# PROTEOME BASIS OF PALE, SOFT, AND EXUDATIVE (PSE) CONDITION IN BROILER MEAT FROM A COMMERCIAL PROCESSING PLANT

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**Abstract – Differences in meat quality attributes (pH, color, cooking loss, and tenderness) and whole muscle proteome of normal and Pale, Soft, and Exudative (PSE) broiler breast meat were evaluated. Normal chicken breast meat had higher pH and a* values (p<0.05), and lower *L** and *b** values, when compared to PSE breast meat (p<0.05). In addition, normal chicken breast meat had lower shear force values than PSE meat (p<0.05). The whole muscle proteomes of normal and PSE chicken breast meat were characterized using two-dimensional electrophoresis and mass spectrometry. Proteome analysis revealed that there were five differentially abundant proteins (p<0.05) between the normal and PSE chicken breast meat samples. Phosphoglycerate mutase 1 and glycogen phosphorylase, are sarcoplasmic proteins that were overabundant (p<0.05) in normal breast meat, whereas beta-enolase and fructose-bisphosphate aldolase C were over abundant (p<0.05) in PSE breast meat. In addition, the myosin heavy chain is the only myofibrillar protein that was overabundant (p<0.05) in PSE breast meat when compared to normal breast meat. Thus, results indicated that differences in meat quality attributes between normal and PSE breast meat could be related to overabundance of beta-enolase, fructose-bisphosphate aldolase C and myosin heavy chain proteins in PSE meat.**

**Key Words– Chicken breast meat, Color, Proteomics**

## I. INTRODUCTION

Pale, Soft and Exudative (PSE) meat is a significant problem in the poultry industry ranging from incidences between 5 and 30% for broilers at different times during the year. This quality defect costs the United States poultry industry more than $200 million in lost revenue per year (Barbut, 1996, Barbut, 1997, McCurdy *et al*., 1996, Owens *et al*., 2000, Woelfel and Sams, 2001, Woelfel *et al.,* 2002, Clements, 2010). PSE is a quality defect in broiler breast meat that originates during rigor mortis when carcasses experience acidic conditions within their muscles at high temperature (Solomon *et al*., 1998). The combination of a pH of approximately 5.8 and a temperature greater than 35°C prior to the onset of rigor mortis can lead to the production of PSE poultry meat (Takahashi *et al*., 2008). PSE meat cannot be used in further-processed products because the proteins (myosin and actin) responsible for the texture of deli meat and frankfurter type products are denatured (non-functional) resulting in product failures such as cracking in deli meats and fatting out in frankfurter type products (Marriott and Schilling, 2003). Myofibrillar and sarcoplasmic proteomes in PSE broiler meat have not been characterized. In the present study, modern-day proteomic tools (two-dimensional gel electrophoresis and mass spectrometry) were used to characterize the proteomes of normal and PSE broiler breast meat. The specific objectives of the present study were: (1) to determine the differences in meat quality traits (pH, color, cooking loss and shear force) of normal and PSE broiler breast meat, and (2) to characterize the whole muscle proteomes in normal and PSE broiler breast meat.

## II. MATERIALS AND METHODS

*Chicken samples*

Normal and PSE chicken breast meat samples were subjectively collected based on visual color from a deboning line in a commercial processing plant on three separate occasions (n=3 replications). Normal (n=12 per replication) and PSE (n=12 per replication) chicken breast samples were analyzed for pH, color, cooking loss, and tenderness at three separate sampling times. Samples from the same normal and PSE breast fillets were frozen at -80°C and the whole muscle proteomes (n=3 per replication) were determined.

*pH measurement*

The pH of the normal (n=12 per replication) and PSE broiler breast meat (n=12 per replication) samples was determined using a pH meter (Model Accumet 61, Fisher Scientific, Hampton, NH, USA) with an attached meat penetrating probe (Penetration tip, Cole Palmer, Vernon Hills, IL, USA), by directly inserting into the breast muscle at three different locations.

*Instrumental color evaluation*

Instrumental color was measured using a Chroma meter (Model CR-400, Minolta Camera Co Ltd, Osaka, Japan) in terms of CIE $L^*$ (lightness), $a^*$ (redness), and $b^*$ (yellowness). PSE breast meat samples were characterized based on pH from 5.5-5.7 and CIE $L^*$ of approximately 60 and normal breast meat samples pH from 5.8-6.2 and CIE L* from 45-55.

*Cooking loss*

Normal (n=12 per replication) and PSE chicken (n=12 per replication) samples were weighed and baked in an oven (Model JBP25DOJ2WH, General Electric, Louisville, KY, USA) to a final internal temperature of 77°C. The internal temperature of the chicken samples was monitored using thermocouples and a data logger (Model UWTR, Omega Engineering, Stamford, Conn., USA). Cooking loss was reported as a percentage and calculated as: (initial weight – final weight) / (initial weight) x 100 (Kin *et al*., 2009).

*Warner Bratzler Shear Force Determination*

Tenderness of Normal (n=12 per replication) and PSE (n=12 per replication) samples was assessed using an objective texture procedure as described in Meek *et al*., (2000). Normal and PSE chicken breast samples that were used for cooking loss determinations were cooled to room temperature and used for shear force determinations. Four to six adjacent 1 cm (width) x 1 cm (thickness) x 2 cm (length) strips were cut from the cooked breast, parallel to the direction of the muscle fibers. Samples were sheared perpendicular to the muscle fibers using a Warner-Bratzler shear attachment that was mounted to an Instron Universal Testing Center (Meek *et al*., 2000).

*Whole muscle proteome isolation and two dimensional gel electrophoresis*

The whole muscle proteome of Normal (n=3 per replication) and PSE (n=3 per replication) samples were isolated from chicken breast meat as per Lametsch *et al*., (2003) with slight modifications. Frozen meat samples were thawed overnight, and muscle tissue (2 g) was homogenized in 8 ml of rehydration buffer (7M Urea, 2M Thiourea, 3% CHAPS, 60 mM DTT, 0.3% carrier ampholyte, and a few grains of bromophenol blue) for 30 sec at low speed. The homogenate was incubated on ice for 30 min. The homogenate was centrifuged at 10,000 ×g for 30 min at 4°C. The supernatant was collected as the whole muscle proteins. Protein concentration was determined using the Bradford assay (Bio-Rad, Hercules, CA, USA). After protein quantification, the protein cleanup was conducted using Bio-Rad ReadyPrep™ 2-D Cleanup Kit (Bio-Rad, Hercules, CA, USA). Cleaned pellet (~325 µg) was suspended in 225 µl of rehydration buffer. Samples were then loaded onto 11cm immobilized pH gradient (IPG) strips with a pH range of 3-10 and subjected to passive rehydration for approx. 13 h. Isoelectric focusing (IEF) was conducted using a protean IEF cell system (Bio-Rad, Hercules, CA, USA). In the first dimension, the IEF system was adjusted to a linear increase voltage to a final total voltage of 25 kVh. After completion of IEF focusing, IPG strips were equilibrated twice for 15 min each in

Equilibration buffer 1 (6M Urea, 30% Glycerol, 2% SDS, 50mM Tris HCl, 2% DTT, few traces of bromophenol blue) and Equilibration buffer 2 (6M Urea, 30% Glycerol, 2%SDS, 50mM Tris HCl, 2.5% Iodoacetamide, few traces of bromophenol blue). In the final step, proteins were resolved in the second dimension on the basis of molecular weight on 12% SDS-PAGE gels using a Criterion gel electrophoresis system (Bio-Rad). After electrophoresis, gels were stained in Coomassie blue for 24 h and were further destained with deionized water.

*Protein gel image analysis and protein identification using Mass Spectrometry (MS)*
Visualization of the gel images was conducted using a Fotodyne imaging system and analyzed by PDQUEST software version 7.0 (Bio-Rad). Spots in normal and PSE meat gels were detected, matched, and normalized by expressing relative quantity of each spot (ppm) as the ratio of individual spot quantity to the total quantity of valid spots (Sayd *et al*., 2006). Spots were considered differentially abundant between the treatments when they exhibited 1.5-fold or more intensity difference associated with 5% statistical significance ($p<0.05$) in the Student's t-test. Differentially abundant spots were subjected to in-gel tryptic digestion, and the peptide extracts were analyzed by tandem mass spectrometry (4800 MALDI TOF-TOF Proteomics Analyzer, Applied Biosystems, Foster City, CA, USA). Tandem mass spectra were analyzed using Protein Pilot 2.0 (Applied Biosystems) in the National Center for Biotechnology Information (NCBI) database to identify the proteins.

*Statistical Analysis*
A randomized complete block design (replications as blocks) with three replications were utilized to test the treatment (Normal vs. PSE) effects ($p<0.05$) on pH, color, cooking loss, and shear force. When differences occurred ($p<0.05$) among treatments, the Fisher's Protected Least Significant Difference (LSD) test was used to separate treatment means.

## III. RESULTS AND DISCUSSION

For normal and PSE chicken breast meat samples, differences were observed in pH, color ($L^*$, $a^*$ and $b^*$), and shear force values ($p<0.05$). The pH of normal breast was higher ($p<0.05$) than that of PSE breast meat. PSE breast meat was lighter and more yellow than normal breast meat, whereas $a^*$ values were lower ($p<0.05$) in PSE meat than normal breast meat. In addition, PSE meat had greater shear force values than normal breast meat. However, no difference ($p>0.05$) was observed in cooking loss between the normal and PSE chicken breast samples (Table 1). For the whole muscle proteome, 5 protein spots demonstrated differential abundance ($p<0.05$) between the normal and PSE breast meat samples. The sarcoplasmic proteins, phosphoglycerate mutase 1 and glycogen phosphorylase were overabundant ($p<0.05$) in normal breast meat, whereas beta-enolase and fructose-bisphosphate aldolase C were overabundant ($p<0.05$) in PSE breast meat. In addition, myosin heavy chain was the only myofibrillar protein that was overabundant ($p<0.05$) in PSE breast meat.

Table 1: The pH, color (L*, a* and b*), shear force and cooking loss of Normal and PSE chicken breast samples (n= 12 per replication)

| Treatments | pH | CIE $L^*$ | CIE $a^*$ | CIE $b^*$ | Shear force (N) | Cooking loss (%) |
|---|---|---|---|---|---|---|
| Normal | $5.85^a$ | $56.00^a$ | $1.00^a$ | $5.71^a$ | $20.36^a$ | $21.84^a$ |
| PSE | $5.60^b$ | $61.45^b$ | $0.62^b$ | $7.17^b$ | $22.73^b$ | $20.04^a$ |

[a-b] Means with the same letter within a column are not significantly different ($p < 0.05$).

## IV. CONCLUSIONS

Differences in meat quality attributes were observed between normal and PSE chicken breast meat. The proteomic approach used in the present study indicated overabundance of sarcoplasmic and myofibrillar proteins in PSE meat, which in future research may help explain the mechanisms for the quality differences between PSE and normal chicken breast meat.

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