Nutrient Characterization of Color Modified
and Unaltered Flaked Turkey Thigh Meat

by

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(ABSTRACT)

Flaked, skinless and boneless turkey thighs were successively washed in 0.03M sodium phosphate buffers at pH 5.8, 7.4 and 8.0. Proximate, mineral and riboflavin composition, as well as protein efficiency ratio (PER) and apparent digestibility (AD) using the rat bioassay technique were determined for three replications. The color modified tissue (CMT) had a higher (P=0.0429) moisture content and less (P=0.0527, 0.1240 and 0.0047, respectively) crude protein, fat and ash than flaked thigh (THI). Percentage of iron, magnesium, phosphorous, potassium and manganese decreased (P=0.0187) after color modification, whereas calcium, zinc and copper concentrations did not change (P=0.1184) and sodium increased (P=0.0058). Riboflavin was

reduced by 30%.

The PER of CMT evaluated was lower (P=0.0318) than THI, but higher (P=0.0001) than either casein or egg albumin diets. AD of CMT was 90.7% which is comparable to other meat products. The overall nutritional evaluation of CMT determined that it has potential as a raw material in further processed foods.

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1.0 INTRODUCTION

1.1 Background

Between 1984 and 1986, turkey production in the United States increased from 1.2 billion kg of processed turkey meat to 1.4 billion kg with a record consumption of 6.1 kg per person in 1986 (USDA, 1987). Much of that increase was due to consumer demand for white turkey meat (USDA, 1987). This white meat preference resulted in an oversupply of 84 million kg of turkey dark meat (Graham et al., 1989).

Early attempts at reducing the dark meat oversupply were to formulate new products using turkey dark meat, such as ham, luncheon meats, frankfurters and smoked turkey drumsticks. However, the manufacture of these products did not eliminate the dark turkey meat oversupply because consumer attitudes about the deleterious effects of fat in red meat (Briedenstein, 1988) and dark poultry meat have reduced demand and consumption. An alternate approach to creating demand for turkey dark meat is the lightening of dark meat color to resemble white meat and formulating it into intermediate value products. The most obvious approach to lightening dark meat is to remove the hemoglobin and myoglobin pigments which are principally responsible for the red color of muscle tissues.

Pigment extraction procedures have been developed to quantitate myoglobin and hemoglobin (Shenk et al., 1934;

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DeDuve, 1948; Fleming et al., 1960). Cold distilled water
was blended with finely comminuted muscle tissue, followed
by centrifugation and filtering in order to extract the
myoglobin and hemoglobin. Since then, researchers have used
various methods to lighten the overall color of dark meat
through pigment extraction.

The seafood industry in the U.S. has recently
introduced analogs which resemble natural seafoods, but are
manufactured from minced fish flesh that has been washed to
remove fat, blood, pigments and odorous substances. This
material is called surimi after a 900 year old Japanese
method used to preserve fresh fish. Surimi manufacturing
techniques have been modified for use with bovine, ovine and
porcine muscle tissues to selectively extract the pigment-
containing protein fraction.

Procedures have been developed to lighten poultry dark
meat using differing particle sizes and washing treatments
(Ball et al., 1984; Bowie, 1985; Hernandez et al., 1986;
Elkhalifa et al., 1988; Dawson et al., 1988). While these
researchers have addressed color change, protein and amino
acid profiles and functional properties of color modified
tissues, data concerning nutrient profiles and nutritional
quality of color modified poultry meat are incomplete.

The removal of water soluble proteins during washing
influences nutritional as well as functional equivalence as
compared to unaltered tissues. Modified amino acid profiles

and composition in color modified tissues (Bowie, 1985; Elkhalifa et al., 1988) are suggestive of changes in protein nutritional quality. In addition, protein loss may affect iron (Fe) absorption. The reduction of myoglobin and hemoglobin coupled with a lowering of fat content can produce a raw material that is less susceptible to lipid oxidation. Loss of Fe in conjunction with myoglobin and hemoglobin reduction may lower lipid oxidation in color modified tissue (Bowie, 1985). However, preliminary studies by Graham et al. (1989) revealed that flavor desirability and thiobarbituric acid values of products manufactured from color modified turkey varied little from unaltered dark turkey products during frozen storage.

Other water soluble components such as vitamins and minerals incur potential leaching during washing as indicated by a reduced ash content (Ball et al., 1983; Bowie, 1985; Elkhalifa et al., 1988). Increased water content of washed tissues and decreased water-holding capacity on the basis of cooking loss may be related to concentration changes of phosphorous (P), sodium (Na) and magnesium (Mg), as well as changes in pH.

Nutritional, as well as functional, alterations may occur in color modified tissues. Meat products are considered to be a good dietary source of iron and other essential minerals which affect membrane permeability, protein interactions and enzyme regulation. In 1984, meat provided 45%

of the adult male's required daily allowance (RDA) of protein (Briedenstein, 1987). In view of these facts, knowledge about the effect of altering the protein and nutrient composition of color modified meat, as it relates to nutritional value, will be necessary before these tissues are formulated into food products.

1.2 Objectives

Meat products are a nutrient dense form of essential amino acids, essential fatty acids, vitamins (especially the B vitamins) and minerals, including iron. If color modified tissues are to be used in marketable food products, nutritional characteristics should be identified. Furthermore, knowledge of the effects of washing on the nutrient profile of these materials will help elucidate explanations for some of the observed functional and sensory changes, such as increased water-holding capacity, juiciness and oxidative rancidity.

This study was designed to provide a comparison of the nutrient content and nutritional equivalence of color modified flaked turkey thigh meat (CMT), as described by Elkhalifa et al. (1988), with unaltered flaked turkey thigh meat (THI). Scientific data gathered in this experiment should assist in determining the value of CMT as a potential raw material in processed turkey products.

Therefore, the specific objectives of this research were to:

(a) provide a nutrient profile indicative of the overall effects of color modification by determining proximate, mineral and riboflavin composition and to

(b) estimate the nutritional equivalence of CMT and THI using protein efficiency ratio (PER) and apparent digestibility (AD).

2.0 REVIEW OF LITERATURE

2.1 Color Modification of Muscle Tissue

While using washed muscle tissue in food products is
new to the poultry and red meat industry, the seafood
industry commercialized washed fish mince for food products
in the early 1960's. The harvest of Alaska pollack was
large in Japan as a result of the commercial value of their
eggs, but the body flesh was wasted because of the drop in
quality due to rapid freeze denaturation (Suzuki, 1981).
This waste of potential food material led researchers to
explore ways of extending the storage quality of Alaska
pollack. The resultant technology produced a washed fish
mince called surimi. In making surimi, fish muscle was
mechanically deboned, water-washed with 7 to 8 times its
volume and dewatered with the addition of cryoprotectants,
such as sucrose, sorbitol, polyphosphates and salt. The
residual washed fish mince was then processed fresh or
frozen in 10 kg blocks.

Washing fish muscle removes fat, blood, pigments and
odorous substances. Typically the tissue absorbs water
during washing and is subsequently strained to provide a
final moisture content of 77 to 80% (Suzuki, 1981). The
remaining 20% of surimi is primarily myofibrillar proteins.
Surimi is basically a tasteless gel that may then be blended
with other ingredients, flavored, formed, used as a stuffing
or extruded (Anonymous, 1988).

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Applying surimi technology to muscle tissue other than fish presents new challenges. Fish muscle fibers are arranged in myotomes one cell layer thick that are connected by thin layers of collagenous connective tissue. In birds and mammals, fibers are arranged in parallel fashion to form bundles and groups of bundles that are sheathed in thick layers of connective tissue (Hultin, 1985). The tight arrangement of the fibers and the barrier provided by connective tissue make it more difficult to wash components from avian and mammalian muscle. According to Suzuki (1981), another advantage that fish flesh has over avian and mammalian tissue in surimi processing is that the red, intermediate and white fibers are more distinctly concentrated and therefore more easily separated. This arrangement makes removal of blood and pigment from fish more efficient.

The red color of fresh avian and mammalian muscle is due largely to the concentrations and chemical state of the heme pigments, myoglobin and hemoglobin. Myoglobin is inherently purplish-red, but when it is oxygenated to oxymyoglobin the color changes to bright red. Oxidation of the iron in heme pigments results in a brown discoloration from metmyoglobin formation, which is reversible if reducing components are available. These pigments are components of the sarcoplasmic protein fraction, which contributes 30-34% to the total protein content of muscle tissue (Bowie, 1985).

The techniques for color modifying dark avian and mammalian muscle tissues have been developed from procedures to extract heme pigments for the estimation of total pigment concentrations of bovine and porcine muscle. The solutions used in extractions have been water and various concentrations of buffers at pH ranges from 4.5 to 8.0.

Ice cold distilled water and multiple extractions of meat slurries followed by filtration were used by Shenk et al. (1934), Drabkin (1950), and Fleming et al. (1960) in chromoprotein concentration estimations. DeDuve (1948) successfully extracted 70-95% of the pigments using a 0.04M acetate buffer at pH 4.5. Following the research by DeDuve (1948), Fleming et al. (1960) used a 0.01N acetate buffer solution at pH 4.5. Warriss (1979) incorporated multiple washings to completely extract the pigments from ground tissue with water and acetate buffer at pH 4.5. This investigator developed a technique for maximum pigment extraction from porcine and ovine muscle using a single washing of 0.04M phosphate buffer at either pH 6.8 and 8.0.

Hernandez et al. (1986) applied the Warriss (1979) technique to mechanically deboned poultry meat (MDPM), a paste-like material used in batter-type products or in combination with white meat in muscle-type products. Using a 0.04M phosphate buffer at pH 8.0, they decreased the a/b ratio, a measure of color change based on redness versus yellowness, from 1.09 to 0.31 or 72%. In comparing the

lightening abilities of water and phosphate buffers with a pH of 6.4, 6.8, 7.2 and 8.0 upon MDPM, these investigators concluded that as pH rose lightness increased and redness decreased.

Dawson et al. (1988) found similar results using tap water at pH 6.8, 0.5% sodium bicarbonate solution at pH 8.5 and 0.1% acetate buffer at pH 5.1 to color modify mechanically deboned chicken meat (MDCM). The recovered product washed in sodium bicarbonate solution (pH 8.5) resulted in the highest Gardner lightness value (L = 64.1) and the lowest redness value (a = 4.5). The lightness and redness values of water and acetate buffer washed MDCM were not different (P>0.05) from each other, although they were lower (P<0.05) than the bicarbonate washed MDCM. These results do not confirm a direct correlation between pH and lightness which was reported by Hernandez et al. (1986). A possible explanation for the discrepancy is that Dawson et al. (1988) varied both the solutions and the pH, whereas Hernandez et al. (1986) used only phosphate buffers and varied the pH.

Smith (1988) applied surimi techniques to mechanically deboned turkey meat (MDTM) using sodium bicarbonate, sodium citrate and potassium phosphate solutions. The treatments increased lightness values by 38.5, 27.9 and 16.9%, respectively. Sodium bicarbonate decreased the mean Hunter "a" (redness) value from 3.66 to -2.24.

Ball et al. (1984) used tap water at pH 7.15, 0.5%
sodium bicarbonate solution at pH 8.45 and 0.05M acetate
buffer at pH 5.25 to reduce pigment content in intact and
blade tenderized deboned chicken broiler thighs. All
treatments that incorporated the Fleming et al. (1960)
method reduced pigment content by 73 to 89% with blade
tenderized, acetate washed thighs having the greatest
reduction as compared to unaltered thighs. This treatment
had the highest lightness value as well. The bicarbonate
wash of blade tenderized thighs, which had the second
largest pigment reduction, did not have a comparable light-
ness value. Ball et al. (1984) explained that as the pH
increases in the muscles, more water is bound which results
in tighter structures that appear to be darker due to a
decrease in scattered incident light. The unwashed tissues
further indicate an inverse relationship between moisture
content and lightness values independent of total pigment
content suggesting that washing procedures alter water and
pigment distribution in muscles. It was also discovered that
all washing treatments exhibited lightness values that were
lighter ($P<0.05$) than unaltered breast meat. Only bicar-
bonate and tenderized acetate treated intact muscle had
redness values that were not lower ($P<0.05$) than breast
tissues. These results led Ball et al. (1984) to conclude
that the consumer would not easily perceive color

differences between washed thigh tissue and breast tissue in fabricated foods.

Chicken thigh strips of average dimensions 4.4cm by 2.4cm by 0.5cm were color modified by Bowie (1985) in solutions containing distilled water and either 1% hydrogen peroxide, 1% sodium bisulfite or 1% ascorbic acid. A tissue-to-solution ratio of 1:2 (weight to volume) was used. Peroxide, bisulfite and ascorbic acid solutions lowered the a/b ratio 62%, 53% and 64%, respectively. All of the washed tissues, excluding the water treatment, received a/b ratios that were not different from the breast tissues. In total color difference, (ΔE), the bisulfite solution washed tissues most closely resembled the breast tissues.

Boneless, skinless turkey thighs were flaked into particles with average dimensions of 7mm by 4mm by 30mm and two washing procedures were used by Elkhalifa et al. (1988). In the first procedure, flaked tissue was washed with three 0.03M potassium phosphate buffers at pH 5.8, 7.4 and 8.0. Following each wash, the tissues were filtered through cheesecloth and centrifuged. The second procedure used a 0.02M sodium acetate buffer at pH 5.8 followed by a 0.03M potassium phosphate buffer at pH 7.4 with filtering and centrifugation after each wash. The phosphate and acetate /phosphate washes reduced the a/b ratio 58 and 49%, respectively. The color modified tissues had a/b ratios that demonstrated 51 and 62%, respectively, of the redness

intensity of breast tissue. Sarcoplasmic proteins were
reduced approximately 89% by both treatments.

2.2 Moisture Retention in Muscle Systems

Water exists in muscle tissue in three major phases
(Hamm, 1986). Less than 0.1% exists as constitutional
water, which is located within the protein molecules.
Another 5 - 15% of the water has restricted mobility and is
located at the surface of proteins in multilayers and small
crevices. This is known as interfacial water. The remain-
ing water is known as bulk phase water and is found in
intracellular and extracellular spaces. Swelling and
shrinkage of muscle fibers causes movement of bulk water
between intracellular and extracellular spaces. According
to Hamm (1986) the water-binding properties of meat can be
determined in terms of the extent to which the bulk phase
water is immobilized within the microstructure of the intact
or comminuted tissue.

Hamm's review (1986) indicates that the extracellular
water is the most easily expressed and that myofibrillar
proteins are the major determinant of water immobilization
in meats. Myosin, which makes up approximately 50% of the
myofibrillar proteins, is known to have a large capacity to
imbibe water and immobilize it intracellularly. Further-
more, the immobilization of water is influenced by the
spatial molecular arrangement of myofibrillar proteins.
Swelling and immobilization of water in a protein gel occur

when attraction between adjacent protein molecules or filaments is decreased. This attraction can be disrupted by increased electrostatic repulsion between similarly charged protein groups or by the weakening of hydrogen or hydrophobic bonds.

Increasing the pH above the isoelectric point of myosin (IP=5.0) causes a net negative charge and, consequently, an electrostatic repulsion which results in protein unfolding (Hamm, 1986; Hultin, 1985; Cheftel et al. 1985). Elkhalifa et al., (1988) reported that this activity caused an increase in moisture content of color modified turkey meat.

In a series of experiments investigating the role of insoluble proteins in meat emulsions, Regenstein and others have established that myosin displays a high degree of waterbinding ability. Huber and Regenstein (1988) discovered that exhaustively washed chicken breast muscle formed a stable emulsion without the presence of soluble proteins. They concluded that the myosin fraction has the ability to form a gel in the aqueous phase which might contribute to the stability of emulsion products. Gaska and Regenstein (1982) established the low emulsion effect of sarcoplasmic proteins in the presence or absence of high-salt solubilized protein. These studies magnify the importance of myofibrillar proteins and the insignificance of sarcoplasmic proteins in waterbinding.

The ionic strength of neutral salts affects the solubility of proteins. "Salting in" of proteins occurs when the salt ions react to decrease the electrostatic attraction of opposite charges between adjacent molecules, therefore increasing the solubility (ability to interact with water molecules) of the proteins. The opposite effect, "salting out", is caused by the salt ions having a greater attraction for water (concentrations greater than 1M) than the proteins. This amplifies the protein-protein interactions and causes them to precipitate out of solution. According to the Hofmeister series (Cheftel et al., 1985), potassium (K), Na, Mg and calcium (Ca) have properties that promote protein unfolding, dissociation and salting in.

Sodium chloride and phosphates have been used extensively to increase the waterbinding capacity of meat products (Hellendoorn, 1962; Schwartz and Mandigo,1976;). Hellendoorn (1962) reported that pyrophosphate and polyphosphates increased the ionic strength of these salts with a concomitant increase of waterbinding in uncooked meat at an alkaline pH. Conversely, when combined with sodium chloride to ionic strengths of 0.48 and 0.51, the phosphates depress waterbinding in uncooked meat. Orthophosphates, as well as pyro- and polyphosphates, are known to increase waterbinding in cooked meat (Hellendoorn, 1962).

2.3 Mineral and Vitamin Retention

in Processed Muscle Systems

Mineral and vitamin losses can be caused by moisture reduction and high cooking temperatures (Procter and Cunningham, 1983; Hultin, 1985). In ice-packed products stored at $2^{\circ}C$ for 14 days, losses ($P<0.05$) of Mg, K and P were noted for whole birds and these same minerals plus Na were lost from broiler breasts due to leaching (Ang et al., 1982). Neither thiamin nor riboflavin concentrations changed significantly for either product. Commercial ice-slush chilling with agitation yielded less P, Na and K ($P<0.01$, 0.01 and 0.05, respectively) than hot-deboning of broilers. These losses may have been due to leaching (Ang and Hamm, 1983). The same study showed no differences ($P>0.05$) in Mg, Ca, riboflavin, niacin or vitamin B_6 contents.

In frankfurters manufactured from beef, pork and chicken, Marriott et al. (1982) reported that measurable element retention ranged from 80.9 - 100% even after washing and boiling in water. A partial explanation was that the casing provided a protective barrier. Contrary to these results, riboflavin and thiamin are considered (Anonymous, 1986) to be susceptible to leaching by water when exposed during processing (i.e. destruction of cells at a cut surface).

In an evaluation of five different cooking methods, Proctor and Cunningham (1983) found that Fe, Mg, Na and zinc

(Zn) content were affected by cooking methods. The same treatments did not affect Ca, P, K, S, Cu and Mn content. Unklesbay et al. (1983) reported significant reductions in P, Na and thiamin and an insignificant loss of riblofavin during infrared and convection heating of pork loin roasts and turkey breasts.

Other factors which may affect vitamin retention during processing include exposure to air, light, pH, natural enzyme systems and the interactions of these factors (Anonymous, 1986). This publication reported that ribo-flavin was unstable in the presence of an alkaline pH (>7), light and heat.

While vitamin and mineral content are important, their value lies in their bioavailability. Davis (1981) defines bioavailability as the "presentation of a nutrient across the intestinal mucosa in a form that can be utilized by the animal or human." Davis (1981) also stated that meat adequately provides the nutritionally required amounts of the essential amino acids and fatty acids, some vitamins and minerals.

Layrisse et al. (1973, 1974, 1975) have established a direct relationship between fortification, vegetable and nonheme iron availability and the inclusion of animal protein in the diet. Interactions between mineral content and mineral availability have been investigated by Zemel and Bidari (1983). They implicated, through fecal examination,

that supplemental calcium reduced polyphosphate phosphorous absorption. In addition, orthophosphate was without effect on Zn, Fe and Cu availability at the 0.53% Ca intake level, but it increased fecal Zn by 11% upon 1.06% Ca intake. While it was not within the scope of this study to determine the effects of orthophosphate buffers on nutrient availibility in CMT, the author recognized that nutrient composition is not equivalent with nutrient availability.

2.4 Protein Quality in Muscle Foods

Protein quality measures the efficiency with which the body can utilize protein for growth and maintenance. It is based upon nitrogen balance, growth and estimated amino acid requirements (Pellett and Young, 1980). Protein requirements are different for rats (the typical bioassay animal) and humans at differing age groups (Pellett, 1978). An important difference is that the protein growth requirement for growing rats is higher than for growing infants. Moreover, both growth requirements are higher than those for the adult rat or for human maintenance (Jansen, 1978). These differences cause rat bioassays to underestimate the protein quality of foods for human consumption (Jansen, 1978; Pellett and Young, 1980).

Proteins from animal sources are typically of higher quality than those from plant sources (Cheftel et al., 1985), apparently due to their balance of the required amino acids. Therefore, whole egg, egg albumin or casein from

milk are the standard reference materials in protein quality assays. Although protein quality studies based on protein efficiency ratio (PER) and protein digestibility have been subjected to much criticism (Pellett, 1978; Jansen, 1978; Hsu et al., 1978), there is evidence that PER bioassays provide sufficient sensitivity to allow the assay of meats and high quality proteins (Staub, 1978; Jansen, 1978). However, Pellett and Young (1980) found a high degree of variability between different laboratories for identical materials and therefore recommended caution in comparing PER values between different studies even when adjusted for casein standards at 2.50.

The PER bioassay has been found sensitive enough to detect differences in protein quality based on processing of meats, as well as differences between food types. Hopkins et al. (1976) reported that lyophilization did not lower the PER of ground beef. MacNeil et al. (1979) evaluated the PER of three types of MDTM with and without antioxidants. They reported no differences between types of MDTM, but treatments with antioxidants were higher (P<0.01) than the casein standard. No conclusions could be drawn about MDTM without antioxidants because of gross errors in the data. Mott et al. (1982) examined three types of mechanically deboned poultry spent layer meat and found similar results that showed a higher PER than the control casein, but not

significantly different among types of mechanically deboned meat.

Sheldon et al. (1980) compared the effect of end-point cooking temperature of processed turkey rolls on nutritive value and composition. Breast and thigh meat were pooled, freeze-dried, formulated into 20% protein diets and fed to mice. Differences (P<0.01) were found between the weights of mice for different end-point temperatures indicating that thermal processing of poultry affects weight gain associated with protein.

Elgasim and Kennick (1980) evaluated the effect of pressurization of pre-rigor beef muscles on protein quality. The PER for the pressurized muscles was not different (P>0.05) from the control, but the digestibility was higher (P<0.05) for pressurized muscle. These workers concluded that protein quality was not adversely affected by pressurization.

Raw MDPM, cooked MDCM and raw MDTM were evaluated for protein quality by Babji et al. (1980). The PER data revealed no differences (P>0.05) among treatments or the reference, casein. Apparent digestibilities had comparable values of approximately 90% with MDTM being the lowest at 87%.

Steinke et al. (1980) showed the effect of autoclaving and replacing inherent meat and plant proteins with isolated soybean protein (ISP) on PER. In both raw and autoclaved

turkey breast samples, the PER was reduced regardless of the percentage of ISP added. When the PER of turkey breast samples with up to 50% ISP were adjusted for a casein value of 2.50, their protein quality was comparable to casein.

Bittel et al. (1981), compared the PER of mechanically separated spleen (MSS) to whole spleen and casein. The PER of MSS did not differ (P>0.05) from casein. This study did not reflect the beneficial results of lowering collagen content as had been previously reported by Hendricks et al. (1977) and Lee et al. (1978). Collagen is known to be deficient in cysteine, tryptophan, tyrosine and methionine, which are essential amino acids. Examining the protein quality of mechanically deboned meat by amino acid analysis and PER, Chang and Field (1977) reported a direct corre-lation between collagen content and reduced protein quality. Lee et al. (1978) varied collagen contents from 4 to 45.8% in blended beef products and found that total essential amino acids and PER decreased linearly with increasing collagen content.

Yield and nutritional characteristics of washed and unwashed mineral rockfish flesh were determined by Adu et al. (1983). Washing the minced flesh did not affect the amino acid composition. The PER value of washed flesh was not different from the unwashed (2.7 $\pm$ 0.4 and 2.7 $\pm$ 0.3, respectively), but both were different from the casein PER of 2.5 $\pm$ 0.3.

The scope of this study includes investigation of the nutrient profile and protein quality of CMT in an attempt to provide a more complete knowledge of washing effects on muscle tissue. Indices of change similar to those discussed in the preceeding pages are incorporated into the evaluation of the nutrient integrity of CMT. The results are interpreted and integrated in view of the knowledge discussed herein, as well as any new information provided by those results.

3.0 MATERIALS AND METHODS

3.1 Raw Material Processing

3.11 Handling and Preparation

Sampling for 54.5 kg of raw, skinless, boneless, intact turkey thigh muscles on each of three days - June 14, 1988, August 12, 1988 and November 10, 1988 - was conducted at a large turkey processing plant in Virginia. Intact thighs were portioned into 18.2 kg lots, placed in polyethylene bags and packed in boxes containing ice. Upon reaching the Muscle Food Products Research Laboratory at Virginia Polytechnic Institute and State University (VPI & SU), all thighs were quartered, placed into white opaque, 33 cm x 66 cm, type L348 Cryovac bags (W.R. Grace & Co.,Duncan,S.C.) to a depth of ca. 5.1 cm and stored at -20°C overnight.

The frozen, quartered thighs were tempered in the bags at 20°C for ca. 30 min or long enough to separate the quartered thighs. The thighs were reduced to 7 mm x 4 mm x 30 mm flakes using a Ross Unicom 1000 (Ross Industries, Inc., Midland, Va.) with blade openings of 8 mm. The flaked material was vacuum packaged using a Foodsaver (Tilia Trust Reg., Italy) and designated for color modification (section 3.12) or stored at -20°C until analyzed.

3.12 Color Modification

Batches of 12, 4 and 4 kg, respectively, of flaked, unaltered thigh tissue (THI) were successively color modified from each of the 54.5 kg acquisitions. The 12 kg

batch of June 14, 1988 was color modified in 4 kg portions and homogenized into one batch.

The batches of THI were color modified with .03 M phosphate buffer using the procedure of Elkhalifa, et al. (1988), which is a modification of surimi processing (Suzuki, 1981). Biotechnical grade mono- and dibasic sodium phosphate (Fisher) was used to prepare 0.03 M phosphate buffers at pH levels of 5.8, 7.4 and 8.0. For each pH level, all batches were washed (1:3 w/v, THI to buffer), for 5 min using a CSE Lab Blender Model CDB-0615-V (Custom Stainless Equipment Co., Inc.,Santa Rosa, Calif.), rested for 15 min and strained through a double layer of 40 grade cheesecloth (American Fiber and Finishing Inc., Burlington, Mass.). The final residue, color modified dark turkey meat (CMT), was vacuum packaged and stored at -20°C until analysis or portioned into 18 oz. Whirl-Pak bags (NASCO) and stored at -4°C for analysis within 24 hr.

3.2 Processed Material Evaluation

3.21 Color Evaluation

Hunter L, a and b values were measured for triplicate samples of THI and CMT using a Hunterlab model D25 Color and Color Difference Meter (Hunter Associates, McLean, Va.). Reference standard white tile C20-1651 was used. Samples were thrice ground and placed in a glass well 15 mm deep (Elkhalifa et al., 1988). ΔE was calculated as the square

root of the sum of the squares of the differences in L, a and b values between CMT and THI.

3.22 Proximate Analysis

Moisture, ether extract and ash determinations were performed according to AOAC (1984) procedures. Crude protein determinations were made with a macro Kjeldahl procedure (AOAC, 1984) modified by diluting the digested solution with 200 mL of H_2O and layering with 70 mL 50% NaOH to neutralize the acid. Quadruplicate samples of THI and CMT were used for moisture analysis and triplicate samples were used for ether extract and protein analyses.

3.23 Elemental Analysis

Duplicate 5 g THI and CMT samples were dry-ashed at 525°C in oven-dried crucibles using a Lindberg series 51000 box (muffle) furnace (Division of Sola Basic Industries, Watertown,Wisc.) following charring at low heat on an electric hotplate according to the AOAC procedure 43.012 (1984). The accuracy of the procedure was verified using National Institute of Standards and Technology Standard Reference Material 1577a Bovine Liver.

After weighing, 5 mL of concentrated HCl (reagent A.C.S. grade, Fisher Scientific Company, Fair Lawn, N.J.) were added to the ash in the dishes and allowed to stand for 30 min. Then, 10, 15, and 20 mL portions of deionized, distilled water were successively added to the ash in HCl at

20, 10 and 5 min intervals, respectively. Final dilution was 10% or 1.2 N HCl.

Duplicate samples of THI and CMT were analyzed at the VPI & SU Soil Testing and Plant Analysis Laboratory by inductively coupled plasma-atomic absorption spectrometry using a Thermo Jarrel-Ash Inductively Coupled Argon Plasma (ICAP) Spectrometer Model 9000 (Waltham,Mass.). Data were recorded as parts per million in solution and converted to μg of element per g of sample.

3.24 Riboflavin Analysis

3.241 Materials

USP reference standard riboflavin (vitamin B_2) was obtained from United States Biochemical Corporation (Cleveland, Ohio) and oven-dried over H_2SO_4 for 24 hr before use in the stock solution. Hydrochloric acid (37.5%) reagent A.C.S. grade, glacial acetic acid reagent A.C.S. grade, potassium permanganate crystals A.C.S. grade, sodium hydrosulfite technical grade, sodium hydroxide beads (molecular biology grade) and Whatman #41 (ashless) filter paper were obtained from Fisher Scientific Company (Fair Lawn, N.J.).

Relative fluorometric units of the standards and samples were determined using a Perkin-Elmer 650-40 Spectrophotofluorometer (Perkin-Elmer Corp., Norwalk, Conn.). Emission and excitation slits were set at 2 and 10 nm, respectively. Wavelengths of emission and excitation were

440 and 520 nm, respectively. An Orion Research model 701A digital ionalyzer (Orion Research Corporation, Cambridge, Mass.) was used to monitor pH of the extract solutions.

3.242 Procedure

Triplicate 5 g samples of THI and CMT were assayed for riboflavin (vitamin B_2) according to a modification of procedure 43.039 - 43.043 Riboflavin (Vitamin B_2) in Foods and Vitamin Preparations Fluorometric Method (AOAC, 1984). Riboflavin stock and intermediate stock solutions were prepared at 25 and 0.5 μg/mL, respectively. The working solution concentration was 0.2 μg/mL. A standard curve was prepared for relative fluorometric unit readings from riboflavin solutions of 0, .05, .10, .20, .40 and .50 μg/mL concentrations. Sample riboflavin concentrations were determined from the standard curve. Subsequently, riboflavin concentrations of the samples (μg/g) were calculated from the formula: riboflavin concentration (μg/mL solution) from standard curve X dilution factor / original sample weight (g).

Solutions of 1% potassium permanganate and hydrogen peroxide were substituted for those formulated at 3% in the AOAC procedure. A 5% sodium hydrosulfite solution in 5% sodium bicarbonate buffer was prepared immediately prior to reading fluorometric values and stored in an ice bath (Augustin, et al., 1985). A 200 μL aliquot was mixed into 2 mL of sample solution in the cuvette for reading "C".

Preliminary trials performed on standard and sample solutions showed that this level of sodium hydrosulfite was adequate to remove the fluorescence of riboflavin.

3.25 Protein Efficiency Bioassay

3.251 Diet Preparation and Formulation

Casein Low Trace Element 30 Mesh, spray dried egg albumin, vitamin diet fortification mixture, USP XVII salt mixture, cottonseed oil and Alphacel Non-nutritive bulk were obtained from ICN Biochemical (Cleveland, Ohio). Specifications are provided in Table 1.

Portions of 6 kg of THI and 12 kg of CMT from the June 14, 1988 processing date were stuffed into 110 mm casings. All stuffed casings were cooked at 71.1°C for 8 hr and 82.2°C for 4 hr in an electrically heated smokehouse (Vortron, Inc., Beloit, Wisc.) to a final internal temperature of 68.3°C. Cooked THI and CMT were freeze dried in a Virtis model no. 10-145MR-BA freeze drier (Gardiner, N.Y.) and powdered using a Cuisinart model DLC-8E food processor (Cuisinarts Inc., Greenwich,Conn.). Proximate analysis (AOAC, 1984) was performed on three samples from freeze-dried THI and CMT (Table 2) for diet adjustments.

Four diets (Table 3) were mixed using a CSE Lab Blender Model CDB-0615-V (Custom Stainless Equipment Co., Inc., Santa Rosa,Calif.) according to AOAC procedure 43.212 (1984) allowing for 10% protein from casein, egg albumin, THI and CMT.

Table 1. Composition of components used in diet formu-
lation.

U.S.P. XVII SALT MIXTURE
COMPOSITION:
Sodium Chloride	13.9300%
Potassium Phosphate Monobasic	38.9000%
Magnesium Sulfate.7H$_2$O	5.7300%
Calcium Carbonate	38.1400%
Ferrous Sulfate	2.7000%
Manganese Sulfate.H$_2$O	0.4010%
Potassium Iodide	0.0780%
Zinc Sulfate.7H$_2$O	0.0548%
Cupric Sulfate.5H$_2$O	0.0477%
Cobalt Chloride.6H$_2$O	0.0023%

VITAMIN DIET FORTIFICATION MIXTURE
COMPOSITION:	gm./kg
Vitamin A Acetate (500,000 I.U./gm)	1.80000
Vitamin D$_2$ (850,000 I.U./gm)	0.12500
DL-alpha-Tocopherol Acetate	22.00000
Ascorbic Acid	45.00000
Inositol	5.00000
Choline Chloride	75.00000
Menadione	2.25000
p-Aminobenzioc Acid	5.00000
Niacin	4.25000
Riboflavin	1.00000
Pyridoxine Hydrochloride	1.00000
Thiamine Hydrochloride	1.00000
Calcium Pantothenate	3.00000
Biotin	0.02000
Folic Acid	0.09000
Vitamin B$_{12}$	0.00135

Table 1.(continued)
EGG ALBUMIN - SPRAY DRIED
COMPOSITION (per 100 g):

Nutrients	Units	Amount	Amino Acid	Unit	Amount
Moisture	g	8.00	Alanine	g	4.96
Calories	kcal	372.00	Arginine	g	4.65
Protein	g	80.20	Aspartic Acid	g	8.22
Fat	g	0.20	Cysteine	g	2.17
Carbohydrates	g	5.70	Glutamic Acid	g	10.54
Fiber	g	0.00	Glycine	g	2.79
Ash	g	5.10	Histidine	g	1.86
Calcium	mg	66.00	Isoleucine	g	4.34
Phosphorous	mg	110.00	Leucine	g	6.82
Iron	mg	1.00	Lysine	g	5.12
Sodium	mg	1103.00	Methionine	g	3.02
Potassium	mg	1000.00	Phenylalanine	g	4.73
Vitamin A	mg	0.00	Proline	g	3.10
Thiamine	mg	0.04	Serine	g	5.50
Riboflavin	mg	1.99	Threonine	g	3.64
Niacin	mg	0.70	Tryptophan	g	1.32
Ascorbic Acid	mg	0.00	Tyrosine	g	3.18
			Valine	g	5.58

CASEIN LOW TRACE ELEMENT 30 MESH - 87% protein

ALPHACEL NON-NUTRITIVE BULK - finely ground alpha-cellulose
                              with minimum nutritional value

PURE CORN STARCH - Argo (Best Foods, Englewood Cliffs, N.J.)
                   Cream (Dial Corporation, Phoenix, Ariz.)

Table 2. Mean values[1] for content by percentage of freeze-
dried samples used in diet formulations.

	Crude Protein		Ether Extract	
Treatment	Mean	S.E.	Mean	S.E.
THI	65.62	0.52	28.87	0.41
CMT	66.06	0.53	30.26	0.01

[1] N = 3 subsamples

Table 3. Percentage composition of diets for the protein
          efficiency ratio (PER).

Diet	Protein	Fat[1]	Salt[2]	Vitamin	Alphacel	Corn Starch	Water
Casein	10	10	5	1	1	68	5
Egg	10	10	5	1	1	68	5
THI	10	10	5	1	1	68	5
CMT	10	10	5	1	1	68	5

[1] Based on inherent fat from the protein source plus the
    difference made up with cottonseed oil.
[2] Based on inherent ash from the protein source plus the
    difference made up with USP XVII salt mixture.

3.252 Bioassay Procedure

Procedure 43.213 - 43.215 Biological Evaluation of Protein Quality (Protein Efficiency Ratio) (32) - Official Final Action (AOAC, 1984) was followed. Two groups of 20 male weanling Sprague Dawley rats (21 days old) from the same colony were received from and housed in individual stainless steel cages at the VPI & SU Laboratory Animal Resources vivarium on June 28, 1988 and July 5, 1988, respectively. The first group was divided into two sets of 10 rats having mean initial weights of 52 g and 54 g and fed the casein and egg albumin (egg) diets, respectively. The second group was divided into two sets of 10 rats having mean initial weights of 45 g each and fed the THI and CMT diets.

Body weight of each rat was recorded on the beginning day and the third and seventh days of each week for four weeks. Diet intake was recorded each day that the diet needed to be replenished and on the days that the rats were weighed. The rats were sacrificed after weighing on the 28th day and the general morphological condition and weights of the liver, spleen and kidneys were recorded. Protein efficiency (PER) was calculated as the ratio of weight gain of the rat after 28 days to the total protein consumed.

3.253 Apparent Digestibility

On the 12th to 16th days of the protein efficiency ratio bioassay, the feces were collected from individual

aluminum trays placed beneath the rat cages. Diet and fecal nitrogen were determined by the macro Kjeldahl method (AOAC, 1984). Apparent digestibility was calculated as the difference between intake nitrogen and fecal nitrogen divided by intake nitrogen times 100 (Pellett and Young, 1980).

3.3 Statistical Evaluation

Proximate, elemental and riboflavin analyses incorporated a randomized block design of two treatments (THI and CMT) evaluated three times as duplicate subsamples for ash and elements, triplicate subsamples for protein, fat and riboflavin and quadruplicate subsamples for moisture. A two-way ANOVA was performed on means for the independent variables, treatments and times, using SAS (1984). Standard errors of the means were reported for all analyses.

Analyses of data from the protein efficiency and apparent digestibility bioassays incorporated a completely randomized design of four treatments (egg, casein, THI and CMT) and 10 or 5 subsamples, respectively. Least square means were analyzed by one-way ANOVA for the independent variable, diet, using SAS (1984). Standard errors of the least square means were reported.

4.0 RESULTS AND DISCUSSION

Nutrient profile and protein quality of CMT were based on the results obtained using the previously described procedures. The effects of color modification on turkey thigh meat were examined in view of results obtained by other investigators and the interrelated observations within the study itself. The following results and discussion serve to elucidate causes for previously described functional attributes of CMT.

4.1 Color, Proximate Analysis and pH

The Hunter color evaluation showed consistency between this study and that previously reported by Elkhalifa et al. (1988). The L values for THI and CMT were reported as 44.85 and 55.87, respectively, by Elkhalifa et al. (1988) and 46.69 and 61.23, respectively, for this study. The ΔE values for CMT as reported by Elkhalifa et al. (1988) and this study were 12.22 and 15.70, respectively.

The proximate compositions of THI and CMT as compared with the mean reference values (USDA, 1979) are shown in Table 4. Tissue pH for THI and CMT was 6.47 and 7.11, respectively. CMT was higher (P=0.0429) in moisture and lower in crude protein, fat (ether extract) and ash (P=0.0537, 0.1280 and 0.0047, respectively) than THI.These results are consistent with the observations reported by Ball et al. (1984), Bowie (1985), Hernandez et al. (1986),

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Table 4. Mean values[1] for proximate content of 100 g edible portions.

Nutrients	Units	Turkey[2] Mean	THI Mean	S.E.	CMT Mean	S.E.	P > F
Proximate:							
Water	g	74.48	72.67	0.42	80.95	1.64	.0429
Protein	g	20.07	19.47	0.85	14.25	0.66	.0537
Fat	g	4.38	7.69	0.52	4.80	0.84	.1280
Ash	g	0.93	0.90	0.13	0.44	0.16	.0047

[1] N = 3 replications of duplicate ash, triplicate fat and protein, and quadruplicate water subsamples.
[2] Turkey, all classes, dark meat without skin (USDA, 1979).

Elkhalifa et al. (1988) and Dawson et al. (1988) when they washed dark poultry tissues.

Water absorption in muscle tissues occurs due to an increase in pH from 6.47 to 7.11. The pH change causes a net negative charge on proteins with a resultant unfolding of the molecule and an entrapment of water into a "gel-like" protein mesh (Hamm, 1986; Hultin, 1985). Hamm (1986) also reported that myosin caused a swelling of muscle tissue by intracellularly imbibing water. According to Elkhalifa et al. (1988), 80% of CMT is myofibrillar protein, therefore it is reasonable that the increased moisture in the CMT of this study was due to intracellular imbibing by myosin.

Washing with the three 0.03 M sodium phosphate buffers potentially influenced the moisture increase in CMT. Residual sodium content increased to 166% of the original value (Table 5). Increasing the ionic strength of sodium promotes protein unfolding, dissociation and "salting in" as predicted by the Hofmeister series (Cheftel et al., 1985). Assuming that the gain of sodium concentration came from the buffer and the 31% reduction in solids, the Na could be in the aqueous phase and bound to the proteins, therefore dissociating the proteins resulting in increased water-holding.

Hellendoorn (1962) reported similar results for ortho-,

Table 5. Mean values[1] for mineral and riboflavin content of 100 g edible portions in THI and CMT.

Nutrients	Units	Turkey[2] Mean	THI Mean	THI S.E.	CMT Mean	CMT S.E.	P > F
Minerals[3]:							
Calcium	mg	17.00	7.46	0.50	8.15	1.36	.5975
Iron	mg	1.75	1.63	0.05	1.08	0.04	.0187
Magnesium	mg	22.00	25.00	0.10	5.00	0.40	.0004
Phosphorous	mg	184.00	226.00	1.00	124.00	2.00	.0001
Potassium	mg	286.00	328.00	4.00	16.00	2.00	.0001
Sodium	mg	77.00	89.00	1.00	148.00	4.00	.0058
Zinc	mg	3.22	3.55	0.10	2.35	0.37	.1184
Copper	mg	0.147	0.199	0.015	0.174	0.012	.3979
Manganese	mg	0.022	0.024	0.001	0.013	0.001	.0035
Vitamins:							
Riboflavin	mg	0.221	0.284	0.011	0.198	0.023	.0626

[1] N = 3 replications of duplicate mineral and triplicate riboflavin subsamples.
[2] Turkey, all classes, dark meat without skin (USDA, 1979).
[3] Accuracy of method was verified using National Institute of Standards and Technology Standard Reference Material Bovine Liver; all values in the acceptable range except that calcium, manganese and zinc were above the range and potassium and copper were below the range.

pyro- and polyphosphates. He showed that the addition of sodium phosphate salts to lean raw beef raised the ionic strength of the tissue from 0.18 to 0.26 and subsequently enhanced waterbinding in this alkaline environment. This enhancement of waterbinding supports the moisture increase noted in CMT. The interaction of myosin, swelling, increased pH, and increased sodium and phosphate ionic strengths may all have contributed to the 8.28% increase in moisture content.

The protein loss was mostly due to selective extraction of the pigments contained in sarcoplasmic proteins. Some of the collagen was observed to stick to the blades and sides of the mixer, along with a perceivable amount of fat, since they had separated from the flaked tissues during mixing. Elkhalifa (1988) reported a reduction of 89% in the sarcoplasmic fraction, 19% in myofibrillar and 26% in the stromal fractions for the procedure used. Bowie (1985) reported similar trends, to a lesser degree, in protein fraction losses, although she did not report the pH of the solutions nor did she change the solution parameters during successive washings.

Fat loss was attributable to its clinging to the mixer. Fat is less dense than water and during mixing it physically separated from the muscle tissues and floated. Some of the fat adhered to the mixing chamber and the cheesecloth filter.

On a dry weight basis, total protein content increased from 69.4% in THI to 73.1% in CMT. As well, fat decreased from 27.4 to 24.6% and ash decreased from 3.2 to 2.3% in CMT. These results indicate an alteration of solids composition due to the color modification process.

4.2 Mineral Content Analysis

The reduction of ash for CMT was due to a loss of minerals into the wash water. Fe, Mg, P, K and Mn sustained losses (P=0.0187) of 34, 80, 45, 95 and 46%, respectively, due to washing (Table 5). Ca, Zn and Cu did not differ (P=0.1184) due to washing and Na showed an increase (P=0.0058) to 166% of the original concentration. This observation reflects a selective mineral loss due to the washing procedure.

The loss of Fe and protein (96% sarcoplasmic protein according to Elkhalifa et al., 1988) was 34 and 27%, respectively. This observation suggests a high association between Fe loss and the hemoglobin and myoglobin containing sarcoplasmic protein loss. Jimenez-Colmenero and Garcia (1981) reported a 32% Fe loss using a water washing technique on pork in similar weight to volume ratios. However, Adu et al. (1983) reported a 71% increase over the original content of Fe when water washing minced rockfish flesh. Their explanation was that the quality of water affected the mineral composition.

Jimenez-Colmenero and Garcia (1981) found trends in Mg, P and K losses similar to those found in CMT, with a minimal simultaneous effect on Ca and Cu. However, the results of Zn and Mn were reversed. Adu et al. (1983) demonstrated similar losses in P and K with increases in Cu and Zn. In contrast to the wash treatment applied to CMT, neither of these studies used a Na containing solution and consequently showed losses in Na. This observation suggests that in CMT the mineral losses were due to leaching by water and that the Na increase resulted from the uptake of Na from the buffers by muscle tissue proteins.

4.3 Riboflavin Assay

A 30% reduction (P=0.0626) of riboflavin was noted (Table 5). The 70% retention level was unexpected as riboflavin is reported to be susceptible to loss through leaching, exposure to air (which happened during mixing) and an alkaline pH (Anonymous, 1986). The results of this study generally agree with Ang et al. (1982) and Ang and Hamm (1983) who showed no losses (P>0.05) of riboflavin due to leaching in chill-holding and ice-slush chilling of broilers. Also, Unklesbay et al. (1983) reported insignificant (P>0.05) losses of riboflavin during infrared and convection heating with drip losses of pork loin roasts and turkey breasts ranging from 2.92 to 9.81%. For other water soluble vitamins, Ball et al. (1984) reported niacin losses of 37 to 68% and thiamine losses of 32 to 50%, while percentage of

pigment losses were 73 to 89. The data reported here coupled with the data cited suggest that muscle food products have some mechanism (such as binding to proteins) for protecting riboflavin and other vitamins from losses due to leaching and destruction from pH changes and oxidation.

4.4 Protein Quality Evaluation

On the twenty-eighth day of the PER study, all 40 rats were sacrificed and their livers, spleens and kidneys were weighed. All rats were examined by the director of the VPI & SU Laboratory Animal Resources vivarium for general condition and for lesions, growths or scar tissue on the organs. None of the rats showed signs of poor health or changes in the morphological conditions of the organs. The mean weight of the liver, spleen and kidneys was directly related to the mean final weight of the rats (Table 6). The trend of final weight of the rats, in ascending order, was the egg albumin, casein, CMT and THI. Bittel et al. (1981) also reported higher mean weights from rats fed test diets versus control diets.

The PER for rats on the THI diet was higher (P=0.0318, 0.0001 and 0.0001) than the CMT, casein and egg diets, respectively (Table 7). Steinke et al. (1980) reported PER values for autoclaved spray-dried egg white, turkey breast, beef, pork and tuna of 2.98, 2.82, 3.22, 3.08 and 2.95, respectively, as compared to a casein reference at 2.5. All PER values for meat-based diets were higher (P<0.05) than

Table 6.   Least square means[1] for final weight (g) and organ
           weights (g) of weanling male rats in a protein
           efficiency ratio bioassay.

Diet	Weight Mean	Weight S.E.	Liver Mean	Liver S.E.	Spleen Mean	Spleen S.E.	Kidneys Mean	Kidneys S.E.
Casein	178[b]	6	8.1[b]	.4	.50[b]	.03	1.4[b]	.1
Egg	134[c]	7	5.6[c]	.4	.30[c]	.04	1.1[c]	.1
THI	218[a]	7	9.8[a]	.4	.60[a]	.04	1.8[a]	.1
CMT	173[b]	7	7.2[bc]	.4	.40[bc]	.03	1.4[b]	.1

[1] N=10 rats per diet.

[abc] Least square means in the same column with identical
      superscripts are not different (P$\geq$0.0286).

Table 7. Least square means[1] for protein efficiency ratio[2] (PER) of male weanling rats on various diets.

| Diet | Mean | S.E. | PROB > \|T\|[3] | | | |
			Casein	Egg	THI	CMT
Casein	2.13	0.11	.	0.6098	0.0001	0.0001
Egg	2.19	0.10	0.6098	.	0.0001	0.0001
THI	3.38	0.11	0.0001	0.0001	.	0.0318
CMT	3.14	0.10	0.0001	0.0001	0.0318	.

[1] N = 10 rats per diet.
[2] PER = Weight gain in g / protein intake in g.
[3] HO : LS means (Diet in vertical) = LS means (Diet in horizontal).

the casein diet and only the turkey breast was not higher (P<0.05)than the egg albumin, which is in agreement with this study. MacNeil et al. (1979) reported PER values for diets containing mechanically deboned poultry meat with antioxidants that were higher (P<0.01) than casein controls, while Babji et al. (1980) reported no relative differences between mechanically deboned poultry meat and a casein control. These studies indicate that based on the growth of weanling rats, protein sources from meat products yield a protein quality comparable to or greater than the casein standard.

The PER of rats on the CMT diet was higher (P=0.0001) than the casein and egg diets, which were not different (P=0.6117) from each other. CMT received higher PER values (3.14 ± 0.10) than those stated for washed minced rockfish tissues by Adu et al. (1983). These workers reported PER values of 2.7 ± 0.4 for minced fish and 2.5 ± 0.3 for reference casein. In a review of a study by Dudek (Anonymous,1988), PER values of surimi and surimi products are reported to be 3.25 and 3.11, respectively, which resemble the values reported in this study for CMT.

The slightly smaller PER value for CMT in comparison to THI (3.14 versus 3.38, respectively) could be explained by a combination of the loss of sarcoplasmic proteins and the increased concentration of collagen proteins (14% in THI and 16% in the CMT, Elkhalifa et al., 1988). Although this

Table 8. Least square means[1] for apparent digestibility[2]
(AD) of male weanling rats on various diets.

| Diet | Mean | S.E. | PROB > \|T\|[3] | | | |
			Casein	Egg	THI	CMT
Casein	92.8	0.9	.	0.2055	0.0612	0.1404
Egg	94.3	0.9	0.2055	.	0.0130	0.0324
THI	90.2	0.9	0.0612	0.0130	.	0.6413
CMT	90.7	0.9	0.1404	0.0324	0.6413	.

[1] N = 5 diet and rat fecal samples.
[2] AD = (intake N - fecal N) / intake N) X 100.
[3] HO : LS means (Diet in vertical) = LS means (Diet in horizontal).

represents only a 2% difference in collagen content, Lee et al. (1978) was able to detect decreases in PER of 0.15 and 0.06 for increases in collagen of 5.7 and 6.3%, respectively. Chang and Field (1977) were able to detect a drop in PER of 0.57 with an increase in collagen of 3.17%. From these results it is feasible to conclude that a 0.24 decrease in PER from THI to CMT could be due to a 2% increase in collagen concentration.

The 94.3% AD value for egg albumin was higher (P=0.0130 and 0.0324) than the THI and CMT diets, respectively, but not different (P=0.2055) from the casein diet (Table 8). The values for AD in this study are comparable to values in other studies. Hsu et al. (1977) reported an in vivo AD of 90.5 for casein similar to the one used in this study and 87.6 for ANRC casein. Babji et al. (1980) reported in vivo digestibility of ANRC casein at 91.2%. The casein diet AD was not higher (P>0.1404) than the CMT and slightly higher (P≤0.0612) than the THI AD.
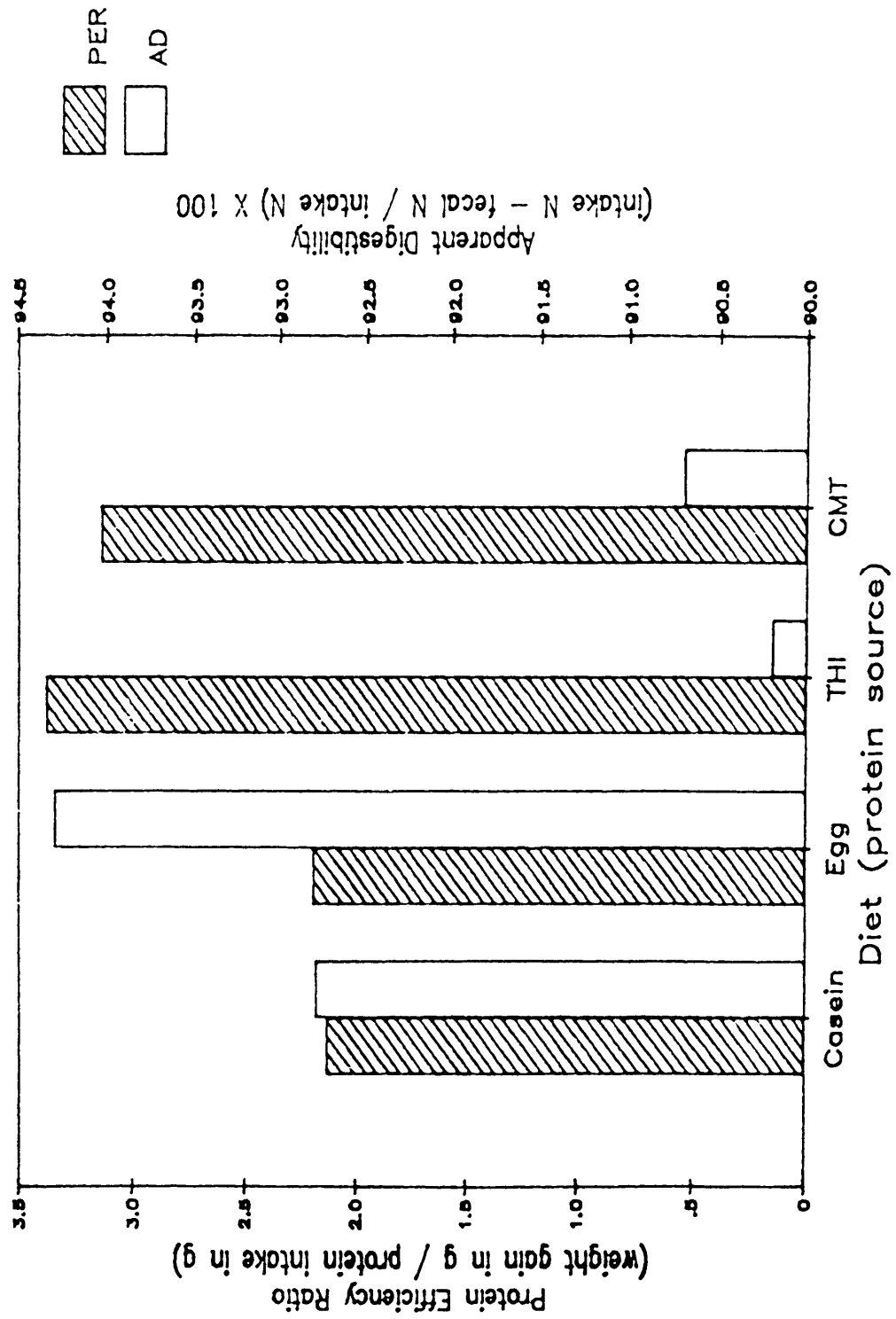
The CMT and THI AD of 90.2 and 90.7, respectively, were not different (P≥0.6413). An experiment by Babji et al. (1980), which yielded AD percentages of 89.92 and 90.11% for raw and cooked mechanically deboned chicken meat, respectively, had AD values comparable to THI and CMT.

Treating beef muscle with pressurization increased (P<0.05) AD from 88.9 ± 0.44 to 90.8 ± 0.43 (Elgasim and Kennick, 1980). These studies support the range for AD

values found for the standard and test diets in this study and also indicate that processing can positively affect apparent digestibility. The results of this study support this finding through demonstrating that CMT has an insignificant ($P=0.6413$), but relative (90.2 to 90.7) increase in AD over THI due to washing.

The PER and AD in this study, were inversely related (Figure 1). This inverse relationship between PER and AD suggests that the amino acid balance in both CMT and THI were more supportive for growth than that of the casein and egg albumin diets. Overall, the color modification process did not cause deleterious effects on the protein quality of flaked turkey thigh meat.

Figure 1. Comparison of least square means for protein efficiency (PER) and apparent digestibility (AD).

5.0 SUMMARY AND CONCLUSIONS

A nutrient profile and protein quality evaluation of color modified turkey thigh (CMT) were successfully obtained. The color of CMT was lighter and less red than unaltered turkey thigh (THI) as determined by Hunter color evaluation. The appearance of CMT was consistent with previously manufactured batches using the technique of Elkhalifa et al. (1988).

Proximate analyses were also consistent with Elkhalifa et al. (1988). With respect to THI, moisture content of CMT increased with concomitant decreases in protein, fat and ash concentration on a wet basis. Composition of solids expressed on a dry weight basis indicate an increased percentage of protein with concurrent decreases in fat and ash concentration.

Increased waterbinding of the CMT had three potential causitive agents. Increasing the tissue pH from 6.47 to 7.11 developed a net negative charge on proteins resulting in their apparent unfolding and entrapment of water in a "gel-like" protein mesh. The increase of sodium to 166% of its original content increased its ionic strength promoting unfolding, dissociation and "salting in" of the myofibrillar proteins. Increasing the ionic strength of phosphate by using orthophosphate wash solutions also contributed to the increased waterbinding.

Protein loss was due primarily to the selective extraction of pigment proteins. A minor reduction also resulted from the loss of collagen clinging to the mixer. Preceded by separation from the tissue due to processing, fat loss was also attributed to its clinging to the mixer.

Ash reduction can be explained by the leaching of some of the minerals during washing. Fe, Mg, P, K, and Mn sustained losses ($P=0.0187$) of 34, 80, 45, 95, and 46%, respectively. Ca, Zn and Cu content did not differ ($P=0.1184$) and Na increased to 166% of its original content. Fe loss was highly associated with the selective extraction of hemoglobin and myoglobin. The uptake by muscle tissue of Na during washing with sodium phosphate buffers accounts for the increased Na concentration.

Due to riboflavin's instability in an alkaline pH and its susceptibility to leaching and oxidation, the 70% retention of riboflavin was unexpected. This observation suggests that muscle tissues have a mechanism to protect from riboflavin loss due to leaching and destruction by alkaline pH, light and air.

The PER of both THI and CMT was higher ($P<0.0001$) than either the casein or egg albumin control diets. The PER of CMT was lower ($P=0.0318$) than THI, but the absolute difference was 0.24. This suggests that PER was not greatly disturbed due to leaching of sarcoplasmic proteins and increased concentration of collagen.

Apparent digestibility (AD) of CMT and THI were not different (P=0.6413). CMT had a relative increase of 0.5% over THI. This concurs with other studies which conclude that processing can have beneficial effects on protein digestibility.

The inverse relationship noted between PER and AD suggests that the amino acid balance in both THI and CMT supported more growth than either the casein or egg albumin diets. Overall, the color modification process did not reduce the protein quality of flaked turkey thigh meat.

Total analysis of the nutrient profile and protein quality of CMT revealed that it could be a viable source of nutrients if used in processed foods. The major detrimental effects on nutrition were the leaching of selected protein and minerals, which could be replaced through fortification, if necessary, and secondly, an increase in Na.

The value of CMT as a potential raw material in restructured meat products is twofold. Firstly, the rearrangement of both the protein structure and profile enhance its functional properties. Secondly, CMT's nutrient profile is maintained at a level that makes it acceptable for use as a basic or additive material.

REFERENCES

Adu, G.A., Babbitt, J.K. and Crawford, D.L. 1983. Effect of washing on the nutritional and quality characteristics of dried minced rockfish flesh. J. Food Sci. 48:1053.

Ang, C.Y.W., Hamm, D. and Searcy, G.K. 1982. Changes in nutrient content during chill-holding of ice-packed and deep-chilled broilers. J. Food Sci. 47:1763.

Ang, C.Y.W. and Hamm, D. 1983. Comparison of commercial processing method vs hot-deboning of fresh broilers on nutrient content of breast meat. J. Food Sci. 48:1543.

Anonymous. 1986. Effects of food processing on nutritive values. Food Technol. 40(12):109.

Anonymous. 1988. Mince, surimi and marine colloids. In "Seafood Products Resource Guide", C. Stott (Ed.), p. 117, Sea Grant and Mid-Atlantic Fisheries Development Foundation, Inc.

AOAC. 1984. "Official Methods of Analysis," 14th ed. Association Official Analytical Chemists, Washington, DC.

Augustin, J., Klein, B.P., Becker, D. and Venugopal, P.B. 1985. "Methods of Vitamin Assay", John Wiley and Sons, Inc., New York, p. 365.

Babji, A.S., Froning, G.W. and Satterlee, L.D. 1980. Protein nutritional quality of mechanically deboned poultry meat as predicted by the C-PER assay. J. Food Sci. 45:441.

Ball, H.R., Sheldon, B.W. and Montejano-Gaitan, J.G. 1984. Evaluation of procedures to remove meat pigments and flavor components from dark poultry meat. North Carolina State University. Unpublished data.

Bittel, R.J., Graham, P.P., Young, R. W. and Bovard, K.P. 1981. Mechanically separated spleen: its composition and protein efficiency ratio. J. Food Sci. 46:336.

Bowie, B.M. 1985. Color properties, oxidative stability and nitrogen fractions of poultry dark tissue subjected to washing treatments. M.S. thesis, Clemson Univ., Clemson, S.C.

Breidenstein, B.C. 1987. Nutrient value of meat. Food & Nutrition News. 59(2):43.

Breidenstein, B.C. 1988. Changes in consumer attitudes toward red meat and their effect on marketing strategy. Food Technol. 42(1):112.

Chang, Y. and Field, R.A. 1977. Protein utilization of mechanically deboned meat by growing rats. J. Nutr. 107:1947.

Cheftel, J.C., Cuq, J.L. and Lorient, D. 1985. Amino acids, peptides, and proteins. In "Food Chemistry", p. 245, O.R. Fennema (Ed.), Marcel Dekker, Inc., New York.

Davis, G.K. 1981. Bioavailibility of nutrients. In "Meat in Nutrition and Health", K.R. Franklin and P.N. Davis (Ed.), p. 75. Interstate Printers and Publishers, Inc., Danville, Ill.

Dawson, P.L., Sheldon, B.W. and Ball, H.R., Jr. 1988. Extraction of lipid and pigment components from mechanically deboned chicken meat. J.Food Sci. 53:1615.

DeDuve, C.A. 1948. Spectrophotometric method for simultaneous determination of myoglobin and hemoglobin in extracts of human muscle. Acta Chem. Scand. 2:264.

Drabkin, D.L. 1950. The distribution of the chromo-proteins, hemoglobin, myoglobin, and cytochrome c, in the tissues of different species, and the relationship of the total content of each chromoprotein to body mass. J. Biological Chem. 182:317.

Elgasim, E.A. and Kennick, W.H. 1980. Effect of pressur-ization of pre-rigor beef muscles on protein quality. J. Food Sci. 45:1122.

Elkhalifa, E.A., Graham, P.P., Marriott, N.G. and Phelps, S.K. 1988. Color characteristics and functional properties of flaked turkey dark meat as influenced by washing treatments. J. Food Sci. 53:1068.

Fleming, H.P., Blumer, T.N. and Craig, H.B. 1960. Quanti-tative estimation of myoglobin and hemoglobin in beef muscle extracts. J. Anim. Sci. 19:1164.

Graham, P.P., Marriott, N.G., Elkhalifa, E.A. and Phelps, S.K. 1989. Characteristics of restructured products made from color modified turkey dark meat. (Unpublished data).

Gaska, M.T. and Regenstein, J.M. 1982. Timed emulsification studies with chicken breast muscle: whole muscle, low-salt washed muscle and low-salt soluble proteins. J. Food Sci. 47:1460.

Hamm, R. 1986. Functional properties of myofibrillar system and their measurements. In "Muscle as Food," P.J. Bechtel (Ed.), p. 135. Academic Press, Orlando, Fla.

Hellendoorn, E.W. 1962. Water-binding capacity of meat as affected by phosphates. I. Influence of sodium chloride and phosphates on the water retention of comminuted meat at various pH values. Food Technol. 16(10):119.

Hendricks, D.G., Mahoney, A.W. and Gillett, T.A. 1977. Influence of removing connective tissue, cooking and nitrite curing on the protein quality of beef shank muscle. J. Food Sci. 42:186.

Hernandez, A., Baker, R.C. and Hotchiss, J.H. 1986. Extraction of pigments from mechanically deboned turkey meat. J. Food Sci. 51:865.

Hopkins, D.T., Steinke, F.H. and Kolar, C.W. 1976. The effect of cooking and water content of ground beef and eggs on the determination of the protein efficiency ratio. J. Food Sci. 41:1426.

Hsu, H.W., Vavak, D.L., Satterlee, L.D. and Miller, G.A. 1977. A multienzyme technique for estimating protein digestibility. J. Food Sci. 42:1869.

Hsu, H.W., Sutton, N.E., Banjo, M.O., Satterlee, L.D. and Kendrick, J.G. 1978. The C-PER and T-PER assays for protein quality. Food Technol. 32(12):69.

Huber, D.G. and Regenstein, J.M. 1988. Emulsion stability studies of myosin and exhaustively washed muscle from adult chicken breast muscle. J.Food Sci. 53:1282.

Hultin, H.O. 1985. Characteristics of muscle tissue. In "Food Chemistry", p. 725, O.R. Fennema (Ed.), Marcel Dekker, Inc., New York.

Jansen, G.R. 1978. Biological evaluation of protein quality. Food Technol. 32(12):52.

Jimenez-Colmenero, F. and Garcia, M.E. 1981. Effect of washing on the properties of mechanically deboned meat. In "Proceedings of the 27th European Meeting of Meat Res. Workers", O. Pranld (Ed.), p. 359. Vienna.

Layrisse, M., Martinez-Torres, C., Cook, J.D., Walker, R. and Finch, C.A. 1973. Iron fortification of food: its measurement by the extrinsic tag method. Blood. 41(3):333.

Layrisse, M., Martinez-Torres, C. and Gonzalez, M. 1974. Measurement of the total daily dietary iron absorption by the extrinsic tag model. Am. J. Clin. Nutr. 27:152.

Layrisse, M., Martinez-Torres, C., Renzy, M. and Leets, I. 1975. Ferritin iron absorption in man. Blood. 45(5):689.

Lee, Y.B., Elliott, J.G., Rickansrud, D.A. and Hagberg, E.C. 1978. Predicting protein efficiency ratio by the chemical determination of connective tissue content in meat. J. Food Sci. 43:1359.

MacNeil, J.H., Mast, M.G. and Leach, R.M. 1979. Protein efficiency ratio and amounts of selected nutrients in mechanically deboned turkey meat. J. Food Sci. 44:1291.

Marriott, N.G., Lopez, A. and Williams, H.L. 1982. Essential elements in unprocessed and processed frankfurters. J. Food Prot. 45(6):707.

Mott, E.L., MacNeil, J.H., Mast, M.G. and Leach, R.M. 1982. Protein efficiency ratio and amounts of selected nutrients in mechanically deboned spent layer meat. J. Food Sci. 47:655.

Pellett, P.L. 1978. Protein quality evaluation revisited. Food Technol. 32(5):61.

Pellett, P.L. and Young, V.R., Ed. 1980. "Nutritional Evaluation of Protein Foods", The United Nations University, Tokyo.

Proctor, V.A. and Cunningham, F.E. 1983. Composition of broiler meat as influenced by cooking methods and coating. J. Food Sci. 48:1696.

SAS. 1984. "SAS User Guide Statistics", A.A. Ray (Ed.). SAS Inst., Inc., Cary, NC.

Schwartz, W.C. and Mandigo, R.W. 1976. Effect of salt, sodium tripolyphosphate and storage on restructured pork. J. Food Sci. 41:1266.

Shenk, J.H., Hall, J.L. and King, H.H. 1934. Spectrophotometric characteristics of hemoglobins. I. Beef blood and muscle hemoglobins. J. Biological Chem. 105:741.

Sheldon, B.W., Essary, E.O., Bovard, K.P. and Young, R.W. 1980. Effect of end-point cooking temperature upon nutritive value and composition of turkey meat. Poultry Sci. 59:2725.

Smith, M.A. 1988. Effect of selected washing treatments on the Hunter L, a_L, and b_L color values of MDTM. In "IFT 88 Program & Abstracts", J.B. Klis (Ed.), p. 189. Institute of Food Technologists, Chicago, Ill.

Staub, H.W. 1978. Problems in evaluating the protein nutritive quality of complex foods. Food Technol. 32(12):57.

Steinke, F.H., Prescher, E.E. and Hopkins, D.T. 1980. Nutritional evaluation (PER) of isolated soybean protein and combinations of food proteins. J. Food Sci. 45:323.

Suzuki, T. 1981. "Fish and Krill Protein: Processing Technology", Applied Science Publishers, Ltd., London.

Unklesbay, N., Davis, M.E. and Krause, G. 1983. Nutrient retention in pork, turkey breast and corned beef roasts after infrared and convective heat processing. J. Food Sci. 48:865.

USDA. 1979. Agriculture Handbook No. 8-5, Composition of Foods, Poultry Products, Raw, Processed, Prepared. p. 213.

USDA. 1987. Livestock and Poultry, Situation and Outlook Report. February.

Warriss, P.D. 1979. The extraction of heme pigments from fresh meat. J. Food Technol. 14:75.

Zemel, M.B. and Bidari, M.T. 1983. Zinc, iron and copper availability as affected by orthophosphates, polyphosphates and calcium. J. Food Sci. 48:567.