

## Quality characteristics of raw and cooked spent hen *Pectoralis major* muscles during chilled storage: Effect of salt and phosphate

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**Abstract:** Quality characteristics of spent hen *Pectoralis major* muscles and effect of NaCl and sodium tripolyphosphate (STPP) on quality changes of raw and cooked spent hen muscles during chilled storage were investigated. Meat from spent hen had nutritional values the same as commercial broiler meat. Treated raw samples with 1.0% NaCl and 10% STPP showed lower drip loss during storage time ( $P<0.05$ ), and lower cooking loss ( $P<0.05$ ) at day 6 and day 9 of storage compared with untreated samples. However, weight loss of cooked-untreated samples exhibited lower ( $P<0.05$ ) than did in cooked-treated samples as storage time increased. NaCl and STPP had more effective to reduce TBARS values and extend shelf-life of cooked samples than did in raw samples during chilled storage. There were no significant differences on other chemical and physical properties of raw and cooked spent hens all storage time, demonstrating the ineffectiveness of NaCl and STPP.

**Keywords:** Quality characteristics, spent hens, storage quality, NaCl, sodium tripolyphosphate, chilled storage

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### Introduction

Excessive expansion of egg industry in Thailand resulted in abundant availability of spent hens. Unfortunately, the toughness prevents spent hens use in whole meat food and reduces the market value in many countries such as Korea, Taiwan, Japan and United state (Sams, 1990; Nowsad *et al.*, 2000a, b; Lee *et al.*, 2003; Li, 2006). However, meat from spent hen is a good protein source (Rhee *et al.*, 1999; Lee *et al.*, 2003), highly enriched with omega-3 fatty acids and lower in cholesterol content in particular breast muscle (Ajuyah *et al.*, 1992) which have been shown to have health promoting benefits. Therefore, additional usages need to be developed to increase the value of spent hen meat. However, there is little information on characteristics and quality of spent hen meat produced in Thailand either raw or cooked meat during chilled storage.

The combination of NaCl and STPP providing greater positive effects on quality of meat and poultry during processing and storage have been reported by many investigators (King *et al.*, 1986; Young and Lyon, 1986; Ahn and Maurer, 1989). Therefore, the objective of this study is to obtain the information on chemical and physical characteristics of spent hen meat and the effect of NaCl and STPP on quality of raw and cooked spent hen meat during chilled storage.

### Materials and Methods

#### Sample preparation and storage conditions

Thirty-seven spent hens aged 72 weeks of  $1.5 \pm 0.2$  kg live weights, obtained from Department of Animal Science, Faculty of Natural Resource, Prince of Songkla University were slaughtered by methods of Wattanachant *et al.* (2004). *Pectoralis major* muscles (breast muscles) were dissected from the carcasses and trimmed of obvious fat and connective tissue after chilling at 4°C for 24 h. Ten fillets were taken randomly and subjected to chemical and physical analysis. Other muscle samples were randomly divided into 4 groups (16 fillets per groups = 64 fillets) of raw-untreated, cooked-untreated, raw-treated and cooked-treated samples. Raw-treated samples were prepared by dipping each muscle for 15 seconds in solution containing 1% NaCl and 10 % STPP with muscle/solution ratio of 1:2 (wt/v) and drained for 15 min on aluminum tray. To prepare cooked-untreated samples, breast muscle were cooked with steam without pressure until an internal temperature reached at 80°C and then allowed to cool and gently blotted with a paper towel to remove surface moisture. The internal temperature was monitored by thermocouple inserted into the thickest part of the samples. To prepare cooked-treated samples, breast muscles were dipped into the same solution before cooked with steam using the same method as described

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previously.

All samples of each group were weighed, placed individually on the supporting mesh and put in the labelled polyethylene plastic bags before sealing. Samples were kept in chilled room at 4°C for 9 days. At 0, 3, 6, and 9 days of storage, four packs of each group were randomized for analysis.

### Chemical properties determinations

#### *Proximate analysis and pH of muscle*

Moisture was determined by oven method; protein was determined by Kjeldahl method; fat was analyzed by the Soxhlet apparatus method; and ash was determined with a furnace 600°C (AOAC, 1999). Raw spent hens were subjected to analyses the pH of muscle as described by Wattanachant (2004).

#### *Fatty acid determination*

The fatty acid composition of the muscle samples was determined after extraction the fat by the method of Bligh and Dryer (1959). The fatty acids present in the extracted lipids were transformed into their methyl esters (Metcalfe *et al.*, 1961). Fatty acid methyl esters were determined by gas chromatography (AOCS, 1991).

#### *Determination of protein composition*

The protein components in muscle samples were fractionated into myofibrillar, sarcoplasmic, stroma protein and alkali-soluble protein according to the method of Hashimoto *et al.* (1979). The nitrogen content of all protein and protein fractions was determined by the Kjeldahl method (AOAC, 1999).

#### *Determination of total and soluble collagen content*

Total collagen content was determined after acid hydrolysis according to the method of Palka and Daun (1999). The hydroxyproline content in the hydrolysate was determined by the procedure of Bergman and Loxley (1963) and converted to collagen content using the factor 7.25. The collagen content was expressed as mg of collagen per g of muscle.

Soluble collagen of samples was determined according to the method of Liu *et al.* (1996). The amount of heat soluble collagen was expressed as a percentage of total collagen (collagen content in sediment plus that in the supernatant).

#### *Determination of myoglobin (Mb) content and metmyoglobin (MetMb) content*

The Mb content was determined by using method of Geileskey *et al.* (1998). The absorbance was read

at 525 nm using a spectrophotometer (Model V-530, Jasco). Mb content was calculated from the millimolar extinction coefficient of 0.132 (Eder, 1996) and a molecular weight of 16,110 (Gomez-Basauri and Regenstein, 1992). The Mb content was expressed as mg per g sample.

The analysis of MetMb was performed as described by Lee *et al.* (1998). The sample solution was prepared in the same manner as that for Mb determination. The supernatant was subjected to absorbance measurement at 700, 572, and 525 nm. The percentage of MetMb was calculated using the following equation (Krzywicki, 1982):

$$\% \text{ MetMb} = \{1.395 - [(A_{572} - A_{700}) / (A_{525} - A_{700})]\} \times 100$$

#### *Determination of TCA-soluble peptides*

The extent of proteolysis was monitored by the method of Morrissey *et al.* (1993). TCA-soluble peptides in the supernatant were measured according to the method of Lowry *et al.* (1951) and expressed as micromole of tyrosine per g muscle.

#### *Determination of TBARS*

Lipid oxidation of samples was quantified using thiobarbituric acid reactive substances (TBARS) determined spectrophotometrically by method of Buege and Aust (1978). The absorbance of the supernatant was measured at 532 nm using a spectrophotometer (Model V-530, Jasco). TBARS value was calculated from the standard curve of malondialdehyde (MDA) and expressed as micromole MDA per g sample.

#### *Determination of cholesterol content*

Cholesterol determination was carried out based on AOAC 994.10 (AOAC, 2005).

### Physical properties determinations

#### *Determination of drip loss and weight loss*

Drip loss of raw and weight loss of cooked samples were determined by calculating the weight loss during chilled as a percentage of the initial weight following the method of Woelfel *et al.* (2002).

#### *Determination of cooking loss*

Cooking loss of raw samples was determined according to the method of Wattanachant *et al.* (2004).

#### *Shear force analysis*

Samples were cut (1.0x3.0x0.5 cm<sup>3</sup>) paralleled with the muscle fiber at the middle portion of the fillets for shear analysis using a texture analyzer (TA-XT2i, Texture Expert Version 1.17; Stable Micro

System, Godalming Surrey, UK) equipped with a Warner-Bratzler shear apparatus (Wattanachant *et al.*, 2004).

#### Determination of muscle color

The color of each muscle samples was determined using a Hunterlab colorimeter and reported as the Complete International Commission on Illumination (CIE) system color profile of Lightness ( $L^*$ ), redness ( $a^*$ ), and yellowness ( $b^*$ ).

#### Determination of thermal transition

Samples were ground and determined for thermal transition according to the method of Wattanachant *et al.* (2005a). The heating rate of the DSC scans was 5°C/min over a range of 25 to 120°C. The onset temperature ( $T_0$ ), temperature of peak transition ( $T_p$ ), and enthalpy of transition ( $\Delta H$ ) were determined from typical thermograms.

#### Microbiological determination

Samples were analyzed for total aerobic mesophilic and psychrophilic microorganisms using aerobic plate count (APC) of colony forming units (CFU) and reported as log CFU per g sample following the method of Avens *et al.* (2002).

#### Statistical analysis

All data were subjected to analysis of variance (ANOVA) and differences between means were evaluated by Duncan's Multiple Range Test (Stell and Torrie, 1980) using analysis software computer.

### Results and Discussion

#### Proximate composition, chemical and physical properties of raw spent hen *Pectoralis major* muscles

Proximate composition of raw spent hen *Pectoralis major* muscles are shown in Table 1. Moisture, protein, and ash contents were in the range of chicken *Pectoralis* muscle as reported previously by many investigators (Xiong *et al.*, 1993; Smith *et al.*, 1993; Van Heerden *et al.*, 2002; Al-Najdawi and Abdullah, 2002; Qiao *et al.*, 2002b; Wattanachant *et al.*, 2004). The fat content was higher than those reported in broiler at 0.68 - 2.78% (Aberni and Bergoglio, 2001; Qiao *et al.*, 2002b; Wattanachant *et al.*, 2004), probably due to difference in breeds and the old age of spent hens used in this study. This finding seemed reasonable because it is generally

recognized that fat content increase with age (Ngoka *et al.*, 1982; Mountney and Parkhurst, 1995). The results performed that spent hen *Pectoralis major* muscle had similar nutritional value to commercial broiler meat except higher fat content.

The low content of cholesterol from current result (Table 1) was consistent with earlier observations in breast muscle of spent hen at 34.29 mg/100 g by Al-Najdawi and Abdullah (2002), 43 mg/100 g by Jantawat and Dawson (1980), and between 50.07-62.64 mg/100 g by Ajuyah *et al.* (1992) which were lower than early report in breast muscle of broiler at 81 mg/100 g by Ang and Hamm (1982). This might confirm health promoting benefits from spent hen meat.

Protein compositions from raw spent hen *Pectoralis major* muscles are shown in Table 1. The spent hen muscle had lower composition of sarcoplasmic protein but higher myofibrillar and stroma protein as compared with broiler muscle reported previously by Wattanachant *et al.* (2004) who found that broiler breast muscle comprised of 43.45% myofibrillar and 48.83% sarcoplasmic protein, respectively. This was probably due to the older age of spent hen. Higher fraction of stroma protein in spent hen meat at  $8.01 \pm 0.84$  % compared with broiler between 1.50 - 1.81% (Lan *et al.*, 1995; Wattanachant *et al.*, 2004) might result in low functional properties of spent hen meat.

Myoglobin content of raw spent hen *Pectoralis major* muscle (Table 1) was observed higher than broiler *Pectoralis* as reported previously between 0.1-0.17 mg/g muscle (Nishida and Nishida, 1985; Boulianne and King, 1995). It was probably due to the higher age of spent hen and difference in breed and feed of animals (Miller, 1994) caused difference in Mb content of muscle. However, it was not shown any differences in color value  $L^*$ ,  $a^*$ , and  $b^*$  of spent hen *Pectoralis major* muscle (Table 1) compared to those of broiler *Pectoralis* muscle, 52.51, -1.18, and 6.96, respectively (Wattanachant *et al.*, 2004).

The collagen content of spent hen *Pectoralis major* muscle in this study (Table 1) was observed higher than broiler *Pectoralis* as reported previously between 1.27-3.86 mg/g (Ruantrakool and Chen, 1986; Smith *et al.*, 1993; Wattanachant *et al.*, 2004) and at 5.09 mg/g for Thai indigenous chicken muscle aged 16 weeks by Wattanachant *et al.*, (2004). Differences in collagen content could be attributed to differences in the age of birds at the time of slaughter (Dawson *et al.*, 1991). Trindade *et al.* (2004) stated that high contents of collagen in any meat can negatively influence its technological and nutritional characteristics, since collagen is a protein

**Table 1.** Chemical composition, pH, C.I.E. values, and shear force value of raw spent hen *Pectoralis major* muscles

Quality characteristics	
Moisture (%)	74.16 ± 0.21
Protein (%)	22.34 ± 0.25
Fat (%)	3.11 ± 0.59
Ash (%)	1.19 ± 0.07
Cholesterol content (mg/100g)	34.6 ± 0.01
Protein composition	
- Myofibrillar protein (%)	55.53 ± 0.33
- Sarcoplasmic protein (%)	34.11 ± 0.50
- Stroma protein (%)	8.01 ± 0.84
- Alkali soluble (%)	2.35 ± 0.03
Myoglobin content (mg/ g sample)	3.47 ± 0.06
Total Collagen (mg/g muscle)	6.47 ± 0.20
Soluble collagen (% of total collagen)	18.76 ± 0.81
Fibre diameter (µm)	32.78 ± 2.80
Sarcomere length (µm)	1.66 ± 0.21
pH	5.78 ± 0.11
L*	52.26 ± 1.29
a*	-2.86 ± 0.46
b*	7.19 ± 2.60
Shear force value (kg)	2.60 ± 0.81

Data are presented as mean ± standard deviation.  
 N=3 for chemical properties, n=8 for physical properties.

with inferior functionality and low nutrition value because of its poor balance of amino acids. Moreover, it has also been shown that the solubility of collagen decreases with increased collagen cross-linking and cross-linking increases as the animal ages (Pearson and Young, 1989; Foegeding and Lanier, 1996). From the result, therefore, spent hen had more highly cross-linked collagen as indicated by the much lower soluble collagen content, compared to that at 31.38 % of total collagen content for younger broiler (aged 38 days) of Wattanachant *et al.* (2004). The high content of stroma protein and highly cross-linked collagen might result in low functional properties of spent hen meat.

The fibre diameters of raw spent hen *Pectoralis major* muscles (Table 1) was higher with those found in spent hen breast muscle at 18.74 µm by Chuaynukool (2007). The average diameter of chicken white fibres has been variously reported as 38 to 46 µm (Smith and Fletcher, 1988), 32.6 µm (Smith *et al.*, 1993) and 26.6 µm (Wattanachant *et al.*, 2004) for broiler and 28.9 µm for Thai indigenous chicken muscle aged 16 weeks by Wattanachant *et al.*, (2004). These differences in muscle fibre diameter may have been due to the differences in age, rate of rigor onset, and degree of sarcomere shortening (Smith and Fletcher, 1988). As shown in Table 1, the sarcomere length of raw spent hen *Pectoralis major* muscles was in consistent with previous report of Chuaynukool (2007) at 1.62 µm. The sarcomere lengths of chicken white muscles have been reported at 1.64 µm for broiler and at 1.61 µm for Thai indigenous chicken muscle by Wattanachant *et al.* (2004).

The ultimate pH of raw spent hen *Pectoralis major* muscle (Table 1) was in the range of an average ultimate pH of poultry breast muscle, 5.76-6.10 (Kahn and Nakamura, 1970; Rasmussen and Mast, 1989; Xiong *et al.*, 1993; Qiao *et al.*, 2002a; and Wattanachant *et al.*, 2004). As shown in Table 1, shear force value obtained from spent hen muscle (2.6 Kg) showed double higher than that of broiler *Pectoralis* (1.20 Kg) in previous report of Wattanachant *et al.*

**Table 2.** Fatty acid composition (% of total fatty acids) of raw spent hen *Pectoralis major* muscles

Fatty acid	% of total fatty acids
Lauric acid (C12:0)	0.60 ± 0.00
Myristic acid (C14:0)	0.90 ± 0.10
Palmitic acid (C16:0)	24.60 ± 0.20
Palmitoleic acid (C16:1)	1.40 ± 0.01
Stearic acid (C18:0)	8.00 ± 0.10
Oleic acid (C18:1)	31.40 ± 0.20
Linoleic acid (C18:2)	17.50 ± 0.10
Linolenic acid (C18:3)	0.40 ± 0.01
Eicosanoic acid (C20:0)	0.10 ± 0.01
Eicosenoic acid (C20:1)	0.20 ± 0.10
<sup>1</sup> SFA	34.27 ± 0.41
<sup>2</sup> MUFA	50.95 ± 0.31
<sup>3</sup> PUFA	17.90 ± 0.11

Data are presented as mean ± standard deviation from duplicate determinations.  
<sup>1</sup>SFA = Total saturated fatty acids (C12:0, C14:0, C16:0, C18:0, and C20:0).  
<sup>2</sup>MUFA = Total monounsaturated fatty acids (C16:1, C18:1, and C20:1).  
<sup>3</sup>PUFA = Total polyunsaturated fatty acids (C18:2 and C18:3).

(2004). This was supported by more stroma protein content and highly cross-linking collagen found in older age of spent hen as discussed earlier. Wattanachant *et al.* (2004) stated that cross-links stabilize the collagen molecule and impart tensile strength to the connective tissue, which is necessary to resist the physical force place on these structure elements. This may partially explain why meat from older animals is tougher than that from younger animals, even though muscle from younger animals generally contain more collagen (Pearson and Young, 1989; Foegeding and Lanier, 1996).

The fatty acid composition of raw spent hen *Pectoralis major* muscle (Table 2) was generally similar to those of fatty acid composition in poultry muscle reported by Smith *et al.* (1993), Qiao *et al.* (2002b), and Wattanachant *et al.* (2004). The results was also in good agreement with recently reported of Liwa (2009) who showed that spent hen breast meat contained 35.67% saturated fatty acids (SFA), 44.57% monounsaturated fatty acids (MUFA), and 19.79% polyunsaturated fatty acids (PUFA), respectively. In addition, the high contents in palmitic acid, stearic acid, oleic acid, and linoleic acid found in current study were similar with those found in spent hen breast meat reported by Liwa (2009). Moreover, the content of linoleic acid (essential fatty acid) obtained in this study was higher than those reported in *Pectoralis* muscle of broiler at 7.63 (% of total fatty acid) by Wattanachant *et al.* (2004) and between 15.62-15.99 (%) by Qiao *et al.* (2002b) elucidated to the high nutritional value of spent hen meat. However, meat from spent hen contained a higher percentage of total MUFA and total PUFA and a lower percentage of SFA as compared with broiler in previous report of Wattanachant *et al.* (2004). Huton (1995) noted that high unsaturated fat intakes may be preferable for human; however, unsaturated fatty acids are more prone to oxidation. This result indicated that meat from spent hen may promote faster oxidation than broiler during processing and storage.

The onset temperature of transition ( $T_o$ ),

maximum thermal transition ( $T_{peak}$ ) temperatures, and denaturation enthalpy ( $\Delta H$ ) were determined for raw spent hen *Pectoralis major* muscle. Five endothermic peaks were obtained at 55.92°C, 62.58°C, 65.08°C, 72.75°C, and 77.83°C. These results were consistent with earlier observations in broiler *Pectoralis* muscle at 54.88°C, 61.66°C, 65.37°C, 70.63°C, and 76.14°C by Wattanachant *et al.* (2004). Water-washed myofibrillar proteins from broiler *Pectoralis minor* muscle have been reported to produce two major thermal transitions at 55.2°C and 77.7°C and corresponded with pure myosin and actin at 57.9°C and 80.8°C, respectively (Kijowski and Mast, 1988). The first and the fifth peaks from these results were most likely belonging to myofibrillar proteins. For sarcoplasmic proteins, only one peak (second peak) in current study was in agreement with Kijowski and Mast (1988) who found 2 main heat transitions at 62.3°C and 68.3°C. Difference results perhaps caused by the authors collected sarcoplasmic proteins as the drip from thawed muscle, which had been frozen at -20°C for 24 h. However, sarcoplasmic proteins of chicken breast muscle have also been reported at 72°C by Wang and Smith (1994) and 71.9°C by Murphy *et al.* (1998). Conversely, Kijowski and Mast (1988) stated that a minor heat transitions at 72.8°C in broiler *Pectoralis minor* muscle could be contributed to thin filaments, such as tropomyosin, troponin, actinin, as well as the other intracellular proteins (connectin). These can be attributed with peak four of the present study. Stroma proteins isolated from breast muscle have been reported at 65.3°C by Kijowski and Mast (1988), and 65.37°C by Wattanachant *et al.* (2004) which was probably associated with the third peak in current result.

### Effect of NaCl and STPP on quality changes of spent hen *Pectoralis major* muscles during chilled storage

#### Changes in chemical properties

##### Changes in TCA-soluble peptides

Proteolysis of raw spent hen *Pectoralis* muscle treated with NaCl and STPP (raw-treated) and untreated samples (raw-untreated) during chilled storage were evaluated by TCA-soluble peptides (Table 3). Significant differences between both samples in TCA-soluble peptides were not found over storage time ( $P \geq 0.05$ ) although raw-treated samples exhibited slightly higher of these values than did find in raw-untreated samples. Both samples showed non-significant increase ( $P \geq 0.05$ ) in TCA-soluble peptides at the beginning of storage. Thereafter, TCA-soluble

peptides of raw-treated samples exhibited gradual increase ( $P < 0.05$ ), while raw-untreated samples showed dramatic increase ( $P < 0.05$ ). It was probably due to the proteolysis in meat during the initial stages caused by endogenous proteases in skeletal muscles (Ouali, 1990; Koohmaraie, 1992; Walker *et al.*, 1995), whereas for longer time of storage might be resulted from proteases of microorganism (Allen *et al.*, 1998). Muscle samples treated with STPP resulted in higher pH near to neutral which could induce the activity of calpains, calcium-dependent protease (Northcutt *et al.*, 1998) leading to the higher rate of proteolysis than untreated samples did at the beginning of storage time. Thereafter, the rate of proteolysis increased with increasing amount of spoilage microorganism in both samples.

**Table 3.** TCA soluble peptides (mmol tyrosine/g muscle) during chilled storage at 4°C of raw-untreated and raw-treated spent hen *Pectoralis major* muscles with NaCl and STPP

Storage time (days)	TCA soluble peptides (mmol tyrosine/g muscle)	
	Raw-untreated	Raw-treated
0	0.0965±0.0038 <sup>a,x</sup>	0.1150±0.0052 <sup>a,x</sup>
3	0.1009±0.0018 <sup>a,x</sup>	0.1160±0.0185 <sup>a,x</sup>
6	0.1242±0.0101 <sup>b,x</sup>	0.1420±0.0096 <sup>b,x</sup>
9	0.1782±0.0004 <sup>c,x</sup>	0.1571±0.0097 <sup>b,x</sup>

Data are presented as mean ± standard deviation from triplicate determinations.

<sup>a,c</sup> Means with differing superscripts in the same column are significantly different ( $P < 0.05$ ).

<sup>x</sup> Means with differing superscripts in the same row are significantly different ( $P < 0.05$ ).

**Table 4.** MetMb content (%) during chilled storage at 4°C of raw-untreated and raw-treated spent hen *Pectoralis major* muscles with NaCl and STPP

Storage time (days)	MetMb content (%)	
	Raw-untreated	Raw-treated
0	73.91±0.73 <sup>a,x</sup>	72.89±0.24 <sup>a,x</sup>
3	73.25±0.71 <sup>a,x</sup>	73.82±1.53 <sup>a,x</sup>
6	75.59±0.65 <sup>b,x</sup>	74.18±0.25 <sup>ab,x</sup>
9	75.73±0.82 <sup>b,x</sup>	75.41±0.15 <sup>b,x</sup>

Data are presented as mean ± standard deviation from triplicate determinations.

<sup>a,b</sup> Means with differing superscripts in the same column are significantly different ( $P < 0.05$ ).

<sup>x</sup> Means with differing superscripts in the same row are significantly different ( $P < 0.05$ ).

#### Changes in MetMb content

The MetMb (oxidized state= $Fe^{3+}$ ) is an oxidized form of Myoglobin (reduced state= $Fe^{2+}$ ) that can be generated during refrigerated storage (Chaijan *et al.*, 2005). The formation of MetMb in spent hen *Pectoralis major* muscles treated with NaCl and STPP (raw-treated) and untreated samples (raw-untreated) during chilled storage are shown in Table 4. No change in MetMb content was observed in both samples up to day 3 of chilled storage ( $P \geq 0.05$ ). A gradual increase in MetMb content of raw-treated muscle samples was found when the storage time increased from day 3 to day 9, whereas MetMb content of raw-untreated increased sharply on day 6 ( $P < 0.05$ ) and then approached a plateau thereafter. The sharp increase in MetMb formation of raw-untreated with extended storage time suggested that myoglobin underwent more oxidation. The constant of MetMb formation in raw-untreated at the end of storage time was probably caused by the lower myoglobin content remaining or bound in the muscle which was in concomitant with the increasing of drip loss (Table 6) resulted in more water loss from muscle leading to

more myoglobin release and could not continue the formation of MetMb. The addition of STPP to meat has been shown to have antioxidation properties by chelating the heavy metal ions and keeping a part of the iron in the reduced state (Stoick *et al.*, 1991; Shahidi *et al.*, 1987), resulting in a gradual increase of MetMb content in raw-treated samples.

**Table 5.** TBARS ( $\mu\text{g MDA/g sample}$ ) during chilled storage at  $4^{\circ}\text{C}$  of raw-untreated, raw-treated, cooked-untreated and cooked-treated spent hen *Pectoralis major* muscles with NaCl and STPP

Storage time (days)	TBARS ( $\mu\text{g MDA/g sample}$ )				
	Raw		Cooked		
	untreated	treated	untreated	treated	
0	16.52 $\pm$ 1.36 <sup>a,x</sup>	16.91 $\pm$ 0.19 <sup>a,x</sup>	45.20 $\pm$ 3.48 <sup>a,x</sup>	41.78 $\pm$ 2.10 <sup>a,x</sup>	
3	24.08 $\pm$ 1.59 <sup>b,x</sup>	22.57 $\pm$ 0.19 <sup>b,x</sup>	57.31 $\pm$ 8.27 <sup>b,x</sup>	52.12 $\pm$ 2.70 <sup>b,x</sup>	
6	39.70 $\pm$ 0.54 <sup>c,y</sup>	35.56 $\pm$ 0.76 <sup>c,x</sup>	99.86 $\pm$ 0.81 <sup>c,y</sup>	82.88 $\pm$ 2.84 <sup>c,x</sup>	
9	38.90 $\pm$ 0.84 <sup>c,x</sup>	36.41 $\pm$ 1.04 <sup>c,x</sup>	94.39 $\pm$ 3.69 <sup>c,y</sup>	81.45 $\pm$ 0.74 <sup>c,x</sup>	

Data are presented as mean  $\pm$  standard deviation from triplicate determinations.

<sup>a-c</sup> Means with differing superscripts in the same column are significantly different ( $P < 0.05$ ).

<sup>x-y</sup> Means with differing superscripts in the same row are significantly different ( $P < 0.05$ ).

### Changes in TBARS

The 2-thiobarbituric acid reactive substances (TBARS) assay is most widely used to determine lipid oxidation in meat which determines malondialdehyde (MDA) content (Broncano *et al.*, 2009). TBARS values of spent hen *Pectoralis major* muscles treated with NaCl and STPP (raw-treated/cooked-treated) and untreated samples (raw-untreated/cooked-untreated) are presented in Table 5. Storage time had influence on lipid oxidation of raw spent hen meat as elucidated by an increase in TBARS values of both samples during chilled storage. As storage time increased from day 0 to day 6, a dramatic increase in TBARS values of both raw-untreated and raw-treated muscle samples were found ( $P < 0.05$ ). Thereafter, no change in TBARS values of both samples was observed ( $P \geq 0.05$ ). An increase in TBARS values in current study seemed reasonable and in agreement with those previous reports in chicken, beef, and turkey by Ang (1988), Ahn *et al.* (1993), and Rhee and Ziprin (2001) who found these values tended to increase with storage time. Lipid oxidation in meat is initiated when polyunsaturated fatty acids (PUFA) react with molecular oxygen, via a free radical chain mechanism, forming peroxides (Gray, 1978). The primary auto-oxidation is followed by a series of secondary reactions which lead to degradation of the peroxidized fatty acid. The susceptibility of meat to oxidative process depends on several factors and one of the most important being the level of highly oxidizable substrates, such as PUFA in the phospholipids fraction of cell membranes (Luciano *et al.*, 2009); where lipid oxidation is initiating (Buckley *et al.*, 1995). The rapid increase in TBARS values in current study probably due to high content of polyunsaturated fatty acids in spent hen meat resulted in the rate of addition of oxygen to lipid molecules to form hydroperoxide was high for spent hen meat at the beginning of

storage. In addition, high phospholipids fraction in spent hen meat could enhance lipid oxidation during chilled storage. Higher content of phospholipids fraction in spent hen breast meat (21.03%) compared with (5.71%) Thai indigenous chicken have been reported by Liwa (2009). Moreover, some chemical composition in meat such as heme pigment could effect on lipid oxidation during chilled storage. Recently studied in different breeds of chicken meat by Liwa (2009) found that the increase of MDA content during chilled storage when extended storage time from 0 to 6 days in each meat sample associated with the increase in non-heme iron content and MetMb formation, and relating to the decrease in heme iron and myoglobin content of chicken meat. The high content of myoglobin in spent hen meat compared to other chicken meat as mentioned earlier might result in high prooxidant compounds in meat system that could directly stimulate lipid oxidation during chilled storage. Adding NaCl and STPP had little effect on retarding lipid oxidation of raw spent hen meat during chilled storage as indicated by raw-untreated samples exhibited higher TBARS values than did in raw-treated muscle samples on day 6 of chilled storage ( $P < 0.05$ ), while no significant differences between both samples was found on other days of storage ( $P \geq 0.05$ ).

As expected, cooked samples exhibited higher TBARS values than raw samples because when meat is cooked and exposed to atmospheric conditions, it becomes rapidly oxidized. Cooking not only disrupts the membrane structure but also facilitates release of iron from the iron carrier proteins or iron storage conditions (Ahn *et al.*, 1993). Under such conditions oxygen can directly react with the ferrous iron in the meat and activate the ground state oxygen to a highly reactive form (Halliwell and Gutteridge, 1990) resulted in rapid oxidation leading to high TBARS value in cooked meat.

For cooked samples, at the beginning of storage (day 0-3), no significant difference in TBARS values of both treated and untreated samples was found ( $P \geq 0.05$ ), thereafter muscle samples treated with NaCl and STPP showed lower values than did found in untreated samples ( $P < 0.05$ ) indicated that NaCl and STPP treated could retard lipid oxidation in cooked spent hen meat. This could be caused by STPP can act like antioxidant as described previously. Storage time also had influence on lipid oxidation of cooked spent hen meat as elucidated by a dramatic increase in TBARS values of both samples were found from day 0 to day 6 ( $P < 0.05$ ) and then approached a plateau thereafter. No change in TBARS value of both raw and cooked samples when extended storage

time perhaps caused by during prolonged storage removal or losses of malondialdehyde that might have occurred due to microbial growth/metabolism (Rhee and Ziprin, 2001).

### Changes in physical properties

#### Drip loss, weight loss, and cooking loss

Drip loss was measured to obtain an overall assessment of the water binding properties of muscle. As expected, increasing storage time caused increasing in drip loss of both raw-untreated and raw-treated samples ( $P<0.05$ ) (Table 6). Similar trend of increasing drip loss during chilled storage of raw breast broiler muscles was also reported by Allen *et al.* (1998). NaCl and STPP treated had influence on drip loss of spent hen meat as indicated by raw-untreated samples exhibited higher drip loss than did in raw-treated samples ( $P<0.05$ ).

by increasing in weight loss as storage time increased (Table 6). However, cooked-treated samples showed higher weight loss than cooked-untreated samples ( $P<0.05$ ). This was probably caused by more water retention in treated muscle samples was carried out during cooking than did in untreated samples. In addition, the treated samples might be more structure changes and expelled more fluid from the muscle fibers due to heat denaturation. Larik and Turner (1992) stated that WHC of muscle tissue was related to the extent of heat denaturation of myofibrillar proteins during thermal processing that leading to structure changes and expelled the sarcoplasmic fluid from the muscle fibers, resulting in water loss from meat tissue (Murphy and Mast, 2000).

Cooking loss is another parameter to evaluate WHC. Because of muscle protein changes with heating, water content within the muscle in the narrow channels between the filaments changes as meat

**Table 6.** Cooking loss (%), drip loss (%), and weight loss (%) of raw-untreated, raw-treated, cooked-untreated, and cooked-treated spent hen *Pectoralis major* muscles with NaCl and STPP during chilled storage at 4°C

Storage time (days)	Raw				Cooked	
	Cooking loss (%)		Drip loss (%)		Weight Loss (%)	
	untreated	treated	untreated	treated	untreated	treated
0	21.60±3.22 <sup>ab</sup>	20.67±2.21 <sup>ab</sup>	-	-	-	-
3	23.38±2.90 <sup>ab</sup>	24.37±1.76 <sup>ab</sup>	0.35±0.02 <sup>xy</sup>	0.24±0.06 <sup>ax</sup>	0.51±0.11 <sup>ax</sup>	0.73±0.08 <sup>xy</sup>
6	25.89±1.83 <sup>bc</sup>	22.25±2.02 <sup>ab</sup>	0.99±0.07 <sup>by</sup>	0.46±0.04 <sup>bx</sup>	0.70±0.11 <sup>bx</sup>	1.02±0.08 <sup>by</sup>
9	27.72±3.28 <sup>c</sup>	20.34±2.58 <sup>ab</sup>	1.48±0.09 <sup>c</sup>	1.29±0.05 <sup>cx</sup>	1.15±0.08 <sup>cx</sup>	1.68±0.13 <sup>c</sup>

Data are presented as mean ± standard deviation from 8 determinations for cooking loss and 4 determinations of drip loss and weight loss.

<sup>ab</sup> Means with differing superscripts in the same column are significantly different ( $P<0.05$ ).

<sup>xy</sup> Means with differing superscripts in the same row are significantly different ( $P<0.05$ ).

**Table 7.** Shear force values (kg) during chilled storage at 4°C of raw-untreated, raw-treated, cooked-untreated and cooked-treated spent hen *Pectoralis major* muscles with NaCl and STPP

Storage time (days)	Shear force values (kg)			
	Raw		Cooked	
	untreated	treated	untreated	treated
0	2.15±0.27 <sup>ab</sup>	2.14±0.32 <sup>ab</sup>	2.25±0.43 <sup>bx</sup>	2.21±0.27 <sup>bx</sup>
3	2.23±0.82 <sup>ab</sup>	2.18±0.38 <sup>ab</sup>	2.02±0.15 <sup>bx</sup>	2.05±0.17 <sup>bx</sup>
6	2.82±0.46 <sup>bx</sup>	2.79±0.70 <sup>bx</sup>	2.58±0.15 <sup>c</sup>	2.93±0.31 <sup>c</sup>
9	2.52±0.81 <sup>ab</sup>	2.16±0.73 <sup>ab</sup>	1.94±0.11 <sup>ax</sup>	1.91±0.20 <sup>ax</sup>

Data are presented as mean ± standard deviation from eight determinations.

<sup>ab</sup> Means with differing superscripts in the same column are significantly different ( $P<0.05$ ).

<sup>xy</sup> Means with differing superscripts in the same row are significantly different ( $P<0.05$ ).

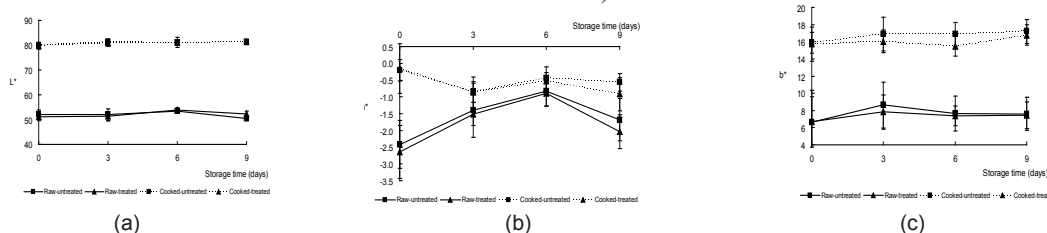
This probably caused by treated samples with NaCl and STPP performed more binding water during storage than did in untreated samples resulted in more water retention in muscle samples leading to lower drip loss. Combinations of NaCl and STPP have been reported to synergistically improve moisture absorption and WHC of poultry meat (Young *et al.*, 1996). The addition of STPP to meat systems increased the meat pH (Jones *et al.*, 1987; Sutton *et al.*, 1997) that moving the meat pH further from the meat protein isoelectric point (about 5.5 in chickens) (Miller, 1998). In addition, NaCl acts to improve WHC by increasing ionic strength of meat proteins (Miller, 1998; Rhee and Ziprin, 2001) and contributes to the swelling of the proteins (Miller, 1998). The swelling provides a higher number of protein side chains that can bind water and therefore WHC increased.

For cooked samples, the same trend was observed

shrinks (Bertola *et al.*, 1994), resulting in cooking loss with heating. The data from Table 6 showed that cooking loss of raw-untreated samples increased gradually throughout chilled storage, whereas that of raw-treated samples slightly increased on day 3 and then decreased gradually until 9 days of storage. It was probably due to degradation of myofibrillar and sarcoplasmic proteins via proteolysis process during chilled storage as indicated by the increase in TCA-soluble peptides (Table 3). Protein degradation resulted in low property of proteins to hold water in muscles and exhibited increase in cooking loss during storage in untreated samples. As described earlier, the STPP treatment increased meat WHC in raw-treated samples than did in raw-untreated samples leading to slightly decline in cooking loss for longer time of storage.

### Changes in texture

Changes in texture during chilled storage of raw and cooked spent hen *Pectoralis major* muscle samples were determined by Warner-Bratzler shear force values as presented in Table 7. NaCl and STPP treatments showed no significance on shear force values of raw and cooked muscle samples throughout storage time ( $P \geq 0.05$ ). However, shear force values of samples were affected by storage time. The highest shear force value was observed on day 6 of storage ( $P < 0.05$ ). The highest value on day 6 was probably



**Figure 1.** Changes in L\* values (a), a\* values (b), and b\* values (c) during chilled storage at 4°C of raw-untreated, raw-treated, cooked-untreated, and cooked-treated spent hen *Pectoralis major* muscles with NaCl and STPP. Bars represent the standard deviations for eight determinations

due to water loss from muscle as indicated by higher drip loss than the early of storage time as described previously. The decrease in this values after prolong storage probably caused by increase in the degree of proteolysis resulted in degradation of protein.

The results showed no improvement on texture of muscle after cooking at 80°C neither NaCl/STPP treated samples ( $P \geq 0.05$ ). Wattanachant *et al.* (2005b) reported a slightly increased in shear value when heated broiler from 60 to 80°C and tended to decrease at higher temperature from 80 to 100°C. The increase in shear value with heating up to 80°C might be due to the combination effect of the denaturation of myofibrillar proteins, the shrinkage of intramuscular collagen, as well as the shrinkage and degradation of actomyosin (Bailey and Light, 1989). Therefore, the results could be demonstrating the ineffectiveness of NaCl and STPP on decreasing toughness of both raw and cooked spent hen muscles during chilled storage. This was due to the spent hen muscle had high content of stroma protein (Table 1) resulted in more difficulty for both additives to penetrate into the muscle. In addition, high collagen cross-linking found in current study leading to less collagen was melt to become gelatin which tenderness of muscle is carried out.

### Changes in color

Changes in surface color (L\*, a\*, and b\* values) during chilled storage of raw and cooked spent hen *Pectoralis major* muscles were presented in Figure 1. There was no significant difference in C.I.E. color values between untreated and NaCl and STPP treated samples for both raw and cooked muscle samples

throughout chilled storage ( $P \geq 0.05$ ). However, L\* and a\* values of samples were affected by storage time ( $P < 0.05$ ), while b\* value was unchanged with storage time ( $P \geq 0.05$ ). Heating tended to increase more light color, less red and more yellow color of meat as indicated by exhibited higher in L\* and b\* values but lower in a\* value of cooked samples than did in raw samples. In addition, the degree of color variation in cooked meat was not pronounced as in raw meat. The L\* value of the meat depends upon the amount of light scattered (Sams and Alvarado, 2004).

Young and West (2001) pointed out that with increasing heating temperature, meat tends to be lighter and also turn to a brown-grey hue and the lightening is due to an increased reflection of light, arising from light scattering by denatured proteins. Lawrie (1991) noted that denaturation of red myoglobin and conversion to brown myohaemochromogen starts at 40°C and is almost completely denatured between 80-85°C. This could explain why cooked meat has increased in b\* values (yellowness) after heating. L\* value of both raw-untreated and raw-treated samples exhibited highest value on day 6 and a slight change was observed in raw-untreated, while no change in L\* value of raw-treated was found on the other days of storage (Figure 1a). It has been indicated that the decrease in pigment concentrations and lower pH increased meat lightness (Boulianne and King, 1995; Fletcher *et al.*, 2000). In general, as drip loss increased, L\* value increased with little effect of pH (Woelfel *et al.*, 2002). The highest L\* value on day 6 was in concomitant with the increasing of drip loss (Table 6), whereas for longer time of storage was effected by higher pH of meat due to rapid growth of spoilage microorganism.

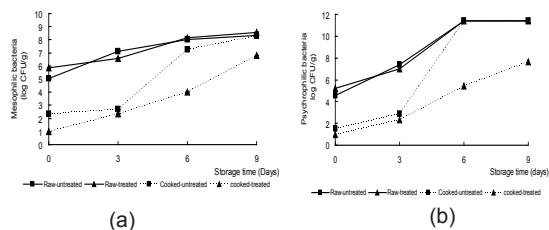
Storage time had no effect on L\* value of cooked-untreated ( $P \geq 0.05$ ), while it showed significantly increase in this value of cooked-treated samples when extended storage time ( $P < 0.05$ ). The color a\* value of raw samples increased gradually from 0 to 6 days and then it slightly decreased thereafter (Figure 1b). The a\* value was positively correlated with total pigment, myoglobin, and iron concentrations (Boulianne and King, 1995) and change in a\* value was correlated well with Mb content that might undergo oxidation



during chilled storage to form MetMb resulting in appear more brownish in color. This result was elucidated by increasing in MetMb when extended storage time (Table 4) as discussed previously. For  $a^*$  value of cooked meat either treated or untreated samples was slightly changed throughout storage ( $P < 0.05$ ). No significant difference in  $b^*$  value was observed for raw and cooked muscle samples during chilled storage ( $P \geq 0.05$ ) (Figure 1c).

### Microbiological changes

The effect of NaCl and STPP on microbiological property of spent hen muscle samples during chilled storage was evaluated mesophilic and psychrophilic bacteria counts as presented in Figure 2a and 2b, respectively.



**Figure 2.** Changes in mesophilic bacteria logCFU/g (a) and psychrophilic bacteria (b) during chilled storage at 4°C of raw-untreated, raw-treated, cooked-untreated, and cooked-treated spent hen *Pectoralis major* muscles. Bars represent the standard deviations for duplicate determinations

The initial microbial loads of raw spent hen muscle in this study were similar range of aerobic plate count (APC) of chicken carcasses as the other reports at 5.4 log by Okolacha and Ellerbroek (2005) and at 5 log CFU/cm<sup>2</sup> by Vareltzis *et al.*, (1997). Mesophilic bacteria (indicator of pathogen) of both raw-untreated and raw-treated samples were increased dramatically with storage time ( $P < 0.05$ ), whereas psychrophilic bacteria (indicator of spoilage) of both samples trended to increase sharply throughout storage ( $P < 0.05$ ). The spoilage of both raw-untreated and raw-treated samples occurred before day 6 of storage as indicated by the microbial guideline for spoilage count of poultry meat which is about exceed 7 logCFU/g (Cox *et al.*, 1975; Lillard and Ang, 1989). Pornaem *et al.* (2005) reported that spoilage of fresh poultry usually begins after 5 to 7 days of refrigerated storage, while, chicken produced by small facility is expected to be 3-5 days of storage. Results obtained from this study concluded that the presence of NaCl and STPP was ineffective to retard microbial growth in raw spent hen muscle during chilled storage. Conflict results have been obtained from other researchers this probably due to difference in the initial load of microbial in poultry and methods use. Some studies had demonstrated an increase in shelf-life of chicken carcasses chilled in phosphate

solution (Elliott *et al.*, 1964; Yoon, 2002) due to the inhibitory action of phosphates to the chelating of metal ions need for microbial growth (Elliott *et al.*, 1964). In contrast, other investigator was unsuccessful at establishing the relationship between the use of phosphates and reduction of bacteria on fresh meat (Zessin and Shelef, 1988). However, the finding of Allen *et al.* (1998) reported that phosphate was not retarded psychrotropic plate counts (PPC) in fresh poultry at day 1 of storage but its showed effective in reduced PPC in samples treated with phosphate after day 7 of storage at 4°C.

As shown in Figure 2, both mesophilic and psychrophilic bacteria counts in cooked-untreated and cooked-treated samples were observed less than 2 logCFU/g at the beginning of storage which showed an effective of heating to reduce microorganism. When storage time increased, both mesophilic and psychrophilic bacteria counts of cooked-untreated samples increased sharply ( $P < 0.05$ ), whereas gradually increased were found in cooked-treated samples ( $P < 0.05$ ). In addition, treated samples with NaCl and STPP showed lower in both mesophilic and psychrophilic bacteria counts than did found in cooked untreated samples throughout storage ( $p < 0.05$ ). The data indicated that cooked-treated sample had longer chilled storage time (9 days) than did in cooked-untreated samples (3 days). This perhaps due to chelating affects of STPP as described earlier. It could be suggested that adding NaCl and STPP in cooked spent meat samples had more effective to extend shelf-life during chilled storage than did in raw samples.

### Conclusions

Spent hen *Pectoralis major* muscles had nutritional value the same as commercial broiler meat. However, further processing of spent hen meat need to be concern due to it meat had high fat and more content of polyunsaturated fatty acid which susceptible to promote lipid oxidation. Adding NaCl and STPP was effective to improve yield and inhibited lipid oxidation of raw spent hen meat but could not improve texture, color and retard microbial growth during chilled storage. This was due to the spent hen muscle had high content of stroma protein and more cross-linking collagen resulted in more difficulty for both additives to penetrate into the muscle to enhance other quality. Cooked spent hen meat could be reduced microorganism and extended shelf-life during chilled storage. However, cooking was ineffective to decrease toughness of the spent hen muscle because of high collagen cross-linking

leading to less collagen was melt to become gelatin which tenderness of muscle is carried out. Therefore, decreasing toughness also need to be developed before processing of the spent hen meat.

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