# Effect of Phosphates on Quality of Spent Hen Muscle Marinated with Tom-Yum Paste

**Pirinya Wongwiwat**

**A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of**

**Master of Science in Food Science and Technology**

**Prince of Songkla University**

**2009**

| | |
|---|---|
| **Thesis Title** | Effect of Phosphates on Quality of Spent Hen Muscle Marinated with Tom-Yum Paste |
| **Author** | Miss Pirinya Wongwiwat |
| **Major Program** | Food Science and Technology |

---

**Major Advisor :**

.....

(Dr. Saowakon Wattanachant)

**Co-advisor :**

.....

(Dr. Sunisa Siripongvutikorn)

**Examining Committee :**

.....Chairperson

(Prof. Dr. Soottawat Benjakul)

.....

(Dr. Saowakon Wattanachant)

.....

(Dr. Sunisa Siripongvutikorn)

.....

(Assoc. Prof. Dr. Kanok-Orn Intarapichet)

The Graduate School, Prince of Songkla University, has approved this thesis as partial fulfillment of the requirements for the Master of Science Degree in Food Science and Technology

.....

(Assoc. Prof. Dr. Krerkchai Thongnoo)

Dean of Graduate School

# CHAPTER 1

# INTRODUCTION

In recent years, consumption of poultry and poultry products has increased rapidly in developing and developed countries resulting in the higher export value of poultry and their product than other meat. However, production of spent hens for egg demand has become a problem in an excessive supply of spent hens. The direct use of meat from spent hen causes problems because their texture are very tough due to high collagen content with increased age, then the toughness prevents their use in whole meat and reduces market values (Nowsad *et al*., 2000).

An approach for product development of spent hen meat into value-added product is achieved in marination technology which is a traditional culinary technique. The major marination methods are still-marination, injection, and tumbling, for the still-marination process requires more space and time than those of marinations. However, the investment cost is much lower than injection and tumbling which need more sophisticated equipment (Lemos *et al*., 1999). The term, "marinade" implies any liquid considered to be tenderizing, flavouring, and enhancing succulence and shelf-life of meat to satisfy consumer demand (Lemos *et al*., 1999; Björkroth, 2005). A marinade may contain such ingredients as salt, sugar, acids, rheology improving additives, antimicrobial agents, spices, and aroma enhancers (Oreskovich *et al*., 1992; Björkroth, 2005). In general, phosphates has been widely used in marination process as a marinade in poultry meat industry to improve the quality of finished products, including reduced cooking loss (Lemos *et al*., 1999; Sheard and Tali, 2004), increased cooking yield (Shahidi and Synowiecki, 1996), tenderized muscle (Zheng *et al.*, 2000), inhibited oxidative off-flavor (Lee *et al*., 1998; Weilmeier and Regenstein, 2004), and prevented microbiological spoilage (Yoon, 2002; Sampathkumar *et al*., 2003). Much of the behaviour of phosphates in improving functional properties of meat was described in increasing ionic strength and pH, sequestering metal ion, and improving buffering capacity of the meat (Gonçalves and Ribeiro, 2008).

Nowadays, the high acid marinade in meat product is more interested since it can incorporate a variety of flavors and alter meat tenderness (Aktaş *et al*., 2003; Burke and Monahan, 2003; Serdaroğlu *et al*., 2006). Famous Thai food cuisine, Tom-Yum, is a hot, sour, and spicy soup normally made from lemon grass, chilli, kaffir lime, and lemon juice. Spices and herbs in Tom-Yum were worldwide attended to be used as natural food additive and added to food to imparting characteristic flavours. Some certain spices and herbs could prolong the shelf-life of food by preventing rancidity through their antioxidant activity or bacteriostatic or bacteriocidal activity (Chattopadhyay and Bhattacharyya, 2007). However, pH of a Tom-Yum marinade classified as acidic, therefore, it may influence meat texture. Since marinades that are highly acidic (pH below 5.0) causes the bond breaks and protein unwind, they bound together into a loose mesh and trapped water molecules within this protein mesh. After a short time, proteins in acidic marinade bonds tighten, water is squeezed out leading to their denaturation, and tissue becomes tough (Brandt, 2003; Corriher, 2009). Therefore, phosphate compounds were studied as pretreatment in acidified spent hen muscle to improve buffering capacity, water holding capacity, and tenderness. The effect of Tom-Yum paste and its ingredient marinades on chemical, physical, and microbiological characteristics of spent hen muscle were determined and the shelf-life of Tom-Yum marinated muscle was evaluated.

## Literature Reviews

### 1. Chicken and spent hen

The dramatic increase in consumption of poultry meat and poultry meat products has been stimulated in part by the public's awareness of nutritional advantage of these products over beef, pork and lamb. Poultry meat has more unsaturated fat, lower fat content (especially in franks and bologna) and fewer calories per serving (Addis, 1986). The proximate compositions of chicken are shown in Table 1. Because of its relatively low cost among meats, chicken is one of the most used meats in the world. It has a fairly neutral flavor and texture.

**Table 1** Proximate compositions of raw chicken meat (/100g edible portion)[a]

| Nutrients | Combined [b] | | Light meat | | Dark meat | |
|---|---|---|---|---|---|---|
| | Flesh and skin | Flesh only | Flesh and skin | Flesh only | Flesh and skin | Flesh only |
| Water (g) | 65.99 | 75.46 | 68.60 | 74.86 | 65.42 | 75.99 |
| Protein (g) | 18.60 | 21.39 | 20.27 | 23.20 | 16.69 | 20.08 |
| Total lipids (g) | 15.06 | 3.08 | 11.07 | 1.65 | 18.34 | 4.41 |
| Ash (g) | 0.79 | 0.96 | 0.86 | 0.98 | 0.76 | 0.94 |
| Food energy (kcal) | 215 | 119 | 186 | 114 | 237 | 125 |

[a] U.S. Department of Agriculture (1979) ; data for broilers or fryers.

[b] Includes both light and dark meat (whole carcass).

**Source:** Bodwell (1986)

Hen is specially a female adult chicken. They can supply eggs for two to three years (usually at 85-100 weeks) before being regarded as spent hens which is mostly underutilized and used in low price product (Nowsad *et al*., 2000). The direct use of meat from spent hen causes problems because collagen content and cross linkage increases with age. Meat from spent hens is generally tough, less tender, and poor in functional properties such as protein solubility and gelling properties (Munira *et al*., 2006). High collagen contents in any meat can negatively influence its functionality

and nutritional characteristics because collagen has poor balance of amino acid (Trindade *et al*., 2004). Nowsad *et al* (2000) found that total collagen content was higher in spent hen muscles compared to broiler muscles (Table 2).

**Table 2** Proximate compositions, pH, and collagen content of chicken mince

| Values[1] | Spent hen | Broiler |
|---|---|---|
| Moisture (%) | 76.2 [a] | 74.5 [a] |
| Protein (%) | 18.7 [a] | 21.1 [b] |
| Lipid (%) | 4.42 [b] | 3.17 [b] |
| Ash (%) | 0.73 [a] | 0.93 [a] |
| pH | 6.5 [a] | 6.4 [a] |
| Collagen of breast (mg/g) | 4.9 [a] | 3.9 [a] |
| Collagen of thigh (mg/g) | 14.7 [b] | 9.3 [a] |

[1] Values are the means of three individual measurements

[a,b] Values within the same row bearing different superscripts are significantly different ($P$<0.05)

**Source**: Nowsad *et al* (2000)

## 2. Muscle proteins

The muscle proteins can be divided into three major groups (Pearson and Young, 1989) depending on

### 2.1 Contractile or myofibrillar proteins

Myofibrillar proteins are the structural proteins that make up the myofibrils, which contain the basic structural unit responsible for contraction in the living animal. The myofibrillar proteins are also involved in the development of rigor mortis following death. Not all of the myofibrillar proteins are believed to be directly involved in the series of events occurring during contraction but may be further subdivided into three subgroups: (1) the major contractile proteins, which include only actin and myosin; (2) the regulartory proteins, which play an important function in initiation and control of contraction; and (3) the cytoskeletal or scaffold proteins, which provide structural support and may function in keeping the myofibrils in alignment or register.

Crude extracts of the myofibrillar proteins can be made by blending fresh muscle with >0.3 µM KCl solution. The KCl solution should be at least 0.3 µM, and may go as high as 0.5 µM to obtain a good extraction. The myofibrillar proteins comprise about 50-60% of the extractable muscle prteins.

Actin and myosin combined account for about 70% of the total myofibrillar proteins, with myosin making up 50% and actin about 20% of the total. Myosin is located in the thick filaments, which are localized in the A-band. Actin is located in the thin filaments, which extend from the Z-line in the I-band into both ends of the A-band where the thick and thin filaments overlap (Figure 1).

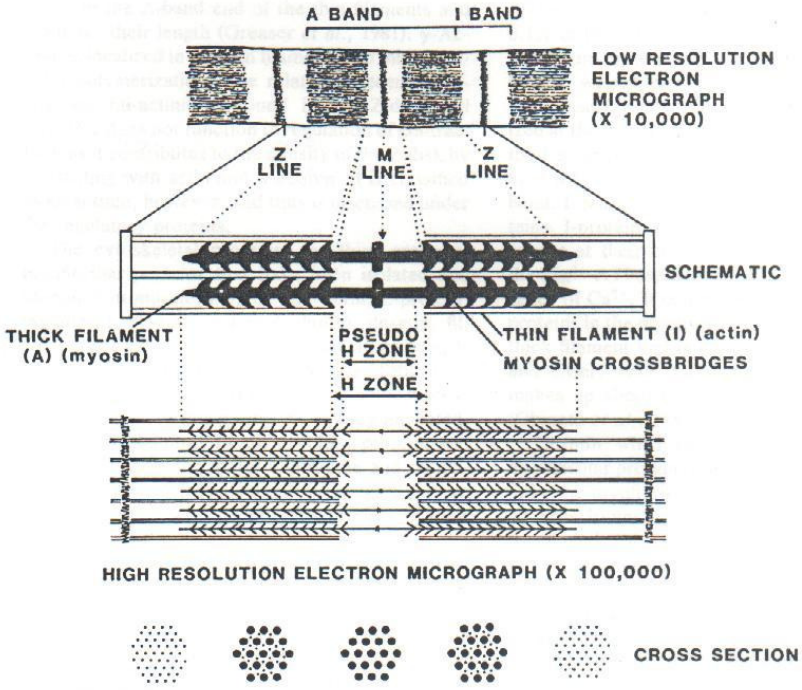



**Figure 1** Fine structure of a single sarcomere was along with portions of two adjacent sarcomeres

**Source**: Pearson and Young (1989)

**2.2 Sarcoplasmic proteins** (Pearson and Young, 1989)

The sarcoplasmic proteins comprise about 30-35% of the total muscle proteins. There are over 100 different sarcoplasmic proteins including most of the enzymes involved in metabolism, which makes it impossible to cover all of them. Not only is the sarcoplasm the fluid medium that contains most of the enzymes involved in energy metabolism, but it also provides a transporting medium for carrying nourishment and oxygen from the blood capillaries to the tissues and the waste products of metabolism, including carbon dioxide, from the tissues back to the capillaries. Hemoglobin in blood and myoglobin in muscle tissues are involved in the exchange of oxygen and carbon dioxide. Other sarcoplasmic proteins involved in high-energy transformations, such as occur in synthesis and breakdown of the high-energy phosphate compounds adenosine triphosphate (ATP), adenosine diphosphate (ADP), and creatine phosphate (CP).

The sarcoplasmic proteins can be removed from muscle by extraction with dilute solutions of an ionic strength of less than 0.1. Water is often used for this purpose, although dilute (<0.1 μM) neutral buffers at pH 6.7-7.5 may also be used.

**2.3 Stromal proteins**

The stromal or connective tissue proteins compose the basic structural elements in the connective tissues of higher animals. The major proteins in the connective tissues are classified as fibrous proteins since they consist of polypeptide chains arranged parallel to each other to form long sheets or fibers. The fibrous proteins are insoluble in water or dilute salt solutions; thus the connective tissue proteins are relatively insoluble and not only impart toughness and shape but also provide to the skeletal muscles. The chief proteins in this group are collagen, elastin, and keratin (Pearson and Young, 1989).

## 3. Water holding capacity of proteins

### 3.1 The mechanism of protein-water interaction

The mechanism of protein-water interactions is important in many natural and formulated foods and different aspects of the physical chemistry of water retention by food protein have been studied. Absorbed water surrounds the protein molecules with several layers of water tightly bound to specific sites in the protein molecules; and further layers of essentially non-structured water, surround the adsorbed layer. A significant part of the water phase consists of water molecules that have lost motional freedom relative to free water. Hydration water is not bound in a true chemical sense, but motionally restricted due to interactions with the macromolecules; it is unfreezable water (Zayas, 1997).

Water interacts with proteins in a number of ways, and significant amounts of water bounded by proteins are retained by hydrogen bonding. Interactions between molecules of water and hydrophilic groups of the protein side chains occur via hydrogen bonding. Structural water is held by hydrogen bonding between polypeptide groups of the proteins. Binding of water to proteins is related to the polar hydrophilic groups, such as imino, amino, carboxyl, hydroxyl, carbonyl, and sulfhydryl groups. The capacity of proteins to retain moisture is affected by the type and number of these polar groups in the protein polypeptide chain. The binding of water is due to the dipolar character of water. Proteins that contain numerous charged amino acids will tend to bind large amounts of water. Water binding of proteins can be predicted from their amino acid composition (Zayas, 1997).

### 3.2 Water holding capacity of muscle proteins

Water holding capacity (WHC) of meat and meat products is the ability to absorb and retain water during mechanical treatment (chopping, coarse grinding, comminution, stuffing), thermal treatment, and subsequent transportation and storage. WHC influences the quality of meat and meat products: juiciness, tenderness, taste, and color, which are important in meat processing (Zayas, 1997). Furthermore, swelling capacity often shows a close correlation with WHC (Hamm, 1986). Swelling capacity of meat is defined as the spontaneous uptake of water from any surrounding fluid, resulting in an increase of weight and volume of muscle fibers.

Water is a dipolar molecule and as such is attracted to charged species like proteins. The definition, “bound water” is water that exists in the vicinity non-aqueous constituents (like proteins) and does not easily move to other compartments. This water is very resistant to freezing and to being driven off by conventional heating. Another fraction of water that can be found in muscles and in meat is termed “trapped (also referred to as immobilized) water”. The water molecules in this fraction may be held either by steric (space) effects and/or by attraction to the bound water. This water is held within the structure of the muscle but is not bound itself to protein. Furthermore, “free water” is water whose flow from the tissue is unimpeded. Weak surface forces mainly hold this fraction of water in meat (Huff-Lonergan and Lonergan, 2005). The state of free or bound water is affected by the molecular arrangement of myofibrillar proteins. Three-dimensional network of filaments in myofibrils provides an open space for water to be immobilized. The decrease of immobilized water is observed as a result of tightening the space between the myofibrils of the network as a result of concentration or protein denaturation. Most of the water in muscular tissue may be termed “immobilized” or “entrapped bulk phase water” which is held in the lattice spaces between the myofibrils. WHC of lean muscular tissue is significantly determined by the spatial arrangement of protein filaments. The main components of meat structure are myofibrils which occupy about 70% of the volume of meat. Consequently, the majority of water is retained in myofibrils in the spaces between the thick and thin filaments (Zayas, 1997). Therefore, changes in WHC of meat will be, in great part, due to variations in the immobilization of bulk phase water in the interfilamental spaces and the filaments themselves (Hamm, 1986).

The immobilization of water in the tissue is apparently determined by the spatial molecular arrangement of the myofibrillar proteins (mainly myosin) or filaments (Figure 2). If the attraction between adjacent molecules or filaments is decreased, as is caused by increasing electrostatic repulsion between similarly charged protein groups or by weakening of hydrogen bonds or hydrophobic bonds, the protein network is enlarged, the swelling increases, and more water can be immobilized within the larger meshes, that is, there is an increase in WHC (e.g. in terms of lower expressible juice or lower cooking loss) (Hamm, 1986).

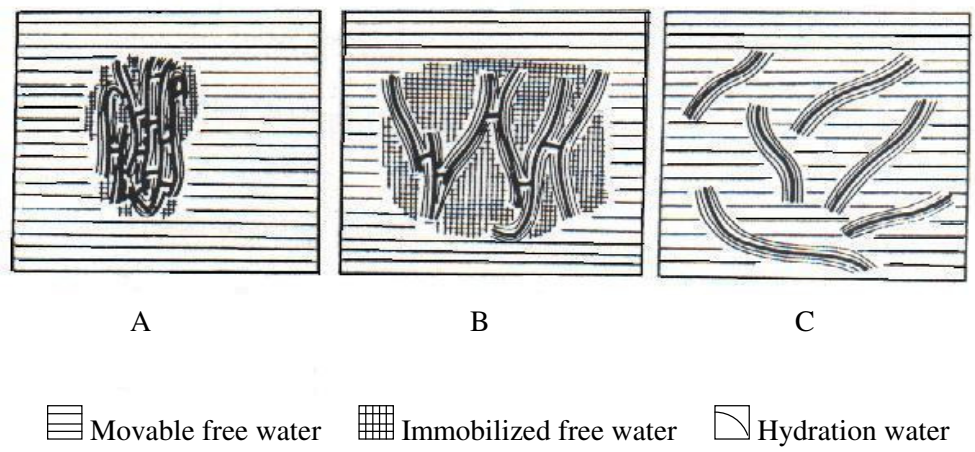



**Figure 2** Influence of cross-linking of proteins or filaments on the water holding capacity (WHC) or swelling of meat

**Source**: Hamm (1986)

**4. pH levels affecting on water holding capacity of meat**

A loosening of the microstructure and, consequently, an increase of immobilized water is caused by raising the protein net charge by the addition of acid and base which results in an increase of interfilament spacing. The pH at which the WHC (or swelling) is at a minimum (pH 5.0) corresponds to the isoelectric point (pI) of myosin or actomyosin, which make up the bulk of myofibrillar proteins. At the pI, the net charge of a protein is at a minimum; expect a maximum of intermolecular salt linkages between positively and negatively charged groups (Figure 3). In the range of pH 5.0-6.5, which is of particular practical interest, a change of pH has a considerable influence on the WHC that must be due to changes in the state of ionization of histidine and to a lesser extent of glutamic acid residues. The change in WHC due to changes in pH in this range are completely reversible whereas in the pH range >10 and <4.5 irreversible changes take place. Differences in WHC of meat between animals of the same species could be related to pH differences; the WHC increases with rising pH provided that the pH variation is larger than the range 5.5-5.8 (Hamm, 1986).

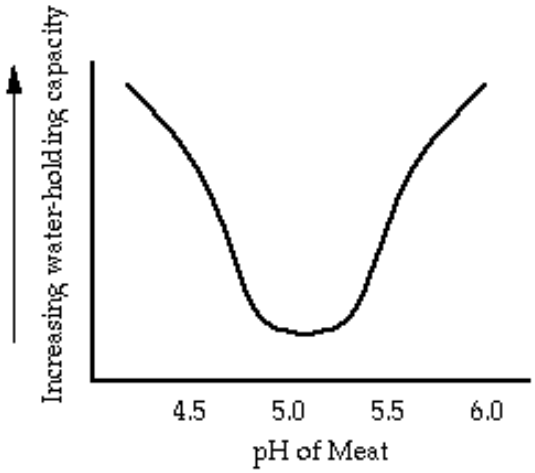



**Figure 3** The relationship between pH and water-holding capacity

**Source**: Meat Science at Texas A&M University (2008)

**5. Salting affecting on water holding capacity of meat**

It is well known that the addition of sodium chloride (NaCl) to meat system causes swelling and increase of WHC. The effect of NaCl on WHC or swelling depends on the pH of the tissue, that is, NaCl increases the WHC at pH > pI and decreases it at pH < pI or around the pI of the myofibrillar system (pH 5) was found that NaCl has no significant influence on WHC (Hamm, 1986). The effect of NaCl is predominantly due to an association of $Cl^-$ ions with positively charged groups of myosin or actomyosin. This adsorption of $Cl^-$ ions cause a weakening of the interaction between oppositely charged groups at pH > pI (Figure 4) and, therefore, an increase of swelling and WHC and it causes a weakening of intermolecular repulsive forces at pH < pI that result in a shrinkage, that is, loss of WHC (Hamm, 1975).

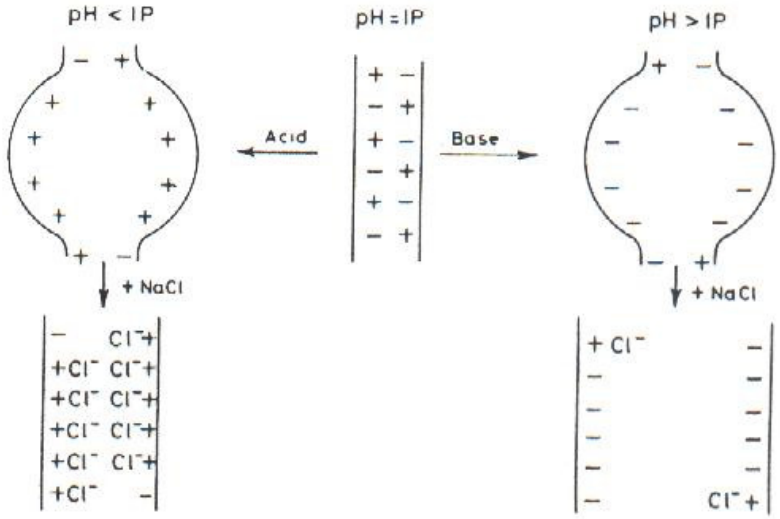



**Figure 4** Influence of NaCl on swelling or WHC of meat at pH values above or below the isoelectric point

**Source**: Hamm (1975)

The addition of NaCl may lead to liberation of bivalent cations ($Mg^{2+}$, $Ca^{2+}$) from muscle proteins, and thus also a loosening of the microstructure of the tissue may take place. It has also been suggested that swelling of the myofilamental system caused by addition of NaCl is due not only to an increase of electrostatic repulsion by $Cl^-$ binding but also to removal of transverse structural constraints (presumably cross-bridges such as the Z-line or M-lines) (Hamm, 1986; Offer and Trinick, 1983).

For the practice of salting and curing it is important that the maximum WHC is achieved at a NaCl addition corresponding to an ionic strength of 0.8-1.0, which mean approximately 5% NaCl, calculated on the meat; at a water addition of 60% the maximum WHC is reach at a NaCl concentration of 8% on the meat. A decrease of WHC at high salt concentration might be due to a reduced repelling activity of carboxyl groups of myosin by the effect of cations and/or to the salting-out phenomenon, that is, to the interaction between nonpolar groups that come to the surface of the protein following a salt-induced unfolding of the peptide chains (Hamm, 1986).

There has been pressured from consumer groups for legislation to reduce the amount of sodium in processed foods due to the possible causal relationship between sodium intake and hypertension. Processed meat and fish products contribute significantly to the total dietary sodium intake (mainly in the form of sodium chloride). Hence, a reduction in salt level of these products will lead to a measurable reduction in total sodium intake. However, in most products just reducing the salt level leads to loss of functionality as exhibited by increased cooking loss and reduced textural properties. However, phosphates can very effectively replace salt in most meat products, their effectiveness depends on the types of phosphate and the conditions under which they are used (Trout and Schmidt, 1984).

**6. Marination technologies**

Marinating is a traditional culinary technique, is used to tenderize and to improve the flavor and succulence of meat to satisfy consumer demand (Lemos *et al*., 1999). A marinade generally refers to a seasoned liquid in which meat, fish, and poultry are soaked to become tenderized and/or absorb flavor. A marinating solution can be so simple as salt, phosphate, and water, or more complex with flavor, seasoning, starches, vegetable or dairy proteins, acids, antimicrobial, and antioxidants (Brandt, 2003). The whole objective of the marination process is to uniformly disperse the functional ingredients in the marinade throughout the muscle. The marination process itself and the incorporation of the appropriate functional ingredients at effective levels are crucial to successful marination. Important processing parameters in marination that affect marinade pickup and retention are

- Marinade temperature is as low as possible.
- Marinade ingredients are completely in solution.
- Pressure and dwell time for needle injectors or appropriate mechanical action, also vacuum and time in the case or tumblers or massagers are adequate.

The major functional ingredients are phosphate and salt. Carrageenan, modified starch, and soy protein isolate have also been added to marinades to improve water binding, but these components by themselves would not generate the desired effect without the salt and phosphate. The latter marinade ingredients modify the myofibril,

the muscle fiber and the fiber bundle opening up spaces for water and the large molecular weight additives to be entrapped. There are several types of phosphates available and an appropriate blend for the type of muscle, hardness of water used in the marinade and time from marination to cooking must be selected (Hamm, 1975; Björkroth, 2005).

Furthermore, marinades are nowadays complex sauces which have a great effect on product appearance and taste. A marinade generally is a combination of herbs and spices mixed with oil and an acidic base such as vinegar, citrus juice, or wine. This combination produced delicious flavors to all kinds of meat (Johnson, 2009). In Finland, they are typically water–oil emulsions containing salt, sugar and acids (acetic, citric), rheology improving additives (such as xanthan and guar gum), antimicrobial agents (such as sorbate and benzoate), spices and aroma enhancers. The pH of these marinades is usually acidic, less than 5.0, so sugar is needed to take the edge off the acidic taste. Basic flavor is often obtained using pepper, onion and tomato base together with other added spices. There is a big selection of different flavors, including curry, Chinese-type, Italian-type, honey and barbecue marinades. The amount of marinade added on meat is variable among product types, 20–30% (w/w) being quite typical for meat strips (Björkroth, 2005).

Lemos *et al* (1999) reported the optimization on still-marinating process to increase weight gain, reduce weight loss during storage and reduce cooking loss. The results showed that marinating times ranging from 8-12 h, salt concentrations ranging from 3-4% and polyphosphates concentration ranging from 2-3% are recommended for the still-marinating process of chicken breast meat. In addition, marinating times ranging from 4-8 h, salt concentration ranging from 3-4% and polyphosphates concentration of about 2% can be suggested for the still-marinating process of chicken legs.

Xiong (2005) indicated that injected brine solution could diffuse into different parts of the meat; the kinetic process and ultimate distribution might be affected by phosphate compounds. Brine migration into chicken filets during tumble marination was highly dependent upon phosphate types. The presence of pyrophosphate and tripolyphosphate greatly facilitated ($P$<0.05) brine penetration when compared with the water-only control, and both phosphates remained effective throughout 30 min

marination period. In contrast, hexametaphosphate (HMP) promoted water pickup by chicken filets only for the first 5 min, with no additional benefit thereafter. Namely, the facile diffusion of pyrophosphate- or tripolyphosphate-containing brine into the muscle tissue resulted from an augmented expansion of the myofibril lattices, the dissociation of the actomyosin complexes, and the removal of the transverse structural myofibrillar proteins. HMP, being a bulky, cyclic compound, was relatively ineffective in promoting marinade penetration in marinated meat, probably due to its low diffusivity and to its lack of interaction with actomyosin.

## 7. Phosphate compounds

### 7.1 Classification and general chemical characteristics of phosphates

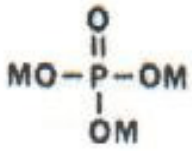
#### 7.1.1 The orthophosphates

The orthophosphate anion is the simplest structure and the basic unit for all phosphates. The phosphate anion is a tetrahedron in which the phosphorus atom around by 4 oxygen atom. The anion is tribasic, and its three valences can be satisfied by hydrogen, metal ion, or combinations of the two. It also can form straight-chain and cyclic polymers (Figure 5) (Ellinger, 1972).

#### 7.1.2 The condensed or polymeric phosphate

The simplest of the condensed phosphate anions is that of pyrophosphate. Its anion contains two phosphorus atom linked through a shared oxygen. The addition of another orthophosphate unit to the pyrophosphate anion forms the next higher straight-chain polymer known as tripolyphosphate. However, it is possible to produce a great variety of condensed phosphates having more than three phosphorus atom linked to each other by shared oxygen. These compounds exist as glassy, amorphous, or fibrous materials. Because they are mixture of a number of polyphosphate of varying chain lengths, they are frequently called or amorphous phosphates. The last form is cyclic metaphosphate, they are two cyclic sodium polyphosphates, but it is not commercially available (Figure 5) (Ellinger, 1972).

**Orthophosphate**



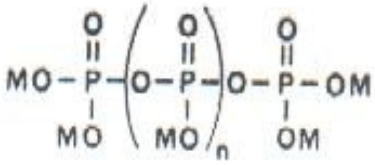
**Condensed phosphates**

Polyphosphates (straight-chain)

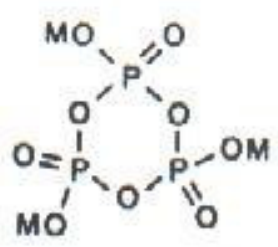
n = 0 pyrophosphate

= 1 tripolyphosphate

> 1 long chain phosphate



Metaphosphate (cyclic)



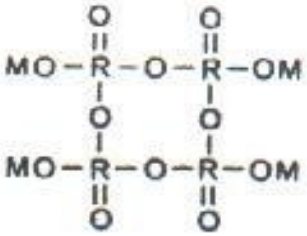


**Figure 5** Structures of phosphates

**Source**: Wong (1989)

Many reactions of the phosphates with components of food systems are explained by the tetrahedral structure of the phosphate anions. For example, this structure allowed the long-chain polyphosphates to coil in the shape of a helix and thus to undergo polyelectrolyte interactions with proteins and gums in food system. This structure also explains the ability of longer-chain polyphosphates to complex or sequesters the metallic ions in food systems (Ellinger, 1972).

**7.2 Phosphate applications in meat processing**

The addition of phosphates, particularly the polyphosphates from the pyrophosphates through the long-chain polyphosphates, or polyphosphate sodium chloride mixtures to meat can produce dramatic effects on tenderness and general palatability of the meat (Ellinger, 1972). Phosphates function by the sequestration of metal ions and the dissociation of the actomyosin complex bringing about on increase

in WHC. Another important reason for using phosphates is their ability to increase meat pH and to retard discoloration by chelating metal ion. However, the amount of phosphates in final product is limited to 5000 $mgkg^{-1}$ (expressed as $P_2O_5$) because they may chelate important metals ions (such as calcium and magnesium) (Ellinger, 1972; Dusêk *et al*., 2003). In practice, different phosphates or combinations of phosphates may be used to impart desired properties to the meat.

**7.2.1 Increasing moisture retention**

The moisture retention, often termed WHC or water binding ability, of muscle tissue is of considerable concern to the meat industry. The WHC of meat tissue increases as the pH of the tissue either decrease or increase away from the pI of approximately 5.0. Since freshly slaughtered meat gradually decreases in pH with the onset of rigor mortis, the WHC of the meat decreases rapidly after slaughter. The combined action of the salt and ATP causes the peptide chains of the protein to unfold; the unfolding leaves such large distances between these chains that bivalent cations, released through the breakdown of ATP, are unable to cross-link these chains. Water can reach the numerous hydrogen bonding sites necessary for complete hydration of the proteins, the polyphosphates are thought to be capable of increasing the WHC of muscle proteins through their ability to cause the expansion of the three-dimensional network of muscle muscle (Ellinger, 1972; Hamm, 1975).

Xiong and Kupski (1999a) explained that phosphates and salt, when used separately, were capable of promoting moisture absorption and reducing the cooking yield. Combination of the two ingredients produced no synergistic or additive, probably resulted from the high concentrations of both compounds being used. The increase of ionic strength resulted in a decrease of bound water and was related to the “salting out” effect involving strong binding of water by the salt and consequent dehydration of the proteins (Zayas, 1997). According to Xiong (2005) showed that the facile diffusion of pyrophosphate- or tripolyphosphate- containing brine into the muscle tissue resulted on expansion of the myofibril lattices, the dissociation of the actomyosin complexes, thereby permitting further water uptake in brine-treated meat.

In addition, Lee *et al* (1998) exhibited that using 0.5% sodium phytate, sodium pyrophosphate, and sodium tripolyphosphate, along with 1% sodium chloride in restructured cooked beef increased cook yield and moisture level ($P<0.05$) compared to control (1% salt). Moreover, tetrasodium pyrophosphate and sodium tripolyphosphate showed a minimum drip volume of mechanically separated seal meat at the 2.5% level (w/w), similarly to cooking yield after thermal processing for 1 h at 95°C. However, sodium hexametaphosphate-treated samples exhibited a minimum drip volume at a 3% level (w/w) of addition (Shahidi and Synowiecki, 1996).

**7.2.2 Color preservation**

The color of meat is dependent on the presence and the chemical reactions of two pigments, myoglobin and hemoglobin. Myoglobin found in muscle tissue, is an iron-protein complex. Factors that affect the color of meat are its pH, the presence of reducing substances, curing salt, and metal ions, and the exposure of meat to oxygen. Fresh aged meat normally has a pH of 5.6-6.6. Low pH values have been found to accelerate the oxidation of fresh meat pigments to form brown metmyoglobin pigments. Optimum stabilization of the redness of meat is obtained at the higher pH values of 6.0-6.6 (Ellinger, 1989).

Smith and Young (2005) showed that the use of phosphates in broiler breast meat greatly increases cook yield (which probably would also improve juiciness of the meat) and slightly lightened the meat. The same as reported by Allen *et al* (1998), to compare color properties of raw and marinated broiler breast fillets (3% sodium tripolyphosphate and 7% sodium chloride). The results showed that L* values were significantly higher and both a* and b* values were significantly lower for both light (L*>50.0) and dark (L*<45.0) marinated fillets than the respective unmarinated control fillets. However, Lee *et al* (1998) indicated that there was a high correlation ($r^2=0.958$) between %metmyoglobin values and Hunter color redness (a) values of raw beef rolls. Using sodium phytate, sodium pyrophosphate, and sodium tripolyphosphate resulted in lower L and b values and higher a values ($P<0.05$).

### 7.2.3 Increasing tenderness

The onset of rigor mortis causes a shortening of the muscle fibers as the contractile muscle proteins slide over each other to form complexes that are highly stabilized by hydrogen bonding. The ability of the phosphates to cause the dissociation of actomyosin into actin and myosin has an important effect on the tenderness of all type of meat (Ellinger, 1972; Trout and Schmidt, 1984; Xiong, 2005).

Zheng *et al* (2000) evaluated the effect of concentrations and types of phosphate on quality of poultry breasts. The results showed that the shear force of breast meat treated with sodium tripolyphosphate (STPP) at 38.0 g $kg^{-1}$ was lower than 12.5 or 25.0 g $kg^{-1}$ STPP. The shear force was about 1.4 times less than for aged-breasts and about 4 times less than for non-aged breasts ($P<0.05$), subsequent studies indicated that the shear force of phosphate treated breast was not significantly different for 38.0 g $kg^{-1}$ STPP, tetrasdium pyrophosphate (TSPP), and hexametaphosphate (GLASS) but it was lower than aged and non-aged breast ($P<0.05$). Additionally, Sheard and Tali (2004) showed that the shear force of injected 5% salt and 5% STPP pork loins was approximately the same, but they were lower than non-injected pork loins ($P<0.05$).

### 7.2.4 Flavor improvement

Phosphates can contribute to the flavor of meats, since they have such significant effects on the retention and other characteristics of the proteins. The proteins also contribute significantly to the development of proper meat flavor through their interaction with proteins and their use in synthetic meat-flavor compositions. In addition, the phosphates able to act as antioxidant synergists prevent oxidation of fats and thus prevent development of rancid flavors in the meat fats. Thus, their inhibition of the development of off-flavors has an important role in flavoring meat products (Ellinger, 1972).

Weilmeier and Regenstein (2004) reported that sodium tripolyphosphate (STPP), added before or after cooking, significantly reduces the formation of thiobarbituric acid-reactive substance (TBARS) only in cooked brook trout muscle throughout the storage period. Perhaps the fact that cooking might inactivate the phosphatases in the fish resulting in polyphosphate could not break down into simpler

phosphate, orthophosphates, which would expect that these orthophosphates have no antioxidant effect, so could explain why STPP was a good antioxidant in the cooked muscle. The results are in agreement with Cheng and Ockerman (2003) who found that roast beef without STPP had significantly highest TBARS values compared to other phosphate levels at day 4 of storage. At day 7, the addition of 0.5% STPP maintained the oxidative stability of pre-cooked roast beef better than those of 0.4% and 0.25% which maintained better TBARS than 0% STPP. Moreover, Lee *et al* (1998) showed that raw restructured beef treated with sodium phytate (SPT), sodium pyrophosphate (SPP), and sodium tripolyphosphate (STPP) had lower TBARS values than the control (1% NaCl). In cooked beef rolls, SPT, SPP, and STPP decreased TBARS formation, and the inhibitory effect of SPT was higher than other phosphate compounds ($P<0.05$).

**7.2.5 Prevention of microbiological spoilage**

An array of methods for reducing the load of potential pathogens on the surfaces of meat products has been developed. These methods use ionizing, organic acid spray, and phosphate (e.g. trisodium phosphate (TSP) and polyphosphate) dips or sprays to reduce the numbers of bacterial pathogens present on raw animal products following processing. TSP in generally recognized as safe by the Food and Drug Administration and has been approved by U.S. Department of Agricultural for use as a food ingredient and for the reduction of *Salmonella* contamination during poultry processing (Federal Register, 1996). As mentioned by Sampathkumar *et al* (2003), the possible modes of action of TSP include (i) exposing microorganisms to high pH, which might particularly affect cell membrane components, (ii) enhancing detachment of bacteria from food surfaces by sequestration of metal ions, and (iii) removing fat from the skin surface, thereby allowing bacteria to be washed from the food surfaces more effectively. However, increasing the concentration of TSP in the treatment solution from 1.5 to 2.5% resulted in a rapid loss of viability of serovar Enteritidis cell, with no detectable survivors after 1 hr (Sampathkumar *et al*., 2003). In addition, Yoon and Oscar (2002) stated that 10% (w/v) NaCl, trisodium phosphate, sodium tripolyphosphate, and tetrapotassium pyrophosphate washing on sterile ground chicken breast patties significantly lowered the survival populations of attached *S. typhimurium*, but did not cause any significant sub-lethal injury of attached *S.*

*typhimurium*, irrespective of storage treatments. During refrigerated storage for 16 days, frozen storage for 10 months, and after 3 freeze-thaw cycles, the TSP washes showed superior effects of removing and inactivated *S. typhimurium* compared to other washing treatments. Chicken breasts dipping in TSP either alone or in combination with NaCl was effective against the proliferation of aerobic microorganism, psychotrophic bacteria, and *Enterobacteriaceae*, therefore, could be utilized successfully to improve the microbial safety, and extend the shelf-life of chicken breast during refrigerated storage (Sallam and Samejima, 2004).

**8. Microbiology of poultry meat**

Microorganisms found on poultry can be divided into two general groups, those which can produce disease in humans, generally referred to as pathogens, and those not associated with a recognized disease which are designated as non-pathogenic organisms.

Poultry are known to be carriers of a number of organisms pathogenic to human. However, despite the large number of pathogenic organisms found in poultry which are transmissible to man, poultry is believed to play a minor role in the transmissible or diseases of human beings. Probably the main reason for the small incidence of human infection is because poultry meat is eaten only after thorough cooking. For non-pathogenic organisms which cause food spoilage produce spoilage by bringing about chemical changes in one or more of the three major nutrients: the carbohydrates, fats, and proteins; in other cases they bring about desirable changes such as fermentation reaction (Mountney and Parkhurst, 1995).

The amount of spoilage bacteria which related to characteristics of spoilage in poultry are explained by Jay (1996). As poultry undergoes spoilage, off-odors are generally noted before sliminess, with the former being first detected when log numbers/ $cm^2$ are about 7.2-8.8. Sliminess generally occurs shortly after the appearance of off-odors, with the log counts/$cm^2$ about 8. Total aerobic plate counts/$cm^2$ of slimy surface rarely goes higher than log 9.5. With the initial growth first confined to poultry surfaces, the tissue below the skin remains essentially free of

bacteria for some time. Gradually, however, bacteria begin to enter the deep tissues, bringing about increased hydration of muscle proteins (Jay, 1996).

Enterobacteriacae, coliforms, faecal coloforms, *Escherichia* coli, and enterococci) are part of the normal intestinal flora of poultry. Some will spread among carcasses even under good processing practices. Some strains of species that fall into the indicator organisms categories (such as certain coliforms and *Enterobacter*) are psychrotrophic and can multiply on refrigerated raw poultry carcasses and products. Microorganisms responsible for spoilage of poultry multiply during refrigeration, even at recommended temperatures of 1 to 4ºC. A commonly recommended microbiological method for estimating shelf-life of chilled poultry, an aerobic plate count (APC) at 0-5ºC, is impracticable for use a port-of-entry because it requires incubation for 10-14 days. A criterion for APC (20ºC), the acceptable limit associated with Good Commercial Practices (GCP) was $5\times10^5$ CFU/g and the limit of safety or quality was$10^7$ CFU/g. This criterion is achievable for processed chicken, but not necessarily turkeys, in modern processing plants that follow good processing practices (ICMSF, 1986).

**9. High acid marinades**

Marinating meat is essential for certain types of meat used to tenderize and also enhances the flavor of meat by the combination of herbs and spices mixed with oil and an acidic base such as vinegar, citrus juice, or wine (Johnson, 2009). Popular ethnic cuisines such as Latino, Asian, and Caribbean incorporate a variety of flavors, including citrus types. Although citrus flavors are gaining in popularity, they are more difficult to incorporate into a marinade system. Typically, marinades in meat processing that are highly acidic (pH below 5.0) generally included of lemon, lime, and orange flavor with blends of lemon pepper, orange coriander, lemon herb and cilantro lime (Brandt, 2003). The acidic in marinade caused the tenderizing was believed to involve several factors including weakening of structures due to swelling of the muscle, increased proteolysis by cathepsins and increased conversion of collagen to gelatin at low pH during cooking (Burke and Monahan, 2003). Tougher meats must marinate (or soak) for a long period of time, preferably overnight, cause a

rapid unfolding of the meat proteins leading to their denaturation. When the meat was cooked in which the proteins have been denatured, the resulting mushy texture allowed for moisture release, resulting in tough or chewy meat (Brandt, 2003; Johnson, 2009).

### 9.1 Tom-Yum characteristics

Tom-yum is characterized by its distinct hot and sour flavors, with fragrant herbs generously used. The basic broth is made of stock and fresh ingredients such as lemon grass, kaffir lime leaves, galangal, shallots, lime juice, fish sauce, tamarind, and crushed chilies. This research applied the garcinia (*Garcinia atroviridis*) instead of lime juice which displayed sour flavor in Tom-Yum paste (Siripongvutikorn *et al*., 2005).

Siripongvutikorn *et al* (2005) reported that garlic in Tom-Yum ingredients exhibited the highest antimicrobial effect on *Pseudomonas fluorescens* ATCC 49839, *Escherichia coli* O157:H7, *Staphylococcus aureus* ATCC 13565 and *Listeria monocytogenes*. While red chili and kaffir lime leaves were main sources of ß-carotene, which was a protector against photo-oxidative processes.

### 9.2 Chemistry of some spices in Tom-Yum

Spices are used to enhance the flavor and palatability of food which consumed in small quantities contained little or no nutritive values compared to vegetables. A complex mixture influences the overall odor quality. Some parts of the volatile oils of spices are lost during the processing of food. The important compounds are not so volatile and are entrapped by fat and proteins in food. In addition to their aroma and pungency factors, spices contain many different compounds such as fat and resin to give the natural flavor spices. Plants contain a variety of compounds called “secondary” plant compounds grouped as glycosides, soponins, tannins, alkaloids, essential oils, organic acids, and others (Ceylan and Fung, 2004).

Flavor compounds of the genus *Allium* (garlic, onion, leek and caucas) have various biologically active sulfer compounds. *Allium* spices were classified into three groups: high proportions of propyl/propenyl cysteinsulfoxide (e.g. onion), high proportions of allyl cysteinsulfoxide (e.g. garlic) and high methyl cysteinsulfoxide (e.g. ornamental *Allium* spices). Characteristic volatile flavor compounds from intact

garlic cloves are enzymatically produced when the garlic tissues are ruptured. The precursor compound alliin (S-allyl-L-cystein-S-oxide) is hydrolyzed by allinase enzyme that produces allicin (2-propenyl-2-propenethiol sulfinate; diallyl thiosulfonate) which contain the principal antimicrobial compound (Ceylan and Fung, 2004).

Sun dried slices of the Garcinia fruits, *Garcinia atroviridis* Griff. Ex T. Anders., are commercially available and are popularly used as a seasoning in curries and sour relish. It was found that, the extracts (methanol) of *G. atroviridis* exhibited strong antimicrobial, antioxidant and antitumour-promoting activities (Mackeen *et al*., 2000). As the finding of Mackeen *et al* (2000) who investigated the phytochemical of *G. atroviridis* by extraction of its fruits using GC-MS, and they identified substances into (-)-hydroxycitric acid, γ-lactone, atroviridin, atrovirisidone and atrovirinone as well as the identification of some organic acids, such as citric, pentadecanoic, octadecanoic, nonadecanoic and dodecanoic acids were found in fruit. Recently, Mackeen *et al* (2002) found two new garcinia acid derivatives, 2-(butoxycarbonylmethyl)-3-butoxycarbonyl-2-hydroxy-3-propanolidc and 1′1″-dibutyl methyl hydroxycitric, were isolated from the fruit of *G. atroviridis*. Both compounds showed selective antifungal activity only against *Cladosporium herbarum* but were inactive against bacteria, other fungi including the yeast *Candida albicans*.

Red pepper obtained from the fruit of *Capsicum frutescens* L. contains minimal quantities of essential oils; the soluble spice of red pepper is dependent solely on the oleoresin. The oleoresin of red pepper, as manufactured, contains 6.38% capsaicin, equivalent to one million Scoville units. The carotenoid pigments capsanthin, cryptoxanthin, carotene, and zeaxnthin contribute most of the color to red pepper (Farrell, 1990).

Lemongrass is unique in its strong, lemon-peellike aroma and flavor. The essential oil content ranges from 0.3 to 0.55%, 70 to 85% of which is citral, also present geraniol (Farrell, 1990).

### 9.3 Effect of spices on shelf-life of products

Apart from microbial spoilage, lipid oxidation is the primary process occurs in quality loss of muscle food. Food deteriorates and poisoning is still a concern for both consumers and food industry despite the use of various preservation methods. Herbal spices have been added to foods since ancient times, not only as flavoring agents, but also as folk medicine and food preservatives. In addition to imparting characteristic flavors, certain spices and herbs prolong the storage life of foods by preventing rancidity through their antioxidant activity, or through bacteriostatic or bacteriocidal activity (Chattopadhyay and Bhattacharyya, 2007).

Shobana and Naidu (2000) found that the degree of antioxidant effect of spices decreased in the order of cloves > cinnamon > pepper > ginger > garlic > mint > onion. The crude extracts (50% alcohol extract) of fresh spices might contain more than one antioxidant and could be responsible for significant inhibition of enzymatic lipid oxidation. The spices mixes (garlic, ginger, and onion) showed cumulative effect indicating synergistic antioxidant activity of spices. Furthermore, boiling garlic, ginger, cloves, cinnamon and pepper extracts at 100ºC for 30 min could retain the antioxidant activity, indicating that the spices constituents were resistant to thermal denaturation. El-Alim *et al* (1999) studied on effectiveness of many dried spices (10 g $kg^{-1}$, w/w) to inhibit lipid oxidation in both fresh and stored ground chicken meat. They found that while the order of potency of dried spices in inhibiting lipid oxidation was marjoram > wild marjoram > caraway > peppermint > clove > nutmeg > curry > cinnamon. Ready-to-cooked spice mix has been studied for their shelf-life (Cheah and Hasim, 2000; Modi et al., 2006). Modi *et al* (2006) indicated that the spice mixed packed in glass bottle is shelf-stable for 6 months at storage temperatures of 27±2 or 37±2 °C. The mean sensory scores for chicken fry (mixed with spice mix) were in the range of 8.3-8.6 which gradually decreased in 6 months to 7.2-7.5 and to 6.8-7.1 for spice mix stored at 27±2 or 37±2 °C, respectively. Moreover, the standard plate count in spice mix increased slowly during 6 months storage and the increase was less than 1 log CFU/g. In addition, Cheah and Hasim (2000) indicated that galangal extract (5 and 10% from crude extract) showed antimicrobial activity in cooked beef, and 10% galangal extract was effective in inhibiting/ minimizing lipid oxidation in minced beef during storage at 4±1°C.

**Objectives**

1. To investigate the effect of phosphates on chemical and physical characteristics and microstructure of spent hen muscle in high acid condition
2. To study the effect of phosphate treatment on quality of spent hen muscle marinated with Tom-Yum paste and Tom-Yum ingredients
3. To study the shelf-life, chemical and physical changes of spent hen muscle marinated with Tom-Yum during refrigerated storage

# CHAPTER 2

# MATERIALS AND METHODS

## 1. Materials

### 1.1 Spent hen muscle sample preparation

The spent hens (ISA brown) aged approximately 80-85 weeks were obtained from a local farm in Phatthalung Province. They were slaughtered conventionally at a slaughter-house and carcasses were chilled in a cold room (4°C) for 24 h. Then the spent hen breast muscle (*Pectoralis major*) was dissected from the carcasses. The obvious fat and connective tissue were removed. The fresh breast muscles were kept in polyethylene bag at 4°C before marinating process (section 3.1). The other parts of spent hen breast muscle were frozen and stored at -20°C for further study (section 3.2 and 3.3). All muscles were cut into size approximately 2x5x0.5 $cm^3$ /slice parallel to the muscle fiber.

Raw spent hen muscle was determined for moisture, protein, fat, and ash contents according to AOAC method (AOAC, 1999). The values were expressed as % (wet weight basis).

### 1.2 Tom-Yum paste preparation

Tom-Yum ingredients consisting of fresh spices (lemon grass, galangal, kaffir lime, coriander root, chilli, garlic, red onion, and garcinia), salt, and sugar were obtained from local market. Tom-Yum paste was prepared by mixing all crushed spices, salt and sugar which was formulated by Siripongvutikorn *et al* (2005).

### 1.3 Chemicals

Phosphates including of disodium orthophosphate (DSP), tetrasodium pyrophosphate (TSPP), and sodium tripolyphosphate (STPP) and citric acid were purchased from High Science, Ltd. Partnership (Songkhla, Thailand) as Food Grade types. Other chemicals were analytical grade. Ammonium molybdate, magnesium chloride, potassium sodium tartrate, sodium acetate, and trisodium citrate were obtained from Ajax Finechem (Wellington, Auckland, New Zealand). Sodium azide and hydrazine sulfate were obtained from Asia Pacific Specialty Chemicals Limited

(Seven Hills, New South Wales, Australia). Glutaraldehyde, propyl gallate, malonaldehyde tetrabutylammonium salt, L-hydroxyproline and albumin from bovine serum were obtained from Fluka Chemical Corp. (Milwaukee, WI, USA). Trichloroacetic acid was obtained from Carlo Erba Reagenti (Strada Rivoltana, Rodano, Italy). Sodium chloride, potassium chloride, calcium chloride, and chloramine T were obtained from Merck Ltd. (Darmstadt, Germany). β-mercaptoethanol (β-ME) and *p*-dimethylamino-benzaldehyde were obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Sodium dodecyl sulfate (SDS) and N,N,N',N'-tetramethyl ethylene diamine (TEMED) were obtained from Bio-Rad Laboratories (Hercules, CA, USA).

**2. Instruments**

Instruments used in this experiment were listed in Table 3.

**Table 3** Instruments used in the experiment

| Instruments | Model | Company/City/Country |
|---|---|---|
| - pH meter | SevenGo SG2 | Mettler Toledo, Schwerzenbach, Switzerland |
| - Homogenizer | PT-MR 2100 | Polytron Kinematica AG, Littau-Lucerne, Switzerland |
| - Water bath | W350 | Memmert, Schwabach, Germany |
| - Refrigerated centrifuge | RC-5B plus | Sorvall, Norwalk CT, USA |
| - Colorimeter | ColorFlex | HunterLab Reston, VA, USA |
| - Texture analyzer | TA-XT2i | Stable Micro Systems, Surrey, UK |
| - Scanning electron microscope | JSW-5200 | JEOL, Akishima, Japan |
| - Differential scanning calorimeter | DSC7 | Perkin Elmer, Calofornia, USA |
| - Electrophoresis apparatus | Mini-Protein II | Bio-Rad, CA, USA |
| - Spectrophotometer | UV-16001 | SHIMADZU, Kyoto, Japan |

## 3. Methods

### 3.1 Study on the effect of phosphate types on chemical and physical characteristics of spent hen muscle in high acid condition

#### 3.1.1 Sample treatments preparation (Figure 6)

Phosphate solutions consisting of disodium orthophosphate (DSP), tetrasodium pyrophosphate (TSPP), and sodium tripolyphosphate (STPP) were prepared at 3% concentration. Breast muscle slices were soaked in various phosphate solutions with a ratio of 1:2 (muscle: solution) at 4°C for 13 h. After that, the muscle slices were drained on sieve for 5 min. A part of muscle slices soaked in each phosphate solution was randomly taken while another part were soaked in 0.1% citric acid solution (pH~2.93; mimic the Tom-Yum condition) for 1 h and drained on sieve for 5 min. The control treatments were raw spent hen muscle without treatment (control 1), muscle slices soaked in water with a ratio of muscle: water to 1: 2 for 13 h at 4°C (water), and muscle soaked in 0.1% citric acid solution for 1 h (control 2). All treatments and controls were stored at 4°C for physical and chemical analyses. The physical characteristics were determined within 3 days of storage.

#### 3.1.2 Analyses

##### 3.1.2.1 Weight gain

Weight gain of muscle slices after soaking in phosphate solution with and without citric acid soaking was determined as described by Xiong and Kupski (1999a). Immediately after marination, slices were drained on a sieve for 5 min and weighed. Weight gain was calculated in grams per 100 g of muscle (%), based on the weight of muscle before ($wt_{before}$) and after marination ($wt_{after}$). The measurements were conducted in ten replications.

$$\text{Weight gain} = (wt_{after} - wt_{before}) / wt_{before} \times 100$$

##### 3.1.2.2 Cooking loss

Cooking loss of muscle slices was determined as described by Murphy and Marks (2000), with slight modification. After heating in water bath (Memmert, W350, Germany) at 80°C for 15 min, samples were cooled in iced water for 10 min. Muscles were removed from the sealed-plastic bag and blotted with a paper towel to remove excess surface moisture and weighed. Cooking loss was

calculated from the weight of slices before and after cooking. The measurements were done in ten replications.

**3.1.2.3 Warner-Bratzler shear force measurement**

Raw and cooked muscle slices were measured for shear force values using the Texture Analyzer equipped with a Warner-Bratzler shear apparatus (Stable Micro System, TA-XT 2i, UK). The operating parameters consisted of a cross head speed of 2 mm/s and a 25 kg load cell (Wattanachant *et al*., 2005). The shear force perpendicular to the axis of muscle fibers was measured in ten replicates for each treatment. The peak of the shear force profile was regarded as the shear force value (Newton).

**3.1.2.4 Surface color measurement**

Raw muscle slices were measured in ten replicates at the inner surface of breast muscle using a Hunterlab colorimeter (ColorFlex, Hunter Lab Reston, USA) and reported as the complete International Commission on Illumination (CIE) system color profiles of lightness (L*), redness (a*), and yellowness (b*).

**3.1.2.5 Differential scanning calorimetry (DSC)**

DSC was performed on a Perkin Elmer DSC7. Thermal analysis was described by Kijowski and Mast (1988a). Minced muscle (10-15 mg) was weighed in aluminum pan which were then sealed with a crimper. In DSC studies, empty aluminum pans were used as a reference and for baseline corrections. Gallium (T=29.8°C, ΔH 80.22 J/g) and Indium (T=156.6°C, ΔH 28.46 J/g) were used as the standards for temperature and enthalpy calibration. The heating conditions were programmed and fully controlled by microcomputer over the range of 20-90°C. A heating rate was set at 10°C/min. The peak transition temperature (Tp) and enthalpy of transition (ΔH) were calculated using a Perkin Elmer Thermal Analysis Data Station.

**3.1.2.6 pH measurement**

The pH was determined directly in the muscle as described by Burke and Monahan (2003) using pH meter (SevenGo SG2, Mettler Toledo, Switzerland). Minced muscle (5 g) was homogenized in 50 ml distilled water for 1 min at 13500 rpm with the Polytron homogenizer (Polytron Kinematica AG, Littau-Lucerne, Switzerland).

### 3.1.2.7 Total collagen and heat-soluble collagen content

Soluble collagen content of muscle was determined by the method of Lui *et al* (1995). Minced breast muscle (2 g) was homogenized for 1 min at 13500 rpm in a 4-fold volume of ¼ strength Ringer's solutions with the Polytron homogenizer (Polytron Kinematica AG, Littau-Lucerne, Switzerland). The homogenate was heated for 30 min at 77°C, and then centrifuged for 30 min at 2000xg with the Sorvall centrifuge (Sorvall, Norwalk CT, USA). The supernatant was decanted, and then the pellet was suspended with the same solution and re-centrifuged. The supernatant solutions were combined and hydrolyzed in 6 N HCl for 24 h at 110°C. For total collagen content, minced breast muscle (0.5 g) was hydrolyzed in 6 N HCl. After clarification with active carbon and neutralization with 10 N NaOH, the amount of hydroxyproline (Hyp) was determined as described by Bergman and Loxley (1963). The amount of heat-soluble collagen was expressed as a percentage of the total amount of collagen. The total amount of Hyp was converted to collagen by a factor of 7.25. The content of collagen was expressed as mg of collagen /g of muscle.

### 3.1.2.8 Myofibrillar protein solubility

Myofibrillar protein of muscle was determined by the procedure of Roussel and Cheftel (1990) with slight modification. Two-gram of minced muscle was added to 20 ml of 0.6 M KCl (for myofibrillar protein) or 0.5 M NaOH (for total protein solubility) and agitated with a magnetic stirrer for 4 h at room temperature. Samples were centrifuged at 12100xg for 30 min at 4°C with a refrigerated centrifuge (Sorvall, Norwalk CT, USA). To the supernatant (4 ml), cold 50% (w/v) TCA was added to a final concentration of 10%. Sample were kept at 4°C for 18 h and then centrifuged at 2500xg for 20 min. The precipitate was solubilised in 0.5 M NaOH. Protein contents were determined by the Biuret method using BSA as a standard. Solubility was expressed as the percentage of total protein extracted by 0.5 M NaOH.

### 3.1.2.9 Phosphate content (as $P_2O_5$)

Phosphate content was determined as $P_2O_5$ by the method of Fiske and Subbarow (1925). Ten-gram of minced breast muscle was homogenized in 20 ml of 10% TCA for 5 min at 13500 rpm with Polytron (Polytron Kinematica AG, Littau-Lucerne, Switzerland). The homogenates were filtrated with Whatman #1 filter

paper and eluted with 10 ml of TCA. The filtrate was neutralized to pH 4.5-5, then made up to 50 ml volume with distilled water. One-milliliter of solution was transferred to 25 ml volumetric flask, and made up to volume with distilled water. The extract (1 ml), distilled water (9 ml) and 1.5% ammonium molybdate (1 ml) was added into test tube and mixed thoroughly. The mixture was heated for 10 min in a boiling water bath, then mixed 1 ml of 1% hydrazine sulfate and heated again for 10 min in a boiling water bath to develop a blue color, cooled with ice water for 20 min. the sample solutions were measured spectrophotometrically at 830 nm. The concentration of phosphate was calculated using the standard curve prepared from potassium phosphate ($KH_2PO_4$).

### 3.2 Study on the effect of soaking time on chemical and physical characteristics of spent hen muscle in high acid condition

#### 3.2.1 Sample treatments preparation

Proper phosphate type was selected from section 3.1 based on water holding capacity. Thereafter muscle slices were soaked in the phosphate solution for 7, 10, and 13 h at 4°C and subjected to soaked in 0.1% citric acid solution as the system of section 3.1.

#### 3.2.2 Analyses

The physical and chemical analyses were determined the same parameters as described in section 3.1.2 except the DSC analysis.

### 3.3 Study on effect of phosphate concentrations on chemical and physical characteristics of spent hen muscle in high acid condition

#### 3.3.1 Sample treatments preparation

The muscle slices were soaked in 2%, 3%, and 4% of the selected phosphate solution for the soaking time which obtained from section 3.2 based on water holding capacity. Each phosphate pretreated muscle were soaked in citric acid solution as mentioned previously. All samples before and after citric acid soaking treatments were kept in zip-locked polyethylene bags at 4°C before physical and chemical analysis as described previously. The control treatments were raw muscle without treatment (control 1), muscle soaked in water for 10 h (water), and muscle soaked in 0.1% citric acid solution for 1 h (control 2).

### 3.3.2 Analyses

The physical and chemical analysis were determined the same parameters as described in section 3.1.2 except the DSC analysis.

## 3.4 Study on microstructure of spent hen muscle pretreated with the proper phosphate solution in high acid condition

### 3.4.1 Sample preparation

The spent hens (ISA brown) aged approximately 80-85 weeks were obtained from Betagro Group Public Company Limited, Songkhla Province, Thailand. They were slaughtered, chilled, and dissected breast muscle (*Pectoralis major*) from the carcasses as mentioned in section 1.1. The obvious fat and connective tissue were removed. A part of breast muscles was subjected to freeze and storage at -20ºC for further study (section 3.5). All spent hen breast muscles were cut into size approximately 2x5x0.5 $cm^3$ /slice parallel to the muscle fiber direction.

Four treatments of spent hen muscle were prepared as followed:

- Raw spent hen muscle without treatment (control)
- Muscles were soaked in 0.1% citric acid solution without phosphate pretreatment
- Muscles were soaked in only phosphate solution which selected from section 3.3
- Muscles pretreated with the phosphate solution was soaked in 0.1% citric acid solution

### 3.4.2 Determination of the microstructure of muscle

The microstructure of spent hen breast muscle was determined by scanning electron microscopy as described by Palka and Duan (1999). The pieces 1x1x0.5 cm were excised from muscle slices and fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.3) for 2 h at room temperature. The specimens were then rinsed with distilled water and dehydrated in 25%, 50%, 70%, 95%, and absolute ethanol (twice) for 1 h in each solution. The samples were cut in liquid nitrogen and critical point dried using liquid carbon dioxide. The fragments of dried specimens were mounted on aluminum stubs and coated with gold. The specimens were examined and photographed in a SEM (JEOL, JSW-5200, Japan), using an accelerating voltage of 10 kV and a working distance of 10-13 mm. The micrographs and video-prints were taken at magnification of x500 for transverse sections. Three

micrographs of each sample were taken and ten measurements of fiber area were made.

**3.5 Study on effect of phosphate and Tom-Yum ingredients on quality changes of marinated spent hen muscle during refrigerated storage**

**3.5.1 Sample preparation**

The spent hen breast muscles were thawed overnight at 4ºC. The muscle slices were prepared as mentioned in section 1.1. After that, muscles were cut into size approximately 2x5x0.5 $cm^3$ /slice parallel to the muscle fiber direction. Raw spent hen muscle was randomly determined the proximate compositions for moisture, protein, fat, and ash contents according to AOAC method (AOAC, 1999). The values were expressed as % (wet weight basis).

Muscle slices were soaked in the selected phosphate solution (results from section 3.3) with a ratio of 1: 2 (muscle: phosphate solution) (w/v) at 4°C for 10 h. After that, muscle slices were drained on sieve for 5 min and kept in zip-locked polyethylene bag at 4ºC for marinating process.

**3.5.2 Marination conditions**

The muscle slices pretreated with and without the phosphate solution were marinated with Tom-Yum ingredients and Tom-Yum paste (prepared as mentioned in section 1.2). The treatments were assigned as shown in Table 4. All treatment samples were put on plastic trays and placed into laminated bag (Nylon/LLDPE) with vacuum seal, then samples were stored at 4°C for 30 days before subjected to analyze the microbiological, physical, and chemical changes every 5 days (day 1, 5, 10, 15, 20, 25, and 30).

**Table 4** Treatments of Tom-Yum marinated spent hen muscle pre-treated with and without selective phosphate solution

| Treatments | Symbol |
|---|---|
| Raw spent hen muscle (without marination) | C1 |
| Muscle without phosphate marinated with Tom-Yum paste | C2 |
| Muscle pretreated with phosphate marinated with salt and sugar | P1 |
| Muscle pretreated with phosphate marinated with garcinia, salt, and sugar | P2 |
| Muscle pretreated with phosphate marinated with Tom-Yum paste except garcinia | P3 |
| Muscle pretreated with phosphate marinated with Tom-Yum paste | P4 |

### 3.5.3 Analyses

The muscle slices of all marinated treatments were rinsed in water for 3 sec. Then muscle slices were blotted the excess surface moisture with a paper towel and wiped off the Tom-Yum ingredients and then bought to chemical and physical analyses.

#### 3.5.3.1 Mesophilic and psychrophilic bacteria

Twenty five-gram of each treatment samples was blended aseptically with 225 ml of 0.1% (w/v) sterile peptone solution for 2 min. The pour plate method (ICMSF, 1986) was used to determine the mesophilic and psychrophilic bacteria with Standard Plate Count Agar (Difco) in suitable serial dilutions. The colonies of mesophilic and psychrophilic were count from the duplicate plate after incubation at 35°C for 48 h and 4°C for 8 to 10 days, respectively, as log colonies-forming units (CFU) per gram.

#### 3.5.3.2 Cooking loss

Cooking loss of muscle slices was determined as described in section 3.1.2.2 by Murphy and Marks (2000).

#### 3.5.3.3 Warner-Bratzler shear force measurement

Raw and cooked muscle slices were measured shear force using the Texture Analyzer equipped with a Warner-Bratzler shear apparatus (Stable Micro System, TA-XT 2i, UK) as mentioned in section 3.1.2.3.

**3.5.3.4 Surface color measurement**

Raw muscle slices were measured in ten replicates using a Hunterlab colorimeter (ColorFlex, Hunter Lab Reston, USA) and reported as the complete International Commission on Illumination (CIE) system color profile of lightness (L*), redness (a*), and yellowness (b*).

**3.5.3.5 Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)**

Minced muscle (3 g) was homogenized in 27 ml of 5% (w/v) SDS at 11000 rpm for 60 s with a homogenizer. The mixture was incubated at 85ºC for 1 h, the extract was then centrifuged at 6100xg for 10 min. The protein content of the supernatant was analyzed according to the Biuret method. SDS-PAGE was carried out by the method of Laemmli (1970). The supernatants were mixed at a ratio of 1:1 (v/v) with the SDS-PAGE sample buffer containing 1% β-ME and boiled for 3 min. The samples (20 µg) were loaded on to the gel made of 4% stacking and 10% separating gels and then subjected to electrophoresis using a minivertical Bio-Rad apparatus (Bio-Rad Laboratories Pty LTd, Reagents Park, Australia). After electrophoresis, the gels were stained with 0.02% Coomassie Brilliant Blue R-250 in 50% methanol and 7.5% acetic acid and destained with 50% methonal and 7.5% acetic acid for 30 min, followed by destaining with 5% methanol and 7.5% acetic acid for 24 h.

**3.5.3.6 pH measurement**

The pH was determined directly in the muscle as described in section 3.1.2.6 by Burke and Monahan (2003) using pH meter (Mettler Toledo, SevenGo SG2-FK2, Switzerland).

**3.5.3.7 Thiobarbituric acid-reactive substances (TBARS)**

The TBARS was determined according to Wang *et al* (2002) with a slight modification. Ten-gram of minced sample was homogenized in 80 ml extract solution (containing 7.5 g trichloroacetic acid, 0.1 g propyl gallate, and 0.1 g EDTA take volume to 100 ml) using the Polytron homogenizer (Polytron Kinematica AG, Littau-Lucerne, Switzerland) for 1 min. The homogenate was centrifuged at 7000 xg for 5 min, the supernatant (2 ml) was mixed with 2 ml of 80 mM TBA solution. The mixture was incubated at 40°C for 90 min. the absorbance was read at 532 nm,

then calculated the value of TBARS using the standard curve constructed from different concentrations of malonaldehyde.

**3.5.3.8 Total collagen and soluble collagen content**

Soluble collagen content of sample was determined by the method of Lui *et al* (1995) as mentioned in section 3.1.2.7.

**3.5.3.9 Phosphate content (as $P_2O_5$)**

Phosphate content was determined as $P_2O_5$ by the method of Fiske and Subbarow (1925) as mentioned in section 3.1.3.9.

**4. Statistics Analysis**

The completely randomized design (CRD) was applied to all experiments. The samples of each treatment replication were randomly taken for physical analysis in 10 replicate determinations and for chemical evaluation in triplicate determinations. Data were analyzed statistically using the SPSS Program for Window 15.0 as one way ANOVA. Significant difference between treatments was analyzed by Duncan's multiple range tests. Significance was accepted at the 5% probability level.

# CHAPTER 3

# RESULTS AND DISCUSSION

## 1. Effect of phosphate types on chemical and physical characteristics of spent hen muscle in high acid condition

### 1.1 Chemical characteristics

The effects of phosphate types including disodium orthophosphate (DSP), tetrasodium pyrophosphate (TSPP), and sodium tripolyphosphate (STPP) on chemical characteristics of spent hen breast muscle are shown in Table 5. The pH of 3% phosphate solutions was 8.88, 10.15, and 8.93 for DSP, TSPP, and STPP, respectively. For the pH of muscle treated with DSP, TSPP, and STPP was 7.34, 7.22, and 7.19, respectively, which was higher than pH of untreated raw muscle (5.92) and muscle soaked in water (5.89). After citric acid soaking, pH of control 2 decreased to 4.92 that was lower than isoelectric point of muscle (pI, approximately 5.0). While pH of muscle pretreated with DSP, TSPP, and STPP and then treated with citric acid decreased to 6.75, 6.42, and 6.35, respectively.

The myofibrillar protein solubility of raw spent hen muscle (control 1) was approximately 31.62% of total protein which was lower than the reports of other researchers. Murphy *et al* (1998) reported that the myofibrillar protein of chicken breast muscle was 62.63% of total protein. While Lan *et al* (1995) found that chicken breast muscle was 56.2% of total protein (base on solubility). Wattanachant (2003) observed that Thai indigenous chicken muscles had lower myofibrillar and sarcoplasmic protein fraction and higher stroma protein compared with that of broiler muscles. The lower myofibrillar protein solubility in this study was found differently from other reports might be contributed to the different sources, age and breeds of chicken. The older age might attribute to the lower content of myofibrillar and sarcoplasmic protein (Wattanachant, 2003). The myofibrillar protein solubility of muscle treated with phosphates before citric acid soaking was significantly greater than without phosphate treatments (control1 and water) ($P<0.05$). It was due to phosphates increased functionality of muscle protein mainly by increasing ionic

strength and pH (Trout and Schmidt, 1984). It was found that myofibrillar protein solubility of muscle treated with TSPP was higher than those of DSP and STPP, respectively ($P<0.05$). Xiong *et al* (2000) indicated that pyrophosphate (PP) and tripolyphosphate (TPP) performed similarly in promoting protein extraction while orthophosphate had no apparent effect, which likely due to pyrophosphate induced change in protein extraction by starting from the both side of the A-band (thick filament) to dissociate the actomyosin complex would enable the myofibril lattices to expand, thereby allowing an increased water uptake. While the protein extraction pattern of orthophosphate was extracted throughout the A-band similar to non-phosphate control sample (Xiong *et* al., 2000). After citric acid soaking, muscle pretreated with all phosphate solutions had more solubility of myofibrillar protein than control 2 ($P<0.05$). In addition the myofibrillar protein solubility of muscle pretreated with STPP and DSP was higher than that of TSPP ($P<0.05$).

It was found that heat-soluble collagen of spent hen muscle (control 1) was 15.23% of total collagen which corresponded to the finding of Vaithiyanathan *et al* (2008) who reported that the soluble collagen of White Leghorn spent hen on 7 days of postmortem ageing was 14.7% of total collagen. The heat-soluble collagen of muscle treated with TSPP and STPP was decreased when compared to control 1 ($P<0.05$) (Table 5). This was probably due to the phosphate-binding to collagen in relationship to precipitation. Weinstock *et al* (1967) indicated that collagen was precipitated much more rapidly and completely from solution in phosphate buffer than in any of the other salt solution. In addition, muscle soaked in water (water) showed the lower soluble collagen ($P<0.05$) than that of control 1 as the same as muscle soaked in TSPP and STPP. Heat-soluble collagen of raw and all phosphate-pretreated muscle significantly increased after soaking in citric acid solution. This explained probably due to the acid treatment disrupted the non-covalent intermolecular bond (hydrogen bonds and dipole or ion-pair interactions and intermolecular cross-links) of intramuscular connective tissue (Voulita, 2009; Oreskovich *et al*., 1992). The control 2 and muscle pretreated with STPP had the highest soluble collagen content compared to DSP and TSPP, respectively ($P<0.05$). Lepetit (2008) concluded that the decrease in meat pH produced a swelling of both connective tissues and muscle fiber. The swelling of collagen fibers affected the

volumed percentage of collagen in fibers which related to the theory of rubber-like elasticity. The stress developed by the fiber was contrastively functioning to the volume fraction of collagen in the denatured collagen fibers resulting in more collagen solubility in high acid condition.

Phosphate content was determined and referred to the amount of phosphate diffused into the muscle (Table 6). The diffusion of phosphate was higher in muscle treated with STPP, DSP, and TSPP ($P<0.05$), respectively. After citric acid soaking, phosphate content of all treatments decreased because of leakage of phosphates during marinating process (Thorarinsdottir *et al*., 2001). Among muscle treated with phosphate solutions, the lowest phosphate content was found in muscle treated with TSPP leading to the lowest myofibrillar protein solubility and heat-soluble collagen content after citric acid soaking. The least diffusion of TSPP into muscle might less improving the functional properties of muscle protein.

**Table 5** Effect of phosphate types on chemical characteristics of spent hen breast muscle before and after soaking in citric acid solution

| Treatments | | pH | Myofibrillar protein solubility (% of total protein) | Soluble collagen (% of total collagen) | Phosphate content (as $P_2O_5$, ppm) |
|---|---|---|---|---|---|
| Before citric soaking [1] | Control 1 | 5.92±0.02 $^{c}$ | 31.62±1.72 $^{d}$ | 15.23±2.79 $^{a}$ | 1558.43±87.42 $^{d}$ |
| | Water | 5.89±0.02 $^{c}$ | 30.99±1.54 $^{e}$ | 9.63±0.87 $^{b}$ | 1308.97±121.02 $^{d}$ |
| | DSP | 7.34±0.03 $^{a}$ | 44.45±1.95 $^{b}$ | 15.07±2.44 $^{a}$ | 4271.24±273.44 $^{b}$ |
| | TSPP | 7.22±0.05 $^{b}$ | 48.29±1.39 $^{a}$ | 10.05±2.87 $^{b}$ | 3155.17±108.96 $^{c}$ |
| | STPP | 7.19±0.02 $^{b}$ | 40.22±2.09 $^{c}$ | 7.98±2.22 $^{b}$ | 4806.22±58.19 $^{a}$ |
| After citric soaking [2] | Control 2 | 4.92±0.02 $^{D}$ | 32.73±0.86 $^{C}$ | 51.70±2.45 $^{A}$ | 1546.19±31.25 $^{C}$ |
| | DSP-C | 6.75±0.03 $^{A}$ | 48.23±0.64 $^{A}$ | 41.59±2.13 $^{B}$ | 2727.07±123.84 $^{B}$ |
| | TSPP-C | 6.42±0.04 $^{B}$ | 43.34±1.82 $^{B}$ | 35.61±2.51 $^{C}$ | 2587.97±95.94 $^{B}$ |
| | STPP-C | 6.35±0.02 $^{C}$ | 52.03±4.88 $^{A}$ | 51.27±3.17 $^{A}$ | 3065.75±23.59 $^{A}$ |

$^{a,b,c,d,e}$ Means within columns before citric soaking treatment with differing superscripts are significantly different at $P<0.05$

$^{A,B,C,D}$ Means within columns after citric soaking treatment with differing superscripts are significantly different at $P<0.05$

[1] Breast muscle soaked in 3% phosphate solutions for 13 h; control 1 = raw muscle without treatment, water = water soaking for 13 h, DSP = disodium orthophosphate, TSPP = tetrasodium pyrophosphate, STPP = sodium tripolyphosphate

[2] Breast muscle soaked in 0.1% citric acid solution for 1 h after soaking in phosphate solutions 13 h; control 2 = citric acid soaking without phosphate

**1.2 Physical characteristics**

The effects of phosphate types on physical characteristics of spent hen breast muscle are shown in Table 6. Muscle treated with phosphates showed dramatically higher weight gain than did the untreated muscle (water soaking). This result related to the final pH of those muscles after marination far away from the pI of muscle which would expand the myofibril lattices thereby permitting further water uptake and immobilization of added water in meat (Hamm, 1975; Xiong *et al*., 2000). The muscle with pH was greater or less than the pI could be progressively increased in weight, in contrast, the muscle pH approached the pI did not exhibit the weight gain (Oreskovich *et al*., 1992). The weight gain of all phosphate treatments was not significantly different ($P \geq 0.05$). The highest cooking loss was found in sample soaked in water ($P < 0.05$), which was attributed to release of fluid (dilute salt solution) of muscle (Hamm, 1986). The result of cooking loss was related to the phosphate content of muscle (Table 6).

Muscle soaked in TSPP which had the lowest phosphate content showed significantly higher cooking loss compared to those soaked in DSP and STPP ($P < 0.05$). This pointed out that phosphate content diffused into muscle did not enough to improve the WHC of muscle. Muscles obtained from DSP and STPP treatments were significantly lower cooking loss than control1. After citric acid soaking, muscle pretreated with STPP had significantly higher weight gain and lower cooking loss compared to control2 ($P < 0.05$). This is probably due to polyanion of STPP could attach to positively charged groups of proteins, while the rest of the molecule can attract water molecules and increase the WHC (Shahidi and Synowiecki, 1996).

Muscle slices treated with all phosphate solutions had shear force values higher than control 1. It meant that all phosphates could diminish toughness of muscle as elucidated by lower shear values than the control. After citric acid soaking, muscle pretreated with phosphates did not influence on shear values compared with control 2 ($P \geq 0.05$). Shear force values of samples after cooking were lower than that of raw samples because of the conversion of collagen to gelatin during cooking (Burke and Monahan, 2003). The toughness of meat was highest where the pH value closet to the pI of meat (Medyński *et al*., 2000). Zheng *et al* (2000) showed that the shear force values were significantly reduced by phosphate treatments (sodium tripolyphosphate,

tetrasodium pyrophosphate, and hexametaphosphate) in injected-marinade of raw chicken breast. There was no significant difference in shear force values of all samples ($P \geq 0.05$) although the heat-soluble collagen of samples after acid soaking increased (Table 5). This might be attributed to the effect of acid solution on myofibrillar protein denaturation occurred greater than the solubility of collagen (Mikel *et al*., 1996).

Phosphate treatments significantly decreased color L* (lightness) and b* values (yellowness) ($P<0.05$) but did not affect on a* value (redness) ($P \geq 0.05$) of muscle compared to control1 and water soaking treatment. This data conflicted with the finding of Allen *et al* (1998), who compared color properties of raw and marinated (3% STPP and 7% NaCl) broiler breast fillets. Those results showed that marinated breast fillets had significantly higher L* but lower a* and b* values compared to unmarinated control fillets. Among phosphate treatments in before and after citric acid soaking, muscle treated with STPP had significantly lower L* and b* values compared to those treated with DSP and TSPP. This was probably due to the higher pH and ionic strength of STPP (pH~7.5) could swell the muscle proteins and alters light reflection resulting in darker muscle color (Önenç *et al*., 2004). The surface color of muscle treated with phosphates were obviously soft grey color leading to lower L*, a*, and b* values compared to controls.

**Table 6** Effect of phosphate types on physical characteristics of spent hen breast muscle before and after soaking in citric acid solution

| Treatments | | Weight gain (%) | Cooking loss (%) | Shear force (N) | | Surface color | | |
|---|---|---|---|---|---|---|---|---|
| | | | | Raw muscle | Cooked muscle | L* | a* | b* |
| Before citric soaking [1] | Control 1 | - | 25.85±2.28 $^{b}$ | 41.51±11.49 $^{b}$ | 35.11±3.46 $^{a}$ | 42.38±1.88 $^{b}$ | -2.13±0.49 | 5.85±0.82 $^{a}$ |
| | Water | 2.40±1.43 $^{b}$ | 42.56±3.02 $^{a}$ | 53.63±12.95 $^{ab}$ | 30.61±4.68 $^{ab}$ | 48.76±1.07 $^{a}$ | -1.87±0.44 | 6.68±0.76 $^{a}$ |
| | DSP | 32.34±6.87 $^{a}$ | 18.42±1.84 $^{c}$ | 58.61±11.05 $^{a}$ | 26.65±4.92 $^{b}$ | 37.44±1.01 $^{c}$ | -2.26±0.29 | 2.88±0.49 $^{b}$ |
| | TSPP | 28.27±5.75 $^{a}$ | 24.94±2.52 $^{b}$ | 57.28±11.55 $^{a}$ | 31.49±5.80 $^{ab}$ | 38.73±0.67 $^{c}$ | -2.03±0.28 | 1.16±0.84 $^{b}$ |
| | STPP | 30.61±6.14 $^{a}$ | 20.03±1.57 $^{c}$ | 53.74±12.09 $^{ab}$ | 27.94±2.85 $^{b}$ | 35.37±1.92 $^{d}$ | -2.13±0.35 | -0.32±2.12 $^{c}$ |
| After citric soaking [2] | Control 2 | 3.02±0.62 $^{B}$ | 41.24±1.46 $^{A}$ | 41.25±11.43 | 39.43±6.80 | 49.67±1.12 $^{A}$ | -1.48±0.37 $^{A}$ | 6.32±0.60 $^{A}$ |
| | DSP-C | 4.64±0.37 $^{B}$ | 36.42±4.24 $^{C}$ | 51.89±13.62 | 33.22±5.28 | 39.52±0.98 $^{C}$ | -2.31±0.23 $^{C}$ | 3.04±0.84 $^{B}$ |
| | TSPP-C | 4.10±2.35 $^{B}$ | 40.02±2.70 $^{AB}$ | 50.24±13.64 | 36.53±5.65 | 43.85±2.12 $^{B}$ | -1.86±0.31 $^{B}$ | 2.44±0.94 $^{B}$ |
| | STPP-C | 8.92±1.72 $^{A}$ | 37.31±2.79 $^{BC}$ | 43.33±13.45 | 32.94±5.83 | 42.83±2.13 $^{B}$ | -2.37±0.40 $^{C}$ | -0.01±1.39 $^{C}$ |

$^{a,b,c}$ Means within columns before citric soaking treatment with differing superscripts are significantly different at $P<0.05$

$^{A,B,C}$ Means within columns after citric soaking treatment with differing superscripts are significantly different at $P<0.05$

[1] Breast muscle soaked in 3% phosphate solutions for 13 h; control 1 = raw muscle without treatment, water = water soaking for 13 h, DSP = disodium orthophosphate, TSPP = tetrasodium pyrophosphate, STPP = sodium tripolyphosphate

[2] Breast muscle soaked in 0.1% citric acid solution for 1 h after soaking in phosphate solutions 13 h; control 2 = citric acid soaking without phosphate

**1.3 Thermal property**

The thermal transitions of spent hen breast muscle treated with various phosphates are shown in Figure 6. The thermogram exhibited three endothermic transitions for all treatments which corresponded to myosin (peak 1), sarcoplasmic and connective tissue protein (peak 2), and actin (peak 3). Kijowski and Mast (1988a) observed five peaks in the thermal curve of breast muscle (*Pectoralis minor*). Those peaks were the two main peaks at 57.1°C (myosin) and 77.7°C (actin), and three additional peaks at 62.5 and 72.8°C (sarcoplasmic protein), and 67.3°C (connective tissue protein). Wattanachant (2003) observed five endothermic peaks at 54.88, 61.66, 65.37, 70.63, and 76.14°C for broiler *Pectoralis* muscle, while only three endothermic peaks were found in Thai indigenous *Pectoralis* muscle at 53.55, 60.70, and 75.94°C. The difference in numbers of peak was likely due to the differences in sources of raw material, type, age, sex, and storage condition of chicken meat (Wattanachant, 2003). Chuaynukool (2008) reported five endothermic peaks of spent hen breast muscle (*Pectoralis major*) at 53.29, 60.79, 65.68, 71.52, and 77.88°C. Similar thermal transition of this study was found in control (untreated raw muscle) and water soaking samples as $T_p$ of peak 1 at 55-57°C, $T_p$ of peak 2 at 65.50-66.84°C, and $T_p$ of peak 3 at 80.17-80.33°C (Table 7).

It was found that muscle treated with phosphates could significantly increase denaturation temperature of peak 1 and peak 2 but decrease of peak 3 compared to control and water treatments ($P<0.05$). The thermal characteristics of proteins muscle treated with phosphate differed from occurring in nature systems due to differences in environmental pH and ionic strength (Kijowski and Mast, 1988a). In addition, first myosin peaks were shifted to 57-61°C, however, the third actin peaks were became 71-77°C for all phosphate treatments (Table 7). This pointed out the stabilized myosin and destabilized actin by phosphates addition. Robe and Xiong (1992) indicated that the addition of ortho-, pyro-, and tripolyphosphate produced similar effects on an increase thermal stability in the protein domains of *Longissimus dosi* salt-soluble protein. The addition of these phosphates generally facilitated the thermal transition as the phosphates bound to myosin subfragment 1 at the same binding site (Robe and Xiong, 1992). Kijowski and Mast (1988b) indicated that the interaction of ions with proteins affected the temperature and enthalpy of denaturation. The negative

polyvalent anions like pyrophosphate and tripolyphosphate were strongly bound to myofibrillar protein and meat systems provided stability to myosin. Additionally, the thermal stabilization of myosin and destabilization of the rest of proteins in the presence of phosphates might consider to be due to phosphates interacted electrostatically (nonspecifically) with charged protein molecules in muscle. However, phosphate also reacted specifically with myosin on the same active binding sites of a molecule as those which reacted with actin. The amount of specifically reacting phosphates was probably restricted to the number of available binding site on the myosin head which usually reacted with actin. The excess ions reacted nonspecifically with oppositely charged group of this protein (myosin) molecules resulting in a decreased thermal stabilization which was observed in muscle treated with STPP. Actin peak of STPP-treated muscle was more destabilized and had lower denaturation temperature compared to other phosphates (Figure 7 and Table 7). It was observed that muscle treated with DSP was higher in denaturation temperature of myosin peak compared to TSPP and STPP, respectively ($P<0.05$). This was probably due to DSP could diffuse into muscle, while TSPP and STPP (polyphosphate) could be dephosphorylized to orthophosphate in muscle to improve the functional property of muscle protein. Furthermore, the unidentified peaks which were observed in muscle treated with phosphate both before and after citric acid soaking might be the proteins extracted by phosphates. The denaturation enthalpy of these unidentified peaks was very low compared to other peaks, therefore, the proteins might be interacted with electrostatic force.

After citric acid soaking, the first peak of myosin in citric treated muscle (citric) was decreased to 55.17°C (Table 7), while the second and the third actin peak were not change ($P\geq0.05$) compared to control. The results exhibited that acidic marinade denatured the muscle proteins. However, the denaturation temperature of peak 1, 2, and 3 of muscle pretreated with all phosphates was not significantly different ($P\geq0.05$). The results showed that phosphate treatments could stabilize the denaturation temperature of myosin even though the muscle soaked in high acid solution. The excess destabilized of actin peak related to the function of STPP (polyanion) in modifying the electrostatic force in proteins, therefore, improving WHC and reducing protein-protein interaction (Shahidi and Synowiecki, 1996; Robe

and Xiong, 1992). This was corresponded to the increase in muscle pH, weight gain, and myofibrillar protein solubility and decrease in cooking loss.

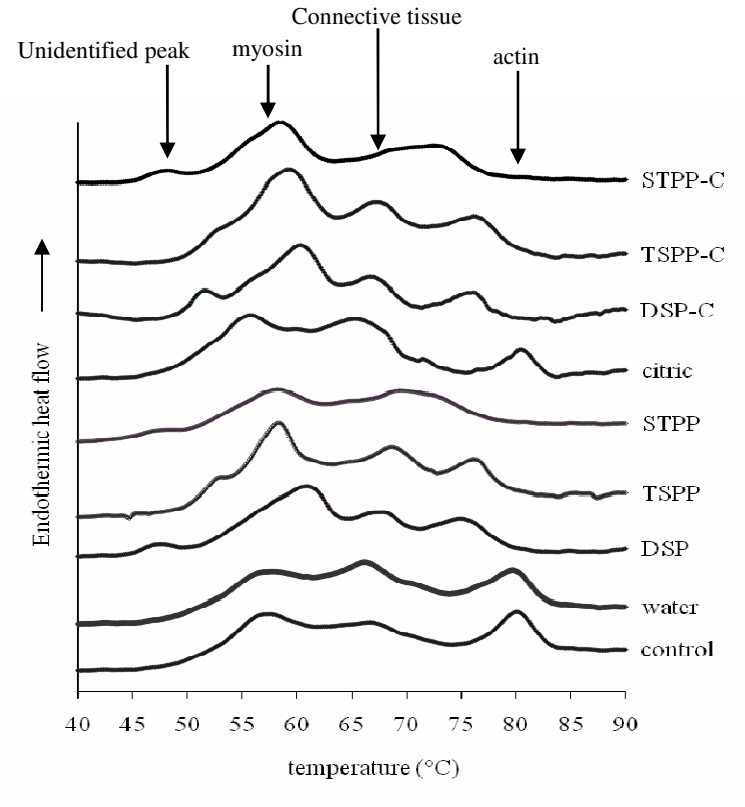



**Figure 7** Effect of phosphate types (3% w/v solution) on thermal transitions of spent hen breast muscle before and after soaking in 0.1% citric acid solution for 1 h

**Note:** control = untreated raw meat, water = water soaking, DSP = disodium orthophosphate TSPP = tetrasodium pyrophosphate soaking, STPP = sodium tripolyphosphate soaking, citric = citric acid soaking, -C = 0.1% citric acid soaking after phosphate pretreatment for 13 h

**Table 7** Effect of phosphate types on transformation temperature and denaturation enthalpy of spent hen muscle before and after soaking citric acid solution

| Treatments * | Tp (°C) | | | | ΔH (J/g) | | | |
|---|---|---|---|---|---|---|---|---|
| | unidentified | 1 | 2 | 3 | unidentified | 1 | 2 | 3 |
| Control | - | 56.67±0.34 $^{de}$ | 66.84±0.23 $^{bc}$ | 80.17±0.50 $^{a}$ | - | 0.49±0.07 $^{cde}$ | 0.13±0.03 $^{b}$ | 0.40±0.02 $^{b}$ |
| Water | - | 56.44±0.10 $^{e}$ | 66.50±0.17 $^{bc}$ | 80.33±0.44 $^{a}$ | - | 0.43±0.10 $^{de}$ | 0.29±0.04 $^{b}$ | 0.35±0.09 $^{bc}$ |
| DSP | 47.33±0.29 $^{c}$ | 60.67±0.44 $^{a}$ | 68.28±0.35 $^{a}$ | 75.83±0.67 $^{b}$ | 0.04±0.01 $^{bc}$ | 0.69±0.12 $^{b}$ | 0.11±0.01 $^{b}$ | 0.23±0.02 $^{d}$ |
| TSPP | 52.78±0.25 $^{a}$ | 58.33±0.17 $^{b}$ | 68.50±0.00 $^{a}$ | 76.50±0.29 $^{b}$ | 0.03±0.01 $^{c}$ | 0.47±0.08 $^{de}$ | 0.27±0.02 $^{b}$ | 0.18±0.02 $^{d}$ |
| STPP | 46.72±0.09 $^{c}$ | 57.17±0.44 $^{cd}$ | - | 71.50±1.30 $^{d}$ | 0.02±0.01 $^{c}$ | 0.39±0.15 $^{e}$ | - | 0.51±0.06 $^{a}$ |
| Citric | - | 55.22±0.25 $^{f}$ | 66.11±0.19 $^{c}$ | 80.11±0.42 $^{a}$ | - | 0.57±0.06 $^{bcd}$ | 0.30±0.10 $^{b}$ | 0.36±0.10 $^{bc}$ |
| DSPC | 51.33±0.17 $^{b}$ | 60.17±0.00 $^{a}$ | 67.22±0.19 $^{b}$ | 76.17±0.17 $^{b}$ | 0.07±0.01 $^{a}$ | 0.62±0.04 $^{bc}$ | 0.13±0.07 $^{b}$ | 0.26±0.05 $^{cd}$ |
| TSPPC | - | 58.33±0.60 $^{b}$ | 68.72±1.25 $^{a}$ | 76.33±0.00 $^{b}$ | - | 1.06±0.04 $^{a}$ | 0.65±0.40 $^{a}$ | 0.27±0.00 $^{cd}$ |
| STPPC | 46.84±0.75 $^{c}$ | 57.78±0.48 $^{bc}$ | - | 72.55±0.25 $^{c}$ | 0.06±0.01 $^{ab}$ | 0.72±0.03 $^{b}$ | - | 0.52±0.02 $^{a}$ |

$^{a,b,c,d,e,f}$ Means within columns with differing superscripts are significantly different at $P<0.05$

* Breast muscle soaked in 3% phosphate solutions for 13 h; control = untreated raw meat, water = water soaking, DSP = disodium orthophosphate soaking 13 h, TSPP = tetrasodium pyrophosphate soaking, STPP = sodium tripolyphosphate soaking, citric = citric acid soaking, -C = 0.1% citric acid soaking after phosphate pretreatment

## 2. Effect of soaking time on chemical and physical characteristics of spent hen muscle in high acid condition

### 2.1 Chemical characteristics

In high acid condition, muscle treated with STPP had the highest efficiency on WHC of muscle compared to other treatments as elucidated by weight gain, cooking loss values and thermal property. The effects of soaking time in 3% STPP solution for 7, 10, and 13 h on chemical characteristics of spent hen muscle are shown in Table 8 which was studied comparatively to untreated raw muscle (control 1) and water soaking (water) for 13 h. It was found that pH of samples was increased when the soaking time increased and was higher than those of the control 1 and water treatments. After citric acid soaking, pH of control 2 treatment was reduced, while muscle pretreated with STPP at each soaking time was slightly changed. Muscle treated with STPP had higher pH far away from the pI of muscle that could improve the WHC of muscle even after citric acid soaking.

It was observed that the myofibrillar protein solubility of all treatments before citric acid soaking was not different ($P \geq 0.05$). After citric acid soaking, the STPP pretreatments had higher solubility than control 2 ($P < 0.05$), but there was no significant difference among soaking time treatments.

Heat-soluble collagen content of all treatments in this section was lower compared to the previous section (Table 5) might be due to the more cross-linked collagen of raw muscle during frozen storage. The soluble collagen content of those soaking time treatments was not significantly different compared to control 1 ($P \geq 0.05$), by which the muscle soaking for 7 h showed the lowest content. After citric acid soaking, muscle pretreated with STPP for 10 and 13 h had higher soluble collagen content compared to control 2. This result might be likely due to the collagen fibers were swelled by pH change at higher soaking time and easily ruptured when soaked in high acid of citric acid solution (Lepetit, 2008; Burke and Monahan, 2003).

Phosphate content of all treatments was reduced after soaking in citric acid solution. The results showed that there was no significance in phosphate content between soaking time 10 and 13 h both before and after citric acid soaking ($P \geq 0.05$) because the phosphate diffusion into muscle was equilibrated at soaking time 10 h.

**Table 8** Effect of soaking time of STPP on chemical characteristics of spent hen breast muscle before and after soaking in citric acid solution

| Treatments | | pH | Myofibrillar protein solubility (% of total protein) | Soluble collagen (% of total collagen) | Phosphate content (as $P_2O_5$, ppm) |
|---|---|---|---|---|---|
| Before citric soaking [1] | Control 1 | 5.74±0.21 [e] | 34.46±3.74 | 5.05±2.33 [ab] | 1285.83 ± 58.14 [c] |
| | Water | 5.78±0.01 [d] | 34.93±2.07 | 6.73±1.24 [a] | 791.98 ± 118.34 [c] |
| | STPP7 | 6.57±0.21 [c] | 33.91±6.72 | 2.04±1.84 [b] | 2713.18 ± 186.48 [b] |
| | STPP10 | 6.76±0.15 [a] | 42.83±6.21 | 8.56±3.00 [a] | 3563.36 ± 602.25 [a] |
| | STPP13 | 6.71±0.01 [b] | 43.35±6.00 | 6.85±2.48 [a] | 3542.60 ± 100.21 [a] |
| After citric soaking [2] | Control 2 | 5.62±0.01 [D] | 34.99±1.86 [B] | 4.30±1.02 [C] | 1152.50 ± 134.77 [B] |
| | STPP7C | 6.64±0.01 [C] | 41.82±4.67 [AB] | 8.22±3.56 [BC] | 2131.52 ± 369.88 [A] |
| | STPP10C | 6.68±0.01 [B] | 49.30±3.65 [A] | 9.81±2.24 [B] | 2489.30 ± 273.35 [A] |
| | STPP13C | 6.72±0.01 [A] | 43.09±6.90 [AB] | 21.49±3.43 [A] | 2553.82 ± 343.80 [A] |

[a,b,c,d,e] Means within columns before citric soaking treatment with differing superscripts are significantly different at $P<0.05$

[A,B,C,D] Means within columns after citric soaking treatment with differing superscripts are significantly different at $P<0.05$

[1] Breast muscle soaked in 3% sodium tripolyphosphate solution; control 1 = raw muscle without treatment, water = water soaking for 13 h, STPP7 = soaking for 7 h, STPP10 = soaking for 10 h, STPP13 = soaking for 13 h

[2] Breast muscle soaked in 0.1% citric acid solution for 1 h after soaking in sodium tripolyphosphate solution for 7, 10, and 13 h; control 2 = citric acid soaking without phosphate

**2.2 Physical characteristics**

Table 9 shows the physical characteristics of spent hen muscle at different soaking time. Before citric acid soaking, sample soaked in water had lower weight gain and higher cooking loss than sample soaked in STPP solution ($P<0.05$). When all samples soaked in citric acid solution, it was observed that control 2 treatment had lower weight gain and higher cooking loss compared to all phosphate treatments. This might be attributed to muscle pH was closet to pI of meat in high acid condition which was responsible for the poor WHC indicates the fraction of bound water retain in the muscle (Alvarado and Sams, 2003; Önenç *et al*., 2004). For phosphate treatments, weight gain of muscle tended to increase when soaking time increased ($P<0.05$). However, weight gain of sample at soaking time 10 and 13 h was not significantly different ($P\geq0.05$) because of the equilibrium of phosphate diffusion into muscle at soaking time 10 h. Moreover, cooking loss of the sample at soaking time 10 h was lower than those at 7 and 13 h. Lemos *et al* (1999) indicated that the effect of marinade composed of salt and polyphosphate at marinating time ranging from 8-12 h were optimized to increase weight gain, reduce weight loss and cooking loss for the still-marinating process of chicken breast meat. After citric acid soaking, muscle samples pretreated with STPP at soaking time 10 and 13 h increased significantly higher weight gain compared to those at soaking time 7 h. It was found that sample at soaking time 13 h was the significantly lowest in cooking loss compared to those at soaking time 7 h and control 2. However, cooking loss of sample at soaking time 10 and 13 h was not significantly different ($P\geq0.05$) as well as weight gain, phosphate content, and myofibrillar protein solubility.

Shear force values of cooked muscle were lower than raw muscle. However, shear values of muscle treated with 3% STPP (before and after citric acid soaking) were not significantly different ($P \geq 0.05$) among soaking time. Additionally, L*, a*, b* values of all phosphate soaking time treatments had no significant difference ($P \geq 0.05$), but they were significantly lower than those of the control.

It was due to the myofibrillar protein solubility, soluble collagen content, phosphate content, weight gain, cooking loss, shear force, and surface color of phosphate pretreated muscles between soaking time 10 and 13 h was not significantly different. Therefore, soaking time for 10 h was the maximum time suitable for phosphate pretreatment of spent hen muscle.

**Table 9** Effect of soaking time of STPP on physical characteristics of spent hen breast muscle before and after soaking in citric acid solution

| Treatments | | Weight gain (%) | Cooking loss (%) | Shear force (N) | | Surface color | | |
|---|---|---|---|---|---|---|---|---|
| | | | | Raw muscle | Cooked muscle | L* | a* | b* |
| Before citric soaking [1] | Control 1 | - | 32.81±1.85 [b] | 65.74±10.86 | 43.54±6.26 [a] | 58.26±0.69 [b] | -1.87±0.52 [b] | 10.08±1.00 [a] |
| | Water | -2.98±1.41 [c] | 41.81±2.15 [a] | 68.98±11.11 | 37.69±7.29 [ab] | 67.65±2.32 [a] | -1.14±0.43 [a] | 10.46±1.01 [a] |
| | STPP7 | 17.86±2.47 [b] | 24.21±1.53 [c] | 60.45±13.45 | 33.99±6.19 [b] | 53.77±2.01 [c] | -2.86±0.46 [c] | 5.02±1.32 [b] |
| | STPP10 | 23.56±3.72 [a] | 22.31±1.43 [d] | 54.52±11.06 | 34.25±6.44 [b] | 52.39±2.18 [cd] | -2.51±0.53 [c] | 5.74±2.25 [b] |
| | STPP13 | 24.53±3.71 [a] | 25.26±2.37 [c] | 55.59±13.12 | 32.09±6.76 [b] | 51.70±2.21 [d] | -2.78±0.69 [c] | 5.86±1.56 [b] |
| After citric soaking [2] | Control 2 | -0.59±1.91 [C] | 37.04±2.39 [A] | 58.22±10.20 | 55.66±8.90 [A] | 63.45±2.15 [A] | -1.42±0.78 [A] | 10.29±1.40 [A] |
| | STPP7C | 3.41±0.89 [B] | 34.74±2.48 [AB] | 62.13±9.16 | 30.96±8.61 [B] | 56.96±2.31 [B] | -2.29±0.96 [B] | 6.17±2.22 [B] |
| | STPP10C | 6.55±0.70 [A] | 33.25±3.34 [BC] | 59.13±11.41 | 31.04±8.29 [B] | 55.84±1.06 [B] | -2.85±0.22 [B] | 5.29±1.20 [B] |
| | STPP13C | 5.62±1.30 [A] | 31.26±3.93 [C] | 56.93±11.98 | 38.77±8.30 [B] | 55.63±1.65 [B] | -2.83±0.46 [B] | 5.68±1.05 [B] |

[a,b,c,d] Means within columns before citric soaking treatment with differing superscripts are significantly different at $P<0.05$

[A,B,C] Means within columns after citric soaking treatment with differing superscripts are significantly different at $P<0.05$

[1] Breast muscle soaked in 3% sodium tripolyphosphate solution; control 1 = raw muscle without treatment, water = water soaking for 13 h, STPP7 = soaking for 7 h, STPP10 = soaking for 10 h, STPP13 = soaking for 13 h

[2] Breast muscle soaked in 0.1% citric acid solution for 1 h after soaking in sodium tripolyphosphate solution for 7, 10, and 13 h; control 2 = citric acid soaking without phosphate

## 3. Effect of phosphate concentrations on chemical and physical characteristics of spent hen muscle in high acid condition

### 3.1 Chemical characteristics

The muscle soaked in phosphate solution at concentrations 2%, 3%, and 4% STPP for 10 h were determined comparatively to control and water soaking treatments. It was found that pH of muscle treated with all STPP concentrations both before and after citric acid soaking was higher than control 1, water, and control 2 treatments, respectively ($P<0.05$) as shown in Table 10. The muscle pH tended to increase when the phosphate concentration increased. After citric acid soaking, pH of muscle without phosphate treatments (control 2) reduced close to the pI, but the muscle treated with phosphate could retard the decrease of muscle pH nearly to pI. The pH of muscle treated with 3% and 4% STPP was not reduced even soaked in citric acid solution ($P\geq0.05$). This probably due to more phosphate anion ($PO_4^{3-}$) associated with increasing phosphate concentration of 3% and 4% STPP could neutralize cation of citric acid (carboxyl group).

It was observed that there was no significant difference in myofibrillar protein solubility of all treatments before citric acid soaking ($P\geq0.05$) (Table 10). This might be related to the raw materials used in this section were frozen for 2 months. However, muscle treated with STPP solution had the solubility higher than control treatments ($P\geq0.05$). Additionally, heat-soluble collagen content could not be detected due to the excess cross-linked collagen during frozen storage of raw materials as explained above.

The phosphate content in muscle treated with STPP increased when phosphate concentration increased. After citric acid soaking, phosphate content in muscle reduced approximately 30.71%, 30.11%, and 17.51% for muscle pretreated with 2%, 3%, and 4% STPP, respectively because of the leaching effect.

**Table 10** Effect of STPP concentrations on chemical characteristics of spent hen breast muscle before and after soaking in citric acid solution

| Treatments | | pH | Myofibrillar protein solubility (% of total protein) | Phosphate content (as $P_2O_5$, ppm) |
|---|---|---|---|---|
| Before citric soaking [1] | Control 1 | 5.69±0.01 [d] | 33.56±2.94 | 1097.19 ± 191.77 [d] |
| | Water | 5.74±0.01 [c] | 34.97±4.97 | 652.19 ± 22.50 [e] |
| | 2STPP | 6.34±0.05 [b] | 39.88±2.94 | 1863.23 ± 933.4 [c] |
| | 3STPP | 6.34±0.01 [b] | 40.97±6.76 | 3018.05 ± 157.41 [b] |
| | 4STPP | 6.63±0.01 [a] | 42.05±3.93 | 3427.37 ± 204.59 [a] |
| After citric soaking [2] | Control 2 | 5.51±0.01 [D] | 34.34±1.26 | 840.51 ± 39.12 [C] |
| | 2STPPC | 6.18±0.01 [C] | 48.45±3.78 | 1141.94 ± 49.43 [C] |
| | 3STPPC | 6.47±0.01 [B] | 51.50±3.15 | 2109.18 ± 161.41 [B] |
| | 4STPPC | 6.63±0.01 [A] | 46.58±1.97 | 2827.27 ± 284.23 [A] |

[a,b,c,d,e] Means within columns before citric soaking treatment with differing superscripts are significantly different at $P<0.05$

[A,B,C,D] Means within columns after citric soaking treatment with differing superscripts are significantly different at $P<0.05$

[1] Breast muscle soaked in sodium tripolyphosphate solutions for 10 h; control 1 = raw muscle without treatment, water = water soaking for 10 h, 2STPP = 2% sodium tripolyphosphate, 3STPP = 3% sodium tripolyphosphate, 4STPP = 4% sodium tripolyphosphate

[2] Breast muscle soaked in 0.1% citric acid solution for 1 h after soaking in sodium tripolyphosphate solutions for 10 h; control 2 = citric acid soaking without phosphate

### 3.2 Physical characteristics

The physical characteristics of spent hen muscle after treated with STPP solution at 2%, 3%, and 4% were evaluated (Table 11). Sample soaking in water increased lower weight gain compared to phosphate treatments. Differences in WHC of meat could be related to pH differences; the WHC increases with rising pH is larger than the range 5.5-5.8 (Hamm, 1975). Among the STPP-treated muscle, 2% STPP had the lowest weight gain compared to 4% STPP and 3% STPP ($P<0.05$), by which the value of 4% STPP was lower than 3% STPP because absorption of marinade above the maximum capacity of muscle is lost during storage (Zheng *et al*., 2000). The cooking loss of control 1 and 2% STPP treatments was not significantly different ($P\geq0.05$), while water treatment had the highest cooking loss compared to control 1 and all phosphate treatments ($P<0.05$). However, muscle treated with 4% STPP had lower cooking loss than that of 3% STPP, which corresponded to L* value. It was found that L* value of 4% STPP was significantly lower than 3% STPP, 2% STPP, control 1, and water treatment. Allen *et al* (1998) found that L* values correlated positively with drip loss and cooking loss. Moreover, a* and b* values were decreased associated with phosphate concentration increased. Also, Alvarado and Sams (2003) reported that STPP approximately pH 9.0 was a significant improvement in L* value and cooking loss of pale fillets (L*>53; pH ≤6) of broiler breast meat. After citric acid soaking, muscle treated with 4% STPP had the highest weight gain compared to 3% STPP, 2% STPP, and control 2, respectively. The cooking loss of control 2 and 2% STPP pretreatments were higher compared to 3% STPP and 4% STPP, respectively ($P<0.05$). It was found that 4% STPP pretreatment was significantly greater WHC than 3% STPP.

The shear force values of cooked breast muscle were lower than its raw counterpart. Before citric acid soaking, the both raw and cooked breast muscle pretreated with 3% and 4% STPP had lower shear force compared to water treatment. After citric acid soaking, muscle pretreated with 4% STPP had the lowest shear force which was not change even though soaked in citric acid solution compared to control. This result was in agreement with Zheng *et al* (2002) who reported that breast injected with 38.0 g/kg STPP had the lowest shear force compared to 25.0 and 12.5 g/kg STPP-injected chicken breast.

**Table 11** Effect of STPP concentrations on physical characteristics of spent hen breast muscle before and after soaking in citric acid solution

| Treatments | | Weight gain (%) | Cooking loss (%) | Shear force (N) | | Surface color | | |
|---|---|---|---|---|---|---|---|---|
| | | | | Raw muscle | Cooked muscle | L* | a* | b* |
| Before citric soaking [1] | Control 1 | - | 34.47±1.70 $^{b}$ | 47.08±8.33 $^{ab}$ | 35.10±6.06 $^{b}$ | 58.38±1.94 $^{b}$ | -1.94±0.73 $^{b}$ | 8.13±1.62 $^{a}$ |
| | Water | 0.90±1.37 $^{d}$ | 43.85±2.03 $^{a}$ | 65.90±9.86 $^{a}$ | 42.81±7.11 $^{a}$ | 64.97±1.98 $^{a}$ | -1.18±0.60 $^{a}$ | 8.85±0.84 $^{a}$ |
| | 2STPP | 15.41±2.66 $^{c}$ | 34.88±1.65 $^{b}$ | 65.35±10.01 $^{a}$ | 30.75±7.72 $^{b}$ | 58.61±1.28 $^{b}$ | -1.84±0.51 $^{b}$ | 7.76±1.06 $^{a}$ |
| | 3STPP | 20.85±3.28 $^{a}$ | 23.88±2.73 $^{c}$ | 53.65±7.88 $^{b}$ | 30.03±5.22 $^{b}$ | 54.16±2.03 $^{c}$ | -2.41±0.61 $^{bc}$ | 5.45±1.19 $^{b}$ |
| | 4STPP | 18.45±2.43 $^{b}$ | 19.87±3.56 $^{d}$ | 54.94±7.74 $^{b}$ | 31.38±5.78 $^{b}$ | 50.25±1.76 $^{d}$ | -2.71±0.68 $^{c}$ | 2.72±2.59 $^{c}$ |
| After citric soaking [2] | Control 2 | -2.39±0.58 $^{B}$ | 40.76±1.83 $^{A}$ | 55.56±6.98 $^{AB}$ | 38.72±8.61 $^{A}$ | 64.02±2.23 $^{A}$ | -1.39±0.61 $^{A}$ | 9.20±1.36 $^{A}$ |
| | 2STPPC | -1.48±0.93 $^{B}$ | 40.05±2.28 $^{A}$ | 62.53±7.75 $^{A}$ | 34.83±7.76 $^{A}$ | 62.35±2.24 $^{A}$ | -1.74±0.51 $^{A}$ | 8.37±1.02 $^{A}$ |
| | 3STPPC | -0.70±3.77 $^{B}$ | 34.87±3.38 $^{B}$ | 55.49±8.13 $^{AB}$ | 33.06±5.91 $^{AB}$ | 58.13±2.11 $^{B}$ | -2.53±0.46 $^{B}$ | 5.78±1.51 $^{B}$ |
| | 4STPPC | 2.22± 1.50 $^{A}$ | 27.45±4.09 $^{C}$ | 49.51±8.26 $^{B}$ | 27.27±5.79 $^{B}$ | 54.63±1.23 $^{C}$ | -3.22±0.44 $^{C}$ | 4.70±1.29 $^{B}$ |

$^{a,b,c,d}$ Means within columns before citric soaking treatment with differing superscripts are significantly different at $P<0.05$

$^{A,B,C}$ Means within columns after citric soaking treatment with differing superscripts are significantly different at $P<0.05$

[1] Breast muscle soaked in sodium tripolyphosphate solution for 10 h; control 1 = raw muscle without treatment, water = water soaking for 10 h, 2STPP = 2% sodium tripolyphosphate, 3STPP = 3% sodium tripolyphosphate, 4STPP = 4% sodium tripolyphosphate

[2] Breast muscle soaked in 0.1% citric acid solution for 1 h after soaking in sodium tripolyphosphate solution for 10 h; control 2 = citric acid soaking without phosphate

## 4. Effect of phosphates on microstructure of spent hen muscle in high acid condition

The microstructure of raw muscle and muscle slices soaked in 4% STPP for 10 h was presented in Figure 7. The changes in microstructure of muscle examined by SEM on the transverse sections after soaking in 4% STPP (Figure 7C) was visible swelling compared to control (Figure 7A) because of the increase in WHC of muscle. The microstructural measurements of muscle are shown in Table 12 as the diameter of muscle fiber. Muscle treated with 4% STPP only (4STPP) had higher fiber diameter than those of soaked in 4% STPP and citric acid (4STPPC), citric acid only (Citric), and control, respectively ($P<0.05$). The fiber diameter of muscle after soaking in citric acid with and without phosphate treatment (Citric and 4STPPC) had no significant difference ($P\geq0.05$). This might be attributed to citric acid solution causing the denaturation of surface muscle and retaining the solution into fibrils. The citric-treated muscle was obvious in stiff muscle as shown in Figure 21 (in the appendixes). The structure of muscle soaked in citric acid solution (Figure 7B) became compact fiber arrangement but that for 4% STPP pretreatment (Figure 7D) was looser because the more water loss, the more gaps between fibers of muscle occurred after citric acid soaking. Mikel *et al* (1996) concluded that organic acids (2% lactic acid and 2% acetic acid mixture; 50:50) appeared to denature muscle microstructure without having any effects on physical attributes, by which acid spray strip loins, on day 0, had less definable H-zones, a loss of M-line and I-band integrity, slight loss of A-band integrity and an weak Z-line. Ke *et al* (2008) indicated that the microstructure of round beef eye muscle was lost upon acidification but reformed upon increasing muscle pH.

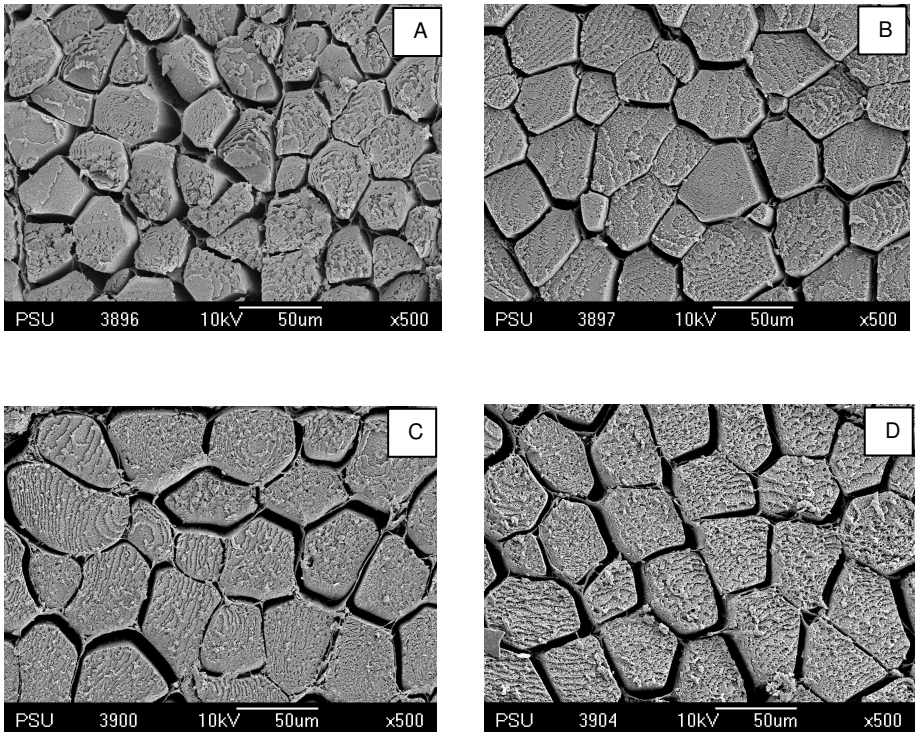



**Figure 7** Scanning electron micrographs of transverse sections of marinated spent hen breast muscle

**Note:** A: raw muscle without treatment (control), B: 0.1% citric acid soaking for 1 h (citric), C: 4% sodium tripolyphosphate soaking for 10 h, and D: 0.1% citric acid soaking for 1 h after soaking in 4% sodium tripolyphosphate for 10 h

**Table 12** Fiber diameter of marinated spent hen breast muscle

| Treatments | Fiber diameter (μm)[1] |
|---|---|
| Control | 17.60±1.39 [c] |
| Citric | 20.19±1.94 [b] |
| 4STPP | 24.32±2.54 [a] |
| 4STPPC | 20.35±2.30 [b] |

[1] means with the standard deviation from 30 determinations

[a,b,c] Means within columns with differing superscripts are significantly different at $P<0.05$

Control = raw muscle without treatment, Citric = citric acid soaking for 1 h, 4STPP = 4% sodium tripolyphosphate soaking for 10 h, and 4STPPC = 0.1% citric acid soaking for 1 h after soaking in 4% sodium tripolyphosphate for 10 h

## 5. Effect of phosphate and Tom-Yum ingredients on quality of marinated spent hen muscle during refrigerated storage

The ingredients such as spices, salt, and sugar (without garcinia) had marinade pH around 5.0-6.0 (Table 13). After adding with garcinia, however, Tom-Yum paste was classified as high acid marinade (pH around 2.53). The marinade pH was the main parameter which strongly affected on the functionality of muscle protein and shelf-life of muscle products.

**Table 13** pH of each marinade treatment

| Marinade treatments | Marinade pH |
|---|---|
| 4% (w/v) STPP solution | 8.57 |
| salt and sugar | 5.90 |
| garcinia, salt, and sugar | 1.27 |
| spices, salt, and sugar | 5.28 |
| Tom-Yum paste | 2.53 |

### 5.1 pH changes

pH of all marinated muscle treatments are shown in Table 14. pH of muscle soaked in 4% STPP (pH~8.57) increased to approximately 6.0. Muscle pretreated with STPP marinated with Tom-Yum paste (P4) had muscle pH closet to pI and significantly higher than that of without phosphate pretreatment (C2, pH~3.98). While the strongly acidic of garcinia mixed ingredients (P2) decreased pH of marinated muscle lower than 5.0. During 5 days of storage, the results showed that STPP could retard reduction in muscle pH in Tom-Yum marinade but not in garcinia mixed ingredients (P2). However, after 5 days storage, STPP did not show the buffering capacity because the muscle pH of all treatments decreased during storage. Presumably, there were no any phosphates that could retard the very low pH of garcinia in marinated muscle.

**Table 14** Effect of phosphate and marinade conditions on pH of marinated spent hen muscle stored at 4ºC

| Treatments | pH | | | | | | |
|---|---|---|---|---|---|---|---|
| | Day 1 | Day 5 | Day 10 | Day 15 | Day 20 | Day 25 | Day 30 |
| C1 | $5.44\pm0.02^{c,D}$ | $6.14\pm0.02^{b,B}$ | $5.48\pm0.01^{c,D}$ | $5.77\pm0.08^{c,C}$ | $6.59\pm0.05^{a,A}$ | - | - |
| C2 | $3.98\pm0.05^{f,C}$ | $4.40\pm0.03^{d,A}$ | $3.77\pm0.02^{f,F}$ | $4.04\pm0.02^{e,B}$ | $3.89\pm0.01^{f,DE}$ | $3.87\pm0.01^{e,E}$ | $3.92\pm0.02^{d,D}$ |
| P1 | $6.14\pm0.04^{b,D}$ | $6.45\pm0.04^{a,A}$ | $6.11\pm0.04^{b,D}$ | $6.21\pm0.02^{a,C}$ | $6.03\pm0.01^{b,E}$ | $6.15\pm0.03^{a,D}$ | $6.34\pm0.02^{a,B}$ |
| P2 | $4.38\pm0.02^{e,A}$ | $4.03\pm0.06^{e,B}$ | $3.86\pm0.04^{e,D}$ | $3.66\pm0.03^{f,E}$ | $3.94\pm0.03^{e,C}$ | $4.04\pm0.04^{d,B}$ | $3.98\pm0.03^{c,BC}$ |
| P3 | $6.25\pm0.02^{a,B}$ | $6.43\pm0.01^{a,A}$ | $6.20\pm0.03^{a,C}$ | $6.14\pm0.01^{b,D}$ | $5.90\pm0.02^{c,F}$ | $5.86\pm0.01^{b,G}$ | $5.96\pm0.01^{a,E}$ |
| P4 | $5.01\pm0.01^{d,A}$ | $5.01\pm0.03^{c,A}$ | $4.49\pm0.06^{d,C}$ | $4.42\pm0.02^{d,D}$ | $4.28\pm0.02^{d,E}$ | $4.58\pm0.04^{c,B}$ | $4.61\pm0.03^{b,B}$ |

$^{a,b,c,d,e,f}$ Mean within column with differing superscripts are significantly different at $P<0.05$

$^{A,B,C,D,E,F,G}$ Mean within row with differing superscripts are significantly different at $P<0.05$

**Note:** C1 = raw muscle without treatment

C2 = muscle marinated with Tom-Yum paste without phosphate treatment

P- = muscle pretreatment with 4% STPP (sodium tripolyphosphate) solution at 4ºC for 10 h and then marinated with salt and sugar (P1), mixed garcinia, salt, and sugar (P2), mixed Tom-Yum ingredients except garcinia (P3), or Tom-Yum paste (P4)

**5.2 microbiological changes**

The effect of STPP and Tom-Yum ingredients on microbial property of marinated spent hen muscle were evaluated the mesophilic and psychrophilic bacteria count as shown in Figure 8 and 9, respectively. On day 1, the number of mesophilic bacteria of all treatments was 4.6-5.5 log CFU/g. The initial microbial loads of raw spent hen muscle in this study were similar range as the other reports. Okolacha and Ellerbroek (2005) found that the aerobic plate count (APC) of chicken carcasses was 5.4 log. Vareltzis *et al* (1997) observed that the initial total mesophilic counts (day 0) on chicken carcarres were approximately 5 log CFU/cm$^2$. The mesophilic bacteria which are normally indicator of pathogen of C1, P1, and P4 were increased, whereas those of C2, P2, and P3 were decreased with the storage time. Okolacha and Ellerbroek (2005) indicated that the APC of whole chicken carcasses were reduced by the acid treatment of 1% lactic acid (Purac®).

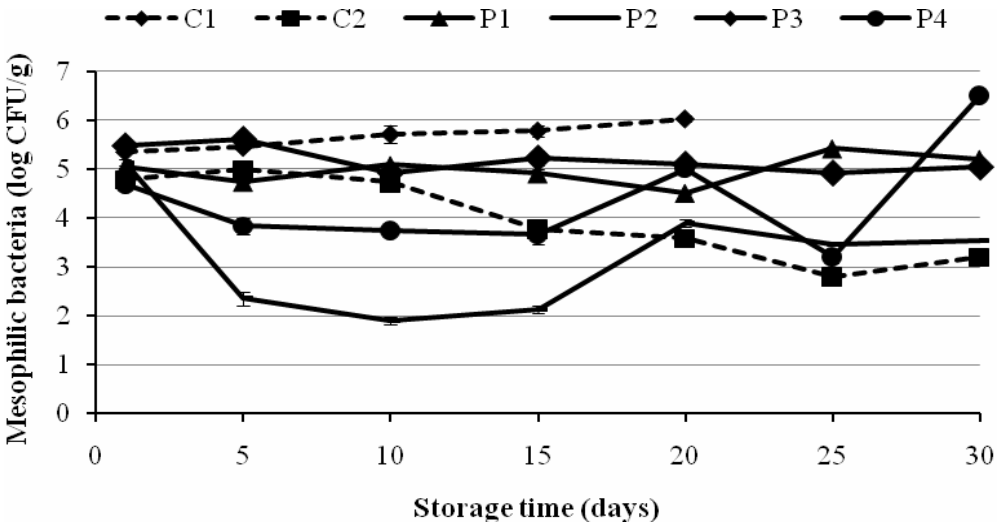



**Figure 8** Effect of phosphate and marinade ingredients on mesophilic bacteria (log CFU/g) of marinated spent hen muscle stored at 4ºC

**Note:** C1 = raw muscle without treatment

C2 = muscle marinated with Tom-Yum paste without phosphate treatment

P- = muscle pretreatment with 4% STPP (sodium tripolyphosphate) solution at 4ºC for 10 h and then marinated with salt and sugar (P1), mixed garcinia, salt and sugar (P2), mixed Tom-Yum ingredients except garcinia (P3), or Tom-Yum paste (P4)

The psychrophilic counts in C1, P1, P3, and P4 treatment on day 1 was found 3.5-4.2 log CFU/g, while C2 and P2 treatments were not found (Figure 9). This explained probably due to the pH of marinade was low enough to inhibit microorganism growth. However, the initial bacterial loads (on day 1) between mesophilic and psychrophilic bacteriac of each treatment were significantly different because psychrophiles had an 8 to 10°C lower minimum growth temperature than mesophiles (Ingraham, 1958). It was found that the psychrophilic bacteria (indicator of spoilage) of all treatments tended to increase when the storage time increased. The numbers of psychrophilic bacteria of P3 treatment at day 3-10 of storage were higher than the others ($P<0.05$), and then it was constant during storage. This was presumably due to a numerous microbial loads in spices resulting in rapidly growth of bacterial. The psychrophilic bacteria of C1, P1, P3, and P4 were higher than those of C2 and P2 treatments. The raw muscle (C1) spoiled on day 5, which the microbial counts exceeded 7 log CFU/g. However, the microbial counts of P1, P3, and P4 treatments did not exceed the criterion (7 log CFU/g) until day 15. It could be explained because the antimicrobial compounds such as garcinia and garlic in spices could inhibit the microorganisms although there were the necessary nutrients for microbial growth like sugar (Chattopadhyay and Bhattacharyya, 2007; Siripongvuttikorn *et al*., 2005). The microbial growth during refrigerated storage of C2, P2, and P4 treatment was respectively lower than other treatments ($P<0.05$) probably due to the main effect of garcinia and spices, especially allicin from garlic ingredient, in marinade. (Ankri and Mirelman, 1999). Mackeen *et al* (2002) revealed that methanol extracts from *Garcinia atroviridis* were almost completely antibacterial which may be attributed to the presence of xanthones and related metabolites. Furthermore, Siripongvutikorn *et al* (2005) reported that garlic in Tom-Yum ingredients exhibited the highest antimicrobial effect on *Pseudomonas fluorescens* ATCC 49839, *Escherichia coli* O157:H7, *Staphylococcus aureus* ATCC 13565 and *Listeria monocytogenes*.

When compare the effect of STPP treatment in Tom-Yum marinating process, it was observed that the psychrophilic bacteria of P4 was higher than C2 treatment along the extended storage time (Figure 9). This may due to the muscle pH of C2 was lower than P4 treatment (Table 14). The depressed pH could inhibit the functioning of

enzymes and the transport of nutrients into the microorganism cells (Jay, 1996). Moreover, Tom-Yum marinated muscle in absence STPP (C2) could inhibit the microorganism growth until day 30, while the marinated muscle in presence STPP (P4) could retard for 20 days. This was due to the acceptable limit by a criterion for aerobic plate count (APC at 20°C) of safety or quality for processed chicken that followed good processing practices was $10^7$ CFU/g (ICMSF, 1986).

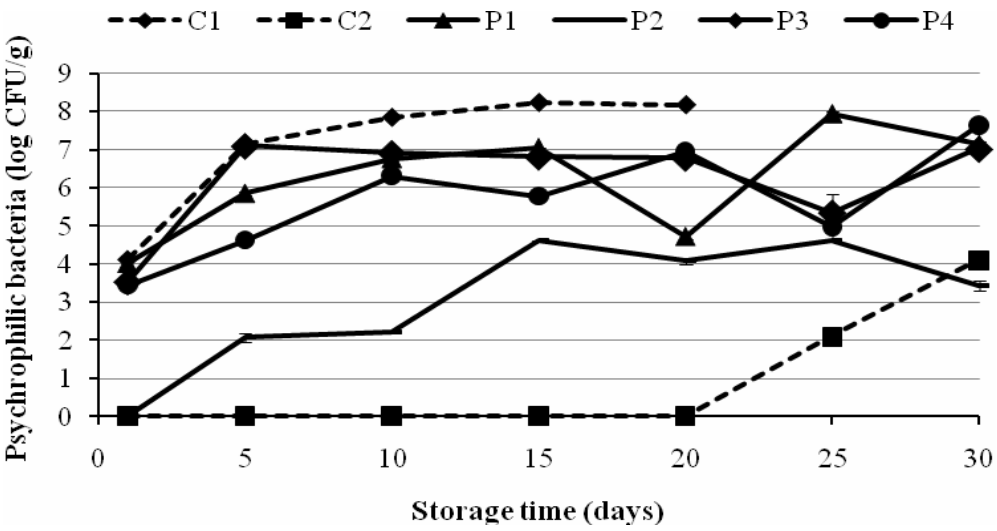



**Figure 9** Effect of phosphate and marinade ingredients on psychrophile bacteria (log CFU/g) of marinated spent hen muscle stored at 4ºC

**Note:** C1 = raw muscle without treatment

C2 = muscle marinated with Tom-Yum paste without phosphate treatment

P- = muscle pretreatment with 4% STPP (sodium tripolyphosphate) solution at 4ºC for 10 h and then marinated with salt and sugar (P1), mixed garcinia, salt and sugar (P2), mixed Tom-Yum ingredients except garcinia (P3), or Tom-Yum paste (P4)

### 5.3 Physical changes

To reduce the effect of ingredients' color, muscle slices of all treatments were rinsed before subjected to evaluate the surface color of muscle by CIE color L*, a*, and b* and results shown as Figure 10, 11, and 12, respectively. The L*, a*, and b* values of each treatment were significantly different ($P<0.05$) during refrigerated storage. On day 1, L* values of P1, P3, and P4 treatments were not significantly different ($P\geq0.05$), but lower than those of C1, C2, and P2 treatments. This was probably due to the higher pH of STPP could swell muscle proteins and alter light reflection resulting in darker and redder muscle (Önenç *et al*., 2004). The reflectance of raw muscle on day 1 was observed in lighter (L*) and greenness (-a*) color. During storage, L* values decreased while a* values increased ($P\geq0.05$). Acid marinated muscle (C2, P2, and P4) had higher L* values than those of P1 and P3 treatment. The L* values of acid treatments tended to increase when the storage time increased ($P<0.05$). This pointed out the more leaching effect on color of meat during storage. Önenç *et al* (2004) indicated that the lower pH in citric acid solution (pH~2.4) leading to denaturation of muscle proteins and a higher light reflection.

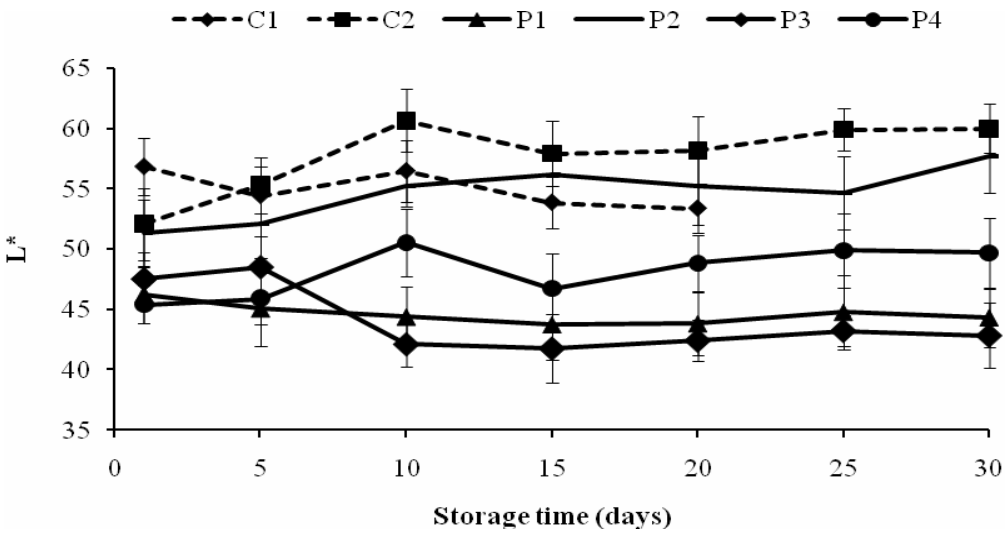



**Figure 10** Effect of phosphate and marinade ingredients on L* values of marinated spent hen muscle stored at 4ºC

**Note:** C1 = raw muscle without treatment

C2 = muscle marinated with Tom-Yum paste without phosphate treatment

P- = muscle pretreatment with 4% STPP (sodium tripolyphosphate) solution at 4ºC for 10 h and then marinated with salt and sugar (P1), mixed garcinia, salt and sugar (P2), mixed Tom-Yum ingredients except garcinia (P3), or Tom-Yum paste (P4)

a* and b* values of all treatments were significantly different during storage (Figure 11 and 12, respectively). This explained probably due to the mixed ingredients in marinade were differently absorbed in muscle. However, a* and b* values of C2, P2, and P4 treatments were higher ($P$<0.05) than other treatments. This might be corresponded to the denaturation of proteins by high acid condition. Additionally, it was found that C2 treatment had the highest L*, a*, and b* values ($P$<0.05). While the lowest values ($P$<0.05) were observed in P1 treatment which remained almost constant along the extended storage time. This could indicate that salt and sugar in Tom-Yum paste had no effect on muscle color change during storage.

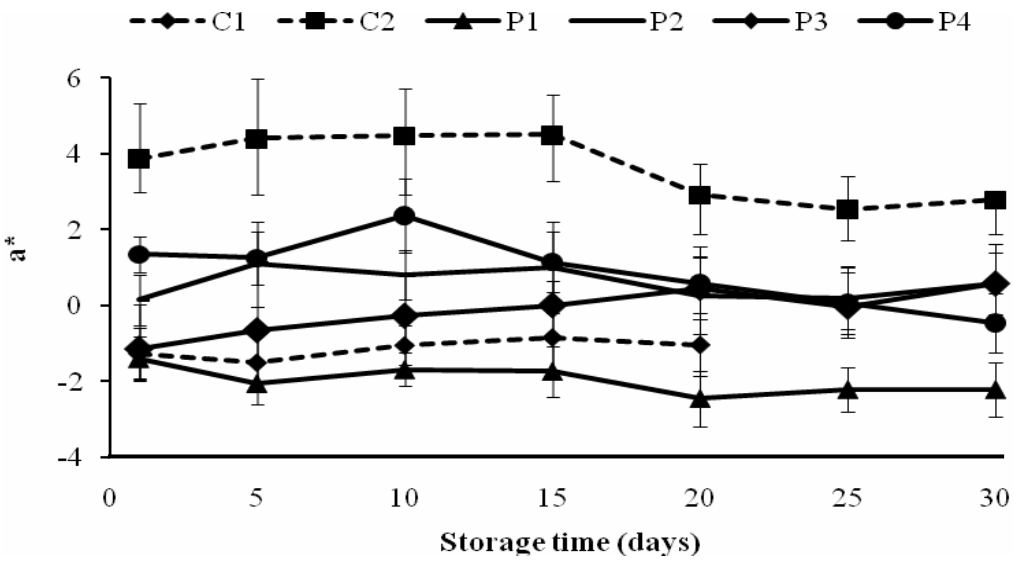



**Figure 11** Effect of phosphate and marinade ingredients on a* values of marinated spent hen muscle stored at 4ºC

**Note:** C1 = raw muscle without treatment

C2 = muscle marinated with Tom-Yum paste without phosphate treatment

P- = muscle pretreatment with 4% STPP (sodium tripolyphosphate) solution at 4ºC for 10 h and then marinated with salt and sugar (P1), mixed garcinia, salt and sugar (P2), mixed Tom-Yum ingredients except garcinia (P3), or Tom-Yum paste (P4)

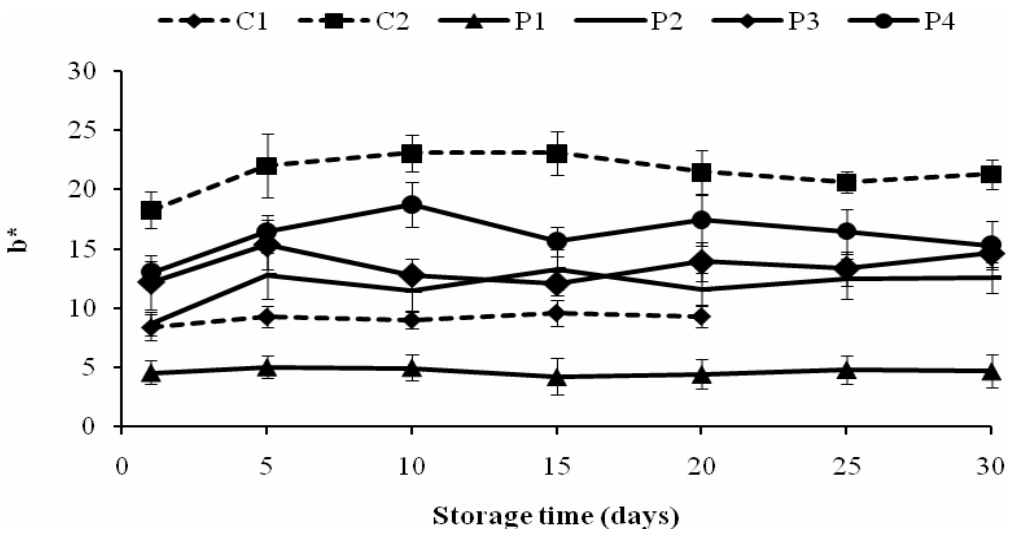



**Figure 12** Effect of phosphate and marinade ingredients on b* values of marinated spent hen muscle stored at 4ºC

**Note:** C1 = raw muscle without treatment

C2 = muscle marinated with Tom-Yum paste without phosphate treatment

P- = muscle pretreatment with 4% STPP (sodium tripolyphosphate) solution at 4ºC for 10 h and then marinated with salt and sugar (P1), mixed garcinia, salt and sugar (P2), mixed Tom-Yum ingredients except garcinia (P3), or Tom-Yum paste (P4)

The cooking losses of marinated spent hen muscles are shown in Figure 13. Initial cooking loss on day 1 of raw muscle (C1) was higher which was not significantly different with P4 treatment. This was probably due to muscle pH of both treatments was in the range of pI (5.0-5.5) (Table 14) resulting in a poor WHC of meat (Hamm, 1975). The C1, C2, P2, and P4 treatments had the same range of cooking loss which was significantly higher values than that of P1 and P3 treatments, respectively ($P<0.05$). The lowest cooking loss of P1 treatments was observed ($P<0.05$) and it was constant during storage. This might be attributed to salt and STPP increased pH and ionic strength of muscle (Thorarinsdottir *et al*., 2001). In P1 treatment, chloride and phosphate ions increased negatively charges on muscle proteins allowing the stronger electrostatic repulsion forces. This was contributed to increase space for water to be held in the muscle and improve WHC of muscle throughout the storage (Thorarinsdottir *et al*., 2001; Xiong *et al*., 2000; Shahidi and Synowiecki, 1997; Hamm, 1975). In addition, the cooking loss of P3 treatment was

rapidly increased after 15 days of storage ($P$<0.05). This might be attributed to bacterial and enzymatic function. All treatments of muscle marinated with garcinia ingredient (C2, P2, and P4) had higher percentage of cooking loss than those of P1 and P3 treatments, and these values were almost constant throughout the storage time. It was probably due to the higher acid marinade leading to decrease muscle pH in the range of pI of meat that contributed in loss of WHC property of muscle (Serdaroğlu *et al*., 2007; Oreskoich *et al*., 1992). These results were similar to the finding of Oreskoich *et al* (1992) who observed that beef cores with a final pH after marination in 0.1 M phosphate buffer (pH 4.24 and 5.38) near the pI of muscle had the highest losses after cooking.

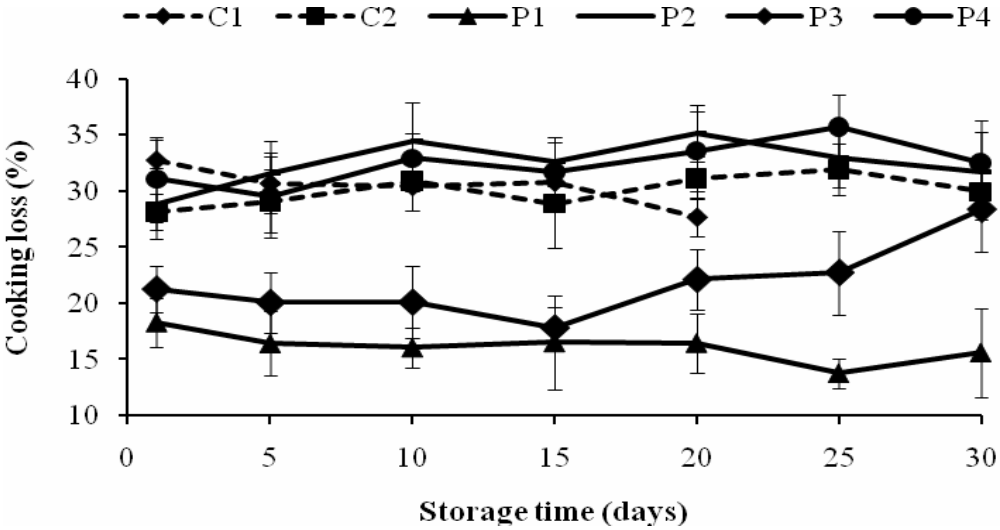



**Figure 13** Effect of phosphate and marinade ingredients on cooking loss (%) of marinated spent hen muscle stored at 4ºC

**Note:** C1 = raw muscle without treatment

C2 = muscle marinated with Tom-Yum paste without phosphate treatment

P- = muscle pretreatment with 4% STPP (sodium tripolyphosphate) solution at 4ºC for 10 h and then marinated with salt and sugar (P1), mixed garcinia, salt and sugar (P2), mixed Tom-Yum ingredients except garcinia (P3), or Tom-Yum paste (P4)

Warner-Bratzler shear force values of marinated spent hen muscle before and after cooking are shown in Figure 14 and 15. There was no tendency of shear value changes during storage of raw marinated spent hen muscle (Figure 14). After cooking, shear force values of all treatments were tended to decrease but had no change with the storage time (Figure 15). It was found that P1 and P3 treatments had the lowest shear values which was corresponded to the least cooking loss (Figure 13) throughout the storage time compared to other treatments ($P<0.05$). This explained probably due to phosphate elevated muscle pH (Table 14) leading to WHC improving and tenderness of meat (Sheard and Tali, 2004). The toughness of breast muscle could be reduced by injection with phosphate and salt as observed in the study of Zheng *et al* (2000). However, P2 treatment for the both of raw and cooked muscle had the largest shear values during storage time because the greater absorption of high acid garcinia hardened myofibrillar proteins (Mikel *et al*., 1996). Shear force values of Tom-Yum marinated muscle (C2 and P4) were lower than raw muscle (C1) during 5-10 days of storage, but after that shear values were higher because of the protein denaturation. However, both Tom-Yum marinated muscle with (P4) and without STPP pretreatment (C2) had no significant difference ($P\geq0.05$) in shear values. It pointed out that 4% STPP could not retain the buffering capacity after muscles were marinated in high acid Tom-Yum paste leading to lower WHC and protein denaturation.

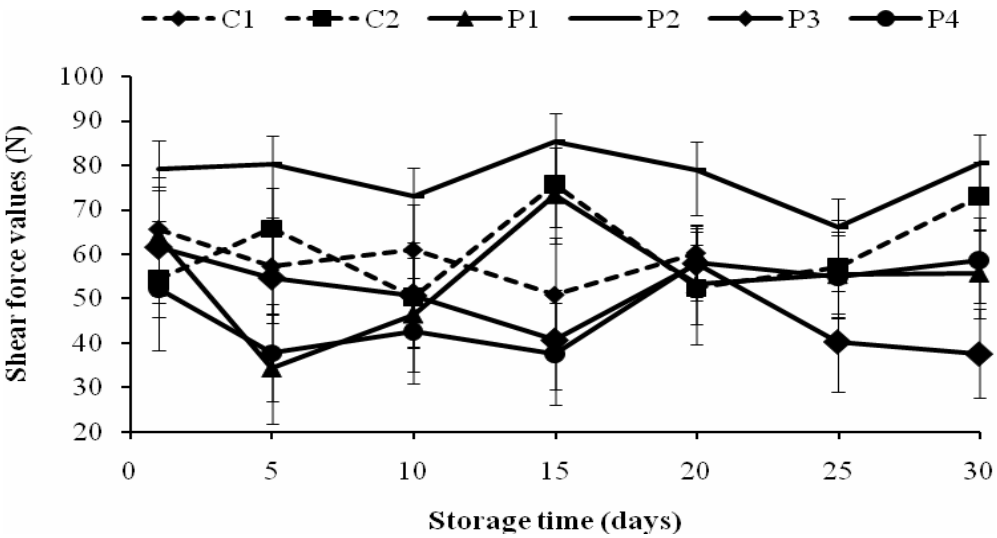



**Figure 14** Effect of phosphate and marinade ingredients on shear force values (N) of raw marinated spent hen muscle stored at 4ºC

**Note:** C1 = raw muscle without treatment

C2 = muscle marinated with Tom-Yum paste without phosphate treatment

P- = muscle pretreatment with 4% STPP (sodium tripolyphosphate) solution at 4ºC for 10 h and then marinated with salt and sugar (P1), mixed garcinia, salt and sugar (P2), mixed Tom-Yum ingredients except garcinia (P3), or Tom-Yum paste (P4)

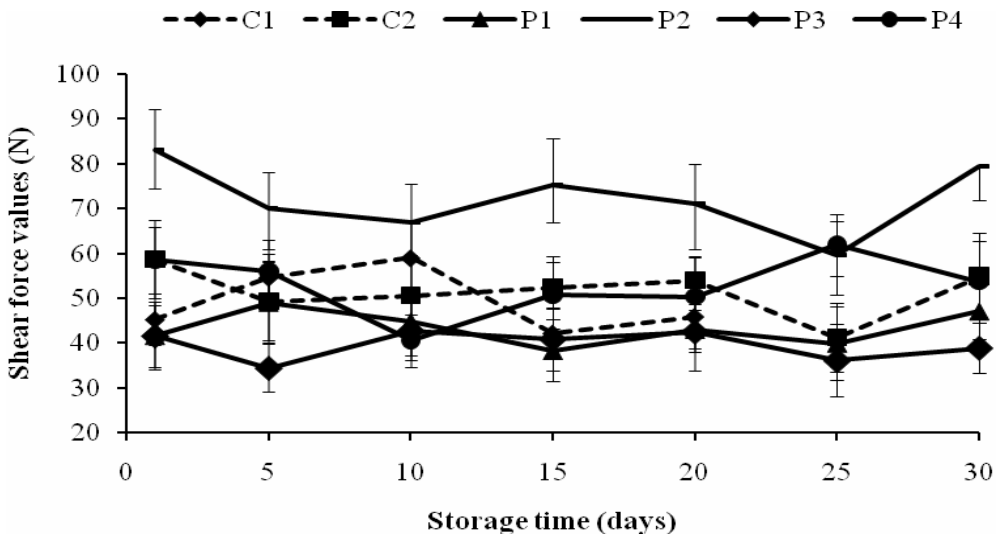



**Figure 15** Effect of phosphate and marinade ingredients on shear force values (N) of cooked marinated spent hen muscle stored at 4ºC

**Note:** C1 = raw muscle without treatment

C2 = muscle marinated with Tom-Yum paste without phosphate treatment

P- = muscle pretreatment with 4% STPP (sodium tripolyphosphate) solution at 4ºC for 10 h and then marinated with salt and sugar (P1), mixed garcinia, salt and sugar (P2), mixed Tom-Yum ingredients except garcinia (P3), or Tom-Yum paste (P4)

### 5.4 Chemical changes

Lipid oxidation determined by TBARS values of spent hen muscle during refrigerated storage are presented in Figure 16. The TBARS values of raw muscle (C1) decreased when the storage time increased. This was contrastive to other researchers probably due to raw spent hen muscle used in this experiment was frozen for 2 months. The TBARS may undergo extensive modification at advanced stages of lipid oxidation. The TBA-test is useful method to measure lipid oxidation (Pomeranz and Meloan, 1994). Petterson *et al* (2004) revealed that storage time influenced the development of rancidity in mechanically deboned turkey meat (MDTM), the significant increase in TBARS values in MDTM stored at -20°C from 1-6 months were detected. The P1 treatment had the highest TBARS values compared to the others due to salt in P1 marinade may act as a prooxidant to accelerate muscle lipid peroxidation by displacing iron ions from binding macromolecules for oxidative reaction (Kanner, 1994; El-Alim *et al*., 1999). At the initial of storage, C2 treatment

had higher TBARS compared to those of P3 and P4 treatments ($P<0.05$) during first 10 days storage. The result might be attributed to STPP and spices in Tom-Yum marinade functions on lipid oxidation of muscle during the marinating process (Weilmeier and Regenstein, 2004). The TBARS values of C2 and P3 treatments tended to decrease when the storage time increased ($P<0.05$). However, P4 treatment was constantly TBARS during storage up to 30 days ($P\geq0.05$). Shobana and Naidu (2000) showed that aqueous plus ethanolic extracts of all spices test (ginger, garlic, onion, mint, cloves, cinnamon, and pepper) affected in inhibition of lipoxygenase-dependent enzymatic lipid peroxidation. This was agreed with the study of El-Alim *et al* (1999) who reported that ethanolic extracts of spices markedly inhibited the oxidation process in raw pork meats pretreated with NaCl during refrigerated and frozen storage.

However, raw spent hen muscle without treatment (C1) had the TBARS value lower than other treatments during storage except P2 treatment ($P<0.05$). This might be attributed to chlorophyll in Tom-Yum paste which obtained from Kaffir lime leaves and lemon grass could be a sensitizer in excitation of oxygen in Type II photo-oxidation, triplet oxygen can be excited by light to singlet oxygen. Therefore, the singlet oxygen formed may react with a polyunsaturated fatty acid to form a hydroperoxide (Gordon, 2001). Besides, oxidative deterioration of pigment may cause the bleaching of foods (Gordon, 2001), especially, carotenoids which was mainly found in red chilli and kaffir lime leaves (Siripongvuttikorn *et al*., 2005). The formation of carotenoid-peroxyl radicals, either autoxidation product of carotenoid CAR-OO$^{\bullet}$ or adduct formed with lipid ROO, ROO-CAR$^{\bullet}$ may have continued the free radical chain reactions and promoted the oxidation of lipids (Haila, 1999).

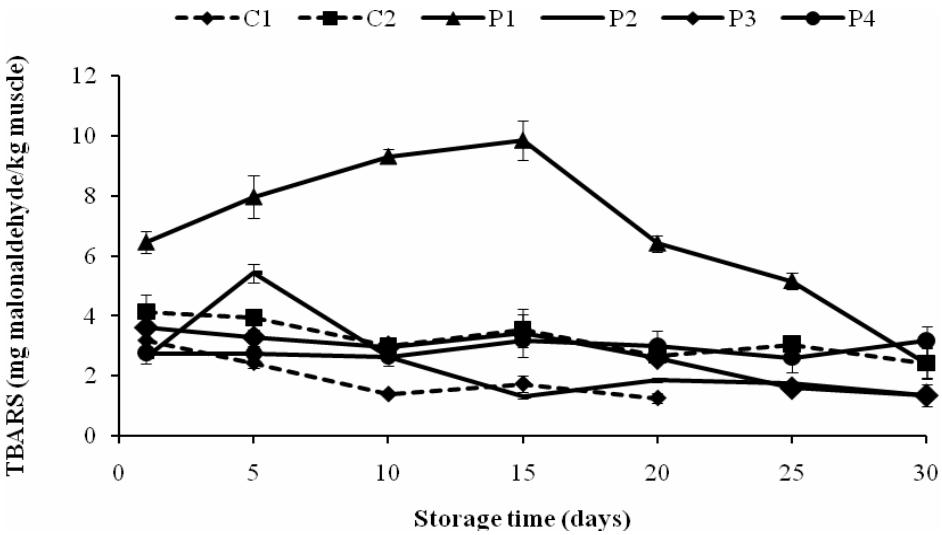



**Figure 16** Effect of phosphate and marinade ingredients on TBARS (mg malonaldehyde/ kg muscle) of marinated spent hen muscle stored at 4ºC

**Note:** C1 = raw muscle without treatment

C2 = muscle marinated with Tom-Yum paste without phosphate treatment

P- = muscle pretreatment with 4% STPP (sodium tripolyphosphate) solution at 4ºC for 10 h and then marinated with salt and sugar (P1), mixed garcinia, salt and sugar (P2), mixed Tom-Yum ingredients except garcinia (P3), or Tom-Yum paste (P4)

Heat-soluble collagen content of marinated spent hen muscle is presented in Figure 17. The results showed that soluble collagen content of P1 and P3 treatments could not be detected after day 15 of storage (Table 26) probable due to the precipitation of collagen-phosphate binding in insoluble forms (Weinstock, 1967). The soluble collagen content of raw muscle (C1) was almost steady during storage ($P \geq 0.05$), while C2, P2, and P4 treatments tended to increase when the storage time extended. This explained probably due to the acid treatment disrupted the non-covalent intermolecular bond (hydrogen bonds and dipole or ion-pair interactions and intermolecular cross-links) of intramuscular connective tissue (Voulita, 2009; Oreskovich *et al*., 1992). The highest soluble collagen content was observed in C2 treatment which attributed to collagen in the higher muscle pH (Table 14) could extremely solubilise in high acid (Voulita, 2009). In the other hand, P4 treatment which marinated with Tom-Yum paste had a higher muscle pH because of STPP treatment, therefore, showed lower soluble collagen content ($P<0.05$) compared to C2

and P2 treatments. Oreskovich *et al* (1992) indicated that the phosphoric acid treatment (pH 1.50) resulted in a reduction of total collagen content in muscle to about 40% of non- treated muscle and an increase in soluble collagen content presumably due to acid hydrolysis. However, the higher soluble collagen content of C2 treatment had no effect on shear values (Figure 14 and 15) compared to P4 treatment. This might be attributed to a more influence of possible hardening sarcoplasmic proteins and denaturation of myofibrillar proteins by high acid condition in Tom-Yum paste. The results could be revealed that change in the collagen solubility was not the primary factor influencing tenderness, the denaturation of myofibrillar proteins might have more effect on toughness of meat although there was an increasing soluble collagen content by acid treatments (Mikel *et al*., 1996).

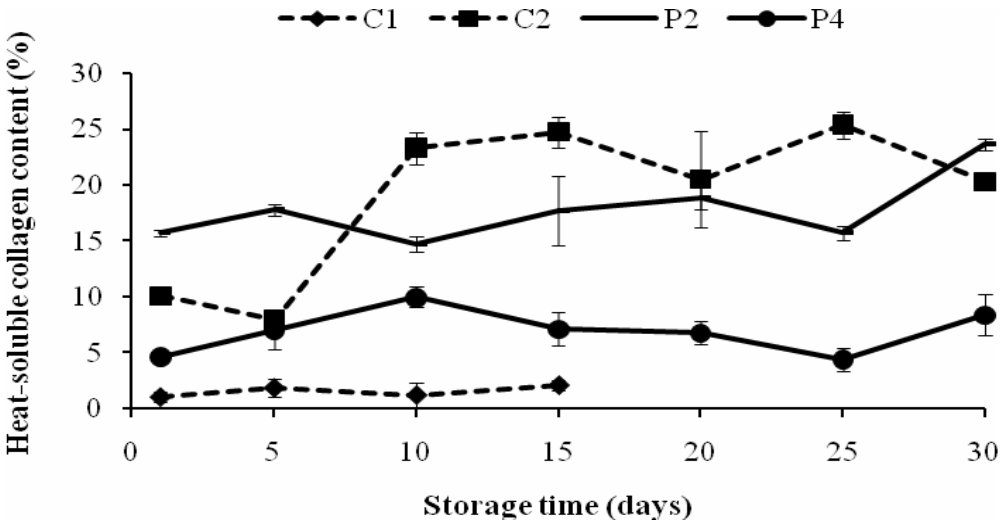



**Figure 17** Effect of phosphate and marinade ingredients on heat-soluble collagen content (% of total collagen) of marinated spent hen muscle stored at 4ºC

**Note:** C1 = raw muscle without treatment

C2 = muscle marinated with Tom-Yum paste without phosphate treatment

P- = muscle pretreatment with 4% STPP (sodium tripolyphosphate) solution at 4ºC for 10 h and then marinated with garcinia, salt and sugar (P2), or Tom-Yum paste (P4)

Phosphate content of all treatments determined in term of $P_2O_5$ are shown in Figure 18. When the storage time increased, the phosphate content in all muscle treatments tended to decreased ($P$<0.05). The maximum phosphate content found in all treatments was not excess the limitation in meat product (5000 ppm as $P_2O_5$). The muscle without STPP treatment (C1 and C2) had lower phosphate content than muscle pretreated with STPP ($P$<0.05). However, C2 was lower than that of C1 treatment ($P$<0.05) because natural phosphate in muscle could be released in moisture loss during high acid marinating process.

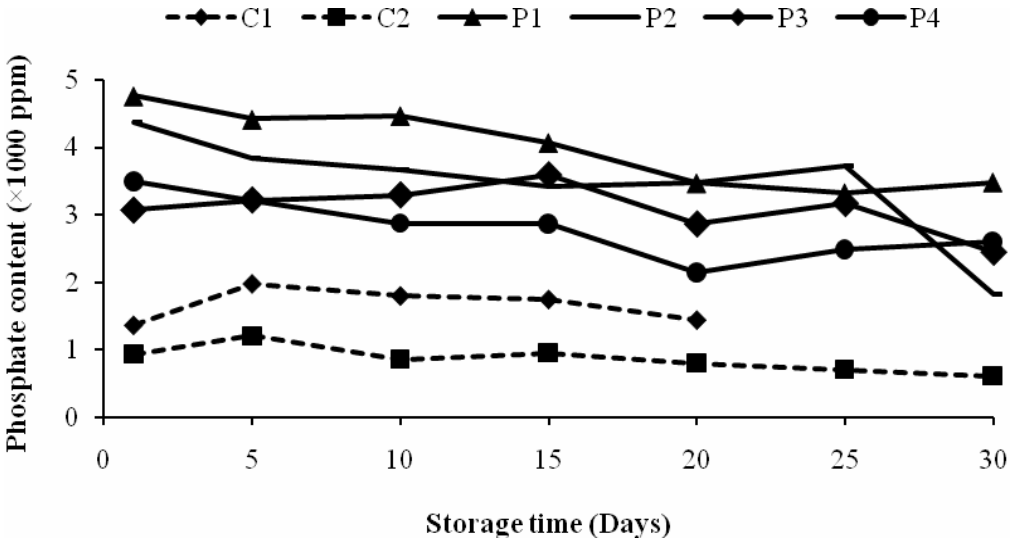



**Figure 18** Effect of phosphate and marinade ingredients on phosphate content (ppm; as $P_2O_5$) of marinated spent hen muscle stored at 4ºC

**Note:** C1 = raw muscle without treatment

C2 = muscle marinated with Tom-Yum paste without phosphate treatment

P- = muscle pretreatment with 4% STPP (sodium tripolyphosphate) solution at 4ºC for 10 h and then marinated with salt and sugar (P1), mixed garcinia, salt and sugar (P2), mixed Tom-Yum ingredients except garcinia (P3), or Tom-Yum paste (P4)

**5.5 SDS-PAGE patterns**

SDS-PAGE patterns of raw muscle and Tom-Yum marinated spent hen muscle pretreated with and without STPP treatment were examined comparatively during refrigerated storage. The intensity of protein bands of all treatments (C1, C2, and P4) were decreased when the storage time increased (Figure 19). Especially, the band intensity of main muscle proteins such as myosin heavy chain (MHC, 200 kDa), α-actinin (106 KDa), actin (46 kDa), and myosin light chain 1 (MLC1, 25kDa) of raw muscle was reduced because of the degradation of protein due to proteolysis (Lametsch *et al*., 2002; Xiong and Brekke, 1989). The α-actinin was a major component of Z filaments and polar links in Z-discs (Papa *et al*., 2004). Protein patterns of muscle marinated with Tom-Tum paste (C2 and P4) were degraded more than that of raw muscle (C1). It was probably due to muscle pH of these treatments was in the range of 3.7-5.0 (Table 14) which was optimum for some proteolytic enzymes. The pH-dependence of muscle protein breakdown during storage was supported that endogenous cathepsins may be the main enzymes responsible for the observed protein degradation (Wang *et al*., 2009). The optimal pH for activity of cathepsins was in the range 3.5-5.0 and hence the lowering of meat pH in an acid marinade may well enhance proteolytic attack by these enzymes (burke and Monahan, 2003). Additionally, protein patterns of P4 treatment could not be identified on day 25 and 30 might be attributed to the degradation of muscle proteins. Figure 20 presents the protein patterns of all treatments comparatively at the same storage day. It was found that the MHC, α-actinin, actin, and MLC1 bands of C1 had more intensity than those of P4 and C2 treatments. The more degradation of the structural proteins which elucidated by SDS-PAGE and the more solubilization of collagen (Figure 17) were observed in C2 than found in P4 treatments. However, these results did not correspond to the tenderness of meat as postulated by no significant difference in shear force values between C2 and P4 treatment (Figure 14 and 15).

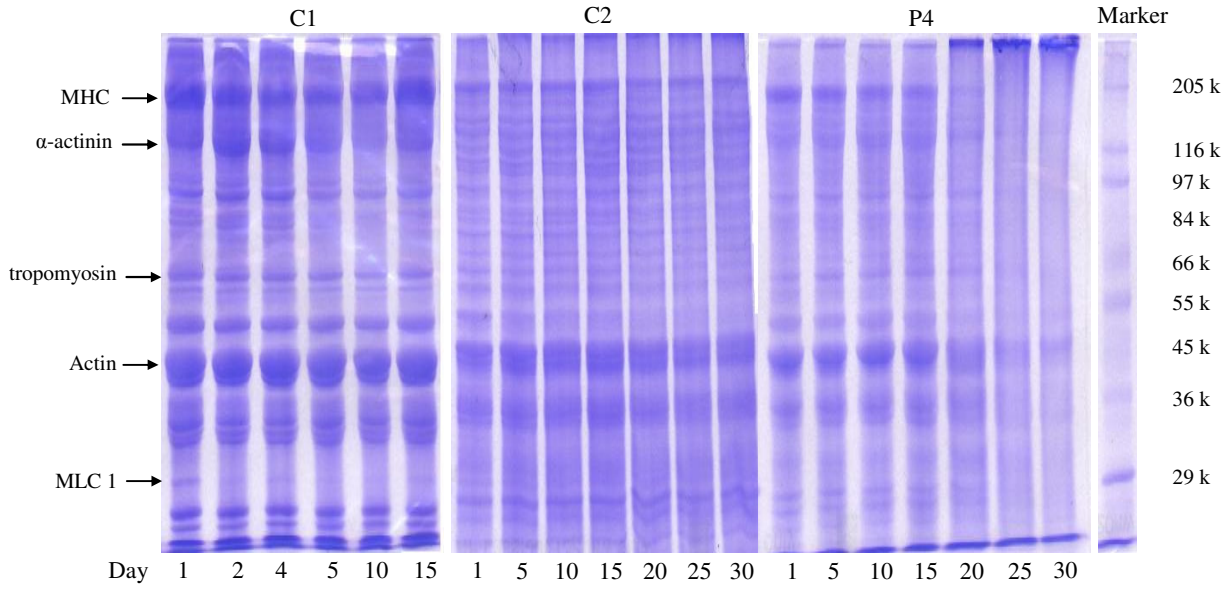



**Figure 19** SDS-PAGE patterns of raw and Tom-Yum marinate spent hen muscle pretreated with and without STPP during storage at 4ºC for 30 days

**Note:** C1 = raw muscle without treatment

C2 = muscle marinated with Tom-Yum paste without phosphate treatment

P4 = muscle pretreatment with 4% STPP (sodium tripolyphosphate) solution at 4ºC for 10 h and then marinated with Tom-Yum paste

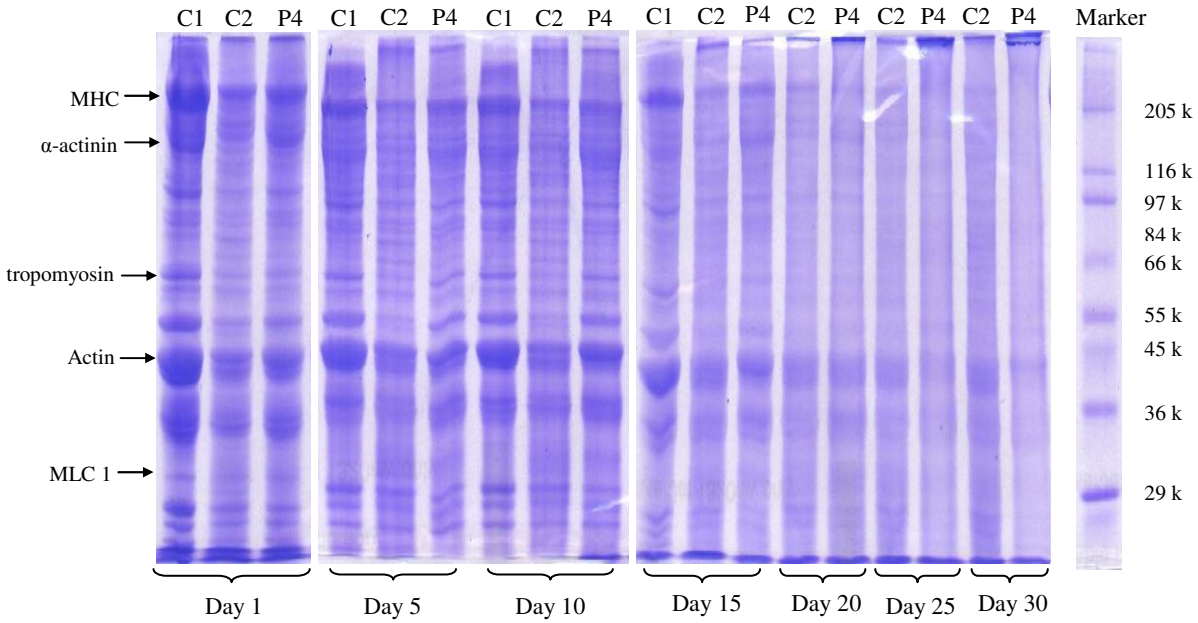



**Figure 20** Effect of phosphate and Tom-Yum marinade on SDS-PAGE patterns of spent hen muscle during storage at 4ºC

**Note:** C1 = raw muscle without treatment

C2 = muscle marinated with Tom-Yum paste without phosphate treatment

P4 = muscle pretreatment with 4% STPP (sodium tripolyphosphate) solution at 4ºC for 10 h and then marinated with Tom-Yum paste

# CHAPTER 4

# CONCLUSIONS

Spent hen breast muscle soaked in phosphate solution was found to increase muscle pH, which resulting in increasing WHC. While, muscle soaked in citric acid solution without phosphate treatment decreased muscle pH nearly to pI of muscle resulting in poor WHC and increasing toughness of meat. However, phosphate pretreatment of muscle in high acid condition could protect the decreased muscle pH close to pI, therefore, buffering capacity of the meat was retained. The STPP pretreatment could act as a polyanion which allowed greater repulsive force that contributed to improve WHC of meat and weight gain of muscle. The lowest WHC of meat was observed in muscle pretreated with TSPP. Additionally, the diffusion of STPP solution into muscle was completely equilibrated at soaking time 10 h. The STPP solution with higher concentration has been associated with higher phosphate anion groups which increased repulsion force of negatively charged group of protein and produced a maximum hydration effect. Muscle soaked in 4% STPP solution for 10 h had the highest final product yield which could improve WHC, shear force, and darkness of surface color of spent hen muscle in high acid condition.

Using garcinia as sour ingredient in Tom-Yum paste produced the high acid marinade resulted in drastical denaturation of myofibrillar proteins in spent hen muscle. Consequentially, there was a high moisture release and high shear force values both in the muscle pretreated with and without STPP. Among Tom-Yum ingredients, salt might act as pro-oxidant to accelerate the lipid oxidation. However, the mixed spices inhibited the lipid oxidation of the marinated spent hen muscle during storage. Additionally, STPP synergized to spices to lower TBARS compared to other marinade treatments. Without STPP pretreatment, garcinia Tom-Yum paste could prolong the shelf-life of the marinated spent hen in term of microbiological

quality up to 30 days. However, the Tom-Yum marinated muscle with STPP pretreatment had a shorter shelf-life for 20 days. The Tom-Yum marinating process, STPP and spices in Tom-Yum paste could maintain the lipid oxidation of marinated spent hen muscle. Contrastively, STPP had no obvious function on WHC, and tenderness after spent hen muscle was marinated with acidic garcinia Tom-Yum paste. Furthermore, STPP could not extend the shelf-life of marinade Tom-Yum product. Therefore, Tom-Yum marinated spent hen muscle could be developed to be ready-to-cook product for value-added chicken product without STPP addition.

# REFERENCES

Addis, P.B. 1986. Poultry Muscle as Food. *In* Muscle as Food (Bechtel, P. J., ed.). p. 371-403. Academic Press, Inc. Olando.

Aktaş, N., Akus, M.İ. and Kaya, M. 2003. The effect of organic acid marination on tenderness, cooking loss and bound water content of beef. J. Muscle Food. 14: 181-194.

Allen, C. D., Fletcher, D. L., Northcutt, J. K. and Russell S. M. 1998. The relationship of broiler breast color to meat quality and shelf-life. Poultry Sci. 77: 361-366.

Alvarado, C. Z. and Sams, A. R. 2003. Injection marination strategies for remediation of pale soft, exudative broiler breast meat. Poultry Sci. 82: 1332-1336.

Ankri, S. and Mirelman, D. 1999. Antimicrobial properties of allicin from garlic. Mcrob. Infect. 2: 125-129.

AOAC. 1999. Official Methods of Analysis of the Association of Official Analytical Chemists. 16th ed. The Association of Official Analytical Chemists, Inc. : Washington D. C.

Björkroth, J. 2005. Microbiological ecology of marinated meat products. Meat Sci. 70: 477-480.

Bodwell, C. E. and Anderson, B. A. 1986. Nutritional Composition and Value of Meat and Meat Products. *In* Muscle as Food (Bechtel, P. J., ed.). p. 321-370. Academic Press, Inc. Olando.

Brandt, L. 2003. Marinades meat challenges (online). Available : http://www.preparefoods.com/CDA/Archives/ff6bfefef70788010VgnVCM100000f932a8c0 [2006, April 14]

Burke, R. M., and Monahan, F. J. 2003. The tenderization of shin beef using a citrus juice marinade. Meat Sci. 63: 161-168.

Ceylan, E. and Fung, D. Y. C. 2004. Antimicrobial activity of spices. J. Rapid Autom. Microbiol. 12: 1-55.

Chattopadhyay, R.R. and Bhattacharyya, S. K. 2007. Hebal spices as alternative antimicrobial food preservatives: An update. Phcog. Rev. 1(2): 235-247.

Cheah, P. B. and Hasim, N. H. A. 2000. Natural antioxidant extract from galangal (*Alpinia galangal*) for minced beef. J. Sci. Food Agric. 80: 1565-1571.

Cheng, J.H. and Ockerman, H.W. 2003. Effect of phosphate with tumbling on lipid oxidation of precooked roast beef. Meat Sci. 65: 1353-1359.

Chuaynukool, K. 2008. Quality of fresh chicken meat and its quality after process in retort pouch Tom Yum soup condition. Degree of Master of Science in Food Technology, Prince of Songkla University.

Corriher, S. 2009. Marinadea add flavor but don't always tenderize (online). Available : http://www.finecooking.com/articles/marinades-flavor-tenderize.aspx [2009, March 22]

Dušek, M., Kvasnička, F., Lukášková, L., and Krátka, J. 2003. Isotachophoresis determination of added phosphate in meat products. Meat Sci., 65: 765–769.

El-Alim, S. S. L. A., Lugasi, A., Hóvári, J. and Dworschák, E. 1999. Culinary herbs inhibit lipid oxidation in raw and cooked minced meat patties during storage. J. Sci. Food Agric. 79: 277-285.

Ellinger, R. H. 1972. Phosphate in Food Processing. *In* CRC Handbook of Food Additives (Furia, T. E., ed.) Vol. I. p. 617-780. CRC Press, Inc. Cleveland.

Farrell, K. T. 1990. Spices, Condiments, and Seasonings. ed. 2nd. AVI Book : New York.

Federal Register. 1996. Use of trisodium phosphate on raw, chilled poultry carcasses. Fed. Regist. 61(146): 39273-39278.

Fiske. C. H. and Subbarow, Y. 1925. The colorimetric determination of phosphorus. J. Biol. Chem. 66: 375-400.

Gonçalves, A. A. and Ribeiro, J. L. D. 2008. Do phosphates improve the seafood quality? Reality and legislation. PANAMJAS. 3(3): 237-247.

Gordon, M. H. 2001. The Development of Oxidative Rancidity in Foods. *In* Antioxidants in Food (Pokorny, J., Yanishlieva, N. and Gordon, M., ed.). p. 7-21. Woodhead publishing Ltd. New York.

Haila, K. 1999. Effects of Carotenoids and Carotenoid-Tocopherol Interaction on Lipid Oxidation *In Vitro* (online). Available : http://ethesis.helsinki.fi/julkaisut/maa/skemi/vk/haila/effectso.html. [2009, May 18]

Hamm, R. 1975. Water Holding Capacity of Meat. *In* Meat (Cole, D. J. A., ed.). p. 321-337. The AVI publishing company. Westport.

Hamm, R. 1986. Functional Properties of the Myofibrillar System and Their Measurements. *In* Muscle as Food (Bechtel, P. J., ed.). p. 135-200. Academic Press, Inc. Olando.

Huff-Lonergan, E. and Lonergan, S. M. 2005. Mechanisms of water-holding capacity of meat: The role of postmortem biochemical and structural changes. Meat Sci. 71: 194–204.

ICMSF, Sampling for Microbiological Analysis: Principles and Specific Applications. *In* Microorganisms in Foods 2. p. 130-156. Blackwell scientific publications. Oxford.

Ingraham, J. L. 1958. Growth of psychrophilic bacteria. J. Bacteriol. 76(1): 75-80.

Jay, J. M. (1996). Modern Food Microbiology. 16th ed. (Jay, J. M., ed.). p. 38-343. International Thomson Publishing. New York

Johnson, L. 2009. Marinades (online). Available : http://www.good-grillin.com/default.aspx [2009, March 22]

Kanner, J. 1994. Oxidative processes in meat and meat products: Quality implications. Meat Sci. 36: 169-189.

Ke, S., Huang, Y., Decker, E. A., and Hultin, H. O. 2008. Impact of citric acid on the tenderness, microstructure and oxidative stability of beef muscle. Meat Sci. 82: 113-118.

Kijowski, J. M. and Mast, M. G. 1988a. Thermal properties of proteins in chicken broiler tissues. J. Food Sci. 53(2): 363-366.

Kijowski, J. M. and Mast, M. G. 1988b. Effect of sodium chloride and phosphates on the thermal properties of chicken meat proteins. J. Food Sci. 53(2): 367-370.

Laemmli, U. K. 1990. Cleavage of structural proteins during the assembly of the head of Bacterio-phage T4. Nature. 227: 680-685.

Lametsch, R., Roepstorff, P., and Bendixen, E. 2002. Identification of protein degradation during postmortem storage of pig meat. J. Agric. Food Chem. 50(20): 5508-5512.

Lan, Y. H., Novakofski, J., McCusker, R. H., Brewer, M. S., Carr, T. R. and McKeith, F. K. 1995. Thermal gelation of myofibrils from pork, beef, fish, chicken and turkey. J. Food Sci. 60: 941-945.

Lee, B. J., Hendricks, G. and Cornforth, D. P. 1998. Effect of sodium phytate, sodium pyrophosphate and sodium tripolyphosphate on physio-chemical characteristics of restructured beef. Meat Sci. 50: 273-283.

Lee, S.O., Min, J. S., Kim, I. S. and Lee, M. 2003. Physical evaluation of poped cereal snacks with spent hen meat. Meat Sci. 64: 383-390.

Lemos, A. L. S. C., Nunes, D. R. M., and Viana, A. G. 1999. Optimization of still-marinating process of chicken parts. Meat Sci. 52: 227-234.

Lepetit, J. 2008. Collagen contribution to meat toughness: Theoretical aspects. Meat Sci. 80: 960-967.

Li, R., Keer, W. L., Toledo, R. and Teng, Q. 2001. $^{31}$P NMR analysis of chicken breast meat vacuum tumbled with NaCl and various phosphates. J. Sci. Food Agric. 81: 576-582.

Lui, A., Nishimura, T. & Takahashi, K. 1996. Relationship between structural properties of intramuscular connective tissue and toughness of various chicken skeletal muscles. Meat Sci. 43: 43-49.

Mackeen, M. M., Ali, A. M., Lajis, N. H., Kawazu, K., Hassan, Z., Amran, M., Habsah, M., Mooi, L. Y. and Mohamed, S. M. 2000. Antimicrobial, antioxidant, antitumour-promoting and cytotoxic activities of different plant part extracts of *Garcinia atroviridis* Griff. Ex T. Anders.. J. Ethnopharmac. 72: 395-402.

Mackeen, M. M., Ali, A. M., Lajis, N. H., Kawazu, K., Kikuzaki, H. and Nakatani, N. 2002. Antifungal garcinia acid esters from the fruits of *Garcinia atroviridis*. Zeitschrift für Naturforschung. 57C: 291-295.

Meat Science at Texus A&M University. 2008. Conversion of muscle to meat (online). Available : http://meat.tamu.edu/conversion.html [2008, November 12]

Medyński, A., Pospiech, E. and Kniat, R. 2000. Effect of various concentrations of lactic acid and sodium chloride on selected physico-chemical meat traits. Meat Sci. 55 : 285-290.

Mikel, W. B., Goddard, B.L. and Bradford, D.D. 1996. Muscle microstructure and sensory attributes of organic acid-treated beef strip loins. J. Food Sci. 6: 1058-1061.

Modi, V. K., Gowda, G. S. S, Sakhare, P. Z. Mahendrakar, N.S. D. and Rao, N. 2006. Pre-processed spice mix formulation and changes in its quality during storage. LWT. 39: 613–620

Mountney, G. J. Parkhurst, C. R. 1988. Poultry meat and egg production. (Parkhurst, C. R., Mountney, G. J., ed.). Van Nostrand Reinhold. New York.

Munira, K. N., Uddin, S., Faruque, S., Parvez, M. S., Miah, M. Y. and Siddiqui, M. S. I. 2006. Comparative study on carcass characteristics of different genetic group of spent hen in Bangladesh. Int. J. Poultry Sci. 5(2): 178-180.

Murphy, R. Y., and Marks, B. P. 2000. Effect of meat temperature on proteins, texture and cook loss for ground chicken breast patties. Poultry Sci. 79: 99-104.

Murphy, R. Y., and Marks, B. P. and Marcy, J. A. 1998. Apparent specific heat of chicken breast patties and their constituent proteins by differential scanning calorimetry. J. Food Sci. 63(1): 88-91.

Nowsad, A. A. K. M., Kanoh, S., and Niwa, E. 2000. Thermal gelation characteristics of breast and thigh muscles of spent hen and broiler and their surimi. Meat Sci. 54: 169-175.

Offer, G. and Trinick, J. 1983. On the mechanism of water holding in meat: The swelling and shrinking of myofibrils. Meat Sci. 8: 245-281.

Okolocha, E. C. and Ellerbroek. 2005. The influence of acid and alkaline treatments on pathogens and shelf-life of poultry meat. Food Control. 16: 217-225.

Önenç, A., Serdaroğlu, M. and Abdraimov, K. 2004. Effect of various additives to marinating baths on some properties of cattle meat. Eur. Food Res. Technol. 218: 114-117.

Oreskovich, D. C., Bechtel, P. J., Mckeith, F. K., Novakofski, J. and Basgall, E. J. 1992. Marinade pH affects textural properties of beef. J. Food Sci. 57(2): 305-311.

O'Sullivan, C.M., Lynch, A.M., Lynch, P.B., Buckley, D.J. and Kerry, J.P. 2004. Assessment of the antioxidant potential of food ingredients in fresh, previously frozen and cooked chicken patties. Int. J. Poultry Sci. 3: 337-344.

Palka, K. and Daun, H. 1999. Changes in texture, cooking losses, and myofibrilla structure of bovine *M. semitendinosus* during heating. Meat Sci. 51: 237-243.

Papa, I., Astier, C., Kwiatek, O., Raynaud, F., Bonnal, C., Lebart, M..C., Roustan, C. and Benyamin, Y. 1999. Alpha actinin–Cap Z, an anchoring complex for thin filaments in Z-line. J. Muscle Res. Cell Motil. 20(2): 187-197.

Pearson, A.M. and Young, R.B. Composition and Structure. *In* Muscle and Meat Biochemistry Academic Press, Inc. Califormia.

Petterson, M. K., Mielnik, M. B., Eie, T., Skrede, G. and Nilsson, A. 2004. Lipid oxidation in frozen, mechanically deboned turkey meat as affected by packaging parameters and storage conditions. Poultry Sci. 83: 1240-1248.

Pomeranz, Y. & Meloan, C. E. 1994. Lipids *In* Food analysis: Theory and practice. 16th ed. pp. 678-732. International Thomson Publishing. New York

Qiao, M., Fletcher, D. L., Smith, D. P. and Northcutt, K. 2002. Effects of raw broiler breast meat color variation on marination and cooked meat quality. Poultry Sci. 81: 276-280.

Robe, G. H. and Xiong, Y. L. 1992. Phosphates and muscle fiber types influence thermai transitions in porcine salt-soluble protein aggregation. J. Food Sci. 57(6): 1304-1310.

Roussel, H. and Cheftel, J. C. 1990. Mechanisms of gelation of sardine proteins: influence of thermal processing and of various additives on the texture and protein solubility of kamaboko gels. Int. J. Food Technol. 25, 260–269.

Sallam, K. and Samejima, K. 2004. Effects of trisodium phosphate and sodium chloride dipping on the microbial quality and shelf life of refrigerated tray-packaged chicken breasts. Food Sci. Biotechnol. 13(4): 425–429.

Sampathkumar, B., Khachatourians, G. G. and Korber, D. R. 2003. High pH during trisodium phosphate treatment caurses membrane damage and destruction of *Salmonella enterica* Serovar Enteritidis. Appl. Envi. Micro. 69(1): 122-129.

Serdaroğlu, M., Abdraimov, K. and Önenç, A. 2007. The effects of marinating with citric acid solutions and grapefruit juice on cooking and eating quality of turkey breast. J. Muscle Food. 18: 162-172.

Shahidi, F. and Synowiecki, J. 1997. Protein hydrolyzates from seal meat as phosphate alternatives in food processing applications. Food Chem. 60: 29-32.

Sheard, P. R. and Tali, A. 2004. Injection of salt, tripolyphosphate and bicarbonate marinade solutions to improve the yield and tenderness of cooked pock loin. Meat Sci. 68: 305-311.

Shobana, S. and Naidu, A. 2000. Antioxidant activity of selected Indian spices. *Prostaglandins*, Leu. and Essen. Fatty Acids, 62, 107-110.

Siripongvutikorn, S., Thummaratwasik, P. and Huang, Y. (2005). Antimicrobial and antioxidation effects of Thai seasoning, Tom-Yum. LWT. 38: 347–352.

Smith, D.P. and Young, L.L. 2005. The effect of pressure and phosphates on yield, shear, and color of marinated broiler breast meat. *In* Proceedings 17th European Symposium on Quality of Poultry Meat. p. 139-144

Thorarinsdottir, K. A., Arason, S., Bogason, S. G. and Kristbergsson, K. 2001. Effects of phosphate on yield, quality, and water-holding capacity in the processing of salted cod (*Gadus morhua)*. J. Food Sci. 66(6): 821-826.

Trindade, M.A., Felício, P. E. and Castillo, C. J. C. 2004. Mechanically separated meat of broiler breeder and white layer spent hens. Sci. Agric. 61(2): 234-239.

Trout, G. R. and Schmidt, G.R. 1984. Effect of phosphate type and concentration, salt level and method of preparation on binding in restructured beef rolls. J. Food Sci. 49: 687–694.

Vaithiyanathan, S., Naveena, B. M., Muthukumar, M. and Girish, P. S. 2008. Biochemical and physicochemical changes in spent hen breast meat during postmortem ageing. Poultry Sci. 87: 180-186.

Vareltzis, K., Soultos, N., Koidis, P., Ambrosiadis, J. and Genigeorgis, C. 1997. Antimicrobial effects of sodium tripolyphosphate against bacteria attached to the surface of chicken carcasses. LWT. 30: 665-669.

Vasavada, M. N., Dwivedi, S. and Cornforth, D. 2006. Evaluation of Garam Malasa spices and phosphates as antioxidants in cooked ground beef. J. Food Sci. 71(5): C292-C297.

Voutila, L. 2009. Properties of intramuscular connective tissue in pork and poultry with reference to weakening of structure. Dissertation. Department of Food Technology. University of Helsinki.

Wang, B., Pace, R.D., Dessai, A.P., Bovell-Benjamin and Phillips, B. (2002). Modified extraction method for determining 2-thiobarbituric acid values in meat with increased specificity and simplicity. J. Food Sci. 67: 2833-2836.

Wang, P. A., Martinez, I. and Olsen, R. L. 2009. Myosin heavy chain degradation during post mortem storage of Atlantic cod (*Gadus morhua* L.). Food Chem. 115: 1228-1233.

Wattanachant, S. 2003. Chemical compositions, properties and structure of muscle affecting textural characteristics of meat from Thai indigenous chicken and broiler. Doctor of Philosophy Dissertation. Prince of Songkla University.

Wattanachant, S., Benjakul, S. and Ledward, D. A. 2005. Effect o heat treatment on changes in texture, structure and properties of Thai indigenous chicken muscle. Food Chem. 93: 337-348.

Weilmeier, D. M. and Regenstein, J. M. 2004. Cooking enhances the antioxidant properties of polyphosphates. J. Food Sci. 69(1): 16-23.

Weinstock, A., King, P.C. and Wuthier, R. E. 1967. The ion-binding characteristics of reconstituted collagen. Biochem. J. 102: 983-988.

Wong, D. W. S. 1989. Additives. *In* Mechanism and Theory in Food Chemistry. AVI publishing. New York.

Xiong, S., Xiong, Y. L., Blanchard, S. P., Wang, B. and Tidwell, J. H. 2002. Evaluation of tenderness in prawns (*Machrobrachium rosenbergii*) marinated in various salt and acid solutions. Int. J. Food Sci. Technol. 37: 291-296.

Xiong, Y. L. and Brekke, C. J. 1989. Changes in protein solubility and gelation properties of chicken myofibrils during storage. J. Food Sci. 54(5): 1141-1146.

Xiong, Y. L. and Kupski, D. R. 1999a. Time-dependent marinade absorption and retention, cooking yield, and palatability of chicken filets marinated in various phosphate solutions. Poultry Sci. 78: 1053-1059.

Xiong, Y. L., and Kupski, D. R. 1999b. Monitoring phosphate marinade penetration in tumbled chicken filets using a thin-slicing, dye-tracing method. Poultry Sci. 78: 1048-1052.

Xiong, Y. L., Lou, X., Wang, C., Moody, W. G. & Harmon, R. J. 2000. Protein extraction from chicken myofibrils irrigated with various polyphosphate and NaCl solutions. J. Food Sci. 65(1): 96-100.

Xiong, Y. L. 2005. Role of myofibrillar proteins in water-binding in brine-enhanced meats. Food Res. Int. 38: 281-287.

Yoon, K. S. 2002. Texture and microstructure properties of frozen chicken breasts pretreated with salt and phosphate solutions. Poultry Sci. 81: 1910-1915.

Yoon, K. S. and Oscar, T.P. 2002. Survival of *Salmonella typhimurium* on sterile ground chicken breast patties after washing with salt and phosphates and durind refrigerated and frozen storage. J. Food Sci. 67(2): 772-775.

Zayas, J. F. 1997. Water Holding Capacity of Proteins. *In* Functionality of proteins in food. (Zayas, J. F., ed.). p. 76-133. Springer-Verlag. Berlin.

Zheng, M., Toledo, R. and Wicker, L. 1999. Effect of phosphate and pectin on quality and shelf-life of marinated chicken breast. J. Food Quality. 22: 553-564.

Zheng, M., Detienne, N. A., Barnes, B. W. and Wicker, L. 2000. Tenderness and yields of poultry breast are influenced by phosphate type and concentration of marinade. J. Sci. Food Agric. 81: 82-87.

# APPENDIX

# ADDITIONAL RESULTS

**Proximate compositions of spent hen breast muscle**

The moisture content was found in the range of 75-77%, while the other compositions were protein 20-22%, fat 2-5%, and ash 1-2% based on wet weight. These results were similar to the study of Nowsad *et al* (2000) who found 76.2% moisture, 18.7% protein, 4.42% fat, and 0.73% ash of spent hen breast muscle (ISA Brown, aged 98 weeks). While, the moisture, protein, fat, and ash content of breast spent hen breast meat (72 weeks old) evaluated by Lee *et al* (2003) was 67.46±3.13%, 24.36±2.81%, 7.15±0.38%, and 1.04±0.09%, respectively. Chuaynukool (2008) determined the proximate compositions of spent hen breast muscle (*Pectoralis major*; *H&M Brown Nick*, aged 52 weeks) and reported at 74.83±0.15%, 20.34±0.72%, 1.64±0.18%, and 0.19±0.84% for moisture, protein, fat, and ash content, respectively.

**Table 15** Proximate compositions (% wet basis) of spent hen breast muscle

| Proximate compositions | Spent hen muscle (% wet weight) |
|---|---|
| Moisture | 78.19±1.26 |
| Protein | 21.50±0.88 |
| Fat | 3.69±1.81 |
| Ash | 1.43±0.41 |

Data are given as mean range from two lots of spent hen materials (n=3×2, triplicate determination×lots)

**Table 16** Effect of phosphate and marinade ingredients on mesophilic bacteria (log CFU/g) of marinated spent hen muscle stored at 4ºC

| Treatments | Mesophilic bacteria (log CFU/g) | | | | | | |
|---|---|---|---|---|---|---|---|
| | Day 1 | Day 5 | Day 10 | Day 15 | Day 20 | Day 25 | Day 30 |
| C1 | $5.35\pm0.03^{b,E}$ | $5.45\pm0.04^{a,DE}$ | $5.72\pm0.19^{a,CD}$ | $5.78\pm0.12^{a,BC}$ | $6.03\pm0.07^{a,A}$ | - | - |
| C2 | $4.79\pm0.00^{d,AB}$ | $4.98\pm0.19^{b,A}$ | $4.23\pm0.63^{c,BC}$ | $3.78\pm0.10^{c,CD}$ | $3.59\pm0.04^{e,D}$ | $2.79\pm0.04^{e,E}$ | $3.19\pm0.02^{d,DE}$ |
| P1 | $5.05\pm0.01^{c,C}$ | $4.73\pm0.01^{b,E}$ | $5.08\pm0.01^{ab,C}$ | $4.91\pm0.09^{b,D}$ | $4.51\pm0.02^{c,F}$ | $5.42\pm0.04^{a,A}$ | $5.21\pm0.00^{a,B}$ |
| P2 | $5.12\pm0.08^{c,A}$ | $2.36\pm0.13^{d,D}$ | $1.90\pm0.0^{d,F}$ | $2.13\pm0.07^{d,E}$ | $3.90\pm0.07^{d,B}$ | $3.45\pm0.01^{c,C}$ | $3.53\pm0.01^{c,C}$ |
| P3 | $5.48\pm0.05^{a,A}$ | $5.61\pm0.09^{a,A}$ | $4.93\pm0.04^{b,C}$ | $5.23\pm0.16^{b,B}$ | $5.10\pm0.01^{b,BC}$ | $4.93\pm0.01^{b,C}$ | $5.05\pm0.11^{b,BC}$ |
| P4 | $4.69\pm0.01^{e,C}$ | $3.84\pm0.17^{c,D}$ | $3.74\pm0.07^{c,D}$ | $3.68\pm0.21^{c,D}$ | $5.01\pm0.08^{b,B}$ | $3.21\pm0.13^{d,E}$ | $6.51\pm0.08^{b,A}$ |

$^{a,b,c,d,e}$ Mean within column with differing superscripts are significantly different at $P<0.05$

$^{A,B,C,D,E,F}$ Mean within row with differing superscripts are significantly different at $P<0.05$

**Note:** C1 = raw muscle without treatment

C2 = muscle marinated with Tom-Yum paste without phosphate treatment

P- = muscle pretreatment with 4% STPP (sodium tripolyphosphate) solution at 4ºC for 10 h and then marinated with salt and sugar (P1), mixed garcinia, salt and sugar (P2), mixed Tom-Yum ingredients except garcinia (P3), or Tom-Yum paste (P4)

**Table 17** Effect of phosphate and marinade ingredients on psychrophilic bacteria (log CFU/g) of marinated spent hen muscle stored at 4ºC

| Treatments | Psychrophilic bacteria (log CFU/g) | | | | | | |
|---|---|---|---|---|---|---|---|
| | Day 1 | Day 5 | Day 10 | Day 15 | Day 20 | Day 25 | Day 30 |
| C1 | $4.13\pm0.04^{a,D}$ | $7.15\pm0.05^{a,C}$ | $7.84\pm0.04^{a,B}$ | $8.23\pm0.01^{a,A}$ | $8.17\pm0.05^{a,A}$ | - | - |
| C2 | $0.00\pm0.00^{c,B}$ | $0.00\pm0.00^{e,B}$ | $0.00\pm0.00^{f,B}$ | $0.00\pm0.00^{f,B}$ | $0.00\pm0.07^{e,B}$ | $2.10\pm0.08^{c,A}$ | $4.11\pm0.01^{c,A}$ |
| P1 | $4.02\pm0.21^{a,F}$ | $5.85\pm0.06^{b,D}$ | $6.76\pm0.01^{c,C}$ | $7.05\pm0.00^{b,B}$ | $4.73\pm0.09^{c,E}$ | $7.94\pm0.08^{a,A}$ | $7.14\pm0.06^{b,B}$ |
| P2 | $0.00\pm0.00^{c,E}$ | $2.08\pm0.11^{d,D}$ | $2.22\pm0.02^{e,D}$ | $4.60\pm0.01^{e,A}$ | $4.07\pm0.08^{d,B}$ | $4.61\pm0.01^{b,A}$ | $3.43\pm0.13^{d,C}$ |
| P3 | $3.54\pm0.01^{b,C}$ | $7.10\pm0.06^{a,A}$ | $6.92\pm0.08^{b,A}$ | $6.81\pm0.13^{c,A}$ | $6.79\pm0.09^{b,A}$ | $5.35\pm0.50^{b,B}$ | $7.02\pm0.01^{b,A}$ |
| P4 | $3.45\pm0.02^{b,G}$ | $4.62\pm0.03^{c,F}$ | $6.32\pm0.01^{d,C}$ | $5.78\pm0.01^{d,D}$ | $6.96\pm0.05^{b,B}$ | $4.98\pm0.40^{b,E}$ | $7.66\pm0.02^{a,A}$ |

$^{a,b,c,d,e}$ Mean within column with differing superscripts are significantly different at $P<0.05$

$^{A,B,C,D,E,F,G}$ Mean within row with differing superscripts are significantly different at $P<0.05$

**Note:** C1 = raw muscle without treatment

C2 = muscle marinated with Tom-Yum paste without phosphate treatment

P- = muscle pretreatment with 4% STPP (sodium tripolyphosphate) solution at 4ºC for 10 h and then marinated with salt and sugar (P1), mixed garcinia, salt and sugar (P2), mixed Tom-Yum ingredients except garcinia (P3), or Tom-Yum paste (P4)

**Table 18** Effect of phosphate and marinade ingredients on L* values of marinated spent hen muscle stored at 4ºC

| Treatments | L* | | | | | | |
|---|---|---|---|---|---|---|---|
| | Day 1 | Day 5 | Day 10 | Day 15 | Day 20 | Day 25 | Day 30 |
| C1 | $56.82\pm2.38^{a,A}$ | $54.41\pm2.42^{a,B}$ | $56.46\pm2.55^{b,A}$ | $53.85\pm2.16^{b,B}$ | $53.36\pm2.02^{c,B}$ | - | - |
| C2 | $52.06\pm2.99^{b,E}$ | $55.29\pm2.32^{a,E}$ | $60.64\pm2.58^{a,A}$ | $57.90\pm2.67^{c,C}$ | $58.17\pm2.82^{a,BC}$ | $59.90\pm1.79^{a,AB}$ | $59.98\pm2.01^{a,AB}$ |
| P1 | $46.25\pm2.36^{cd,A}$ | $45.03\pm3.06^{d,AB}$ | $44.36\pm2.49^{d,AB}$ | $43.72\pm2.89^{d,B}$ | $43.80\pm2.57^{e,B}$ | $44.75\pm3.12^{d,AB}$ | $44.30\pm2.42^{d,AB}$ |
| P2 | $51.32\pm2.81^{b,C}$ | $52.07\pm2.78^{b,C}$ | $55.15\pm1.60^{b,B}$ | $56.15\pm2.10^{a,AB}$ | $55.23\pm3.27^{b,B}$ | $54.64\pm3.00^{b,B}$ | $57.65\pm2.98^{b,A}$ |
| P3 | $47.55\pm2.16^{c,A}$ | $48.53\pm2.49^{c,A}$ | $42.13\pm1.83^{e,B}$ | $41.78\pm2.87^{e,B}$ | $42.43\pm1.66^{e,B}$ | $43.25\pm1.29^{d,B}$ | $42.85\pm2.72^{c,B}$ |
| P4 | $45.41\pm1.56^{d,B}$ | $45.92\pm2.14^{d,B}$ | $50.54\pm2.81^{c,A}$ | $46.71\pm2.92^{c,B}$ | $48.83\pm2.32^{d,A}$ | $49.89\pm3.06^{c,A}$ | $49.68\pm2.89^{c,A}$ |

$^{a,b,c,d,e}$ Mean within column with differing superscripts are significantly different at $P<0.05$

$^{A,B,C,D,E}$ Mean within row with differing superscripts are significantly different at $P<0.05$

**Note:** C1 = raw muscle without treatment

C2 = muscle marinated with Tom-Yum paste without phosphate treatment

P- = muscle pretreatment with 4% STPP (sodium tripolyphosphate) solution at 4ºC for 10 h and then marinated with salt and sugar (P1), mixed garcinia, salt and sugar (P2), mixed Tom-Yum ingredients except garcinia (P3), or Tom-Yum paste (P4)

**Table 19** Effect of phosphate and marinade ingredients on a* values of marinated spent hen muscle stored at 4ºC

| Treatments | a* | | | | | | |
|---|---|---|---|---|---|---|---|
| | Day 1 | Day 5 | Day 10 | Day 15 | Day 20 | Day 25 | Day 30 |
| C1 | -1.27±0.67$^{d,AB}$ | -1.51±0.75$^{d,B}$ | -1.05±0.51$^{e,AB}$ | -0.84±0.87$^{d,A}$ | -1.04±0.82$^{c,AB}$ | - | - |
| C2 | 3.87±0.88$^{a,A}$ | 4.39±1.47$^{a,A}$ | 4.48±1.58$^{a,A}$ | 4.50±1.24$^{a,A}$ | 2.91±1.04$^{a,B}$ | 2.53±0.81$^{a,B}$ | 2.78±0.90$^{a,B}$ |
| P1 | -1.41±0.58$^{d,A}$ | -2.06±0.56$^{d,BC}$ | -1.69±0.44$^{e,AB}$ | -1.74±0.67$^{e,AB}$ | -2.45±0.73$^{d,C}$ | -2.22±0.57$^{c,C}$ | -2.22±0.70$^{d,C}$ |
| P2 | 0.15±0.66$^{c,B}$ | 1.09±1.12$^{b,A}$ | 0.81±0.66$^{c,AB}$ | 0.99±1.01$^{b,A}$ | 0.25±0.62$^{b,B}$ | 0.18±0.81$^{b,B}$ | 0.58±1.05$^{b,AB}$ |
| P3 | -1.14±1.01$^{d,C}$ | -0.66±0.61$^{c,BC}$ | -0.28±1.04$^{d,B}$ | 0.00±0.65$^{c,AB}$ | 0.45±0.84$^{b,A}$ | -0.04±1.06$^{b,AB}$ | 0.57±0.81$^{b,A}$ |
| P4 | 1.34±0.47$^{b,B}$ | 1.25±0.71$^{b,B}$ | 2.37±0.97$^{b,A}$ | 1.14±0.80$^{b,BC}$ | 0.59±0.95$^{b,CD}$ | 0.05±0.81$^{b,DE}$ | -0.47±0.78$^{c,E}$ |

$^{a,b,c,d,e}$ Mean within column with differing superscripts are significantly different at $P<0.05$

$^{A,B,C,D,E}$ Mean within row with differing superscripts are significantly different at $P<0.05$

**Note:** C1 = raw muscle without treatment

C2 = muscle marinated with Tom-Yum paste without phosphate treatment

P- = muscle pretreatment with 4% STPP (sodium tripolyphosphate) solution at 4ºC for 10 h and then marinated with salt and sugar (P1), mixed garcinia, salt and sugar (P2), mixed Tom-Yum ingredients except garcinia (P3), or Tom-Yum paste (P4)

**Table 20** Effect of phosphate and marinade ingredients on b* values of marinated spent hen muscle stored at 4ºC

| Treatments | b* | | | | | | |
|---|---|---|---|---|---|---|---|
| | Day 1 | Day 5 | Day 10 | Day 15 | Day 20 | Day 25 | Day 30 |
| C1 | 8.40±1.12$^{c,B}$ | 9.28±0.91$^{d,A}$ | 9.02±0.71$^{e,AB}$ | 9.61±1.08$^{e,A}$ | 9.32±0.93$^{e,A}$ | - | - |
| C2 | 18.25±1.54$^{a,D}$ | 22.01±2.66$^{a,AB}$ | 23.04±1.56$^{a,A}$ | 23.06±1.87$^{a,A}$ | 21.47±1.88$^{a,BC}$ | 20.59±0.90$^{a,C}$ | 21.29±1.23$^{a,BC}$ |
| P1 | 4.57±1.00$^{d}$ | 5.03±0.91$^{e}$ | 4.98±1.08$^{f}$ | 4.21±1.54$^{f}$ | 4.43±1.21$^{f}$ | 4.81±1.23$^{d}$ | 4.68±1.42$^{d}$ |
| P2 | 8.68±1.03$^{c,C}$ | 12.73±1.95$^{c,AB}$ | 11.47±1.79$^{d,B}$ | 13.26±1.72$^{c,A}$ | 11.57±1.36$^{d,B}$ | 12.49±1.68$^{c,AB}$ | 12.56±1.29$^{c,AB}$ |
| P3 | 12.19±2.28$^{b,D}$ | 15.31±2.09$^{b,A}$ | 12.77±1.37$^{c,CD}$ | 12.07±1.02$^{d,D}$ | 13.88±1.67$^{c,BC}$ | 13.32±1.48$^{c,CD}$ | 14.58±1.17$^{b,AB}$ |
| P4 | 13.00±0.94$^{b,D}$ | 16.41±1.45$^{b,BC}$ | 18.72±1.89$^{b,A}$ | 15.62±1.22$^{b,C}$ | 17.43±2.14$^{b,B}$ | 16.46±1.88$^{b,BC}$ | 15.29±2.08$^{b,C}$ |

$^{a,b,c,d,e,f}$ Mean within column with differing superscripts are significantly different at $P<0.05$

$^{A,B,C,D}$ Mean within row with differing superscripts are significantly different at $P<0.05$

**Note:** C1 = raw muscle without treatment

C2 = muscle marinated with Tom-Yum paste without phosphate treatment

P- = muscle pretreatment with 4% STPP (sodium tripolyphosphate) solution at 4ºC for 10 h and then marinated with salt and sugar (P1), mixed garcinia, salt and sugar (P2), mixed Tom-Yum ingredients except garcinia (P3), or Tom-Yum paste (P4)

**Table 21** Effect of phosphate and marinade ingredients on cooking loss (%) of marinated spent hen muscle stored at 4ºC

| Treatments | Cooking loss (%) | | | | | | |
|---|---|---|---|---|---|---|---|
| | Day 1 | Day 5 | Day 10 | Day 15 | Day 20 | Day 25 | Day 30 |
| C1 | $32.74\pm1.83^{a,A}$ | $30.69\pm2.70^{a,A}$ | $30.42\pm2.18^{b,A}$ | $30.78\pm2.08^{ab,A}$ | $27.69\pm1.68^{c,B}$ | - | - |
| C2 | $28.15\pm1.59^{c,C}$ | $29.11\pm2.80^{caBC}$ | $30.91\pm0.95^{b,AB}$ | $28.90\pm3.98^{b,BC}$ | $31.17\pm1.92^{b,AB}$ | $31.95\pm2.27^{b,A}$ | $29.95\pm2.47^{ab,ABC}$ |
| P1 | $18.28\pm2.22^{e,A}$ | $16.40\pm2.88^{c,AB}$ | $16.02\pm1.78^{d,AB}$ | $16.50\pm4.18^{c,AB}$ | $16.44\pm2.64^{e,AB}$ | $13.74\pm1.36^{d,B}$ | $15.59\pm3.97^{c,AB}$ |
| P2 | $28.87\pm3.16^{bc,C}$ | $31.57\pm2.92^{a,BC}$ | $34.50\pm3.39^{a,AB}$ | $32.63\pm2.15^{a,AB}$ | $35.14\pm2.53^{a,A}$ | $32.93\pm2.58^{b,AB}$ | $31.74\pm3.51^{ab,BC}$ |
| P3 | $21.25\pm2.05^{d,B}$ | $20.05\pm2.72^{b,BC}$ | $20.07\pm3.20^{c,BC}$ | $17.78\pm1.84^{c,C}$ | $22.10\pm2.68^{d,B}$ | $22.73\pm3.74^{c,B}$ | $28.36\pm3.74^{b,A}$ |
| P4 | $31.04\pm3.81^{ab,BC}$ | $29.47\pm3.57^{a,C}$ | $32.88\pm2.28^{b,C}$ | $31.68\pm2.67^{ab,BC}$ | $33.56\pm3.59^{ab,AB}$ | $35.70\pm2.88^{a,A}$ | $32.48\pm3.85^{a,ABC}$ |

$^{a,b,c,d,e}$ Mean within column with differing superscripts are significantly different at $P<0.05$

$^{A,B,C}$ Mean within row with differing superscripts are significantly different at $P<0.05$

**Note:** C1 = raw muscle without treatment

C2 = muscle marinated with Tom-Yum paste without phosphate treatment

P- = muscle pretreatment with 4% STPP (sodium tripolyphosphate) solution at 4ºC for 10 h and then marinated with salt and sugar (P1), mixed garcinia, salt and sugar (P2), mixed Tom-Yum ingredients except garcinia (P3), or Tom-Yum paste (P4)

**Table 22** Effect of phosphate and marinade ingredients on shear force values (N) of raw marinated spent hen muscle stored at 4ºC

| Treatments | Shear force values (N) | | | | | | |
|---|---|---|---|---|---|---|---|
| | Day 1 | Day 5 | Day 10 | Day 15 | Day 20 | Day 25 | Day 30 |
| C1 | $65.61\pm9.56^{ab,A}$ | $57.29\pm10.91^{b,AB}$ | $60.98\pm10.26^{ab,AB}$ | $50.91\pm12.75^{b,B}$ | $60.28\pm6.34^{a,AB}$ | - | - |
| C2 | $54.31\pm8.62^{b,BC}$ | $65.68\pm9.16^{b,B}$ | $50.36\pm11.27^{bc,C}$ | $75.63\pm9.51^{a,A}$ | $52.42\pm12.65^{b,C}$ | $57.10\pm10.45^{a,ABC}$ | $73.00\pm7.73^{a,A}$ |
| P1 | $63.97\pm13.40^{b,AB}$ | $34.22\pm12.40^{c,D}$ | $46.35\pm12.79^{bc,CD}$ | $73.19\pm10.84^{a,A}$ | $53.09\pm8.96^{b,BC}$ | $55.25\pm9.76^{a,BC}$ | $55.57\pm10.03^{b,BC}$ |
| P2 | $79.10\pm11.62^{a,A}$ | $80.02\pm13.43^{a,A}$ | $72.92\pm11.54^{a,AB}$ | $85.15\pm9.37^{a,A}$ | $78.85\pm10.16^{a,A}$ | $66.02\pm9.43^{a,B}$ | $80.41\pm6.50^{a,A}$ |
| P3 | $61.59\pm12.60^{b,A}$ | $54.70\pm10.15^{b,AB}$ | $50.73\pm11.95^{bc,ABC}$ | $40.69\pm11.23^{bc,BC}$ | $57.92\pm8.28^{b,A}$ | $40.33\pm11.34^{b,BC}$ | $37.61\pm10.00^{c,C}$ |
| P4 | $52.12\pm13.80^{b,AB}$ | $37.74\pm11.02^{c,C}$ | $42.70\pm11.85^{c,AB}$ | $37.55\pm11.40^{c,C}$ | $57.94\pm7.73^{b,A}$ | $54.87\pm9.19^{a,AB}$ | $58.56\pm9.50^{b,A}$ |

$^{a,b,c}$ Mean within column with differing superscripts are significantly different at $P<0.05$

$^{A,B,C,D}$ Mean within row with differing superscripts are significantly different at $P<0.05$

**Note:** C1 = raw muscle without treatment

C2 = muscle marinated with Tom-Yum paste without phosphate treatment

P- = muscle pretreatment with 4% STPP (sodium tripolyphosphate) solution at 4ºC for 10 h and then marinated with salt and sugar (P1), mixed garcinia, salt and sugar (P2), mixed Tom-Yum ingredients except garcinia (P3), or Tom-Yum paste (P4)

**Table 23** Effect of phosphate and marinade ingredients on shear force values (N) of cooked marinated spent hen muscle stored at 4ºC

| Treatments | Shear force values (N) | | | | | | |
|---|---|---|---|---|---|---|---|
| | Day 1 | Day 5 | Day 10 | Day 15 | Day 20 | Day 25 | Day 30 |
| C1 | 45.25±5.72$^{c,B}$ | 54.63±5.13$^{b,A}$ | 58.97±7.99$^{a,A}$ | 42.18±5.59$^{c,B}$ | 45.92±8.00$^{bc,B}$ | - | - |
| C2 | 58.67±8.64$^{b,A}$ | 49.32±8.87$^{b,AB}$ | 50.58±9.03$^{b,AB}$ | 52.37±7.05$^{b,A}$ | 54.07±5.33$^{b,A}$ | 41.20±7.71$^{b,B}$ | 54.89±9.75$^{b,A}$ |
| P1 | 41.59±7.51$^{c,ABC}$ | 48.99±8.95$^{b,A}$ | 44.79±7.72$^{bc,ABC}$ | 38.26±6.88$^{c,C}$ | 42.98±4.32$^{c,ABC}$ | 39.89±8.23$^{b,BC}$ | 47.06±6.23$^{bc,AB}$ |
| P2 | 83.06±8.62$^{a,A}$ | 70.00±9.01$^{a,BCD}$ | 66.96±8.09$^{a,CD}$ | 75.29±8.41$^{a,ABC}$ | 71.02±10.24$^{a,BC}$ | 59.52±8.84$^{a,D}$ | 79.41±7.67$^{a,AB}$ |
| P3 | 41.52±6.92$^{c}$ | 34.40±5.39$^{c}$ | 42.66±6.39$^{bc}$ | 40.82±6.92$^{c}$ | 42.43±8.50$^{c}$ | 36.08±8.02$^{b}$ | 38.85±5.54$^{c}$ |
| P4 | 58.45±7.39$^{b,AB}$ | 55.79±7.14$^{b,AB}$ | 40.49±5.87$^{c,C}$ | 50.62±7.51$^{b,B}$ | 50.23±8.81$^{bc,B}$ | 61.88±6.87$^{a,A}$ | 53.63±9.10$^{b,AB}$ |

$^{a,b,c}$ Mean within column with differing superscripts are significantly different at $P<0.05$

$^{A,B,C,D}$ Mean within row with differing superscripts are significantly different at $P<0.05$

**Note:** C1 = raw muscle without treatment

C2 = muscle marinated with Tom-Yum paste without phosphate treatment

P- = muscle pretreatment with 4% STPP (sodium tripolyphosphate) solution at 4ºC for 10 h and then marinated with salt and sugar (P1), mixed garcinia, salt and sugar (P2), mixed Tom-Yum ingredients except garcinia (P3), or Tom-Yum paste (P4)

**Table 24** Effect of phosphate and marinade ingredients on TBARS (mg malonaldehyde/ kg muscle) of marinated spent hen muscle stored at 4ºC

| Treatments | TBARS (mg malonaldehyde/kg muscle) | | | | | | |
|---|---|---|---|---|---|---|---|
| | Day 1 | Day 5 | Day 10 | Day 15 | Day 20 | Day 25 | Day 30 |
| C1 | $3.19\pm0.06^{cd,A}$ | $2.44\pm0.17^{e,B}$ | $1.39\pm0.07^{d,D}$ | $1.74\pm0.28^{c,C}$ | $1.26\pm0.17^{d,D}$ | - | - |
| C2 | $4.14\pm0.58^{b,A}$ | $3.95\pm0.23^{c,A}$ | $3.02\pm0.23^{b,BC}$ | $3.56\pm0.48^{b,AB}$ | $2.68\pm0.12^{b,BC}$ | $3.05\pm0.20^{b,C}$ | $2.42\pm0.50^{b,C}$ |
| P1 | $6.45\pm0.37^{a,C}$ | $7.97\pm0.72^{a,B}$ | $9.32\pm0.24^{a,A}$ | $9.86\pm0.66^{a,A}$ | $6.42\pm0.65^{a,C}$ | $5.17\pm0.27^{a,D}$ | $2.42\pm0.53^{b,E}$ |
| P2 | $2.63\pm0.22^{e,B}$ | $5.42\pm0.30^{b,A}$ | $2.63\pm0.09^{c,B}$ | $1.32\pm0.08^{c,D}$ | $1.86\pm0.04^{c,C}$ | $1.74\pm0.07^{c,C}$ | $1.35\pm0.10^{c,D}$ |
| P3 | $3.60\pm0.12^{c,A}$ | $3.28\pm0.27^{d,A}$ | $2.95\pm0.13^{bc,AB}$ | $3.43\pm0.79^{b,A}$ | $2.59\pm0.12^{b,B}$ | $1.60\pm0.17^{c,C}$ | $1.36\pm0.37^{c,C}$ |
| P4 | $2.77\pm0.11^{de}$ | $2.76\pm0.09^{cd}$ | $2.65\pm0.32^{d}$ | $3.18\pm0.24^{b}$ | $2.99\pm0.44^{b}$ | $2.61\pm0.50^{b}$ | $3.17\pm0.49^{a}$ |

$^{a,b,c,d,e}$ Mean within column with differing superscripts are significantly different at $P<0.05$

$^{A,B,C,D,E}$ Mean within row with differing superscripts are significantly different at $P<0.05$

**Note:** C1 = raw muscle without treatment

C2 = muscle marinated with Tom-Yum paste without phosphate treatment

P- = muscle pretreatment with 4% STPP (sodium tripolyphosphate) solution at 4ºC for 10 h and then marinated with salt and sugar (P1), mixed garcinia, salt and sugar (P2), mixed Tom-Yum ingredients except garcinia (P3), or Tom-Yum paste (P4)

**Table 25** Effect of phosphate and marinade ingredients on total collagen content (mg/g muscle) of marinated spent hen muscle stored at 4ºC

| Treatment | Total collagen content (mg/g muscle) | | | | | | |
|---|---|---|---|---|---|---|---|
| | Day 1 | Day 5 | Day 10 | Day 15 | Day 20 | Day 25 | Day 30 |
| C1 | $7.00\pm1.74^{b,C}$ | $12.97\pm1.01^{a,A}$ | $7.76\pm1.43^{cd,BC}$ | $8.92\pm0.30^{b,BC}$ | $9.24\pm0.28^{d,B}$ | - | - |
| C2 | $9.40\pm0.15^{a,CD}$ | $10.77\pm0.27^{b,AB}$ | $8.81\pm0.45^{c,D}$ | $10.57\pm1.10^{a,BC}$ | $11.25\pm0.08^{b,AB}$ | $12.01\pm0.35^{a,A}$ | $11.34\pm0.11^{a,AB}$ |
| P1 | $6.35\pm0.14^{b,B}$ | $5.33\pm0.17^{c,BC}$ | $8.95\pm0.22^{c,A}$ | $4.69\pm0.00^{c,C}$ | $5.86\pm0.13^{e,BC}$ | $5.23\pm0.02^{c,BC}$ | $5.86\pm1.28^{bc,BC}$ |
| P2 | $9.92\pm0.28^{a}$ | $9.76\pm0.25^{b}$ | $10.96\pm0.09^{b}$ | $11.55\pm0.27^{a}$ | $10.76\pm0.23^{c}$ | $10.84\pm0.46^{b}$ | $10.18\pm2.54^{a}$ |
| P3 | $4.15\pm0.66^{c,BC}$ | $4.85\pm0.14^{c,B}$ | $6.19\pm0.29^{d,A}$ | $4.61\pm0.02^{c,BC}$ | $3.86\pm0.18^{f,C}$ | $4.76\pm0.74^{c,B}$ | $4.57\pm0.10^{c,BC}$ |
| P4 | $9.09\pm0.37^{a,D}$ | $13.28\pm0.20^{a,A}$ | $13.29\pm0.27^{a,A}$ | $11.00\pm0.13^{a,C}$ | $13.90\pm0.20^{a,A}$ | $12.35\pm0.22^{a,B}$ | $7.20\pm0.59^{b,E}$ |

$^{a,b,c,d,e,f}$ Mean within column with differing superscripts are significantly different at $P<0.05$

$^{A,B,C,D,E}$ Mean within row with differing superscripts are significantly different at $P<0.05$

**Note:** C1 = raw muscle without treatment

C2 = muscle marinated with Tom-Yum paste without phosphate treatment

P- = muscle pretreatment with 4% STPP (sodium tripolyphosphate) solution at 4ºC for 10 h and then marinated with salt and sugar (P1), mixed garcinia, salt and sugar (P2), mixed Tom-Yum ingredients except garcinia (P3), or Tom-Yum paste (P4)

**Table 26** Effect of phosphate and marinade ingredients on heat-soluble collagen content (%) of marinated spent hen muscle stored at 4ºC

| Treatment | Heat-soluble collagen content (%) | | | | | | |
|---|---|---|---|---|---|---|---|
| | Day 1 | Day 5 | Day 10 | Day 15 | Day 20 | Day 25 | Day 30 |
| C1 | $1.03\pm0.46^{d}$ | $1.84\pm0.84^{c}$ | $1.20\pm1.05^{e}$ | $2.07\pm0.41^{d}$ | ND | ND | ND |
| C2 | $10.09\pm0.27^{b,C}$ | $7.96\pm0.78^{a,C}$ | $23.29\pm1.45^{a,AB}$ | $24.71\pm1.37^{a,A}$ | $20.48\pm4.32^{b,B}$ | $25.36\pm1.20^{a,A}$ | $20.28\pm0.63^{a,B}$ |
| P1 | $9.56\pm1.30^{b,A}$ | $5.59\pm0.40^{b,B}$ | $4.46\pm0.27^{d,B}$ | ND | ND | ND | ND |
| P2 | $15.71\pm0.29^{a,BC}$ | $17.77\pm0.55^{a,BC}$ | $14.68\pm0.70^{b,C}$ | $17.70\pm3.10^{b,BC}$ | $18.78\pm0.98^{a,B}$ | $15.71\pm1.01^{b,BC}$ | $23.63\pm0.52^{a,A}$ |
| P3 | $8.42\pm1.34^{b,A}$ | $5.42\pm1.51^{b,B}$ | $2.41\pm0.41^{e,C}$ | $2.81\pm0.40^{d,C}$ | ND | ND | ND |
| P4 | $4.66\pm0.48^{c,CD}$ | $7.01\pm1.68^{ab,BC}$ | $9.95\pm0.92^{c,A}$ | $7.09\pm1.50^{c,BC}$ | $6.78\pm1.06^{c,BCD}$ | $4.38\pm0.23^{c,D}$ | $8.38\pm181^{b,AB}$ |

$^{a,b,c,d}$ Mean within column with differing superscripts are significantly different at $P<0.05$

$^{A,B,C,D}$ Mean within row with differing superscripts are significantly different at $P<0.05$

**Note:** C1 = raw muscle without treatment

C2 = muscle marinated with Tom-Yum paste without phosphate treatment

P- = muscle pretreatment with 4% STPP (sodium tripolyphosphate) solution at 4ºC for 10 h and then marinated with salt and sugar (P1), mixed garcinia, salt and sugar (P2), mixed Tom-Yum ingredients except garcinia (P3), or Tom-Yum paste (P4)

ND = not detectable

**Table 27** Effect of phosphate and marinade ingredients on phosphate content (as $P_2O_5$; ppm) of marinated spent hen muscle stored at 4ºC

| Treatment* | Phosphate content (ppm) | | | | | | |
|---|---|---|---|---|---|---|---|
| | Day 1 | Day 5 | Day 10 | Day 15 | Day 20 | Day 25 | Day 30 |
| C1 | $1356.88\pm191.44^{d,C}$ | $1964.88\pm154.34^{c,A}$ | $1787.61\pm100.55^{d,A}$ | $1734.96\pm184.20^{d,AB}$ | $1432.65\pm320.22^{d,BC}$ | - | - |
| C2 | $931.51\pm22.75^{e,B}$ | $1200.48\pm100.75^{d,A}$ | $849.66\pm236.26^{e,AB}$ | $948.30\pm48.40^{e,B}$ | $792.17\pm68.25^{e,BCD}$ | $697.46\pm108.30^{d,CD}$ | $607.33\pm59.77^{d,D}$ |
| P1 | $4758.56\pm476.34^{a,A}$ | $4412.56\pm188.27^{a,AB}$ | $4465.53\pm124.13^{a,AB}$ | $4068.37\pm226.67^{a,BC}$ | $3468.63\pm583.88^{a,CD}$ | $3322.88\pm407.09^{b,D}$ | $482.61\pm433.28^{a,CD}$ |
| P2 | $44370.89\pm192.32^{a,A}$ | $3828.70\pm876.52^{ab,AB}$ | $3662.99\pm520.69^{b,AB}$ | $3419.41\pm575.81^{bc,B}$ | $3472.47\pm121.04^{a,B}$ | $3726.52\pm312.93^{a,AB}$ | $1818.06\pm193.56^{c,C}$ |
| P3 | $3067.49\pm132.06^{c,BC}$ | $3217.74\pm256.55^{b,BC}$ | $3293.29\pm178.07^{b,AB}$ | $3591.85\pm479.34^{ab,A}$ | $2861.38\pm177.81^{b,C}$ | $3166.39\pm89.71^{b,BC}$ | $2442.14\pm36.59^{b,D}$ |
| P4 | $3500.96\pm307.48^{b,A}$ | $3219.6\pm191.59^{b,AB}$ | $2871.37\pm148.03^{c,BC}$ | $2866.90\pm415.75^{c,BC}$ | $2132.92\pm145.40^{c,D}$ | $2486.66\pm259.64^{c,CD}$ | $2597.94\pm339.51^{b,C}$ |

$^{a,b,c,d,e}$ Mean within column with differing superscripts are significantly different at $P<0.05$

$^{A,B,C,D}$ Mean within row with differing superscripts are significantly different at $P<0.05$

**Note:** C1 = raw muscle without treatment

C2 = muscle marinated with Tom-Yum paste without phosphate treatment

P- = muscle pretreatment with 4% STPP (sodium tripolyphosphate) solution at 4ºC for 10 h and then marinated with salt and sugar (P1), mixed garcinia, salt and sugar (P2), mixed Tom-Yum ingredients except garcinia (P3), or Tom-Yum paste (P4)

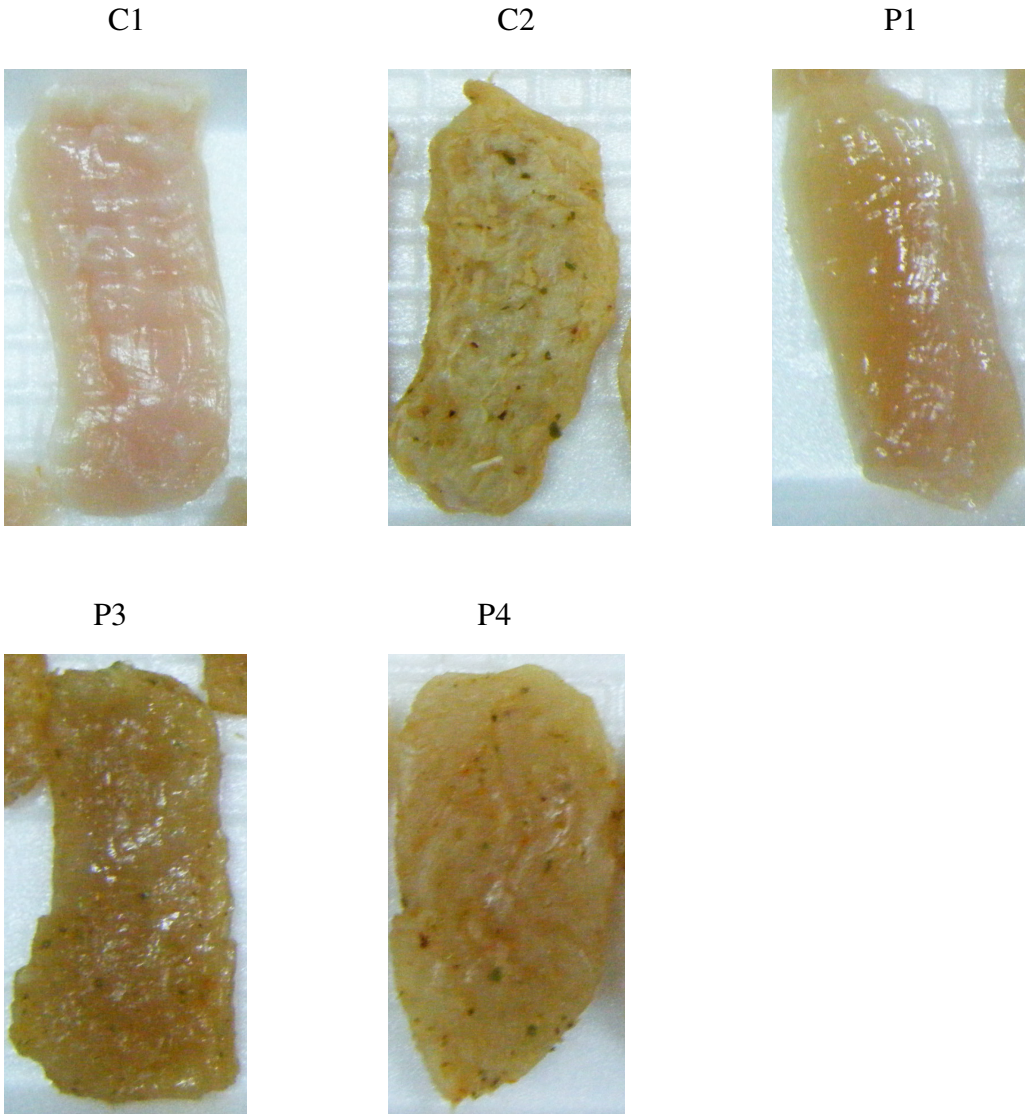



**Figure 21** Appearance of marinated spent hen muscle

**Note:** C1 = raw muscle without treatment

C2 = muscle marinated with Tom-Yum paste without phosphate treatment

P- = muscle pretreatment with 4% STPP (sodium tripolyphosphate) solution at 4ºC for 10 h and then marinated with salt and sugar (P1), mixed Tom-Yum ingredients except garcinia (P3), or Tom-Yum paste (P4)


**Figure 22** Tom-Yum paste marinated spent hen muscle product

# VITAE

**Name** Miss Pirinya Wongwiwat

**Student ID** 4911020019

**Educational Attainment**

| Degree | Name of Institution | Year of Graduation |
|---|---|---|
| Bachelor of Science (Agro-industry, Second Class Honor) | Prince of Songkla University | 2005 |

**Scholarship Awards during Enrolment**

The Scholarship for Development of Agro-Industry to the Excellence from Faculty of Agro-Industry, Prince of Songkla University

**List of Publication and Proceedings**

Wongwiwat, P., Yanpakdee, S. and Wattanachant, S. 2007. Effect of mixed spices in lemon glass marinade cuisine on changes in chemical physical and microbiological quality of ready-to-cook Thai indigenous chicken meat during chilled storage. Songklanakarin J. Sci. Technol. 29(6): 1619-1632.

Wongwiwat, P. 2008. Effect of phosphate types on physicochemical characteristics of spent hen muscle in high acid condition. In The sixth Regional IMT-GT UNINET Conference. The Gurney Hotel, Penang, Malaysia. 28-30 August 2008. P. 275-278.