Effects of Pale, Normal, and Dark Chicken Breast Meat on Microstructure, Extractable Proteins, and Cooking of Marinated Fillets

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ABSTRACT The effects of chicken breast meat lightness value (L*) on microstructure, protein extraction, and marinating and tumbling was investigated. Pale soft, and exudative (PSE) meat (L* = 57.7, pH 5.72) showed significantly lower salt soluble protein extraction with less heavy myosin chains compared with dark, firm, and dry (DFD) meat (L* = 44.8, pH 6.27). The PSE meat showed larger intercellular spaces among muscle fibers and bundles compared with normal and DFD meat. Marinated and tumbled PSE breast fillets had higher unbound brine compared with the other meats. Further cooking resulted in lower yield and higher shear force values for the PSE meat compared with normal and DFD fillets.

(*Key words*: chicken meat; pale, soft, exudative; microstructure; poultry; protein)

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INTRODUCTION

Meat quality can be defined in various ways depending on the segment of the industry. For the grower, a high percentage of grade A is desired; however, low microbial count will be considered high quality by the fast food segment. For the meat processor, attributes such as water- and fat-holding capacity are of utmost importance.

Pale, soft, and exudative (PSE) meat is characterized by low moisture retention, soft texture, and light appearance and affects meat from different species. The pork industry has worked extensively over the last 3 decades on the problem and has developed several approaches to reduce the problem (e.g., stress reduction, genetic selection). Numerous studies on the effects of susceptible breeds, preslaughter stressors, and slaughtering methods have been published in the pork meat area (Bendall and Swatland, 1988; Backstrom and Kauffman, 1995). One of the most important items has been the finding of a genetic marker, currently used to remove susceptible pigs from herds. To date, poultry geneticists have not identified such a marker, and the industry has to rely on more conventional methods such as stress reduction. It should be mentioned that the pork industry has been working on the PSE issue for the past 3 decades, whereas the poultry industry has started focusing on the problem only over the past decade.

Marination is a popular technique used to tenderize and improve the flavor and succulence of meat (Lemos et al., 1999). Additional tumbling (during or after marinating) can have an important role in obtaining good distribution of brine, enhancing brine absorption, and facilitating protein extraction (Barbut, 2002). There are many reports on the effects of tumbling, but the results are sometimes contradictory. According to Froning and Sackett (1985), the presence of salt during tumbling significantly reduces expressible moisture and cooking losses but does not affect shear values of turkey rolls. Overall, the presence of salt and polyphosphates during tumbling improved the sensory properties. Maki and Froning (1987) reported that tumbling with a salt solution significantly reduced cooking loss and shear values as well as increased proteins in the exudates but did not significantly affect expressible moisture of turkey breast muscles. However, tumbling did not have any significant effect on sensory properties.

The effects of marinating and tumbling on PSE; normal; and dark, firm, and dry (DFD) poultry meats have been reported by several authors. Qiao et al. (2002), vacuum-tumbled (–25 kPa, 25 rpm, 20 min) broiler breast meat in brine [5% salt, 2.5% sodium tripolyphosphate (STPP); target 20% addition] and reported significant differences in marinade absorption, cook yield, and shear value among the PSE, normal, and DFD fillets. Allen et al. (1998) vacuum-tumbled (–80 kPa, 20 min) broiler breast fillets and reported that dark fillets had significantly higher marinade pick-up, a higher proportion of bound moisture, and lower drip and cooking losses than light fillets. However, they reported no significant differences in shear

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Abbreviation Key: DFD = dry, firm, dark; L* = lightness; PSE = pale, soft, exudative; STPP = sodium tripolyphosphate.

values between the color groups. Fletcher (1995) found no significant differences in shear values among broiler breast muscles of various lightness, redness, and pH values. Woelfel and Sams (2001) reported no significant difference in marinade uptake and drip loss between pale and normal vacuum-tumbled (–68 kPa, 30 min) chicken fillets that were marinated in brines adjusted to pH 9 and 11. However, they reported higher cook loss for the pale meat at pH 9 but not at pH 11. The results of marinating and tumbling chicken breast meat fillets are often variable. Thus, objectives of this study were to further investigate the relationships among microstructure, protein extraction, water retention, and the effect of marinating and tumbling on PSE, normal, and DFD chicken meat.

MATERIALS AND METHODS

Sample Collection

Boneless, skinless chicken breast meat (obtained from broilers 40 ± 1 d old, average BW 2.2 ± 0.1 kg) was collected from the deboning line of a large local commercial processing plant. Meat was collected within 9 ± 2 h postmortem on 6 different occasions. Samples were put on ice and shipped to the university laboratory. Drip loss during transportation (2 to 3 h in plastic bags) was calculated as weight of moisture loss divided by weight of the original meat.

Color and pH Measurements

The lightness value (L*) of the raw fillets was determined with a color meter.[2] The pH of individual fillets was measured at 14 to 16 h postmortem by inserting the probe of a hand-held pH/mv/temperature meter[3] into the middle of the pectoralis muscle.

Light Microscopy

Samples (about 2.0 × 2.0 × 0.5 mm) were cut from the centers of raw muscle samples classified as PSE (L* > 53, pH < 5.7), DFD (L* < 46, pH > 6.1), and normal (46 < L* < 53, 5.7 < pH < 6.1). Values are based on data from Barbut (1997) and Woelfel et al. (1998), indicating that meats in these ranges represent the 3 categories. Samples were placed in 10% formalin for 10 h to prefix the structure and then were dehydrated with a series of increasing alcohol solutions followed by xylene; an automated system was used.[4] Samples were then embedded in paraffin, cut into 4 to 6 μm thick sections, allowed to float on water, and transferred onto a glass slide. Slides were dried, stained with hematoxylin-eosin, and observed using a light microscope[5] at 100× magnification. Pictures were captured by a computerized image analysis system.[6]

Protein Extraction and Protein Content Determination

Proteins were extracted on 6 different occasions from chopped chicken breast meat (60 s in a food processor) classified as PSE, DFD, or normal. Ten grams of each sample was mixed with 20 mL of 5% NaCl solution, incubated (4°C) for 1 h, and centrifuged at 30,000 × g (14,000 rpm) for 20 min (Gordon and Barbut, 1992). The Bio-Rad protein assay (Bradford, 1976) was used to determine protein concentration in the salt soluble protein extracts.

SDS-PAGE

Polyacrylamide gel electrophoresis[7] on a 20% gradient gel was performed after boiling (5 min) 30 μL of the protein extractions with 160 μL of SDS buffer. The molecular weights of the protein bands were determined from a regression plot of the relative mobilities (Rt) of a protein standard kit[8] (α-lactalbumin, 14.4 kDa; soybean trypsin inhibitor, 20.1 kDa; carbonic anhydrase, 30 kDa; ovalbumin, 43 kDa; bovine serum albumin, 67 kDa; and phosphorylase b, 94 kDa) against their $\log_{10}$ molecular weights.

Marinating and Tumbling

Five samples from each color group were used in each of the 6 trials. Samples were trimmed of all visible connective tissue and fat, weighed, and put into individual plastic bags[9] with 27.3% cold brine (93.24% water, 4.66% salt, and 2.10% sodium tripolyphosphate). Air was blown into the bags prior to sealing. The bags were tumbled[10] under vacuum (–50 kPa, 5 rpm, 30 min, 4°C). The meat was left to rest overnight, and an additional 30 min of tumbling was carried out the next morning.

Calculation of Unbound Brine and Brine Uptake

After being tumbled, the samples were removed from the bags and drained for 2 min. Unbound brine was calculated as weight of liquid released from tumbled fillets × 100/weight before marinating. Percentage of brine uptake was calculated as (weight after tumbling and holding – weight before tumbling) × 100/weight before tumbling.

Cooking and Cook Loss

Marinated meat was vacuum-packed[11] under –950 mbar and cooked in an 80°C water bath[12] until internal

[2]Model CR200B, Minolta Corp., Osaka, Japan.
[3]Model IQ150, IQ Scientific Instruments, San Diego, CA.
[4]Model 5, Tissue-Tek VIP, Sakura Finetechnical Co., Tokyo.
[5]System BX60, Olympus Optical Ltd., Tokyo.
[6]Image-Pro Plus, Version 4.5-1.29, Media Cybernetics Inc., Silver Spring, MD.
[7]Phast System, Pharmacia Biotech, Montreal, QC, Canada.
[8]Molecular Weight Standard, Pharmacia Biotech, Montreal, QC, Canada.
[9]Ziploc Freezer Bag, S.C. Johnson and Son Ltd., Brantford, ON, Canada.
[10]Model 46 115-1-60-3A, Lyco, Columbus, WI.
[11]Model A300/16, Multi-Vac, Knud Simonsen Ltd., Toronto, ON, Canada.
[12]Model W26, Haake, Dieselstr, Germany.

temperature reached 74°C. A thermocouple unit[13] was used to monitor the internal temperature. The bags were removed from the water bath and then put under tap water for 20 min, and the released liquid was poured off and weighed. Cooking loss was calculated as released liquid × 100/weight before cooking.

Shear Test

The cooked samples were kept at 4°C overnight. Shear test was performed on the following day using a texture analyzer.[14] Five to seven strips (1 cm wide, 1 cm thick, and 5 cm long) were obtained from each sample by cutting with a special knife (i.e., along the muscle fibers). A rectangular blade with an inverted V cut (60°, 30 mm high, 2 mm thick blade) at the bottom edge was used (i.e., the Warner Bratzler shear test blade). The blade descended at 5 mm/s and sheared the sample that was positioned on a slotted platform with muscle fibers positioned perpendicular to the blade. As the blade descended through the slot, the samples were sheared, and the maximum force and distance to shear were recorded (Lyon and Lyon, 1996).

Statistical Analyses

The experiment was a complete randomized block design with 6 independent replicates. Data were analyzed by ANOVA[15] with $P < 0.05$ considered as significant. Meat color groups were tested as main effects. Means were separated using the least significant difference test.

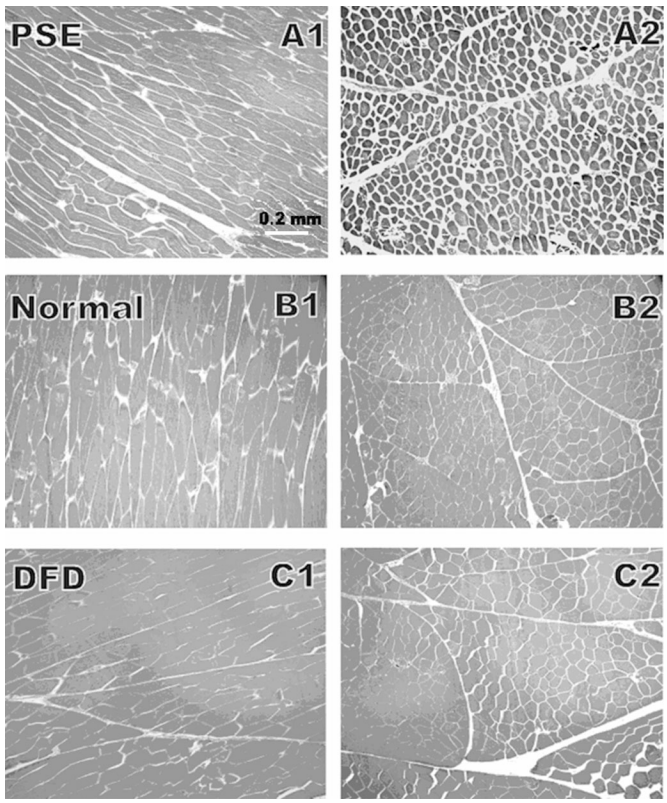

FIGURE 1. Light micrographs of longitudinal and transversal sections of skeletal muscle of chicken breast meat with different color. A1 = pale, soft, exudative (PSE) longitudinal; A2 = PSE transversal, B1 = normal longitudinal; B2 = normal transversal; C1 = dry, firm, dark (DFD) longitudinal, C2 = DFD transversal.

RESULTS AND DISCUSSION

Color and Microstructure

The average lightness (L*) and pH values of the raw broiler breast meat samples were significantly different among the PSE, normal, and DFD groups (Table 1). These results are consistent with previous data (Barbut, 1997; Woelfel et al., 1998; Fletcher et al., 2000) showing that lighter chicken breast meat has lower pH and water-holding capacity values compared with normal and DFD meats.

The microstructure images (Figure 1) revealed morphological differences among the PSE, normal, and DFD meats. The normal meat appeared to have a fairly loose microstructure with no abnormalities. The muscle fibers in the DFD group were arranged in a much denser and more compact manner. There are much smaller open spaces among muscle fibers compared with the PSE muscle (shown in the transversal and longitudinal sections). The normal and DFD groups had muscle fibers that almost entirely filled the endomysial network and muscle bundles that filled the perimysial network. However, more intercellular open spaces could be observed in the PSE group. Sijacki et al. (1991) compared the microstructure of PSE and normal pig muscles and found that PSE muscle samples, whether early or late PSE, had large intracellular gaps, some structural irregularities, and the presence of giant fibers. The chicken PSE muscles (Figure 1, A1 and A2) also had gaps of variable widths between fiber bundles but no structural irregularities. Offer and Cousins (1992) observed a gap formation between fiber bundles and the perimysial network in some muscles. Consequently, they concluded that the fluid expelled due to myofibrillar shrinkage accumulated in the extracellular spaces. Offer (1984) suggested that the fluid collected in these wide channels might be the source of drip loss, which could explain the significantly higher drip loss from the PSE meats than from normal and DFD chicken meats (Table 1).

Extractable Protein

Extraction of salt soluble proteins from PSE meat yielded 76.99 mg/mL for PSE meat, 100.62 mg/mL for normal meat, and 108.49 mg/mL for DFD meat; the amount for PSE was lower ($P < 0.05$) than for DFD and normal. Protein concentration (or protein solubility) can

[13]Model 52 K/J, Fluke Mfg. Co., Everett, WA.
[14]Model TA.XT2, Stable MicroSystems, Texture Technologies Corp., New York.
[15]SAS User's Guide, GLM Procedure, Version 8.02, SAS Institute Inc., Cary, NC.

TABLE 1. The effects of marinating and tumbling on PSE,[1] normal and DFD chicken breast meat

	Meat color group		
Measurement	PSE	Normal	DFD
L*	57.70[a]	49.71[b]	44.88[c]
pH	5.72[a]	6.02[b]	6.27[c]
Drip loss (%)	1.65[a]	1.00[b]	0.69[b]
Unbound brine (%)	1.58[a]	1.16[b]	0.90[b]
Brine uptake (%)	12.60[a]	13.56[ab]	14.96[b]
Cooking loss (%)	14.59[a]	11.25[b]	11.01[b]
Marinated yield (g/100 g of fresh meat)	112.60[a]	113.55[ab]	114.97[b]
Final yield (g/100 g of fresh meat)	96.23[a]	100.78[b]	102.40[b]
L* after cooking	80.75[a]	79.73[ab]	78.71[b]
Cooked shear force (N)	11.31[a]	9.65[b]	8.69[c]

[a–c]Means (n = 30) within a row with no common superscript differ significantly ($P < 0.05$).

[1]PSE = pale, soft, exudative; DFD = dry, firm, dark; L* = lightness.

be used as a measure of protein denaturation due to PSE (Van Laack et al., 2000). Joo et al. (1999) found a similar trend in extracted proteins from pork with 111 mg/mL for PSE, 169 mg/mL for normal, and 173 mg/mL for DFD meat. PSE pork muscles tend to exhibit lower protein extraction and solubility than normal meat. In turkey breast meat samples, Pietrzak et al. (1997) reported that less myosin could be solubilized from PSE versus normal myofibrils. Qiao et al. (2002) reported that light chicken breast meat has significantly lower total protein value than normal or dark meat (23.27 vs. 22.58%). The gel electrophoresis profiles (Figure 2) show bands in similar locations for the 3 meats, except that PSE meat has some missing bands in the high molecular weight protein area (i.e., see the 151 kDa region where the heavy myosin chain segments are missing from the 2 PSE lanes). In another study Rathgeber et al. (1999), used SDS-PAGE followed by Western blot to identify the 152 kDa band they obtained by extracting turkey breast meat proteins from rapid post mortem glycolysis and delayed chilling treatments. They used an anti-myosin heavy chain antibody (F 27) and showed that it was part of the myosin heavy chain. The absence of the 151-kDa band points out the partial protein denaturation in the PSE meat. This finding agrees with that of Pietrzak et al. (1997), who reported that less myosin could be solubilized from PSE versus normal turkey myofibrils. In their study, they also extracted the proteins from PSE and normal turkey breast muscles, ran SDS gel electrophoresis, and identified the bands by Western blotting. They suggested that the irreversible myosin insolubility, due to low pH and high temperature, is decisive in the development of PSE meat. In addition to poor myosin solubility, they observed that the phosphorylase enzyme becomes closely associated with myofibrils in PSE muscles; the latter was not evaluated in the present study.

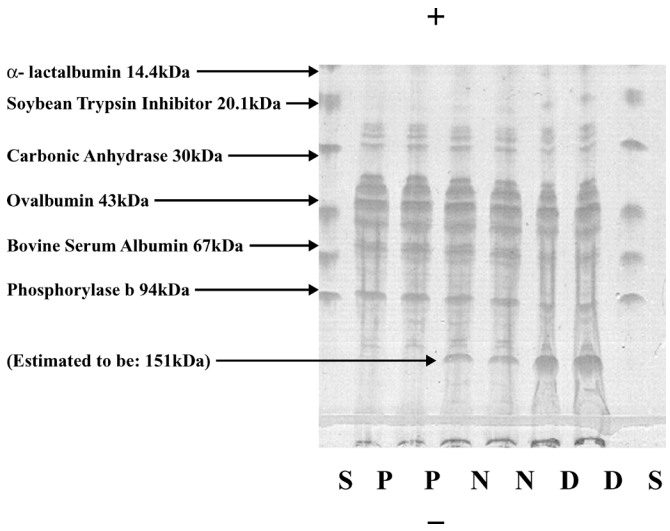

FIGURE 2. SDS-polyacrylamide gel of myofibril fractions subjected to salt (3.3% NaCl solution) extraction. Molecular weights for standards are identified on the left. S = standard; P = pale, soft, exudative meat; N = normal meat; D = dry, firm, dark meat.

Van Laack et al. (2000) reported high correlations between the amount of extracted sarcoplasmic proteins from broiler breast meat and L* (r = 0.71), moisture uptake (r = 0.66), and cooking yield (r = 0.66). However, correlations between total protein solubility and moisture uptake and cooking yield were not significant. Joo et al. (1999) investigated the relationship among sarcoplasmic and myofibrillar protein solubility to color and water-holding capacity in pork. They reported color to be highly correlated with precipitation of sarcoplasmic proteins (r = –0.84) and total protein solubility (r = –0.70). Drip loss was correlated with sarcoplasmic protein (r = 0.72) and total protein solubility (r = 0.64). Several other authors have also reported that pork myofibrillar and sarcoplasmic protein solubilities are highly correlated with water retention measurements, such as drip loss and moisture uptake (see review by Bendall and Swatland, 1988). These reports demonstrate that protein solubility affects some of the physical properties of the meat and can explain why the actual proteins extracted from PSE meat have poor functionality. However, Camou and Sebranek (1991) suggested that loss of functionality, due to PSE, is more than simply lower solubility and also involves some losses of molecular functionality. They indicated that

when protein concentration is adjusted, the average gel strength of proteins extracted from PSE pork is only 45% of that obtained from protein extracted from normal meat. The different state of the protein indicates that native proteins from PSE muscle are less functional even after solubilization. Similar information about poultry meat has not been found in the literature.

Marinating and Tumbling

The light raw PSE meat showed a significantly higher drip loss, or lower ability to hold its water, compared with normal and DFD meats (Table 1). Later, the cooking treatment decreased the color variation among the 3 groups. There was no significant difference between the cooked PSE and normal meat; PSE and DFD meats remained different. Fletcher et al. (2000) also showed that cooking reduced the degree of color variation among PSE, normal, and DFD meats (i.e., L* values of cooked normal and DFD meats were not significantly different, but PSE and DFD were). Qiao et al. (2002) indicated that cooking reduced L* value differences among PSE, normal, and DFD breast meat, but L* values were still significantly different among the 3 groups.

The poor ability of PSE meat to hold its water in the raw state (i.e., higher drip loss during transportation) also persisted during marinating, showing more unbound brine after tumbling (Table 1). Normal and DFD meat did not show a significant difference in percentage of unbound brine. Cooking loss was highest in PSE meat and lowest in DFD meat. The high cooking loss of PSE meat indicated poor water retention compared with DFD and normal meat, a characteristic that could be attributed to the lower pH and protein extractability of PSE poultry meat (Woelfel and Sams, 2001; Barbut 2002). The results demonstrate that marinating and tumbling (with salt and phosphate) did not remedy the consequences of using PSE meat (i.e., PSE still showed low marinated yield and higher cooking losses).

There was a significant difference in shear force values among the 3 meat groups with PSE showing the highest and DFD the lowest. This result was most probably due to the higher moisture loss from PSE before and after cooking and might also be associated with more protein toughening due to denaturation (Brewer et al., 1999). Overall, texture is very important for product acceptability because of consumer demand for tender chicken fillets. Brewer et al. (1999) evaluated the effects of phosphate/salt solution (99.45% water, 0.3% STPP, and 0.25% NaCl at 10% injection rate) on the quality characteristics of PSE, normal, and DFD pork. They reported that drip loss was higher in PSE and lower in normal and DFD muscles; cooked DFD muscles were juicier and more tender than normal or PSE muscles. Allen et al. (1998) evaluated the relationships among broiler breast meat color, quality, and shelf life. They reported that dark fillets had a significantly greater marinade (90% water, 3% STPP, and 7% NaCl) uptake and significantly lower drip and cooking losses. However, no differences were observed in shear value among the color groups. Qiao et al. (2002) investigated the effect of raw broiler breast meat color on marinated (92.5% water, 5% NaCl, and 2.5% phosphate) and cooked meat quality. They reported that darker meat absorbed significantly less brine (16.3%) than normal (17.1%) and lighter (17.6%) groups; light and normal meats were not significantly different. Cooked meat yield was significantly greater in the dark and normal groups fillets compared with PSE meat, whereas shear force was different among color groups. Woelfel and Sams (2001) compared the effect of marination (94% water, 3.5% NaCl, 2.5% STPP) on pale and normal broiler breast meat and found no significant difference between pale and normal fillets for marinade uptake; however, the pale fillets had significantly higher cooking loss compared with normal fillets. They suggested that the PSE condition could not be reversed by marination.

In summary, the microstructure of the DFD meat showed a denser and more compact muscle fiber arrangement, whereas PSE meat showed more intercellular open spaces and gaps of variable width between fiber bundles compared with DFD. The SDS gel electrophoresis confirmed that protein denaturation was more extensive in PSE meat than in DFD meat. Combined with water retention data, it was shown that drip loss and unbound brine in the raw state were higher with increasing protein denaturation. The marinating and cooking treatments also resulted in lower moisture retention in the PSE fillets, indicating lower protein functionality in the PSE meat.

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