INFLUENCE OF PRESLAUGHTER INJECTIONS OF ADRENALIN AND IODOACETATE ON pH, DRIP AND CERTAIN TURKEY MUSCLE PROTEIN CHANGES DURING FROZEN STORAGE

Thesis for the Degree of Ph. D.
MICHIGAN STATE UNIVERSITY
JOSEPH CARTER CRIGLER
1968

This is to certify that the

thesis entitled

INFLUENCE OF PRESLAUGHTER INJECTIONS OF ADRENALIN AND IODOACETATE ON pH, DRIP AND CERTAIN TURKEY MUSCLE PROTEIN CHANGES DURING FROZEN STORAGE

presented by

Joseph Carter Crigler

has been accepted towards fulfillment
of the requirements for

Ph. D. degree in Food Science

L E Dawson
Major professor

Date November 18, 1968

O-169

ABSTRACT

INFLUENCE OF PRESLAUGHTER INJECTIONS OF ADRENALIN AND IODOACETATE ON pH, DRIP AND CERTAIN TURKEY MUSCLE PROTEIN CHANGES DURING FROZEN STORAGE

By

Joseph Carter Crigler

This study was undertaken to evaluate the effects of a high ultimate pH of turkey muscle, obtained by injection of either adrenalin or iodoacetate, and frozen storage on the percentage drip, changes in certain muscle protein fractions and amino acid changes of drip. The use of gas chromatography for analyses of amino acids from protein hydrolysates was also investigated.

Five turkeys received a 1.5 mg subcutaneous injection of adrenalin per kg of body weight and were fasted 20 hours prior to bleeding. A second group of five turkeys received a 200 mg intravenous injection of iodoacetate per kg body weight three minutes prior to bleeding. After processing, light and dark meat was removed from each carcass, ground and formed into slabs prior to freezing. Drip was collected separately from light and dark meat during a thawing period of 36 hours at 5°C after frozen storage periods of 0, 30 and 90 days at -20°C.

High ultimate pH values for light and dark meat from the adrenalin treated turkeys (6.78 and 7.05, respectively) and the dark meat from the iodoacetate treated turkeys (6.97) were obtained. pH values for the control light and dark meat were 5.96 and 6.16, respectively. A high ultimate pH reduced the amount of drip during thawing. Control meat samples yielded 3.3 and 1.2 percent drip from light and dark meat, respectively, before storage. Insufficient drip was obtained from the light and dark meat from the adrenalin treated birds for measurement before storage. Percentage drip from the iodoacetate treated birds before storage, from the light and dark meat, was 10.3 and 1.1 percent, respectively. Light meat had a higher percentage drip than dark, and the percentage drip from both light and dark meat increased during storage, except for the percentage from light meat from the iodoacetate treated turkeys.

Gas chromatography was found to be a satisfactory means of separating and quantitating the amino acids from a mixture of pure amino acids. Results of analyses from a hydrolysate of purified protein (bovine serum albumin) were less satisfactory, with threonine and phenylalanine giving high recoveries and leucine and aspartic acid giving low recoveries. Amino acid analyses of a water soluble protein extract varied considerably from the results of amino acid analyzer. Satisfactory recovery was obtained

for only six of the 14 amino acids investigated, namely, alanine, valine, proline, glutamic acid, tyrosine and lysine.

Sarcoplasmic proteins did not change in solubility as a result of frozen storage up to 90 days. Non-protein nitrogen in the water soluble fraction was higher from light meat than from dark meat, and values remained constant during storage. A decrease in myofibrillar proteins with storage was found to occur only in meat samples from the control light meat and dark meat from the iodoacetate treated birds. Similar percentages of myofibrillar nitrogen were obtained from meat samples with high ultimate pH values and no apparent change occurred with 90 days frozen storage.

Percentage denatured protein from the control light and dark meat samples increased with storage, and light meat contained more denatured protein than dark meat. Only slight changes were found in meat samples from the adrenalin treated birds during storage. Denatured protein values from the light meat of the iodoacetate treated birds were considerably higher than from dark meat, and higher than values from both light and dark meat samples from the control and adrenalin treated birds, and increased with storage.

Stroma nitrogen values varied between treatments and decreased during storage for all samples. A high

ultimate pH was found to result in higher percentages of soluble sarcoplasmic and myofibrillar proteins and lower percentages of denatured and stroma proteins. Meat samples with an ultimate pH of 6.2 or higher contained larger amounts of free amino acids than samples with an ultimate pH below 6.2.

The amount of amino acids in the drip increased with storage time of the meat and the amino acid content of the drip from light meat was higher than from dark meat from all samples. A dilution effect was observed in the amino acid concentration of drip after 30 days storage but was not apparent in the meat stored for 90 days. The additional storage period allowed proteolysis to continue and resulted in a high concentration of amino acids in the drip. It was concluded that a high ultimate pH of the muscle was of major significance in reducing the amount of fluid exuded during thawing, since there was a direct relationship between amount of drip and total amount of amino acids released from tissues.

INFLUENCE OF PRESLAUGHTER INJECTIONS OF ADRENALIN AND IODOACETATE ON pH, DRIP AND CERTAIN TURKEY MUSCLE PROTEIN CHANGES DURING FROZEN STORAGE

By

Joseph Carter Crigler

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Food Science

1968

ACKNOWLEDGMENTS

The author deeply appreciates and is sincerely grateful to Dr. L. E. Dawson for his devoted assistance and guidance throughout this course of study. I would also like to extend a special word of thanks to Dr. Dawson for his prompt and critical review of the manuscript, which was of tremendous value in the completion of my course of study in light of my military obligation.

The author would like to express his appreciation to Dr. B. S. Schweigert and the faculty and students of the Department of Food Science for their cooperation given throughout my graduate program; to Dr. A. M. Pearson, Dr. R. K. Ringer, Dr. C. M. Stine, and Dr. D. E. Ullrey for their cooperation and prompt and critical review of the manuscript.

A special note of thanks to Dr. J. R. Brunner and Ursula Koch for their advice and assistance in amino acid analyses, and to Mrs. Maurice Ritchey for assistance in typing and preparation of the manuscript.

To my wife Marilyn, insufficient words are available to me to express my appreciation for the encouragement, understanding and help given through this trying period.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

For many years, most of the turkeys produced have been fresh-frozen for both the retail and institutional trade. Since most adult turkeys are marketed in late summer or early fall, it is essential to preserve them for consumption throughout the year. There has been a marked increase in use of turkey and chicken in convenience foods such as turkey or chicken rolls, roasts and frozen dinners. Further processing of turkey and chicken in Federally inspected plants rose from 105 to 335 million pounds for turkey and 295 to 481 million pounds for chicken between 1961 and 1966 (U.S.D.A., 1967).

During freezing ice crystal formation results in expansion of the product. As these ice crystals form, an ice front moves through the product and results in the formation of a concentrated salt solution in the interstitial spaces. The severity of the damage resulting from this expansion, the formation of ice crystals and the concentration of salt in solution affects the quality of the frozen product.

During thawing, fluid is released from the tissue and collects as drip. The amount of fluid exuded during

thawing is affected by the pH of the meat. Hamm (1953, 1958) reported that the amount of drip was closely related to the water-holding capacity and Whitaker (1959) reported that pH affects the water-holding capacity by modifying the charges of the protein. Kuprianoff (1952) found that there may be no drip on defrosting, especially when meat is in the pH range 6.3-6.4.

Chicken meat proteins undergo both denaturation and proteolysis during frozen storage. Since beef contained a protease active at freezing temperatures (Balls, 1938), it was concluded that a similar enzyme could possibly be present in chicken muscle (Khan, et al., 1963).

While results of fractionation data and amount of drip from frozen turkey meat has been reported by a few investigators in the past, little work has been reported on the effect of a high ultimate pH by pretreatment of the birds. Since pH has been shown to affect the amount of drip and water-holding capacity, protein characteristics could be different.

The use of the amino acid analyzer has proven to be a satisfactory method for evaluation of amino acid composition. In the past few years amino acid analyses by gas chromatography for biological systems has been investigated. Information concerning the accuracy of this method is limited except for a few purified proteins.

The purposes of this study were: 1. to obtain a high ultimate pH in turkey meat by treatment of the birds with either adrenalin or iodoacetate before slaughter; 2. to evaluate the effects of high ultimate pH and storage on the amount of drip, on certain muscle protein fractions, on changes in amino acid content of drip and on changes in the water soluble protein fraction; 3. to evaluate the use of gas chromatography as a means of amino acid quantitation and to compare the results with those obtained by an amino acid analyzer.

LITERATURE REVIEW

Changes in Meat During Freezing and Thawing

Histological structure of frozen poultry was investigated by Koonz and Ramsbottom (1939a). They reported that in small pieces of meat which were frozen almost instantaneously, the frozen water appeared within the fibers as minute evenly distributed ice columns, whereas in tissue frozen more slowly there were fewer, larger ice columns with a peripheral distribution. With a further decrease in freezing temperature a single centrally located ice column resulted. However, when the freezing process was sufficiently prolonged, a temperature was reached at which water was lost from the fibers; freezing externally to the fibers.

The explanation of drip formation during thawing of fish or meat includes more than the rupturing of cells by the expansion of ice crystals (Taylor, 1930). He stated that there were certain physical-chemical changes related to the conditions under which colloidal protein systems were converted from liquid to gel or gel to liquid and form heterogeneous systems that have a role in the formation of drip. Three means by which the cell jelly could

be converted to a liquid were suggested by Taylor: 1) the salting out of proteins by strong brine in the frozen cells, 2) the contraction of the jelly and squeezing out of the then dilute liquid, and 3) autolysis of the cell membrane. The cell membrane would have remained undamaged by quick-freezing, and the juice would have been unable to escape through the membrane. The process of autolysis offered a possible explanation since it might account for digestion of the cell membrane or increased membrane permeability.

Denaturation of protein in fish muscle and fish muscle juice was reported by Riddell et al. (1937). The extent of denaturation was governed largely by storage temperature and length of the holding period. Later Reay (1933, 1934) emphasized the denaturation phenomenon by investigating the influence of freezing temperature on haddock. He found that muscle protein solubility in salt solutions decreased after the fish were held in frozen storage. The globulin (actomyosin) fraction was most affected and the change was greatest in range -2 to -6°C. He also concluded that the denaturation and deterioration, which occurred during thawing was similar to that which occurred during freezing.

Plank (1925) emphasized the importance of two effects of freezing: 1) the damage to the protoplasmic structure by formation of ice crystals, and 2) the

consequent dehydration of the colloid. It was pointed out by Callow (1952) that the salts in muscle juice become more concentrated during the formation of ice in muscle. Callow (1955) and Luijpen (1957) found that high concentrations of salt may be capable of denaturing some cellular proteins.

Dyer (1951) stated that the formation of ice crystal fragments in frozen fish was attributed to the dehydration of tissue cells. He also reported that the proteins present in the sol form in the fresh tissue were converted to gel form at the eutectic point by denaturation of the salts in the fresh tissue.

Smorodintsev and Bystrov (1937) reported that low temperature freezing (-25°C) resulted in no denaturation of muscle proteins as determined by ATP-ase activity or by the solubility and swelling of tissue in salt solution. Rapid freezing and thawing together with storage at low temperatures may be important in denaturation, since these conditions minimize changes due to denaturation of the proteins by strong salt solutions (Finn, 1932).

Deatherage and Hamm (1960) found that quick freezing and thawing of beef caused no significant decrease in hydration and no change in the isoelectric point of the meat; however, small but significant changes in acidic and basic groups of muscle proteins were noted.

While the overall extent of denaturation depended on the length of storage (Love, 1955) large differences in

protein denaturation were caused by different freezing rates of fish. Love (1956) concluded that an important factor which affected denaturation was the mode of ice formation, distribution and the resulting concentration patterns of tissue components rather than actual mechanical damage.

Love (1958a), in re-examining the question of tissue damage, reported that the appearance of deoxypentose-nucleic acid (DNA) in the expressible fluid from muscle indicated rupture of the sarcolemmas with liberation of nucleic materials. The presence of DNA provided a means of assessing the degree of cellular damage. As fish fillets were frozen more rapidly, the expressible nucleic acid suddenly reached a maximum; this was thought to correspond with the point of formation of intracellular ice crystals. The great damage reported in the ultra-rapid freezing range was thought to be due to a different type of cell damage, an expansion of the interior during cooling, and subsequent cracking of the hard outer shell which had formed earlier.

Using different freezing methods (solid CO_2 and plate at -78°C) Love (1958a) further examined the action of intercellular ice on muscle cells. He showed that DNA content in the drip corresponded with actual rupture of the sarcolemmas, both from histological evidence (Love, 1958a) and from the presence of cell particles in the expressible fluid (Love, 1958b). A zone of minimum damage

was found with a freezing rate of about 115 minutes, (Love, 1958a) the freezing time at which all ice was able to form in the intracellular spaces. At slower freezing rates (200 to 500 minutes) cell damage was found to be at a maximum value and then decreased. With very slow freezing, the strong salt solutions, which were created by the freezing out of water from the weak saline interstitial fluids, exercised a considerable solvent effect on the fibers without actually rupturing the cells. An increase in the protein concentration in the interstitial fluid tended to increase during very slow freezing (approximately 750 minutes).

Love (1962a) found that denaturation was less when the quantity of ice present at a given temperature was reduced, since a reduction in the amount of ice reduced the concentration of tissue salts. These results further supported the theory (Love, 1958c) that denaturation results directly or indirectly from the action of concentrated salts on the proteins.

Chicken muscle proteins undergo both denaturation and proteolysis during frozen storage according to Khan et al. (1963). Stepwise denaturation of actomyosin decreased ATP-ase activity and protein solubility. Proteolysis also occurred in both leg and breast muscle at -18, -10 and -4°C, and the rate of change depended directly on storage temperature and time. Bandack-Yuri and Rose (1961) reported that muscle proteases have a pH optimum of 4 to 7

and temperature optimum of 37°C. Since beef contains a protease active at freezing temperatures (Balls, 1938), it has been concluded that a similar enzyme system could possibly be present in chicken muscle (Khan et al., 1963). Khan and van den Berg (1965), in further investigation of changes occurring during storage, showed that there was a proteolytic enzyme active during frozen storage. This conclusion was based on an analysis of cooked meat stored at temperatures between -10 and -40°C for two years. They reported that since there was no formation of protein-breakdown products, as in raw meat, the enzymes must have been inactivated by heat.

Sawant and Magar (1961) studied the denaturation of proteins in frozen fish and concluded that denaturation was restricted to the actomyosin fraction of protein while the sarcoplasmic fraction remained unchanged. Dyer (1951) observed that the albumin fraction was not denatured during frozen storage, while Seagram (1958, 1959) suggested that the sarcoplasmic fraction was not associated with the formation of drip.

Denaturation and autolytic changes in compounds of muscle tissue during cooling and freezing of meat were studied by Pavlovskii (1962). Autolysis of muscle was characterized by a degradation resulting from the activity of ATP-ase and glycolytic conversion and resynthesis of adenasine triphosphate. Since freezing increased NaCl,

myosin and ATP-ase concentration, he concluded that denaturation of frozen muscle was characteristic of meat previously autolyzed before freezing.

Proteolysis in poultry during frozen storage was indicated by an increase in non-protein and soluble nitrogen of both light and dark meat (Swanson and Sloan, 1953). They also noted a decrease in amino acid nitrogen which suggested that some metabolic processes were being continued in the frozen state resulting in additional degradation of the amino acids formed during proteolysis.

The protein components of autolyzed muscle tissue were studied during cooling and freezing of meat by Pavlovskii and Grigor'eva (1963a). The amount of readily extractible protein from the tissues decreased during cooling and freezing when compared to that of unfrozen meat. However, during the initial storage of frozen meat the soluble protein increased, but after three months this fraction decreased.

Tarr (1947) found that undesirable changes in fish (formation of large ice crystals ruptured the cells during freezing) took place during freezing, with much of the deterioration resulting from a change (denaturation) in the myosin, the most important single protein present. This denaturation rendered the myosin insoluble. Denaturation occurred most rapidly between the temperatures of 30°F and 23°F although denaturation occurred slowly at much

lower temperatures. Tarr concluded that "the only practical method of retarding protein denaturation is by very rapid freezing and storage at a constant low temperature, preferably below -40°F."

Khan (1964) reported that the nonprotein nitrogen fraction of chicken increased noticeably during storage for 45 weeks at temperatures of -5 and -40°C. He found about 80 percent of the increase resulted from an increase in amino acids, mostly acidic, aromatic and sulfur containing amino acids. He also believed that limited proteolysis occurred as a result of the release of enzymes due to freezing.

Amount and Composition of Drip

Quantity of Drip

Cook et al. (1926) reported drip to be the clear, reddish-brown colored fluid which exudes from meat after it has been frozen and thawed. Sair and Cook (1938a) also reported that removal of skin, cutting of meat and particularly mincing the meat increased the susceptibility of frozen-defrosted tissues to drip. Reay (1934) reported that the quantity of expressible fluid from frozen fish depended on the number of cuts made in the flesh. However, they reported that the major factor determining the amount of drip in haddock was the time spent in the temperature range of -1 to -5°C, either during thawing or freezing.

Koonz and Ramsbottom (1939b) reported that the temperature of freezing affected the amount of drip obtained from frozen-defrosted poultry meat. This was based on the fact that the rate of freezing determined the size, distribution and number of ice nuclei formed. They reported that white meat frozen at 8°F was much more suceptible to drip loss than when frozen at -50°F. Callow (1952) and Empey and Howard (1954) similarly reported that slow freezing resulted in more drip.

The quantities of drip obtained from thawed beef decreased as freezing temperatures were lowered from 18 to -114°F (Hiner et al., 1945). Protein reabsorbtion of a large proportion of the water originally frozen out was believed to be the cause of reduced drip since there was increased intrafibrillar freezing and rupturing of the fibers at lowered freezing temperatures.

Pearson and Miller (1950) studied steaks which had been frozen at various temperatures. They reported that increased frozen storage time resulted in increased drip, which became apparent after 90 days storage. However, they found that rate of freezing did not alter the amount of expressible fluid. Ramsbottom and Koonz (1940) reported that the amount of drip from steaks was increased by increasing the period of time between slaughter and freezing.

Time of storage, at any one temperature, had little effect on the amount of drip (Moran and Hale, 1932) but

temperature of storage affected the amount of drip. They found no variation in drip due to thawing rate when using 10° for rapid thawing and 1°C for slow thawing. Moran (1932) reported that maximum drip was recorded when frozen tissues were stored 80 days at -2 to -3°C. He also found a denaturation effect on the protein at -1.5°C and at temperatures lower than -3°C.

Marion and Stadelman (1958) using different methods of freezing (brine, plate, and moving air) found that the method of freezing exerted no significant effect on the amount of drip from chicken fryers, fowl, turkey fryers and mature tom turkeys. They reported that drip from chicken fryers ranged from 5.3 to 5.8 percent; fowl, 5.2 to 5.6 percent; turkey fryers 4.3 to 5.3 percent; and mature turkeys, 2.3 to 3.0 percent.

According to Ramsbottom and Koonz (1939) small rapidly frozen steaks retained more drip on defrosting since intrafiber freezing occurred. Whereas in steaks which were slowly frozen, extrafiber freezing took place and upon defrosting more fluid was lost as drip before it could be reabsorbed by the partially dehydrated fibers. They also reported that irrespective of freezing temperature small rib cuts lost more fluid as drip than large rib cuts due to area of the cut surface in relation to the volume.

Moisture loss during the defrosting of turkey was affected significantly by the cooling and freezing treatment

(Spencer et al., 1956). They found that losses from air-cooled carcasses averaged lower than losses from carcasses cooled in ice water. They also found a higher weight loss from turkeys frozen at 0°F than from turkeys frozen at lower temperatures.

Bouton et al. (1958) found a gradual decrease in the amount of drip from psoas and l. dorsi muscles when the pH increased above 5.8. Maximum drip was obtained from meat in the pH range between 5.4 - 5.8. Howard and Lawrie (1956) found that the amount of drip exuded from l. dorsi was approximately twice that from psoas at the same pH. They concluded that different muscle proteins may be affected differently by the same pH during thawing.

The quantity of drip from meat frozen at a constant rate was affected by the pH of the tissue and the period of time between slaughter and freezing (Sair and Cook, 1938a). The quantity of drip was determined primarily by the amount of acid contained in the tissue, and increased as pH fell below 6.0. Maximum drip was obtained at pH 5.2 - 5.5, and when freezing rates were faster than 3 days (meat temperature changed from 0°C to -5°C) there was a reduction in the amount of drip obtained. The reduced amount of drip was attributed to the high water-retaining capacity of tissue proteins at pH 6.4 and was not affected by the size or number of ice crystals formed during freezing. A decrease in water-retaining power of the proteins occurred

at pH 5.2, and resulted in increased moisture losses. Rapid freezing reduced these losses due to the production of smaller ice crystals and a more uniform distribution of water during defrosting. The reduced moisture-retaining capacity at pH 5.2 - 5.5 was due to isoelectric conditions rather than to accelerated denaturation.

Ramsbottom and Koonz (1940) found that thawed beef with pH values between 6.2 and 6.5 produced only 0.7 percent drip compared with 4.3 percent drip for beef thawed at pH 5.7 - 5.9. Empey (1933) reported that pH was the major factor that influenced the amount of drip, and the lower the pH, the greater the volume of drip.

After examining the effects of freezing on the swelling of tissues in a buffered solution, Smorodintsev and Bystrov (1937) concluded that the quantity of fluid secreted from the tissue varied inversely with the degree of swelling. The swelling of frozen tissues increased with acidity of the medium (starting at pH 3.4) and a decrease was noted at pH values higher than 6.0.

According to Koonz and Ramsbottom (1939b) less drip was obtained from ground dark chicken meat than from ground white meat regardless of the freezing temperatures (-45.5°C, -26.1°C, -13.3°C). The pH value for meat 2.3 hours after slaughter was 5.7 for the white meat and 6.1 for dark meat, and after 22 days storage the pH values were 6.2 and 6.3,

respectively. The greater reabsorption capacity exhibited by the dark muscle was thought to be due to the higher pH values.

Wladyka (1965) reported that larger amounts of drip were obtained from white meat than dark meat and that the amount of drip increased with storage. He found that the percentage of drip obtained from white meat after 30 and 90 days storage was 5.4 and 9.6 percent respectively, while that obtained from dark meat was 4.8 and 7.9 percent, respectively.

Kaloyereas (1947) found that rapid freezing was less effective in decreasing drip loss from products with high boundwater content than from products with low boundwater content.

The amount of drip obtained is closely related to the water-holding capacity (WHC) of meat, Hamm (1953, 1958). When a high WHC was observed in meat before freezing, comparable WHC was noted after defrosting. Whitaker (1959) found that pH affects the water-holding capacity of the meat by modifying the charges of the protein. Meat with a pH in the range of its isoelectric point (5.0 - 5.5) had a greater drip loss after freezing and thawing than meat with a higher pH (Kuprianoff, 1952). He also found little or no drip from meat defrosted in the pH range of 6.3 - 6.4.

Drip losses from frozen aged meat were much less than those from frozen rigor muscles (Wierbicki et al.,

1957b; Bouton et al., 1958) since the aging of meat resulted in increased water-holding capacity (Hamm, 1960).

When whole animals or quarters of beef were infused with NaCl, the water-holding capacity of the meat was markedly increased so that little or no drip occurred after freezing and thawing (Wierbicki et al., 1954).

The effect of added cations on meat shrinkage at 70°C was studied by Wierbicki et al. (1957a). They found that the cations sodium, calcium, potassium, and magnesium increased the water-holding capacity of meat and that NaCl, added to the meat prior to freezing, decreased the amount of drip. They also stated that the pH shift toward alkalinity, produced by the addition of NaCl, decreased after freezing and thawing, indicating a possible protein modification due to freezing.

Howard and Lawrie (1957) reported that there were two ways to reduce drip from frozen beef muscle on thawing, depending on biochemical factors. Their importance was suggested from fundamental work in vitro (Empey, 1933; Bendal and Marsh, 1951; Marsh, 1952a, 1952b). Apparently these factors enhance the capacity of muscle proteins to retain fluid. The factors were:

a. Reduction in the rate of ATP breakdown during the onset of rigor mortis.

b. Restriction of postmortem production of lactic acid from glycogen which resulted in a high ultimate pH of the muscle tissue.

Radouco-Thomas et al. (1959) reported on the injection of epinephrine on skeletal muscles of rat, rabbit, sheep and pigs. They found that epinephrine prevented the autolytic process from setting in and prevented an increase in build up of lactic acid, pH drop, and reduced exudation. Anglemier et al. (1961), deFremery (1963), deFremery and Pool (1963) found similar results with pork and poultry respectively.

Bendall and Lawrie (1962) reported the effects of ante-mortem injections of various drugs on post-mortem changes in muscle. Injection of adrenalin 4 hours before slaughter significantly depleted muscle glycogen in rabbits and raised ultimate muscle pH. Hedrick et al. (1957) reported that injection of epinephrine 24 and 12 hours prior to slaughter significantly elevated the ultimate pH of bovine muscle. pH of the control animals ranged from 5.45 to 5.7 and for the adrenalin treated animals, pH ranged from 6.0 to 6.15. The accepted explanation for this effect is that adrenalin causes activation of phosphorylase so that glycolysis proceeds more rapidly along the anaerobic pathway to lactic acid (Cori and Illingworth, 1956).

Iodoacetate was shown by Padieu and Mommaerts (1960) to inhibit the action of the enzyme phosphoglyceraldehyde dehydrogenase and therefore eliminate glycolysis. deFremery (1963) and deFremery and Pool (1963) investigating factors affecting poultry tenderness, found a high ultimate pH

after an injection of iodoacetate and a reduction in the amount of fluid exuded during defrosting. deFremery and Pool (1960) found similar results using sodium monobromoacetate which inhibited glycolysis.

Partmann (1963) reported that when muscle tissue was frozen immediately after death, the ATP present at that moment persisted. When the temperature increased sufficiently after thawing, the ATP was split quickly by contraction of the tissue and produced thaw rigor. The muscle contracted during thawing to about 50 percent of its former length and was accompanied by a high drip loss. Marsh and Thompson (1957) suggested that this was the result of cell membrane rupture. Love (1962b) reported that pre-rigor frozen fragments had about 60 percent lower soluble-protein values after thawing, and suggested that the exudation resulted from a change in the proteins which reduced their ability to bind water.

Composition of Drip

Godeaux (1957) stated that the fluid which escaped from muscle during thawing, due to cell membrane destruction, contained large amounts of proteins (myogen and globulin x). Pearson et al. (1959) reported that the amino acid content of drip from thawed pork indicated losses ranging from 7.15 percent for tryptophane to 11.08 percent for isoleucine. They also stated that the losses did not

appear to be associated with the water solubility of the individual amino acids which indicated that the losses in drip may have been due in part to the leaching of more complex substances.

The concentration of essential amino acids in the drip from dark and light chicken meat increased as the time of frozen storage increased (Wladyka, 1965). Larger quantities of essential amino acids were detected in drip from frozen white meat than from frozen dark meat after both 30 and 90 days of storage. When reported as the percentage of total protein contained in the drip, arginine values ranged from 2.80 to 6.05 percent, and histidine from 4.70 to 12.83 percent after 30 and 90 days storage, respectively. Arginine and histidine content of drip from light meat varied from 3.63 to 7.26 percent, respectively after 30 days storage, whereas after 90 days storage, the values varied from 8.25 to 16.45 percent for isoleucine and histidine, respectively.

Khan and Lentz (1965) reported on the influence of pre-rigor, rigor, and post-rigor freezing on drip losses and protein changes in chicken meat. They found an average of 0.8 ml drip released per 100 grams carcass and 5.9 mg total nitrogen released per 100 grams of carcass in post-rigor chicken muscle. Nonprotein nitrogen accounted for 35 percent and protein nitrogen, 65 percent of the total nitrogen.

According to Wladyka (1965) the drip from the white meat contained larger quantities of protein than drip from dark meat, after storage periods of 30 or 90 days. He reported that the drip from white meat held for 90 days contained 10.92 percent protein nitrogen and that stored 30 days, 9.17 percent. Similarly, the protein nitrogen content of drip from dark meat stored for 90 days (6.26 percent) was larger than the protein content of drip from dark meat stored for 30 days (5.16 percent).

Love (1958a) investigated cell damage in fish muscle during slow freezing and reported solids and ash content. Solid matter varied from 7.29 to 8.47 grams per 100 grams of expressible fluid with freezing rates of 2 and 47 minutes, respectively. Ash content in the expressible fluid did not vary greatly regardless of the freezing rate (1.27 to 1.34 grams per 100 grams of expressible fluid with freezing rates of 2 and 42 minutes, respectively). Love (1955) reported the solids and nitrogen content of expressible fluid from unfrozen fillets used as controls. Total solids and nitrogen were 8.20 and 1.15 grams per 100 ml of expressible fluid, respectively.

Proteolytic changes in meat during storage and during defrosting were studied by Pavlovskii and Grigor'eva (1963b). Electrophoretic patterns were similar for the extractible proteins of meat and drip. Loss of protein in the drip was 15-22 percent greater for meat subjected to

preliminary autolysis before freezing than for quick-frozen meat. The proteins of the myoplasmic tissue increased in the drip during the initial storage period. After three months of storage, no increase in volume of drip was observed, and the amount of myoplasmic proteins in the drip decreased.

Seagram (1958) reported that drip in fish was due, at least in part, to the denaturation of proteins which normally hold the water in the muscle. Using dilution techniques and electrophoresis, he showed a definite similarity between the protein composition of drip and extracts of low ionic strength from fish muscle. He found the contractile protein, actomyosin, absent in drip from frozen and thawed rockfish. He concluded that the sarcoplasmic fraction of fish muscle was not closely associated with the origin of drip, and suggested that drip formation and texture changes, as a result of freezing and thawing, were due in part to actomyosin denaturation by a dehydration process.

Howard et al. (1960) found a higher concentration of both hemoglobin and myoglobin in drip than in whole muscle. Higher hemoglobin presumably came from the residual fluid in the vascular system. This hemoglobin containing fluid moved more readily through the sample than fluid contained within the cells and interstitial spaces. A similar increase in concentration of myoglobin was expected when

the pigment was confined to a portion of the cell fluid which moved more readily than that associated with the fibrillar protein.

Paper electrophoresis and ultracentrifugation results indicated that drip has patterns similar to those from low ionic strength extracts of muscle (Howard et al., 1960). Since freezing and thawing modified the solids content of the drip, they did not modify the composition of these solids. Modification brought about by freezing were largely restricted to structural proteins which were insoluble at the ionic strength of the drip fluids.

Gas Chromatography of Amino Acids

A great number of gas chromatographic methods and techniques have been investigated to determine amino acid composition of products, both qualitatively and quantitatively. Hunter et al. (1956) established the beginning of extensive gas chromatographic analyses of amino acids by using oxidation with ninhydrin to prepare volatile aldehydes from several aliphatic amino acids. Later the procedure was developed (Zlatkis, 1960) into a fully automated method for the analysis of seven amino acids. Numerous other approaches have been evaluated, including decarboxylation to give volatile amines (Bier and Teitelbaum, 1959), and conversion to methyl-chloro esters (Melamed and Renard, 1960). Bayer et al. (1957) reported that methyl esters of

a number of aliphatic amino acids, prepared by the Fischer method, could be separated by gas chromatography. Successful chromatography of the ester hydrochlorides of 14 amino acids, by adding ammonia to the carrier gas, was reported by Saroff et al. (1962). Nicholls et al. (1963) explored the possibility of chromatographing the acid salts of amino acid methyl esters by operating the flash heater at a sufficiently high temperature to cause disassociation of the free esters. Youngs (1959) was one of the first to recognize the advantages of N-substituted esters of amino acids for gas chromatographic analysis. He reported that chromatographic peaks had been obtained for the n-butyl N-acetyl esters of glycine, alanine, valine, leucine, isoleucine, and proline. Johnson et al. (1961) investigated the preparation and gas chromatographic separation of the n-amyl N-acetyl esters of amino acids. Chromatographic peaks were obtained for the derivatives of 36 amino acids: however, difficulty was experienced with tryptophan, histidine, cystine, and arginine. An important part of the investigation was the use of columns containing small percentages of liquid phase at relatively low temperatures, to give fair separation.

Graff et al. (1963) considered the n-propyl N-acetyl esters of the amino acids to be the best derivatives for chromatography because of their ease of separation. However, after examination of the properties of a number of the

derivatives, Blau and Darbre (1963) found that the n-amyl N-trifluoroacetyl esters were the most satisfactory. It was reported by Zomzely et al. (1962) that the n-butyl N-trifluoroacetyl esters of 22 naturally occurring amino acids could be prepared and that they could be chromatographed on a single column by means of temperature programming. Separation of the derivatives was fairly complete, however, no quantitative evaluation of the procedure to prepare the derivatives was made.

Pisano et al. (1963) and Landowne and Lipsky (1963) investigated the gas chromatographic behavior of the phenylthiohydrantions and methyl 2,4-dinitrophenyl esters; the latter derivatives were investigated using an electron capture detector. Trimethyl N-trimethylsilyl amino acid derivatives were separated by Rühlmann and Giesecke (1961) but the derivatives were not sufficiently stable for quantitative analysis. Shahrokhi and Gehrke (1967 and 1968) used trimethyl esters to analyze for sulfur and iodine containing amino acids and Ruyle et al. (1967) quantatively analyzed for purine and pyrimidine bases as the trimethylsilyl derivatives. An ultramicromethod for analysis of amino acids with a minimum detectable limit of 5×10^{-12} g injected was also developed by Ertingshauser (1967). He used the N-diethyl phosphate amino acid methyl esters of the amino acids.

Methyl N-trifluoracetyl esters were investigated by Weygand et al. (1960), Saroff and Karmen (1960) and Hagen and Black (1964). All of the common protein amino acids and ornithine were chromatographed by Cruickshank and Sheehan (1964) after conversion to their methyl N-trifluoroacetyl esters. They did not, however, study the quantitative aspect of the derivative procedure.

Analyses of amino acids as their N-trifluoroacetyl n-butyl esters by Lamkin and Gehrke (1965) and later by Gehrke and Stalling (1967) has led to a most important improvement and advancement in the analyses of amino acids by gas chromatography. They were able to quantatively analyze the 20 common amino acids with fair separation on 0.5% neopental glycol sebacate. However, Stafanovic and Walker (1967), using 0.35% ethylene glycol adipate were able to obtain better separation for 17 amino acids and by using an OV-17 column in conjunction, they were able to obtain good separation, as well as good resolution. Satterlee and Lillard (1967) reported on GLC analysis of free amino acids in meats using the method of Lamkin and Gehrke (1965). They found the procedure satisfactory for the analyses of amino acids after they had removed all the carbohydrates, proteins and 98 percent of the inorganic salts. Consistancy in their work was acceptable, however there was no comparison made to the accepted method of the amino acid analyzer. Another application of GLC analysis

for protein was made (Ward et al., 1967) using the n-amyl N-acetyl derivatives for analysis of the amino acids in actinomycin hydrolysates. They reported that the minimal detectable limit was at least 10^{-9}mole.

Ikehawa et al. (1966) reported on a method for the separation and estimation of DNP derivatives for 13 amino acids of serum by GLC. However, no information was given as to the accuracy of the method.

EXPERIMENTAL PROCEDURE

Experimental Animals

Broad Breasted White turkeys, 20 weeks of age, and raised on the same ration at the Michigan State University Poultry Farm were used in this study. One group of eight birds received no treatment prior to slaughter and served as the controls. Two other groups of five birds each were treated with either adrenalin (as the hydrochloride; Parke, Davis and Company) or iodoacetate (as the sodium salt; Eastman Kodak) prior to bleeding to minimize post-mortem glycolysis (deFremery, 1963). Each bird in one group received a 1.5 mg subcutaneous injection of adrenalin per kg of body weight and was fasted for 20 hours prior to bleeding. Each bird in the other group received a 200 mg intravenous injection of iodoacetate per kg body weight, prior to bleeding.

All turkeys were subjected to an electric shock prior to bleeding to prevent excessive activity, bled, scalded in a Rotomatic Scalder, and defeathered in an automatic rubber fingered picker. All the birds were then eviscerated, washed, and chilled in slush ice for 24 hours. After the chilling period the Pectoralis major and minor

muscles and the thigh muscles were carefully removed from the birds to insure a minimum of connective tissue (tendons) in the samples. After removal of the meat from each bird it was pooled into samples of all breast and all thigh meat and ground with an auger type meat grinder through a 64mm (1/4 inch) plate. The breast and thigh meat samples were formed into slabs approximately 15 x 20 x 4 cm (6 x 8 x 1 1/2 inch) and vacuum packed separately in Cryovac bags. The samples were frozen and stored at -20°C for further analyses. Samples were held in storage for periods of 0, 30 and 90 days to evaluate the effect of storage on protein change.

General Methods Used

Nitrogen Determinations

All nitrogen values were determined using the micro-Kjeldahl method outlined by the American Instrument Company (1961). Nitrogen contents were reported as mg of nitrogen per ml of solution or per g of tissue. All nitrogen determinations were run in duplicate.

pH

All pH measurements were made with a Corning Model 10, Expanded Scale pH Meter and read to the nearest one-hundredth unit. A 5g sample of meat was blended with

40 ml of deionized water for 1 minute and the pH of the sample read.

Collection of Drip

The method of drip collection was similar to that reported by Pearson et al. (1959), Wladyka (1965) and Crigler (1968). The collection apparatus consisted of a V-shaped trough lined with aluminum foil, which served as a funnel for the collection of the drip in a beaker. A wire grid was placed over the funnel to hold the meat above the aluminum foil and allow the drip to be exuded. The entire device was then placed in a disconnected freezer chest which had a layer of water on the bottom to provide a saturated atmosphere.

The previously frozen white and dark meat samples were placed on the grids for thawing after the packaging material had been removed and the samples were weighed. After the thawing period of 36 hours at 5°C, each meat sample was reweighed and the amount of drip measured to the nearest ml. The drip was then frozen and held for further analyses.

Muscle Protein Fractionation

After thawing, the meat samples were again throughly mixed. A modified extraction procedure of Fischer (1963) and Maier and Fischer (1966) was used to fractionate the

muscle proteins of turkey meat (Figure 1). Five g samples of meat were weighed and blended at high speed with a Model 1042, two speed Delux Waring-Blendor. Blending was conducted in a semi-micro waring blendor jar with approximately 40 ml of deionized water. A 50 ml volumetric flask was placed in the blendor jar to prevent the formation of a vortex, which leads to excessive protein denaturation. The blended samples were then transferred to a 250 ml centrifuge bottle and the blendor jar washed twice with approximately 30 ml of deionized water to make a final volume of approximately 100 ml. The centrifuge bottles were placed in a refrigerated Model RC2-B Sorvall centrifuge and centrifuged in a Type GSA head for 10 minutes at 10,000 rpm. After centrifugation, the supernate was decanted into a 100 ml graduated cylinder and read to the nearest 1.0 ml. The residue from the water soluble fraction was placed in a semi-micro Waring Blendor jar and approximately 40 ml of KCl-PO_4 buffer (ionic strength of 1.00, pH 7.50, 0.01 Mole KF/liter) was added and the sample blended for 3 - 5 seconds at low speed. The blendor jar was washed several times with the buffer solution and the washings were added to the blended portion of the residue in a 250 ml centrifuge bottle to give a final volume of approximately 200 ml. After standing for 4 hours at 1°C the samples were centrifuged at 3,000 rpm for 10 minutes. The supernate was decanted off, measured to the nearest

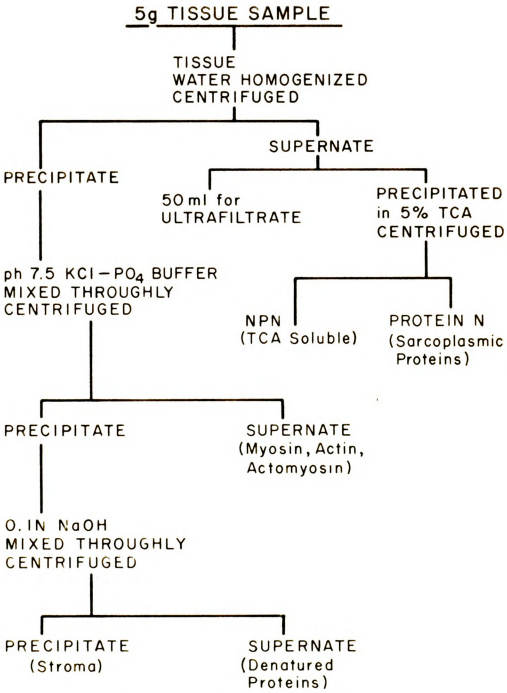


Figure 1. Outline for the fractionation of turkey muscle proteins.

1.0 ml, and the residue suspended in approximately 100 ml of 0.1 N NaOH. The NaOH soluble fraction was separated from the NaOH insoluble fraction, after standing 4 hours, by centrifugation at 3,000 rpm for 10 min. The volume of supernate was read to the nearest 1.0 ml.

As a means of quantitation of each fraction, and to determine the difference between treatments, total nitrogen was determined on each fraction and each meat sample.

In an attempt to follow the change in non-protein nitrogen (NPN) during storage, and to evaluate treatment differences, NPN was determined on the water soluble fraction. NPN was determined by pipetting out 10 ml of the water soluble fraction and adding 10 ml of 10 percent trichloracetic acid (TCA) to give a final concentration of 5 percent TCA. After mixing, the above mixture was centrifuged at 3,000 rpm in a Type SS-34 Sorvall centrifuge head in 50 ml centrifuge tubes for 10 min.

To determine how proteolytic activity (which occurres during storage) was affected by treatment, an ultrafiltrate of the water soluble fraction was obtained. The ultrafiltrate was obtained by pipetting 50 ml of the water soluble fraction into a 4.76 cm (1 7/8 inch) dialysis tube and the dialysis tube was placed in a 250 ml polyethylene screw cap centrifuge bottle with the tapered part of the bottle removed. To increase the surface area and the speed of diffusion of the free amino acids and peptides

through the tube, approximately 4 cm of 6 mm glass beads were placed in the bottom of the centrifuge bottle. After placing the filled dialysis tube in the centrifuge bottle the sample was centrifuged at 3,000 rpm in a Model CS International Centrifuge for approximately 5 hr or until a volume of 10 - 15 ml was collected. The samples were centrifuged (in a walk-in cooler) at 1°C. After collection of the ultrafiltrate, it was placed in sample containers and stored at -20°C for later amino acid analysis by GLC.

Amino Acid Analyses by Gas-Liquid Chromatography

Preliminary Investigations

An F & M, model 810, gas chromatograph (GLC) equipped with dual hydrogen flame detectors was used for the amino acid analysis by GLC in this study. Temperature data, program rate, and column packing material parameters were obtained from Gehrke and Stalling (1967). From the parameters obtained from their work the following optimum conditions for gas chromatography were determined and are reported below:

Column: 3-mm I.D. x 6 ft coiled borosilicated glass

Column packing: 0.5% neopentyglycol sebacate (Analabs) on 80/100 mesh Chromosorb G (Johns-Manville)

Conditions: Column temperature: Initial 70°C, final 220°C
Program rate: 2°C/min
Injection port temperature: 230°C
Detector temperature: 250°C
Carrier gas and flow rate: Helium, 40 ml/min
Hydrogen flow rate: 50 ml/min
Air flow rate: 550 ml/min
Sensitivity: 10/4
Chart speed: 0.63 cm/min

Commercially prepared N-trifluoroacetyl n-butyl esters (Regis Chemical Company) of the 14 amino acids to be investigated in this study were used to determine the elution sequence. The elution sequence was determined by injecting one amino acid and then recording the position of elution. Amino acids were injected in the order of expected elution by the work of Gehrke and Stalling (1967). A second amino acid was then added to the first and mixed, after which the position of the second peak was recorded. This procedure was continued until the position of each amino acid in the sequence was determined.

Calibration of the detector and recorder systems used for quantitative analysis was necessary before evaluation of the procedure could be conducted. This was done by calculating factors which related peak height per weight of internal standard to the peak height per weight of amino acid to be analyzed. The commercially prepared N-trifluoroacetyl n-butyl derivatives of the amino acids which contained a known concentration (0.05M) were mixed with the internal standard, butyl stearate (1 mg/ml

n-butanol), and analyzed by GLC. An appropriate factor for each amino acid was calculated from the resulting peak heights of the N-trifluoroacetyl n-butyl derivatives by the following equations:

$$\text{Factor} = \frac{\text{wt. of amino acid x peak height of internal standard}}{\text{peak height of amino acid x wt. of internal standard}}$$

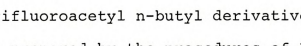
To quantitate, the equation was rearranged as shown below:

$$\text{Wt. of amino acid} = \frac{\text{peak height wt. of}}{\text{factor x of amino acid x internal standard}} \Big/ \text{peak height of internal standard}$$

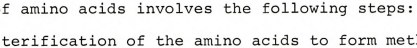
Derivative Formation

The N-trifluoroacetyl n-butyl derivatives of the amino acids were prepared by the procedures of Lamkin and Gehrke (1965) and Gehrke and Stalling (1967). The derivatization of amino acids involves the following steps:

1. Esterification of the amino acids to form methyl ester hydrochlorides.



2. Interesterification of the methyl ester hydrochlorides to form n-butyl ester hydrochlorides.



3. Acylation of n-butyl ester hydrochlorides with trifluoroacetic anhydride to form N-trifluoroacetyl n-butyl esters.

$$(CF_3CO)_2O + \underset{\underset{NH_3^+Cl^-}{|}}{RCHCOOC_4H_9} \xrightarrow[5\ min]{150°C} \underset{\underset{NHCOCF_3}{|}}{RCHCOOC_4H_9}$$

Methyl ester formation was necessary to achieve solubility of lysine in n-butanol.

The following procedure was used to form derivatives:

1. Esterification of the amino acids with methanol.
   a. To a dried amino acid sample 10 ml of 1.25N HCl in methanol (Regis Chemical Company) was added for each 60 mg of amino acids.
   b. A 24/40 standard tapered erlenmeyer flask was stoppered and stirred with a magnetic stirrer for 30 minutes at room temperature.
   c. The methanol HCl was removed with a rotary evaporator at 60°C.

2. Interesterification of methyl ester hydrochlorides.
   a. 10 ml of 1.25N HCl in n-butanol (Regis Chemical Company).
   b. Add an exact amount of internal standard (butyl stearate).
   c. The flask was placed in 100°C oil bath with a cold water condensor positioned in the flask and a $CaSO_4$ drying tube in the end exposed to the air. The temperature was maintained and the sample agitated for 2 1/2 hours by a Fisher Thermomix magnetic hot plate.
   d. The butanol HCl was removed with a rotary evaporator at 60°C.

3. Acylation of n-butyl ester hydrochlorides.
   a. 4 ml of 25% by volume of trifluoroacetic anhydride in methylene chloride (Regis Chemical Company) were added to the flask.
   b. Sample was stirred at room temperature for 15 minutes.

c. One ml of the solution was transferred to a high-temperature acylation tube and heated for 5 minutes at 150°C in an oil bath (immersed only to the sample liquid level).
d. After cooling to room temperature the sample was ready for analysis by GLC.

Recovery of Amino Acid Derivatives

An amino acid standard solution and a protein hydrolysate of each bovine serum albumin and a water soluble protein extract of turkey meat were used to quantitatively evaluate the derivative formation as follows:

1. Amino acid standard solution

The accuracy of the method was evaluated by first preparing a standard solution of amino acids (Nutritional Biochemicals Corporation) with a known concentration of 0.5 μM per ml. Five ml of the standard solution were pipetted into a 125 ml standard tapered erlenmeyer flask and lyophilized.

The accuracy of the method was determined for the amino acid standard solution using the factors obtained from the commercially prepared N-trifluoroacetyl n-butyl derivatives and the procedure outlined previously. In addition, the amino acid standard solution was analyzed by the amino acid analyzer and the percentages of amino acids recovered by each method were determined.

A Beckman/Spinco, Model 120, Amino Acid Analyzer was used to obtain the amino acid ion-exchange values in this study. Samples were prepared according to the method

of Spackman (1960). Protein hydrolysates containing 3 to 4 mg protein were transferred quantitatively to a 25 ml pear-shaped flask fitted with a ground glass joint. Norleucine was added to the hydrolysate before transferring in order to check for transfer losses. The hydrolysate was evaporated to dryness on a rotary evaporator. The residue was taken up in a small amount of deionized water and redried. Five ml of citrate-HCl buffer (pH 2.2) was added to the dried hydrolysate. When all the soluble material had dissolved the sample was ready for analysis on the amino acid analyzer. Standard amino acid mixtures were analyzed with the same ninhydrin solution within four or five days.

2. Purified protein

The amino acid content of the purified protein was determined by the GLC procedure, and values obtained were compared with ion-exchange data from similar samples. A 100 ml solution containing 4 mg of bovine serum albumin (Nutritional Biochemicals Corporation) per ml of water was prepared to evaluate the method. Two ml of the solution were pipetted into a 20 ml ampoule with enough concentrated HCl added to give a final concentration of 6N HCl. After making to 6N with HCl the ampoule was evacuated and sealed under vacuum. The sample was hydrolyzed in a forced air oven at 110°C for 20 hours. Before analysis by GLC, the sample was analytically transferred from the ampoule to a

125 ml standard tapered 24/40 erlenmeyer flask. The HCl was removed on a rotary evaporator using a 60°C water bath. Derivative formation of the amino acids was conducted on the entire 2 ml sample in the same manner as for the amino acid standard solution. A short Dowex 50 column was used to determine if the recovery of the amino acids from bovine serum albumin could be improved by removing interferring substances. The use of a Dowex 50 column for biological systems was recommended by Gehrke and Regis Chemical Company (1967).

3. Water soluble fraction of light turkey meat

After evaluation of the GLC method for the purified protein, it was applied to the water extractible protein fraction from light turkey meat. A 125 g sample of breast meat from turkey was blended in a Waring Blendor with 1500 ml of water. After blending, the suspension was centrifuged in a Model RC2-B Sorvall Automatic Refrigerated Centrifuge using a Type GSA head at 10,000 rpm. The supernate was decanted off, frozen and held at -20°C for further analysis.

The water extracted protein was analyzed by the amino acid analyzer to determine the concentration of the various amino acids. Amino acid analysis by GLC was then conducted on 3 ml samples of the water soluble protein, which were hydrolyzed with 6N HCl in an evacuated ampoule for 20 hours at 110°C. HCl was removed from the sample by

a rotary evaporator at 60°C and analyzed by GLC after derivative formation. Since there were numerous interferring substances which resulted in extraneous peaks and poor recovery of the amino acids, a purification procedure was required. In an attempt to clean up the samples prior to GLC analyses, and obtain better quantitative results, the following procedures were tried:

1. a. Ether extraction of protein hydrolysate
   b. Ether extracted protein hydrolysate placed on a short Dowex 50W-X12 (H^+ form) column to remove acids, sugars and other neutral contaminants by absorbing the amino acids on the column and eluting with an aqueous ammonium hydroxide.

2. The effluent from 1 was placed on a short Dowex 2-X8 (OH^-form) column and eluted with HCl.

3. Steam was bubbled through the hydrolyzed samples to remove amines and other volatiles.

4. Samples were mixed with activated charcoal and filtered through Whatman No. 2 filter paper.

The above purification procedures did not result in satisfactory recovery of all amino acids when compared to the results obtained from the amino acid analyzer. However, since the recovery data obtained by the use of activated charcoal was comparable to other purification procedures, and required less sample preparation time, it was used in this study.

The entire hydrolyzed sample was filtered through the activated charcoal and dried with the aid of a rotary evaporator at 60°C to remove the water and HCl. Analysis

was then carried out on the protein hydrolysate using the same procedure as for the amino acid standard solution.

Amino Acid Analyses of Samples from Experimental Animals

Water Soluble Fraction

Ten ml of the ultrafiltrate were pipetted into a 20 ml ampoule and hydrolyzed in 6N HCl at 110°C for 20 hours. The hydrolysate was then mixed with activated charcoal and filtered into a 125 ml standard tapered erlenmeyer flask. To insure complete transfer of the hydrolysate to the flask, the ampoule and charcoal were washed with 5 separate 10 ml washings of deionized water which were collected in the flask. The sample was dried using a rotary evaporator and water bath at 60°C. After drying, the sample was analyzed by GLC to quantitate the amino acids as outlined in the derivative formation section.

Drip

Five ml of the drip were pipetted into a 20 ml ampoule and hydrolized in 6N HCl at 110°C for 20 hours. The hydrolysate was then mixed with activated charcoal and filtered into a 100 ml volumetric flask and made up to volume after 5 separate 10 ml washings with deionized water. A 3 ml aliquot from the 100 ml was dried and analyzed by GLC using the procedure for derivative formation previously outlined.

RESULTS AND DISCUSSION

Evaluation of Amino Acid Analysis by Gas-Liquid Chromatography

Preliminary Investigations

The elution sequence of the amino acids and internal standard on a column packed with Chromosorb G and coated with 0.5 percent neopentylglycol sebacate is shown in Figure 2. Good separation and resolution were obtained for all the amino acids investigated, except for the tendency of glycine and isoleucine to overlap with increased use of the column. Good separation was obtained by Stafanovic and Walker (1967) using a dual type column system consisting of one column packed with 0.325 percent ethylene glycol adipate on Chromosorb G and a second column containing 2.5 percent OV-17 on Chromosorb G. Samples injected on the first column were programmed at 4°C per minute to 210°C and then the temperature reduced to 125°C and another portion of the sample injected on the second column and programmed to 215°C at 6°C per minute. This procedure, although it required a two column system, has been shown to have substantial advantages over the other systems investigated for complete amino acid analysis.

Figure 2. Chromatogram of the sequence and temperature of elution for 14 N-trifluoroacetyl n-butyl amino acid derivatives and internal standard.

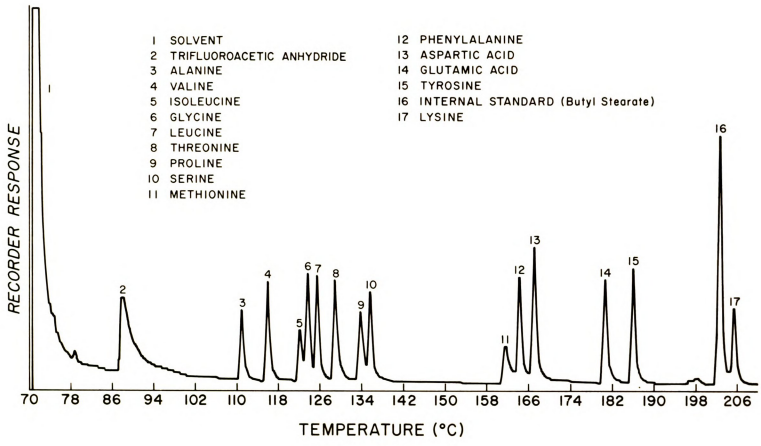

1 SOLVENT
2 TRIFLUOROACETIC ANHYDRIDE
3 ALANINE
4 VALINE
5 ISOLEUCINE
6 GLYCINE
7 LEUCINE
8 THREONINE
9 PROLINE
10 SERINE
11 METHIONINE
12 PHENYLALANINE
13 ASPARTIC ACID
14 GLUTAMIC ACID
15 TYROSINE
16 INTERNAL STANDARD (Butyl Stearate)
17 LYSINE
RECORDER RESPONSE
70 78 86 94 102 110 118 126 134 142 150 158 166 174 182 190 198 206
TEMPERATURE (°C)

Figure 2

Quantitation factors for the amino acids were obtained using the commercially prepared N-trifluoroacetyl n-butyl derivatives of the amino acids. These factors are reported in Table 1. The use of these factors for quantitation should give repeatable results since an extremely small standard deviation was obtained among replicate samples for all amino acid derivatives except leucine, threonine and methionine. Relatively low standard deviation among replicate samples were obtained for these latter amino acids.

Evaluation of Procedure

The accuracy and precision of the method for quantitating amino acids were evaluated using a standard amino acid solution containing 0.5 μM of each amino acid per ml of solution. The amino acid analyzer was used to determine the actual concentration by an established and accepted procedure. Results of the amino acid concentrations by both GLC and the amino acid analyzer are shown in Table 2. Both procedures gave good recoveries of each amino acid when values were compared to the actual amount added. Variation among replicate samples was small when analyzed by GLC, as shown by the small standard deviation obtained for each amino acid. The least variation among replicates was found for glycine and largest variation for isoleucine.

Table 1. Experimentally derived factors used for quantitation of specific amino acids by GLC

Amino acid	Factor 1/	Standard deviation
Alanine	1.010	.010
Valine	.934	.010
Isoleucine	2.059	.231
Glycine	.505	.052
Leucine	1.034	.390
Threonine	.921	.271
Proline	1.272	.064
Serine	.920	.089
Methionine	1.587	.307
Phenylalanine	1.088	.104
Aspartic acid	.713	.094
Glutamic acid	1.006	.058
Tyrosine	1.046	.034
Lysine	1.227	.106

1/ Average of eight independent determinations.

Table 2. Percentage recovery of specific amino acids from a standard solution by GLC and amino acid analyzer

Amino acid	mg added [1]	Amino acid recovered by GLC: Av. mg [2]	Percent	Amino acid recovered by ion exchange: Av. mg [3]	Percent
Alanine	44.5	44.2 ± .055	99.3	43.6	98.0
Valine	58.6	58.8 ± .089	100.3	57.3	97.8
Isoleucine	65.6	67.1 ± .780	102.3	65.3	99.5
Glycine	37.5	36.8 ± .013	98.1	35.9	95.7
Leucine	65.6	64.3 ± .092	98.0	65.0	99.1
Threonine	59.6	56.0 ± .074	94.0	54.8	91.9
Proline	57.6	52.8 ± .195	91.7	54.5	94.6
Serine	52.5	54.0 ± .113	102.9	54.1	103.1
Methionine	74.6	71.4 ± .169	95.7	72.2	96.8
Phenylalanine	82.6	83.7 ± .147	101.3	82.9	100.4
Aspartic acid	66.6	65.4 ± .135	98.2	63.6	95.5
Glutamic acid	73.6	63.4 ± .059	86.1	72.5	98.5
Tyrosine	90.6	90.8 ± .065	100.2	87.6	96.7
Lysine	58.5 [4]	55.8 ± .101	95.4	58.8	100.5

1/ 0.5 μM per ml solution of standard amino acids.

2/ Average of seven replicates.

3/ Average of two replicates.

4/ Corrected value since Lysine was added as L-Lysine monohydrochloride.

Recovery and quantitation of the amino acids from bovine serum albumin were more variable between GLC and amino acid analyzer procedures (Table 3) than results obtained from the standard amino acid solution. The recovery of threonine and phenylalanine (neutral and aromatic, respectively) were not accurate since values were 147.2 and 243.7 percent, respectively. Recovery of leucine (62.7 percent) and aspartic acid (50.0 percent); neutral and dicarboxlyic, respectively, on the other hand, were incomplete with low values obtained. The reasons for the high recoveries of threonine and phenylalanine, or the low values for leucine or aspartic acid are not known since the amino acids were from hydrolysates of a purified protein, hydrolized under the same conditions for both methods of analysis. These results differ from results of Gehrke and Stalling (1967) based on comparisons between ion exchange and GLC values. Their GLC values were within 1 percent absolute of the analyzer results for bovine serum albumin, kappa casein and purified soy-protein.

Amino acid values obtained from the water soluble fraction of light turkey meat by GLC and the amino acid analyzer (Table 4) varied considerably. Figure 3 shows a chromatogram of the separation and resolution of the amino acids from the water soluble protein extract. Only 6 of the 14 amino acids were recovered with satisfactory results. These 6 amino acids were alanine, valine, proline, glutamic

Table 3. Percentage recovery of specific amino acids from a solution of bovine serum albumin by GLC and amino acid analyzer

Amino acid	Av. mg by amino acid analyzer [1/2/]	Av. mg by GLC [1/3/]	Percent recovery [4/]
Alanine	26.45	21.11 ± .264	79.8
Valine	24.84	23.72 ± .137	95.5
Isoleucine	8.99	9.16 ± .280	101.9
Glycine	6.27	4.60 ± .151	73.4
Leucine	41.35	25.91 ± .192	62.7
Threonine	18.61	27.40 ± .377	147.2
Proline	26.05	21.32 ± .308	81.8
Serine	13.77	10.40 ± .257	75.5
Methionine	1.31	1.16 ± .05	88.5
Phenylalanine	23.54	57.37 ± .165	243.7
Aspartic acid	36.20	18.11 ± .186	50.0
Glutamic acid	54.55	51.93 ± .168	95.2
Tyrosine	19.03	19.44 ± .281	102.2
Lysine	44.70	46.45 ± .358	103.9

1/ 4mg/ml of bovine serum albumin.

2/ Average of two independent analyses.

3/ Average of five independent analyses.

4/ GLC values as a percentage of amino acid analyzer values.

Table 4. Percentage recovery of specific amino acids from the water soluble fraction of light turkey meat by GLC and amino acid analyzer

Amino acids	Av. mg by amino acid analyzer 1/2/	Av. mg GLC 3/4/	Percent recovery by GLC 5/
Alanine	18.34	14.91 ± .166	81.3
Valine	19.00	15.66 ± .198	82.4
Isoleucine	15.26	33.75 ± .505	222.6
Glycine	20.53	5.14 ± .069	25.0
Leucine	24.60	12.67 ± .188	51.5
Proline	12.67	11.58 ± .108	91.4
Serine	13.17	6.09 ± .115	46.2
Methionine	2.90	4.54 ± .115	156.5
Phenylalanine	13.16	32.02 ± .318	243.3
Aspartic acid	29.93	6.46 ± .092	21.6
Glutamic acid	35.10	29.29 ± .261	83.4
Tyrosine	7.76	6.53 ± .077	84.1
Lysine	22.19	18.82 ± .360	84.8

1/ Average of two samples.

2/ mg/100 ml of water soluble fraction.

3/ Average of 10 independent samples.

4/ mg/100 ml of water soluble fraction.

5/ GLC values as a percentage of amino acid analyzer value.

Figure 3. Chromatogram of a water soluble protein hydrolysate from light turkey meat.

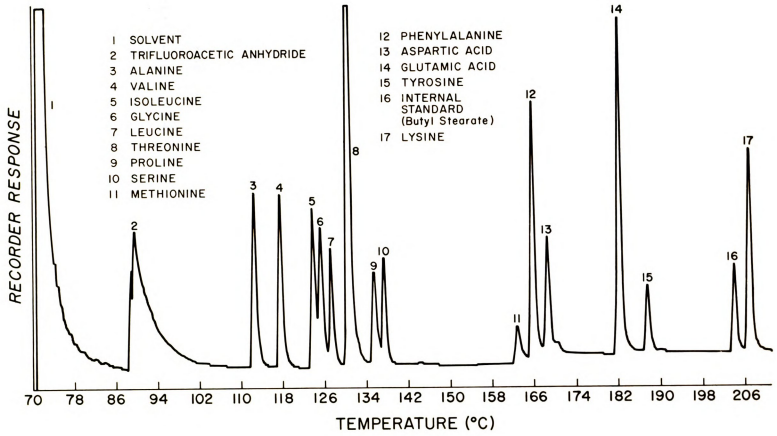

1 SOLVENT
2 TRIFLUOROACETIC ANHYDRIDE
3 ALANINE
4 VALINE
5 ISOLEUCINE
6 GLYCINE
7 LEUCINE
8 THREONINE
9 PROLINE
10 SERINE
11 METHIONINE
12 PHENYLALANINE
13 ASPARTIC ACID
14 GLUTAMIC ACID
15 TYROSINE
16 INTERNAL STANDARD (Butyl Stearate)
17 LYSINE
RECORDER RESPONSE
70 78 86 94 102 110 118 126 134 142 150 158 166 174 182 190 198 206
TEMPERATURE (°C)

Figure 3

acid, tyrosine and lysine with recoveries of 81.3, 82.4, 91.4, 83.5, 84.2 and 84.8 percent, respectively, when compared with values for the amino acid analyzer.

To improve the percentage recovery by GLC in comparison to the values obtained by the amino acid analyzer, several techniques were tried. Ether extraction, as suggested by Gehrke and Regis Chemical Company (1967) in conjunction with a short Dowex 50W-12X (H^+ form) column to remove acids, sugars and other neutral contaminants, was not effective. This procedure did not reduce the high recovery values for isoleucine, methionine or phenylalanine. In addition, this procedure did not remove substances which may have interferred with esterification or acylation, since the low percentage recoveries for these amino acids were not improved. When elution of the amino acids from a Dowex 2W-X8 (OH^- form) or bubbling live steam through the protein hydrolysates were used, no improvement in the percentage recovery could be detected. This indicated that neither amines nor volatiles were causing a significant effect on the recoveries of the amino acids.

Filtration through activated charcoal was used to clarify the samples to determine if amino acids from a protein hydrolysate could be more effectively recovered. This method proved to be as satisfactory as any of the methods tried and required less time, thus making it the most desirable. Since the percentage recovery for threonine

was approximately 500-600 percent, no further attempt was made to quantitate or collect data for this amino acid from biological systems.

The chemical classification of the amino acids had little effect on the percentage recoveries. Recoveries of aliphatic, aromatic, sulfur-containing, secondary, dicarboxylic and basic amino acids were similar. At least one amino acid from each classification (except sulfur-containing) gave satisfactory results. However, it should be noted that there was only a small amount of methionine present in the water soluble fraction and this could have affected actual quantitation and resulted in high values.

Thèse results have shown that amino acids in standard solutions and hydrolysates of purified protein extracts can be satisfactorily recovered and quantitated using the GLC procedure. However, when attempting to separate and recover these amino acids from a complex meat hydrolysate, the results were quite variable and in most cases, not closely related to the values obtained from the amino acid analyzer. Since the use of an amino acid analyzer has been an accepted procedure, it is assumed to be more accurate than these procedures for separating and quantitating the amino acids in complex meat hydrolysates. However, bearing in mind the consistancy of the values obtained, the GLC procedure can still provide a valuable means in detecting differences or changes in samples.

Effects of Adrenalin and Iodoacetate on pH and Related Changes in Certain Muscle Fractions

pH of Muscle

Turkeys were given several different treatments before slaughter to alter the ultimate pH of the muscle after processing. The pH values for the tissue from the control turkeys and those treated with adrenalin and iodoacetate are shown in Table 5. pH of the light and dark meat samples from control birds 24 hours after slaughter and after 0, 30, and 90 days storage are within the expected range (Wladyka, 1965). The pH of the meat from adrenalin treated birds was also as expected since the pH values of the dark and light meat were 7.05 and 6.87, respectively, 24 hours after slaughter. These results from the light meat agree with those reported by deFremery (1963), who found that the pH of the light meat was 6.94, however, no comparable data were found for dark meat. pH values of 6.04 and 7.03 for light and dark meat, respectively, were obtained for meat from the iodoacetate treated turkeys. The pH values obtained for dark meat were as expected, however, the results for light meat differ from those reported by deFremery (1963), who found, using the same treatment, a pH of 6.76 for the light meat.

A possible explanation for the low pH of the light meat from the birds treated with iodoacetate is the time required for complete mixing of the blood in the bird.

Table 5. pH values of muscle from adrenalin and iodoacetate treated turkeys 24 hours after slaughter and after frozen storage

Treatment	24 hours after slaughter	Days of Storage		
		0	30	90
		pH values		
CL[1/]	5.95 ± .10[2/]	5.96 ± .09[3/]	5.95 ± .07	5.90 ± .12
CD	6.25 ± .13	6.16 ± .08	6.12 ± .12	6.14 ± .09
AL	6.87 ± .16[4/]	6.78 ± .12	6.80 ± .15	6.81 ± .11
AD	7.05 ± .19	7.05 ± .11	6.91 ± .15	6.97 ± .09
IL	6.04 ± .21	6.01 ± .19	5.96 ± .11	5.87 ± .15
ID	7.03 ± .22	6.97 ± .11	6.90 ± .15	6.99 ± .24

1/ CL - Control-Light
CD - Control-Dark
AL - Adrenalin-Light
AD - Adrenalin-Dark
IL - Iodoacetate-Light
ID - Iodoacetate-Dark

2/ CL and CD - Average of individual determinations on muscle samples from eight birds.

3/ Average of three independent analyses on each pooled sample.

4/ AL, AD, IL, and ID - Average of individual determinations on muscle samples from five birds.

Pino et al. (1951) reported that birds required from 2 to 3 minutes for complete mixing of the blood. Blood mixing time could have affected results since the birds were slaughtered 3 minutes after injection to prevent death. Further confirmation of insufficient mixing time was obtained from George and Berger (1966). They reported that each white fiber, of which light meat of the domestic fowl mainly consist, receives considerably less blood per unit area than red fibers which makes up the larger portion of the dark meat. Insufficient time for the iodoacetate to be distributed within the light tissue may have resulted. As a result sufficient iodoacetate may not have been present to inhibit the action of the phosphoglyceraldehyde dehydrogenase, which was reported by Padieu and Mammaerts (1960) to be the limiting enzyme in anaerobic glycolysis within the muscle complex. These factors could have a direct effect on the results, since birds in this study were slaughtered 3 minutes after injection of the iodoacetate, whereas deFremery (1963) slaughtered 3 - 6 minutes after injection.

In addition, there is a higher concentration of glycogen in white than red muscle fibers, and anaerobic glycolysis is predominant in the white meat (white muscle fibers) according to George and Berger (1966). Thus, it would seem that a higher concentration of

phosphoglyceraldehyde dehydrogenase would exist requiring more iodoacetate for inactivation.

The pH values obtained showed that treatment had a marked effect on the ultimate pH values of the tissue. The light and dark meat from the adrenalin treated, and the dark meat from the iodoacetate treated birds showed marked changes in ultimate pH, and were sticky and gummy.

Drip

The effects of the various treatments and storage times on the percentages of drip obtained are shown in Table 6. Percentages of drip from the control light meat samples at 0, 30 and 90 days of storage were 3.3, 5.8 and 9.1 percents, respectively. Control dark meat samples had 1.2, 7.9 and 9.4 percents of drip as storage time increased. The percentages of drip from the dark meat after 30 and 90 days of storage were higher than from light meat, which according to previous literature would not be expected. Kuprianoff (1952) reported that at the isoelectric point of meat (pH 5.0 to 5.5) maximum amount of fluid would be exuded. Since the pH of the dark meat was higher than that of the light meat, it would be expected that maximum drip would be obtained from the light meat.

Percentage drip from the light and dark meat of the adrenalin treated turkeys and from control samples increased with storage time. Insufficient drip

Table 6. Percentage drip from muscles of adrenalin and iodoacetate treated turkeys after frozen storage

Treatment	Days of Storage		
	0	30	90
	Percent		
CL[1/]	3.3	5.8	9.1
CD	1.2	7.9	9.4
AL	0.0[2/]	2.0	2.9
AD	0.0[2/]	0.5	1.1
IL	10.3	8.4	8.4
ID	1.1	1.3	2.0

1/ CL - Control-Light
CD - Control-Dark
AL - Adrenalin - Light
AD - Adrenalin-Dark
IL - Iodoacetate-Light
ID - Iodoacetate-Dark

2/ Insufficient drip for measurement.

(less than 1 ml) was obtained at 0 time to determine percent loss of drip. Percentages of drip obtained from the dark meat were 0.0, 0.5 and 1.1 percent and from the light meat 0.0, 2.0 and 2.9 percent for samples evaluated after 0, 30 and 90 days storage, respectively.

Dark meat samples from iodoacetate treated turkeys had an increase in the percentage drip released with increased storage. Percentage drip from dark meat after 0, 30 and 90 days of storage was 1.1, 1.3 and 2.0 percent, respectively. The percentage drip from light meat did not follow the same pattern with the highest percent obtained before storage (10.3 percent). No effect of storage could be determined since 8.4 percent drip was obtained from samples stored for 30 and 90 days.

The large amount of initial drip from the light meat can be explained as follows: The iodoacetate treatment caused the birds to go into rigor immediately (within 2 minutes after bleeding), as determined by the stiffness of the carcass, and poor bleeding occurred. This resulted in high fluid retention by the tissue. Also, since the iodoacetate treatment did not affect the pH of the light meat, optimum pH conditions for maximum fluid release or minimum water-holding capacity were approached. No evidence is available to explain why percentage drip released did not increase with longer storage periods, as it did from the other samples. One possibility is that a physical and/or

biochemical phenomenon occurred in the frozen samples during storage due to the presence of iodoacetate in the white muscle fibers.

A direct relationship between pH values and percentage drip was found in this study and has been confirmed by a number of workers. Empey (1933) demonstrated that a high ultimate pH was associated with reduced drip loss from beef muscles. Maximum amount of drip was obtained at about pH 5.2 and as the pH increased the amount of drip decreased to zero at about pH 6.4 (Sair and Cook, 1938b). Meat with a pH near its isoelectric point (5.0 to 5.5) has a larger drip loss after freezing and thawing than meat at a higher pH (6.3 to 6.4) according to Kuprianoff (1952).

Nitrogen Released in Drip

The mg of nitrogen released per 100 g of tissue was greater from light meat than dark meat control samples and increased with storage for both light and dark meat (Table 7). Mg of nitrogen released per 100 g of light and dark meat were 16.7 and 4.2, respectively, before storage.

Effects of storage times of 30 and 90 days on percentages of drip from meat samples of the adrenalin treated birds are shown in Table 6. Insufficient drip was obtained for analysis from both light and dark meat before storage. As with the control samples, the mg

Table 7. Nitrogen content in drip from muscles of adrenalin and iodoacetate treated turkeys after frozen storage

Treatment	Days of Storage		
	0	30	90
	Grams		
CL[1/]	16.74[2/]	27.98	43.58
CD	4.19	25.25	40.81
AL	0.00[3/]	10.89	14.58
AD	0.00[3/]	1.94	4.20
IL	56.90	45.24	38.42
ID	4.05	4.88	6.66

1/ CL - Control-Light
CD - Control-Dark
AL - Adrenalin-Light
AD - Adrenalin-Dark
IL - Iodoacetate-Light
ID - Iodoacetate-Dark

2/ Mg of N released in drip per 100 grams of tissue.

3/ Insufficient drip for determination (less than 1 ml obtained).

nitrogen released per 100 g tissue was greater from the light meat than dark meat and both showed an increase with storage. The amount of nitrogen released per 100 g of tissue for light meat was 10.9 and 14.6 mg for 30 and 90 days storage, respectively, and from the dark meat 1.9 and 4.2 mg, respectively.

Results of 0, 30 and 90 days storage on the mg nitrogen released per 100 g of tissue from the iodoacetate treated turkeys are shown in Table 7. Mg nitrogen released per 100 g of tissue for the light meat is considerably higher than from the dark meat. Values of 56.9, 45.2 and 38.4 mg nitrogen per 100 g light meat and 4.1, 4.9 and 6.7 mg nitrogen per 100 g dark meat, were obtained for 0, 30 and 90 days storage, respectively. The higher nitrogen values obtained for light meat at 0 and 30 days storage are the direct result of a higher percentage drip obtained from these samples. The amount of nitrogen released after 30 and 90 days of storage is of interest. Percentage drip released after 30 and 90 days of storage was approximately the same (Table 6), however, the amount of nitrogen decreased with storage. This could indicate that the protein in solution (drip) for this treatment (iodoacetate) was being retained by the meat, since dilution was not accounting for the decreased nitrogen release. Based on these observations some unknown relationship may be responsible

for the greater amount of drip and nitrogen released at 0 time.

Wladyka (1965) reported that the protein nitrogen increase, which occurred in drip from dark and light meat, although of limited proportion, does indicate the possibility of denaturation and proteolysis in the chicken meat during frozen storage. According to Khan et al. (1963) denaturation and proteolysis occurred in chicken muscle proteins during frozen storage. Swanson and Sloan (1953) concluded that proteolysis occurred in poultry meat during frozen storage as evidenced by an increase in soluble nitrogen and non-protein nitrogen of both dark and light meat.

Muscle Protein Fractionation

Muscle protein fractionation data of dark and light meat proteins from the control, adrenalin and iodoacetate treated birds are shown in Tables 8 - 10. The water soluble protein extract (sarcoplasmic proteins) of light and dark meat from the control birds remained relatively constant during storage, however, more sarcoplasmic protein was extracted from light than dark meat. The amount of sarcoplasmic protein of tissue from adrenalin and iodoacetate treated birds was also constant during storage. These results are in agreement with those reported by Sawant and Magar (1961) and Khan et al. (1963)

Table 8. Protein components of light and dark meat from control turkeys after frozen storage

Protein component	Days of Storage: 0 — Nitrogen g/100 g tissue 1/	0 — % of total	30 — Nitrogen g/100 g tissue 1/	30 — % of total	90 — Nitrogen g/100 g tissue 1/	90 — % of total
			LIGHT MEAT			
Total N	3.86	100.0	3.91	100.0	3.95	100.0
Water Sol. N	0.78	20.1	0.71	18.1	0.76	19.2
NPN of Water Sol.	0.46	12.1	0.48	12.2	0.49	12.4
Salt Sol.	1.48	38.3	1.41	36.0	1.33	33.7
NaOH Sol.	0.62	16.2	1.12	28.6	1.04	26.4
NaOH Insol.	0.98	25.4	0.67	17.1	0.79	19.9
			DARK MEAT			
Total N	3.32	100.0	3.38	100.0	3.36	100.0
Water Sol. N	0.58	17.4	0.57	17.0	0.58	17.3
NPN of Water Sol.	0.38	11.5	0.34	10.1	0.33	9.9
Salt Sol.	1.27	38.3	1.45	42.8	1.23	36.6
NaOH Sol.	0.75	22.6	1.04	30.7	1.12	33.3
NaOH Insol.	0.72	21.7	0.33	9.7	0.43	12.7

1/ Each value is based on duplicate nitrogen determinations from each of three independent extractions.

Table 9. Protein components of light and dark meat from adrenalin treated turkeys after frozen storage

Protein component	Days of Storage: 0 Nitrogen g/100 g 1/ tissue	0 Nitrogen % of total	30 Nitrogen g/100 g 1/ tissue	30 Nitrogen % of total	90 Nitrogen g/100 g 1/ tissue	90 Nitrogen % of total
			LIGHT MEAT			
Total N	3.71	100.0	3.79	100.0	3.78	100.0
Water Sol. N	1.16	31.3	1.19	31.4	1.18	31.1
NPN of Water Sol.	0.53	14.3	0.43	11.3	0.49	13.1
Salt Sol.	1.75	47.1	1.80	47.6	1.91	50.5
NaOH Sol.	0.53	14.4	0.49	13.0	0.45	12.0
NaOH Insol.	0.27	7.2	0.30	8.0	0.24	6.3
			DARK MEAT			
Total N	3.42	100.0	3.33	100.0	3.40	100.0
Water Sol. N	0.81	23.7	0.89	26.6	0.80	23.5
NPN of Water Sol.	0.37	10.8	0.31	9.2	0.34	10.1
Salt Sol.	1.75	51.4	1.56	47.9	1.76	51.7
NaOH Sol.	0.44	12.9	0.58	17.4	0.51	14.9
NaOH Insol.	0.41	12.0	0.31	9.2	0.34	9.9

1/ Each value is based on duplicate nitrogen determinations from each of three independent extractions.

Table 10. Protein components of light and dark meat from iodoacetate treated turkeys after frozen storage

Protein component	Days of Storage: 0 Nitrogen g/100 g tissue 1/	0 Nitrogen % of total	30 Nitrogen g/100 g tissue 1/	30 Nitrogen % of total	90 Nitrogen g/100 g tissue 1/	90 Nitrogen % of total
			LIGHT MEAT			
Total N	3.84	100.0	3.80	100.0	3.82	100.0
Water Sol. N	0.71	18.5	0.70	18.4	0.74	19.5
NPN of Water Sol.	0.42	12.3	0.51	13.5	0.47	12.2
Salt Sol.	1.36	35.5	1.09	28.8	1.02	26.6
NaOH Sol.	1.31	34.2	1.47	38.6	1.58	41.3
NaOH Insol.	0.55	14.3	0.54	14.1	0.48	12.7
			DARK MEAT			
Total N	3.37	100.0	3.20	100.0	3.32	100.0
Water Sol. N	0.81	23.9	0.79	24.7	0.76	23.0
NPN of Water Sol.	0.31	9.1	0.36	11.4	0.31	9.2
Salt Sol.	1.54	45.6	1.64	51.2	1.41	45.0
NaOH Sol.	0.52	15.3	0.49	15.4	0.72	21.8
NaOH Insol.	0.51	15.1	0.28	8.7	0.34	10.3

1/ Each value is based on duplicate nitrogen determinations from each of three independent extractions.

who reported that the sarcoplasmic fraction (water-soluble nitrogen) remained unchanged except after long periods of storage (50 weeks).

The amount of sarcoplasmic nitrogen of light meat from the control and iodoacetate treatments were similar with approximately 19 percent of the total nitrogen extracted. However, light meat from the adrenalin treated birds contained a much higher percent (31%) of sarcoplasmic nitrogen. Sarcoplasmic nitrogen values for dark meat from birds treated with adrenalin and iodoacetate (24 percent of total nitrogen) were higher than the extractable nitrogen values from the control birds (17 percent of total nitrogen).

Nonprotein nitrogen (NPN) of the water soluble fraction from all samples did not change as a result of storage. Conflicting results were reported by Khan (1964) who found that NPN increased during storage as a result of limited proteolysis. However, his results were reported at 45 weeks of storage compared to a 13 week storage period used in this study. Results of this investigation compare favorably with those of Marion and Forsythe (1962) who found no increase in TCA-soluble nitrogen after 60 days storage.

Percentage of NPN of total nitrogen was higher from light meat (approximately 12 percent) than dark meat (approximately 10 percent) for samples from all treatments.

These results agree with Scharpf and Marion (1964) who found that NPN of light meat was higher than NPN of dark meat.

The salt soluble fractions (myofibrillar proteins) of light meat from the control and iodoacetate treated birds were the only myofibrillar proteins with decreased solubility during storage. Myofibrillar nitrogen from the control samples decreased from 38.1 to 33.7 percent, and nitrogen in extracts of tissue from the iodoacetate treated samples decreased from 35.5 to 26.6 percent during storage. This indicates that denaturation or alteration of the proteins occurred during storage, which agrees with results reported by Seagram (1958) and Khan et al. (1963). However, the same phenomenon was not detected in meat extracts from dark meat from control birds where the amount of myofibrillar nitrogen remained relatively constant. No detectable change in solubility occurred in samples from treated turkeys where the ultimate pH values of the samples were considerably higher (adrenalin-light and -dark and iodoacetate-dark meat samples).

Percentage myofibrillar nitrogen from light and dark meat samples with high ultimate pH values were similar. Myofibrillar nitrogen content of control light and dark meat samples were approximately the same before storage, but dark meat myofibrillar nitrogen was higher in stored samples. Light meat from the iodoacetate treated turkeys had the

lowest percentage solubility in $KCl-PO_4$ buffer for light meat from all treatments. This indicated that the iodoacetate treatment seemed to affect the fibrillar proteins in some manner, possibly by denaturation. Light and dark meat from adrenalin treated birds and dark meat from the iodoacetate treatment, which had high ultimate pH values, contained similar percentages of myofibrillar nitrogen.

Nitrogen in the NaOH soluble fraction (denatured proteins) from both light and dark meat control samples increased during storage and dark meat contained more denatured protein nitrogen than light meat. The increase in denatured protein was expected since numerous investigators have reported that denaturation occurs during storage (Dyer, 1951; Love, 1955a; Khan et al., 1963 and Khan, 1964).

Percentage denatured proteins from both light and dark meat samples from the adrenalin treated birds changed slightly during storage. Results were similar from samples of both light and dark meat and indicate that the high ultimate pH, induced by the adrenalin treatment, reduced protein denaturation and resulted in minimum denaturation during storage. Further evidence that a high ultimate pH reduces initial and storage denaturation, is apparent in the data for dark meat samples from the birds treated with iodoacetate. Although more denatured protein was found,

the values are lower than those from control samples indicating less change during storage.

The percentage denatured protein in both dark and light meat from the iodoacetate treated birds increased during storage. Values from light meat samples were considerably higher than from dark meat and for both light and dark meat samples from the control and adrenalin treated birds. The high percentage of denatured protein for light meat samples from the iodoacetate treated birds is further explanation for the large amount of initial drip obtained.

The NaOH insoluble fraction (stroma) varied between treatments, and decreased during storage for all samples. Control samples had the highest percentage of stroma nitrogen before storage, with values of 25.4 and 21.7 percent of the total nitrogen, for light and dark meat respectively. The samples from the adrenalin treated birds had the lowest percentage of stroma nitrogen with values of 7.2 and 12.0 percent of the total nitrogen, light and dark meat, respectively. Samples from the iodoacetate treated birds had intermediate values.

Khan (1962) reported that NaOH insoluble fraction (stroma) was 10.3 percent from light meat of 4 month old chickens and 18.8 percent from dark meat of 10 week old chickens. Stroma nitrogen was reported by Maier and Fischer (1966) to be 13 percent of total nitrogen for

light meat. Both Khan (1962) and Scharpf and Marion (1964) found that the percentage stroma (of total nitrogen) increased with age. Values obtained from the control birds were considerably higher for light meat and similar for the dark meat. Results of this study indicate that the treatments resulting in a high ultimate pH affected the extraction of stroma nitrogen. This was indicated by a large amount of stroma nitrogen from the control light meat. Light meat from the iodoacetate treated bird, however, had a low ultimate pH, went into rigor rapidly, and did not have the same high percentage of stroma nitrogen.

From an evaluation of these results, pH affected the solubility or insolubility of all fractions. A high ultimate pH resulted in high percentages of soluble sarcoplasmic and myofibrillar proteins and low percentages of denatured and stroma proteins. This was expected since a drop in pH could cause denaturation of the proteins.

Amino Acid Analyses of Ultrafiltrate

Amino acid compositional data from the ultrafiltrate of the water soluble protein fractions from light and dark turkey meat are shown in Tables 11 - 16 for all treatments. These tables separate the amino acid data into two groups. The first six are amino acids whose recoveries by GLC (from water soluble protein fraction; Procedure Evaluation) were most closely related to those obtained from the amino acid

Table 11. Amino acid composition of the ultrafiltrate of the water soluble fraction of light meat from control turkeys after frozen storage

Amino acids	Days of Storage		
	0	30	90
	mg/100 g tissue[1/]		
Alanine	6.18	9.13	9.06
Valine	2.33	4.02	3.68
Proline	2.47	3.74	2.88
Glutamic acid	18.06	21.24	21.32
Tyrosine	2.09	2.31	1.95
Lysine	5.76	5.24	7.12
Sub-total	36.89	45.68	46.01
Isoleucine	63.15	72.42	72.32
Glycine	2.45	2.77	2.74
Leucine	3.71	4.89	4.77
Serine	3.57	4.09	4.98
Methionine	2.13	2.20	1.90
Phenylalanine	6.25	8.44	7.91
Aspartic acid	1.05	1.27	1.01
Sub-total	82.31	96.08	95.63
TOTAL	119.20	141.76	141.64

1/ Average of three replicates from independent extractions.

Table 12. Amino acid composition of the ultrafiltrate of the water soluble fraction of the dark meat from the control turkeys after frozen storage

Amino acid	Days of Storage		
	0	30	90
	mg/100 g tissue 1/		
Alanine	18.45	17.92	15.16
Valine	4.48	3.83	3.18
Proline	4.96	5.69	4.35
Glutamic acid	64.61	37.51	52.97
Tyrosine	2.08	2.31	1.73
Lysine	6.15	5.50	4.72
Sub-total	100.73	72.76	82.11
Isoleucine	77.68	77.57	57.86
Glycine	3.29	3.81	1.85
Leucine	5.74	6.58	5.39
Serine	11.54	11.34	10.03
Methionine	0.91	2.23	1.89
Phenylalanine	11.73	11.92	12.00
Aspartic acid	1.17	0.97	1.14
Sub-total	112.06	104.42	90.18
TOTAL	212.79	187.18	172.29

1/ Average of three replicates from independent extractions.

Table 13. Amino acid composition of the ultrafiltrate of the water soluble fraction of the light meat from the adrenalin treated turkeys after frozen storage

Amino acid	Days of Storage		
	0	30	90
	mg/100 g tissue[1/]		
Alanine	16.56	16.74	18.48
Valine	11.90	12.51	13.47
Proline	11.80	11.64	12.41
Glutamic acid	47.94	47.44	48.75
Tyrosine	5.07	6.59	3.70
Lysine	19.16	20.41	20.95
Sub-total	112.43	115.33	117.76
Isoleucine	80.47	80.55	91.02
Glycine	4.60	4.78	6.70
Leucine	14.38	14.69	15.52
Serine	10.13	10.27	10.05
Methionine	4.23	5.53	8.20
Phenylalanine	21.88	24.29	25.50
Aspartic acid	4.08	4.79	5.05
Sub-total	140.04	144.90	162.04
TOTAL	252.47	260.23	279.80

1/ Average of three replicates from independent extractions.

Table 14. Amino acid composition of the ultrafiltrate of the water soluble fraction of the dark meat from the adrenalin treated turkeys after frozen storage

Amino acid	Days of Storage		
	0	30	90
	mg/100 g tissue 1/		
Alanine	17.52	19.23	13.54
Valine	6.50	6.98	4.66
Proline	8.56	7.67	5.48
Glutamic acid	65.48	56.31	53.88
Tyrosine	2.51	2.41	1.34
Lysine	9.37	10.65	8.00
Sub-total	109.94	103.25	86.90
Isoleucine	92.15	82.41	64.31
Glycine	4.96	3.57	2.15
Leucine	9.18	8.94	6.13
Serine	12.92	13.32	8.52
Methionine	3.11	2.68	2.19
Phenylalanine	17.62	18.32	14.08
Aspartic acid	2.23	2.17	1.73
Sub-total	142.17	131.41	99.11
TOTAL	252.11	234.66	186.01

1/ Average of three replicates from independent extractions.

Table 15. Amino acid composition of the ultrafiltrate of the water soluble fraction of the light meat from the iodoacetate treated turkeys after frozen storage

Amino acid	Days of Storage		
	0	30	90
	mg/100 g tissue[1/]		
Alanine	7.03	11.26	9.25
Valine	2.02	2.97	2.50
Proline	2.74	3.35	1.89
Glutamic acid	15.64	19.37	16.18
Tyrosine	0.91	1.76	1.85
Lysine	2.65	3.31	3.27
Sub-total	30.99	42.02	34.94
Isoleucine	58.65	78.40	62.64
Glycine	1.79	2.76	0.97
Leucine	2.16	3.90	2.77
Serine	3.99	5.28	3.61
Methionine	0.93	1.49	1.64
Phenylalanine	5.41	6.74	5.41
Aspartic acid	0.41	0.93	0.69
Sub-total	73.34	99.50	78.73
TOTAL	104.33	141.52	112.67

1/ Average of three replicates from independent extractions.

Table 16. Amino acid composition of the ultrafiltrate of the water soluble fraction of the dark meat from the iodoacetate treated turkeys after frozen storage

Amino acid	Days of Storage		
	0	30	90
	mg/100 g tissue[1/]		
Alanine	20.32	21.88	19.51
Valine	2.58	3.22	2.83
Proline	5.98	7.17	7.42
Glutamic acid	63.01	64.56	65.28
Tyrosine	1.28	1.53	1.31
Lysine	4.10	4.57	5.29
Sub-total	92.27	102.93	101.64
Isoleucine	66.33	67.71	72.28
Glycine	2.24	2.21	1.90
Leucine	5.06	5.22	5.38
Serine	10.71	11.23	11.59
Methionine	1.41	1.76	2.08
Phenylalanine	13.80	14.67	14.91
Aspartic acid	0.74	0.91	0.95
Sub-total	105.29	103.80	109.09
TOTAL	197.56	206.73	210.73

1/ Average of three replicates from independent extractions.

analyzer. The other group of seven amino acids, whose recoveries did not compare favorably with results from the amino acid analyzer, followed the same trends as the group of amino acids with satisfactory recoveries.

The ultrafiltrate of the dark meat samples from control and iodoacetate treated birds contained a higher total amino acid content than the corresponding light meat extracts for each treatment. Total amino acid values from both light and dark meat from the adrenalin treated birds were higher than from comparable control samples. The largest variation between treatments was obtained from the total amino acid content of the ultrafiltrate from light meat samples.

Only slight random changes due to storage time are indicated by these data. This was expected since the NPN of the water soluble fraction showed only minor changes with storage.

In this experiment, meat samples with an ultimate pH of 6.2 or higher (control-dark, adrenalin-light and -dark and iodoacetate-dark) contained larger amounts of free amino acids than did samples with an ultimate pH below 6.2. This indicates that the proteolytic enzymes have an optimum pH above 6.2.

Amino Acid Analyses of Drip

The amino acid values in drip from all samples (mg per 100 g tissue and mg per 100 ml drip) are reported in Tables 17 - 22. In addition to the total values for all amino acids, data are summarized for the six amino acids whose recovery and quantitation by GLC most closely corresponded to amino analyzer results. The sum of values for these six amino acids is approximately one-half the sum of all amino acids for every sample analyzed.

The amino acid values from light meat control samples were considerably higher than from dark meat samples. When evaluated as mg amino acids released per 100 g tissues, total values for light meat increased with storage time. However, when comparing results on the basis of mg per 100 ml drip, the values for both dark and light meat decreased after meat samples were stored 30 days but increased after 90 days storage.

Amino acid content of drip reported as mg per 100 g tissue was expected to increase with storage time since there was an increase in the amount of drip exuded. This has been shown by an increase in the quantity of drip and nitrogen released (Tables 6 and 7). An increase in amino acids released in drip after storage was reported by Wladyka (1965). When the amino acids were reported as mg per 100 ml drip a dilution effect was apparent after 30 days storage.

Table 17. Amino acid composition of drip from the light meat of the control turkeys after frozen storage

Amino acid	Days of Storage			Days of Storage		
	0	30	90	0	30	90
	mg/100 g tissue 1/			mg/100 ml drip 1/		
Alanine	3.87	6.41	17.24	127.93	108.13	189.80
Valine	4.55	7.78	20.24	148.60	133.40	222.80
Proline	2.89	4.69	13.33	98.13	80.40	146.73
Glutamic acid	6.12	10.07	28.30	196.40	172.60	311.53
Tyrosine	0.28	0.64	0.77	9.60	11.00	8.47
Lysine	5.04	7.64	23.08	153.47	131.00	254.06
Sub-total	22.75	37.22	102.96	734.13	636.53	1133.39
Isoleucine	6.92	11.18	31.57	210.67	191.67	347.53
Glycine	1.41	2.37	6.39	42.87	90.60	70.33
Leucine	3.47	5.29	14.02	115.60	90.73	54.33
Serine	1.52	2.31	6.95	56.40	39.67	76.53
Methionine	1.03	1.74	5.08	31.33	39.87	55.93
Phenylalanine	8.52	10.27	41.27	269.40	244.67	454.33
Aspartic acid	0.31	0.68	1.67	9.60	11.60	18.40
Sub-total	23.18	33.85	102.94	664.87	648.81	1176.38
TOTAL	45.93	71.07	205.90	1399.00	1285.34	2310.77

1/ Average of two independent analyses of the drip.

Table 18. Amino acid composition of drip from the dark meat of the control turkeys after frozen storage

Amino acid	Days of Storage			Days of Storage		
	0	30	90	0	30	90
	mg/100 g tissue 1/			mg/100 ml drip 1/		
Alanine	0.89	4.38	7.77	72.33	55.47	82.73
Valine	1.08	5.46	17.52	87.87	69.13	186.67
Proline	0.76	4.46	6.25	61.93	56.53	66.60
Glutamic acid	1.87	9.69	15.89	153.20	122.73	169.27
Tyrosine	0.03	0.17	0.26	2.60	2.13	2.73
Lysine	1.18	6.48	10.18	95.53	61.40	108.47
Sub-total	5.81	30.64	57.87	473.46	387.39	447.20
Isoleucine	1.72	8.34	14.58	139.47	105.67	155.33
Glycine	0.32	1.56	2.78	25.87	19.80	29.67
Leucine	0.78	3.91	7.21	63.13	49.47	76.80
Serine	0.42	2.07	3.58	34.07	26.27	37.60
Methionine	0.12	1.17	1.58	9.67	14.87	16.87
Phenylalanine	2.24	11.62	19.10	181.40	147.20	203.47
Aspartic acid	0.14	0.18	0.29	11.80	2.33	3.13
Sub-total	5.76	28.84	49.12	565.41	365.61	692.14
TOTAL	11.57	59.48	106.99	938.87	753.00	1139.34

1/ Average of two independent analyses of the drip.

Table 19. Amino acid composition of drip from the light meat of the adrenalin treated turkeys after frozen storage[1/]

Amino acid	Days of Storage			
	30	90	30	90
	mg/100 g tissue[2/]		mg/100 ml drip[2/]	
Alanine	2.21	3.51	108.73	120.20
Valine	2.70	4.34	132.60	148.80
Proline	1.73	2.64	84.87	90.67
Glutamic acid	2.68	5.91	180.67	202.67
Tyrosine	0.15	0.11	7.60	3.93
Lysine	3.00	4.49	197.33	153.93
Sub-total	12.47	21.00	661.80	720.20
Isoleucine	3.97	6.18	195.07	211.86
Glycine	0.83	1.43	40.60	48.87
Leucine	1.88	2.81	92.07	96.40
Serine	0.90	1.31	44.27	45.07
Methionine	0.44	0.66	21.53	22.73
Phenylalanine	5.13	7.32	252.07	251.00
Aspartic acid	0.29	0.20	14.13	6.93
Sub-total	13.44	19.91	659.74	682.86
TOTAL	25.91	40.91	1321.54	1403.06

1/ Insufficient drip for analysis at 0 days storage.

2/ Average of two independent analyses of the drip.

Table 20. Amino acid composition of drip from the dark meat of the adrenalin treated turkeys after 90 days frozen storage[1/]

Amino acid	mg/100 g tissue [2/]	mg/100 ml drip [2/]
Alanine	0.79	73.40
Valine	1.01	94.07
Proline	0.78	72.07
Glutamic acid	1.78	164.93
Tyrosine	0.02	2.00
Lysine	1.15	106.60
Sub-total	5.53	513.07
Isoleucine	1.55	144.00
Glycine	0.31	28.67
Leucine	0.74	68.87
Serine	0.38	35.20
Methionine	0.20	18.27
Phenylalanine	2.13	197.60
Aspartic acid	0.03	2.47
Sub-total	5.34	495.08
TOTAL	10.87	1008.15

1/ Insufficient drip for analysis at 0 and 30 days storage.

2/ Average of two independent analyses of the drip.

Table 21. Amino acid composition of drip from the light meat of iodoacetate treated turkeys after frozen storage

Amino acid	Days of Storage			Days of Storage		
	0	30	90	0	30	90
	mg/100 g tissue 1/			mg/100 ml drip 1/		
Alanine	10.05	7.92	10.14	97.13	94.47	120.07
Valine	11.53	10.01	11.54	111.40	119.40	136.60
Proline	7.38	5.63	7.92	71.33	67.20	93.80
Glutamic acid	16.33	13.34	14.60	157.80	159.20	172.87
Tyrosine	0.00 2/	0.25	0.79	0.00 2/	3.00	9.40
Lysine	12.99	10.48	13.93	125.53	125.00	164.93
Sub-total	58.28	47.63	58.92	563.19	568.27	697.67
Isoleucine	18.00	14.17	19.15	173.87	169.00	226.73
Glycine	3.67	2.88	3.80	35.47	34.40	45.00
Leucine	8.96	6.92	9.08	86.60	82.60	107.53
Serine	4.49	3.41	4.58	43.40	40.67	54.27
Methionine	4.11	3.67	5.12	39.67	43.73	60.60
Phenylalanine	23.08	17.90	20.17	223.00	213.53	238.80
Aspartic acid	0.54	0.85	1.97	5.27	10.13	23.27
Sub-total	62.85	49.80	63.87	607.28	593.96	756.30
TOTAL	121.13	97.43	122.79	1170.47	1162.23	1453.97

1/ Average of two independent analyses of the drip.

2/ Insufficient tyrosine for detection.

Table 22. Amino acid composition of drip from the dark meat of iodoacetate treated turkeys after frozen storage

Amino acid	Days of Storage			Days of Storage		
	0	30	90	0	30	90
	mg/100 g tissue 1/			mg/100 ml drip 1/		
Alanine	1.02	1.21	1.36	93.33	96.13	69.47
Valine	1.21	1.49	1.61	110.53	118.67	82.00
Proline	0.73	0.94	1.09	67.07	74.87	55.80
Glutamic acid	1.75	2.40	2.73	159.53	191.67	139.27
Tyrosine	0.00 2/	0.00 2/	0.00 2/	0.00 2/	0.00 2/	0.00 2/
Lysine	1.07	1.49	1.78	97.53	118.93	90.87
Sub-total	5.78	7.53	8.57	527.99	600.27	437.41
Isoleucine	0.96	2.28	2.50	87.33	182.20	127.73
Glycine	0.35	0.40	0.42	32.33	13.87	21.33
Leucine	0.84	0.93	1.09	77.14	74.47	55.80
Serine	0.45	0.48	0.59	40.87	38.53	30.00
Methionine	0.08	0.12	0.21	7.80	9.67	10.73
Phenylalanine	2.29	2.82	3.38	209.40	224.48	172.53
Aspartic acid	0.03	0.04	0.06	3.07	3.47	2.87
Sub-total	3.93	5.58	8.25	457.94	546.69	420.79
TOTAL	9.71	13.11	16.82	985.93	1146.96	858.20

1/ Average of two independent analyses of the drip.

2/ Insufficient tyrosine for detection.

These results would indicate that little proteolytic activity occurred between 0 and 30 days storage based on the observation that total amino acid content of the drip decreased with an increase in amount of drip. Further, the amino acid content after 90 days storage was higher than for either 0 or 30 days storage. Therefore, it would appear that 30 days of frozen storage is not sufficient time for major proteolytic activity to occur.

Since muscles from adrenalin treated birds exuded very little drip (except after storage), the results from meat stored 90 days are most reliable. Likewise the few values based on mg tissue are low. The drip from the light meat contained only about 20 percent as much total amino acids as was found in drip from the controls. Drip from dark meat, however, contained only about 10 percent of the amount of total amino acids as found in comparable dark meat control samples. When reported as mg per 100 ml drip, the composition of drip was similar for dark meat from adrenalin treated and control samples. Whereas the drip from light meat of adrenalin treated birds contained approximately 60 percent as much total amino acids as found in comparable controls. The large difference in amino acid content, as mg per 100 g tissue, was due to differences in pH values. The reduced amount of drip from the adrenalin treated turkeys was caused by the higher pH.

Total amino acid values, were less variable when based on mg per 100 ml drip, than when based on mg per 100 g tissue.

Changes in amino acid composition of drip from both light and dark meat samples for birds treated with iodoacetate varied less during storage than did the controls. Amino acid content, as mg per 100 g tissue, from the light meat had values similar to those from the comparable control light meat samples. However, the dark meat with a high ultimate pH, had amino acid values similar to those obtained from the adrenalin treated turkeys. When amino acid values were reported as mg per 100 ml drip from light and dark meat, the values from control samples were similar to those from the adrenalin treated turkeys.

Light meat gave the highest amount of total amino acids released for all treatments based on both methods of reporting composition of the drip (mg per 100 g tissue or mg per 100 ml drip). These results agree with those reported by Wladyka (1965) who found that the concentration of essential amino acids was higher in drip from light meat than from dark meat. This would also be expected since the nitrogen content of drip from light meat was higher than that from dark meat in this study. Similar results were reported by Wladyka (1965).

The results of this experiment indicate that pH had a marked effect on the total amount of amino acids

lost from tissue but does not have as great an effect on the amino acid concentration in the drip. Thus, it would appear that the high ultimate pH is of major importance in reducing the amount of fluids (containing amino acids) which are lost during thawing. This high ultimate pH, however, resulted in more free amino acids in the water soluble fraction and indicates greater proteolytic activity.

SUMMARY

Turkeys were injected with either iodoacetate or adrenalin to effect an increase in the ultimate pH of the meat tissues. Meat samples were ground, formed into slabs, frozen and stored up to 90 days. The influence of the high ultimate pH of meat and frozen storage on amount of drip, changes in certain protein fractions of light and dark turkey meat and amino acid composition of drip were evaluated. The use of GLC as a means of determining the amino acid composition of protein hydrolysates was also investigated.

Amino acid analyses by GLC were found to give excellent results for pure amino acid mixtures when compared to those obtained by the amino acid analyzer. Evaluation of the GLC method of analysis for a purified protein (bovine serum albumin) proved to be less satisfactory with threonine and phenylalanine having high recoveries and leucine and aspartic acid having incomplete recoveries. Amino acid values obtained from a water soluble fraction of light turkey meat by GLC varied considerably from the results of the amino acid analyzer. Only six of the 14 amino acids

(alanine, valine, proline, glutamic acid, tyrosine and lysine) were recovered with satisfactory results.

High ultimate pH values were obtained from adrenalin treated turkeys from both light and dark meat (6.78 and 7.05, respectively) and from dark meat (6.97) for the iodoacetate treated turkeys. These high ultimate pH values had a marked effect on the percentage of drip exuded during thawing. Percentage drip was higher from light meat than from dark meat. Control meat samples had 3.3 and 1.2 percent drip from light and dark meat, respectively, before storage. Meat samples from adrenalin treated turkeys before storage had low (unmeasurable) quantities of drip from both light and dark meat. Light and dark meat samples from the iodoacetate treated birds had 10.3 and 1.1 percent drip, respectively, before storage. The percentage drip was found to increase with storage for all samples regardless of ultimate pH.

A high ultimate pH was also found to result in a high percentage of soluble sarcoplasmic and myofibrillar proteins and low percentage of denatured and stroma proteins. This effect could be attributed to the decrease in pH during rigor which could be associated with denaturation or alteration of the proteins.

The sarcoplasmic proteins were not influenced by storage treatments. Non-protein nitrogen (NPN) in the water soluble fraction was apparently not affected by

frozen storage periods of 90 days; however, light meat contained more NPN than dark meat for all treatments. Myofibrillar proteins from the control dark and light meat and light meat from the iodoacetate treated birds were the only ones which decreased in solubility during storage. Percentages of myofibrillar nitrogen were similar from light and dark meat samples with high ultimate pH values, and were apparently not affected by 90 days frozen storage.

Light meat control samples contained more denatured proteins than dark meat, and the percentage of denatured protein increased in both meat types during storage. Denatured protein in meat samples from the adrenalin treated birds changed slightly during storage. Values of denatured protein were considerably higher in the light meat than in dark meat from the iodoacetate treated birds and in both light and dark meat samples from the control and adrenalin treated birds. The percentage of denatured protein in both light and dark meat increased during storage.

Stroma nitrogen values varied between treatments and decreased during storage for all samples. Meat samples with an ultimate pH of 6.2 or higher contained larger amounts of free amino acids than samples with an ultimate pH below 6.2.

The amount of amino acids in the drip increased with storage time of the meat and the amino acid content of the drip from light meat was higher than from dark

meat from all samples. A dilution effect was observed in the amino acid concentration of the drip after 30 days storage but was not apparent in the meat stored for 90 days. The additional storage period allowed proteolysis to continue and resulted in a high concentration of amino acids in the drip. It was concluded that a high ultimate pH of the muscle was of major significance in reducing the amount of fluid exuded during thawing, since there was a direct relationship between amount of drip and total amount of amino acids released from tissues.

LITERATURE CITED

LITERATURE CITED

American Instrument Co., 1961. The determination of nitrogen by the Kjeldahl procedure including digestion distillation and titration. Reprint No. 104.

Anglemier, A. F., D. L. Crawford, R. F. Cain, W. K. Johnston, Jr., and J. F. Bone, 1961. Tenderness and juiceness of pork as affected by pre-slaughter injection of epinephrine. Proc. Western Sect., Am. Soc. Animal Production. 12:XXIX.

Balls, A. K., 1938. Enzyme action in food products at low temperatures. Ice and Cold Storage. 41:85, 101, 143. In R. J. Whitaker, 1959. Chemical changes associated with aging of meat with emphasis on the proteins. In Advances in Food Research, v. 9:1. Academic Press, Inc., New York.

Bandack-Yuri, S., and D. Rose, 1961. Proteases of chicken muscle. Food Technol. 15:186.

Bayer, E., K. H. Reuther, and F. Born, 1957. Analysis of amino acids by GLC. Angew. Chem. 69:640.

Bendall, J. R., and B. B. Marsh, 1951. The biochemistry of muscular tissue in relation to loss of drip during freezing. Proc. 8th Intern. Congr. Refrig., London, p. 351.

Bendall, J. R. and R. A. Lawrie, 1962. The effect of pre-treatment with various drugs on post-mortem glycolysis and the onset of rigor mortis in rabbit skeletal muscle. J. Comp. Path. Therop. 72:118.

Bier, M., and P. Teitelbaum, 1959. Gas chromatography in amino-acid analysis. Ann. N. Y. Acad. Sci. 72, Art. 13:641.

Blau, K., and A. Darbre, 1963. Preparation of volatile derivatives of amino acids for gas chromatography. Biochem. J. 88:8P.

Bouton, P. E., A. Howard and R. A. Lawrie, 1958. Studies on beef quality. VII. The influence of certain holding conditions on weight losses and eating quality of fresh and frozen beef carcasses. Gt. Brit. Dept. Sci. Ind. Research Special Repts. No. 67.

Callow, E. H., 1952. Frozen meat. J. Sci. Food Agric. 3:145.

Callow, E. H., 1955. Meat and refrigeration. Bull. inst. intern. froid, Suppl. 35-23. In R. Hamm, 1960. Biochemistry of meat hydration. In Advances in Food Research, 10:355. Academic Press, Inc., New York.

Cook, G. A., E. F. Love, J. R. Vickery and W. J. Young, 1926. Studies on the refrigeration of meat. I. Investigations into the refrigeration of beef. Aust. J. Exp. Biol. and Med. Sci., 3:15. Abstr. in Chem. Abstr. 1926. 20:2028.

Cori, G. T. and B. Illingworth, 1956. The effect of epinephrine and other glycogenolytic agents on the phosphorylase a content of muscle. Biochem. et Biophy. Acta. 21:105.

Crigler, J. C., and L. E. Dawson, 1968. Cell disruption in broiler breast muscle related to freezing time. J. Food Sci. 33:248.

Cruickshank, P. A. and J. C. Sheehan, 1964. Gas chromatographic analysis of amino acids as N-trifluoroacetyl amino acid methyl esters. Anal. Chem. 36:1191.

Deatherage, F. E., and R. Hamm, 1960. Influence of freezing and thawing on hydration and changes of the muscle proteins. Food Research, 25:623.

deFremery, D., 1963. Relation between biochemical properties and tenderness of poultry. Campbell Meat Tenderness Symposium, p. 99.

deFremery, D., and M. F. Pool, 1960. Biochemistry of chicken muscle as related to rigor mortis and tenderization. J. Food Sci. 25:73.

deFremery, D., and M. F. Pool, 1963. The influence of post-mortem glycolysis on poultry tenderness. J. Food Sci. 28:173.

Dyer, W. J., 1951. Protein denaturation in frozen and stored fish. Food Res. 16:522.

Empey, W. A., 1933. Studies on the refrigeration of meat. Conditions determining the amount of drip from frozen and thawed muscle. J. Soc. Chem. Ind. 52:230T.

Empey, W. A., and A. Howard, 1954. Drip formation in meat and fish. Food Preserv. Quart. 14(2):33.

Ertingshausen, G., C. W. Gehrke and W. A. Aue, 1967. Ultramicro determination of amino acids by GLC. Abs. of papers presented at the 154th Meeting ACS. Chicago, Ill. Sept. 11, abs. 152.

Finn, D. B., 1932. Denaturation of proteins in muscle juice by freezing. Proc. Royal Soc. B 111, 396.

Fischer, R. L., 1963. Changes in the chemical and physcial properties of protein during aging of meat. In Campbell Meat Tenderness Symposium, p. 71.

Gehrke, C. W. and Regis Chemical Company, 1967. "Regis Lab Notes." Regis Chemical Company, Chicago, Ill.

Gehrke, C. W., and D. L. Stalling, 1967. Quantitative analysis of the twenty natural protein amino acids by gas-liquid chromatography. Separation Science 2:101.

George, J. C., and A. J. Berger, 1966. Avian Myology Academic Press, N. Y. and London. p. 42.

Godeaux, J., 1957. Composition du suc de decongelation du muscle strie de la grenouille. Experimentia 13:443.

Graff, J., J. P. Wein, and M. Winity, 1963. Quantitative determination of α-acids by gas-liquid chromatography. Federation Proc. 22:244.

Hagen, P., and W. Black, 1964. A method for the quantitative determination of the composition of a mixture of 19 amino acids by gas chromatography of their N-trifluoroacetyl methyl esters. Federation Proc. 23:371.

Hamm, R., 1953. Water-binding capacity of meat and its commercial importance. Deut. Lebensm. Rund. schau 49:153. Abstr. in Chem. Abstr. 1953. 47:21683 a.

Hamm, R., 1958. Adenosinetriphosphate and its importance for meat quality. II. Function of in post-mortem changes of muscle. Flelschwirtschaft. 10:80. Abstr. in Chem. Abstr. 1958. 52:7560 c.

Hamm, R., 1960. Biochemistry of meat hydration. In Advances in Food Research. v. 10:355. Academic Press, Inc., New York.

Hedrick, H. B., D. E. Brady and C. W. Turner, 1957. Effect of ante-mortem stress on post-mortem beef characteristics. Proc. 9th Research Conf. p. 9. American Meat Institute Foundation, Chicago, Ill.

Hiner, R. L., L. L. Madsen and O. G. Hankins, 1945. Histological characteristics, tenderness and drip losses of beef in relation to temperature of freezing. Food Res. 10:312.

Howard, A., and R. A. Lawrie, 1956. Studies on beef quality. III. Influence of various pre-slaughter treatments on weight losses and eating quality of beef carcasses. C.S.I.R.O. Aust. Div. Food Pres. Transp. Tech. Pap. No. 2.

Howard, A., and R. A. Lawrie, 1957. Studies on beef quality. V. Further observations on biochemical and physiological responses to pre-slaughter treatments. C.S.I.R.O. Aust. Div. Food Pres. Transp. Tech. Pap. No. 4.

Howard, A., R. A. Lawrie and C. A. Lee, 1960. Studies on beef quality. VIII. Some observations on the nature of drip. C.S.I.R.O. Aust. Div. Food Pres. Transp. Tech. Pap. No. 15.

Hunter, I. R., K. R. Dimick, and J. W. Corse, 1956. Determination of amino acids by ninhydrin oxidation and GC separation of leucine and isoleucine. Chem. Ind. (London), 294.

Ikekawa, N., O. Hashino, and R. Watanuki, 1966. Gas chromatographic separation of DNP amino acids and its application to the analysis of serum amino acids. Anal. Biochem. 17:16.

Johnson, D. E., S. J. Scott and A. Meister, 1961. GLC of amino acid derivatives. Anal. Chem. 33:669.

Kaloyereas, S. A., 1947. Drip as a constant for quality control of frozen foods. Food Res. 12:419.

Khan, A. W., 1962. Extraction and fractionation of proteins in fresh chicken muscle. J. Food Sci. 27:430.

Khan, A. W., L. van den Berg and C. P. Lentz, 1963. Effects of frozen storage on chicken muscle proteins. J. Food Sci. 28:425.

Khan, A. W., 1964. Quality deterioration in frozen poultry. Changes in nonprotein nitrogenous constituents of chicken breast muscle stored at below freezing temperatures. Agr. Food Chem. 12:378.

Khan, A. W., and C. P. Lentz, 1965. Influence of prerigor, rigor and postrigor freezing on drip losses and protein changes in chicken meat. J. Food Sci. 30:787.

Khan, A. W., and L. van den Berg, 1965. Changes in chicken muscle proteins during cooking and subsequent frozen storage, and their significance in quality. J. Food Sci. 30:151.

Koonz, C. H., and J. M. Ramsbottom, 1939a. Susceptibility of frozen defrosted poultry meat to drip. Food Res. 4:485.

Koonz, C. H., and J. M. Ramsbottom, 1939b. A method for studying the histological structure of frozen products. I. Poultry. Food Res. 4:117.

Kuprianoff, J., 1952. Neuere Erkenntnisse uber die Veranderungen von Fleisch bein Kuhlen und Gerrieren. Kaltetechik 4:156. In R. Hamm 1960. Biochemistry of meat hydration. In Advances in Food Research v. 10:355.

Lamkin, W. M., and C. W. Gehrke, 1965. Quantitative gas chromatography of amino acids, preparation of n-butyl N-Trifluoroacetyl esters. Anal. Chem. 37:383.

Landowne, R. A., and S. R. Lipsky, 1963. High sensitivity detection of amino acids by gas chromatography and electron affinity spectrometry. Nature, London 199:144.

Love, R. M., 1955. The expressible fluid of fish fillets. I. Nucleic acid as an index of cell damage in fillets frozen from both sides. J. Sci. Food Agric. 6:30.

Love, R. M., 1956. Influence of freezing rate on the denaturation of cold-stored fish. Nature, London 178:988.

Love, R. M., 1958a. The expressible fluid of fish fillets. VIII. Cell damage in slow freezing. J. Sci. Food Agric. 9:257.

Love, R. M., 1958b. The expressible fluid of fish fillets. IX. Other types of cell damage caused by freezing. J. Sci. Food Agric. 9:262.

Love, R. M., 1958c. Studies on protein denaturation in frozen fish. III. The mechanism and site of denaturation at low temperatures. J. Sci. Food Agric. 9:609.

Love, R. M., 1962a. New factors involved in the denaturation of frozen cod muscle. J. Food Sci. 27:544.

Love, R. M., 1962b. Protein denaturation in frozen fish. VII. Effect of onset and resolution of rigor mortis on denaturation. J. Sci. Food Agric. 13:534.

Luijpen, A. F. M. G., 1957. Denaturation of fish proteins. Nature, London 180:1422.

Maier, G. E., and R. L. Fischer, 1966. Acrylamide gel disc electrophoretic patterns and extractability of chicken breast muscle proteins during post-mortem aging. J. Food Sci. 31:482.

Marion, W. W., and W. J. Stadelman, 1958. Effect of various freezing methods on quality of poultry meat. Food Technol. 72:367.

Marion, W. W., and R. H. Forsythe, 1962. Nitrogen distribution in turkey meat: Estimation of amino, TCA soluble, protein, and total soluble fractions (Abstr.) Poultry Sci. 41:1663.

Marsh, B. B., 1952a. Rigor mortis in whale muscle. Biochim. et. Biophys. Acta 9:127. Abstr. in Chem. Abstr. 1953. 47:2858g.

Marsh, B. B., 1952b. The effects of adenosinetriphosphate on the fiber volume of a muscle homogenate. Biochim. et Biophys. Acta. 9:247. Abstr. in Chem. Abstr. 1953. 47:1264e.

Marsh, B. B., and J. F. Thompson, 1957. Thaw rigor and the delta state of muscle. Biochim. et Biophys. Acta. 24:2427. Abstr. in Chem. Abstr. 1957. 51:14071c.

Melamed, N., and M. Renard, 1960. Analysis of mixtures of amino acids by GC. J. Chromatog. 4:339.

Moran, T., 1932. Rapid freezing. Critical rate of cooling. J. Soc. Chem. Ind. 51:16t.

Moran, T., and H. P. Hale, 1932. Rapid freezing. Temperature of storage. J. Soc. Chem. Ind. 51:20t.

Nicholls, C. H., S. Makisumi, and H. A. Saroff, 1963. Gas chromatography of the methyl esters of the amino acids as the free base and by disassociation of their acid salts. J. Chromatog. 11:327.

Padieu, P., and W. F. H. M. Mommaerts, 1960. Creatine phosphoryl transferase and phosphoglyceraldehyde dehydrogenase in iodoacetate poisoned muscle. Biochim. et Biophys. Acta. 37:72.

Partmann, W., 1963. Post mortem changes in chilled and frozen muscle. J. Food Sci. 28:15.

Pavlovskii, P. E., 1962. Autolytic and denaturation changes of the compounds of muscle tissue during cooling and freezing of meat. Izv. Vysshikh. Uchebn. Zavedenii. Pishchevaya Tekhnol. No. 5:52. Abstr. in Chem. Abstr. 1963. 58:9557 a.

Pavlovskii, P. E., and M. F. Grigor'eva, 1963a. Changes in protein components of autolyzing muscle tissues during cooling and freezing of meat. Izv. Vysshikh. Uchebn. Zavedenii. Pishchevaya Tekhnol. 1963, No. 1, 24. Abstr. in Chem. Abstr. 1963. 59:2103 h.

Pavlovskii, P. E., and M. P. Grigor'eva, 1963b. Changes in the protein of meat muscle tissue during defrosting. Izv. Vysshikh. Uchebn. Zavedenii. Pishchevaya Tekhnol. 1963(2), 47. Abstr. in Chem. Abstr. (1963). 59:8050 f.

Pearson, A. M., and J. I. Miller, 1950. The influence of rate of freezing and storage upon the quality of beef of known origin. J. Animal Sci. 9:13.

Pearson, A. M., R. G. West and R. W. Luecke, 1959. The vitamin and amino acid content of drip obtained upon defrosting frozen pork. Food Res. 24:515.

Pino, J. A., H. S. Weiss, P. D. Sturkie, and R. J. Defalco, 1951. Blood volume determinations in the fowl using diphtheria antitorid. Proc. IX World's Poultry Cong. 3:102.

Pisano, J. J., W. J. A. VandenHeuvel and E. C. Horning, 1963. Gas chromatography of phenyltheohydantoin and dinitrophenyl derivatives of amino acids. Biochim. Biophys. Res. Commun. 7:82.

Plank, R., 1925. Neue Untersuchunger uber die Konservierung von Fleisch und Fischen durch das Gefierverfahren. 12 Mittelung. Zur. theorie der Gerfierveranderunger in Tierschen Geweben. Stschr. f. die Kalt-Industrie 32:109. In P. Paul and A. M. Child, 1937. Effect of freezing and thawing beef muscle upon press fluid, losses and tenderness. Food Res. 2:339.

Radouco-Thomas, C., C. Lataste-Dorolle, R. Zender, R. Busset, H. M. Meyer, and R. F. Mouton, 1959. The anti-autolytic effect of epinephrine in skeletal muscle: non-additive process for preservation of meat. Food Res. 24:453.

Ramsbottom, J. M., and C. H. Koonz, 1939. Freezing temperature as related to drip of frozen defrosted beef. Food Res. 4:425.

Ramsbottom, J. M., and C. H. Koonz, 1940. Relationship between time of freezing beef after slaughter and amount of drip. Food Res. 5:423.

Reay, G. A., 1933. Influence of freezing temperatures on haddock muscle. J. Soc. Chem. Ind. 52:265 T.

Reay, G. A., 1934. The influence of freezing temperature on haddock's muscle. II. J. Soc. Chem. Ind. 53:413 T.

Riddell, W. A., H. N. Brocklesby and L. I. Pugsley, 1937. Frozen fish research; chemical and biochemical studies of halibut. Ice and Cold Storage 40:189. In M. H. Swanson and H. J. Sloan, 1953. Some protein changes in stored and frozen poultry. Poultry Sci. 32:643.

Rühlmann, K., and W. Giesecke, 1961. GC of silysated amino acids. Angew. Chem. 73:113.

Ruyle, C. D., C. W. Gehrke, and D. L. Stalling, 1967. Quantitative GLC analysis of nucleic acid constituents as TMS derivatives. Abs. of papers presented at the 154th Meeting ACS. Chicago, Ill. Sept. 11, abs. 41.

Sair, L., and W. H. Cook, 1938a. Effect of precooling and rate of freezing on the quality of dressed poultry. Can. J. Res. 16D:139.

Sair, L., and W. H. Cook, 1938b. Relation of pH to drip formation in meat. Can. J. Res. 16D:255.

Saroff, H. A., and A. Karmen, 1960. GC of the trifluoracetyl esters of the amino acids. Anal. Biochim. 1:344.

Saroff, H. A., A. Karmen and J. W. Healy, 1962. Gas chromatography of the amino acid esters in ammonia. J. Chromatog. 9:122.

Satterlee, L. D., and D. A. Lillard, 1967. A procedure for gas chromatographic analysis of free amino acids in meats. J. Food Sci. 32:682.

Sawant, P. L., and N. G. Magar, 1961. Studies on frozen fish. I. Denaturation of proteins. J. Food Sci. 26:253.

Scharph, L. G., Jr. and W. W. Marion, 1964. Extraction of fibrillar and sarcoplasmic proteins of turkey muscle. J. Food Sci. 29:303.

Seagram, H. L., 1958. Analysis of the protein constituents of drip from thawed fish muscle. Food Res. 23:143.

Seagram, H. L., 1959. Analysis of fluids obtained from mechanically disrupted and frozen fish muscle. Food Res. 24:681.

Shahrokhi, F., and C. W. Gehrke, 1967. Quantitative gas-liquid chromatography of iodine containing amino acids. Abs. of papers presented at the 154th Meeting ACS. Chicago, Ill. Sept. 11, abs. 156.

Shahrokhi, F. and C. W. Gehrke, 1968. Quantitative gas-liquid chromatography of sulfur containing amino acids. J. Chromatog. 36:31.

Smorodintsev, I. A., and S. P. Bystrov, 1937. The effect of freezing on the swelling of tissue. Compt. rend. acad. sci. U.S.S.R. 14, 369. Abstr. in Chem. Abstr. 1937. 31:6266.

Spackman, D. H., 1960. Instruction Manual and Handbook, Beckman/Spinco Model 120 Amino Acid Analyzer. Beckman Instruments, Inc., Palo Alto, California.

Spencer, J. V., W. E. Matson, W. J. Stadelman and M. C. Ahrens, 1956. The effect of cooling and freezing procedures on consumer acceptability factors of turkey meat. Food Technol. 10:16.

Stafanovic, M., and B. L. Walker, 1967. Effect of stationary phase-support ratio on the gas chromatographic separation of trifluoracetyl amino acid butyl esters. Analytical Chemistry 39(7):710.

Swanson, M. H., and H. J. Sloan, 1953. Some protein changes in stored and frozen poultry. Poultry Sci. 32:643.

Tarr, H. L. A., 1947. Preservation of quality of edible fish products. Fisheries Research Board Can., Pacific Coast Star., Progr. Repts. 71:15.

Taylor, H. F., 1930. Solving problems of rapid freezing. Food Industries 2:146.

U.S.D.A., 1967. Poultry and egg situation. PES-245, United States Dept. of Agri., Washington, D.C.

Ward, M. A., T. E. McCarthy and J. S. Nishimurce, 1967. Gas liquid chromatography of derivatives of several amino acids that occur in the actinomycins. Anal. Biochim. 20:275.

Weygand, F., B. Kalb, A. Prox, M. A. Tilak and I. Tomida, 1960. Gaschromatographischi Trennung von N-TFA-Dipeptid-methylestern. Z. Phyziol. Chem. 322:38.

Whitaker, J. R., 1959. Chemical changes associated with aging of meat with emphasis on the proteins. In Advances in Food Research v. 9:1. Academic Press Inc., New York.

Wierbicki, E., L. E. Kunkle and F. E. Deatherage, 1954. The relation of tenderness to protein alterations during post mortem aging. Food Technol. 8:506.

Wierbicki, E., V. R. Cahill and F. E. Deatherage, 1957a. Effects of added sodium chloride, potassium chloride, calcium chloride, magnesium chloride, and citric acid on meat shrinkage at 70°C and of added sodium chloride on drip losses after freezing and thawing. Food Technol. 11:74.

Wierbicki, E., L. E. Kunkle and F. E. Deatherage, 1957b. Changes in the water holding capacity and cationic shifts during the heating and freezing and thawing of meat as revealed by a simple centrifugal method for measuring shrinkage. Food Technol. 11:69.

Wladyka, E. J., 1965. Changes in the amino acid composition of chicken meat and drip during frozen storage. Ph.D. Thesis. Michigan State University.

Youngs, C. G., 1959. Analysis of mixtures of amino acids by GLC. Anal. Chem. 31:1019.

Zlatkis, A., J. F. Oro and A. P. Kimball, 1960. Direct amino acid analysis by GC. Anal. Chem. 32:162.

Zomzely, C., G. Marco and E. Emery, 1962. Gas chromatography of the n-butyl N-trifluoroacetyl derivatives of amino acids. Anal. Chem. 34:1414.