PROCESSING STRATEGIES FOR LOW-SALT, LOW-FAT BOLOGNA

A Thesis Submitted

To the College of Graduate Studies and Research

In Partial Fulfillment of the Requirements

For the Degree of Master of Science

In the Department of Food and Bioproduct Sciences

University of Saskatchewan

By

Marilyn Garcia Edrosolam

2013

PERMISSION TO USE

In presenting this thesis in partial fulfillment of the requirement of a postgraduate degree from the University of Saskatchewan, I agree that the libraries of the university may make it freely available for inspection. I further agree that the permission for copying of this thesis in any manner, in whole or in part, for scholarly purpose may be granted by the professor who supervised my thesis work or in their absence, by the Head of the Department or the Dean of the College in which, my thesis work was done.

It is understood that any copying or publication or use of this thesis or parts thereof for financial gain shall not be allowed without my written permission. It is also understood that due recognition shall be given to me and to the University of Saskatchewan in any scholarly use, which may be made of any material in my thesis.

Request for permission to copy or to make other use of material in this thesis in whole or part should be addressed to:

Head of the Department of Food and Bioproduct Sciences,
University of Saskatchewan,
51 Campus Drive
Saskatoon, SK
Canada, S7N 5A8

ABSTRACT

Two studies on potential approaches for processing low-salt, low-fat (LSLF) bologna were completed. In study 1, the effects of three factors, namely salt type (sea salt vs. regular NaCl), NaCl concentration (0.75%, 1.00%, 1.25% and 2.00%) and holding of stuffed batter before cooking (cooked immediately (CI) vs. delayed cooking (DC)), on the quality of LSLF bologna were investigated. There was no difference between salt type for most of the parameters measured. The holding factor significantly improved the water holding capacity (WHC) and texture of bologna samples containing 0.75% NaCl, as shown by lower (p<0.05) expressible moisture. However, holding factor did not affect WHC and instrumental texture of samples with 1.00%, 1.25% or 2.00% NaCl. A NaCl level by hold effect (p<0.05) was observed for texture profile analysis (TPA) in which there was significant improvement in the texture of samples containing 0.75% NaCl that were subjected to DC, but no effect at other NaCl levels. Panelists were able to detect the positive effect (p<0.05) of DC on the texture of samples with 0.75% or 1.00% NaCl. This study showed that DC is effective in improving the texture of bologna samples with extremely low NaCl (0.75%) content. The biggest challenge in this first study was the difficult sample handling experienced during slicing. Since bologna is commonly sold as thin slices, the bologna must be firm enough for ease of slicing.

The second study focused on improving bologna firmness by the addition of microbial transglutaminase (MTG), known for its functionality as a protein cross-linker, and of flaxseed meal (FSM), known for its excellent water holding capacity. The physico-chemical and sensory characteristics of 12 treatment combinations (0, 0.15% and 0.30% MTG; 0, 0.5%, 1.0% and 1.5% FSM) were determined. In general, results showed that MTG significantly improved the textural quality of bologna, but resulted in a higher purge loss during storage of vacuum packaged slices. On the other hand, FSM significantly reduced the expressible moisture content and purge loss of the product. In terms of product colour, MTG had no effect but FSM when added to the formulation at level as low as 0.5%, affected the colour as determined by both instrumental and sensory evaluation.

The overall results of the project indicated that texture in LSLF bologna is not a major issue, since processing conditions and combinations of ingredients can be manipulated to improve texture. The biggest challenge, however, is in the area of flavour – improving the flavour of low-salt processed meats warrants further research.

ACKNOWLEDGEMENT

I would like to express my sincere gratitude to Dr. Phyllis Shand, my supervisor, for giving me the chance to work in her lab and for mentoring me throughout the course of this study. Also, special thank for her support to our family especially during our early months in Canada. We will forever treasure her personal kindness to our family.

I would also like to thank my advisory committee, Dr. P. J. Shand, Dr. G. Arganosa, Dr. D. Korber, and Dr. R. Tyler for valuable advice and contributions. A special thanks to Dr. Greg Penner for serving as my external examiner.

I acknowledge the technical assistance of H. Silcox, G. Maynard, and T. Kwan of the Department of Food and Bioproduct Sciences, University of Saskatchewan during this study. I am also grateful to the members of sensory panel, Yuanlong Cao, Chang Chang, Clara Olivia, Da Wang, Kornsulee Ratanapariyanuch, Maria Martinez, Lamlam Cheung, Praiya Unatrakarn, Sanjie Jiang, Subhanie Pathiratne, Shayamali Saha, Geun Pio, Stuart Johnston, and Michelle Nie of the Department of Food and Bioproduct Sciences and Department of Plant Sciences. Their time and full participation contributed to the completion of this project.

My sincere thanks to the Saskatchewan Ministry of Agriculture and the Alberta Livestock and Meat Agency for funding this project. A special thanks also to the University of Saskatchewan-Department of Food and Bioproduct Science's Devolved Scholarship for financial assistance.

I would also like to thank my fellow students, faculty, and staff of the Department of Food and Bioproduct Sciences in the College of Agriculture and Bioresources for the encouragement and friendship throughout the course of my program.

Also, thanks a million to my parents whom from my childhood inculcated in me the value of education. My biggest and warmest thanks to my husband and to my two lovely daughters for their love, understanding, and for everything.

Last, but certainly not the least, to our Almighty Creator whom is the source of wisdom, strength, and all the blessings. *Maraming maraming salamat po!* (Thank you so much).

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

1 INTRODUCTION

1.1 General

Although there are a number of factors (e.g., stress, genetic susceptibility of individuals, lack of physical activity, age, some forms of illness and others) that increase the risk of developing high blood pressure, a reduction in dietary salt (sodium chloride, NaCl) intake has been one of the major approaches to reduce this diet-related health illness. According to the World Health Organization report, "to prevent essential hypertension and chronic diseases, the population NaCl intake should be <5.0 g/day" (WHO, 2003). Each country has its own target and ways to achieve specific NaCl reduction goals. Strategies include consideration of the population dietary pattern. Since consumption of processed foods is a major source (70% - 77%) of sodium in the diet (specifically in the Western part of the world) in the form of NaCl, sodium reduction in processing is always included in a sodium reduction strategy (Brown et al., 2009). For example, processed meat alone accounts for 9.0% of the total sodium intake of the Canadian population (Health Canada, 2012). Thus, Canada's strategy to achieve the interim intake goal of 2,300 mg sodium/ day (100 mmol sodium/day or 5.847 g salt/ day) by 2016 includes sodium reduction in processed meats.

Several approaches to NaCl reduction in processed meats have been investigated. These included partial substitution of NaCl by other chloride salts (KCl, $MgCl_2$), the use of non-chloride salts to the increase ionic strength of the mixture (e.g., phosphates), and processing modifications which include addition of flavour enhancers (Seman et al., 1980; Trout and Schmidt, 1984; Barbut et al., 1988; Ruusunen and Poulanne, 2005; Desmond, 2006; Weiss et al., 2010). Although some of these studies have shown promising results, none can deliver functionalities equivalent to those of NaCl in meat processing when considering product taste and texture. Therefore, sodium reduction remains an area of challenge in the food industry and ranks as 13^{th} of the 14^{th} greatest challenges in nutrition research for the next 30 years (Katan et al., 2009).

In addition to hypertension, high consumption of meat, processed meat in particular, has been related to other health problems, such as obesity, cardiovascular diseases and some types of cancer (e.g., stomach) and postulated to be associated to high fat and sodium content in processed meats (Biesalski, 2005; Demeyer et al., 2008). The World Health Organization

recommended an urgent call to reduce fat intake in the human diet (WHO, 2003). Moreover, the World Cancer Research Fund recommended that one of the 10 universal guidelines for healthy nutrition is to "limit meat intake and avoid processed meat" (WCRF, 2007). Therefore, in this project, a low-fat model system was used for two reasons. The first reason was basically to create a "healthy (low-fat) product". The second reason was to create a "stressed matrix" by replacing fat with water on an equal basis (without any fillers or binders). This "stressed matrix" is believed to be an ideal system to show the effectiveness of treatment factors.

In the present study, potential processing strategies (holding the stuffed batter at 1°C for 20 h before cooking) and the use of additional ingredients (flaxseed meal (FSM) and microbial transglutaminase (MTG)) for manufacturing of low-salt processed meat in a low-fat (replaced with water) model system were investigated. The effectiveness of extended holding and added ingredients were determined by evaluating protein solubility, processing, instrumental and sensory characteristics of the cooked low-salt, low-fat bologna.

1.2 Study hypothesis and objectives

Study 1: Effectiveness of extended holding of stuffed batter at 1°C before cooking as a simple reduction strategy in processing low-salt, low-fat bologna

Hypothesis: Extended holding before cooking can maximize solubilization of myofibrillar proteins and stabilize oil-protein-water interactions

Objectives: To determine the effectiveness of extended holding on the texture, water holding capacity and sensory characteristics of low-salt, low-fat bologna

Study 2: Potential utilization of flaxseed meal and microbial transglutaminase in processing low-salt, low-fat bologna

Hypothesis: The cross-linking functionality of MTG in combination with FSM will increase the firmness, water holding capacity, processing yield and sensory characteristics of low-salt, low-fat bologna.

Objectives: To determine the effects of MTG and FSM combinations in improving water holding capacity, texture and sensory characteristics of low-salt, low-fat bologna

2 REVIEW OF LITERATURE

2.1 Sodium chloride in human lives

Sodium chloride (NaCl), known as common salt, table salt or halite, has been an important part of human lives since ancient times. Some of the early quotes found in the article of de Lozy and Stare (1980) describing the importance of NaCl in human history includes: "*it may well be that some seek not gold, but there lives not a man who does not need salt*" (5th century Goth administrator); "*first there is salt, without which practically nothing is eatable*" (Plutarch); "*Heaven knows a civilized life is impossible without salt*" (Pliny). Historically, salt was a precious commodity and in many cultures it was the equivalent of money. The word "salary" is derived from the Latin *salaries* or salt money (de Lozy and Stare, 1980). Salt influenced the establishment of trade routes and cities, provoked and financed wars, secured empires and inspired revolutions in the course of history (Kurlansky, 2002). One of the most important roles of salt, especially before the invention of refrigeration/cold storage, was its ability to preserve food. It eliminated the dependence on the seasonal availability of food, and allowed for the transport of foods over long distances (AkzoNobel Salt Specialties, 2012).

The NaCl peculiar elemental composition (60.663% chlorine and 39.337% sodium) and chemical properties (e.g., low freezing point, pure salty taste and others) is responsible for its myriad applications, from foods, feeds, fertilizers, medical uses, highway de-icing, to manufacturing of glass, textiles and others (Salt Institute, 2012). It has 14,000 known uses (Salt Institute, 2012). Only around 20% of the NaCl produced is used in human foods and animal feeds. The majority is used as raw material for the chemical industry such as production of chlorine, soda ash (Na_2CO_3) and sodium hydroxide (NaOH, caustic soda), which are basic chemicals for glass, paper, polyvinyl chloride, and aluminum metal manufacturing (Salt Institute, 2012).

In general, NaCl is a widespread, low-value, bulk commodity (Canadian Minerals Yearbook, 2008). It is relatively easy to extract, and transportation represents a significant proportion of the total delivered price (Canadian Minerals Yearbook, 2008). Many global markets are served by neighboring salt-producing countries; therefore, long-distance trade is

limited. Canada is the 5th largest NaCl producer in the world and the largest NaCl consumer (basically due to winter conditions).

Once salt was so precious and considered a very high commodity basically due to its limited availability. The improved technology of mining underground salt and improved processing of extracting salt from the ocean made this commodity readily available and affordable. The ready availability and widespread individual practice of using salt for better taste in foods has contributed to the global wide health issue of hypertension.

2.2 Production of NaCl

The purity of NaCl is affected by source and production process. There are two main sources of NaCl: sea water and rock salt (underground salt deposit). During ancient times, commercialized NaCl was primarily produced through evaporation of sea water. In the second half of the 19th century, industrial mining and drilling techniques were discovered leading to commercialization of rock salt (AkzoNobel Salt Specialties, 2012). Currently, these NaCl production processes are widely used. Mining is done by either solution or excavation mining (Salt Institute, 2012). In solution mining, a drill is inserted into an underground salt dome, water is pumped into the hole, and then brine is pumped to the surface, and transported by pipeline to an evaporating plant, consisting of a series of boilers and vacuum pumps (Salt Institute, 2012).

The NaCl for human foods (purest grade of salt) is usually produced by evaporation (solar, open pan, or under partial vacuum evaporation) and involves addition of additives such as iodine (potassium iodide or iodate) and anti-caking agents (magnesium carbonate, calcium silicate, calcium phosphate, magnesium silicate, and calcium carbonate) (World Food Programme, 2009). The iodine is intentionally added to prevent iodine deficiency disorder (IDD), including goiter, a disease of the thyroid gland (World Food Programme, 2009).

Drake and Drake (2011) demonstrated that harvest location and process had an effect on mineral contents. These mineral contents can influence taste perception. For example, the zinc salt is characterized to have higher astringency and umami sensation; magnesium and calcium salts can elicit bitter sensations; and iron compounds are usually perceived to have metallic flavour (Yang and Lawless, 2005).

2.3 NaCl intake and hypertension

The global-wide endeavor to reduce NaCl intake of individuals is related to association of sodium with high-blood pressure, commonly known as hypertension. This is a condition where the pressure inside the blood vessels is higher ($\geq$140/90 mmHg) than normal ($\leq$120/80 mmHg) (Cheung and Lam, 2003). In measuring blood pressure, two numbers are reported: systolic (pressure in the blood vessels when the heart pumps) and diastolic (the pressure when the heart relaxes between pumps). There is abundant evidence showing the relationship between high NaCl intake and development of high blood pressure (Dahl, 1972; Bing et al., 1979; James et al., 1987; Haddy, 1991; Cheung and Lam, 2003; He and MacGregor, 2009). The Chinese emperor Huang Ti (~4000 years ago) is credited with being the first to recognize this negative health consequence of high NaCl intake when he characterized this illness as a "hardness of the pulse" (Morris et al., 2009). The international study known as INTERSALT which involved 32 countries (n = 10,079, using standardized methods of measuring blood pressure and 24-h urinary sodium assessments) found a strong significant correlation (r=0.56) between adjusted systolic, diastolic and adjusted sodium excretion (Intersalt Cooperative Research Group, 1988). This further supported the findings of several animal studies, clinical evidence, and several epidemiologic studies linking salt and high blood pressure (He and MacGregor, 2009). Moreover, high NaCl intake was also linked to the development of stomach cancer, stroke, and increase in left ventricular mass (Wardener and MacGregor, 2002; He and MacGregor, 2009).

Although chronic high NaCl intake has been considered an important risk factor in developing hypertension, both in hypertensive and normotensive individuals, and consequently increasing risk of cardiovascular disease in humans (Weller, 1980; Pearson and Wolzak, 1982), a small amount of NaCl plays many essential physiological roles in the body. In addition to potassium, the sodium and chloride ions are the most common electrolytes in the human body which regulate nerves and muscles and maintain acid-base balance (Nelson and Cox, 2008). Several animal studies showed the important roles of sodium in animal growth. For example, Fine et al. (1987) reported that rats subjected to low sodium diets exhibited decreased bone and muscle weights. They hypothesized that this could be due to lower feed efficiency (high food intake in search to satisfy sodium needs causing a diet induced thermogenesis) and reduction of extracellular fluid volume that may control cell growth. Bursey and Watson (1983) found that sodium restriction during gestation in rats

increased the number of stillborn pups, led to smaller brain size, lower amount of protein per unit of wet brain tissue, and decreased total brain RNA.

However, the current mean sodium intake of the North Americans were 7x-18x higher than the physiological requirements (180-500 mg sodium/day vs. 3400 mg sodium intake/day) and more than the upper tolerable intake by 150% (2300 mg sodium/day vs. 3400 mg sodium intake/day) (Health Canada, 2012). This high sodium intake is believed to be an important environmental factor that cause increase in blood pressure of both normotensive and hypertensive individuals. When NaCl intake of an individual reaches more than the body's metabolic requirement, the salt is excreted in the urine. Haddy (1991) proposed that the defect in renal sodium excretion leads to extracellular volume expansion which the increases circulating levels of an inhibitor sodium/potassium pump inhibitor and atrial natriuretic peptide/factor (ANF). The sodium pump inhibitor increases cardiac contractility and peripheral vascular resistance, raising blood pressure. The ANF can modulate these effects by vasodilation but cannot overcome.

2.4 Sodium reduction strategy in Canada

Sources and intake of dietary sodium vary largely worldwide. In Asian countries for example, the average intake of sodium was estimated to be more than 4,600 mg sodium/day (200 mmol sodium/day) in which the greatest proportion comes from NaCl added during cooking and sauces (soy sauce and miso (in Japan) (Brown et al., 2009). In European and North American countries, sodium intake is dominated by sodium added in manufactured foods (approximately 75% of intake) (Brown et al., 2009). Therefore to make the sodium reduction strategies effective, the main source of dietary sodium and specific eating habit of the population should be considered in policy development and implementation (Brown et al., 2009).

In Canada, a group known as the "Sodium Working Group (SWG), created in October 2007, was mandated to develop a population-health strategy to reduce sodium in the diets of Canadians. In June 2012 they came up with recommendations on how to meet the intake goal of 2,300 mg sodium/day by 2016 (Health Canada, 2012). Currently, the actual mean intake of Canadians is approximately 3,400 mg per day (Health Canada, 2012). Therefore, a 32.3% reduction for sodium intake is expected to be achieved within the next 4 yrs. The 2,300 mg sodium interim goal is the tolerable upper intake level (TL) which is believed to have no

significant negative effect on human health. Shown in **Table 2-1** is the sodium AI and UL for various ages.

The SWG believes that the success of sodium reduction strategy for Canada will depend on implementation of the recommendations in four areas: food supply (processors, restaurants, regulatory agencies, and stakeholders), awareness and education (continuous information dissemination about high sodium intake and related health consequences), research and recommendations (i.e., increased resources to the granting councils and the relevant science based departments and agencies) and lastly monitoring and evaluation recommendations (i.e., development of a comprehensive sodium monitoring and evaluation plan) (Health Canada, 2012).

Table 2-1 Sodium adequate intake (AI) and tolerable upper intake (UL) levels for various age groups

Age group (yr)	AI (mg sodium/day)	UL (mg sodium/day)
1-3	1,000	1,500
4-8	1,200	1,900
9-13	1,500	2,200
14-18	1,500	2,300
19-30	1,500	2,300
31-50	1,500	2,300
51-70	1,300	2,300
Over 70	1,200	2,300
Pregnant	1500	2,300

Source: Health Canada (2012)

2.5 Functional roles of NaCl

There are three functional roles of NaCl in foods: flavour, antimicrobial, and processing properties (Desmond, 2006).

2.5.1 Flavour

In general, humans prefer a salty taste thus foods are usually prepared with NaCl (Beauchamp and Cowart, 1990). The factors responsible for high NaCl preferences are not well understood, although it has been suggested that they are the result of conditioning particularly during childhood (Dahl, 1972). Perceived saltiness of NaCl in food is mainly due to the Na^+ cation with the Cl^- anion modifying the perception of foods (Miller and Barthoshuk, 1991). This concept of the role of Cl^- is supported by the work of Ugawa et al. (1992) which showed that the flavour enhancing effect of Na phosphate was much less

compared to NaCl at equimolar Na^+, suggesting that both the anion and cation contribute to the flavour modification effect of NaCl.

Sodium and lithium are the only two minerals known to elicit a pure salty taste. Other minerals (potassium) can elicit some salty taste however it is often mixed with metallic or bitter flavour (Lindsay, 2007). Lithium is toxic when ingested in moderate quantities and therefore is not added to foods (Lindsay, 2007).

In addition to salty taste, NaCl increases palatability of foods by intensifying perception of sweetness, umami, and suppression of bitterness (Gillete, 1985; Mitchell et al., 2011). Ugawa et al. (1992) reported that the presence of NaCl greatly enhances the sweet taste of single amino acids (glycine, alanine and serine). This response enhancement is concentration and amino acid species dependent (Ugawa et al., 1992). For example, the response to lysine and leucine are greatly enhanced by 100 mM NaCl, while those to asparagine and glutamine are little enhanced. Furthermore, this response enhancement to amino acids did not affect the threshold of sweet taste of amino acids which suggests that salt did not change the affinity of the amino acids to receptor sites. Ugawa et al. (1992) speculated that the binding of cation and anion of NaCl on the receptor membranes induces exposure of the receptor sites for amino acids available for binding of amino acids. Salt can also modify bitterness in foods. Brewer et al. (1995) reported as the NaCl was increased in the sample, the bitterness (of potassium lactate) decreased. Breslin and Beauchamp (1997) confirmed this bitter-suppressing functionality of salt. It was hypothesized that more palatable flavours are released as a result of bitterness suppression (Breslin and Beuchamp, 1997).

Mitchell et al. (2011) showed shifts of some sensory characteristics of vegetable soup after salt was reduced. Significant reduction in salt flavour, overall flavour, overall flavour complexity, aftertaste, and carrot aroma was perceived when the formulation was changed from standard full NaCl to reduced NaCl (30.0% reduction).

In a more complex system, such as processed meats, similar results to the soup experiment were published. Increasing level of NaCl resulted in increasing saltiness and flavour intensity and juiciness of sausages (Ruusunen et al., 2003; Ventanas et al., 2010). Perez-Juan et al. (2008) demonstrated the concepts of flavour-protein binding in pork and flavour release as affected by salts. In their study, pork proteins (both sarcoplasmic and myofibrillar) can bind volatile flavour compounds. The presence of sarcoplasmic proteins led to significant reduction of some free volatile compounds (3-methylbutanal, 2-methylbutanal,

hexanal, methional and octanal). Interestingly, when NaCl was added to the solution containing sarcoplasmic proteins there was a significant increase of the free percentage of all the volatiles compounds. This shows that NaCl affects the release of flavour and thus influences flavour perception. This is attributed to modifications to the polarity of surface proteins and to protein denaturation effects (Ruusunen et al., 2005; Perez-Juan et al., 2008).

2.5.2 Antimicrobial effect

The antimicrobial effect of NaCl is achieved through reduction of water activity (a_w) in food systems. However, this seems to apply when NaCl is added in greater quantities which were significant enough to lower a_w. In the case of ready-to-eat meat products, a_w remains high enough to support the growth of microorganisms. Thus, other mechanisms may explain the antimicrobial activity of NaCl in environments with high a_w. Smith et al. (1987) suggested that NaCl prevents transport of substrates into the bacterial cells (e.g., *Staphylococcus aureus* 196E). They hypothesized that bacterial cells are using greater amounts of energy by excluding Na^+ leading to less energy available for substrate transport across the cell membrane. Limiting oxygen availability and interference with enzymes are other potential mechanisms of NaCl antimicrobial activity (Smith et al., 1987). There was little change in microbial numbers on reducing NaCl from 2.4% to 1.6% in frankfurters (Whiting et al., 1984a). They also found storage temperature to be a more important factor than NaCl in controlling growth of *Clostridium sporogenes* and *Staphylococcus aureus*. However, in turkey frankfurters 50% substitution of 2.5% NaCl with KCl or $MgCl_2$ resulted in faster production of *Clostridium botulinum* toxin (Barbut et al., 1986) indicating that NaCl was better than KCl or $MgCl_2$ for inhibiting *C. botulinum* toxin production in turkey frankfurters.

2.5.3 Technological functionalities in meat processing

2.5.3.1 Water holding capacity

Meat contains 70-80% water with the majority of this water present within the myofibrils (in the spaces between the thick and thin filaments) (Offer and Trinick, 1983; Hamm, 1986). This water can be either bound or in a free form (Hamm, 1986; Strasburg et al., 2007). Bound water is tightly associated with proteins through hydrogen bonds, which is influenced by the surface charge and polarity of protein (Strasburg et al., 2007). Free water is

held via capillary forces in different compartments of the muscle tissue (Strasburg et al., 2007).

In addition to this inherent water, meat is also capable of imbibing added water at elevated salt concentrations (Offer and Trinick, 1983). This ability of meat to retain its own water and added water during application of any force is known as water holding capacity (WHC). This WHC can be measured in several ways as proposed by Hamm, (1986):

- Drip loss which is the formation of exudate from meat or meat systems without application of external forces;
- Thawing loss which is formation of exudate from meat or meat systems after freezing and thawing without application of external forces;
- Cooking loss which is the release of fluid after heating of meat or meat systems either without or with application of external forces (centrifugation or pressing); and
- Expressible juice which is the release from meat or meat systems during application of external forces such as pressing, centrifugation methods, or suction methods

Both in fresh meat and processed meat, the myofibrillar system plays a dominant role in water holding capacity (Hamm, 1986). Changes in the water holding capacity of meat can be explained by changes in the volume of myofibrils resulting from changes in interfilament spacing (Offer and Trinick, 1983; Hamm, 1986) and water imbibing power of the thick filaments (Hamm, 1986).

Myofibrillar proteins are composed of various protein fractions and are further classified into two sub-groups: contractile and regulatory proteins (Strasburg et al., 2007). Contractile proteins are composed of myosin and actin which are directly involved in muscle contraction and relaxation in living animals. On the other hand, regulatory proteins include troponin, tropomyosin, M-protein, A-bands, Z-disk, I-band which are all indirectly involved in muscle contraction. They are structural proteins that maintain integrity of the muscle. After the animal is exsanguinated, changes in these structural proteins may explain quality of fresh meat and effect of raw materials in processed meats. For example, degradation of regulatory proteins allows swelling of myofibrils. The highly swollen myofibrillar matrix plays an important role in determining the consistency of the final processed product since this material solidifies to a firm gel upon heating, resulting to binding of all components (including fat and water) together to form a coherent elastic sausage texture (Hamm, 1986).

Numerous works have been published showing an increase in WHC of meat when NaCl or brine is added in meat (Hamm, 1972; Offer and Trinick, 1983; Wilding et al., 1986). According to Hamm (1972) this is due to an adsorption of ions to myofibrillar proteins rather than osmotic effects. Winger and Pope (1981) supported this theory showing the stronger swelling of muscles when soaked in NaCl or KCl solutions than in mannitol solutions of the same osmolality. The effect of NaCl on WHC is due to an association of chloride ions with positively charged groups of myosin or actomyosin and is greatly influenced by the pH of the meat (Hamm, 1986). This adsorption of chloride ions causes weakening of the interaction between oppositely charged groups increasing the repulsive force between filaments and tending to cause expansion/swelling of the myofibril lattice (Hamm, 1986). Swelling of a protein network means an increase of distances between molecules or protein aggregates - the larger the distance, the lower the interactions (such as attractive and repulsive forces between molecules) (Hamm, 1986). Eventually, these forces approach zero, that is, the proteins are solubilized. Thus swelling and dissolution of a protein system are different states of the same event. There is a continuous transition between both states (Hamm, 1986). In addition to increasing charge repulsions between adjacent myofilaments, high concentrations of NaCl (>2.5%) are able to dissociate myosin filaments, creating a bulky polypeptide matrix for moisture retention (Hamm, 1986).

Offer and Trinick (1983) showed the changes in structures when isolated myofibrils were irrigated with different concentrations of NaCl through contrast and electron microscopy. At 0.5 M NaCl, pH 5.5 there was a change in diameter or band patterns and showed that the Z-line structure of the myofibril was weakened. Increasing salt concentration to 0.6 M resulted in substantial increase in diameter of both A- and I- bands, the middle of the A-band was extracted and the Z-line was completely dissolved. At 1 M NaCl, nearly all of the A-band was extracted. The increase in the diameter of the myofibril when irrigated with sodium chloride solutions from 0.1 M to 1 M was 2.8x. Their observations showed the crucial factor in swelling is contributed by the removal at a critical NaCl concentration of one or more transverse structural constraints in the myofibril (probably crossbridges, the M-line or the Z-line) allowing the filament lattice to expand until solubilized at higher salt concentration. Addition of NaCl caused weakening of the muscle structure and could be related to liberation of bivalent cations (Mg^{2+}, Ca^{2+}) from muscle proteins, and thus loosened the microstructure of the muscle tissues favoring swelling and protein solubilization (Hamm, 1986).

2.5.3.2 Water retention and gelation

When processing sausages, water and fat retention are key elements to prevent excessive losses and product failure (Foegeding, 1988). The gelling ability of proteins, basically myosin (extracted myosin after salt addition and mechanical action), allows them to immobilize fat and water giving desirable texture in processed meats.

Gelation in heated muscle foods takes place as three sequential processes (Strasburg et al., 2007) and this explains fat and water immobilization in the sausage matrix:

- Initial unfolding or denaturation - this exposes reactive surfaces of neighboring protein molecules. It is believed that the S-1 region of the heavy meromyosin (HMM) (head) gets unfolded upon exposure at 35°C.
- Aggregation is initiated as a result of hydrophobic association or head to head interaction of the myosin. At 48°C, oligomers coalesce and form intermolecular disulfide bonds.
- Crosslinking – as temperature approaches 50-60°C, the light meromyosin (LMM) (tail) undergoes conformational changes leading to exposure of hydrophobic regions and side groups of amino acids. This then will create a tail-tail interaction and further heating leads to formation of permanent strands which is further stabilized by disulfide bonds. When sufficient bonding occurs, a three dimensional network is formed, resulting in permanent gel formation.

Coagulation of swollen actomyosin is another mechanism explaining water retention in heated meat batters or sausage (Hamm, 1986). Myofibrillar proteins have been implicated as most important in thermally-induced gelation. Of all the myofibrillar proteins, myosin can form excellent gels and the entire myosin molecule is required to develop desirable gel strengths (Sun and Holley, 2011). Although actin is a poor candidate for gelation (Samejima et al., 1982) several reports showed that it can enhance myosin heat-induced gelation (Samejima et al., 1969; Yasui et al., 1980. The difference in gelation capability between actin and myosin is due to their difference in structure and size. The myosin has a large length-to-diameter ratio (approximately 100 nm in length and 1.5-2 nm in diameter) capable of forming highly viscoelastic gel while actin is a globular protein of about one-tenth of the myosin size (Strasburg et al., 2007).

Protein concentration plays a key role on the quality of meat protein gels. Generally, gel firmness and hardness linearly increase with increasing amount of protein regardless of protein source (Foegeding et al., 1986; Brewer et al., 2005). Brewer et al. (2005)

hypothesized that the increase in hardness as a result of increasing protein concentration was caused by an increase in the net matrix area occupied by the protein. However, the critical protein concentration value for gel formation may change depending on the protein source (Sun and Holley, 2011). The gelation of plant protein occurs normally when protein content ranges between 5% to 15% protein (Arntfield et al., 1990; Sun and Holley, 2011) whereas for myofibrillar protein as low as 0.5% is sufficient to produce heat-induced gelation.

Heating rate also affects protein thermal gelation. A slow heating rate allows favorable protein-protein interactions to occur, producing a stronger, better-ordered three dimensional gel (Foegeding et al., 1986; Camou et al., 1989). Going back to the process of gelation, holding the product at lower temperature (i.e., water temperature of 50°C) will provide sufficient time for proteins to denature (step 1 in the process, unfolding of proteins) resulting to a more favorable head-to-head interaction and aggregation to form the gel structure.

2.6 Ionic strength (μ) and pH

The ionic strength, μ, of a salt solution is determined by the equation:

$$\mu = 0.5\Sigma C_i Z_i^2$$

where C_i is concentration of an ion and Z_i is valence of an ion

Ionic strength influences the solubility of proteins through a charge screening effect. At low ionic strength, ions neutralize charges at the surface of proteins (Damodaran, 2007). The increase in solubility is caused by a decrease in the ionic activity/electrostatic repulsive forces of the protein (Damodaran, 2007; Kristinsson and Hultin, 2003).

Increasing the amount of NaCl linearly increases the ability of myosin to bind meat pieces primarily by solubilizing the protein (Siegel and Schmidt, 1979). However, Kristinsson and Hultin (2003) showed that strong water-absorbing gels can be produced at low ionic strengths when pH of the meat system is increased to 7.4. At low ionic concentration, the negative charge of the muscle proteins is the driving force for water uptake and retention (Kristinsson and Hultin, 2003). This pH driven electrostatic repulsion is similar in theory with NaCl addition. At the isoelectric point (pI), proteins have a net charge of zero and retain the least hydration. At pI, the myofibrillar proteins aggregate and are least soluble resulting to poor gelation (Smith, 2001). However, a pH of 7.4 in "real" meat system (e.g., meat processing condition) rarely exists, unless one utilized prerigor meat (~pH just below 7) with phosphates added (Smith, 2001).

2.7 Challenges in reducing NaCl in processed meats

Reducing NaCl in sausage production will result in lower extracted protein which may have negative effects on processing and texture characteristics. In a study by Sofos (1983), reduction in the NaCl level from 2.5% to 2.0%, 1.5%, and 1.0% in frankfurters resulted in lower smokehouse yield, a less stable emulsion (higher total fluid losses during cooking), poor peelability and appearance. Based on that study, salt concentration in the range of 2.0-2.5 % appeared necessary for manufacturing commercial frankfurters in the absence of any other ingredients that may supplement the effects of NaCl. Barbut (1988) showed the effect of processing, specifically, long vs short chopping treatments on sodium reduced sausage. Salt at 1.5% was sufficient to produce stable coarse-type sausage (short chopping) while 2.0% is required to stabilize a finely chopped meat batter. The former is a more sensitive system in which a high degree of binding and fat emulsification is necessary. Currently, the recommended level of sodium for ready-to-eat processed meats is set to a maximum of 360 mg Na^+/55 g serving. To achieve this target, the level of salt in the formulation should be limited to a maximum of 1.66%. Based on published report, use of 1.66% NaCl or lower in coarse-type would not be as difficult as in emulsified-type sausages. This implies that NaCl reduction is still a challenge specifically for emulsified products such as bologna.

2.8 NaCl reduction studies

A number of approaches for processing low-salt emulsified sausages have been investigated and some are commercially used by the meat processors (i.e., phosphates, cross-linking enzymes, pressure treatment, salt replacers, and others).

2.8.1 Phosphates

Phosphates have been shown to work synergistically with NaCl to improve the yield and texture of meat products (Bendall, 1954; Siegel and Schmidt, 1979; Knipe et al., 1985; Whiting, 1984a; Barbut et al., 1988; Barbut, 1988). There are three major roles of phosphate relevant to meat texture: it has the ability to move meat pH away from the isoelectric point of protein, it increase ionic strength and it involves in dissociating actomyosin (Hamm, 1986). This dissociation effect can free myosin to participate in a greater number of molecular interactions upon heating (Siegel and Schmidt, 1979). However, the degree of phosphate functionality in a meat system is greatly influenced by phosphate type and concentration,

chain length, degree of dissociation, and sodium chloride concentrations (Trout and Schmidt, 1984; Knipe at al., 1985; Barbut, 1988; Barbut et al., 1988). Knipe et al. (1985) found that tetrapotassium pyrophosphate was significantly superior in solubilizing proteins compared to other inorganic phosphates (potassium pyrophosphate, sodium pyrophosphate, tetrasodium phosphate). This finding was attributed to the anion contribution of tetrapotassium pyrophosphate in increasing pH of the meat system. The amount of NaCl necessary to achieve maximum protein swelling and extraction was significantly lower in the presence of phosphate (Bendall, 1954).

In Canada, the Food and Drug Regulations permit the addition of phosphate salts in meat products with the maximum permitted level of 0.5% calculated as sodium phosphate dibasic. A conversion factor chart is provided to guide processors when using different forms of phosphates (CFIA, 2012a).

2.8.2 Pressure treatment

Application of high hydrostatic pressure is another approach for NaCl reduction. It causes depolymerization of actin and myosin and promotes solubilization of other myofibrillar proteins, and thus changes gelation properties of these proteins (MacFarlane, 1974; Cheftel and Culioli, 1997).

Increased solubilization of myofibrillar proteins led to improved gelation at <400 MPa (Lee et al., 2007). Yamamoto et al. (2002) observed that hydrostatic pressure at 200-300 MPa induced gelation of chicken myofibrils (40 mg/mL protein) and increased gel strength of chicken myofibrils in low ionic strength at 0.1 M NaCl. Crehan et al. (2000) reported that texture of frankfurters with 1.5% NaCl was improved after application of 150 MPa. Another advantage of high hydrostatic pressure is its microbial killing effect (Cheftel and Culioli, 1997). However, some major drawbacks of this technology include capital equipment cost and undesirable meat color changes (Sun and Holley, 2011).

2.8.3 NaCl substitutes

Numerous studies on substituting NaCl with other chloride salts (potassium chloride (KCl), calcium chloride ($CaCl_2$), and magnesium chloride ($MgCl_2$)) have been published. For example, Seman et al. (1980) showed that 50% substitution of NaCl with KCl in processing sausages resulted in comparable characteristics as the control. However when substitution was 65%, there were detectable changes in the flavor profile of the sausage. Furthermore,

they reported that divalent magnesium in the form of $MgCl_2$ as a NaCl substitute resulted in significantly more fat, gel-liquid, and total cook out compared to treatments with NaCl alone, NaCl + KCl, or NaCl + phosphate. A recent study in dry-cured hams by Alino et al. (2010) further confirmed that divalent cation forms of $CaCl_2$ or $MgCl_2$ were not a suitable NaCl substitute in meat processing. Alino et al. (2010) reported that divalent cations had more difficulty in penetrating the muscle during the pile-salting process in dry cured hams and thus delayed the decrease in water activity. This had an impact on the salting process as it needed a longer post-salting period to achieve desired water activity for shelf-stability and ideal texture of dry-cured hams. Horita et al. (2011) reported that use of divalent cation in the form of $CaCl_2$ to substitute 25% NaCl in the formulation of reduced NaCl mortadella resulted in poor emulsion stability. The presence of divalent cations in the formulation may reduce the extraction of myofibrillar proteins (Hamm, 1986). Therefore, the current NaCl substitutes could not replace NaCl at a higher level due to their effect on taste (i.e., bitterness of KCl at higher levels of substitution) and in emulsion stability (i.e., negative effects of divalent cations - $MgCl_2$ or $CaCl_2$), thus there is a continuous need to investigate other alternatives that can be utilized in processing low-salt meat products.

In culinary cooking, the use of sea salt is becoming more popular (Drake and Drake, 2011). Everyday cooks and top chefs have been using this gourmet salt claiming that this is a healthier option since it contains less sodium and carries several minerals which are limited in common refined salt. In a recent study that compared salt intensity and mineral profiles of salts (five salt substitutes, two table salts (with and without iodine) and 38 commercial sea salts collected around the world), Drake and Drake (2011) found that, although not statistically significant, most of the sea salts (23/38) were numerically lower in sodium compared to reference table salt, and only 7.9% (3/38) of the sea salts tested had statistically ($p<0.05$) less sodium (30.0% less) compared to a reference table salt. However, some salts (8/38) were relatively higher in sodium compared to the reference salt. Salts were also different in specific minerals (calcium, magnesium, iron and zinc). Trained panelists evaluated sample solutions and found that some sea salts had volatile flavors (green/herbal, smoky, earthy). Salty taste intensity was not different when samples were adjusted to an equivalent sodium basis. However, time-intensity profiles for salty taste (equal sodium concentrations = 8.0 g/L) of samples were significantly different. This time-intensity test was conducted to determine if the differences in other minerals could influence the time intensity of salty taste. Drake and Drake (2011) concluded that the minerals present in some sea salts

could have contributed to some distinct salty taste intensity of sea salts over time. However, in a complex system such as meat, effect of these minerals on saltiness may or may not be detectable. Therefore to generate valid proof whether there are additional benefits in using sea salt to reduce sodium intake, proper investigation on the potential of sea salt in complex food systems is necessary.

2.8.4 NaCl enhancers

Use of flavour enhancers in manufacturing low-salt products has been investigated. By definition, flavour enhancers are additives based on amino acids and nucleotides, glycine salts, guanylic acid salts, inosinic acid salts, certain organic acids and herbs and spices that have the functionality to supplement, enhance, modify the original taste and/or aroma of a food (Mitchell et al., 2011). Amino acids enter into the Maillard reaction when heated with reducing sugars (Reineccius, 2005). This initiates a series of interrelated reactions resulting in a complex mixture of highly-aromatic compounds and a characteristic brown colour (Reineccius, 2005).

Monosodium glutamate (MSG) is the best-known and most widely used amino acid based flavour enhancer (glutamine) (Burdock, 2009; Marcus, 2009). It has the ability to enhance the presence of other taste-active compounds (Loliger, 2000). In most Asian countries, MSG is part of everyday cooking. Fish, pork, beef, chicken, and vegetable menus (cooked in different ways such as roasting, stewing, stir frying, or barbequing) would not be complete without MSG. As shown in **Table 2-2**, there is a higher intake of MSG/day of most Asians compared to other nations.

Table 2-2 Daily intake of monosodium glutamate (MSG)

Countries	Intake of MSG g/d
USA	0.55
The Netherlands	0.66
Thailand	1.50
Japan	1.42
Indonesia	0.60
Korea	1.57

Adapted from Loliger, J. (2000).

Addition of MSG to food enhances several specific flavour characteristics, such as impact, body of fullness, continuity, mouth fullness, mildness and complexity (Burdock, 2009). Studies also have shown that MSG enhances savouriness of soup with various NaCl

concentrations (Yamaguchi and Takahashi, 1984; Roininen et al., 1996; Okiyama and Beauchamp, 1998). In a mathematical model of palatability, Yamaguchi and Takahashi (1984) reported that the optimal levels of MSG and NaCl in a clear soup were 0.38% and 0.81%, respectively. They suggested that to maintain a high palatability score with restricted sodium intake, the MSG concentration should be kept at an optimum and the NaCl concentration reduced. MSG is commonly produced by a fermentation process using glucose (often sugar molasses) as a starting substance. Once the glucose is converted to glutamic acid, it is filtered, dissolved and converted to MSG by neutralization with sodium hydroxide (Burdock, 2009). However, MSG itself contains sodium. If the usage is 0.38% for example, it provides an additional source of ~23 mmol/L sodium in the diet. In order to eliminate additional sodium, Ball et al. (2002) investigated calcium diglutamate and found similar effect as MSG in soup. Sensory data showed that 50 to 85 mM NaCl in combination with 21-43 mM calcium diglutamate had the same rating in terms of flavour intensity, natural fullness, and richness of taste as the reference sample which contained 150 mM NaCl.

Even though MSG has been used in Asian countries for several decades, an Asian study on consumers' perception towards use of MSG in food products showed that respondents were willing to pay premium price for food products with "No added MSG" (Radam et al., 2010). This consumers' reaction could be partly influenced by health-related issues on adverse effects of MSG such as "Chinese restaurant syndrome (such as broncho-constriction in asthmatics)" (Raiten et al., 1995). In North America, use of glutamate containing ingredients (e.g., tomato, cheese, soy sauce) is preferred over MSG addition.

2.8.5 Preblending process

Preblending is a process which involves subjecting a portion of meat block (lean part) with NaCl, cure, phosphate and a portion of the water for several hours or days prior to actual sausage manufacture (Pearson and Tauber, 1984; Hand et al., 1987; Knipe et al., 1990). This preblending process offers advantages both in logistics and quality of processed products. In terms of logistics, during preblending there is a sufficient time to collect and analyze (i.e., proximate composition) samples and thus ensure accurate product formulation (Eilert and Mandigo, 1995) and control of composition of the finished product (Knipe et al., 1990). In terms of quality, since the meat block (lean portion) is subjected to high salt concentration, it favors myofilament swelling and protein solubilization (Pearson and Tauber, 1984; Hand et al., 1987; Knipe et al., 1990). However, the magnitude of swelling and solubilization effects

on the finished products depends on multiple factors which could explain why several studies reported different results. Lamkey et al. (1991) found a significant reduction in expressible moisture after 24 h storage of presalted meat at 1^{o}C prior to sausage manufacture. Ockerman and Crespo (1982) found improvement in water holding capacity and viscosity of beef extract after preblending ground lean beef with 20.0% water and either 3.0 and 6.0% NaCl. However, Hermansson (1982) reported that preblending had no effect on fat- and water binding of frankfurter type sausages in a commercial scale experiment. Moreover, Knipe et al. (1990) looked into the effect of preblending and type of inorganic phosphates in reduced sodium chloride (0.75%) meat emulsions. Meat was preblended with sodium chloride and appropriate phosphates for 16 h at -1.7^{o}C prior to chopping. The emulsion was formulated to contain 27% fat. They found that preblending did not give any advantage in emulsion stability as measured by total cookout, gel-liquid, soluble proteins, cohesiveness, and hardness.

A paper by Gumpein and Sørheim (1987) may explain the conflicting results. They investigated the interaction effect of chopping and preblending time on water holding capacity and found that preblending is advantageous only in coarse type sausage. There was a significant reduction in cook loss when presalted meat was subjected to a short period of chopping. However, in their work they found that the effectiveness of preblending in improving cook loss decreased as period of chopping time increased (exceeding 10-15 min at low chopper speed). According to them, this prolonged chopping time was enough to complete swelling of myofibrils and extraction of proteins. This result was confirmed by Frye et al. (1991) showing that the addition of sodium tripolyphosphates to fine-cut sausage, regardless of the point-of-addition (i.e., preblended or non-preblended) resulted in minimal improvement of finished product characteristics. However, for coarse-ground sausages, preblending with sodium tripolyphosphates improved all finished product characteristics.

2.8.6 Transglutaminase

Another approach for processing low-salt meat products is the utilization of enzymes. Enzymes are proteins that increases rate of chemical reactions. There are several catalytic mechanisms on how enzymes efficiently do their work: acid-base catalysis, covalent catalysis, metal-ion catalysis, electrostatic catalysis, proximity and orientation effects, and preferential binding of the transition state complex (Nelson and Cox, 2008). Exogeneous

transglutaminase (TG) has shown to modify texture of protein based foods such as fish, meat, bakery, and dairy products (Kuraishi et al., 2001).

Transglutaminases are present in mammals, plants, fish, and bacteria and play diverse roles physiologically (de Jong and Koppelman, 2002). For example, in plants, TG is involved in the formation of the cytoskeletal and cell wall structures (De Jong and Koppelman, 2002) and in human blood, TG, known as factor XIII, helps to stop bleeding by forming crosslinks of fibrin molecules and stabilizes fibrin polymers (Kuraisi et al., 2001). In bacteria, TG is involved in cell wall synthesis (De Jong and Koppelman, 2002). Because of the ability of endogenous TG to crosslink proteins, it was hypothesized that their application in food systems can alter functionalities of food proteins and thus can improve texture of protein-based foods (De Jong and Koppelman, 2002). However, during the early years of investigating the potential applications of TG in food systems, cost, and availability were the main constraints. In 1989, microbial TG (MTG) was isolated from *Streptoverticillium sp*. and since then it has been commercialized as a food enzyme preparation by Ajinomoto Co., Inc. (Kuraishi et al., 2001). MTG is active over a wide range of temperatures from 0 to 65°C (optimal activity at 55°C), stable between pH 5-9 (optimal activity at pH 6-7), calcium independent, and destroyed during cooking of food to internal temperature above 70°C (Kuraishi et al., 2001). Functionalities of MTG are based on deamidation, acyl-transfer, and cross-linking reactions (De Jong and Koppelman, 2002). These reactions modify functional properties of food proteins. The cross-linking reaction is the most important and dominant reaction catalyzed by TG in food systems resulting in the formation of isopeptide bonds between glutamine and lysine proteins, ultimately producing high-molecular weight polymers (De Jong and Koppelman, 2002). In meat systems, formation of these covalent crosslinks between proteins led to improved rigidity and elasticity of meat products (Muruguma et al., 2003).

Uses of MTG in muscle foods to reduce either phosphate or salt have been investigated. Min and Green (2008) examined the functionalities of MTG in processing patties made of minced catfish with low levels of NaCl (1.0%) and without phosphate. A significant increase in textural hardness was observed in the treatment with 0.7% MTG added. However, the cooking yield of MTG treatment was significantly lower compared to the control. This reduction in water binding caused by MTG addition was due to an increase in protein-protein interactions and a decrease in water-protein interactions (Ramirez et al., 2002; Carballo et al., 2006). However, the MTG effect on cook loss of muscle protein based

food is not consistent; some researchers reported no effect, some showed enhanced cook yield, and some had reduced cook loss.

Although addition of MTG in muscle-based food systems improved texture properties of low-salt products, it was shown to not work if NaCl is completely eliminated from the formulation (Colmenero et al., 2005). The salt-soluble myofibrillar proteins are a good substrate for cross-linking reactions with MTG (Kuraishi et al., 1997) and in the absence of NaCl there will be insufficient protein substrate. Thus, samples with MTG + 0 NaCl resulted in poorer water- and fat-binding properties and a softer texture (Colmenero et al., 2005). Several researchers have indicated that it is not feasible to obtain muscle products using MTG alone as binding agent (O'Kennedy, 2000; Pietrasik and Li-Chan, 2002). As a result, MTG is generally used in combination with salt and/or various food proteins and polysaccharides in raw (Kuraishi et al., 1997; O'Kennedy, 2000) and cooked (Kerry et al., 1999; Pietrasik and Li-Chan, 2002; Kilic, 2003; Muguruma et al., 2003) meat products. **Table 2-3** shows some published literature investigating the effect of MTG in muscle-based foods.

2.9 Formulation and quality of processed meats

2.9.1 Low-fat model system

In addition to sodium reduction, reducing fat content is another challenging area in meat processing. Commercially available emulsion products typically contain 30.0% fat (Matulis et al., 1995) or can go as high 37.0% in some sausages type (e.g., dry, cured pork salami) (Weiss et al., 2010). However, fat (saturated fatty acids, cholesterol) in muscle-based foods negatively affect human health. It is linked to obesity, cardiovascular diseases, and certain types of cancer (e.g., colon-, breast-, and prostate cancer) (Biesalski, 2005). In 2003, the World Health Organization recommended an urgent call to reduce fat intake in the human diet (WHO, 2003) and part of the strategy is reducing fat in processed meat. However, reduction of fat in finely-ground meat products is extremely challenging and difficult in terms of product appearance, flavour and texture (Weiss et al., 2010). Fat interacts with other ingredients to develop texture, mouthfeel, and assist in the overall sensation of lubricity of foods (Giese, 1996). In addition to that, fat content was found to affect water binding in meat products (Giese, 1992). Reducing the fat content resulted in a decrease in water holding capacity thus affecting cook yield and purge in vacuum packages (Keeton, 1993).

Table 2-3 Summary of research showing the influence of transglutaminase in muscle-based protein foods

Muscle	Conditions	Results	References
Catfish (minced patties)	1.0% NaCl, phosphate free, 0.7% MTG	Significant increase in TPA hardness, no effect on product color, significant increase in cook loss	Min and Green, 2008
Pork, chicken, lamb (meat batter)	1.5 % MTG/caseinate tested in batter with 0% NaCl, or 1.5% NaCl	High cook loss in treatments with no salt in all the muscle species, significant increase in TPA hardness in samples either with salt or no salt	Carballo, Ayo, and Colmenero, 2006
Pork (meat gel)	2.0% curing mixture (99.5% NaCl +0.5% sodium nitrite) 0.6% MTG	Significant increase in TPA hardness, reduced-cook loss and expressible moisture	Pietrasik, Jarmoluk, and Shand, 2007
Chicken and beef (sausages, batch weight 82.61g)	1.70% NaCl 0.25% Phosphate 0.372% MTG (3.72 mg/mL)	Significant increase in breaking strength	Ahhmed, Kawahara, Ohta, Nakade, Soeda, Muguruma, 2007
Chicken (meat balls)	1.0% salt 0.2% phosphate 0-1.0% MTG	Significant increase in gel strength and cook yield, but no effect on product color	Tseng, Liu, Chen, 2000
Beef (gel)	2.0% NaCl 0.5% MTG	Significant increase in purge loss, no effect on cook loss, increase lightness (L*) but did not affect redness (a*) and yellowness (b*), significant increase in gel hardness	Pietrasik and Li-Chan, 2002
Chicken (doner kebab)	2.0% NaCl 1.0% MTG (use as cold set binder)	No effect on cooking yield, sensory characteristics, and hardness, however hardness increased when caseinate was added to MTG treatment	Kilic, 2003
Chicken (Restuctured poultry)	1.5% NaCl 0.7% MTG+1.5% Caseinate (use as cold set binder)	No effect on total loss (cooking and purge loss), no effect on product color, slight reduction in overall flavor acceptability	Cofrades, Lopez-Lopez, Ruiz-Capillas, Triki, Jimenez-Colmenero, 2011
Pork (frankfurter, <1.0 kg batch)	0.7% MTG (either alone or in combination with KCl, or fiber, or caseinate), 0.0% NaCl, 0.18% phosphate vs. control (2.5% salt)	All treatments with MTG in combination with salt replacers (KCl, fiber, or caseinate) had significantly higher cook loss, low TPA hardness (N) compared to control with 2.5% salt	Colmenero, Ayo, and Carballo, 2005

Therefore, a common approach in salt reduction is the use of hydrocolloids with high water-binding capacity which are able to promote the formation of gels (Weiss et al., 2010). **Table 2-4** shows a list of some published works which investigated functionality of plant-based ingredients as fat replacers in meat systems.

Table 2-4 Summary of research showing plant-based fat replacers in meat system

Product	Condition	Fat replacers	Results	References
Low-fat pork bologna	Salt content: 1.57% Fat content: 1.0% Added water: 38.7% (control) Treatments: all formulations had 1.0% deheated mustard; added water was substituted with fat replacers (1:1)	1.0% soy protein concentrate 0.25% kappa carrageenan 4.0% various cereals (wheat flour, normal starch barley or flour, waxy starch barley meal or flour, potato starch).	Addition of barley particularly hull-less waxy barley meal provided greatest purge control	Shand, 2000
Low-salt and low-fat bologna	Fat content: 12 and 16% Salt content: 1.1,1.35, 1.6 All formulations contain: 6.8% pork skin, 6% potato flour, 2% sodium caseinate + soya isolate, added water 34%	0.25-0.5% : Sodium citrate, carboxylmethyl cellulose, and carrageenan	At 1.4% NaCl, addition of those fat replacers increased firmness, juiciness, saltiness, and flavour intensity	Ruusunen et al., 2003
Low-fat, low salt franks	Salt content: 1.0% and 2.0 % Fat content: 21.1% Added water: 17.7% and 15.4% 5 Treatments: Control, reduced fat 10.5% and 19.3% fat were replaced with konjac gel; 2 low formulations in which seaweed was added at 3.3% level	Konjac flour : 10.5% and 19.3% Seaweeds (sea spaghetti): 3.3%	Combination of sea weed and konjac gel negatively affect the product: increase cook loss and reduced emulsion stability. Product colour was also affected.	Colmenero et al., 2010
Reduced-fat turkey franks	Salt level: 0.61% + 2.82% seasoning Added water: 20-35.07%	Modified corn starch	Level of starch affected cohesiveness and colour of the product. Recommended that for optimal physical and sensory characteristics starch level should be limited to 2.3% and water 33.6%	Beggs et al., 1997

Modified starches are often used to maintain juiciness and tenderness in low-fat meat products (Giese, 1992). Several plant fibers have been also tested, such as citrus fiber (Gines et al., 2003) and soy fiber (Cofrades et al., 2000) in bologna, oat bran (Chang and Carpenter,

1997), rice bran (Choi et al., 2009) and peach dietary fiber in frankfurters. Oat products provide textural enhancement through increased moisture retention and improved mouthfeel (Dawkins et al., 2001). Other oat products like oat gum and oat trim improved protein content of meat-based patties (Dawkins et al., 2001).

In a study of pea fiber, 10% (wt/wt) usage significantly increased both fat retention and cooking yield in high-fat ground beef. Anderson and Berry (2001) hypothesized that the fat holding capacity of fibers is physical in nature. Fiber interacts with the protein in meat batter matrix and acts like a wall, thus hindering the potential coalescence and migration of fat out of the product.

The effect of fat replacers on perceived flavour is not consistent. Some of the fat replacers negatively affected flavor profile of the product. For example, Berry and Wergin (1993) reported that addition of modified pregelatinized potato starch (MPPS) resulted in reduction of beef flavour intensity both in low- and high-fat formulations and panelists further commented occasional acid flavors from samples with MPPS. Moreover, algin and sugarbeet fiber also resulted in reduced of beef flavour intensity in low-fat beef patties (Troutt et al., 1992; Bullock et al., 1995). However, formulations with iota carrageenan had improved beef flavour intensity (Egbert et al., 1992).

The release of flavour from a food matrix and human perception during mastication involve a complex process. Pangborn and Szczesniak (1974) reported that both polarity and the volatility of compound appear to play role in determining how flavour intensity is affected by the addition of hydrocolloids. Hydrocolloids can cause changes in concentration of flavour volatiles released from the gel system. Moreover, hydrocolloids retained more water after cooking and therefore can cause dilution of the flavour compounds (Bullock et al., 1995).

2.9.2 Potential functionalities of flaxseed meal in low-fat bologna

Flaxseed (*Linumusitatissimum* L.) is one of the most important oilseed plant crops in Canada. It provides several agri-based products, such as oil from seed, meal after oil extraction, and other parts as fiber source (Wanasundara and Shahidi, 1994).

After oil extraction, the protein content in flaxseed meal (FSM) increased 1.7 times (203 to 345 g/kg) and the oil content decreased about 7.7 times (438 to 57 g/kg) (Oomah and Mazza, 1993). The FSM also contains non-starch polysaccharides known as mucilage, trace amounts of cyanogenic glycosides, phytic acid, trypsin, chymotrypsin inhibitors, linatine (a

vitamin B6 antagonist), lignans (phyto-estrogens), minerals and vitamins (Oomah and Mazza, 1998). Factors such as genetics, growing conditions and processing may influence composition of FSM.

Flaxseed by-products can be potential ingredients in food processing owing to their various functional properties (Rabetafika et al., 2011). Mazza and Biliaderis (1989) examined physical and functional properties of flaxseed mucilage and reported that it contained less carbohydrates, more minerals and more protein than commercial locust bean and guar gums. Its solubility was higher than locust bean and guar gums and lower than gum Arabic. Based on functionality data (solubility, foam stability and viscosity), they suggested that flaxseed mucilage could be used as a substitute for gum Arabic in food formulations. Dev and Quensel (1989) investigated functional properties of flaxseed proteins (with different levels of mucilage) and found that compared to soybean proteins, flaxseed protein exhibited favorable water absorption, oil absorption, emulsifying activity, and emulsion stability.

Those flaxseed technological functionalities were usually investigated in a model system (e.g., soybean oil-in-water emulsions (Wang et al., 2010)) and few studies were conducted in complex food systems such as in meat. Bilek and Turan (2009) investigated addition of flaxseed flour in enhancing nutritional status of beef patties. Addition of flaxseed led to significant reduction of product cook loss, increased PUFA/SFA ratio, however it increased caloric content and affected the sensory scores if addition was higher than 6.0%. Pelser et al. (2007) studied the effect of addition of flaxseed oil in Dutch style fermented sausages and suggested that it is possible to replace part of animal fat to improve fatty acid profile of the product.

Numerous animal model studies have shown positive health effects of flaxseed such as hypotriglyceridemic and hypocholesterolemic effects in rats and suggested it can be a potential new therapeutic strategy to reduce hypertriglyceridemia and fatty liver (Bhathena et al., 2003). Velasquez et al. (2003) reported that dietary protein substitution with defatted flaxseed meal reduces proteinuria and glomerular and tubule interstitial lesions in obese rats. These initial studies indicate that flaxseed meal is more effective than soy protein in reducing proteinuria and renal histologic injury in this model and that this beneficial effect is independent of the amount of protein intake and glycemic control. Flaxseed has many phytochemicals which may explain positive health effects of flax meal such as mammalian lignan precursor (secoisolariciresinol diglucoside), fiber, and polyunsaturated fatty acids

(omega-3 and omega-6). Since defatted flaxseed meal was used in their feeding trial, Velasquez et al. (2003) hypothesized that lignans were the most probable phytochemical responsible in protecting kidneys of those obese rats.

According to Wang et al. (2010), flaxseed protein concentrate containing mucilage extracted from defatted meal is used in the food industry in China, mainly in the meat product and ice-cream industries. However, no one has investigated/documented potential use of FSM in LSLF.

Glutamine is the major amino acid component of FSM (18.27%-26.4% of crude protein (Bhatty and Cherdkiatgumchai, 1990; Eastwood, 2008; Marambe, 2011). This high glutamine content of FSM can be helpful in processing LSLF bologna especially when combined with cross-linking enzyme (e.g., MTG).

2.10 Summary

Several studies on NaCl reduction in processed meat have been published. These used various strategies, including NaCl substitutes, uses of different phosphates, application of pressure, and potentials of flavour enhancers and others. However, reducing NaCl to a level low enough to meet some levels set by the health community (e.g., < 360 mg sodium/serving of bologna) remains challenging especially in low-fat emulsified model system. Therefore, research on this area is still necessary.

3 EFFECTIVENESS OF EXTENDED HOLDING OF STUFFED BATTER AT 1°C BEFORE COOKING AS A SIMPLE REDUCTION STRATEGY IN PROCESSING OF LOW-SALT, LOW-FAT BOLOGNA

3.1 Abstract

The effects of three factors, namely salt type (sea salt vs. regular NaCl), NaCl concentation (0.75%, 1.00%, 1.25% and 2.00%) and holding of stuffed batter before cooking (cooked immediately (CI) vs. delayed cooking (DC)), on the quality of LSLF bologna were investigated. All meat batters were formulated to contain 10.0% fat and 11.0% protein. Effects of treatments on water holding capacity (WHC) (vacuum purge, expressible moisture, and cook loss), texture profile analysis (TPA-hardness, cohesiveness, springiness, and chewiness), torsional gelometry (shear stress and shear strain), and sensory characteristics (firmness, springiness, juiciness, flavour, and saltiness) using 13 semi-trained panelists were determined. Sodium and potassium contents of the cooked product were verified using flame atomic absorption spectroscopy (FAAS).

In general, there was no difference between sea salt and regular salt on most of the parameters measured ($p>0.05$). The extended holding of batter before cooking significantly improved WHC and texture of samples with 0.75% NaCl as shown by lower ($p<0.05$) expressible moisture. However, extended holding did not affect WHC and instrumental texture of samples with 1.00%, 1.25%, and 2.00% NaCl ($p>0.05$). There was a NaCl level by hold interaction ($p<0.05$) observed in TPA in which there was significant improvement in samples with 0.75% NaCl subjected to extended holding before cooking. Panelists were able to detect the positive effect ($p<0.05$) of DC on texture both in samples with 0.75% and 1.00% NaCl.

This study shows that extended holding of batter was effective in improving texture of samples with extremely low NaCl (0.75%) content when manufacturing LSLF bologna. Low-fat bologna containing 1.00% NaCl had similar texture than those with higher NaCl levels and this could be due to combinations of favorable conditions during processing of LSLF bologna.

3.2 Introduction

The reduction of sodium intake ranks as 13th of the 14 greatest challenges in nutrition research for the next 30 years (Katan et al., 2009). Reducing sodium remains a big challenge for the meat industry (Desmond, 2006) as processed meat is a significant source of sodium in the human diet (Ruusunen et al., 2005; CTAC, 2009-2010). The desire to reduce sodium in foods is due to the reported link between excessive sodium intake and the incidence of hypertension. Sodium chloride plays a number of functional roles in meat processing including, flavor, antimicrobial, and extraction of myofibrillar proteins for gelation and structure development (Desmond, 2006).

There are several approaches that are currently used to produce low-sodium processed meats. Most common is replacement of NaCl with potassium chloride (KCl) (Mojet et al., 2004; Kremer et al., 2009). However, limitations with the use of KCl include metallic taste (Kremer et al., 2009). Another approach is the use of phosphate (Trout and Schmidt, 1984; Barbut et al., 1988; Barbut, 1988). Its functionalities are related to its ability to change pH of the aqueous phase, to increase ionic strength, to bind to meat proteins (align with oppositely charged groups of protein molecules which can increase the volume of open spaces and subsequently enhance water holding capacity), and to dissociate actomyosin into actin and myosin (Hamm, 1972). There are many types of phosphates and the extent of effectiveness depends on the types (chain length) and concentration (Trout and Schmidt, 1984). Pyrophosphate (n=2) is the smallest polyphosphate and is usually formed after breakdown of larger polyphosphate (Li et al., 1993) and is most effective in increasing water retention (Knipe et al., 1985; Offer and Knight, 1988). In restructured beef rolls, pyrophosphate was the most effective r**e**sulting in highest tensile strength and highest cook yield at all salt levels tested (0.6-2.00%), followed by tripolyphosphate, tetrapolyphosphate, and hexamethaphosphate (Trout and Schmidt, 1984). Knipe et al. (1985) found that tetrasodium or tetrapotassium pyrophosphate resulted in higher raw meat batter pH, greater protein solubility, and improved emulsion stability and water holding capacity than sodium tripolyphosphate.

Some studies also showed the potential of preblending in producing low-sodium processed products. Preblending is a process which involves subjecting a portion of meat materials with NaCl, cure (sodium nitrite or nitrate) and a portion of the water for several hours or days prior to actual sausage processing (Hand et al., 1987; Knipe et al., 1990). Hypothetically, since the meat block is subjected to initial high ionic strength during

preblending, it is expected that extraction of myofibrillar protein is maximized. However, results on the advantage of preblending in terms of product texture are conflicting. Some authors (Acton and Saffle, 1969; Hand et al., 1990) found positive effects of preblending on product texture while Hermansson (1982) reported that preblending had no effect on fat- and water- binding of frankfurter type sausages in a commercial scale experiment. Gumpein and Sørheim (1987) investigated the interaction effect of chopping and preblending time on water holding capacity. They reported that preblending is advantageous in coarse type sausage only when presalted meat was subjected to a short period of chopping. However, as period of chopping time was increased (exceeding 10-15 min at low chopper speed), in emulsion type, no positive effect on texture was observed. They hypothesized that prolonged chopping time was enough to complete swelling of myofibrils and extraction of proteins.

In this study, the potential of three factors, namely, NaCl types, NaCl levels, and DC (which was considered as a modified version of preblending), in processing LSLF bologna were investigated. It was hypothesized that DC could enhance protein solubility in the emulsion and stabilize protein-water-fat interaction thus resulting in better product texture. Sea salt was included in the study based on many marketing advertisements and anecdotal claims that it gives a saltier perception and has a balanced mineral content (Miller, 2009) and therefore its inclusion could be a potential approach for processing low-sodium processed meats. The presence of some metallic minerals could have contributed to some distinct salty taste intensity of some sea salts over time (Drake and Drake, 2011). These treatment factors were all tested in a low-fat (high water added) meat matrix using some favorable conditions based on published papers (i.e., use of tetrapotassium pyrophosphate (TKPP) (Knipe et al., 1985) and using a slow heating rate during cooking (Foegeding et al., 1986). The effects of all treatment factors were determined on the texture, water holding capacity, and sensory characteristics of LSLF bologna.

3.3 Materials and Methods

3.3.1 Materials

For each replication, chilled fresh lean (1-2 d post-mortem) pork leg muscles (22.47% protein, 3.20% fat, 1.87% ash, and 71.60% moisture, and pH 5.71) were obtained from a commercial meat packing company through a local meat purveyor. The meat was cut into cubes, minced through a 6.5 mm hole plate (Biro Grinder, Marblehead, OH, USA, model AMFG-24), transfer in 30-lb capacity vacuum bags (mylar/polyethylene vacuum pouch, 3

mm thick, with oxygen permeability of 7.7 cc/m2/24 h), vaccum packaged (-0.9 bar, Roshermatic Type VM-20, Osnabruck, Germany) and kept frozen at -30^{o}C prior to use. Non-meat ingredients such as sea salt (SS), regular salt (RS), tetrapotassium pyrophosphate (TKPP 7320-34-5), sodium nitrite (5-200), and sodium erythorbate were obtained from Cargill Salt (Minneapolis, MN, USA), The Canadian Salt Company Ltd (Pointe-Claire, QC, CA), Innophos (Lowbanks, ON., CA), Griffith Laboratories (Scarborough, ON, CA), and from Unipack Packaging Products, LTD (Edmonton, AB, CA), respectively.

3.3.2 Sausage manufacture

Frozen meat was thawed for two days at 1°C before processing and was reground using a 3.9 mm hole grinder. Each bologna treatment was formulated to produce meat batters with 11.0% protein (in compliance with Canadian regulations for minimum meat protein content) and 10.0% fat. The levels of meat, backfat, TKPP, sodium erythorbate, and sodium nitrite of all batches were held at 48.95%, 11.10%, 0.50%, 0.05%, 0.0192%, respectively. The NaCl levels were varied according to treatment and water was adjusted based on changes in NaCl levels (**Table 3-1**). No spices/seasonings were added in the formulation to eliminate flavor complexity in the matrix and potential interference in saltiness perception during sensory evaluation.

Table 3-1 Composition (%, w/w) of bologna with different levels of NaCl

Ingredients	NaCl (%)			
	0.75	1.00	1.25	2.00
Pork leg muscle, ground, %	48.95	48.95	48.95	48.95
Pork backfat, ground, %	11.10	11.10	11.10	11.10
Ice water, %	38.63	38.38	38.13	37.38
Salt, NaCl, %	0.75	1.00	1.25	2.00
Sodium nitrite, %	0.0192	0.0192	0.0192	0.0192
TKPP*, %	0.50	0.50	0.50	0.50
Na erythorbate, %	0.05	0.05	0.05	0.05

*tetrapotassium pyrophosphate

The bologna processing procedure was carried out in a refrigerated pilot plant (~4.0°C) at the University of Saskatchewan. The finely ground meat was chopped with NaCl, TKPP, sodium nitrite, with a portion of the ice water in a 35-L top bowl chopper (Hobart, Troy, OH, USA, model #84181D) for 1 min with a machine setting of bowl speed #2, knife

speed #4. Thereafter the pork backfat and remaining water were added and chopped for another 3 min. The total chopping time was 4 min and meat batter temperature did not exceed 9°C. The emulsification process was completed by passing the chopped mixture through an emulsion mill (Type 1E-75F, Alexanderwerk, Remscheid, DE) twice. The meat batter final temperature did not exceed 14°C. To release the air trapped in the meat batter matrix, the batter was vacuum tumbled (Model VSM-150H., Glass, Frankfurt, DE) for 3 min and was repeated twice. The batter was then stuffed (~1 kg) into 63 mm diameter, moisture-proof plastic casing (Walsroder K plus CT black, Art-No.: 40101236, CaseTech GmbH and Co., Walsrode, DE) using a hydraulic stuffer (Model EL-20, Mianca Equipamientos Carnicos, SL, Barcelona, ES). The stuffed batter (chubs) were twisted by hand, clipped with aluminum clips, and washed to clean the outside of the casings. They were then randomly assigned for holding treatment, half of the set for cooked immediately (representing CI) and the other half for delayed cooking (DC, kept at 1°C for 20 h before cooking).

Cooking of bologna was done by immersing the bologna chubs in an agitated water bath (~200 L) and cooked using a four-stage thermal processing schedule: 30 min at 50°C (initial water temperature), 30 min at 60°C, 30 min at 70°C and finally at 75°C until sample core temperature reached 71°C. The total cooking time was approximately 2 h. Product internal temperature was monitored using both a hand-held Fluka digital thermometer (Type T Fluke 51 II thermometer, Fluke Corp., Everett, WA, USA) with a chromel-alumel thermocouple probe and a 8 channel data logger (Model 692-0000 Barnant scanning Thermocouple Thermometer, Barnant Co., Barrington, IL, USA) with copper constantan thermocouples positioned in the geometric center of the bologna chubs.

Immediately after cooking, samples were cooled in an ice and water mixture for one hour and stored at 1°C until analyses.

3.3.3 Elemental composition of salts

Concentration of elements such as calcium, lithium, magnesium, manganese, potassium, rubidium, sodium and strontium of SS and RS were determined using an inductively coupled plasma (ICP) technique (SRC Analytical, Saskatoon, SK, CA).

3.3.4 Batter viscosity

During stuffing of raw batter into casings, small portions of meat batter (~200 g) from each treatment were stuffed into two 250-mL plastic cups. One set was used to represent CI

and the other set was held overnight at 1°C to represent delayed cooking (DC). The apparent viscosity in centipose unit (cP) of each sample was measured following the procedure outlined by Shand (2000). Measurement was done at the same time as the chubs were cooked.

3.3.5 Protein solubility

Using the same slurries used for viscosity, the amount of salt soluble protein was determined following the stirring method by Steinmann and Fischer (1993). Briefly, 3 g of batter was diluted with 30 mL soluble protein extraction solutions (made up to match salt/phosphate concentrations found in the aqueous phase of each treatment, **Table 3-2**), mixed for 30 sec using a stirring magnetic bar and vortex mixer (full speed, Fisher vortex, NY, USA), and centrifuged for 15 min at 31,920 x *g* at 2^{o}C.

Table 3-2 Molar concentrations of NaCl and TKPP in protein extraction solutions

NaCl level	NaCl (M)	TKPP (M)
0.75%	0.174	0.020
1.00%	0.233	0.021
1.25%	0.292	0.021
2.00%	0.469	0.021

The supernatant was collected and weighed. An aliquot was taken and protein content was evaluated using the Biuret method (Gornall et al., 1949) using bovine serum albumin (BSA) as a standard. Soluble protein was expressed as % protein of the total protein in the matrix.

$$\% \text{ Soluble protein} = \frac{\text{Concentration}^{\epsilon} * \left[\frac{\text{wt. of extract} * \text{dilution factor}}{\text{wt. of sample}}\right] * 100}{\text{Protein content of the matrix}}$$

$^{\epsilon}$ determined using a standard curve

3.3.6 pH of raw and cooked bologna

The pH of the raw batter and ground cooked samples was determined by homogenizing 20 g of the samples with 80 g of deionized water for 3 min using a stomacher (Seward Lab Stomacher Lab Blender 400 Model No BA6021, London, England). The pH of

the resulting slurry was determined using a calibrated pH glass electrode (model 915 Fisher Accumet, Fisher Scientific Ltd., ON, CA).

3.3.7 Proximate composition

The proximate compositions of the cooked samples as % moisture, % ash, % protein, and % fat were determined using AOAC (1990) methods.

3.3.8 Salt (%), sodium, and potassium contents of cooked bologna

The percentage (w/w) of salt was determined using a mercuric nitrate method in which titration was simplified by using a Hach digital titrator (Hach Company, Loveland, CO, USA) and standard reagents (Hach Company, Loveland, CO, USA). Using this method, mercuric nitrate reacts with chloride ion to form mercuric chloride. The excess mercuric ions react with the indicator forming a pink-purple complex color (mercury-diphenylcarbazone) signaling the end of titration. Ten grams of ground sample from each treatment were weighed, added with 100 mL deionized water, homogenized for 2 min, filtered using # 40 ashless Whatman filter paper (VWR Canlab, Mississauga, ON, CA), and approximately 20 mL of the filtrate collected. From the collected filtrate, 10 mL was transferred to a 250 mL erlenmeyer flask, diluted with deionized water to make up 100 mL total volume, a diphenylcarbazone reagent powder pillow added (HACH cat. No. 836-96), and swirled to mix. The mixture was titrated using a digital titrator with 2.256 N mercuric nitrate (HACH cat. No. 921-01) until the color changed from yellow to pink. The % salt was determined using this formula:

% salt = digits from titrator x 0.0165

The sodium and potassium contents of the cooked samples from each replicate were measured in duplicate using flame atomic absorption spectroscopy (FAAS). In the FAAS method, a solution containing small amounts of metallic elements is converted into a vapor containing free atoms. A light source (hollow cathode lamp made from the element to be measured by a detector) emits radiation which is characteristic of the element to be determined and this radiation is directed through a flame. The atoms are dispersed in the flame and will absorb some of the radiation. This results in a decrease in the intensity of radiation leaving the flame and this decrease is measured by a detector. A monochromator is

included in the system so that energy of the desired wavelength can be isolated from that of neighboring wavelengths emitted from the source.

The elements (Na and K) in cooked bologna were extracted following a water extraction procedure standardized by Health Canada (Health Canada, 1983 (Laboratory Procedure LPFC-125, 1983)). Briefly, ten grams of ground sample were diluted with 200 g of deionized water (Millipore), homogenized using a polytron (27,400 rpm, 90 sec), centrifuged for 20 min at 1000 x *g*, filtered using # 40 ashless Whatman filter paper (VWR Canlab, Mississauga, ON, CA), and finally diluted several fold with 0.37% hydrochloric acid as needed. The absorbance of the filtrate was measured using FAAS wavelength settings of 589.6 nm (for sodium) and 769.9 nm (for potassium) and concentration was determined against the sodium and potassium standard curves. The sodium and potassium contents of the samples were then computed by multiplying the concentration of the filtrate with the dilution factor.

3.3.9 Slicing of cooked bologna for WHC and instrumental texture measurement

Shown in **Figure 3-1** is the slicing procedure used to obtain samples for WHC and instrumental texture measurement. This slicing was followed throughout the course of the study.

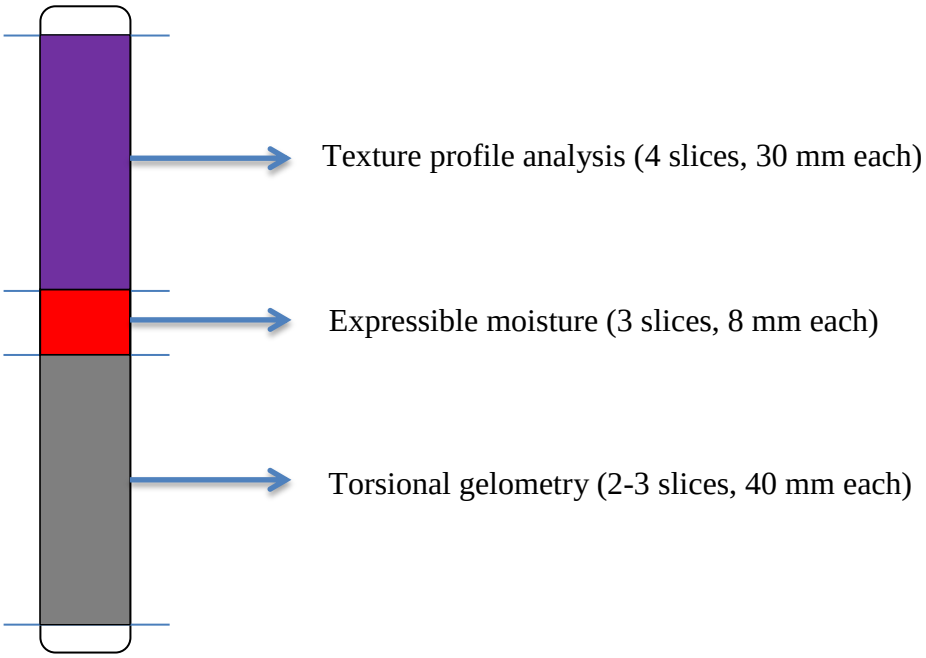

Figure 3-1 Schematic diagram of cooked bologna chub illustrating cutting lines to obtain appropriate portions for various tests.

3.3.10 Water binding capacity

3.3.10.1 Cook loss

The effect of treatments on product cooking loss was determined using three bologna chubs from each treatment. Each intact bologna chub was carefully opened by cutting one side with the use of clean scissors, and blotted by rolling over a paper towel before being weighed.

$$\% \text{ Cook loss} = \frac{\text{Sample weight}^{¥} - \text{blotted weight}}{\text{Sample weight}} * 100$$

¥sample weight = weight of intact cooked bologna – (weight of casing + metal clips)

3.3.10.2 Expressible moisture (EM)

Expressible moisture of cooked samples was determined in triplicate using a modified method as outlined by Shand (2000). A meat core sample (1.0-1.5 g) was placed in the thimble shaped filter papers (Whatman # 3 and 5 in a 50-mL Falcon plastic tube), centrifuged (15 min at 750 x *g*, Sorvall RC-6 Plus TM Superspeed Centrifuge, Thermo Fischer, Scientific, Ashville, NC, U.S.A), and weighed. Expressible moisture was expressed as the percentage of moisture lost after centrifugation relative to the initial sample weight.

3.3.10.3 Vacuum purge loss

Purge during simulated display was monitored in twelve slices (two stacks of six 2 mm bologna slices) per treatment placed in pre-weighed vacuum pouches (mylar/polyethylene vacuum pouch, 3 mm thick, with oxygen permeability of 7.7 cc/m^2/24 h), vacuum packaged (-0.9 bar, Roshermatic Type VM-20, Osnabruck, Germany), and kept at 4ºC for 14 days. Thereafter, the slices were blotted with paper towel and weighed. Vacuum purge was expressed as the percentage of fluid lost from the initial sample weight.

3.3.11 Instrumental texture

3.3.11.1 Texture profile analysis (TPA)

This was determined using a TMS-Pro Texture Press (Food Technology Corp., Rockville, MD, USA) according to Bourne (1978). Eight cores measuring 35 mm in diameter and 25 mm height were obtained from two bologna chubs (4 slices (cores) from each chub) of each treatment and allowed to equilibrate to room temperature for 1 h. All samples were compressed two times between unlubricated parallel metal surfaces to 50% of their original

height at a crosshead speed of 50 mm/min using a 250 N (25.5 kg) capacity load cell. The following parameters were obtained using Texture Lab pro software:

- **hardness** (peak force of the first compression of the product),
- **springiness** (degree to which a product physically springs back after it has been deformed during the first compression)
- **cohesiveness** (refers to the degree to which the sample withstands a second deformation relative to how it behaved under the first deformation)

3.3.11.2 Torsional gelometry

From each treatment, 12 core meat samples (28.7 mm length, 19.0 mm diameter) were taken, glued to two slotted plastic styrene discs using cyanoacrylate glue (Loctite® 404 instant adhesive, Loctite Corp., Newington, CT, USA), reduced to a dumbbell shape by rotation against a sharp rotating grinding wheel (Model KCI-24A2, Bodline Electric Co., Chicago, IL, USA) to a minimum 12.5 mm diameter at the midsection. The dumbbell shaped sample was then placed in a bottom torsion fixture attached to a Brookfield digital viscometer (Model DV-I+, Brookfield Engineering Laboratories, Inc., MA, USA). The bottom disk was kept stable while allowing the upper disk to rotate at 2.5 rpm. The specimen was twisted until failure. Shear stress and shear strain at failure were recorded.

3.3.12 Sensory evaluation

Sensory evaluation of LSLF bologna was conducted using a 13-member panel. Series of screening tests and trainings of panelists were conducted following the procedure of American Meat Science Association (1995).

Initially, 21 individuals received a simple screening test by ranking different concentrations of NaCl for intensity (Meilgaard et al., 1999) (**Figure 3-2**).

After two screening tests, those panelists who ranked the samples correctly or inverted ranking only in adjacent pairs were selected and chosen as potential members of the semi-trained panel.

A consecutive two-week training (20-30 min per day, four consecutive days a week) was given to 16 potential panelists. During the training sessions, panelists were given representative samples of bologna treatments (e.g., lowest and highest salt level, CI and DC) and asked to evaluate using sensory terminologies. The final panel was composed of nine

females and four males, aged 21-40 years old, staff and students from the Departments of Food and Bioproducts Sciences and Plant Sciences.

Name: _____
Date: _____
Instruction: You are given liquid samples in coded cups. Please rank them in ascending order of saltiness.

	Code
Least salty	_____
	_____
	_____
	_____
More salty	_____

Thank you.

Figure 3-2 Scorecard used for ranking test for NaCl intensity

The samples were prepared by slicing the 60 mm diameter bologna to 12.5 mm thickness. Slices were manually cut using a dual scalpel blade knife to give 12.5 mm x 12.5 mm cubed samples. Five cubed samples were placed into covered white plastic cups coded with 3-digit random numbers and kept cold (4°C) until served to panelists. Trays of samples were evaluated using a descriptive sensory analysis. The panels were conducted between 1:30 – 2:30 p.m. in a room equipped with individual booths, running water, and indoor fluorescent white lighting. Samples of bologna were scored (**Figure 3-3**) for firmness (8 as extremely firm---1 as extremely soft), springiness (8 as extremely elastic--- 1 as extremely inelastic), juiciness (8 as extremely juicy ----1 as extremely dry), flavor (8 as extremely intense --- 1 as extremely bland), and saltiness (6 as extremely salty ---- 1 as no detectable saltiness). The sensory data are means of 13 panelists * 3 replications.

The sensory protocol for this study was accepted on ethical grounds (**BEH # 11-111**) by the University of Saskatchewan Behavioral Research Ethics Board.

Name: Booth #:
Panelist #:

Instruction: Please carefully evaluate each sample for saltiness and textural characteristics. Please write the score (under each sample code) which describe most the characteristic of each product. Please follow this rinse protocol between samples: rinse your mouth with water, have a bite of unsalted cracker, then rinse your mouth with water again)

SCORE	FIRMNESS	SPRINGINESS	JUICINESS	FLAVOUR	SALTINESS
8	Extremely firm	Extremely elastic	Extremely juicy	Extremely intense	[redacted]
7	Very firm	Very elastic	Very juicy	Very intense	
6	Moderately firm	Moderately elastic	Moderately juicy	Moderately intense	Extremely salty
5	Slightly firm	Slightly elastic	Slightly juicy	Slightly intense	Very salty
4	Slightly soft	Slightly inelastic	Slightly dry	Slightly bland	Moderately salty
3	Moderately soft	Moderately inelastic	Moderately dry	Moderately bland	Slightly salty
2	Very soft	Very inelastic	Very dry	Very bland	Very slightly salty
1	Extremely soft	Extremely inelastic	Extremely dry	Extremely bland	No detectable saltiness

SCORES: SAMPLES

CHARACTERISTICS	Reference	459	932	378	561	603
Firmness	_____	_____	_____	_____	_____	_____
Springiness	_____	_____	_____	_____	_____	_____
Juiciness	_____	_____	_____	_____	_____	_____
Flavour	_____	_____	_____	_____	_____	_____
Saltiness	_____	_____	_____	_____	_____	_____

COMMENTS:_____

Thank you very much for your time and cooperation!!!!

Figure 3-3 Scorecard for evaluating low-salt, low-fat bologna

3.3.13 Statistical analysis

This study was repeated three times. Observed data were analyzed as a Randomized Complete Block Design using the Proc Mixed Procedure of SAS (SAS, Inst. Inc., Cary, NC). Variations contributed by meat materials used in each replicate were considered as block. Within each block, the order of processing of each treatment (bologna) was completely randomized. Treatments and interactions were written in the model and considered as fixed effects and block as random effect. Means were analyzed and separated with the least significant difference (LSD) procedure of SAS and a pdmix SAS macro was used to convert mean separation output to letter groupings (Saxton, 1998). Significance was declared at $p<0.05$. The experimental layout and sources of variation with corresponding degrees of freedom are presented in **Tables 3-3** and **3-4**.

Table 3-3 Experimental layout showing three blocks and combinations of three factors

Salt type	Salt level	Holding	Trt comb.	Block 1	Block 2	Block 3
Sea salt	0.75	CI	S0.75CI	*	*	*
		DC	S0.75DC	*	*	*
	1.00	CI	S1.00CI	*	*	*
		DC	S1.00DC	*	*	*
	1.25	CI	S1.25CI	*	*	*
		DC	S1.25DC	*	*	*
	2.00	CI	S2.00CI	*	*	*
		DC	S2.00DC	*	*	*
Regular salt	0.75	CI	R0.75CI	*	*	*
		DC	R0.75DC	*	*	*
	1.00	CI	R1.00CI	*	*	*
		DC	R1.00DC	*	*	*
	1.25	CI	R1.25CI	*	*	*
		DC	R1.25DC	*	*	*
	2.00	CI	R2.00CI	*	*	*
		DC	R2.00DC	*	*	*

Table 3-4 Sources of variation with corresponding degrees of freedom

Sources of variation	Degrees of freedom
Block	3-1=2
Salt type (T)	2-1 =1
Salt level (L)	4-1 =3
Holding (H)	2-1 =1
T * L	1*3=3
T * H	1 *1 =1
L* H	3*1=3
T * L * H	1*3*1=3
Error (T*L*H-1) * (b-1)	(2*4*2-1)(3-1)=30
Total	(2*4*2*3-1)=47

3.4 Results

3.4.1 Elemental salt composition

Presented in **Table 3-5** is the elemental salt composition of sea salt and regular salt used in this study. Regular salt had higher contents of divalent cations than the sea salt. On the other hand, sea salt had higher sodium and potassium contents than regular salt. These values were slightly different from the specification sheet submitted by the suppliers (The Canadian Salt Company Limited, 2009; Cargill Salt Technical Information, 2008). For example, calcium content of regular salt was reported to be 140 – 240 µg/g calcium (supplier's information), but determined to be slightly higher (280 µg/g calcium measured using an ICP technique). Sea salt was declared to contain 30 µg/g calcium but using ICP it

was only 10 ppm. Nonetheless, based on this elemental analysis, sea salt was purer than the regular industrial salt in terms of the presence of divalent cations.

Table 3-5 Elemental analysis of regular and sea salt

Element	Regular salt (µg/g)	Sea salt (µg/g)
Calcium	280	10
Magnesium	10	<0.1
Potassium	30	50
Sodium	366,000	392,000

3.4.2 Batter viscosity

Table 3-6 shows the average apparent viscosity of raw bologna as affected by experimental factors. The NaCl levels, holding factor, and interaction effect (level x hold) significantly affected (p<0.05) apparent batter viscosity however, salt type did not account for any difference in batter viscosity of the treatments (see **Appendix A-1**).

Table 3-6 Effects of salt type, NaCl level, and holding of batter before cooking on the apparent viscosity [1]

Salt type	Level (%)	Hold[2]	Viscosity (cPs x 10^4)	Batter temp (°C)
Sea salt	0.75	CI	7.9±0.66^{bc}	4.5±0.20^{a}
		DC	8.3±0.52^{ab}	1.7±0.36^{b}
	1.00	CI	7.8±0.80^{cd}	5.1±0.66^{a}
		DC	8.6±0.55^{a}	1.8±0.17^{b}
	1.25	CI	6.6±0.44^{e}	4.6±1.05^{a}
		DC	7.5±0.51^{d}	1.6±0.13^{b}
	2.00	CI	6.0±0.23^{f}	4.6±0.30^{a}
		DC	7.0±0.28^{e}	2.1±0.25^{b}
Regular salt	0.75	CI	8.1±0.10^{bc}	5.0±0.85^{a}
		DC	8.3±0.15^{ab}	1.7±0.18^{b}
	1.00	CI	7.5±0.88^{cd}	4.9±0.93^{a}
		DC	8.6±0.64^{a}	1.7±0.05^{b}
	1.25	CI	6.6±1.31^{e}	5.1±1.31^{a}
		DC	7.4±0.29^{d}	1.6±0.09^{b}
	2.00	CI	5.9±0.26^{f}	5.1±0.71^{a}
		DC	6.8±0.44^{e}	2.3±1.02^{b}

[a-b]Means with different letters in the same column are significantly different (p<0.05)
[1]Values are means of three replicates ± standard deviation
[2]CI – cooked immediately; DC – delayed cooking

A graphical presentation showing the interaction effect is presented in **Figure 3-4**. The results show that DC significantly increased apparent viscosity of raw batter from all treatments except in treatments with 0.75% NaCl. Interestingly, samples with higher NaCl (1.25 and 2.00%) had lower (p<0.05) apparent viscosity than samples with lower NaCl. There is no trend as to how batter viscosity may influence sample textural quality. For example, highest apparent viscosity was obtained from DC treatments with 0.75% or 1.0% NaCl, followed by CI treatments with 0.75% NaCl, while samples with 2.0% CI had the lowest viscosity.

The effect of DC on batter temperatures was observed in all treatments. There was significantly lower (p<0.05) batter temperatures as a result of holding at 1°C (from 5.42 to 2.36°C). This decrease in temperature could partially explain the observed increase in batter viscosity after holding in most of the treatments.

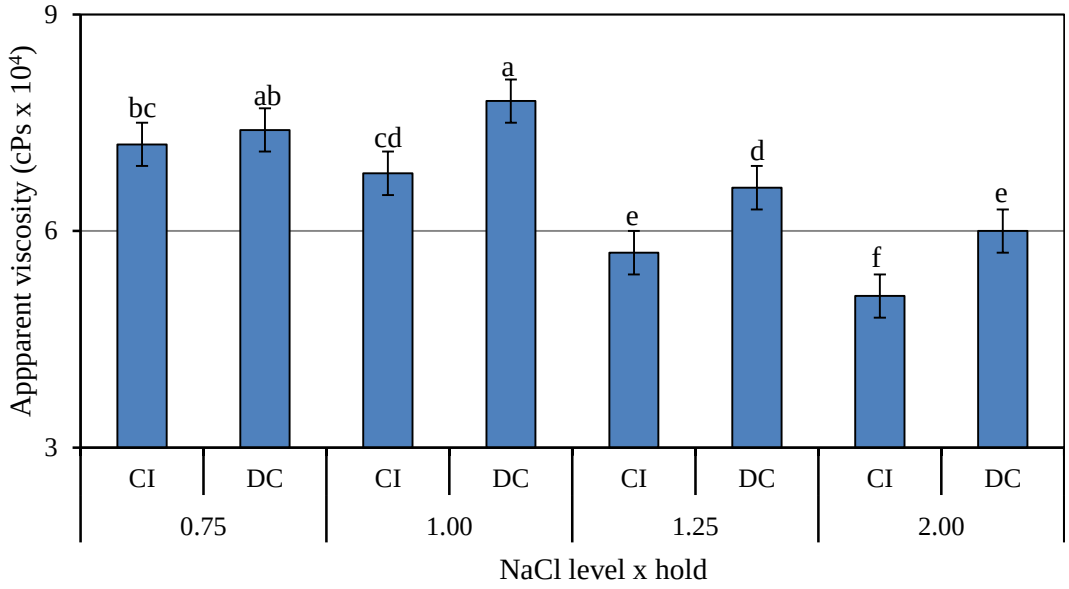

Figure 3-4 NaCl level x hold interaction effects on the apparent viscosity (CI-cooked immediately; DC- delayed cooking). [a-f] Means with different letters are significantly different (p<0.05)

3.4.3 Protein solubility

The average measured soluble protein in the raw batter is presented in **Table 3-7**. Salt type did not affect amount of soluble protein present in the meat batter (p>0.05). However, NaCl level and DC significantly increased (p<0.05) protein solubility but there was no significant interaction effect (see **Appendix A-2**).

Table 3-7 Effects of salt type, NaCl level and holding of batter before cooking on the solubility of protein[1]

Salt type	Level (%)	Hold[2]	Soluble protein (%)
Sea salt	0.75	CI	52.90±4.33[b]
		DC	60.39±5.30[a]
	1.00	CI	56.10±3.64[ab]
		DC	63.96±7.34[a]
	1.25	CI	59.20±3.88[ab]
		DC	61.33±5.71[a]
	2.00	CI	63.34±2.40[a]
		DC	66.54±4.59[a]
Regular salt	0.75	CI	48.85±4.26[b]
		DC	55.35±5.45[a]
	1.00	CI	57.23±3.06[ab]
		DC	69.81±6.18[a]
	1.25	CI	58.36±0.60[ab]
		DC	62.55±5.43[a]
	2.00	CI	65.08±1.28[a]
		DC	63.49±1.28[a]

[a-b]Mean values in the same column with different superscripts are significantly different ($p < 0.05$)
[1]Values are means of three replicates ± standard deviation
[2]CI – cooked immediately; DC- delayed cooking

As the NaCl level increased, soluble protein also increased (**Figure 3-5)** and in general DC increased soluble protein (p<0.05; 58.1% vs. 62.9%).

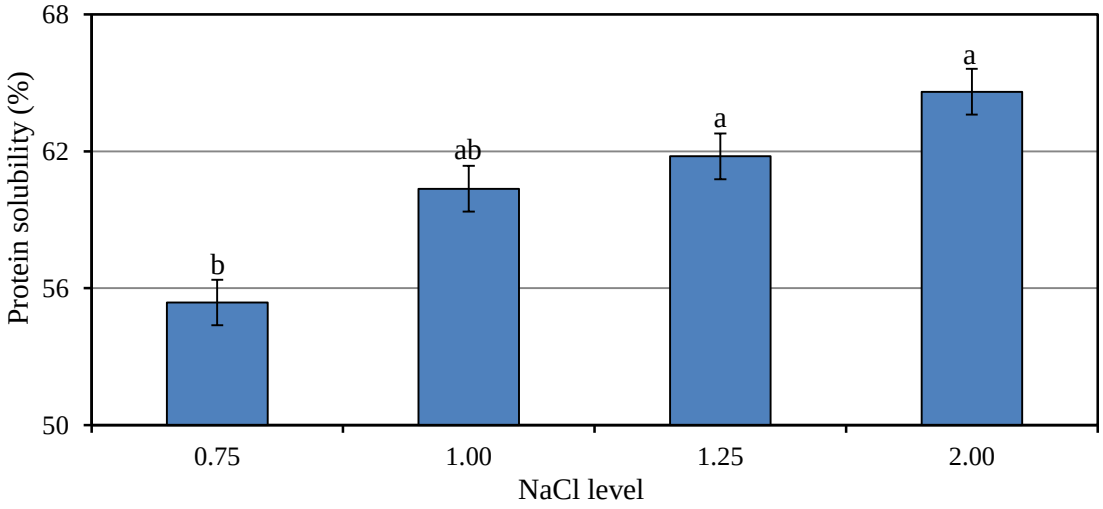

Figure 3-5 NaCl level effects on protein solubility. [a-b] Means with different letters are significantly different (p<0.05)

However, samples with 1.00% NaCl had statistically the same solubilized protein as treatments with 1.25% and 2.00% NaCl. It seems that as low as 1.0% NaCl with 0.5% potassium pyrophosphate in combination with processing conditions (grinding, chopping, emulsion milling) were enough to effect instant solubilization of proteins in the bologna meat matrix.

3.4.4 pH of raw batter and cooked bologna samples

Results showed that NaCl type, NaCl level, and holding factor had no effect (p>0.05) on pH of the samples (**Table 3-8**). The pH of raw batter ranged from 6.40 to 6.43. This is expected since lean meat used in processing was from post rigor meat and all treatments were added with 0.50% TKPP which increased the pH of the mixture from 5.71 (fresh ground meat) to ~6.40 (raw bologna batter).

Table 3-8 Effects of salt type, NaCl level and holding of batter before cooking on the pH of raw batter and cooked bologna[1]

NaCl type	NaCl level (%)	Hold	pH	
			Raw[ns]	Cooked[ns]
Sea salt	0.75	CI	6.43±0.04	6.53±0.02
		DC	6.40±0.03	6.51±0.04
	1.00	CI	6.41±0.04	6.49±0.03
		DC	6.40±0.05	6.49±0.03
	1.25	CI	6.41±0.02	6.50±0.05
		DC	6.41±0.04	6.50±0.05
	2.00	CI	6.40±0.02	6.51±0.03
		DC	6.40±0.04	6.50±0.04
Regular salt	0.75	CI	6.42±0.04	6.48±0.05
		DC	6.40±0.06	6.51±0.03
	1.00	CI	6.42±0.05	6.48±0.01
		DC	6.41±0.05	6.50±0.02
	1.25	CI	6.41±0.03	6.51±0.04
		DC	6.40±0.04	6.51±0.05
	2.00	CI	6.41±0.01	6.49±0.04
		DC	6.40±0.04	6.49±0.04

[ns]not significant (p>0.05)
[1]Values are means of three replicates ± standard deviation
[3]CI – cooked immediately; DC – delayed cooking

There was a consistent increase of pH after cooking, with the pH of cooked samples ranging from 6.49 - 6.53. Several authors also reported that pH of cooked sausage was higher than that of raw sausage (Xiong et al., 1999; Choi et al., 2007). Several hypotheses with supporting evidence suggests that the increase in pH after heating of sausages is due to the exposure of the imidazole groups or could be due to protonation of some basic amino acid residue side chains (Xiong et al., 1999; Choi et al., 2007).

3.4.5 Proximate composition of cooked bologna samples

Presented in **Table 3-9** is the proximate composition of cooked bologna samples. The products were formulated to give 10.0% fat and 11.0% protein. Although there were slight variations from the targets (ranging from 0.3–1.4%), based on the results, the desired contents were achieved. This was made possible by determining proximate composition (AOAC methods) of raw materials prior to processing. The variations from the targets can be explained by variation in the product cook loss and uncontrollable experimental error especially during sampling and sample preparation (i.e., during grinding of cooked bologna, fat accumulated and was stuck on the top and bottom of the blender blades).

The percentage moisture content of bologna samples ranged from 75.66–77.03%. Samples with 1.25% NaCl had the highest moisture content while samples with 2.00% and 0.75% NaCl had the lowest moisture among samples. This observation could be due to the difference in added water which was used to balance the changes in NaCl (see product formulation in Table 3-1) and cook loss. The samples with 0.75% NaCl had the highest added water in the formulation but also had the highest cook loss. On the other hand, although sample with 2.00% NaCl had the lowest cook loss, they had the lowest added water resulting to lowest cooked sample moisture content.

In terms of protein content, treatments with 0.75% NaCl had the highest protein content among cooked bologna samples. This treatment had the highest cook loss and thus concentrated the amount of proteins. For the ash content, as expected, increasing the NaCl content by increasing the NaCl in the formulation resulted to higher amount of ash in the product.

Table 3-9 Effects of salt type, NaCl level and holding of batter before cooking on the proximate composition[1,2] of cooked bologna

Salt type	Level (%)	Hold[3]	Moisture (%)	Fat (%)	Ash (%)	Protein (%)
Sea salt	0.75	CI	76.0±1.2^{b}	10.2±0.3^{a}	1.8±0.0^{d}	11.7±0.2^{a}
		DC	76.2±0.4^{b}	9.9±0.3^{a}	1.8±0.0^{d}	11.4±0.2^{a}
	1.00	CI	76.6±0.7^{ab}	9.0±0.9^{b}	1.9±0.2^{c}	11.3±0.6^{ab}
		DC	76.3±0.2^{ab}	9.2±0.9^{b}	2.0±0.0^{c}	11.3±0.8^{ab}
	1.25	CI	76.8±0.9^{a}	9.8±1.1^{ab}	2.3±0.0^{b}	11.5±0.5^{ab}
		DC	76.8±0.5^{a}	10.2±1.8^{ab}	2.9±0.1^{b}	11.7±0.9^{ab}
	2.00	CI	75.7±0.6^{b}	10.0±1.8^{ab}	3.0±0.0^{a}	10.6±0.5^{b}
		DC	75.8±0.5^{b}	9.6±1.5^{ab}	3.0±0.1^{a}	11.1±0.9^{b}
Regular salt	0.75	CI	75.7±1.2^{b}	10.2±0.8^{a}	1.8±0.0^{d}	12.0±0.6^{a}
		DC	76.0±0.8^{b}	9.6±0.8^{a}	1.8±0.1^{d}	11.9±0.6^{a}
	1.00	CI	76.7±1.1^{ab}	9.1±0.7^{b}	2.0±0.0^{c}	11.2±0.3^{ab}
		DC	76.0±0.6^{ab}	8.9±0.8^{b}	2.0±0.1^{c}	11.4±0.6^{ab}
	1.25	CI	77.0±0.8^{a}	8.6±0.9^{ab}	2.3±0.0^{b}	10.7±0.1^{ab}
		DC	76.8±0.5^{a}	9.1±0.8^{ab}	2.3±0.0^{b}	11.2±0.6^{ab}
	2.00	CI	76.1±0.3^{b}	9.8±1.5^{ab}	3.0±0.0^{a}	11.1±0.5^{b}
		DC	75.7±0.4^{b}	9.9±1.0^{ab}	3.2±0.2^{a}	11.4±0.4^{b}

$^{a-b}$Means with different superscripts within each column are significantly different ($p < 0.05$)
[1]Values are means of three replicates ± standard deviation
[2]Protein was calculated as total nitrogen x 6.25
[3]CI – cooked immediately; DC – delayed cooking

3.4.6 Percentage salt, sodium and potassium contents of cooked bologna

Neither delayed cooking nor salt type affected the salt (%), sodium, and potassium contents of bologna samples and as expected NaCl level affected ($p<0.05$) these components.

The sodium content increased as the NaCl level in the formulation increased while the potassium contents were similar in all samples (**Table 3-10**). This trend is expected since all samples were formulated with equal amount of tetrapotassium pyrophosphate (0.05%) while sodium varied as a result of increasing NaCl contents in the formulation. The percentage salt using mercuric nitrate method confirmed that the amount of NaCl added in the formulation was correct according to the desired NaCl level in each treatment. Based on the result of sodium analysis, to meet the recommended target sodium in bologna, which is 360 mg/55 g or 655 mg/100g (Heart and Stroke Foundation, 2012), the bologna product should be formulated with less than 2.00% NaCl.

Table 3-10 Effects of salt type, NaCl level and holding of batter before cooking on the salt (%), Na, and K contents of cooked bologna[1]

Salt type	Level (%)	Hold[2]	% Salt[3]	Sodium (mg/100g)	Potassium[ns] (mg/100g)
Sea salt	0.75	CI	0.79±0.14[d]	300±11[d]	340±6
		DC	0.80±0.14[d]	320±31[d]	350±30
	1.00	CI	1.04±0.17[c]	390±30[c]	360±45
		DC	1.06±0.17[c]	400±32[c]	350±16
	1.25	CI	1.26±0.17[b]	470±6.0[b]	360±16
		DC	1.25±0.17[b]	510±36[b]	360±25
	2.00	CI	1.94±0.17[a]	720±6[a]	360±22
		DC	1.92±0.17[a]	730±44[a]	360±16
Regular salt	0.75	CI	0.76±0.25[d]	320±10[d]	380±15
		DC	0.79±0.15[d]	330±23[d]	380±5
	1.00	CI	1.03±0.15[c]	410±24[c]	370±16
		DC	1.05±0.12[c]	400±31[c]	360±42
	1.25	CI	1.26±0.22[b]	500±34[b]	370±10
		DC	1.25±0.18[b]	490±37[b]	360±70
	2.00	CI	1.95±0.17[a]	710±38[a]	380±28
		DC	1.94±0.21[a]	730±24[a]	360±18

[a-d]Means in the same column with different superscripts are significantly different ($p< 0.05$)
[ns]Not significant
[1] Overall means from three replications ± standard deviation
[2]CI – cooked immediately; DC – delayed cooking
[3]% total chlorides

3.4.7 Water holding capacity (WHC)

The effects of salt types, NaCl levels, and DC on water holding capacity measured in cooked bologna are presented in **Table 3-11** (see also **Appendix A-3**)

3.4.7.1 Cook loss

Among the three main factors, only NaCl level significantly influenced ($p<0.05$) the cook loss and neither salt type nor delayed cooking had an impact on cook loss (**Table 3-11**). Samples with lowest NaCl (0.75% both CI and DC) had the highest cook loss. However, samples with 1.00% NaCl had the same cook loss as samples with 1.25% and 2.00% NaCl (**Figure 3-6**). Although not statistically significant, DC had the tendency ($p=0.09$) to reduce cook loss of the products (2.28% cook loss from CI samples vs. 1.89% cook loss from DC samples).

Table 3-11 Effects of salt type, NaCl level and holding of batter before cooking on the water holding capacity of bologna determined by three different methods[1]

NaCl type	Level (%)	Holding[2]	Cook loss (%)	Expressible moisture (%)	Purge loss (%)
Sea salt	0.75	CI	5.4±0.5[a]	30.0±2.1[a]	8.1±1.0[a]
		DC	4.6±0.2[a]	22.8±1.5[b]	7.2±1.0[a]
	1.00	CI	1.1±0.2[b]	22.7±1.5[b]	6.6±1.4[b]
		DC	1.0±0.2[b]	22.3±1.8[b]	6.3±1.1[b]
	1.25	CI	1.3±0.8[b]	21.0±1.4[b]	6.6±1.0[b]
		DC	0.9±0.1[b]	21.3±1.9[b]	6.9±1.0[b]
	2.00	CI	0.6±0.1[b]	20.8±1.7[b]	6.6±1.2[b]
		DC	0.5±0.1[b]	22.2±1.9[b]	6.6±0.9[b]
Regular salt	0.75	CI	6.5±0.8[a]	34.1±2.0[a]	7.3±1.6[a]
		DC	4.8±0.9[a]	25.4±1.8[b]	7.4±1.3[a]
	1.00	CI	1.2±0.1[b]	21.5±1.6[b]	6.7±2.0[b]
		DC	1.2±0.2[b]	20.8±0.7[b]	6.1±1.0[b]
	1.25	CI	1.2±0.8[b]	22.1±2.6[b]	6.5±1.1[b]
		DC	1.1±0.5[b]	21.9±1.4[b]	6.1±1.6[b]
	2.00	CI	0.9±0.2[b]	21.2±1.6[b]	6.0±1.0[b]
		DC	0.9±0.1[b]	22.0±1.1[b]	6.3±1.1[b]

[a-b]Means in the same column with different superscripts are significantly different ($p< 0.05$)
[ns]not significant ($p>0.05$)
[1] Overall means from three replications ± standard deviation
[2]CI – cooked immediately; DC – delayed cooking

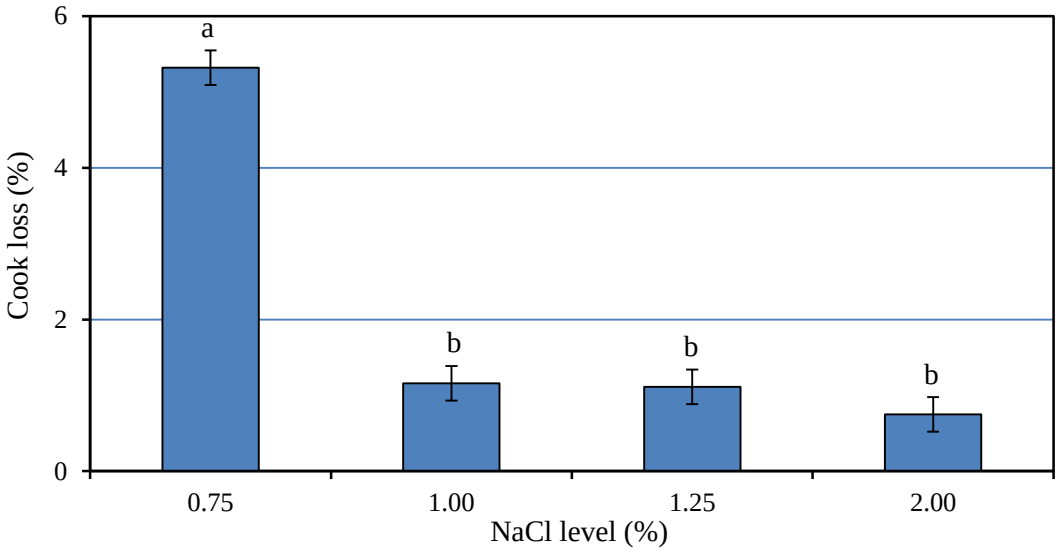

Figure 3-6 NaCl level effects on cook loss. [a-b] Means with different letters are significantly different ($p<0.05$)

3.4.7.2 Expressible moisture

Similar to cook loss, salt type did not influence (p>0.05) expressible moisture loss of the product. However, there was a significant NaCl level by hold interaction effect (p<0.05) (**Figure 3-7**).

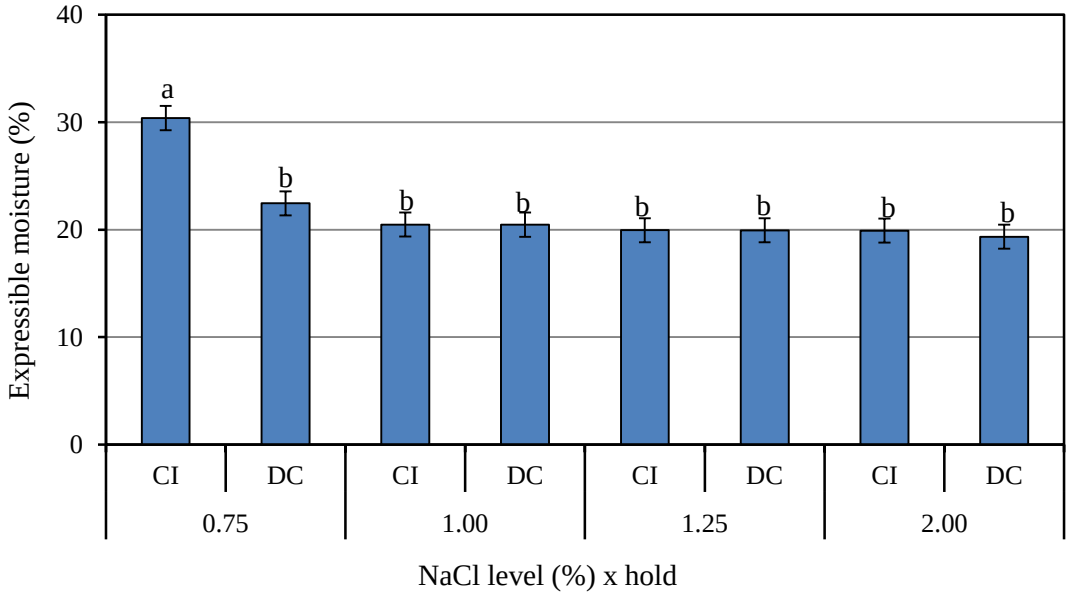

Figure 3-7 NaCl level x hold interaction effects on expressible moisture (CI-cooked immediately; DC-delayed cooking). [a-b] Means with different letters are significantly different (p<0.05)

By looking closely at **Figure 3-7**, the interaction effect of NaCl level by hold on expressible moisture was confined only to 0.75% NaCl with DC resulting in a reduction of expressible moisture that was not different from samples containing 1.0–2.0% NaCl (CI and DC).

3.4.7.3 Purge loss

There was a similar trend as to cook loss, with only NaCl level significantly affecting purge loss (p<0.05) and neither salt type nor holding affected purge loss. Samples with 0.75% NaCl had the highest purge loss while 1.00% NaCl had the same purge loss as 1.25% and 2.00% NaCl (**Figure 3-8**).

Interestingly, holding affected WHC (expressible moisture) of samples only at extremely low NaCl level (0.75% NaCl). This observation could be due to the increase in protein solubility in DC samples with 0.75% NaCl. This additional soluble protein significantly participated in water and fat retention in the matrix. However, the effect of DC was not statistically significant in cook loss (but tended to be significant, p=0.09) or purge

loss. The absence of any observed effect of DC in purge loss could be due to a very minimum force (gravitational) as compared to centrifugal force applied in expressible moisture or sensitivity of the measurements.

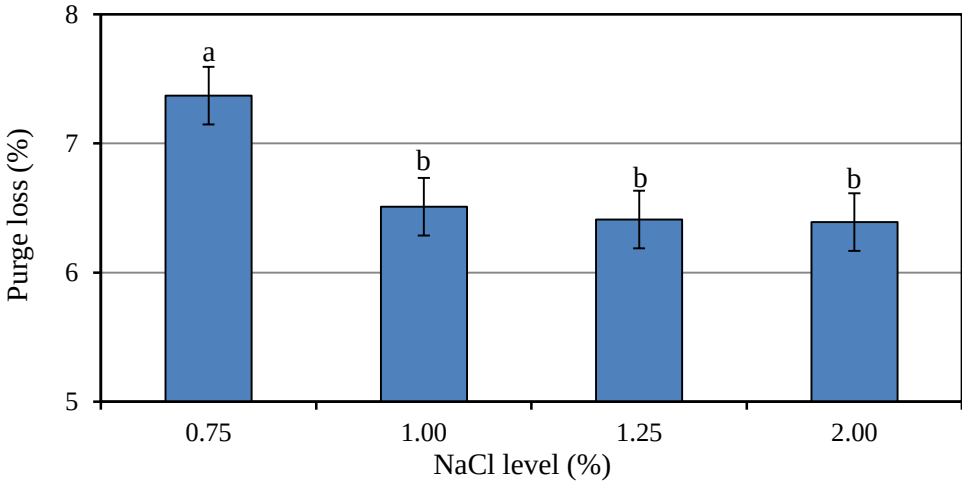

Figure 3-8 NaCl level effects on purge loss. [a-b] Means with different letters are significantly different (p<0.05)

3.4.8 Instrumental texture

3.4.8.1 Texture profile analysis (TPA). The effects of treatment factors on the TPA characteristics of samples are presented in **Table 3-12**. Salt type had no significant effect while NaCl level and hold significantly affected TPA characteristics of the samples. There was a significant NaCl level by hold interaction effect (p<0.05) (see **Appendix A-4**).

3.4.8.1.1 TPA hardness

TPA hardness has been shown to be a good instrumental texture measurement to predict sensory characteristics of solid foods (Montejano et al., 1985; Monaco et al., 2008). Thus among the parameters in TPA, hardness is one of the most useful parameters. In the present study, there was a significant NaCl level by hold interaction effect for hardness (**Figure 3-9**).

Interestingly, the interaction effect was confined only to 0.75% NaCl level wherein there was a significant increase in TPA hardness as a result of delayed cooking of meat batter. Although not statistically different, the numerical TPA value for 0.75% NaCl DC was slightly higher compared to the rest of the samples. This observation could be related to the effect of two factors: higher cook loss of DC samples with 0.75% NaCl compared to treatments with higher salt levels, and structure formation as a result of delayed cooking.

Table 3-12 Effects of salt type, NaCl level and holding of batter before cooking on the TPA of bologna samples[1]

Salt type	Level	Hold[2]	Hardness (N)	Springiness (%)	Cohesiveness (-)	Chewiness (N)
Sea salt	0.75	CI	38.2±6.5^{b}	66.35±0.4^{c}	0.30±0.02^{c}	95.3±19.7^{b}
		DC	67.8±4.1^{a}	77.52±0.6^{b}	0.48±0.02^{b}	317.3±26.4^{a}
	1.00	CI	62.2±9.3^{a}	79.25±1.6^{ab}	0.53±0.00^{a}	332.5±58.0^{a}
		DC	61.7±7.7^{a}	81.17±0.2^{a}	0.54±0.00^{a}	338.0±33.0^{a}
	1.25	CI	60.4±4.4^{a}	80.35±2.0^{a}	0.53±0.01^{a}	321.5±32.8^{a}
		DC	60.1±6.0^{a}	80.08±1.3^{a}	0.53±0.02^{a}	316.9±31.5^{a}
	2.00	CI	59.3±6.0^{a}	80.50±1.5^{a}	0.53±0.01^{a}	315.5±33.8^{a}
		DC	60.1±2.2^{a}	80.54±0.8^{a}	0.53±0.00^{a}	321.6±16.6^{a}
Regular salt	0.75	CI	33.5±7.3^{b}	63.87±2.6^{c}	0.26±0.02^{c}	73.8±19.9^{b}
		DC	61.2±6.7^{a}	77.14±2.7^{b}	0.49±0.03^{b}	286.4±61.0^{a}
	1.00	CI	59.9±1.2^{a}	80.10±0.2^{ab}	0.53±0.02^{a}	321.5±18.3^{a}
		DC	62.2±2.9^{a}	81.13±0.9^{a}	0.55±0.04^{a}	348.0±12.5^{a}
	1.25	CI	55.7±7.1^{a}	80.33±0.8^{a}	0.53±0.00^{a}	299.7±42.1^{a}
		DC	56.2±6.2^{a}	80.69±0.4^{a}	0.55±0.00^{a}	309.5±40.5^{a}
	2.00	CI	58.4±4.6^{a}	80.36±1.2^{a}	0.54±0.00^{a}	315.4±33.2^{a}
		DC	56.5±2.8^{a}	80.32±0.4^{a}	0.55±0.02^{a}	312.2±18.6^{a}

$^{a-c}$Means in the same column with different superscripts are significantly different ($p< 0.05$)
[1]Overall means from three replications ± standard deviation
[2]CI – cooked immediately; DC – delayed cooking

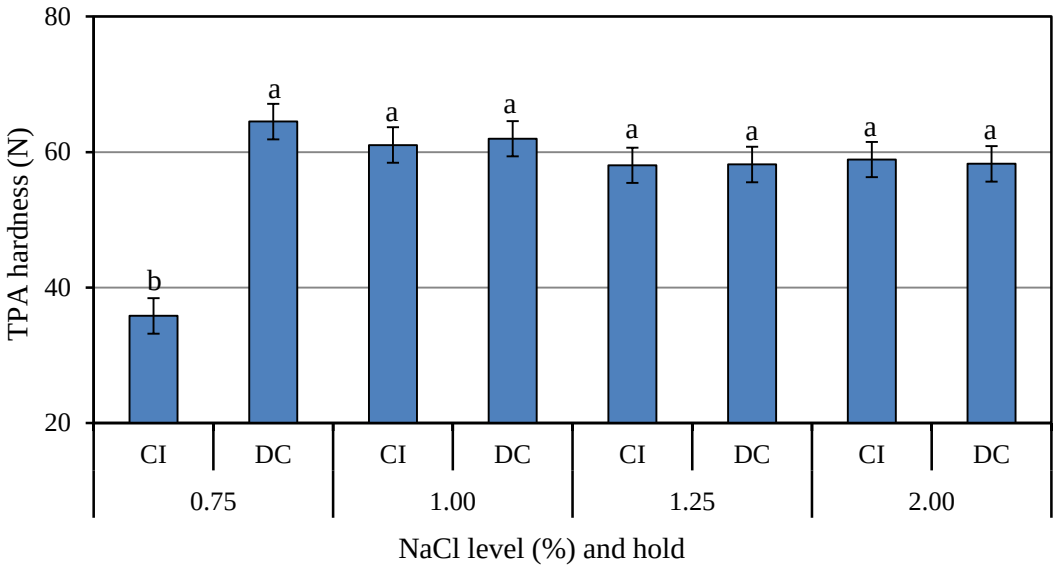

Figure 3-9 NaCl level x hold interaction effects on the TPA hardness (CI-cooked immediately; DC-delayed cooking). $^{a-b}$ Means with different letters are significantly different ($p<0.05$)

TPA hardness of CI or DC samples with 1.0% NaCl was not statistically different from samples containing higher NaCl levels (1.25% and 2.00%). All these TPA hardness values were lower compared to typical bologna samples found in the literatures (similar core sample size, 35 mm diameter and 25 mm height). For example, Thushan Sanjeewa et al. (2010) reported TPA hardness of low-fat bologna with 1.5% NaCl and 13.9 % protein ranges from 100 – 137 N with and without flours (chickpea). Shand (2000) reported that low-fat pork bologna with different binders had TPA hardness of 104-172 N. This only shows that the matrix in this study despite higher NaCl levels (2.0% NaCl) was soft compared to typical bologna formulations.

3.4.8.1.2 TPA Springiness

There was a NaCl level by hold interaction effect observed in TPA springiness of bologna samples. Interaction of NaCl level and DC effect was observed in samples with 0.75% and 1.00% (**Figure 3-10**). Nonetheless, the data shows that samples with 1.00% NaCl did not differ statistically from samples of higher NaCl levels.

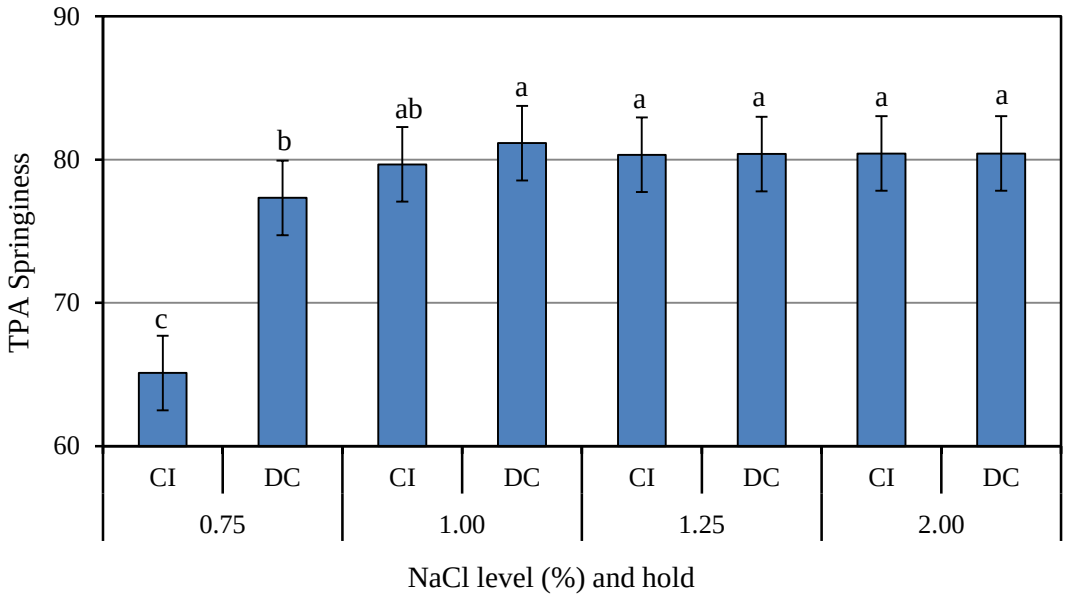

Figure 3-10 NaCl level x hold interaction effects on TPA springiness (CI-cooked immediately; DC-delayed cooking). [a-c] Means with different letters are significantly different ($p<0.05$)

Similar to TPA hardness, TPA springiness was found to accurately predict rheologic properties of solid samples (Monaco et al., 2008).

3.4.8.1.3 TPA cohesiveness

Similar to other TPA properties (hardness and springiness), a significant NaCl level by hold interaction effect was observed in bologna samples (**Figure 3-11**).

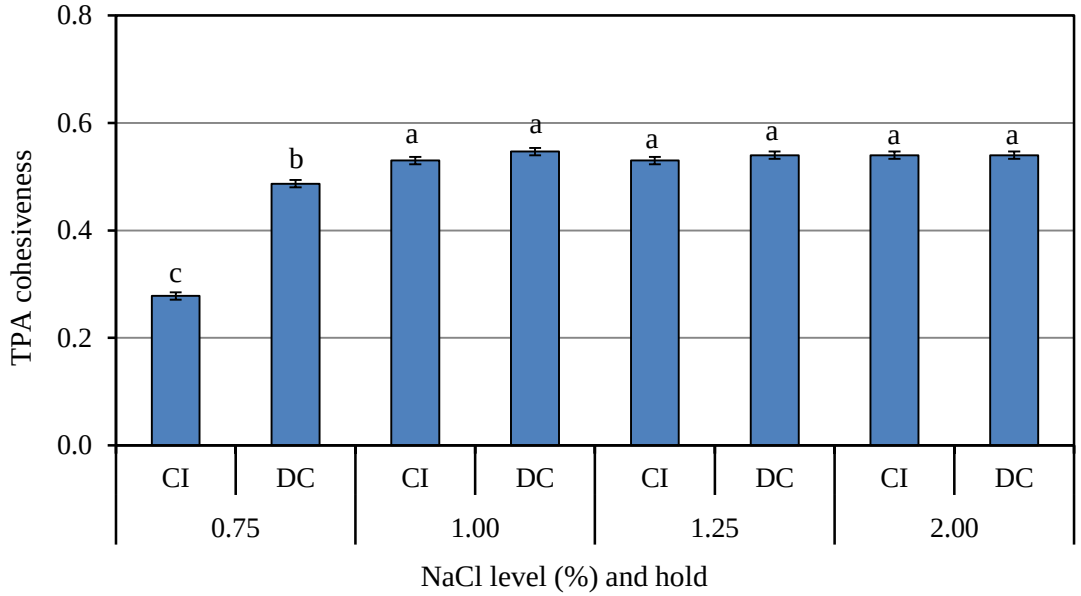

Figure 3-11 NaCl level x hold interaction effects on TPA cohesiveness (CI-cooked immediately; DC-delayed cooking). [a-c] Means with different letters are significantly different (p<0.05)

3.4.8.2 Torsional gelometry

In this texture measurement, shear strain and stress at failure of samples were measured and results showed that salt type did not affect torsion characteristics of the samples. Numerical value of shear stress is strongly influenced by protein types and concentrations, processing condition, and ingredients while shear strain is affected by protein quality and denotes protein functionality in the food matrix (Hamann, 1988). The effects of treatment factors on the torsion characteristics of bologna are presented in **Table 3-13 (**see also **Appendix A-5)**.

3.4.8.2.1 Shear stress

Shear stress of bologna samples was affected by NaCl level, delayed cooking, and interaction effect of these two main factors (**Table 3-13**). However, similar to other observations (e.g., TPA hardness), the interaction effect was only observed in treatments with 0.75% NaCl. Delayed cooking of bologna was effective in increasing shear stress only at 0.75% NaCl samples and no further effect was seen at higher levels (**Figure 3-12**).

Table 3-13 Effects of salt type, NaCl level and holding of batter before cooking on the torsional properties of bologna [1]

NaCl type	Level	Hold[2]	Shear Stress (kPa)*	Shear strain [£]
Sea salt	0.75	CI	15.5±2.1^{b}	1.34±0.12^{bz}
		DC	24.9±0.8^{a}	1.61±0.10^{by}
	1.00	CI	23.7±1.6^{a}	1.65±0.0.06^{abz}
		DC	27.9±1.8^{a}	1.72±0.10^{aby}
	1.25	CI	26.9±0.3^{a}	1.71±0.07^{az}
		DC	25.0±1.9^{a}	1.70±0.04^{ay}
	2.00	CI	24.2±3.7^{a}	1.59±0.08^{abz}
		DC	24.5±2.8^{a}	1.63±0.13^{aby}
Regular salt	0.75	CI	13.8±2.4^{b}	1.22±0.13^{bz}
		DC	22.1±4.9^{a}	1.56±0.18$^{a\by}$
	1.00	CI	24.8±2.7^{a}	1.61±0.14^{abz}
		DC	26.5±2.6^{a}	1.73±0.06^{aby}
	1.25	CI	23.4±0.9^{a}	1.63±0.03^{az}
		DC	25.1±2.7^{a}	2.20±0.99^{ay}
	2.00	CI	22.8±3.1^{a}	1.55±0.10^{abz}
		DC	23.9±3.0^{a}	1.64±0.11^{aby}

*$^{a-b}$ Means in the same column with different superscripts are significantly different (p< 0.05)
£ $^{a-b}$ Means in the same column with different superscripts are significantly different (p< 0.05) as affected by salt level and $^{y-z}$ as affected by DC
[1]Overall means from three replications ± standard deviation
[2]CI – cooked immediately; DC- delayed cooking

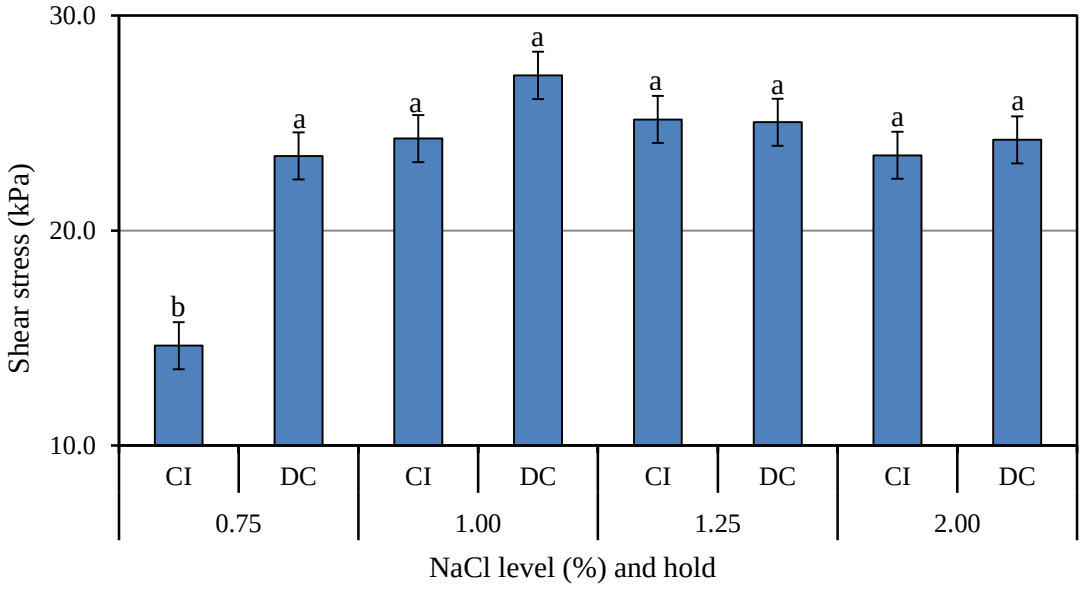

Figure 3-12 NaCl level x hold interaction effects on shear stress (CI-cooked immediately; DC-delayed cooking). $^{a-b}$ Means with different letters are significantly different (p<0.05)

3.4.8.2.2 Shear strain

Both NaCl level and DC affected the shear strain of samples (**Figures 3-13**) but there was no interaction effect. DC increased the strain of bologna samples by 12.3% (1.54 to 1.73).

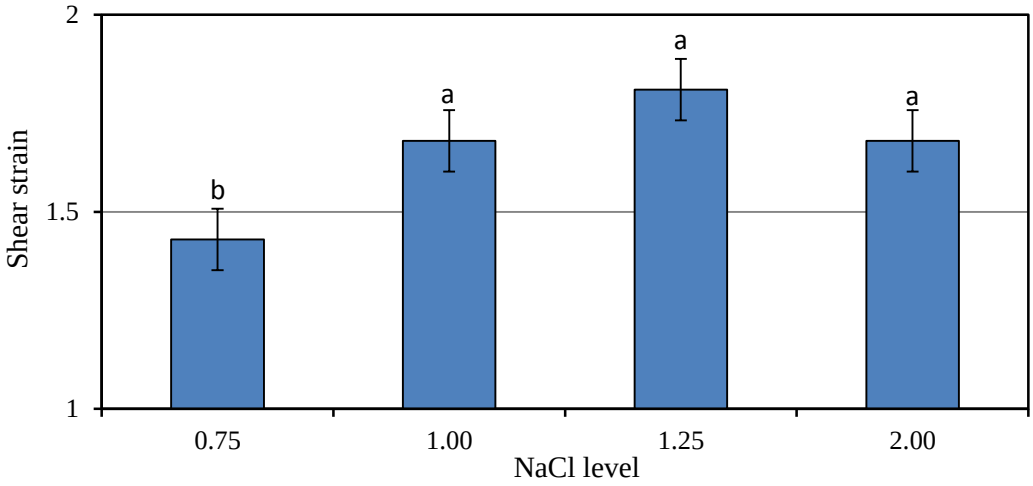

Figure 3-13 NaCl level effects on shear strain. [a-b] Means with different letters are significantly different (p<0.05)

Shear stress was plotted against shear strain using Lanier (1986) mapping to describe mechanical texture properties (mushy brittle, rubbery, and tough) of protein samples (**Figure 3-14**).

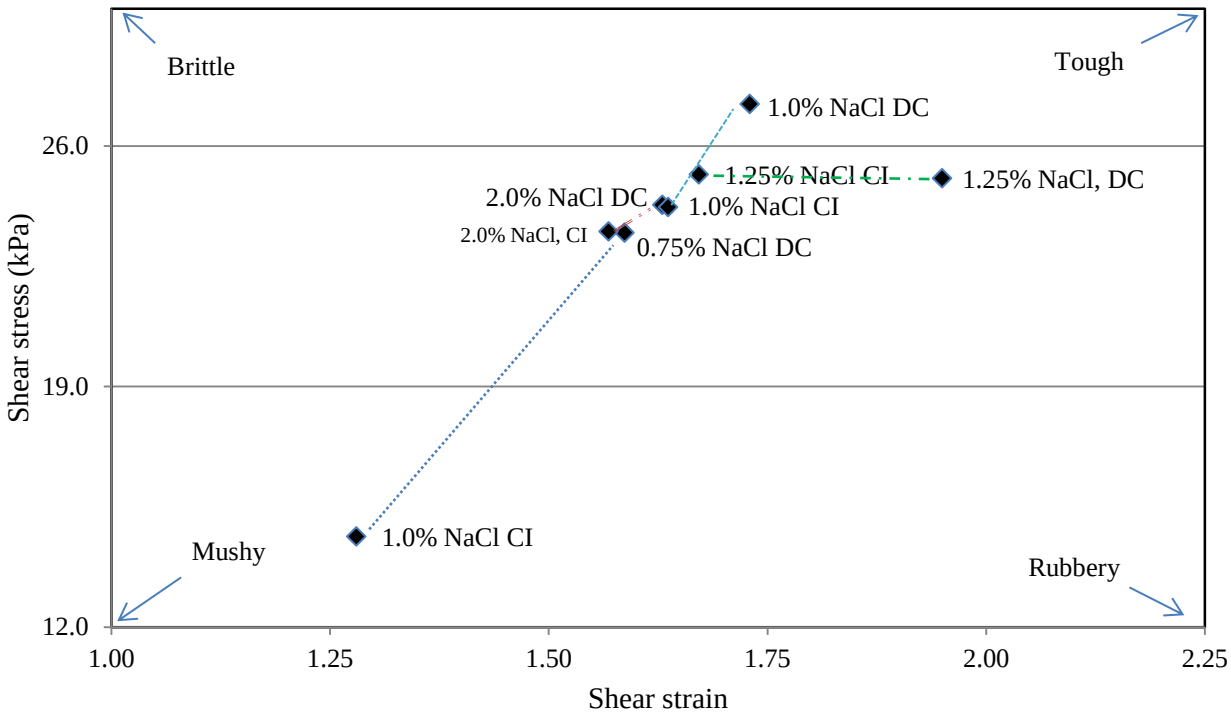

Figure 3-14 Torsion texture map of low-salt, low-fat bologna as affected by NaCl level and hold (CI-cooked immediately; DC-delayed cooking).

Results show that the only clear mechanical property of the samples found in this study is the "mushy" characteristic of samples with 0.75% NaCl CI. In general, DC affected torsion properties of all samples. Interestingly, the magnitude effect of DC varied according to NaCl level. DC increased shear stress of samples with 0.75%, 1.00% and 2.00% NaCl but not samples with 1.25% NaCl. A significant increase in shear strain was noted at 1.25% NaCl tending to show a textural characteristic of between rubbery and tough. However, this observation for 1.25% NaCl DC does not agree with other measurements (e.g., TPA hardness, strain, or even WHC). The most probable reason could not be explained but can be related to high standard deviation of the reading for regular salt 1.25% NaCl DC (see **Table 3-13**, 2.20±0.99) that could have influenced the overall effect of DC on this specific NaCl level. Nonetheless, general results showed the positive effect of DC in gel stress and slight increase in gel strain.

3.4.9 Sensory evaluation

A thirteen member semi-trained sensory panel evaluated bologna samples for firmness, springiness, juiciness, flavour, and saltiness. Salt type did not affect the firmness and springiness of samples but interaction of NaCl level and DC significantly influenced these sensory parameters. In general, results for firmness and springiness were consistently similar to instrumental texture measurement except that panelists perceived improvement contributed by the holding factor in samples both with 0.75 and 1.00% NaCl. A significant NaCl level x hold effect was confined only at 0.75% and 1.00% NaCl, where DC significantly affected sensory firmness (see **Appendix A-6**).

In terms of juiciness, samples with 0.75% NaCl (CI) received comments of being wet and not juicy; while samples with 1.00%, 1.25, and 2.00 % did not differ in sensory juiciness scores (**Table 3-14**). This wetness could be due to weak protein-water interaction and 0.75% NaCl seemed a critical level for a stable meat emulsion formation.

The perception of flavour was significantly affected by NaCl ($p< 0.05$). Samples with 0.75% NaCl had the lowest flavour score and panelists commented that this sample (CI) had an "unpleasant flavour". The perception of flavour depends on the degree or intensity of volatile and non-volatile flavour compounds released from the food (Ross, 2009). The release of compounds from the food matrix is influenced by the matrix structure, chemical and physical properties. Sample with 2.00% NaCl had the highest flavour intensity. This effect of salt on meaty flavour perception agrees with the published reports of Barbut et al. (1988) and

Matulis et al. (1995) showing that as the salt increased, the flavour intensity also increased. These observations could be due to the flavour enhancement effect of NaCl (Gillette, 1985) and can be related to bitterness-suppressing functionality of NaCl (Breslin and Beauchamp, 1995). The unpleasant flavour detected in sample with 0.75% NaCl could be attributed to poor physical property/texture of the sample as well as effect of ingredients not tightly bound in the emulsion matrix (e.g., phosphate, known to have soapy flavor). Furthermore, the 0.75% NaCl may not be enough to mask bitterness from phosphate in a very poor emulsion matrix.

Table 3-14 Effects of salt type, NaCl level and holding of batter on the sensory characteristics of bologna [1]

NaCl Type	Level (%)	Hold[2]	Sensory parameters[3]				
			Firmness	Springiness	Juiciness	Flavour	Saltiness
Sea	0.75	CI	2.9±1.3^{d}	2.8±1.2^{d}	4.1±1.7^{c}	3.7±1.5^{d}	2.0±1.0^{d}
		DC	4.8±1.6^{c}	4.5±1.4^{c}	4.8±1.2^{b}	4.0±1.2^{d}	1.9±1.0^{d}
	1.00	CI	5.4±1.2^{b}	4.9±1.4^{ab}	5.3±0.8^{a}	4.2±1.2^{c}	2.4±0.9^{c}
		DC	6.1±1.0^{a}	5.4±1.3^{a}	5.2±1.0^{a}	4.2±1.0^{c}	2.4±1.0^{c}
	1.25	CI	5.4±1.4^{b}	5.1±1.3^{ab}	5.4±0.9^{a}	4.6±0.9^{b}	4.0±1.0^{b}
		DC	5.7±1.3^{b}	5.2±1.4^{ab}	5.3±0.8^{a}	4.9±1.2^{b}	3.8±1.2^{b}
	2.00	CI	5.4±1.6^{b}	4.9±1.2^{ab}	5.5±0.7^{a}	5.2±1.2^{a}	4.2±1.2^{a}
		DC	5.4±1.0^{b}	4.9±1.3^{b}	5.3±1.0^{a}	5.4±1.2^{a}	4.5±0.8^{a}
Regular	0.75	CI	2.6±1.5^{d}	2.4±1.0^{d}	3.7±1.6^{c}	3.5±1.4^{d}	1.9±0.9^{d}
		DC	4.9±1.5^{c}	4.2±1.6^{c}	4.5±1.3^{b}	4.0±1.2^{d}	1.8±0.9^{d}
	1.00	CI	5.8±1.3^{b}	5.2±1.4^{ab}	5.2±0.9^{a}	4.4±1.0^{c}	2.5±1.1^{c}
		DC	6.3±0.9^{a}	5.5±1.6^{a}	5.1±1.0^{a}	4.2±1.1^{c}	2.4±1.1^{c}
	1.25	CI	5.4±1.0^{b}	5.2±1.3^{ab}	5.3±0.8^{a}	4.5±1.2^{b}	3.0±1.0^{b}
		DC	5.5±1.1^{b}	5.3±1.5^{ab}	5.5±0.7^{a}	4.7±1.0^{b}	3.9±0.9^{b}
	2.00	CI	5.4±1.5^{b}	5.2±1.4^{ab}	5.4±0.9^{a}	5.3±1.2^{a}	4.2±1.1^{a}
		DC	5.4±1.3^{b}	4.9±1.3^{b}	5.4±0.9^{a}	5.3±1.0^{a}	4.2±1.0^{a}

$^{a-c}$ Means in the same column with different superscripts are significantly different (p<0.05)
[1]Overall means from thirteen panelists with three replications (13 panelists x 3) ± standard deviation
[2]CI – cooked immediately; DC – delayed cooking
[3]Scores: 8 (extremely firm, elastic/springy, juicy, intense flavour) ----1 (extremely soft, inelastic/not springy, dry, bland flavour); 6 (extremely salty --- 1 no detectable saltiness)

Similar to flavour, only NaCl level influenced the saltiness perception. Panelists scored 0.75% treatment to be very slightly salty, while bologna sample with 2.00% NaCl to be very to moderately salty. Panelists easily detected the saltiness differences of samples with different levels of NaCl in the formulations.

3.5 Discussion

In the present study, the bologna matrix had a low-fat content (10.0%) at constant protein level (11.0%). Formulating LSLF emulsified type products is beset with challenges. Fat is a critical part in a food matrix for both flavor and texture properties and thereby affecting overall acceptability of the product (Ventanas et al., 2010). Fat reduction is commonly achieved either with the use of leaner meats (but costly) or "dilution effect" from substances like low or noncaloric ingredients or water (Claus et al., 1989). The latter was used in the present study with the assumption that the high amount of added water stressed the meat system thus one can fully elucidate the potential of each experimental factor in improving quality of low-salt bologna.

The use of sea salt is commonly marketed (anecdotal) as a healthier option in cooking. The presence of "trace minerals" which is lacking in regular refined salt is said to be responsible for its flavour-enhancing effect and said to boost the immune system (Adams, 2012). This common marketing claim about sea salt may mislead the consumer. Although only one type of sea salt was used in this study (basically "commercialized" sea salt from Cargill Salt, Minneapolis, MN, USA), it is clear that the composition of sea salt is very variable and highly dependent on harvest process. Based on the supplier's technical data, both salts used in this study were produced by a vacuum evaporation process of purified brine. The difference was the harvest location. The purified brine of sea salt was from the Pacific ocean near the San Francisco Bay, USA (Cargill Salt, 2008) while the purified brine of regular salt (mined) was harvested and processed in Canada (The Canadian Salt Company Limited, 2009). The commercial sea salt used in the present study was purer than the common refined salt as it contained less divalent cations (calcium, magnesium) and contained higher sodium and potassium. According to Hamm (1986), divalent cations such as Ca^{2+} and Mg^{2+} may have a negative effect on meat. Binding of cations reduces the electrostatic repulsion between the negatively-charged groups by screening effects; therefore, the protein structure is tightened and shrinking occurs. This concept was further confirmed by Seman et al. (1980) in which they found that a combination of NaCl + $MgCl_2$ resulted in higher total liquid-, gel-, cook-loss than treatments with NaCl + KCl or NaCl + phosphate. However, in the present study, it seems that the difference of divalent cation between sea salt and regular salt was not large enough to chemically affect the quality of the meat batter (**Table 3-5**). According to Xiong and Brekke (1991) effects of magnesium and calcium as NaCl replacers on the extractability of proteins and gelation is concentration dependent. In an extracted

chicken myofibril, extractability of salt soluble proteins and myofibril gel strength were increased by calcium and magnesium chloride at less than 5 mM but were decreased at greater than 10 mM. The divalent contribution of each salt type in the meat batter is shown in **Table 3-15**.

Table 3-15 Concentration of divalent cations (by calculation) present in the meat batter as affected by salt type and NaCl level

NaCl level (%)	Sea salt		Regular salt	
	Calcium (µg/g)	Magnesium (µg/g)	Calcium (µg/g)	Magnesium (µg/ g)
0.75	0.075	<0.0075	2.10	0.075
1.00	0.100	<0.0010	2.80	0.100
1.25	0.125	<0.00125	3.50	0.125
2.00	0.200	<0.0020	5.60	0.200

The current indicates that SS and RS imparted similar saltiness in cooked bologna as perceived by panelists. Drake and Drake (2011) reported that the presence of some minerals in sea salts may play a role in salty taste perception as they found that time-intensity profiles for salty taste (in aqueous solution) were distinct among sea salt samples. They reported that some sea salts had volatile (green/herbal, smoky, and earthy) flavors compared to reference table salt. However, our methodology was limited to the determination of overall flavor and overall saltiness intensity and panelists did not perceive any difference between sea salt and regular industrial salts within the same salt level. The purity of sea salt in the present study and complexity of the sample matrix (meat system) eliminated the potential role of minerals in sea salt for saltiness perception.

In this particular experiment, batter viscosity was not an effective tool to characterize the effect of investigated treatments on the quality of LSLF bologna. One of the possible explanations for significantly higher viscosity in this 0.75% CI sample was the higher proportion of uncoated fat cells in the meat batter. Since there was insufficient protein to coat the fat, those small fat particles tended to coalesce causing mechanical stress during measurement giving higher apparent viscosity numbers. Another probable reason is the incompatibility of the bologna matrix with the rotational viscometer measurement as use of the rotating spindle may create a channel affecting the real viscosity of the sample (Brookfield Engineering Laboratories, 2012).

Due to cost and time associated in conducting sensory evaluation, several studies compared the most appropriate instrumental texture measurement that can be used to predict sensory texture characteristics (Montejano et al., 1985; Monaco et al., 2008). For example, in protein gels (egg white, pork, beef, turkey, surimi gels), Montejano et al. (1985) reported a high correlation of TPA hardness and shear stress in measuring the strength characteristics of the gels. In this study, sensory firmness was predicted better (based on correlation analysis) by shear stress than by using TPA hardness (R^2= 0.80 and 0.69, respectively).

It was consistently observed in this study that the overall texture quality of samples with 1.00% NaCl was not statistically different than those samples with higher salt levels (1.25 and 2.00%). This result generally was not supported by the previous published reports. For example, Barbut et al. (1988) reported that texture and physical properties of frankfurters prepared with 1.0% salt were extremely defective. Sofos (1983) observed that frankfurters with 1.0% salt were very soft and crumbly and even those with 1.5% salt were still soft. Moreover, Hand et al. (1987) reported that low-fat frankfurters with 1.5% NaCl had softer consistency than those containing 2.0% or 2.5% NaCl. The mechanisms explaining the effect of NaCl on WHC are known and explained in many papers. For example, when NaCl is added to meat, the chloride ions will react to positively-charged groups of myosin or actomyosin, eventually causing weakening of the interaction between oppositely-charged groups of the muscle proteins or increasing the repulsive force between filaments (electrostatic repulsion) and expansion/swelling of the myofibril lattice (Hamm, 1972; Hamm, 1976; Offer and Trinick, 1986; Wildings et al., 1986). With greater swelling (bigger spaces in the myofilament), more exogenous water can fill the filament lattice. The second part of the mechanism is how this water can be retained in the hydrated system. When electrostatic repulsion reached 100%, the proteins become solubilized (Hamm, 1986). These solubilized myofibrillar proteins when subjected to heat form a three-dimensional gel trapping matrix which retains water and fat.

In general, our model system had favorable high pH and sufficient ionic strength (IS, $\mu = 0.5\sum CiZi^2$) explaining the comparable characteristics of samples with 1.00% NaCl to those samples with 1.25%, and 2.00% NaCl. Shown in **Table 3-16** is the total ionic strength contributed by salt and phosphate considering the aqueous component in the formulation (71.6% moisture content of meat and 38.63% added water for 0.75% NaCl treatment).

Table 3-16 Total ionic strength (by calculation) of meat batter at each NaCl level

NaCl level	NaCl (μ, aqueous)	TKPP (μ, aqueous)	Total
0.75	0.174	0.205	0.379
1.00	0.233	0.206	0.439
1.25	0.292	0.207	0.499
2.00	0.469	0.242	0.711

It is important to note however that during initial step of chopping (the first 2 min in bowl chopper), lean meat was exposed to even higher ionic values (**Table 3-17**). Again this is due to considerably less water (25% of total added water or 10% of the recipe formulation) during initial chopping. In commercial processing of sausage, when using the "build-up method", ~1/3 of the total required added water is initially mixed with the meat, NaCl, phosphate and sodium nitrite (Heinz and Hautzinger, 2007). This exposes the meat to high ionic strength during the first few minutes of mixing allowing swelling of the myofilament lattice and extraction of proteins (Hamm, 1986).

Table 3-17 Ionic strength (by calculation) of meat during initial chopping

Salt Level	IS NaCl (μ, aqueous)	IS TKPP (μ, aqueous)	IS during initial chopping
0.75	0.287	0.338	0.625
1.00	0.383	0.339	0.722
1.25	0.480	0.340	0.820
2.00	0.817	0.361	1.178

There was a significant correlation (R^2=0.79) between initial ionic strength and percentage soluble protein (**Figure 3-15**). It seems that the critical limit is between 0.625 to 0.722 initial ionic strengths. There was a sudden failure at 0.75% NaCl and sudden structure development at 1.00% NaCl.

Based on the overall data, it seems that 2 min exposure of meat to 0.625 IS (0.75% NaCl) mixture was not enough to produce a stable emulsion. However, holding during delayed cooking for 20 h of this treatment with total final IS of 0.379 was enough to create a firm gel. This can be related to the effect of DC in increasing protein solubility which seems to support the theory of Bendall (1954) that some time is needed to reach the full potential of added phosphates, as cited by Knipe et al. (1985).

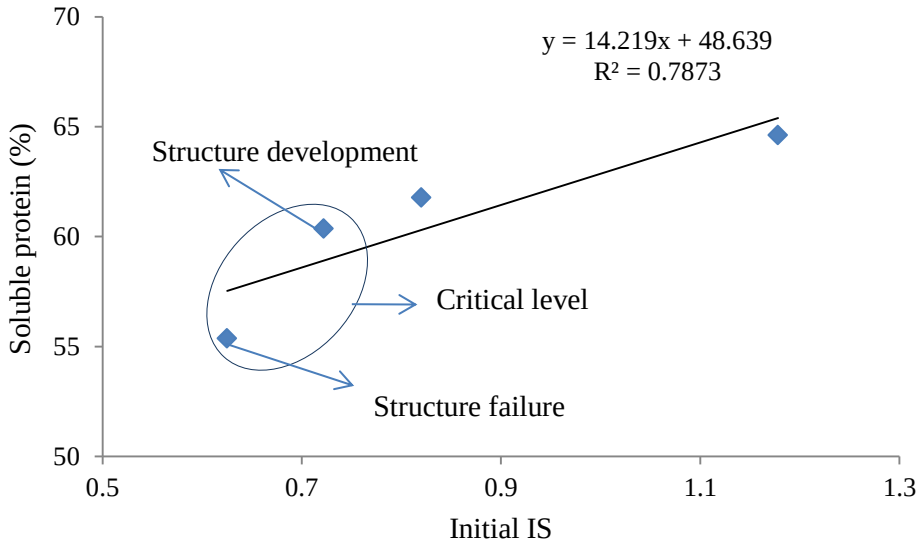

Figure 3-15 Initial ionic strength (IS) and solubility of myofibrillar proteins (p <0.05)

In addition to ionic strength, there are many factors that may contribute to the difference of our observation compared to other papers. Formulations and processing conditions may partially explain such differences.

The first reason could be due to differences among meat raw materials. In this study, pork leg muscle was used. Barbut (1988) used turkey, and Sofos (1983) and Hand et al. (1983) used a combination of pork and beef ground meat (pork trimmings and chuck, respectively). According to Barbut and Mittal (1990) there are differences between the proteins of beef, pork, and poultry meat in gelation patterns and responses to salt. Lan et al. (1995) investigated thermal gelation properties of protein fractions and found that pork myosin had higher gel strength and lower cooking loss compared to chicken breast myosin at 7.0% protein, 6.0 pH, 2.0% NaCl and cooked to 70ºC. The turkey breast had the lowest gel strength among the species tested (pork, chicken, beef, turkey) (Lan et al., 1995).

The second reason could be the presence of phosphate. None of the above published papers used tetrapotassium pyrophosphate. Based on our results, the combination of 1.00% NaCl and 0.50% tetrapotassium pyrophosphate with 3 min chopping and passing through emulsion mill twice was enough to produce similar water holding capacity characteristics and texture (hardness, springiness, and chewiness) as with those samples with higher NaCl concentration (1.25–2.00%). Whiting (1984b) found that water exudate of frankfurter was increased when NaCl concentration was reduced. However, addition of either tripolyphoshate or pyrophosphate to 1.5% NaCl batter reduced water exudate to values less than the 2.5% NaCl batters. Graham and Trout (1984) evaluated different types of phosphates and found

that the most effective was tetrasodium pyrophosphate, followed by sodium tripolyphosphate, then sodium tetrapolyphosphate, and the least effective was sodium hexametaphosphate. Tetrasodium pyrophosphate gave the highest effect in increasing the ionic strength of the batter at different salt levels. In their study, maximum tensile strength was obtained at 1.3% NaCl (using tetrasodium pyrophosphate) and there was no significant difference between tensile strength values at 1.3%, 1.65% and 2.00% NaCl (p>0.05). A similar result was reported by Knipe et al. (1985) where they compared the effect of four inorganic phosphates (sodium tripolyphosphate, tetrasodium pyrophosphate, potassium tripolyphosphate, and tetrapotassium pyrophosphate) on protein solubility, stability, and pH of meat emulsions. Based on their observation, tetrapotassium pyrophosphate was significantly superior in solubilizing proteins resulting in a stable emulsion. This effect was due to anion difference between tripolyphosphates and pyrophosphates. Although K^+ and Na^+ pyrophosphates have basically similar anions, NaPP had relatively smaller effect on soluble protein. Moreover, doubling the amount of phosphates (0.15% to 0.30%) resulted in a significant increase in soluble protein (Knipe et al., 1985). In the present study, usage of phosphate was near the upper concentration permitted. In Canada, the Food and Drug Regulations permit the addition of phosphate salts in meat products with the maximum permitted level of 0.5% calculated as sodium phosphate dibasic. A conversion chart is provided to guide processors when using different forms of phosphate. The 0.5% (w/w) TKPP used in this study was below the maximum when converted to sodium phosphate dibasic (0.43%). This amount of phosphate could be the main contributory factor that allows formulation with 1.00% NaCl to have similar textural properties to those with higher levels of NaCl.

The third reason the present results were different than other literature reports could be the favorable heating condition for gelation formation. The cooking schedule used in this study was a slow heating rate (30 min at 50°C, 30 min at 60°C, 30 min at °70°C, and 75°C until internal temperature reached 71°C). Heating rate plays a key role in determining the properties of the resulting gel (Sun and Holley, 2011). Several authors reported that slower heating rates increase the strength of the myosin gels (Foegeding et al., 1986), salt soluble protein (Camou et al., 1989) and different muscle systems (Lan et al., 1995). Cofrades et al. (1997) found that frankfurters (low- and high-fat) heated at slow rate exhibited greater hardness, cohesiveness, springiness, and chewiness than those heated at either medium or high heating rates. A similar observation was reported by Barbut and Mittal (1990) for meat batters and Saliba et al. (1987) for frankfurters. The effect of heating rate on the

characteristics of emulsified-type sausages is explained by the longer time for protein to unfold, thus providing more favorable conditions for proteins to interact (Foegeding et al., 1986) and form more favorable protein-protein interactions, producing a stronger, better-ordered three-dimensional gel (Camou et al., 1989).

The advantage of DC, especially at a lower NaCl concentration (0.75%), could be related to the theory of Bendall (1954) that states that additional time is needed after the phosphate has been mixed with meat to reach its full functionalities (i.e., cleaving actomyosin) allowing maximum protein extractability. The DC factor significantly increased soluble protein at this extremely low salt level. However, extraction was immediate at higher salt levels and thus no further improvement was observed. Fukazawa et al. (1961) and Samejima et al. (1969) proved that adequate extraction of myosin was necessary to create the gel formation that provides the product texture. The combination of high NaCl level (high ionic strength) and processing (chopping) was adequate to extract soluble proteins instantaneously responsible for cooked product texture while holding time was necessary to further extract soluble proteins when batter matrix has low ionic strength. However, in addition to soluble proteins, the swollen myofibrils may contribute to WHC and gel strength. Coagulation of swollen actomyosin influenced immobilization of water in a heated sausage batter in addition to gelation of solubilized myosin (Hamm, 1986). Interestingly, increasing the amount of extracted myofibrillar proteins does not necessarily means better gel strength (samples with 1.25% and 2.00% NaCl had higher soluble protein but similar texture as did samples with 1.00% NaCl). The overall result of this study support the postulate of Trout and Schmidt (1984) that only a certain amount of extracted myofibrillar protein is needed to produce a cohesive bond in meat products, and any additional extracted protein has no beneficial effect.

3.6 Conclusion

Results of this study indicate that it is possible to formulate LSLF bologna (minimum of 0.75% and optimum of 1.00% NaCl concentration) provided that the stuffed batter is held at 1°C for 20 h prior to cooking and 0.50% TKPP is included in the formulation. Bologna at these NaCl levels had sodium contents of 324 mg/100 g serving and 400 mg/100 g serving, respectively, which is significantly below the target of 360 mg sodium/55 g (or 655 mg sodium/100 g) serving as set by the government and criteria set by Heart and Stroke Foundation (CTAC 2009-2010).

The NaCl type (sea salt vs conventional salt) had no effect on water holding capacity, instrumental and sensory texture, flavour, and saltiness of low-fat bologna samples. Increasing NaCl in the formulation resulted in improved water holding capacity only from 0.75% to 1.00% NaCl. The DC was effective at 0.75% NaCl level and to some extent at 1.00% NaCl level. This study showed the usefulness of holding stuffed batter at 1°C for 20 h prior to thermal processing in formulating low-sodium (0.75% NaCl), low-fat bologna (11% protein, 10% fat).

Although the texture of the 1.00% NaCl DC treatment was similar to as bologna with 1.25 and 2.00% NaCl, the challenge may be to increase the firmness of the product for ease of slicing. As bologna is commonly sold as thin slices (2–3mm), its firmness is a critical factor both in product appearance and processing (specifically during this slicing step).

3.7 Connection to the Next Study

Although numerous papers have been published on the effectiveness of microbial transglutaminase (MTG) in low-salt meat products (such as chicken kebob, chicken sausage), none has been done on LSLF at the pilot-scale level with processing procedures that exactly mimic commercial processing (e.g., >5.0 kg batch, using sausage bowl chopping machine, emulsion mill and stuffing into large water proof diameter casing (60 mm)). A second study was therefore carried out to examine the usefulness of MTG in combination with FSM on the processing, chemical, and sensory characteristics of LSLF bologna.

4 POTENTIAL UTILIZATION OF FLAXSEED MEAL AND MICROBIAL TRANSGLUTAMINASE IN PROCESSING OF LOW-SALT, LOW-FAT BOLOGNA

4.1 Abstract

The effects of addition of flaxseed meal (FSM, different levels: 0.0, 0.5, 1.0, and 1.5%) and microbial transglutaminase (MTG, 0.0, 0.15, 0.3%) on the physicochemical properties of low-salt, low-fat bologna (LSLF) were investigated. Both FSM and MTG addition did not affect moisture and fat contents of cooked bologna. However, percentage protein was higher in treatments with 0.3% MTG while percentage ash was higher in samples with 1.0-1.5% FSM. Residual nitrite of all the samples was more than 50% of the input amount. Interestingly, addition of 1.5% FSM resulted in a significant reduction of residual nitrite (reduced by 7.8%).

In terms of water holding capacity (WHC), FSM reduced (p<0.05) expressible moisture and purge loss during display and did not affect cook yield while MTG had no effect on expressible moisture but significantly increased purge loss and had the tendency to decrease cook yield (p=0.057). Addition of FSM in samples with MTG improved water holding as compared to samples with MTG alone. In terms of product texture, as low as 0.15% MTG significantly increased TPA hardness, cohesiveness and springiness of bologna samples. Likewise, the sensory panel perceived this significant increase in firmness of samples with MTG addition. The use of 1.5% FSM increased instrumental texture of the samples but this increase was not detected by the sensory panel. FSM addition negatively affected (p<0.05) the color of samples both using instrumental and sensory colour evaluation. In terms of flavour-related perceptions, panelists did not perceive any foreign flavour or even reduction of spice intensity as contributed by MTG or FSM. However, saltiness perception was reduced (p<0.05) in samples with 1.5% FSM.

This study shows that MTG was superb in improving texture of LSLF bologna but caused greater purge. To maximize its technological application, additional ingredients that are capable of increasing water binding of the mixture must be added. Although FSM can be used in combination with MTG, its application is limited due to its high mucilage component

causing sliminess that eventually led to some handling problems (slicing step). Furthermore, FSM affected product surface colour. Therefore, to commercialize use of flax in meat processing, reduction of the mucilage component of FSM or an alternative form of flax product (e.g., use of flax protein isolate) warrants further research.

4.2 Introduction

In Chapter 3, it was shown that extended holding of stuffed batter at 1°C for 20 h before cooking was effective ($p<0.05$) in improving texture of samples with 0.75% NaCl. The texture of bologna with 1.00% NaCl was generally similar to treatments with 1.25% and 2.00% NaCl. Evidence showed that 1.00% NaCl with 0.50% tetrapotassium pyrophosphate was enough to form a stable meat emulsion matrix. However, because the model emulsion system contained higher added water (>37%), the cooked product was not very firm (TPA hardness = ~60-62 N). One of the most critical characteristics of bologna is "firmness", both for processing (slicing) and sensory appeal. Therefore, as a sequel to the previous study, use of ingredients such as FSM and MTG to increase firmness of LSLF bologna was investigated.

The enzyme transglutaminase (protein-glutamine γ-glutamyl transferase) can form ε-(γ-glutamyl)-lysine bonds in various proteins (Motoki and Kumazawa, 2000). This protein polymerization changes protein functionalities, ultimately leading to significant product texture improvement (Motoki and Kumazawa, 2000). Use of this cross-linking enzyme may reduce amount of salt required in meat processing (Nielsen et al., 1995).

Application of transglutaminase either alone or in combinations with some non-meat ingredients like soya, casein, konjac flour, κ-carrageenan (Kilic, 2003; Pietrasik, 2003; Colmero et al., 2005; Carballo et al., 2006) have been effective in increasing gel strengths both in pork and beef model systems and in some processed products (frankfurters, chicken kebab, chicken meat balls and others). However, none have evaluated the potential of MTG in combination with FSM as potential way to improve yield and firmness of LSLF bologna. The FSM is a by-product of oil extraction and contains a moderate amount of protein (~30%) and is high in glutamic acid (~18% of crude protein) (Eastwood, 2008; Marambe, 2011). This glutamine in FSM can serve as the substrate for the MTG-mediated cross-linking reaction.

Based on preliminary work, a level of 0.5% MTG in the formulation produced an extremely firm product and was evaluated as not pleasant to consume by panelists. Since

transglutaminase is an expensive ingredient (~CAD $200/kg) determining the lowest optimum level is beneficial in reducing production cost. Therefore the objective of this study was to determine the effect of MTG, FSM and their combination in improving water holding capacity, texture, and sensory characteristics of LSLF bologna.

4.3 Materials and methods

4.3.1 Materials

For each replication, chilled fresh lean (<7 d post-mortem) pork leg muscles (21.40% protein, 3.66% fat, 1.21% ash, and 73.7% moisture, and pH 5.71±0.01) were obtained from a commercial meat packing company through a local meat purveyor. The meat was cut into cubes and minced through a 6.5 mm hole plate (Biro Grinder, Marblehead, OH, USA, model AMFG-24). Non-meat ingredients included in the formulation are the following: sea salt (SS, Cargill Salt, Minneapolis, MN, USA), tetrapotassium pyrophosphate (TKPP 7320-34-5, Innophos, Lowbanks, ON, CA), sodium nitrite (Griffith Laboratories, Scarborough, QN, CA), sodium erythorbate (Unipack Packaging Products LTD, Edmonton, AB, CA), MTG (Ajinomoto, Itasca, IL, USA), and FSM (Bioriginal, Saskatoon, SK, CA).

4.3.2 Hydration capacity of FSM

The water hydration of FSM was measured by the method of Association of American Cereal Chemists (AACC 1999). This test is used to determine the maximum amount of water that 1 g of material can imbibe and retain under low-speed centrifugation (AACC International Method 56-30.01).

Briefly, a 5-g (as-is) FSM sample was weighed into a preweighed 50-mL centrifuge tube, added with a small amount of distilled water, and centrifuged at 2000 x *g* for 10 min at room temperature. The resulting supernatant was discarded and the content was weighed. If no supernatant appeared, more distilled water was added, and the procedure was repeated. The approximate water-hydration capacity (WHC) was calculated as:

$$\text{Approximate WHC, mL/g} = \frac{\text{(weight of tube + sediment)} - \text{(weight of tube + 5.0)}}{5}$$

The water hydration capacity was determined more accurately by repeating the above procedure except that quantity of FSM and water to be added were determined following this formula:

$$\text{Weight of FSM} = \frac{15^{¥}}{\text{Approximate WHC} + 1}$$

¥15 is desired total weight of sample and water;

Water to be added = (15 – g of FSM) ± 1.5, 0.5

The content of each tube (total of four tubes where amount of water was adjusted: +1.5, +0.5, -1.5, -0.5) was vigorously mixed with a stirring rod for 2 min and then centrifuged as before. The two tubes, one with and one without supernatant, represented the limits within which the WHC values occurred. The WHC values were presented as the mid-point between these two volumes divided by the mass of the material in grams.

4.3.2 Gel electrophoresis

Since MTG has been shown to cross-link meat protein (myosin heavy chain and actin) as reported in numerous papers (Ramirez-Suarez and Xiong, 2003; Xiong et al., 2008; Hong et al., 2012), gel electrophoresis in this study was done only to investigate if MTG can cross-link flaxseed protein. Changes in isolated flaxseed protein (sample obtained from Marambe, 2011; 86.01 % protein, 0.55 % lipid, 8.01% total dietary fiber, 5.98% moisture, 4.13% ash) after 6 and 20 h incubation with MTG were examined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) according to Laemmli (1970). Flaxseed protein (0.2%, w/w, using 0.17 mM NaCl + 50 mM phosphate buffer) and MTG enzyme (0.1%, w/w using 0.17 mM NaCl + 50 mM phosphate buffer) were mixed and incubated for 0, 6, and 20 h at room temperature. After incubation, samples were mixed with one part of a Laemmli sample buffer (25% glycerol, 8%, SDS, 10% β-mercaptoethanol and 62.5 mM Tris-HCl (pH 6.8), Bio-rad Laboratories, Inc., Hercules, CA, USA), boiled for 4 min, centrifuged at 15,000 rpm (~21,000 x *g*) for 1 min (Eppendorf 5424, Thermo Scientific, Asheville, NC, USA) to remove precipitate. Aliquots of 10 µg of protein per lane were loaded onto acrylamide gradient (4-20%) Mini-Protean® TGX™ gels (Bio-RAD, Cat #456-1099). Electrophoresis was run with a Mini-PROTEAN III mini system apparatus (Bio-Rad Laboratories, Hercules, CA, USA) under a constant voltage of 105V.

4.3.2 Sausage manufacture

Each bologna treatment was formulated to produce meat batters with 11.0% protein (in compliance with Canadian regulations for minimum protein content) and 10.0% fat. The levels of meat, backfat, NaCl, seasonings, TKPP, sodium erythorbate and sodium nitrite of all batches were held constant at 48.95%, 11.10%, 1.00%, 0.60%, 0.50%, 0.05%, and 0.0192%, respectively. The levels of FSM and MTG were varied according to treatments and water was adjusted based on the variations of these main effects (**Table 4-1**). The bologna processing procedure was similar to the previous study.

Table 4-1 Formulation (%, w/w) of bologna with different levels of MTG and FSM

INGREDIENTS	TREATMENTS											
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
Pork meat, ground	48.95	48.95	48.95	48.95	48.95	48.95	48.95	48.95	48.95	48.95	48.95	48.95
Pork fat	11.1	11.1	11.1	11.1	11.1	11.1	11.1	11.1	11.1	11.1	11.1	11.1
Added water	37.78	37.28	36.78	36.28	37.63	37.13	36.63	36.13	37.48	36.98	36.48	35.98
Sodium chloride	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Sodium nitrite	.0192	.0192	.0192	.0192	.0192	.0192	.0192	.0192	.0192	.0192	.0192	.0192
Tetrapotassium pyrophosphate	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Sodium erythorbate	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Seasonings	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60
Transglutaminase	0	0	0	0	0.15	0.15	0.15	0.15	0.30	0.30	0.30	0.30
Flax meal	0	0.5	1.0	1.5	0	0.50	1.0	1.5	0	0.5	1.0	1.5
TOTAL	100	100	100	100	100	100	100	100	100	100	100	100

4.3.3 Batter viscosity

The procedure for measuring batter viscosity is detailed in the previous chapter (3.3.4).

4.3.4 Instrumental colour

Initial colour of sliced LSLF bologna was determined using a Hunterlab Miniscan XETM (Hunter Associates Laboratory, Inc., Reston, VA, U.S.A.) using illuminant A and observer 10. The instrument was standardized using black and white tiles. The colour test was performed on day 1 of the same set sample used for purge loss. Colour measurement taken

for L* = lightness, a* = redness, and b* = yellowness through the intact packages (2 packages/treatment). The samples were then rotated 90° and readings were repeated.

4.3.5 pH of raw and cooked bologna

Refer to chapter 3 (3.3.6) for the procedure details.

4.3.6 Proximate composition of cooked bologna

Refer to chapter 3 (3.3.7) for the procedure details.

4.3.7 Sodium, potassium, and approximate salt contents of cooked bologna

Refer to chapter 3 (3.3.8) for procedure details.

4.3.8 Residual nitrite content of cooked bologna

Residual nitrite contents of cooked samples were analyzed following the procedure of Agriculture Canada (Residue Unit, 1993). This method is based on the Shinn reaction in which the anion in the presence of sulfanilamide and N-(1-napthtyl)-ethylenediamine dihydrochoride produces a colored compound by diazotization and coupling reaction and color changes is monitored at 538 nm (Oliveira et al., 2004).

Briefly, approximately 100 g of cooked bologna samples were ground on the day of analysis. Five grams of each sample was weighed into 200 mL volumetric flask, treated and mixed with 15 mL of 2.0% (w/v) NaOH, 12 mL of 12% (w/v) $ZnSO_4$, and diluted with 50 mL distilled water. The pH of the mixture was measured and maintained between pH 7– 8 with 0.1 N NaOH. The mixture was then incubated in a 50°C water bath for 30 min, cooled to room temperature, and diluted with distilled water to make up to 200 mL volume. The mixture was then filtered using Whatman #40 filter paper. Afterwards, 1 mL of the clear filtrate was transferred into a 50 mL volumetric flask, treated with 6 mL of 0.5% (w/v) sulfanilic acid. The chemical reaction was allowed to take place for 5 min and then 4 mL of 0.1% (w/v) N-(1-naphthyl)-ethylenediamine dihydrochoride solution was added to complete the color reaction. The solution was diluted with water to make up to 50 mL volume and absorbance at 538 nm was read after 15 min. The concentration of residual nitrite was determined using a sodium nitrite standard curve (2-14 ppm). A nitrite stock standard solution was prepared by drying approximately 1 g of sodium nitrite at 100^{o}C for 1 h and cooled inside a dessicator. Thereafter, 150 mg of sodium nitrite was dissolved and made to 1

L with nitrite free distilled water. Nitrite working solutions for the standard curve were then prepared.

4.3.9 Water holding capacity

Refer to chapter 3 (3.3.10) for the detailed procedure.

4.3.10 Texture profile analysis (TPA)

Refer to chapter 3 (3.3.11) for the detailed procedure.

4.3.11 Sensory evaluation

Sample preparation was as described in chapter 3 (3.3.12). During the sample evaluation, the 12 panelists were asked to score samples for surface colour (8 extremely pinkish red --- 1 extremely brown), initial firmness (8 as extremely firm---1 as extremely soft), chewiness (8 as extremely hard to chew--- 1 as extremely easy to chew), overall juiciness (8 as extremely juicy ----1 as extremely dry), saltiness (6 as extremely salty ---- 1 as not detectable), spice flavour intensity (8 as extremely intense --- 1 extremely weak), and foreign flavour (8 as no foreign flavour --- 1 extremely intense) (**Figure 4-1**). The sensory data are means of 12 panelists x 3 replications.

4.3.12 Statistical analysis

This study was repeated three times. Observed data were analyzed as a Randomized Complete Block Design using the Proc Mixed Procedure of SAS (SAS, Inst. Inc., 1989). Variations contributed by meat materials used in each replicate were considered as a block. Treatments and interactions were written in the model and considered as fixed effects and block as random effect. Means were analyzed and separated with the least significant difference (LSD) procedure of SAS and pdmix SAS macro was used to convert mean separation output to letter groupings (Saxton, 1998). Significance was declared at $p<0.05$.

Name:_____

Date: February 15, 2012

Sample No.: **329**

Instruction: Please evaluate the samples in the order that the scorecards are arranged. For each characteristic, **circle** the descriptor that best describes your impression. Feel free to provide any comments as well. Please take a drink of water before beginning and between samples. Unsalted crackers are also available as needed.

DESCRIPTORS	**SCORE**							
	8	**7**	**6**	**5**	**4**	**3**	**2**	**1**
Surface appearance: Colour	Extremely pinkish red	Very pinkish red	Moderately pinkish red	Slightly pinkish red	Slightly brown	Moderately brown	Very brown	Extremely brown
Texture: Initial firmness	Extremely firm	Very firm	Moderately firm	Slightly firm	Slightly soft	Moderately soft	Very soft	Extremely soft
Chewiness	Extremely hard to chew	Very hard to chew	Moderately hard to chew	Slightly hard to chew	Slightly easy to chew	Moderately easy to chew	Very easy to chew	Extremely easy to chew
Overall juiciness	Extremely juicy	Very juicy	Moderately juicy	Slightly juicy	Slightly dry	Moderately dry	Very dry	Extremely dry
Flavour: Saltiness	[redacted]		Extremely salty	Very salty	Moderately salty	Slightly salty	Very slightly salty	Not detectable
Spice flavour intensity	Extremely intense	Very intense	Moderately intense	Slightly intense	Slightly weak	Moderately weak	Very weak	Extremely weak
Foreign flavour	No foreign flavor	Very weak	Moderately weak	Slightly weak	Slightly intense	Moderately intense	Very intense	Extremely intense
Overall acceptability	Extremely acceptable	Very acceptable	Moderately acceptable	Slightly acceptable	Slightly unacceptable	Moderately unacceptable	Very unacceptable	Extremely unacceptable

Comments: _____

Thank you very much!!!

Figure 4-1 Score card for bologna (study 2)

4.4 Results and Discussion

4.4.1 Characteristics of FSM

The composition of FSM used in the study is shown in **Tables 4-2** and **4-3**. The presence of sodium in FSM can potentially affect the sodium content of cooked product if it is used at a higher level. However, in this study the highest level of FSM in the formulation was 1.5% and thus it is expected to be an insignificant contributor of sodium (~1.2 mg/100 g) to the final product.

Table 4-2 Proximate composition and hydration capacity of FSM

Proximate composition	Value
Moisture, %	5.57±0.15
Fat, %	7.77 ±0.25
Protein, %	32.71 ±0.11
Ash, %	5.51±0.16
pH	5.72±0.03
Hydration capacity (mL/g)	6.17± 0.08

Table 4-3 Additional information on FSM*

Carbohydrate (%)	42.0
Dietary fiber (%)	41.0
Soluble (%)	23.0
Insoluble (%)	18.0
Sugars	1.0
Minerals (mg/g)	
Sodium	0.83
Calcium	3.63
Iron	0.07
Potassium	10.0
Phosphorus	7.44
Magnesium	4.91
Copper	0.03
Zinc	0.07
Vitamins (µg/g)	
Thiamine	10.00
Riboflavin	5.00
Niacin	9.00
Lignans (%)	2.00

* information provided by BioOriginal, Saskatoon (source of FSM)

One of the most important components of FSM which influenced batter viscosity and water binding properties of cooked bologna is fiber (both soluble (gum/mucilage) and insoluble polysaccharides). Although fiber is beneficial to human health, bologna formulations in the present study cannot be declared as a fiber source due to its limited

amount in the formulation. The Canadian Food Inspection Agency requires that food must contain at least 2 g per serving to be declared as a source of fiber (CFIA, 2012b). To meet this regulation, FSM must be added in a processed meat product at ~8.0% level. The level of FSM usage in this study is limited to its technological purpose only (water and fat retention) owing to its fiber content (41.0%).

One of the most important components of FSM which influenced batter viscosity and water binding properties of cooked bologna is fiber (both soluble (gum/mucilage) and insoluble polysaccharides). Although fiber is beneficial to human health, bologna formulations in the present study cannot be declared as a fiber source due to its limited amount in the formulation. The Canadian Food Inspection Agency requires that food must contain at least 2 g per serving to be declared as source of fiber (CFIA, 2012b). To meet this regulation, FSM must be added in a processed meat product at ~8.0% level. The level of FSM usage in this study is limited to its technological purpose only (water and fat retention) owing to its fiber content (41.0%).

The FSM water hydration/binding capacity or the amount of water that remains bound to the hydrated fibers following application of external force (centrifugation in this study) was 6.15 mL/g (**Table 4-2**). This value was within the range of flaxseed meal hydration capacity as reported by Wanasundara and Shadidi (1994), both in laboratory prepared meal (5.2-6.5) and commercial meal (6.1-6.3) samples. The hydration capacity of FSM was much higher than that of other oilseed meals (canola meal=2.1 and soybean meal=1.8) (Bhatty and Cherdkiatgumchai, 1990). This may be due to the presence of mucilage in FSM and also to the existing differences in the conformational characteristics of its proteins (Wanasundara and Shadidi, 1994).

Flaxseed is also known to be a good source of protein. After oil extraction, protein content becomes concentrated in FSM. The FSM material used in this study was contained 32.71% protein. According to Marambe (2011) protein content of flaxseed meal can be as high as 35-40%. Plant genetics, environmental factors, and processing can influence composition of flaxseed and by-products (Oomah and Mazza, 1993; Oomah, 2001). Glutamic acid is the main amino acid in flax protein meal ranging from 18.27% - 26.4% of crude protein while lysine ranges from 2.12% - 3.85% (Bhatty and Cherdkiatgumchai, 1990; Eastwood, 2008; Marambe, 2011). Glutamine and lysine are thus available to serve as the substrates for MTG cross-linking reaction.

4.4.2 MTG and FSM cross-link reaction

Shown in **Figure 4-2** is the SDS-PAGE profile of flaxseed protein incubated with MTG for various time. There was a consistent reduction of the lower molecular weight band intensity of flax protein after 4 and 20 h incubation time with MTG and simultaneous formation of large molecular size protein bands. This preliminary data shows that flaxseed protein can be a good substrate for MTG cross-linking reaction. Several studies have also shown that some non-meat proteins such as sodium caseinate (Kuraishi et al., 1997; Kilic, 2003; Pietrasik et al., 2007), blood plasma protein (Pietrasik et al., 2007), soybean proteins and whey protein isolate (Murugama et al., 2003) were good substrates for MTG mediated cross-linking. A prerequisite for the cross-linking with TG is sufficient exposure of the lysines and glutamines in protein (De Jong and Koppelman, 2002). For example, casein and gelatin are known to have readily available glutamine and lysine and thus can be easily cross-linked by bacterial TG (De Jong and Koppelman, 2002).

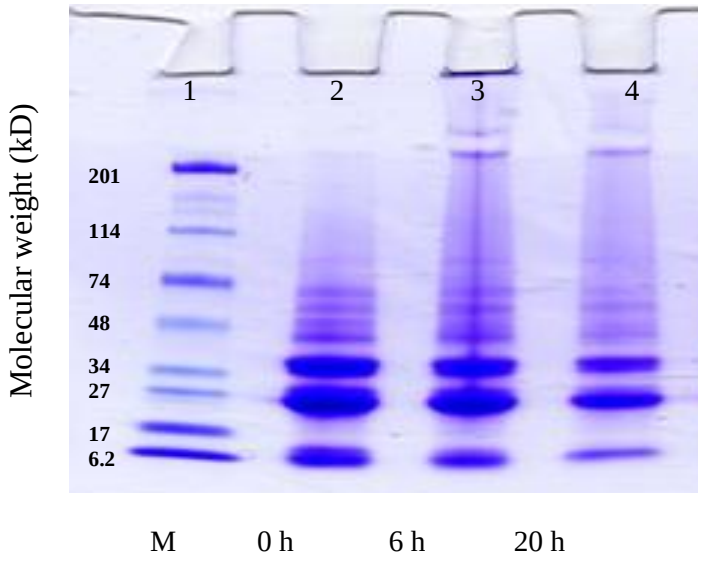

Figure 4-2 SDS-PAGE profile of flax protein incubated with MTG. Lane 1- molecular weight marker, lane 2- MTG + Flax (0 h), lane 3- MTG + Flax (6 h), lane 4- MTG + Flax (20 h)

Although lysine accounts for only a small proportion in FSM protein (2.12%), this SDS-PAGE profile showed a clear cross-linking reaction after a few hours of incubation with MTG. There is another mechanism of MTG-mediated protein modification in proteins containing high glutamine and low lysine levels (e.g., in wheat gluten, ~70% GLU and 2% LYS) through the process of deamidation (Ohtsuka et al., 2001). In this chemical process, the water reacts as nucleophile resulting in deamidation of glutamines which may lead to changes

in protein functionalities (i.e., enhanced protein solubility, emulsification, foaming, and gelation properties) (Ohtsuka et al., 2001). This deamidation process in addition to cross-linking can contribute to the protein modification in flaxseed protein treated with MTG.

4.4.3 Batter viscosity

The temperature of the batter during processing was monitored and all the treatments were of similar temperature after passing through the emulsion mill (11-12.5^{o}C). During preliminary trials, it was observed that there was an increase in emulsion temperature after the 2nd or 3rd batch of processing regardless of the treatments. This rise in temperature was contributed by equipment (bowl chopper and emulsion mill) and thereby use of ice cold water for rinsing from one batch to the next batch was practiced to cool down the processing machines.

Both MTG and FSM levels affected the viscosity of the raw batter (p<0.05) but no interaction effect was observed (**Figures 4-3 and 4-4; Appendix B-1**). The addition of MTG increased the apparent viscosity of meat emulsion. This observation agrees with previous reports by Beriain (2009) indicating polymerization of proteins has occurred. Furthermore, it was reported that change in apparent viscosity of meat emulsion was noted 2 h post-processing (Beriain, 2009). MTG is active over a wide range of temperatures. Its optimal activity is at 50°C, inactivated at 70°C, and retains some activity at temperatures above the freezing point (Motoki and Suguro, 1998). Thus, in the present study, change of batter viscosity was observed even before cooking since MTG mediated cross-linking the moment it was added into the meat.

An increase in viscosity contributed by FSM addition was as expected and can be explained by the presence of mucilage in flax. Flaxseed mucilage accounts for 8% of the seed (Mazza and Biliaderis, 1989; Warrand et al., 2005) and is even higher (23%) in defatted flaxseed meal. Polysaccharide gums have many applications in foods including viscosity enhancement (Mazza and Biliaderis, 1989) and emulsion stabilization (BeMiller, 1973). In sausage manufacture, ease of handling during stuffing of raw batter is one of the benefits of having higher viscosity but the direct effect of raw batter viscosity on cooked sausage quality is not known yet (Shand, 2000).

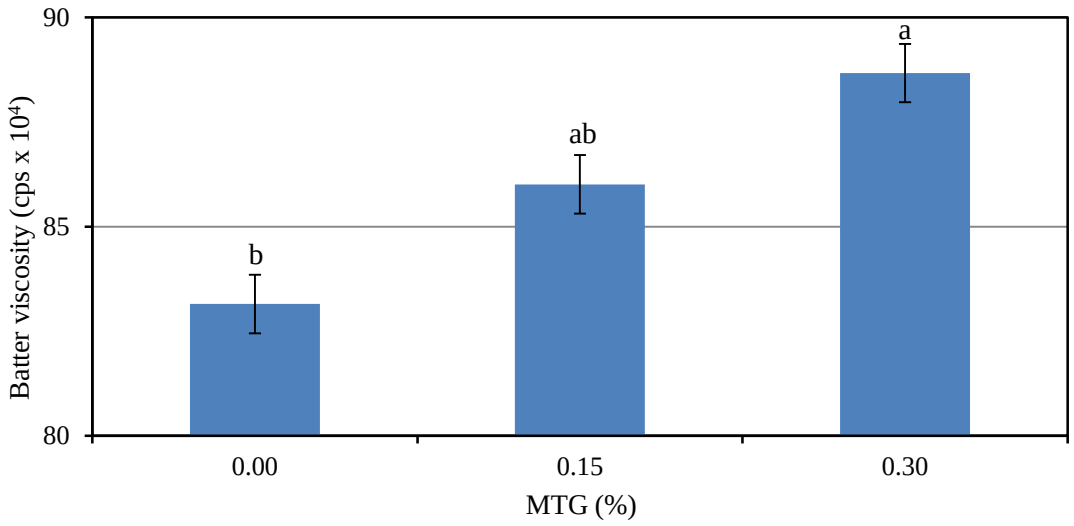

Figure 4-3 Effects of MTG level on batter viscosity. [a-b] Means with different letters are significantly different (p<0.05).

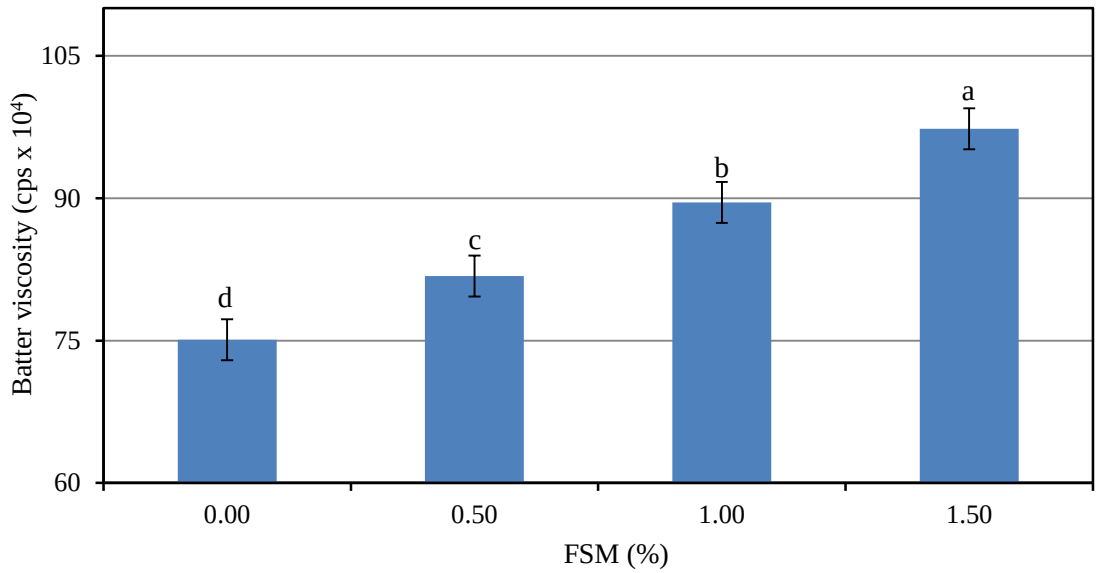

Figure 4-4 Effects of FSM level on batter viscosity. [a-d] Means with different letters are significantly different (p<0.05)

4.4.4 pH of raw batter and cooked bologna samples

Result showed that pH of the raw batter was neither affected by main effects (MTG and FSM) nor interaction factors (**Table 4-4**).

Table 4-4 Effects of MTG and FSM on the pH of raw batter and cooked bologna [1]

MTG (%)	FSM (%)	Raw batter[ns]	Cooked bologna
0.0	0.0	6.47±0.06	6.53±0.06[a]
	0.5	6.40±0.10	6.53±0.06[a]
	1.0	6.40±0.10	6.50±0.00[a]
	1.5	6.43±0.06	6.50±0.12[a]
0.15	0.0	6.40±0.10	6.47±0.10[b]
	0.5	6.43±0.12	6.47±0.06[b]
	1.0	6.37±0.06	6.43±0.06[b]
	1.5	6.40±0.00	6.47±0.12[b]
0.30	0.0	6.40±0.10	6.47±0.12[b]
	0.5	6.37±0.06	6.43±0.06[b]
	1.0	6.40±0.00	6.43±0.06[b]
	1.5	6.40±0.06	6.43±0.06[b]

[ns] not significant (p>0.05); MTG - microbial transglutaminase; FSM-flaxseed meal
[a-b]Means with different letters in the same column are significantly different (p<0.05)
[1]Values are means of three replicates ± standard deviation

However, similar to the previous study (Chapter 3) the trend shows that there was a consistent increase in pH after cooking regardless of the treatments (**Table 4-4**). This increase in pH was also reported in many papers (Xiong et al., 1999; Morin et al., 2002; Choi et al., 2007) and it can be hypothesized to be due to exposure (as a result of protein denaturation during cooking) of amino acids with basic side chains (lysine, arginine, and histidine). Interestingly, when pH of cooked samples was compared, addition of MTG caused a significant reduction in pH of cooked bologna (**Table 4-4**, **Figure 4-5**). This could be related to participation of lysine (one of the amino acids with basic side chains) in MTG mediated cross-linking reactions. The formation of a covalent bond between lysine and glutamine made the lysine basic side chain unavailable to contribute to a change in pH of cooked meat batter.

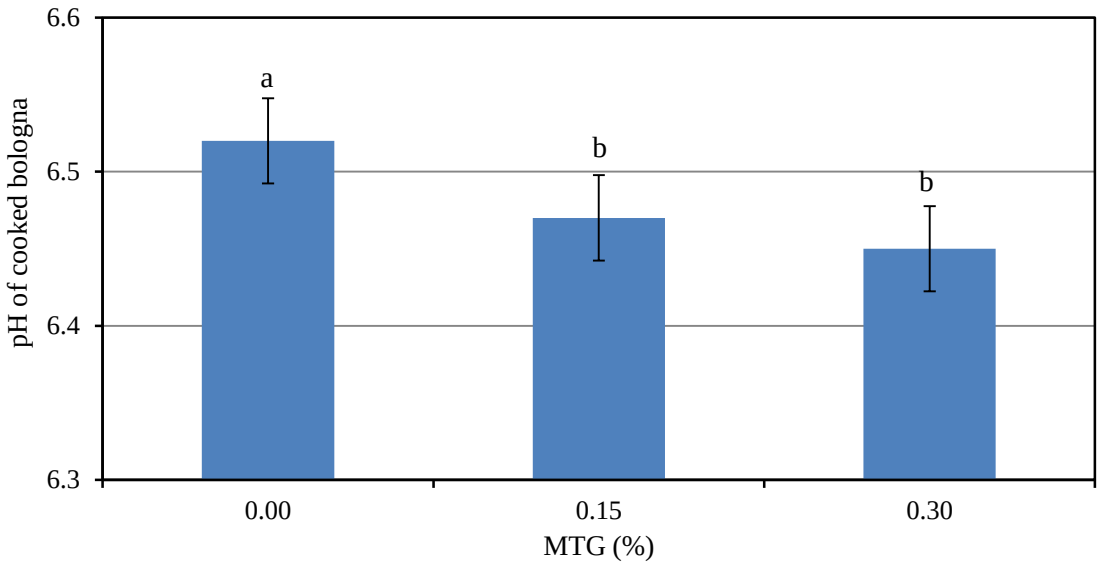

Figure 4-5 Effects of MTG level on the pH of cooked bologna. [a-b] Means with different letters are significantly different (p<0.05)

4.4.5 Proximate composition of cooked bologna

Shown in **Table 4-5** is the proximate composition of all samples. The protein level in the cooked products ranged from 10.97% (in samples with no MTG and no FSM) to 11.60% (in samples with 0.15% MTG and 1.50% FSM).

Table 4-5 Effects of MTG and FSM on the proximate composition (%) of cooked bologna[1,2]

MTG	FSM	Protein	Moisture[ns]	Fat[ns]	Ash
0.0	0.0	10.97±0.15[b]	76.23±0.47	10.40±0.26	2.00±0.01[b]
	0.5	11.13±0.23[b]	75.77±0.25	10.53±0.72	2.03±0.06[b]
	1.0	11.07±0.11[b]	75.40±0.10	10.73±0.25	2.13±0.06[a]
	1.5	11.03±0.57[b]	74.97±0.15	9.73±1.42	2.10±0.10[a]
0.15	0.0	11.03±0.40[ab]	76.67±1.04	9.70±2.58	2.03±0.06[b]
	0.5	11.27±0.29[ab]	77.07±1.46	9.93±1.87	2.00±0.01[b]
	1.0	11.23±0.50[ab]	76.13±1.13	9.63±1.93	2.17±0.06[a]
	1.5	11.60±0.17[ab]	74.60±0.35	9.60±1.73	2.17±0.06[a]
0.30	0.0	11.33±0.30[a]	75.30±3.83	9.70±3.00	2.07±0.06[b]
	0.5	11.23±0.15[a]	75.83±1.10	9.50±1.58	2.07±0.01[b]
	1.0	11.57±0.57[a]	75.60±0.96	9.70±1.25	2.10±0.10[a]
	1.5	11.43±0.42[a]	75.13±0.74	10.5±0.62	2.10±0.01[a]

[ns] not significant (p>0.05); MTG- microbial transglutaminase; FSM-flaxseed meal
[a-b]Means with different superscripts within each column are significantly different (p < 0.05)
[1]Values are means of three replicates ± standard deviation
[2]Protein was calculated as total nitrogen x 6.25

In all samples, meat protein level was held constant (11.0%). Interestingly, the addition of FSM did not affect the protein level of cooked product (p >0.05) but the addition of MTG as low as 0.30% affected the protein content of the cooked samples (p< 0.05) (**Figure 4-6**). It was expected that addition of 1.5% FSM would add 0.45% protein to the product while MTG contributed a maximum of 0.3% protein. The significantly higher protein level of treatments with MTG and no effect in treatments with FSM could be related to the amount of cook loss (will be discussed under water holding capacity). Those treatments with MTG had the tendency (p=0.0567) to have higher cook loss thus affecting (concentrating) the protein content of the cooked product.

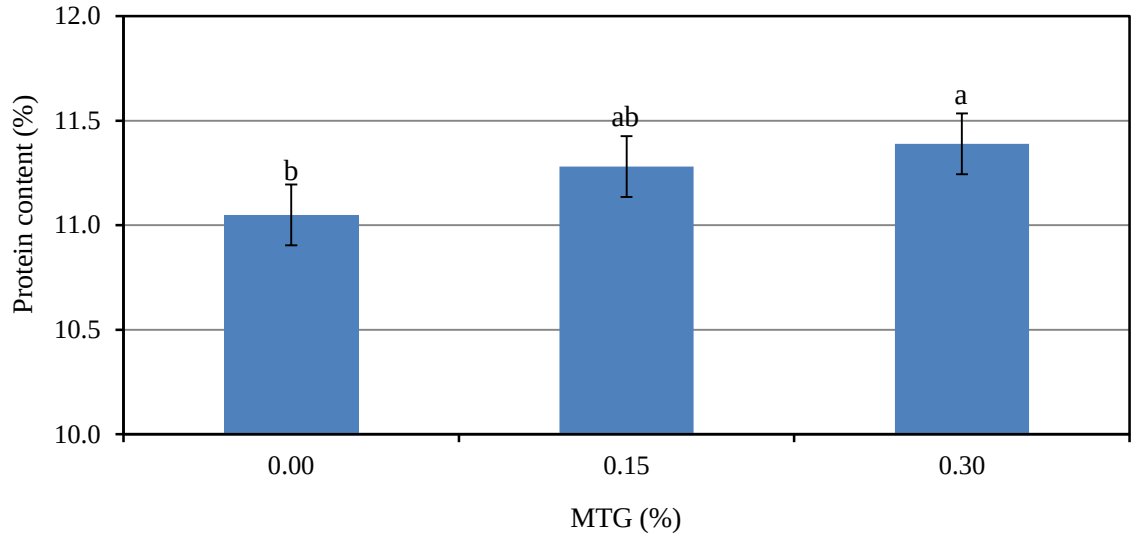

Figure 4-6 Effects of MTG level on the protein content of cooked bologna.
[a-b] Means with different letters are significantly different (p<0.05)

The ash levels were higher in samples added with 1.0% or 1.5% FSM. Minerals present in FSM (e.g., magnesium, calcium, phosphorus, iron, and others (list in **Table 4-3**) is the contributory factor to higher ash of samples with FSM.

4.4.6 Sodium and potassium composition of cooked bologna

Results showed that the sodium and potassium content of cooked bologna were not affected by treatment factors (**Table 4-6**). The amount of sodium in FSM was 0.83 mg/g and expected not to affect the total sodium content of the product. For example, the highest level of FSM in this study was 1.5% by computation; this theoretically contributed 1.2 mg Na^+/100 g bologna. Nonetheless, the cooked bologna in this study had lower amount of sodium (430-

470 mg sodium/100 g) than the target Health Check criteria established by the Heart and Stroke Foundation at 654 mg sodium/100 g (Health Canada, 2012).

Table 4-6 Effects of MTG and FSM on the sodium and potassium contents of bologna[1]

MTG	FSM	Sodium (mg/g)	Potassium (mg/g)
0.0	0.0	4.51±0.06	3.60±0.06
	0.5	4.71±0.10	3.81±0.06
	1.0	4.52±0.10	3.58±0.00
	1.5	4.48±0.06	3.61±0.12
0.15	0.0	4.46±0.10	3.43±0.10
	0.5	4.63±0.12	3.40±0.06
	1.0	4.46±0.06	3.82±0.06
	1.5	4.56±0.00	3.68±0.12
0.30	0.0	4.62±0.10	3.70±0.12
	0.5	4.37±0.06	3.60±0.06
	1.0	4.46±0.00	3.56±0.06
	1.5	4.60±0.06	3.72±0.06

[1]Values are means of three replicates ± standard deviation
[ns]not significant (p>0.05); MTG- microbial transglutaminase; FSM-flaxseed meal

Bologna in this study was lower in potassium content than those commercially available "enhanced" fresh meat cuts as reported by Sherman and Mehta (2009) where potassium content of 25 enhanced or additive added samples ranged from 692 mg K/100 g to 930 mg K/100 g. High intake of potassium can be beneficial or bad for human health. Patients who are undergoing dialysis are advised to limit their potassium intake. A dialysis patient who eats a 200-g portion of "enhanced" meats (containing around 2 g potassium) would be at increased risk for the development of hyperkalemia (Sherman and Mehta, 2009). On the other hand, it has been reported that potassium intake may play a protective role in preventing the development of essential hypertension and/or ameliorating when hypertension is established (MacGregor, 1983; Treasure and Ploth 1983; Adrogue and Madias, 2007). The protective effect of an increase in potassium intake on sodium-induced hypertension could be explained by several proposed mechanisms: by natriuretic nature of potassium (inhibit the tubular reabsorption of sodium ions from glomerular filtrate, thereby resulting in greater amounts of that ion in the urine) and by relaxing vascular smooth muscle and reducing peripheral vascular resistance directly (MacGregor, 1983; Treasure and Ploth, 1983).

Although 0.5% tetrapotassium pyrophosphate was added, the bologna in this study had a potassium content comparable to that found in non-enhanced fresh meat cuts (<387 mg/100g) as reported by Sherman and Mehta (2009). The 0.5% TKPP added in the bologna was expected to add 73.5 mg K/100 g to the cooked product. However since the raw material (ground pork leg muscle) in the formulation was only ~49.0%, the finished cooked product resulted in a lower potassium content as compared to those additive added fresh meats and comparable to non-enhanced fresh meats. Therefore, the addition of 0.5% TKPP in bologna will not be a problem in terms of causing hyperkalemia in a certain group of the population. In terms of phosphate, this 0.5% TKPP is 0.07% below the maximum phosphate (0.5% as sodium phosphate dibasic) that can be added in processed meats allowed by the Canadian Food Inspection Agency considering the conversion factor.

4.4.7 Water holding capacity

Three parameters (cook loss, expressible moisture, and purge loss) were used to determine the effect of FSM and MTG on water holding properties of LSLF bologna. These measure WHC after application of three forms of force to the meat matrix, namely, heat for cook loss, centrifugal force for expressible moisture, and gravitational for purge loss. Purge loss or storage loss is an important quality characteristic of sliced bologna not only in terms of weight loss but also product appearance during display. The overall effect of treatments on the WHC is shown in **Table 4-7** (see also **Appendix B-2)**.

4.4.7.1. Cook loss

The addition of FSM did not affect cook loss while MTG tended to increase cook loss (p=0.057) (**Figure 4-7**). There was no interaction effect observed. The effect of MTG on cook loss based on previous works and in this study was inconsistent. For example, Kilic (2003) reported that the addition of 1.0% MTG had no effect on cook yield in chicken kebab while Tseng et al. (2000) reported a lower cook loss in sample with 0.5% - 1.00% crude pig plasma transglutaminase in low-salt chicken meat balls. Our observation of the tendency of MTG to increase cook loss is in agreement with Beriain (2009) who found that cook loss was higher in low salt meat emulsion with 0.3% MTG and Hong et al. (2012) that the addition of MTG reduced the cooking yields of the myofibrillar emulsion gel.

Table 4-7 Effects of MTG and FSM on the WHC of bologna[1]

MTG (%)	FSM (%)	Cook loss (%)	Expressible moisture (%)	Purge loss* (%)
0.0	0.0	0.49±0.10	21.0±0.3^{a}	4.9±0.3^{bz}
	0.5	0.39±0.01	18.5±1.5^{b}	4.1±0.6^{by}
	1.0	0.34±0.01	17.5±1.8^{b}	2.8±0.3^{bx}
	1.5	0.41±0.01	14.6±3.0^{c}	2.8±0.3^{bx}
0.15	0.0	0.37±0.10	21.1±1.9^{a}	6.1±0.8^{az}
	0.5	0.43±0.12	18.8±2.6^{b}	4.8±0.3^{ay}
	1.0	0.49±0.10	16.4±1.5^{b}	4.1±0.2^{ax}
	1.5	0.51±0.10	14.5±0.9^{c}	3.5±0.2^{ax}
0.30	0.0	0.39±0.10	19.8±1.0^{a}	5.9±0.5^{az}
	0.5	0.56±0.06	17.4±2.6^{b}	5.1±0.2^{ay}
	1.0	0.65±0.05	15.8±1.4^{b}	4.4±0.8^{ax}
	1.5	0.66±0.03	14.5±1.1^{c}	3.9±0.4^{ax}

^{ns} not significant (p>0.05); MTG- microbial transglutaminase; FSM-flaxseed meal
$^{a-b}$means with different letters in the same column are significantly different (p<0.05)
* $^{a-b}$means with different letters in the same column are significantly different (p<0.05) as affected by MTG and $^{x-z}$ as affected by FSM
[1]Values are means of three replicates ± standard deviation

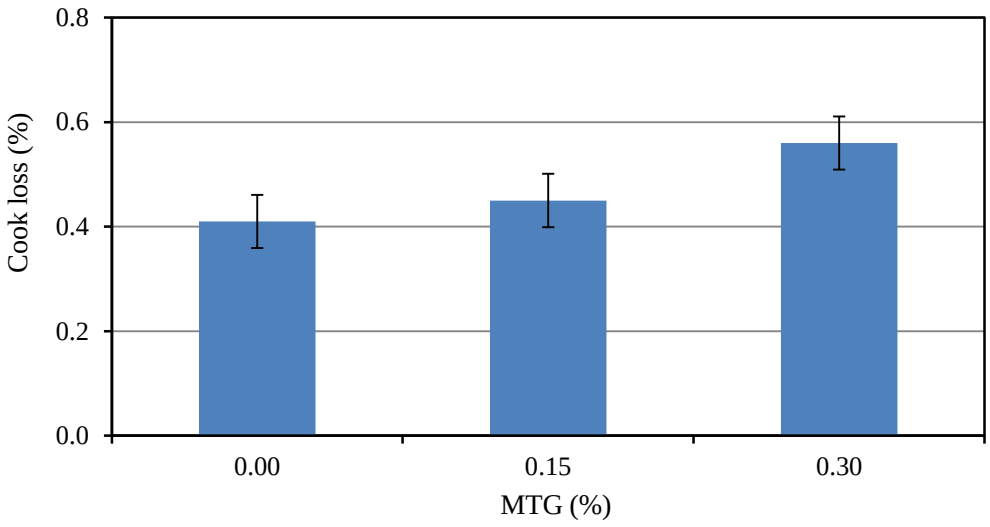

Figure 4-7 Effects of MTG level on cook loss (p=0.057).

During measurement of cook loss, it was observed that samples with FSM had a small amount of viscous liquid on both ends of bologna chub. This viscous liquid was formed due to the presence of flaxseed mucilage/gum. In general, mucilage/gums has good water holding capability and water binding ability (Fedeniuk and Biliaderis, 1994) but forms a weak gel and

therefore extra free viscous fluid (not trapped or participating in the emulsion matrix system) appeared as viscous exudate in cooked bologna. Furthermore, when the casing was removed, the bologna surface of treatments with 1.5% FSM was slimy/slippery and caused some handling difficulty (tended to slide from hands especially during slicing). The formation of this viscous liquid after cooking and the imparted sliminess may limit the application of FSM in meat processing.

4.4.7.2 Expressible moisture

The addition of MTG had no effect (p>0.05) while addition of FSM resulted in reduction of moisture loss during application of centrifugal force (p<0.05) (**Figure 4-8**). There was no interaction effect. Our observation was not in agreement with some of the published papers. Pietrasik (2003) reported that addition of 0.5% MTG significantly decreased EM.

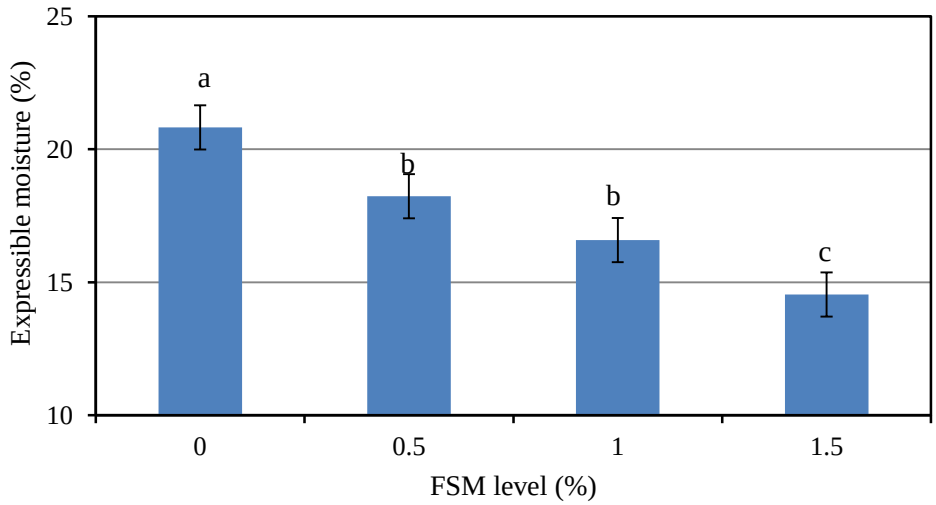

Figure 4-8 Effects of FSM level on expressible moisture. [a-c] Means different letters are significantly different (p<0.05)

Similar to other fillers and binders, addition of FSM reduced expressible moisture and purge loss during simulated retail display and this can be explained by the presence of insoluble polysaccharides in flax seed meal. In dietary fibers, water binding improvement is more of a function of insoluble polysaccharides than soluble polysaccharides (Thebaudin et al., 1997). Water can bind to this polysaccharide by either surface tension in the pore matrix (capillary effect) and by hydrogen bonds, ionic bonds, and/or hydrophobic interactions (Thebaudin et al., 1997).

4.4.7.3. Purge loss

Purge loss of bologna samples was higher (p<0.05) in treatments with MTG (**Figure 4-9**) while the addition of FSM resulted in reduced (p<0.05) purge loss (**Figure 4-10**). There was no interaction effect (p>0.05).

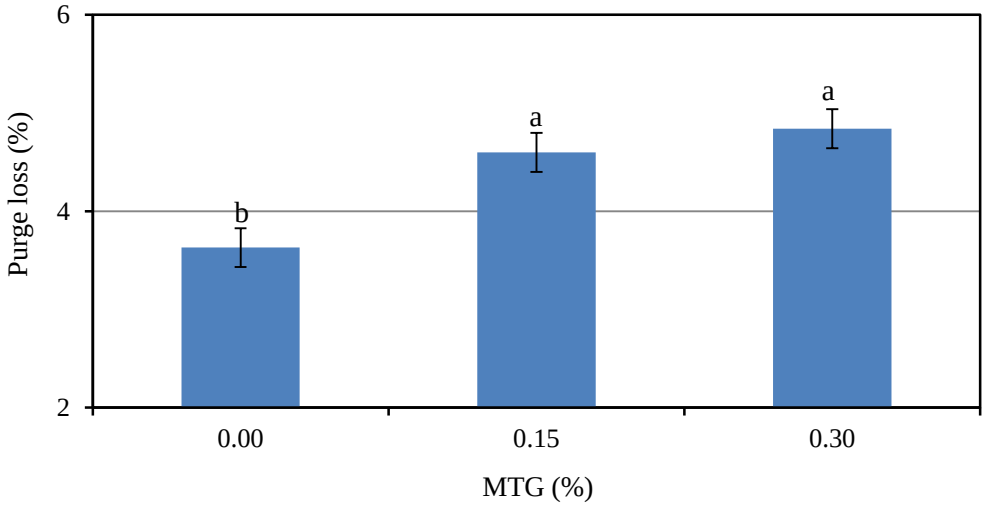

Figure 4-9 Effects of MTG level on purge loss. [a-b] Means with different letters are significantly different (p<0.05)

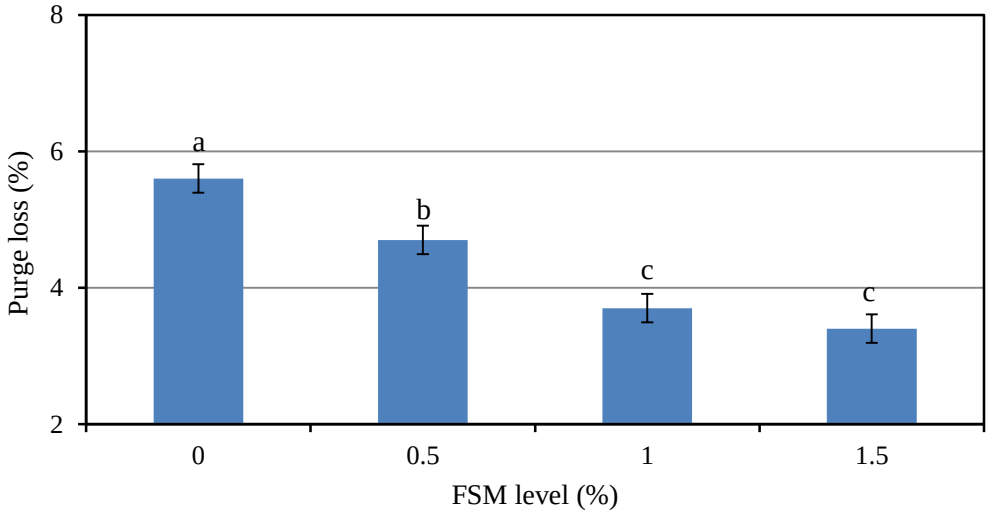

Figure 4-10 Effects of FSM level on purge loss. [a-c] Means with different letters are significantly different (p<0.05)

The negative effect of MTG addition on purge loss could be due to very strong protein-protein interactions and decreased protein-water interactions (Ramirez et al., 2002; Dondero et al., 2006) such that water was not tightly held in the matrix and therefore released during storage as purge. Addition of FSM significantly reduced this purge loss. Our

observation support the suggestion of Cofrades et al. (2011) that other means need to be used along with MTG to promote the protein-water interactions required for adequate water holding in fresh and cooked products.

4.4.8 Instrumental colour

The colour of cooked meat products are affected by many factors such as usage level of some spices with strong colour (cayenne, paprika, turmeric, saffron, mace, and others), or pigment of meat (chicken vs pork vs beef), or level of protein (myoglobin content) (Martin and Rogers, 1991), or variations of pigment conversion rates occurring on cooking (Pietrasik, 2003), or fat (as fat content is lowered, the products becomes darker or redder) (Barbut and Mittal, 1996; Pietrasik and Janz, 2008), added water, and other non-meat ingredients (Pietrasik and Janz, 2008; Shand, 2000).

In this experiment, level of spices/seasonings and protein level were held constant and therefore any change in colour can be explained by either MTG or FSM addition (see **Appendix B-3** for the tests of fixed effects). Similar to other works (Nielsen et al., 1995; Tseng et al., 2000). MTG addition did not affect L* or lightness of cooked samples (**Figure 4-11**). However, as low as 0.5% addition of FSM resulted in reduction of lightness of the bologna colour. This effect on product lightness was likely due to the light brown color of FSM. There was no MTG by FSM interaction effect on L* of bologna.

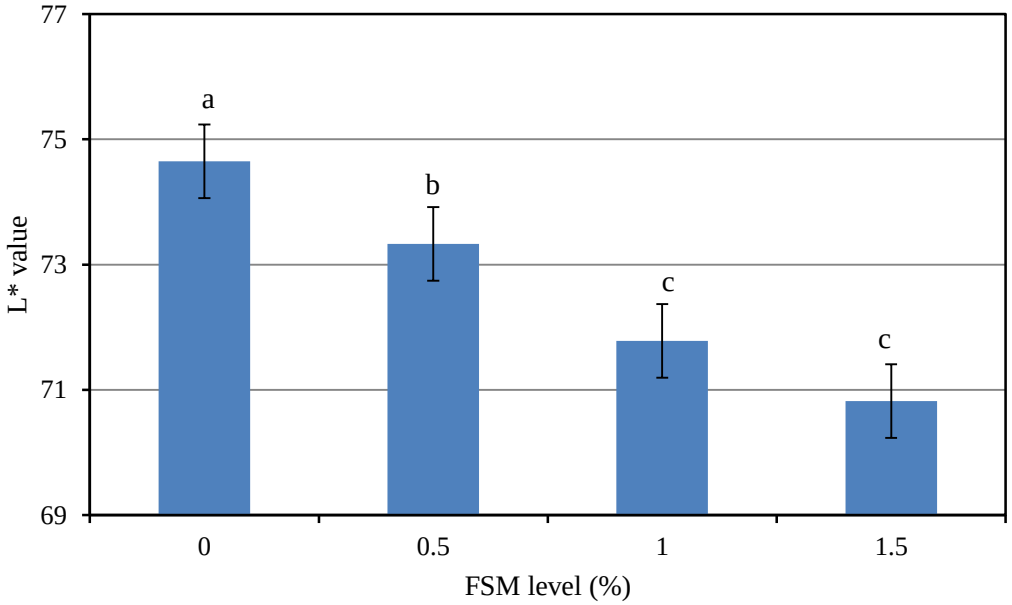

Figure 4-11 Effects of FSM level on L* (lightness) of bologna. [a-c] Means with different letters are significantly different (p<0.05)

A similar trend was observed for a* (redness) values of bologna, MTG had no effect but FSM addition resulted in significant reduction (p<0.05) in redness (**Figure 4-12**).

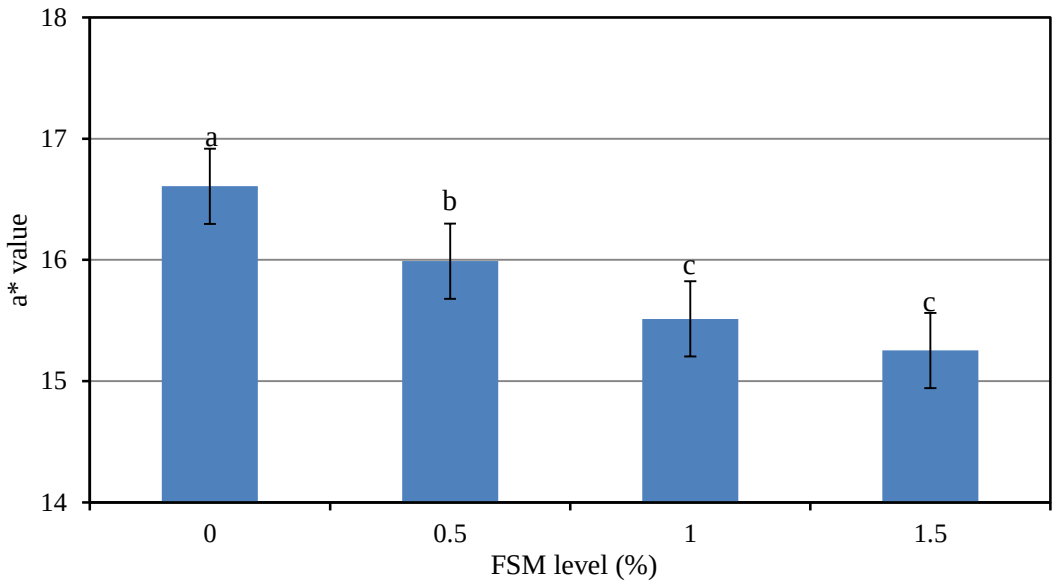

Figure 4-12 Effects of FSM level on a* (redness) of bologna. [a-c] Means with different letters are significantly different (p<0.05)

The b* (yellowness) were higher (p<0.05) in samples with FSM (**Figure 4-13**) and MTG had no effect. There was no interaction effect.

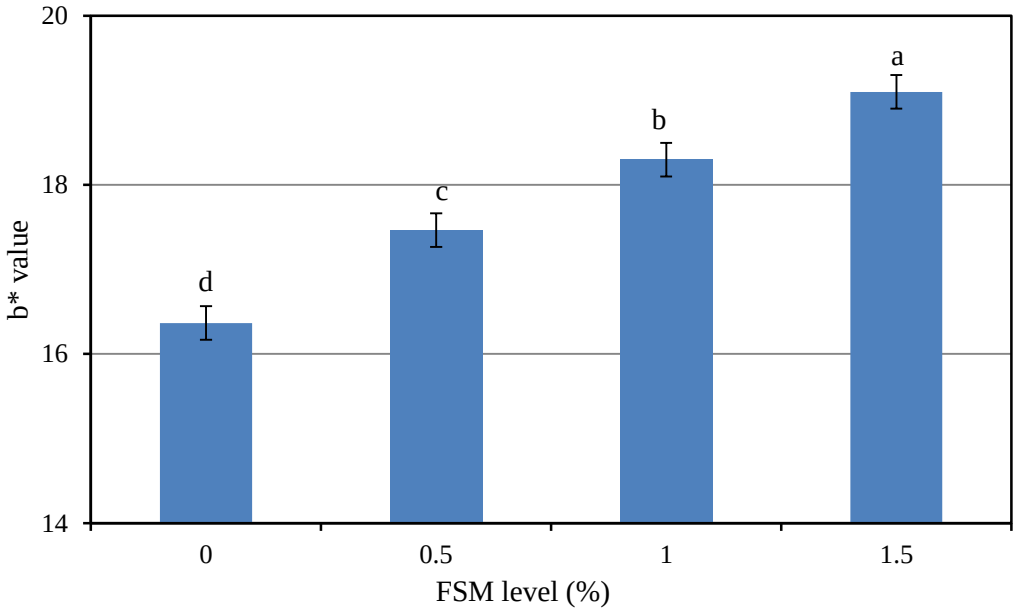

Figure 4-13 Effects of FSM level on b* (yellowness) of bologna.
[a-d] Means with different letters are significantly different (p<0.05)

In general, MTG did not affect sample colour and this observation agrees with several published reports (Nielsen et al., 1995; Tseng et al., 2000). The addition of FSM, on the other hand, a level as low as 0.5% in the formulation had a big impact on colour. This change in colour due to addition of FSM was also observed in bakery products (muffins and breads) and attributed to the natural pigment of FSM. Minor effect of some fillers or binders on the colour of emulsified sausages had been reported. For example, Shand (2000) reported that bologna added with soy protein concentrate and wheat flour resulted to lighter colour. Wheat fiber affected yellowness of beef burgers (Mansour and Khalil, 1999). Dzudie et al. (2002) found a similar pattern of colour changes as in this study; addition of bean flour to beef sausages resulted in lighter colour, reduced redness and more yellowness than those samples without bean flour.

In product development, any negative impact of non-meat ingredients can be manipulated/masked using some processing/ingredients adjustments, for example by increasing the amount of certain ingredients like paprika or modification of the proportion of meat block. However, this manipulation can be justified only if the non-meat ingredient to be added has more beneficial effects on the quality, and its effect on colour is just a slight drawback.

4.4.9 Residual nitrite

In 2007, the World Cancer Research Fund published the 10 guidelines for healthy nutrition. One of its recommendations is to "limit intake of red meat and avoid processed meat" in order to reduce the risk of developing colon cancer (WRCF, 2007; Demeyer et al., 2008). Numerous papers explained the potential mechanisms of association of consumption of meat and processed meat with colon cancer risk (Demeyer et al., 2008; Fergusson, 2010; Corpet 2011) wherein one of the pathways explains that the presence of residual nitrite in processed meat which eventually (in the intestine and stomach) is converted to DNA damaging N-nitrosocompounds (Corpet, 2010). If it is true, then, a reduction in the residual nitrite in processed products will have a positive health impact. In this study, presence of both MTG and FSM ingredients resulted in a reduction ($p<0.05$) in the residual nitrite content of the bologna (**Figures 4-14** and **4-15; Appendix B-4**). There was no interaction effect ($p<0.05$).

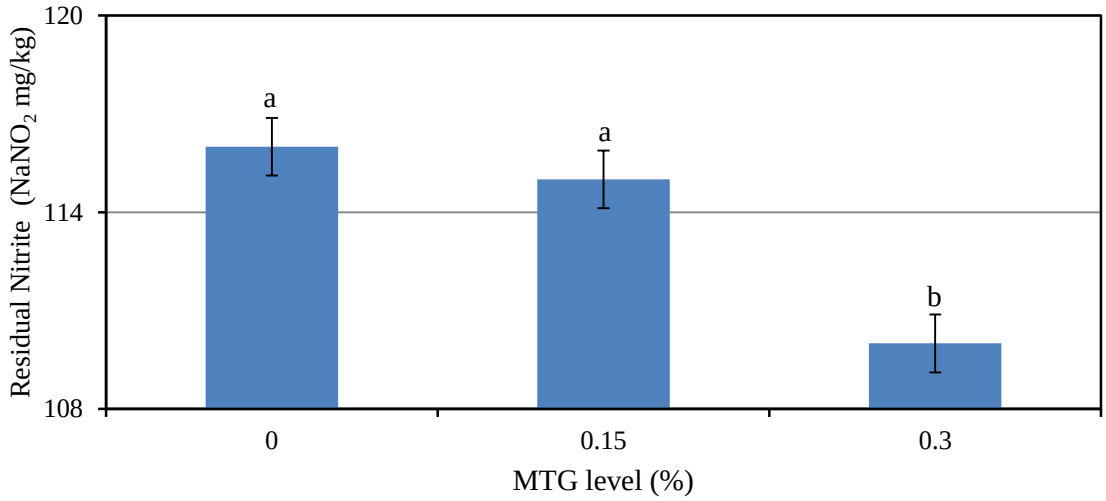

Figure 4-14 Effects of MTG level on the residual nitrite of bologna. [a-b] Means different letters are significantly different (p<0.05)

How MTG and flaxseed meal contributed to the lowering of the residual nitrite is unknown but could be related to the reducing sugar (maltodextrin) as a carrier in the MTG product. The oxidation of free aldehydes in maltodextrin could have contributed to the reduction of nitrite.

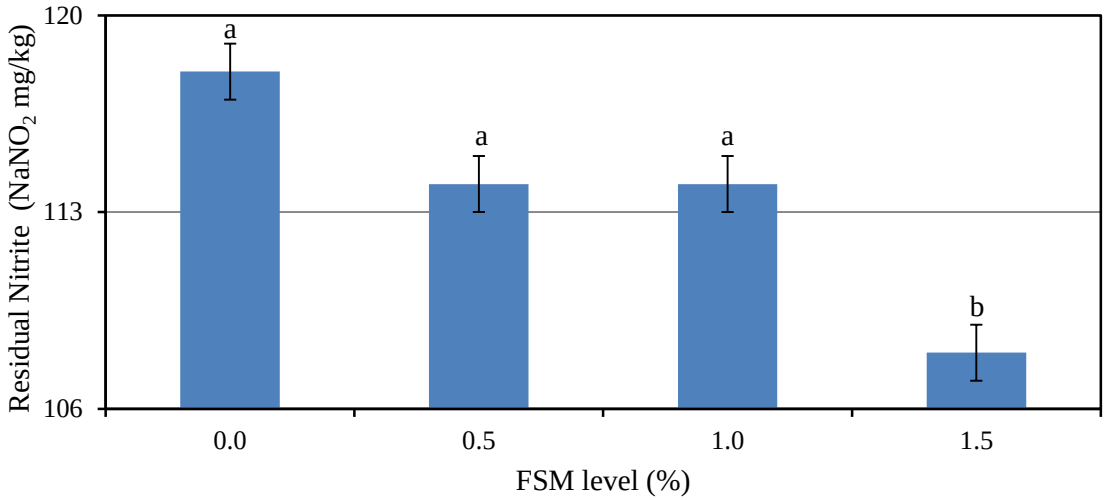

Figure 4-15 Effects of FSM level on the residual nitrite of bologna. [a-b] Means with different letters are significantly different (p<0.05)

According to Cassens (1997), when nitrite is added to the biologically complex system of meat, it reacts with or binds to various naturally occurring chemical components

such as protein and lipids. So, in addition to the protein component in raw meat, there could be a possibility that the nitrite in the formulation became bound to FSM.

What is interesting in our findings is the high concentration of detected residual nitrite in all the samples (>50% of the originally added nitrite). The heating conditions during cooking of sausages speeds up the curing reaction leading to reduction of unreacted or free nitrite in which after processing, only about 10-20% of the originally added nitrite is normally detected analytically (Russ and Gould, 2003). Peres-Alvarez and Fernandez-Lopez (2006) enumerated factors affecting depletion of sodium nitrite such as pH, initial nitrite concentration, process and storage temperatures, presence of reductants and meat to protein ratio. The formulations in the present study have some similarities to commercial processing for low-fat emulsified type sausages (e.g., 0.05% sodium erythorbate as a reducing agent, 0.5% phosphate, controlled temperature during processing). However, our formulation had a higher amount of added water (> 37%), slightly higher sodium nitrite input (~192 ppm), absence of reducing sugars (e.g., no dextrose was added which is commonly found as part of the formulation of processed meats) and absence of oxygen which could explain higher residual nitrite in the cooked products. Previous reports have shown that the amount of fat in the formulation affected the residual nitrite. Higher residual nitrite levels have been reported in low-fat sausages than in high-fat products (Jimenez-Colmenero et al., 2010; Pando et al., 2011). Therefore, to eliminate the potential problem of high residual nitrite in low-fat products, sodium nitrite input should be based on the meat block portion and not on the total batch weight and reductants (in addition to sodium erythorbate) should be part of the formulations.

4.4.10 Texture profile analysis (TPA)

There are many factors affecting texture of emulsified type sausages. In general, as fat is reduced by increasing the proportion of water and keeping the amount of protein essentially constant, a low-fat system becomes softer (Johnson et al., 1977; Grigelmo-Miguel et al., 1999; Jimenez-Colmenero, 2010). And thus firmness is one common problem in fat reduced processed meats. Shown in **Table 4-8** is the TPA hardness, cohesiveness, springiness, and chewiness of low-salt, low-fat bologna.

Table 4-8 Effects of MTG and FSM on the TPA of bologna[1]

MTG (%)	FSM (%)	Hardness (N)	Cohesiveness -	Springiness (%)
0.0	0.0	71.3±4.7^{cz}	0.51±0.01^{b}	79.0±1.0^{c}
	0.5	76.7±3.5^{cz}	0.51±0.01^{b}	81.3±1.2^{c}
	1.0	80.0±3.5^{cz}	0.50±0.01^{b}	80.0±4.4^{c}
	1.5	86.0±3.6^{cy}	0.48±0.04^{b}	78.7±2.5^{c}
0.15	0.0	116.3±12.0^{bz}	0.62±0.01^{a}	85.7±0.6^{b}
	0.5	119.7±11.9^{bz}	0.61±0.02^{a}	84.7±0.6^{b}
	1.0	125.0±7.9^{bz}	0.60±0.01^{a}	84.3±0.6^{b}
	1.5	125.0±14.0^{by}	0.58±0.02^{a}	83.0±1.7^{b}
0.30	0.0	124.0±3.6^{az}	0.66±0.02^{a}	87.0±0.8^{a}
	0.5	136.3±6.4^{az}	0.64±0.02^{a}	86.3±1.5^{a}
	1.0	145.3±5.8^{az}	0.63±0.01^{a}	86.3±1.2^{a}
	1.5	151.3±2.6^{ay}	0.60±0.02^{a}	86.7±1.2^{a}

^{ns} not significant (p>0.05); MTG- microbial transglutaminase; FSM-flaxseed meal
$^{a-c}$means with different letters in the same column are significantly different as affected by MTG (p<0.05) while $^{y-z}$ as affected by FSM
[1]Values are means of three replicates ± standard deviation

TPA characteristics of samples were affected (p<0.05) by FSM and MTG but there was no significant interaction effect (see **Appendix B-5**), therefore only main effects are presented (**Figures 4-16 and 4-17**). The addition of 0.50% and 1.00% FSM did not affect (p>0.05) TPA hardness while addition of 1.50% FSM produced harder bologna than the control.

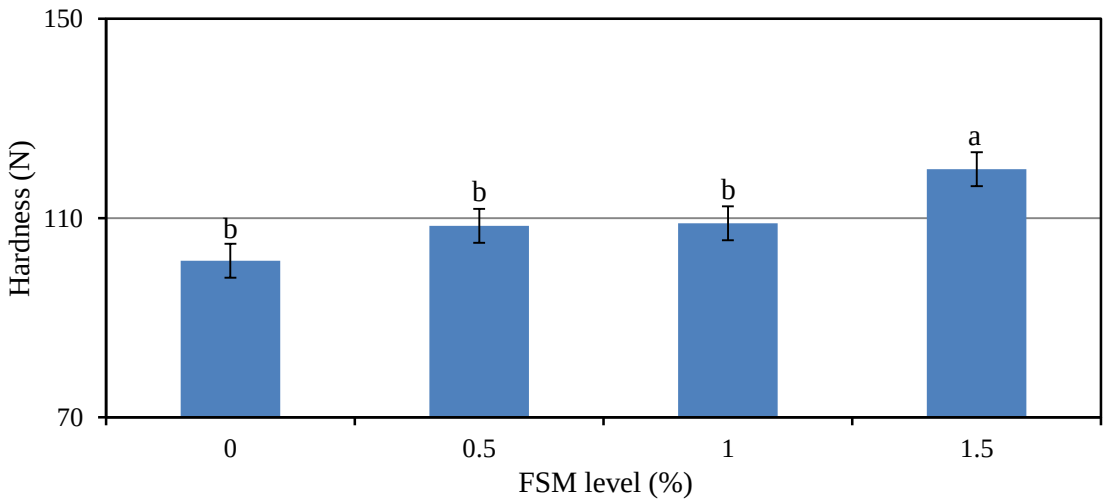

Figure 4-16 Effects of FSM level on TPA hardness of bologna. $^{a-b}$ Means with different letters are significantly different (p<0.05)

A significant increase (p<0.05) in hardness was noted as MTG was added, even at concentration as low as 0.15% of the formulation (**Figure 4-17**).

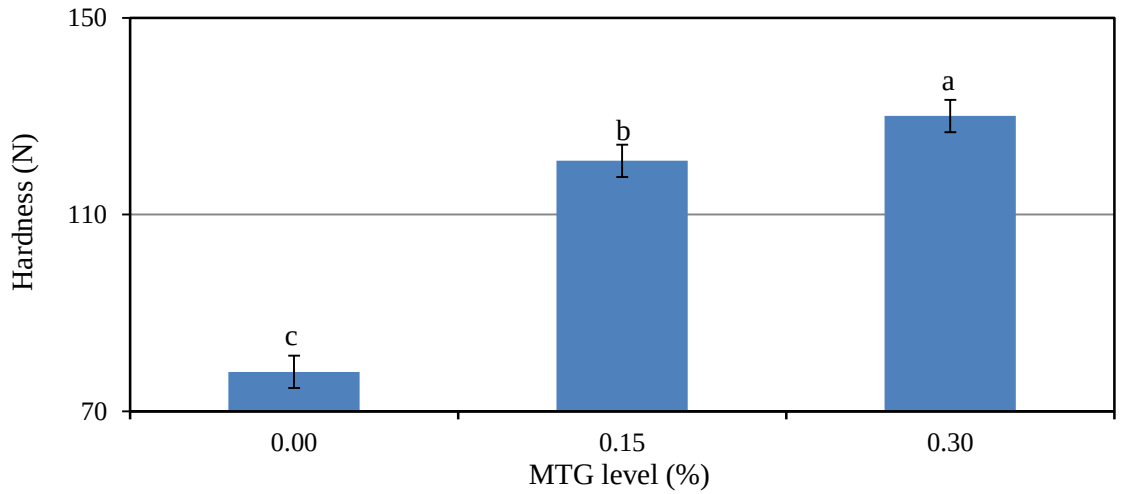

Figure 4-17 Effects of MTG level on TPA hardness of bologna. [a-c] Means with different letters are significantly different (p<0.05)

The effects of different binders/fillers on the hardness of emulsified sausages have been reported. Jimenez-Colmenero et al. (2010) reported that addition of konjac gel and seaweed in low-fat frankfurters significantly increased hardness of samples. Likewise, 1.0 % orange dietary fiber (Viuda-Martos et al., 2010) and rice bran fiber (Choi et al., 2009) also led to a significant increase in hardness of high-fat mortadella and in low-fat emulsion system, respectively. Upon addition of these non-meat ingredients (fibers) in the formulation, they are incorporated in the complex meat system and favorably strengthen the matrix during cooking (Viuda-Martos et al., 2009) resulting in increased hardness. This increase in hardness contributed by FSM could be a potential indication of meat protein-FSM protein interaction or could be due to its WHC.

Cohesiveness refers to a measure of the degree of difficulty of breaking down the internal structure of the sausages (Horita et al., 2011). This internal structure is dependent on the degree of all interactions (hydrophobic interactions, disulfide bonds, and others) which formed during thermal gelation or cooking process. MTG affected (p<0.05) cohesiveness of samples (**Figure 4-18**). However, addition of FSM, though it increased hardness, it did not influence (p>0.05) cohesiveness of samples. There was no interaction effect.

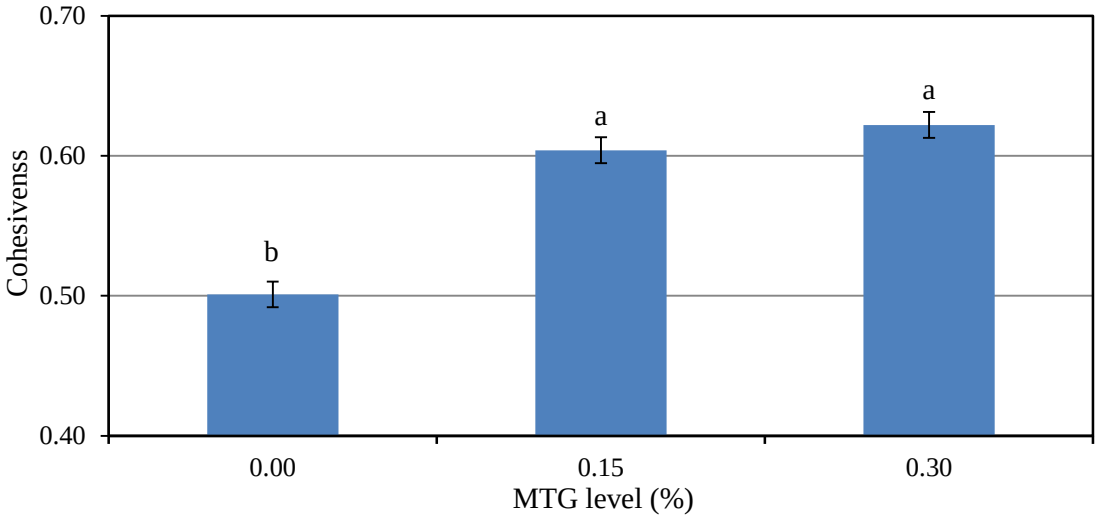

Figure 4-18 Effects of MTG level on TPA cohesiveness of bologna. [a-b] Means with different letters are significantly different (p<0.05)

In terms of springiness, similar trend as to cohesiveness was observed only with MTG affecting (p<0.05) the samples (**Figure 4-19**). FSM did not affect (p>0.05) springiness and there was no interaction effect.

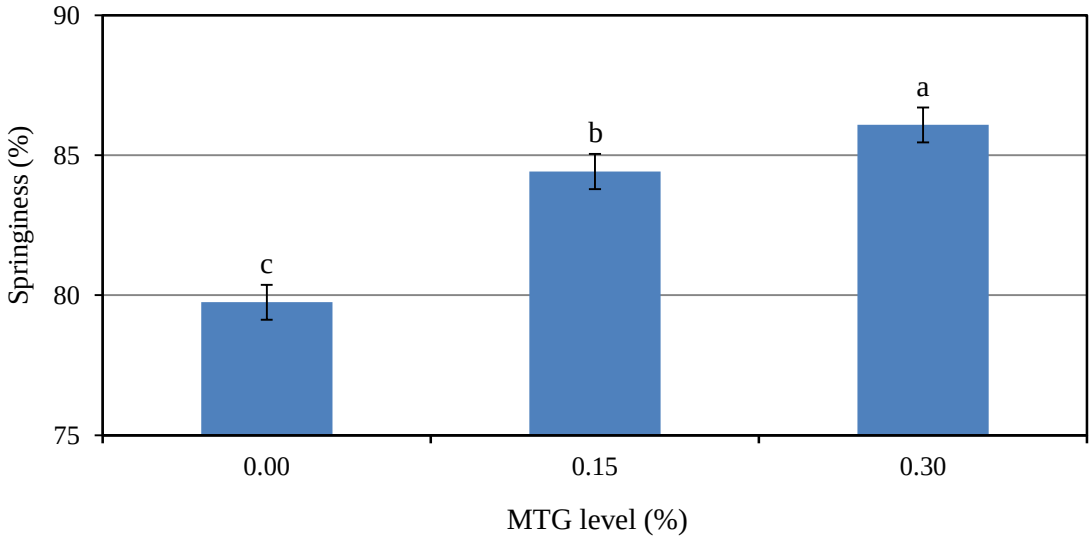

Figure 4-19 Effects of MTG level on TPA springiness of bologna. [a-c] Means with different letters are significantly different (p<0.05)

MTG addition in meat products as one way to reduce salt in processed meats has been previously investigated. Tseng et al. (2000) reported that it was possible to produce low salt (1.0%) chicken meat balls with similar or even higher gel strength as samples with regular

salt content (2.0%) by addition of different levels of MTG. However, MTG was incapable of improving texture of frankfurters (Colmenero and Carballo, 2005) and meat gels (Soeda et al., 2006) when no salt was added. Improving texture of meat products by MTG is due to its ability to cross-link meat proteins, increasing molecular weight of the proteins. Gelation is favored by the protein size, since large molecules formed extensive networks by cross-linking in three dimensions, and by the flexibility and ability of the proteins to denature (Oakenfull and Burley, 1997). The salt-soluble myofibrillar proteins are good substrates for the cross-linking reaction (Kuraishi et al., 1997). In the absence of salt in the meat matrix there will be insufficient substrate resulting in less protein aggregation to form a strong protein network (Colmenero and Carballo, 2005). It is recommended then that it is not feasible to obtain muscle products using transglutaminase alone as the binding agent (O'Kennedy, 2000; Pietrasik and Li-Chan, 2002; Tellez-Luis et al., 2002). In the present study, MTG at as low as 0.15% addition level was sufficient to produce a firm product and 1.00% salt in the formulation and 0.50% TKPP were sufficient to maximize solubilization of myofibrillar proteins providing sufficient substrate for MTG.

4.4.11 Sensory evaluation

4.4.11.1 Colour

Panelists did not detect any change in color of the samples due to addition of MTG (p>0.05). However, addition of FSM affected the color of bologna (**Figure 4-20;** see also **Appendix B-6**).

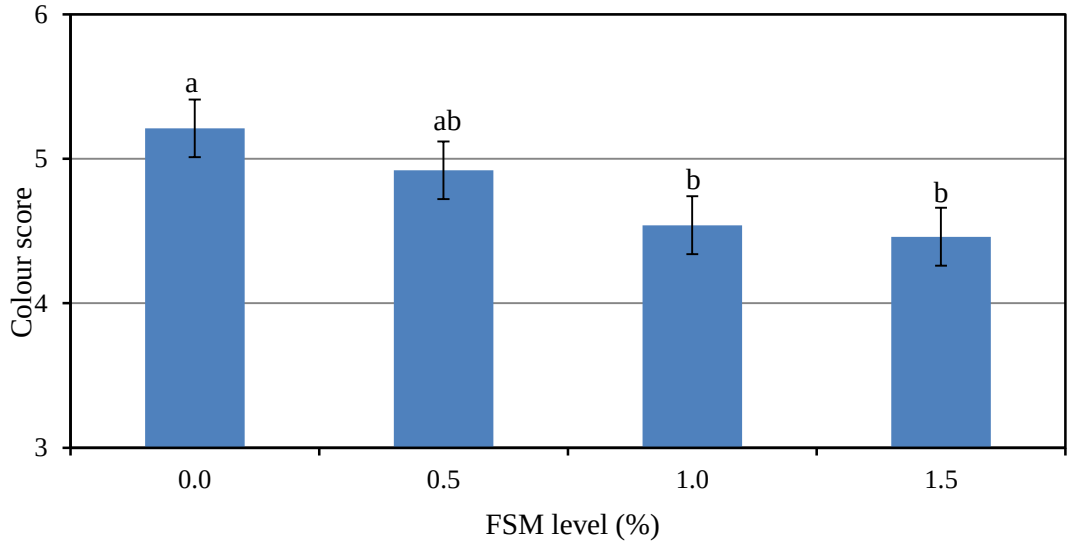

Figure 4-20 Effects of FSM level on the sensory colour of bologna. [a-b] Means different letters are significantly different (p<0.05)

Similar to instrumental colour measurement, the addition of MTG had no effect (p>0.05) on sample colour while addition of 0.5% FSM affected (p<0.05) the sensory colour of bologna. This observation about the lack of colour effect of MTG agrees with Kilic (2003) who found that the addition of MTG did not change sensory colour of chicken kebab. Likewise, in baked goods, Lipilina and Ganji (2009) reported darker colour of the breads and muffins crust and crumb when FSM was added (30% and 50% FSM). This darkening effect on colour was due to the presence of natural pigments in FSM.

4.4.11.2 Sensory texture

Similar to instrumental hardness results, panelists detected the effect of MTG addition on the firmness of the samples (**Table 4-9**). A major increase in firmness and chewiness was detected upon addition of 0.15% MTG and no further increase was noted at 0.30% MTG. The FSM contribution to firmness and chewiness was not detected by panelists (p>0.05). There was no observed interaction effect (see **Appendix B-7**).

Table 4-9 Effects of MTG and FSM on the sensory texture of bologna [1]

MTG	FSM	Sensory parameters[2]		
		Firmness	Chewiness	Juiciness[ns]
0	0	3.7±1.1[b]	3.5±0.8[b]	4.97±0.1
	0.5	4.1±1.0[b]	2.5±0.9[b]	5.03±0.2
	1.0	4.8±1.1[b]	4.300.9[b]	4.80±0.2
	1.5	4.5±0.7[b]	3.9±0.6[b]	5.07±0.2
015	0	6.0±0.7[a]	5.3±0.7[a]	5.00±0.2
	0.5	5.9±0.7[a]	5.2±0.6[a]	5.03±0.2
	1.0	5.9±0.7[a]	5.2±0.6[a]	4.87±0.1
	1.5	5.9±0.8[a]	5.2±0.6[a]	4.70±0.3
0.30	0	6.0±1.0[a]	5.2±0.8[a]	5.03±0.2
	0.5	6.0±1.2[a]	5.20±0.9[a]	5.03±0.2
	1.0	5.2±0.1[a]	4.6±0.1[a]	4.77±0.2
	1.5	5.9±1.1[a]	5.1±1.0[a]	4.70±0.3

[a-b]Means in the same column with different superscripts are significantly different as affected by MTG (p < 0.05)
[ns]No significant difference among means at the same column
[1]Overall means from 12 panelists with three replications (12 panelists x 3) ± standard deviation
[2]Scores: 8 (extremely firm, hard to chew, juicy) ----1 (extremely soft, easy to chew, dry)

Despite differences in firmness and chewiness among samples, all samples had similar juiciness scores (**Table 4-9**). Juiciness in cooked sausages is defined as the amount of moisture or juice perceived during mastication (Matulis et al., 1995) which is then related to the ability of meat proteins (Ventanas et al., 2010) and binders (e.g., carrageenan) to entrap water and affect moisture release during the initial bite (Matulis et al., 1995). In the present study, even though addition of MTG resulted to changes in protein functionalities (led to very firm texture) the moisture is readily released from the product upon chewing thus panelists did not see any change in juiciness among samples. Likewise, FSM water binding did not alter moisture release during the initial bites.

4.4.11.3 Flavour-related perception

The addition of MTG and FSM did not affect (p>0.05) the spice flavour intensity and foreign flavour perception (**Table 4-10**). Interestingly, panelists detected saltiness reduction of in samples with the highest level of FSM (**Figure 4-21**).

Table 4-10 Effect of MTG and FSM on the flavour-related perception of bologna

		Sensory parameters		
MTG	FSM	Spice intensity[ns]	Foreign flavour[ns]	Saltiness
0	0	4.3±0.3	6.3±0.3	2.8±0.2[a]
	0.5	4.2±0.1	6.3±0.6	2.7±0.1[a]
	1.0	4.1±0.1	6.4±0.1	2.6±0.0[ab]
	1.5	4.2±0.1	6.3±0.4	2.5±0.2[b]
015	0	4.3±0.4	6.5±0.2	2.7±0.0[a]
	0.5	4.0±0.1	6.5±0.2	2.7±0.1[a]
	1.0	4.2±0.1	6.4±0.1	2.6±0.0[ab]
	1.5	4.0±0.1	6.4±0.1	2.5±0.2[b]
0.30	0	4.2±0.1	6.4±0.1	2.7±0.1[a]
	0.5	4.0±0.2	6.4±0.1	2.7±0.1[a]
	1.0	4.0±0.2	6.3±0.2	2.6±0.2[ab]
	1.5	4.0±0.2	6.3±0.2	2.5±0.2[b]

[a-b]Means in the same column with different superscripts are significantly different as affected by FSM (p< 0.05)
[ns]No significant difference among means at the same column
[1]Overall means from 12 panelists with three replications (12 panelists x 3) ± standard deviation
[2]Scores: 8 (extremely intense, no foreign flavour) ----1 (extremely weak, extremely intense off-flavour); 6 (extremely salty) ---- 1 (no detectable saltiness)

In addition to NaCl level, saltiness perception can be affected and explained by many factors. For example, Ventanas et al. (2010) reported positive correlation between juiciness and saltiness of bologna type sausage. Saltiness was perceived to be more intense and for a longer time in the juiciest samples. However, in this study correlation of juiciness and saltiness was not observed. Panelist detected all samples to have comparable juiciness but addition of FSM affected saltiness perception even though all samples were formulated with an equal amount of NaCl. For foreign flavour, panelists scored the samples to have a moderately weak to very weak foreign flavour. The MTG and FSM addition did not contribute to any foreign flavor (p>0.05).

In some of the papers, changes in flavour profile due to addition of hydrocolloids are partly explained by dilution effect. For example, Beggs et al. (1997) reported a reduction of saltiness in turkey frankfurters formulated with high starch and this high amount of starch tended to reduce spiciness. Morris (1987) speculated that hydrocolloids effect on flavour could be due to inefficient mixing as the polymer chains became obstacles to diffusion. Interestingly, panelists did not detect change in spice flavour intensity but detected changes in saltiness perception. If FSM affected diffusion of flavour or caused dilution effect of flavour, panelists could have detected both changes in spice intensity and saltiness. Therefore, there could be another mechanism that may explain changes in saltiness due to FSM addition or this dilution and entangling effect of polymers may apply but because the spice flavour in our formulation was quite intense, a slight intensity reduction had no impact on the overall spice flavour intensity thus was not perceived by the panel.

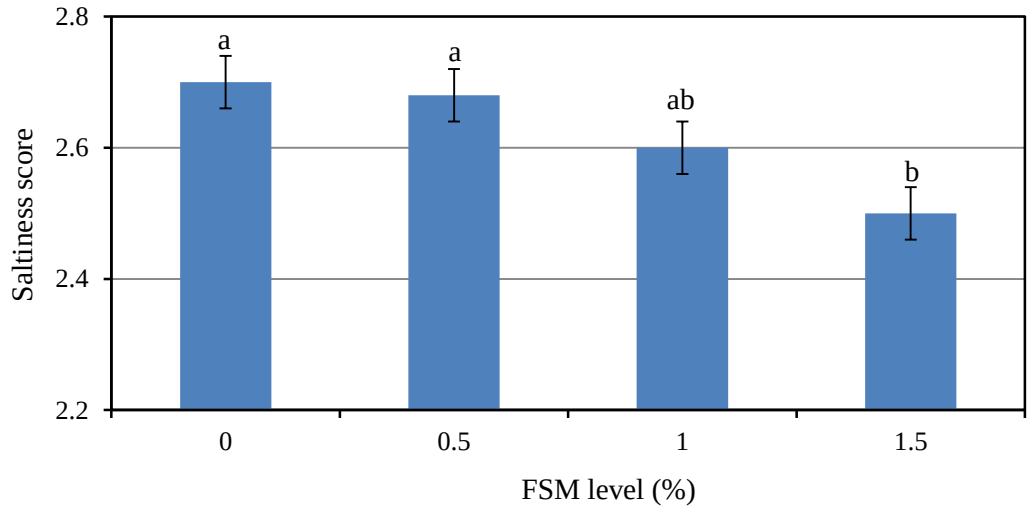

Figure 4-21 Effects of FSM level on the saltiness of bologna. [a-b] Means with different letters are significantly different (p<0.05)

4.4.11.4 Overall acceptability

Although experienced panelists are not the best panel to rate the overall acceptability of the product, this parameter was included in this study for the purpose of generating general information on the effect of treatment factors on overall acceptability. Results showed that MTG did not affect acceptability of the samples ($p>0.05$) but 1.5% of FSM addition reduced ($p<0.05$) the acceptability score of the cooked bologna samples (**Figure 4-22**). The FSM (1.5% level) effect on acceptability could be related to its effect on product colour and saltiness (**Figures 4-23 and 4-24**).

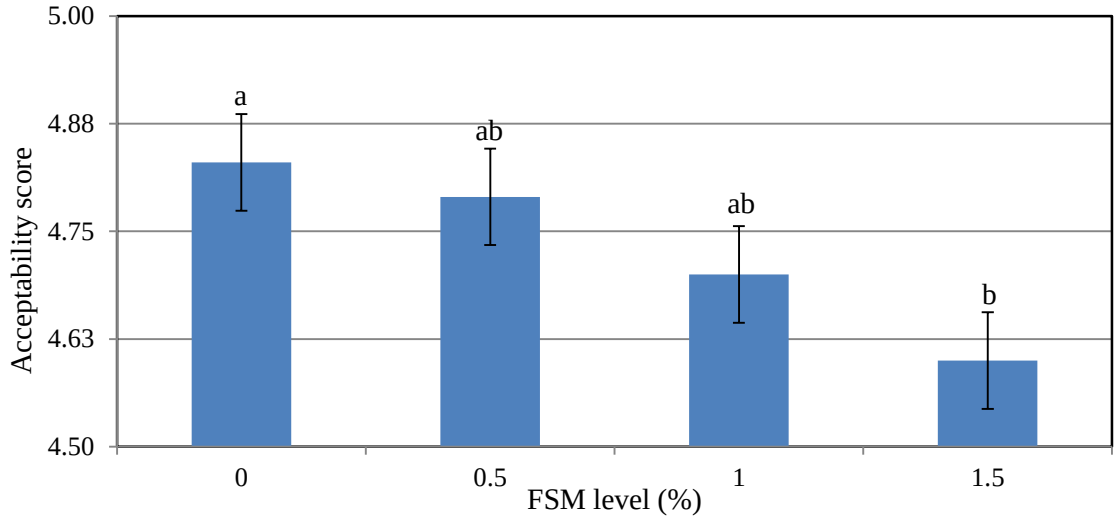

Figure 4-22 Effects of FSM level on the overall acceptability of bologna.
[a-b] Means with different letters are significantly different ($p<0.05$)

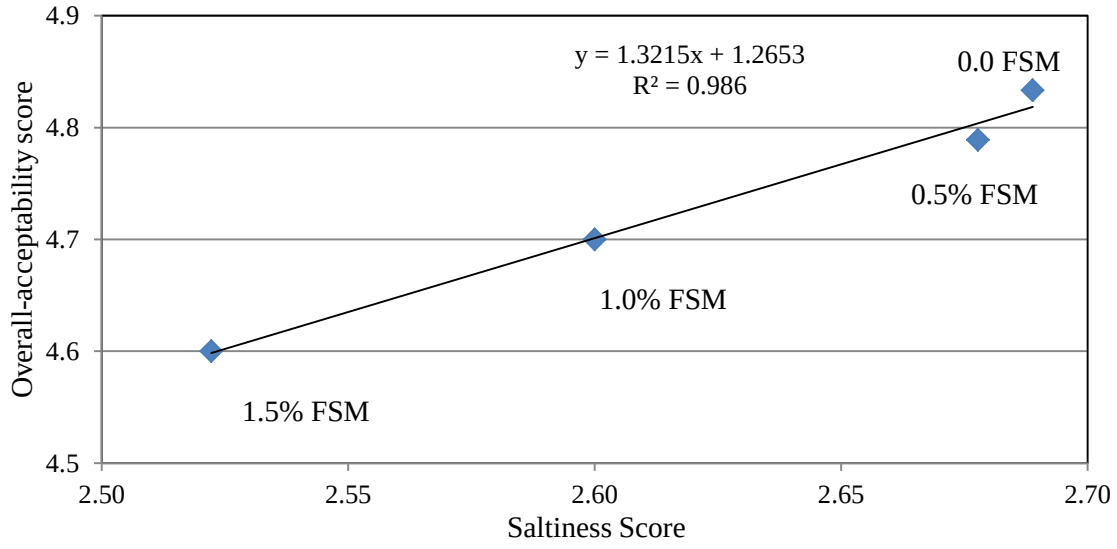

Figure 4-23 Linear relationship of overall acceptability score and saltiness score ($p<0.05$)

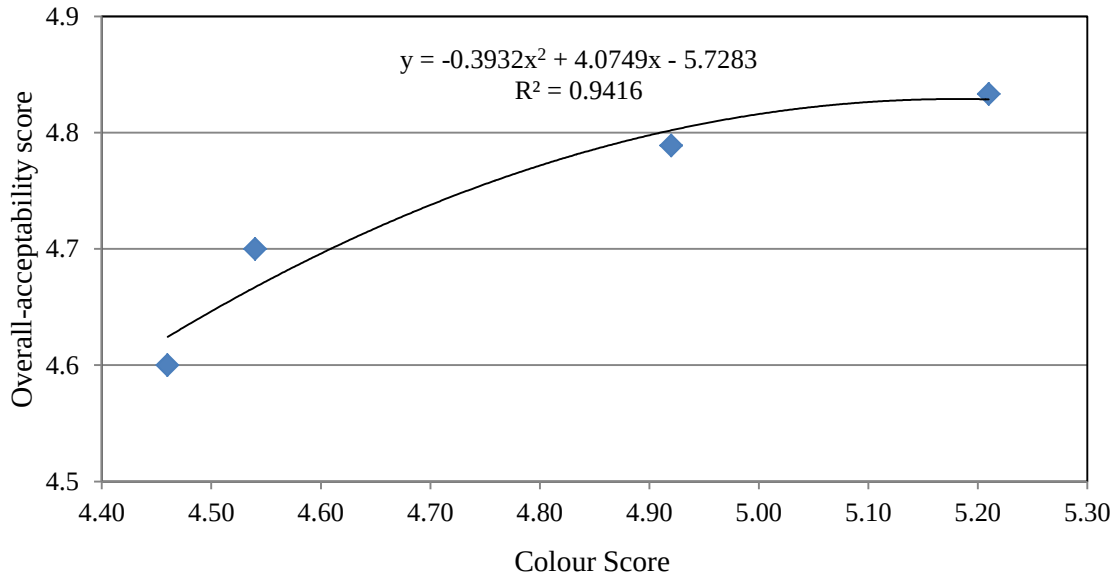

Figure 4-24 Polynomial relationship of overall acceptability score and colour score ($p<0.05$)

In general, this study confirms that 1.00% NaCl and 0.5% TKPP in a low-fat bologna formulation was sufficient to solubilize myofibrillar proteins such that they could serve as a substrate for MTG crosslinking reactions resulting in a firm gel. Shang and Xiong (2010) suggested that 0.3% was an appropriate (cost effective) level of transglutaminase application for the improvement of both textural properties of myofibrillar protein gels and their water-holding capacity. However, in this study, even half of the suggested level was proven effective to produce a firm gel in a meat matrix composed of >37% water, 11% protein, and 10% fat.

4.5 Conclusion

This study confirmed the superb capability of MTG to improve TPA hardness of LSLF bologna. The addition of 0.15% MTG in the formulation increased TPA hardness by 163%. Therefore use of MTG in producing processed products like our model system is highly recommended but it should be used with other ingredients capable of increasing water binding of the matrix to overcome the purge loss during display. Although had good water binding ability, good nutrient profile, ability to reduce residual nitrite without influencing flavor, its application in meat processing will be limited due to several factors. Usage of as low as 0.5% FSM affected color and at 1.5% produced a slimy texture which will eventually become a problem during handling (specifically during the slicing step). This study shows

that utilization of ingredients rich in mucilage/gums in combination with MTG in processed products with high amount of added water (>37%) is limited to a low level of addition. The other forms of flax seed by products (e.g., flax protein isolate) would be worth testing.

The overall result of this study agrees with the previous published papers recommending the use of binders to increase protein-water interaction when MTG is added (MTG basically promotes only protein-protein interaction expelling water in the matrix). Therefore, in low-salt, low-fat formulations, a small amount of MTG is very beneficial in improving texture. However to maximize profitability and quality, a small amount of binder should be added to eliminate high purge loss during display.

5 GENERAL DISCUSSION

The overall objective of this project was to examine potential processing alternatives for the manufacture of low-salt bologna. Low-fat bologna was used as a matrix for the entire study. Fat reduction is normally achieved with either the use of leaner (but more costly) meats and the "dilution effect" from substances like low or noncaloric ingredients or by replacing a portion of the fat with water (Claus et al., 1989). The former was used for the entire project, on the assumption that this created a "stressed matrix system" which would be efficient in showing the potentials of experimental factors for improving the quality of low-salt bologna. For example, the effect of treatment factors on purge loss can be observed much more easily, as previous reports showed that purge loss is a common problem with this highly added-water meat system (Ruusunen et al., 2003).

In the first phase of this project (Chapter 3), the effects of three different factors on WHC, instrumental texture and sensory characteristics were investigated. The factors were the salt types (sea salt vs. regular salt), NaCl level (0.75%, 1.00%, 1.25%, 2.00%) and the effect of holding stuffed meat batter (2 h vs 20 h) before cooking. The general idea in choosing these experimental factors was based on some published papers, with modifications. For the salt types, sea salt was included as a treatment factor based on the observation of Drake and Drake (2011) that some minerals in sea salt affected the time-intensity profiles for salty taste at equal sodium concentrations (8.0 mg/L). The sea salt used in present study was obtained from Griffith Laboratories (one of the largest sea salt suppliers for food processors) and was manufactured by Cargill Company (Cargill Salt, Minneapolis, MN, USA). In terms of NaCl level, 2.00% NaCl (the highest level) was chosen as this is the common NaCl level in commercial meat products, but it may go as high as 3% (Claus et al., 1994); 0.75% was chosen as the lowest based on our preliminary study showing that low-fat bologna produced with less than 0.75% NaCl was extremely soft and the preparation of samples (i.e., slicing, cutting, and gluing for torsional gelometry) for texture measurement was extremely difficult. In terms of holding or delayed cooking, this factor was considered a modified version of preblending/presalting which was effective in coarsely-ground type sausages (Gumpen and Sørheim, 1987).

The uniqueness of this study was the combination of three different factors in low-fat matrix and the utilization of tetrapotassium pyrophosphate (TKPP) which was reported as a superior type of phosphate to solubilize myofibrillar proteins (Knipe et al., 1985). Furthermore, the cooking method used was slow heating which was also reported to be effective in producing firmer gels (Foegeding et al., 1986). This study was also conducted on a bigger scale (>5.0 kg), which was large enough to simulate commercial processing. A total of 16 treatments were prepared in which the experimental unit was represented by the cooked bologna chub. All samples were formulated to contain 11.0% protein, 10.0% fat, 0.5% TKPP, 0.0192% sodium nitrite, and 0.05% sodium erythorbate and were all cooked following a four-stage process schedule.

Elemental composition of regular salt and sea salt showed the highly variable nature of salt as affected by production process and harvest location. This sea salt was purer in that it contained less amount of contaminants (i.e., Ca^{2+}, Mg^{2+}) compared to regular salt. However, despite the difference in composition between the two salt types, the magnitude of difference was low and thus did not influence the general physical, chemical, and sensory characteristics of low-sodium, low-fat bologna. Overall, the refined sea salt behaved the same as the commercial refined salt.

Salt level was the main factor affecting cook loss and purge loss. Samples with 0.75% NaCl had the poorest WHC but this was overcome by delayed cooking. The salt level by holding effect interaction was significant for expressible moisture. This interaction effect was observed only in samples with 0.75% NaCl level. In general, WHC of 1.00%, 1.25%, and 2.00% samples were statistically similar. A similar trend was observed for instrumental texture. Texture of bologna samples with 0.75% NaCl was poor but delayed cooking effectively improved its texture, both measured using TPA and torsional gelometry ($p<0.05$). The effect of delayed cooking is related to the theory of Bendall (1954) that ample time is necessary to maximize functionalities of phosphates (i.e., increase solubility of protein).

One of the most interesting findings in the present study is the consistent observation that texture qualities (instrumental and sensory) of samples with 1.00% NaCl were similar to those samples with 1.25% and 2.00%. This general observation was different from some published reports (Sofos, 1983; Hand et al., 1987; Barbut et al., 1988) cited in this thesis. It seems that many favorable conditions such as use of high amount of TKPP (0.5% w/w), good quality meat proteins, optimum chopping time, and favorable heating rate during cooking were provided and utilized in this study. The use of TKPP increased the pH of from 5.71

(fresh meat) to 6.40-6.43 (raw meat batter). The advantageous pH effect on low NaCl sausages has been reported (Knipe, 1985; Hamm, 1986; Knipe, 1990). For example, Puolanne et al. (2001) reported that similar water-holding capacity with 2.5% NaCl in pH 5.7 can be achieved in pork sausage with 1.5% NaCl when pH of the matrix is pH 6.1 and above.

Also, the raw material utilized in the present study was composed of good quality protein (pork leg muscle) when compared to composite or trimmings (from different parts of muscles) which is commonly used in those cited papers. In addition, the desired target protein was achieved in the present study. The actual numbers generated from proximate composition of raw materials were utilized in formulating the product and not just estimating the content based on published papers. Another factor is the slow heating rate during cooking which has been reported to favor protein gelation (Foegeding et al., 1986; Saliba et al., 1987; Camou et al., 1989; Barbut and Mittal, 1989; Lan et al., 1995; Cofrades et al., 1997)

A greater magnitude effect of DC in improving texture quality of LSLF was observed only at 0.75% NaCl and had no effect at higher NaCl levels. This general observation can be related to the Bendall postulate in which full functionalities of phosphates were achieved after following a longer time after it was added to the meat. This phosphate functionality was clearly demonstrated by the increased protein solubility.

Although we have a slightly different matrix the general observation in the present study agrees with the previous findings of Gumpein and Sørheim (1987). They reported that salt diffusion, protein dissolution, and myofibril swelling in pork and beef batters with 1.8% NaCl were immediate in finely chopped (exceeding 10-15 min at low chopper speed) treatments and pre-salting had no effect. In the present study, protein solubility was immediate for treatments even as low as 1.00% NaCl. Holding was effective only at 0.75% NaCl.

In this first study, texture quality of low-fat bologna with 0.75% NaCl subjected to holding was significantly improved and became comparable to treatments with higher NaCl levels. However, based on the panelist scores and comments, it seems that it is not practical to formulate sausages at this extremely low NaCl level. Furthermore, in general all the samples were generally softer (TPA hardness = 56-62 N). Bologna is a popular product which is normally sold as thinly slices (2-3mm), thus firmness is especially important for the slicing procedure. Thus in product formulation, it is critical to investigate addition of ingredients capable of increasing firmness of low-fat, low-sodium bologna. This was the basis for conducting the second study where MTG (well-known for its ability to cross-link

proteins to proteins including myosin leading to modification of protein functionalities as previously reported by Kuraishi et al., 1997; De Jong and Koppelman, 2002; Kilic, 2003; Dondero et al., 2006; Ahhmed et al., 2007; Cofrades et al., 2011) was combined with different levels of flaxseed meal (FSM) (also known for its superb water hydration capacity according to Mazza and Biliaderis, 1989; Bhatty and Cherdkiatgumchai, 1990; Wanasundara and Shadidi, 1994). Although MTG has been proven effective in improving texture of low-sodium products, to our knowledge, no research has been conducted examining its potential combination with FSM in LSLF emulsified model system at a scale big enough to mimic the industry's actual process. The processing conditions were similar to the first study (i.e., 0.5% TKPP was added, 11.0% protein, 10.0% fat, use of pork leg muscle, and cooked using four-stage process schedule). Parameters to measure effectiveness of treatment factors were also similar to the first study.

Interestingly, when quality of bologna with 1.0% NaCl (cooked immediately) in study 1 (chapter 3) is compared to the quality of bologna with no MTG and no FSM in study 2 (chapter 4), there was an eighteen percent difference in TPA hardness (59.9-62.2 vs. 71.3 N, respectively). Bologna in study 2 was slightly firmer. This observation can be attributed to the effect of freezing on meat (frozen meat was used in study 1 while chilled fresh was used in study 2) and perhaps the difference in the amount of added water by 0.6%. In study 2, fresh chilled meat was used and 0.6% spice was added in the formulation. The WHC of the bolognas were very comparable.

In general, result of study 2 shows that MTG as low as 0.15% significantly improved firmness of LSLF bologna (from 62.2 to 116.3 N) however, this resulted in higher purge loss, likely due to excessive protein-protein interaction. This observation supported the recommendation of Cofrades et al. (2011) that other means need to be used along with MTG to promote the protein-water interactions required for adequate binding in fresh and cooked products. The magnitude of purge loss was reduced with the addition of FSM. Although FSM generally can be a good candidate because of its good water binding ability, good nutrient profile, ability to reduce residual nitrite without influencing flavour, its application in meat processing has some limitations. Usage of as low as 0.5% affected colour and at 1.5% produced a slimy texture which will eventually become a problem during handling (specifically during slicing step). It also affected perception of saltiness (at 1.5% FSM), and overall acceptability (at 1.5% FSM) of the product.

This study shows that utilization of ingredients rich in mucilage/gums in combination with MTG in processed products with high amount of added water (>37%) is limited to small amount/level. Other forms of flaxseed by-products (e.g., isolate) would be worth testing.

6 GENERAL CONCLUSION

This study was conducted to investigate potential strategies in processing LSLF bologna which can be used in commercial processing plants. The meat matrix system used for the entire project was formulated to give a final composition of 11.0% protein, 10.0% fat. Water was used to replace fat, thus >37.0% water was added during processing.

Although the general concept that is globally accepted is that texture of processed meat is affected by total NaCl, this project illustrated that this concept is true only at a critical total (NaCl + TKPP) ionic strength range (i.e., < 0.4 M or 0.75% NaCl) given the conditions in this study. At this extremely low NaCl level (0.75%), delayed cooking can be introduced during processing to improve the texture quality of the processed product. A consistent observation was that the texture and water holding properties of samples with 1.00% NaCl were statistically not different from samples with 1.25% or 2.00% NaCl. However, it is important to note that even at a 2.00% NaCl level, when the formulation of emulsified-type sausage is composed of that high amount of water with no added fillers or binders, its texture is soft for "sliced bologna". Therefore, additives or ingredients to increase product firmness are necessary in producing low-fat bologna. Although MTG alone at a low level can be utilized to achieve ideal product firmness, additional ingredients capable of increasing water-protein interaction in the matrix must be added to reduce the problem of purge accumulation during display.

Collectively, the data from this project clearly demonstrates that modifications of processing (delayed cooking) can be introduced in processing extremely low-salt (0.75% NaCl) and available functional ingredients can be added to increase firmness/hardness of low-fat emulsified product. The perception of overall product flavour and saltiness is highly dependent on the levels of NaCl, and this remains an important issue with respect to sodium reduction. Increasing flavour in low-salt meat products warrants further research.

7 REFERENCES

ACCC. (1999). Approved Methods of the American Association of Cereal Chemists. 9th Ed. St. Pau, MN.

Acton, J. C. & Saffle, R. L. (1969). Preblended and prerigor meat in sausage emulsions. Food Technology , 23 (3), 367.

Adams, M. (2012). Top 20 ways to boost immunity to colds and flu without using vaccines. Internet accessed on November 10, 2012 at http://truththeory.com/2012/10/12/top-20-ways-to-boost-your-immunity-to-colds-and-flu-without-using-vaccines/

Adrogue, H. J. & Madias, N. E. (2007). Sodium and potassium in the pathogenesis of hypertension. The New England Journal of Medicine, 356, 1966-1978.

AOAC. (1990). Official Methods of Analysis. 15th ed. Association of Official Analytical Chemists, Washington, DC.

Ahhmed, A. M., Kawahara, S., Ohta, K., Nakade, K., Soeda, T., & Muguruma, M. (2007). Differentation in improvements of gel strength in chicken and beef sausages induced by transglutaminase. Meat Science, 76, 455-462.

Akzonobel Salt Specialties. (2012). History of salt through the ages. Retrieved on May 25, 2012 from http://www.akzonobel.com/saltspecialties/salt/salt/index.aspx.

Alino, M., Grau, R., Toldra, F., & Barat, J. M. (2010). Physicochemical changes in dry-cured hams salted with potassium, calcium, and magnesium chloride as partial replacement for sodium chloride. Meat Science, 86, 331-336.

American Meat Science Association. (1995). Research guidelines for cookery, sensory, evaluation and instrumental tenderness measurements of fresh meat. American Meat Science Association (Ames, Iowa) in cooperation with the National Livestock and Meat Board. pp. 48

Anderson, E. T. & Berry, B. W. (2001). Effects of inner pea fiber on fat retention and cooking yield in high fat ground beef. Food Research International, 34, 689-694.

Arntfield, S. D, Murray, E. D., & Ismond, M. A. H. (1990). Dependence of thermal properties as well as network microstructure and rheology on protein oncentration for ovalbumin and vicilin. Journal of Texture Studies, 21, 191-212.

Asghar, A., Samejima, K., & Yasui, T. (1985). Functionality of muscle proteins in gelation mechanisms of structured meat products. CRC Critical Reviews in Food Science and Nutrition, 22, 27-106.

Ball, P., Woodward, D., Beard, T, Shoobridge, D., & Ferrier, M. (2002). Calcium diglutamate improves taste characteristics of lower-salt soup. European Journal of Clinical Nutrition, 56, 519-523.

Barbut, S. (1988). Microstructure of reduced salt meat batters as affected by polyphosphates and chopping time. Journal of Food Science, 53, 1300-1304.

Barbut, S., Maurer, A. J., & Lindsay, R. C. (1988). Effects of reduced sodium chloride and added phosphates on physical and sensory properties of turkey frankfurters. Journal of Food Science, 53, 62-66.

Barbut, S. & Mittal, G. S. (1989). Effect of salt reduction on the rheological and gelation properties of beef, pork, and poultry meat batters. Meat Science, 26, 177-191.

Barbut, S. & Mittal, G. S. (1996). Effect of three cellulose gums on the texture profile and sensory properties of low fat frankfurters. International Journal of Food Science and Technology, 31, 241-247.

Barbut, S. & Mittal, G. S. (1990). Effect of heating rate on meat batter stability, texture and gelation. Journal of Food Science, 55, 334-337.

Barbut, S., Tanaka, N., & Maurer, A. J. (1986). Effects of varying levels of chloride salts on *Clostridium botulinum* toxin production in turkey frankfurters. Journal of Food Science, 51, 1129-1131.

Beauchamp, G. K. & Cowart, B. J. (1990). Preference for high salt concentrations among children. Developmental Psychology, 26, 539-545.

Beggs, K., Bowers, J., & Brown, D. (2006). Sensory and physical characteristics of reduced-fat turkey frankfurters with modified corn starch and water. Journal of Food Science, 62, 1240-1244.

BeMiller, J. N. (1973). Quince seed, psyllium seed, flaxseed and okra gums. In: Ayad, A. (2010). Characterization and properties of flaxseed protein fractions. Ph. D. Thesis, McGill University. Internet accessed on September 30, 2012 at http://digitool.Library.McGill.CA:80/R/-?func=dbin-jump-full&object_id=92214&silo_library=GEN01

Bendall, J. R. (1954). The swelling effect of polyphosphates on lean meat. Journal of the Science of Food and Agriculture, 5, 468-475.

Beriain, J. (2009). Microbial transglutaminase effects on texture properties of low-salt meat emulsions. Proceedings of the 55th International Congress of Meat Science and Technology (ICoMST), Copenhagen, Denmark, 16-21 August 2009, 762-764.

Berry, B. W. & Wergin, W. P. (1993). Modified pregelatinized potato starch in low-fat ground beef patties. Journal of Muscle Foods, 4, 305-320.

Bhathena, S., Ali, A., Haudenschild, C., Latham, P., Ranich, T., Mohammed, A. I., Hansen, C. T., & Velasquez, M. T. (2003). Dietary flaxseed meal is more protective than soy protein concentrate against hypertriglyceridemia and steatosis of the liver in an animal model of obesity. Journal of the American College of Nutrition, 22, 157-164.

Bhatty, R. S. & Cherdkiatgumchai, R. (1990). Compositional analysis of laboratory-prepared and commercial samples of linseed meal and of hull isolated from flax. Journal of American Oil Chemical Society, 67, 79-84.

Biesalski, H. K. (2005). Meat as a component of a healthy diet – are there any risks or benefits if meat is avoided in the diet? Meat Science, 70, 509-524.

Bilek, A. & Turan, S. (2009). Enhancement of the nutritional status of beef patties by adding flaxseed flour. Meat Science, 82, 472-477.

Bing, R. F., Thurston, H., & Swales, J. D. (1979). Salt intake and diuretic treatment of hypertension. The Lancet, 314, 121-123.

Bourne, M. C. (1978). Texture profile analysis. Food Technology, 32 (7), 66–72.

Breslin, P. A. & Beauchamp, G. K. (1995). Suppression of bitterness by sodium. Chemical Senses, 20, 609-623.

Breslin, P. A. & Beauchamp, G. K. (1997). Salt enhances flavor by suppressing bitterness. Nature, 387, 563.

Brewer, S., Gills, L. A., & Vega, J. D. (1995). Sensory characteristics of potassium lactate and sodium chloride in a model system. Journal of Sensory Studies, 10, 73-87.

Brewer, M. S., Peterson, W. J., Carr, T., McCusker, C., & Novakofski, J. (2005). Thermal gelation properties of myofibrillar protein and gelatin combinations. Journal of Muscle Foods, 16, 126-140.

Brookfield Engineering Laboratories. (2012). Helipath stand for non-flowing substance. Internet accessed on June 30, 2012 at http://www.brookfieldengineering.com/products/accessories/helipath-stand.asp

Brown, I. J., Tzoulaki, I., Candeias, V., & Elliott, P. (2009). Salt intakes around the world: implications for public health. International Journal of Epidemiology, 38, 791-813.

Bullock, K. L., Huffman, D. L., Egbert, W. R., Bradford, D. D., Mikel, W. B., & Jones, W. R. (1995). Nonmeat ingredients for low-fat ground beef patties. Journal of Muscle Foods, 6, 37-46.

Burdock, G. A. (2009). Fenaroli's handbook of flavor ingredients. 6th ed. CRC Press Taylor and Francis Group, Florida, USA, pp. 1440-1445.

Bursey, R. G. & Watson, M. L. (1983). The effect of sodium restriction during gestation of offspring brain development. American Journal of Clinical Nutrition, 37, 43-51.

Camou, J. P., Sebranek, J. G., & Olson, D. G. (1989). Effect of heating rate and protein concentration on gel strength and water loss of muscle proteins gels. Journal of Food Science, 54, 850-854.

Canadian Food Inspection Agency. (2012a). Chapter 4 (Annex C): Use of Phosphate Salts and Nitrites in the Preparation of Meat Products. Internet accessed on May 25, 2012 at http://www.inspection.gc.ca/english/fssa/meavia/man/ch4/annexce.shtml

Canadian Food Inspection Agency. (2012b). Chapter 7 (24) Nutrient Content Claims on Fiber. Internet accessed on May 25, 2012 at http://www.inspection.gc.ca/english/fssa/labeti/guide/ch7be.shtml

Canadian Minerals Yearbook (CMY). (2008). Salt. Retrieved on May 25, 2012 from http://www.nrcan.gc.ca/minerals-metals/business-market/canadian-minerals-yearbook/2008-review/commodity-reviews/3413

Carballo, J., Ayo, J., & Colmenero, F. J. (2006). Microbial transglutaminase and caseinate as cold set binders: Influence of species and chilling storage. LWT-Food Science and Technology, 39, 692-699.

Carballo, J., & Colmenero, F. J. (2006). Microbial transglutaminase and caseinate as cold set binders: Influence of species and chilling storage. LWT-Food Science and Technology, 39, 692-699.

Cargill Salt Technical Information. (2008). Purified salt – untreated. Cargill Salt, Minneapolis, MN.

Cassens, R. G. (1997). Residual nitrite in cured meat. Food Technology, 51, 53-55.

Chang, H. C. & Carpenter, J. A. (1997). Optimizing quality of frankfurters containing oat bran and added water. Journal of Food Science, 62, 194-202.

Cheftel, C. J. & Culioli, J. (1997). Effects of high pressure on meat: a review. Meat Science, 46, 211-236.

Cheung, B. M. Y. & Lam, T. C. (2003). Hypertension and diet. In: Caballero, B. (ed). Encyclopedia of Food Sciences and Nutrition, 2nd ed. Elsevier Science Ltd, Academic Press, pp. 3194-3199.

Choi, Y. S., Choi, J. H., Doo, J. H., Hack, Y. K., Mi, A. L., So, Y. S., Jeong, J. H., Chung, H. J., & Cheon, J. K. (2007). Effects of replacing pork back fat with vegetable oils and rice bran fiber on the quality of reduced-fat frankfurters. Meat Science, 84, 557-563.

Choi, Y. S., Choi, J. H., Han, D. J., Kim, H. Y., Lee, M. A., & Kim, H. W. (2009). Characteristics of low-fat meat emulsion systems with pork fat replaced by vegetable oils and rice bran fiber. Meat Science, 82, 266–271.

Claus, J. R., Hunt, M. C., & Kastner, C. L. (1989). Effects of substituting added water for fat on the textural, sensory, and processing characteristic of bologna. Journal of Muscle Foods, 1, 1-21.

Cofrades, S., Carballo, J., & Jiménez-Colmenero, F. (1997). Heating rate effects on high-fat and low-fat frankfurters with a high content of added water. Meat Science, 47, 105-114.

Cofrades, S., Guerra, M. A., Carballo, J., Fernandez-Martin, F., & Colmenero-Jimenez. (2000). Plasma protein and soy fiber content effect on bologna sausage properties as influenced by fat level. Journal of Food Science, 65, 281-287.

Cofrades, S., Lopez-Lopez, I., Ruiz-Capillas, C., Triki, M., & Jimenez-Colmenero, F. (2011). Quality characteristics of low-salt restructured poultry with microbial transglutaminase and seaweed. Meat Science, 87, 373-380.

Colmenero, F. J., Ayo, M. J. & Carballo M. J. (2005). Physicochemical properties of low sodium frankfurter with added walnut: effect of transglutaminase combined with caseinate, KCl and dietary fiber as salt replacers. Meat Science 69, 781-788.

Colmenero, F. J., Cofrades, S., Lopez, I. L., Capillas, C. R., Pintado, T., & Solas, M. T. (2010). Technological and sensory characteristics of reduced/low-fat, low-salt frankfurters as affected by the addition of konjac and seaweed. Meat Science, 84, 356-363.

CTAC- Conseil de la transformation agroalimentaire et des produits de consummation. (2009-2010). Reformulation of products to reduce sodium: salt reduction guide for the food industry. Project funded by the Department of Agriculture and Agri-Food (DAA) through its Advancing Canadian Agriculture and Agri-Food Program (ACAAF) and

through the regional adaptation council, the Fonds de developpement de la transformation alimentaire Inc. (FDTA).

Corpet, D. E. (2011). Red meat and colon cancer: should we become vegetarians, or we can make meat safer. Meat Science, 89, 310-316.

Crehan, C. M., Troy, D. J., & Buckey, D. J. (2000). Effect of salt level and high hydrostatic pressure processing on frankfurters formulated with 1.5 and 2.5% salt. Meat Science, 55, 123-330.

Dahl, L. K. (1972). Salt and hypertension. American Journal of Clinical Nutrition, 25, 231-244.

Damodaran, S. (2007). Amino acids, peptides, and proteins. In: Damodaran, S., Parkin, K.L. & Fennema, O. R. (eds). Fennema's Food Chemistry. 4th ed., CRC Press Taylor and Francis Group, FL, USA, pp. 217-329.

Dawkins, N. L., Gager, J., Cornillon, J. P., Kim, Y., Howard, H., & Phelps, O. (2001). Comparative studies on the physicochemical properties and hydration behavior of oat bran and oatrim in meat-based patties. Journal of Food Science, 66, 1276-1282.

De Jong, G. A. H. & Koppelman, S. J. (2002). Transglutaminase catalyzed reactions: impact on food applications. Journal of Food Science, 67, 2798-2806.

De Lozy, M. & Stare, F. J. (1980). Salt in the diet. In: Moses, C. (ed). Sodium in medicine and health A monograph. Reese Press, Inc., MD, USA, pp. 55-61.

Demeyer, D., Honikel, K., & De Smet, S. (2008). The World Cancer Research Fund report 2007: A challenge for the meat processing industry. Meat Science, 80, 953-959.

Desmond, E. (2006). Reducing salt: A challenge for the meat industry. Meat Science, 74, 188-196.

Dev, D. K. & Quensel, E. (1989). Functional properties of linseed protein products containing different levels of mucilage in selected food systems. Journal of Food Science, 54, 183-186.

Drake, S. L. & Drake, M. A. (2011). Comparison of salty taste and time intensity of sea salt and land salts from around the world. Journal of Sensory Studies, 26, 25-34.

Dondero, M., Figueroa, V., Morales, X., & Curotto, E. (2006). Transglutaminase effects on gelation capacity of thermally induced beef protein gels. Food Chemistry 99, 546-554.

Dzudie, T., Scher, J., & Hardy, J. (2002). Common bean flour as an extender in beef sausages. Journal of Food Engineering, 52, 143-147.

Eastwood, L. (2008). The nutritional value of flaxseed meal for swine. M. Sc. Thesis. University of Saskatchewan.

Egbert, W. R., Huffman, D. L., Bradford, D. D., & Jones, W. R. (1992). Properties of low-fat ground beef containing potassium lactate during aerobic refrigerated storage. Journal of Food Science, 57, 1033-1037.

Eilert, S. J. & Mandigo, R. W. (1996). Use of phosphates and salt to improve lean meat water retention in high-moisture preblends. Journal of Muscle Foods, 7, 1-16.

Fedeniuk, R. W. & Biliaderis, C. G. (1994). Composition and physicochemical properties of linseed (*Linumusitatissimumm L.)* mucilage. Journal of Agricultural and Food Chemistry, 42, 240-247.

Fergusson, L. (2010). Meat and cancer. Meat Science, 84, 308-313.

Fine, B. P., Ty, A., Lestrange, N., & Levine, R. (1987). Sodium deprivation growth failure in the rat: alterations in tissue composition and fluid spaces. Journal of Nutrition, 117, 1623-1628.

Foegeding, E. A., Allen, C. E., & Dayton, W. R. (1986). Effect of heating rate on thermally formed myosin, fibrinogen, and albumin gels. Journal of Food Science, 51, 104-108, 112.

Foegeding, E. A. (1988). Gelation in meat batters. Reciprocal Meat Conference Proceedings, Volume 41, 44-47. Internet accessed on May 30, 2012 at http://meatsci.osu.edu/as555/555Au2008/FoegedingGelation.pdf

Frye, C. B., Myers, K. L., Knipe, C. L., & Sebranek, J. G. (1991). Methods of sodium tripolyphosphate addition in preblended pork and its effects on fine-cut and coarse-ground pork sausage product characteristics. Journal of Muscle Foods, 2, 37-48.

Fukazawa, T., Hashimoto, Y., & Yatsui, T. (1961). The relationship between the components of myofibrillar protein and the effect of various phosphates that influence the binding quality of sausage. Journal of Food Science, 26, 550-555.

Giese, J. (1992). Developing low-fat meat products. Food Technology, 76 (4), 100-108.

Giese, J. (1996). Fats, oils and fat replacers. Food Technology, 50 (4), 78–84.

Gillette, M. (1985). Flavor effects of sodium chloride. Food Technology, 39 (6), 47–52.

Gines, J., Fernandez, J., Barbera, E., Sendra, E., & Alvarez, J. (2003). Effect of storage conditions on quality characteristics of bologna sausages made with citrus fiber. Journal of Food Science, 68, 710-715.

Gornall, A. G., Batdawill, C. J., & David, M. (1949). Determination of serum proteins by means of the biuret reagent. Journal of Biological Chemistry, 177, 751.

Grigelmo-Miguel, N., Abadias-Seros, M. I., & Martin-Belloso, O. (1999). Characterisation of low-fat high-dietary fiber frankfurters. Meat Science, 52, 247-256.

Gumpein, A. A., & Sørheim, O. (1987). Presalting effects on water retention of pork and beef batters. Food Chemistry, 23, 235-243.

Haddy F. J. (1991). Roles of salt, potassium, calcium, and natriuretic factors in hypertension. Hypertension, 18(suppl III):179-183.

Hamann, D. D. (1988). Rheology as a means of evaluating muscle functionality of processed foods. Food Technology, 42 (6), 66-71.

Hamm, R. (1986). Functional properties of the myofibrillar system and their measurement. In: Bechtel, P. J. (ed.). Muscle as Food Academic Press, Inc., San Diego, CA, USA, pp. 135-199.

Hamm, R. (1972). Kolloidchemie des fleishes. In: Ruusunen, M. and Puolanne, E. (2005). Reducing sodium intake from meat products. Meat Science, 70, 531-541.

Hand, L. W, Hollingsworth, C. A., Calkins, C. R., & Mandigo, R. W. (1987). Effects of preblending, reduced fat, and salt levels on frankfurter characteristics. Journal of Food Science, 52, 1149-1151.

Hand, L. W., Mandigo, R. W., & Calkins, C. R. (1990). The effects of preblending time on physical and textural properties of coarse ground sausages. Meat Science, 31, 13-24.

He, F. J. & MacGregor, G. A. (2009). A comprehensive review on salt and health and current experience of worldwide salt reduction programmes. Journal of Human Hypertension, 23, 363-384.

Health Canada. (1983). A rapid method for the determination of sodium and potassium. Health Protection Branch Laboratories, Ottawa. LPFC-125, June 1983.

Health Canada. (2012). Guidance for the food industry on reducing sodium in processed foods. Internet accessed on June 10, 2012 at http://www.hc-sc.gc.ca/fn-an/alt_formats/pdf/legislation/guide-ld/2012-sodium-reduction-indust-eng.pdf.

Heinz, G. & Hautzinger, P. (2007). Meat Processing Technology. Internet accessed on June 20, 2012 at http://www.fao.org/docrep/010/ai407e/ai407e00.htm

Hermansson, A.-M. (1982). The effect of presalting of meat on the properties of finely comminuted meat products. In: Gumpein, A. A., & Sørheim, O. (1987). Presalting effects on water retention of pork and beef batters. Food Chemistry, 23, 235-243.

Hong, G. P., Mon, S. G. & Chin, K. B. (2012). Emulsion properties of pork myofibrillar protein in combination with microbial transglutaminase and calcium alginate under various pH conditions. Meat Science, 90, 185-193.

Horita, C. N., Morgano, M. A., Celeghini, R. M. S., & Pollonio, M. A. R. (2011). Physico-chemical and sensory properties of reduced-fat mortadella prepared with blends of calcium, magnesium and potassium chloride as partial substitutes for sodium chloride. Meat Science, 89, 426-433.

Intersalt Cooperative Research Group. (1988). Intersalt: an international study of electrolyte excretion and blood pressure. Results for 24 hour urinary sodium and potassium excretion. British Medical Journal, 297, 319-328.

James, W. P., Ralph, A., & Sanchez-Castillo, J. (1987). The dominance of salt in manufactured food in the sodium intake of affluent societies. The Lancet, 329, 426-429.

Jimenez-Colmenero, F., Cofrades, S., Lopez-Lopez, I., Ruiz-Capillas, C., Pintado, T., & Solas, M. T. (2010). Technological and sensory characteristics of reduced/low-fat, low-salt frankfurters as affected by the addition of konjac and seaweed. Meat Science, 84, 356-363.

Joffres, M., Campbell, N., Manns, B., & Tu, K. (2007). Estimate of the benefits of a population-based reduction in dietary sodium additives on hypertension and its related health care costs in Canada. Canadian Journal of Cardiology, 23, 437-443.

Johnson, H. R., Aberle, E. D., Forrest, J. C., Hague, C. G., & Judge, M. D. (1977). Physical and chemical influences on meat emulsion stability in a model emulsitator. Journal of Food Science, 42, 522-525.

Katan, M. B., Boekscoten, M. V., Connor, W. E., Mensink, R. P ., Seidell, J., Vessby, B., & Willet, W. (2009). Which are the greatest recent discoveries and the greatest future challenges in nutrition? European Journal of Clinical Nutrition, 63, 2-10.

Keeton, J. T. (1993). Low-fat meat products – technological problems with processing. Meat Science, 36, 261-276.

Kerr, W.L., Wang, X., & Choi, S. G. (2005). Physical and sensory characteristics of low-fat Italian sausage prepared with hydrated oat. Journal of Food Quality, 28, 62–77.

Kerry, J. K., O'Donnell, A., Brown, H., Kerry, J. P., & Buckley, D. J. (1999). Optimization of transglutaminase as a cold set binder in low-salt beef and poultry comminuted meat products using response surface methodology. In: Carballo, J., Ayo, J., & Colmenero, F. J. (2006). Microbial transglutaminase and caseinate as cold set binders: Influence of species and chilling storage. LWT-Food Science and Technology, 39, 692-699.

Kilic, B. (2003). Effect of microbial transglutaminase and sodium caseinate on quality of chicken doner kebab. Meat Science 63, 417-421.

Knipe, C. L., Rust, R. E., & Olson, D. G. (1990). Some physical parameters involved in the addition of inorganic phosphates to reduced-sodium meat emulsions. Journal of Food Science, 55, 23-25.

Knipe, C. L., Olson, D. G., & Rust, R. E. (1985). Effects of selected inorganic phosphates, phosphate levels, and reduced sodium chloride levels on protein solubility, stability, and pH of meat emulsions. Journal of Food Science, 50, 1010-1013.

Kremer, S., Mojet, J., & Shimojo, R. (2009). Salt reduction in foods using naturally brewed soy sauce. Journal of Food Science, 74, 255-262.

Kristinsson, H.G. & Hultin, H.O. (2003). Role of pH and ionic strength on water relationships in washed minced chicken-breast muscle gels. Journal of Food Science, 68, 917-922.

Kuraishi, C., Sakamoto, J., Yamazaki, K., Sosa, Y., Kuhara, C., & Soeda, T. (1997). Production of restructured meat using microbial transglutaminase without salt or cooking. Journal of Food Science, 62, 88-90, 515.

Kuraishi, C., Yamazaki, K., & Susa, Y. (2001). Transglutaminase: its utilization in food industry. Food Reviews International, 17, 221-246.

Kurlansky, M. (2002). Salt: A world history. In: Morris, M. J, Na, E. S., & Johnson, A. K. (2009). Salt craving: the psychobiology of pathogenic sodium intake. Physiology and Behavior, 94, 709-721.

Laemmli, U.K. 1970. Cleavage of structural proteins during assembly of the head of bacteriophage T4. Nature 227 (5259), 680-685.

Lan, Y. H., Novakofski, J., McCusker, R. H., Brewer, M. S., Carr, T. R., & McKeith, F. K. (1995). Thermal gelation properties of protein fractions from pork and chicken breast muscles. Journal of Food Science, 60, 742-747.

Lanier, T. C. (1986). Functional properties of surimi. Food Technology, 49 (3), 107-114.

Lee, E. J., Kim, Y. J., Lee, N. H., Hong, S. I., & Yamamoto, K. (2007). Differences inproperties of myofibrillar proteins from bovine semitendinosus muscle after hydrostatic pressure or heat treatment. Journal of the Science of Food and Agriculture, 87, 40-46.

Li, W., Bowers, J., Craig, J., & Peng, S. (1993). Sodium tripolyphosphate stability and effect in ground turkey meat. Journal of Food Science, 58, 501-504.

Lindsay, R. C. (2007). Flavors. In: Damodaran S., Parkin, K., Fennema, O. R. (eds) Fennema's Food Chemistry. 4th ed. CRC Press, Taylor and Francis Group, Boca Raton, FL,USA, pp. 639-687.

Lipilina, E. & Ganji, V. (2009). Incorporation of ground flaxseed into bakery products and its effect on sensory and nutritional characteristics – a pilot study. Journal of Foodservice, 20, 52-59.

Loliger, J. (2000). Function and importance of glutamate for savory foods. The American Society for Nutritional Sciences, 130, 915-920.

Lyon, C. E., Lyon, B. G., Davis, C. E., & Townsend, W. E. (1980). Texture profile analysis of patties made from mixed and flaked-cut mechanically deboned poultry meat. Poultry Science, 59, 69-76.

MacFarlane, J. J. (1974). Pressure-induced solubilization of meat proteins in saline solution. Journal of Food Science, 39, 542-547.

MacGregor, G. A. (1983). Sodium and potassium intake and blood pressure. Hypertension, Supplement III, 5, 79-84.

Mansour, E. H. & Khalil, A. H. (1999). Characteristics of low fat beef burgers as influenced by various types of wheat fibers. Journal of the Science of Food and Agriculture, 79, 493-498.

Marambe, H. (2011). Generation and characterization of bioactive peptides from seed proteins of flax (*Linum usitatissimum* L.). Ph. D. Thesis, University of Saskatchewan.

Marcus, J. (2009). Unleashing the power of umami. Food Technology, 63, (11). Internet accessed on May 30, 2012 at http://www.ift.org/knowledge-center/learn-about-food-science/food-facts/unleashing-the-power-of-umami.aspx

Martin, J. W. & Rogers, R. W. (1991). Cure levels, processing methods, and meat source effects on low-fat frankfurters. Journal of Food Science 56, 59-61.

Matulis, R. J., McKeith, F.K., Sutherland, J., & Brewer S. (1995). Sensory characteristics of frankfurters as affected by fat, salt, and pH. Journal of Food Science, 60, 42-47.

Mazza, G., & Biliaderis, C. G. (1989). Functional properties of flaxseed mucilage. Journal of Food Science, 54, 1302-1305.

Meilgaard, M., Civille, G. V., & Carr, B. T. (1999). Sensory Evaluation Techniques. 3rd ed. CRC Press LLC, Florida, USA. pp.123-158.

Miller, I. J., & Barthoshuk, L. M. (1991). Taste perception, taste bud distribution and spatial relationship. In: Desmond, E. (2006). Reducing salt: A challenge for the meat industry. Meat Science, 74, 188-196.

Miller, V. (2009). The Truth About Salt. (2009). Internet accessed on August 20, 2012 at http://naturalbias.com/the-truth-about-salt/

Min, B. & Green, B. W. (2008). Use of microbial transglutaminase and nonmeat proteins to improve functional properties of low NaCl, phosphate-free patties made from channel catfish (*Ictalurus punctatus*) belly flap meat. Journal of Food Science, 73, 218-226.

Mitchell, M., Brunton, N. P., & Wilkinson, M. G. (2011). Impact of salt reduction on the instrumental and sensory flavor profile of vegetable soup. Food Research International, 44, 1036-1044.

Mojet, J., Heidema, J., & Christ-Hazelhof, E. (2004). Effect of concentration on taste-taste interactions in foods for elderly and young subjects. Chemical Senses, 29, 671-681.

Monaco di, R., Cavella, S., & Masi, P. (2008). Predicting sensory cohesiveness, hardness, and springiness of solid foods from instrumental measurements. Journal of Texture Studies, 39, 129-149.

Montejano, J. G., Hamann, D. D, & Lanier, T. C. (1985). Comparison of two instrumental methods with sensory texture of protein gels. Journal of Texture Studies, 16, 403-424.

Motoki, M. & Kumazawa, Y. (2000). Recent trends in transglutaminase technology for food processing. Food Science Technology and Research, 6, 151–160.

Motoki, M. & Suguro, K. (1998). Transglutaminase and its use for food processing. Trends in Food Science and Technology, 9, 204-210.

Morin, L. A., Temelli, F., & McMullen, L. (2002). Physical and sensory characteristics of reduced-fat breakfast sausages formulated with barley beta-glucan. Journal of Food Science, 67, 2391–2396.

Morris, E. R. (1987). Organoleptic properties of food polysaccharides in thickened systems. In: Hollowood, T. A., Linforth, R. ST., and Tayor, A. J. (2002). The effect of viscosity on the perception of flavor. Chemical Senses, 27, 583-591.

Morris, M. J, Na, E. S., & Johnson, A. K. (2009). Salt craving: the psychobiology of pathogenic sodium intake. Physiology Behavior, 94, 709-721.

Muruguma, M., Tsuruoka, K., Katayama, K., Erwanto, Y., Kawahara, S., Yamauchi, K., Sathe, S. K., & Soeda, T. (2003). Soybean and milk proteins modified by transglutaminase improves chicken sausage texture even at reduced levels of phosphate. Meat Science, 63, 191-197.

Nelson, D. L. & Cox, D. L. (2008). Lehninger's Principles of Biochemistry. 5th ed. W. H. Freeman Publishers, NY, USA, pp. 20-40.

Nielsen, G. S., Peterson, B. R., & Møller, A. J. (1995). Impact of salt, phosphate and temperature on the effect of a transglutaminase (F XIIIa) on the texture of restructured meat. Meat Science, 41, 293-299

Nu-tek Salt. 2012. Nu-Tek Potassium Chloride. Internet accessed on May 10, 2012 at http://www.nu-teksalt.com/

Oakenfull, J. P. & Burley, R. W. (1997). Protein gelation. In: S. Damodaran, A. Paraf (Eds.). Food proteins and their applications, Marcel Dekker, Inc., New York, USA, pp. 111-142

Ockerman, H. W. & Crespo, F. L. (1982). Physicochemical changes occurring during storage of precured beef blends at different temperatures and two levels of salt. Journal of Food Science, 47, 849-851.

Offer, G. & Knight, P. (1988). The structural basis of water holding in meat. In: Lawrie R. (ed). Developments in Meat Science. London, UK: Elsevier Applied Science Publishers, pp. 63-172.

Offer, G. & Trinick, J. (1983). On the mechanisms of water holding in meat: the swelling and shrinking of myofibrils. Meat Science, 8, 245-281.

Ohtsuka, T., Umezawa, Y., Nio, N., & Kubota, K. (2001). Comparison of deamidation activity of transglutaminase. Journal of Food Science, 66, 25-29.

O'Kennedy, B. (2000). Use of novel dairy ingredients in processed meats. In: Carballo, J., Ayo, J., & Colmenero, F. J. (2006). Microbial transglutaminase and caseinate as cold set binders: Influence of species and chilling storage. LWT-Food Science and Technology, 39, 692-699.

Okiyama, A. & Beauchamp, G. K. (1998). Taste dimensions of monosodium glutamate (MSG) in a food system: role of glutamate in young American subjects. Physiology and Behavior, 65, 177-181.

Oliveira, S. M., Lopes, T. I. M. S., & Rangel, A. O. S. (2004). Spectrophotometric determination of nitrite and nitrate in cured meat by sequential injection analysis. Journal of Food Science, 69, 690-694.

Oomah, D. (2001). Flaxseed as a functional food source. Journal of the Science of Food and Agriculture, 81, 889-894.

Oomah, D. & Mazza, G. (1993). Flaxseed proteins review. Food Chemistry, 48, 109-114.

Pando, G. D., Cofrades, S., Capillas, C., Solas, M. T., Triki, M., & Colmenero, F. J. (2011). Low-fat frankfurters formulated with a healthier lipid combination as functional ingredient: microstructure, lipid oxidation, nitrite content, microbiological changes, and biogenic amine formation. Meat Science, 89, 65-71.

Pangborn, R. & Szczesniak, A. S. (1974). Effect of hydrocolloids and viscosity on flavor and odor intensities of aromatic flavor compounds. Journal of Texture Studies, 4, 467-482.

Pearson, A. M. & Wolzak, A. M. (1982). Salt-its use in animal products- a human health dilemma. Journal of Animal Science, 54, 1263-1268.

Pearson, A. M. & Tauber, F. W. (1984). Processed Meats. 2nd ed. AVI Publishing Co Inc., Westport, CT, USA, pp. 53-77

Pelser, W. M., Linssen, J. P., Legger, A., & Houben, J. H. (2007). Lipid oxidation in n-3 fatty aid enriched Dutch style fermented sausages. Meat Science, 75, 1-11.

Perez-Alvarez, J. A. & Fernandez-Lopez, J. (2006). Chemistry and biochemistry of color in muscle foods. In: Hui, Y. H. (ed). Food Biochemistry and Food Processing. Blackwell Publishing, IA, USA, pp. 337-351.

Perez-Juan, M., Flores, M., & Toldra, F. (2008). Effect of meat proteins on the binding of the volatile compounds. Food Chemistry, 108, 1226-1232.

Pietrasik, Z. (2003). Binding and textural properties of beef gels processed with k-carrageenan, egg albumin, and microbial transglutaminase. Meat Science, 63, 317-324.

Pietrasik, Z. & Janz, J. A. (2008). Influence of freezing and thawing on the hydration characteristics, quality, and consumer acceptance of whole muscle beef injected with solutions of salt and phosphate. Meat Science, 81, 523-532.

Pietrasik, Z., Jarmoluk, A., & Shand, P. J. (2007). Effect of non-meat proteins on hydration and textural properties of pork meat gel enhanced with microbial transglutaminase. LWT-Food Science and Technology, 40, 915-20.

Pietrasik, Z. & Li-Chan, E. C. Y. (2002). Binding and textural properties of beef gels as affected by protein, k-carrageenan and microbial transglutaminase addition. Food Research International, 35, 91-98.

Poulanne, E., Ruusunen, M., & Vainionpaa, J. (2001). Combined effects of NaCl and raw meat pH on water-holding in cooked sausage with and without added phosphate. Meat Science, 58, 1-7.

Rabe, S., Krings, U., & Berger, R. G. (2003). Initial dynamic flavor release from sodium chloride solutions. European Food Research and Technology, 218, 32-39.

Rabetafika, H., Remoortel, V., Danthine, S., Paquot, M., & Blecker, C. (2011). Flaxseed proteins: food uses and health benefits. International Journal of Food Science and Technology, 46, 221-228.

Radam, A, Yacob, M., Bee, T., & Selamat, J. (2010). Perceptions, attitudes and willingness to pay towards food products with "no added MSG" labeling. International Journal of Marketing Studies, 2, 65-77.

Raiten, D. J., Talbot, J. M., & Fisher, K. D. (1995). Executive summary from the report: Analysis of adverse reactions to monosodium glutamate (MSG). The Journal of Nutrition, 125, 2891-2906.

Ramirez, J. A., Uresti, R., Tellez, S., & Vasquez, M. (2002). Using salt and microbial transglutaminase as binding agents in restructured fish products resembling hams. Journal of Food Science, 67, 1778-1784.

Reineccius, G. (2005). Flavor Chemistry and Technology. 2nd ed. CRC Press Taylor and Francis Group, FL, USA, pp. 313-315.

Roininen, K., Lahteenmaki, L., & Tuorila, H. (1996). Effect of umami taste on pleasantness of low-salt soups during repeated testing. Physiology and Behavior, 60, 953-958.

Russ, N. J., G. W. Gould (eds.). (2003). Food Preservatives. 2nd Ed. Kluwer Academic/Plenum Publishers, NY, USA, pp. 102-115.

Ruusunen, M. & Puolanne, E. (2005). Reducing sodium intake from meat products. Meat Science, 70, 531-541.

Ruusunen, M., Sarkka-Tirkkonen, M., & Poulannne, E. (1999). The effect of salt reduction on taste pleasantness in cooked 'bologna-type' sausages. Journal of Sensory Studies, 14, 263-270.

Ruusunen, M., Vainionpaa, J., Puolanne, E., Lyly, M., Lähteenmäki, L., Niemistö, L., &Ahvenainen, R. (2003). Effect of sodium citrate, carboxymethyl cellulose and carrageenan levels on quality characteristics of low-salt and low-fat bologna type sausages. Meat Science, 64, 371-381.

Ruusunen, M., Vainionpaa, J., Lyly, M., Lahteenmaki, L., Niemisto, M., Ahvenainen, R., & Poulanne, E. (2005). Reducing the sodium content in meat products: the effect of the formulation in low-sodium ground meat patties. Meat Science, 69, 53-60.

Saliba, D. A., Foegeding, E. A. & Hamann, D. D. 1987. Structural failure and nondestructive rheological analyses of frankfurter batters: Effects of heating rates and sugars. Journal of Texture Studies, 18, 241-259.

Salt Institute. (2012). Production and industry. Internet accessed on April 3, 2012 at http://www.saltinstitute.org/production-industry.

Samejima, K., Hashimoto, Y., Yasui, T., & Fukazawa, T. (1969). Heat gelling properties of myosin, actin, actomyosin and myosin-subunits in a saline model system. Journal of Food Science, 34, 242-245

Samejima K, Ishioroshi M, & Yasui T. (1982). Heat induced gelling properties of actomyosin: effect of tropomyosin and troponin. In: Sun, X. D. & Holley, R. A. (2011). Factors influencing gel formation by myofibrillar proteins in muscle foods. Comprehensive Reviews in Food Science and Food Safety, 10, 33-51.

Sanai, T. & Kimura, G. (1996). Renal function reserve essential hypertension and sodium sensitivity in essential hypertension. Journal of Laboratory and Clinical Medicine, 128, 89-97.

SAS Institute, Inc. (1989). SAS Users Guide: Basics, Version 6.06 Edition. Statistical Analysis System Institute Inc., Cary, NC, USA.

Saxton, A.M. (1998). A macro for converting mean separation output to letter groupings in Proc mixed. In: Proceedings 23rd SAS Users Group International, SAS Institute, Cary, NC, USA, pp.1243-1246.

Searby, L. (2006). Pass the salt. International Food Ingredients (February/March), 6-8.

Seman, D. L., Olson, D. G., & Mandigo, R. W. (1980). Effect of reduction and partial replacement of sodium on bologna characteristics and acceptability. Journal of Food Science, 45,1116-1121.

Shand, P. J. (2000). Textural, water holding, and sensory properties of low-fat pork bologna with normal or waxy starch hull-less barley. Journal of Food Science, 65, 101-107.

Shang, Y. & Xiong, Y. 2010. Xanthan enhances water binding and gel formation of transglutaminase-treated porcine myofibrillar proteins. Journal of Food Science, 75, 178-185.

Sherman, R. A. & Mehta, O. (2009). Phosphorus and potassium content of enhanced meat and poultry products: Implication for patients who receive dialysis. Clinical Journal of American Society of Nephrologist, 4, 1370-1373.

Siegel, D. G. & Schmidt, G. R. (1979). Ionic, pH, and temperature effects on the binding ability of myosin. Journal of Food Science, 44, 1686-1689.

Skaara, T. & Regenstein, J. M. (1990). The structure and properties of myofibrillar proteins in beef, poultry, and fish. Journal of Muscle Foods, 1, 269-291.

Smith, D. M. (2001). Functional properties of muscle proteins in processed poultry products. In: Sun, X. D. & Holley, R. A. (2011). Factors influencing gel formation by myofibrillar proteins in muscle foods. Comprehensive Reviews in Food Science and Food Safety, 10, 33-51.

Smith, J. L., Maurer, M. J., Bencivengo, M. M., & Kunsh, C. A. (1987). Effect of sodium chloride on uptake of substrate by *Staphylococcus aureus* 196E. In: Davidson, P. M.,

Sofos, J. N. & Branen, A. L. (eds). (2005). Antimicrobials in Food. 3rd ed. CRC Press Taylor and Francis Group, FL, USA, pp. 573-578

Sofos, J. N. (1983). Effects of reduced salt (NaCl) on the stability of frankfurters. Journal of Food Science, 48, 1684-1691.

Soeda, T., Kasagi, T., Hokazono, A., Yamazaki, K., & Muruguma, M. (2006). Effects of salt and polyphosphate on physical properties of meat gel treated with microbial transglutaminase. Nippon Shokuhin Kagaku Kaisi, 53, 109-113.

Steinmann, R. & Fischer, A. (1993). Frankfurter-type sausage manufacture. Protein solubility and stability in frankfurter-type sausage as a function of salt concentration, nitrogen cooling, and diphosphate. In: Eilert, S. J. and Mandigo, R. W. (1996). Use of phosphates and salt to improve lean meat water retention in high-moisture preblends. Journal of Muscle Foods, 7, 1-16.

Strasburg, G., Xiong, Y., & Chiang, W. (2007). Physiology and chemistry of edible muscle tissues. In: Damodaran, S., Parkin, K., Fennema, O. R. (eds). Fennema's Food Chemistry. 4th ed. CRC Press Taylor and Francis Group, FL, USA, pp.923-973

Sullivan, G. A. (2011). Naturally cured meats: Quality, safety, and chemistry. Ph. D. Thesis. Internet accessed on May 30, 2012 at http://lib.dr.iastate.edu/etd/12208

Sun, X. D. & Holley, R. A. (2010). High hydrostatic pressure effects on the texture of meat and meat products. Journal of Food Science, 75, 17-23.

Sun, X. D. & Holley, R. A. (2011). Factors influencing gel formation by myofibrillar proteins in muscle foods. Comprehensive Reviews in Food Science and Food Safety, 10, 33-51.

Tellez-Luis, S. J., Uresti, R. M., Ramirez, J.A., Vazquez, M. (2002). Low-salt restructured fish products using microbial transglutaminase as binding agent. Journal of the Science of Food and Agriculture, 82, 953-959.

Thebaudin, J. Y., Lefebvre, A. C., Harrington, M., & Bourgeois, C. M. (1997). Dietary fibers: nutritional and technological interest. Trends in Food Science, 8, 41-48.

Thushan Sanjeewa, W. G., Wanasundara, J. D. J., Pietrasik, Z., & Shand, P. J. (2010). Characterization of chickpea (*Cicer arietinum L*.) flours and application in low-fat pork bologna as a model system. Food Research International, 43, 617-626.

The Canadian Salt Company Limited (Windsor Salt). (2009). Hi grade granulated and hi Grade granulated untreated salt: Regina, Saskatchewan production.
Poite-Claire, Quebec.

Tobin, B. D., O'Sullivan, M. G., Hamill, R. M., & Kerry, J. P. (2012). Effect of varying salt and fat levels on the sensory quality of beef patties. Meat Science 91, 460-465.

Treasure, J. & Ploth, D. (1983). Role of dietary potassium in the treatment of hypertension. Hypertension, 5, 864-872.

Troutt, E. S., Hunt, M. C., Johnson, D. E., Claus, J. R., Kastner, C. L., & Kropf, D. H. (1992). Characteristics of low-fat ground beef containing texture modifying ingredients. Journal of Food Science, 57, 19-24.

Trout, G. R. & Schmidt, G. R. (1984). Effect of phosphate type and concentration, salt level, and method of preparation on binding in restructured beef rolls. Journal of Food Science, 49, 687-694.

Trout, G. R. & Schmidt, G. R. (1986). Water binding ability of meat products: Effect of fat level, effective salt concentration, and cooking temperature. Journal of Food Science, 51, 1061-1062.

Tseng, T. F., Liu, D. C., & Chen, M. T. (2000). Evaluation of transglutaminase on the quality of low-salt chicken meat balls. Meat Science 55, 427-431.

Ugawa, T., Knonosu, S., & Kurihara, K. (1992). Enhancing effects of NaCl and Na-phosphate on human gustatory responses to amino acids. Chemical Senses, 17, 811-815

Velasquez, M. T., Bhathena, S. J., Ranich, T., Schwartz, A. M., Cardon, D.E., Ali, A. A., Haudenschild, C. C., & Hansen, C. T. (2003). Dietary flaxseed meal reduces proteinuria and ameliorates nephropathy in an animal model of type II diabetes mellitus. Kidney International, 64, 2100-2107.

Ventanas, S., Puolanne, E., & Tuorila, H. (2010). Temporal changes of flavor and texture in cooked bologna type sausages as affected by fat and salt content. Meat Science, 85, 410-419.

Viuda-Martos, M., Ruiz-Navajas, Y., Fernandez-Lopez, J., & Perez-Alvarez, J. A. (2009). Effect of orange dietary fiber, oregano essential oil and packaging conditions on shelf-life of bologna sausages. Food Control, 21, 436-443.

Viuda-Martos, M., Ruiz-Navajas, Y., Fernandez-Lopez, J., & Perez-Alvarez, J. A. (2010). Effect of added citrus fiber and spice essential oils on quality characteristics and shelf-life of mortadella. Meat Science, 85, 568-576.

Wanasundara, J. & Shadidi, F. (1994). Functional properties and amino-acid composition of solvent-extracted flaxseed meals. Food Chemistry, 49, 45-51.

Wang, B., Li, D., Wang, L. J., & Ozkan, N. (2010). Effect of concentrated flaxseed protein on the stability and rheological properties of soybean oil-in-water emulsions. Journal of Food Engineering, 96, 555-561.

Wardener, H. E. & MacGregor, G. A. (2002). Harmful effects of dietary salt in addition to hypertension. Journal of Human Hypertension, 16, 213-223.

Warrand, J., Michaud, P., Picton, L., Muller, G., Courtois, B., Ralainirina, R., & Courtois, J.(2005). Structural investigations of the neutral polysaccharide of *Linumusitatissimum* L. Seed mucilage. International Journal of Biological Macromolecules, 35, 121-125.

Weller, J. M. (1980). Sodium, its importance in health and disease. Sodium and hypertension. In: Moses, C. (ed). Sodium in medicine and health A monograph. Reese Press, Inc. Baltimore, USA. pp. 55-61.

Weiss, J., Gibis, M., Schuh, V., & Salminen, H. (2010). Advances in ingredient and processing systems for meat and meat products. Meat Science, 86, 196-213.

Whiting, R. C. (1984a). Addition of phosphates, proteins, and gums to reduced-salt frankfurter batters. Journal of Food Science, 49, 1355-1357.

Whiting, R. C. (1984b). Stability and gel strength of frankfurter batters made with reduced NaCl. Journal of Food Science, 49, 1350-1354.

Wilding, P., Hedges, N., & Lillford, P. J. (1986). Salt-induced swelling of meat: the effect of storage time, pH, ion-type, and concentration. Meat Science, 18, 55-75.

WCRF. (2007). Policy and action for cancer prevention. Food, nutrition, and physical activity: A global perspective. Washington, DC.

World Food Programme. (2009). Salt production quality control. Internet accessed on May 8, 2012 at http://foodquality.wfp.org/FoodProcessing/Saltproduction/tabid/332/Default.aspx

WHO. (2003). Diet, Nutrition and the prevention of chronic diseases. In:WHO Technical report series 916. Geneva: World Health Organisation /Food and Agricultural Organisation. Internet accessed on June 15, 2012 at http://libdoc.who.int/trs/WHO_TRS_894.pdf

Xiong, Y. L. & Brekke, C. J. (1991). Gelation properties of chicken myofibrils treated with calcium and magnesium chlorides. Journal of Muscle Foods, 2, 21-36.

Xiong, Y. L., Noel, D. C., & Moody, W. G. (1999). Textural and sensory properties of low-fat beef sausages with added water and polysaccharides as affected by pH and salt. Journal of Food Science, 64, 550-554.

Yamaguchi, S. & Takahashi, C. (1984). Interactions of monosodium glutamate and sodium chloride on saltiness and palatability of a clear soup. Journal of Food Science, 49, 82-85.

Yamamoto, K., Yoshida, T., & Iwasaki, K. (2002). Hydrostatic pressure-induced solubilization and gelation of chicken myofibrils. Progress in Biotechnology, 19, 461-468.

Yang, H. L. & Lawless, H. T. (2005). Descriptive analysis of divalent salts. Journal of Sensory Studies, 20, 97-113.

Yasui, T., Ishioroshi, M., & Samejima, K. (1980). Heat-induced gelation of myosin in the presence of actin. Journal of Food Biochemistry, 4, 61-78.

Appendix A. Summary of statistical analyses for study 1

A-1 Type 3 tests of fixed effects for batter viscosity of meat batter

Effects	Probability
Salt	0.8432
Level	<0.0001
Hold	<0.0001
Salt*level	0.7634
Salt*Hold	0.7679
Level*Hold	0.0023
Salt*Level*Hold	0.4342

A-2 Type 3 tests of fixed effects for protein solubility of meat batter

Effects	Probability
Salt	0.9277
Level	0.0002
Hold	0.0009
Salt*level	0.4385
Salt*Hold	0.7769
Level*Hold	0.0918
Salt*Level*Hold	0.4620

A-3 Type 3 tests of fixed effects for water holding capacity of bologna samples

Effects	Probability		
	Cook loss	Purge loss	Expressible moisture
Salt	0.1957	0.4448	0.2407
Level	<0.001	0.0494	<0.001
Hold	0.0965	0.4082	<0.005
Salt*level	0.7863	0.9400	0.0816
Salt*Hold	0.8139	0.7746	0.5537
Level*Hold	0.2099	0.7593	<0.001
Salt*Level*Hold	0.7403	06436	0.9865

A-4 Type 3 tests of fixed effects for TPA of bologna samples

Effects	Probability		
	Hardness	Cohesiveness	Springiness
Salt	0.0514	0.2964	0.5638
Level	<0.0001	<0.0001	<0.0001
Hold	<0.0001	<0.0001	<0.0001
Salt*level	0.6718	0.1092	0.3477
Salt*Hold	0.9349	0.0867	0.5808
Level*Hold	<0.0001	<0.0001	<0.0001
Salt*Level*Hold	0.9008	0.4140	0.5909

A-5 Type 3 tests of fixed effects for shear stress and shear strain of bologna samples

Effects	Probability	
	Shear stress	Shear strain
Salt	0.0847	0.7556
Level	<0.0001	0.0072
Hold	0.0001	0.0134
Salt*level	0.7576	0.5023
Salt*Hold	0.8932	0.1910
Level*Hold	0.0004	0.5482
Salt*Level*Hold	0.4724	0.4866

A-6 Type 3 tests of fixed effects for sensory characteristics of bologna

Effects	Probability				
	Firmness	Springiness	Juiciness	Flavour	Saltiness
Salt	0.8792	0.5846	0.1437	0.9365	0.8178
Level	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Hold	<0.0001	<0.0001	0.0551	0.1522	0.3334
Salt*level	0.4209	0.0748	0.2926	0.6816	0.2249
Salt*Hold	0.6485	0.3773	0.2007	0.4737	0.8178
Level*Hold	<0.0001	<0.0001	<0.0001	0.2131	0.6246
Salt*Level*Hold	0.6555	0.7206	0.7799	0.4330	0.7096

Appendix B. Summary of statistical analyses for study 2

B-1 Type 3 tests of fixed effects for batter viscosity of meat batter

Effects	Probability
MTG	0.0359
FSM	<0.0001
MTG*FSM	0.4148

B-2 Type 3 tests of fixed effects for water holding capacity of bologna

	Probability		
Effects	Cook loss	Purge loss	Expressible moisture
MTG	0.0569	0.1644	0.1644
FSM	0.4967	<0.0001	<0.0001
MTG*FSM	0.4376	0.8310	0.8573

B-3 Type 3 tests of fixed effects for instrumental color of bologna samples

	Probability		
Effects	L*	a*	b*
MTG	0.1114	0.0670	0.0.0958
FSM	<0.0001	<0.0001	<0.0001
MTG*FSM	0.6929	0.9885	0.8051

B-4 Type 3 tests of fixed effects for residual nitrite of cooked bologna

Effects	Probability
MTG	0.0017
FSM	0.0003
MTG*FSM	0.9117

B-5 Type 3 tests of fixed effects for TPA of bologna samples

Effects	Probability		
	Hardness	Cohesiveness	Springiness
MTG	<0.0001	<0.0001	<0.0001
FSM	<0.0001	0.0002	0.0680
MTG*FSM	0.1563	0.8653	0.6367

B-6 Type 3 tests of fixed effects for sensory colour, spice intensity, saltineass and overall acceptability of bologna samples

Effects	Probability				
	Colour	Spice intensity	Foreign flavor	Saltiness	Overall acceptability
MTG	0.5470	0.0824	0.4434	0.2609	0.0905
FSM	0.0069	0.0960	0.7442	0.0484	0.0324
MTG*FSM	0.9045	0.7713	0.9080	0.7361	0.7874

B-7 Type 3 tests of fixed effects for sensory of bologna samples

Effects	Probability		
	Firmness	Chewiness	Juiciness
MTG	0.0002	0.0002	0.2609
FSM	0.9742	0.98889	0.0584
MTG*FSM	0.6341	0.7030	0.7361