

Proteins Associated with Thermally Induced Gelation of Turkey Breast Meat

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ABSTRACT: Principal component analysis was performed on turkey breast muscle that originated from a pool of genetically distinct turkeys by separating the salt-soluble from the salt-insoluble proteins. The salt-soluble proteins (SSP) formed thermally induced meat gels and the viscoelastic properties of the gels were analyzed. The storage modulus (G') at 80 °C of thermally induced gels derived from the SSPs ranged from 3 to 400 Pa. Each protein fraction was analyzed using 5% to 20% sodium dodecyl sulfate polyacrylamide gel electrophoresis. Reverse stepwise regression analysis of the electrophoretic fingerprints of the protein fractions showed that G' at 80 °C was associated with the 2 protein bands from the salt-soluble fraction ($R^2 = 0.87$, $P < 0.01$) and 1 protein from the salt-insoluble fraction ($R^2 = 0.27$, $P < 0.05$). Primary sequence analysis identified one of the SSPs positively associated with G' at 80 °C as α -tropomyosin and the other SSP negatively associated with G' at 80 °C as pyruvate kinase. The salt-insoluble protein identified as triose phosphate isomerase was negatively associated with G' at 80 °C. These findings are unique in that they show that proteins other than myosin and actin participate in the mechanisms of forming thermally induced meat gels.

Keywords: functionality, proteomics, rheology, thermally induced meat gel, turkey breast meat

Introduction

The modern commercial turkey is largely the end result of genetic selection for body weight (BW) and increased relative size of the *Pectoralis major* breast muscle. The practice of artificial insemination within both the primary breeder and commercial segments of the industry has allowed for increased genetic pressure on a few traits of major economic importance compared with more traditional, natural breeding programs (Nestor 1977; Lilburn and Nestor 1991).

Genetic increases in breast yield inadvertently may have resulted in generating a negative effect on collateral protein functionality important to the quality of further processed breast meat products (Liu and Xiong 1997). One anomaly that is becoming more common in today's commercial turkeys, especially in birds exposed to adverse rearing conditions such as heat stress, is the condition of pale, soft, and exudative (PSE) meat. While the meat exhibiting this condition is still highly nutritious, it is less acceptable to the consumer and ultimately results in a processed product that exhibits reduced water-holding capacity (WHC) and reduced textural quality of the thermally induced meat gel evaluated in terms of dynamic rheological properties G' (storage modulus) and G'' (loss modulus) (Barbut 1996; McKee and Sams 1997; Updike and others 2005). Processed turkey products are prepared by mixing comminuted turkey meat with salt solution in order to extract the salt-soluble proteins (SSP), which in turn form a thermally induced meat gel upon heating. The meat gel holds the comminuted pieces of turkey together. The storage modulus (G'), a commonly used property to describe the solid characteristics of gels, is a measure of the elasticity of the thermally induced meat gel.

The present research was designed to study the variation among individual birds that came from varied genetic backgrounds. The

RBC2 line represents the genetics of commercial turkeys from the 1960s, when fewer functional problems were reported. The C-line birds are modern commercial turkeys that have been selected for both rapid growth and increased relative weight of the *Pectoralis major*, the breast muscle that accounts for more than 80% of total breast meat. The F-line represents a genetic intermediate, it has the growth characteristics (BW) of the C-line but the same breast yield as the RBC2-line. Breast yield in the C-line is approximately 25% greater than in the RBC2 or F-line turkeys.

The viscoelastic properties of thermally induced meat gels derived from the 3 phenotypically distinct lines of turkeys have been previously examined (Updike and others 2005). A trend ($P = 0.09$) of higher storage modulus (G') was observed for thermally induced gels from R- and F-line birds in comparison with the birds from C-line, although large bird-to-bird variation within the lines existed. Furthermore, even for the SSP samples with the similar myosin heavy chain (MyHC) concentrations, there were 10-fold differences in the elasticity of the corresponding thermally induced meat gels. Therefore, the objective of this study was to employ statistical and electrophoretic analyses to identify the protein/peptide profile of SSP and salt-insoluble proteins from breast muscle obtained from genetically distinct turkey lines contributing to the elasticity of thermally induced meat gels.

Materials and Methods

Experimental design

Market weight turkeys ($n = 15$), from 3 genetically distinct lines previously described in Updike and others (2005), were exsanguinated at 16 wk of age. Immediately following picking of the feathers, the breast muscles from the animals were removed, split into right and left halves, and placed in an ice water bath for 24 h. The breast muscles were then stored at -20 °C.

Brine extraction of pectoralis major

The breast muscles were thawed and 25 g of muscle tissue were minced. Brine (75 mL) (1.4 M NaCl, 0.01 M Na tripolyphosphate,

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pH 7.6) (Li and Wick 2001) was added and the homogenate agitated for 1 h at 4 °C. The homogenate was centrifuged for 15 min at 10000 × g. The supernatant containing the SSP was removed and filtered through cheesecloth. The samples were stored at 4 °C for use within 24 h. The pellet (salt-insoluble proteins) from the centrifugation was stored at −20 °C for future electrophoretic analysis.

Dynamic rheological analysis

The SSP in brine was used for dynamic rheological measurements. The gelation of SSP solutions was studied using a controlled stress rheometer (AR 1000-N, TA Instruments, Newcastle, Del., U.S.A.). The SSP solution (4 mL) was placed on the rheometer plate. A 60-mm acrylic cone was used for analysis. The edge of the sample was coated with mineral oil to prevent evaporation of the water from the sample during heating. A solvent trap was placed over the cone geometry to prevent evaporation. Temperature ramps from 40 to 80 °C were performed at a heating rate of 1 °C/min for each sample. The frequency was set at 1 Hz and a constant stress of 0.1768 Pa was maintained during all dynamic measurements. Storage modulus G' and loss modulus G'' were calculated. G' at 80 °C was used for analysis with the peptide profile. Corresponding strain at 80 °C changed between 0.001 and 0.003, satisfying the linear viscoelastic region condition. Measurements were performed in duplicate or in triplicate for each sample.

Electrophoretic analysis

Electrophoresis was conducted as previously reported (Sawdy and others 2004) with modifications. Briefly, SSP and the salt-insoluble proteins were resolved by SDS-PAGE according to Laemmli (1970). The SSPs or saltinsoluble proteins were mixed with an equal volume of dissociation buffer (60 mM Tris buffer, pH 6.8, containing 2% SDS, 15% glycerol, 350 mM DTT, and 0.1% bromophenol blue), boiled for 5 min, and an equal volume of sample (approximately 10 µg of protein) was loaded onto each lane. A 1.0 mm × 12 cm × 14 cm polyacrylamide slab gel consisting of 20% to 5% gradient resolving gel (30 : 0.8, acrylamide: N, N'- bis-methylene acrylamide) and a 3% stacking gel containing 1% SDS were used. Electrophoretic separation was carried out at a constant voltage of 10 V/cm. After electrophoretic separation, gels were fixed with 20% trichloroacetic acid for 12 h and then stained with colloidal Coomassie blue stain (0.1% [w/v] Coomassie blue G250 in 20% [w/v] trichloroacetic acid) for 3 h. The gels were destained with deionized water. Gels were scanned on a Scanmaker 5900 scanner (Microtek, Carson, Calif, U.S.A.). The images were analyzed in Phoretix 1D™ (Nonlinear Dynamics, Newcastle upon Tyne, U.K.). The bands were identified and then analyzed as the percent that the staining intensity of each band contributed to the total staining intensity of all the bands.

Statistical analysis

A prediction model had been previously developed by Sawdy and others (2004). This model was analyzed using a reverse stepwise multiple regression within Proc Reg in SAS v. 8.0 (Draper and Smith 1998; SAS Institute Inc. 2004). The following statistical model was fitted to elastic modulus values to determine which bands were contributing to the variable:

$$Y_i = \mu + \beta_j \text{Band}_{ij} + \text{error}_{ijkl}$$

where Y_i is the differential variable: viscoelastic strength at 80 °C,

μ is the overall mean, β_j is the regression coefficient for the effect of the percent contribution of Band $_k$ ($k = 1, \dots, n$), and error_{ijkl} are the random errors due to birds and are assumed independent and normal. Insignificant ($P > 0.10$) bands were removed iteratively from the model.

Peptide sequencing

Bands identified by the regression model as being associated with G' at 80 °C were submitted for characterization by mass spectrometry. All mass spectrometry was performed at the Campus Chemical Instrument Center of The Ohio State Univ. using established methods. Briefly, excised bands were digested with sequencing grade trypsin (Promega, Madison, Wis., U.S.A.) using a Montage In-Gel Digestion Kit (Millipore, Bedford, Mass., U.S.A.) following manufacturer's protocols. Subsequently, 1 µL of matrix (α -cyano-4-hydroxy cinamic acid) and 1 µL of sample were thoroughly mixed together and 0.5 µL of this was spotted on the target plate and then allowed to dry. Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) was performed on a Bruker Reflex III (Bruker, Bremen, Germany) mass spectrometer operated in linear, positive ion mode with a N₂ laser.

Capillary-liquid chromatography-nanospray tandem mass spectrometry (nano-LC/MS/MS) was performed on a Micromass hybrid quadrupole time-of-flight Q-ToF™ II (Micromass, Wythenshawe, UK) mass spectrometer equipped with an orthogonal nanospray source (New Objective Inc., Woburn, Mass., U.S.A.) operated in positive ion mode. The LC system was a Waters Alliance 2690 Separation Module (Waters, Milford, Mass., U.S.A.). Mass spectra were recorded using MassLynx 3.5 (Micromass) automatic switching functions. Sequence information from the MS/MS data was processed using the MassLynx 3.5 Biolynx software.

Results and Discussion

Rheological properties

A typical temperature sweep showing the change of G' and G'' as a function of temperature is shown in Figure 1. Figure 1 shows that the gel formed can be described as a strong gel because its elastic (storage) modulus is approximately 15 times larger than its viscous (loss)

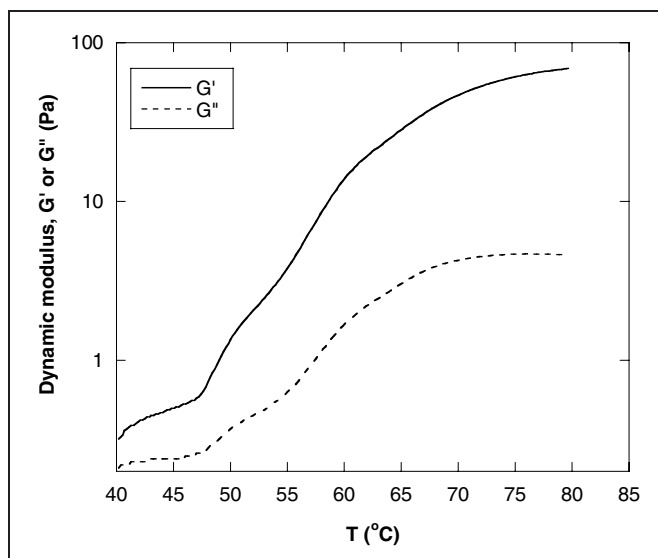


Figure 1—Representative dynamic modulus data as a function of temperature during gel formation. Frequency: 1 Hz, oscillation stress: 0.1768 Pa.

modulus at 80 °C. For all the samples tested this ratio varied between 7 and 21, indicating all of the gels formed are strong, however the strength of the gels varied among the genetic lines as well as within the genetic line. As previously reported in Updike and others (2005) the elastic modulus at 80 °C ranged from 3 to 400 Pa for the gels produced from all turkey lines. The RBC2-line had G' of 131 ± 42 Pa, the F-line had G' of 89 ± 39 Pa, and the C-line had G' of 54 ± 42 Pa ($P = 0.09$) at 80 °C. Similar to gel strength, a bird-to-bird variation within the genetic lines was also apparent for G' values.

The variation observed for gel elasticity at 80 °C may indicate that SSPs other than myosin and actin may contribute to the functional properties of thermally induced meat gels because our statistical analysis showed that the staining intensity of neither the MyHC band nor the actin band was associated with variation in gel elasticity ($P > 0.05$). In these lines of birds, only adult MyHC is present in 16-wk birds, suggesting that MyHC isoform differences are not the cause of variation (Updike and others 2005).

Previous research by Wu and others (1991) showed that even with the same myosin concentration, the magnitude of the shear modulus varied among individual birds of same type. They attributed differences to seasonal change or genetic variation among birds; however, they did not further evaluate the origin of the variation among individual birds. The dependence of G' on protein composition as

well as protein concentration was also reported for spectrin-actin gels (Schanus and others 1985), indicating the correlation between gel elasticity and protein composition.

SDS-PAGE analysis and peptide sequencing

Reverse stepwise regression analysis of the electrophoretic fingerprints of the protein fractions showed that 2 protein bands from the salt-soluble fraction ($R^2 = 0.87$, $P < 0.01$) (Figure 2 and 3) and 1 protein band from the salt insoluble fraction ($R^2 = 0.27$, $P < 0.05$) (Figure 4 and 5) were associated with G' . The 58-kDa band in the SSP identified as pyruvate kinase (Figure 6) was negatively associated with G' at 80 °C. The 32-kDa band in the SSP identified as α -tropomyosin (Figure 7) was positively associated with G' at 80 °C. The 26-kDa band identified as triose phosphate isomerase (Figure 8) from the salt-insoluble fraction was negatively associated with G' at 80 °C. Alpha tropomyosin had a band percent range of 3.2% to 12.5%, pyruvate kinase had a band percent range of 2.0% to 9.0%, and triose phosphate isomerase had a band percent range of 0.4% to 5.1%.

Tropomyosin is a regulatory protein with an alpha-helical coiled-coil region, similar to the myosin rod region. Tropomyosin regulates muscle contraction by uncovering the myosin-binding site on F-actin after signaling from the troponin complex (Monterras and others 1982; Bandman 1992). Contribution of α -tropomyosin to gel stiffness could be due to interaction between α -tropomyosin and MyHC in the formation of thermally induced meat gels. The rod region of the MyHC is known to aggregate during the formation of thermally induced meat gels (Samejima and others 1969). Goldman (2000) also reported increased storage modulus in actin gels with incorporations of tropomyosin and troponin. Goldman proposed that tropomyosin addition reduced the internal chain dynamics, causing an increase in the rigidity of actin gels. A similar mechanism may be responsible as a result of interaction between tropomyosin and MyHC during gel formation.

It was also suggested that α -tropomyosin may have a regulatory speed or sensitivity that is different from the other isoform

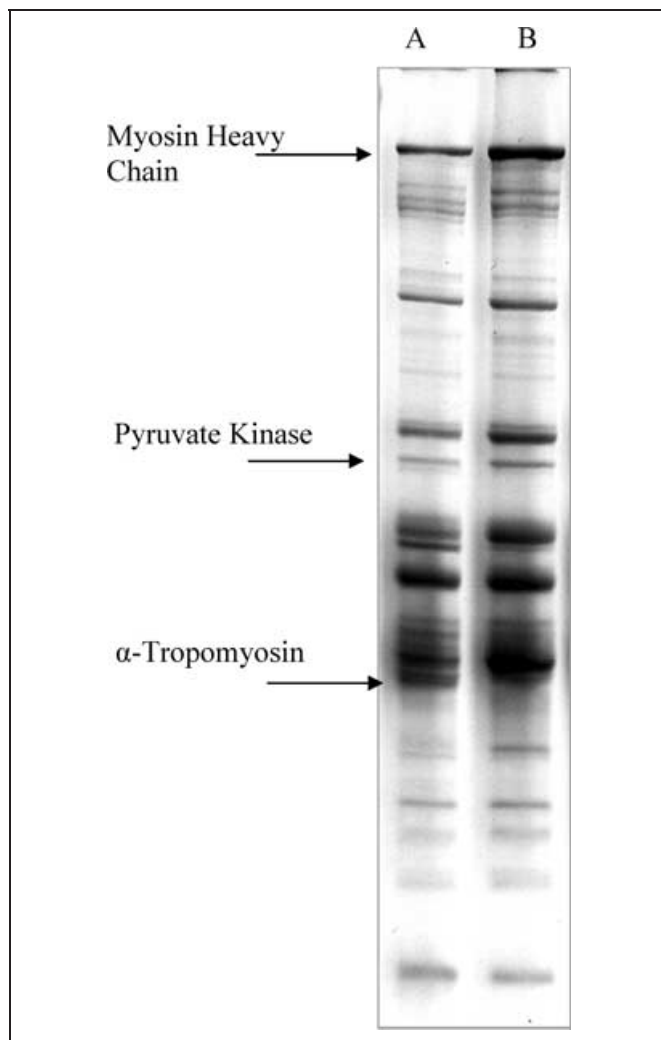


Figure 2—Representative lanes from 5% to 20% SDS-PAGE of the SSP fraction with lane A having a high G' and lane B having a low G'

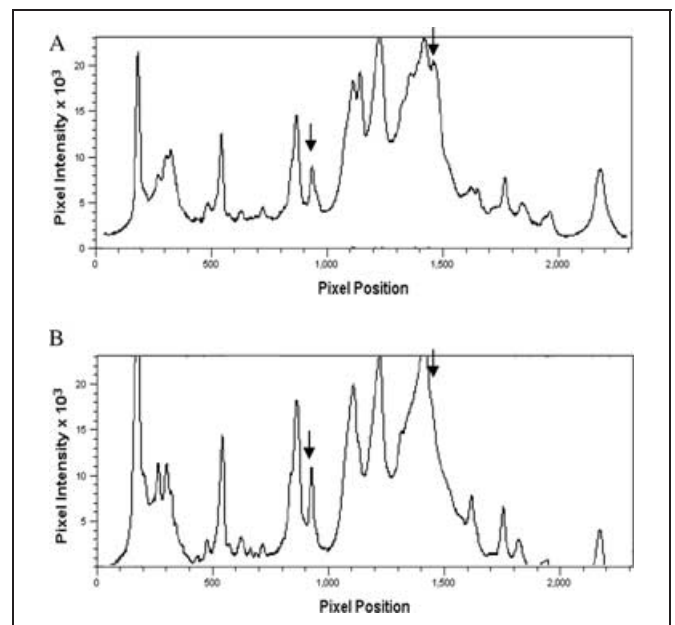


Figure 3—The electropherograms of the 2 lanes from Figure 2. The sample with a high G' value (A) has a small pyruvate kinase peak and a large α -tropomyosin peak. The sample with a low G' value (B) has a larger pyruvate kinase peak and a smaller α -tropomyosin peak.

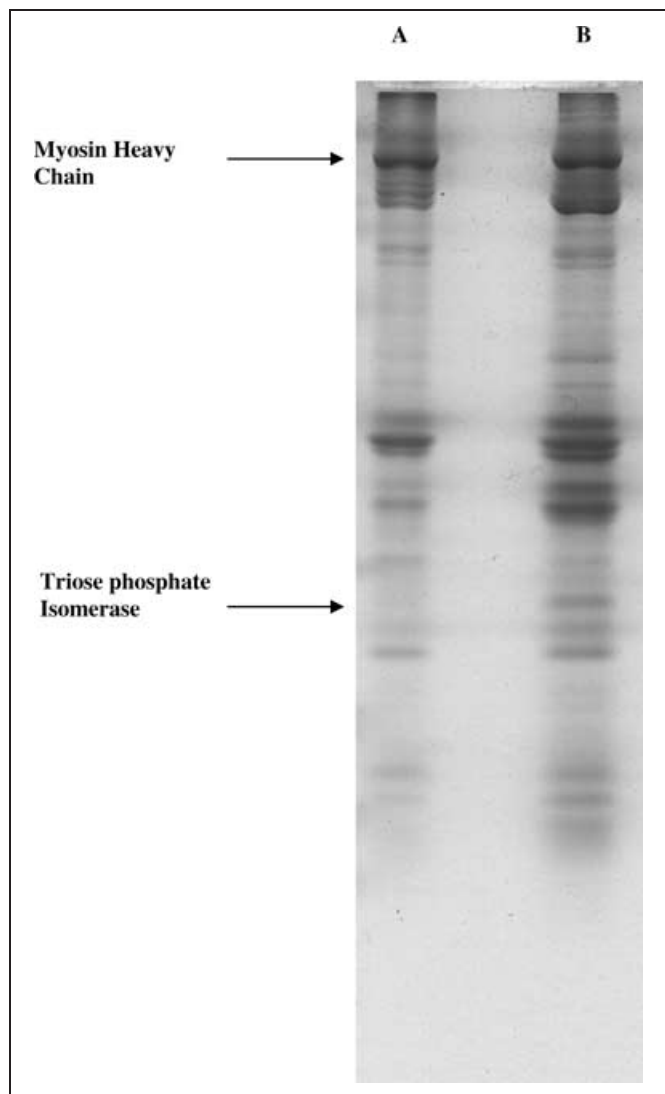


Figure 4—Representative lanes from 5% to 20% SDS-PAGE of the SSP fraction with lane A having a high G' and lane B having a low G'

of tropomyosin (Jagatheesan and others 2003; Gaffin and others 2004). This could lead to an increased rate of ATP hydrolysis by myosin before rigor. If ATP is hydrolyzed more slowly post-mortem, then glycolysis and lactic acid production may occur at a slower rate. A slower postmortem metabolic rate could result in a slower rate of pH decline that would result in higher pH values before the carcass reached the final chill temperature. This potential mechanism may be related to PSE meat (Pietrzak and others 1997).

The fingerprinting analyses also showed a negative association between pyruvate kinase and the elasticity of thermally induced meat gels and a negative association between triose phosphate isomerase and the elastic property of the thermally induced meat gels. Both pyruvate kinase and triose phosphate isomerase are enzymes in the glycolytic pathway. The finding that an increased presence of pyruvate kinase in the SSP is associated with decreased gel elasticity and the finding that an increased presence of triose phosphate isomerase in the salt-insoluble fraction is associated with decreased gel elasticity support the hypothesis that metabolic activity postharvest affects the ultimate functionality of meat. Pyruvate kinase is proposed to be one of the rate-limiting steps in glycolysis (Beatty and others 1976). In addition, reduced oxygen levels have been hypothesized to slow the biodegradation rate of pyruvate kinase (Hance and others 1980). Potentially, an increased concentration of these glycolytic enzymes postmortem may cause an increased rate of glycolysis that would lead to a lowered pH that is associated with PSE meat.

Triose phosphate isomerase has previously been reported to be correlated to porcine meat quality. The authors suggested this correlation may be due to altered flux through glycolysis (Lametsch and others 2003).

Numerous studies have shown a relationship between a rapid decline in pH immediately postmortem and lowered meat quality. In pork, this pH decline is triggered due to a mutation in the ryanodine receptor. Although, as of yet, the biochemical mechanism for rapid pH decline in poultry remains to be elucidated, this research reports a potential metabolic mechanism responsible for the rapid postmortem pH decline that negatively affects processed turkey quality. Increased concentrations of glycolytic enzymes may increase the glycolytic potential of muscle cells and allow for more flux through glycolysis and a resulting increased concentration of lactic acid postmortem.

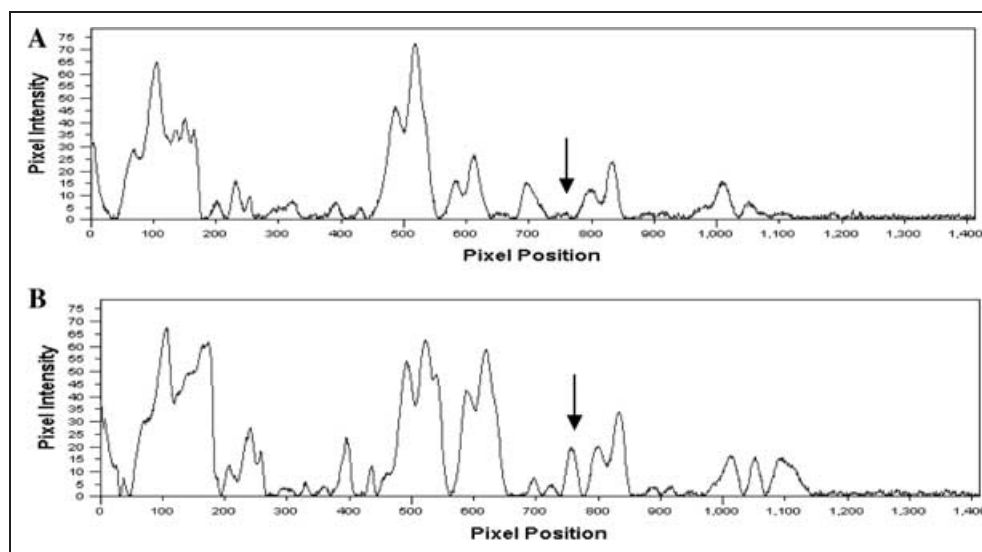


Figure 5—The electropherograms of the 2 lanes from Figure 4. The sample with a high G' value (A) has a small triose phosphate peak and the sample with a low G' value (B) has a larger triose phosphate peak.

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MSKHH DAGTA  FIQTQQLHAA  MADTFLEHMC  RLDIDSEPTI  ARNTGIICTI
GPASRSVDKL  KEMIKSGMNV  ARLNFSHGTH  EYHEGTIKNV  REATESFASD
PITYRPVAIA  LDTKGPEIRT  GLIKSGTAE  VELKKGAALK  VTLDNAFMEN
CDENVLWVDY  KNLIKVIDVG  SKIYVDDGLI  SLLVKEGKGD  FVMTEVENGG
MLGSKKGVNL  PGAAVDLPV  SEKDIQDLKF  GVEQNVDMVF  ASFIRKAADV
HAVRVKLVGK  GKHIKIISKI  ENHEGVRRFD  EIMEASDGIM  VARGDLGIEI
PAEKVFLAQK  MMIGRCNRAG  KPIICATQML  ESMIKKPRPT  RAEGSDVANA
VLDGADCIML  SGETAKGDYP  LEAVRMQHAI  AREAEAAMPH  RQQFEEILRH
SVHHRPADA  MAAGAVEASF  KCLAAALIVM  TESGRSAHLV  SRYRPRAPII
AVTRNDQTAR  QAHLRYGVFP  VLCKQPAHDA  WAEDVDLRVN  LGMNVGKARG
FFKTGDLVIV  LTGWRPFGSY  TNTMRVVPV
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Figure 6 – Peptide sequence of the 58 kDa protein positively associated with G'. This band was identified as pyruvate kinase. The underlined and bold amino acids are the tryptic fragments identified by mass spectrometry.

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MDAIKKKMQM  LKLDKENALD  RAEQAEADKK  AAEERSKQLE  DELVALQKKL
KGTEDELDKY  SESLKDAQEK  LELADKKATD  AEEVASLNR  RIQLVEEELD
RAQERLATAL  QKLEEAEEKAA  DESERGMKVI  ENRAQDEEK  MEIQEIQLKE
AKHIAEEADR  KYEEVARKLV  IIEGDLERA  ERAELSESK  AELEELKTV
TNNLKSLEAQ  AEKYSQKEDK  YEEEIKVLTD  KLKEAETRAE  FAERSVTKLE
KSIDDEDEL  YAQRLKYKAI  SEELDHALND  MTSI
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Figure 7 – Peptide sequencing of the 32 kDa protein negatively associated with G'. This band was identified as α -tropomyosin. The underlined and bold amino acids are the tryptic fragments identified by mass spectrometry.

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MAPRKFFVGG  NWKMNGDKKS  LGELIHLTLG  AKLSADTEVV  CGAPSIYLD
ARQKLDKAG  VAAQNCYKVP  KGAFTGEISP  AMIKDIGAAW  VILGHSERRH
YFGESDELIG  QKVAYHALAEG  LGVIACIGEK  LDEREAGITE  KVVFQTKAI
ADNVKDWSKY  VLAYEYPWAI  GTGKTATPOQ  AOEVHEKLRG
WLKSHVSDAV  AQSTRIIYGG  SVTGGNCKEL  ASQHDVDGFL  VGGASLKPEF
VDIINAKH
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Figure 8 – Sequence corresponding to triose phosphate isomerase accession gi|45382061. Sequences underlined and in bold correspond to tryptic peptide fragments identified by mass spectrometric analysis as being derived from triosephosphate isomerase.

Conclusions

This research demonstrates that other proteins may be acting alone or in concert with myosin and actin in the formation of thermally induced meat gels. This suggests that current product-quality problems in the turkey industry may be due to expression levels of multiple proteins. Future study needs to be performed again on a larger number of turkeys to give more degrees of freedom to the statistical analysis so that more protein bands may be identified. In addition, future studies will be done to discern the mechanism that causes an association between the identified proteins and asymptotic elastic modulus at the end of heating.