A STUDY OF THE RELATIONSHIP BETWEEN FREE AMINO ACID CONTENT AND TENDERNESS OF CHICKEN MUSCLE TISSUE

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ABSTRACT

A STUDY OF THE RELATIONSHIP
BETWEEN FREE AMINO ACID CONTENT
AND TENDERNESS OF CHICKEN MUSCLE TISSUE

by Julius Henry Miller

The free amino acid content of breast and thigh meat of fresh-chilled and refrigerator stored broilers (tender) and hens (tough) was analyzed for the purpose of establishing a possible relationship between the relative amounts of free amino acids and tenderness in chicken muscle. This information should also help to clarify the nature of the chemical processes which occur during aging and help to resolve the contradictory reports in this area currently in the literature.

Two groups consisting of three broilers and three hens each, all of the same strain and sex, were processed by a standard commercial procedure and chilled in slush ice. All broilers were approximately the same age and weight and had been fed a commercial broiler ration. All hens were approximately the same age and weight and had been fed a commercial laying ration. Samples from one group of birds were prepared after chilling for 18 hours. Birds of the second group were removed from slush ice after 18 hours, vacuum packaged in Cryovac bags and refrigerated at $35^{\circ} \pm 2^{\circ}F$ for one week prior to sample preparation.

Fat and connective tissue free samples were prepared from the right sides only, essentially according to the method of Spackman (1960). The

12.50 ± .02 g samples were cut into 1-3 g portions, ground with 1% picric acid and the resulting protein precipitate was removed by centrifugation. Excess picric acid was removed on an ion exchange resin. After concentrating the clear effluent, aliquots were removed and frozen for storage. A sulfiting procedure converted glutathione, cysteine and cystine to forms which did not emerge as identifiable peaks, and therefore, did not interfere with the chromatography. Two 2-ml aliquots were used for analysis on a Beckman/Spinco Model 120 Amino Acid Analyzer; one aliquot for the basic free amino acids and the other for the neutral and acidic free amino acids.

In general, ammonia nitrogen remained fairly constant throughout the study with little difference between broilers and hens, fresh-chilled or refrigerator stored. Storage resulted in general increases in free amino acid concentration with proline a major exception. Light meat showed lower free amino acid concentrations than dark meat with major exceptions being lysine and histidine. Broilers had a higher concentration of free amino acids than hens in most cases. No relationship was found between tenderness and the general pattern of free amino acid concentration nor between tenderness and the concentration of any single free amino acid.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF APPENDICES

INTRODUCTION

Most people would agree that tenderness is one of the most important factors in determining the quality of meat. Extensive research has been conducted to seek out the causes of tenderness in meat and methods by which tenderness may be evaluated. However, many factors have been considered responsible for tenderness and no objective method of measuring tenderness has yet been devised that is as reliable an indicator as the subjective method which utilizes taste panels.

It has been known for many years that the aging or ripening process for meat is associated with tenderization. This knowledge has been employed by the meat industry much to the pleasure of the consumer. It is also well known that certain changes occur in the muscle proteins during the aging of meat. A review of the literature prior to 1952 by Swanson and Sloan (1953) however, showed that research involving studies of chemical changes in stored, frozen meats, fish and poultry were rather limited and the results reported were quite contradictory in nature. This was especially true, they found, in the area concerned with protein changes.

A review of the literature since the Swanson and Sloan (1953) report revealed a continuing lack of information in this area especially insofar as poultry is concerned. Many contradictions apparently have not been resolved.

With this information in mind, it was thought that a study of free amino acids in the muscles of chicken, both young (tender) and old (tough),

fresh and aged, might show some properties or constituents of the meat that would reflect quality as represented by tenderness.

Specifically, the purposes of this study were: to determine if a relationship existed between the relative amounts of free amino acids and tenderness in chicken muscle; to help clarify the nature of the chemical processes which occur during aging; and to resolve the contradictory reports in this area currently in the literature.

REVIEW OF THE LITERATURE

I. Factors affecting the tenderness of poultry meat

A. General

Tenderness is the foremost factor considered in meat acceptability (Miyada and Tappel, 1956; Parrish et al., 1962). Optimum tenderness, together with optimum flavor, is fundamentally important in the acceptance of meat type chickens (Pool et al., 1959). Even when meat has good flavor, it is still undesirable if it is tough (Deatherage and Reiman, 1946). Although tenderness was once considered a problem only with hens and roosters, research has established that young chicken fryers or broilers may reach the consumer's table with less than optimum tenderness if the effects of modern processing and utilization practices are not taken into account and suitable adjustments made (Pool et al., 1959).

The factors affecting poultry meat tenderness are breed, class, strain, age, sex, diet and grade; muscle structure, composition and location; and post mortem treatment including cooking (Carlson et al., 1962; Dodge and Stadelman, 1959; Harrison et al., 1959). A review of the factors affecting poultry palatability with emphasis on histological post mortem changes was presented by Lowe (1948). An up-to-date comprehensive review of all factors relating to meat tenderness was published by Campbell Soup Company (1963).

B. Muscle characteristics

1. Structure

Schmitt (1944) presented a complete review of structural protein in tissues and Bourne (1960) reviewed the structure and function of muscle. Koonz and Robinson (1946) reported on variations existing within the principal muscles composing the poultry carcass. Strandine et al., (1949) in a chemical and histological study of 50 of the principal beef muscles and 12 principal chicken muscles demonstrated existing variations between muscles within the same species and in different species. Carlson et al., (1962) attributed some variability in Lee-Kramer shear-force values and tenderness scores of a panel to differences between muscles of Broad Breasted Bronze turkeys.

A general correlation between the fasciculi-connective tissue patterns and tenderness was found by Strandine et al., (1949) tenderness being associated with muscles having indistinct fasciculi. A suspected correlation between tenderness in beef and observed microscopic changes in the tissue was reported by Wang and Maynard (1955).

Whitaker (1959) in his review of the chemical changes associated with aging of meat, emphasized the proteins and included discussions on the structure of skeletal muscle and muscle fiber as well as extracellular proteins.

In following the chemical and histological changes which occur during proteolysis of stored beef, Locker (1960) noted that the histological arrangement of muscle fibers must be considered in the tenderness

of beef. Hostetler and Cover (1961) reported a relationship between muscle fiber extensibility and tenderness. Although the role of muscle fibers in meat tenderness is not yet fully understood, it is becoming apparent that not all individual muscle fibers are alike. Differences between them contribute to variations in meat tenderness.

2. Composition

Koonz and Robinson (1946) reported the histological and chemical composition of 12 of the more important muscles which collectively compose approximately 70% of the muscle tissue of the poultry carcass. Various muscles showed some variations in the amount and distribution of connective tissue and fat and in the size and arrangement of muscle bundles. White muscles had relatively little fat, were low in moisture and high in protein. pH of white muscles was lower than that of dark muscles.

Strandine et al., (1949) noted variations in pH, protein, fat and moisture content in beef and chicken muscles, but the variations did not correlate with tenderness values of the muscles.

Of the biochemical factors studied by Husaini et al., (1950 a and b) alkali insoluble protein and muscle plasma as represented by muscle hemoglobin (myoglobin) seemed most closely correlated with changes in tenderness of beef.

A relationship between the amount of nitrogen extractible by buffer solution and tenderness of beef was reported by Wierbicki et al., (1954). Paul et al., (1958) found that a correlation between tenderness

score and percent nitrogen extractible by buffer solution was high enough to be statistically significant but too low to indicate decided usefulness of this means for measuring tenderness of chicken.

Muscle shear values, as determined by a Warner-Bratzler shear press, of birds with the highest muscle glycogen concentrations were lower than values of corresponding birds of lowest glycogen concentrations (Mellor et al., 1958).

Parrish et al., (1962) noted that of all factors influencing tenderness, perhaps connective tissue is the constituent in many beef cuts most responsible for tenderness variations. According to Cover et al., (1962a) connective tissue in beef muscle is made up of collagen, elastin, reticulin and the ground substances. Collagen is present in the largest amount and is heat labile. Ritchey and Cover (1962) reported that it is residual collagen which is important in tenderness of meat. It should be noted that Koonz and Robinson (1946) found elastic connective tissue almost completely absent in poultry muscle.

Ma et al., (1961) used paper chromatography to study variations in the free amino acid content of beef muscle. An analysis of 11 cuts representing nine muscles from a cow showed, in general, that the more tender cuts contain more leucine-isoleucine than the less tender cuts. This finding was confirmed in a comparative study of these same muscles from seven beef animals. In each of these seven animals, the amounts of these amino acids increased from the less tender to the more tender muscles.

A study of the amino acid composition of the protein

mixtures of 10 edible muscle meats including beef, veal, lamb, pork, chicken, turtle, codfish, salmon, frog legs and shrimp was reported by Beach et al., (1943). The protein mixture which makes up the voluntary muscle tissues was found to be similar in Mammalia, Aves, Amphibia, Pisces and Crustacea with respect to the amino acids investigated. Since muscle tissues of these various classes of animals do not differ widely in their amino acid patterns, the findings support the belief that the same or closely similar amino acid composition of muscle proteins is repeated throughout the animal kingdom.

The amounts of 18 amino acids in six different cuts of fresh and cooked choice and utility grades of beef were determined by Greenwood et al., (1951). The percentages of the amino acids in the crude protein of the different cuts and grades were similar. Further, the amino acid composition of crude protein of beef cuts was found to be similar to that of pork and lamb.

Szkutnik (1958) performed studies on the amino acid composition of trypsinized and acid protein hydrolyzates of cattle, sheep, swine and horse meat by paper chromatography. After 24 hours or tryptic or acid digestion, the qualitative and quantitative amino acid composition of all the meats was very nearly the same.

No reports in the literature of microbiological determinations of amino acids in chicken meat were found by Millares and Fellars (1948). Only eight essential and two non-essential amino acids were determined by chemical methods and reported prior to 1948. Their

analyses by microbiological methods indicated that chicken meat is an excellent source of the "indispensible" amino acids and is equivalent to beef, pork, lamb and veal meats on the basis of content of these amino acids. Light chicken meat had a higher protein content than dark. Results reported by Kik (1962) on the protein efficiency of light and dark chicken meat agreed essentially with those of Millares and Fellars (1948).

The percentages of amino acid and basic nitrogen in fresh broilers were found to be 1.02 for light meat and 0.82 for dark meat by Hepburn (1950). In roasters these figures were 1.22 and 0.90, respectively. The percentage of protein was slightly higher in roasters as well.

Scott (1959) compared the amino acid values of turkey meat with those of chicken, beef and pork and showed the essential amino acids were present in similar proportions in all of these meats. Methionine and cystine values showed the largest differences. No significant differences in methionine and cystine of chicken meat were found between individual birds (Fry and Stadelman, 1960). Methionine was highest in light meat.

The amino acid composition of breast and leg muscle from both male and female turkeys was reported to be remarkably constant when the amino acids are expressed as percentages of protein in these tissues (Scott, 1959). Histidine was the only amino acid which appeared to be present in different proportions in leg meat as compared with breast meat. Histidine content was higher in breast meat. Ito (1957-1958), on the other hand, microbiologically determined the amino acid composition

of the muscles of six aquatic animals and reported that red muscle contained approximately twice the amount of histidine found in ordinary muscle.

3. Location and functional specialization

Hanson et al., (1942) reported a correlation between tenderness in broiler breast and thigh muscles and microscopic post mortem changes. Thigh meat was consistently judged to be more juicy and less tender than breast meat.

An apparent difference between the all "white" meat of chicken and turkey breast muscles and the "dark" meat of goose breast was noted by Peters and Dodge (1959) while studying the changes in pH and temperature in poultry breast muscles at slaughter.

The rate of post mortem breakdown process including the interval between death and onset of rigor mortis varies from muscle to muscle in beef (Howard and Lawrie, 1957). These differences appear to be due to the different functional specialization of these muscles in the animal.

Koonz et al., (1954) reported that the tenderness of muscles from one side of a bird compared with muscles from the other side. De Fremery and Pool (1960) found that paired muscles from the same bird were remarkably alike in tenderness if they underwent identical treatments. However, May et al., (1962b) reported differences in tenderness between the right and left breasts for 72-week old birds aged at 0°C and Marion and Stadelman (1958) noted a significant difference in tenderness between the left and right Pectoralis major muscles of chicken fryers. The right half was more tender.

C. Carcass treatment

1. Ante-mortem treatment

Among the ante-mortem treatments devised to affect increased tenderness in poultry meat have been injections of crystalline and/or crude papain (Huffman et al., 1961), withholding of feed for 24 hours and brief exercise immediately before slaughter (Koonz and Robinson, 1946; Lineweaver, 1955), additions of tranquilizer to the feed (Dodge and Stadelman, 1960a) and anesthesia before slaughter (Stadelman and Wise, 1961). Ante-mortem treatments have shown varying degrees of success. Injections of papain resulted in overtenderization of the breast muscle (Huffman, 1961) while the administration of even a high level of tranquilizer by Dodge and Stadelman (1960a) produced almost no measurable affect.

2. Processing

a. Slaughter

Perhaps more important than ante-mortem treatment as a factor influencing the tenderness of poultry meat is processing technique (Pool et al., 1959). Goodwin et al., (1960) conducted two studies to determine the effect of five humane methods and one conventional method of slaughter on post mortem tenderness. They found that humane slaughter affected the tenderness scores.

Dodge and Stadelman (1960a) performed three experiments on 288 birds to determine the effect of struggling on post mortem tenderization and found that under normal processing conditions, struggling does not exert any effect. Gainer et al., (1951) reported that the muscles of birds which struggled during slaughter scored more tender than muscles from birds of the same lot that did not struggle.

b. Scald

Variations in scalding temperature were found to have no effect on tenderness of roasted muscles of Broad Breasted Bronze turkeys (Klose and Pool, 1954). In the case of roasted skin, however, increases in scalding temperature produced marked increases in toughness and wrinkling. Certain modifications in cooking method were found to reduce toughness of roasted skin from turkeys scalded at high temperatures.

Klose et al., (1956a) reported that elevated scalding temperatures and prolonged scalding times did have adverse effects on tenderization of chicken and turkeys. Klose et al., (1959) found that either increased scalding temperature or increased scalding time within commercial ranges had significant but small toughening effects on turkeys. Similar effects were found to be true in chickens (Pool et al., 1959).

Shannon et al. (1957), within the limits of their study, found that increasing time of scald and temperature of scald significantly reduced tenderness of poultry meat as did the interaction of time with temperature. The effect of time was greater than that of temperature.

The effects of various scald-time-temperature combinations on the tenderness characteristics of the Pectoralis major muscle at several distances from the surface were determined by Wise and Stadelman (1959). Resistance to shear was related at a highly significant level to the depth at which the samples were taken, to the temperature of the scald water and to the scald time duration. Under the conditions of their experiment, the toughening effect of the high temperature long time scald

is related to the depth to which the scald heat penetrates the muscle tissue.

c. Beating and picking

Gainer et al., (1951) reported that the muscles of machine picked and hand massaged groups of roasters aged 30 minutes scored more tender than those hand picked. Roasters aged 60 minutes after killing and before cooking was started, were scored more tender than those held 30 minutes, the difference being significant at the 1% probability level.

Klose et al., (1956b) reported that toughness induced in chickens and turkeys by excessive beating cannot be completely resolved by prolonged aging; and that the effects of beating are cumulative and may be reduced by limiting the beating action to that barely essential for complete feather removal.

Turkey fryers and some turkey roasters were subjected to various conditions of feather picking by Klose et al., (1959). Machine picking, such as they employed, resulted in cooked meat about twice as tough as for hand picked controls. The toughening effects of individual picking machines on a commercial line of machines were accumulative. Differences in shear values between machine picked and hand picked birds were essentially unaltered by extending chill period. Thus, earlier findings were substantiated.

In studies similar to those just mentioned, Pool et al., (1959) found the ultimate toughness after aging of chickens increased

with extent of beating action incurred by the carcass during feather removal. Beating action exerted its greatest toughening effect when applied immediately after slaughter. Beating delayed 1 to 3 hours after slaughter had less effect.

Significant differences were also found by Wise and Stadelman (1957) among the different methods of picking and among the treatments within a method. In general, the more severe the beating or the longer the beating period, the more adversely the tenderization process was affected.

Goodwin and Stadelman (1962) reported on the toughening effect of hand massaging turkeys. Massaging was done in tap water for 1 hour before cooking and in slush ice for 1/2, 1 and 2 hours before cooking. Two hours of muscle flexing and massaging increased shear values. Massaging for shorter times did not affect hens as badly as toms or fryers.

d. Irradiation

The minor effect of gamma irradiation on tenderness of poultry meat was reported by Stadelman and Wise (1961). De Fremery and Pool (1960) found that electron irradiation prior to rigor made chicken tougher in texture than samples irradiated after rigor.

e. Polyphosphates

May et al., (1962a) and Spencer and Smith (1962) reported that chilling chickens in a solution of polyphosphates resulted in significant increases in tenderness.

f. Excising the muscle

Lowe (1948) and Koonz et al., (1954) reported that excising muscle before rigor induces a toughness which would be only partially resolved by aging. De Fremery and Pool (1960) substantiated these findings and reported that muscles excised pre-rigor are suitable for studies of additional treatment even though excising induces a moderate degree of toughness.

3. Aging

a. General

As early as 1907, Lehman reported that aging increases the tenderness of beef. Since that time many investigators have studied the effects of aging on tenderness in poultry meat. Most agree that tenderness in poultry meat increases rapidly with the passing of rigor with a maximum tenderness reached in 24 hours at 35°F. However, it has been noted that the exact time required for the same degree of tenderness varies from muscle to muscle and bird to bird (Koonz et al., 1954; Klose et al., 1956a; Dawson et al., 1958; Dodge and Stadelman, 1959; Weinberg and Rose, 1960).

Various experiments designed to shorten the chill time for maximum tenderization have failed to produce positive results (Kahlenberg et al., 1960; Klose et al., 1960; Klose et al., 1961; and Goodwin and Stadelman, 1962).

b. Rigor mortis

Dawson et al., (1958) reported that lack of tenderness,

frequently called "toughness" of chicken meat is connected primarily with muscle fibers and the bio-physical changes which take place following slaughter. Immediately after slaughter, the pliable yet viscous muscle fibers of the living animal pass into a state of turgidity known as "rigor mortis." With resolution of rigor, the muscles become pliable again and normal aging changes proceed. These changes apparently coincide with the development and resolution of rigor but may or may not be directly responsible.

A review of the physiology and chemistry of rigor mortis with special reference to the aging of beef was reported by Bate-Smith (1948). Lowe (1948) reviewed post mortem changes and rigor in poultry muscle. A more comprehensive review of the latest theory on post mortem changes in muscle was presented by Bendall (1960, 1963).

De Fremery and Pool (1960) studied the rate of development of rigor mortis and related biochemical changes in chicken muscle in relation to their effect on the ultimate tenderness of the cooked muscle. Every treatment that resulted in a more rapid loss of ATP (adenosine triphosphate), more rapid drop of pH and more rapid loss of glycogen also induced increased toughness. Injection of sodium monobromoacetate which causes rapid loss of ATP but only a slight decrease in pH and glycogen failed to induce toughness. They postulated that the relative toughness of cooked muscle in otherwise uniform groups increases with increasing rate of onset of rigor mortis or with some factor closely related to it.

This postulation helps explain results of studies by Mellor et al., (1958) on the influence of glycogen on the tenderness of broiler meat as noted above, and by Koonz and Robinson (1946), Peters and Dodge (1959), Dodge and Peters (1960), and Dodge and Stadelman (1960b) on changes in pH and temperature in poultry early post mortem.

c. Muscle plasma changes

Much evidence has been presented in recent years which indicates that increases in tenderness with post mortem age involve, to a great extent, changes in muscle plasma (Wierbicki et al., 1954). Perhaps the muscle plasma proteins of greatest interest are myosin, actin and actomyosin which account for about half of the muscle proteins and are usually considered of primary importance in contraction. Weinberg and Rose (1960) suggested that tenderization is not merely random autolysis but results from a specific cleavage of an action association responsible for the maintenance of the muscle matrix.

d. Oxidizing reactions

Another possibility reported as a factor which may be important for chicken meat tenderization during post mortem aging is the role of certain oxidizing reactions (Chajuss and Spencer, 1962 a,b).

e. Proteolysis

In general, it appears that changes brought about by aging can be associated with either one or a combination of the following factors: 1) changes in the connective tissues, 2) dissolution of actomyosin, 3) increased hydration of the proteins and 4) proteolysis (Whitaker, 1959).

After the death of an animal, the enzymes of the muscles are still quite active. Proteolytic enzymes in tissues hydrolyze the peptide bonds of the proteins. These enzymes are called cathepsins to distinguish them from those of the digestive tract. They are amply supplied with substrate and at least one has been found which is active in frozen meat and has a pH optimum approximately 4.1 (Balls, 1938).

The exact role played by these enzymes in the increase in tenderness associated with aging meat is rather obscure (Whitaker, 1959). Enzymes and their influence on meat tenderness were discussed by Landmann (1963).

Classical approaches to get evidence on the effect of cathepsin in the proteolytic aging process have yielded negative or at best inconclusive results (Wierbicki et al., 1954). The increases in non-protein nitrogen that should occur during proteolysis or autolysis have not always been found though increases in free amino acids have been reported (Locker, 1960).

Paper partition chromatography was used by Ichikawa and Hojo (1950) to study hydrolyzed samples of beef putrified at room temperature in summer for various intervals. The beef after putrefaction for 48 hours still contained all the essential amino acids and significant changes were not observed in the number of amino acids detected. Unidentified spots increased suddenly after 48 hours and most of them were thought to be amines due to their colors with ninhydrin and positions on the chromatogram.

Swanson and Sloan (1953) reviewed the contradictory reports in the literature and reported results of protein changes in stored frozen poultry which indicated that proteolysis occurred during frozen storage. Increases in soluble nitrogen and non-protein nitrogen of both leg and breast muscle were noted. Decreases in amino nitrogen suggested that certain metabolic processes must be continuing in the frozen state causing further breakdown of the amino acids formed by proteolysis. Although the analysis of variance showed that the observed changes were highly significant, F values also indicated that there was a significant difference among birds in the rate of proteolysis.

Wierbicki et al., (1954) confirmed an earlier report by Husaini et al., (1950a) that there is no increase in non-protein and TCA (trichloroacetic acid) soluble nitrogen during post mortem tenderization.

Monzini (1953 a,b) studied quick frozen beef and veal and found that ammonia nitrogen remained low as in fresh meat, amino nitrogen increased to 5-6% of total nitrogen compared with minute quantities present in fresh meat. Cathepsinic and trypsinic activities of the enzymatic extracts of beef were increased after freezing. Although the freezing temperature had no influence, repeated freezing and defrosting increased the intensity of the enzymatic activities. In 1955 these studies were extended to run 48 months and a reduction in the amino nitrogen was noted while ammonia increased.

Ginger et al., (1954) reported that aging increased the amino nitrogen of the non-protein nitrogen fraction of both raw and

cooked meat. The amount in cooked steak plus drippings was always greater than that of raw meat which shows that some proteolysis occurred during cooking. Aging samples had little effect on the arginine, leucine and tyrosine content. A greater percentage of arginine, leucine and tyrosine was found in the drippings and non-protein nitrogen fractions after 2 weeks aging than in paired steaks not aged. Less than 3% of the total amount of these amino acids were found in the drippings or non-protein nitrogen fraction. Bound forms of leucine, tyrosine, glutamic acid and lysine were present in the drippings and non-protein nitrogen fractions. Up to 31% of the total histidine was found in the non-protein nitrogen fraction of the samples and the nitrogen from histidine accounted for 26% of the non-protein nitrogen in the extracts. Aging in the cut increased the free amino acid nitrogen of raw rib steaks.

Colombo and Gervasini (1954, 1955 a,b) detected the following free amino acids in the extracts of the leg muscles of just butchered animals: rabbit: alanine, phenylalanine, glycine, leucine, proline, tyrosine, valine and aspartic acid; ox: same as rabbit plus tryptophan; wild goat: same as ox plus serine.

Alanine, glycine, serine, leucine, tyrosine, valine, lysine, arginine, glutamic acid, aspartic acid and cystine could be detected by chromatography in the muscle of recently butchered beef, pork and horse meat without differences for the various species. The alanine, glutamic acid, cystine and leucine contents of fresh meat were respectively: 0.676, 0.900, 1.964 and 0.261; of 6 day old meat: 0.690, 0.900,

2.060 and 0.312; and of 12 day old meat: 0.708, 0.946, 2.100, and 0.335 mg/g.

Colombo and Gervasini (1956) reported on the chromatographic investigation of free amino acids in fresh, refrigerated and frozen stored fowl meat. Cystine, lysine, arginine, glycine, serine, glutamic acid, threonine, alanine, tyrosine, valine and leucine were found with quantitative variations between chest and limb muscles and with storage conditions. For example, limb and chest muscle leucine and limb and chest muscle glutamic acid averaged (mg/g) 0.083, 2.00, 0.73 and 0.33 in fresh; 2.06, 3.08, 3.23 and 7.00 in refrigeration stored (2-5^{o}C); and 1.26, 2.03, 2.83 and 2.73 in frozen (-12^{o}C) meat.

Holding first grade and canner beef carcasses under practical conditions at 0^{o}C and 20^{o}C was not associated with any change in soluble nitrogen, alkali insoluble protein or hydroxyproline (Bouton et al., 1958). Holding was associated with a reduction in the amount of peptides, carnosine (β-alanyl-L-histidine) and anserine (β-alanyl-1-methyl-L-histidine) present in muscle extracts.

It would appear that the changes brought about by holding treatment or freezing, which are associated with changes in eating quality and water holding capacity, are also associated with change in the protein molecules (increases in -SH groups). On the other hand, it is obvious that no intensive breakdown into smaller units took place since no significant increase in soluble nitrogen occurred and, at least in the case of the holding treatments studied, amino acid changes are

limited to a breakdown of carnosine/anserine. This supports earlier contentions that proteolysis plays little part in post mortem conditioning. This view was upheld again by Bandack-Yuri and Rose (1961) after investigating the proteases of chicken breast muscle.

Marion and Forsythe (1962) reported values for amino, TCA soluble protein and total soluble nitrogen from samples of Pectoralis superficialis and Biceps femoris of Bronze turkey males during: 1) Pre-rigor (10 minutes after slaughter), 2) post rigor (after 26 hours in slush ice), 3) following rapid freezing and thawing or 4) after 60 days storage at -29°C. Average values for pre-rigor muscles (an average of both muscles) were, respectively: 380, 1663, 1733 and 3396 mg/100g of dry tissue. No significant changes occurred as muscles underwent rigor and subsequent storage. A pronounced difference existed between the two muscles with results from the Pectoralis superficialis being consistently higher.

f. Aseptic autolysis

The changes that occur when cod muscle is allowed to spoil during storage in ice are different from those produced by autolysis alone (Hodgkiss and Jones, 1955 and Shewan and Jones, 1957). With spoilage, there are two factors to be considered: 1) leaching losses due to the ice melt water and 2) the transformation by both bacterial and autolytic enzymes.

Little change occurred in most of the free amino acids during storage at 0°C due to autolysis. Individual acids varied somewhat

in the extent of the change but the overall patterns were similar. In sterile cod muscle glycine remained unchanged while alanine and cystine both fell in amount and glutamic acid increased by over 300%.

In spoiling cod muscle, the behavior of the remaining free amino acids is quite different from that in the sterile autolyzing muscle. Several show a slight fall, probably due to leaching over the first few days (e.g. glycine, alanine and glutamic acid), but afterwards they all increase until the 10th day after which they again fall or remain stationary. Lysine on the other hand increases steadily all the time, particularly over the first 10-12 days. The precise reasons for these changes are not known but are obviously linked with bacterial activity. It is probable that the steady increase in lysine is caused by the splitting off of the terminal lysine of the fish protein by the proteolytic bacteria.

Zender et al., (1958) using strict methods to retain naturally sterile conditions, allowed lamb and rabbit muscles to undergo proteolysis in an anaerobic environment. A steady increase in the level of free amino acids and a decrease of glycine-soluble protein was noted during storage at both 25° and 38°C. According to electrophoretic studies, it appeared that the protein first split into large fractions and later into amino acids. Quantitatively speaking, however, the protein affected by the proteolytic process appeared to be small.

Van den Berg et al., (1963) reported that breast meat became less tender after 5 weeks of aseptic storage under nitrogen at 0°C. The tenderness and juiciness of leg meat increased during the first week.

Proteolysis in both breast and leg meat was appreciable resulting in the formation of free amino acids and other breakdown products. Non-protein nitrogen increased with storage as indicated by the accumulation of amino acid nitrogen as determined by the ninhydrin method.

g. Free amino acid changes

Many recent investigators have continued to follow the changes occurring in meat by similar studies. Niewiarowicz (1956) chromatographically determined the free amino acids present after beef and pork were aged for 18 days at 4°C. Most common amino acids with the exception of tryptophan were detected on the first day: α-alanine 18, glycine 12 and others 1-3 mg%. Free amino acids increased with aging. Tryptophan was found on the 12th or 15th day. Taurine, glutathione and carnosine were also detected. Larger amounts of amino acids were found in HCl (hydrochloric acid) hydrolyzates except for tyrosine.

Leinati (1957) used electophoresis and chromatography to show that 6-12 day old meat from slaughtered cattle, sheep, horses and swine contained larger quantities of alanine, cystine, leucine and glutamic acid than did fresh meat. Cooled chicken breast and thigh contained less leucine and glutamic acid than did corresponding frozen parts.

One-dimensional paper chromatography was used by Grau and Böhm (1958) to study free amino acids in "green" and cured meats. There were no differences in the amino acids found between the raw meat and those treated with various curing solutions.

Massi (1958) reported that no amino acid increases take place in frozen meat. However, proteolysis occurred very rapidly during and after defrosting leading to the destruction of the molecular structure. Massi (1959) showed that the free amino acids in meat frozen 2 months increased by one-third. After defrosting, in 8-10 days, the free amino acids doubled and in 15-20 days quadrupled.

Rat, horse, pork, chicken, Cypriners carpio, Katsuonus sp. and Neothunnus macropterus were analyzed by Sasaki et al., (1959) for the presence of free amino acids immediately after death. Glycine, alanine, glutamic acid and taurine were found. The amounts and kinds of free amino acids in meats were more than in pork, Katsuonus and N. macropterus than in chicken and C. carpio. The amounts and kinds of amino acids increased during aging of meat and threonine, leucine, isoleucine and phenylalanine appeared.

Babin and Lazarev (1960) used paper chromatography to follow the change in free amino acid composition in beef after various periods of aging and storage. After 4 hours free cystine, lysine, histidine, arginine, serine, glycine, glutamic acid, alanine, methionine and valine were all found during the 30 day experiment. During the first 7 days at $-2^{o}-1^{o}C$ little change in amino acid composition was noted. Serine and alanine remained constant for 30 days. Cystine decreased after 20 days. Glutamic acid and valine appeared after 20 days. Aspartic acid, arginine, threonine and leucine were not present at 4 hours but the first two increased gradually and the last two were present after 30 days.

In a second experiment by these authors, meat was aged at 2-4°C for 1-13 days, frozen at -27°C and stored at -12 - -15°C for 53 months. At the end of storage arginine had remained nearly constant, alanine increased as ripening increased to 13 days then decreased with longer ripening. A trace of phenylalanine was found in meat aged 13 days. Tyrosine was not found the first day but was found in significant amounts in meat aged 13 days.

The pattern of release from native beef protein of free alanine, arginine, aspartic acid, glutamic acid, glycine, histidine, leucine, methionine, phenylalanine, proline, serine, threonine, tyrosine and valine was reported by Thompson et al., (1961). In general, leucine, valine, alanine, glutamic acid, proline and tyrosine were identified in fresh, untreated beef and the levels of these amino acids increased at each period of storage to 60 days at 34°F. In fresh, untreated beef, either no free amino acids or trace amounts were initially found for the other amino acids. Histidine appeared at 30 days, serine, glycine, threonine and phenylalanine at 45 days and aspartic acid and methionine at 60 days. No free lysine, tryptophan or cystine was found in beef samples stored 60 days at 34°F.

Much work remains to be done before the contribution of the proteolytic enzymes to the increase in tenderness of meat can be evaluated (Whitaker, 1959). Many of the reported changes in the various nitrogen fractions were obtained with meat held longer than the normal aging period. In most of the work, proteolysis was determined by measuring

the amino acids produced. It is apparent that a protein can be extensively degraded before many amino acids are liberated. Also, extensive release of a few amino acids could occur with little total protein breakdown.

h. Freezing effects

There is disagreement in the literature on the effect of freezing on tenderness. Some investigators have reported that the aging process continues in frozen meat and results in tenderization (Carlin, 1949; Carlin et al., 1949) or in chemical changes associated with tenderization (Hepburn, 1950; Swanson and Sloan, 1953; Monzini, 1953 a,b; Colombo and Gervasini , 1956). Others have reported that freezing halts the tenderization process (Koonz et al., 1954; Spencer et al., 1956; Rose and Lentz, undated) or chemical changes associated with tenderization (Bouton et al., 1958 and Massi, 1958).

4. Cooking

A final factor which affects tenderness and requires discussion is cooking method and time. Mickelberry and Stadelman (1960) reported that cooking prior to freezing reduced tenderness of chicken meat. Baking aluminum foil wrapped birds in a convection heated oven yielded a more tender product than other methods studied. Deep fat fried birds were observed to be less tender than birds from other methods of cookery.

Boiling, simmering and pressure cooking old fowl in salt solutions had no advantage over cooking in water with respect to tenderness (Kahlenberg and Funk, 1961). Pressure cooking gave significantly lower Kramer shear force values of cooked breast meat than did either

boiling or simmering. Differences in shear values as a result of either boiling or simmering were not significant.

Goodwin et al., (1962b) reported that method of cooking had no statistical effect on shear values of turkey meat. The methods studied were cooking by microwave oven, deep fat frying, steam pressure, rotary reel oven, a combination of steam and deep fat frying and a combination of deep fat frying and microwave oven. However, May et al., (1962b) reported that cooking chicken in an electronic oven consistently resulted in a product with higher shear readings than an identical product cooked in boiling water.

Turkey meat cooked to 55°C had significantly higher shear values than meat cooked to 77°C or above. The rate of cooking had no significant effect on shear values (Goodwin et al., 1962a).

Cover et al., (1962 a,b,c,d) also reported on the effect of cooking temperature and method on beef and Paul (1963) reviewed some of the more recent studies on the influence of methods of cooking on meat tenderness.

II. Methods of evaluating tenderness

A. General

Pearson (1963) discussed the complexities involved in attempted measurements of tenderness including the factors which must be taken into account in a tenderness evaluation. Deatherage (1963) wrote that "... tenderness means different things to different people." All methods of measuring tenderness must be based on sensory tests, yet the

many drawbacks of sensory evaluation have led to the development of both chemical and mechanical methods (Pearson, 1963).

B. Subjective and objective methods

A review of the sensory methods of tenderness evaluation by consumer panels and laboratory panels along with the advantages and disadvantages of each was presented by Pearson (1963). He also reviewed the objective methods of evaluation including chemical, histological and mechanical developments, the latter being more widely accepted.

The possibility of using free amino nitrogen as an indicator of tenderness was suggested by Pearson (1963) since it increases as proteolysis proceeds. Even though there is little difference in free amino nitrogen per unit of total nitrogen in meat that may differ greatly in tenderness, in a given muscle from the same carcass, free amino nitrogen may be a good indicator of proteolysis and subsequently tenderness.

METHODS AND MATERIALS

I. General

Free amino acid analyses were conducted on samples from the breast and thigh meat of fresh-chilled and refrigerator stored broilers (tender) and hens (tough).

Six broilers and six hens of the same strain and sex were divided into two equal groups of three broilers and three hens each. All broilers were fed a commercial broiler ration and were approximately the same age and weight. All hens were fed a commercial laying ration and were approximately the same age and weight.

II. Processing

All birds were hung on a killing wheel for 2 minutes, bled by the so-called "Kosher" method (outside cut), placed in a Roto-matic scalder containing water maintained at a temperature of $138^{o}\pm2^{o}F$, scalded for 20 seconds and machine picked in an automatic rubber fingered picker. The birds were then hung on shackles, pinned, eviscerated, washed and placed in slush ice for 18 hours.

Samples from birds of Group I were prepared at the end of the 18-hour chill period. Birds of Group II were removed from the slush ice after the 18-hour chill period, vacuum packaged in Cryovac bags and refrigerated at $35^{o}\pm2^{o}F$ for 1 week prior to sample preparation.

III. Sample preparation

Sample preparation was done essentially according to the method of Spackman (1960). Samples of fat and connective tissue

free muscle of 12.50 ± 0.02 g were taken from the right sides only. Breast meat samples were prepared by taking a cross-section of the Pectoralis major and minor muscles; thigh meat, by taking the same representative muscles in every case.

After weighing, the samples were cut into 1-3 g portions and ground in a Waring blender with 125 ml of 1% picric acid for 2 minutes. The resulting precipitate was promptly removed by centrifugation at 30,000 rpm for 30 minutes. The supernatant liquid was passed through a Dowex 2-X10 (chlorine form) resin bed. The resin was packed 4 cm high in a 2 by 20 cm chromatograph tube and covered with glass wool. Prior to use, the resin bed was washed with five 6 ml-portions of 1N HCl and then with water until the effluent was neutral.

Once the supernatant fluid was passed through the resin bed, the walls of the tube and the bed were washed with five 6-ml samples of 0.02N HCl. The clear effluent and washings were concentrated in a rotary evaporator under vacuum to about 5 ml and made up to 25 ml. A 5-ml aliquot was removed, diluted to 15 ml and frozen until the night preceeding analysis.

At that time, the sample was brought to room temperature and the solution adjusted to a pH of 7.2-7.5 with 1N NaOH (sodium hydroxide). One ml of a freshly prepared 0.5M solution of sodium sulfite was added and the pH again adjusted to the above range. The solution was allowed to stand, open to the air, for 4 hours and stirred periodically. The pH of the sample was then adjusted to 2.0-2.2 with

1N HCl, the sample was diluted to 25 ml and frozen overnight. Two 2-ml aliquots were used after thawing for analysis on a Beckman/Spinco Model 120 Amino Acid Analyzer; one aliquot for the basic free amino acids and the other for the neutral and acidic free amino acids.

RESULTS AND DISCUSSION

The Beckman/Spinco Model 120 Amino Acid Analyzer provides for the complete 24-hour separation and quantitative analyses of the amino acid content of unknown mixtures. The basic principles underlying the analysis performed by the Model 120 are elution chromatography from buffered columns of ion exchange resin followed by colorimetric determination of the separated components by the ninhydrin reaction. The amount of each component amino acid in a sample analyzed by the Model 120 is determined by measuring the area enclosed by its corresponding peak on a chromatogram and comparing it to that of a standard.

Details on the theory and operation of the Model 120, including the methods of integration used in calculating peak areas of the 24 samples analyzed, are discussed in the Appendix.

The average concentration in micromoles of free amino acids found in the various samples are listed in Tables 1 and 2. Increases in free amino acids during storage were noted in this study with few exceptions, the main ones in broilers being that taurine decreased slightly in dark meat, proline showed a decrease in both light and dark meat and lysine and histidine decreased in the light meat only; in hens, taurine again decreased in the dark meat while proline remained fairly constant. These expected increases are generally in agreement with previous reports by Ginger et al., (1954) Colombo and Gervasini (1954; 1955 a,b; 1956), Niewarowicz (1956) and Thompson et al.,(1961). Ammonia

Table 1. Average free amino acid concentration[a] of fresh-chilled broilers and hens (Group I).

Free amino acid	Broilers		Hens	
	dark meat	light meat	dark meat	light meat
Lysine	1.21	3.23	0.709	3.44
Histidine	0.484	2.30	0.550	3.10
Ammonia nitrogen	1.27	1.55	1.27	1.48
Arginine	0.0902	0.0554	0.0598	0.0294
Taurine	2.82	0.102	4.26	0.0463
Aspartic acid	0.0496	0.0249	0.0745	0.0116
Threonine	0.143	0.0848	0.115	0.0624
Serine	0.973	0.174	0.612	0.110
Glutamic acid	0.305	0.127	0.394	0.150
Proline	0.121	0.0998	0.111	0.0758
Glycine	0.284	0.119	0.209	0.0726
Alanine	0.426	0.155	0.432	0.0999
Valine	0.0417	0.0411	0.0437	0.0407
Methionine[b]	0.0344	0.0486	0.0356	0.0193
Isoleucine	0.0196	0.0238	0.0277	0.0158
Leucine	0.0452	0.0418	0.0479	0.0311
Tyrosine	0.0392	0.0378	0.0214	0.0281
Pnenylalanine	0.0222	0.0215	0.0235	0.0139

[a]Average concentration in micromoles of three 0.20 g samples.

[b]Total free methionine and free methionine sulfoxide concentration (see Appendix Tables 1 and 2).

Table 2. Average free amino acid concentration[a] of refrigerator stored broilers and hens (Group II).

Free amino acid	Broilers		Hens	
	dark meat	light meat	dark meat	light meat
Lysine	1.10	2.89	1.06	3.68
Histidine	0.637	1.60[b]	0.942	3.81
Ammonia nitrogen	1.36	1.26	1.33	1.73
Arginine	0.112	0.0705	0.0876	0.0791
Taurine	2.62	0.265	3.47	0.199
Aspartic acid	0.117	0.0674	0.0859	0.0401
Threonine	0.168	0.141	0.0779	0.0840
Serine	0.961	0.355	0.788	0.257
Glutamic acid	0.576	0.258	0.455	0.251
Proline	0.115	0.0742	0.189	0.0753
Glycine	0.357	0.207	0.305	0.127
Alanine	0.522	0.312	0.454	0.209
Valine	0.0863	0.106	0.0587	0.0812
Methionine[c]	0.0469	0.0896	0.0328	0.0654
Isoleucine	0.0506	0.0721	0.0306	0.0477
Leucine	0.0944	0.135	0.0628	0.0987
Tyrosine	0.0525	0.0760	0.0268	0.0557
Phenylalanine	0.0359	0.0549	0.0216	0.0404

[a]Average concentration in micromoles of three 0.20 g samples.

[b]Average of two samples only.

[c]Total free methionine and free methionine sulfoxide concentration (see Appendix Tables 3 and 4).

nitrogen remained fairly constant throughout this study with little difference between broilers and hens, fresh-chilled or refrigerator stored.

In comparing the amounts of free amino acids in light and dark broiler meat lysine, histidine, methionine and isoleucine were found to be higher in light meat and the others lower. In hens lysine, histidine and tyrosine were higher in the light meat. After storage, lysine and histidine remained higher in the light meat of both hens and broilers and valine, methionine, isoleucine, leucine, tyrosine and phenylalanine were also higher. The biggest difference in composition in the light and dark meat of both broilers and hens was in the taurine concentration. It was much higher in dark meat.

Generally, the amount of free amino acids in broilers was higher than in hens; however, many exceptions were noted. In the dark meat of fresh chilled birds, the concentrations of many of the free amino acids were higher in hens; in the light meat of both fresh-chilled and refrigerator stored birds, the concentrations of lysine and histidine were higher in hens; and in refrigerated birds, the proline concentration was higher in hens.

Variations between birds were expected and observed. The variations were more pronounced in hens than in broilers. The fact that some of the hens used in this study were in production while others were not may explain some of the variation.

Poor resolution or incomplete separation between lysine and histidine and between threonine and serine proved to be a

problem. In one case, the histidine concentration could not be calculated (see Appendix Table 3). Although the concentration of these amino acids in the other runs were calculatable, the poor resolution may explain some of the variations noted in the tables. The only unknown peak of any significance occurred prior to taurine.

These data lead the author to the conclusion that Bouton et al., (1958), Bandack-Yuri and Rose (1961) and Zender et al., (1958) were correct in suggesting that proteolysis plays little part in the post mortem conditioning of meat. No relationship could be found between tenderness and the general pattern of free amino acid concentration nor between tenderness and any single free amino acid.

SUMMARY AND CONCLUSIONS

Free amino acid analyses were conducted on the muscle tissues of chickens, both young (tender) and old (tough), fresh and aged, in an attempt to show some properties or constituents of the meat which would reflect quality as represented by tenderness and to elucidate the nature of the chemical processes which occur during aging.

Six broilers and six hens were divided into two groups of three broilers and three hens each. All birds were processed in a standard commercial procedure. The eviscerated, washed birds were placed in slush ice for 18 hours.

Samples of fat and connective tissue free muscle were prepared from the breast and thigh meat of the right sides of birds essentially according to the method of Spackman (1960). Birds of Group I were sampled at the end of the 18 hour chill period. Birds from Group II were removed from the slush ice after the 18 hour chill period, vacuum packaged in Cryovac bags and refrigerated at $35^{o} \pm 2^{o}F$ for 1 week prior to sample preparation. A Beckman/Spinco Model 120 Amino Acid Analyzer was used for the analyses.

In general, ammonia nitrogen remained fairly constant throughout the study with little difference between broilers and hens, chilled or stored. Storage resulted in general increases in free amino acids with proline a major exception. Light meat showed less free amino acids than dark meat with major exceptions being lysine and histidine. Broilers had more free amino acids than hens in most cases.

Further attempts should be made to determine the pattern of release of amino acids from chicken meat post mortem. Larger samples are required since the variations between birds exert a significant influence on results. Studies should be conducted to determine whether or not samples from the same carcass could be used for analysis after varying periods and conditions of storage. The changes which occur during autolysis should be compared to changes during spoilage. The effect of diet on the free amino acid concentration should be determined.

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APPENDICES

APPENDIX A

The Beckman/Spinco Model 120 Amino Acid Analyzer[1]

Ion-Exchange Chromatography

"The Model 120 effects the separation of the amino acids in a sample by chromatography on ion-exchange resins. When an amino acid is placed on a column of the sodium salt of a polysulfonic resin, ion-exchange takes place. Polysulfonic resin is a cation exchanger having negatively charged sulfonic acid groups; the amino acid molecule is attracted to the resin primarily through ionic forces by means of its positively charged amino group. This is a reversible reaction and equilibrium takes place. The amount of a given quantity of an amino acid which is bound to the ion-exchange resin relative to that remaining in solution at equilibrium and under a given set of conditions is usually expressed as a distribution coefficient, K, and the magnitude of this coefficient depends on the structure of the individual amino acid.

"As the amino acid still in solution filters down through the resin, the balance of equilibrium at the top of the column is destroyed, resulting in that portion of the resin releasing more of the amino acid molecules to restore the balance. As the amino acid solution travels down the column, the balance of equilibrium at each point is first established, then destroyed. The rate at which the zone of each individual amino acid moves down the column depends upon the distribution coefficient, K, providing the resin particle size is sufficiently small, relative to the flow rate of the eluting buffer, to allow equilibrium conditions to prevail. This overall separation of the components in the sample is dependent on the chemical composition, resin particle size and resin pore size (degree of cross linking) of the resin; the diameter and length of the packed column; the charge and side group of the amino acid; the pH, ionic strength and flow of the eluting buffer and the temperature of the operation. Providing the capacity of the resin is not exceeded, each amino acid in the sample moves down the column in an individual and independent zone and with the appropriate control of the foregoing variables, conditions are established to allow each amino acid to be separated from each other amino acid by the time it emerges from the column.

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[1]Spackman, Darrel H. Instruction Manual & Handbook. Beckman/Spinco Model 120 Amino Acid Analyzer. Beckman Instruments, Inc. 1960. p. 1-2, 1-3, 10-2. Palo Alto, California.

"The basic amino acids (those amino acids which contain an extra basic group such as histidine, arginine, tryptophan and lysine) have the strongest affinities for the resin while the acidic amino acids (those containing an extra carboxyl group such as aspartic acid and glutamic acid) have the weakest. The ionic bond strengths of the remaining neutral acids (the monoaminomonocarboxylic acids such as glycine, alanine, valine, isoleucine and leucine; the hydroxyamino acids such as methionine; the aromatic acids such as phenylalanine and tyrosine and the pyrrolidyl acids such as proline) lie between these, with the aromatic amino acids having the strongest of the neutral amino acids.

"If the process of separation were to be limited to the flow of buffers through the columns by gravity or moderate air pressure, the duration of the period required for complete analysis would be unwieldly. In the Model 120, buffers are forced through the columns by positive displacement pumps working at several atmospheres pressure to allow a complete protein or peptide analysis to be carried out in 24 hours. The amino-acid-containing sample is chromatographed on the resin columns with acidic sodium-citrate buffers. In the analysis of protein and peptide hydrolyzates, two buffers are used with automatic change from the first to the second (which was a higher pH) midway through the analysis of the neutral and acidic amino acids. A third buffer with a higher pH and higher ionic strength is used with a second column for the analysis of the basic components in the sample."

Color Development and Photometric Determination

"A number of polycarbonyl compounds undergo extensive reaction with amino acids. The most studied of these reagents, which lead to the formation of a colored compound, is ninhydrin, triketohydrinene hydrate. Ninhydrin, with an amino acid, participates in a deaminative oxidative decarboxylation and then condenses further to give a blue compound, diketohydrindylidene-diketohydrindamine (DYDA), which can be crystallized in pure form. The color formed from the reaction with the imino acids proline and hydroxyproline is yellow.

"The Model 120 makes use of this reaction to make a quantitative colorimetric analysis of each amino acid. By maintaining constant environmental factors, the color formation can be made proportional to only the quantity of the amino acid present. Using a colorimeter containing three photometer units each consisting of a light source, a lens, an interference filter, a slit, the cuvette and a photovoltaic cell, an electrical current is generated proportional to the density of the color in the effluent-ninhydrin mixture.

"The electrical current is then used to drive a conventional multipoint recorder which plots the results of the analysis as absorbance versus

time. As the DYDA from each amino acid passes through the colorimeter, light to the voltaic cell is reduced, resulting in a reduction of electrical output and a movement on the recorder pen. Three multipoint curves are plotted simultaneously consisting of a series of peaks, each peak corresponding to a specific amino acid."

Calculations

"The amount of each component amino acid in a sample analyzed by the Model 120 is determined by measuring the area enclosed by its corresponding peak on the chromatogram... . The [chromatogram] chart travels at a rate of 3 inches per hour and is marked along the length with a light line every 0.1 inch and a heavy line every 1.0 inch. At column elution rates of 30 ml/hr, each light line along the chart is equivalent to 1.0 ml elution volume. The chart is calibrated across in absorbance on a log scale from zero to infinity. The recorder prints a dot every 5 seconds; a dot on each of the three printed curves is thus printed every 15 seconds. On each of the three curves, every fourth dot is black; this pattern assists in the integration of the area under each peak... . Thus on each curve and for each 1.0 ml effluent volume (i.e., each 0.1 inch) there are printed 8 dots, two of them being black.

"Two methods are used to integrate these peak areas: the height-width (HW) method and the absorbance method. Both methods are accurate. The HW method is the faster of the two and hence is used for the integration of the majority of peaks. The absorbance method is used for peaks which are 1) markedly askew or asymmetric...; or 2) are incompletely separated from adjoining peaks...; or 3) are very small such as the methionine sulfoxides... .

"In using the HW method, the height of the peak is multiplied by the width which is measured at half the height. The height of the peak is easily determined from the chart and since the chart scale is a log scale, the proportional accuracy with which a height value can be read is about the same over all of the usable part of the scale. The width of a peak is measured in terms of time by counting the number of dots printed above the half-height of the peak. To facilitate the half-height of the peak. To facilitate the counting of the dots, every fourth dot of each of the three curves is black.

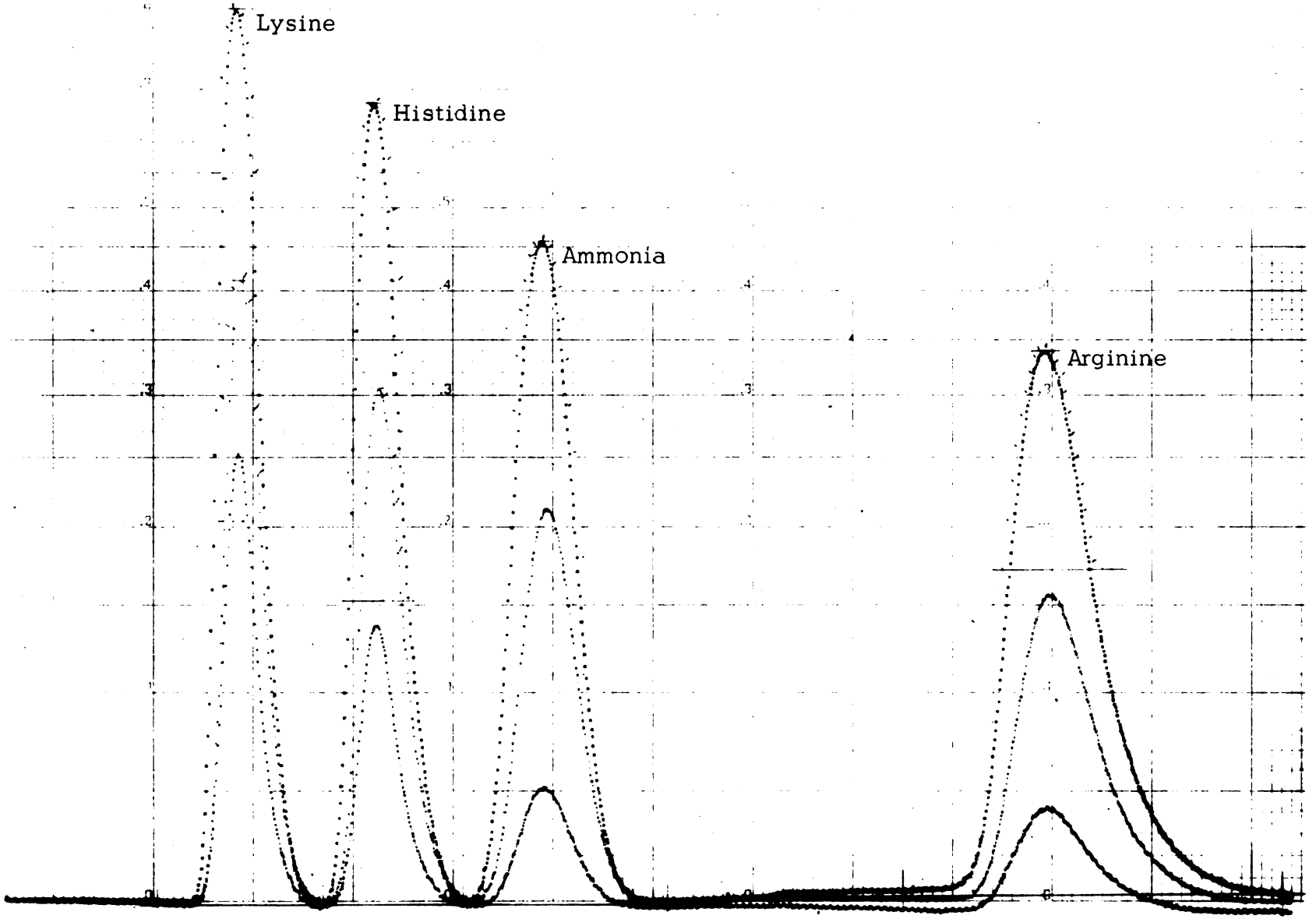
"In using the absorbance method, the absorbance values read at each 1 ml increment under the peak are added. The sum is then converted to micromoles concentration by dividing by the product of the HW constant and the absorbance conversion constant."

Standardization is accomplished by analyzing aliquots of a standard amino acid calibration mixture of known concentrations. "...From the standardization

runs, HW constants (C_{HW}) are calculated from each individual amino acid. These constants are then used for the calculation of unknown components in other samples. As part of the initial standardization, the alternate 570 mμ conversion constant and the absorbance conversion constant are also determined.

"The former of these relates the peak areas under the normal 570 mμ curve and the alternate 570 mμ curve and is used when alternate 570 mμ peaks are integrated. The absorbance conversion constant is used whenever integration by the absorbance method is carried out.

"The results of integrations and further calculations are recorded on the back side of the data sheets [see sample data sheet, page 55]... . The 440 mμ curve (yellow) is used for proline and hydroxyproline. The normal 570 mμ curve (red, normally the tallest) is used for all other peaks whose height on the normal 570 mμ curve exceeds 1.40, the alternate (suppressed) 570 mμ curve (green) is used. In the latter case, areas integrated using the alternate 570 mμ curve are converted to equivalent normal 570 mμ values by multiplying by the alternate 570 mμ conversion constant... ."



APPENDIX B

Typical chromatogram of the basic amino acids and ammonia

APPENDIX C

50° RUN DATA SHEET

BECKMAN/SPINCO
MODEL 120

CHROMATOGRAM No. _____

SAMPLE _____

Date _____

Analysis No. _____

Operator _____

Calculations _____

Checked _____

Total Sample Volume (ml) _____

Volume on Col. 2 _____

Volume on Col. 1 _____

NOTES _____

	Col. 2	Col. 1
Ninhydrin in at		
Recorder on at		
Buffer Timer Set		
Temp. Timer Setting		
Shutdown Timer Set		
Height of Resin		

NINHYDRIN

Age _____ days

Notes _____

PUMP and HELIPOT DATA

		Total Vol (Reac. coil)	Pump 1			Pump 2			Ninhydrin Pump			Helipots		
			Set	P	Vol	Set	P	Vol	Set	P	Vol	1	2	3
Column 2	Start													
	End													
Column 1	Start													
	End													

Amino Acid	Base Line	Height	Half Height	H Net Height	W Width (Dots)	H x W	Micro moles $\frac{H \times W}{C}$						
Lysine													
Histidine													
Ammonia													
Arginine													
Aspartic Acid													
Threonine													
Serine													
Glutamic Acid													
Proline													
Glycine													
Alanine													
Half Cystine													
Valine													
Methionine													
Isoleucine													
Leucine													
Tyrosine													
Phenylalanine													

APPENDIX D

Table 1. Free amino acid concentration[a] of fresh-chilled broilers.

Free amino acid	dark meat samples 1	2	3	light meat samples 1	2	3
Lysine	1.30	1.27	1.07	3.51	2.70	3.49
Histidine	0.526	0.510	0.418	2.30	2.80	1.79
Ammonia nitrogen	1.19	1.37	1.25	1.62	1.46	1.57
Arginine	0.0650	0.0999	0.106	0.0433	0.0439	0.0791
Taurine	3.01	3.13	2.33	0.0780	0.149	0.0795
Methionine sulfoxide	0.0355	0.0166	0.0191	0.0197	0.0171	0.0681
Aspartic acid	0.0432	0.0431	0.0625	0.0278	0.0132	0.0337
Threonine	0.190	0.107	0.131	0.0999	0.0596	0.0949
Serine	1.09	0.778	1.05	0.194	0.162	0.165
Glutamic acid	0.289	0.233	0.394	0.114	0.155	0.112
Proline	0.117	0.124	0.123	0.0851	0.0762	0.138
Glycine	0.303	0.283	0.267	0.107	0.112	0.137
Alanine	0.417	0.471	0.392	0.136	0.153	0.175
Valine	0.0433	0.0404	0.0415	0.0493	0.0345	0.0394
Methionine	0.0126	0.0121	0.00745	0.0146	0.0119	0.0142
Isoleucine	0.0231	0.0186	0.0172	0.0265	0.0217	0.0232
Leucine	0.0456	0.0493	0.0408	0.0498	0.0392	0.0363
Tyrosine	0.0427	0.0339	0.0409	0.0586	0.0284	0.0265
Phenylalanine	0.0242	0.0170	0.0254	0.0410	0.0154	0.00798

[a]Concentration in micromoles per 0.20 g sample.

Table 2. Free amino acid concentration[a] of fresh-chilled hens.

Free amino acid	dark meat samples 1	2	3	light meat samples 1	2	3
Lysine	0.459	0.817	0.852	3.01	3.24	4.06
Histidine	0.205	0.613	0.833	2.43	3.24	3.64
Ammonia nitrogen	1.03	1.37	1.42	1.51	1.45	1.47
Arginine	0.0398	0.0530	0.0866	0.0388	0.0166	0.0328
Taurine	4.04	4.12	4.62	0.0688	0.0121	0.0580
Methionine sulfoxide	0.0121	0.0510	0.0185	0.0152	0.0139	0.00634
Aspartic acid	0.0484	0.0802	0.0950	0.0113	0.0121	0.0113
Threonine	0.0815	0.186	0.0788	0.0528	0.0965	0.0379
Serine	0.450	0.621	0.766	0.133	0.0955	0.103
Glutamic acid	0.325	0.404	0.454	0.131	0.134	0.186
Proline	0.107	0.119	0.106	0.120	0.0565	0.0508
Glycine	0.119	0.263	0.244	0.0775	0.0757	0.0647
Alanine	0.335	0.459	0.502	0.1073	0.0976	0.0948
Valine	0.0352	0.0516	0.0471	0.0447	0.0390	0.0354
Methionine	0.00816	0.00958	0.00745	0.00280	0.0105	0.00911
Isoleucine	0.0388	0.0234	0.0210	0.0132	0.0176	0.0166
Leucine	0.0491	0.0494	0.0453	0.0283	0.0344	0.0307
Tyrosine	0.0180	0.0240	0.0222	0.0200	0.0392	0.0252
Phenylalanine	0.0243	0.0313	0.0149	0.0147	0.0123	0.0147

[a]Concentration in micromoles per 0.20 g sample.

Table 3. Free amino acid concentration[a] of refrigerator stored broilers.

Free amino acid	dark meat samples			light meat samples		
	1	2	3	1	2	3
Lysine	1.14	0.819	1.33	3.25	2.80	2.63
Histidine	0.580	0.486	0.844	- -[b]	1.78	1.42
Ammonia nitrogen	1.32	1.39	1.36	1.41	1.16	1.20
Arginine	0.121	0.0708	0.144	0.166	0.0467	0.0689
Taurine	2.16	3.52	2.20	0.363	0.180	0.252
Methionine sulfoxide	0.0380	0.0102	0.0248	0.0650	0.0349	0.0376
Aspartic acid	0.161	0.118	0.0723	0.111	0.0409	0.0502
Threonine	0.169	0.161	0.177	0.202	0.110	0.110
Serine	0.911	1.01	0.958	0.503	0.273	0.290
Glutamic acid	0.765	0.609	0.352	0.383	0.206	0.186
Proline	0.102	0.128	0.114	0.0907	0.0776	0.0544
Glycine	0.412	0.299	0.361	0.315	0.151	0.154
Alanine	0.564	0.475	0.527	0.470	0.241	0.224
Valine	0.118	0.0597	0.0811	0.167	0.0750	0.0768
Methionine	0.0263	0.0135	0.0281	0.0647	0.0347	0.0320
Isoleucine	0.0774	0.0295	0.0450	0.114	0.0485	0.0536
Leucine	0.120	0.0659	0.0969	0.199	0.107	0.100
Tyrosine	0.0648	0.0335	0.0593	0.105	0.0574	0.0653
Phenylalanine	0.0460	0.0271	0.0347	0.0816	0.0400	0.0431

[a]Concentration in micromoles per 0.20 g sample.

[b]Not calculatable due to incomplete resolution.

Table 4. Free amino acid concentration[a] of refrigerator stored hens.

Free amino acid	dark meat samples			light meat samples		
	1	2	3	1	2	3
Lysine	0.810	1.01	1.36	3.45	3.57	4.00
Histidine	0.658	1.33	0.843	3.18	4.66	3.59
Ammonia nitrogen	1.37	1.25	1.36	1.65	1.84	1.71
Arginine	0.0834	0.0941	0.0852	0.0822	0.0875	0.0676
Taurine	4.46	2.85	3.10	0.303	0.119	0.175
Methionine sulfoxide	0.00761	0.0101	0.0266	0.0245	0.0293	0.0325
Aspartic acid	0.0888	0.0996	0.0694	0.0460	0.0386	0.0356
Threonine	0.0769	0.0750	0.0817	0.0852	0.0745	0.0922
Serine	0.740	0.966	0.659	0.267	0.244	0.260
Glutamic acid	0.586	0.499	0.281	0.212	0.290	0.250
Proline	0.120	0.322	0.126	0.0656	0.0976	0.0628
Glycine	0.270	0.382	0.264	0.125	0.120	0.137
Alanine	0.423	0.456	0.484	0.198	0.205	0.225
Valine	0.0567	0.0681	0.0514	0.0790	0.0854	0.0792
Methionine	0.0189	0.0224	0.0126	0.0309	0.0376	0.0444
Isoleucine	0.0288	0.0349	0.0282	0.0443	0.0467	0.0522
Leucine	0.0589	0.0722	0.0572	0.0946	0.0936	0.108
Tyrosine	0.0305	0.0214	0.0284	0.0635	0.0455	0.0582
Phenylalanine	0.0202	0.0225	0.0221	0.0432	0.0354	0.0427

[a]Concentration in micromoles per 0.20 g sample.