REDUCTION OF FAT CONTENT IN PROCESSED MEATS USING
HOT-BONING AND COLD-BATTER MINCING TECHNOLOGY

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ABSTRACT

Reduction of fat content in processed meats using hot-boning and cold-batter mincing technology

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Processed meats have received negative publicity due to high fat contents that have been linked to adverse effects on human health. Fat is an essential ingredient in many processed meat products, so reducing this all while maintaining the desired characteristics of the product is a challenge. The purpose of this study was to generate low-fat meat products using a combination of hot-boning/crust-freeze-air-chilling (HB-CFAC) and cold-batter mincing (CBM) technologies. Pork hams were subjected to HB-CFAC or chill-boning/crust-freeze-air-chilling (CB-CFAC) prior to 3 min pre-mincing and 6 min mincing for control gels with back-fat addition or low-fat gels with water addition instead of the reduced back-fat. Raw meat quality, protein functionality and textural properties were analyzed through various analyses. The pH values of HB muscle and cooked gels were significantly higher than those of CB muscle and cooked gels. The fat and moisture contents of control gels was higher and lower, respectively, than those of low-fat gels, regardless of HB or CB. The protein functionality and gel forming ability of HB muscle were superior to those of the CB muscle, regardless of fat content. These results indicate that fat can be reduced with no loss of textural quality because cold-batter mincing of the HB-CFAC muscle resulted in higher gel forming ability than that of CB-CFAC muscle.

Key words: hot-boning, crust-freezing, cold-mincing, low-fat, protein functionality
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1. INTRODUCTION

1.1 Statement of Problem

Meat and meat products are generally recognized as good sources of protein, B vitamins, minerals and a part of a healthy diet. More specifically, processed meats are popular food items throughout the world because they are convenient, nutritious, and tasty. In fact, in 2019, Americans spent 6.2 billion dollars on hot dogs and sausages in supermarkets (National Hot Dog and Sausage Council, 2019). However, their image to consumers is relatively negative and products like hot dogs have received less health recognition due to high fat contents that have been linked to adverse effects on human health (de Vries, 2007). While there are plenty of these products to choose from on grocery shelves, a low-fat pork product is hard to find and least favorable. Fat is an essential ingredient in many processed meat products for flavor, juiciness, and overall liking, so reducing this all while maintaining the desired characteristics is a challenge (Bolumar et al., 2015).

Cold-batter (meat paste) mixing is an emerging technology that can be used for fat reduction in processed meats while maintaining desirable sensory characteristics. Mixing meat proteins for an extended period, at or near freezing temperature, may cause the proteins to slightly unfold in a manner that would improve protein binding functionality and generate creamy-like mouth feeling. It is postulated that internal hydrophobic protein residues are subtly twisted outward and exposed to the surface during cold-mixing, thereby inducing fat-like creaminess (Eisner et al., 2006). However, no fundamental research has been conducted to elucidate the underlying structural, functional, and organoleptic changes, or the interactions between chemical and
physical factors (e.g., shear power intensity or shear rate) during the mixing. As the effect of cold mixing on protein structure alternation is better understood, this novel technology can be more readily applied to production of low-fat meat products in a variety of ways. Thus, implementation of the results of this project will have a significant positive national public health impact as the result of reduced fat consumption.

1.2 Purpose of Study

In this study, low-fat meat products were made using a combination of hot-boning/crust-freeze-air-chilling (HB-CFAC) and cold-batter mincing technologies. The purpose of this research was to evaluate the effect of HB-CFAC and cold-batter mincing on the gel-forming properties of control and low-fat gels using whole pork ham muscles. For this study, control batters were made from pork, salt, starch, backfat, and water/ice, whereas low-fat batters were made similarly except the portion of the fat reduction that was replaced with water. The experiment was replicated three times in order to evaluate raw meat quality, protein functionality, and textural properties of protein gels at California Polytechnic State University (Cal Poly, San Luis Obispo, CA). We hypothesized that HB muscles would demonstrate superior raw meat quality to CB muscles and that the combination of HB-CFAC and CBM could improve protein functionality, thereby replacing fat with water for the generation of low-fat products with no textural quality loss.
Chapter 2

2. LITERATURE REVIEW

2.1 Processed Meat Consumption

Pork is notably the most consumed meat on a worldwide basis and processed meats are popular food items; in fact, twenty-two percent of the meat consumed in the U.S. is processed (Zeng et al., 2019). Processed pork products are pork transformed by grinding, curing, smoking, or seasoning prior to wholesale or retail sale. The processed pork category includes several items such as lunch meats, hot dogs, bacon, sausage, and smoked ham. While all of these products are in high demand throughout the world, they are also linked by their high fat contents. In fact, the fat content of fresh pork sausage could be up to 50% and as much as 30% in cooked and cooked/smoked sausages (Safe Practices for Sausage Production, 2014).

Over-consumption of animal fat (primarily saturated fat) is associated with a higher risk of obesity, arteriosclerosis, and coronary heart disease (de Vries, 2007). Accumulating evidence also links excessive consumption of processed meat with an increased risk of diabetes, and even some cancers (Zeng et al., 2016). As a result, health conscious consumers demand low-fat meat products, and the Food and Drug Administration (FDA) recommends to limiting consumption meats that are high in saturated fat (McGuire, 2016).

According to the report of U.S. Surgeon General (2010), about two-thirds of adults and one in three children in the United States are overweight or obese, which contributes to an estimated 112,000 preventable deaths each year. The consumption of foods high in saturated fats contribute to this high number. And while there are “healthy” alternatives such as turkey dogs, there are very few “low fat” pork hot dogs on the market.
2.2 Chill-Boning of Carcasses as a Traditional Method

In the commercial swine industry, pork carcasses are chilled to near 34°F (1°C) with an air velocity of 1.5 to 3 feet/second for 24 hours (Huff-Lonergan, 2005). The removal of heat directly impacts the quality of the meat product. If the carcass heat is not removed quickly enough and the pH declines rapidly, the potential to produce pale, soft and exudative (PSE) product exists. This PSE product has a lower water holding capacity compared to the product with a normal temperature and pH decline (Jones et al., 1988). These negative qualities can make it difficult to further process the meat into other products like hot dogs and sausages.

However, if the temperature declines too quickly, the potential for development of a tougher product exists, although the rapid chilling can decrease microbial growth of spoilage organisms and pathogens on the product. Conventional chill-boning of carcasses is widely accepted in the industry because it allows for a high number of harvests per day and the processing line can keep up with the speed. This is due to the fact that the carcasses are chilled before being further broken down, so the line can move quickly and efficiently with no worry of getting backed up.

2.3 Hot-Boning of Carcass as an Alternative Method

Hot-boning is defined as the removal of muscles from the carcass before chilling (West, 1983). The method of hot-boning has economic advantages due to savings in space, energy and labor, as well as improvements in functional properties of the meat (Pisula and Tyburcy, 1996). Also, hot-boning has the potential to remove each muscle prior to the development of rigor
mortis and therefore possessing the property of pre-rigor meat (White et al., 2006). The pre-
rigor meat is more susceptible to shortening due to the loss of muscle-bone attachment, and rapid
chilling/freezing would enhance the likelihood of cold shortening (Devine et al., 2004; Savell et
al., 2005). Cold shortening is rare in pork carcasses but is more likely to occur with product that
is hot-boned meat. To prevent cold shortening, it is recommended to not chill pork carcasses
below 41°F (5°C) while the muscle pH is greater than 6.0. When hot boning, it is recommended
to not drop the temperature of the meat below 59°F (15°C) while the muscle pH is greater than
6.2

Hot-trimming or skinning of carcasses prior to chilling allows for a more rapid chill of
the carcass (Milligan et al., 1998) thus reducing the rate of pH decline. Meade and Miller (1990)
showed that trimmed carcasses had a more rapid temperature decline for the first three hours. In
hot boned beef carcasses, they further observed that hot-boning of the muscle significantly
increased metmyoglobin-reducing activity of the interior portion of the muscle and subsequently
increased meat color stability compared with that of traditionally cold-boned muscles (Honikel,
1999).

Hot-boning of pork has not completely been adopted world-wide, mostly due to the
logistics of converting all plants to this technique and also to the potential negative impact on
tenderness due to the rapid drop in muscle temperature. Another challenge of hot-boning is the
line speed within the processing plant; traditionally carcasses would be chilled and then further
broken down but by removing the muscle while the carcass is still hot, it must be broken down
immediately which could cause technical problems on a large scale.
However, despite these challenges, past studies have shown that hot-boned meat is superior to chill-boned. HB muscle produces superior quality meats compared with chill-boned (CB) muscle (Kastner, 1977; Sørheim et al., 2006). In past research, the mincing of HB muscle showed that higher amounts of protein were extracted, and water-holding capacity was greater than that obtained from CB muscle (Bernthal et al., 1989). Furthermore, meat batter made with HB meat exhibited both greater emulsifying capacity and emulsion stability than that of CB muscle (Froning and Neelakantan, 1971). Wyche and Goodwin (1974) reported a higher cooking yield for hot-boned broiler than for the chill-boned broilers.

2.4 Crust-Freeze Air Chilling

Crust freeze-air chilling (CFAC) is a technique that reduces meat temperature at a high chilling rate which causes freezing of the meat surface. This method, depending on the muscle, temperature, and speed of air, can take less than an hour to achieve the desired low internal temperature, which can reduce synchronizing issues between the hot-boning line and further processing line (Herbert and Smith, 1980).

Previously, turkey breasts were subjected to CFAC and the results indicated that CFAC maintained the quality of pre-rigor muscle and sub-zero mincing of CFAC improved gel-forming ability, water holding capacity, and cooking yield of the cooked gels (Medellin-Lopez et al., 2014). The purpose of CFAC is to stop the biochemical mechanisms of rigor mortis so that the muscle can possess the quality of pre-rigor meat, without an issue of thaw-rigor that occurs when thawing the hot-boned and deep-frozen meats.
2.5 Traditional vs. Cold Batter Mincing

Traditionally, hot dog batters are minced in a cold room (4 – 6 °C) to the end-mincing temperature of 12 – 22 °C (Kletner, 1987; Gadea De Lopez and Hand, 1993; Reichert et al., 1986). However, cold batter mincing was conducted in a freezing room (-23 °C) for an extended period so that the resulting batter has more extracted protein with slight unfolding in a manner that would improve protein binding functionality. It is postulated that internal hydrophobic protein residues are subtly twisted outward and exposed to the surface during cold-mixing, thereby inducing fat-like creaminess (Eisner et al., 2006). Until today, little research has been carried out to evaluate the effect of cold-batter mixing on low-fat protein gels using hot-boned (HB) and crust-freeze air chilled (CFAC) meat.

Previously, Brauer (1993) reported that “firm and rubbery properties in low-fat frankfurter sausage” were prevented when the cutting/mixing process was extended for an unspecified time, and the batter temperature did not exceed 10°C in sausage mixing. Bard (1965) also reported that the amount of muscle protein extracted from pre-rigor meat mixed for 15 min was greater than that of post-rigor muscle extracted for 15 h. In addition, protein extraction dramatically increased in the range of -5 to 2°C compared to the temperatures higher than 2°C.

Cold-batter mixing is an emerging technology that improves protein functionality when meat is minced at subfreezing temperatures for an extended period (Lee et al., 2014). In a recent study, turkey breasts were HB, quarter-sectioned, and crust-freeze-air-chilled (HB-1/4 CFAC) until the internal temperatures reached approximately 4 °C. When these fillets were chopped using cold-batter mincing or traditional mincing, the best gel-forming ability was seen in the HB-1/4 CFAC fillet gels from cold-batter mincing, followed by HB fillet gels from traditional
mincing, and CB fillet gels from traditional mincing (Medellin-Lopez et al., 2014). With this technology of sub-zero chopping, more protein can be extracted because of the more open space within the muscle fibers. If there is more protein extracted, these proteins can interact more resulting in a batter or mixture with higher protein functionality.

2.6 Implications of Fat Reduction

Reduction of fat content creates numerous technological and practical challenges for meat processors (Bolumar et al., 2015). Fat not only contributes to desirable characteristics of juiciness, mouth-feel, and flavor (Yang et al., 2001), but it also maintains textural qualities such as particle binding, high cooking yield, and uniform texture (Keeton, 1994, Brewer, 2012).

There has been a movement among food producers, processors, and manufacturers in the United States toward lowering the fat content of animal products (National Research Council (US) Committee on Technological Options to Improve the Nutritional Attributes of Animal Products, 1988). An advantage of this is a healthier product for the consumer because the negative health risks linked to high fat consumption would dissolve. However, fat plays an important role in processed meat products, so implementing new technology on a wide-scale level could make several problems to arise.

The knowledge and technology gained from this project could be implemented to meat packing plants where pre-rigor meats can be prepared and meat processing plants where cold-mixing technology can be applied for low-fat products. This technology could not only be applied to processed pork products but also meats from other species, like beef. Combining the use of pre-rigor meat (open structure) and cold-batter mixing instead of the conventional post-
rigor meat (closed structure) and traditional mixing would further improve the processing efficacy and product quality. By applying the results of this project to the industry, there could be a significant positive public health impact as the result of reduced fat consumption.

2.7 Combination of Technologies

In past studies, the combination of hot-boning/crust-freeze-air-chilling (HB-CFAC) and cold-batter mincing (CBM) technologies has shown to improve meat turnover time, raw meat quality, and gel-forming ability in turkey protein gels (Lee et al., 2014). Crust-freeze-air-chilling (CFAC) can induce a rapid chilling of hot-boned (HB) muscles and improve both protein quality and functionality. Cold-batter (meat paste) mixing is an emerging technology that can extract meat protein to a maximal level during the extended mixing with a minimal protein denaturation at cold temperatures (Lee et al., 2014).

Based on these studies, it is expected that mincing of meat batters for an extended period at or near freezing temperature can produce cold-denatured protein structures that results in batter with improved protein binding functionality. By combining these technologies, this study aims at producing a low-fat product from a mechanical standpoint, which would induce fat-like creaminess without having traditional amounts of fat to products.

Using similar technology, researchers at the Fraunhofer Institute in Germany produced “a juicy and succulent mouth-feel sausage” comprising less than 3% fat (Eisner et al., 2006). Eisner was able to succeed with this technology and abided by very strict protocols in order to produce a low-fat sausage without a quality loss. Recently, the technology of HB-CFAC and CBM has recently been used to improve protein quality and salt reduction in turkey protein gels (Lee et al.,
The superior raw meat quality is expected due to the combination of hot-boning and rapid chilling, and the improved protein functionality after cold-batter mincing is presumed due to the cold-batter mixing at near sub-zero temperatures up to 6 min. Previously, when HB muscle was minced, more protein was extracted and higher water-holding capacity was obtained than that of CB muscle (Bernthal et al., 1989).

**2.8 pH**

When an animal is harvested, the muscle goes through many physiological changes during the time of converting the muscle to meat. Scientifically, the animal is dead after stunning and the completion of exsanguination, but the muscle is still alive and responsive to nerve stimuli. As a result, the muscle cells shift their energy (ATP) production from aerobic to anaerobic process. Without sufficient ATP, the onset phase of rigor mortis occurs (Huff-Lonergan, 2005). Lactic acid is created by the anaerobic metabolism of glycogen and the pH starts to decline. The pH of living tissue is about 7.0, but after the completion of rigor mortis, the pH of meat is normally about 5.5 to 5.7.

The extent of pH decline is primarily affected by the quantity of glycogen in muscle at the time of slaughter. The amount of glycogen present in the muscle at slaughter is directly proportional to the potential amount of lactic acid. Therefore, the more glycogen present in the muscle, the greater the potential for lower ultimate pH. Proteins are responsible for many of the functional and quality characteristics of fresh and processed pork. Because proteins are influenced by the rate and extent of pH decline in pork, pH can have a large effect on fresh and processed meat quality (Huff-Lonergan, 2005). This change in pH during the conversion of
muscle to meat affects many chemical, physical, and sensory traits of meat products including water holding capacity.

2.9 Water Holding Capacity

Water holding capacity of meat is defined as the ability of postmortem muscle (meat) to retain water even though external pressures are applied to it. Proteins are an important aspect of water holding capacity in pork because muscle is made of 75% water. More precisely, the myofibrillar proteins, both actin and myosin that bind to water are directly affected by pH (Huff-Lonergan, 2005). A rapid pH decline can create the condition of a low pH and high temperature. This combination denatures myosin and less protein is available to bind water. As the onset of rigor occurs, cross-bridges between the thick and thin filaments form which also reduce available space for water to be in the muscle. Water is a dipolar molecule that is most likely to bind to proteins that have a net charge. Meat proteins have no net charge at pH 5.1 (Huff-Lonergan, 2005). This point is referred to as the isoelectric point. As the pH of meat approaches, the level of 5.1, the water holding capacity drops drastically (Figure 1).

Figure 1. Relationship between pH and WHC
2.10 Protein Functionality

Protein functionality refers to any property of the protein that affects the attributes of the final product, such as water and fat binding, emulsifying capacity, and solubility. Protein functionality is an important aspect of processed meat products. The protein to protein interactions found within these products not only effects the final product’s gelation ability, but also the sensory aspect of mouthfeel. A study completed by Camou and Sebranek (1991) compared gelation characteristics of muscle proteins from PSE pork to proteins from normal pork. Their conclusion was that the gel strength of PSE extracts was only 45% of the controls at equivalent protein concentration (Camou and Sebranek, 1991). This difference in PSE and normal pork was considered to be from reduced solubility and loss of molecular functionality. An earlier study had confirmed that the loss of functionality by PSE muscle was due to protein denaturation resulting from the combination of high muscle temperature and rapid lactic acid production that occurs post-mortem (Park et al., 1975).

2.11 Rheology

The rheology of food is dependent on both the structure and composition of products. The matrices of food rheology have a significant influence on development of products, quality control, sensory evaluation and equipment process design and evaluation. Oscillatory rheology measurements are regularly used to investigate the gelling or viscoelastic behavior of a system, since it is recognized that a solid can be differentiated from a liquid based on the frequency dependency of the storage and loss modulus of the system (Diofanor Acevedo Correa et al.,
Fundamental rheology tests provide vital information about time-dependent viscoelastic behavior and the molecular mechanisms that surround changes in structure when a protein undergoes gelification.

Furthermore, rheology tests exemplify the changes of microstructure in the emulsified samples. During tests, the mass of the minced muscle tissue represents a mixture of fragments of fiber with an intact structure of myofilaments, membranes and sub-cellular particles, fat and connective tissue particles and the liquid properties of these suspended fragments. The more water that is added to this homogenized mixture, the lower the performance and viscosity the product will have (Payne and Rizvi, 1988). This is because increasing the water content is expected to decrease interactions among particle surfaces and reduce the viscosity of the liquid phase. The rheology of emulsified meats strongly depends on pH, and both yield value and viscosity show a minimum between pH 5.0 to 5.5 (Payne and Rizvi, 1988). The pH of this minimum corresponds to the isoelectric point of myosin and actomyosin.

Processing deviations can often be detected more rapidly using rheological measurements rather than chemical tests (Escher, 1983). The texture of a product can often be related to its rheological properties (Sherman, 1970; Szczesniak and Farkas, 1962), and an understanding of a product’s sensory attributes may require a fundamental knowledge of its rheology (Payne and Rizvi, 1988).
2.12 Textural Profile Analysis

Texture Profile Analysis (TPA) is a well-known double compression test for determining the textural properties of foods. During a TPA test, samples are compressed twice using a texture analyzer to provide understanding into how samples behave when chewed. The TPA test is frequently called the "two bite test" because the texture analyzer simulates the biting action of the mouth. Primary TPA characteristics include hardness, cohesiveness, springiness, and resilience.

![Figure 2: Texture Profile Analyzer on hot dog sample](image)

Hardness is defined as the force that occurs during the first “bite” and measured as the maximum force of the first compression. Cohesiveness is how well the product withstands a second deformation relative to its resistance under the first deformation. It is measured by the peak force value that occurs during the first compression. This is measured by the area of work during the second compression divided by the area of work during the first compression. A
product is cohesive when it adheres to itself under some compressive or tensile stress. A piece of pork, for example, is highly cohesive when it takes a great many chews to break down.

Springiness is defined as how well a product physically springs back after it has been deformed during the first compression and has been allowed to wait for the target wait time between strokes. The spring back is measured at the down-stroke of the second compression. Springiness is expressed as a ratio or percentage of a product's original height. Springiness is measured by the distance of the detected height during the second compression divided by the original compression distance. The more a product is destroyed, the less springiness it will exhibit.

Gumminess is measured as hardness multiplied by cohesiveness. Chewiness is measured as hardness times cohesiveness times springiness. The TPA method is a powerful tool that can provide highly meaningful insights into product texture.
2.13 Raw Meat Quality

Raw meat quality factors and meat batter properties such as pH, batter temperature, and protein solubility can help to predict the effectiveness of processing and the overall quality of completed meat products. Conditions that affect the structural integrity of postmortem muscle will ultimately affect overall meat quality and functionality of the product (Huff-Lonergan, 2015).

Low muscle pH is associated with low water-holding capacity, due to structure changes and reduced charges in muscle proteins which is directly related to raw meat quality (Guerrero-Legorreta and Hui, 2010). Since the extent of pH decline has an impact on fresh (uncooked) pork firmness and on water holding capacity, it stands to reason that it will impact sensory tenderness. Brown and Toledo (1975) recommended that batter-mincing temperature should not be greater than 15 degrees C after chopping for a superior quality of protein extraction. Upon reaching over 16 degrees C, both water and fat are released from the batter, which resulted in quality loss of finished products (Deng et al., 1976).

Protein functionality and solubility are directly related to pH and batter temperature of the product. Of the myofibrillar proteins in meat, myosin and actin contribute most to the development of desirable gel characteristics in processed meat products. The heat-induced gelation of myosin results in the formation of a 3-dimensional structure that holds water in a less mobile state (Yasui et al. 1979). During the formation of this network, water and fat retention are improved which consequently impact the yield, texture, cohesion and ultimate gelling ability of the product. It is widely accepted that gel firmness and hardness increase with increasing protein
concentration regardless of protein source (Ishioroshi et al., 1979; Foegeding and Ramsey, 1986; Fretheim et al., 1986).

2.14 Surface Hydrophobicity ($S_0$)

An increase in the surface hydrophobicity of proteins leads to protein-protein interactions and their aggregation during thermal treatment, although the primary structure of proteins is often not affected (Li et al., 2015). During traditional mincing, meat and water are chopped around 4-6°C until they reach 12-15°C.

Protein unfolding and surface hydrophobicity play an important role in meat gelation. Gelation is a multistep thermodynamic process that involves protein unfolding, aggregation, and formation a 3-dimensional network resulting in an elastic gel (Lesiow and Xiong, 2001). The increased protein unfolded and the exposure of hydrophobic residues to the water molecules contributed to the gelling process (Li et al., 2015).

During cold-batter mincing, the chopping time is extended while the temperature is lower and there is less protein denaturation within the batter. At this moment, hydrophobic residues are exposed to the outside of the product. So, the more the protein is denatured like in traditional mincing, the more hydrophobic residue is found on the outside. However, when you chop the batter at sub-zero temperatures, there is less protein denaturation and less hydrophobic residue present. Meat that has been CFAC has a significantly lower temperature than that of traditionally chilled meat and combining these two technologies could possibly yield future industry changes.
Chapter 3

3. MATERIALS AND METHODS

All procedures were approved by the Institutional Animal Care and Use Committee of California Polytechnic State University (AUF # 1712).

3.1 Pig Slaughter and Ham Muscle Preparation

Twelve commercial pigs with live weight of 113 ± 5 kg (4 pigs/each of 3 replications) were obtained locally and delivered to the Meat Processing Center at California Polytechnic State University (Cal Poly). Upon arrival, pigs were electrically stunned for 8 s (1.25 A, 60 Hz, 220 V) and exsanguinated after sticking the neck. Following bleeding, the pigs were subjected to scalding/dehairing at 59 °C for 4 min in a combined scalding and dehairing machine (JWE 25-1900, JWE BAUMANN FOODTEC, Aalen-Oberalfingen, Germany). Immediately after evisceration and washing, each carcass was split in half; the right side was subjected to hot boning (HB) without chilling and the left half-carcass was chill deboned (CB) after conventional chilling at 4 °C overnight (Figure 3).

Figure 3. Pork carcass split for hot boning (HB, right) and chill boning (CB, left).
3.2 Carcass Temperature and pH

At 60 min postmortem, muscle pH was recorded by inserting a pH electrode (model 13-620-631, Fisher Scientific Inc., Houston, TX) attached to a pH meter (Accumet AR15, Fisher Scientific Inc., Pittsburgh, PA) into the muscle between the 10th and 11th posterior rib. Similarly, muscle temperature was recorded by inserting a thermometer (Digital Meat Thermometer, Nexgrill Industries, Inc., Chino, CA) to the center of whole ham. The ham muscles were then hot-boned from the right half-carcasses while the other ham muscles were subjected to chilling overnight at 4 °C for chill-boning. The resulting hams were deskinned, deboned, and fat trimmed (Figure 4).

3.3 Crust-Freeze Air Chill, Meat Batter and Cooked Gel Preparations

Skinless and boneless ham muscles were cut into strips of 2 - 2.5 cm width and 0.5 - 0.75 cm thickness in various lengths. The strips were hung using a bacon hook and were crust-freeze-air-chilled to internal temperatures of -4 to -5 °C in a freezer room at -29 °C prior to cutting into 3 - 4 cm length (Figure 5). The meat cuts (7.2 kg, 65% of a batch) were pooled and pre-chopped for 3 min using a 6-knife headed bowl cutter at 1420 rpm (K15, TALSA, EU) with salt (2% w/w), starch (2% w/w), pork back-fat (15% w/w), and ice (16% w/w) for a control-fat gel, while the
same ingredients were used for a low-fat gel except that ice (31%, w/w) was used instead of pork back-fat (15%)/ice (16% w/w).

After the pre-chopping (3 min), the meat batters were minced for 6 min in the same bowl cutter at 2840 rpm, regardless of HB or CB meats (Figure 6). At the end of mincing, batters were stuffed into a sausage casing, weighed, and cooked in a water bath (Isotemp 2310, Fisher Scientific, Marietta, OH) to the internal temperature of 75 °C using a heating rate of 0.5 °C/min. On the following day, the gels in casings were reweighed for the determination of cooking yield, and the cooked gels were used for pH and textural analysis.
Moisture and fat contents were determined using a drying oven (model DX 400, Yamato Scientific, Tokyo, Japan) and fat extractor (Soxtec™ 2043, FOSS, Eden Prairie, MN), respectively, according to AOAC International methods 950.46B and 991.36, respectively (Official Methods of analysis of AOAC, 2005).
3.5 Expressible Moisture (EM, %)

Expressible moisture was determined using the procedure of Jauregui et al. (1981). Briefly, a sample (1.5 g) of cooked protein gel was weighed, covered with filter paper (Whatman #3), and centrifuged at 1,000 × g for 15 min (Centrifuge 5810 R, Eppendorf AG, Hamburg, Germany). Expressible moisture (EM, %) was calculated using the equation: EM (%) = (weight of expressed water in filter paper/weight of sample) × 100 (Jauregui et al., 1981).

3.6 Protein Solubility Determination (%)

For protein solubility determination, duplicate 3-g batter samples were added to a 27 mL solubilizing buffer (0.6 M KCl in 20 mM Tris-HCl, pH 7) and blended for 60 sec with a homogenizer. The resulting meat pastes were centrifuged at 20,000 × g for 30 min at 4 °C. Protein concentrations from the resulting supernatants were determined using the Bradford protein assay and a nitrogen analyzer (model FP 2000, LECO Corp., St. Joseph, MI), respectively (Bradford, 1976).

3.7 Texture Profile Analysis (TPA)

Texture profiles of gel samples (12.5 mm diameter and 13 mm height) were evaluated using puncturing apparatus and Texture Analyzer (TA-XT2, Texture Technologies Corp., Scarsdale, NY) of Stable Micro Systems (Haslemere, England). In a two-cycle compression test, sample height was compressed by 75% at a rate of 1.67 mm/sec using a 25 kg load cell for the measurement of gel hardness (N), springiness (cm), cohesiveness, gumminess, and chewiness (Bourne, 1978).
3.8 Rheological and Thermal Properties

Rheological and thermal properties (sol to gel process) of meat pastes were assessed to compare viscosities (Pa) among treatments using a rheometer (Discovery Hybrid Rheometer, DHR-2, TA Instruments, New Castle, DE). According to the method of Xiong and Blanchard (1994), meat paste samples were placed between two flat parallel plates (1 mm) where the perimeter was coated with a thin layer of silicone oil to prevent dehydration during heating. The resulting samples were heated from 35 °C to 85 °C with a heating rate of 1°C/min while they were continuously sheared in an oscillatory mode at a fixed frequency of 0.1 Hz and 0.02 strain (Lee et al., 2019).

3.9 Surface Hydrophobicity

Surface hydrophobicity ($S_0$) of protein was determined using 1-anilinonaphthalene-8-sulfonic acid (ANS) as a fluorescence probe according to the method of Alizadeh-Pasdar and Li-Chan (2000). The excitation and emission slits were set at 5 nm each, and the excitation and emission wavelengths were 390 nm and 470 nm, respectively (Luminescence Spectrometer LS 50 B; Perkin Elmer, Inc., Norwalk, CT). An aliquot (3 g) of meat batter was homogenized with 27 mL of 0.6-M KCl, 20-mM Tris-HCl buffer (pH 7.0). After centrifugation at 20,000 × g for 30 min at 4 °C, the protein concentrations of the supernatant were adjusted to 0.1, 0.2, 0.3 and 0.4 mg/mL, respectively. Four milliliters of each sample were mixed with 20 µL of ANS stock solution (8 x 10^{-3} M ANS in 0.1-M phosphate buffer, pH 7.4). After 10 min incubation at 4 °C, the relative fluorescence intensity (RFI) of each solution was measured. The fluorescence values were corrected for light scattering using a protein blank with no ANS. The RFI for the blank was subtracted from the sample with ANS to obtain corrected fluorescence values. The change in
fluorescence intensity was used to represent the change in hydrophobicity resulting from protein
denaturation as a function of boning types (Alizadeh-Pasdar and Li-Chan, 2000).

3.10 Surface-reactive Sulfhydryl (SRS) and Total Sulfhydryl (TSH) Determination
Surface-reactive sulfhydryl content (SRS) was determined using Ellman’s reagent [5-5’-
dithiobis (2-nitrobenzoic acid); DTNB] as described by Hamada et al. (1994) with slight
modifications. The meat batter was solubilized in 0.6 M NaCl and 20 mM phosphate buffer pH
7.0. The protein contents of the supernatants were adjusted to 1.0 mg/mL using the same
phosphate buffer (Hamada et al., 1994). For total sulfhydryl (TSH), the resulting solutions were
mixed with 2 mL of 8 M urea and 0.2 M Tris-HCl buffer (pH 7.0), followed by addition of 50 µL
of 10 mM DTNB in 0.1 M sodium phosphate buffer, pH 7.2 containing 0.2 mM
ethylenediaminetetraacetic acid (EDTA). For SRS, the samples were vortexed and incubated at
40 °C for 15 min after which absorbance were measured at 412 nm using a UV
spectrophotometer. Surface reactive sulfhydryl content and total sulfhydryl content were
determined using a molar extinction coefficient of 13,600 M⁻¹cm⁻¹ (Riddles et al., 1979).

3.11 Statistical Analysis
All experiments were replicated 3 times. Data were evaluated by one-way ANOVA (analysis of
variance), using PASW 18 statistic program (SPSS Inc., Chicago, IL) and a completely
randomized design. A post-hoc analysis was performed using Duncan’s multiple range test to
evaluate difference among treatments at $P < 0.05$. 
4. RESULTS AND DISCUSSION

4.1 Raw Meat

The mean temperatures of internal ham and the pH of loin muscle (between the 10th and the 11th rib) were 41 °C and 6.27 at 60 min postmortem, respectively. Both values were significantly reduced to 4.2 °C and pH 5.63 after overnight chilling (P < 0.05) (Table 1). Kim et al. (2016) reported that the temperature and pH values of pork *longissimus dorsi* at 45 min postmortem was in the range of 36.4 - 40.4 °C and pH 6.1 - 6.8, respectively, which was reduced to 5.0 - 6.3 °C and pH 5.8 - 5.9 at 24 h postmortem, respectively. When pre-rigor porcine muscle was incubated at the temperatures of 0, 10, 20, 30, and 40 °C to 6 h post mortem, the muscle incubated at 40 °C showed a significant decrease of sarcoplasmic protein solubility, lower shear force, and rapid reduction of muscle pH, all of which are consistent with development of PSE (pale, soft, exudative) meat (Liu *et al.*, 2014).

**Table 1.** Muscle temperature of internal ham and loin pH between the 10th and 11th rib before and after chilling

<table>
<thead>
<tr>
<th>Parameter/Chilling</th>
<th>Before chilling&lt;sup&gt;1&lt;/sup&gt;</th>
<th>After chilling&lt;sup&gt;2&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature (°C)</td>
<td>41.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.2&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>pH</td>
<td>6.27 ± 0.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.63 ± 0.11&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>Means within a row with unlike superscripts are different (P < 0.05).
<sup>1</sup>Measured after 1 h postmortem (number of observations in each chilling, n = 9).
<sup>2</sup>Measured after 24 h postmortem (number of observations in each chilling, n = 9).
4.2 Meat Batter Properties

When the ham muscles were strip-cut, crust-freeze-air-chilled, and minced for 6 min, the batter temperatures of HB/crust-frozen muscle (hereafter, identified as HB muscle) were lower than those of CB/crust-frozen muscle (hereafter, identified as CB muscle), regardless of back fat addition, whereas the batter pH of HB muscle was higher than that of CB muscle ($P < 0.05$) (Table 2). The reason for the higher pH in HB batters is expected from the higher muscle pH compared to the CB muscle. A similar pattern was observed in turkey breast meat batters, in which cold-batter mincing of HB muscle for 6 min resulted in a higher pH than that of CB batter (Lee et al., 2014).

Table 2. Batter temperature and pH after mincing hot boned (HB) or chilled boned (CB) ham muscle for control and low-fat gels

<table>
<thead>
<tr>
<th>Parameter/Mincing</th>
<th>HB control-fat&lt;sup&gt;1&lt;/sup&gt;</th>
<th>HB low-fat&lt;sup&gt;2&lt;/sup&gt;</th>
<th>CB control-fat&lt;sup&gt;3&lt;/sup&gt;</th>
<th>CB low-fat&lt;sup&gt;4&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature ($^\circ$C)</td>
<td>-2.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-2.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>pH</td>
<td>6.02 ± 0.11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.00 ± 0.11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.73 ± 0.05&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.67 ± 0.08&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a,b</sup>Means within a row with unlike superscripts are different ($P < 0.05$).

<sup>1</sup>Minced HB muscle with 15% pork back-fat/16% ice (number of observations in each chilling, n = 9).

<sup>2</sup>Minced HB muscle with 31% ice (number of observations in each chilling, n = 9).

<sup>3</sup>Minced CB muscle with 15% pork back-fat/16% ice (number of observations in each chilling, n = 9).

<sup>4</sup>Minced CB muscle with 31% ice (number of observations in each chilling, n = 9).
4.3 Cooked Gel Properties

The moisture content of control-fat gels (containing 15% back-fat/16% ice) was lower than that of low-fat gels (having 31% ice), regardless of HB or CB (P < 0.05) (Table 3). Similarly, the fat content of control-fat gels including back-fat was higher than the low-fat gels having no back-fat for both HB and CB (P < 0.05) (Table 3). In expressible moisture, HB low-fat gels showed significantly lower values than CB low-fat gels showing higher water holding capacity of HB gels, while no difference was found between HB and CB control-fat gels. The lack of difference between the control-fat gels was expected based on the lower moisture content (64-65%) in the initial stage compared with the higher moisture content (76-78%) in the low-fat gels.

Table 3. Proximate compositions (moisture and fat %) and functional properties (expressible moisture content and cooking yield, %) of cooked pork gels prepared with hot boned or chill boned ham muscle with 15% pork back fat or water

<table>
<thead>
<tr>
<th>Parameter/Mincing</th>
<th>HB control-fat</th>
<th>HB low-fat</th>
<th>CB control-fat</th>
<th>CB low-fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture (%)</td>
<td>64.8 ± 1.07b</td>
<td>77.7 ± 1.22a</td>
<td>65.3 ± 1.91b</td>
<td>75.9 ± 2.31a</td>
</tr>
<tr>
<td>Fat (%)</td>
<td>11.2 ± 1.35a</td>
<td>1.61 ± 0.35b</td>
<td>11.8 ± 0.39a</td>
<td>2.95 ± 0.90b</td>
</tr>
<tr>
<td>Expressible moisture (%)</td>
<td>10.1 ± 0.66c</td>
<td>21.5 ± 2.41b</td>
<td>14.5 ± 3.75bc</td>
<td>32.0 ± 5.71a</td>
</tr>
<tr>
<td>Cooking yield (%)</td>
<td>95.2 ± 1.41a</td>
<td>90.5 ± 1.34ab</td>
<td>89.1 ± 2.44b</td>
<td>76.7 ± 3.24c</td>
</tr>
</tbody>
</table>

Means within a row with unlike superscripts are different (P < 0.05).

Mincing treatments: same as in Table 2 (number of observations in each chilling, n = 9).

Higher cooking yields were observed in the HB gels than the CB gels, regardless of fat content, with no significant difference observed between HB low-fat and CB control-fat gels.

Again, these results indicated that HB gels possessed higher water holding capacity than CB gels.
presumably due to greater protein extraction and less protein denaturation during the batter mincing at subzero temperatures. These results support the previous findings of higher cooking yields in pre-rigor meat gels than those of post-rigor meat gels (Medellin-Lopez et al., 2014, Sørheim et al., 2006). The hamburger patties made with pre-rigor meat showed significantly higher juiciness and overall acceptability mainly due to higher water holding capacity compared with post-rigor patties (Akwetey, 2012). Kim et al. (2015) reported that the protein gels of pre-rigor broiler breast significantly improved water holding capacity, cooking yield, protein solubility, and gel hardness compared with those of post-rigor gels.

### 4.4 Textural Properties

In evaluation of textural properties of cooked gels, higher hardness values were observed in HB gels than CB gels, regardless of fat addition \( (P < 0.05) \) (Table 4). The hardness values indicate the maximum force of the first compression in the two-cycle compression test. During cooking, the structure of extracted myofibrillar proteins unfolds and interacts with neighboring proteins to form a three-dimensional gel network. When the protein gels are cross-linked primarily by covalent bonds, they display a rubbery elastic-like behavior with a less viscous element (Lanier et al., 2004). Pre-rigor meat produces firmer patty textures than post-rigor meat while fast-chilled meat produced firmer gels than slow-chilled meat (Sørheim et al., 2006, Rathgeber et al., 1999). The combination of hot-boning, rapid chilling, and cold-batter mincing technologies in this study is expected to contribute to improving protein functionality and textural firmness that were similarly observed previously (Lee et al., 2014).
Table 4. Textural properties of pork gels prepared with hot boned or chill boned ham muscle with 15% pork back fat or water

<table>
<thead>
<tr>
<th>Parameter/Mincing</th>
<th>HB control-fat</th>
<th>HB low-fat</th>
<th>CB control-fat</th>
<th>CB low-fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hardness (N)</td>
<td>57.0 ± 9.17a</td>
<td>53.5 ± 3.50a</td>
<td>30.6 ± 4.29b</td>
<td>24.1 ± 6.59b</td>
</tr>
<tr>
<td>Cohesiveness (ratio)</td>
<td>0.30 ± 0.03b</td>
<td>0.43 ± 0.06a</td>
<td>0.36 ± 0.09b</td>
<td>0.26 ± 0.01c</td>
</tr>
<tr>
<td>Springiness (ratio)</td>
<td>0.88 ± 0.03a</td>
<td>0.78 ± 0.11a</td>
<td>0.89 ± 0.04a</td>
<td>0.78 ± 0.03a</td>
</tr>
<tr>
<td>Gumminess</td>
<td>8.18 ± 0.08ab</td>
<td>16.4 ± 4.98a</td>
<td>6.70 ± 3.64b</td>
<td>4.57 ± 1.50b</td>
</tr>
<tr>
<td>Chewiness</td>
<td>7.20 ± 0.30ab</td>
<td>13.0 ± 4.93a</td>
<td>5.95 ± 3.30ab</td>
<td>3.58 ± 1.22b</td>
</tr>
</tbody>
</table>

a–c Means within a row with unlike superscripts are different (P < 0.05).

1Mincing treatments: same as in Table 2 (number of observations in each chilling, n = 9).
2The maximum force of the 1st compression.
3The area of work during the 2nd compression/the area of work during the 1st compression.
4The distance of the detected height during the 2nd compression/the distance of the detected height during the 1st compression.
5Hardness x cohesiveness.
6Hardness x cohesiveness x springiness.

Cohesiveness is the ratio of the area under the second cycle compression curve over the first cycle compression. The highest cohesiveness value was observed in HB low-fat gels, followed by HB/CB control-fat and CB-low-fat gels. When non-covalent interactions such as hydrogen bonds and hydrophobic interactions are removed, the heat-induced gels are likely losing their viscous elements (Niwa et al., 1988). The cohesiveness is related with gel deformability and serves as an indicator of protein quality that is not affected within a certain range of moisture content from 75 to 81% (Hamann and McDonald, 1992, Kim et al., 2004). The reason for the highest cohesiveness values in HB low-fat gels compared to CB gels is the combination of high protein quality (or less denatured protein) and deformability. The lower cohesiveness value in HB control-fat gels (65% moisture) than HB low-fat gels (78% moisture)
is expected from the lower moisture content rather than lower levels of protein quality, resulting in less deformability or flexibility.

No significant difference was observed in springiness, regardless of boning condition or fat addition. Higher values in gumminess and chewiness were found in HB low-fat gels than CB low-fat gels, with no significant difference observed between HB and CB control-fat gels (Table 4). Again, these results generally indicate that the protein quality and gel-forming ability of HB gels are better than those of CB gels.

4.5 Dynamic Rheological Properties

The dynamic viscoelastic properties of HB and CB meat batters were observed by monitoring elastic property of a gelling system (G’, Pa) during the heating from 35 to 85 °C. Hot boned meat batters showed a rapid increase of G’ value in the initial heat at 35 to 41 °C (HB low-fat) - 43 °C (HB high-fat), which was expected from the combination of more protein extraction, more sticky batter, and higher muscle pH than CB batters (Figure 7). HB batters usually absorb more energy than CB batters due to open-structure proteins and become more susceptible to thermal unfolding for protein gelation (Lee et al., 2019, Claus and Sørheim, 2006, Acton et al., 1983). Chan et al. (2011) compared rheological properties of protein gels made with pale (pH ≤ 5.7), normal (5.9 pH < 6.1), and dark (pH ≥ 6.3) turkey breast muscles, referred to as low, normal, and high pH gel, respectively. A maximum G’ value increased as the gel pH increased from low to normal, and to high at 56.6 °C, while a maximum rate-increase in G’ value was observed in the temperature range of 30 to 45 °C, regardless of pH level.
As the heating process continued, the storage value of HB control-fat meat batter rapidly decreased with one peak between 53 and 58 °C, followed by a rapid increase from 58 to 85 °C. Similar results were observed in the HB low-fat batter. The pattern of storage modulus change was similarly reported in a pork meat paste (Kang et al., 2014, 2019) and a myofibrillar protein fraction (Wu et al., 2009, 2018, Siegel and Schmidt, 1979). During the heating of HB pastes from 43 to 56 °C (P-I and II), protein became denatured and protein-protein interaction was initiated. More specifically, S1 sub-fragments in myosin head become primarily denatured and associated as heating is continued (Wu et al., 2009, Xiong and Brekke, 1990a, 1990b).

In the continuous heating from 56 to 85 °C (P-III and IV), the G’ of the HB control-fat batter decreased between 56 °C and 59 °C, followed by a sharp increase. These rheological transitions have been explained by stepwise structural changes and interactions of myofibrillar proteins. The decrease of G’ is mainly due to the denaturation of myosin tails (Wu et al., 2009,
and the rapid increase is explained by the conversion of viscous sol to elastic gel (Alvarez et al., 2012). Using differential scanning calorimetry (DSC), a typical thermal curve of meat paste has been reported for conformational changes (denaturation) of proteins during heating (Martens and Vold, 1976). The first transition has been attributed to myosin denaturation between 54 to 58 °C while the second and third transitions were explained by the denaturation of collagen and sarcoplasmic protein between 65 to 67 °C, followed by actin denaturation between 80 to 83 °C (Wright and Wilding, 1984, Stabursvik and Martens., 1980).

Unlike HB meat batters, CB meat batters showed little change of G’ value in the initial heat up to 46 °C. During the continuous heating from 46 to 85 °C, the G’ decreased slowly to 53 °C (P-I) and increased slightly to 55 °C (P-II), followed by a slow decrease (P-III) to 58 and a rapid increase to 85 °C (P-IV) (Figure 7). These types of rheological behaviors have been explained in the HB gels with four phases of thermodynamic properties (P-I to IV).

In general, both HB gels showed higher G’ values than CB gels except the intermediate value of CB control-fat gel after 59 °C. When comparing the results of fat addition, both HB gels showed higher G’ values than CB gels up to 59 °C, whereas both control-fat gels marked higher G’ values between 60 and 85 °C. In case of myofibrillar protein, G’ stepwise increased as fat (lard) was added from 0% to 15% over the entire heating range (20 to 80 °C), especially at the final heating temperatures (Wu et al., 2009). It was explained that the fat globules participated and induced permanent protein-fat globule interactions and subsequently aggregated at the final heat control, which was similarly observed in frankfurters (Theno and Schmidt, 1978). These results support the findings of textural properties that both HB-control and HB-low fat gels had higher hardness value than CB gels, with intermediate value of CB-control fat gel observed for cohesiveness, gumminess, and chewiness (Table 4).
In case of loss modulus, both HB batters showed higher $G''$ values during heating from 35 to 58 °C than cold batters with a maximum $G''$ values observed at 42 – 48 °C while the cold batters showed flat lines with lower $G''$ values. During the heating from 58 to 85 °C, the $G''$ values increased and showed a flat line, indicating the stabilization of elastic gels, regardless of boning type and fat level (Figure 8). Chan et al. (2011) showed a continuous increase of loss modulus during heating from 30 to 80 °C, with no detection of early peak probably due to no hot-boned gels.

Figure 8. Loss modulus ($G''$) of control-fat or low-fat batters using crust-freeze air chill ham muscles after hot-boning (HB) or chill-boning (CB).

1Phase I – IV for hot-boned batter
2Phase I – IV for chill-boned batter
3Chilling, mincing, and formulation conditions are same as in Figure 5
4.6 Protein Solubility

The solubilized protein of HB batters ranged between 44 to 46% which was significantly higher than the range (37- 40%) of CB batters, with higher values for control-fat gels than low-fat gels (Figure 9). These results are consistent with the previous report of Lee et al. (2014) showing that HB turkey batter had higher protein solubility than CB batter, regardless of salt level. Proteins in pre-rigor muscle are likely extracted more easily than those in post-rigor muscle due to the open status with less actomyosin formations (Claus and Sørheim, 2006, Saffle and Galbreath, 1964, Bernthal et al., 1989).

Figure 9. Protein solubility (%) of control-fat or low-fat batters using crust-freeze air chill ham muscles after hot-boning (HB) or chill-boning (CB).

a-d Means with unlike superscripts are different (P < 0.05)

1Chilling, mincing, and formulation conditions are same as in Figure 4 (number of observations in each chilling, n = 6)
4.7 Surface Hydrophobicity (S₀)

The surface hydrophobicity of HB control-fat batters was significantly lower than that of CB batters, regardless of fat content ($P < 0.05$) (Figure 10). These results were expected because the proteins of HB muscle have been subjected to less enzymatic hydrolysis with no overnight chilling and less physical disruption during mincing (3 min pre- + 6 min mincing) than CB muscle (Wheeler et al., 2000, Kang and Kim, 2017). No significant difference between HB low-fat batter and CB batters has been found. The result is potentially explained by more structural exposure during chopping due to more mechanical friction with less fat or lubricant. These results can support the finding of low storage modulus and loss modulus values in HB low-fat paste compared to HB control-fat paste in the beginning of heating (Figures 7 and 8).

Figure 10. Surface hydrophobicity (S₀) of control-fat or low-fat batters using crust-freeze air chill ham muscles after hot-boning (HB) or chill-boning (CB).

$^{a-d}$ Means with unlike superscripts are different ($P < 0.05$)

$^{1}$Chilling, mincing, and formulation conditions are same as in Figure 4 (number of observations in each chilling, n = 6)
In native protein, hydrophobic side chains are buried inside protein molecules. However, some of these hydrophobic side chains become exposed to the protein surface by temperature abuse or physical disruption, resulting in deformation of three-dimensional structure of the protein and loss of protein functionality (Wagner and Anon, 1985). Lee et al. (2014) reported that the temperature of 2% NaCl turkey batter was higher than that of 1% NaCl batter, regardless of HB or CB, explaining that more protein was extracted, and more mechanical friction occurred during the chopping. The surface hydrophobicity of pork myofibrillar protein (Longissimus dorsi) showed a peak value as cooking temperature was increased to 70 °C, and pronounced unfolding of myosin heads was observed when bovine muscle has been frozen at lower freezing rate (Bax et al., 2012, Yuan et al., 2011).

Protein hydrophobicity is an important parameter that is related with protein denaturation or structural unfolding (Bax et al., 2012). In meat processing, the extraction of myofibrillar proteins at cold temperature (no deep-freezing) may result in subtle exposure of hydrophobic residues or minimal denaturation (Li-Chan et al., 1984) that might be more favorable to improvement of protein-protein interaction and protein-lipid interaction (Kundu and Guputasarma, 1999, London, 1986). However, over exposure of protein structure and undesirable protein denaturation resulted in loss of fat and water binding capacity that were observed in the prolonged mincing at elevated batter temperatures > 15 °C (Barbut, 1990, Brown and Toledo, 1975).
4.8 Surface Reactive Sulfhydryl (SRSH) and Total Sulfhydryl (TSH) contents

Figure 11 indicated that the values of SRSH and TSH in HB batters were lower than CB low-fat batter, with an intermediate value observed for the CB control fat gel. The content of SH group is affected by the level of protein unfolding and disulfide bond formation through the oxidation of SH groups. It has been known that sulfhydryl groups increased with heating of myofibrils to 60 °C, potentially due to an unfolding of protein molecules that expose buried SH groups to the surface during heat denaturation (Tinbergen, 1970; Jacobsen and Henderson, 1973). As for the results of hydrophobicity of HB and CB meat batters (Figure 10), the HB control-fat batter showed the least SRSH and TSH value followed by HB low-fat/CB control-fat batters and CB low-fat batter. Using fish muscle, Thawornchinsombut and Park (2004) reported that more disulfide bonds and lysinoalanine cross-links occurred when the pH of muscle protein was adjusted to 12. In our study, the lower values of SRSH and TSH in HB batters are expected from less protein unfolding (or denatured) during the batters mincing.

Figure 11. Surface reactive sulfhydryl (SRSH, A) and total sulfhydryl (TSH, B) of control-fat or low-fat batters using crust-freeze-air-chilled ham muscles after hot-boning (HB) or chill-boning (CB).

Means with unlike superscripts are different (P < 0.05)

Chilling, mincing, and formulation conditions are same as in Figure 4 (number of observations in each chilling, n = 6)
Chapter 5

5. CONCLUSION

This study demonstrated that the crust-freeze air chilling of hot-boned (HB) muscle maintained the pre-rigor meat quality such as higher muscle pH and water holding capacity. In the implementation of HB technology in industry, however, synchronization of processing speeds between hot-boning line and further processing line is a challenge because the speed of HB is usually faster than the speed of additional processing, resulting in loss of pre-rigor meat quality. Crust-freezing air chilling and cold-batter mincing of hot-boned meats demonstrated rapid processing, improved protein functionality, and better gel-forming ability over the cold-batter mincing of chill-boned meat. Preservation of protein integrity and functional properties during deboning and mincing is important to produce low-fat meats with high moisture contents. Results of this study indicate that the combination of hot-boned/crust-freeze-air-chilled muscle and cold-batter mincing technologies could enable to produce low-fat protein gels with no functional and textural quality loss.
Chapter 6

6. AREAS FOR FURTHER STUDY

The following topics are recommended for future study:

- Sensory analysis and evaluation of finished products.
- Additional tests in pilot and plant scales are required to implement the new processing technique of HB-CFAC and cold-batter mincing.
REFERENCES CITED


Brauer, H. Fat-reduced frankfurters-type sausage: technology for preventing too firm and rubbery a bite. Fleischwirtschaft 1993. 73, 64-65.


Herbert E. F., and M. G Smith. 1980. Hot-boning of meat: refrigeration requirements to meet microbiological demands. Food Res Quart, Sydney, AU.


Kang, Z.; Li, B.; Ma, H.; Chen, F. Effect of different processing methods and salt content on the physicochemical and rheological properties of meat batters. Int. J. Food Prop. 2016. 19, 1604-1615.


Kim, B. Y.; Park, J. W.; Yoon, W. B. Rheology and texture properties of surimi and surimi-based foods. In Surimi and surimi seafood; Park, J. W.; Ed.; Taylor & Francis: New York, 2004; p 491-582.


Safe Practices for Sausage Production. The U.S. Department of Agriculture (USDA), Food Safety and Inspection Service (FSIS), and The Association of Food and Drug Officials (AFDO), 2014.


