Journal of Nutrition & Food Sciences
ISSN: 2155-9600

Singh and Deshpande, J Nutr Food Sci 2018, 8:4
DOI: 10.4172/2155-9600.1000709

Research Article Open Access

Thermally Induced Changes in Quality of Chicken Breast Meat Protein Fractions

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Abstract

Functional properties of chicken breast meat are affected by heat treatments and the condition of meat, i.e., normal or pale, soft exudative (PSE) meat. The two main fractions of meat proteins, myofibrillar and sarcoplasmic are affected differently by the treatments and the condition of the meat. The aim of this study was to extract myofibrillar and sarcoplasmic proteins from the chicken breast meat and determine differences in protein extractability between normal and PSE meat, and determine temperature transitions in whole muscle and its constituent proteins. The protein concentration of sarcoplasmic protein was less than that of myofibrillar protein in chicken breast muscle. It was also found that the protein solubility differed in normal and PSE muscle. The PSE muscle showed lower protein solubility as compared to the normal muscle which was assumed to be due to the denaturation of some of the protein fractions in PSE muscle. The results of SDS-PAGE did not show much variance in the protein profiles of the PSE and normal samples indicating that the solubilized protein in PSE and normal samples was similar. Thermal denaturation determined using differential Scanning Calorimeter, identified thermal transition peaks which could be of value in the design a scheduled heating sequence for cooking of chicken breast.

Keywords: PSE muscle; Myofibrillar protein; Water holding; Denaturation

Introduction

Poultry meat is comprised of about 20-23% protein. Muscle proteins are divided into three categories based mainly on their solubility as myofibrillar, sarcoplasmic and stromal proteins. Myofibrillar or the salt soluble proteins (SSP) comprise about 50-56% of the total skeletal muscle protein and are insoluble in water, but can be extracted with concentrated salt solutions. Myosin is the predominant salt soluble protein and is 50-55% followed by actin which is about 22% of the total myofibrillar protein [1]. Proteins mainly soluble in water are called as sarcoplasmic or water soluble proteins (WSP). These comprise of Creatine kinase, myoglobin and other enzymes. Stromal proteins which constitute 3-5% of the total protein mainly consist of collagen and elastin [2].

Functional properties of poultry meat such as texture and water holding capacity (WHC) are very important for consumer. Loss in functionality of poultry breast meat is often associated with pale meat called as pale, soft, exudative (PSE) or woody breast (WB) meat. Factors affecting the color values of the breast meat include inadequate aging before deboning, time the meat was stored before examination, and thickness of the meat. Several researches have examined the differences in characteristics of normal and pale fillets selected visually or using L^*(lightness) values [3]. Aside from color, PSE meat is characterized by low moisture retention, softness and lack of springiness when poked with the finger, and more reflective of light [4]. In another study, it was observed that compared with normal meatballs, PSE-like and woody meatballs showed inferior functional properties [5].

Heating of meat results in the development of textural, color and flavor properties characteristic of a cooked product. These changes result from thermally induced changes in proteins and interactions with fats in the meat. Myosin and actin (myofibrillar proteins) and also collagen (main protein in connective tissue) are the major structural proteins in muscle foods. Thermal transitions of these proteins, the time span across and the high temperature hold time after these transitions, have major influences on the resulting texture of the cooked meat [6]. These effects include protein denaturation, dissociation of myofibrillar proteins, shrinkage in length and width of meat fibers, aggregation and gel formation of sarcoplasmic proteins and solubilization of connective tissue [7-10]. The solubilization of connective tissue improves meat tenderness and heat denaturation of myofibrillar proteins causes toughening [11]. Most of the water in muscle is held by capillary action by the myofibrils. When muscle is heated, proteins denature and release the entrapped juice. The release of water and dissolved ions cause a detectable change in dielectric properties, which can be used as a method to measure the protein denaturation [12].

Conformational changes on heating of protein are usually called denaturation and the temperature where these changes occur is called the denaturation temperature. Differential Scanning Calorimeter (DSC) has been used to determine the denaturation temperatures of meat proteins [13]. The amount of denatured proteins differs in normal and PSE muscle. The PSE muscle has a high drip loss, a very pale color and contains denatured myofibrillar or sarcoplasmic proteins. The soluble sarcoplasmic proteins are lower in PSE compared to normal muscle. Denatured muscle proteins are less soluble than that of native proteins because insoluble aggregates are formed on denaturation. It has been suggested that the light reflective and pale color of PSE muscle is due to precipitation of denatured sarcoplasmic proteins. The precipitated proteins mask the red color of the sarcoplasm producing the pale color.

Heat induced changes in proteins changes water-holding capacity of meat. Meat can shrink in two dimensions (length and width) and

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Received May 30, 2018; **Accepted** June 15, 2018; **Published** June 29, 2018

Citation: Singh RK, Deshpande D (2018) Thermally Induced Changes in Quality of Chicken Breast Meat Protein Fractions. J Nutr Food Sci 8: 709. doi: 10.4172/2155-9600.1000709

expand in the third dimension. The extent of meat shrinkage and expansion varies with different muscles and meat temperature. Loss of moisture also contributes to changes in sarcomere length and juiciness as meat temperatures increases [8].

The objectives of this study were: (i) To apply known procedures for extraction of myofibrillar and sarcoplasmic proteins from the chicken breast meat and determine differences in protein extractability between normal and PSE meat; and (ii) To determine temperature transitions in un- marinated whole muscle and its constituent proteins.

Material and Methods

Chicken meat samples

The chicken breast fillets were obtained from a local processor (Wayne Farms-DQH, Pendergras, GA). The pH and the L^*(lightness), a^*(redness), b^*(yellowness) values were measured 24 h post mortem. The L^*, a^*, b^* values for the PSE samples were 60.55, 12.03 and 10.59 respectively and pH=5.58. The normal sample had L^*, a^*, b^* values of 57.87, 11.97 and 13.92 respectively and pH=5.85. The samples were chopped in a food processor prior to extraction. Extracts of sarcoplasmic and myofibrillar proteins were stored in the freezer until used in further investigations and analysis.

Protein solubility

To determine the solubility of sarcoplasmic and myofibrillar proteins two extractions were conducted with some modifications of procedure described in the literature [14]. Sarcoplasmic proteins were extracted from 2 g of meat sample using 20 ml of 0.025M sodium phosphate buffer (Buffer A, pH=7.2). The samples were homogenized with a PRO300A Proscientific homogenizer (Sparks Technologies, Buford, GA) on ice with low setting for 1 min. The samples were then centrifuged in a Sorvall RC-5B refrigerated super-speed centrifuge with an SLC 1500 rotor (Du Pont Instruments, Wilmington, DE) at 8000 x g for 20 min at 4°C. The supernatant was collected and labeled as sarcoplasmic or water soluble proteins. During extraction, the samples were kept under cold conditions so as to prevent the denaturation of proteins due to increase in temperature as well as to avoid the inconsistency in results.

The pellets obtained were mixed with 0.025 M of sodium phosphate buffer, pH=7.2 containing 0.6 M of NaCl (Buffer B) and was used for the extraction of Myofibrillar proteins. This was followed by similar process of homogenization of the samples with low setting on ice for 1 min. To ensure proper solubilization of the salt soluble proteins, the samples were left on a Corning PC 620 hot plate/stir plate for 4 h. The samples were placed on ice during stirring. Then they were centrifuged at 8000 x g for 20 min at 4°C and the supernatants were labeled as myofibrillar or salt soluble proteins.

Protein quantification

The concentration of proteins in the supernatants was determined by the Better Bradford Assay (Pierce™ Coomassie Plus (Bradford) Assay Kit, Thermo Scientific™) with some changes described as follows. The samples were diluted in the ratio of 1:20. About 400 µl of the sarcoplasmic and myofibrillar protein sample was mixed with 7600 µl of buffer A and buffer B, respectively. The protein standards were prepared using BGG (Bovine Gamma Globulin) 2 mg/ml (Product # 23212, Pierce™, Thermo Scientific). BGG was used instead of BSA to get more accurate results. Instead of mixing the protein standard and samples with the Coomassie Plus protein assay Reagent (product # 1856210, Pierce™) on the micro plate, they were initially mixed in test tubes with the reagent (20 µl of standard and sample with 600 µl of Coomassie Plus reagent). Then about 310 µl of the above mixture was put on the micro plate and the absorbance was measured on a micro-plate reader using the Micro-plate Manager (Bio-Rad model 550 micro-plate reader, Hercules, CA) at 595 nm. The Better Bradford Assay was used to compare the protein concentration in normal and PSE muscle.

Molecular weight determination by Sodium Dodecyl Sulphate (SDS) gel electrophoresis

Once the protein concentration was determined using the Better Bradford Assay, the samples were diluted to 8 mg/ml. Dilution was carried out by mixing the sarcoplasmic and myofibrillar supernatants with calculated amount of Buffer A and Buffer B, respectively. The stock standard sample buffer contained 8 M urea, 2 M thiourea, 3% (w/v) SDS, 75 mM D1-dithothreitol, 25 mM Tris-HCl at pH=6.8 and 0.1% Bromophenol blue. This was mixed with β-mercaptoethanol (25 µl in 475 µl of stock sample buffer) and was prepared immediately before use. The samples were mixed 1:1 with standard sample buffer (300 µl of sample and 300 µl of sample buffer) and were heated at 100°C for 5 min in boiling water bath, cooled to room temperature, centrifuged for a minute (Model 5412, Brinkmann Instruments, N.Y) and applied on to the gel.

The gel electrophoresis was carried out using the Pharmacia Biotech Phast System, (Amersham Biosciences, Sweden). The system consisted of a separation and control unit and a development unit. The Fast Coomassie staining technique was used for detecting proteins in Phast gel separation media using Phast gel blue R which is Coomassie R 350 dye in a tablet form. The SDS buffer strips and the gradient gel were obtained from GE healthcare biosciences AB, Sweden. The solutions for the electrophoresis were prepared as follows: Stain was made of 0.1% PhastGel Blue R solution in 30% methanol and 10% acetic acid in distilled water (mili Q water). The stock solution was prepared initially and the final solution was made fresh and used immediately. Destain consisted of 30% methanol and 10% acetic acid in distilled water (mili Q water). The Preserving solution was prepared with 10% glycerol and 10% acetic acid in mili Q water. 4-15% gradient gel was used for separation of myofibrillar proteins while the 8-25% was used for the sarcoplasmic proteins. This was done because 8-25% gradient gel had smaller pore size which was suitable for low molecular weights of sarcoplasmic proteins. While 4-15% gradient gels had larger pore size which was more suitable for high molecular weight proteins like myosin and actin.

The molecular weights of the protein subunits were determined using a densitometer (Model GS-700, Imaging Densitometer, Bio-Rad Laboratories) by comparison of the relative mobility of migration with those of protein molecular weight standards. The relative migration distance (Rf) values were calculated as shown in Equation 1:

Rf = (Distance of band from origin) / (Distance from the origin to the reference point) (Equation 1).

The distances were measured using a ruler. The gels were scanned using the Molecular Analyst software (Windows software for Bio-Rad's image analysis systems version 1.5). Scanning densitometers with software installed readily integrated the stained band or spot relative to the total staining intensity of the entire gel or a part of it [15].

Protein denaturation using differential scanning calorimeter DSC

Extracts of the constituent proteins (i.e. water soluble, salt soluble proteins and collagen) were removed from the freezer, allowed to thaw under refrigeration, and dialyzed. The dialyzed retentate samples were stored at -80°C for couple of weeks. The samples were thawed before using them for DSC.

The DSC (Mettler Toledo) was calibrated with indium before measuring the denaturation temperatures of chicken breast meat and its constituent proteins. Hermetic aluminum pans were used for sample measurement for DSC. Approximately, 10-15 mg of the sample was placed in the pan. Empty aluminum pans were used as reference. The samples were subjected to a heating rate of 10°C /min for the range of 10-100°C. The DSC curves were obtained and the peak denaturation temperatures were determined by analyzing three replicates for each sample using the Mettler Toledo Star E software.

Results and Discussion

The procedure used for extraction of sarcoplasmic and myofibrillar proteins from the normal and PSE muscle was relatively easy to perform and ample quantities of protein were recovered in the extracts. Stirring the homogenized mixture on ice for 4 h increased the amount of extracted myofibrillar proteins.

The extract of PSE muscle was found to have less protein concentration as compared to the normal muscle extract. The concentration of sarcoplasmic proteins and myofibrillar proteins were 12.2 mg/ml and 16.6 mg/ml, respectively in the PSE muscle extract. On the other hand, concentration in the normal muscle extract was 13 mg/ml and 20.4 mg/ml, respectively for sarcoplasmic and myofibrillar proteins. The concentration of sarcoplasmic proteins (WSP) was lower as compared to that of myofibrillar proteins (SSP) in both the normal and PSE samples which is in agreement with the data given in the literature. In a separate study, it was found that increasing the salt (NaCl) content to 3% or more increased the solubility of myofibrillar protein and the extraction of salt soluble proteins, which improved cooking yield of chicken meat [16]. Both, normal and woody breast (WB) meat showed different protein profiles, with myosin heavy chain exhibiting a higher intensity at ≥ 3% salt level [16].

Protein solubility affects some of the physical properties of the meat, therefore the differences between PSE and normal meats show the consequences of processing procedures on these two types of muscles' response. It has been reported that light chicken breast meat has significantly lower total protein value than normal or dark meat [17].

The type of protein extracted was investigated by performing SDS-PAGE electrophoresis. Using Equation 1 and the calibration curve, the molecular weights for the unknown protein sub fractions were determined and are shown in Tables 1 and 2. The scanned picture of the gels is shown in Figure 1a shows the electrophoresis profile for sarcoplasmic (WSP) proteins. The bands were observed in similar locations for all the four samples in this experiment except for the intensity of the bands in different lanes. Lane 2 and 4 are the electrophoretic patterns of extracts from pale and normal samples respectively using the extraction procedure previously described. Lane 3 and 5 are the patterns for extracts of pale and normal samples respectively when a different extraction procedure was used. Thus, the first band is darker for lanes 2 and 4 as compared to the first band in lanes 3 and 5. For myofibrillar proteins as seen in Figure 1b, slight difference was observed in the position of one band for the PSE and normal sample. The estimated molecular weights of the two distinctly dark bands seen for both the PSE and normal samples were approximately 210 kDa and 35 kDa (Table 2). The molecular weight of heavy chain Myosin was 200 kDa and that of actin in its monomeric form of G-actin had molecular weight of 42 kDa [8]. Hence the first dark protein band for both the PSE and normal samples in this experiment could be assumed as Myosin heavy chain (MW 210) followed by the myosin light chains. Actin is not degraded by proteolytic enzymes and is resistant to denaturation. Hence, the amount of actin present in the sample is directly related to the amount of sample spotted in the electrophoresis gel. In another study [18], it was found that the extracts of pale and normal breast meat contained similar quantities of phosphorylase in the myofibrillar fraction indicating that protein denaturation had occurred in pale and normal muscles. It was reported that the myofibril fragments in extracts of PSE chicken breast meat subjected to SDS-PAGE had some missing bands in the area corresponding to the high molecular weight

Distance (cm)	Rf	Log Mw	Mw (kDa)
1.2	0.245	5.01	102.8
1.7	0.347	4.82	66.2
1.8	0.367	4.78	60.6
2.1	0.429	4.67	46.5
2.2	0.449	4.63	42.6
2.3	0.469	4.59	39.0
2.8	0.571	4.40	25.1
2.9	0.592	4.36	23.0
3.1	0.633	4.28	19.3

Rf = Relative migration distance on the gel

Table 1: Molecular weights as estimated for the unknown sarcoplasmic protein subunits for gel # 1 in Figure 1.

Distance PSE meat (cm)	Rf	Log Mw	Mw	Distance Normal meat (cm)	Rf	Log Mw	Mw (kDa)
1.4	0.286	5.32	210.5	1.4	0.286	5.32	210.2
2.3	0.469	4.97	93.7	2.3	0.469	4.97	93.7
2.4	0.49	4.93	85.6	2.4	0.49	4.93	85.6
3.4	0.694	4.54	34.9	3.4	0.694	4.54	34.9
3.6	0.735	4.46	29.1	3.6	0.735	4.46	29.1
3.9	0.796	4.35	22.3	3.9	0.796	4.35	22.3
4	0.816	4.31	20.3	4.1	0.837	4.27	18.6
4.3	0.878	4.19	15.5	4.3	0.878	4.19	15.5
4.6	0.939	4.07	11.9	4.6	0.939	4.07	11.9

PSE = Pale, soft and exudative

Rf = Relative migration distance on the gel

Table 2: Molecular weights (Mw) as estimated for the unknown myofibrillar protein subunits for gel # 2 in Figure 1.

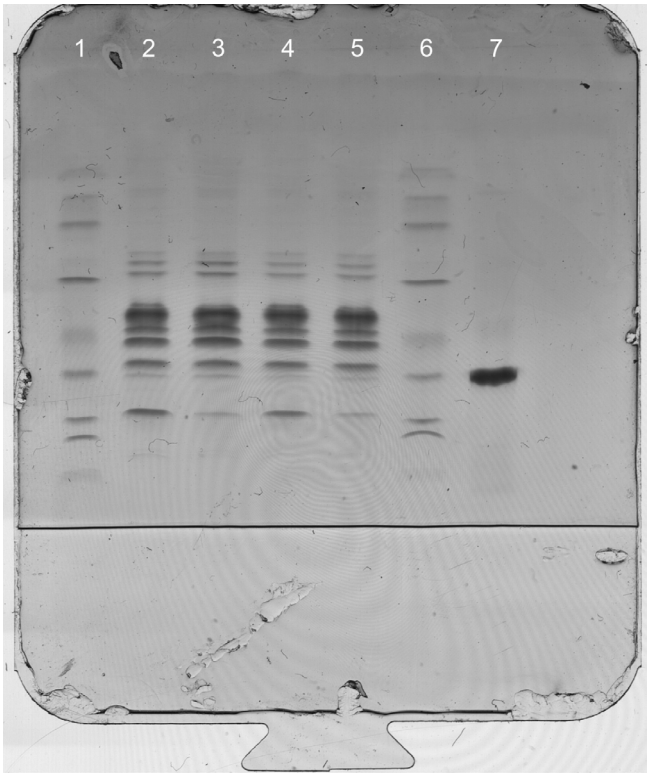

Figure 1a: Gel electrophoretic pattern of water soluble proteins (WSP): Lane 1&6 Standards, Lane 7-Bovine serum albumin (BSA), Lanes 2&3- Pale, soft and exudative (PSE) samples.

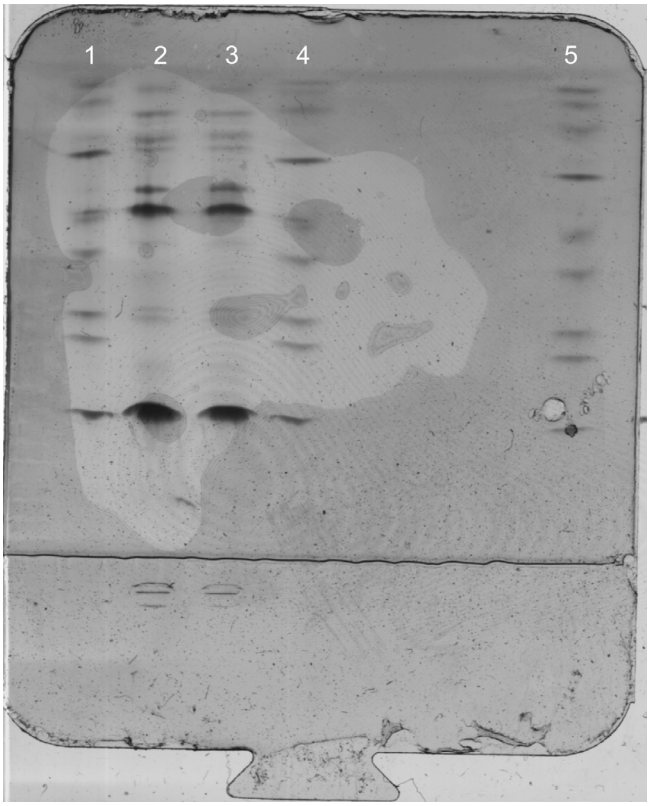

Figure 1b: Gel electrophoretic pattern of salt soluble proteins (SSP): lane 1,4 & 8 – Standards, 2- pale, soft and exudative (PSE) sample, lane 3, 4&5 – normal samples.

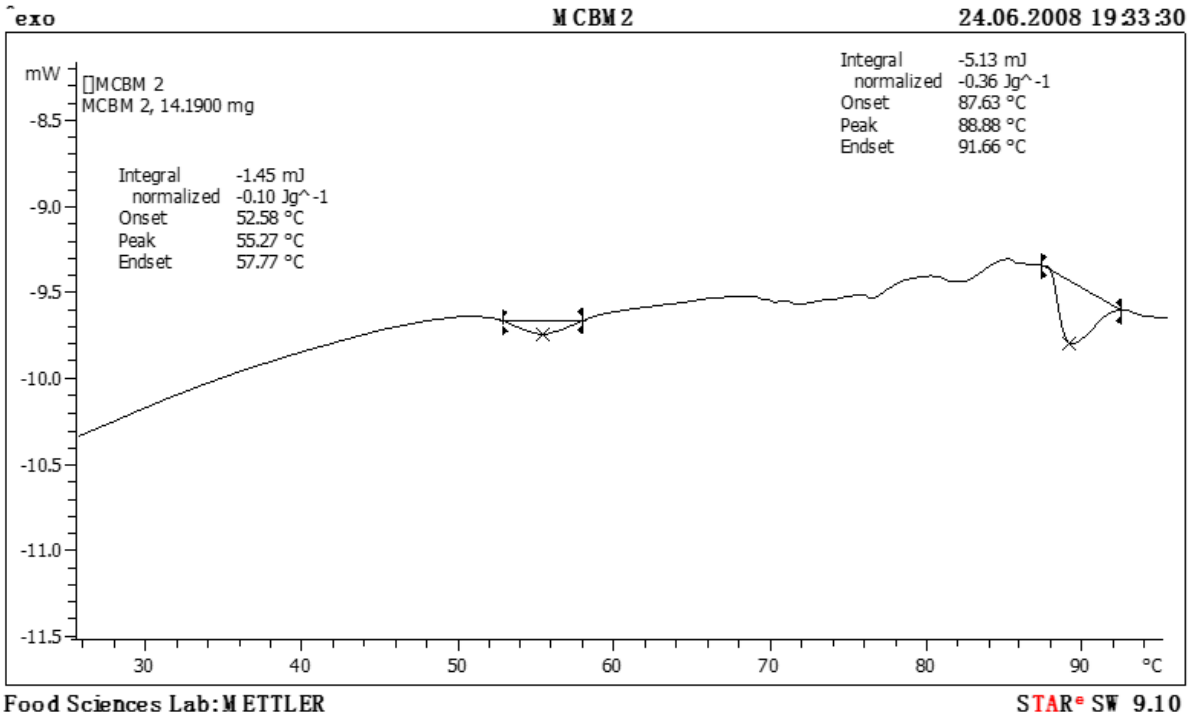

Figure 2: DSC of marinated chicken breast meat showing peak denaturation temperature at 55.27˚C.

	Tp1 (°C)	Tp2 (°C)	Tp3 (°C)
1. Fresh chicken breast meat	58.08	77.77	--
2. WSP(water soluble proteins)	27	52.47	75.84
3. SSP (salt soluble proteins)	59.57	--	--
4. Collagen	--	--	--
5. Marinated chicken breast meat	55.68	--	--

Table 3: Peak denaturation temperatures for chicken breast meat and its constituent proteins.

protein [4]. This could be because the extent of soluble myosin was lower in PSE compared to that in the normal muscle. From the results of SDS-PAGE, they confirmed that the protein denaturation was more extensive in PSE meat than in dark meat. Another study on the pork samples, showed that the pre-rigor conditions in PSE muscle caused precipitation of the sarcoplasmic proteins which are most sensitive to pH and temperature conditions after post mortem and led to the hypothesis that the early conditions (pH and temperature) after slaughter play an important role in determining the color of the meat [14]. They further confirmed that the pork color was associated with the precipitation of sarcoplasmic proteins on the myofibrillar proteins [14].

The results of denaturation study of chicken breast meat and its constituent proteins are given in Table 3 and Figure 2. The DSC curves showed that the fresh chicken breast meat had two peak transitions, one at 58°C and the other at 77°C. However, three peak transitions were obtained for sarcoplasmic protein fractions, at 27°C, 52°C and 75°C. For myofibrillar proteins, there was a single large peak obtained at around 59°C. The stromal proteins failed to show a peak transition in the DSC curve obtained. The thermal denaturation study for marinated chicken breast meat (stored for 24 h) showed a peak transition at 55°C.

A study was done using DSC to obtain the apparent specific heat of chicken breast meat and its constituent proteins [19]. These researchers found that chicken breast meat yielded three endothermic transitions, with peak transition temperatures of 53, 70 and 79°C. These endothermic transitions in chicken breast patties corresponded to the denaturation of myofibrillar (53°C) and sarcoplasmic (70 and 79°C) proteins.

Another researcher [13] stated that most of the sarcoplasmic proteins aggregate between 40 and 60°C, but for some of them the coagulation can extend up to 90°C. For myofibrillar proteins in solution, unfolding starts at 30-32°C, followed by protein-protein association at 36-40°C and subsequent gelation at 45-50°C. At temperatures between 53 and 63°C, the collagen denaturation occurs, followed by collagen fiber shrinkage.

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