>
<td>Green, pungent, sweet</td>
</tr>
<tr>
<td><strong>Sulfur compounds:</strong></td>
<td></td>
</tr>
<tr>
<td>Disulfide dimethyl</td>
<td>Very strong, sulfurous, garlic, onion</td>
</tr>
<tr>
<td>Thiophene 2-methyl</td>
<td>Green, Sweet</td>
</tr>
</tbody>
</table>
contributors to the flavor of meat products. Furan and thiophene are degradation products from the thermal destruction of thiamine which is involved in the development of "meaty" flavors in cooked meat products (Tannenbaum and Young, 1985; Ang et al., 1975). A number of reports (Hirai et al. 1973; Persson et al. 1973) indicated that the furans are identified and related to volatile beef flavor. In this study, two kinds of furans (2-ethyl and 2-pentyl) were found and they were also identified in pork liver (Mussinan and Walradt, 1974). Moreover, there is little doubt that many of the most important compounds contributing to meat flavor are derived from the myofibrillar proteins. Sulfur-containing volatile compounds are formed principally from the sulfur-containing amino acids in meat protein. Among the products of cysteine and methionine pyrolysis are mercaptoethylamine and \( \text{H}_2\text{S} \) from the former and methional and methanethiol from the latter. The dimethyl disulfide found in this study, is a further degradation product of methanethiol (Lindsay, 1985).

4.13. Sensory Evaluation of Chinese Style Dry-cured and Aged Hams

Table 12 shows the sensory score of Chinese style hams
Table 12. The effects of electrical stimulation\(^1\) and aging times\(^2\) on the mean sensory parameters (standard error) of fresh ham and Chinese dry-cured and aged hams

<table>
<thead>
<tr>
<th>Sensory parameters(^3)</th>
<th>Ham dry-cured and aged</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 mon</td>
</tr>
<tr>
<td>Ham flavor intensity</td>
<td></td>
</tr>
<tr>
<td>(ES)</td>
<td>4.7(^{a})</td>
</tr>
<tr>
<td>(NES)</td>
<td>4.5(^{a})</td>
</tr>
<tr>
<td>Tenderness (ES)</td>
<td>4.6(^{a})</td>
</tr>
<tr>
<td>(NES)</td>
<td>4.6(^{a})</td>
</tr>
<tr>
<td>Red color (ES)</td>
<td>4.7(^{a})</td>
</tr>
<tr>
<td>(NES)</td>
<td>5.7(^{a})</td>
</tr>
<tr>
<td>Texture (ES)</td>
<td>4.8(^{a})</td>
</tr>
<tr>
<td>(NES)</td>
<td>4.7(^{a})</td>
</tr>
<tr>
<td>Overall Acceptability</td>
<td>5.7(^{a})</td>
</tr>
</tbody>
</table>

1. There is no difference (p>0.05) between treatments of non-electrical stimulation (NES) and electrical stimulation (ES).

2. \(a, b, c\) - Means within row with different superscript letters are significantly different (p<0.05) with aging times.

3. Based on a nine point hedonic scale; 9 = extremely intensive ham flavor, tender, dark red in color, fine texture, and acceptable; 1 = extremely bland ham flavor, tough, light red in color, coarse texture, and unacceptable.
aged 0, 2, 4, and 6 months. The results of sensory parameters including ham flavor intensity, tenderness, red color, texture, and overall acceptability showed that the values increased (p<0.05) with aging times. However, no differences (p>0.05) were found by using ES. These sensory characteristics are discussed as follows:

4.13.1. Ham flavor intensity

Flavor is a complex of sensations resulting from the stimulation of the sense of odor, taste, feel, and sometimes vision and audition (Kurtz, 1959). The unique ham flavor of cured aged ham derives from (1) the volatiles flavor and carbonyl compounds released from the cured ham; (2) the precursors for meaty flavor, glycoprotein and inosinic acid, which are water soluble and are converted to flavor compounds during cooking of the meat; (3) the characteristic differences in flavor are due to the components derived from lipids; and (4) the degradation products of protein and fat.

In the current investigation, Table 13 demonstrated that high correlation coefficients between ham flavor intensity and non-protein nitrogen, total free amino acids, total nucleosides, total free fatty acids, and carbonyl compounds which have been recognized as important to the formation of cured ham.
Table 13. Correlation coefficients between ham flavor intensity and biochemical measurements related to flavor characteristics of Chinese ham.

<table>
<thead>
<tr>
<th>Ham flavor intensity</th>
<th>R²</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-protein nitrogen</td>
<td>0.89</td>
<td>0.003</td>
</tr>
<tr>
<td>Total free amino acids</td>
<td>0.96</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total nucleosides</td>
<td>0.70</td>
<td>0.050</td>
</tr>
<tr>
<td>Total free fatty acids</td>
<td>0.79</td>
<td>0.021</td>
</tr>
<tr>
<td>Carbonyl compounds</td>
<td>0.89</td>
<td>0.002</td>
</tr>
</tbody>
</table>
Table 14  The correlation coefficients of individual volatile compounds between ham flavor intensity and overall acceptability.

<table>
<thead>
<tr>
<th>Volatile compounds</th>
<th>Ham flavor intensity</th>
<th>Overall acceptability</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R²</td>
<td>P</td>
</tr>
<tr>
<td>Pentane</td>
<td>0.581</td>
<td>0.151</td>
</tr>
<tr>
<td>Heptane</td>
<td>0.629</td>
<td>0.132</td>
</tr>
<tr>
<td>Octane</td>
<td>0.596</td>
<td>0.145</td>
</tr>
<tr>
<td>2-propanone</td>
<td>0.718</td>
<td>0.099</td>
</tr>
<tr>
<td>2-butanone</td>
<td>0.964</td>
<td>0.012</td>
</tr>
<tr>
<td>2-ethyl furan</td>
<td>0.955</td>
<td>0.015</td>
</tr>
<tr>
<td>2-pentanone</td>
<td>0.894</td>
<td>0.036</td>
</tr>
<tr>
<td>Methyl benzene</td>
<td>0.955</td>
<td>0.015</td>
</tr>
<tr>
<td>Dimethyl disulfide</td>
<td>0.466</td>
<td>0.206</td>
</tr>
<tr>
<td>Hexanal</td>
<td>-0.381</td>
<td>0.718</td>
</tr>
<tr>
<td>2-methyl thiophene</td>
<td>0.874</td>
<td>0.043</td>
</tr>
<tr>
<td>2-Heptanone</td>
<td>0.711</td>
<td>0.101</td>
</tr>
<tr>
<td>2-Pentyl furan</td>
<td>0.573</td>
<td>0.154</td>
</tr>
</tbody>
</table>
flavor either as flavor compounds or flavor precursors increased during postmortem aging. In addition, Table 14 indicated that volatile compounds such as 2-butanone, 2-ethyl furan, 2-pentanone, methyl benzene, and 2-methyl thiophene were significantly (p<0.05) correlated with ham flavor intensity. Compared between Table 10 and 14, only 2-methyl thiophene was affected by ES.

### 4.13.2. Tenderness

Consumers consider tenderness as the most important palatability characteristics of meat products. With adequate tenderness, consumers appreciate the more subtle characteristics of flavor and juiciness (Bratzler, 1978). The overall impression of meat tenderness comes from three factors: (1) the ease with which the teeth sink into the meat when chewing begins; (2) the ease with which meat breaks into fragments (friability or mealiness); and (3) the amount of residue remaining after chewing. Friability may well reflect muscle fiber resistance to breakage perpendicular to its axis while the amount of residue is thought to reflect the amount of collagen or connective tissue present in the meat (Bratzler, 1978).
Table 15. Correlation coefficients between tenderness, texture and relative activity of CDP-I and myofibril fragmentation index.

<table>
<thead>
<tr>
<th></th>
<th>Tenderness</th>
<th></th>
<th>Texture</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$R^2$</td>
<td>$P$</td>
<td>$R^2$</td>
<td>$p$</td>
</tr>
<tr>
<td>Relative activity of CDP-I</td>
<td>0.68</td>
<td>0.012</td>
<td>0.70</td>
<td>0.01</td>
</tr>
<tr>
<td>Myofibril fragmentation index</td>
<td>0.82</td>
<td>0.002</td>
<td>0.94</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
There were high correlation coefficients between tenderness and CDP-I activity and MFI (Table 15). It indicated the tenderness increased (p<0.05) with aging time, possible reasons may be due to proteolytic activity and related to the changes in myofibrillar proteins.

4.13.3. Red color

Nitric oxide is the important decomposition product, from the added nitrite in the curing formula, which enters directly into the "color fixation" reaction with myoglobin. The nitric oxide combines with myoglobin to become nitric oxide myoglobin which is a red color. After heating, the nitric oxide myoglobin is converted to nitrosyl hemochrome and is pink in color (Ockerman, 1983). However, sodium chloride present in meat may promote oxidation from nitro oxide myoglobin to metmyoglobin. The metmyoglobin is converted to denatured metmyoglobin by the heating process. Both metmyoglobin and denatured metmyoglobin are brown or dark red in color (Greene and Price, 1975).

Table 12 demonstrates that the hams became dark red in color during aging. Two reasons can interpret this result (1) sodium nitrite residue rapidly disappeared (Figure 9) which then could not convert into nitro oxide and form nitro oxide
myoglobin; and (2) due to the oxidation from nitro oxide myoglobin to metmyoglobin.

4.13.4. Texture

The texture of muscle may be assessed by an estimation of the apparent muscle bundle size (fibers grouped by perimysial connective tissue). The size of the muscle bundles is positively associated with the visible coarseness of a cross-sectional area of the muscle. Increase in thickness of connective strands and large size of the muscle fibers and bundles may contribute to coarse texture (Bratzler, 1978).

Table 12 shows that the longer the aging times, the finer the texture became. Table 15 also shows a high correlation between texture and CDP-I activity and MFI. The major reason for this is the possible continuous function of the indigenous muscle proteases on the muscle fibers and bundles and subsequently fine texture appeared on the cut surface of the muscle.

4.13.5. Overall acceptability

Sensory evaluation is a composite of sensations resulting from the stimulation of the senses of odor, taste, feel and ease
of mastication. The overall acceptability is decided by general impressions of flavor, tenderness, texture, and color (Bratzler, 1978). Table 12 indicated that the hams aged 4 and 6 months resulted in higher overall acceptability than hams aged 0 and 2 months. Table 14 also indicated that the volatile compounds such as 2-propanone, 2-butanone, 2-ethyl furan, methyl benzene, and 2-methyl thiophene were significantly (p<0.05) related to overall acceptability.
CHAPTER V

SUMMARY AND CONCLUSIONS

The first experiment was to investigate the effect of electrical stimulation (electrical stimulation ES vs non-electrical stimulation NES) and aging periods (0, 2, 4, and 6 months) on the changes of myofibrillar proteins of Chinese-style dry-cured and aged hams by evaluating the ham's pH values, relative activity of low-calcium-requiring calcium-dependent proteases (CDP-I), SDS-polyacrylamide gel electrophoresis (SDS-PAGE), myofibril fragmentation index (MFI), and protein concentration. Results are as follows:

1. Electrical stimulation on pork carcasses increased the rate of tissue pH decline by accelerating glycolysis. There was an approximate 0.2 unit of pH difference between ES and NES carcasses at 30 min postmortem.
2. Both the pH value and the relative activity of CDP-I were increased (p<0.01) during the aging periods and significantly (p<0.01) affected by ES. The higher the pH values, the more the relative activity of CDP-I occurred. The activity and relative contributions of CDP-I to the aging process may be determined by the ultimate postmortem pH value of the muscle system. CDP-I retained 27% to 40% of its activity which would be a sufficient level of activity to produce the myofibrillar changes observed during aging.

3. The SDS-PAGE electrophotogram of myofibrils showed that the myosin, C-protein, α-actinin, troponin-I, myosin light chain, and tropomyosin were completely degraded during six months of postmortem aging. The degradation polypeptides with molecular weights of 150 K, 110 K, 95 K, 82 K, 75 K and 65 K dalton have been found. Troponin-T (37 K dalton) was also degraded; polypeptides with molecular weights of 34 K, 30 K and 25 K dalton appeared. But the actin was not affected.

4. The longer aging times, the higher (p<0.01) MFI became. The MFI is an important indicator related to the changes in myofibrillar proteins and to the reduction in toughness during postmortem tenderization.
5. Crude porcine protein was utilized for the preparation of a standard protein curve rather than using bovine serum albumin. The protein concentration was significantly different (p<0.01) among fresh ham, as a control, and hams aged 0, 2, 4, and 6 months.

6. However, the treatment of ES seemed too weak to make a difference (p>0.05) in MFI, protein concentration, and SDS-PAGE electrophotogram.

The second experiment has focused on the effect of electrical stimulation and aging (0, 2, 4, and 6 months) on the biochemical changes which are associated with the formation of ham flavor of Chinese-style dry-cured and aged hams. The efficiencies of these treatments were evaluated by proximate analysis, non-protein nitrogen, free amino acids, total nucleotides and nucleosides, total free fatty acids, carbonyl compounds, sodium nitrite residues, T.B.A. values, volatile compounds and sensory evaluation. Results are as follows:

1. The moisture content decreased (p<0.05) quickly during dry-curing, salt equalization and during the first two months of aging. An increase (p<0.05) was observed in crude protein and crude fat due to loss of water. In addition, there was a difference (p<0.05) in crude ash and salt between fresh hams
and aged hams due to added cure during the dry-curing process.

2. Non-protein nitrogen increased ($p<0.05$) during aging which may be due to the continuous action of muscle proteases, peptidases, nucleosidase systems and deaminase on myofibrillar proteins during the dry-curing and aging of hams.

3. The individual free amino acids increased ($p<0.05$) among fresh ham and aged hams (0, 2, 4, and 6 months). Free amino acids play an important role in producing meaty flavor or enhanced the meaty flavor. SDS electrophoresis demonstrated that degradation products of myofibrillar proteins were the major source of increased non-protein nitrogen and free amino acids during postmortem aging.

4. The total nucleotides decreased ($p<0.05$), while the total nucleosides increased ($p<0.05$) in Chinese-style hams during the aging periods. The nucleotides progressively break down to hypoxanthine and ribose which have been associated the aged ham flavor.

5. The total free fatty acids and carbonyl compounds increased ($p<0.05$) during the aging periods. Changes in free
fatty acids due to lipolysis of the lipid fraction and changes in carbonyl compounds due to oxidation of the unsaturated fatty acids were observed during aging.

6. T.B.A. values greatly increase (p<0.05) during aging, while the sodium nitrite residue rapidly decreased (p<0.05) between 0 month and 2 months of aging. Nitrite served as a metal chelator for trace metals in meat. Thus, the nitrite level quickly disappeared and could not effectively retard lipid oxidation.

7. There were 13 volatile compounds identified by dynamic headspace analysis (DHA) and related to flavor characteristics of Chinese style aged ham (Table 9, 10 and 11). Heating increased (p<0.05) the quantity of volatile compounds.

8. Sensory characteristics including ham flavor intensity, tenderness, red color, texture and overall acceptability of Chinese style hams increased (p<0.05) during aging times.

9. There was no effect (p>0.05) due to electrical stimulation on proximate analysis, non-protein nitrogen, free amino acids, total nucleotides and nucleosides, total free fatty acids, carbonyl compounds, sodium nitrite residues, T.B.A. values.
and sensory parameters. Only one volatile compound, 2-methyl thiophene, correlated to ham flavor intensity and overall acceptability which affected by ES.
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