GELATION AND BIOCHEMICAL PROPERTIES OF MECHANICALLY SEPARATED PORK

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ABSTRACT

Mechanical separation of pork serves to recover more protein from a carcass but may compromise texture of processed meat products. To increase utilization of mechanically separated pork (MSP) as a partial substitute of regular pork in comminuted meat products, it's critical to understand the physicochemical and biochemical properties of MSP.

The first objective for this study was to investigate the chemical composition, physicochemical and gelation properties of MSP and the effect of chilling rate and frozen storage on properties of MSP. MSP from pork picnic bones had lower collagen content, higher pH value, and was higher in calcium and iron content ($p<0.05$) than pork picnic (PP). The natural actomyosin (NAM) from MSP showed decreased viscoelasticity. At the cellular level, the MSP had highly disrupted structure with randomly oriented, curved and twisted myofibrils. The myosin heavy chain (MHC) was partially degraded after the mechanical separation process as confirmed by immunoblotting with MHC antibodies. The ratio of myofibrillar protein to sarcoplasmic protein decreased ($p<0.05$) in MSP compared with PP. With the heat and pressure generated through the process, MSP had higher ($p<0.05$) lipid oxidation level throughout the frozen storage period (1 to 13 months) compared with ground PP.

The second objective was to evaluate the potential utilization of MSP as a substitute of regular pork in bologna processing and the effects of initial chilling rate and -18 °C frozen storage on gelation properties. Substitution of 7.5% MSP didn't lead to a significant negative influence on the texture of a low fat (14%) bologna product. The bologna with 15% MSP was dark red, soft and mushy in texture with very low hardness and chewiness, which is not acceptable. The initial chilling rate and the length of frozen storage didn't play a significant role in the physicochemical properties of MSP and bologna formulated with 7.5% or 15% MSP.

Overall, the results indicated that the mechanical separation process generated highly disrupted pork muscle cell ultrastructure, degraded MHC, and compromised gelation properties of MSP. Following up to 13 months of frozen storage, the colour of MSP was slightly deteriorated and lipid oxidation increased. The approach to chill MSP more quickly didn't help preserve the gelation ability significantly and typical delays in chilling also had no effect. The replacement of MSP at 7.5% level didn't negatively influence bologna texture significantly, but 15% replacement did.

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LIST OF ABBREVIATIONS

a*	Redness
AOAC	Association of Official Agricultural Chemists
b*	Yellowness
BF	Pork Back Fat
BM	Meat Trim from Bone
BM-7.5	Bologna Formulated with 7.5% of Meat Trim from Bone
BM-15	Bologna Formulated with 15% of Meat Trim from Bone
BR	Bone Residue
CFIA	Canadian Food Inspection Agency
CIE	International Commission on Illumination
CON	Control
δ	Phase Angle
DL-7.5	Bologna Formulated with 7.5% of Delay Chilled Mechanically Separated Pork
DL-15	Bologna Formulated with 15% of Delay Chilled Mechanically Separated Pork
ECL	Enhanced Chemiluminescence
G’	Storage Modulus
G”	Loss Modulus
HIS	High Ionic Strength
HSS	High Salt Solution
L*	Lightness
LIS	Low Ionic Strength
LSS	Low Salt Solution
MDA	Malondialdehyde
MHC	Myosin Heavy Chain
MLC	Myosin Light Chain
MSP	Mechanically Separated Pork
MSP-DL	Delay Chilled Mechanically Separated Pork
MSP-PC	Prechilled Mechanically Separated Pork

MSP-STD	Standard Mechanically Separated Pork
MSM	Mechanically Separated Meat
NAM	Natural Actomyosin
NHI	Non-Heme Iron
PBS	Phosphate Buffered Saline
PBST	Phosphate Buffered Saline with Tween 20
PC-7.5	Bologna Formulated with 7.5% of Prechilled Mechanically Separated Pork
PC-15	Bologna Formulated with 15% of Prechilled Mechanically Separated Pork
PP	Pork Picnic
PSE	Pale, Soft, Exudative
SAS	Statistical Analysis System
SDS-PAGE	Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis
SSS	Standard Salt Solution
STD-7.5	Bologna Formulated with 7.5% of Standard Mechanically Separated Pork
STD-15	Bologna Formulated with 15% of Standard Mechanically Separated Pork
STPP	Sodium Tripolyphosphate
TBARS	Thiobarbituric Acid Reactive Substances
TCA	Trichloroacetic Acid
TEM	Transmission Electron Microscopy
TMP	1,1,3,3-Tetramethoxypropane
TPA	Texture Profile Analysis
WHC	Water Holding Capacity

1 INTRODUCTION

1.1 Overview

Bones are a coproduct of meat slaughtering and processing. Mechanically separated meat (MSM) is meat mechanically removed from bones by applying pressure to separate soft muscle tissue and attached bones (CFIA, 2019a). Pork carcasses could provide about 170,000,000 kg of recovered tissue annually (Ockerman et al., 1981). The picnic, ham and blade bones which represent 60-65% bones are available for mechanically deboning and will produce 0.91-1.27 kg of deboned meat per animal (Ockerman et al., 1981). As an underused low value by-product, investigations have been conducted to study the characteristics and possibility of transforming it into more market valuable products.

Muscle protein is the major functional and structural component in meat (Bandman, 1987). MSP has around 15% protein, while regular PP has about 18%. Myofibrillar proteins are a group of salt soluble protein which could be extracted in intermediate or high ionic strength buffer. They account for up to 60% of total protein and are known to be responsible for texture of processed meat products (Xiong, 2004). Gelation is the association or crosslinking of polymer chains into three-dimensional network (Glicksman, 1982). Gelation of myofibrillar protein is very important for the thermally induced gelation process, since it's responsible for immobilization of water and fat, binding meat pieces together (Miyaguchi et al., 2000).

MSM has typically pasty texture with high quantity of pulverized muscle fibre residue and de-structured muscle fibres (Hui, 2012a). The jelly-like texture and high fat content of MSP limit its use to the manufacture of emulsion products (Guerra-Daros et al., 2005). The mechanical separation process that the bones go through breaks meat cells, de-structures muscle fibres, increases lipids and heme content, and denatures protein which leaves this recovered meat with low nutritional and textural properties (Hui, 2012a). It's reported that the hardness of fermented sausage product decreased as level of MSP substitution of hand deboned regular pork increased (Kramer & Sebranek, 1990). Compared with mechanically separated chicken, mechanically separated red meats have much high level of hemo-proteins (hemoglobin and myoglobin) and lower polyunsaturated fatty acid content, which is said to be responsible for difference in storage

characteristics (Ockerman et al., 1981). MSM also has higher bone content than manually deboned meat as a methodological problem of the mechanical deboning process. These adverse characteristics of MSM limit usability as a valuable ingredient in processed meat products.

Making bologna by replacing pork in part with MSP is a direct approach to explore the potential utilization of MSP in meat processing. Previous studies have shown that substitution was limited to 30% or less due to the texture softening issue (Kramer & Sebranek, 1990). To increase the value of MSP in meat processing and explain the textural changes in bologna, it's essential to understand why MSP behaves differently from regular pork in comminuted products. The investigation was established to explore the difference between MSP and PP, with focus on the compositional difference and protein gelation properties which are believed to determine the texture of comminuted meat products, and the effect of frozen storage on the quality of MSP proteins and lipids, which is an important part of the shelf life study. Accelerated chilling is believed to reduce the deteriorating effect of freezing on meat quality (Murano, 2003). Whether the accelerated chilling could help improve the gelling behaviour of MSP in industrial application is of interest. The current research is on the evaluation of chemical composition, physicochemical, biochemical and gelation properties of MSP and the effect of chilling rate and frozen storage on physicochemical and gelation properties of MSP. The potential utilization of MSP as a substitute of regular pork in bologna processing was evaluated based on bologna processing and instrumental textural analysis with samples at different chilling rates following frozen storage.

1.2 Study hypotheses and objectives

Study 1:

- Evaluation of chemical composition, physicochemical, biochemical and gelation properties of MSP.
- Evaluation of the effect of chilling rate and frozen storage on physicochemical and gelation properties of MSP.

Hypotheses:

- MSP has different chemical composition to PP, such as the concentration difference of collagen and myofibrillar protein.
- MSP has different muscle fibre structure, and biochemical properties of actomyosin.
- MSP has weaker gelation properties than regular pork.

- Accelerated chilling could better preserve functionality of myofibrillar protein in MSP.

Objectives:

- To investigate the chemical composition and physicochemical difference between MSP and regular ground pork.
- To study the ultrastructure of MSP muscle fibre and biochemical properties of myofibrillar protein.
- To investigate the gelation properties of MSP.
- To evaluate the effect of chilling rate and frozen storage on the quality of MSP.

Study 2:

- Potential utilization of MSP as a substitute of regular pork in bologna processing.

Hypotheses:

- Increased substitution level of MSP will lead to textural failure of bologna product.
- Accelerated chilling rate of MSP helps improve the textural properties of bologna.
- Frozen storage of raw meats will decrease the texture desirability of bologna.

Objectives:

- To evaluate the effects of MSP substitution level, chilling rate, and frozen storage on the texture of bologna.

Study 3:

- Evaluation of the effects of accelerated chilling and prolonged frozen storage on physicochemical and gelation properties of MSP.

Hypotheses:

- Accelerated chilling through heat exchanger can better preserve gelation properties of MSP.
- Prolonged frozen storage will lead to worse textural properties of bologna.

Objectives:

- To determine the effect of accelerated chilling and prolonged frozen storage on the quality of MSP.

2 LITERATURE REVIEW

2.1 Muscle protein

Muscle protein is the major functional and structural component in meat (Bandman, 1987). The properties of the muscle protein directly determine the quality of raw meat and resulting processed meat products (Smyth et al., 1999). Influencing factors for muscle protein quality include but are not limited to animal species, muscle type, as well as post-mortem changes from muscle to meat.

Meat muscle protein can be divided into three categories according to their structure and solubility, namely sarcoplasmic proteins, myofibrillar proteins, and stromal proteins (Xiong, 2004). These three categories of proteins have very different gelation properties based on their structural difference.

2.1.1 Sarcoplasmic proteins

Sarcoplasmic proteins are the metabolic proteins that are soluble in water or low ionic strength solution (Hemung & Chin, 2013). They represent about 35% of total muscle proteins, and include metabolic enzymes and myoglobin (Xiong, 2004; Hemung & Chin, 2013). They are relatively small in size, mostly globular or rod-shaped, with high density of exposed charged polar side chains (Asghar et al., 1985). Myoglobin at 17,000 Da, is considered the most important sarcoplasmic protein in the sarcoplasm as it is responsible for meat colour (Xiong, 2004). The effect of sarcoplasmic protein on myofibrillar gelation ability was not conclusive (Hemung & Chin, 2013). Sarcoplasmic proteins could help with binding with myosin at low ionic strength, however, their assistance is negligible or even deteriorating to the gel formation of myosin at high ionic strength (Schmidt, 1987). They have low water holding capacity (WHC) and weak gelation properties thus have little contribution to binding strength of cooked sausage products observed (Miyaguchi et al., 2000). Fish sarcoplasmic protein was reported to inhibit the gelling process of myofibrillar protein (Hashimoto, et al., 1985). But fish protein gel strength was found increased by addition of sarcoplasmic protein (Morioka & Shimizu, 1990; Piyadhammaviboon & Yongsawatdigiul, 2009).

2.1.2 Myofibrillar proteins

Myofibrillar proteins are salt soluble proteins which could be extracted in intermediate or high ionic strength buffer. They account for up to 60% of total protein and are known to be responsible for texture of processed meat products (Xiong, 2004). Gelation of myofibrillar protein is very important for gelation induced by thermal processing, since it's responsible for immobilization of water and fat, binding meat pieces together (Miyaguchi et al., 2000). Myosin and actin are two major proteins of myofibrils, and account for more than 70% of total myofibrillar protein (Xiong, 2004).

2.1.2.1 Myosin

Myosin is the major component of thick myofilaments with about 4,500 amino acids subunits (Xiong, 2004). It is composed of a total of six subunits, two large MHC and four small myosin light chains (MLC). MHC is the key element for myosin gel strength development while MLC plays a negligible role (Sano et al., 1990). The two MHC form the hydrophilic α-helical rod portion (tail) and part of the hydrophobic globular portion (head) while two MLC are located on head portion of myosin molecule (Xiong, 2004). This structure makes myosin molecules (thick filament), whose length and diameter vary between species, able to assemble themselves to form muscle filaments with actin (thin filament), which is responsible for generating contractile force (Harrington & Rodgers, 1984). In muscle sarcomeres, myosin tails form the backbone of the thick filament while heads are arranged on the surface with actin and ATPase binding sites (Harrington, & Rodgers, 1984). Myofibrillar protein is the most important protein responsible for gel formation (Xiong, 2004; Doerscher et al., 2003), while the MHC is the key structural component. According to Asghar et al. (1985), the myosin rod is responsible for gelling potential, rather than globular heads, and increased cross-link formation by addition of actomyosin could affect gelling ability of myosin (Asghar et al., 1985).

The isoelectric point of myosin is around 5.3, lower than pH of meat during processing around 6, which makes myosin negatively charged, and be able to bind with water (Mark & Stewart, 1948). Salt is usually added to extract myosin in meat processing in order to enhance gel strength and WHC by causing molecular swelling and water uptake, and shifts of isoelectric points (Offer & Trinick, 1983). The addition of pyrophosphate reduces substantially the sodium concentration requirement for maximum swelling (Offer & Trinick, 1983).

2.1.2.2 Actin

Actin is a major structural protein of thin myofilaments, and accounts for 22% of myofibrillar protein and usually is bound to myosin postmortem (Yates & Greaser, 1983). Actin is a globular protein with binding sites for tropomyosin and troponin. The molecular weight of globular actin (G-actin) is approximately 42,000 Da, and each thin filament contains about 400 actin molecules (Pearson & Young, 1989). Muscle movement is achieved by binding of myosin head to actin filaments with hydrolysis of ATP (Pearson & Young, 1989). Actin is also important for reinforcing myosin gel structure (Wang et al., 2009). Actin could influence the gelation property of actomyosin by altering denaturation of myosin structural domains, and the ratio of myosin and actin will influence the gel strength and gelation property of meat product, and free myosin to actomyosin ratio is more determinate than the myosin to actin ratio (Wang & Smith, 1995; Yasui & Samejima, 1990).

2.1.2.3 Actomyosin

In post rigor meat, actomyosin which is formed by cross-linked myosin and actin is the dominant protein complex form in meat (Xiong, 2004). Without a supply of ATP, actomyosin in post-rigor muscle become relatively permanent (Huff-Lonergan & Lonergan, 2005). Studying actomyosin rather than myosin could better reflect the real gelation ability of pork muscle proteins (Wang et al., 2009). The NAM consists of myosin, actin, tropomyosin, troponin and α-actinin (Asghar et al., 1985). According to Ishioroshi et al. (1980), addition of PPi into actomyosin before heat treatment led to dissociation of actomyosin which results in decreased gel strength (Ishioroshi et al., 1980). In the myosin-actin system, the maximum gel strength could be obtained with 80% of free myosin and 20% actomyosin (Yasui & Samejima, 1990).

2.1.3 Stromal proteins

Stromal protein is also known as connective tissue protein and includes collagen, elastin and lipoproteins, etc. (Xiong, 2004). Collagen is the predominant stromal protein and responsible for the toughness of meat (Xiong, 2004). Collagen is formed by three helical polypeptide chains. It has little gelation ability as a collagen fraction will not coagulate until 80 °C which is higher than processing temperature of processed meat at 72 °C. It was reported that 10% replacement of myofibrillar protein with collagen could help improve the WHC without interrupting protein gel

strength formed by myofibrillar protein (Doerscher et al., 2003). But 20% or more collagen replacement led to the slower gel formation rate (Doerscher et al., 2003). However, products made without stromal protein could also be a problem, as the texture would become soft, rubbery and low in cohesiveness (Schmidt, 1987).

2.2 Thermally induced meat protein gelation

Gelation is the association or crosslinking of polymer chains into a three-dimensional network (Glicksman, 1982). Protein gelation is important in formation of meat product structure, water-holding and appearance (Hermansson, 1985). The emulsion capacity of soluble protein determines the water and fat binding ability (Nuckles et al., 1990). There are many influencing factors of protein gelation. Protein gelation can be triggered by different pathways, while thermally induced gelation is one of the most commonly used processing procedures in the meat processing industry. And protein concentration, degree of denaturation, pH, temperature, etc. would contribute to determination of gelation characteristics (Totosaus et al., 2002).

The process of protein gelling could be divided into approximately four steps as described by Ferry (1948). First, heat or other factors provoke the protein matrix to unfold and dissociate. Second, dissociated proteins associate and aggregate to form a three-dimension network and interact and immobilize water, fat and other proteins. Myosin gelation follows four steps to be specific: (1) local conformation change at enzyme active site in head region of myosin; (2) myosin head aggregation; (3) unfolding of rod secondary structure above 40 °C; (4) complete intermolecular cross-linkages through exposed hydrophobic residues (Yasui & Samejima, 1990). The disassociation of myosin rods starts with the α-helical region unfolding (Visessanguan & An, 2000). The denatured and unfolded protein molecules interact with other protein molecules around and form a new ordered 3-D network which immobilize moisture and other ingredients in the protein matrix (Visessanguan & An, 2000).

Myofibrillar protein is the most important protein fraction responsible for gel formation (Xiong, 2004; Doerscher et al., 2003). The property and strength of gel directly determine the texture, juiciness and stabilization of fat.

2.2.1 Protein denaturation and aggregation during gelation

Protein denaturation is the process of protein molecule polypeptide chains spatially rearranging to form a more disordered arrangement (Asghar et al., 1985). This process involves protein molecule secondary, tertiary, or quaternary structure change which assists in gel formation, water holding, protein solubility and emulsification afterwards (Ziegler & Foegeding, 1990). During protein denaturation, more hydrophobic sites are exposed on the surface and results in protein aggregation. The hydrogen bonds, disulphide linkages, peptide bonds, electrostatic and hydrophobic interactions between denatured protein molecules stabilize the gel (Wang et al., 2009).

Protein-protein interaction is mainly responsible for physico-chemical changes during processing and storage of meat products (Xiong et al., 2009). Muscle proteins associate and aggregate by noncovalent forces or covalent bonds to form a viscoelastic gel matrix that immobilize water and stabilizes fat particles in the comminuted protein matrix (Xiong et al., 2009).

Both head and tail portions of myosin are involved in meat gel formation (Chan et al., 1993). The gelling capacity is mostly determined by the condition of myosin and species (Visessanguan & An, 2000). The transition temperature differs depending on muscle type, batter composition, ionic strength, pH and etc.

2.2.2 Factors influencing protein gelation property

Protein gelation is highly dependent on the protein molecular interactions and interactions with surrounding environment. The textural, sensory, and nutritional quality of a meat product is highly determined by the protein composition and functionality during processing (Sun et al., 2011; Visessanguan et al., 2004). The following are major influencing factors of protein gelation.

2.2.2.1 Protein type

The main proteins responsible for gelation are myofibrillar proteins (Xiong, 2004). The characteristics of myofibrillar protein determine the gelation property. Myosin and actomyosin are the most essential components for developing binding ability, and the interaction between actin and myosin is of great importance in terms of formation of viscoelastic gel (Siegel & Schmidt, 1979). Water soluble proteins and some salt soluble proteins, including actin and tropomyosin plays a role in gel stability preservation without any direct effect on gel formation (Samejima et al., 1969). Macfarlane et al. (1977) found that in salt concentration up to 1 M, myosin had superior binding

strength than that of actomyosin. Also, in different species and or muscle types, gel strength and WHC could be different. For example, white and red muscle fibre have different isoforms of myofibrillar proteins. Chicken breast muscle myosin formed stronger gel compared with leg muscle, and red muscle myosin forms gel networks with finer filamentous structures than white muscle (Asghar et al., 1984). Fretheim and others (1986) agreed that white bovine myosin has superior gelling ability than red myosin.

2.2.2.2 Protein concentration and solubility

The appropriate concentration of protein is essential for gel formation and gel strength as well as WHC. In the heat induced gelation process for meat proteins, the salt soluble protein gel strength is in a log-log pattern to the increase of protein concentration in the system from 10 to 50 mg/mL (pH 5.5-5.7) (Camou et al., 1989). The minimum protein concentration should be met to form gel network as stated by Ferry (1948). The gel formed with 10 mg/mL of salt soluble protein was very soft according to Camou et al. (1989). Acton et al. (1981) reported that at least 6 mg/mL NAM was needed to form a gel. Barreto et al. (1996) found that higher protein levels in pork meat batter formed more rigid gels. Li-Chan et al. (1985) reported that protein that experienced mild denaturation without aggregation would not have obvious change in sulfhydryl but increased surface hydrophobicity. The reduced muscle protein solubility after protein denaturation could be a result of the formation of hydrophobic bonds rather than disulfide bonds (Farouk et al., 2003).

2.2.2.3 Protein oxidation

Proteins are readily oxidized during food preparation by reactive oxygen species, which could lead to changes such as modification of amino acid side chains, structural changes and protein fragmentation or polymerization (Li et al., 2012; Chanarat et al., 2015). A number of studies have proved that protein aggregation modified by radicals and secondary products of lipid oxidation from meat, could promote cross-linking of myosin (Xiong et al., 2009). Myosin oxidation in meat forms cross-linked heterogeneous myosin with varying functional properties (Xiong & Decker, 1995; Chanarat et al., 2015), including decreased gelation properties, decreased protease activity and proteolysis even at very low levels of oxidation (Huff-Lonergan & Lonergan, 2005). It was reported that oxidation of meat resulted in decreased gel strength (Decker et al. 1993; Srinivasan & Hultin, 1997). In the presence of reactive oxygen species, transition metals and reducing sugars,

protein is subject to oxidative damage (Estévez, 2011). Oxidation could induce protein structural changes, and reduce conformational stability reflected by the increased carbonyl and disulfide contents (Li et al., 2012; Visessanguan et al., 2004). Myofibrillar proteins have enriched sulfhydryls that is susceptible to oxidative degradation in a dose dependent manner by hydroxyl radicals that is generated in the presence of H_2O_2 (Chen et al., 2016). The oxidation products include disulfide bonds and other derivatives, such as sulfone and sulfoxide (Chen et al., 2016). Non-heme iron (NHI) with high catalytic activity is one of the most important oxidation promoters in meat (Estévez & Cava, 2004). Thermal processes have been shown to increase NHI in meat (Lombardi-Boccia et al., 2002). The breakdown of the heme molecule, myoglobin, during cooking or storage decreases heme iron and releases NHI (Gómez-Basauri & Regenstein, 1992a, 1992b).

2.2.2.4 Protein degradation

Protein degradation involves the process of disruption of peptide bonds that may further influence protein functionality. Proteolytic degradation of myofibrillar proteins, especially myosin, takes place during post-mortem storage and processing of meat with results of undesirable flavor and texture alterations (Visessanguan & An, 2000). The sequential unfolding of protein structural domains and the ordered protein-protein interaction are essential for the formation of gels with high viscoelasticity (Sharp & Offer, 1992). The conformational changes of proteins, especially secondary structure, and intermolecular bonds are correlated with protein gelation properties (Liu et al., 2008; Liu et al., 2011). According to a study conducted on Arrowtooth flounder fillet, the decreased level of myofibrillar protein and increased moisture retention are accompanied by proteolytic degradation (Visessanguan & An, 2000). According to Visessanguan & An (2000), the initial degradation of myosin most often occurs in the tail region. The structural disruption decreased myosin gelling ability and gel rigidity. Pale, soft, exudative (PSE) pork showed increased myosin degradation compared with regular pork during frozen storage with the appearance of an extra protein band at the size of 95 to 100 kDa (Wang et al., 2005). In addition, the NAM from PSE pork showed inferior rheological properties which is usually linked to decreased gelation ability (Wang et al., 2009).

2.2.2.5 Endogenous enzymes

Gel weakening during processing of surimi products could be induced by muscle protein deterioration while endogenous proteinases remain active postmortem (Jiang, 1999). The myofibrillar protein content was reported to be closely related to muscle gel texture (Park et al., 1996), and especially myosin was reported to have higher binding strength than that of actomyosin (Siegel & Schmidt, 1979). According to Park et al. (1996), the intact MHC was key to maintain best function of heat-induced myosin gels.

Myosin reduces molecular weight and loses structural domains, which are essential for binding, after proteolytic degradation (Visessanguan & An, 2000). It is essential to understand the properties of enzymes that are involved in the proteolysis of myofibrillar protein. The two major proteases related to myofibrillar protein degradation are cathepsin and calpain.

Cathepsins are a group of proteolytic enzymes in lysosomes that maintain intracellular homeostasis (Turk et al., 2012). It's widely reported that heat-stable proteinases, cathepsins B, H, L and L-like are responsible for gel softening or inducing modori in some fish muscle (Jiang, 1999; An et al., 1996). Cathepsins B, D, and L are usually involved in muscle structure breakdown with textural changes as a result (Godiksen et al., 2009). The optimal pH for cathepsins falls in the acidic range, and following release from lysosomes, they are involved in myofibril degradation (Hui, 2012b). Cathepsin D was reported to be relatively stable below 70 °C in duck meat, and it had significant effect on the dissociation of actomyosin (Wang et al., 2013). In the live animal, cathepsins are restricted inside lysosomes and inactive. But after death, the lysosomes can become disrupted and the cathepsins can be released from the lysosomes and come into contact with their substrates (Chéret et al., 2007).

Calpains are another major enzyme group responsible for degradation of myofibrils in muscle at pH around 7.0 and above 0.1 mM Ca^{2+} *in vitro* (Du & McCormick, 2009). These cysteine proteases have two forms, calpain I (μ-calpain) and calpain II (m-calpain), and requires reduced environment, sensitive to muscle temperature, 50 to 100 μM Ca^{2+} and 1 to 2 mM Ca^{2+} for maximal activity respectively (Mohrhauser et al., 2014; Lametsch et al., 2008; Etherington, 1984). The requirement of Ca^{2+} level was much higher than Ca^{2+} level found in living muscle cells (Etherington, 1984). μ-calpain and m-calpain are heterodimers composed of a 80 kDa subunit which goes through autolysis from the N-terminus into a 78 kDa intermediate then finally 76 kDa, and a 28 kDa subunit for regulation (Lametsch et al., 2008). The active site includes a cysteine unit

that is sensitive to oxidants with reduced activity as a result (Lametsch et al., 2008). Elevated temperature could increase the μ-calpain activation in lamb and beef (Du et al., 2017). The autolysis of μ-calpain is usually used as an indicator of calpain activation in postmortem muscles (Huff-Lonergan et al., 2010). Calpastatin was reported to be the endogenous inhibitor of calpain in meat which could reduce calpain II activity by binding Ca^{2+} ions to calpains I and II (Du & McCormick, 2009). The rate and extent of myofibrillar protein proteolysis and μ-calpain autolysis could be limited by increased amount of calpastatin during incubation (Geesink & Koohmaraie, 1999).

Besides those above, myofibril-bound serine proteinase from carp was reported to readily degrade MHC after incubation at 55 °C for 1 hour which is known as "modori" effect (Cao et al., 1999).

2.2.2.6 Ionic strength

Addition of salt will enhance salt-soluble protein, myofibrillar protein by enhancing interaction between protein and water (Huxley, 1963). The protein solubility, extractability, and water binding capacity will increase as the addition of salt (Huxley, 1963). The myosin is in thick filament style at low ionic strength environment (< 0.3 M) and neutral pH. It will disperse in higher ionic strength environment as monomers (Sano et al., 1990). Generally, 2-3% salt in processed meat is usually used in commercial products. Pork gel stability and WHC were increased by 1.5, or 3% salt, however, further increases in salt content to 4.5 or 6% didn't increase the gel hardness (Park et al., 1996). Addition of alkaline phosphates is also commonly used to enhance protein gel formation (Barbut, 1988). They can not only increase protein solubility, but increase pH of meat batter about 0.2 to favor dissociation of the actomyosin complex (Trout & Schmidt, 1986). Phosphates usually used in meat processing are sodium acid pyrophosphate, tetra sodium phosphate, and sodium triphosphate (Whiting, 1988).

Further, the ionic strength rises while muscle is converted to meat (Huff-Lonergan & Lonergan, 2005). Along with pH decrease post-mortem, these may cause conformational change of enzymes and protein substrates (Huff-Lonergan & Lonergan, 1999).

2.2.2.7 pH value

The pH value of meat could be very important for meat gel formation. Low pH meat, PSE for example, will have higher protein denaturation prior to processing, which results in weak gel

strength and poor WHC (Torley et al., 2000). PSE pork is usually linked with poor functional properties of processed meat products (Torley et al., 2000). The rapid post-mortem glycolysis with high carcass temperature (≥38 °C) and low pH are characteristics of PSE pork. The pH value of normal pork is usually around 5.6-6, while in PSE pork, the pH could be lower than 5.5, and could cause protein denaturation while meat is still warm. The pH of muscle tissue influences the myofibrillar protein denaturation and the resulting gel formation (Torley et al., 2000; Westphalen et al., 2005). Myosin in PSE meat tends to have smaller head size (Offer et al., 1989), decreased enthalpy of denaturation (Honikel & Kim, 1986), and lower solubility (Sung et al., 1976).

According to Lan et al. (1995), muscles from pork, beef, fish, chicken, and turkey all showed higher gel strength (force required to rupture the gel and force required to move plunger through the gel) at pH 6.0 than pH 5.5, 6.5, or 7.0. The isoelectric point of myosin is 5.3, where it has equal number of positive and negative charged amino acid side chains (Honikel, 2004). The attraction force maximizes while approaching myosin isoelectric point, and decreases the space between myofilaments where water is immobilized, resulting in reduced WHC and swollen gel.

2.2.2.8 Processing prior to cooking

In meat processing, bowl chopping and emulsification are widely used to manufacture comminuted products. The process is conducted with the presence of salt to reduce particle size, extract myofibrillar protein, induce swelling and water binding, and achieve desirable fat particle size and distribution (Acton et al., 1982). Heat and pressure could be generated with chopping, mixing, grinding, homogenizing, etc. The warming of meat batter is beneficial to release soluble protein, and accelerate cured colour development (Aberle et al., 2012). A temperature rise during chopping up to around 14 °C for pork is a common practice in the industry to reach the ideal fat particle size in emulsion. However, it's widely acknowledged that chopping pork at temperature above 18 °C will result in dramatic cook loss or complete emulsion breakdown (Townsend et al., 1971; Brown & Ledward, 1987). Controlling the processing temperature is essential to the texture formation and cook yield of products (Aberle et al., 2012).

As for the effect of high pressure treatment, it was suggested that proteins subject to structural modification by disruption of hydrophobic and electrostatic interactions through high pressure processing (Molina et al., 2001). Fernández-Martín (2007) reported that actin was the

protein most sensitive to pressure (400 MPa) followed by myosin and sarcoplasmic protein based on studies on poultry meat.

Heat during the process usually leads to protein denaturation and low protein solubility. The temperature and time of these processing procedures should be controlled to optimize gel property, and the optimal processing condition varies among different products. However, according to Tamilmani and Pandey (2016), high-pressure processed porcine blood plasma was degraded to a higher extent than spray dried, indicating that proteins are more susceptible to pressure than to elevated temperature. Villamonte et al. (2016) stated that high pressure (> 200 MPa) treated sarcoplasmic protein has increased surface hydrophobicity and reactive sulfhydryl group. Using ultra-high pressure homogenization decreased soy protein solubility (Liu & Kuo, 2016). For mechanically separated chicken, Saricaoglu et al. (2017) reported that high pressure (100 MPa) homogenization treated samples had decreased particle size and stronger electrostatic repulsion, which improved water solubility, emulsifying and foaming properties of protein suspensions, but created a soft gel network behaviour. Macfarlane and Mckenzie (1976) and Tokifuji et al. (2013) reported that high-pressure treated myofibrils have increased solubility due to the fragmentation effect. In general, pre-treatment of raw meat could alter the physical and chemical properties of muscle proteins, and subsequently influence the protein functionality and cooking behaviour.

2.2.2.9 Heating rate and temperature of cooking

The heating rate and cooking temperature are of significance to the gel product texture. Heat induced gelation is developed in two steps. The first one is protein unfolding and dissociation, while the second one is the reassociation and aggregation of protein molecules, and 3D spatial network formation (Ferry, 1948). The second step is very temperature sensitive, so the temperature increase should remain slow to ensure protein arrangement and rebinding. Low heating rate under low temperature helps gel aggregate, while high temperature or fast heating rate could weaken crosslinking of myosin gel (Wang & Smith, 1995; Camou et al., 1989). The rational is that the low temperature setting period could help order the gel matrix which is believed to have higher gel strength by localized exposure and interaction of hydrophobic amino residues (Acton & Dick, 1984; Lanier et al., 1982; Samejima et al., 1981).

In fish surimi type product, the protein gel has gel-strengthening at low cooking temperature (suwari), and gel-softening at intermediate temperature (modori) effect (Wang & Xiong, 1998). The phenomenon is believed to be related to endogenous enzymes, thus incorporating protease inhibitors or controlling cooking temperature could help improve myofibrillar protein gelation in surimi (Lee, 1986).

2.2.3 Meat emulsion

A meat emulsion is a system in which animal fat is finely distributed in the meat protein matrix by comminuting the meat with ice and salt (Lee, 1985). The formation of a meat emulsion includes two components: 1) swelling of proteins to form a viscous matrix for heat induced gel formation; 2) emulsification of fat droplets and air by the protein matrix (Aberle et al., 2012). The property of meat emulsion is determined by the meat species, fat type, and level of other ingredients including salt, water, and processing parameters (Lee, 1985). A major concern with meat emulsions is fat and water destabilization within the emulsion system, which might result in undesirable texture and sensory properties (Lee, 1985). The myofibrillar proteins have the superior capacity to solubilize and absorb water at the appropriate ionic strength and act as the emulsifying agent (Aberle et al., 2012). The dispersed spherical fat particles are coated with soluble protein in matrix. Fat particle size decreases during chopping until the ideal temperature is reached with the increase of surface area. The over-chopped meat batter loses its stability for the lack of soluble protein to completely coat the surfaces (Aberle et al., 2012). Addition of emulsifiers into meat emulsion is one of the choices to help increase emulsion stability and water binding. Sodium caseinate is one of the widely used non-meat protein emulsifiers in meat products (Cáceres et al., 2008). Unlike meat proteins, the emulsifying capacity of non-meat emulsifiers is not sensitive to heat (Hoogenkamp, 1987). Pre-emulsified fat or oil is commonly prepared in the meat industry the day before processing to increase the availability of salt soluble proteins for better gelation and water holding (Hoogenkamp, 1985; Hoogenkamp, 1987). Air could compete with fat particle for protein coating during blending, chopping, and emulsification. Vacuum processing steps such as vacuum tumbling could help control the air incorporation for desirable emulsion stability and texture (Aberle et al., 2012).

2.2.4 Water holding capacity (WHC)

Water is a bipolar molecule that is attracted to charged proteins in meat in forms of bound water, entrapped water, and free water (Huff-Lonergan & Lonergan, 2005). WHC and texture are two major considerations for formulating meat products (Thomsen & Zeuthen, 1988), among which WHC has been an index for palatability, microbial quality and manufacturing potential of muscle products influencing the formulation, processing, cooking, and freezing processes since it's related to weight loss and ultimate quality of product (Abdullah & Al-Najdawi, 2005; Field, 1988). The myofibrillar proteins and their structure determined the WHC of meat products through binding and entrapping water (Huff-Lonergan & Lonergan, 2005). The influencing factors for WHC include pH, presence of iron, copper, calcium and magnesium from bone, skin and collagen content, and the processes of cooking and freezing (Abdullah & Al-Najdawi, 2005).

2.3 Methods for study of protein denaturation and degradation

2.3.1 Protein solubility

Protein solubility is the amount of protein that dissolves into a solvent from a sample (Zayas, 1997). As myofibrillar proteins play an essential role in binding and water holding, and sarcoplasmic proteins are responsible for meat colour, the solubility of those functional proteins could be an indicator of protein denaturation which is related to the meat quality and the gelation capacity (Sayre & Briskey, 1963). PSE pork was reported to have lower sarcoplasmic and myofibrillar and total protein solubility compared with reddish-pink, soft, exudative, reddish-pink, firm, non-exudative, and dark, firm, and dry pork (Joo et al., 1999). The pork colour is highly influenced by the precipitation of sarcoplasmic proteins, and the WHC is decreased as a result of the denaturation of myofibrillar protein and lower ultimate pH in PSE pork (Joo et al., 1999).

The protein solubility is assessed by extracting prepared muscle samples with buffer at optimum temperature, pH and ionic strength for a period of time that is sufficient to complete extraction. For myofibrillar proteins, it is generally accepted that extraction conducted at -5 °C to 2 °C, a sodium chloride concentration of 10%, and long extraction time up to 15 h gives the maximum protein solubility (Schmidt, 1987). However, Gillett et al. (1977) found that the optimum extraction temperature was 7.2 °C for different extraction conditions they adopted. Increasing the pH or adding polyphosphate along with salt could increase the extractability of salt soluble protein (Schmidt, 1987).

2.3.2 Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE is one of the most commonly used techniques to separate and characterize proteins. The discontinuous electrophoretic system was developed by Ulrich K. Laemmli to separate proteins with molecular mass of 5 to 250 kDa (Laemmli, 1970). The sodium dodecyl sulphate is a negatively charged amphipathic detergent that denatures proteins. One sodium dodecyl sulfate with anionic head and lipophilic tail non-covalently binds to two amino acids in protein, and causes unfolding of both polar and nonpolar protein sections and dissociation of non-covalent bonds. The proteins become negatively charged, and the intrinsic charge of protein is masked. The negatively charged proteins migrate towards anode during electrophoresis, and the rate of migration is determined by molecular mass which makes it possible to monitor protein characteristics and degradation of meat protein (Ninfa & Ballou, 1998).

2.3.3 Immunoblotting

Immunoblotting also known as western blotting or protein blotting is a very useful and powerful method for immunodetection of post-electrophoresis protein (Kurien & Scofield, 2006). The method evolved from DNA blotting (Southern) and RNA blotting (Northern) (Alwine et al., 1977). The protein from an SDS-PAGE gel is transferred to an absorbent membrane, usually nitrocellulose or polyvinylidene fluoride or polyvinylidene difluoride membranes (Towbin et al., 1979). Antibody probes are directed against the membrane bound proteins to make them detectable and help with protein characterization (Kurien & Scofield, 2006). Simple diffusion, vacuum blotting, and electroblotting are three common methods for protein transfer from gel to membrane. Semi-dry transfer is one of electroblotting methods that can blot gels simultaneously, with a low power and cheap equipment requirement (Kurien & Scofield, 2006). The gel-membrane sandwich is placed between carbon plate electrodes covered with two layers of thick filter paper which are soaked in transfer buffer at both sides. After transfer, radioactive and enzyme-linked reagents could be incubated with the membrane for immunodetection. During incubation, enzyme-labeled antibodies, normally horseradish peroxidase antibodies, attach to the primary antibody that bound with membrane protein and react with soluble substrates to yield detectable insoluble pigments (Knecht & Dimond, 1984). However, this method is unable to provide quantitative results, and difficult to obtain high quality protein photographs due to poor contrast between protein stains and membrane background (Kurien & Scofield, 2006).

2.4 Methods for gelation properties and texture measurement

2.4.1 Texture profile analysis (TPA)

TPA is the use of computer programmable equipment that bridge the instrumental evaluation and sensory characteristics without incorporating a series of descriptive terms or bias (Friedman et al., 1962). The TPA equipment mimics the masticating action of the human mouth with a flat-bottomed cylinder to compress a piece of food to 50% of its original height two times. A force-time curve is plotted to illustrate the entire force history of simulated masticatory activity, which reflects the fracturability, hardness, cohesiveness, adhesiveness, springiness, gumminess, and chewiness of the food sample. The mathematical formulation was developed through experimentation considering the mechanical parameters of the sample and measurement conditions (Friedman et al., 1962). Friedman et al. (1962) gave out the interpretation for TPA typical texturometer curve. The hardness is measured by the height of first chew. Cohesiveness is expressed as the ratio of the area under the second chew and the first chew. The adhesiveness is measured as the area of negative peak beneath the base line. Elasticity is expressed as the time constant for a completely inelastic standard, e.g. clay, of the instrument minus the time between initial sample contact to the contact on the second chew. Springiness is measured as the ratio of the time between the compression force above zero to maximum compression in the second chew to the time in the first chew (Bourne et al., 1978). Chewiness is a mathematical measurement based on the hardness, cohesiveness and elasticity parameters (hardness × cohesiveness × springiness) (Bourne et al., 1978). However, the instrumental TPA was unable to give a complete physical description of texture, and it can only provide partial mechanical properties (Friedman et al., 1962).

2.4.2 Dynamic oscillatory rheology

Dynamic oscillatory rheology is an approach to dynamically measure viscoelastic behaviour of gels as a function of time and temperature at low strains without sample destruction (Hamann, 1987). Low frequency oscillations are applied to reflect molecular structures of the sample, including rigidity, shear modulus, storage modulus (G'), loss modulus (G"), rather than sample destruction (Foegeding, 1988; Savoie & Arntfield, 1996). In the typical experiment, the sample is placed on top of a stationary temperature-controlled plate while the top surface is covered by a motor rotated top plate. The sinusoidal shear deformation is applied to the sample in the linear viscoelastic range while the stress response was measured by the top plate connected to the sensor.

Temperature sweep is the procedure used while temperature is basically the only thermodynamic parameter in biopolymers gelation process (Lopes da Silva et al., 1998). In the temperature sweep cycle, the G' and G" are measured at a constant frequency.

The viscoelastic behaviour of protein gel is described by G': the solid-like contribution to stress response, and G", the liquid-like contribution to stress response. The phase angle (δ) is the phase lag between stress and strain, and ranges between 0 to 90. The tan δ is calculated as the ratio of G" to G' (Loppes da Silva et al., 1998). A strong gel (more elastic than viscous) is usually associated with the property that G' > G" (Douglas, 2018), or indicated by lower δ.

Measuring the viscoelastic behaviour of gel at its threshold is the inherent difficulty of this measurement while the sol system has very low viscosity even below the instrument resolution of the small strain oscillatory instruments (Loppes da Silva et al., 1998).

2.4.3 Torsional gelometry

Torsional gelometry is a method to test the shear stress and shear strain of protein gels (Hamann, 1991). Shear stress is the force applied in parallel to the cross section of sample (surface), and shear strain is the deformation of sample (Daubert & Foegeding, 1998). In the torsion test, the protein gel sample is milled into a capstan shape to ensure that sample fractures at the narrowest centre (Tunick & Van Hekken, 2002). The capstan-shaped sample is twisted in a viscometer to fracture, and the stress and strain generated at the point of fracture is measured with shear stress related to protein type and concentration, processing conditions, and ingredients, and shear strain related to protein quality (Hamann, 1988 & 1991). The measurement of shear stress and shear strain could give insight of effects of ingredient and processing conditions on protein gel product texture (Truong & Daubert, 2001). Fracture of gel could happen in different modes, namely compression, tension, and shear (Hamann et al., 2006). TPA alone is not sufficient to describe textural properties of gel samples. Compared with TPA, torsional gelometry shows high correlations between TPA hardness and shear stress, and TPA cohesiveness and shear strain (Montejano et al., 1986). The torsion textural map (**Figure 2-1**) could help link the sensory texture with shear stress and strain measurements.

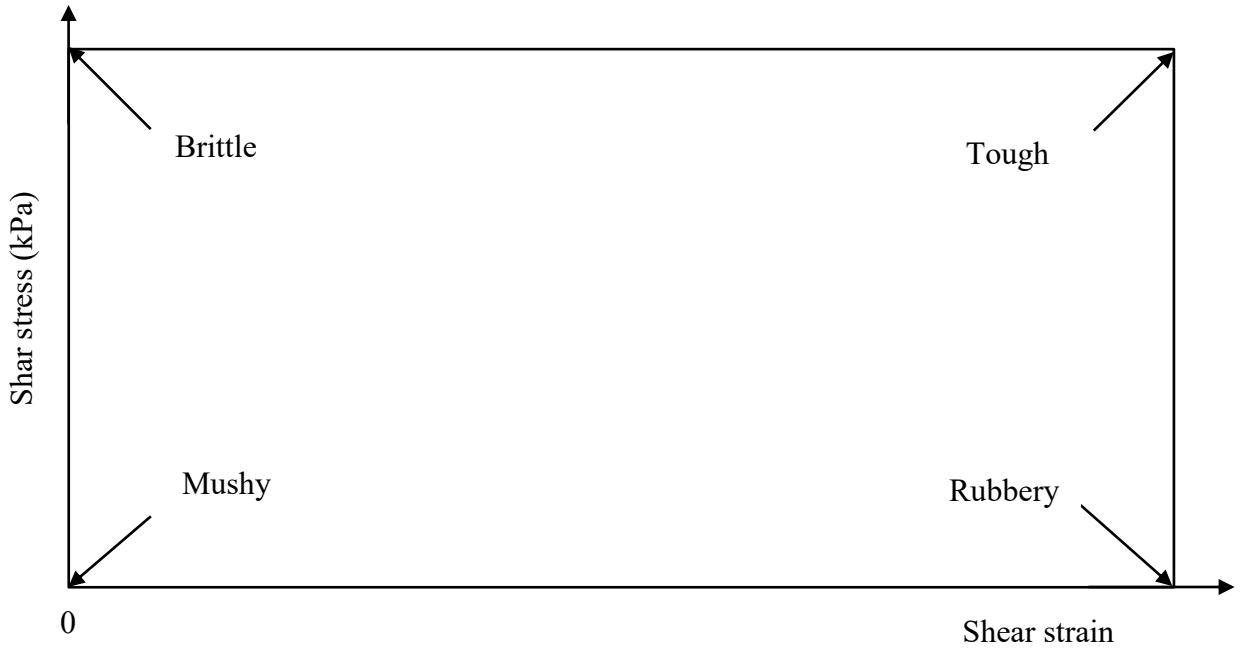

Figure 2-1 Torsional textural map. Modified from Lanier (1986).

2.5 Mechanically separated pork (MSP)

The world is seeking more protein for human consumption, and protein recovered after hand boning of meat could be a valuable source of animal protein. It was reported that 2 million metric tons of mechanically deboned meat could be possibly recovered annually on a world basis (Field, 1976).

Bone with some adhering tissue is the coproduct of meat slaughtering and processing. The bone weight comprises about 16-20% of total carcass weight (Ockerman et al., 1981). Mechanical separation technology makes it possible to recover soft meat tissues from hard bones, and serve as substitute ingredients for intact meat in food processing with lower cost (Hui, 2012a). As an underused low value by-product, investigations have been conducted to study the characteristics and possibility of transforming it into more market valuable products.

The raw materials for mechanical separation vary. The mechanical deboning process could be applied to any species including manually boned animal bones (sheep, goat, pork, beef), and poultry carcasses (chicken, duck, turkey), necks, backs in particular (Hui, 2012a).

However, the mechanical separation process breaks meat cells, destructures muscle fibres, increases lipids and heme content, and denatures protein, which leaves the recovered meat with low nutritional and textural property (Hui, 2012a). These adverse characteristics of MSMs limited usability as valuable ingredients in processed meat products.

Unlike MSM from poultry or fish that have been successfully used in further processed meat products, the use of mechanically separated red meats has been restricted for both labeling requirements and its undesirable quality change, textural softness in particular (Kramer & Sebranek, 1990). For the type of bones used in the separation process, round bones are the least desirable material as they have little lean tissue attached to the bones filled with marrow. Bone from vertebral column, ribs, and sternum were more preferred for the process for the higher lean tissue content (Field, 1976).

2.5.1 Regulation of MSP

According to Canadian Food Inspection Agency (CFIA) (2019a), mechanically separated and finely textured meat is meat that has been mechanically deboned by applying pressure to separate soft muscle tissue and attached bones. Meat with bones is forced to pass through sieve like surface, during which soft meat goes through while bones pass over.

MSM is the soft muscle obtained by mechanically removing bones, and should have no more than 0.027% of calcium for every 1% protein, no bone particles larger than 2 mm, and more than 10% protein content (CFIA, 2019a). MSM should have more than 14% protein content for retail purpose. The bones after boning should be stored in no higher than 10 °C environment, and go through mechanical separation within 5 hours, or be refrigerated to lower temperature and mechanically separated afterwards within certain storage hours.

2.5.2 Characteristics of MSP

MSP is the by-product of the pork carcass deboning process. Pork carcasses could provide about 170 million kg of recovered tissue annually worldwide (Ockerman et al., 1981). The picnic, ham and blade bones which represent 60-65% of bone weight are available for mechanical deboning, and will produce 0.91-1.27 kg of deboned meat per animal (Ockerman et al., 1981).

MSP has typically pasty texture with high quantity of pulverized muscle fibre residue and destructured muscle fibres (Hui, 2012a). The jelly-like texture and high fat content make it mostly suitable for use in manufacturing of emulsion products (Guerra-Daros et al., 2005). The mechanical separation process makes the MSP special for the considerable cellular level disruption, increased surface area, lipid, heme oxidation and protein denaturation, incorporation of more blood, calcium, and iron, and elevated pH to a more neutral value (Krautil & Tulloch, 1987). MSP quality and

composition are highly dependent on recovery process condition, machine type, meat cut, as well as previous treatment including freezing and trimming (Hui, 2012a). But it still shares some common characteristics such as higher collagen content, lipid content, and microorganism load than hand deboned pork (Hui, 2012a).

The protein content of MSP is dependent on meat portion and species, and varies from 11.4% to 20.6% (Hui, 2012a). It's known that high collagen content in meat products could adversely affect meat functionality, and give low nutritional value for its unbalanced amino acid profile (Trindade et al., 2004). It was reported by Abdullah and Al-Najdawi (2005) that collagen in MSM could give detrimental effects to its emulsifying capacity, but no influence was observed on WHC. According to Field et al. (1976), MSM has lower protein and higher fat content compared with similar hand deboned meat due to the extra bone marrow extracted and removal of connective tissue. The composition of MSP was also dependent on the different separation systems used.

Higher lipid content up to about 30% is also characteristic of MSP. The extra lipids in MSP are mainly phospholipids from bone marrow and tissue with high percentage of polyunsaturated fatty acids (Trindade et al., 2004; de Azevedo Gomes et al., 2003). This could make MSP more prone to lipid oxidation, and result in undesired sensory quality after storage (Trindade et al., 2004). The rapid onset of oxidative rancidity results in undesired flavor and odor. The high level of polyunsaturated fatty acid may have adverse physiological effects on human health for their (per) oxidation products (Estévez & Xiong, 2019). Also, the metal ions from deboning equipment and calcium and phosphorous from bone could facilitate heme oxidation which would further stimulate lipid peroxidation (Field, 1988) and develop undesired aromas in meat products. Another potential concern of MSP is the higher cholesterol content released from bone marrow, body fat, and skin (Demos & Mandigo, 1995). It was reported that the cholesterol values in MS beef were 90 mg/100 g, much higher than those from manually deboned beef, with values of 50 mg/100g (Serdaroúlu et al., 2005).

Also, MSP has higher bone content than manually deboned meat as a methodological consequence of the mechanical deboning process (Field, 1982). High level of calcium is observed in MSP as small particles of bone pass through separating equipment (Mayer et al., 2007). Field (2000) indicated that calcium and ash contents could be used as indicators of bone fragment contents in MSM. The effect of calcium in meat emulsions can be controversial. On the one hand, calcium carbonate or calcium-citrate-malate complex fortified beef emulsion (500-1000 mg/45 g

frankfurter) showed softer and less springy or chewy texture than control frankfurters (Boyle et al., 1994). On the other hand, calcium can strengthen gel texture by forming bridges with carboxyl groups in protein (Comfort & Howell, 2003; Mulvihill & Kinsella, 1988).

For the utilization of MSP in comminuted meat products, it's been reported that frankfurters containing 25% or more MSP had less desirable texture according to sensory panels (Marshall et al., 1977). The substitution of hand deboned meat with MSP in comminuted meat product could be a challenge for the MSP application due to the compromised texture, lipid oxidation level and colour of the final products. However, meat emulsion containing MSM could be improved with the addition of other ingredients, such as salt, sodium tripolyphosphate (STPP), sodium caseinate, and potato starch (Defreitas & Molins, 1991).

2.5.3 Lipid oxidation

Lipid oxidation is one of the important reasons for food quality loss and short shelf life (Sakanaki et al., 2005), including undesirable colour, flavour, texture change, and loss of nutritional value (Park et al., 2012). The extent of lipid oxidation depends on the temperature, the presence of prooxidants and antioxidants, and the nature of lipid in the product (Nawar, 1996). Compared with MS chicken, MS red meats have much higher level of hemo-proteins (hemoglobin and myoglobin) and lower polyunsaturated fatty acid content, which is said to be responsible for difference in storage characteristics (Ockerman et al., 1981). Protein-lipid interactions is another phenomenon that is usually observed in meat complexes (Guyon et al., 2016). According to Zhou et al. (2016), the heme iron plays a role in the co-oxidation of lipids and proteins in a dose dependent manner with oxymyoglobin acting as a pro-oxidant. The aldehydes from lipid peroxidation could promote the formation of metmyoglobin from myoglobin oxidation, the oxidation product reversely induce the lipid peroxidation (Chaijan, 2008). Wang et al. (2018) found out that acrolein, a lipid oxidation product, has the potential to impair myofibrillar protein gelling ability from rabbit meat. To understand the chemistry underlying the lipid and protein interaction, it was proposed that proteins act as lipid oxidation inhibitors at the expense of their own oxidation (thiol oxidation etc.) (Estévez & Xiong, 2019). Yang and Xiong (2018) found that myofibrillar protein in emulsion system could inhibit lipid oxidation by radical-scavenging or metal chelating activities. Higher lipid content is observed in MSP. The mechanical separation process further reduces the particle size of lipid with increased surface area to air and muscle proteins, besides the raised meat temperature during

process. It was reported that lipid oxidation would cause rapid deterioration of MSP during frozen storage with initial thiobarbituric acid reactive substances (TBARS) values increased from 0.2 malondialdehyde (MDA)/ kg meat initially to 5.0-8.0 after 6 weeks (Defreitas & Molins, 1991). The lipid oxidation level of MSP could limit its use in meat products manufacturing and their shelf life.

2.5.4 Processing technique

2.5.4.1 Mechanical separation process

There are three kinds of recovery machines used to separate residual meat, namely by sieve screen, by using a stripper disk, and by using centrifugal forces (Hui, 2012a). Different techniques used under different processing conditions will affect properties of MSM (Hui, 2012a).

One of the machines based on the belt-drum system is mainly for fish bone recovery. The bones on a rubber belt are forced against a perforated drum with the holes about 5 mm in diameter. Only meat will pass through holes with bone and skins remaining on the belt (Aberle et al., 2012; Hui, 2012a).

The second type of deboner is an auger type that is used mostly in the fish and poultry industry. The meat is forced through orifices (Ø ~ 1 mm) in stainless steel cylinders by rotating augers inside, while the bones remain inside and are augured out (Aberle et al., 2012; Hui, 2012a).

The machine for pork and beef separation uses a hydraulically powered press. The bones are pressed at high pressure in a chamber with holes in the walls and the pressing head. The meat is pulled off the bones and filtered through an outlet and bones are discarded. The meat with small bone particles and connective tissues then passes through a belt and drum separator with holes 1.0-1.3 mm in diameter where the sinew, cartilage and bone particles are removed (Aberle et al., 2012; Hui, 2012a).

The bone particle size is determined by the deboning machine, operation and the size of filter, and care should be taken when assembling equipment to avoid unacceptable particle size (Hui, 2012a). According to Koolmees et al. (1986), the MSM from pressure force machine had higher cartilage and less calcium content than sieve screen machines. The calcium and iron in MSM are also affected by the pressure applied, as it is adjusted for a certain yield. According to Barbut (2002), the calcium and iron content in MSM from poultry increased from 582 ppm and 10.0 ppm to 764 ppm and 17.9 ppm respectively when the pressure was raised from 300 to 1000 kPa.

2.5.4.2 Chilling and freezing process

Chilling and freezing are two commonly used processes for meat preservation. As required by CFIA (2019a), meat bones should be stored at refrigeration temperature within several hours before the deboning process, and freezing may be applied after deboning (Hui, 2012a). Refrigeration is the most commonly used method to extend shelf life of fresh meats from 5-7 days, for temperature below 3 °C could inhibit growth of pathogenic bacteria (Murano, 2003). Frozen storage could further extend shelf life of meats by stopping growth and reproduction of pathogenic microorganisms, and slowing down deleterious chemical reactions, but the rate of freezing, length of frozen storage, and air in package could influence the quality of meat (Murano, 2003). The previous freezing and frozen storage process could decrease the protein emulsifying or gelling capacity by altering specific protein characteristics (Jiménez-Colmenero & Borderías, 1983). Wagner & Añon (1985) found that freezing would have denaturing effect on myofibrillar protein from bovine, the slower the freezing rate, the greater loss as a result of partial unfolding of the myosin head. The increased ionic strength as a result of myofibrils dehydration and water migration from myofibrillar space would result in myosin head unfolding (Wagner & Añon, 1985) as the previous protein water interaction was replaced with protein-protein associations or other interaction (Fennema, 1977; Hamm, 1975). Meat fibre orientation, freezing rate and temperature, and time all influence the efficiency of freezing (Tamilmani & Pandey, 2016).

Commonly used freezing processes include blast freezing and contact freezing. In blast freezing, cold air is blown over meats by fans inside the cold room, while contact freezing cools down meat in contact with refrigerated surfaces (Murano, 2003). Fast chilling is believed to reduce chilling time, moisture evaporation, and microbial population on the meat surface (Xu et al., 2012). Usually, a faster chilling rate could better protect meat tissue from cell membrane rupture and large ice crystal formation (Murano, 2003). Lower freezing rate increases myofibrillar protein denaturation (Wagner & Añon, 1985) by partial unfolding of myosin head which is a result of local ionic strength increase due to the water migration from myofibrillar space or dehydration of myofibrillar protein during freezing. The protein unfolding leads to protein aggregation and denaturation by increased exposure of hydrophobic groups (Wagner & Añon, 1985). It was reported that using plate freezer could reduce the time needed to reach -20 °C core temperature by 51% compared with an air blast freezer (Lakshmisha et al., 2008).

However, the effect of ultra-fast freezing and low storage temperature on meat protein functional properties remains controversial. It was reported by Farouk et al. (2003) that freezing rate alone had no effect on protein solubility of beef muscles studied. Compare with ultra-fast freezing, the current practice of freezing and storage temperature in industry is sufficient to retain the protein functional properties of beef. It is the storage time and the interaction between storage time and freezing rate that influences the quality of meat protein.

2.5.5 Application of MSP

MSM is mostly used in making emulsion products due to their fine consistency and lower cost. MSM is a good substitute for regular meat in products that do not require fibrous texture but high emulsion stability and natural colour (Huxley, 1963). Recovered poultry meat is the most often used substitute for red meat in industry. It was reported that MSP could be substituted for regular hand deboned pork in fermented snack sausage (Kramer & Sebranek, 1990). Substitution of 20% MSP got the highest acceptance rating than 0, 10 or 30% MSP substitution. However, the softness and level of oxidative rancidity increased as the increase of MSP. In their study, partial substitution of MSP for hand deboned pork didn't decrease the acceptability of fermented snack sausage.

3 STUDY 1: EVALUATION OF CHEMICAL COMPOSITION, PHYSICOCHEMICAL, BIOCHEMICAL AND GELATION PROPERTIES OF MSP AND THE EFFECT OF CHILLING RATE AND FROZEN STORAGE ON PHYSICOCHEMICAL AND GELATION PROPERTIES OF MSP

3.1 Abstract

The chemical composition, physiochemical, biochemical and gelation properties of MSP from pork arm bones were examined. The MSP was darker and redder in colour, had higher pH value (6.4-6.5) (p<0.05), and was higher (p<0.05) in sodium, calcium, and iron content than PP. At the cellular level, the MSP had highly disrupted structure with curved and twisted myofibrils. The gelation ability of MSPs was decreased by the mechanical separation process, which was indicated by decreased G' of NAM after heat induced gelation. The SDS-PAGE of NAM from MSP showed that part of MHC was degraded to smaller molecular mass peptides and confirmed by immunoblotting with a monoclonal antibody to MHC. The ratio of myofibrillar protein to sarcoplasmic protein was around 1.05 for MSP after mechanical separation process, while the ratio for BM and PP was 1.24, and 1.32 respectively. The effect of chilling rate and frozen storage (up to 4 months) on lipid oxidation, protein solubility and dynamic rheological profiles of MSP was analyzed. With the heat and pressure generated through the process, MSP had significantly higher (p<0.05) lipid oxidation level (~ 0.76 mg MDA/kg meat) throughout the frozen storage period compared with ground PP (~ 0.46 mg MDA/kg meat). Faster chilling didn't necessarily help preserve the gelation properties or retard lipid oxidation of MSP, as MSP didn't show high sensitivity to current chilling rate difference. The protein solubility of MSP was relatively stable during 1 to 4 months of frozen storage.

3.2 Introduction

Meat bones after deboning are a low value by-product of meat slaughtering and processing. The soft muscle tissue adhering to bones could be a valuable source of animal proteins for human consumption and a potential solution to food security. Mechanical separation technology has been applied on manually boned sheep, pork, beef and poultry carcasses (Hui, 2012a).

However, meat cells were broken, and destructured as bones go through the process, leaving the meat with reduced nutritional and textural properties (Hui, 2012a).

The most common application of MSP is in emulsion products since they do not require fibrous texture but high emulsion stability and natural colour (Huxley, 1963). It was reported that MSP could be substituted for regular hand deboned pork in fermented snack sausage with the highest acceptance rating at 20% substitution level (Kramer & Sebranek, 1990). The softness and level of oxidative rancidity increased as the increase of MSP level up to 30%. The mushy texture of products made with MS red meats limits their use, and the reasons remain unclear. One of the assumptions is the higher collagen in MSP that could give detrimental effects to its emulsifying capacity (Abdullah & Al-Najdawi, 2005). However, Field et al. (1976) found that the connective tissue could be removed from the meat during mechanical separation. Another assumption is the conformational changes of myofibrillar protein due to the proteolysis of myofibrillar protein after the cell structure disruption or the protein denaturation caused by the pressure and heat generated through the deboning process. It is necessary to understand the principle of these changes in MSP for better MSP utilization.

Higher lipid content up to 30% is one of the characteristics of MSP. The extra lipids in MSP are from fatty tissue attached to bones, phospholipids from bone marrow (de Azevedo Gomes et al., 2003; Trindade et al., 2004;). The metal ions from deboning equipment and calcium and phosphorous from bones could facilitate heme oxidation, and further stimulate lipid peroxidation (Estévez & Xiong, 2019). The rapid onset of oxidative rancidity would develop undesired aromas in meat products.

Chilling and freezing are two common processes for meat preservation. The rate of freezing and the length of frozen storage, and the amount of air in package could influence the quality of meat (Murano, 2003). It was reported that previous freezing and frozen process could decrease the protein emulsifying or gelling capacity by altering specific protein characteristics (Jiménez-Colmenero & Borderías, 1983). The slower the freezing rate, the greater loss of myofibrillar protein was observed as a result of partial unfolding of myosin head (Wagner & Añon, 1985). Faster chilling could be a potential strategy to retard rapid lipid oxidation and preserve the protein gelation ability of MSP.

The present study focused on the physicochemical, biochemical, and gelation difference between MSP and PP from the chemical composition, ultracellular structure, to molecular mass of

myofibrillar protein in order to explain the gelation capacity difference between MSP and PP and improve its utility. The effect of initial chilling rate and frozen storage on the gelation properties and lipid oxidation level has also been investigated.

3.3 Hypotheses

MSP has different chemical composition, such as higher collagen and fat level, higher calcium and iron content than PP. MSP has different muscle fibre structure and biochemical properties of actomyosin, and higher lipid oxidation level than PP. MSP has weaker gelation properties due to the deteriorated quality of myofibrillar protein. Accelerated chilling could better preserve functionality of myofibrillar protein in MSP. Frozen storage has effect on the functionality of MSP.

3.4 Materials and methods

3.4.1 Materials

3.4.1.1 Mechanical separation process

The MSP, regular PP meat cuts, and hand deboned PP bones were obtained from Maple Leaf Foods Inc., Brandon, MB. Samples were collected onsite and shipped to Saskatoon, SK, under temperature-controlled conditions. The samples were collected at 3 different production dates as 3 replications.

The pigs were slaughtered the previous day and cuts were hand deboned at 24-30 h post-mortem into boneless PP and pork arm bones with some adhering meat and cartilage before mechanical recovery. The MSP sample was recovered from PP bones with big bone pieces removed by a linear press meat harvest system (ProTEN, Marel, Iceland). Soft muscle tissue, namely MSP, was further separated from the small bone pieces, cartilage and connective tissue through a BAADER 605 soft separator (BAADER, Germany). The mixture of small bone pieces, cartilage and connective tissue was collected as bone residue (BR).

MSP samples (about 25 kg per box) were packed into 56 (L) × 36(W) × 15 cm^3 wax-lined cardboard boxes, and frozen in a blast freezer. Two different chilling rates were applied in this study. The MSP-DL (MSP-delay) samples were MSP that was tempered at production floor temperatures (4-6 °C) for 4 h before being moved to the blast freezer for a slower chilling effect and to simulate the time delay possible during production. The MSP-STD (MSP-standard) samples

were moved to the blast freezer right after processing. Three boxes of each product were frozen and returned to the University of Saskatchewan.

The bone meat (BM) which is the soft muscle tissue trimmed from arm bone manually was collected onsite and brought back to Saskatoon under refrigerated condition for further processing.

3.4.1.2 Sample collection

Day 0 MSP samples were collected after mechanical deboning, and after 4 h tempering before being moved into the blast freezer (at -35 °C) in the processing plant. Samples were wrapped in labelled foil, frozen and stored in liquid nitrogen for transport back to the University of Saskatchewan. They were then moved to the -80 °C freezer before analysis. Temperature profiles of MSP-DL, MSP-STD with 2 different chilling rates were tracked with iButton temperature trackers (Maxim Integrated, San Jose, CA, U.S.A) on both the surface and centre of MSP blocks from one box per treatment. Frozen MSP samples were shipped under frozen condition with temperature of samples tracked by iButton. PP used in this study was fresh vacuum packed PP manufactured on the same date, and shipped in refrigerated condition to Saskatoon. Frozen MSP samples were collected after boxes were commercially shipped to campus.

3.4.1.3 Sample preparation

PP was cut into large cubes and ground through Ø 0.95 cm hole plate (Biro Grinder, Marblehead, OH, USA, model AMFG-24) 7 days postmortem. The ground PP sample was packed in 13 × 20 cm^2 bags, and stored at -18 °C with air exposure before analysis. BM sample was coarsely ground through 0.95 cm hole plate, and finely ground through Ø 0.32 cm plate twice to mimic the particle size of MSP. The ground samples were packed with air exposure and stored at -18 °C before analysis.

MSP-DL and MSP-STD samples were stored at -30 °C after receiving, and subsampled while frozen by cutting into about 1 kg slabs (2 × 15 × 36 cm) with a band saw (Fleischerei-Bandsäge, model 4210, Reich, Germany). The MSP-DL and MSP-STD samples were stored in -18 °C freezer with air exposure for study of the effects of extended frozen storage.

Samples of PP, MSP-DL, and MSP-STD were taken following 1 and 4 months of frozen storage for analysis. The internal samples of each treatment were cut into about 10 g cubes, and

vacuum packed (model 550A, Sipromac, Drummondville, QC, Canada) in vacuum bags (polyethylene vacuum pouch, 3 μm thick, with oxygen permeability of 7.7 cc/m^2/24h) and stored in -80 °C freezer before analysis.

3.4.2 Methods

3.4.2.1 Proximate composition

a) Moisture

The moisture content of samples was measured by Association of Official Analytical Chemists (AOAC) Method 950.46 (AOAC, 2000) with a slight modification. Three to four grams of sample was accurately weighed in duplicate in pre-dried aluminum dishes, and air oven-dried at 105 °C for 16-18 h (overnight). The moisture content was calculated as follows:

$$\text{Moisture\%} = \frac{\text{weight of sample} - \text{weight of dried sample}}{\text{weight of sample}} \times 100 \quad (3.1)$$

b) Crude protein

The crude protein content was measured by AOAC 981.10 Kjeldahl method (AOAC, 2000), with 6.25 as the nitrogen conversion factor. Around 1.2 g of sample was accurately weighed and enveloped inside weighing paper to be put into digestion tubes. Two tablets of catalyst, Kjel-Tabs (Fisher Scientific Ltd., ON, CA), and 25 mL concentrated sulfuric acid were added into digestion tubes followed by heat treatment for about 1-2 hour until all solution inside tubes become clear. After tubes were cooled to room temperature, 50 mL distilled water was added to dilute acid. Thirty mL of 4% boric acid was poured into Erlenmeyer flask with 5-6 drops of indicator (bromocressol green and methyl red). Distillation was performed by Buchi K355 steam distillation unit with addition of about 90 mL of 30% NaOH solution and the distillate was collected. The distillate was titrated with about 0.2 N HCl solution, and the end point was indicated by the colour of indicator that changed from green to pink. The protein content was calculated based on amount of HCl titrated and sample weight.

$$\text{Nitrogen\%} = \frac{(\text{mL 0.2 N HCl sample} - \text{mL HCl blank}) \times \text{N HCl} \times 0.014}{\text{sample weight}} \times 100 \quad (3.2)$$

$$\text{Protein\%} = \text{Nitrogen\%} \times 6.25 \quad (3.3)$$

c) Crude fat

The crude fat content was evaluated by AOAC Method 960.39a (AOAC 2000) using a Gerhardt 6 place Soxtherm Extractor and Multistat (C. Gerhardt GMBH & CO. KG, Germany). About 3.0 g of sample were weighed inside a thimble, mixed with about 1.5 g of sand before drying overnight in an oven at 105 °C. The extraction beaker weight was recorded before the extraction. The thimble was placed inside the extraction beaker and extracted with 85 mL of petroleum ether. After extraction and cooling down to room temperature, the extraction beaker with fat in it was weighed again to calculate the fat content. The crude fat content was calculated as a ratio of weight difference of the extraction beaker to sample weight.

d) Ash

The ash content was determined by AOAC 920.153 (AOAC, 2000). Approximately 2.5 g of sample were weighed into dry pre-ashed crucibles, and dried in an air oven at 105°C overnight. The dried crucibles were incinerated at 550°C in a muffle furnace overnight. The ash content of sample was calculated as weight difference of the crucible.

3.4.2.2 Bone fragment content

Bone fragment testing was conducted on the MSP samples according to Casas (2015). About 100 g of meat sample was mixed with 250 mL 12% potassium hydroxide denatured ethyl alcohol solution (90%). Sample was digested on a hot plate (Isotemp, Fisher Scientific Ltd., ON, CA) with constant stirring (low speed) at 160 °C until thoroughly digested. The remaining solution was diluted with methanol and decanted until a clear solution was obtained. The solution was filtered through glass fibre filter paper (GF 8) under vacuum condition with a Buchner funnel. The filter paper was dried for 30 min, and the bone fragments left on the filter paper were weighed afterwards. Number 10 (2 mm), number 14 (1.4 mm), and number 18 (1.0 mm) sieves (Fisher Scientific Ltd., ON, CA) were used in order to sieve the bone fragments. The bone size and mass was reported if any fragment remains in number 10, 14 and 18 sieves.

3.4.2.3 Collagen content

The collagen content analysis was conducted on MSP, BR, and PP samples by an outside laboratory. The hydroxyproline content was analyzed according to Bergman and Loxley (1963). Freeze-dried meat samples (100 mg) were hydrolysed with 6 mL of 5M HCl for 20 hours with heat,

then cooled down for 10 min in ice water bath. The precipitate was collected and dried after filtration through No. 4 filter paper. The precipitate was reconstituted with 10 mL water then 500 µL (250 µL for BR) was mixed with 500 µL (750 µL for BR) to make 1 mL for hydroxyproline estimation. The isopropanol (2.0 mL) and oxidation solution (mixture of 7% (w/v) chlororamine T and acetate/citrate buffer (0.42 M sodium acetate, 0.13 M trisodium citrate, 0.03 M citric acid and 38.5% isopropanol), at a ratio of 1:4 (v/v)) was combined and mixed thoroughly; 13 mL of Ehrlich's reagent solution (mixture of solution A; 2 g of p-dimethylaminobenzaldehyde in 3 mL of 60% (v/v) perchloric acid (w/v)) was added into the mixture. The mixture was mixed and heated to 60 °C for 25 min in water bath, followed by cooling for 5 min in ice water. The solution was diluted to 50 mL with isopropanol and distilled water was used to replace sample as blank. The hydroxyproline content was measured by reading absorbance against blank at 558 nm with hydroxyproline standard concentrations ranging from 2.5 to 40 µg/mL. Hydroxyproline content was calculated and expressed as µg/g raw meat sample. The conversion factor of 7.14 was used to calculate the collagen content in raw meat samples according to the assumption that the hydroxyproline content is 14% of meat collagen (Stanton & Light, 1987).

3.4.2.4 Iron, magnesium, sodium, calcium content

Iron, magnesium, sodium and calcium contents of MSP and PP samples were analyzed using the AOAC 2015.01 method. Samples were sent to a commercial lab for analysis.

3.4.2.5 Raw meat pH

The pH values of raw meat samples during storage was evaluated by diluting 20 g of ground sample with 80 mL of distilled water in duplicate, and well mixed by stomacher (Stomacher Lab-Blender, Model BA6021, Seward Limited, Edmunds, UK) for 3 min. The pH value of the slurry was measured by pH meter (Model 915 Fisher Accumet, Fisher Scientific Ltd., ON, CA).

3.4.2.6 Raw meat colour

The raw ground PP, MSP-DL and MSP-STD samples were pressed into plastic petri dishes (0.8 cm depth), and covered with 1 layer of oxygen permeable film and stored at 4 °C for at least 2 h before analysis. The colour of samples was analyzed with HunterLab MiniScan XETM (Hunter Associates Laboratories Inc., Reston, VA) expressed as International Commission on Illumination

(CIE) lightness (L*) redness (a*) yellowness (b*) by using illuminant A and 10° standard observer. The instrument was calibrated with black and white tiles, and the reproducibility was monitored by testing a pink tile as standard. The measurement was taken through the film. The second reading for one sample was taken after 90° rotation clockwise of sample, and the measurement was done on duplicate petri dishes per treatment.

3.4.2.7 Lipid oxidation

The lipid oxidation of raw meat samples was measured as TBARS reactive substances according to Bedinghaus and Ockerman (1995) on frozen samples during frozen storage. Each meat sample (about 2.5 g) was extracted with 25 mL extraction solution (20% w/v trichloroacetic acid (TCA) with 1.6% v/v phosphoric acid), then blended with 25 mL of cold distilled water (Stomacher Lab-Blender, Model BA6021, Seward Limited, Edmunds, UK). The slurry was filtered through Whatman #1 filter paper into 50 mL volumetric flask. After bringing to volume, a 5 mL aliquot was mixed with 5-mL of 0.02 M TBARS reagent, and heated in boiling water bath for 35 min to facilitate the formation of pink coloured thiobartituric acid-malonaldehyde complex. After cooling down the mixture, the absorbance was measured at 532 nm by Spectronic Genesys 5 Spectrophotometer (Spectronic Instruments, Inc., Rochester, NY). The extent of lipid oxidation was expressed as mg malonaldehyde/kg sample.

A standard curve was created by mixing 0.75, 1.5, and 2.25 mL of 2×10^{-7} mol/mL of 1,1,3,3-tetramethoxypropane (TMP) with 10% TCA extracting solution (cold) and made to volume of 50 mL. The absorbance was measured with the mixture of 5 mL of TMP/TCA mixture and 5 mL of TBARS reagent. The standard curve was plotted by absorbance as a function of TMP concentration. The recovery rate was around 80% with current experiment facilities.

3.4.2.8 Transmission electron microscopy (TEM)

The TEM images of MSP and PP were collected on frozen MSP samples and PP muscle samples according to Peng and others (2013) and Lee and others (2012).

3.4.2.9 Protein solubility

Protein solubility analysis was conducted on PP, MSP-DL, MSP-STD, and BM samples that were frozen in the plant and during 1 and 4 months of -18 °C frozen storage according to Joo

and others (1999) and Helander (1957). Soluble sarcoplasmic protein was extracted with low ionic strength buffer (LIS: 0.03 M potassium phosphate buffer, 2 mM EDTA, pH 7.4) by mixing 20 mL of LIS with 2 g of meat sample. The mixture was homogenized with Polytron homogenizer (Polytron PT 10/35 Brinkman Instruments, Mississauga, ON) for 15 sec at 15,000 rpm, and shaken at 4 °C overnight (D-91126 Schwabach, Heldolph Instruments, Germany). The mixture was centrifuged at 6,000 × g for 15 min (Sorvail RC 6+ Centrifuge, Thermo Fisher Scientific Ltd., ON, Canada) to collect supernatant as sarcoplasmic protein extract. The pellet was washed with 10 mL of LIS by shaking at 4 °C for 2 h and followed by centrifugation at 6,000 ×g for 15 min. The supernatant was carefully decanted, and the washing step repeated. The collected pellet was homogenized with 20 mL high ionic strength buffer (HIS: 0.1 M potassium phosphate buffer, 2 mM EDTA, 1.1 M KI, pH 7.4) by Polytron homogenizer for 15 sec at 15,000 rpm, and shaken at 4 °C overnight. The myofibrillar protein extract (supernatant) was collected after the mixture was centrifuged at 6,000 ×g for 15 min. The protein content was analyzed by Kjeldahl method (AOAC, 2000).

3.4.2.10 NAM extraction

Myofibrillar protein was extracted according to Wang and others (2005). Eighteen grams of ground raw meat were homogenized with 90 mL of standard salt solution (SSS: 50 mM KCl, 20 mM potassium phosphate buffer, pH 7.0) using a blender (Osterizer, Sunbeam Corporation (Canada) LTD.) for 15 sec, followed by centrifugation at 10,000 × g for 5 min. The pellet was collected after centrifugation, and these steps were repeated. The final pellet was homogenized with 180 mL high salt solution (HSS: 0.6 M KCl, 20 mM potassium phosphate buffer, pH 7.0) for 15 sec, followed by centrifugation at 10,000 ×g for 5 min. The supernatant was filtered through 3 layers of cheese cloth (Veratec Cotton Cheesecloth, Simpsonville, SC, U.S.A) and diluted with 1080 mL of low salt solution (LSS: 20 mM potassium phosphate buffer) and stirred for 30 min. The protein aliquot was centrifuged at 10,000 × g for 10 min, and pellet was collected and resuspended with 15 mL of SSS to be washed. After centrifugation at 10,000 × g for 10 min, the pellet collected was myofibrillar protein for further analysis. The whole extraction process was carried out at 4 °C.

3.4.2.11 Protein assay

The protein content of myofibrillar protein extract was measured by Bradford method (Bradford, 1976) in duplicate. Bovine serum albumin (BSA, Millipore-Sigma, St. Louis, MO, U.S.A) was used to make standard curve.

3.4.2.12 SDS-PAGE of NAM

Each NAM aliquot was standardized to 3 mg/mL, and mixed with 2× laemmli (Laemmli, 1970) loading buffer (Bio-Rad Laboratories, Inc., Hercules, CA, U.S.A) at 1:1 ratio. Samples were heated to 90 °C for 10 min with mixing rate of 1,000 rpm by Thermal Mixer (Isotemp Fisher Scientific Ltd., ON, Canada) followed by centrifugation at 10,000 rpm (Eppendorf centrifuge 5424, Canada) for 8 min after they had cooled down. Mini-PROTEAN TGX Gels from Bio-Rad were used to separate protein extract with 4-20% resolving gel at voltage of 125 V, and 12 µg of sample has been loaded into each well. After the dye front reached the gel bottom, the gels were gently removed from cassettes and rinsed with deionized water, then the proteins in the gel were fixed with staining solution (1% Coomassie brilliant blue R-250, 10% glacial acetic acid, and 40% methanol). The Precision plus protein standards (Bio-Rad Laboratories, Inc.) was used as molecular weight markers ranging from 10 to 250 kDa.

3.4.2.13 Immunoblotting of NAM

SDS-PAGE gel of NAM (0.6 µg protein each lane) was transferred in transfer buffer (25 mM Tris base, 192 mM Glycine, 20% methanol) to the nitrocellulose membrane (0.2 µm, Bio-Rad Laboratories, Mississauga, ON, Canada) at 25 V for about 30 min with a semi-dry Transblot cell (Bio-Rad Laboratories, Mississauga, ON, Canada). After transfer, the membrane was blocked with phosphate buffered saline (PBS) with 5% skim milk and 0.05% tween 20 (phosphate buffered saline with tween 20: PBST) for about 1 h at room temperature. The membrane was then incubated with monoclonal MHC antibody (Developmental Studies Hybridoma Bank, Iowa City, IA, U.S.A) with 5% milk PBST for 2 h at room temperature. After binding, the membrane was washed with 5% milk PBST 3 times (10 min each). After washing, the membrane was incubated with the secondary antibody (anti-mouse horseradish peroxidase conjugated antibody) in 5% milk PBST for 0.5 h at room temperature. After incubation, the membrane was washed with 5% PBST twice (10 min each), and rinsed with PBST twice (5 min each). The membrane was treated with ECL

(enhanced chemiluminescence) reagent and the image of the membrane was developed with Bio-Rad VersaDoc TM Imaging System and Quantity One – 4.6.9 program.

3.4.2.14 Dynamic oscillatory rheology

The dynamic rheological properties of protein samples (20 mg/mL in 0.6 M KCl, 20 mM KH_2PO_4/K_2HPO_4 buffer, pH 7.0) were analyzed by AR-1000 advanced rheometer (TA Instruments, New Castle, DE, USA) with a parallel plate measuring system (40 mm diameter) as described by Wang and others (2009) with modification. Around 2 g of sample solution was applied to the lower plate and the gap between the lower and upper plate was set as 1 mm under control of a programmed procedure (AR 1000 Rheometer Solution Software, AR 1000 Module, V1.1.7, New Castle, DE, USA). The sample was heated at 1 °C/min from 25 to 85 °C. The oscillatory measurements were made at 0.1 Hz, and strain of 0.01 within the linear range. The G', G", and δ ($\tan\delta = G"/G'$) were recorded for analysis by software (TA Instruments, New Castle, DE, USA).

3.4.2.15 Statistical analysis

The three replications of data were analyzed by Mixed Procedure of Statistical Analysis System (SAS) (SAS, Inst. Inc., Cary, NC) with Randomized Complete Block Design. The variations from meat materials of different production dates were considered as blocks. Within each block, the treatments and storage times were considered as fixed effects. The means of data was compared using Tukey procedure and the degree of freedom for means was approximated using a Kenward-Roger adjustment on standard errors. The significance level chosen in this study was $\alpha=0.05$.

3.5 Results and discussion

3.5.1 Temperature profile of MSP-DL and MSP-STD samples

Figure 3-1 shows the surface and centre temperature profile of MSP-DL and STD sample blocks during the first 80 h after the mechanical separation process. The data (collected by iButtons) shown was the average of temperature profiles of 3 sample replications. During the freezing process, the sample was initially frozen in a large blast freezer (operating at -40 to -30 °C) for about 48 h for the fast temperature drop to as low as -27 °C and then moved into -20 °C cold room for storage or shipping.

As shown in **Figure 3-1**, the beginning temperature of MSP-DL and MSP-STD was 7.6 ± 0.15 °C. The mechanical deboning process including the deboning and separating steps generated heat and raised meat temperature from 2 °C (temperature of meat on bones before deboning) to around 8 °C. The MSP-DL was tempered at around 4 °C in the staging area for 4 h before moving to the blast freezer, while MSP-STD was moved to the blast freezer right after packaging. The initial internal temperature of MSP-DL sample block was higher than that of MSP-STD before temperature reached 0 °C. From the data collected, it took 13.8 ± 0.84 h for the temperature of the centre of MSP-DL block to reach 0 °C and 11.9 ± 1.42 h for MSP-STD. It took 5.0 ± 0.46 h for the temperature of the surface of MSP-DL block to reach 0 °C and 1.4 ± 0.40 h for MSP-STD. After around 27 h post processing, the meat temperature started to decrease faster again, indicating cooling of already frozen meat. After 41.0 (MSP-DL) or 37.9 (MSP-STD) h post-processing, all MSP samples were chilled to around -20 °C, reflecting that the 4 hours delay had an influence on the temperature decline of the MSP-DL. A delay in chilling is a common occurrence in production settings as each pallet of boxed MSP takes some time to fill in the staging area before it is transported into the freezing facility. The time interval between processing and freezing did play a role in the freezing rate of meat product.

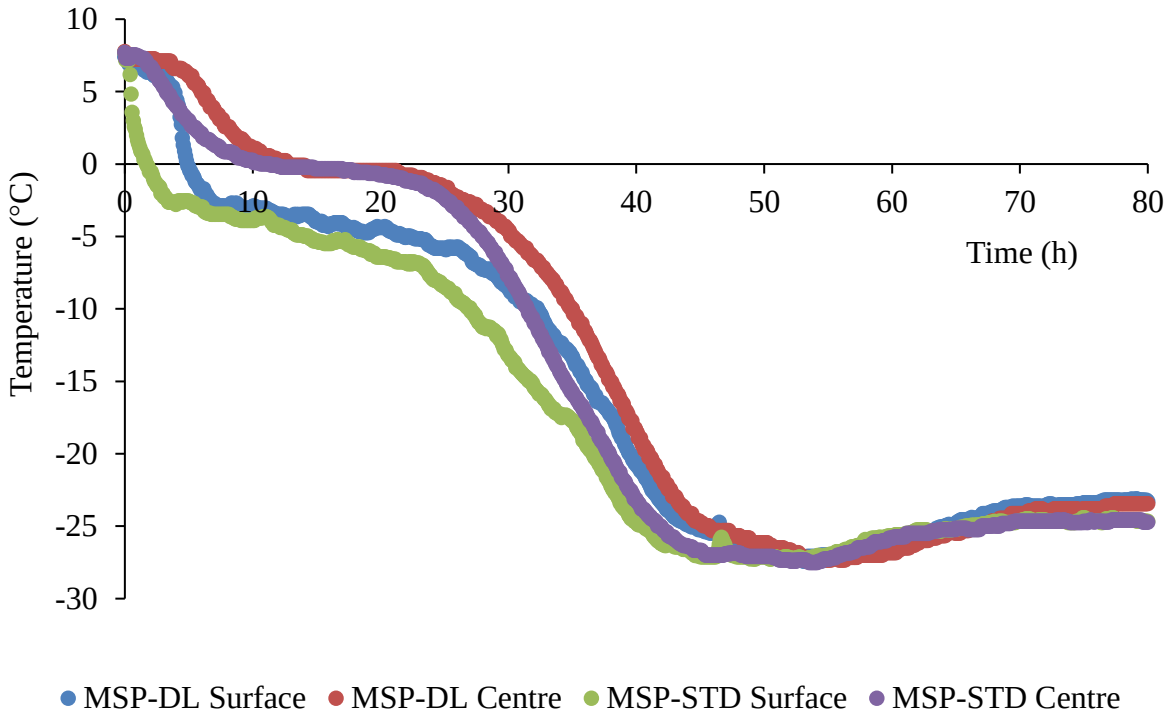

Figure 3-1 Surface and centre temperature profile of delay chilled mechanically separated pork (MSP-DL) and standard mechanically separated pork (MSP-STD) sample blocks during first 80 hours of chilling and freezing process (Average of 3 replicates).

3.5.2 Proximate composition of raw meats

Table 3-1 shows the proximate composition of PP, BM, MSP-DL, and MSP-STD. The PP and BM had higher moisture content than MSP, but lower fat content (p<0.05). The protein content of MSP that has been collected was variable (values ranging from 13.4% to 16.9%), due to the heterogenous nature of the raw material and possibly different equipment settings on different days. The protein content of MSP in general was lower than that of PP (~ 18%). BM was higher in moisture and protein, but lower in fat content than MSP even though both are from the same part of bone. The compositional difference between BM and MSP could be the result of effective selection in the mechanical separation system. The partial soft tissue removal from broken bones by the linear press system and further removal of connective tissue from the soft tissue led to accumulation of fat and decreased protein content in MSP compared with BM for the reason that fat and muscle tissue were easy to pass the separator, but the removal of connective tissue decrease the total protein content in MSP. The ash content of MSP, PP and BM was not different. Field (1976) suggested that the composition of MSP depended on the material entering the machine and

the setting and type of separation equipment. The MSP has lower protein, but higher fat content than similar hand deboned meat (Field et al., 1976). Our result agreed with other reports in the literature.

Table 3-1 Proximate composition of pork picnic (PP), meat trim from bone (BM), MSP-delay (MSP-DL) and MSP-standard (MSP-STD).[1]

Sample[2]	Moisture (%)	Protein (%)	Fat (%)	Ash (%)
PP	66.15±0.73^{a}	17.75±0.26^{ab}	14.61±0.13^{b}	0.94±0.05^{ab}
BM	67.60±0.45^{a}	18.65±2.80^{a}	12.59±2.52^{b}	0.91±0.01^{b}
MSP-DL	63.01±1.03^{b}	15.02±1.78^{b}	20.19±1.53^{a}	1.03±0.02^{a}
MSP-STD	62.41±1.87^{b}	14.98±1.13^{b}	20.71±2.05^{a}	1.00±0.05^{ab}

$^{a-b}$Means with different letters in the same column are significantly different (p<0.05)
[1]Values are means of 3 replicates ± standard deviation
[2]PP: pork picnic; BM: meat trim from bone; MSP-DL: delay chilled mechanically separated pork; MSP-STD: standard mechanically separated pork

3.5.3 Bone fragment content

The bone fragment content was tested on MSP samples with total weight recorded (**Table 3-2**). No bone fragment larger than 1.0 mm in diameter was observed. The regulation for bone fragments in MSM according to CFIA (2019a) is no bone particles larger than 2 mm, or no more than 10% of protein content. The bone particle size (**Figure 3-2**) and content of MSP sample met the regulation requirement. This observation agreed with Field (1981) that fine bone particles go through holes of the deboner.

Table 3-2 Bone fragment weight and size of mechanically separated pork (MSP).[1]

Storage period	Total bone fragment (g/kg meat)^{ns}	Pass CFIA regulation
MSP-DL[2]	0.573±0.172	Y
MSP-STD	0.576±0.059	Y

^{ns}not significant (p>0.05)
[1]Values are means of 3 replicates ± standard deviation
[2]MSP-DL: delay chilled mechanically separated pork; MSP-STD: standard mechanically separated pork

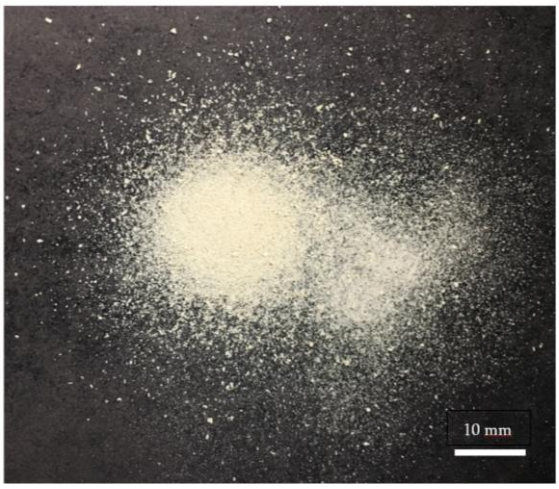

Figure 3-2 Bone fragments in mechanically separated pork (MSP).

3.5.4 Collagen content

The collagen content of MSP was much lower than PP (p<0.05) (**Table 3-3**). Since more connective tissue is needed to support the muscle movement of an animal, theoretically more collagen content is expected to be collected in MSP. However, the separation process by the soft tissue separator effectively screened out tough connective tissue, bone fragments or cartilage. So that the removed part which was collected and named as BR had significantly higher collagen content than MSP or PP. This result agreed with Field et al. (1976) that connective tissue could be removed from the meat during mechanical separation. Thus this material is actually lower in collagen than regular pork which may influence protein gelation. Doerscher et al. (2003) concluded that addition of pork collagen (10%) in purified pork myofibrils resulted in significantly increased WHC of myofibrillar protein gels and similar gel firmness compared with pure myofibrillar protein gels (cooked to 70 °C). The decreased collagen content in MSP might influence the WHC of bologna product in study two.

Table 3-3 Collagen content of pork picnic (PP), bone residue (BR) and mechanically separated pork (MSP).[1]

Sample[2]	Total collagen (mg/g meat)
PP	12.54±0.76[b]
BR	72.47±0.51[a]
MSP	4.18±0.30[c]

[a-c]Means with different letters in the same column are significantly different (p<0.05)
[1]Values are means of 3 replicates ± standard deviation
[2]PP: pork picnic; BR: bone residue; MSP: mechanically separated pork

3.5.5 Mineral content

Sodium, calcium, magnesium, and iron content were analyzed to compare PP and MSP (**Table 3-4**). MSP had nearly doubled sodium content, and nearly 6 times higher calcium content, and 5 times higher iron content than PP. However, the magnesium content of MSP was lower than that of PP. Pork semimembranosus muscle was reported to contain about 598 ppm of sodium (390-827 ppm), 266 ppm of magnesium (244-294 ppm), and 118 ppm calcium (98-155 ppm), and 14.2 ppm iron (10.0-27.9 ppm) with some variation among pig breeds (Tomović et al., 2011). The values are comparable to PP results with variation probably due to the difference in muscle type, pig breed, pig diet, etc. The possible reason for the difference of minerals between MSP and PP could be the incorporation of blood and other soft tissue that were leaked from the bone marrow cavity while the bone was partially broken by the mechanical force. By going through the separation process, the proportion of proteins in MSP was different from normal pork muscle. Hemoglobin from blood could increase the iron content in MSP. The mechanical separation process was reported to increase the hemoglobin and NHI content (Ockerman et al., 1981). Another reason is that collagen, which is mostly devoid of iron, was partially removed from the MSP. It was reported that calcium content of MSM was always higher than hand deboned meat as the fine bone particles go through the deboner and are left in the MSM soft tissue (Field, 1982). The calcium content in MSM is increased with the increase of yield (Field, 1982), which is possibly related to the pressure or hole size setting of the deboner. The maturity of bone also affects the bone content in MSM, as mature animals have higher calcium in bones (Field, 1982).

Table 3-4 Sodium, calcium, magnesium, and iron content in pork picnic (PP) and mechanically separated pork (MSP).[1]

Mineral (ppm)[2]	Sodium	Calcium	Magnesium	Iron
PP	431±24[b]	45±2[b]	167±4[a]	7±1[b]
MSP	838±27[a]	729±191[a]	126±12[b]	35±4[a]

[a-b]Means with different letters in the same column are significantly different ($p<0.05$)
[1]Values are means of 3 replicates ± standard deviation
[2]PP: pork picnic; MSP: mechanically separated pork

3.5.6 Raw meat pH

The pH of postmortem meat is usually around 5.8, and varies by species, muscle type and genotype, living conditions, etc. (Hambrecht et al., 2005; Puolanne et al., 2002).

As shown in **Table 3-5**, the pH of the PP was significantly lower (p<0.05) than MSP. Even though the BM and MSP were recovered from the same bone material, the MSP was higher in pH value compared with BM. One might expect that the pH of BM and MSP could be the same and higher than the PP as the inner muscle attached to bones could have slightly higher pH than the muscles of boneless picnic as pork picnic consists of multiple different muscles. Another explanation for the higher pH is the incorporation of bone marrow or tissue fluid from the bone cavity which has higher pH (~ 6.8-7.4) (Ockerman et al., 1981). The higher pH of MSP agreed with findings of Ockerman et al., (1981). They believed that the higher pH was due to the incorporation of bone marrow (pH 6.8-7.4). The higher pH of MSP could also be a result of higher basic free amino acid released by protein dissociation and degradation during separation process or following chilling and frozen storage (Braggins et al., 1999).

Table 3-5 pH of pork picnic (PP), meat trim from bone (BM), MSP-delay (MSP-DL) and MSP-standard (MSP-STD) during frozen storage.[1]

Storage period	1 month	4 months
PP[2]	5.93±0.06[b]	5.93±0.02[b]
BM	6.24±0.23[ab]	-
MSP-DL	6.43±0.13[a]	6.46±0.10[a]
MSP-STD	6.42±0.13[a]	6.49±0.12[a]

[a-b]Means with different letters in the table are significantly different (p<0.05)
[1]Values are means of 3 replicates ± standard deviation
[2]PP: pork picnic; BM: meat trim from bone; MSP-DL: delay chilled mechanically separated pork; MSP-STD: standard mechanically separated pork

The pH of pork samples was relatively stable during frozen storage for three months, with that of MSP ranging from 6.4 to 6.5. The pH of MSP from pork backbone was reported as 6.3 and was relatively stable during storage, while hand-deboned semimembranosus muscle was 5.9 according to Field (1982). The basic calcium phosphate in powdered bone in the MSP proved of little effect on meat pH (Field, 1982). The results in this study were comparable.

3.5.7 Raw meat colour

The BM and PP were similar in colour (**Table 3-6**), but BM was slightly dull and pale. The MSP samples all showed much lower L*, but higher a* and b* than BM and PP (p<0.05). This could be due to the different amount of hemoglobin and myoglobin in MSP, and higher infusion of oxygen inside finely minced muscle of MSP. According to **Table 3-4**, the MSP had about 5 times

higher iron content than PP indicating higher amount of heme containing proteins in MSP which consists of hemoglobin and myoglobin that are red in colour.

Table 3-6 Colour of pork picnic (PP), meat trim from bone (BM), MSP-delay (MSP-DL) and MSP-standard (MSP-STD) during frozen storage.[1]

Treatments[2]	Storage (month)	L*	a*	b*
PP	1	59.53±1.62^{a}	24.31±2.20^{b}	21.48±1.45^{bc}
	4	58.53±2.16^{a}	24.60±1.70^{b}	21.16±1.48^{bc}
BM	1	59.12±2.61^{a}	22.36±1.00^{b}	20.68±0.22^{c}
MSP-DL	1	48.51±5.82^{b}	34.11±1.03^{a}	27.67±1.78^{a}
	4	48.34±0.49^{b}	31.47±2.02^{a}	25.58±1.22^{a}
MSP-STD	1	49.34±3.50^{b}	35.79±2.05^{a}	29.24±2.12^{a}
	4	48.87±0.56^{b}	31.82±1.72^{a}	25.18±1.17^{ab}

$^{a-c}$Means with different letters in the same column are significantly different (p<0.05)
[1]Values are means of 3 replicates ± standard deviation
[2]PP: pork picnic; BM: meat trim from bone; MSP-DL: delay chilled mechanically separated pork; MSP-STD: standard mechanically separated pork

According to Wismer-Pedersen (1959), the apparent meat colour, light reflectance or light scattering could be affected by the muscle structure without any pigment content changes. Goldspink (1964) has a theory that the precipitated sarcoplasmic protein masks the red colour of sarcoplasm which makes the meat pale in colour in PSE pork. MSP analyzed in this study had darker and redder colour which could be a combination of enriched pigment proteins and the muscle structural change. Similarly, Field (1982) reported that MSM was darker in colour with 25-35% higher colour level than that of pork and beef muscle of similar protein and fat level. Sanchez et al. (1979) tested the total pigment of MSM and hand deboned meat; they found out that total pigment of MSM (7-10 mg/g meat) was much higher than that of hand deboned meat (1.5-6.7 mg/g meat). The colour intensity of MSM has logical relationship with the pH, since the marrow (pH 7.0-7.4) increased the pH and colour intensity of MSM simultaneously (Field, 1982). It was suggested that MSM from regions with little red marrow (hemoglobin) would present similar colour to hand deboned meat (Field, 1982). The colour of fresh MSM was bright red colour, but dull brownish red in colour after pigment oxidation (Field, 1982). Ockerman et al. (1981) suggested that the darker colour was attributed to the higher heme pigments from bone marrow and removal of connective tissue. Our result agreed with what has been reported in the literature.

During 4 months of frozen storage, the L* of PP and MSPs remained stable, however the a* and b* decreased (p<0.05). No colour difference was observed due to the chilling rate. According to the protein solubility results in section 3.4.10, the percent sarcoplasmic protein in meats was relatively stable during storage and with slight decrease after 4 months of storage (p>0.05). The colour change of meat during frozen storage is usually correlated to the oxidative stress with decreased a* and b* observed, for the pro-oxidants formed through lipid oxidation can promote the transition of oxymyoglobin to metmyoglobin which is dark brownish in colour (Frankel, 1998). The colour change of raw meats agreed with the literature.

3.5.8 Lipid oxidation

The lipid oxidation levels of PP, BM and MSP were not significantly different during both 1 and 4 months of frozen storage (**Table 3-7**). The BM was finely ground to mimic the very fine particle size reduction of MSP. Thus, it is reasonable that the TBARS value of BM was numerically closer to that of MSP. The extent of lipid oxidation of PP and MSP-DL and -STD was quite stable during frozen storage. The lipid oxidation level of all samples at two storage periods were not significantly different (p>0.05), which meant there was no interaction between sample treatments and storage periods. However, when comparing PP to MSP across both storage periods, MSP-DL and MSP-STD were significantly higher in lipid oxidation level compared with PP with p=0.0284 and standard error of 0.1395. The reasons for higher lipid oxidation in MSP include: higher lipid content in MSP, and higher iron and sarcoplasmic protein including myoglobin and hemoglobin in MSP which can catalyse lipid oxidation according to Zhou et al. (2016). From the processing perspective, the deboning process increased the meat temperature by generating friction and heat, and increased surface area of MSP by mincing it into fine particles. This process increased the contact area of oxygen to meat, which made it more prone to oxidation. The higher TBARS value of BM than PP could be explained by its greater surface area and resulting increased oxygen exposure and by the heat generated during hand trimming and heat generated in the grinder with the finer hole size to mimic the fine particle size of MSP. The rate of lipid oxidation is closely related to the unsaturated fatty acid content in MSM (Field, 1982).

Table 3-7 Lipid oxidation (mg malondialdehyde/kg meat) of pork picnic (PP), meat trim from bone (BM), MSP-delay (MSP-DL) and MSP-standard (MSP-STD) during frozen storage.[1]

Storage period[2]	1 month[ns]	4 months[ns]
PP	0.49±0.07	0.43±0.03
BM	0.71±0.24	-
MSP-DL	0.85±0.28	0.73±0.29
MSP-STD	0.73±0.33	0.73±0.42

[ns]not significant (p>0.05)
[1]Values are means of 3 replicates ± standard deviation
[2]PP: pork picnic; BM: meat trim from bone; MSP-DL: delay chilled mechanically separated pork; MSP-STD: standard mechanically separated pork

MSP was reported to show higher TBARS values during 18 weeks frozen storage compared with mechanically separated beef or mutton which is usually associated with decreased taste acceptability (Meiburg et al., 1976). According to Kramer & Sebranek (1990), increasing the level of MSP in fermented snack sausage significantly increased level of TBARS values of product ($p<0.05$), however, the TBARS value didn't exceed 1.1 even at 30% MSP substitution after 84 days of storage. NHI is regarded as the most important oxidation promoter in meat (Kanner et al., 1991). Silberstein & Lillard (1978) found that mechanical deboning process increased hemoglobin and NHI content in mechanically deboned fish but showed little effect on the amount of myoglobin in flesh. Myoglobin was reported to have greater catalytic effect on lipid oxidation than hemoglobin, and hemoprotein has direct influence on oxidative activity in fish muscle extracts (Silberstein & Lillard, 1978). Thermal processes and refrigerated storage of cooked meats could break down heme iron from porphyrin ring of myoglobin to NHI (Estévez & Cava, 2004). Heme oxidation would further stimulate lipid peroxidation (Field, 1988) and develop undesired aromas in meat products. As MSP had higher iron content as analyzed in this study, the possible explanation for higher lipid oxidation level than PP could be the NHI and heme oxidation catalyzed lipid oxidation. The increased heme and calcium content, and air exposure were assumed to have helped increase the level of lipid oxidation of MSP.

3.5.9 Transmission electron microscopy (TEM)

The TEM imaging (**Figure 3-3**) revealed that PP tissue had well preserved sarcomere structure (A & B: arrowheads) indicated by closely packed actin-myosin filaments bundles with

sharp borders, and clear z-line (B: double arrowheads). But MSP showed randomly oriented, curved and twisted actin-myosin filaments indicating high level of ultrastructural disruption (E & F: arrowheads). The enlarged myofibrils in disrupted myocyte were separated by large, debris-filled spaces (E: double arrowheads). It was reported that high pressure (520 MPa for 260 s at 10 °C) treated bovine muscles had increased lysosomal enzyme activity by interrupting the lysosome membrane, and the increased activity was maintained during aging (Jung et al., 2000). Simply observing the TEM images of MSP is not enough to assume how much lysosomal protease has been activated, but support my hypothesis that the amount of muscle cellular level disruption caused by the mechanical deboning could lead to increased enzyme activity in muscle tissue. Future research on this concept is warranted.

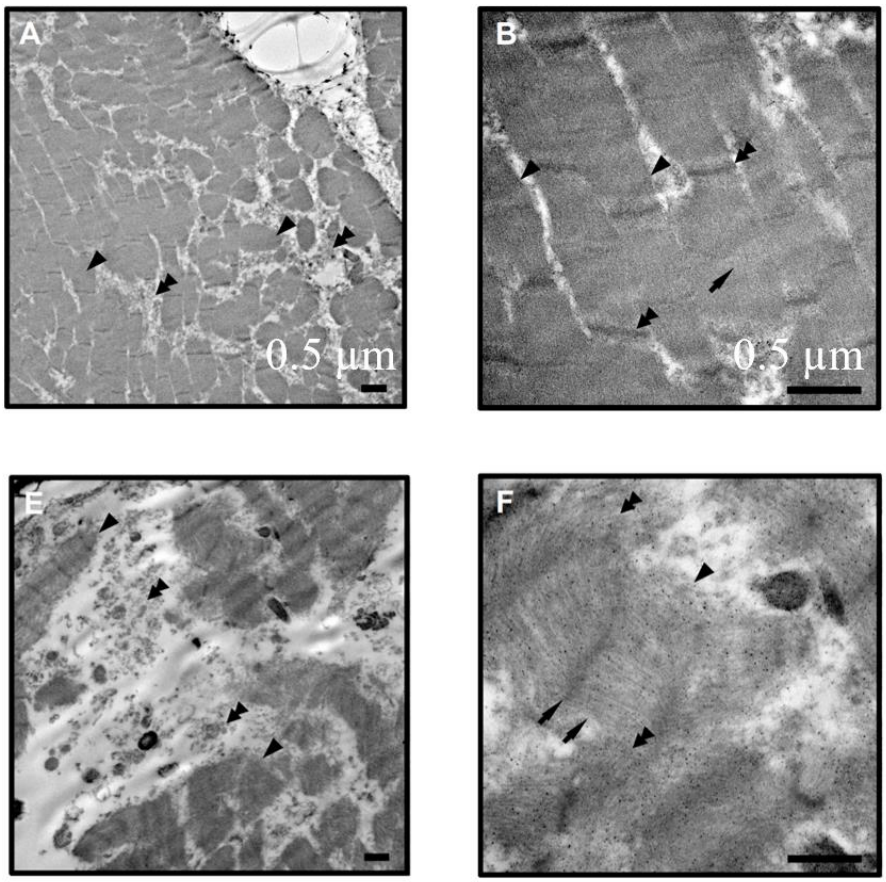

Figure 3-3 Images of pork picnic (PP) muscle and mechanically separated pork (MSP) at 5,000 × and 20,000 × magnification by transmission electron microscopy (TEM). A. PP at 5,000 × magnification; B. PP at 20,000 × magnification; E. MSP at 5,000 × magnification; F. MSP at 20,000 × magnification

3.5.10 Protein solubility

Protein solubility is an indicator of protein denaturation (Miller et al., 1980). **Table 3-8** shows protein solubility of meats during 1-4 months of frozen storage. **Table 3-9** and **3-10** show the protein solubility of meats across storage time, and the effects of frozen storage respectively. The total protein solubility of PP was around 81% at 0 to 1 month of frozen storage, much higher than that of BM (~72%). The main reason could be the higher amount of connective tissue in BM (**Table 3-3**). MSP had higher protein solubility than BM which could be explained by the lower amount of connective tissue in MSP samples by the selective separation during the mechanical separation process. Part of the connective tissue in BM would have been too tough and big in size to pass through the sieve of the soft tissue separator, which was screened out with cartilage and big pieces of bone fragments when MSP was made.

Table 3-8 Sarcoplasmic protein and myofibrillar protein solubility of pork picnic (PP), meat trim from bone (BM), MSP-delay (MSP-DL) and MSP-standard (MSP-STD) during frozen storage.[1]

Treatments[2]	Storage (month)	Sarcoplasmic protein (mg/g meat)[ns]	%Sarcoplasmic protein of total protein	Myofibrillar protein (mg/g meat)	%Myofibrillar protein of total protein[ns]	Total soluble protein (mg/g meat)[ns]	%Total soluble protein of total protein[ns]	Ratio of myofibrillar protein to sarcoplasmic protein (w/w)
PP	0	35.95±8.14	35.95±4.03^{ab}	84.48±10.67^{a}	47.56±5.36	148.36±18.67	83.51±9.29	1.32±0.04^{ab}
	1	63.26±8.47	35.60±4.21^{ab}	85.17±8.88^{a}	47.95±4.26	148.44±17.36	83.58±8.44	1.35±0.04^{a}
	4	58.33±1.56	32.87±0.54^{ab}	74.63±4.29^{ab}	42.05±2.23	132.97±5.67	74.95±2.78	1.28±0.05^{abc}
BM	0	58.08±9.04	33.29±3.91^{ab}	74.45±1.24^{ab}	40.41±5.01	128.19±2.81	69.55±8.30	1.24±0.27^{abcd}
	1	56.17±6.03	30.23±1.22^{b}	69.83±3.93^{ab}	38.16±7.21	126.01±2.14	73.47±14.79	1.26±0.19^{abc}
MSP-DL	0	63.84±4.98	41.52±6.20^{ab}	61.59±6.31^{b}	40.10±6.87	125.42±11.09	81.63±12.99	0.96±0.04^{d}
	1	61.30±6.72	40.83±0.35^{ab}	67.50±6.48^{ab}	45.03±1.84	128.80±13.06	85.87±2.07	1.11±0.04^{abcd}
	4	57.32±2.96	38.50±4.71^{ab}	60.37±3.84^{b}	40.76±7.24	117.69±5.07	79.25±11.71	1.05±0.08^{bcd}
MSP-STD	0	61.44±4.80	40.56±5.19^{ab}	62.96±6.75^{b}	41.82±8.19	124.40±10.12	82.38±13.08	1.03±0.10^{cd}
	1	65.04±5.95	43.39±0.84^{a}	71.05±2.50^{ab}	47.56±2.61	136.09±7.92	90.95±1.78	1.10±0.08^{abcd}
	4	59.12±6.81	39.68±5.81^{ab}	61.38±7.12^{b}	41.32±7.24	120.49±13.28	81.00±12.77	1.04±0.07^{cd}

$^{a-d}$Means with different letters in the same column are significantly different (p<0.05)

^{ns}not significant (p>0.05)

[1]Values are means of 3 replicates ± standard deviation

[2]PP: pork picnic; BM: meat trim from bone; MSP-DL: delay chilled mechanically separated pork; MSP-STD: standard mechanically separated pork

Table 3-9 Sarcoplasmic protein and myofibrillar protein solubility of pork picnic (PP), MSP-delay (MSP-DL) and MSP-standard (MSP-STD).[1]

Treatments[2]	Sarcoplasmic protein (mg/g meat)[ns]	%Sarcoplasmic protein of total protein[ns]	Myofibrillar protein (mg/g meat)	%Myofibrillar protein of total protein[ns]	Total soluble protein (mg/g meat)	%Total soluble protein of total protein[ns]	Ratio of myofibrillar protein to sarcoplasmic protein (w/w)
PP	61.82±1.99	34.81±1.98	81.43±2.77[a]	45.85±2.76	143.25±4.07[a]	80.68±4.61	1.32±0.03[a]
MSP-DL	60.82±1.99	40.38±1.98	63.15±2.77[b]	41.96±2.76	123.72±4.07[b]	82.25±4.61	1.04±0.03[b]
MSP-STD	61.86±1.99	41.21±1.98	65.13±2.77[b]	43.57±2.76	132.72±4.07[b]	84.78±4.61	1.05±0.03[b]

[a-b]Means with different letters in the same column are significantly different (p<0.05)
[ns]not significant (p>0.05)
[1]Values are means of 3 replicates ± standard deviation
[2]PP: pork picnic; MSP-DL: delay chilled mechanically separated pork; MSP-STD: standard mechanically separated pork

Table 3-10 Effect of frozen storage on sarcoplasmic protein and myofibrillar protein solubility of pork picnic (PP), MSP-delay (MSP-DL) and MSP-standard (MSP-STD).[1]

Storage[-]	Sarcoplasmic protein (mg/g meat)[ns]	%Sarcoplasmic protein of total protein[ns]	Myofibrillar protein (mg/g meat)[ns]	%Myofibrillar protein of total protein	Total soluble protein (mg/g meat)	%Total soluble protein of total protein[ns]	Ratio of myofibrillar protein to sarcoplasmic protein (w/w)
0 month	63.05±1.99	39.35±1.74	69.67±2.72	43.16±1.86[ab]	132.72±4.07	82.50±3.97	1.10±0.02[b]
1 month	63.20±1.99	39.94±0.83	74.58±2.17	46.85±1.86[a]	137.77±4.07	86.80±1.71	1.19±0.02[a]
4 months	58.26±1.99	37.01±1.44	65.46±1.76	41.38±1.86[b]	123.72±4.07	78.40±3.38	1.12±0.02[b]

[a-b]Means with different letters in the same column are significantly different (p<0.05)
[ns]not significant (p>0.05)
[1]Values are means across treatments ± standard error

The soluble sarcoplasmic, myofibrillar protein, and total soluble protein in PP, and MSP as a percentage of total protein were not different (p>0.05). The soluble sarcoplasmic protein in PP and MSPs was not different (p>0.05). However, the PP had higher soluble myofibrillar protein and total soluble protein than MSPs. This could be partially explained by the slightly higher total protein content in PP (**Table 3-1**). The ratio of myofibrillar protein to sarcoplasmic protein for BM and PP were around 1.24 and 1.32 respectively. However, the ratio for myofibrillar protein to sarcoplasmic protein for MSPs was around 1.05, significantly (p<0.05) lower than that of PP (**Table 3-9**). The lower ratio of myofibrillar protein in MSPs might be explained by the migration of water-soluble protein (blood) from bone marrow or degradation products of salt soluble protein, but more analysis is needed to clarify the phenomenon. During frozen storage, the ratio of myofibrillar protein to sarcoplasmic protein increased after 1 month of storage for all pork samples possibly due to the freezing effect on the extractability of myofibrillar protein, and decreased during 1-4 months of frozen storage, which meant that the myofibrillar protein was more susceptible to frozen storage with decreased solubility as a result.

During frozen storage, the protein solubility of sarcoplasmic protein and myofibrillar protein were relatively stable during the four-month frozen period. MSP-STD samples showed slightly higher sarcoplasmic and myofibrillar protein solubility than MSP-DL, but the differences were not statistically significant. Farouk et al. (2003) reported that beef muscle sarcoplasmic protein solubility increased during frozen storage at -18 °C, while myofibrillar protein solubility decreased. The length of frozen storage and freezing rate interaction would influence the protein functional properties. Our results showed that the solubility of sarcoplasmic protein was not influenced by 4 months of frozen storage, minor change of myofibrillar protein solubility was observed. Increasing replications and length of storage may give more information on the change of protein solubility over time.

According to Chen et al. (2016), the myofibrillar protein from PSE pork had smaller particle size after endogenous protease degradation and oxidation, which enhanced the solubility. This might help explain that the solubility of myofibrillar protein in MSP is not necessarily directly correlated with the gelation ability of MSP. It has been reported that high oxygen packaging could induce lipid and myoglobin oxidation and aggregation of myosin (Kim et al., 2010). In MSP, the fine particle size of meat created by the mechanical separation process increased the meat surface area and oxygen exposure, which might influence the protein solubility as a result.

3.5.11 SDS-PAGE of NAM

A representative (Rep 3) NAM profile of PP and MSP before and after one month of -18 °C frozen storage is shown in **Figure 3-4**. Compared with PP, the NAM of MSP showed proteolytic change of certain bands, especially the MHC, which is known to be responsible for meat protein gelation (Xiong, 2004). Multiple intense bands around 130 to 150 kDa were observed in the NAM of MSP, and they were slightly different in molecular weight and more intense than what had been observed in NAM of PP. More unidentified bands were distributed in lanes of NAM from MSP giving darker background. Bands around 17 and 9 kDa were only observed in NAM from MSP samples. The MHC intensity of PP at both 0 month and 1 month was higher than that of MSP-DL, MSP-STD indicating the degradation of MSP.

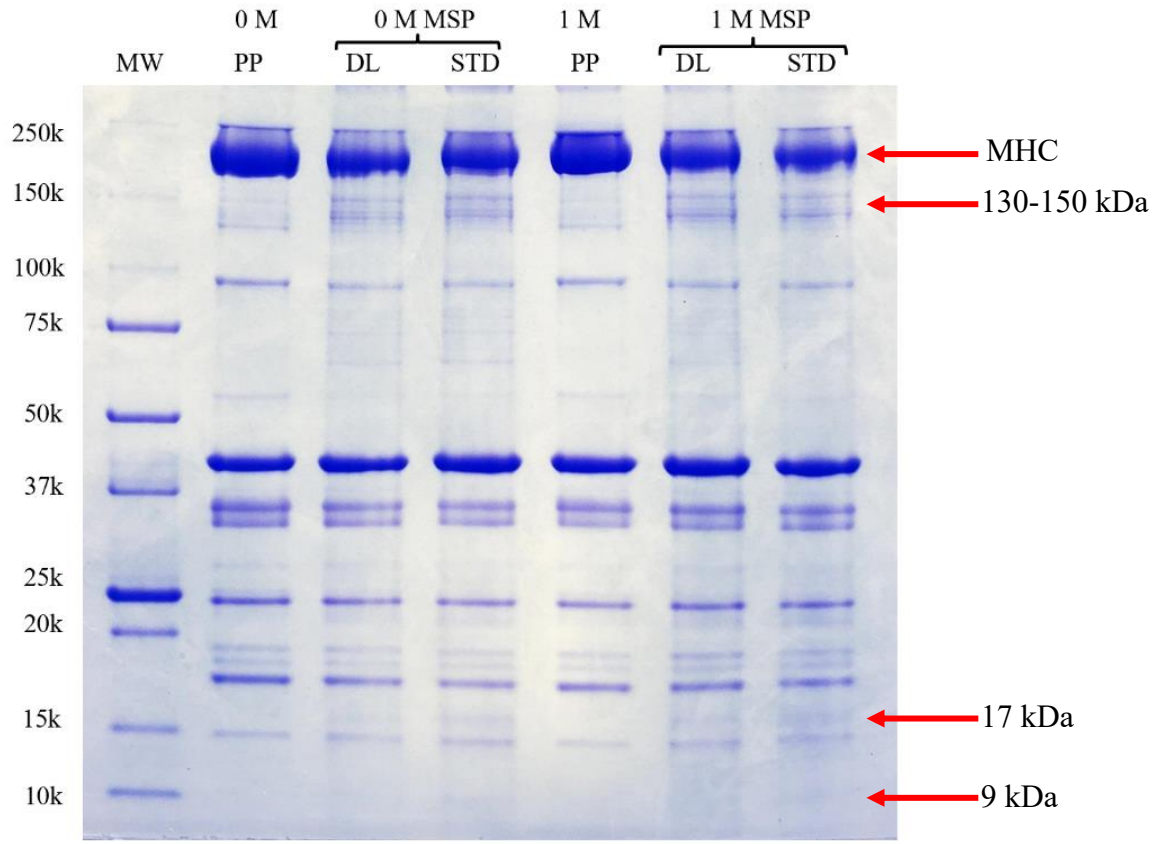

Figure 3-4 Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (4-20% gel) profile of natural actomyosin (NAM) (12 µg protein loaded in each lane) extracted from pork picnic (PP) and delay chilled mechanically separated pork (MSP-DL) and standard mechanically separated pork (MSP-STD) at 0 and 1 month of frozen storage at -18 °C. MW: molecular weight marker; 0 M: before blast freezing; 1 M: 1 month of frozen storage; MHC: myosin heavy chain

According to Park et al. (1996), pork myosin went through proteolytic degradation after the surimi like pork was incubated at 50 °C for up to 90 min in a salt dependent manner at pH of 6.8-7.5. The MHC band decreased upon heating with new bands at 138, 128, 120, 86, 77, 64 kDa, and cleavage was site specific. Our result was similar to what they have reported, however the cleavage was more random with certain more intense new bands observed. Wang and Xiong (1998) also reported that bovine cardiac muscle had gel-weakening effect after 2 h (pH 6.0) preincubation at 50 °C, the MHC was partially hydrolyzed after incubation with new polypeptides around 130 kDa. The addition of several kinds of protease inhibitors successfully inhibited degradation of MHC, indicating that the degradation was a result of endogenous protease proteolysis, most likely lysosomal proteases (cathepsins). Boles et al. (2000) reported that myosin recovered from beef bone went through degradation with 4% sodium chloride, 4% sodium chloride with 0.3% STPP, 0.3% tetrasodium pyrophosphate or 0.05 M sodium hydroxide. In their study, the bones were sliced and chopped into 2.5-5 cm pieces for protein extraction. The reason for degradation in the current study was not clear at this point. The suspected explanation is either unknown proteolytic factor from bone cavity or crushed muscle cells, or physical cell damage resulting from the high pressure generated by the mechanical deboning equipment. As stated in the literature review, calpain is active at neutral pH with certain Ca^{2+} concentration, but it goes through autolysis quickly post-mortem, while several cathepsins are only active at acidic pH. In our case, the condition of MSP was not perfect for either of these types of enzyme. More specific analysis is needed to further clarify the phenomenon.

3.5.12 Immunoblotting of NAM

In order to confirm the degradation of the MHC bands around 200 kDa in size, the NAM of PP and MSP during frozen storage was separated on a 4-20% SDS-PAGE gel, and probed with monoclonal MHC antibody from mouse. **Figure 3-5-A** shows MHC of PP and MSP-STD after processing, and after 1-7 months of frozen storage, and **Figure 3-5-B** shows MHC of MSP-DL and MSP-STD during frozen storage. The MHC from MSP had multiple degraded bands at lower molecular mass of around 150 kDa, but MHC from PP didn't show any clear sign of degradation. This analysis confirmed the assumption that MHC from MSP went through protein degradation to certain extent. The effect of chilling rates and frozen storage on the degradation of MHC was not obvious, indicating that degradation occurred early in the separation process and did not appear to

increase with time. This phenomenon was similar to what has been reported by Boles et al. (2000). They found the MHC in salt soluble protein extracted from beef bones had proteolytic degradation during the extraction process as indicated by myosin antibodies.

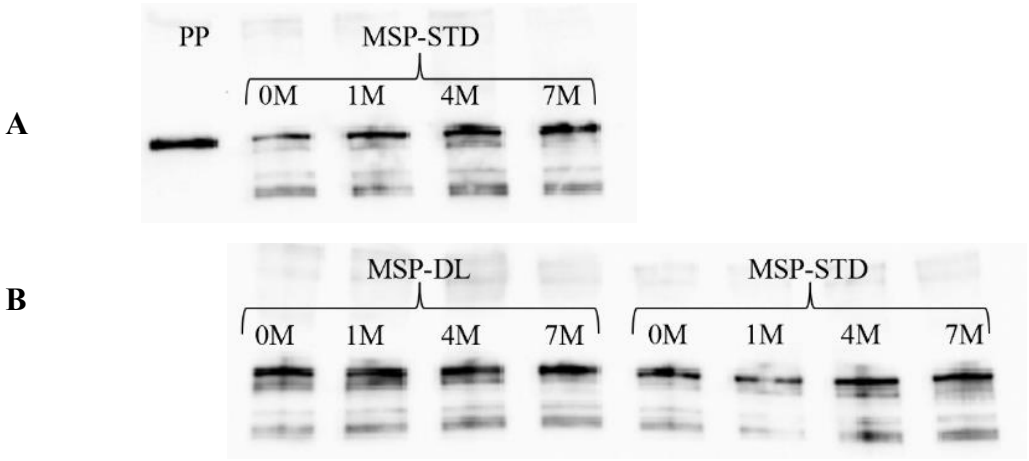

Figure 3-5 A. Western blot of myosin heavy chain (MHC) from natural actomyosin (NAM) extracted from pork picnic (PP) and standard mechanically separated pork (MSP-STD) after 0-7 months of frozen storage; B. Western blot of MHC from NAM extracted from delay chilled mechanically separated pork (MSP-DL) and MSP-STD after 0-7 months of frozen storage. Proteins were transferred from 4-20% SDS-PAGE gel and probed with MHC antibody from mouse and anti-mouse horseradish peroxidase conjugated antibody. 0M: after MSP processing; 1, 4, 7M: 1, 4, 7 months of frozen storage

3.5.13 Dynamic oscillatory rheology

Dynamic oscillatory rheology was conducted on NAM (20 mg/g) from PP and MSPs following 1, 4 and 7 months of frozen storage and MSP right after manufacturing (0 M MSP-STD). The G' (elastic behaviour), G" (viscous behaviour) and δ of NAM during the heating cycle from 25 to 85 °C have been graphed showing the viscoelastic properties of NAM.

The G' of PP (**Figure 3-6**) increased with the increase of temperature at the beginning, shouldered at 35 °C and peaked around 42 °C. According to Sano et al. (1990), and Xiong and Blanchard (1994), the increase of G' at 30-45 °C was due to the interaction among myosin molecules, which helped with development of gel elasticity. The G' decreased considerably after further heating until the lowest value (6.43 Pa) was observed at 58-59 °C, which is known as a typical denaturation temperature of myosin (Doerscher et al., 2003). According to Wang et al. (2009), the decline of G' between 43 and 59 °C is often associated with the loss of α-helical structure, which accompanies disruption of hydrogen bonds. After this point, the G' steadily

increased to 57.85 Pa. The G' profile of NAM from PP was similar to the pork NAM profile that has been reported by Wang et al. (2009). After temperature went above 59 °C, further irreversible elastic protein network development through protein unfolding and cross-linking was reflected by increased G'. The influence of 1-7 months of frozen storage on rheological profile of NAM from PP was negligible. Compared with PP, 0M MSP-STD (**Figure 3-7**) had very comparable G' profile. However, after one month of frozen storage, the profile shifted. The G' slightly increased with the increase of temperature at the beginning up to around 32 °C, then began to slightly decrease until around 42 °C. Upon further heating, the G' peaked around 45 °C, followed by a sharp decrease until the lowest value was reached at 58 °C. The slope of G' of NAM from MSPs was much lower than that of PP after 58 °C, showing the lower elasticity of gel and poorer development of a 3-D network. Prolonged frozen storage up to 7 months decreased G' value over all heating cycles but with the shape of curve remaining unchanged. The MSP-STD and DL (**Figure 3-7, 3-8**) showed comparable profiles and MSP-DL had even flatter curve after 58 °C. The final G' values for MSP-STD and MSP-DL were 30.46, and 10.69 Pa respectively after 1-month frozen storage.

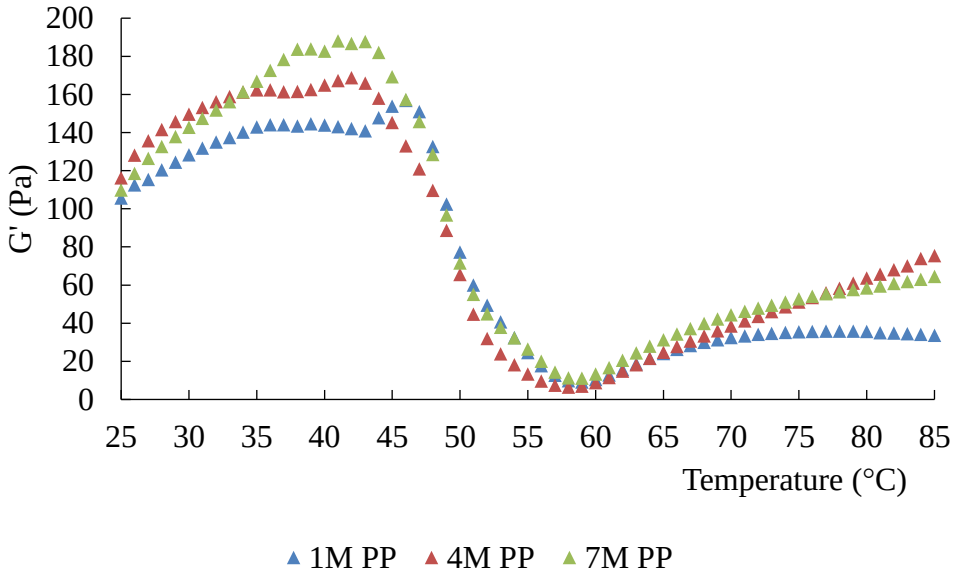

Figure 3-6 Storage modulus (G') of natural actomyosin (NAM) from pork picnic (PP) after 1, 4, and 7 months of frozen storage with heating rate of 1 °C/min. M: months of frozen storage

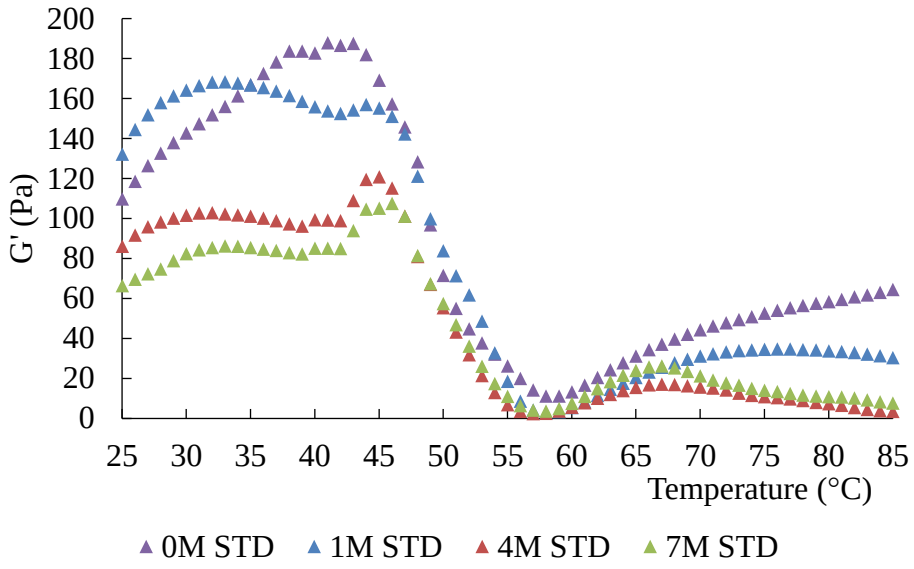

Figure 3-7 Storage modulus (G') of natural actomyosin (NAM) from standard mechanically separated pork (MSP-STD) before frozen storage and after 1, 4, and 7 months of frozen storage with heating rate of 1 °C/min. M: months of frozen storage

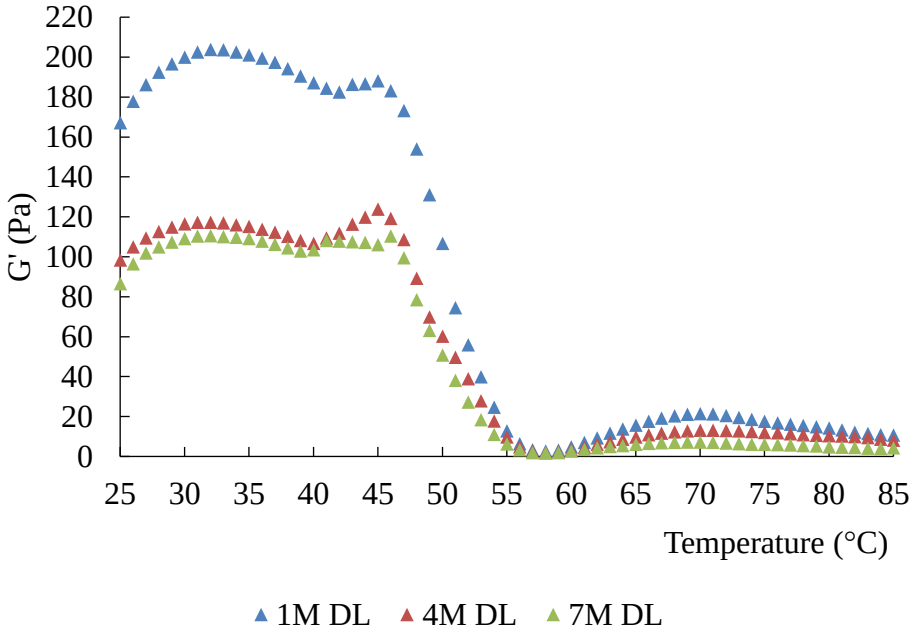

Figure 3-8 Storage modulus (G') of natural actomyosin (NAM) from delay chilled mechanically separated pork (MSP-DL) after 1, 4, and 7 months of frozen storage with heating rate of 1 °C/min. M: months of frozen storage

According to Yasui and Samejima (1990), the thermal-gelation of myosin consists of two steps: (1) aggregation of myosin head at 43 °C; (2) myosin tail helix-coil transition forms cross-

linking at 55 °C. In **Figure 3-7, 3-8**, the G' increased after heating from 25 to 33 °C, and started to decrease before 43 °C unlike PP (**Figure 3-6)**, where the G' kept increasing until 42 °C. This phenomenon suggested a structural change of myosin from MSP, with possible changes in the myosin head region.

The actomyosin gel from MSP was less heat-stable than that from regular hand deboned PP. According to Visessanguan and An (2000), degraded myosin is less heat-stable after losing its structural domains. It has weaker gel structures than intact myosin, as indicated by the lower gel modulus; despite that it still has cross-links and deposition of small fragments. In this study, the partially fragmented MHC presented by SDS-PAGE and immunoblotting helped explain the decreased G' during heat-induced gelation. The loss in elasticity of myofibrillar protein gel from MSP would negatively decrease the gel firmness of comminuted meat product which was shown in Study two in the next chapter.

The G" of PP (**Figure 3-9**) and 0M MSP-STD peaked around 47 °C, but the G" of frozen stored MSPs peaked around 45 °C. Further heating led to quick decrease of G" until the lowest value was observed at around 58 °C, which is in accordance with the lowest point for G'. The NAM gel lost its viscous property but developed an elastic gel after further heating (Wang et al., 2009). Frozen storage of MSP (**Figure 3-10, 3-11**) led to decreased G" value over heating curve. The difference between MSP-DL and MSP-STD was not obvious.

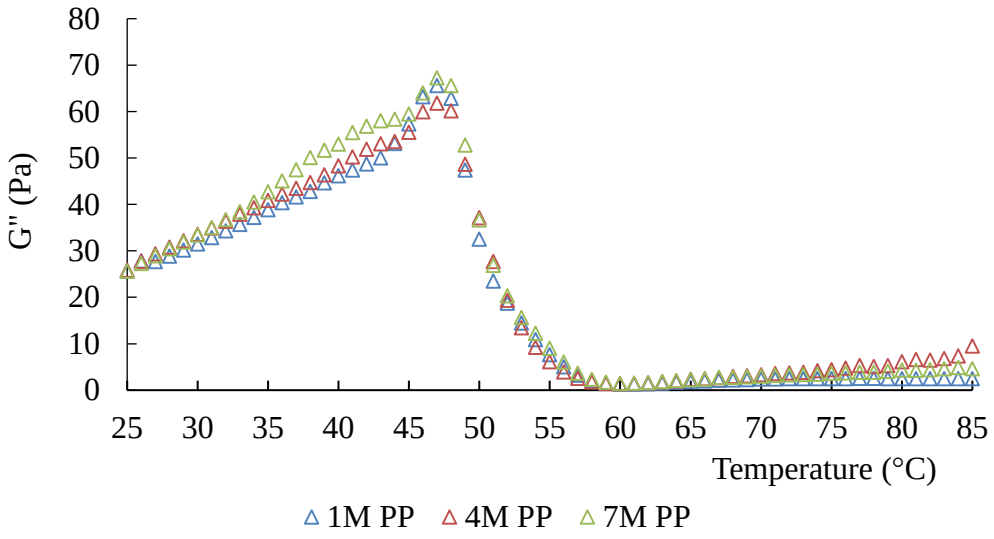

Figure 3-9 Loss modulus (G") of natural actomyosin (NAM) from pork picnic (PP) after 1, 4, and 7 months of frozen storage with heating rate of 1 °C/min. M: months of frozen storage

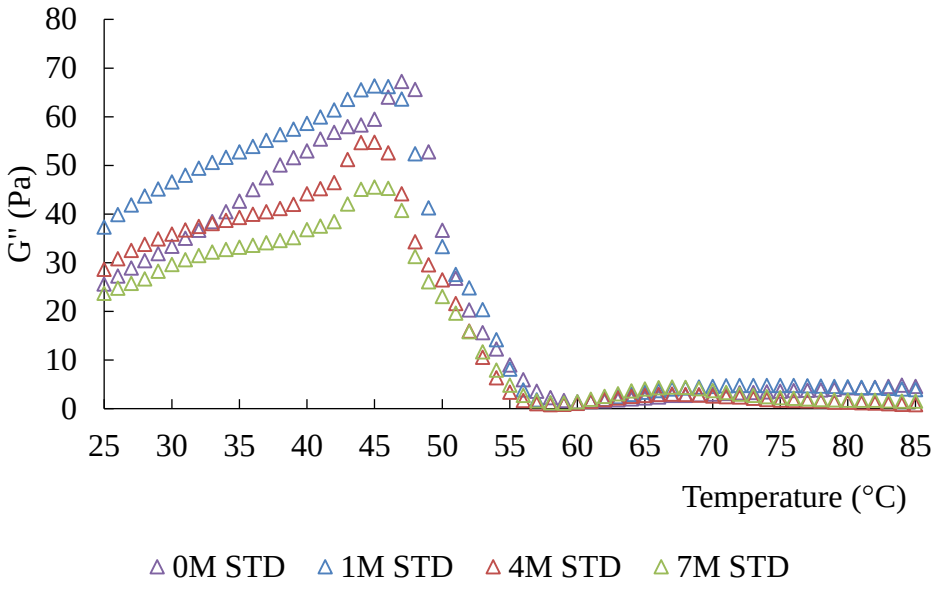

Figure 3-10 Loss modulus (G”) of natural actomyosin (NAM) from standard mechanically separated pork (MSP-STD) before frozen storage and after 1, 4, and 7 months of frozen storage with heating rate of 1 °C/min. M: months of frozen storage

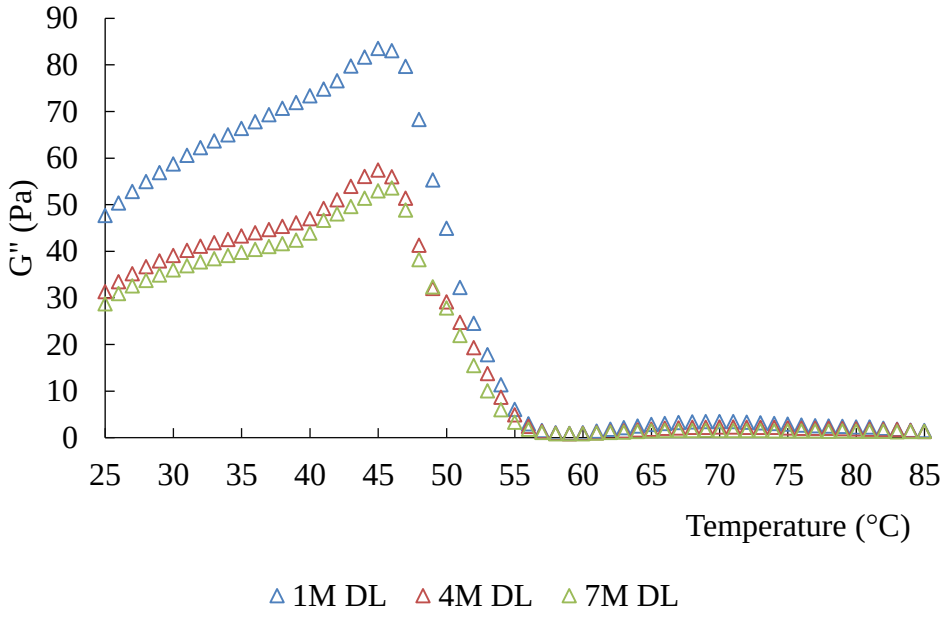

Figure 3-11 Loss modulus (G”) of natural actomyosin (NAM) from delay chilled mechanically separated pork (MSP-DL) after 1, 4, and 7 months of frozen storage with heating rate of 1 °C/min. M: months of frozen storage

The viscoelastic behaviour of fluid is indicated by δ. It is equal to the shear strain rate lag to the changes in the causative force. The lower tan δ indicated the more solid like gel (Niraula et al., 2003). The δ of PP (**Figure 3-12**) and 0M MSP-STD (**Figure 3-13**) both increased upon heating followed by a sharp decrease between 49 to 63 °C showing the gel networking formation process, then became stable afterwards. The δ of MSP-STD and DL (**Figure 3-14**) after frozen storage was higher than that of PP between 25 to 46 °C and 55 to 85 °C, which meant MSP had higher viscosity and lower elasticity during these temperature ranges. As temperature increased from 46 °C, the δ rather than continuing to increase like PP, went slightly down and then was maintained at around 49 °C which was the peak for PP and 0M MSP-STD. Another peak of δ was observed at around 56 °C followed by a quick decrease. Compared with δ of PP, after the temperature reached 58 °C, the NAM from MSP after frozen storage formed a less elastic but more viscous network. The difference between MSP-DL and MSP-STD was not obvious.

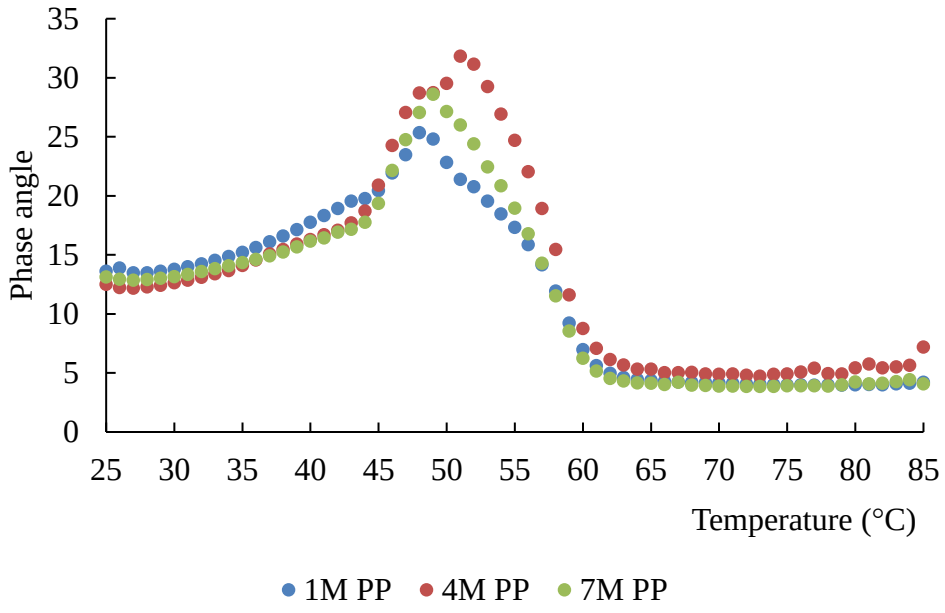

Figure 3-12 Phase angle (δ) of natural actomyosin (NAM) from pork picnic (PP) and after 1, 4, and 7 months of frozen storage with heating rate of 1 °C/min. M: months of frozen storage

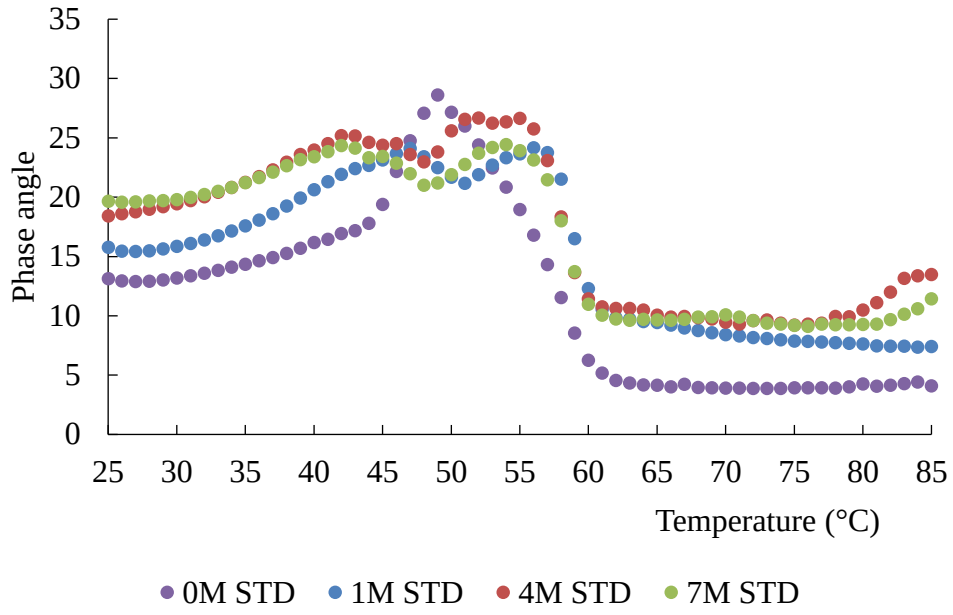

Figure 3-13 Phase angle (δ) of natural actomyosin (NAM) from standard mechanically separated pork (MSP-STD) before frozen storage and after 1, 4, and 7 months of frozen storage with heating rate of 1 °C/min. M: months of frozen storage

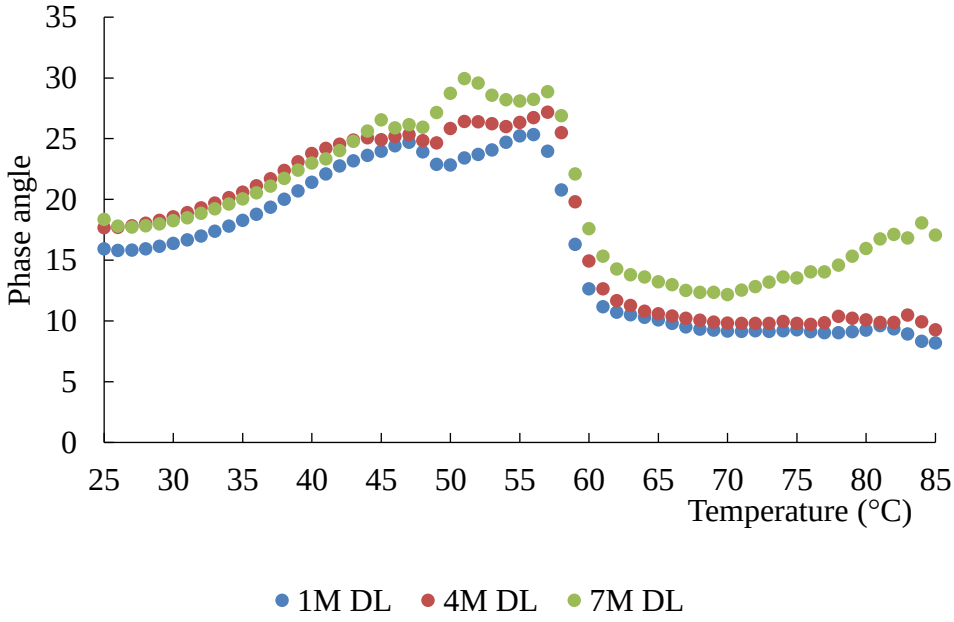

Figure 3-14 Phase angle (δ) of natural actomyosin (NAM) from delay chilled mechanically separated pork (MSP-DL) and after 1, 4, and 7 months of frozen storage with heating rate of 1 °C/min. M: months of frozen storage

In conclusion, the NAM from frozen stored MSP showed different G' pattern than PP while the NAM from MSP before storage (0M MSP-STD) was comparable. The NAM from frozen MSP had more viscous than elastic property than that of PP, especially after the denaturation of myosin and the formation of irreversible 3-D protein network after 58 °C. The difference could be possibly induced by the changes in the myosin head region, and the following heat instability due to the myosin degradation, even though the gel still had cross-links. The difference in heating pattern between MSP-DL and MSP-STD was not obvious, indicating the limited effect of initial chilling rate on the gelling ability of MSP. The down shift of G' was observed in both PP and MSPs after frozen storage, with rapid change observed during the initial 4 months of frozen storage.

3.6 Conclusion

MSP had less connective tissue, nearly two times of sodium, 16 times of calcium, and five times the iron content of PP. It was darker red in colour with higher pH (6.4-6.5) and higher level of lipid oxidation. The ratio of myofibrillar to sarcoplasmic protein in MSP was significantly lower than that of PP regardless of storage time. The mechanical separation process disrupted pork muscle tissue structure and led to degradation of MHC, which is believed to be responsible for the heat induced gelation in meat products. The dynamic rheological profile of NAM from MSP showed decreased G' compared with that of PP after heat induced crosslinking of NAM. The 4 months of frozen storage of MSP further decreased viscoelasticity of NAM, and the redness and yellowness of MSP, however other properties remained stable. The initial freezing rate of MSP didn't play a major role in preserving the myofibrillar protein in terms of protein degradation and rheological property of NAM. The lipid oxidation level of MSP remained stable during the initial 4 months of frozen storage and was not influenced by the initial chilling rate.

3.7 Connection to the next study

MSP has very fine particle size, darker colour, higher fat content, and lower gelation properties. The most common application for MSP is in comminuted emulsion type products, including frankfurter and bologna, which do not require intact muscle pieces. Analysis of the physicochemical, biochemical, and gelation properties in a meat system would help with understanding the functionalities of MSP as an ingredient in meat product manufacturing.

The next study was focused on the effect of MSP substitution levels, the frozen storage of MSP and the initial chilling rate of MSP on the texture and quality of bologna products. WHC and instrumental analysis on appearance, including colour, and texture, including TPA and torsional gelometry have been conducted. The effect of BM on bologna texture was also explored to have a better understanding of the effect of mechanical deboning process compared to physical removal of the same tissue.

4 STUDY 2: POTENTIAL UTILIZATION OF MSP AS A SUBSTITUTE OF REGULAR PORK IN BOLOGNA PROCESSING

4.1 Abstract

The objective of study two was to analyze the effect of chilling rate, standard fast chilling and delay chilling, and 4 months of frozen storage of MSP on the texture of bologna type products made with partial substitution of MSP for PP. Two levels, 7.5% and 15%, were selected to evaluate the effect of MSP addition, and the bologna treatments were formulated with the same level of total protein (12%) and fat (14%). The colour, WHC of cooked products as well as textural profile, and torsional shear values were analyzed.

The replacement of PP with 7.5% MSP did not lead to a significant negative influence on bologna texture and appearance, including WHC, torsional gelometry and TPA texture profile. The addition of MSP in formula increased (p<0.05) redness and yellowness, and decreased (p<0.05) lightness of bologna. The bologna made with 15% MSP was soft and mushy in texture with a large decrease in TPA hardness and chewiness, and red and dull in colour compared with the pink colour of the CON. The fast chilling of MSP did not help improve the gelation ability of MSP and the texture of bologna products. The effect of 1-4 months of frozen storage of MSP on the cook loss, and purge loss of bologna was not significant, but the longer storage led to the decreased (p<0.05) shear strain and increased (p<0.05) shear stress of bologna.

4.2 Introduction

MSP typically has pasty texture with pulverized and destructured muscle fibre residue, high fat content, and increased surface area. Partial substitution of regular pork with MSP is commonly used to add value. However, the amount of MSP that could be added is strictly limited by the labeling requirement and undesirable textural softness (Kramer & Sebranek, 1990). It was reported that addition of 25% or more MSP in frankfurthers led to less desirable texture according to sensory panels (Marshall et al., 1977). Kramer & Sebranek (1990) found out that 20% MSP

level in fermented snack sausage was most accepted amount 10-30% MSP substitution. Increasing the level of MSP made sausage darker and redder, and the total work required to shear through the product decreased (Kramer & Sebranek, 1990). McMillin et al. (1980) found that frankfurters with more than 30% MSP incorporation led to softer texture. However, increasing the level of MSP from 10-50% in frankfurters increased WHC (McMillin et al., 1980). In order to explore the potential utilization of MSP in bologna, it is important to understand the effect of MSP on the texture and appearance of bologna and the appropriate substitution level. Protein content is more critical in bologna texture determination, than fat content or cooking conditions (Colmenero et al., 1995). The higher protein in bologna led to tough product (Colmenero et al., 1995). It was reported that addition of 0.5 g or higher fish sarcoplasmic protein in 100 g of fish myofibrillar protein decreased the gel strength. According to Study One, MSP had lower ratio of myofibrillar protein to sarcoplasmic protein than PP. MHC from MSP was partially degraded, and the NAM showed decreased viscoelasticity than that from PP. The less desirable soft texture of bologna may be due to the extremely fine mincing process of MSP, removal of connective tissue, and poor quality of myofibrillar protein in MSP (Ockerman et al., 1981).

Chilling and frozen storage is commonly used to extend shelf life by stopping the growth and reproduction of pathogenic microorganisms, and slowing down the deleterious chemical reactions (Murano, 2003). Fast chilling is believed to reduce chilling time, moisture evaporation, and microbial population on the meat surface (Xu et al., 2012). Lower freezing rate increases myofibrillar protein denaturation (Wagner & Añon, 1985) by partial unfolding of myosin head which is a result of local ionic strength increase due to the water migration from myofibrillar space or dehydration of myofibrillar protein during freezing. Since fast chilling has the potential to help preserve the protein from further damage, which could influence the protein emulsifying or gelling capacity (Jiménez-Colmenero & Borderías, 1983), it's important to investigate the quality change and performance of MSPs initially chilled at two different rates (standard fast chilling and delayed chilling) during the 4 months frozen storage in the real meat system. The physicochemical, biochemical, and gelation properties have been analysed in Study One to help understand the role MSP plays in bologna. The present study focused on the appearance and texture changes of bologna made with 7.5% or 15% MSP-STD and MSP-DL during the initial 4 months of frozen storage.

4.3 Hypotheses

The 7.5% substitution of MSP in bologna batter would not decrease the TPA hardness of bologna. Increased substitution level of MSP would lead to textural failure of bologna product. Accelerated chilling rate of MSP (MSP-STD) helped improve the textural properties of bologna and the texture of bologna, so that using fast chilling method could increase the MSP substitution level without decreasing the texture acceptability. Increasing the length of frozen storage (shelf life of MSP for meat processing) would lead to decreased textural properties.

4.4 Materials and methods

4.4.1 Materials

4.4.1.1 Meat ingredient preparation

PP and frozen MSP used in this bologna processing were collected from Maple Leaf Foods Inc., Brandon, MB. The vacuum packaged regular PP cuts were shipped fresh to Saskatoon while the two kinds of MSP were shipped frozen.

Upon receiving (about 7 days postmortem), the PP was coarsely ground through Ø 0.95 cm hole size plate once (model AMFG-24, Biro Grinder, Marblehead, OH, USA), and well mixed in a 150 L tumbler (Glass VSM 150 Vacuum mixing machine, Rostfrei, Germany). The ground PP was vacuum packed in 46×56 cm^2 vacuum bags (polyethylene vacuum pouch, 3 μm thick, with oxygen permeability of 7.7 $cc/m^2/24h$) at 95% with vacuum packer (model 550A, Sipromac, Drummondville, QC, Canada), and stored at -30 °C before processing.

BM was treated as described in Study 1. MSP-DL and MSP-STD samples were initially stored at -30 °C after receiving, and subsampled by cutting into about 1 kg slabs (2.5×15×36 cm^3) with a band saw as described in Study 1. The sample slabs were randomly allocated into 5 groups using Latin square design for 1 and 4 months of -18 °C frozen storage study. Samples for proximate composition analysis was evenly taken from the whole MSP blocks for bologna formula development.

Frozen pork back fat (BF) was collected from Maple Leaf Inc., Brandon to adjust fat level of bologna formulations as needed.

4.4.1.2 Dry ingredient preparation

Non-meat ingredients included salt, Prague powder (6.4% sodium nitrite in salt), STPP, sodium eyrthorbate. They were obtained from the Canadian Salt Company Ltd (Pointe-Claire, QC, Canada), Innophos (Lowbanks, ON, Canada) and Unipack Packaging Products, Ltd. (Edmonton, AB, Canada), respectively.

4.4.2 Methods

4.4.2.1 Bologna formula

The formula for all bologna products manufactured included 2.23% salt, 0.29% Prague powder (6.4% sodium nitrite, 93.6% salt), 0.30% STPP, and 0.05% sodium eyrthorbate. The target protein and fat concentration of the final product were set to 12 and 14% respectively. For each MSP (MSP-DL, MSP-STD) or BM treatments, the percentage of PP and water needed was adjusted based on proximate composition of each meat ingredient to meet the protein and fat targets for final products. The control (CON) treatment was made with only PP cut as the meat ingredient. The 2 MSP treatments or BM were added at 7.5% or 15% levels of the total batter weight, the rest of meat protein and fat came from PP and BF. The batch size of each bologna formulation was around 8 kg and produced 4 chubs of bologna (~1 kg each). The processing treatment formulations are shown in **Table 4-1**. The processing was conducted following 1 and 4 months of -18 °C frozen storage of the MSP. The PP was kept at -30 °C to delay any changes to highlight effects of MSP addition, and its changes during frozen storage.

Table 4-1 Typical bologna processing formula.

Treatments/Ingredients[2] (%, w/w)	MSP/ BM	PP	BF	Ice water
CON[1]	0	65.2	5.4	26.5
BM-7.5	7.5	55.9	6.2	27.5
BM-15	15	46.7	7.0	28.5
DL-7.5	7.5	58.3	4.9	26.4
DL-15	15	51.4	4.4	26.3
STD-7.5	7.5	58.7	4.7	26.3
STD-15	15	52.2	3.9	26.0

[1]CON: bologna formulated with only regular pork as meat ingredient; BM-7.5/15: bologna formulated with 7.5 or 15% of meat trim from bone; DL-7.5/15: bologna formulated with 7.5 or 15% of delay chilled mechanically separated pork; STD-7.5/15: bologna formulated with 7.5 or 15% of standard mechanically separated pork

[2]MSP: mechanically separated pork; BM: meat trim from bone; PP: pork picnic; BF: pork back fat

Dry ingredients: 2.50% salt, 0.19% sodium nitrite, 0.30% STPP; 0.05% sodium erythorbate

4.4.2.2 Bologna processing

The frozen meat ingredients were thawed at 1 °C for 3 days ahead of bologna processing. The processing was carried out in a refrigerated meat processing room. The PP and BF were finely ground through Ø 0.48 cm hole plate once, and 3 MSP treatments were ground through Ø 0.96 cm hole plate to help homogenize the meat sample. The sequence of treatments for processing was randomized.

The lean meat ingredients were mixed with dry ingredients (salt, Prague powder, STPP, sodium erythorbate) in a bowl chopper before chopping (Model #8418D, Hobart, Troy, OH, U.S.A) and ice water was added while chopping at knife speed #1 for 1 min. The BF was added during the chopping at knife speed #3 for 2 min. After chopping, the batter was transferred to an emulsion mill (Type 1E75-F, Alexander, Remscheid, DE) to be emulsified twice. The temperature of batter was checked during chopping and milling to be kept lower than 14 °C. Before stuffing, the batter was de-aired in a vacuum tumbler (Glass VSM 150 Vacuum mixing machine, Rostfrei, Germany) until the pressure went above 27 mmHg, then tumbled for 1 minute to mix and scrap the batter to the bottom. The batter was stuffed (~ 1 kg) into water-proof casings (Walsroder KFS MATT yellow, CaseTech GmbH, Walsrode, Germany) with 63 mm stuffing diameter by a hydraulic stuffer (EI-20, Equipamientos Carnicos, S.L., Barcelona, Spain). The stuffed bologna chubs were sealed with cotton string and stored in a 1 °C cooler overnight before cooking.

4.4.2.3 Bologna cooking

Stuffed bologna chubs were cooked in an air agitated water bath (~200 L) using a five-stage thermal processing schedule: 30 min at 40 °C as the initial water temperature, increased temperature to 50 °C and held for 30 min, held at 60 °C for 30 min, then held at 70 °C for 30 min and finally set water temperature to 75 °C, cooked until internal temperature of bologna reached 72 °C and held for 5 min. The water temperature and product internal temperature was monitored using a digital thermometer (Omega TC-08 Thermocouple, Omega Engineering, Canada) with copper thermocouples (Type T) inserted in the geometric centre of one of the bologna chubs and in the water bath. The bologna chubs were removed right after cooking, and cooled in iced water for 45 min, then stored in a 1 °C cooler overnight before slicing.

4.4.2.4 Slicing of cooled bologna

Two cooled bologna chubs of each treatment were sliced for analysis based on the schematic slicing diagram below.

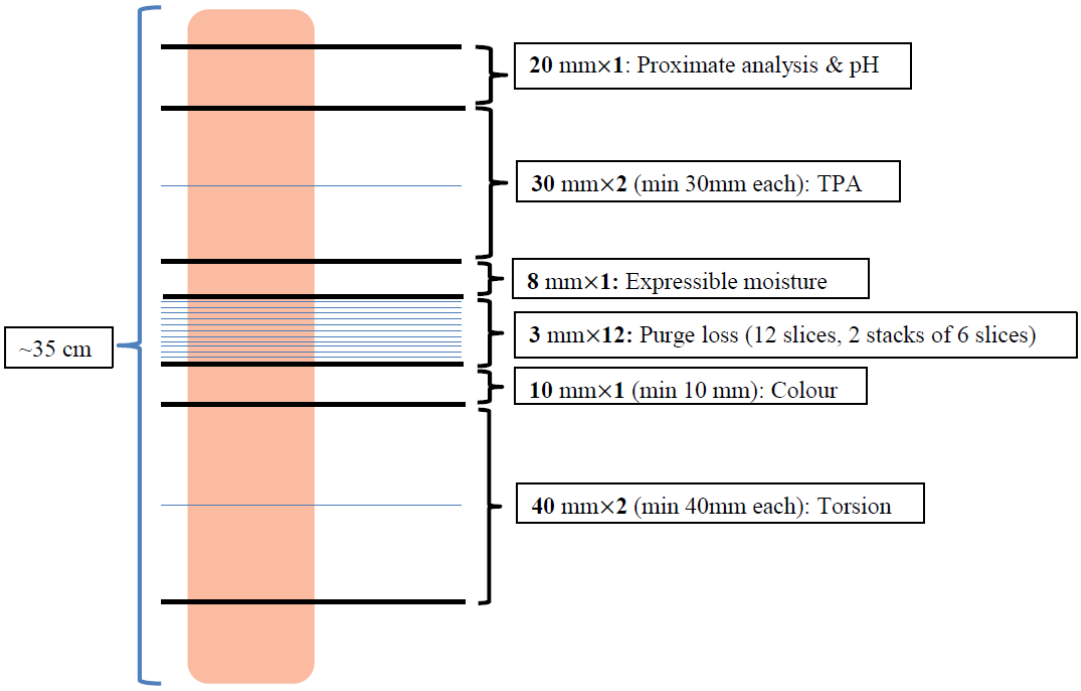

Figure 4-1 Cooked bologna schematic slicing diagram for various analysis.

The sliced bologna slices were either vacuum packed (model 550A, Sipromac, Drummondville, QC, Canada) or sealed in plastic bags (polyethylene vacuum pouch, 3 µm thick,

with oxygen permeability of 7.7 cc/m^2/24h) to be stored in 1 °C, or 4 °C for different analysis purposes.

4.4.2.5 Proximate composition of cooked bologna

Proximate composition of cooked bologna was measured according to methods described in section 3.4.2.1.

4.4.2.6 Batter viscosity

Viscosity of the meat batter was measured by stuffing each meat mixture into a 250 mL plastic cup and measured in quarduplicate using a Brookfield Synchro-Lectric viscometer (Model RVT; Brookfield Engineering, Stoughton, MA, USA). The reading was taken after inserting # 7 spindle into the meat batter for 30 sec and the batter temperature was recorded at the same time.

4.4.2.7 pH of raw and cooked bologna

The pH of raw bologna was measured by diluting 20 g of meat batter with 80 mL of distilled water following the procedure described in section 3.3.2.5 above. For cooked bologna samples, the sample was finely ground in a food processor (Cuisinart, Woodbridge, ON, Canada), then the procedure followed as described for raw bologna.

4.4.2.8 Water holding capacity (WHC)

a) Cook loss

The raw bologna chubs were weighed before cooking. Cooked bologna chubs were stored in 1 °C cooler overnight, and carefully opened by cutting one side with knife, and blotted by rolling over paper towel before weighing. The measurement was conducted in duplicate, and cook loss was calculated as per the formula below.

$$\text{Cook loss\%} = \frac{\text{sample weight} - \text{blotted weight}}{\text{sample weight}} \times 100 \quad (4.1)$$

b) Expressible moisture

The expressible moisture of cooked bologna samples was measured using a method described by Shand (2000). The bologna chubs were cut into 8 mm thick slices. The meat core

sample with weight around 1.0-1.5 g was placed in thimble shaped filter paper (Whatman # 3 and 5 in 50 mL Falcon plastic tube), and centrifuged for 15 min at 750×*g* using Eppendorf centrifuge (Eppendorf Centrifuge 5810 R, Canada). The expressible moisture was measured in quadruplicate and calculated as % moisture loss after centrifugation against initial sample weight.

c) Purge loss

Cooked bologna samples were cut into 3 mm thick slices as shown in **Figure 4-1**. Six slices were stacked together with a total of two stacks (weighing about 100-120g). The 2 stacks of sample were weighed on the day of slicing, and vacuum packed (model 550A, Sipromac, Drummondville, QC, Canada) in plastic pouches (polyethylene vacuum pouch, 3 µm thick, with oxygen permeability of 7.7 cc/m^2/24h) in duplicate. After being stored at 4 °C for 14 days, the cooked sample slices were blotted with paper tower to remove liquid on sample surface, and the sample weight was taken as blotted weight. The purge loss of cooked products was calculated as follows:

$$\text{Purge loss}\% = \frac{\text{sample weight} - \text{blotted weight}}{\text{sample weight}} \times 100\% \quad (4.2)$$

4.4.2.9 Colour of cooked bologna

For colour, cooked bologna samples were cut into 10 mm thick slices and vacuum packed (model 550A, Sipromac, Drummondville, QC, Canada) with 13*20 cm^2 vacuum bags (polyethylene vacuum pouch, 3 µm thick, with oxygen permeability of 7.7 cc/m^2/24h) to exhaust oxygen from bags. They were stored in 4 °C cooler for at least 48 h (to deplete any residual oxygen) before being measured by HunterLab MiniScan XETM (Hunter Associates Laboratories Inc., Reston, VA) expressed as CIE L*a*b* by using illuminant A and 10° standard observer. The measurement was taken through the film. The second reading for one sample was taken after 90° rotation of sample, and the measurement was done on duplicate packages per treatment.

4.4.2.10 Instrumental texture analysis

a) Texture profile analysis (TPA)

The TPA texture of cooked bologna samples was measured by applying TMS-Pro Texture Press (Food Technology Crop., Sterling, VA) with compression flat cylindrical probe (55 mm diameter). The bologna chubs were cut into 4-30 mm thick slices. Four cylindrical core samples

(22 mm in diameter, and 25 mm in height) were taken from the centre of each slice for each treatment. After tempering at room temperature for 30 min, each core was compressed to 50% of its original height for 2 cycles. The hardness, adhesiveness, cohesiveness, springiness, and chewiness of cooked bologna were measured in this analysis.

b) Torsional gelometry

The shear stress and shear strain at failure were tested by torsional gelometry utilizing a special fixture on a viscometer (Brookfield Digital Viscometer Model DV-I, Gel Consultants Inc., Raleigh, NC, U.S.A). The samples were cut into eight 19 mm diameter and 28.7 mm long cylinders. Styrene discs were attached to both ends of cylinders using cyanoacrylate glue (Loctite 404, Instant Adhesive, Loctite Corporation, Newington, CT, U.S.A), and milled to capstan-shape with center diameter of 10 mm by a modified bench grinder (KCI-24A2, Bodine Electric Company, Chicago, IL, U.S.A). The milled cylinders were stored at 4 °C before analysis and brought to room temperature prior to analysis. The samples were vertically mounted and twisted to failure with the bottom end fixed at a speed of 2.5 rpm on the torsional gelometer according to the method described by Kim et al. (1986).

4.4.2.11 Statistical analysis

The three replications of the experiment were analyzed by Mixed Procedure of SAS (SAS, Inst. Inc., Cary, NC) with Randomized Complete Block Design. The variations from meat materials of different production dates were considered as blocks. Within each block, the allocation of sample storage time and the order of processing of each bologna treatment were completely randomized. The treatments and storage times were considered as fixed effects. The means of data were compared using Tukey procedure and the degree of freedom for means was approximated using a Kenward-Roger adjustment on standard errors. The significance level chosen in this study was $\alpha=0.05$.

4.5 Results and discussions

4.5.1 Proximate composition of cooked bologna

Table 4-2 shows the proximate composition of all bologna treatments. The protein level of cooked products ranged from 10.98 (in BM-15) to 11.71% (in CON). The fat level of cooked products ranged from 12.31 (in DL-15) to 15.54% (in DL-15). The target protein and fat level of

all treatments were 12% and 14% respectively, which was slightly higher than values reached, but none of these parameters were statistically different. The variation in proximate composition of final product could be the introduction of moisture from the equipment during processing, the nonhomogeneous components distribution of raw meat samples (PP, MSP, BF), and the error from the proximate analysis of raw meat ingredients.

Table 4-2 Proximate composition of cooked bologna.[1]

Treatments[2]	%Moisture [ns]	%Ash [ns]	%Protein [ns]	%Fat [ns]
CON	70.08±0.52	3.39±0.03	11.58±0.31	13.61±0.92
BM-7.5	70.06±0.90	3.41±0.05	11.41±0.15	13.73±1.14
BM-15	70.33±0.75	3.36±0.10	11.11±0.13	13.25±0.77
DL-7.5	69.98±0.84	3.43±0.03	11.47±0.15	13.37±0.34
DL-15	69.70±0.42	3.44±0.03	11.04±0.08	13.95±1.61
STD-7.5	70.64±0.76	3.42±0.03	11.47±0.20	13.50±1.47
STD-15	70.24±0.31	3.39±0.05	11.10±0.08	13.23±0.50

[ns]not significant (p>0.05)

[1]Values are means of 3 replicates ± standard deviation

[2]CON: bologna formulated with only regular pork as meat ingredient; BM-7.5/15: bologna formulated with 7.5 or 15% of meat trim from bones; DL-7.5/15: bologna formulated with 7.5 or 15% of delay chilled mechanically separated pork; STD-7.5/15: bologna formulated with 7.5 or 15% of standard mechanically separated pork

4.5.2 Batter viscosity

The apparent viscosity of the raw meat batter made following one to four months of frozen storage is presented in **Table 4-3**, and did not differ statistically among treatments and storage time or treatment and storage time interaction. After four months frozen storage of the meats prior to use, the apparent viscosity of all formulations stayed stable compared to one month frozen storage. The difference between MSP-DL and MSP-STD substitution at either level was not significant (p>0.05). The viscosity of CON formulation was comparable to the bologna batter viscosity reported in other studies conducted in our research group with the same processing and analysis equipment (Wei, 2019). In his study, the viscosity was 10.8±0.3 cPs × 10^4 in pork bologna formulated with 13% protein and 10% fat, and 1.6% salt.

Table 4-3 Effects of freezing rates, addition levels, and frozen storage length of mechanically separated pork (MSP) on the bologna batter apparent viscosity.[1]

Treatments[2]	1 month		4 months	
	Viscosity (cPs × 10^4)^{ns}	Batter temperature (°C)^{ns}	Viscosity (cPs × 10^4)^{ns}	Batter temperature (°C)^{ns}
CON	11.1±0.4	11.5±1.4	11.2±0.8	9.0±0.7
BM-7.5	10.8±0.3	11.0±1.8	-	-
BM-15	10.5±0.9	10.8±1.4	-	-
DL-7.5	10.0±0.8	10.4±1.8	10.6±1.3	8.2±0.0
DL-15	10.1±0.6	11.0±1.8	10.0±1.6	8.7±1.3
STD-7.5	10.3±0.5	11.2±1.3	10.7±1.0	9.0±1.9
STD-15	10.0±0.8	11.5±1.0	10.1±1.9	9.1±0.6

^{ns}not significant (p>0.05)

[1]Values are means of 3 replicates ± standard deviation

[2]CON: bologna formulated with only regular pork as meat ingredient; BM-7.5/15: bologna formulated with 7.5 or 15% of meat trim from bones; DL-7.5/15: bologna formulated with 7.5 or 15% of delay chilled mechanically separated pork; STD-7.5/15: bologna formulated with 7.5 or 15% of standard mechanically separated pork

4.5.3 pH of raw and cooked bologna

The pH of raw bologna batters (**Table 4-4**) fluctuated around 6.2 for most of the sample formulas after 1 and 4 months of meat frozen storage. The storage effect and treatment effect on the raw batter pH were significant (p<0.05), but the interaction was not. The raw batter pH of CON was significantly lower than that of treatments incorporated with MSP, and those treatments with addition of MSPs were not different (**Table 4-5**). The reason for slightly higher pH values in raw batter of MSP substituted treatments could be the raw meat ingredients added into the formula, as pH of PP was 5.93 compared with MSP-DL and MSP-STD with pH values of 6.43 and 6.42 respectively (Section 3.5.6). However, phosphate addition likely increased the pH of all treatments slightly (Aberle et al., 2012). The 4 months frozen storage of meats significantly increased the pH of raw bologna batter (**Table 4-6**).

Table 4-4 Effects of freezing rates, addition levels, and frozen storage length of mechanically separated pork (MSP) on the pH of raw and cooked bologna.[1]

Treatments[2]	1 month		4 months	
	Raw[ns]	Cooked[ns]	Raw[ns]	Cooked[ns]
CON	6.02±0.14	6.39±0.02	6.17±0.06	6.36±0.06
BM-7.5	6.13±0.06	6.41±0.03	-	-
BM-15	6.06±0.07	6.41±0.01	-	-
DL-7.5	6.15±0.08	6.43±0.05	6.22±0.05	6.36±0.03
DL-15	6.08±0.12	6.45±0.05	6.28±0.08	6.43±0.01
STD-7.5	6.16±0.06	6.41±0.03	6.25±0.10	6.38±0.04
STD-15	6.13±0.13	6.44±0.06	6.30±0.06	6.42±0.03

[ns]not significant (p>0.05)

[1]Values are means of 3 replicates ± standard deviation

[2]CON: bologna formulated with only regular pork as meat ingredient; BM-7.5/15: bologna formulated with 7.5 or 15% of meat trim from bones; DL-7.5/15: bologna formulated with 7.5 or 15% of delay chilled mechanically separated pork; STD-7.5/15: bologna formulated with 7.5 or 15% of standard mechanically separated pork

Table 4-5 Effects of freezing rates and addition levels of mechanically separated pork (MSP) on the pH of raw and cooked bologna .[1]

Treatments[2]	Raw	Cooked[ns]
CON	6.09±0.02[b]	6.38±0.02
DL-7.5	6.19±0.02[a]	6.40±0.02
DL-15	6.18±0.02[a]	6.44±0.02
STD-7.5	6.21±0.02[a]	6.40±0.02
STD-15	6.21±0.02[a]	6.43±0.02

[a-b]Means with different letters in the same column are significantly different (p<0.05)

[1]Values are means across storage time ± standard error

[2]CON: bologna formulated with only regular pork as meat ingredient; DL-7.5/15: bologna formulated with 7.5 or 15% of delay chilled mechanically separated pork; STD-7.5/15: bologna formulated with 7.5 or 15% of standard mechanically separated pork

Table 4-6 Effects of frozen storage length of mechanically separated pork (MSP) on the pH of raw and cooked bologna.[1]

Storage	Raw	Cooked
1 month	6.11±0.03[b]	6.43±0.01[a]
4 months	6.24±0.02[a]	6.39±0.01[b]

[a-b]Means with different letters in the same column are significantly different (p<0.05)
[1]Values are means across treatments ± standard error

The pH of cooked bologna was around 6.4. The pH of cooked bologna significantly decreased with the storage of raw meats for 1 to 4 months (**Table 4-6**). The effect of the interaction between treatment and storage on the pH of cooked bologna was not significant, but the treatment had the trend (p=0.057) to significantly influence the pH of cooked bologna (**Table 4-5**).

4.5.4 WHC of cooked bologna

The effects of MSP freezing methods, levels, and frozen storage on WHC were determined by cook loss of bologna during cooking, the water holding under centrifugal stress (expressible moisture) and the purge loss after 14 days refrigerated storage as presented in **Table 4-7**.

Table 4-7 Effects of freezing rates, addition levels, and frozen storage length of mechanically separated pork (MSP) on the cook loss, expressible moisture, and purge loss of cooked bologna.[1]

Treatments	Cook loss (%)[ns]		Expressible moisture (%)[ns]		Purge loss (%)[ns]	
	1 month	4 months	1 month	4 months	1 month	4 months
CON[2]	0.6±0.4	0.5±0.2	12.4±0.7	12.4±0.8	3.4±0.5	3.5±0.8
BM-7.5	0.5±0.1	-	12.2±0.5	-	3.2±0.6	-
BM-15	0.5±0.1	-	11.9±0.6	-	3.1±0.6	-
DL-7.5	0.6±0.1	0.5±0.1	11.8±0.6	13.2±1.1	3.2±0.3	3.4±0.5
DL-15	0.4±0.1	0.5±0.1	16.1±5.0	17.0±5.2	2.7±0.3	2.9±0.2
STD-7.5	0.5±0.1	0.5±0.1	12.1±1.1	12.6±1.5	3.2±1.5	3.4±0.5
STD-15	0.6±0.1	0.5±0.1	15.9±4.8	17.4±4.8	2.6±0.4	2.4±0.2

[ns]not significant (p>0.05)
[1]Values are means of 3 replicates ± standard deviation
[2]CON: bologna formulated with only regular pork as meat ingredient; BM-7.5/15: bologna formulated with 7.5 or 15% of meat trim from bones; DL-7.5/15: bologna formulated with 7.5 or 15% of delay chilled mechanically separated pork; STD-7.5/15: bologna formulated with 7.5 or 15% of standard mechanically separated pork

4.5.4.1 Cook loss

According to the cook loss measurement, the addition of MSP in formula did not increase the cook loss compared with CON formulation (p>0.05). And the initial chilling rate of MSPs, 1-4 months of frozen storage, or the interaction of treatment and frozen storage didn't play a significant role on cook loss (**Table 4-7**). Products were formulated to have very little cook loss, and cooked in waterproof casing, so that the macro composition of all treatments remained the same. The cook loss of bologna with 2.5% salt and at 11-12% protein level was comparable to the cook loss of bologna made with 2% salt and at 11-12% protein level in previous studies conducted in our research group with the same equipment (Edrosolam, 2013).

4.5.4.2 Expressible moisture

Higher expressible moisture indicates poor WHC of bologna. The 4 months frozen storage of raw meats significantly increased expressible moisture of bologna compared to one month (**Table 4-8**). The expressible moisture of 15% MSP substitution formulation has the trend to be higher than that of the CON formula or 7.5% MSP formula (p=0.21), and the effect of initial chilling rate of MSPs, and the treatment and storage time interaction were not significant. The expressible moisture of 7.5% MSP or BM treatments were comparable with the CON formula. High variation of expressible moisture in 15% MSP substitution formulation at both storage times was observed. The possible reason could be the formulas with MSP-DL and MSP-STD from the 3rd replicate showed better bologna texture in processing compared with the first two replicates due to the quality variation of raw MSPs. Different batches of pig carcasses, previous processing condition, and mechanical separation equipment settings might lead to the variation in sample quality, and similar phenomenon was observed during preliminary studies.

Table 4-8 Effects of frozen storage length of mechanical separated pork (MSP) on the expressible moisture of bologna.[1]

Storage	Expressible moisture (%)
1 month	13.67±0.79[b]
4 months	14.53±0.79[a]

[a-b]Means with different letters in the same column are significantly different (p<0.05)
[1]Values are means across treatments ± standard error

4.5.4.3 Purge loss

For the purge loss analysis, the effect of the interaction of treatment and storage was not significant (p>0.05). The purge loss of bologna with 15% MSP showed a trend (p=0.08) to be significantly lower than that of CON, DL-7.5, and STD-7.5. Storage time didn't show any effect on the purge loss of bologna samples.

Based on these various measurements of WHC, no major treatment and storage interaction was found on the WHC of bologna formulated with 7.5 or 15% MSP. The initial chilling rate didn't show effect on the WHC of bologna. The cook loss was not influenced by addition of MSP at either level, or storage length. However, 15% MSP addition in formula had a trend to decrease the purge loss (p=0.08) and increase the expressible moisture (p=0.21) of bologna. WHC of formulas with 7.5% MSP showed comparable results with that of CON or BM incorporated treatments.

It was proposed that increasing the level of fish sarcoplasmic protein helped reduce the cook loss of myofibrillar protein gel and smooth the microstructure of gel surface (Hemung & Chin, 2013). McMillin et al. (1980) reported that WHC of frankfurters made with 10-50% MSP increased with the increasing level of MSP. The results in this study agreed with literature that purge loss of bologna gels decreased with the increased level of MSP. The sarcoplasmic proteins might act as water holding agents in the myofibrillar gel system. The increased expressible moisture of formulas with 15% MSP could be explained by the poor gel strength and soft texture of bologna samples that failed to hold their shape under centrifugal force. The initial chilling rate of the MSP didn't show an effect on the WHC of the bologna.

4.5.5 Colour of cooked bologna

The colour change of bologna is shown in **Table 4-9**, and **Figure 4-2** shows pictures of bologna slices. Main effects of treatment, and storage time were significant, but not the treatment by storage time interaction. The addition of MSP decreased the L* value, but increased a* and b* values, and the extent of change was proportional to the addition of MSP added (**Table 4-10**). The initial chilling rate had no effect on bologna colour. The MSP added samples all showed dark red colour compared with CON formula which was a light pink colour regardless of storage time. The results could be explained by the darker and redder colour of MSP compared with PP (**Table 3-6**). The colour of BM-7.5 or 15 was not different to the CON formula. Ockerman et al. (1981) found that the mechanical separation process darkened the raw MSP and finished product. Kramer &

Sebranek (1990) found that increasing the level of MSP in fermented snack sausage decreased L* value and increased a* value, which indicate that the sausage became darker and redder in colour. Their sensory study showed that panelists prefer the colour of sausage substituted with higher lever (30%) of MSP. Our results of bologna colour agreed with what has been observed in previous studies.

Table 4-9 Effects of freezing rates, addition levels, and frozen storage length of mechanically separated pork (MSP) on the colour of cooked bologna.[1]

Treatments[2]	Storage (month)	L*	a*	b*
CON	1	72.74±2.00^{a}	15.98±0.60^{e}	12.25±0.16^{de}
	4	71.27±0.70^{ab}	17.43±0.36^{de}	13.06±0.12^{cd}
BM-7.5	1	71.88±2.17^{ab}	16.12±0.55^{e}	12.17±0.21^{e}
BM-15	1	71.59±1.24^{ab}	16.11±0.32^{e}	12.17±0.48^{e}
DL-7.5	1	67.81±1.07^{cde}	18.76±0.23^{cd}	13.01±0.45^{cde}
	4	66.79±0.18^{de}	19.97±0.10^{bc}	13.83±0.21^{bc}
DL-15	1	64.56±1.10^{ef}	21.06±0.58^{ab}	14.03±0.58^{ab}
	4	62.63±1.60^{f}	21.89±0.79^{a}	14.57±0.25^{ab}
STD-7.5	1	68.48±1.64^{bcd}	18.33±0.39^{d}	12.85±0.68^{de}
	4	66.77±0.98^{de}	20.30±0.47^{b}	13.94±0.14^{ab}
STD-15	1	64.36±0.55^{ef}	21.10±0.50^{ab}	14.13±0.58^{ab}
	4	62.78±0.70^{f}	22.09±0.70^{a}	14.77±0.46^{a}

[a-f]Means with different letters in the same column are significantly different (p<0.05)
[1]Values are means of 3 replicates ± standard deviation
[2]CON: bologna formulated with only regular pork as meat ingredient; BM-7.5/15: bologna formulated with 7.5 or 15% of meat trim from bones; DL-7.5/15: bologna formulated with 7.5 or 15% of delay chilled mechanically separated pork; STD-7.5/15: bologna formulated with 7.5 or 15% of standard mechanically separated pork

Table 4-10 Effects of freezing rates and addition levels of mechanically separated pork (MSP) on the colour of cooked bologna.[1]

Treatments[2]	L*	a*	b*
CON	72.00±0.48[a]	16.70±0.28[c]	12.66±0.20[c]
DL-7.5	67.30±0.48[b]	19.36±0.28[b]	13.42±0.20[bc]
DL-15	63.59±0.48[c]	21.48±0.28[a]	14.30±0.20[ab]
STD-7.5	67.62±0.48[b]	19.31±0.28[b]	13.39±0.20[bc]
STD-15	63.57±0.48[c]	21.59±0.28[a]	14.45±0.20[a]

[a-c]Means with different letters in the same column are significantly different (p<0.05)
[1]Values are means across the storage time ± standard error
[2]CON: bologna formulated with only regular pork as meat ingredient; DL-7.5/15: bologna formulated with 7.5 or 15% of delay chilled mechanically separated pork; STD-7.5/15: bologna formulated with 7.5 or 15% of standard mechanically separated pork

Figure 4-2 Bologna slices processed with meats after 1 month of frozen storage.
*CON: bologna formulated with only regular pork as meat ingredient; BM-7.5/15: bologna formulated with 7.5 or 15% of meat trim from bones; DL-7.5/15: bologna formulated with 7.5 or 15% of delay chilled mechanically separated pork; STD-7.5/15: bologna formulated with 7.5 or 15% of standard mechanically separated pork

Bologna prepared from meats frozen for 4 months had significantly lower L* value, but increased a* and b* values for all treatment groups than bologna made from meats with one month storage (**Table 4-11**). This result means that all bologna became darker, redder, and yellower in colour after the extended (4 months) frozen storage of meat ingredients.

Table 4-11 Effects of frozen storage length of mechanically separated pork (MSP) on the colour of cooked bologna.[1]

Storage	L*	a*	b*
1 month	67.59±0.30[a]	19.04±0.13[b]	13.25±0.13[b]
4 months	66.05±0.30[b]	20.33±0.13[a]	14.04±0.07[a]

[a-b]Means with different letters in the same column are significantly different (p<0.05)
[1]Values are means across treatments ± standard error

4.5.6 Instrumental texture analysis

4.5.6.1 Texture profile analysis (TPA)

Table 4-12 showed TPA of bologna in terms of TPA hardness, adhesiveness, cohesiveness, springiness, and chewiness. The BM incorporated formula showed nearly identical performance in TPA texture properties as of CON formula, indicating that it's the mechanical separation not the meat trim near bones that led to compromised gelation properties of MSP. The effect of treatment and storage time interaction and initial chilling rate of MSPs were not significant for all TPA texture properties. Substitution of 7.5% MSP didn't change the TPA texture of bologna very much, however, further increasing the level of MSP had a trend to decrease the hardness (p=0.10), cohesiveness (p=0.06), springiness (p=0.10), and chewiness (p=0.09) of bologna. High variation of TPA hardness, cohesiveness, springiness and chewiness in 15% MSP substitution formulation at both storage times was observed. The possible reason could be the formulas with MSP-DL and MSP-STD from the 3rd replicate showed better bologna texture in processing compared with the first two replicates due to the quality variation of raw MSPs. The storage length has no effect on the bologna hardness, cohesiveness, springiness, or chewiness, however, 4 months frozen storage of meats significantly increased adhesiveness of bologna (p<0.05), which means samples became more adhesive on surface. The average adhesiveness across treatments at one month storage was 0.77, and the adhesiveness increased to 0.92 after 4 months of frozen storage.

Table 4-12 Effects of freezing rates, addition levels, and frozen storage length of mechanically separated pork (MSP) on the texture profile analysis (TPA) of pork bologna.[1]

Treatments	Storage (month)	Hardness (N)[ns]	Adhesiveness (-)[ns]	Cohesiveness (-)[ns]	Springiness (%)[ns]	Chewiness (N)[ns]
CON[2]	1	128.5±27.1	0.9±0.3	0.52±0.03	75.9±3.2	724±206
	4	134.0±22.9	0.9±0.3	0.53±0.03	78.3±2.1	746±110
BM-7.5	1	123.2±21.3	0.9±0.2	0.54±0.01	80.5±1.0	738±138
BM-15	1	126.6±18.9	0.9±0.3	0.55±0.01	82.0±0.7	760±125
DL-7.5	1	120.8±23.7	0.8±0.2	0.53±0.02	79.3±2.2	657±116
	4	120.9±7.7	1.0±0.1	0.53±0.01	78.5±1.1	667±66
DL-15	1	72.5±52.5	0.6±0.3	0.33±0.18	62.7±16.5	306±435
	4	68.7±47.0	0.8±0.2	0.33±0.17	59.8±17.2	258±325
STD-7.5	1	115.9±27.5	0.9±0.2	0.53±0.03	80.8±0.7	659±164
	4	117.6±15.5	1.0±0.2	0.54±0.01	78.7±0.2	664±104
STD-15	1	64.2±47.9	0.6±0.3	0.32±0.16	59.2±17.9	260±347
	4	58.5±44.5	0.9±0.1	0.33±0.17	56.9±19.1	232±318

[ns]not significant (p>0.05)

[1]Values are means of 3 replicates ± standard deviation

[2]CON: bologna formulated with only regular pork as meat ingredient; BM-7.5/15: bologna formulated with 7.5 or 15% of meat trim from bones; DL-7.5/15: bologna formulated with 7.5 or 15% of delay chilled mechanically separated pork; STD-7.5/15: bologna formulated with 7.5 or 15% of standard mechanically separated pork

According to the findings in Study One, the decreased gelation ability of NAM in MSPs, decreased ratio of myofibrillar protein to sarcoplasmic protein, and decreased collagen content in MSPs might be the reason for the decreased hardness and springiness in bologna texture. It was reported by Hemung & Chin (2013) that addition of 0.1 g fish sarcoplasmic protein into 100 g of fish myofibrillar protein didn't decrease the gel strength but adding 0.5 g/ 100g or higher (1.0 g/100 g) decreased the gel strength. The slightly higher low ionic strength soluble protein level from MSP may play a role on decreasing the gel strength of MSP incorporated meat gel. According to Colmenero et al. (1995), protein content is the critical determinant of bologna texture rather than fat content or cooking temperature, higher protein content (16.3%) led to too tough product. In this experiment, the quality of protein determined the texture of bologna product. The removal of collagen could increase the softness and reduce the cohesiveness of bologna (Schmidt, 1987). The less desirable soft texture of bologna may due to the changes and disruption during mechanical separation process of MSP, including removal of connective tissue (Schmidt, 1987) and poor quality of myofibrillar protein in MSP (Ockerman et al., 1981). Increasing the ratio of MSP myofibrillar protein with lower gelling ability as the increase of MSP substitution level from 7.5% to 15% could reduce the hardness of bologna. McMillin et al. (1980) reported that frankfurters with more than 30% MSP incorporation led to softer texture. The texture softness was observed only at 15% MSP substitution level, the difference might come from the bone type, pig breeds, previous processing condition, mechanical separation equipment, and bologna processing condition.

4.5.6.2 Torsional gelometry

The effects of mechanically separated pork (MSP) freezing methods, levels, and frozen storage on shear stress and shear strain at failure of pork bologna is shown in **Table 4-13**. The treatment and storage time interaction and the initial chilling rate of MSPs had no effect on the shear stress or shear strain at failure of pork bologna. The BM incorporated formula showed nearly identical performance in torsional gelometry analysis as control formula, confirming the fact that it's the mechanical separation process that led to compromised gelation properties of MSP. The bologna shear strain decreased ($p<0.05$), and shear stress increased ($p<0.05$) during 1-4 months frozen storage of raw meats.

Table 4-13 Effects of freezing rates, addition levels, and frozen storage length of mechanically separated pork (MSP) on the shear stress and shear strain at failure of pork bologna.[1]

Treatments[2]	Shear stress (kPa)[ns]		Shear strain[ns]	
	1 month	4 months	1 month	4 months
CON	23.64±1.23	25.10±1.92	1.51±0.17	1.41±0.07
BM-7.5	22.58±0.97	-	1.47±0.11	-
BM-15	24.93±1.62	-	1.56±0.25	-
DL-7.5	19.02±4.79	25.37±5.38	1.42±0.02	1.31±0.06
DL-15	12.28±7.81	13.94±10.05	1.19±0.26	0.99±0.30
STD-7.5	20.53±1.60	24.25±4.96	1.42±0.13	1.33±0.04
STD-15	11.16±7.42	12.91±9.94	1.07±0.21	0.96±0.21

[ns]not significant (p>0.05)

[1]Values are means of 3 replicates ± standard deviation

[2]CON: bologna formulated with only regular pork as meat ingredient; BM-7.5/15: bologna formulated with 7.5 or 15% of meat trim from bones; DL-7.5/15: bologna formulated with 7.5 or 15% of delay chilled mechanically separated pork; STD-7.5/15: bologna formulated with 7.5 or 15% of standard mechanically separated pork

The MSP substitution level significantly influenced the bologna shear strain (p<0.05), and the increase of substitution level showed the trend to decrease the shear stress at failure (p=0.09) compared with the control treatment (**Table 4-14**). The high variation of shear stress and strain of bologna at 15% MSP level was observed due to the quality variation of raw MSP ingredient in the third sample replicate as discussed in section 4.5.4.2. The shear strain and stress of MSP at 7.5% level were not significantly different from CON. The shear stress of 15% MSP substitution treatments was around 12-13 kPa, and trended (p=0.09) to be lower than 24 kPa of CON formulated with pure PP. The shear strain of 15% MSP-STD substitution treatment was around 1.01, significantly lower than that of CON (1.46).

Table 4-14 Effects of freezing rates and addition levels of mechanically separated pork (MSP) on the shear stress and shear strain at failure of pork bologna.[1]

Treatments[2]	Shear stress (kPa)[ns]	Shear strain
CON	24.37±3.55	1.46±0.09[a]
DL-7.5	22.20±3.55	1.37±0.09[ab]
DL-15	13.11±3.55	1.09±0.09[ab]
STD-7.5	22.39±3.55	1.38±0.09[ab]
STD-15	12.03±3.55	1.01±0.09[b]

[a-b]Means with different letters in the same column are significantly different (p<0.05)

[1]Values are means across storage time ± standard error

[2]CON: bologna formulated with only regular pork as meat ingredient; DL-7.5/15: bologna formulated with 7.5 or 15% of delay chilled mechanically separated pork; STD-7.5/15: bologna formulated with 7.5 or 15% of standard mechanically separated pork

Figure 4-3 shows the texture map of bologna. The DL-15 and STD-15 showed mushy texture while DL-7.5, STD-7.5, BM-7.5, BM-15 showed texture closer to CON. The three additional months of frozen storage of raw meats made bologna mushier and slightly more brittle in texture, indicating the increase of hardness and decrease of elasticity of bologna. It was reported that the mechanical separation process decreased the tenderness desirability of sausage (Ockerman et al., 1981). Bologna made with 15% MSP in our study was less desirable due to its mushy and soft texture compared to CON. According to Nuckles et al. (1990), the stress and strain of cooked meat batters increased with the increase of percent myosin and actin in the meat while it decreased with the increase of low ionic strength soluble protein. This helped explain the reduced shear stress and strain at failure of bologna made from MSP compared with bologna made with BM or PP. As discussed in Study One, the MSP had degraded MHC with decreased gelling ability compared with intact myosin (Park et al., 1996). The bologna incorporated with MSP had less intact MHC even though the total protein or total soluble myofibrillar protein was not different to bologna made with pure PP. As the MSP substitution level increased, the bologna batter might have less functional myofibrillar protein to form a gel that reaches the firmness level of bologna made with PP that has more functional myofibrillar protein. The decreased collagen content in MSP may further increase the softness and reduce the cohesiveness of bologna (Schmidt, 1987).

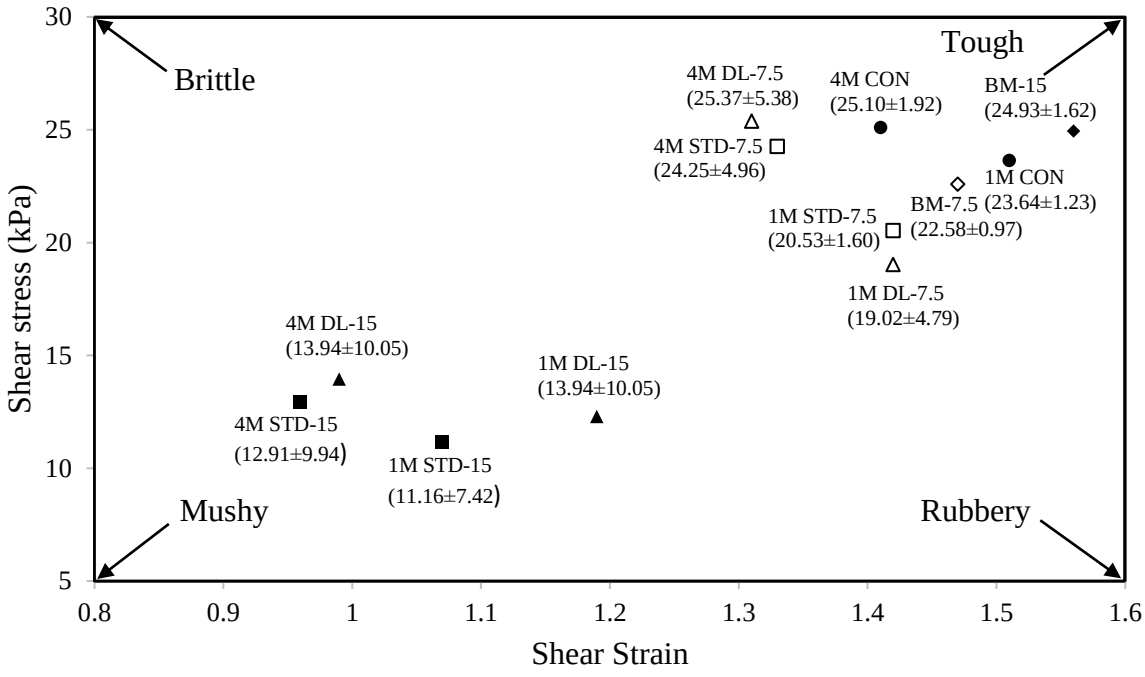

Figure 4-3 Torsion texture map of bologna processed with meats after 1 or 4 months of frozen storage. 1M: 1 month of frozen storage; 4M: 4 months of frozen storage; CON: bologna formulated with only regular pork as meat ingredient; BM-7.5/15: bologna formulated with 7.5 or 15% of meat trim from bones; DL-7.5/15: bologna formulated with 7.5 or 15% of delay chilled mechanically separated pork; STD-7.5/15: bologna formulated with 7.5 or 15% of standard mechanically separated pork

4.6 Conclusion

The replacement of PP with 7.5% MSP didn't lead to a significant negative influence on bologna texture and WHC. However, replacement of 15% MSP was not acceptable in terms of texture and appearance of bologna product. The colour of bologna with 15% MSP was dark red, compared with the light pink colour of regular bologna. The bologna made with 15% MSP was soft and mushy in texture with a trend to show decreased TPA hardness and chewiness. A decrease in shear strain was also observed in bologna with 15% MSP. This could be mainly due to the structural changes of myofibrillar protein in MSP such as the degradation of MHC found in the previous study. The effects of initial freezing rates of MSP on the bologna texture was not significant. Fast chilling didn't help with preserving the gelation capacity of MSP. Bologna had darker and redder colour, higher expressible moisture, increased TPA adhesiveness, increased

shear stress and decreased shear strain at failure after frozen storage of MSP for 4 months compared with 1-month frozen storage.

4.7 Connection to the next study

The quality of MSP was not significantly improved with fast chilling rate according to the previous two studies with the initial MSP temperature at around 8 °C when it was boxed. Fast temperature drop through a heat exchanger after processing could decrease the MSP temperature to as low as 1 °C before blast freezing. It was interesting to explore the effect of MSP prechilling with the newer technology on the quality of MSP, and the texture of bologna made with up to 15% of MSP.

For the frozen storage effect on the quality change of MSP, initial 1-4 months of frozen storage didn't show significant influence on the quality and lipid oxidation level of MSP, however, MSP usually has about 1 year of shelf life under commercial frozen storage condition. Longer term frozen storage could have an effect on the gelation properties or lipid oxidation level of MSP and the texture and appearance of bologna made with it. The initial freezing rate also could have quality preservation effect on the longer stored samples.

Study three was conducted to evaluate the effect of accelerated post mechanically deboning chilling and prolonged frozen storage on physicochemical and gelation properties of MSP. Note that due to equipment breakdown, only one lot of meat was available.

5 STUDY 3: EVALUATION OF THE EFFECTS OF ACCELERATED CHILLING AND PROLONGED FROZEN STORAGE ON PHYSICOCHEMICAL AND GELATION PROPERTIES OF MSP

5.1 Abstract

The objective of this study was to analyse the effects of accelerated chilling rate (through a pre-chilling system) and prolonged frozen storage (1-13 months) on the physiochemical, biochemical, and gelation properties of MSP, and the effects on the WHC, texture, and appearance of bologna made with 7.5 or 15% MSP.

The prechilling or fast freezing didn't obviously influence the physicochemical, biochemical and gelation properties of MSP, and the properties of bologna treatments with MSP substitution at either level. However, the prechilling process showed the potential of decreasing the speed of lipid oxidation of MSP after 7-13 months long-term frozen storage by quickly reducing the initial meat temperature prior to freezing. The redness and yellowness of MSPs decreased with extended frozen storage, but pH or protein solubility remained unchanged. The WHC of bologna treatments was not influenced by frozen storage of MSPs, but the redness decreased after 4-13 months of storage. The 1-13 months frozen storage of MSPs didn't show noticeable effect on the texture of bologna treatments with 7.5% MSP, however, there was slightly decreased TPA hardness, springiness, chewiness, and shear strain at failure of bologna treatments with 15% MSP substitution. Replication of this study with additional lots of meat is needed to confirm these findings.

5.2 Introduction

Frozen storage is commonly used to increase meat shelf life and the frozen storage duration is crucial for maintaining meat quality and preventing spoilage, in addition to the frozen storage temperature and freezing rate (Leygonie et al., 2012; Kim et al., 2018). The routinely applied export storage temperature (-18 °C) is sufficient to maintain the sensory quality or storage duration (Hagyard et al., 1993; Farouk et al., 2003; Estévez, 2011). The low storage temperature effectively decreases water activity (<0.9) to prevent microbial growth, and prevent oxidative

rancidity (Coombs, et al., 2017). The issues with frozen storage of raw meats are diminished WHC and liquid loss upon thawing (Añón & Calvelo, 1980; Winger & Hagyard, 1994; Vieira et al., 2009) as a result of muscle fibres disruption and ice crystal formation (Rahelić et al., 1985). Short term frozen storage prevented negative effects on meat quality traits (Coombs et al., 2017). Effect of long-term frozen storage on colour varies (Coombs et al., 2017). Colour stability of beef was proven to be affected by frozen storage, and the instrumental colour parameters (L*, a*, b*) decreased as the increase of storage duration (Farouk & Swan, 1998; Vieira et al., 2009). Prolonged frozen storage was reported to significantly decrease the shear force of lamb (Muela et al., 2015). Most studies on the effects of frozen storage focused on intact meat muscle. MSP, as a complex system could have different performance during the -18 °C frozen storage, in terms of colour stability, lipid oxidation level, protein gelation ability and functionality. And the effect of these property changes on the texture and appearance of bologna would be of interest.

Freezing rate has a substantial effect on the quality attributes of frozen meat (Kim et al., 2018). Fast freezing is believed to reduce thaw loss through the formation of more, smaller, and intracellular ice crystals rather than big ice crystals formed during slow freeing (Petrović et al., 1993). The lipid and protein oxidation could also be minimized by fast freezing (Kim et al., 2017). Blast freezing and contact freezing are two commonly used freezing processes. The contact freezing cools down the meat surface by contact with a refrigerated surface for efficient heat transfer while blast freezing chills meat by blowing cold air inside the room. In this study, the MSP (MSP-PC) was prechilled by a heat exchanger using the principle of contact chilling prior to boxing to quickly drop the initial MSP temperature before the immediate blast freezing. The MSP-STD was chilled in the blast freezer immediately after production, and the MSP-DL was held at 4 °C environment for 4 hours before moving into the blast freezer. In real MSP manufacturing practices, it is normal to see around one to four hour delay in chilling of packed MSPs while they are waiting to be transferred into the freezing area. It is of practical meaning to study the effects of super-fast chilling (MSP-PC), standard fast chilling (MSP-STD), and delayed chilling (MSP-DL) on the quality and gelation ability of MSP, and the interaction with frozen storage duration of up to 13 months.

5.3 Hypotheses

Accelerated chilling through heat exchanger can better preserve gelation properties of MSP and retard lipid oxidation. Prolonged frozen storage of MSP would result in worse textural properties of bologna, and higher level of lipid oxidation.

5.4 Materials and methods

5.4.1 Materials

5.4.1.1 Mechanical separation process

The MSP, regular PP cuts, hand deboned pork picnic bones were collected as described in 3.4.1.1. The MSP-PC samples were fresh MSP that went through SPX scraped surface heat exchanger (Delavan, WI) and was prechilled to about 1 °C before packaging and blast chilling. They were moved into blast freezer right after processing for fast chilling effect. Three boxes of each product were frozen and returned to the University of Saskatchewan. Due to major equipment (SPX scraped surface heat exchanger) breakdown, only one lot of the MSP-PC was available for this study.

5.4.1.2 Sample collection

Day 0 MSP samples were collected after mechanical deboning, after prechilling through the SPX scraped surface heat exchanger, and after 4 h tempering before being moved into blast freezer in the processing plant, frozen and stored in liquid nitrogen. They were moved to the -80 °C freezer before analysis. Temperature profiles of MSP-DL, MSP-STD, MSP-PC with 3 different chilling rates were tracked with iButton temperature trackers (Maxim Integrated, San Jose, CA, U.S.A) on both the surface and centre of the MSP block. Frozen MSP samples were shipped under frozen condition with temperature of samples tracked by iButton. PP used in this study was fresh vacuum packed PP manufactured on the same date, and shipped in refrigerated condition to Saskatoon. MSP samples were collected after shipping (about 7 days postmortem).

5.4.1.3 Sample preparation

Rep 1 samples were prepared as described in 3.4.1.3. Storage samples was taken at 1, 4, 7, and 13 months of frozen storage time for analysis. Frozen BF was collected from Maple Leaf Inc., Brandon, MB to adjust fat level of bologna formulations as needed.

5.4.1.4 Dry ingredient preparation for meat processing

Non-meat ingredients included salt, Prague powder (6.4% sodium nitrite in salt), STPP, sodium eyrthorbate. They were obtained from the Canadian Salt Company Ltd (Pointe-Claire, QC, Canada), Innophos (Lowbanks, ON, Canada) and Unipack Packaging Products, Ltd. (Edmonton, AB, Canada), respectively.

5.4.2 Methods

5.4.2.1 Proximate composition

Described in section 3.4.2.1.

5.4.2.2 pH

Described in section 3.4.2.5.

5.4.2.3 Raw meat colour

Described in section 3.4.2.6.

5.4.2.4 Lipid Oxidation

Described in section 3.4.2.7.

5.4.2.5 Protein solubility

Described in section 3.4.2.9.

5.4.2.6 NAM extraction

Described in section 3.4.2.10.

5.4.2.7 Protein Assay

Described in section 3.4.2.11.

5.4.2.8 SDS-PAGE of NAM

Described in section 3.4.2.12.

5.4.2.9 Dynamic oscillatory rheology

Described in section 3.4.2.14.

5.4.2.10 Bologna formula

The formula for all bologna products manufactured included 2.23% salt, 0.29% Prague powder, 0.30% STPP, and 0.05% sodium eyrthorbate. The target protein and fat concentrations of final product were set to 12 and 14%, respectively. For each MSP (MSP-DL, MSP-STD, MSP-PC) or BM treatments, the percentage of regular pork and water needed was adjusted based on proximate composition of each meat ingredient to meet the protein and fat targets for final products. The control treatment was made with only PP cut as the meat ingredient. The 3 MSP treatments or BM were added at 7.5% or 15% levels of the total batter weight, the rest of meat protein and fat came from PP and BF. The batch size of bologna product was around 8 kg of total raw weight for 4 chubs of bologna (~1 kg). The processing treatment formula was shown in **Table 5-1** below. The processing was conducted following 1, 4, 7 and 13 months of -18 °C frozen storage.

Table 5-1 Typical bologna processing formula (rep 1)

Treatments/Ingredients[1] (%, w/w)	MSP/ BM	PP	BF	Ice water
CON[2]	0	65.2	5.4	26.5
BM-7.5	7.5	55.9	6.2	27.5
BM-15	15	46.7	7.0	28.5
DL-7.5	7.5	58.3	4.9	26.4
DL-15	15	51.4	4.4	26.3
STD-7.5	7.5	58.7	4.7	26.3
STD-15	15	52.2	3.9	26.0
PC-7.5	7.5	57.7	5.3	26.7
PC-15	15	50.2	5.1	26.8

[1]MSP: mechanically separated pork; BM: meat trim from bone; PP: pork picnic; BF: pork back fat

[2]CON: bologna formulated with only regular pork as meat ingredient; BM-7.5/15: bologna formulated with 7.5 or 15% of meat trim from bone; DL-7.5/15: bologna formulated with 7.5 or 15% of delay chilled mechanically separated pork; STD-7.5/15: bologna formulated with 7.5 or 15% of standard mechanically separated pork; PC-7.5/15: bologna formulated with 7.5 or 15% of prechilled mechanically separated pork

Dry ingredients: 2.23% salt, 0.29% Prague powder, 0.30% STPP; 0.05% sodium erythorbate

5.4.2.11 Bologna processing

Described in section 4.4.2.2.

5.4.2.12 Bologna cooking

Described in section 4.3.2.3.

5.4.2.13 Slicing of cooked bologna

Described in section 4.3.2.4.

5.4.2.14 Proximate composition of cooked bologna

Proximate composition of cooked bologna was measured according to methods described in section 3.3.2.1.

5.4.2.15 Batter viscosity

Described in section 4.3.2.5.

5.4.2.16 pH of raw and cooked bologna

Described in section 4.3.2.6.

5.4.2.17 Water holding capacity (WHC)

Described in section 4.3.2.7.

5.4.2.18 Colour of cooked bologna

Described in section 4.3.2.8.

5.4.2.19 Instrumental texture analysis

Described in section 4.3.2.9.

5.4.2.20 Statistical analysis

One replication of experiment was analyzed. Means were computed, but no further statistical analysis was run on data collected.

5.5 Results and discussion

5.5.1 Temperature profile of MSP-DL, MSP-STD, and MSP-PC samples

As shown in **Figure 5-1**, the starting temperature of MSP-PC was around 1 °C, much lower than 8 °C of MSP-DL and MSP-STD. The mechanical deboning process including the deboning and separating steps generated heat and raised meat temperature from 2 °C (temperature of meat on bones before deboning) to around 8 °C. The heat exchanger chilled the MSP efficiently with extra churning inside the chamber. The MSP-DL was tempered at around 4 °C for 4 h before moving to the blast freezer, while MSP-STD and PC were moved to blast freezer right after packaging. From the data collected, it took about 14.2 h for the internal temperature of MSP-DL to reach 0 °C, 11.4 h for MSP-STD, and 7.9 h for MSP-PC. The heat exchanger significantly decreased the time needed for the sample to reach the frozen temperature. After around 28 h post processing, the meat temperature started to decrease faster again, indicating cooling of already frozen meat now. At this time, internal temperature of MSP-DL decreased the most rapid, followed by MSP-STD, and MSP-PC. After around 56 h post-processing, all MSP samples were chilled to around -20 °C.

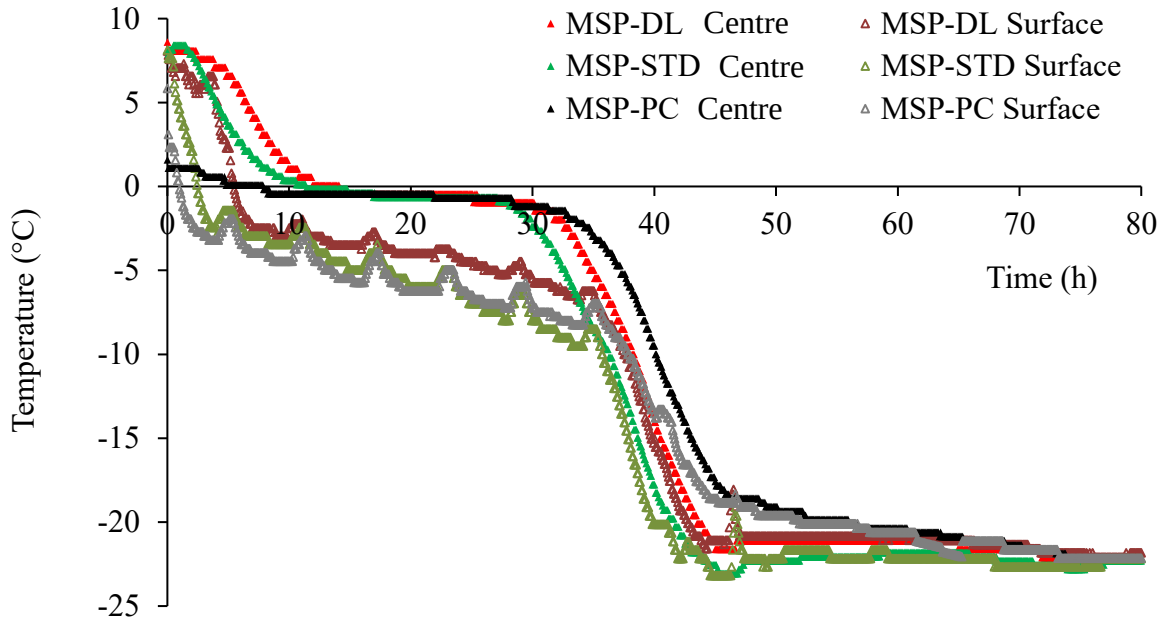

Figure 5-1 Surface and centre temperature profile of delay chilled mechanically separated pork (MSP-DL), standard mechanically separated pork (MSP-STD), prechilled mechanically separated pork (MSP-PC) samples blocks during first 80 hours of chilling and freezing process.

5.5.2 Proximate composition of raw meats

Table 5-2 showed the proximate composition of PP, BM, MSP-DL, MSP-STD, and MSP-PC. The protein (16.07-18.13%) and fat (9.70-21.03%) contents of MSPs varied due to the variation of tissue composition adhering to bones. The high protein and low fat content of BM was a result of hand removal of tough connective tissue for the purpose of simulating the composition of MSP.

Table 5-2 Proximate composition of pork picnic (PP), meat trim from bone (BM), MSP-delay (MSP-DL), MSP-standard (MSP-STD), MSP-prechilled (MSP-PC).

	Moisture (%)	Protein (%)	Fat (%)	Ash (%)
PP*	65.31	18.05	14.59	1.00
BM	68.12	21.89	9.70	0.92
MSP-DL	61.98	16.91	19.21	1.01
MSP-STD	60.47	16.07	21.03	0.95
MSP-PC	64.38	18.13	16.48	0.97

*PP: pork picnic; BM: meat trim from bone; MSP-DL: delay chilled mechanically separated pork; MSP-STD: standard mechanically separated pork; MSP-PC: prechilled mechanically separated pork

5.5.3 Raw meat pH

As shown in **Table 5-3**, the PP had lowest pH value compared with BM and MSP as discussed in section 3.5.6. The pH of MSP-PC was not different from MSP-DL or MSP-STD. The pH of pork samples was relatively stable during storage period, with that of MSP ranged from 6.31 to 6.58. The results agreed with Field (1982) that the pH of MSP was stable during frozen storage.

Table 5-3 pH of pork picnic (PP), meat trim from bone (BM), MSP-delay (MSP-DL), MSP-standard (MSP-STD), MSP-prechilled (MSP-PC) during frozen storage.

Storage period	1 month	4 months	7 months	13 months
PP*	5.87	5.95	5.94	5.96
BM	6.09	-	-	-
MSP-DL	6.36	6.50	6.31	6.38
MSP-STD	6.32	6.58	6.34	6.35
MSP-PC	6.34	6.56	6.40	6.38

*PP: pork picnic; BM: meat trim from bone; MSP-DL: delay chilled mechanically separated pork; MSP-STD: standard mechanically separated pork; MSP-PC: prechilled mechanically separated pork

5.5.4 Raw meat colour

Table 5-4 shows the colour of PP, BM, and MSPs after 1, 4, 7, and 13 months of frozen storage. The colour of MSP-PC was comparable to the colour of MSP-DL and MSP-STD. During 1 to 13 months of frozen storage, the a* and b* values of all meat samples had a tendency to decrease with the storage time as a result of myoglobin oxidation and denaturation (Field, 1982). According to Field (1982), the colour of MSM turned to dull brownish red after pigment oxidation. The increase of storage length was reported to decrease the instrumental colour (L*, a*, b*) of beef

(Farouk & Swan, 1998). The increased lipid oxidation level as shown in section 5.5.5 might also contribute to the colour change of MSP during long-term frozen storage. The MSP had higher iron than PP. The heme iron plays a role in the co-oxidation of lipids and proteins (Zhou et al., 2016). The lipid peroxidation products could promote the myoglobin oxidation in return (Chaijan, 2008).

Table 5-4 Colour of pork picnic (PP), meat trim from bone (BM), MSP-delay (MSP-DL), MSP-standard (MSP-STD), MSP-prechilled (MSP-PC) during frozen storage.

Treatments*	Storage (month)	L*	a*	b*
PP	1	59.65	26.64	23.06
	4	58.48	25.22	21.87
	7	55.87	23.17	20.14
	13	62.60	22.91	20.54
BM	1	56.24	22.18	20.68
MSP-DL	1	41.79	33.68	26.47
	4	48.90	32.02	25.39
	7	42.59	31.37	25.30
	13	43.51	27.03	22.28
MSP-STD	1	45.35	36.87	30.00
	4	49.39	33.27	26.05
	7	44.30	28.61	23.82
	13	44.83	28.19	23.67
MSP-PC	1	47.99	36.56	29.26
	4	50.68	34.31	27.43
	7	44.56	28.63	23.80
	13	47.89	28.76	24.38

*PP: pork picnic; BM: meat trim from bone; MSP-DL: delay chilled mechanically separated pork; MSP-STD: standard mechanically separated pork; MSP-PC: prechilled mechanically separated pork

5.5.5 Lipid oxidation

The BM and MSP had numerically higher lipid oxidation compared with PP (**Table 5-5**), which could be explained by the increased surface area, and increased temperature during processing and before frozen storage which increased the chance of lipid oxidation. The formation of metmyoglobin as a result of protein oxidation during frozen storage could reversely induce the lipid peroxidation (Chaijan, 2008). The extent of lipid oxidation of PP slightly increased with frozen storage until 7 months, followed by slight decrease at 13 months. However, the MSP-DL,

STD, PC were quite stable in terms of lipid oxidation during frozen storage until 7 months. The extent of lipid oxidation dramatically increased after 13 months of frozen storage. Compared with MSP-DL and MSP-STD samples, the MSP-PC sample showed much lower lipid oxidation value at the beginning of storage probably due to the decreased amount of time that MSP-PC stayed at high temperature (~8 °C) before frozen storage. After 13 months of frozen storage, the MSP-PC showed much lower lipid oxidation level than the other 2 kinds of MSP indicating the fact that prechilling of MSP could decrease the level of lipid oxidation throughout the frozen storage period especially after long term frozen storage. But more replicated data should be collected to further confirm this observation.

Table 5-5 Lipid oxidation (mg malondialdehyde/kg meat) of pork picnic (PP), meat trim from bone (BM), MSP-delay (MSP-DL), MSP-standard (MSP-STD), MSP-prechilled (MSP-PC) during frozen storage.

Storage period	1 month	4 months	7 months	13 months
PP*	0.42	0.47	0.56	0.39
BM	0.98	-	-	-
MSP-DL	1.17	1.06	1.21	3.05
MSP-STD	1.11	1.22	0.97	4.90
MSP-PC	0.81	0.86	0.89	1.48

*PP: pork picnic; BM: meat trim from bone; MSP-DL: delay chilled mechanically separated pork; MSP-STD: standard mechanically separated pork; MSP-PC: prechilled mechanically separated pork

5.5.6 Protein solubility

Table 5-6 shows protein solubility of meat samples followed 0-13 months of frozen storage. The solubility of sarcoplasmic protein and myofibrillar protein stayed relatively stable through out the storage period, while MSP-PC showed slightly higher protein solubility level than MSP-DL or STD at the same condition. The possible reason could be that prechilling delayed lipid oxidation and protein oxidation during frozen storage. The beef myofibrillar protein solubility decreased during frozen storage (Farouk et al., 2003). The same phenomenon has been observed in PP sample after 1-7 months storage length, however, the myofibrillar protein solubility of MSPs didn't change over time.

Table 5-6 Sarcoplasmic protein and myofibrillar protein solubility of pork picnic (PP), meat trim from bone (BM), MSP-delay (MSP-DL), MSP-standard (MSP-STD), MSP-prechilled (MSP-PC) during frozen storage.

Treatments*	Storage (month)	Sarcoplasmic protein (mg/g meat)	%Sarcoplasmic protein of total protein	Myofibrillar protein (mg/g meat)	%Myofibrillar protein of total protein	Total soluble protein (mg/g meat)	%Total soluble protein of total protein	Ratio of myofibrillar protein to sarcoplasmic protein (w/w)
PP	0	73.04	40.46	95.43	52.87	168.48	93.33	1.31
	1	73.04	40.46	95.43	52.87	168.48	93.33	1.31
	4	59.80	33.13	76.57	42.42	136.38	75.56	1.28
	7	54.51	30.20	68.84	38.14	123.36	68.34	1.26
	13	56.35	31.22	70.03	38.80	126.38	70.02	1.24
BM	0	55.50	25.36	75.80	34.63	131.30	59.98	1.37
	1	63.12	28.83	65.34	29.85	128.46	58.68	1.04
MSP-DL	0	58.12	34.38	54.55	32.27	112.67	66.65	0.94
	1	68.37	40.44	74.93	44.32	112.09	66.28	1.10
	4	55.92	33.07	56.17	33.22	112.09	66.28	1.00
	7	55.38	32.75	51.01	30.17	106.39	62.92	0.92
	13	55.04	32.55	56.62	33.48	111.66	66.03	1.03
MSP-STD	0	58.64	34.68	55.16	32.63	113.80	67.31	0.94
	1	70.33	43.77	73.92	46.00	144.25	89.78	1.05
	4	53.24	33.13	53.22	33.12	106.46	66.25	1.00
	7	54.12	33.68	52.23	32.50	106.35	66.18	0.97
	13	54.16	33.70	56.52	35.17	110.68	68.87	1.04
MSP-PC	0	68.51	37.80	63.68	35.14	132.19	72.93	0.93
	1	81.53	44.98	80.91	44.64	162.44	89.62	0.99
	4	62.57	34.51	62.29	34.36	124.86	68.87	1.00
	7	63.47	35.01	61.07	33.69	124.54	68.69	0.96
	13	63.39	34.97	67.23	37.08	130.62	72.05	1.06

*PP: pork picnic; BM: meat trim from bone; MSP-DL: delay chilled mechanically separated pork; MSP-STD: standard mechanically separated pork; MSP-PC: prechilled mechanically separated pork

5.5.7 SDS-PAGE of NAM

The NAM profile of PP and MSPs during 4-7 months of frozen storage is shown in **Figure 5-2**. Comparing with PP, the NAM of MSP showed proteolytic change of certain bands, especially the MHC which is known to be responsible for meat protein gelation (Xiong, 2004). The MSP-PC didn't show any obvious difference in terms of MHC degradation compare with MSP-DL or STD.

The effect of storage length on the protein degradation could not be quantified in this analysis. However, no noticeable difference has been observed between samples stored for 4 or 7 months.

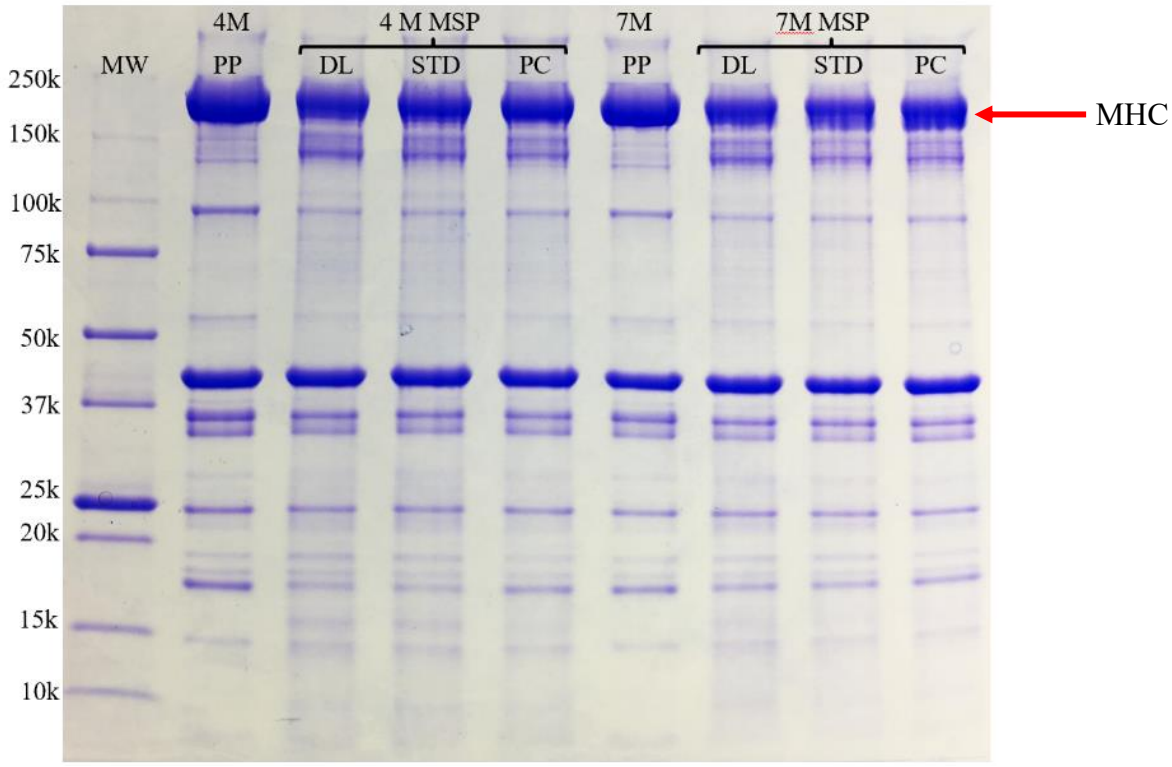

Figure 5-2 Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (4-20% gel) profile of natural actomyosin (NAM) extracted from pork picnic (PP) and delay chilled mechanically separated pork (MSP-DL), standard mechanically separated pork (MSP-STD), prechilled mechanically separated pork (MSP-PC) after 4 and 7 months of frozen storage at -18 °C. MW: molecular weight marker; 4M: 4 months of frozen storage; 7M: 7 months of frozen storage; MHC: myosin heavy chain

5.5.8 Dynamic oscillatory rheology

Dynamic oscillatory rheology has been conducted on NAM from PP and MSPs following 4 and 7 months of frozen storage. The G', G" and δ of NAM during heating cycle from 25 to 85 °C has been graphed showing the viscoelastic property of NAM.

Figure 5-3, 5-4, 5-5 showed rheological property of NAM from PP, MSP-DL, MSP-STD, MSP-PC after 4 months of frozen storage. The G' of PP increased with the increase of temperature at the beginning, shouldered at 35 °C and peaked around 42 °C. The G' decreased considerably after further heating until the lowest value (6.43 Pa) was observed at 58 °C, which is known as a typical denaturation temperature of myosin (Doerscher et al., 2003). After this point, the G' steadily increased to 75.36 Pa. Compared with PP, MSPs had lower G' value at the beginning of heating, the G' slightly increased with the increase of temperature up to around 32 °C, then began to slightly decrease until around 41 °C. Upon further heating, the G' peaked at 45-46 °C, followed by sharp decrease until the lowest value was reached at 58 °C. The slope of G' of NAM from MSPs was much lower than that of PP after 58 °C, showing the lower elasticity of gel and poorer development of a 3-D network. The final G' values for MSP-DL, MSP-STD, MSP-PC were 8.24, 3.50, and 21. 70 Pa respectively. The G" of PP peaked around 47 °C, but the G" of MSPs peaked around 45 °C. Further heating led to quick decrease of G" until the lowest value was observed at around 58 °C. The δ of PP and MSPs both increased upon heating followed by sharp decrease between 51 to 63 °C showing the gel networking formation process, then became stable afterwards. The δ of MSP-DL and STD was higher between 25 to 46 °C, and 55 to 85 °C compared with PP, which meant MSP had higher viscosity and lower elasticity during these temperature ranges. The δ of MSP-PC after 55 °C was very close to the value of PP, even though the slope of G' and G" was lower.

The NAM from MSP-PC had highest G' and G" value followed by MSP-DL, and MSP-STD at 4 months of frozen storage.

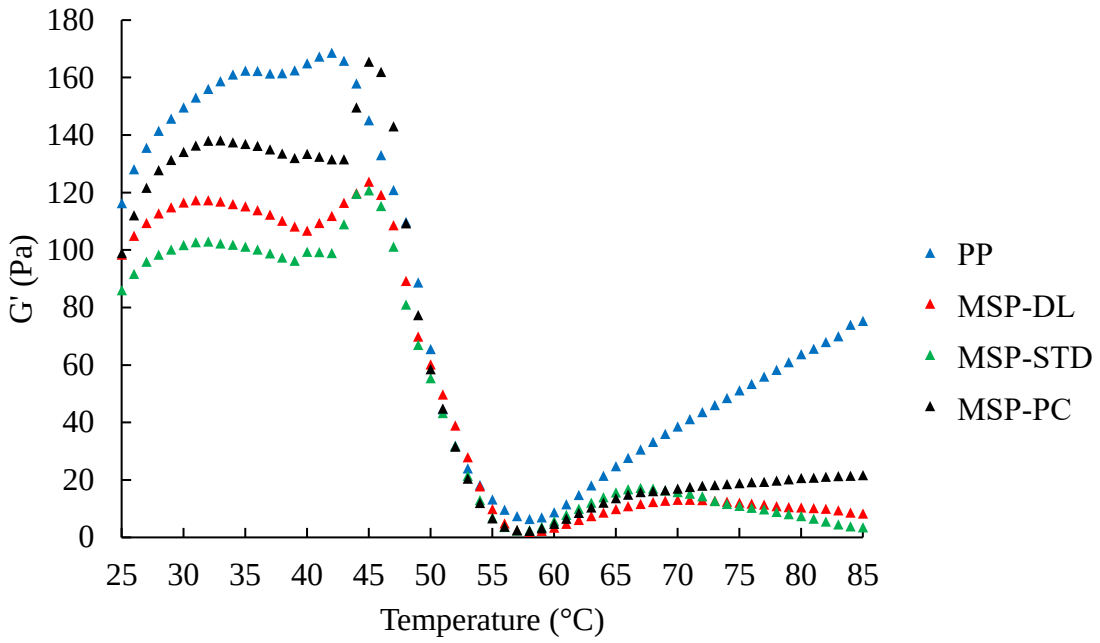

Figure 5-3 Storage modulus (G') of natural actomyosin (NAM) from pork picnic (PP) and delay chilled, standard, prechilled mechanically separated pork (MSP-DL, MSP-STD, MSP-PC) after 4 months of frozen storage with heating rate of 1 °C/min.

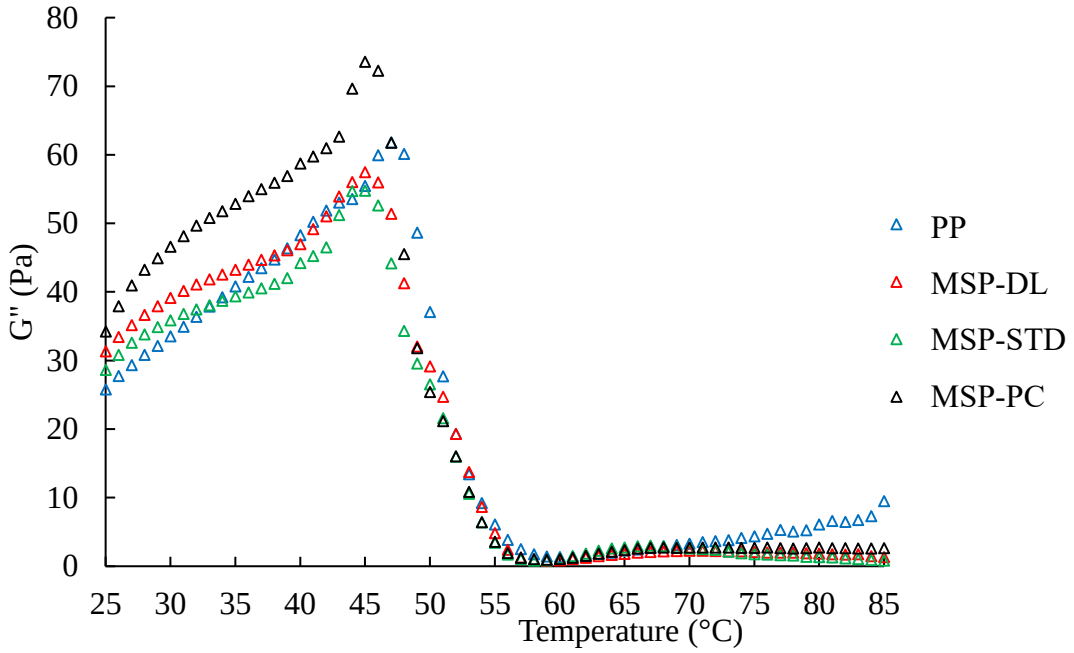

Figure 5-4 Loss modulus (G") of natural actomyosin (NAM) from pork picnic (PP) and delay chilled, standard, prechilled mechanically separated pork (MSP-DL, MSP-STD, MSP-PC) after 4 months of frozen storage with heating rate of 1 °C/min.

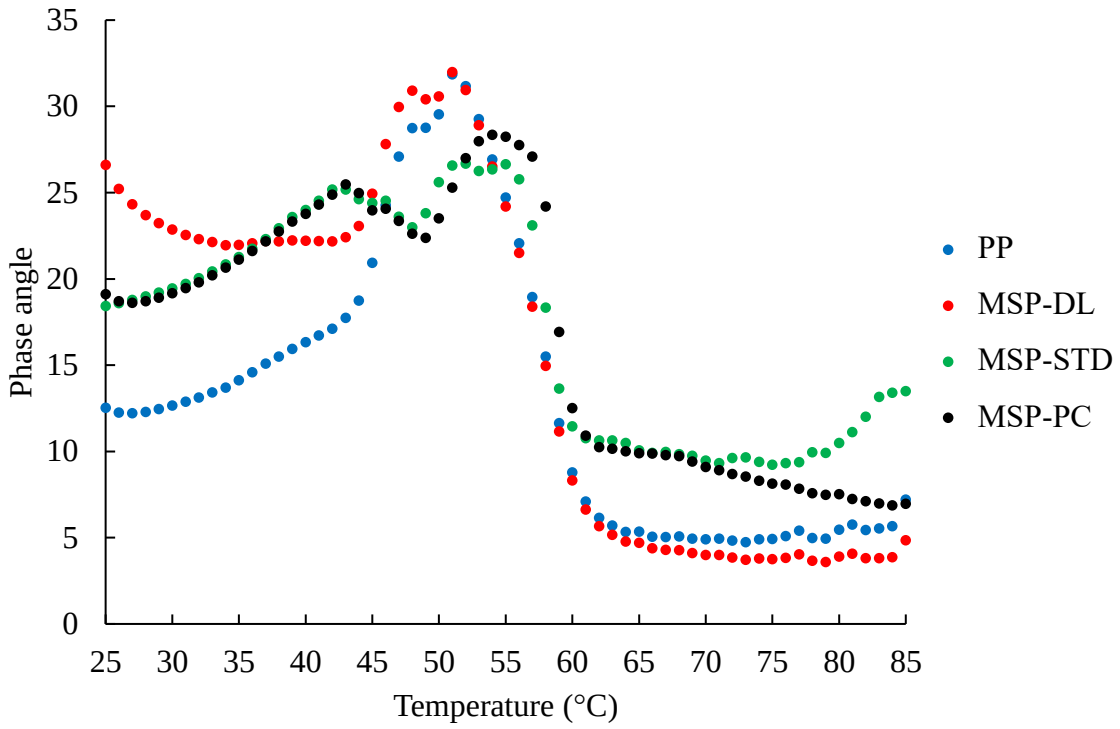

Figure 5-5 Phase angle (δ) of natural actomyosin (NAM) from pork picnic (PP) and delay chilled, standard, prechilled mechanically separated pork (MSP-DL, MSP-STD, MSP-PC) after 4 months of frozen storage with heating rate of 1 °C/min.

Figure 5-6, 5-7, 5-8 show the viscoelastic properties of NAM after 7 months frozen storage of the meat samples. Compared with 4 months of frozen storage, decrease in G' and G" value has been observed in MSP samples, but not in PP. The viscoelasticity of NAM from MSPs decreased as the increase of frozen storage. The effect of initial chilling rate on G' and G" decreased after 7 months of frozen storage.

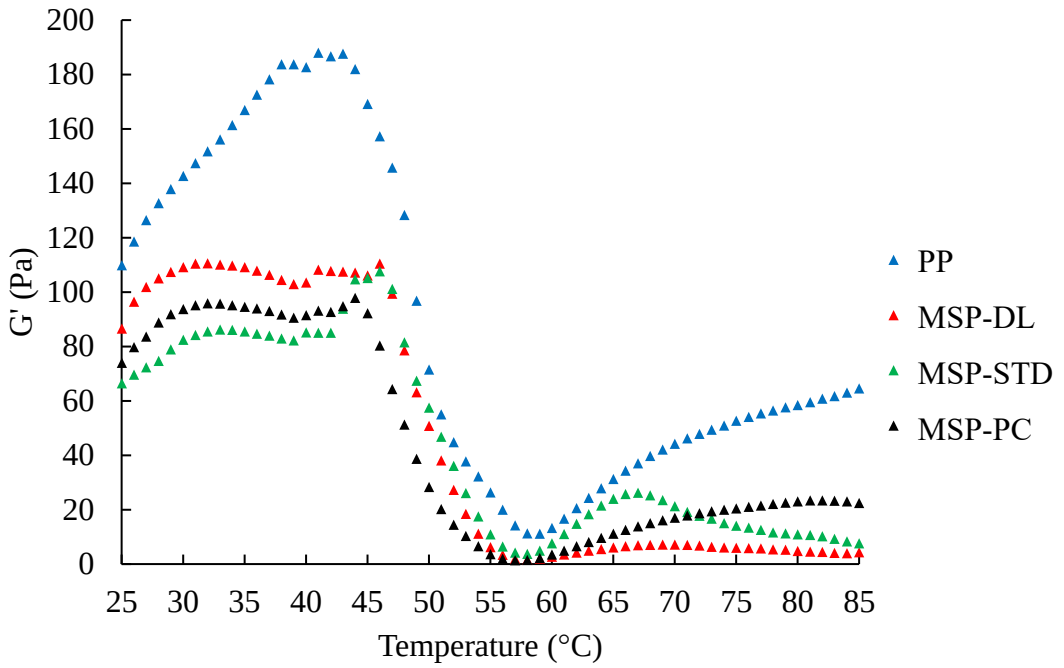

Figure 5-6 Storage modulus (G') of natural actomyosin (NAM) from pork picnic (PP) and delay chilled, standard, prechilled mechanically separated pork (MSP-DL, MSP-STD, MSP-PC) after 7 months of frozen storage with heating rate of 1 °C/min.

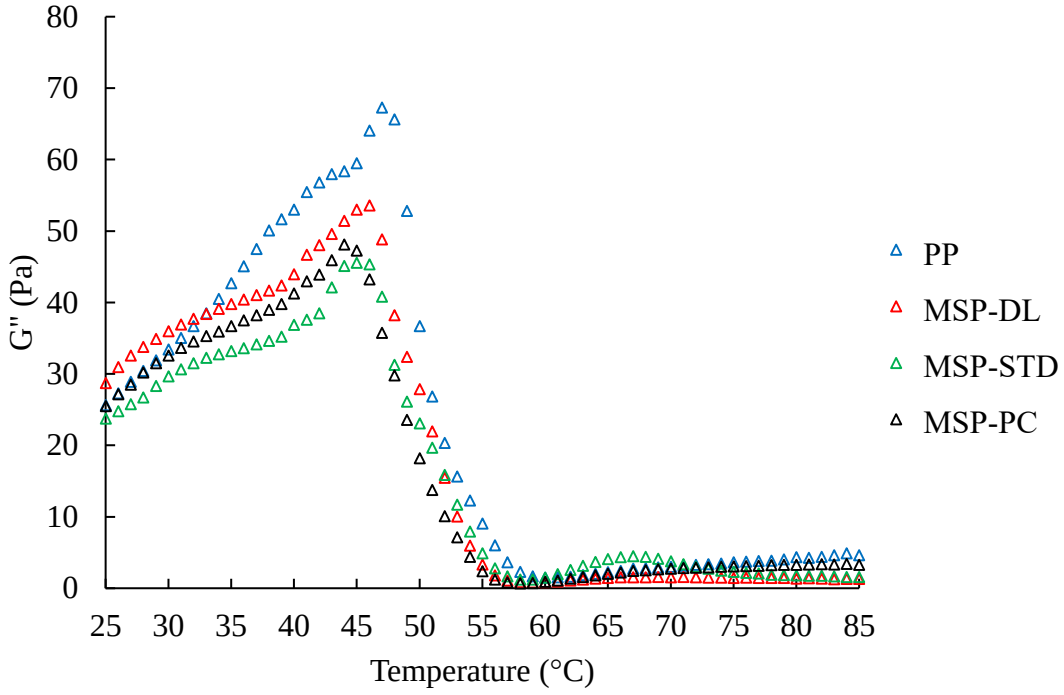

Figure 5-7 Loss modulus (G") of natural actomyosin (NAM) from pork picnic (PP) and delay chilled, standard, prechilled mechanically separated pork (MSP-DL, MSP-STD, MSP-PC) after 7 months of frozen storage with heating rate of 1 °C/min.

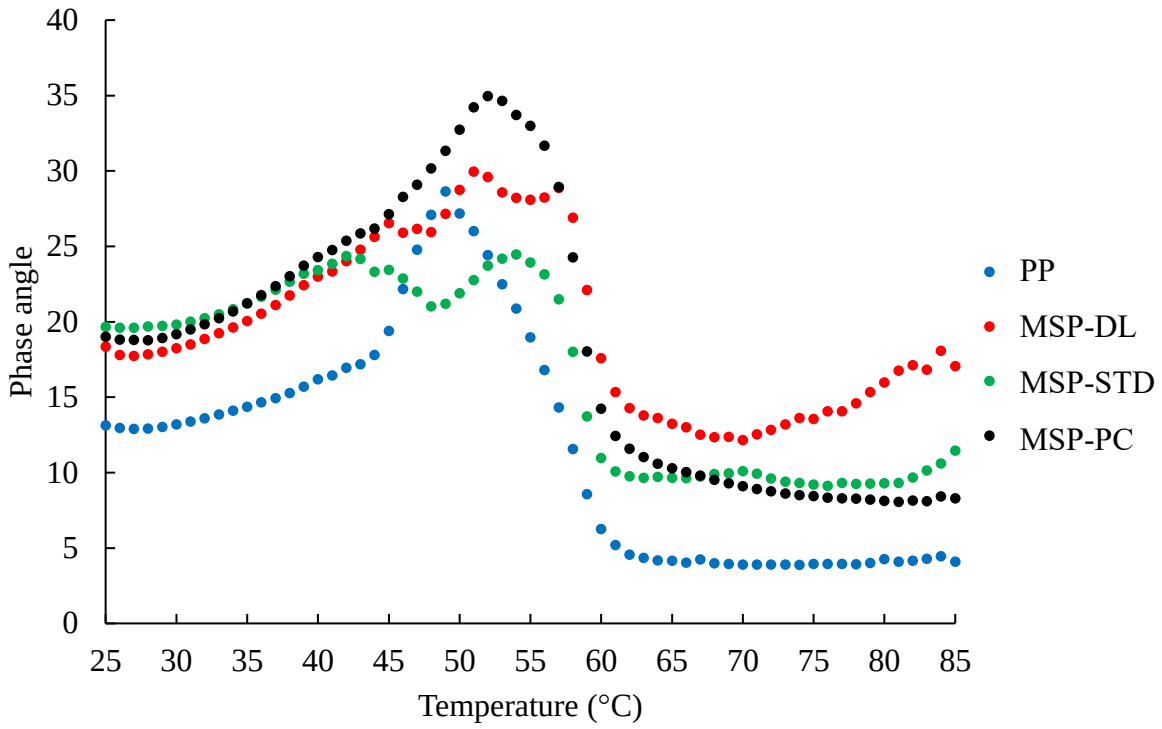

Figure 5-8 Phase angle (δ) of natural actomyosin (NAM) from pork picnic (PP) and delay chilled, standard, prechilled mechanically separated pork (MSP-DL, MSP-STD, MSP-PC) after 7 months of frozen storage with heating rate of 1 °C/min.

5.5.9 Proximate composition of cooked bologna

Table 5-7 shows the proximate composition of bologna samples. The protein level in cooked products ranged from 10.79 to 11.43%, the fat level in cooked products ranged from 12.55 to 15.54%. The proximate composition of bologna products slightly deviated from target protein (12%) and fat (14%) levels.

5.5.10 Batter viscosity

The apparent viscosity of meat batters is presented in **Table 5-8**. The difference between MSP-DL, MSP-STD, MSP-PC substitution at either level was not obvious with limited data replication collected. The 13 months long-term storage of MSPs didn't result in decreased batter viscosity.

Table 5-7 Proximate composition of cooked bologna.

Treatments*	%Moisture	%Ash	%Protein	%Fat
CON	69.90	3.38	11.09	14.44
BM-7.5	70.10	3.40	11.28	14.39
BM-15	71.10	3.25	11.23	12.55
DL-7.5	69.68	3.44	11.31	13.33
DL-15	70.03	3.47	11.00	15.54
STD-7.5	70.08	3.43	11.28	15.19
STD-15	70.42	3.43	11.01	13.79
PC-7.5	70.09	3.44	11.43	13.32
PC-15	69.66	3.39	10.79	14.64

*CON: bologna formulated with only regular pork as meat ingredient; BM-7.5/15: bologna formulated with 7.5 or 15% of meat trim from bones; DL-7.5/15: bologna formulated with 7.5 or 15% of delay chilled mechanically separated pork; STD-7.5/15: bologna formulated with 7.5 or 15% of standard mechanically separated pork; PC-7.5/15: bologna formulated with 7.5 or 15% of prechilled mechanically separated pork

Table 5-8 Effects of freezing rates, addition levels, and frozen storage length of mechanically separated pork (MSP) on bologna batter apparent viscosity.

Treatments*	1 month		4 months		7 months		13 months	
	Viscosity ($cPs \times 10^4$)	Batter temperature (°C)	Viscosity ($cPs \times 10^4$)	Batter temperature (°C)	Viscosity ($cPs \times 10^4$)	Batter temperature (°C)	Viscosity ($cPs \times 10^4$)	Batter temperature (°C)
CON	11.5	13.0	10.6	8.8	13.6	12.4	11.6	9.6
BM-7.5	10.5	13.0	-	-	-	-	-	-
BM-15	9.4	12.0	-	-	-	-	-	-
DL-7.5	9.2	12.0	9.1	8.2	12.6	11.1	10.6	8.6
DL-15	9.4	13.0	8.2	7.2	12.0	11.6	9.5	11.6
STD-7.5	9.7	12.7	9.5	6.8	11.3	12.0	10.0	8.2
STD-15	9.1	12.4	7.9	8.4	10.5	11.4	9.5	8.4
PC-7.5	10.9	12.8	8.7	7.6	12.2	9.2	9.9	9.5
PC-15	9.6	13.0	8.3	7.6	11.8	11.2	9.4	10.3

*CON: bologna formulated with only regular pork as meat ingredient; BM-7.5/15: bologna formulated with 7.5 or 15% of meat trim from bones; DL-7.5/15: bologna formulated with 7.5 or 15% of delay chilled mechanically separated pork; STD-7.5/15: bologna formulated with 7.5 or 15% of standard mechanically separated pork; PC-7.5/15: bologna formulated with 7.5 or 15% of prechilled mechanically separated pork

5.5.11 pH of raw and cooked bologna

The pH of raw bologna batters fluctuated around 6.2 for most of the sample formulas after 1-13 months storage (**Table 5-9**). For cooked bologna samples, the pH was around 6.4. The pH values of raw batter formulated with MSP were slightly higher than those of CON. The reason could be the higher pH value of MSPs. The storage length of MSPs didn't show clear effect on the pH of raw bologna batter.

Table 5-9 Effects of freezing rates, addition levels, and frozen storage length of mechanically separated pork (MSP) on pH of raw and cooked bologna.

Treatments	1 month		4 months		7 month		13 months	
	Raw	Cooked	Raw	Cooked	Raw	Cooked	Raw	Cooked
CON*	6.18	6.41	6.10	6.31	6.25	6.43	6.08	6.44
BM-7.5	6.18	6.41	-	-	-	-	-	-
BM-15	6.14	6.42	-	-	-	-	-	-
DL-7.5	6.24	6.38	6.16	6.34	6.29	6.49	6.11	6.44
DL-15	6.22	6.40	6.19	6.43	6.29	6.48	6.20	6.56
STD-7.5	6.19	6.39	6.15	6.38	6.25	6.47	6.12	6.44
STD-15	6.28	6.43	6.18	6.42	6.27	6.48	6.21	6.53
PC-7.5	6.17	6.41	6.18	6.40	6.29	6.47	6.15	6.44
PC-15	6.25	6.45	6.20	6.46	6.29	6.49	6.22	6.49

*CON: bologna formulated with only regular pork as meat ingredient; BM-7.5/15: bologna formulated with 7.5 or 15% of meat trim from bones; DL-7.5/15: bologna formulated with 7.5 or 15% of delay chilled mechanically separated pork; STD-7.5/15: bologna formulated with 7.5 or 15% of standard mechanically separated pork; PC-7.5/15: bologna formulated with 7.5 or 15% of prechilled mechanically separated pork

5.5.12 WHC of cooked bologna

The effects of MSP freezing methods, levels, and frozen storage on WHC were determined by cook loss of bologna during cooking, and the water holding under centrifugal stress (expressible moisture) and the purge loss after 14 days refrigerated storage as presented in **Table 5-10**.

Table 5-10 Effects of freezing rates, addition levels, and frozen storage length of mechanically separated pork (MSP) on cook loss, expressible moisture, and purge loss of cooked bologna.

Treatments	Cook loss (%)				Expressible moisture (%)				Purge loss (%)			
	1 M[2]	4 M	7 M	13 M	1 M	4 M	7 M	13 M	1 M	4 M	7 M	13 M
CON[1]	1.1	0.7	0.5	0.5	12.4	13.3	12.6	13.3	3.9	4.4	3.5	3.4
BM-7.5	0.6	-	-	-	12.5	-	-	-	3.8	-	-	-
BM-15	0.6	-	-	-	12.6	-	-	-	3.8	-	-	-
DL-7.5	0.7	0.4	0.5	0.5	11.8	14.3	14.2	13.3	3.5	3.9	3.8	3.4
DL-15	0.4	0.6	0.5	0.6	20.0	22.4	22.1	22.2	3.0	2.7	2.1	2.3
STD-7.5	0.6	0.4	0.5	0.5	12.6	14.3	13.3	13.7	3.3	4.0	3.9	3.2
STD-15	0.7	0.5	0.5	0.7	19.4	22.4	20.3	21.1	2.4	2.3	2.4	1.9
PC-7.5	0.4	0.4	0.5	0.4	13.8	14.7	13.3	14.6	3.4	3.5	3.9	3.2
PC-15	0.5	0.5	0.5	0.7	20.3	21.9	22.4	21.1	2.5	2.1	2.7	1.9

[1]CON: bologna formulated with only regular pork as meat ingredient; BM-7.5/15: bologna formulated with 7.5 or 15% of meat trim from bones; DL-7.5/15: bologna formulated with 7.5 or 15% of delay chilled mechanically separated pork; STD-7.5/15: bologna formulated with 7.5 or 15% of standard mechanically separated pork; PC-7.5/15: bologna formulated with 7.5 or 15% of prechilled mechanically separated pork
[2]M: month

5.5.12.1 Cook loss

The cook loss was not influenced by the storage length of MSPs and PP, and the initial MSP chilling rates. However, after 13 months of frozen storage, more jelly like surface of bologna was observed.

5.5.12.2 Expressible moisture

The expressible moisture of bologna samples was not influenced by initial chilling rates of MSPs, or the 1-13 months of frozen storage of raw meat ingredients. However, the bologna formulated with 15% MSPs showed much higher expressible moisture throughout the storage study. The result agreed with the results discussed in section 4.5.4.2.

5.5.12.3 Purge loss

For the purge loss analysis, the addition of 15% MSP to bologna decreased purge loss after 14 days refrigerated storage, which was observed at all storage times of the meat prior to processing. The purge loss of bologna was not influenced by the storage length of meats or the initial chilling rate of MSPs.

5.5.13 Colour of cooked bologna

Figure 5-9 shows the inter sections of bologna treatments made with meats frozen stored for 7 months. The colour change of bologna is shown in **Table 5-11** below. The results showed that the colour of bologna was relatively stable during the 1 to 13 months of raw meat frozen storage. The L* decreased during 1 to 4 months of frozen storage, and then remained stable. The a* values of bologna treatments slightly increased after 4 months frozen storage of meats, then gradually decreased after extended frozen storage for up to 13 months. b* values remained stable through out the frozen storage. The colour of bologna was not influenced by the initial chilling rate of MSPs.

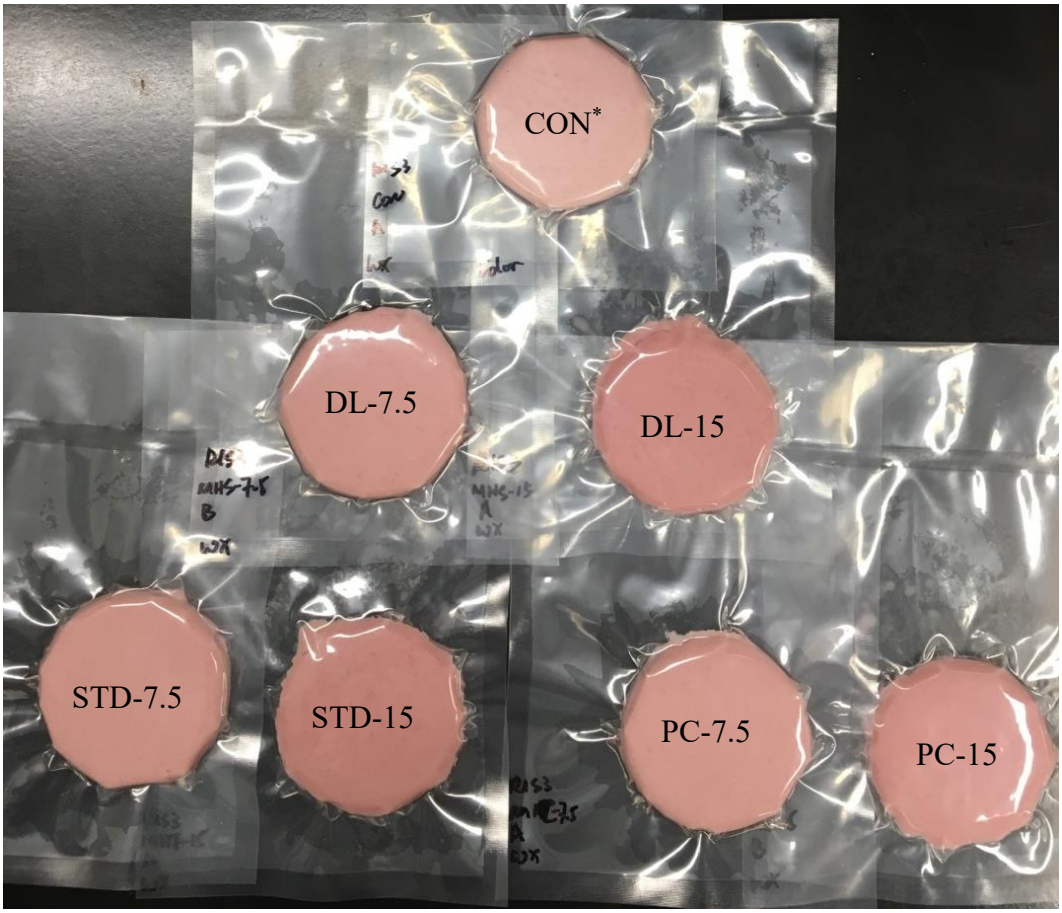

Figure 5-9 Bologna slices processed with meats after 7 months of frozen storage.
*CON: bologna formulated with only regular pork as meat ingredient; DL-7.5/15: bologna formulated with 7.5 or 15% of delay chilled mechanically separated pork; STD-7.5/15: bologna formulated with 7.5 or 15% of standard mechanically separated pork; PC-7.5/15: bologna formulated with 7.5 or 15% of prechilled mechanically separated pork

Table 5-11 Effects of freezing rates, addition levels, and frozen storage length of mechanically separated pork (MSP) on colour of cooked bologna.

Treatments*	Storage (month)	L*	a*	b*
CON	1	75.03	15.41	12.28
	4	72.05	17.03	12.96
	7	72.45	16.95	12.89
	13	71.23	15.78	12.13
BM-7.5	1	74.32	15.53	12.40
BM-15	1	73.01	16.14	12.72
DL-7.5	1	69.03	19.01	13.52
	4	66.59	20.05	13.83
	7	66.54	20.00	13.84
	13	66.21	19.11	13.90
DL-15	1	65.44	21.54	14.68
	4	60.79	22.76	14.73
	7	61.72	22.00	14.76
	13	63.30	20.15	14.72
STD-7.5	1	70.18	18.63	13.53
	4	65.67	20.81	14.09
	7	67.66	18.95	13.54
	13	66.90	18.17	13.39
STD-15	1	64.84	21.67	14.77
	4	62.03	22.87	15.30
	7	62.64	21.96	15.12
	13	65.90	18.63	13.58
PC-7.5	1	71.39	18.20	13.38
	4	67.25	20.23	14.11
	7	68.07	18.94	13.23
	13	68.73	17.69	12.95
PC-15	1	66.85	20.14	13.94
	4	63.13	22.10	14.60
	7	64.82	20.71	14.04
	13	62.39	19.65	13.88

*CON: bologna formulated with only regular pork as meat ingredient; BM-7.5/15: bologna formulated with 7.5 or 15% of meat trim from bones; DL-7.5/15: bologna formulated with 7.5 or 15% of delay chilled mechanically separated pork; STD-7.5/15: bologna formulated with 7.5 or 15% of standard mechanically separated pork; PC-7.5/15: bologna formulated with 7.5 or 15% of prechilled mechanically separated pork

5.5.14 Instrumental texture analysis

5.5.14.1 Texture profile analysis (TPA)

Table 5-12 shows TPA of bologna in terms of TPA hardness, adhesiveness, cohesiveness, springiness, and chewiness. Substitution of 7.5% MSP didn't change the hardness of bologna very much, however, further increasing the addition level of MSP led to a dramatic decrease of bologna hardness, cohesiveness, springiness, and chewiness. For 15% MSP formulations, the hardness, springiness, and chewiness of bologna slightly decreased after frozen storage (1-13 months), however, the effects of storage on texture of bologna chubs made with 7.5% MSP or pure PP were not obvious in this replicate. The effect of initial chilling rate of MSPs on the texture of bologna during long-term frozen storage was negligible according to the data collected.

As discussed in section 4.5.6.1, the soft and mushy texture of bologna made with 15% MSP could be the result of poor gelling ability of MSP, lack of high quality myofibrillar proteins from PP, and reduced collagen content. The G' of NAM from MSPs decreased with the increase of frozen storage duration was discussed in section 5.5.8. The further decreased functionality of NAM in MSPs could help explain the textural changes of bologna made with 15% MSPs.

Table 5-12 Effects of freezing rates, addition levels, and frozen storage length of mechanically separated pork (MSP) on the texture profile analysis (TPA) of pork bologna.

Treatments*	Storage (month)	Hardness (N)	Adhesiveness (-)	Cohesiveness (-)	Springiness (%)	Chewiness (N)
CON	1	97.3	0.6	0.49	74.6	487.9
	4	107.6	0.7	0.57	80.3	619.7
	7	120.6	0.7	0.51	77.8	678.2
	13	124.2	0.9	0.54	79.5	726.1
BM-7.5	1	105.0	0.6	0.53	79.4	626.6
BM-15	1	107.1	0.5	0.56	81.2	631.0
DL-7.5	1	98.4	0.7	0.51	77.0	532.6
	4	113.5	0.9	0.53	77.2	595.2
	7	110.1	0.7	0.53	77.7	606.7
	13	101.1	0.8	0.46	76.0	507.3
DL-15	1	35.5	0.4	0.21	49.8	52.3
	4	30.9	0.9	0.22	43.6	37.4
	7	25.9	1.0	0.23	43.9	34.0
	13	25.8	0.9	0.20	38.7	27.9
STD-7.5	1	84.2	0.8	0.55	81.1	482.3
	4	100.4	0.8	0.55	78.9	547.7
	7	80.3	0.8	0.55	72.5	456.3
	13	98.8	0.6	0.48	75.1	513.0
STD-15	1	26.6	0.3	0.20	44.4	32.3
	4	24.5	1.0	0.21	40.9	26.0
	7	20.9	0.9	0.22	38.2	23.5
	13	18.3	1.2	0.22	38.4	20.0
PC-7.5	1	105.6	0.6	0.50	78.0	552.2
	4	103.6	0.8	0.54	78.7	575.5
	7	95.8	0.8	0.54	80.7	537.8
	13	86.9	0.7	0.54	76.5	476.5
PC-15	1	29.0	0.3	0.22	50.9	44.0
	4	24.5	1.0	0.22	43.8	30.5
	7	21.4	1.1	0.22	40.1	24.7
	13	17.8	1.1	0.20	36.2	17.6

*CON: bologna formulated with only regular pork as meat ingredient; BM-7.5/15: bologna formulated with 7.5 or 15% of meat trim from bones; DL-7.5/15: bologna formulated with 7.5 or 15% of delay chilled mechanically separated pork; STD-7.5/15: bologna formulated with 7.5 or 15% of standard mechanically separated pork; PC-7.5/15: bologna formulated with 7.5 or 15% of prechilled mechanically separated pork

5.5.14.2 Torsional gelometry

According to **Table 5-13**, the substitution of 7.5% MSP in bologna slightly decreased the shear stress and shear strain at failure compared with the CON. After increasing the amount of MSP to 15%, the shear stress and shear strain decreased dramatically. The shear stress of 15% MSP substitution treatments was around 6 kPa compared with 23 kPa of CON formulated with pure PP (74% decrease). The initial freezing methods didn't show any influence on the shear stress or strain at failure of bologna. The shear strain of all treatments showed a trend of decreased shear strain after 13 months of raw meat frozen storage indicating that the texture of bologna became mushier with the storage of meats.

Table 5-13 Effects of freezing rates, addition levels, and frozen storage length of mechanically separated pork (MSP) on the shear stress and shear strain at failure of pork bologna.

Treatments	Shear stress (kPa)				Shear strain			
	1 month	4 months	7 months	13 months	1 month	4 months	7 months	13 months
CON*	23.90	22.94	20.70	22.12	1.70	1.41	1.58	1.40
BM-7.5	21.60	-	-	-	1.60	-	-	-
BM-15	26.55	-	-	-	1.84	-	-	-
DL-7.5	13.56	21.63	18.27	20.05	1.40	1.36	1.44	1.30
DL-15	6.54	6.84	5.22	5.29	1.16	0.78	0.73	0.78
STD-7.5	18.79	19.93	15.76	17.87	1.55	1.38	1.26	1.26
STD-15	5.51	5.66	4.87	5.05	1.05	0.82	0.75	0.92
PC-7.5	16.25	18.98	18.70	16.06	1.49	1.39	1.47	1.21
PC-15	6.90	5.86	5.23	4.60	1.14	0.83	0.96	0.75

*CON: bologna formulated with only regular pork as meat ingredient; BM-7.5/15: bologna formulated with 7.5 or 15% of meat trim from bones; DL-7.5/15: bologna formulated with 7.5 or 15% of delay chilled mechanically separated pork; STD-7.5/15: bologna formulated with 7.5 or 15% of standard mechanically separated pork; PC-7.5/15: bologna formulated with 7.5 or 15% of prechilled mechanically separated pork

5.6 Conclusion

The initial freezing rate of MSPs didn't show obvious influence on the physicochemical, biochemical and gelation properties. But the prechilling process (MSP-PC) showed the potential to retard lipid oxidation of MSP by reducing the initial meat temperature through pre-chilling process using the heat exchanger. The WHC, texture and appearance of bologna were not influenced by the initial freezing rates.

The pH and protein solubility of MSP didn't change with the storage length (1-13 months). The redness and yellowness of MSPs decreased as storage progressed. The MSPs had stable lipid oxidation level during the initial 7 months of frozen storage, however, the TBARS level quickly increased during 7 to 13 months of frozen storage. The viscoelasticity of NAM slightly decreased during the initial 7 months of storage. For the effect of frozen storage on bologna properties, the WHC was not influenced, but bologna redness was found to decrease after 4 to 13 months of raw meats' frozen storage. The effect of MSP frozen storage on bologna formulas at 7.5% substitution level was negligible. However, the trend of decreased TPA hardness, springiness, and chewiness, and decreased shear strain at failure was observed in bologna formulas with 15% MSP.

6 GENERAL DISCUSSION

Meat primary processing involves removal of intact meat cuts without getting bone splinters either by removing manually or using machines. There is a considerable amount of muscle protein remaining on bones that is valuable if they were carefully removed to make the most of the animal. MSM gradually has gained people's attention as the meat industry gets more mechanised than previous decades and produces large volumes at each plant, even though there is a concern about public health risk, microbial hazards for example. The mechanical separation process is an efficient and inexpensive strategy to help decrease the cost of meat production and increase the utilization of animal proteins. Currently, the mechanical separation process has been applied on pork, beef, poultry carcasses, and fish etc. to recover soft muscle tissues (Hui, 2012a).

Instead of using only a screwing and sieving action commonly involved in mechanically separated poultry processing, squeezing or rubbing action is used more in red meat processing (Hui, 2012a). In this study, the linear press system was used to squeeze the flesh adhering to pork arm bones under the pressure adjusted to give a target yield without breaking many bones. The big bone pieces were discarded, and the removed soft tissue together with crushed bone particles, cartilage, and sinews were subjected to a sieving process operated by a belt separator from which the soft muscle and fat tissues with small amount of very fine bone particles were sieved through a cylindrical filter and produced as MSP. MSM usually has lower protein content and higher fat content depending on factors including species and bone types (Field et al., 1976). In this study, the MSP had about 63% moisture, 15% protein, 20% fat, and 1.02% ash, while PP had about 66% moisture, 18% protein, 15% fat, and 0.94% ash. Due to the separation process, the calcium content is higher in MSM in general compared to fresh meat (Mayer et al., 2007). The calcium content is usually considered as a criterion to discriminate MSM, but it varies with bone type, species, feeding, age of the animal, and the separation pressure (Stenzel & Hildebrandt, 2006). As separation pressure increases, the calcium and iron, and total ash content increase (Barbut, 2002). According to a study conducted on the effect of deboner head pressure on the chemical composition and yield of MSM from poultry, the calcium and iron content increased from 582 ppm and 10.0

ppm to 764 and 17.9 ppm, respectively, as the pressure increased from around 300 kPa to 1000 kPa (Barbut, 2002). In the current study, the calcium and iron content in MSP was 729 and 35.1 ppm respectively, comparable to what has been reported considering red meats naturally have higher iron in muscle tissue, and the size of bone fragments was no larger than 1.0 mm in diameter. The calcium and iron content in MSP were significantly higher than those in PP, with 45 and 6.9 ppm respectively. The increased iron content helps explain the darker and redder colour of MSP as the incorporation of heme pigment from bone and blood.

The collagen content of MSM has been controversial. Field et al. (1976) reported that the connective tissue had been removed during the separation, while others found that collagen in MSM was higher than normal meats (Hui, 2012a). Our study agreed with Field et al. (1976) that collagen has been partially removed from MSP as the connective tissue such as sinews was too tough and large to go through the separator. The BR, which was the discarded soft tissue after MSP separation process, had much higher collagen content than PP or MSP and further confirmed the removal of connective tissue from MSP during the separation process. The pH of MSP was more neutral (6.4) than that of PP at 5.9, possibly due to the incorporation of bone marrow and blood. Higher lipid rancidity is another characteristic of MSP. MSP in this study showed higher lipid oxidation level (0.76 mg MDA/kg meat) at one month frozen storage than PP (0.49 mg MDA/kg meat). The reason could be the higher lipid content of MSP, and higher iron in MSP from hemeproteins (Zhou et al., 2016). Besides, the meat temperature increased through the separation process by the friction and heat generated in the equipment, and decreased meat particle size could facilitate the lipid peroxidation.

The mechanical separation process makes the MSP unique for its considerable cellular level disruption (Krautil & Tullock, 1987) which is likely the cause of the impaired gelation behaviour later. The TEM imaging revealed that PP had well preserved sarcomere structure with clear Z-lines, and sharp edges of actin-myosin filaments bundles. But MSP showed randomly oriented, curved and twisted actin-myosin filaments as a result of high-level cellular disruption. The interruption of lysosome membrane by high pressure was reported to increase the lysosomal enzyme activity in bovine muscles (Jung et al., 2000), similarly may have induced the proteolysis observed in the present study. However, the effects of the mechanical separation process on the protease activity in MSP remains unclear as we were not able to test this experimentally. Other evidence showed that the process led to the decreased ratio of myofibrillar protein to sarcoplasmic

protein (MSP: 1.05; PP: 1.32) and decreased gelation ability in MSP, even though the content of myofibrillar protein in MSP and PP were not significantly different. The dynamic oscillatory rheology profile of NAM from MSP showed reduced gel elasticity after denaturation of actomyosin and formation of 3-D gel network.

Unlike MSM from poultry or fish that have been successfully used in meat processing, the utilization of mechanically separated red meats has been restricted by its undesirable quality change, and textural softness in comminuted products (Kramer & Sebranek, 1990). The sarcoplasmic proteins have low WHC and weak gelation properties thus little contribution to binding strength of cooked sausage products was observed (Miyaguchi et al., 2000). It is widely accepted that myofibrillar proteins are responsible for gelation in meat products (Xiong, 2004), and the gelling capacity is mostly determined by the condition of myosin and species (Visessanguan & An, 2000). The intact myosin with its structural domains and natural conformation is essential for gel formation (Visessanguan & An, 2000). The different gel-forming ability was attributed to differences in the cross-linking of MHC in fish muscle from different species (Chan et al., 1992), but may not lead to the proper 3-D structure needed for strong gel texture.

Protein is subject to oxidative damage in the presence of reactive-oxygen species and transition metals (Estévez, 2011). Protein aggregation by radicals and secondary products of lipid oxidation could promote the cross-linking of myosin (Xiong et al., 2009), as myofibrillar proteins have enriched sulfhydryls that are susceptible to oxidative degradation (Chen et al., 2016). The protein oxidation of NAM could be a possible reason for the decreased gelation capacity of MSP. The SDS-PAGE analysis of NAM better characterized the difference between MSP and PP at the molecular level. The NAM from MSP showed proteolytic changes of certain bands, especially the MHC even before frozen storage. Multiple intense bands around 130 to 150 kDa were observed in NAM from MSP, and were confirmed to be the degradation products of MHC by immunoblotting. As the intact myosin molecule is the key to maintain best function of heat-induced myosin gels (Park et al., 1996), the partly degraded myosin in MSP could decrease the gelation capacity and functionality of MSP as a substitution for regular pork in comminuted meat products.

The common utilization of MSP is to substitute for normal meat in emulsion products that don’t require muscle fibre structure. However, the amount of MSP that could be incorporated into the bologna type products remains unclear for the mushy texture and decreased sensory acceptance due to the poor gelling capacity of MSP (Kramer & Sebranek, 1990). In this study, the MSP was

formulated at 0 (control), 7.5 or 15% in bologna formulas to replace part of PP. BM was added into the formula at 7.5 and 15% levels to have a direct comparison of the effects of MSP on the bologna texture, to better understand the effect of mechanical separation process on the gelation ability of MSP. The total protein (12%) and fat (14%) for bologna treatments were controlled to the same level for better comparison of texture, and to meet the CFIA (2019b) regulation for minimum meat protein content (9.5%) and total protein content (11%) in bologna.

As hypothesized, the bologna colour, texture, and WHC were influenced by the addition of MSP, and the addition level of MSP needs to be carefully tested before commercial utilization. The colour intensity of bologna increased with the increasing addition of MSP. The control bologna showed light pink colour as well as the BM incorporated treatments. The colour became darker and redder after the addition of MSP, since the MSP had more intense red colour pigments. The addition of 7.5 or 15% MSP didn't significantly affect the meat batter viscosity, cook loss, expressible moisture, and purge loss, but 15% MSP substitution showed a trend to increase the expressible moisture and decrease the purge loss of bologna. The high variation in expressible moisture and purge loss observed in 15% MSP formulas was due to the quality variation of MSPs from the third sample replicate, and the variation between meat lots also influenced the results of TPA and torsional gelometry analysis. The 15% MSP substitution had a trend to decrease the hardness, cohesiveness, springiness, and chewiness of bologna. The 7.5% MSP substitution level showed comparable WHC and TPA texture to the control treatment. The torsional gelometry texture map showed that bologna at 15% MSP level had mushy and soft texture indicated by decreased shear strain, while bologna made with 7.5% MSP, bologna made with BM at 7.5% and 15% levels all showed comparable shear stress and strain value to that of CON. According to Nuckles et al. (1990), the shear stress and strain increased with the increase of myosin and actin in meat while they decreased with the increase of low ionic strength protein. The observation agreed with literature, and the results of protein composition and functionality of MSP shown in this study helped explain the effect of MSP on bologna texture. In summary, the replacement of PP with 7.5% MSP didn't compromise the bologna texture, however, replacement of 15% MSP was not acceptable due to the mushy texture and undesirable appearance. Industry needs to be highly cautious in using high levels of MSP, as the products have very different texture and appearance. The texture of bologna formulated with BM at both levels was comparable to the control treatment,

which further confirmed that the decreased quality of MSP was the result of mechanical separation process rather than the intrinsic properties of muscle tissues.

In the meat industry, MSP is processed directly after short term refrigerated storage, or subjected to frozen storage for up to one year. Refrigeration is commonly used to extend shelf life of fresh meats from 5-7 days, and frozen storage could further extend shelf life by stopping the growth and reproduction of pathogenic microorganisms, and slowing down the deleterious chemical reactions (Murano, 2003). The previous freezing and frozen storage could decrease the protein emulsifying and gelling capacity (Jiménez-Colmenero & Borderías, 1983). Slow freezing rate could have a large denaturing effect on myofibrillar protein as a result of partial unfolding of the myosin head (Wagner & Añon, 1985). The unfolding is induced by the replacement of previous protein-water interaction with protein-protein associations or other interaction as a result of water migration during dehydration of myofibrils (Hamm, 1975; Fennema, 1977). The process of rapid freezing has little effect on meat colour, flavour, odour, or juiciness after cooking, however, frozen storage gradually decreases odour and flavour acceptability (Urbain & Campbell, 1987). The freezing temperature and condition, and air exposure has significant effect on meat quality (Urbain & Campbell, 1987). Since fast chilling has the potential to better protect protein from aggregation and denaturation, in this study MSP was chilled at standard and delayed rates to test the effects of initial chilling rates and frozen storage on the physicochemical and gelation properties of MSP.

After the mechanical separation process, the temperature of MSP was raised to about 7.6 °C. In the third study, ultra fast chilling was achieved by having MSP-PC go through a heat exchanger right after the separation process. The temperature of MSP-PC dropped to around 1 °C before boxing and it was blast chilled immediately for the ultrafast chilling effect (one sample replicate collected due to the major equipment breakdown). The MSP-STD was blast chilled immediately after boxing for the fast chilling effect, while MSP-DL was held at refrigeration temperature for 4 hours before blast chilling for the delayed freezing effect. From the data collected, it took 13.8 h for MSP-DL, 11.9 h for MSP-STD, 7.9 h for MSP-PC to be chilled below 0 °C. The blast frozen MSPs were stored at -18 °C for up to 13 months to investigate the effect of frozen storage and the synergistic effect of initial chilling rate and frozen storage.

The results showed that MSP-STD and MSP-DL were not different in pH, colour, lipid oxidation level, protein solubility, and viscoelasticity of NAM. The initial chilling rates didn't show influence on the colour, texture and WHC of bologna made from these meats. Basically, fast

chilling didn't play a major role in preserving the gelation ability of MSP likely because the damage had already occurred in the mechanical separation process. The pH, lipid oxidation level, and protein solubility of MSP was relatively stable during the initial 4 months of frozen storage. The redness and yellowness of MSPs decreased as the increase of storage length for up to 13 months, however the pH and protein solubility remained unchanged. The G' of NAM from MSP decreased during the 1 to 7 months of frozen storage, and it was relatively stable during the 4 to 7 months frozen storage period. The texture, cook loss, and purge loss of bologna treatments made with MSPs frozen stored for one to four months were stable. However, the expressible moisture significantly increased after 4 months frozen storage of raw meats compared with 1-month frozen storage. The redness and yellowness of bologna significantly increased, and lightness decreased during 1 to 4 months raw meat frozen storage. But the redness tended to decrease during 4-13 months storage of meats, while lightness and yellowness didn't show any trend. There was only one replicate of sample that had been stored for 4 to 13 months; more data is needed to further confirm the colour change of bologna during frozen storage of meats. The lipid oxidation level of MSP was stable for the first 7 months, however, a rapid increase to values up to 4.90 mg MDA/kg meat was observed at 13 months in MSP-STD. According to the data currently available, the MSP-PC had lower lipid oxidation level at the beginning of frozen storage compared to that of MSP-STD and MSP-DL, and the lipid oxidation level of MSP-PC (1.48 mg MDA/kg meat) was much lower than those of MSP-DL (3.05 mg MDA/kg meat) or MSP-STD (4.90 mg MDA/kg meat) after 13 months of frozen storage. More replicated data is needed to confirm the observation. For the texture and appearance of bologna made with MSP, the WHC, and texture of bologna treatments were relatively stable for up to 13 months of frozen storage of the meats, with the trend of decreased TPA hardness, springiness, chewiness, and shear strain along with storage observed in 15% MSP substitution treatments. Future study is needed to confirm these results.

In summary, MSP showed compromised gelation ability compared to PP due to the protein degradation and disruption during separation process, while the mechanism for protein damage remains unclear. The initial freezing rate and 1-4 months frozen storage showed limited effects on MSP properties, and textural features of bologna. Further increasing the length of frozen storage resulted in increased lipid oxidation level and undesirable colour change. The 7.5% substitution level of MSP in bologna showed comparable textural properties to control bologna, and frozen

storage length showed negligible influence on the bologna texture. However, 15% MSP substitution deteriorated the texture, and would not be a recommended level of addition.

7 OVERALL CONCLUSION AND FUTURE OUTLOOK

MSP is the soft muscle tissue recovered from bones by pressure. It provides nutritional values and could be transformed into more market valuable products. To explore the potential of utilizing MSP as a replacement of regular ground pork in bologna type products processing, the thesis focused on the gelation properties of MSP. The investigation was designed to understand MSP from the raw ingredient properties to its effects on bologna product textural features based on three hypotheses: 1) MSP is different from PP in chemical composition, and biochemical and gelling properties; 2) Accelerated chilling rate of MSP helps improve the quality of MSP; 3) Frozen storage has effects on the properties of MSP.

MSP in general has lower protein, but higher fat content than PP. It contains a small amount of fine size (Ø < 1 mm) bone fragments, and has higher iron and calcium content but same total ash content to PP. The mechanical separation process removes part of connective tissue, and leaves MSP with lower ratio of myofibrillar protein to sarcoplasmic protein than PP. BM, which consists of the same source of soft muscle tissue as MSP, has the same colour and pH as PP. But MSP is dark red in colour with higher pH and higher level of lipid oxidation. According to the second study, the bologna formulated with up to 15% BM has comparable texture to 100% PP bologna (CON). Hence, the changes to the physicochemical and gelation properties in MSP happen during the separation process. The mechanical separation process disrupts pork muscle tissue structure and leads to the degradation of myofibrillar proteins which can result in decreased gelation properties. This helps explain why the bologna formulated with 7.5% MSP does not have compromised texture, but 15% does. The MSP in bologna formula at 15% level made bologna dull and redder in colour and had a trend to decrease the TPA hardness, cohesiveness, springiness, and chewiness. The torsion texture map showed that 15% MSP substituted bologna had mushy texture with lower shear stress and strain, while the texture of 7.5% MSP substituted bologna was comparable to the bologna made with PP (CON).

It is concluded that MSP has degraded myofibrillar protein and poor gelation capacity compared with regular pork. MSP substitution level at 15% in bologna processing is not recommended due to the undesirable texture and appearance. However, the mechanism of

myofibrillar protein degradation remains unclear. More future studies are needed in several areas: to test the enzyme activity in the bone marrow or bone extract, to test the myofibrillar protein profile of MSP processed at different pressure or with different equipment. Due to the limited experimental conditions, the analysis on MSP was mostly conducted on previously frozen MSP samples. It would be valuable to explore the NAM properties of fresh MSP.

In this project, the MSP out of production line was about 8 °C. MSP-DL was delay chilled for 4 hours after processing, MSP-STD was chilled immediately after manufacturing, and MSP-PC (1 rep data collected) was chilled even faster through a heat exchanger to drop the MSP initial temperature to around 1 °C. They were subjected to frozen storage at -18 °C for up to 13 months. The initial chilling rate showed no effect on pH, colour, protein solubility, lipid oxidation level of MSP-DL and MSP-STD during the first 4 months of frozen storage. However, MSP-PC had lower lipid oxidation level in the beginning of storage (1 month), and faster chilling did help with slowing down the increase of lipid oxidation level after long-term frozen storage for 7 to 13 months. Replicated data is needed in future studies to confirm this result. The chilling rate of MSP had no effect on the meat batter viscosity and pH. And the WHC, colour, TPA, and torsional texture of bologna made with MSP frozen stored for 1-13 months were not influenced by the initial chilling rate. In summary, the initial freezing rate didn't play a major role in gelation, but help decrease the level of lipid oxidation during the extended frozen storage period.

In general, the MSP was relatively stable during frozen storage for up to 13 months, however, there were still some noticeable quality changes in MSP and some further influence on the bologna texture made from such meats. The frozen storage of MSP up to 4 months didn't show influence on the pH, lightness, oxidation level, and protein solubility, but exhibited a trend of decreased viscoelasticity and slightly decreased redness and yellowness. The colour of MSP showed a trend of further decrease in redness and yellowness after 4-13 months of frozen storage. The bologna batter viscosity was not influenced by frozen storage, however the pH of raw batter increased and the pH of cooked bologna decreased after one to four months frozen storage of MSPs. The expressible moisture of bologna increased after 1-4 months frozen storage of MSPs, but the cook loss or purge loss remained unchanged. The WHC of bologna was stable during 4-13 months frozen storage of raw meats. The texture of bologna made with frozen stored MSP (1-13 months) was relatively stable with slight decrease in hardness over time in 15% MSP formulas. Future

studies are still required to confirm the effects of long term frozen storage (13 months) on the MSP with replicated data.

The most important finding was related to the changes observed in the MHC. Further work on quantifying the degradation of MHC over storage time is necessary to better explain the changes of MSP gelation ability and the effects of the subsequent frozen storage. The myofibrillar protein oxidation is another possible reason for the poor gelation ability of MSP. As MSP was found to have higher lipid oxidation level, more analysis on myofibrillar protein oxidation level is necessary to better understand the damage to myofibrillar protein during the mechanical separation process.

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