

5-2014

EFFECT OF BROMELAIN ON DUCK BREAST MEAT TENDERIZATION

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EFFECT OF BROMELAIN ON DUCK BREAST MEAT TENDERIZATION

A Thesis
Presented to
the Graduate School of
Clemson University

In Partial Fulfillment
of the Requirements for the Degree
Master of Science
Food, Nutrition, and Culinary Sciences

by
Ahmet Buyukyavuz
May 2014

Accepted by:
Dr. Paul L. Dawson, Committee Chair
Dr. Julie Northcutt
Dr. Doug Smith

ABSTRACT

This study was conducted to determine the effect of bromelain on duck breast meat tenderization. Duck breasts were marinated with different concentrations (0, 1.5, 3 and 4.5%; C2, B1.5, B3 and B4.5, respectively) of bromelain and a solution composed of 92% refrigerated water, 6% salt and 2% sodium tripolyphosphate by using a vacuum food tumbler machine. A second non-marinated control (C1) was also included. Marination tumbling was operated at 8 RPM for 8 minutes. Physico-chemical and quality parameters were determined on raw and cooked samples. A significant reduction ($p < 0.05$) in pH was observed in all bromelain treated samples when compared to C2. Although there was a significant reduction in pH of B1.5 when compared to C1, there was no significant difference among B3, B4.5 and C1. A significant increase was observed in water holding capacity of B3 and B4.5 when compared to C1. While a significant reduction was observed in lightness (L^*), no differences were observed in redness (a^*) and yellowness (b^*) values of marinated samples (C2, B1.5, B3 and B4.5) when compared to C1. However, a significant increase in lightness (L^*), and a significant decrease in redness (a^*) and yellowness (b^*) were observed in cooked bromelain treated samples. No differences were observed in razor shear forces, moisture content and water activity between treatments. There was no significant difference in cook yield of all marinated samples (C2, B1.5, B3, and B4) when compared to the C1. A significant reduction ($p < 0.05$) in Warner-Bratzler shear force values was observed in all marinated samples when compared to untreated control (C1). Based on results, use of bromelain in marination solutions under the conditions tested here would not be cost effective.

ACKNOWLEDGMENTS

I would like to express my appreciation to my advisor, Dr. Paul L. Dawson, Professor, Department of Food, Nutrition, and Packaging Sciences for continuous guidance, support and understanding.

I am thankful to Dr. Inyee Y. Han, Research Specialist, Department of Food, Nutrition, and Packaging Sciences for her wonderful assistance and continuous help in the Lab throughout my research work.

Appreciation is also expressed to my committee members Dr. Julie Northcutt, Professor, Department of Food, Nutrition, and Packaging Sciences and Dr. Doug Smith, Associate Professor, Department of Poultry Science for their support and excellent suggestions.

Finally, I would like to thank my parents, Hasan and Nazli Buyukyavuz, for their prayers, support and encouragement throughout my life.

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CHAPTER 1

INTRODUCTION

Commercial poultry processors cut-up and debone carcasses as soon after chill as possible to minimize economical loss from carcass storage (energy, floor space) and yield loss from water that exudes during holding (Fletcher, 2002). However, previous research has shown that rapid chilling and early deboning of carcasses may have negative impact on other meat qualities, such as toughness, moisture content (Wakefield et al., 1989; Lyons et al., 1992; Dunn et al., 1993). It has been reported that earlier deboning times, shorter aging times (Craig et al., 1999) and early removal of breast from the carcass (Obanor et al., 2005) led to increased toughness in poultry breast meat. Moreover, rapid chilling before the completion of rigor mortis can adversely affect the tenderization process (Bendall, 1978). Tenderness has been described as the most significant quality attribute affecting consumer satisfaction and product perception (Savell et al., 1989). Tenderness is influenced by the length of the sarcomeres, structural integrity of the myofibrils that influence the actomyosin toughness and the integrity of the connective tissue that affects the background toughness (Chen et al., 2006). Meat from older animals is generally tougher than meat from young animals since old animals have more collagen cross-linking (Koohmaraie, 1992). Conventional aging with endogenous enzymes (calpains and cathepsins) is time consuming and may still result in toughness (Koohmaraie, 1994; Pohlman et al., 1997).

Several methods have been tested to improve the tenderness of meat by the use of enzymes (Melendo et al., 1997; Ashie et al., 2002; Wada et al., 2002; Naveena et al.,

2004; Pawar et al., 2007), salts, phosphates (Pietrasik et al., 2010) and calcium chloride (Gerelt et al., 2000). Moreover, there have been some physical methods to tenderize meat, such as pressure treatments (Palka, 1999), ultrasound (Pohlman et al., 1997; Jayasooriya et al., 2007), electrical stimulation (Claus et al., 2001) and blade tenderization (Pietrasik et al., 2010). Proteolytic enzymes are commonly used in meat tenderization. These enzymes can be obtained from plant, bacterial and fungal sources. There are five exogenous enzymes (papain, ficin, bromelain, *Aspergillus oryzae* protease, and *Bacillus subtilis* protease) recognized by United States federal agencies as Generally Recognized as Safe (GRAS) for meat tenderization. Apart from these GRAS enzymes, others have been evaluated including ginger rhizome (Sullivan and Calkins, 2010). However, proteolytic enzymes extracted from plant such as papain, bromelain, and ficin have been more commonly used as meat tenderizers in the world (Naveena et al., 2004) because bacterial derived enzymes mainly lead to safety concerns (Chen et al., 2006).

Although duck meat is usually considered as tough by consumer (Smith and Fletcher 1992), there is very little research on duck meat tenderization when compared to the other poultry meats (Naveena and Mendiratta, 2001; Serdaroglu et al., 2006; Claus et al., 2001). Therefore, this study was undertaken to determine the effect of commercial bromelain on duck breast meat tenderization.

CHAPTER 2

REVIEW OF LITERATURE

1. Muscle structure

Skeletal muscle contains a large number of single muscle fibers. The entire skeletal muscle is wrapped by the epimysium, which is a thin cover of connective tissue extending over the tendon. Each muscle includes several muscle fiber bundles, which are wrapped by the perimysium, another thin cover of connective tissue. Muscle fiber bundles include a varying number (30-80) of individual muscle fibers up to a few centimeters long with a diameter of 50 microns. Each bundle is surrounded by the endomysium, the other layer of connective tissue. Muscle fibers contain about 1,000 myofibrils, which are responsible for muscle contraction and relaxation. Myofibrils are composed of thin filaments (actin) and thick filaments (myosin) (Cassens, 1994; Toldrá, 2002; Heinz and Hautzinger, 2007).

The sarcomere is the smallest contractile unit of a muscle fiber and is approximately 2-3 μm long (Toldrá, 2002). Sarcomeres are linked end-to-end within a muscle fiber. There are thick and thin filaments within each sarcomere. The filaments are oriented an overlapping arrangement, which results in dark (A) and light (I) bands giving appearance of striations. The area which actin and myosin overlap is the A band. The area in the A band which includes no thin filaments is the H zone while the region with no thick filaments is the I band (Huxley, 1972). While I bands are bisected by Z-lines, A bands are bisected by M-lines.

Myofilaments are connected to the net-like structured cell membrane, known as the sarcolemma. Muscle fibers are filled with intracellular sarcoplasm (cellular fluid), which is a liquid made up of approximately 80% water as well as proteins, enzymes, lipids, carbohydrates, and inorganic constituents (Aberle et al., 2001; Chan, 2011).

2. Muscle protein composition

A mammalian skeletal muscle is composed of approximately 75% water, 18-20 % protein, 0.5-1% fat (minimum), 1% of carbohydrate and 3-5% other soluble material (non-protein and non-carbohydrate) (Duke, 1943). These percentages may differ, especially in fat content, depending on species, amount of fattening and, inclusion of the adipose tissue. (Pearson and Young, 1989) reported all the range of different chemical constituents found in muscle (Table 1).

Table 1 Proximate Composition of Muscle (Pearson and Young, 1989).

Constituent	Range (%)
Water	75-78
Protein	15-22
Lipid	0.5-3
Minerals	1-2
Carbohydrate	1-2
Vitamins	Traces
Nitrogenous non-protein extractives	1.5-1.8

Muscle proteins have significant roles for the structure, function, and integrity of the muscle. Proteins incur changes during the conversion of muscle to meat that affect tenderness; and additional changes occur during further processing, through the

formation of peptides and free amino acids as a result of the proteolytic enzymatic activity (Toldrá and Reig, 2006). A typical adult mammalian muscle is approximately composed of 19% proteins which are sarcoplasmic (5.5%), myofibrillar (11.5%) and connective tissue (2%) (Lawrie, 1979). Based on their function in a muscle and solubility in aqueous solvents, proteins are generally categorized into three main groups: myofibrillar, sarcoplasmic and stromal proteins (Goll et al., 1970).

2.1 Myofibrillar proteins: Myofibrillar proteins are salt-soluble proteins making up about 45 - 50% of the total proteins in skeletal muscle. They can be classified into three main groups: (1) contractile proteins (myosin and actin), (2) regulatory proteins (tropomyosin and troponin), and (3) cytoskeletal proteins (titin, nebulin, C-protein, H-protein, and M-protein). Myosin and actin are the two most plentiful contractile proteins, comprising about 55 and 23% of the myofibrillar proteins, respectively. They provide support to the myofibril and are responsible for contraction-relaxation of the muscle. Myosin is the main protein of the thick filament having a long rod shape. Each myosin has a tail and two globular heads (Whitaker and Tannenbaum, 1977). The two globular heads, relatively hydrophobic, are able to bind to actin. However, the rod part, relatively hydrophilic, exhibits the montage of myosin into thick filaments (Xiong, 1997). The molecular weight of myosin is about 500,000 Da (Lawrie, 1979). Actin, another contractile protein, is the main component of thin filaments. Actin can exist in two forms: one, globular form, is (G-actin) which has a molecular weight approximately 70,000 Da and the other, fibrous form, is (F-actin) (Lawrie, 1979). Tropomyosin and troponin, regulatory proteins, are the other components of the thin filaments. Tropomyosin is a rod

shaped protein and surrounds the chain of actin. It comprises approximately 6% of the myofibrillar proteins. Troponin is a globular shaped protein and also composed of approximately 6% of the myofibrillar proteins. There are three subunits of troponin named Troponin T (Tn-T), troponin C (Tn-C), and troponin I (Tn-I). Tn-T binds to tropomyosin, Tn-C binds Ca^{+2} and starts the contractile process, and Tn-I is inhibitory protein. Actin-myosin interaction is blocked by the combination of tropomyosin and Tn-I (Hopkins, 2006; Urich, 1994). Titin and nebulin are two large proteins located between Z-lines and thin filaments. They provide myofibrils durability and elasticity (Robson et al., 1997).

2.2 Sarcoplasmic proteins: Sarcoplasmic proteins are water-soluble proteins, making up about 30-35% of the total proteins in muscle (Goll et al., 1970). They contain metabolic enzymes (mitochondrial, lysosomal, microsomal, nucleus or free in the cytosol), hemoglobin, myoglobin, and cytochromes (Toldrá, 2002; Wang, 2006). Some of these enzymes have important activity in postmortem meat and during further processing. The main sarcoplasmic protein is myoglobin which is responsible for the red meat color. The amount of myoglobin depends on the fiber type, age of animal and animal species. For example, beef and lamb meat include more myoglobin than pork and poultry. In general, the amount of myoglobin increases with the age of the animal (Toldrá and Reig, 2006).

2.3 Stromal proteins: Stromal proteins are water- and salt-insoluble proteins, comprising about 10 - 15% of the total protein in skeletal muscle (Goll et al., 1970). The main components of stromal proteins are collagen, reticulin, and elastin (Toldrá and Reig,

2006). Stromal proteins predominantly contain collagen providing strength and support to the muscle structure (Toldrá, 2002). The skeletal muscle becomes tougher with age because the number of crosslinks increase in the collagen fibers. This is the reason why meat tenderness decreases in older animals (Toldrá and Reig, 2006). There are two kinds of connective tissue proteins which are called as proper and supportive. The connective tissue layers, epimysium, perimysium and endomysium, are known as connective tissue proper (Alvarado and Owens, 2006). Supportive connective tissue provides structural support because it contains bones and cartilages. Elastin is found in lower level, generally in capillaries, tendons, nerves, and ligaments (Toldrá and Reig, 2006). Stromal proteins are significant to food technologist due to its undesirable effects on meat quality: (1) Connective tissue proteins reduce meat tenderness; (2) Stromal proteins reduce emulsifying capacity of meat; (3) Due to their low content of charged and hydrophilic amino-acids, stromal proteins decrease of water holding capacity of meat; (4) due to having a low rate of nutritionally essential amino-acids, stromal proteins cause to lower nutritive value of meat (Whitaker and Tannenbaum, 1977).

3. Muscle fiber types

There are many factors affecting fiber type composition, such as species, muscle types (Klont et al., 1998), gender (Ozawa et al., 2000), age (Candek-Potokar et al., 1998), breed (Ryu et al., 2008), hormones (Florini et al., 1996), and physical activity (Jurie et al., 1999). Fiber types can be differentiated depending on their appearances (red or white), physiological behavior (speed of contraction), biochemical properties (myoglobin content), and histochemical staining properties (glycolytic or oxidative) (Pearson and

Young, 1989). In order to describe the characteristics of muscle and determine various muscle fibers, histochemical staining techniques can be used (Morita et al., 2000 and Ozawa et al., 2000).

The most commonly accepted form of classification of muscle fibers is based on the difference in sensitivity of ATPase activity under different pH conditions. Based on this principle, Brooke and Kaiser (1970) displayed the method with a range of pH pre-incubations and three fiber types were determined in adult skeletal muscle, i.e. types I, IIA and IIB fibers. Although fiber type I indicated a stable, high ATPase activity after pre-incubation at either pH 4.3 or 4.6, it indicated a low ATPase activity after pre-incubation at pH 10.4. On the other hand, fibers type IIA lost ATPase activity after pre-incubation at pH 4.3 or 4.6 although it showed a strong reaction pre-incubation at pH 10.4. Fiber type IIB indicate a strong reactivity after pre-incubation at pH 10.4, but it had no a reaction after acid pre-incubation at pH 4.3 (Lind and Kernell, 1991).

Muscle fibers also can be classified as red, intermediate, and white fibers (Dubowitz and Pearse, 1960; Jurie et al., 1999). In poultry (chicken and turkey), breast meats are mainly composed of white fibers. Therefore, they are referred to as white meat. However, leg meat, which is mainly made up of red fibers, is referred to as red meat (Pearson and Young, 1989). Red fibers have a greater amount of myoglobin and hemoglobin, higher oxidative metabolism, lower levels of glycolytic enzymes, and lower glycogen content compared to white fibers. On the other hand, intermediate fibers have characteristics between that of red and white fibers having both oxidative and glycolytic capacities (Dubowitz and Pearse, 1960; Jurie et al., 1999). White fibers fatigue faster

compared to red fibers because the contraction speed of white fibers is faster (Pearson and Young, 1989). White fibers also display more susceptibility to the development of PSE meat than red fibers due to their predisposition to anaerobic glycolysis (Solomon et al., 1998).

Pette and Staron (1997) classified fiber types as type I and type II which correspond to slow and fast muscle fiber. Peter et al. (1972) classified the muscle fibers based on contractile speed and patterns of metabolic enzymes as slow-twitch oxidative (SO), fast-twitch oxidative glycolytic (FOG) and fast-twitch glycolytic (FG). There is a good correlation between type I and SO fibers, but the correlations between type IIA and FOG and type IIB and FG fibers are more different (Hamalainen and Pette, 1995; Pette and Staron, 1997).

4. Conversion of muscle to meat

After animal slaughter, a range of biochemical events occurs during the conversion of muscle to meat affecting final meat quality. One of the most important events in the conversion of muscle to meat is glycolysis. In the living tissue, adenosine triphosphate (ATP), the primary energy source is produced through aerobic glycolysis for cellular functions. After death, blood flow is terminated, and energy metabolism in the muscle is switched into anaerobic pathway for the production of ATP due to the lack of oxygen. The muscle is able to produce 12 moles of ATP per mole of glucose under normal aerobic conditions, while it is only able to produce 2 moles of ATP per mole glucose under anaerobic conditions. As glycogen is depleted, lactic acid will accumulate in the muscle until the pH decreases to a level that inhibits further glycolysis and ATP

production stops (Valin et al., 1992; Greaser, 1986; Tarté and Amundson, 2006). With the accumulation of lactic acid, muscle pH reduces generally from 7.2 (physiological pH of meat muscle proteins) to an ultimate pH of 5.3 - 5.8 (Kerry et al., 2002). Figure 1 shows the rate and extent of postmortem pH decline of chicken breast muscle for PSE, normal, and DFD meat. The rate of pH reduction and the ultimate pH effects on meat quality and color development.

Dark, firm and dry (DFD) meat, a quality defect, is often the result of animals exposed to long term stress prior to slaughter (Warriss, 2000). Stress and exercise deplete the animal's glycogen reserves, and, therefore, the pH drop will be minimal, and the ultimate pH will remain high (Viljoen et al., 2002; Kannan et al., 2002). DFD meat has a higher water holding, a dark color and a dry appearance capacity (Zhang et al., 2005; Mounier et al., 2006). These meats must be processed with extreme attention because they are more susceptible to foodborne pathogens (Toldrá and Reig, 2006).

Red, firm, normal meat (RFN) has a pH drop to around 5.8–6.0 at 2 hours postmortem.

Pale, soft, exudative (PSE) meat is the result of a rapid drop in the pH while the meat temperature is still high. This combination can result in the denaturation sarcoplasmic and myofibrillar proteins (especially myosin), causing excessive drip loss, decreased water holding capacity, pale appearance and soft texture (Warriss and Brown, 1987; Santos et al., 1994; Penny, 1969).

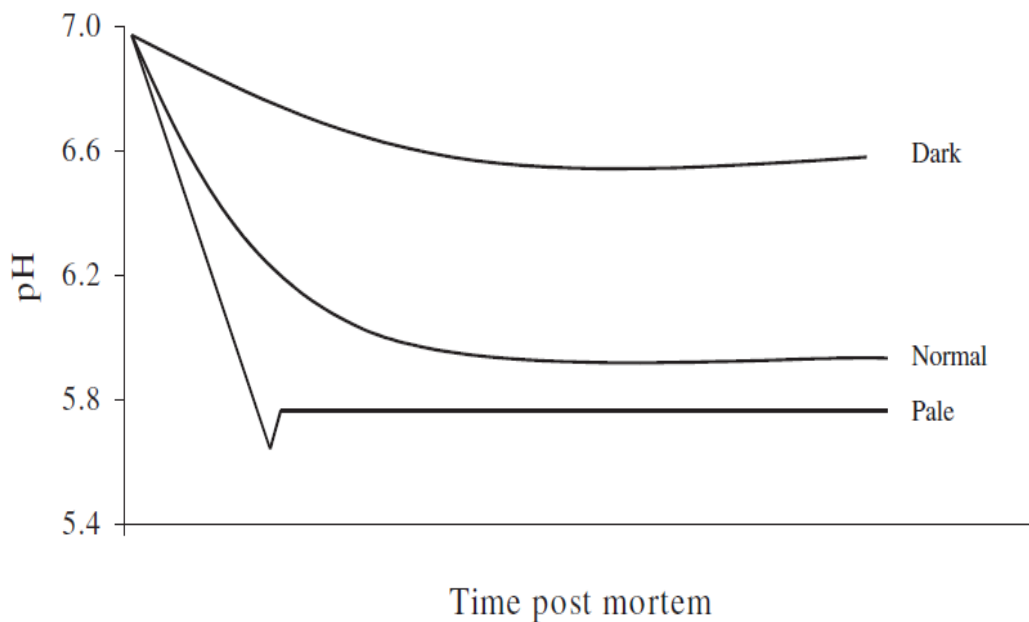


Figure 1: Rate and extent of postmortem pH decline of chicken breast meat (Barbut, 2002).

When the ATP concentration is depleted below $1 \mu\text{M} / \text{g}$ of tissue, the process of rigor mortis (stiffness of death) begins and the actomyosin cross-bridges are established between actin and myosin. The muscle remains contracted since there is no more energy for relaxation. The forming of actomyosin complexes continue until the ATP concentration reaches $0.1 \mu\text{M} / \text{g}$ of tissue at which time rigor is developed (Offer, 1991; Pietrzak et al., 1997). The length of rigor time depends on the species, muscle, fiber type, holding temperature, rate of glycolysis and the extent of struggling at the time of death (Greaser, 1986). Although the conversion of muscle to meat is similar in avian and mammalian species, glycolysis and rigor mortis happen considerably faster in poultry when compared to the red meat species (Grey et al., 1974; Grey and Jones, 1977; Addis, 1986). For example, rigor completion continues more than 24 h in sheep muscle

(Wheeler and Koochmarraie, 1994) and less than 6 h in chicken breast muscle (Schreurs, 2000). The rigor process generally contains two distinct phases including a delay phase and a rapid phase (Bate Smith and Bendall, 1949). The time between slaughter and the onset of rigor mortis is delay phase. The muscle encounters a longer period of resolution following maximum rigor (Li et al., 2010).

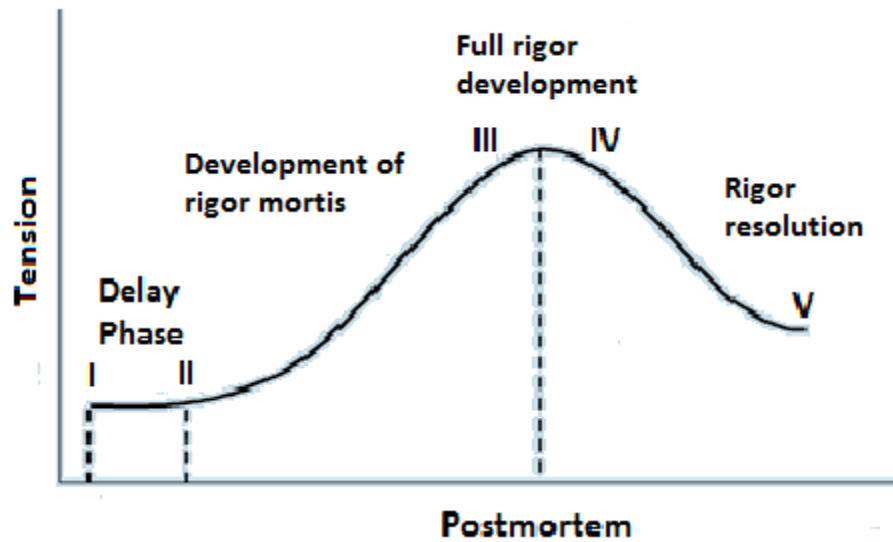


Figure 2: Development of Rigor Mortis Expressed as Muscle Tension Over time. Modified from (Hedrick et al., 1994).

There are a great variety of enzymes in the muscle. Some of them have a significant role in biochemical activities during the meat aging process. For example, calpains and cathepsins, activated by the release of calcium, play a significant role in meat tenderization. Both enzymes are found in muscle tissues naturally (Tarté and Amundson, 2006). Calpains are placed in the cytosol, around the Z-line area. There are basically two forms of calpains. Calpain I is also named μ -calpain because it requires micromolar levels (50-70 M) of Ca^{2+} to be active. Likewise, calpain II is also named m-

calpain because it demands millimolar levels (1-5 mM) of Ca²⁺ for activation (Toldrá and Reig, 2006). Calpains have maximum activity around pH 7.5. Calpain activity diminishes very quickly with decreasing pH to 6.0, with no activity at pH 5.5 (Etherington, 1984). Calpains are very effective in degradation of titin, nebulin, troponins T and I, tropomyosin, C-protein, filamin, desmin, and vinculin, which relate to the fiber structure. However, they do not affect myosin, actin, α -actinin and troponin C (Goll et al., 1983; Koohmaraie 1994). Calpastatin acts as an endogenous reversible and competitive inhibitor of calpain in the living tissues. It regulates the activity of calpains in postmortem tissues (Koohmaraie et al., 1987).

Cathepsins are a set of acidic proteinases located in the lysosomes (Xiong, 2004). They are efficient in proteolysis which happens at a later time postmortem during the aging cycle (>4 days) (Taylor et al., 1995). There are a variety of cathepsins and the most important ones are cathepsins B, H, L, and D. The optimal pH for activity is pH around 6.0 for cathepsins B and L, pH around 4.5 for cathepsin D, and 6.8 for cathepsin H (Toldrá et al., 1992). Cathepsins affect degradation of different myofibrillar proteins. Cathepsins D and L are very effective in degradation of myosin, titin, M and C proteins, tropomyosin, and troponin T and I (Matsakura et al., 1981, Zeece and Katoh, 1989). Cathepsin L affect degradation of titin and nebulin while Cathepsin B can degrade myosin and actin (Schwartz and Bird, 1977). Cathepsin H shows both endo- and aminopeptidase activity (Okitani et al., 1981). Although cathepsins degrade actin and myosin, it is thought that they have minimal participation in meat postmortem meat tenderization because they are not very active at refrigerated temperatures, not released

from the lysosomes during postmortem aging, and need a low pH for optimal activity (3.5-4.5) (Huff-Lonergan and Lonergan, 1999; Xiong, 2004).

Another important factor affecting meat quality during the post mortem is temperature. An optimum temperature during the postmortem process is between 15-20 °C. For this reason, it is commonly suggested that the muscle temperature should be dropped as soon as possible after slaughtering. However, when the muscle is frozen before it goes into rigor, a condition known as thaw rigor can affect meat tenderness. Thaw rigor leads to moisture loss, muscle shortening, and muscle toughening (Hedrick et al., 1994). On the other hand, when the temperature is decreased to below 15 °C, but above freezing, before the beginning of rigor, a less severe problem, cold shortening can occur (Bilgili et al., 1989). Heat rigor can also create tenderness problems when the muscle temperature is held at relatively high temperatures (up to 50 °C) during the rigor. However, this is not common as the other muscle shortening problems (Aberle et al., 2001).

During thaw rigor, due to high level of ATP in the muscle, muscle contraction occurs by a sudden release of calcium from sarcoplasmic reticulum into the sarcoplasm and that results in a physical shortening of 60-80% of original length. Similarly, the sarcoplasmic reticulum is unable to hold the calcium due to the quick chilling during cold shortening. Therefore, calcium is released into sarcoplasm and muscle contraction takes place while ATP is still available. During the heat rigor, a rapid consuming of ATP is observed in the muscle (Aberle et al., 2001).

5. The enzymes used in meat tenderization

5.1 Papain

Papain is extracted from latex of *Carica papaya* fruits. Papain, a cysteine hydrolase, is highly stable and active under a wide range of conditions (Cohen et al., 1986). Due to its proteolytic property, it is extensively used in the food industry to tenderize meat and as an ingredient in flour and in beer manufacturing (Schmidt, 1995; Khanna and Panda, 2007). Papain also has antifungal, antibacterial and anti-inflammatory properties (Chukwuemeka and Anthoni, 2010). Vo and Huynh (2009) reported that the activity of papain is strongly affected by type of substrate, the ratio of enzyme to substrate, pH, temperature, and period of hydrolyzing process. For instance, optimal pH for casein was found to be 7-7.5 and, 4.5-7.1 for albumin (Duc Luong Nguyen, 2004). Vo and Huynh (2009) conducted a study on protein of *Pangasianodon hypophthalmus*; using the Kunitz method (1947) to determine enzyme activity. This method uses Ortho-phthaldialdehyde to measure the amount yielded amino acid. It was reported that optimum pH was 6.0 and optimum temperature was 55°C for papain. The enzyme had very low activity at pH 4 and 10 and at temperatures of 4 and 95°C. In two other studies, Khaparde and Singhal (2001) reported that papain had a pH optimum of 6.0 and temperature optimum at 70 °C, while Katsaros et al. (2009) reported that 95% inactivation of papain was achieved at 900MPa and 80 °C, after 22 minutes of processing. White and White (1997) gave some specific information for papain:

Molecular weight: 23,000 Da

Optimal pH: 6.0-7.0

Isoelectric point (pI): 8.75

Stable pH: 6-7.5

(White and White, 1997)

Activators (White and White, 1997)

- Cysteine
- Sulfide and sulfite
- Heavy metal chelating agents like EDTA
- N-bromosuccinimide

Inhibitors (White and White, 1997)

- PMSF
- TLCK, TPCK
- α 2-Macroglobulin
- Hg²⁺ and other heavy metals
- AEBSF
- Antipain
- cystatin
- E-64
- Leupeptin
- Sulfhydryl binding agents
- Carbonyl reagents
- Alkylating agents

5.2 Bromelain

There are two different bromelains extracted from pineapple plant (*Ananas comosus*), stem and fruit bromelain. Fruit bromelain is not commercially available. Bromelain is composed of cysteine proteases and non-protease components (Heinecke and Gortner, 1957; Rowan et al., 1990; Larocca et al., 2010). This proteolytic enzyme is mainly used for meat tenderization (Melendo et al., 1997; Kolle et al., 2004). It is also used as a nutritional additive to assist digestive health, an anti-inflammatory (Wen et al., 2006), anti-edematous (Seltzer, 1964), absorption facilitator of antibiotic drugs (Neubauer, 1961), an anti-thrombotic (Metzig et al., 1999), an inhibitor of tumor cell reproduction (Batkin et al., 1988), a debrider (Klaue et al., 1979) and an immunogenic agent (Hale et al., 2002).

Preferential cleavages of bromelain are lysine, alanine, tyrosine and glycine. Therefore, amino acid composition of meat is also important in the tenderization effect of bromelain. Amino acid composition (g/100 g protein) of Peking and Muscovy duck breast meat is displayed in table 2.

Table 2 Amino acid composition (g/100 g protein) of Peking and Muscovy duck breast meat (Aronal et al., 2012).

Type of amino acid	Peking duck breast	Muscovy duck breast
Essential		
Cystine	2.65	0.07
Histidine	3.23	2.96
Isoleucine	7.61	3.44
Leucine	2.79	7.63
Lysine	9.21	9.41
Methionine	7.09	6.15
Phenylalanine	3.22	3.9
Threonine	4.65	4.96
Tyrosine	1.84	3.7
Valine	4.58	3.49
Total	46.87	45.71
Non-essential		
Arginine	7.07	7.28
Alanine	6.21	6.62
Aspartic acid	9.57	10.01
Glutamic acid	15.21	15.62
Glycine	6.26	5.57
Proline	4.23	4.31
Serine	4.56	4.87
Total	53.11	54.28

As with many other enzymes, bromelain activity depends on some intrinsic and extrinsic factors. Among those, temperature and pH significantly affect bromelain activity (Truc et al.). Corzo et al. (2012) conducted a study characterizing the proteolytic activity of fruit bromelain using five different substrates: azocasein and azoalbumin (pH 3–10 at 20–70 °C), casein and sodium caseinate (pH 2–10 at 20–70 °C), and hemoglobin (pH 2–6.5 at 30–60 °C). In this study, fruit bromelain had its optimum activity at pH 7.5 for azoalbumin and at 6.5 for azocasein, at 55 °C for azoalbumin and at 50 °C for

azocasein. When casein and sodium caseinate were used as a substrate, its optimum activity was at 59 °C for both substrates, while the optimum pH was 7.7 for casein and 6.5 for sodium caseinate. Fruit bromelain showed its optimum activity at the temperature of 37 °C, and at pH 2.9 with hemoglobin.

Corzo et al. (2012) also determined the Michaelis–Menten K_m constant, maximum reaction velocity V_{max} , and the reaction specificity V_{max}/K_m for azocasein, azoalbumin, casein, sodium caseinate and hemoglobin. They found that azocasein (0.104) is the most suitable substrate for the fruit bromelain activity, followed by azoalbumin (0.096), casein (0.022), sodium caseinate (0.020), and hemoglobin (0.014). They strongly recommended using azocasein and azoalbumin to determine fruit bromelain activity at optimal reaction conditions due to the low K_m .

In the other studies, Yoshioka et al. (1991) found that commercial bromelain from pineapple stems was completely inactivated by heating for 30 min at 60°C; while Gupta et al. (2007) indicated that bromelain lost 50% of its activity by heating for 20 min at 60°C. Liang et al. (1999) reported that bromelain from pineapple fruit juice concentrate lost 50% of its initial activity by heating 60 min at 60°C. Jutamongkon and Charoenrein (2010) studied the effect of incubation temperature on fruit bromelain activity at 40, 50, 55, 60, and 80 °C using casein as a substrate. They indicated that there was no fruit bromelain activity loss for up to 60 min at 40°C, while at 50°C almost 83% of activity remained. Fruit bromelain retained 51% activity after heating 8 min at 60°C. However, fruit bromelain activity was almost completely eliminated after 8 min at 80°C. They also

reported that bromelain from pineapple juice or fruit is more stable when encountering heating than commercial bromelain obtained from pineapple stems.

Optimum activities of fruit bromelain with casein as the substrate were also reported in a temperature range of 10°-75°C and pH range of 4.6-9.5. Optimum temperature was determined as 55 °C and optimum pH was determined as 7.1. In neutral environment (pH 6.8 - 7.1), fruit bromelain activity was active at temperature range of 40 - 60°C. At basic environment (pH 7.5 - 9.5), optimal temperature of the enzyme was at range of 25 - 40°C. At lower pH (4.6 - 5.2), its activity was maximum at 10 - 25°C. It was entirely inactivated at above 70°C (Truc et al.). However, Pardo et al. (2000) reported that bromelain was almost inactivated after 60 min at 75°C. Suh et al. (1992) reported that the optimum conditions for fruit bromelain activity with casein were pH 8.0 at 50 °C, while Liang et al. (1999) reported that the optimum temperature for the activity of bromelain was found to be at about 55°C.

Furthermore, it was shown that bromelain extracts stored under frozen conditions (at -30 °C) were more stable than those stored at cold conditions (4 °C). With cold storage, bromelain activities began to decrease after ten days. According to previous studies, rapid freezing at below -20°C (the best is in -70°C or using liquid nitrogen) was ideal to protect enzymatic activity (Copeland, 2000).

5.3 Ficin

Ficin represents to the endoproteolytic enzymes from trees of the genus *Ficus*. The ficins that have been isolated from the latex of *Ficus glabrata* and *Ficus carica* are the most widely studied ficins. However, recently a less known ficin from the latex of *Ficus*

racemosa has been identified with a molecular weight of $44,500 \pm 500$ Da, optima pH between 4.5 and 6.5 and maximum activity at $60 \pm 0.5^\circ\text{C}$. These unique properties make it distinct from other known ficins and give it application in many sectors (Deveraj et al., 2008).

5.4 Ginger rhizome

Ginger rhizome is a source of plant proteolytic enzyme. The ginger protease shows optimum activity at 60°C and rapid denaturation at 70°C . Its proteolytic activity on collagen was greater than it was on actomyosin (Thompson et al., 1973; Naveena and Mendiratta, 2001). It has been reported that ginger extract has antioxidant and antimicrobial characteristics as well as its tenderizing properties (Lee et al., 1986; Kim and Lee 1995; Mendiratta et al., 2000).

Several examples of proteases (plant, bacterial and fungal) application in meat products can be found in the literature. Sullivan and Calkins (2010) studied the tenderization effect of five GRAS enzymes (papain, ficin, bromelain, *Bacillus subtilis* protease, two variations of *Aspergillus oryzae* proteases which are *Aspergillus oryzae* concentrate and *Aspergillus oryzae* 400) and homogenized fresh ginger on beef muscles. All enzymes except ginger extracts displayed a considerable tenderization effect, but the lowest shear forces were obtained from the meat treated by papain. Bromelain degraded collagen more than the contractile proteins. Ginger showed tenderization effect, but higher levels of ginger result in flavor issues. *Bacillus subtilis* protease and both *Aspergillus oryzae* proteases indicated more degradation effect on myofibrillar than

collagen proteins, while ficin was showing the most balanced degradation effect on both myofibrillar and collagen proteins.

Ketnawa and Rawdkuen (2011) studied the effect of bromelain extract on beef, chicken and squid. These meat products were sprinkled with a powdered bromelain extract (0, 3%, 7%, 10% and 20% (w/w)). After mixing thoroughly, they were kept at room temperature for 1 h prior to analyses. They reported shear force values, pH, moisture content, water holding capacity, cooking yields decreased while TCA-soluble peptides' content significantly increased in all of samples marinated with bromelain extract when compared to the control.

Naveena and Mendiratta (2001) tested ginger extract (GE) on spent hen meat. In the study, in order to find optimum concentration, post-chill spent hen breast meat chunks were marinated with different concentrations (0%, 1%, 3% and 5% v/w) of ginger extract for 24 h at $4^{\circ}\pm 1^{\circ}\text{C}$ and cooked to an internal temperature of 70°C . The volume of marinate used for all treatments was 10% v/w. Based on yield, pH, moisture, shear force values and sensory attributes of the cooked samples, optimum concentration of GE for tenderization of spent hen meat was determined as 3% v/w. Later, spent hen meat chunks were marinated both at pre- and post-chilled stage using 3% v/w concentration of GE and evaluated after 24 h of treatment. They found that tenderness scores were better in samples treated at post-chilled stage (at $4^{\circ}\pm 1^{\circ}\text{C}$ for 24 h).

Naveena and Mendiratta (2004) reported that treatment with 5% v/w GE was the optimum for tenderization of buffalo meat based on shear force values and sensory evaluation values. In this research, buffalo carcasses that were chilled for 24 h at $4\pm 1^{\circ}\text{C}$

were cut into 3 x 3 x 3 cm uniform sized chunks. They were randomly divided into four groups (500 g each) and marinated with different concentrations (0, 3, 5 and 7% v/w) of ginger extract. The required volume of GE was diluted with distilled water and the mixture was sprayed at 15% v/w of meat chunks (15ml/100 g meat). For example, in order to prepare 3% of GE solution, 3 ml ginger extract and 12 ml distilled water were used. After mixing, the samples were kept in polyethylene bags at 4 ± 1 °C for 48 h. Later, they were washed, drained and cooked to an internal temperature of 75 ± 1 °C before evaluating for cooking yield, pH, moisture, and shear force.

Naveena et al. (2004) conducted a study to compare two plant proteolytic enzymes from *Cucumis trigonus* Roxb (Kachri) and *Zingiber officinale* roscoe (Ginger rhizome) with papain in point of tenderizing effect. After 2-3 h post-slaughter, buffalo meat samples were packed in low density polyethylene (LPDE) and stored at 4 °C for 24 h. Next, they were cut into 3 x 3 x 3 cm size and sprayed with distilled water (control), 2% (w/w) powdered cucumis extract, 5% (w/v) GE or 0.2% (w/w) papain. Next, the samples were held in polyethylene bags at 4 ± 1 °C for 24 h for marination. Later, they were washed, drained and cooked. They reported that all enzyme-treated samples made an improvement in flavor, juiciness, tenderness and overall acceptability scores. However, the samples treated with GE received better scores while the samples treated papain and cucumis received almost the same scores.

Tsai et al. (2012) indicated that besides its antioxidant effect, GE had a great effect on degradation of several major cytoskeletal/myofibrillar proteins (titin, myosin heavy chain, troponin-T, desmin and α -actinin). In this research, Muscovy duck breast

muscles were marinated with GE for 14 days at 5 °C. Samples were taken at the end of 0, 1, 3, 7 and 14 days of the marination.

Qihe et al. (2006) conducted a research for the tenderization feasibility of a microbial enzyme, elastase from *Bacillus* sp. EL31410, comparing with papain. In this research, meat tenderization was done by dipping the beef meat cut in different enzyme solutions (0.1% papain, 1% papain and 1% of a new elastase from *Bacillus* sp. EL31410) after freeze-dehydration. After 4 h treatment, the samples were stored at 4 °C for 24, 48, and 72 h before analyses. As a result, the elastase from *Bacillus* sp. had the same tenderization effect on beef meat as papain. However, it was reported that there were some problems when the elastase was used, such as elastase safety and elastase stabilization in the meat tendering process.

Cold-adapted collagenolytic protease MCP-01 is another enzyme tested for meat tenderization in the literature. A cold-adapted enzyme, MCP-01, is isolated from the deep-sea psychrophilic bacterium *Pseudoalteromonas* sp. SM9913 (Chen, Xie et al., 2007). This enzyme nearly has high activity at 0-25 °C, and is unstable at temperature higher than 40 °C. Since meat tenderization is usually achieved at room temperature before cooking, an ideal meat tenderizer should have high activity at room temperature and be comfortably inactivated during cooking. Due to their high activity at 0-30 °C and losing their activity at temperatures higher than 50 °C, cold-adapted proteases may be functional as meat tenderizers (Zhao et al., 2012). Therefore, Zhao et al. (2012) studied on the tenderization effect and the mechanism of a cold-adapted collagenolytic enzyme MCP-01 on beef meat at low temperatures. In this research, it was found that cold

adapted collagenolytic enzyme MCP-01 (10 U caseinolytic activity) and bromelain with 10 U of caseinolytic activity showed almost the same tenderization ability at low temperature (4 C) with the reduction of shear forces 22% and 23%, respectively when they compared with control. The water loss of the meat treated by papain and bromelain was 3-4 folds higher than the water loss of the meat treated by MCP-01. Moreover, MCP-01 showed better freshness, color features, and better tenderization effect than papain and bromelain.

CHAPTER 3

MATERIAL AND METHODS

Source of meat

Fresh boneless duck breast meat was obtained from a commercial duck producer, shipped overnight to the laboratory in an insulated cooler with ice packs then held at -20 °C until prepared for sampling. 24 to 48 hours before sampling, packages were thawed at 4 °C. After removing the skin, the breasts were marinated in different concentrations of bromelain (1.5% (B1.5), 3% (B3), and 4.5% (B4.5)).

Marination

The experiment was repeated 3 times using 10 whole duck breasts per replication. The duck breasts were randomly divided into 5 groups (2 controls, 3 bromelain treatments) of 2 whole breasts for each group. Two control treatments were used during the study. The first control was un-treated (C1) and the other was treated with a solution composed of 92% refrigerated water, 6% salt and 2% sodium tripolyphosphate (C2). The same base solution was used for the bromelain treated samples with commercial bromelain (Sigma Chemical Co. St.Louis, MO) added at different concentrations (1.5% (B1.5), 3% (B3), and 4.5% (B4.5)). Fillets were marinated under a vacuum in a tumble marinator (Flavor-Maker™ model F-8, Ramm Master, Westfield, WI) at 8 RPM for 8 min and a pressure of 17-18 mm-Hg. The ml of marinade added to the tumbler was 10% of the weight (g) of breast meat placed in the tumbler. Prior to marination, each sample was weighed to determine the amount of marination solution needed. The weight of C1 was not weighed because it was not marinated. Moreover, the sample weights in the first group were not determined because they were not used for the calculation of cook yield. Samples were dried on a paper towel after marinating to remove residual solution (Table 3).

After tumbling, residual surface moisture was removed from each fillet with a paper towel and the fillets were placed on two different trays and immediately put in the

refrigerator before the second treatment completed tumbling. Thus, one sample from each treatment was used for the analyses of water holding capacity, moisture content, pH and water activity after marination. The second sample from each treatment was used for color, cooking yield, Warner-Bratzler (WB) and razor blade analyses on the second day with samples held at refrigerated temperature ($4\pm 2^{\circ}\text{C}$) overnight. Therefore, all analyses for the first trial were completed in two days. The same procedures were followed for the second and third trials with analyses completed in six days.

Table 3 Weights for duck breasts before and after marination and after cooking

Trial 1			
Treatment	Raw weight (g)	Marinated weight (g)	Cooked weight (g)
C1	unknown	161.6	117.1
C2	$\frac{160.7}{149}$	143.5	106.3
B1.5	$\frac{156.1}{167.7}$	151	110.3
B3	$\frac{162}{159.2}$	159.2	105.9
B4.5	$\frac{173.9}{179.8}$	169.1	114.4
Trial 2			
Treatment	Raw weight (g)	Marinated weight (g)	Cooked weight (g)
C1	unknown	154.1	106
C2	$\frac{171.3}{155.1}$	154.2	114.8
B1.5	$\frac{168.2}{178.9}$	173.9	117.2
B3	$\frac{160.5}{175.1}$	157.5	109.6
B4.5	$\frac{174.4}{167.1}$	162.5	114
Trial 3			
Treatment	Raw weight (g)	Marinated weight (g)	Cooked weight (g)
C1	unknown	169.6	121.5
C2	$\frac{172.7}{157.4}$	174.1	130.7
B1.5	$\frac{168.9}{146.1}$	169.7	118.5
B3	$\frac{165.5}{169.1}$	164.6	113.8
B4.5	169.2	158	108.4

1. pH

Approximately 10 g of minced duck breast meat was homogenized with 100 ml distilled water using a tissue homogenizer (Osterizer Pulse matic, Milwaukee, WI) for 1 min. The pH values were determined using a digital pH meter (Orion pH meter, model 420A).

2. Moisture Content and Water Activity (Aw)

Moisture content was determined by using a Halogen Moisture Analyzer (HB43-S, Mettler Toledo, Switzerland). Approximately 2.6-3.5 gram of minced duck breast meat was spread evenly over the sample pan.

Water activity was determined by using an AquaLab Lite Water Activity Meter (Deacon Devices, WA). Minimum sample amount was evenly covered bottom of sample cup as no more than half-full of sample.

3. Water Holding Capacity

Water holding capacity (WHC) was determined according to the procedure described by Wardlaw et al. (1973). Approximately 10 g samples of meat were stirred for 1 min with 15 ml of 0.6 M NaCl solution in a 45 ml centrifuge tube. The tube then was held at 4° C for 15 min, stirred again for 1 min, placed in a centrifuge (Centrifuge 5804 R Eppendorf AG, Hamburg, Germany) and centrifuged at 10,000 x G at 4° C for 15 min. After centrifugation, the volume of the supernatant was measured using a 25 ml volumetric cylinder and the results were reported as the proportion the fluid retained by the sample according to the following equation:

$$\text{WHC (\%)} = ((\text{Initial volume} - \text{Volume of supernatant}) / \text{Initial volume}) \times 100.$$

4. Cooking yield

One fillet from each group was cooked in an oven at 176.7 °C on the aluminum trays lined with aluminum foil to an internal temperature was reached of 76.7 °C. The temperature was checked using a spear-tipped thermocouple inserted in the center of the

two bigger samples of five samples in the tray to make sure that internal temperature of the samples was reached of 76.7 °C. Then, the samples were cooled at room temperature, covered with foil, and held overnight at 4 °C. The following day the cooked samples were blotted dry, re-weighed, and measured again for color before texture analyses. The cooking yield was calculated by the following formula:

$$\text{Cooking Yield (\%)} = (\text{Weight of cooked breast} / \text{Weight of raw breast}) \times 100$$

5. Color

Color measurements of the skinless muscle surfaces were determined using a HunterLab UltraScan PRO Color Spectrophotometer (Hunter Associates Laboratory Inc., Reston, VA USA) and readings of C.I.E. L* (lightness), a* (redness), and b* (yellowness) and L*C*h* (lightness, Chroma, hue) values at 2 different locations on the fillet surface were measured. The spectrophotometer was calibrated with a standard white ceramic tile (L* = 56.95, a* = -21.94, b* =10.06) immediately before the measurements were taken. Before the measurements, the fillet surfaces were dried using a paper towel to minimize and standardize surface gloss.

6. Razor Blade

Razor blade shear force (N) were determined using a Texture Analyzer (Model TA-XT2i; Texture Technologies, Scarsdale, N.Y., U.S.A.) according to the method of Cavitt et al. (2004) with a 5-kg load cell using a razor blade (height 24 mm; width 8 mm) set to a penetration depth of 20 mm. Crosshead speed was set at 10 mm/s, and the test was triggered by a 10-g contact force. The samples were placed on the platform of the machine and muscle fibers were sheared perpendicular to fiber. Four observations were recorded per breast. Two observations were on connective tissue (tendon), one was on cranial part and the other was on caudal part of fillet (Figure 3). Tendon texture was determined using the method of Smith et al. (2014b). The razor blade shear force (N) was calculated as the maximum force recorded.

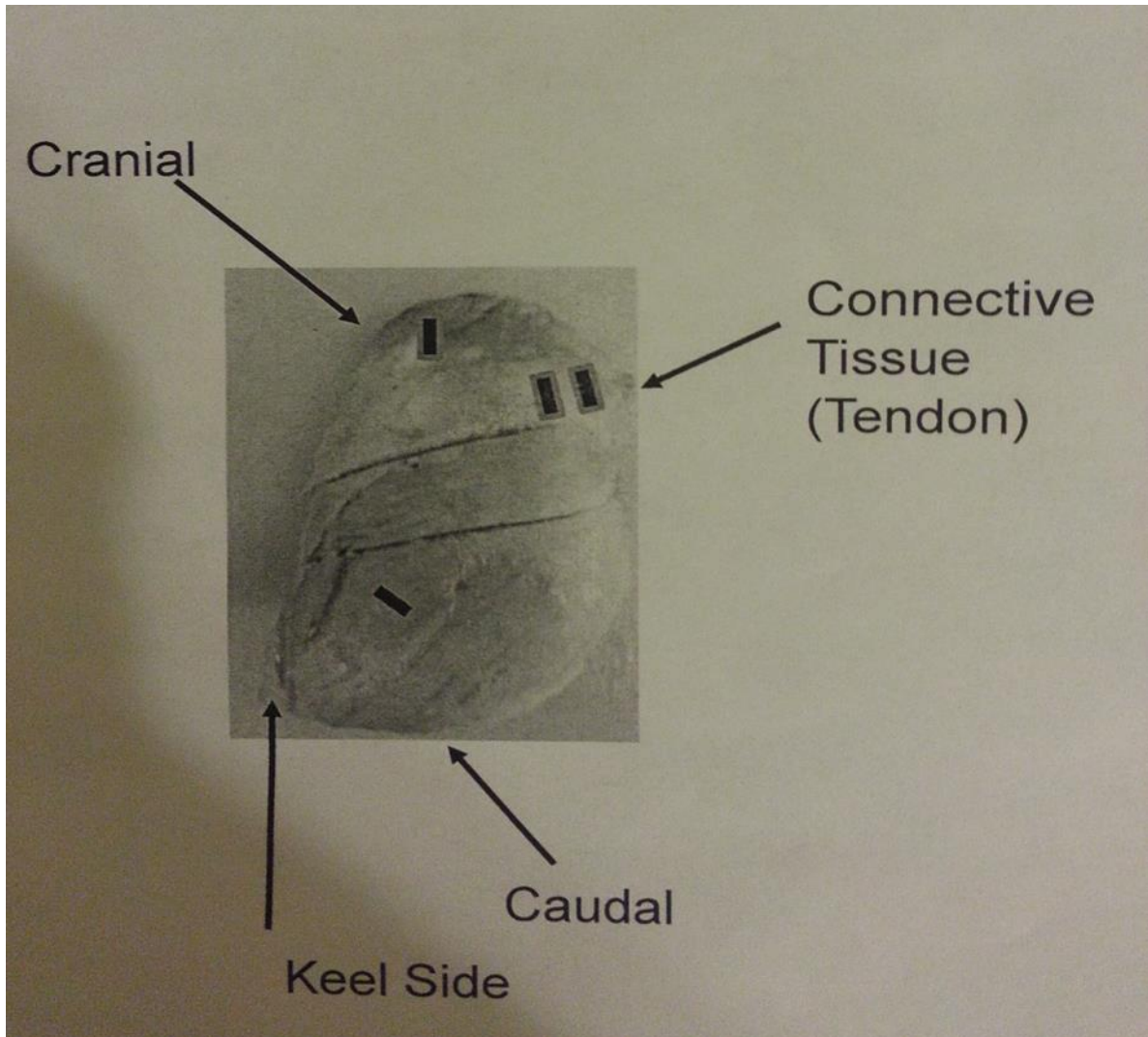


Figure 3 Diagram of duck breast fillet (right side) and 4 locations sheared by razor blade (Smith et al., 2014b).

Warner-Bratzler Shear Force

Both razor blade and WB shear force instrumental tests were performed on the same breast fillet. The samples sheared by razor blade were cut into three strips (head, middle and tail) parallel to the muscle fibers using a metal stick which is in 1.9 cm width. Each

strip was sheared two times perpendicular to muscle fiber direction using Warner-Bratzler, Model 235 6x (Chicago, IL).

7. Statistical Analysis

The experiment was repeated with 3 different trials with each trial considered as a replication. All data was analyzed using the ANOVA option of the general linear model (GLM) procedure of SAS. Tukey's studentized range (HSD) test was used to determine the significant differences among means for different treatments. The accepted level of significance for all comparisons was $P < 0.05$.

CHAPTER 4

RESULTS AND DISCUSSION

pH

The pH values for all treatments ranged from 6.16 to 5.97 (Table 4). A significant reduction ($p < 0.05$) in pH was observed in all bromelain treated samples when compared to the marinated control (C2) and meat from the B1.5 treatment also was lower than both control treatments (C1 and C2). No significant difference was observed in pH among bromelain treated samples. Ketnawa and Rawdkuen (2011) reported a significant decrease in pH for bromelain treated chicken samples. In the Ketnawa and Rawdkuen study, there was a significant reduction in pH for the 20% bromelain treated samples, while the 3, 7, 10% bromelain treated samples did not differ. However, in the current research the greatest pH decrease was observed when the lowest level (1.5%) of bromelain was applied. Furthermore, there was no significant difference between controls although C2 had slightly higher pH than C1. This may have been due to the higher pH (7.74) in the C2 solution because sodium tripolyphosphate in the solution used for C2 was more basic. The pH for the treatments of B1.5, B3 and B4.5 was 7.47, 7.32, and 7.18, respectively. The pH is one of the most important factors affecting other physico-chemical and quality parameters such as water holding capacity, tenderness and juiciness (Goli et al., 2007; Ketnawa and Rawdkuen, 2011). For example, minimum WHC is observed at the isoelectronic point of the major proteins, especially myosin (pH=5.4). The number of positive and negative charged groups of proteins is equal and the net charge is zero at isoelectric point. This results in a reduction in WHC. With increasing

the net charge upon increasing or decreasing of pH, there is a related repulsion of fibrillar proteins resulting in swelling of the matrix and increase in WHC. This continues until reaching very low or very high pH levels. Due to the protein denaturation at very high and low pH, WHC reduces again (Offer, 1991).

Table 4 Physico-chemical properties of duck breast meat not-marinated and marinated in solutions containing bromelain and without bromelain.

Parameters	Treatment				
	C1	C2	B1.5	B3	B4.5
pH	6.09±0.05 ^{ab}	6.16±0.07 ^a	5.97±0.02 ^c	6.04±0.09 ^{bc}	6.01±0.08 ^{bc}
WHC (%)	16.44±2.34 ^b	26.11±18.70 ^{ab}	25.05±10.65 ^{ab}	31.83±11.35 ^{ab}	38.63±6.48 ^a
Moisture Content (%)	77.27±0.43	76.81±0.50	76.62±0.82	77.26±0.97	76.57±0.95
Water Activity (Aw)	0.995±0.006	0.997±0.006	0.987±0.007	0.989±0.008	0.992±0.010

Means±S.D from triplicate determinations.

Means within column with different letters are significantly different (P<0.05).

Number of observation=6

C1, Control 1 (untreated control); C2, Control 2 (treated control with solution only); B1.5, 1.5% of bromelain; B3, 3% of bromelain; B4.5, 4.5% of bromelain.

Water Holding Capacity (WHC)

WHC values ranged from 16.44% to 38.63% and was increased significantly by marination (Table 4). WHC was highest to lowest for treatments; B4.5, B3, C2 (meat and solution), B1.5 and C1 (meat), respectively however did not significantly differ between C2, B1.5, B3 and B4.5. Breast from C2 had a higher WHC than C1 breasts. This could be due to the salts and phosphates in the control marination solution. Hamm (1960) reported that inorganic salts increased WHC by increasing charge of the muscle proteins. Moreover, NaCl is commonly used to promote water retention and consequent swelling in myofibrils (Honikel, 1987). WHC slightly increased as the bromelain concentration increased. However, there was only a significant difference in WHC of B3 and B4.5 when compared to C1. Karakaya and Ockerman (2002) reported that the addition of papain, bromelain and ficin increased the WHC of beef as compared to untreated controls. In contrast to these studies, Ketnawa and Rawdkuen (2011) reported that WHC decreased as the bromelain concentration increased in beef, chicken, and squish. They stated that the lower WHC in the bromelain treated samples was probably due to denaturation of myofibrillar proteins. However, Chan et al. (2011) reported that although low and normal pH turkey breast meat showed the same amount of protein denaturation, low pH meat had lower WHC than normal pH meat. On the other hand Huff-Lonergan and Lonergan (2005) reported that myofibrillar proteins lost the ability of to bind water as they closed to their isoelectric point. Therefore, there could be different reasons for the lower water retention ability of low pH meat instead of protein denaturation (Van Laack and Lane, 2000). Abdullah and Matarneh (2010) reported that WHC, color, and chemical

composition of broiler breast fillet were not affected by carcass weight, broiler sex, and post chill carcass aging duration. It was reported that lower WHC in poultry meat could depend on phospholipase A2 (PLA2) enzyme activity in muscle (Velleman, 2000; Chan et al., 2011).

Moisture Content and Water Activity (Aw)

Both moisture content and water activity did not differ significantly among treatments (Table 4). Aw of the samples ranged from 0.987 to 0.997 and moisture content of the samples ranged from 76.57 to 77.27. Ketnawa and Rawdkuen (2011) reported that increased bromelain concentration led to a decrease in moisture content.

Color

Breasts from treatments C2, B1.5, B3 and B4.5 were darker (lower L* values) as compared to untreated samples (C1) (Table 5). However, no differences were observed in redness (a*) and yellowness (b*) values of uncooked meat samples compared to marinated treatments. L* values ranged from 45.44 to 38.45, a* values ranged from 13.71 to 11.17 and b* values ranged from 10.30 to 8.50 for uncooked duck breasts. Smith et al. (2014a) also reported that uncooked marinated fillets were darker than control fillets (44.4 versus 46.3, respectively). Breasts from B1.5 cooked samples were had significantly higher L* values than C1. However, there was no difference in L* values of cooked C1, C2, B3 and B4.5. All cooked bromelain treated samples (B1.5, B3 and B4.5) had significantly lower a* values than untreated cooked control (C1). Redness (a*) values of cooked samples ranged from 7.61 to 5.84. The lowest a* values of cooked samples was observed in B1.5, B3, B4.5, C2 and C1. There was a significant decrease in b* values

of bromelain treated cooked samples (B1.5, B3 and B4.5) when compared to the control C1. Significantly lower b^* values were observed in all bromelain treated cooked samples as compared to cooked C1. The lowest b^* value was observed in B3 treated samples.

On the other hand, when the cooked and uncooked samples were compared in terms of color values (CIE L^* , a^* , and b^*), all cooked samples had higher L^* and b^* and lower a^* values than raw samples. These results are in agreement with the study of Smith et al. (2014a). They reported that cooked marinated L^* values were higher for marinated (60.2) as compared to control (58.6) fillets.

Table 5 Color values (C.I.E. Lightness, L*, redness, a*, and yellowness, b*) of uncooked and cooked duck breast fillets not-marinated and marinated in solutions containing bromelain and without bromelain.

Treat ment	Uncooked			Cooked		
	Lightness	Redness	Yellowness	Lightness	Redness	Yellowness
	(L*)	(a*)	(b*)	(L*)	(a*)	(b*)
C1	45.44 ± 4.72 ^a	11.17 ± 3.04	9.45 ± 1.87	49.51 ± 5.23 ^b	7.61 ± 0.95 ^a	18.35 ± 1.18 ^a
C2	40.21 ± 3.36 ^b	12.18 ± 2.48	8.50 ± 3.21	52.33 ± 3.71 ^{ab}	6.70 ± 1.66 ^{ab}	17.12 ± 1.14 ^{ab}
B1.5	41.50 ± 3.03 ^b	13.47 ± 1.93	10.12 ± 2.31	55.87 ± 3.45 ^a	5.31 ± 0.95 ^c	15.65 ± 1.53 ^{bc}
B3	38.45 ± 2.95 ^b	13.71 ± 1.64	10.30 ± 2.40	52.66 ± 3.38 ^{ab}	5.47 ± 0.47 ^{bc}	14.56 ± 1.31 ^c
B4.5	41.09 ± 1.44 ^b	12.84 ± 2.17	9.28 ± 2.35	52.40 ± 1.96 ^{ab}	5.84 ± 0.89 ^{bc}	15.64 ± 1.96 ^{bc}
P value	0.0167	0.3339	0.7278	0.0947	0.0042	0.0013

Means±S.D from triplicate determinations.

Means within column with different letters are significantly different (P<0.05).

Number of observation=6

C1, Control 1 (untreated control); C2, Control 2 (treated control with solution only);

B1.5, 1.5% of bromelain; B3, 3% of bromelain; B4.5, 4.5% of bromelain.

No significant difference was observed in the C* values of all uncooked treatments (ranged from 17.20 to 14.66) (Table 6). However, the only significant difference in the hue values of all raw samples was between controls. Raw C1 had significantly higher hue value (40.74) than raw C2 (33.00). On the other hand, there was no significant difference in C* values of cooked samples between C1 and C2. However, there was a significant decrease in C* values of bromelain treated cooked samples when compared to the controls. No significant difference was observed in hue values of all cooked samples.

Table 6 Color values (Chroma, C*, and Hue, h) of uncooked and cooked duck breast fillets not-marinated and marinated in solutions containing bromelain and without bromelain.

Treatment	Uncooked		Cooked	
	Chroma	Hue	Chroma	Hue
	(C*)	(h)	(C*)	(h)
C1	14.66 ± 3.40	40.74 ± 4.41 ^a	19.89 ± 0.97 ^a	67.39 ± 3.31
C2	14.93 ± 3.68	33.00 ± 7.26 ^b	18.44 ± 1.24 ^a	68.72 ± 4.86
B1.5	16.91 ± 2.62	36.60 ± 5.08 ^{ab}	16.54 ± 1.63 ^b	71.29 ± 2.59
B3	17.20 ± 2.49	36.56 ± 4.81 ^{ab}	15.57 ± 1.14 ^b	69.25 ± 2.98
B4.5	15.88 ± 3.00	35.51 ± 3.87 ^{ab}	16.71 ± 2.03 ^b	69.48 ± 2.48
P-value	0.5226	0.2298	0.0002	0.3983

Means±S.D from triplicate determinations.

Means within column with different letters are significantly different (P<0.05).

Number of observation=6

C1, Control 1 (untreated control); C2, Control 2 (treated control with solution only);

B1.5, 1.5% of bromelain; B3, 3% of bromelain; B4.5, 4.5% of bromelain.

Cook Yield

Mean cook yield values ranged from 74.5% to 68.4% and meat marinated without bromelain (C2) had a higher cook yield (p<0.05) when compared to all samples (Table 7). C1, B1.5, B3 and B4.5 did not differ in cook yield values. Therefore, bromelain treated samples did not affect cook yield when compared to C1. However, both B3 and B4.5 had lower cook yield when compared to C2. These results were in disagreement

with the study in Ketnawa and Rawdkuen (2011) which treated 3, 7, 10, and 20% bromelain. They reported that marinated samples with bromelain extract decreased the cook yield significantly. However, the results were in agreement with the study by Smith et al. (2014a). They reported that marination solution including water, salt and sodium phosphate significantly increased the cooking yield.

Table 7 Cook yield of duck breast meat not-marinated and marinated in solutions containing bromelain and without bromelain.

Treatment	N	Cook yield (%)
C1	3	71.0 ± 1.91 ^{ab}
C2	3	74.5 ± 0.52 ^a
B1.5	3	71.8 ± 3.97 ^{ab}
B3	3	68.4 ± 1.68 ^b
B4.5	3	68.8 ± 1.28 ^b
P-value		0.0392

Means±S.D from triplicate determinations.

Means within column with different letters are significantly different (P<0.05).

Number of observation=3

C1, Control 1 (untreated control); C2, Control 2 (treated control with solution only);

B1.5, 1.5% of bromelain; B3, 3% of bromelain; B4.5, 4.5% of bromelain.

Warner Bratzler Shear Force

There was a significant reduction in shear values of marinated samples (C2, B1.5, B3 and B4.5) when compared to unmarinated samples (C1) (Table 8). A significant reduction was also found in shear force values of the duck breast from B3 when compared to C2. Other bromelain treated samples (B1.5 and B4.5) did not differ in WB shear values with C2. The shear force ranged from 3.54 to 1.89 kg while previous research on duck breast reported WB shear force values from 3.2 kg to 4.2 kg (Smith et al., 2014a; Kim et al., 2012; Ali et al., 2008). Smith et al. (2014a) reported that marination with a solution including water, salt, and sodium phosphate of duck breast significantly reduced shear force when compared to the unmarinated duck breast. In this research, all bromelain treated samples did not differ in WB shear force although B3 samples were the only bromelain treatment to exhibit lower WB shear force compared to C1 and C2.

Tenderization is affected by rapid chilling, early deboning, gender, age, carcass weight, aging time, and bird breed. Chartrin et al. (2006) and Smith et al. (2014a) reported that duck breeds (Pekin, Muscovy, Hinny, and Mule) showed differences in color, cooking loss, and shear values. Omojola (2007) and Smith et al. (2014a) reported that WB shear of duck breast meat from males was significantly lower than meat from females. However, Smith et al. (2014a) reported that WB shear of cooked duck breast meat was not affected by gender or size of bird. However, Abdullah and Matarneh (2010) showed that the fillets from male broiler were more tender than those from female broiler. They also reported that tenderness decreased with carcass weight, and increased

with aging time. Musa et al. (2006) reported that breast fillets from females had more tender meat than those from males. However, Northcutt et al. (2001) reported that bird gender did not significantly affect breast fillet tenderness. Dransfield and Sosnicki (1999) reported that the meat obtained from bird with a rapid growth rate was more tender than the meat coming from bird with a slow growth rate. It was suggested that at least four hours of aging was required for broiler carcasses before deboning to have more tender breast fillets (Stewart et al., 1984; Lyon et al., 1985; Dawson et al., 1987).

Table 8 Warner-Bratzler shear force (kg) mean values of cooked duck breast meat not-marinated and marinated in solutions containing bromelain and without bromelain.

Treatment	N	Warner-Bratzler Shear
		(kg)
C1	16	3.54 ± 0.74 ^a
C2	16	2.73 ± 0.74 ^b
B1.5	16	2.26 ± 0.63 ^{bc}
B3	16	1.89 ± 0.58 ^c
B4.5	16	2.33 ± 0.69 ^{bc}
p-value		<.0001

Means±S.D from triplicate determinations.

Means within column with different letters are significantly different (P<0.05).

Number of observation=16

C1, Control 1 (untreated control); C2, Control 2 (treated control with solution only);

B1.5, 1.5% of bromelain; B3, 3% of bromelain; B4.5, 4.5% of bromelain.

Although shear forces obtained by head part of the duck breast were slightly lower (2.2 kg) than those obtained by middle (2.65 kg) and tail (2.67kg) part of the duck meat, no significant difference was observed in the shear values of the locations (Table 9). However, Smith et al. (2014b) reported that the part sheared nearer the keel of the breast meat showed significantly lower shear value (2.4 kg) than the part sheared closer to the wing insertion (3.0 kg).

Table 9 Warner-Bratzler shear force (kg) mean values by location for cooked duck breast meat not-marinated and marinated in solutions containing bromelain and without bromelain.

Location	N	Warner-Bratzler Shear
		(kg)
Head	20	2.20 ± 0.75
Middle	30	2.65 ± 0.90
Tail	30	2.67 ± 0.86

Means±S.D from triplicate determinations. Means within column with different letters are significantly different (P<0.05). N=number of observation.

Head, Cranial; Middle, between Cranial and Caudal part of breast meat; Tail, Caudal.

Razor Blade Shear Force

No significant difference was observed in razor shear values between treatments (Table 10). However, there was a slight difference between the razor shear values of meat only (M) and meat with tendon (M+T). Smith et al. (2014b) reported that the force needed to shear (M+T) was more than 150% above the force needed to shear M for unmarinated duck breasts, and was more than 125% higher than M values for marinated duck breasts. They also reported that marination and the gender of animal were effective on razor shear values, while size was not effective. Breast meat from male ducks was less tender than breast meat from female ducks.

These small differences between (M) and (M+T) razor shear values in this research could be due to bromelain, namely, bromelain could be very effective on tendon. Kang and Rice (1970) studied the effect of various enzymes on beef meat protein fractions. They reported that bromelain was more effective on degrading the connective tissue than myofibrillar proteins of the muscle. However, papain and ficin were more effective on degrading the myofibrillar fraction than the connective tissue. Wismer-Pederson (1972) also reported that bromelain was effective for tenderizing the connective tissue proteins.

Although texture analyses were conducted with the same samples, shear forces decreased in the samples treated by bromelain when they were sheared by WB method and shear forces were the same in all samples when sheared by razor blade. That could be related to the size of the razor. Cavitt et al. (2005) sheared the broiler breast meat with Allo-Kramer (10 blade), needle puncture (2 mm diameter), and razor blade (8.9 mm

width) shear to measure texture. They reported that Allo-Kramer and razor blade shear tests detected the significant differences in breast meat toughness. However, needle puncture could not detect significant differences in tenderness.

Table 10 Razor shear force (Newton) mean values of cooked duck breast meat not-marinated and marinated in solutions containing bromelain and without bromelain.

Treatment	Razor Shear (Newtons)		
	Meat Only (M)	Meat + Tendon (M+T)	Difference (Tendon) (T)
C1	6.3	6.9	0.6
C2	5.9	6.3	0.4
B1.5	5.8	5.7	- 0.1
B3	5.9	6.2	0.3
B4.5	6.8	6.2	- 0.6

N=number of observation =6.

C1, Control 1 (untreated control); C2, Control 2 (treated control with solution only);

B1.5, 1.5% of bromelain; B3, 3% of bromelain; B4.5, 4.5% of bromelain.

M=meat without tendon; M+T=meat with tendon; T=Tendon, difference in (M+T) and (T)

CHAPTER 5

CONCLUSION

While B3 and B4.5 did not affect pH, B1.5 significantly reduced pH of duck breasts when compared to C1. WHC significantly increased by B3 and B4.5. However, B1.5 did not influence WHC. Marination was effective on lightness (L^*). A significant reduction was observed in lightness (L^*), no differences were observed in redness (a^*), yellowness (b^*) and Chroma (C^*) values of all raw meat samples. Cooking process also changed the color. A significant increase in lightness (L^*), and a significant decrease in redness (a^*) and yellowness (b^*) were observed in all samples. There was no significant difference in moisture and water activity values of all samples. No significant difference was observed in cook yield among (C1, B1.5, B3 and B4.5). However, cook yield significantly decreased in B3 and B4.5 when compared to C2. No significant difference in razor shears, but a significant decreased in WB shears due to bromelain, was observed. However, even though there is significant difference in Warner shear force ranged from 3.54 to 1.89, this small difference would likely not be noticed by consumers because these averages would not be considered tough by the consumers based on sensory panel test connected with objective shear results (Lyon and Lyon, 1991). Therefore, the application of commercial bromelain on duck breast meat is not technologically worthy.

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