

BAKSO (TRADITIONAL INDONESIAN MEATBALL) PROPERTIES WITH  
POSTMORTEM CONDITION AND FROZEN STORAGE

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## ABSTRACT

Bakso is a finely comminuted boiled Indonesian meat product that is traditionally made of starch, salt and emulsified prerigor or early postmortem meat and often sold from street vendors. Recently processors have begun to commercially manufacture bakso. This research was conducted to investigate the substitution of early postmortem meat with postrigor meat to allow more efficient manufacturing and raw material procurement. The first experiment was to determine the properties of bakso with three tapioca starch concentrations added to early or late postmortem beef. No differences ( $p < 0.05$ ) were observed in bakso properties of composition and texture, though bakso made of post-rigor meat had slightly less elasticity, strength and shear. These disadvantages were compensated by incorporating 15% starch concentration, indicating that the replacement of early postmortem meat with postrigor meat was applicable in industrial mass production of bakso. The second phase of experiments was to investigate the properties of bakso with different frozen storage times of raw postrigor meat and after different times of frozen storage for bakso made from postrigor and early postmortem meat. Postrigor meat substitution for early postmortem meat in bakso with 15% tapioca starch resulted in minimum composition and textural differences ( $p > 0.05$ ). Meat stored frozen for 2 and 4 months was still suitable as raw material for bakso production. The decreased oxidative stability in bakso made from postrigor meat after frozen storage of 2 and 4 months should be addressed with antioxidant ingredients or procedures to minimize potential off-flavors of the precooked bakso products stored frozen.

## CHAPTER 1. INTRODUCTION

Food is an integral part of human society, providing nourishment and cultural pleasure. People of developed countries with sufficient calorie intake are faced with concerns of obesity and related diseases that are caused by high calorie intake. In other parts of the world that are less developed, food supplies limit intake of adequate calories. A well-balanced agriculture provides variety in food type availability and consequently a potentially well-balanced diet (Romans *et al.*, 1994).

Romans *et al.* (1994) stated that meat and meat products, as foods, are dense in nutrients, where the major sources of calories are fats and proteins. Lawrie (1991) defined meat as animal flesh that is used as food. Meat consumption in general is of muscles, but organs such as livers, kidneys, brains and other edible tissue are also prepared as delicacies in certain parts of the world (Lawrie, 1991).

Variability in preparation and meat eating are different in different parts of the world and are also affected by the needs and demands of the consumers (Lawrie, 1991; Romans *et al.*, 1994). The growing complexity and hectic metropolitan life have developed demands for ready-to-eat prepared foods described as convenience foods (Roman *et al.*, 1994). Increasing popularity of convenience food has a new impact in the retail food industries and the food service industries (Romans *et al.*, 1994).

The speed or ease of preparation is a defining factor in convenience foods (Sloan, 2003). Convenience foods can be of several types. Processed meats are in a form more readily used by consumers than unprocessed meats. Raw processed meat require more preparation time than pre-cooked meat that only requires reheating before serving. Ready-to-eat (RTE) items can be consumed directly from the package without any additional

preparation although these usually are heated to some extent. Increasing amounts of meat are being processed into more usable forms. Example of this would be patties or meatballs formed from ground meat, cooking of roast beef, and slicing of luncheon meats for packaging and consumer purchasing in these forms.

Meatballs are very well known worldwide, such as the polpetta of Italy, Königsberger Klöße or “meatballs in lemon and caper” of Germany, Nunh Hoa of Vietnam, Curried Koftas of India and Chinese meatballs of China (Fulton, 1983; Duong and Kiesel, 1991). The popularity of meatballs and fishballs extends throughout Asia. Meatballs in Indonesia are known as bakso. Traditionally, Indonesian bakso are produced from prerigor or early postmortem meat by grinding the meat and emulsifying with salt, starch (tapioca) and garlic. The batter is shaped and formed into balls and cooked in boiling water. Bakso is commonly served with boiled chicken stock/soup and distributed by peddlers from a pushcart at street corners (Pandisurya, 1983; Triatmojo, 1992).

Bakso popularity in Indonesia has attracted interest in the traditional food as a business opportunity by the Indonesian food industry. Several Indonesian food companies are integrating bakso into their production lines and bringing bakso into full industrial scale while providing food safety attributes for bakso consumers. In order to fulfill the increasing demand, processors have upscaled from home industry type production to mass production of bakso packed in vacuum packages and sold frozen at supermarkets or grocery stores. Traditional bakso are produced from prerigor or early postmortem meat obtained from traditional butchers and local traditional market-places. It is difficult for large-scale industries to obtain sufficient quantities of fresh prerigor or early postmortem meat due to the slaughter frequency in small meat plants. The use of late postmortem meat would be a solution for

large scale manufacture of bakso by allowing bakso producers to have supplies of raw material in inventory and limit the need for daily purchase of recently slaughtered early postmortem raw material. The additional requirements of raw material space and freezing facilities for late postmortem meat are already possessed by most meat producers. The storage and distribution of bakso in the frozen state provides consistent product supplies for consumers.

The objectives of this research were to evaluate the properties of bakso after substitution of late postmortem meat for early postmortem meat in bakso produced with three levels of tapioca starch and to determine the influence of frozen storage time of late postmortem meat before processing and frozen storage time of finished products on the properties of bakso.

## CHAPTER 2. LITERATURE REVIEW

### General

Processed meats are products that have been altered in form, size, shape, function or palatability to provide a more highly desired product by consumers. There are many different types of processing, including particle size reduction, freezing, curing, tenderizing and forming. Depending on processing methods, particle size reduction and structuring result in flaked, formed, chunked, sectioned and formed, or chopped and formed products.

Comminuted meat products are mixtures of protein, fat particles, water and carbohydrate (Barbut, 1995). During processing, the meat is mixed with ingredients, commonly salt, phosphate, and protein or carbohydrate binders that will bind the particles back together directly or indirectly. The mixture is formed to the desired shape and the formed shape will be maintained after freezing or cooking (Romans *et al.*, 1994).

Finely comminuted products include various sausages, frankfurters, bologna, and some meat loaves (Barbut, 1995). These products demonstrate a homogenous appearance in structure when sliced. Meatballs are processed meats that fall into the category of restructured meat products. Meatballs are very popular among some countries within the Asian region and certain European cuisine. Indonesian meatballs (bakso) also fall into the category of finely comminuted or emulsified meat products. The Asian type meatballs are commonly produced by emulsifying ground meat with salt and starch, mixing herbs specific to the ethnic cuisine, and finally shaping into balls. The balls are cooked by steaming, boiling, or deep-frying, depending on the cuisine. Bakso is traditionally made from prerigor meat or before it has undergone rigor mortis (Purnomo, 1990).

## **Rigor Mortis**

### **Factors Influencing Rigor Mortis**

When oxygen is permanently removed from the muscle postmortem, irreversible anaerobic glycolysis will occur and the muscle becomes inextensible. This is the stiffness of death or rigor mortis. Muscle contraction and relaxation requires energy and ATP provides the energy that the muscle needs to function (Lawrie, 1991). In the absence of oxygen, the enzymatic systems of the cell that are maintaining the levels of ATP will use creatine phosphate (CP) to generate ATP from ADP, since the aerobic circulation of metabolites is ceased. The major source of energy in these circumstances is through the breakdown of glycogen via glycolysis producing ATP and lactate (Lundberg and Vogel, 1987).

Koohmaraie *et al.* (1984) reported that aging of meat after rigor mortis has been practiced for many years to improve tenderness. Muscle proteolysis appears to be the major cause of changes, where the two most-likely indigenous proteolytic systems that may be major contributors are the  $\text{Ca}^{2+}$  dependent proteases and the cathepsins (Koohmaraie *et al.*, 1988). The degradation and structural changes of the Z-line resulting in the fragmentation of myofibrils and the appearance of 30,000 dalton components are the major changes observed in postmortem aging. These changes in the muscle during postmortem aging are considered due to calcium-activated factors known as the calpain system (Koohmaraie *et al.*, 1984; Ahn *et al.*, 2003). The calpain system is responsible for the initiation of myofibrillar turnover in muscle. Calpain activity is proportionally inverse to the postrigor activity of calpastatin, the endogenous inhibitor of calpain (Doumit *et al.*, 1996). Degradation of the z-line and fragmentation of myofibrillar proteins during postmortem aging correlates to decreasing

shear force values and has been consistently related to tenderization of meat (Ahn *et al.*, 2003).

### **Prerigor and Postrigor Meat in Restructured Meats**

Muscle (meat), either in pre- or postrigor state, can be used in meat processing. Processing in the different states results in different processing characteristics, including water-binding capacity, fat binding, emulsification properties, stability against oxidation, and product color. All of these attributes contribute to the acceptability of the product (Pearson and Young, 1989).

According to Lawrie (1991), reformed meat or restructured meat can be prepared from prerigor meat, where high water holding capacity of prerigor meats would be an advantage in the manufacture of restructured meat products. The comminution process would induce rigor mortis, but conducting the comminution process in the presence of salt immediately postmortem can reduce the event of rigor. Unfortunately, employing this treatment can be difficult in industry situations because of the time needed to debone the meat before rigor onset. An increase in pH of postrigor meat by addition of alkaline compounds improves processing properties, but not to the same level as with prerigor meat (Lawrie, 1991).

Pearson and Young (1989) described the distinct advantages of prerigor meat over postrigor meat as higher water binding capacity, greater ability to emulsify fat, the formation of a more stable fat emulsion, a more stable red color, and a lower susceptibility to oxidation. Pre rigor-meat has greater fat emulsifying capacity than postrigor meat, since gel strength decreases on the aging of myosin at ionic concentrations of less than 0.3 M KCl (Pearson and Young, 1989).

During postmortem storage, textural and structural changes in muscle occur and are generally due to the interactions of the myofibrillar proteins in muscle tissues.

Transformations of unattached actin and myosin (prerigor) to the actomyosin complex (postrigor) result in changes of the protein properties, also affecting final meat products (Xiong and Brekke, 1991).

### **Comminuted Meats**

Meat comminution is the process of reducing or chopping meat to a finely particulated state (Acton *et al.*, 1983). Blending comminuted meats along with other non-meat ingredients forms a coarse dispersion of water, fat and protein (Acton *et al.* 1983; Foegeding and Ramsey, 1986). In sausage production, meat is comminuted in the presence of salt to provide it with the adequate ionic strength to induce swelling, water binding and partial extraction of myofibrillar protein components (Acton *et al.*, 1983; Barbut *et al.*, 1988). Applications of thermal energy convert the finely comminuted batter of protein sol matrix into a viscoelastic solid. This can be viewed as a protein gel network filled with fat particles as protein-protein interactions occur (Acton *et al.*, 1983; Foegeding and Ramsey, 1986). The aggregated filamentous network forms a structure that entraps moisture and fat (Acton *et al.*, 1983).

The comminution process disrupts the muscle tissues by damaging the sarcolemma, endomycium and the integrity of muscle fibers (Smith, 1988). The most important group of muscle proteins contributing to comminuted batter stability is the myofibrillar proteins, mainly consisting of actin and myosin (Barbut, 1995; Lan *et al.*, 1995a). Myosin proteins alone are excellent gel formers, while actin proteins may have a synergistic or antagonistic effect on protein gel formation, depending on the myosin:actin ratio (Lan *et al.* 1995a).

Myosin is the major protein contributing to the interfacial protein film (IPF) that forms around small fat globules. This film of myosin has been shown to be an excellent emulsifier in an oil-in-water emulsion. The amount of myosin and other proteins forming the IPF correlates with the batter stability, thus the formation of a thin and flexible IPF is desirable (Barbut, 1995). Lan *et al.* (1995b) implied that myofibrillar proteins serve as the most important factors in thermally induced gelation, where myosin is the major factor in prerigor meat and actomyosin in postrigor meat.

Gelation of muscle proteins during thermal processing is critical to the three dimensional structure and the formation of the desired rheological attributes (Sherman, 1979; Hamann, 1987; Lan *et al.*, 1995a,b). Gelation of muscle protein is a dynamic process that involves the unfolding and aggregation prior to the formation of the three dimensional network structures while exhibiting complex changes in rheological characteristics (Xiong and Blanchard, 1994).

The native structural characteristics of the proteins influence flow properties and dispersions, thus influencing the three dimensional structure and rheological properties (Sherman, 1979). The three dimensional structure of myofibrillar protein that provides structural rigidity is a result of gel formation from intermolecular interactions (Fennema, 1976; Lan *et al.*, 1995a). Gel network formation involves protein solubilization, dissociation, swelling and denaturation. Dissociation and denaturation prior to aggregation result in a desirable gel network when the proteins involved are globular (Sherman, 1979). Protein gelation is a two-stage process that consists of protein unfolding and the exposure of binding sites, while the second stage is gel matrix formation by aggregation (Lan *et al.*, 1995a).

Protein aggregation also disrupts the gel network during heating, resulting in large capillaries in the network while slow heating further enhances gel formation (Barbut and Mittal, 1990).

### **Non-meat Ingredients in Comminuted Meats**

Within meat products, fat and water (moisture) tend to have an inverse relationship (Eilert *et al.*, 1996). Replacing fat with water while maintaining the texture in reduced fat products can be done by addition of hydrocolloids, which will bind loose water molecules in a food system. DeFreitas *et al.* (1997a) stated that fat could be replaced by water and non-meat ingredients such as hydrocolloids (carrageenan, starch, maltodextrin, alginates) during manufacture of low-fat meat products, which could assist the stability of the food system and improve rheological properties. Chin *et al.* (1998) also mentioned that polysaccharides have been used in emulsified meat systems due to the gelling properties and their ability to bind water, since moisture to protein ratio in emulsified meat products is related to its juiciness and its texture as well as the tenderness and firmness.

Comminution processes permit the incorporation of polysaccharides, such as alginate, which can contribute to cohesiveness of the reformed meat and lower its cost of production (Lawrie, 1991). The utilization of polysaccharide gums like alginate and carrageenan in meat products has been of great interest to the meat industry due to demands of consumers for leaner and lower cost meat products (DeFreitas *et al.*, 1997c). The implementation of carrageenan has been reported in roasted turkey breast and pork sausages (Bater *et al.*, 1992; DeFreitas *et al.*, 1997a, b, c). Asyahari (1993) reported that tapioca starch in bakso would contribute to gels with the function of a thickening agent and a binding agent. Supporting that statement, Purnomo (1995) explained that non-meat additions before the heating or cooking

process could increase the ability of binding meat (muscle pieces). This caused a more compact and slight elastic texture to the final product.

DeFreitas *et al.* (1997a) reported that carrageenan, especially  $\kappa$ -carrageenan, increased gel strength and water retention of salt-soluble meat protein in model systems. Though molecular interaction due to the addition of the stabilizing/destabilizing reagents was not observed, physical rearrangements of  $\kappa$ -carrageenan and salt-soluble protein molecules observed through electron microscopy suggested improvements in water retention and texture. Chin *et al.* (1998) observed higher moisture contents and shear force values ( $p < 0.05$ ) in a model system of low-fat bologna with 0.5% konjac blend compared with a model system with 0.1% konjac blend. A 0.5% konjac blend that resulted in a system with a moisture:protein ratio of 5.5-6.0 had similar textural properties to that of a regular bologna system with 30% fat. Konjac is a polysaccharide powder classified as a glucomannan hydrocolloid that is composed of mannose and glucose units weighing approximately 1,000,000 daltons. Acetyl groups are randomly distributed on side chains, which maintain control of its solubility in water (Keeton, 1996). Additions of hydrocolloids and starches also provide fat-lowering while maintaining the textural and sensorial characteristics through gelling properties and interactions with the protein matrix of a restructured meat system (Chin *et al.*, 1998).

### **Bakso Indonesian Traditional Meatballs**

Purnomo (1997a, b) and Astawan and Astawan (1989) reported that bakso, traditional Indonesian meatball, is a meat product made of ground meat, flour, and spice; formed; and boiled. Bakso has earned popularity among every social class level of the Indonesian society

and is found produced traditionally and distributed by peddlers on a pushcart or in small outlets along the pedestrian walk paths on street corners throughout Indonesia.

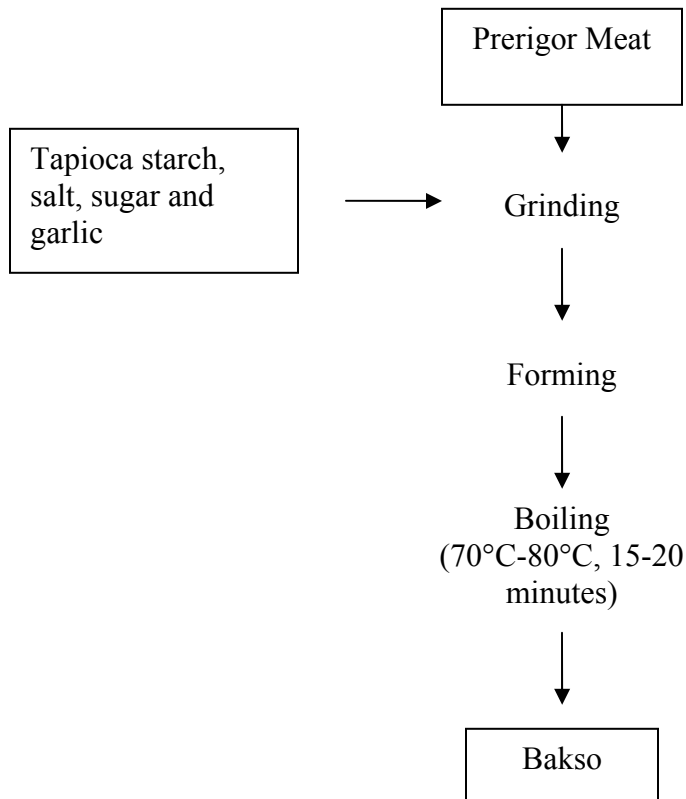


Fig 1. Traditional Bakso Process (Astawan and Astawan, 1989).

Bakso is traditionally prepared of ground meat, tapioca starch, garlic and salt. All ingredients would be blended and the mixture or batter formed into a ball ranging in size from a marble to a ping-pong ball and then cooked in boiling water (Triatmojo, 1992).

According to Triatmojo (1992), price ranges and quality of bakso in the market are also influenced by the amount of filler or binder that is added. Commonly added fillers of bakso are starches with gelation properties. Triatmojo (1992) further suggested that the

maximum acceptance of starch addition would comprise up to 50% of the main ingredient (meat). Substitutions exceeding this amount would compromise the product composition, physical qualities, and organoleptic properties of the product.

Tapioca starch is isolated from cassava and considered a good source of carbohydrate. According to Tjokroadikusumo (1993), tapioca starch consists of high amylopectin, which provides it with the gelatinizing properties and its stickiness. Once gelatinized, tapioca starch granules don't easily break nor go through a syneresis process and have a relatively low gelation temperature. Tapioca is flavorless, which makes it a good choice as an ingredient functioning as a binder or a stabilizer.

### **Non-meat Ingredients in Bakso**

The texture of bakso is very much dependent upon the filler that is being added. Modified potato starch has a very stable gelling property and a low gelling temperature, while also enduring high temperature abuse. These properties allow the practice of high retort temperature in the canning process of bakso (Yuliati, 1999).

Hidayati (2002) concluded that sodium alginate had a gelling property that prevented shrinkage and decreased the hardness of bakso, causing the product to lose its original texture. Alginate appeared to improve texture in beef patties slightly better than carrageenan (Kuo and Keeton, 1998). When combined alginate-carrageenan was used, the patties appeared to have a higher yield and percent moisture although the shear force value was lower than alginate or carrageenan alone. Carrageenan has the tendency to release more free water than alginate after being heated and reheated (Kuo and Keeton, 1998).

Purnomo (1997b) reported that soy flour used as a substitute for tapioca starch raised the protein content. It was not advised to incorporate soy flour because lipooxygenase

enzymes were released and activated after contact with water and oxygen, forming ethyl-phenyl-ketones, an unpleasant odor that was not favored by the panelists in the studies.

Addition of tapioca did not reduce this unpleasant odor. It was concluded that tapioca alone is the most suitable hydrocolloid filler and texture improving agent for bakso.

### **Relationship of Objective Texture to Sensory Properties**

Stanley *et al.* (1971) noted that texture in meat products is used to express the mechanical properties and not the coarseness or fineness, and can be measured objectively and organoleptically as perceived during mastication. de Man *et al.* (1976) implied that to measure and evaluate texture, the various characteristics of texture must be categorized in groups classified by the actions or works. Texture of foods, including meat and meat products is a sequential phenomenon which changes during the food eating process (de Man *et al.*, 1976). Textural characteristics of foods are perceived in 4 stages:

1. During initial perception, where visual appearance, sampling and slicing characteristics, spreading, creaming characteristics and pourability are observed.
2. During initial perception in the palate, where observation of primary and secondary characteristics takes place. Primary characteristics include analytical characteristics, particle size and distribution, particle shape, air content and air cell size, distribution and shape. The secondary characteristics include the basic rheological parameters such as elasticity, viscosity and adhesion form.
3. During mastication; this involves a complex observation of two or more attributes like hardness, fracturability, chewiness and gumminess.
4. Residual mastication impression that includes oiliness, greasiness and coating of the palate.

Stanley *et al.* (1971) revealed measurements of meat texture properties by the means of a given force toward the shearing properties and elasticity of the meat. Elasticity, according to de Man *et al.* (1976), is the property of a material that after deformation and upon removal of stress would regain part or all of original size, shape or both. Adhesiveness is the work necessary to overcome attractive forces between the surfaces of food and other materials which the food comes in contact with such as tongue, teeth or palate. Applied to a single beef muscle fiber, the correlation of extensibility to organoleptic tenderness was highly and negatively significant (Stanley *et al.* 1971).

### **Meat Texture Measurement**

Texture profile parameters of foods can be obtained by measuring the force deformations and the textural parameters derived from their measurements by the Universal Testing Machine (Peleg, 1976). Cohesiveness was defined as  $A_1$  divided by  $A_2$ , given  $A_1$  and  $A_2$  are areas under force-deformation curves of the first and the second bites; hardness as the force measurement at the major second peak of the first bite; gumminess as calculated by the multiplication of hardness by cohesiveness; springiness which correlates to the width of the second bite curve, and chewiness or the multiplication results of gumminess and springiness (Peleg, 1976).

Measuring texture of cooked meat products may also involve applying a certain amount of force to shear the sample via a blunt edged probe, where stress is given on immediate contact by the shearing bar (Stanley *et al.*, 1971). Shear force measures a criterion that breaks muscle elongation or the longest length of the muscle fibers. Through the methods of obtaining shear force, elasticity can also be measured, if the muscle is regarded as viscoelastic so that it resembles a combination of elastic solids and viscous fluid (Stanley *et*

*al.*, 1971). In postmortem meat, the absence of ATP after death causes the loss of the calcium pump system and myosin heads are bound to actin forming rigid chains of acto-myosin (Lawrie, 1991). Texture of restructured or comminuted meat products correlates closely with the ability to bind muscle particles and the amount of extracted myofibril proteins, where product will be more elastic with more actin-myosin bonds developed (Elviera, 1988).

Numerous attempts have been made to develop an accurate instrumentation for measuring meat tenderness (Wheeler *et al.*, 1997). Wheeler *et al.* (1996, 1997) demonstrated that Warner-Bratzler is still the most accurate and popular method of measuring shear force, but variations could still occur due to the differences in execution of the protocol. A development of a simplified technique to determine shear force in meat was developed by Shackelford *et al.* (1999), which is referred as the slice shear force (SSF) that seemed to be more accurate than Warner-Bratzler shear force (WBSF). The slice shear force technique was also repeatable and as accurate to assess pork longissimus tenderness in belt grilled cookery as for beef (Shackelford *et al.*, 2004). Hidayati (2002) performed texture analysis in studies on bakso with Shackelford-slice shear force and found that they were repeatable.

## CHAPTER 3. BAKSO PROPERTIES WITH MEAT POSTMORTEM CONDITION AND LEVEL OF STARCH CONCENTRATION

### Introduction

Postmortem or rigor-mortis associated events are primarily due to a series of chemical changes of anaerobic glycolysis, the conversion of glycogen to lactic acid (Briskey *et al.*, 1966). Rigor-mortis of muscle, converting muscle to meat, affects tenderness and textural traits of meat and its emulsified products (Lawrie, 1991). Fragmentation of myofibrillar proteins due to the structural changes in z-disk postmortem causes meat tenderization and decreasing shear values in postrigor meat (Ahn *et al.*, 2003). Textural traits of comminuted meats and other different types of meat products are affected by the inclusion of connective tissue and adipose tissue, air, water droplets, and melted fat. Extractability of the myofibrillar proteins of prerigor meat is greater than that of postrigor meat (Bailey, 1984).

Bakso is an Indonesian meat product made of starch, salt, and emulsified meat, traditionally from prerigor or early-postmortem sources. Most meat products target increased tenderness, but bakso is more preferred by panelists with higher elastic and shear texture values (Hidayati, 2002). Gelation of starches and the interaction of myofibrils and starch molecules that fill the spaces in the myofibril matrices result in a rigid structure and increase hardness of myofibril gels (Hidayati, 2002). Starch gelation may replace the loss of elasticity from muscle protein degradation in the rigor mortis process. Tapioca starch has a stringy texture and is highly viscous (McWilliams, 1997). Other starches with similar potential qualities of tapioca are corn and sago, but these starches result in bakso texture that is not as acceptable to consumers as bakso with tapioca starch (Triatmojo *et al.*, 1995). Tapioca starch up to 25% is acceptable (Asyhari, 1992), while Triatmojo (1992) suggested that bakso can

incorporate 50% starch and remain organoleptically acceptable, though nutritional concerns for decreased protein content would arise.

The objective of this project was to compare traits of bakso produced with meat from different postmortem times and three tapioca concentrations.

## **Materials and Methods**

Bakso balls were made of meat from local 2-4 year old Ongole crossbred grass-fed cattle. The cattle were slaughtered at RPH Pegirian (Pegirian Slaughter House, Surabaya, East Java, Indonesia). Semimembranosus and Semitendinosus muscles were deboned from carcasses and transported to the meat plant at PT.Eloda Mitra (Sidoarjo, East Java, Indonesia). Meat collection was approximately 3 hours in total and transportation to PT. Eloda Mitra was about a 1 hour drive. Immediate after collection meat was stored chilled (approximately 10 °C) until transportation. A refrigerated truck was used to transport the meat to the processing plant. The 160kg of collected meat was randomly divided into groups of 80kg and assigned to early postmortem and postrigor. Early postmortem meat was ground for immediate usage. Postrigor was held chilled at 10°C for approximately 24 hours until usage. Early postmortem ground meat pH was 5.30-5.79, while the postrigor ground meat had pH of 5.06-5.59. Added ingredients (table 1) were tapioca starch (National<sup>®</sup> 7, National Starch and Chemical, Singapore) with properties of a fine powder, white to off-white in color and a bland taste, sodium tripolyphosphate (STPP, Na<sub>5</sub>P<sub>3</sub>O<sub>10</sub>, Albert & Wilson Phosphate Groups, Indonesia), salt (NaCl) from local markets, regular cane sugar obtained from local markets, and monosodium glutamate (MSG, PT. Ajinomoto, Indonesia).

The equipment, facilities, formulation and processing procedures of PT. Eloda Mitra were used to manufacture bakso. Procedures were:

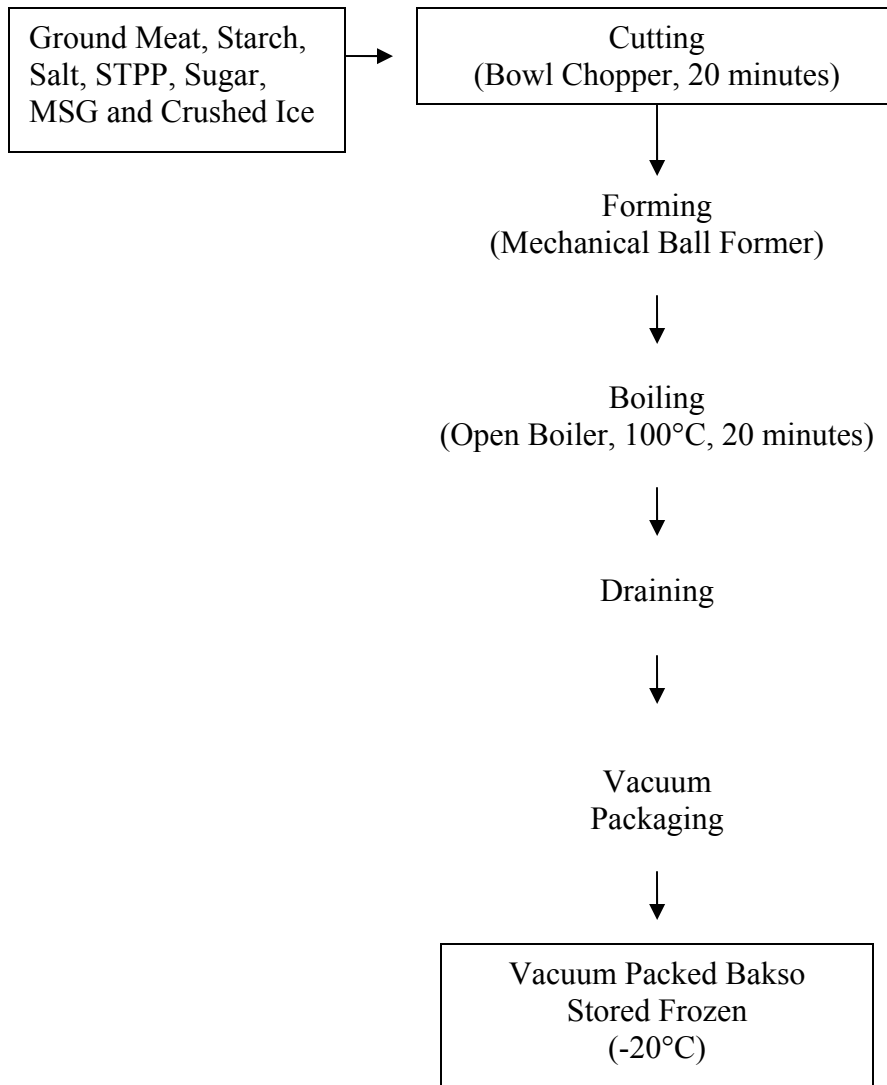


Fig 2. Flow diagram of bakso processing.

A total 80 kg of ground meat was divided into 8.9kg batches of ground meat. Depending on starch concentration an amount of ground meat was taken from the 8.9 kg and chopped in a bowl chopper (K.G. Wetter, Germany) for 20 minutes. Salt was added, followed by STPP, and then tapioca starch was added at 5, 10, 15% levels to early

postmortem and late postmortem meat batches (Table 2). Each experiment was replicated 3 times. Crushed ice was added (up to approximately 5% of total ingredients) to maintain batter temperature at approximately 15°C. Batter was formed mechanically into balls of approximately 14g with a meatball former (Chuang Zong Baller, Taiwan). The balls were then boiled at 100°C for 20 minutes in an open boiler (PT. Mastrada, Indonesia) or until balls were floating in the boiling water. The cooked bakso balls were removed and drained on perforated aluminum trays to remove excess water (bakso balls appeared dry on the outer surface). The bakso balls were packed in bags (limited-low-density polyethylene, 25 x 160mm, 0.15mm thickness; Top Printing Indonesia Co., Indonesia) at 20 balls/bag, vacuum packaged (Henkelman H-800 Double Chamber, Netherlands) and kept frozen at -20°C. Bakso balls were thawed at room temperature (32°C) for 20-30 minutes before sampling for analysis.

Table 1. Bakso formulation with starch treatments

Ingredients *	5% Starch	10% Starch	15% Starch
Early / postrigor ground meat	91.4	86.4	81.4
Tapioca starch	5	10	15
Sodium tripolyphosphate	0.6	0.6	0.6
Salt (NaCl)	1.6	1.6	1.6
Sugar	0.6	0.6	0.6
Monosodium glutamate	0.8	0.8	0.8
Total	100%	100%	100%

\*Crushed ice is added to approximately 5% of total ingredients

Table 2. Experiment design.

	5% Tapioca starch (TS1)	10% Tapioca starch (TS2)	15% Tapioca starch (TS3)
Early postmortem (C1)	MC1TS1	MC1TS2	MC1TS3
Postrigor (C2)	MC2TS1	MC2TS2	MC2TS3

For the proximate analysis of bakso, moisture content was analyzed by method number 950.46 and fat content by method number 960.39 (AOAC, 1990) at Laboratorium Sentral Pangan (Central Food Lab, University of Brawijaya, Malang, East Java, Indonesia).

Texture analysis was done with Lloyd Machine Model Universal Testing Instrument according to Hidayati (2002) and Yuliati (1999) at Pusat Antar Universitas Pangan dan Gizi (Center Inter-University Food and Nutrition, Gajah Mada University, Yogyakarta, Indonesia). The Lloyd universal testing machine was warmed up for 10 minutes. Before texture analysis, a program was called and set for elasticity, gel strength and shear force readings. The upper cycle limit was set to 4.0mm, while the lower cycle limit was set to 3.0mm; the mode was set to compression and test speed was set to 60.0mm/minute, chart speed was set 20.0mm/minute, and the sample width was set to 10.0mm, depth to 10.0mm and gauge length to 10.0mm. The bakso sample was prepared by cutting into a cube of 10.0mm x 10.0mm x 10.0mm. The sample was placed under the slice shear force probe and the machine was operated. Texture measurement was read on the monitor in measurements

of minutes/gram for elasticity and in newtons for gel strength (hardness) and shear force values.

Samples for scanning electron microscope procedure were prepared according to Hidayati (2002) and Yuliati (1999) at UPT Mikroskopi Elektron (University of Airlangga, Surabaya, East Java, Indonesia). A sample of the bakso was sliced 2 to 3 mm thick with a razor blade. Samples were fixed with 2% glutaraldehyde in a phosphate buffer of 7.3 pH and dried using critical point drying (CPD, Sumdri-780 Sample Drying, USA) for 72 hours and then placed on a holder made of brass plate. The sample on the holder was coated with 24 carat gold with an ion sputter-fine coater (JEOL-GLE4X, JEOL Technic Co. Ltd., Japan) for 1.5 minute or achieving an approximate thickness of 0.25  $\mu$ m. The coated sample was observed under a scanning electron microscope unit (JEOL GSM-T100 Scanning Electron Microscope, JEOL Technic Co. Ltd., Japan) at 1500 times magnification.

### **Statistical Analysis**

Statistical analysis was performed by the general linear model (GLM) procedures for complete randomized design (SAS, 1998). Data were analyzed for the main effects of postmortem condition, tapioca starch concentration, replications and interactions, and least mean squares differences in analyses of variance at probability value of less than 0.05. The experiments were executed in 3 replicated experiments with a single batch of bakso as an experimental unit.

The statistical model was:

$$Y_{ijk} = \mu + \alpha_i + \delta_j + \alpha\delta_{ij} + \varepsilon_{ijk}$$

Where: Y = observed value of  $k^{\text{th}}$  measurement on bakso made of the meat i, and j starch concentration,

$\mu$  = overall mean,

$\alpha_i$  = effect of treatment i, i = meat condition (early or postrigor),

$\delta_j$  = effect of treatment j, j = starch concentration (5,10, or 15%),

$\alpha\delta_{ij}$  = effect of treatment i and j interaction,

$\varepsilon_{ijk}$  = experimental error.

## Results and Discussion

Replications in this experiment were significantly different ( $p < 0.05$ ) for fat content and shear force (table 3). Differences observed in the replications of bakso were most likely an effect of the poorly homogenized condition of the batter during the cutting process. A difference in source (animal) for the meat batches was also assumed to affect replication.

Table 3. Means for bakso from early and postrigor meat with batch replication.

Replication	Moisture (%)	Fat (%)	Elasticity (min/g)	Gel strength (N)	Shear force (N)
1	73.39	0.31 <sup>a</sup>	0.55	37.49	21.09 <sup>b</sup>
2	73.64	0.16 <sup>b</sup>	0.48	36.18	21.89 <sup>a</sup>
3	73.59	0.16 <sup>b</sup>	0.58	37.50	20.08 <sup>c</sup>
SEM	0.58 <sup>nd</sup>	0.03	0.05 <sup>nd</sup>	0.88 <sup>nd</sup>	0.13

Data represents means and standard errors (SEM) of the 3 replicates. Different superscripts among means indicate differences, while nd=no difference ( $p < 0.05$ ).

Bakso from early postmortem meat had slightly higher moisture and slightly lower fat than bakso from postrigor meat (table 4). Early postmortem meat had more extracted myofibrillar protein (recovery) and improved water retention than postrigor meat (Xiong and Brekke, 1991). This would explain the slightly higher water content of bakso from early postmortem than bakso from postrigor meat.

Bakso to meet the Indonesian National Standards (Board of Nasional Standardization, 1995) has approximately 70% moisture content and a maximum fat content of 2%.

Table 4. Proximate analysis and rheological means of bakso from early and postrigor meat.

Meat Conditions	Moisture (%)	Fat (%)	Elasticity (min/g)	Gel strength (N)	Shear force (N)
Postrigor	73.40	0.22	0.499	36.365	20.604 <sup>a</sup>
Early postmortem	73.68	0.20	0.573	37.747	21.433 <sup>b</sup>
SEM	0.474 <sup>nd</sup>	0.024 <sup>nd</sup>	0.041 <sup>nd</sup>	0.720 <sup>nd</sup>	0.107

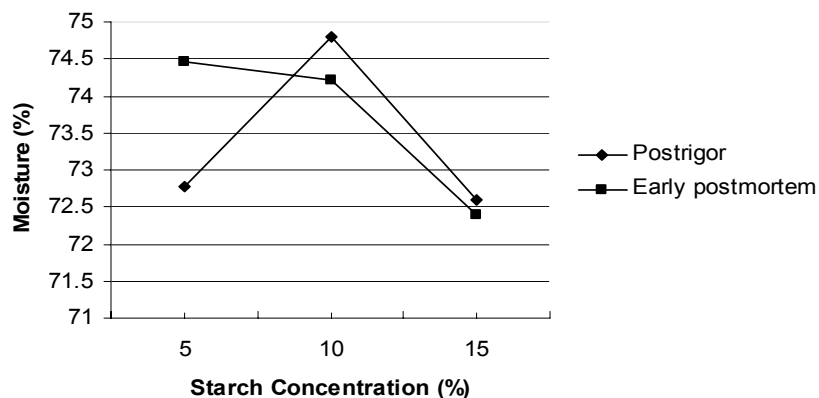
Data represents means and standard errors (SEM) of 3 replicate experiments. Different superscripts among means indicates significant differences, while nd=no difference ( $p < 0.05$ ).

However, any differences in moisture might have been minimized by the effects of the 0.6% STPP in both of the formulations. Eilert *et al.* (1996) used 0.5% STPP in frankfurters made of frozen flaked connective tissues and reported effective control over processing yield loss and recovery of emulsion stability. Use of early postmortem meat increased bakso textural measurements compared with postrigor meat. This was attributed to increased protein cohesion and binding in early postmortem meat (Acton *et al.*, 1983).

The range of moisture content of 72% to 75% was not significant among treatments. Figure 3 illustrates slightly higher moisture content for bakso of early postmortem meat than postrigor at 5% starch concentration, while early postmortem and postrigor meat bakso demonstrated very similar moisture content with 10% and 15% starch. At 5% starch concentration, most water binding effects are probably due to myofibrillar protein gelation and the small amount of starch did not influence water binding as greatly as higher levels of starch. The findings were consistent with the increased water retention of early postmortem meat than postrigor meat (Xiong and Brekke, 1991).

The high moisture and the stable moisture content of bakso were probably influenced by the water-binding effect of actomyosin gelation after extraction with salt (1.6% of the formula) and STPP (0.6%) and also by tapioca starch gelation. NaCl (sodium chloride)

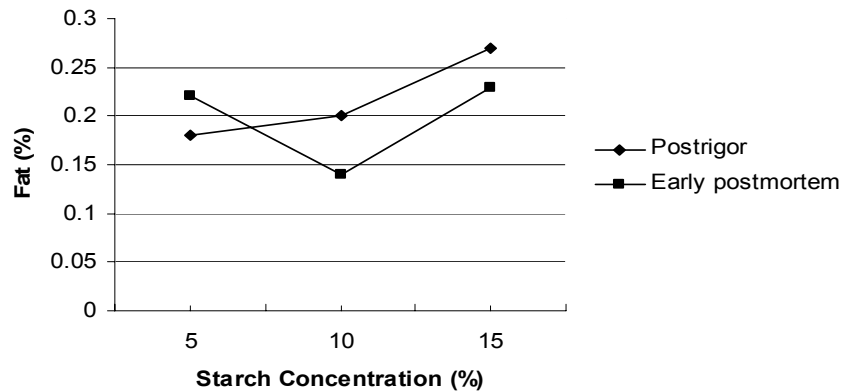
extracts the actomyosin proteins, exposing chemical groups for binding. Increasing NaCl from 1.5% to 2.5% doubles the amount of extractable proteins resulting in more stable batters in the case of comminuted products (Barbut, 1995). The moisture content of both early postmortem and postrigor bakso was lower with 15% starch than 5 or 10% starch. With 15% starch, there is a lower proportion of meat in the product, and thus less myofibrillar proteins (myosin, actin or actomyosin) are available for water holding capacity and gelation in the product. The gelation of 15% starch concentration may have helped to maintain moisture content almost to the level in bakso with higher amounts of meat protein. Starch granule swelling and gelatinization contribute to the prevention of emulsion breakdown, loss of viscosity or elasticity and the prevention of syneresis during refrigeration, frozen storage, or thawing (Hood *et al.*, 1974a). Hood *et al.* (1974b) reported that complete absence of water-holding-capacity of gels of tapioca starch and milk was observed after the gels were cycled through a freeze-thaw cycle 42 times during 60 days of frozen storage at -32°C.



SEM=0.820

Fig. 3 Meat and starch concentration interaction with moisture as the dependent variable.

Fat content was similar ( $p>0.05$ ) in bakso from early and postrigor meat. No differences ( $p>0.05$ ) were observed among the starch treatments (5%, 10%, and 15%; Figure 4). The interactions of starch with rigor type showed only small variations in fat of bakso balls.



SEM=0.041

Fig 4. Meat and starch concentration interaction with fat as the dependent variable.

Fat in the meat batter is stabilized by the formation of a protein film around the fat globule known as the interfacial protein film (IPF). The film acts as a barrier that prevents coalescence of water and fat in the meat batter. Under heating, fat loss is followed by moisture loss. The low fat content was probably due to the fat lost under heating or due to low initial fat in the raw meat, which was not measured. Fat, moisture retention and binding are important in the formation of a stable batter (Barbut, 1995).

Bakso rheological properties were determined by the elasticity, gel strength (hardness) and shear force values. The elasticity and gel strength (hardness) of bakso balls made from early postmortem beef were slightly higher, but not different ( $p<0.05$ ), than bakso from postrigor meat (table 4). The gel shear force values of bakso were higher ( $p<0.05$ ) with early than postrigor meat (table 4).

The tougher texture of bakso from early postmortem meat suggested that early postmortem meat had slightly improved emulsifying capacity and more extracted myosin than postrigor meat. Lan *et al.* (1995a) reported that myofibrillar proteins are responsible for the three dimensional network of protein fibers and promote the structural rigidity. These myofibrillar proteins consist of free myosin in prerigor meat and actomyosin in postrigor meat. Free myosin forms excellent gels while free actin implicates the gels in a synergistic manner or in an antagonistic manner depending on the significance of its presence. Ramírez *et al.* (2000) noted that myosin is the protein fragment that is responsible for gelation, while other fragments, such as actin, do not form gel, but regulate the viscoelastic properties of the gel.

Hidayati (2002) studied the effects of sodium tripolyphosphate and sodium alginate on the rheological properties of bakso and obtained elasticity ranging from 0.5183 to 0.540 minutes/gram using Loyd Universal Testing Instruments. Hardness of bakso ranged between 24.237 to 59.410N (Hidayati, 2002). The results of texture analysis in those studies were also relatively uniform, which concurred with the present results.

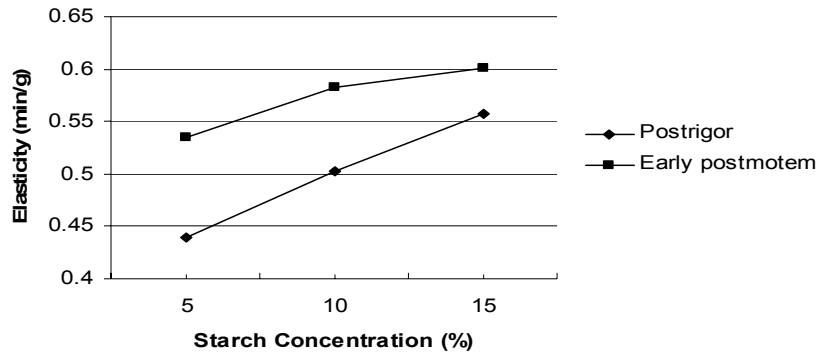
Though the effects of tapioca starch concentration in bakso texture were significant ( $p < 0.05$ ) only for shear force, the trends were noted that texture increased as starch concentration increased (Figure 5). Elasticity of postrigor bakso (fig 5a) increased with increased starch concentration. Early postmortem bakso had higher elasticity, gel strength and shear force than postrigor bakso. Gel strength values (hardness) also demonstrated an increasing trend throughout the increments of starch concentration (fig 5b). Postrigor bakso hardness value increased steadily from 5% to 15% while early postmortem increased slightly

more in the 10% to 15% starch concentration (fig 5b). Shear force of bakso also increased as tapioca starch concentration increased (fig 5c).

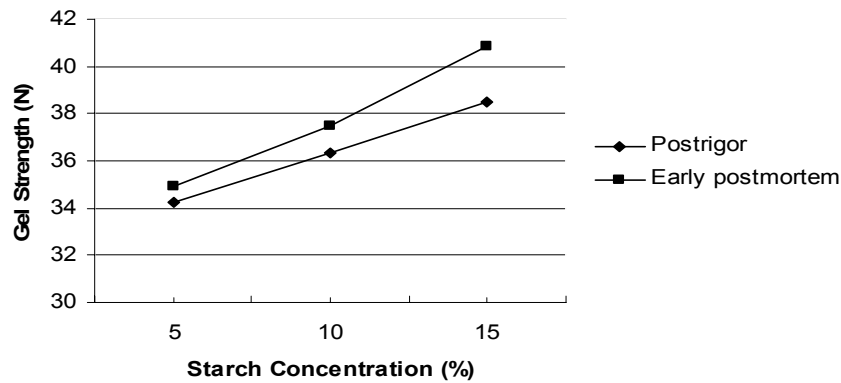
Yuliati (1999) reported that bakso texture was also very much influenced by the starch that was added. Sensory panelists indicated that tougher bakso with more elastic properties was more desirable, since that expectation for bakso texture has been culturally integrated (Yuliati, 1999). Gelation and granule swelling of starches in the presence of water is critical to emulate fat, reduce moisture loss and improve freeze thaw stability in emulsified meat products. Hydrocolloids and starches may provide synergistic effects to benefit textural attributes of low-/no-fat meat products (Keeton, 1996). The effect of mechanical treatments on the texture of comminuted emulsion type meatballs was studied by Wu and Ockerman (1982). Hammering increased emulsifying capacity, stability, and viscosity, but decreased water holding capacity. Wu and Ockerman (1982) reported that 4 minutes of chopping produced acceptable texture, and thus could replace conventional hammering/tumbling in comminuted meatball production.

Micrographs of bakso illustrate the physical appearance of bakso's spongy three dimensional structures. Figure 6-MC1TS1 is a micrograph of bakso from early portem meat with 5% tapioca starch at 1500x magnification. The micrograph demonstrates the myosin and actomyosin network and the tapioca granules. The myosin and actomyosin networks appear as thin thread lines or protein strands that interconnect, forming a web-like net matrix, while the tapioca granules appear as dense granules aggregated into one another. Yuliati (1999) also reported appearances of starch granules as spherical aggregates in a study on the effects of canning on bakso quality. The dense, aggregate, and non-swelled appearance of tapioca starch granules may be an effect of the high vacuum of the scanning electron microscope

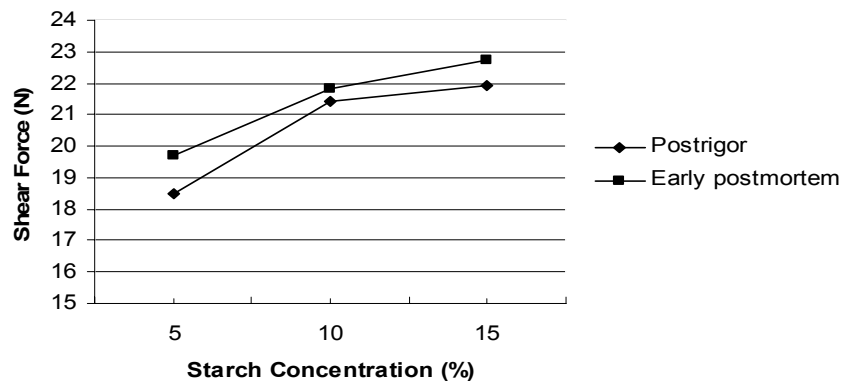
column (Hood *et al.*, 1974a). Barbut (1995) demonstrated the protein matrix as a sponge-like matrix that consists of protein strands interconnected to form a coherent three dimensional structure.



SEM=0.071 a.



SEM=1.248 b.



SEM=0.186 c.

Fig 5. Elasticity, gel strength (hardness) and shear force of early and postrigor bakso with 5%, 10% and 15% tapioca starch (a=Elasticity, b=Gel strength, c=Shear force).

The second micrograph (figure 6-MC1TS2) pictures the actomyosin network of bakso from prerigor meat and 10% tapioca starch concentration at 1500x magnification. Starch granules appear rough and sparsely cover the actomyosin matrix. Unlike the first micrograph (figure 6-MC1TS1), this micrograph captures a section of the bakso that is not as dense in starch, where the actomyosin matrix is demonstrated more effectively. Protein strands in the three dimension sponge structure are shown clearly in this micrograph (figure 6-MC1TS2). The lack of uniformity of starch distribution is assumed to be an effect of less homogenized cutting and mixing of the batter during the cutter steps of processing bakso. This poorly homogenized condition also explains the differences observed in the replications of bakso texture measurements.

The more open space and coarse structure of the three dimension network are assumed to be the remaining set and physical rearrangement of the actomyosin network. The “fat out” phenomena of the emulsion structure leaving an empty space of interfacial film protein (IFP) and the effects of cooking shrinkage to the network proteins are suggested to be the reasons for this matrix rearrangement. Considering the emulsion theory of comminuted products, Smith (1988) reported that in finely comminuted products the role of emulsification becomes important as fat cells are disrupted; fat melts and spaces of various droplet sizes are formed as the matrix protein sets.

Figure 6-MC1TS3 is a micrograph of bakso from early postmortem meat with 15% tapioca starch at 1500x magnification. This micrograph captures an unhomogenized section where actomyosin matrix and starch granules are present. Again, in this micrograph, fat spaces are present, leaving strands of protein forming the three dimensional actomyosin networks. Throughout emulsification, fat dispersion and IFP were considered as an aspect

that is responsible for comminuted meat emulsion stability, but fat dispersion and IFP are not sole aspects responsible for stability. Acton *et al.* (1983) reported that water binding that occurs through myofibrillar extraction and during entrapment within the gel matrix is another aspect that should be taken into account in terms of emulsion stability.

Figures 6-MC2TS1, MC2TS2 and MC2TS3 show scanning electron micrographs of 5%, 10% and 15% starch concentration, respectively, in bakso from postrigor meat.

Micrographs of bakso from postrigor meat illustrate a less complex protein networking and pores appear to have more large voids than in bakso from early postmortem meat. Bakso from postrigor meat has a spongy structure that appears to have less threaded lines of protein strand to build a network unlike bakso from early postmortem meat that appear to be more

web-like with many smaller void spaces. Prerigor meat or meat that has not been aged is considered to have better emulsifying properties than postrigor meat or aged meat, due to the loss of myosin filament-forming ability during aging and the loss of textural qualities (Smith, 1988).

### **Implications**

The results of phase one suggest that use of early postmortem meat in bakso production gave slightly more desired textural properties than postrigor meat. The results also indicated that bakso with 15% starch had the highest elasticity, which is sought as a bakso rheological trait. Combinations of both factors (postmortem condition and starch concentration) indicated that postrigor meat can be used to produce bakso with sufficient textural traits if 15% tapioca starch was incorporated. Replacement of early postmortem meat with postrigor meat in bakso would be applicable for industrial mass production.

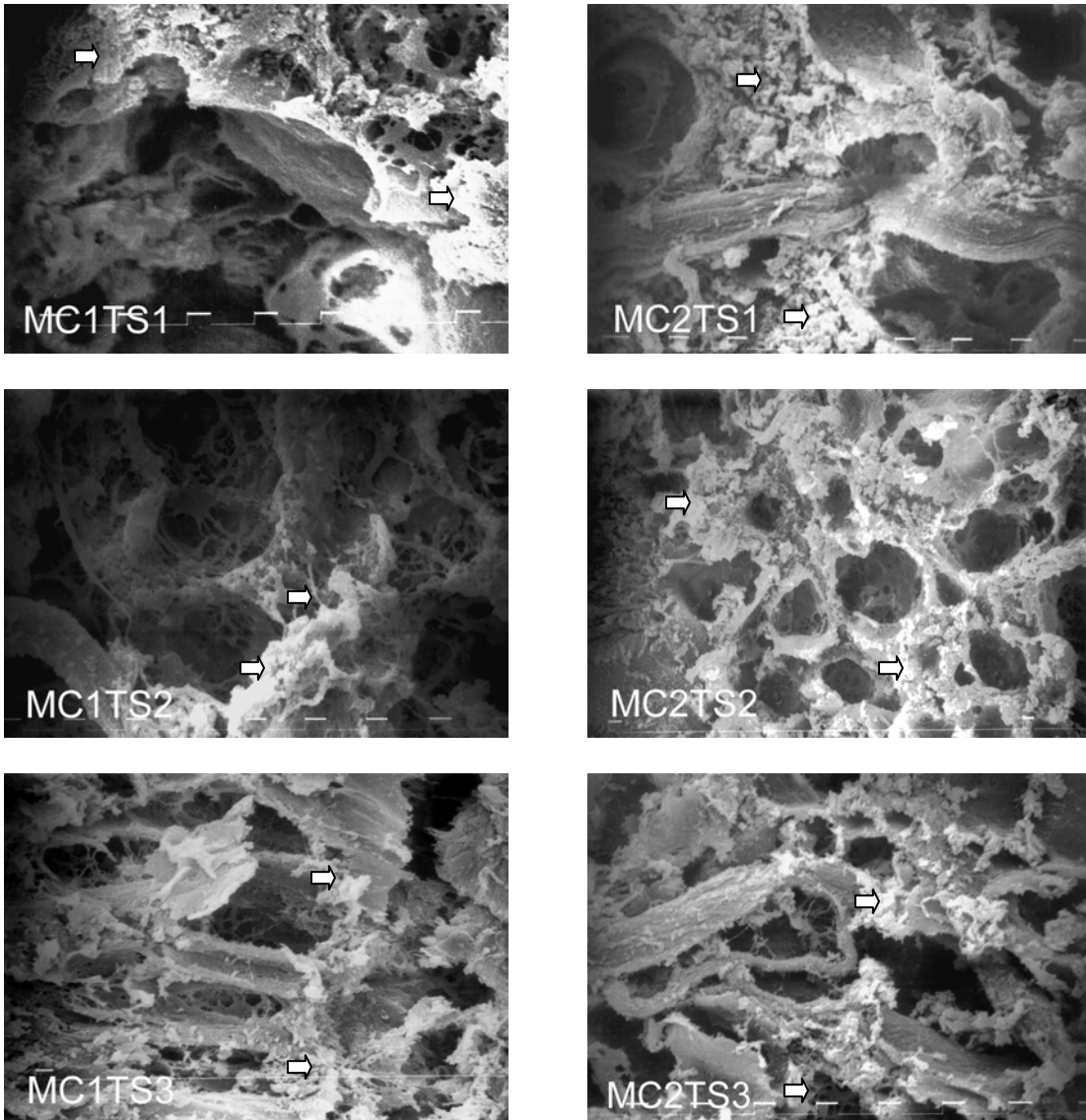


Fig 6. Scanning electron microscope of bakso from early and postrigor meat at 1500x magnification (MC1TS1= early postmortem and 5% tapioca starch; MC1TS2= early postmortem and 10% tapioca starch; MC1TS3= early postmortem and 15% tapioca starch; MC2TS1= postrigor and 5% tapioca starch; MC2TS2= postrigor and 10% tapioca starch; MC2TS3= postrigor and 15% tapioca starch; arrows indicate starch granules).

## **CHAPTER 4. EFFECTS OF MEAT STORAGE AND PRODUCT STORAGE ON PROXIMATE ANALYSIS, RHEOLOGY AND MATRIX STRUCTURE OF BAKSO**

### **Introduction**

Ready-to-eat and more convenient foods are becoming increasingly popular. The consumer demands influence the food service industry. Demands for convenient foods also influence the processed meats industry. Increasing amounts of meats are reprocessed to various products, such as patties and meatballs, requiring less preparation time (ready-to-eat products) (Romans *et al.*, 1994; Sloan, 2003). The Indonesian bakso ball is a traditional emulsified meat product that is usually made with early postmortem meat, starch and salt. Bakso is often sold from street vendors; recently processors have begun commercial manufacturing of bakso. Previous experiments indicated that raw-chilled postrigor meat could be substituted for early postmortem meat in production of bakso to allow for more efficient manufacturing and raw material procurement.

Commercial bakso production requires increased time for distribution and storage of product so investigations have been made on bakso preservation with canning, vacuum packaging and freezing (Purnomo, 1997a,b; Yulianti, 1999; Hidayati, 2002). Freezing is considered to be one of the best methods of food preservation (Jiang *et al.*, 1987). Frozen storage of muscles from vertebrates induces a decrease in the functional properties of myofibrillar proteins, including solubility, gelling capacity and water holding capacities (Visessanguan *et al.*, 2000). Myofibrillar instability during frozen storage has been associated with changes in myosin stability as the major contractile protein of skeletal muscle (Ramírez *et al.*, 2000). The use of frozen late postrigor meat would further improve inventory control and allow purchase of materials at lower prices or for use at a later time.

Chemical and physical changes of muscle food during storage, especially oxidative processes, result in the deterioration of quality that includes discoloration, development of off-flavors, loss of nutrients, textural changes, and progression of spoilage and/or pathogenicity (McMillin, 1996). Declining quality of long term freezer-stored meats and meat products, such as the brown (metmyoglobin) color and off-flavors, is mainly due to lipid and pigment oxidation (Zaehringer *et al.*, 1959). Furthermore, Judge and Aberle (1980) reported that the objectionable changes in meat quality during frozen storage are a result of phospholipid breakdown, because phospholipids are susceptible to oxidation. The release of fatty acids from phosphoglycerides by lipolysis is suspected to be the initiator of the oxidation process. Xiong (1996) stated that frozen storage of meats and comminuted meat products causes protein denaturation, recrystallization, lipid oxidation, protein oxidation and sublimation, which result in the degradation of muscle protein functionality. The bakso properties with incorporation of frozen stored postrigor meat into bakso and the frozen storage time of cooked bakso are unknown.

The objective of this experiment was to investigate the properties of bakso with different times of frozen storage of raw postrigor meat and after different frozen storage times of bakso made from postrigor and early postmortem meat.

### **Materials and Methods**

Meat for the bakso balls in this phase was from local grass fed Ongole crossbred cattle 2-4 years of age. The cattle were slaughtered at RPH Pegirian (Pegirian Slaughter House, Surabaya, East Java, Indonesia). Semimembranosus and semitendinosus muscles were obtained from carcasses, deboned and transported to PT. Eloda Mitra. Meat collection required approximately 3 and 3/4 hours. Immediately after collection, meat was stored

chilled at 10°C and maintained during transportation in a refrigerated truck to PT Eloda Mitra processing plant (about a 1 hour drive.) For each batch, 200kg of the collected meat was divided randomly, assigning 25kg to early postmortem for immediate usage, 25kg for postrigor 0 months storage that was chilled overnight for usage the following day, 75kg for postrigor 2 months storage that was stored frozen for usage after 2 months of storage, and 75 kg for postrigor 4 months storage that was also stored frozen for usage after 4 months of storage. Each 75 kg meat batch was divided into 25kg portions for 0, 2, and 4 months of bakso storage in each frozen stored postrigor meat treatment. Early postmortem meat pH was 5.42-5.74, while the late postmortem meat had pH of 5.08-5.62. Other ingredients for bakso were tapioca starch (National<sup>®</sup> 7 white to off-white and bland taste, National Starch and Chemical, Singapore), sodium tripolyphosphate (STPP, Na<sub>5</sub>P<sub>3</sub>O<sub>10</sub>, Albert & Wilson Phosphate Groups, Indonesia), salt (NaCl) obtained from local markets, regular cane sugar (local markets), and monosodium glutamate (MSG, PT. Ajinomoto, Indonesia).

Bakso was made with the formulation in table 5 (15% starch formulation) and according to the procedures of PT.Eloda Mitra (Sidoarjo, East Java, Indonesia; figure 7). Weighed ground meat (20.51kg) was chopped (bowl chopper, K.G. Wetter, Germany) for 20 minutes. Salt was added, followed by STTP, and tapioca starch. Crushed ice was added (approximately up to 5% of total ingredient) to maintain batter temperature at approximately 15°C. Batter was formed mechanically with a meatball former (Chuang Zong Baller, Taiwan) into balls of approximately 14 g/ball. The balls were then boiled at 100°C for 20 minutes in an open boiler (PT. Mastrada, Indonesia) or until balls were floating in the boiling water. The cooked bakso balls were removed and drained on perforated aluminum trays to remove excess water until bakso balls appeared dry on the outer surface.

Table 5. Bakso formulation

Ingredients*	%
Early postmortem or postrigor ground meat	81.4
Tapioca starch	15
Sodium tripolyphosphate	0.6
Salt (NaCl)	1.6
Sugar	0.6
Monosodium glutamate	0.8
Total	100%

\*Crushed ice added to approximately 5% of total ingredients

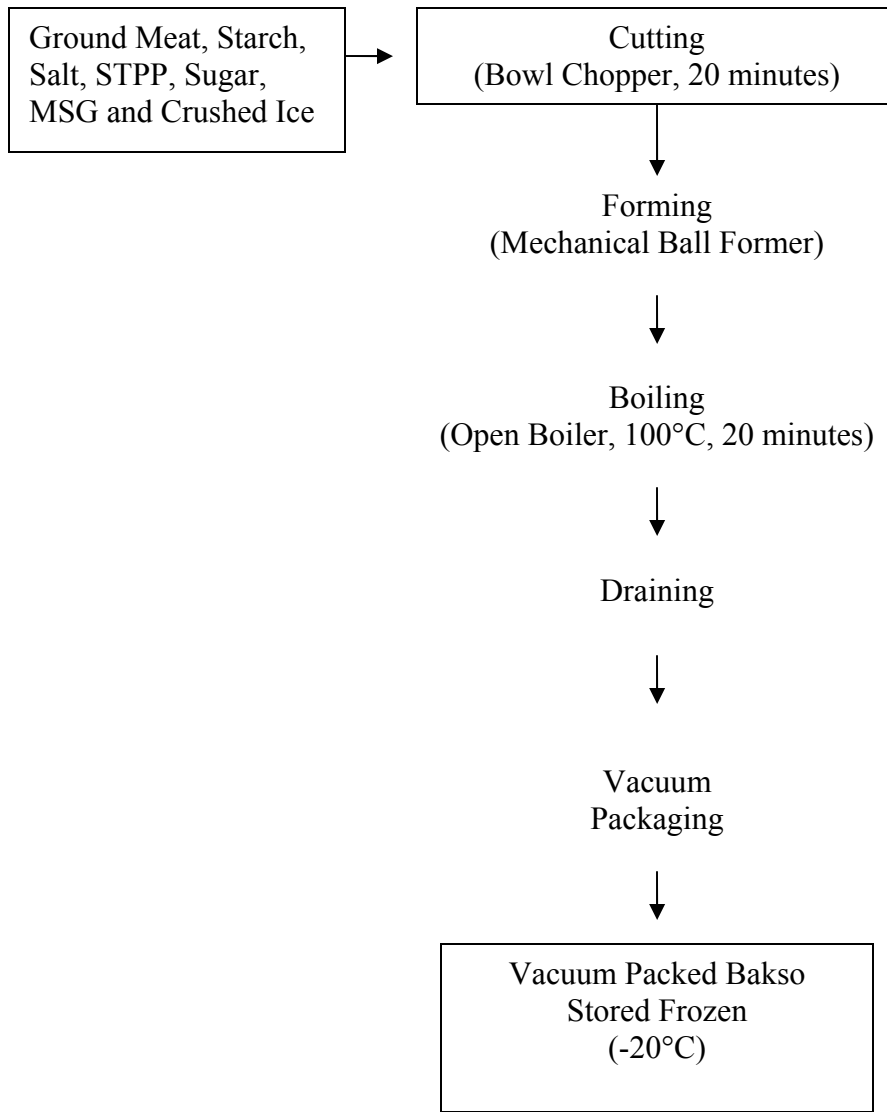


Figure 7. Flow diagram of bakso processing

The bakso balls were packed in polyethylene bags (limited low density 0.15 mm thickness; 25 x 160mm, Top Printing Indonesia Co., Indonesia) at 40 balls/bag, and vacuum packaged (Henkelman H-800 Double Chamber, Netherlands).

Three packs of early postmortem bakso and 3 packs of postrigor bakso were sampled for 0 months analysis. The remaining packs were frozen at -20°C. After 2 and 4 months of frozen storage, 3 packs representative of early postmortem and postrigor bakso were thawed and tested.

After 2 months of frozen meat storage, the meat was thawed at room temperature (approximately 28 °C) for 2 hours, 61.54 kg were weighed, ground and processed into cooked bakso balls that were packed as previously described. Three packs were tested initially and the remaining packs were stored at -20°C for an additional 2 and 4 months before testing. After 4 months of frozen meat storage, the remaining 75 kg of stored meat were thawed, and 61.54 kg were weighed, ground and processed into bakso. Three packs were tested initially after production and the remaining packs were stored frozen at -20°C before testing for 2 and 4 months bakso storage.

Moisture content of bakso was analyzed by method number 950.46 and fat content analysis by method number 960.39 (AOAC, 1990). Lipid oxidation was evaluated by TBARS values with the method described by Wu *et al.* (2000). These analyses were executed at Laboratorium Sentral Pangan (Central Food Lab, University of Brawijaya, Malang, Indonesia).

Texture analysis was done with a Lloyd Machine Model Universal Testing Instrument (Lloyd Universal Testing Model-1000s; England) according to Hidayati (2002) and Yuliati (1999) at Pusat Antar Universitas Pangan dan Gizi (Center Inter-University Food and Nutrition, Gajah Mada University, Yogyakarta, Indonesia). The universal testing machine was warmed up for 10 minutes before the program was called and set for elasticity, gel strength and shear force readings. The upper cycle limit was set to 4.0mm, while the

lower cycle limit was set to 3.0mm; the mode was set to compression, and test speed was set to 60.0mm/minute, speed was set 20.0mm/minute, the sample width was 10.0mm, depth 10.0mm and gauge length 10.0mm. The bakso sample was prepared by cutting a cube of 10.0mm x 10.0mm x 10.0mm, placing it under the slice shear force probe and the measurement started. Texture measurement was read on the monitor in minutes/gram for elasticity and in newtons for gel strength (hardness) and shear force values.

Samples for scanning electron microscope procedure were prepared according to Hidayati (2002) and Yuliati (1999) at UPT Mikroskopi Elektron (University of Airlangga, Surabaya, East Java, Indonesia). A sample of the bakso was sliced 2 to 3-mm thick with a razor blade. Samples were fixed with 2% glutaraldehyde in a phosphate buffer of 7.3 pH and dried using critical point drying (CPD, Sumdri-780 Sample Drying, USA) for 72 hours and then placed on a holder made of brass plate. The sample on the holder was coated with 24 carat gold with an ion sputter-fine coater (JEOL-GLE4X, JEOL Technic Co. Ltd., Japan) for 1.5 minute or approximately after achieving a thickness of 0.25mm. The coated sample was observed under a scanning electron microscope unit (JEOL GSM-T100 Scanning Electron Microscope, JEOL Technic Co. Ltd., Japan) at 1500 times magnification.

### **Statistical Analysis**

Statistical analysis was performed by the general linear model (GLM) procedures for complete randomized design (SAS, 1998). Data were analyzed for the main effects of different meat storage and different bakso storage times, interactions and replications, and least squares mean differences in analyses of variance at probability value of less than 0.05. The data was analyzed in 2 separate designs. The first design was for bakso made of postrigor meat after meat frozen storage time of 0, 2, and 4 months and bakso frozen storage

time of 0, 2, and 4 months using a 3 x 3 factorial arrangement. To substantiate the results of the first design, another model was used to analyze the data. This model analyzed the main effects of frozen meat storage time and frozen bakso storage time in terms of the nine different treatment combinations. The second design was a 2 x 3 factorial design to analyze bakso from early postmortem and postrigor meat after 0, 2, and 4 months of frozen bakso storage. The experiments were executed in 6 replicated experiments with a single batch of bakso treated as an experimental unit.

The statistical models for each design were:

- First design :  $Y_{ijk} = \mu + \alpha_i + \delta_j + \varepsilon_{ijk}$

Where: Y = observed value of  $k^{\text{th}}$  measurement on bakso made of the  $i^{\text{th}}$  months of frozen meat storage time stored frozen for j months,

$\mu$  = overall mean,

$\alpha_i$  = effect of treatment i, i = frozen meat storage time (0, 2, and 4 months),

$\delta_j$  = effect of treatment j, j = frozen storage time of bakso (0, 2, and 4 months),

$\varepsilon_{ijk}$  = experimental error.

- Verification model :  $Y_{ij} = \mu + \alpha_i + \varepsilon_{ij}$

Where: Y = observed value of  $j^{\text{th}}$  measurement on bakso made of the  $i^{\text{th}}$  months of frozen meat storage time and stored frozen,

$\mu$  = overall mean,

$\alpha_i$  = effect of treatment i, i = frozen meat storage time and bakso storage time,

$\varepsilon_{ij}$  = experimental error.

- Second design :  $Y_{ijk} = \mu + \alpha_i + \delta_j + \alpha\delta_{ij} + \varepsilon_{ijk}$

Where: Y = observed value of k<sup>th</sup> measurement on bakso made of the meat i,  
stored frozen for j months,

$\mu$  = overall mean,

$\alpha_i$  = effect of treatment i, i = meat condition (early postmortem or  
postrigor),

$\delta_j$  = effect of treatment j, j = frozen storage time of bakso (0, 2, and 4  
months),

$\alpha\delta_{ij}$  = effect of treatment i and j interaction,

$\varepsilon_{ijk}$  = experimental error.

## Results and Discussion

### Effects of Meat Storage Time on Bakso Properties

Replication in this study had no effects on any properties ( $p < 0.05$ ; appendix page 80).

The length of frozen storage time of postrigor beef had no effects ( $p < 0.05$ ) on any composition or texture properties of bakso (table 6), but 0, 2, and 4 months of bakso storage influenced ( $p < 0.05$ ) fat content and TBARS values (table 7).

Table 6. Properties of bakso from postrigor meat stored frozen for 0, 2, and 4 months.

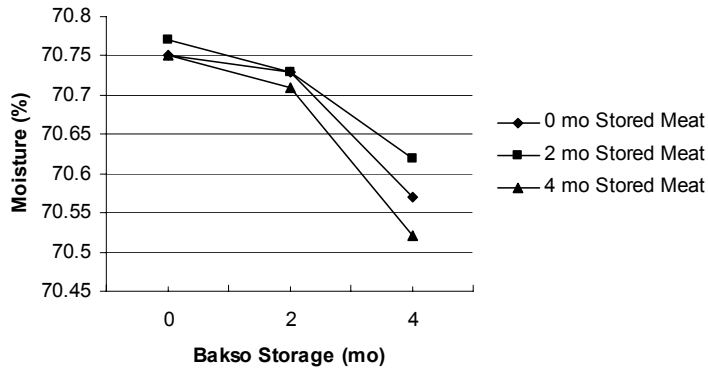
Meat storage (months)	Moisture (%)	Fat (%)	TBARS (mg/kg)	Elasticity (min/g)	Gel strength (N)	Shear force (N)
0	70.68	0.21	2.540	0.542	38.822	22.238
2	70.71	0.21	2.471	0.533	38.598	22.220
4	70.66	0.20	2.431	0.525	38.513	22.143
SEM	0.195 <sup>nd</sup>	0.004 <sup>nd</sup>	0.137 <sup>nd</sup>	0.008 <sup>nd</sup>	0.107 <sup>nd</sup>	0.247 <sup>nd</sup>

Data are means and standard errors (SEM) of 6 replicated experiments. SEM with nd superscripts indicate no significant differences among corresponding means ( $p < 0.05$ ). TBA=Thiobarbituric acid reactive substances.

Table 7. Properties of bakso stored frozen for 0, 2, and 4 months.

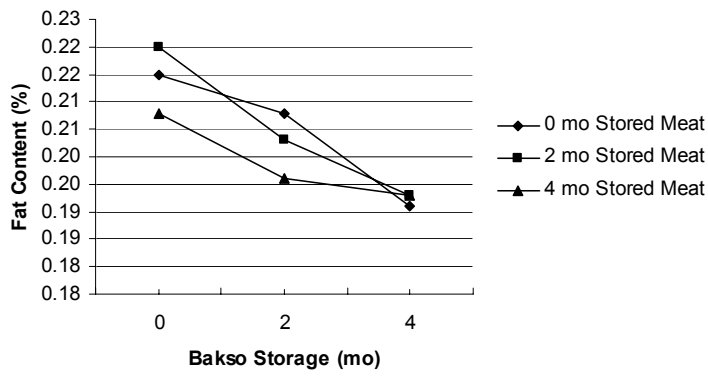
Bakso Storage (Months)	Moisture (%)	Fat (%)	TBA (mg/kg)	Elasticity (min/g)	Gel strength (N)	Shear force (N)
0	70.75	0.21 <sup>a</sup>	1.551 <sup>b</sup>	0.544	38.721	22.222
2	70.72	0.20 <sup>a</sup>	2.859 <sup>a</sup>	0.530	38.615	22.189
4	70.57	0.19 <sup>b</sup>	3.031 <sup>a</sup>	0.526	38.598	22.189
SEM	0.19 <sup>nd</sup>	0.004	0.137	0.008 <sup>nd</sup>	0.107 <sup>nd</sup>	0.247 <sup>nd</sup>

Data are means and standard errors (SEM) of 6 replicated experiments. SEM's with nd superscripts indicate no significant differences among corresponding means ( $p < 0.05$ ). TBARS=Thiobarbituric acid reactive substances.



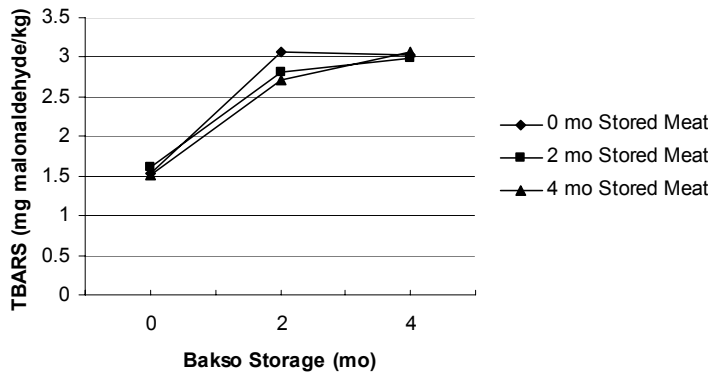
SEM=0.369

a.



SEM=0.008

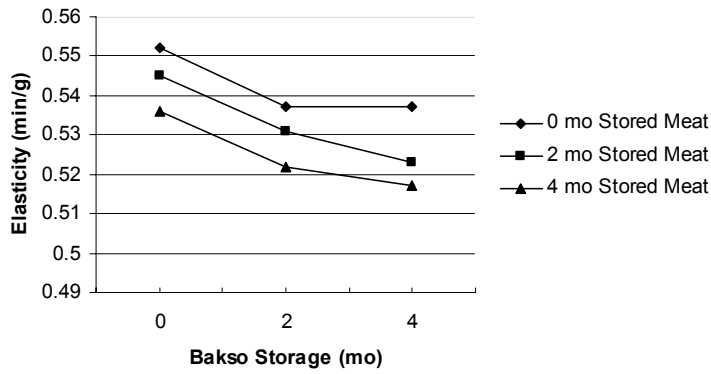
b.



SEM=0.255

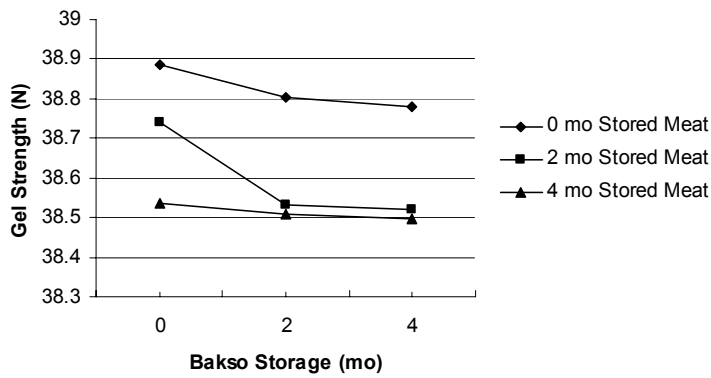
c.

Fig 8. Moisture (a), fat (b) and TBARS values (c) of bakso made of postrigor meat stored frozen for 0, 2, and 4 months and after 0, 2, and 4 months of frozen bakso storage.



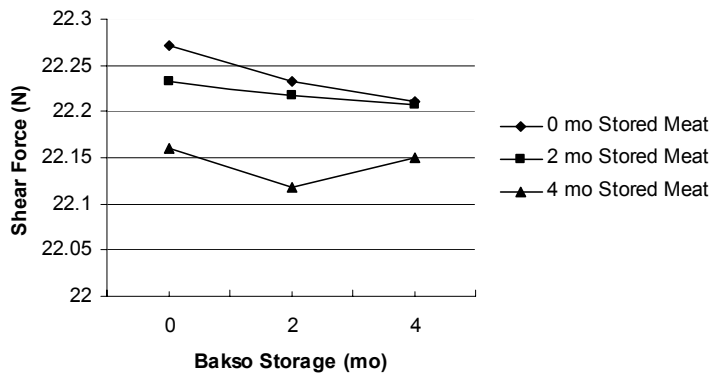
SEM=0.016

a.



SEM=0.202

b.



SEM=0.468

c.

Fig 9. Texture properties of bakso made of postrigor meat stored frozen for 0, 2, and 4 months and after 0, 2, and 4 months of frozen bakso storage (a=Elasticity, b=Gel strength and c=Shear force).

Neither frozen storage time of postrigor meat from frozen stored meat nor frozen storage time of frozen bakso for 0, 2, and 4 months significantly affected the properties of bakso, except for fat content and TBARS. Further observations of the mean trends revealed that throughout frozen storage of bakso made of frozen postrigor meat, there was a trend of decreasing moisture content, fat content, and texture properties. Only TBARS values increased during frozen bakso storage time (Fig 8; Fig 9).

The slight decrease of moisture content was probably an effect of the decreasing functionalities of the myofibrillar proteins due to frozen storage. Myofibrillar instability that induces the loss of water holding capacity during frozen storage has been associated with the stability changes in myosin. However, the mechanism involving frozen-induced aggregation of myosin is still unclear (Ramírez *et al.*, 2000).

The decrease of fat content in the frozen stored meat and in bakso with frozen storage might also have possibly been due to losses of triglycerides. Pearson and Dutson (1987) reported that triglycerides might decrease, in raw and in cooked beef during frozen storage while phospholipids were relatively stable. The minor differences in fat content in the present study could be due to small differences in triglyceride changes. The decline in quality of freezer-stored meats is principally due to fat oxidation (Zaehring *et al.*, 1959; Kanner *et al.*, 1988; Liu *et al.*, 1994). The TBARS values of bakso increased as the total fat content decreased during frozen storage. Pearson and Dutson (1987) reported that as the percentage of total fat content decreased, the proportion of phospholipids increased and triglycerides decreased. Phospholipids are as susceptible to rapid oxidation breakdown as triglycerides in ground meat. The increase in TBARS values during frozen-stored bakso

concluded with the results of Ho *et al.* (1995) in pork frankfurters and Wu *et al.* (2000) in cooked beef steaks.

The fact that the bakso formulation had uniform 0.6% phosphate and 1.6% salt added for all treatments might have been the reason for the stability and the lack of variability during storage of bakso and also the lack of variability of bakso products made of different lengths of stored meat.

Phosphates have been reported to be effective water-binding agents that improve emulsion stability and texture among most comminuted products (Eilert *et al.*, 1996; Seman *et al.*, 1980). The presence of phosphate induces solubilization of actomyosin, which stabilizes water holding capacity, texture, and reduces cooking loss with or without addition of hydrocolloids (Defreitas *et al.*, 1997c). Trius *et al.* (1994) reported the addition of 0.5% of STPP in pork sausages increased hardness, even in the absence of carrageenan, while Barbut *et al.* (1988) reported an increase in firmness of turkey frankfurters that contained 0.4% phosphate with either 1.5% or 2.0% salt.

The role of hydrocolloid in comminuted meat products was also taken into consideration as a factor that prevented loss of bakso properties and maintained its properties throughout storage. The presence of tapioca starch in bakso may also contribute in compensating for the loss of meat myofibrillar properties due to frozen meat storage. Tapioca starch gelation may aid in maintaining the properties and textural stability of bakso during bakso storage. In agreement, DeFreitas *et al.* (1997a, b, c) reported that hydrocolloids such as kappa- and iota- carrageenan may be useful for increasing freeze/thaw stability of cooked meat products.

Table 8. Properties of bakso made of postrigor meat frozen for 0, 2, and 4 months after 0, 2, and 4 months of frozen bakso storage.

Meat Storage	Bakso Storage	Moisture (%)	Fat (%)	TBARS (mg/kg)	Elasticity (min/g)	Gel strength (N)	Shear force (N)
0	0	70.75	0.22	1.523b	0.553	38.885	22.272
0	2	70.73	0.20	3.060a	0.537	38.803	22.232
0	4	70.57	0.19	3.035a	0.537	38.778	22.210
2	0	70.77	0.22	1.612b	0.545	38.740	22.233
2	2	70.73	0.20	2.810a	0.532	38.535	22.218
2	4	70.62	0.19	2.990a	0.522	38.520	22.208
4	0	70.75	0.20	1.518b	0.535	38.538	22.160
4	2	70.71	0.20	2.710a	0.523	38.507	22.118
4	4	70.52	0.19	3.068a	0.518	38.495	22.150
SEM		0.33nd	0.01nd	0.232	0.015nd	0.206nd	0.386nd

Data are means and standard errors (SEM) of 6 replicated experiments. SEM's with nd superscripts indicate no significant differences among corresponding means ( $p < 0.05$ ). TBA=Thiobarbituric acid reactive substances.

When the nine treatments were compared in a different design, the treatments of frozen meat storage and frozen bakso storage had no effect ( $p < 0.05$ ) on moisture content, fat content, elasticity, hardness and shear force values of bakso (table 8). Significant differences were only observed for TBARS values ( $p < 0.05$ ; table 8). Fresh cooked bakso made of frozen stored meat for 0, 2, and 4 months did not reveal differences in TBARS, but bakso that was stored for 2 and 4 months were different in TBARS values compared to 0 months of bakso storage. The results indicated that oxidative deterioration was not significant throughout frozen meat storage, while the frozen storage of bakso (cooked product) resulted in high ( $p < 0.05$ ) lipid oxidation. Oxidation of lipids (rancidity), especially phospholipids, occurs much faster in cooked meat than in stored uncooked meat (Lawrie, 1991).

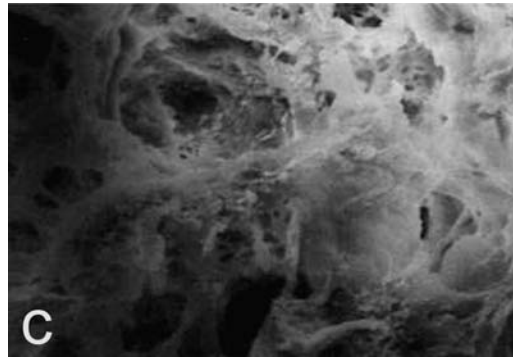
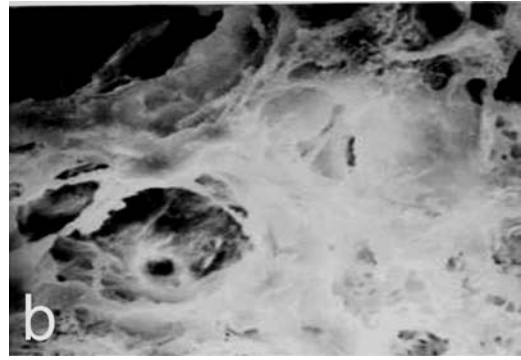


Fig 10. Scanning electron microscopy at 1500x magnification of bakso made of postrigor meat stored frozen 0 month after 0 (a), 2 (b), and 4 (c) months of frozen bakso storage.

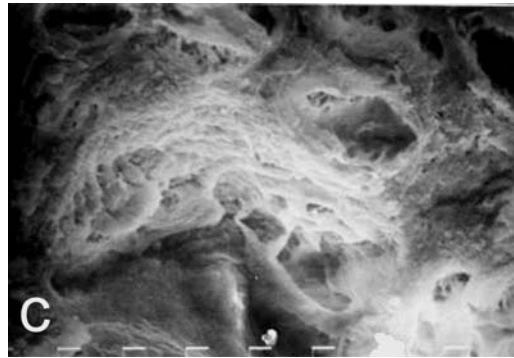
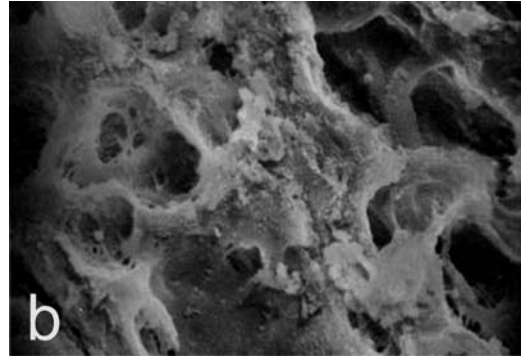
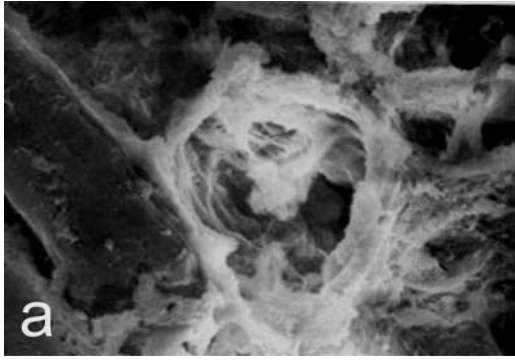


Fig 11. Scanning electron microscopy at 1500x magnification of bakso made of postrigor meat stored frozen 2 months after 0 (a), 2 (b), and 4 (c) months of frozen bakso storage.

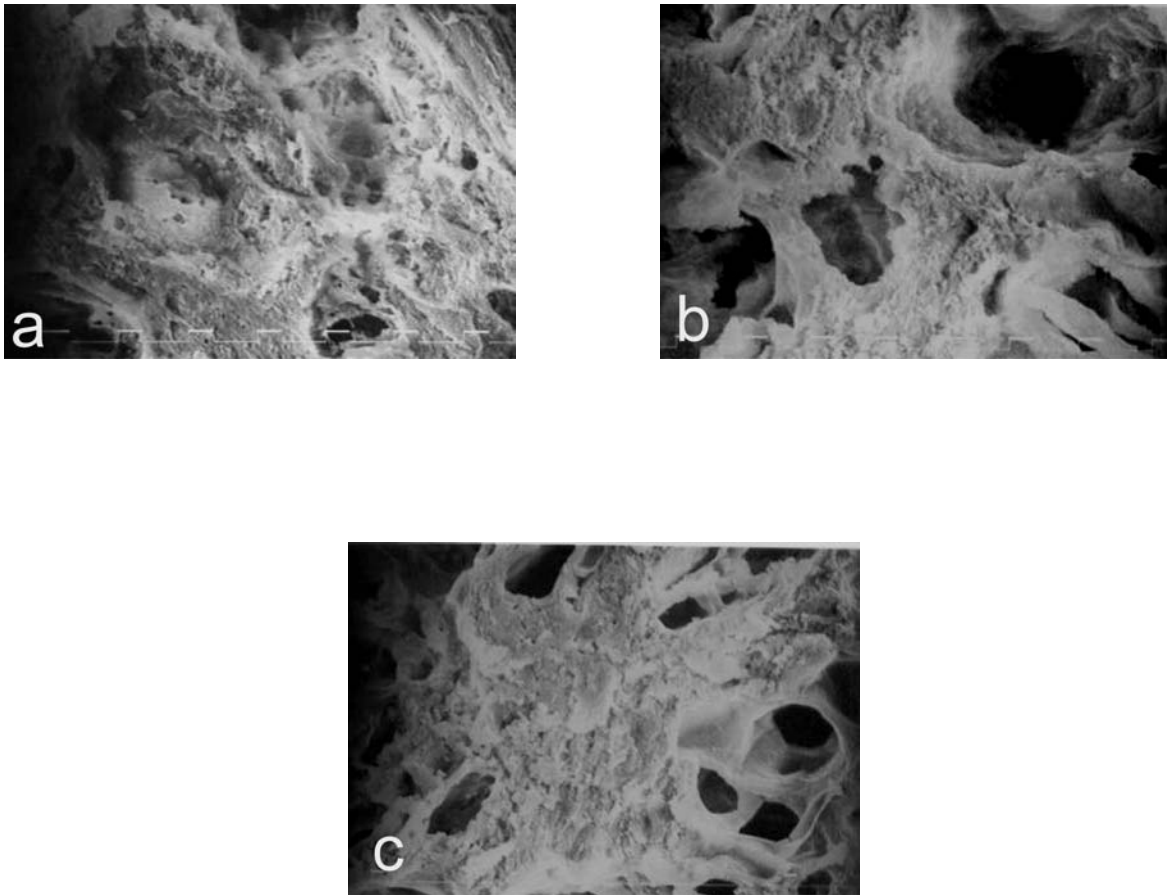


Fig 12. Scanning electron microscopy at 1500x magnification of bakso made of postrigor meat stored frozen 4 months after 0 (a), 2 (b), and 4 (c) months of frozen bakso storage.

Bakso made of 0 month frozen stored postrigor meat (Fig 10) appeared to have a more web-like protein strand network than that of bakso made of 2 months and 4 months frozen stored meat (fig 11 and 12).

Micrographs demonstrated a difference in bakso matrix structure of 0 months of frozen meat storage compared with 2 and 4 months of frozen meat storage. At 4 months of meat storage, the bakso appeared to show large empty voids of space (Fig 12) unlike 0

months of meat storage or early postmortem bakso, which had more protein strands filling the spaces of the large voids (Fig 10). Differences in these micrographs were suspected to be caused by an interruption in myofibrillar gelation. Interruptions of gelation can be due to small actomyosin fragments from the degradation of actomyosin with the presence of muscle proteinase during storage. The length of frozen storage and, more specifically, the degree of protein denaturation occurring during frozen storage have been shown to influence gel properties of beef, chicken and fish muscle protein (Smith, 1988). Visessanguan *et al.* (2000) reported that actomyosin gel forming in the presence of proteinase such as cathepsin L had a detrimental effect due to fragments of myofibrillar proteins that disrupted the initial structure formation during gel setting, even though crosslinks and depositions of small fragments might occur.

#### **Effect of Frozen Storage Time on Bakso from Early Postmortem and Postrigor Meat**

Replications had no effects on any properties ( $p < 0.05$ ; appendix page 82). The moisture and fat content of bakso were not different with meat postmortem condition ( $p > 0.05$ ) throughout frozen bakso storage of 0, 2 and 4 months (table 9). Though statistically insignificant, the moisture content of bakso from postrigor meat was lower than that from meat obtained early postmortem, while the fat content was higher in bakso from postmortem meat. The TBA values of bakso from postrigor meat were higher ( $p < 0.05$ ) than bakso from early postmortem meat.

Bakso storage and meat postmortem condition also affected ( $p < 0.05$ ) bakso texture properties. Bakso from early postmortem meat throughout storage had higher ( $p < 0.05$ ) elasticity values, gel strength and shear force values than bakso from postrigor meat.

Table 9. Effect of meat postmortem condition on bakso stored frozen for 0, 2, and 4 months

Rigor condition	Moisture (%)	Fat (%)	TBARS (mg/kg)	Elasticity (min/g)	Gel strength (N)	Shear force (N)
Postrigor	70.683	0.21	2.540 <sup>a</sup>	0.542 <sup>a</sup>	38.822 <sup>a</sup>	22.237 <sup>a</sup>
Early postmortem	70.700	0.19	1.388 <sup>b</sup>	0.630 <sup>b</sup>	40.548 <sup>b</sup>	23.162 <sup>b</sup>
SEM	0.100 <sup>nd</sup>	0.01 <sup>nd</sup>	0.069	0.009	0.129	0.158

Data represents means and standard errors (SEM) of six replicates of bakso from postrigor and early postmortem meat that was stored frozen from 0-4 months. Means with different superscripts are significantly different ( $p < 0.05$ ), while nd=not significantly different ( $p < 0.05$ ). TBARS=Thiobarbituric acid reactive substance.

Moisture content of bakso from early postmortem and postrigor meat decreased throughout the length of frozen storage from 0 months to 4 months ( $p < 0.05$ ; Fig 13a). Moisture content for bakso from both early postmortem and postrigor meat demonstrated a slight decrease after 2 months storage ( $p < 0.05$ ), but the greatest decrease was after 4 months of the storage period ( $p < 0.05$ ; fig 13a). The slight decrease of moisture content during storage of bakso may have been caused by water holding capacity loss from freeze-thaw, as the samples were thawed before testing, but weight loss was not measured in this study. Water crystallization during freezing may have damaged cells and gel structure causing moisture to purge as the samples were thawed. Meat freezing usually results in an increased exudate during thawing (Barbut and Mittal, 1990). Oxidation of muscle proteins are also suspected to affect water holding capacity, causing the slight decrease in moisture content. Oxidation of muscle protein is common in frozen stored meat and processed meats, especially meat subjected to grinding or comminution processes, where lipid peroxidation is extensive. Oxidation processes alter the functional behaviors of muscle proteins and can

influence gel forming and water holding capacity and hence texture, binding, stability, and palatability of the product (Xiong, 1996).

Bakso from early postmortem meat was slightly higher in moisture than bakso from postrigor meat. It is possible that the differences of bakso moisture content were due to the fact that early postmortem meat has more free-myosin than postrigor meat, where more myosin are bound in actomyosin. Free myosin is known to have better gelling properties and thus water holding capacity (Xiong and Brekke, 1991). TBARS values with postrigor meat at 0 months of bakso frozen storage (fig 13c) were slightly higher than bakso from early postmortem meat. Bakso from postrigor meat experienced a strong increase in TBARS value after 2 months of frozen storage and stabilized ( $p < 0.05$ ) after 4 months of frozen storage, while bakso from early postmortem meat had a slightly stable and almost constant increase ( $p < 0.05$ ) throughout the storage period (0-4 months; fig 13c). According to Wu *et al.* (2000), prerigor and postrigor steaks had similar TBA values until 1 month of frozen storage. Wu *et al.* (2000) reported lipid oxidation increased during 1 to 3 months of postrigor storage while a decline in TBA values after 3 months of storage and stabilization through further frozen storage up to 6 months was postulated to be due to further decay of lipid oxidation products. Ho *et al.* (1995) reported an increase in TBARS values in frozen pork sausages through 8 weeks and 16 weeks storage time. Furthermore, Ho *et al.* (1995) reported that methods of packaging influenced oxidative rancidity during frozen storage. Vacuum packaging retarded oxidative rancidity, but compared to products with antioxidants, the TBARS values were still high.

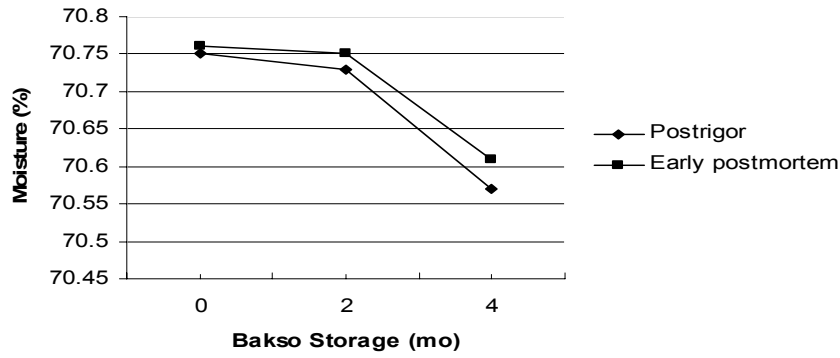
Comminution and cooking process of bakso balls were also suspected to cause the significant TBARS values. Comminution and heating processes were also reported to

influence initiation of lipid peroxidation (Harel and Kanner, 1985; McMillin, 1996; Xiong, 1996). During cutting, chopping, or grinding, oxyhemoglobin and oxymyoglobin pigments are oxidized. This leads to the formation of metmyoglobin or methemoglobin and the dismutation of  $O_2^-$  to  $H_2O_2$  (Harel and Kanner, 1985). The presence of  $H_2O_2$  activates metmyoglobin to a porphyrin cation radical which initiates lipid peroxidation, causing oxidation of unsaturated fatty acids. Cooking or heating processes destroy catalase that is present in uncooked meats. Catalase in uncooked meat partially prevents the activation of metmyoglobin. Metmyoglobin during the heating process still retains its capacity to be activated by  $H_2O_2$ . Therefore, the heating process does not prevent or retard lipid oxidation and this might be the reason why cooked meat products are more susceptible to lipid oxidation than uncooked meat products (Harel and Kanner, 1985; McMillin, 1996).

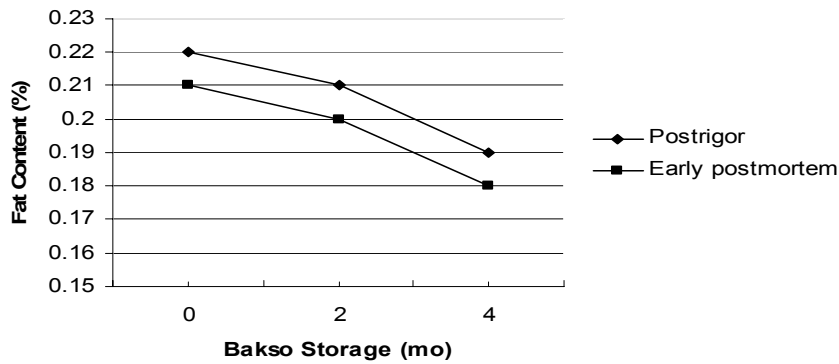
Texture of bakso from both early postmortem and postrigor meat decreased only slightly throughout the frozen bakso storage period. The decrease of textural properties throughout the storage period was relatively consistent in elasticity, gel strength, and shear force values ( $p < 0.05$ ).

The textural properties of bakso from early postmortem meat had trends toward higher, more desirable values than bakso from postrigor meat, while micrographs illustrated the differences in three dimensional structures of bakso from early postmortem and postrigor meat. These differences are possibly due to the myofibrillar protein of early postmortem meat that consists of more free-myosin than actomyosin, while the myofibrillar protein in late postmortem is assumed to consist of more actomyosin than free myosin. Vissesanguan *et al.* (2000) reported that both myosin and actomyosin play an important role in gel network formation. Myosin is recognized as the major protein of the muscle tissue in respect to gel-

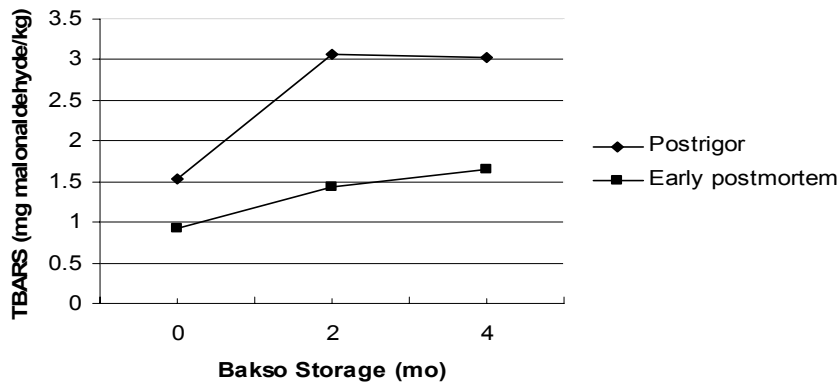
forming abilities, while other myofibrillar proteins such as actin and other regulatory and skeletal proteins do not form gels even though they also have influence on viscoelasticity (Lee and Lanier, 1995; Ramírez *et al.*, 2000).



SEM=0.172 a.

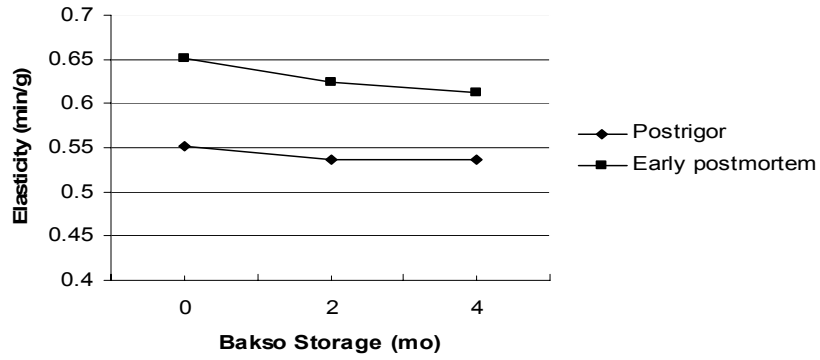


SEM=0.012 b.

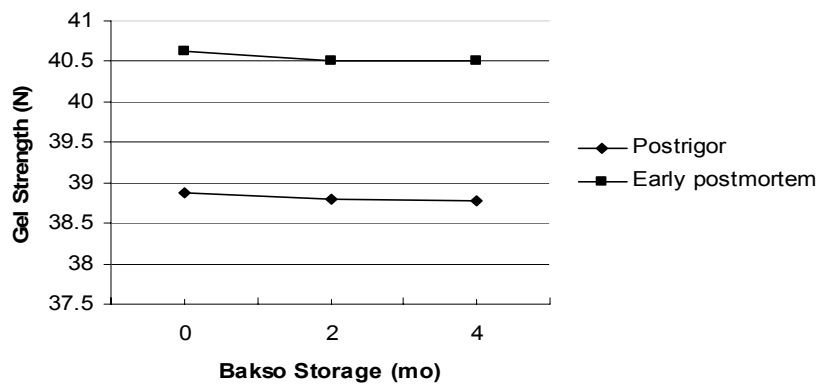


SEM=0.119 c.

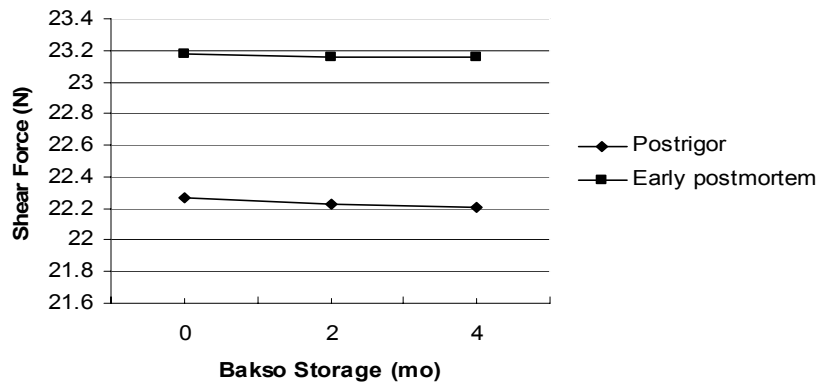
Fig 13. Moisture (a), fat content (b) and TBARS values (c) of bakso from early postmortem and postrigor meat after frozen bakso storage of 0, 2, and 4 months.



SEM=0.016 a.



SEM=0.224 b.



SEM=0.274 c.

Fig 14. Texture properties of bakso from early postmortem and postrigor meat after frozen bakso storage of 0, 2, and 4 months (a=Elasticity, b=Gel strength and c=Shear force).

Early postmortem bakso

Postrigor bakso

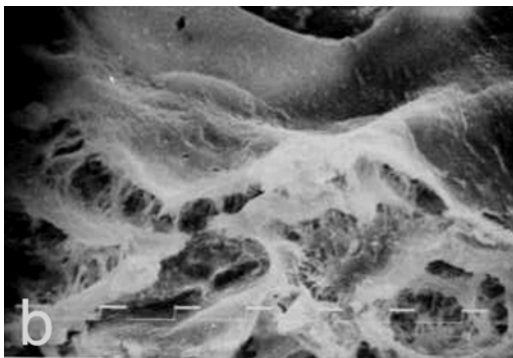
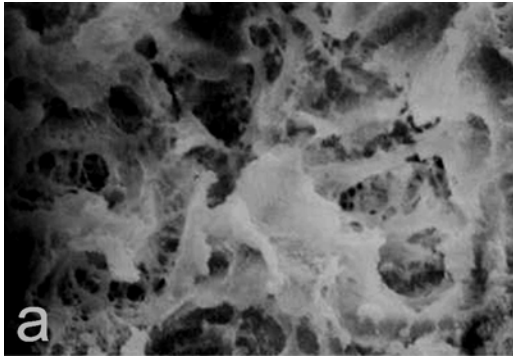


Fig 15. Scanning electron microscopy at 1500x magnification of bakso from early postmortem and postrigor meat after 0 (a), 2 (b), and 4 (c) months of frozen storage (early postmortem on the left and postrigor on the right).

Acton *et al.* (1983) noted that based on emulsification properties and stability, myosin and actomyosin are the principal myofibrillar proteins. In the absence of other myofibrillar proteins, myosin is preferentially absorbed in the system. The actomyosin complex is the major gel-forming agent in postrigor meat as myosin is in early postmortem meat (Acton *et al.*, 1983).

The micrographs from scanning electron microscopy (SEM) of bakso from early postmortem meat for 0 months storage time (fig 15a) demonstrated a well distributed network of the three dimension matrix of protein strands and starch. There appeared to be less protein strands holding the structure and the structure is more supported by the gelatinized tapioca starch than the myofibrillar protein gelation with 2 and 4 months of frozen storage.

Bakso from postrigor meat had less protein strands supporting the three dimensional network. Pores of the spongy-like structure appear empty when observed closely as in fig 15 (late postmortem), and the whole structure seems to be more supported by the gelatinized starch than the myofibrillar network. Bakso from postrigor meat after 2 months and 4 months frozen storage time did not show major differences in structure, but did have less spongy void structure.

### **Implications**

The substitution of postrigor meat for early postmortem meat in bakso production using 0.6% STPP, 1.6% NaCl, and 15% starch tapioca resulted in bakso with minimal composition and texture differences. The decrease in oxidative stability of bakso made from postrigor meat after 2 and 4 months of frozen storage compared with the use of early postmortem meat or bakso stored frozen for 0 months must be addressed by inclusion of

commercial ingredients to minimize potential off-flavors of the precooked bakso products.

Meat stored frozen for 2 and 4 months was still suitable as raw material for bakso production.

## CHAPTER 5. SUMMARY AND CONCLUSION

Two phases of experiments were performed to study the replacement of early postmortem meat with postrigor meat in industrial scale bakso production. The objectives of the first phase were to determine if postrigor meat use would be applicable in bakso production and to evaluate levels of tapioca starch concentration to compensate for the diminished gelation capacity of postrigor meat. Results indicated no difference in composition of bakso balls made from either early postmortem or postrigor meat. Further observations of the data revealed that postrigor meat did have some disadvantages, especially in texture of bakso. This disadvantage was compensated by the 15% tapioca starch in combination with the basic formula of 0.6%STPP and 1.6% NaCl in bakso manufacture.

The possibilities of using postrigor meat for bakso production led to investigations of bakso properties after frozen storage of meat and after frozen storage of the finished products. Storing the meat frozen at -20°C for 2 months and 4 months resulted in no differences in bakso quality from meat at 0 month, although storage had a slightly detrimental effect on gel forming capabilities and the final matrices of protein networks. These structural changes were not great enough to diminish the advantages of using frozen postrigor meat. Reducing slaughter frequencies required to obtain sufficient quantities of early postmortem raw material for bakso production and longer times of raw material storage with frozen rather than refrigerated storage of postrigor meat creates opportunity for the industry to reduce production costs and raise profit margins while maintaining bakso quality traits. Further investigations are suggested to determine the maximal time that postrigor raw material can be stored frozen and result in desirable properties and shelf life of the bakso.

Research on various antioxidants that could be incorporated to reduce the off-flavors of cooked bakso products after long periods of chilled or frozen storage should be addressed. Studies on the usage of frozen postrigor meat imported from Australia or the U.S. on the properties of bakso should also be conducted. Investigations on the implementation of preblending with salt or phosphate to provide additional protein extraction before bakso manufacture can also be suggested to accompany the usage of postrigor meat.

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**APPENDIX A. ANOVA TABLES FOR PHASE 1 (CHAPTER 3)**

ANOVA Table with moisture content as dependent variable

<b>Source</b>	<b>Df</b>	<b>Mean Square</b>	<b>F-value</b>	<b>p-value</b>
<b>Model</b>	<b>17</b>	<b>4.22</b>	<b>1.05</b>	<b>0.46</b>
Postmortem state	1	0.76	0.19	0.67
Starch	2	12.16	3.01	0.07
Replication	2	0.22	0.05	0.95
Postmortem*Starch	2	4.37	1.08	0.36
Rep.*Postmortem	2	9.96	2.47	0.11
Rep.*Starch	4	1.01	0.25	0.91
Rep.*Postmortem*Starch	4	3.38	0.84	0.52
<b>Error</b>	<b>18</b>	<b>4.04</b>		
<b>Corrected Total</b>	<b>35</b>			

Mean : 73.54

Coefficient of Variance : 2.73

ANOVA Table with fat content as dependent variable

<b>Source</b>	<b>Df</b>	<b>Mean Square</b>	<b>F-value</b>	<b>p-value</b>
<b>Model</b>	<b>17</b>	<b>0.02</b>	<b>2.05</b>	<b>0.07</b>
Postmortem state	1	0.003	0.32	0.58
Starch	2	0.02	2.03	0.16
Replication	2	0.09	9.13	0.002
Postmortem*Starch	2	0.01	0.83	0.45
Rep.*Postmortem	2	0.02	2.18	0.14
Rep.*Starch	4	0.001	0.13	0.97
Rep.*Postmortem*Starch	4	0.01	1.41	0.27
<b>Error</b>	<b>18</b>	<b>0.01</b>		
<b>Corrected Total</b>	<b>35</b>			

Mean : 0.21

Coefficient of Variance : 48.33

ANOVA Table with elasticity as dependent variable

Source	Df	Mean Square	F-value	p-value
<b>Model</b>	<b>17</b>	<b>0.0003</b>	<b>5.63</b>	<b>0.0003</b>
Postmortem state	1	0.0000	0.10	0.76
Starch	2	0.0020	34.34	<0.0001
Replication	2	0.0040	6.71	0.01
Postmortem*Starch	2	0.0001	0.76	0.48
Rep.*Postmortem	2	0.0001	1.20	0.32
Rep.*Starch	4	0.0001	1.47	0.25
Rep.*Postmortem*Starch	4	0.0001	0.92	0.47
<b>Error</b>	<b>18</b>	<b>0.0001</b>		
<b>Corrected Total</b>	<b>35</b>			

Mean : 0.072

Coefficient of Variance : 10.82

ANOVA Table with gel strength as dependent variable

Source	Df	Mean Square	F-value	p-value
<b>Model</b>	<b>17</b>	<b>0.01</b>	<b>3.63</b>	<b>0.005</b>
Postmortem state	1	0.01	4.28	0.05
Starch	2	0.004	2.82	0.09
Replication	2	0.003	2.38	0.12
Postmortem*Starch	2	0.01	9.97	0.001
Rep.*Postmortem	2	0.003	1.97	0.17
Rep.*Starch	4	0.001	0.58	0.68
Rep.*Postmortem*Starch	4	0.01	5.22	0.01
<b>Error</b>	<b>18</b>	<b>0.001</b>		
<b>Corrected Total</b>	<b>35</b>			

Mean : 0.29

Coefficient of Variance : 12.72

ANOVA Table with shear force as dependent variable

<b>Source</b>	<b>Df</b>	<b>Mean Square</b>	<b>F-value</b>	<b>p-value</b>
<b>Model</b>	<b>17</b>	<b>16.44</b>	<b>0.35</b>	<b>0.98</b>
Postmortem state	1	3.29	0.07	0.79
Starch	2	10.08	0.21	0.81
Replication	2	110.87	2.34	0.12
Postmortem*Starch	2	5.32	0.11	0.89
Rep.*Postmortem	2	1.31	0.03	0.97
Rep.*Starch	4	3.31	0.07	0.99
Rep.*Postmortem*Starch	4	1.96	0.04	1.00
<b>Error</b>	<b>18</b>	<b>47.42</b>		
<b>Corrected Total</b>	<b>35</b>			

Mean : 9.97  
 Coefficient of Variance : 69.07

## APPENDIX B. ANOVA TABLES FOR PHASE 2 (CHAPTER 4)

### Experiment 1:

ANOVA Table with moisture content as dependent variable

Source	Df	Mean Square	F-value	p-value
<b>Model</b>	<b>29</b>	<b>0.4675</b>	<b>0.69</b>	<b>0.8343</b>
Meat storage	2	0.0128	0.02	0.9814
Bakso storage	2	0.1795	0.26	0.7706
Replication	5	0.5010	0.74	0.6043
Replication*Meat storage	10	0.5747	0.84	0.5939
Replication*Bakso storage	10	0.4923	0.72	0.6962
Error	24	0.6814		
<b>Corrected Total</b>	<b>53</b>			

Mean : 70.68

Coefficient of Variance : 1.17

ANOVA Table with fat content as dependent variable

Source	Df	Mean Square	F-value	p-value
<b>Model</b>	<b>29</b>	<b>0.0007</b>	<b>2.18</b>	<b>0.0272</b>
Meat storage	2	0.0002	0.67	0.5187
Bakso storage	2	0.0021	6.95	0.0042
Replication	5	0.0002	0.60	0.7017
Replication*Meat storage	10	0.0008	2.70	0.0226
Replication*Bakso storage	10	0.0006	1.81	0.1127
Error	24	0.0003		
<b>Corrected Total</b>	<b>53</b>			

Mean : 0.20

Coefficient of Variance : 8.58

ANOVA Table with TBARS values as dependent variable

Source	Df	Mean Square	F-value	p-value
<b>Model</b>	<b>29</b>	<b>1.0487</b>	<b>3.09</b>	<b>0.0031</b>
Meat storage	2	0.0550	0.16	0.8513
Bakso storage	2	11.7892	34.75	<0.0001
Replication	5	0.3345	0.99	0.4468
Replication*Meat storage	10	0.1351	0.40	0.9344
Replication*Bakso storage	10	0.3700	1.09	0.4071
Error	24	0.3393		
<b>Corrected Total</b>	<b>53</b>			

Mean : 2.481

Coefficient of Variance : 23.48

ANOVA Table with elasticity as dependent variable

Source	Df	Mean Square	F-value	p-value
<b>Model</b>	<b>29</b>	<b>0.0014</b>	<b>1.14</b>	<b>0.3746</b>
Meat storage	2	0.0013	1.06	0.3628
Bakso storage	2	0.0017	1.39	0.2676
Replication	5	0.0007	0.54	0.7418
Replication*Meat storage	10	0.0009	0.72	0.6979
Replication*Bakso storage	10	0.0022	1.82	0.1103
Error	24			
<b>Corrected Total</b>	<b>53</b>			

Mean : 0.533

Coefficient of Variance : 6.564

ANOVA Table with gel strength as dependent variable

<b>Source</b>	<b>Df</b>	<b>Mean Square</b>	<b>F-value</b>	<b>p-value</b>
<b>Model</b>	<b>29</b>	<b>0.2629</b>	<b>1.27</b>	<b>0.2793</b>
Meat storage	2	0.4589	2.21	0.1314
Bakso storage	2	0.0808	0.39	0.6818
Replication	5	0.5460	2.63	0.0493
Replication*Meat storage	10	0.1387	0.67	0.7421
Replication*Bakso storage	10	0.2427	1.17	0.3571
Error	24			
<b>Corrected Total</b>	<b>53</b>			

Mean : 38.64

Coefficient of Variance : 1.18

ANOVA Table with shear force as dependent variable

<b>Source</b>	<b>Df</b>	<b>Mean Square</b>	<b>F-value</b>	<b>p-value</b>
<b>Model</b>	<b>29</b>	<b>0.4816</b>	<b>0.44</b>	<b>0.9821</b>
Meat storage	2	0.0459	0.04	0.9591
Bakso storage	2	0.0062	0.01	0.9943
Replication	5	0.3028	0.28	0.9216
Replication*Meat storage	10	0.5306	0.48	0.8840
Replication*Bakso storage	10	0.7042	0.64	0.7637
Error	24	1.0964		
<b>Corrected Total</b>	<b>53</b>			

Mean : 22.200

Coefficient of Variance : 4.716

## Experiment 2:

ANOVA Table with moisture content as dependent variable

Source	Df	Mean Square	F-value	p-value
<b>Model</b>	<b>25</b>	<b>0.3264</b>	<b>1.82</b>	<b>0.1620</b>
Postmortem state	1	0.0038	0.02	0.8871
Bakso storage	2	0.1008	0.56	0.5871
Postmortem*Bakso storage	2	0.0005	0.00	0.9973
Replication	5	0.0395	0.22	0.9456
Replication*Postmortem	5	0.4243	2.37	0.1156
Replication*Bakso storage	10	0.5634	3.14	0.0426
Error	10	1.1794		
<b>Corrected Total</b>	<b>35</b>			

Mean : 70.69

Coefficient of Variance : 0.60

ANOVA Table with fat content as dependent variable

Source	Df	Mean Square	F-value	p-value
<b>Model</b>	<b>25</b>	<b>0.0007</b>	<b>0.85</b>	<b>0.6463</b>
Postmortem state	1	0.0013	1.59	0.2365
Bakso storage	2	0.0023	2.73	0.1135
Postmortem*Bakso storage	2	0.0000	0.05	0.9492
Replication	5	0.0008	0.97	0.4789
Replication*Postmortem	5	0.0004	0.45	0.8073
Replication*Bakso storage	10	0.0006	0.71	0.7016
Error	10	0.0008		
<b>Corrected Total</b>	<b>35</b>			

Mean : 0.20

Coefficient of Variance : 14.64

ANOVA Table with TBARS values as dependent variable

Source	Df	Mean Square	F-value	p-value
<b>Model</b>	<b>25</b>	<b>1.1727</b>	<b>13.83</b>	<b>&lt;0.0001</b>
Postmortem state	1	13.0020	153.30	<0.0001
Bakso storage	2	4.6453	54.77	<0.0001
Postmortem*Bakso storage	2	0.8560	10.09	0.0040
Replication	5	0.1009	1.19	0.3796
Replication*Postmortem	5	0.1543	1.82	0.1968
Replication*Bakso storage	10	0.4035	4.76	0.0107
Error	10	0.0848		
<b>Corrected Total</b>	<b>35</b>			

Mean : 1.939  
Coefficient of Variance : 15.018

ANOVA Table with elasticity as dependent variable

Source	Df	Mean Square	F-value	p-value
<b>Model</b>	<b>25</b>	<b>0.0034</b>	<b>2.39</b>	<b>0.0753</b>
Postmortem state	1	0.0700	47.83	<0.0001
Bakso storage	2	0.0024	1.66	0.2377
Postmortem*Bakso storage	2	0.0004	0.26	0.7793
Replication	5	0.0004	0.28	0.9139
Replication*Postmortem	5	0.0005	0.36	0.8646
Replication*Bakso storage	10	0.0007	0.50	0.8566
Error	10	0.0014		
<b>Corrected Total</b>	<b>35</b>			

Mean : 0.586  
Coefficient of Variance : 6.484

ANOVA Table with gel strength as dependent variable

Source	Df	Mean Square	F-value	p-value
<b>Model</b>	<b>25</b>	<b>1.3060</b>	<b>4.34</b>	<b>0.0097</b>
Postmortem state	1	26.8065	89.16	<0.0001
Bakso storage	2	0.0483	0.16	0.8537
Postmortem*Bakso storage	2	0.0016	0.01	0.9948
Replication	5	0.4196	1.40	0.3050
Replication*Postmortem	5	0.0768	0.26	0.9274
Replication*Bakso storage	10	0.3261	1.08	0.4502
Error	10	0.3006		
<b>Corrected Total</b>	<b>35</b>			

Mean : 39.685

Coefficient of Variance : 1.382

ANOVA Table with shear force as dependent variable

Source	Df	Mean Square	F-value	p-value
<b>Model</b>	<b>25</b>	<b>0.5081</b>	<b>1.13</b>	<b>0.4424</b>
Postmortem state	1	7.6914	17.07	0.0020
Bakso storage	2	0.0054	0.01	0.9880
Postmortem*Bakso storage	2	0.0012	0.00	0.9973
Replication	5	0.1435	0.32	0.8909
Replication*Postmortem	5	0.0927	0.21	0.9526
Replication*Bakso storage	10	0.3817	0.85	0.6010
Error	10	0.4507		
<b>Corrected Total</b>	<b>35</b>			

Mean : 22.700

Coefficient of Variance : 2.957

### APPENDIX C. LSMEAN TABLES FOR PHASE 1 (CHAPTER 3)

Proximate analysis and rheological means of bakso from early postmortem and postrigor meat with 0, 5, and 15% starch concentration

Meat rigor Condition	Starch (%)	Moisture (%)	Fat (%)	Elasticity (min/g)	Gel strength (N)	Shear force (N)
Postrigor	5	72.78	0.18	0.439	34.269 <sup>b</sup>	18.505 <sup>f</sup>
Postrigor	10	74.80	0.20	0.503	36.335 <sup>a</sup>	21.401 <sup>cd</sup>
Postrigor	15	72.59	0.27	0.557	38.490 <sup>a</sup>	21.905 <sup>b</sup>
Early	5	74.45	0.22	0.535	34.901 <sup>b</sup>	19.720 <sup>e</sup>
Early	10	74.21	0.14	0.582	37.467 <sup>a</sup>	21.817 <sup>bc</sup>
Early	15	72.39	0.23	0.601	40.871 <sup>a</sup>	22.762 <sup>a</sup>
SEM		0.82 <sup>nd</sup>	0.04 <sup>nd</sup>	0.071 <sup>nd</sup>	1.248	0.186

Data are means and standard errors (SEM) of 3 replicated experiments. SEM's with nd superscripts indicate no significant differences among corresponding means (p<0.05).

Proximate analysis and rheological means of bakso with 0, 5 and 15% starch concentration

Starch (%)	Moisture (%)	Fat (%)	Elasticity (min/g)	Gel strength (N)	Shear force (N)
5	73.62	0.20	0.487	34.585 <sup>b</sup>	19.112 <sup>c</sup>
10	74.51	0.17	0.542	36.901 <sup>ab</sup>	21.609 <sup>b</sup>
15	72.50	0.25	0.579	39.680 <sup>a</sup>	22.333 <sup>a</sup>
SEM	0.58 <sup>nd</sup>	0.03 <sup>nd</sup>	0.050 <sup>nd</sup>	0.882	0.131

Data are means and standard errors (SEM) of 3 replicated experiments. SEM's with nd superscripts indicate no significant differences among corresponding means (p<0.05).

## APPENDIX D. LSMEAN TABLES FOR PHASE 2 (CHAPTER 4)

### Experiment 1

Replications of means of bakso made of postrigor meat stored frozen for 0, 2, and 4 months after 0, 2, and 4 months of frozen bakso storage

Replication	Moisture (%)	Fat (%)	TBARS (mg/kg)	Elasticity (min/g)	Gel strength (N)	Shear force (N)
1	70.50	0.21	2.836	0.526	38.898	22.244
2	70.99	0.21	2.501	0.527	38.960	21.917
3	70.94	0.20	2.323	0.527	38.649	22.131
4	70.48	0.20	2.491	0.548	38.491	22.123
5	70.73	0.20	2.430	0.533	38.552	22.360
6	70.47	0.20	2.303	0.537	38.317	22.424
SEM	0.28 <sup>nd</sup>	0.01 <sup>nd</sup>	0.194 <sup>nd</sup>	0.012 <sup>nd</sup>	0.152 <sup>nd</sup>	0.349 <sup>nd</sup>

Data are means and standard errors (SEM) of 6 replicated experiments. SEM's with nd superscripts indicate no significant differences among corresponding means (p<0.05). TBARS=Thiobarbituric acid reactive substances.

Properties of bakso made of postrigor meat stored frozen for 0, 2, and 4 months after 0, 2, and 4 months of frozen bakso storage

Meat Storage (mo)	Bakso Storage (mo)	Moisture (%)	Fat (%)	TBARS (mg/kg)	Elasticity (min/g)	Gel strength (N)	Shear force (N)
0	0	70.75	0.22	1.524 <sup>c</sup>	0.552	38.884	22.272
0	2	70.73	0.21	3.061 <sup>a</sup>	0.537	38.804	22.232
0	4	70.57	0.19	3.036 <sup>a</sup>	0.537	38.778	22.210
2	0	70.77	0.22	1.612 <sup>bc</sup>	0.545	38.742	22.233
2	2	70.73	0.20	2.811 <sup>ab</sup>	0.531	38.532	22.218
2	4	70.62	0.19	2.989 <sup>a</sup>	0.523	38.519	22.208
4	0	70.75	0.21	1.519 <sup>c</sup>	0.536	38.537	22.160
4	2	70.71	0.20	2.706 <sup>ac</sup>	0.522	38.507	22.118
4	4	70.52	0.20	3.069 <sup>a</sup>	0.517	38.496	22.150
SEM		0.37 <sup>nd</sup>	0.01 <sup>nd</sup>	0.255	0.016 <sup>nd</sup>	0.202 <sup>nd</sup>	0.468 <sup>nd</sup>

Data are means and standard errors (SEM) of 6 replicated experiments. SEM's with nd superscripts indicate no significant differences among corresponding means (p<0.05). TBARS=Thiobarbituric acid reactive substances.

## Experiment 2

Replications of means of 0, 2, and 4 months frozen stored bakso made from early postmortem and postrigor meat

Replication	Moisture (%)	Fat (%)	TBARS (mg/kg)	Elasticity (min/g)	Gel strength (N)	Shear force (N)
1	70.71	0.21	2.095	0.595	39.814	22.765
2	70.80	0.20	1.974	0.595	39.866	22.622
3	70.63	0.21	1.848	0.585	39.889	22.430
4	70.57	0.20	2.072	0.574	39.557	22.868
5	70.73	0.19	1.758	0.581	39.778	22.783
6	70.71	0.17	1.879	0.587	39.199	22.731
SEM	0.17 <sup>nd</sup>	0.01 <sup>nd</sup>	0.119 <sup>nd</sup>	0.016 <sup>nd</sup>	0.224 <sup>nd</sup>	0.274 <sup>nd</sup>

Data are means and standard errors (SEM) of 6 replicated experiments. SEM's with nd superscripts indicate no significant differences among corresponding means ( $p < 0.05$ ). TBARS=Thiobarbituric acid reactive substances.

Properties of 0, 2, and 4 months frozen stored bakso made of early postmortem and postrigor meat

Meat Condition (mo)	Bakso Storage (mo)	Moisture (%)	Fat (%)	TBARS (mg/kg)	Elasticity (min/g)	Gel strength (N)	Shear force (N)
Postrigor	0	70.75	0.22	1.524 <sup>bc</sup>	0.552 <sup>bcd</sup>	38.884 <sup>b</sup>	22.272
Postrigor	2	70.73	0.21	3.061 <sup>a</sup>	0.537 <sup>cde</sup>	38.805 <sup>b</sup>	22.232
Postrigor	4	70.57	0.19	3.036 <sup>a</sup>	0.537 <sup>c</sup>	38.778 <sup>b</sup>	22.210
Early	0	70.76	0.21	0.922 <sup>d</sup>	0.651 <sup>a</sup>	40.632 <sup>a</sup>	23.175
Early	2	70.75	0.20	1.438 <sup>bcd</sup>	0.624 <sup>ab</sup>	40.507 <sup>a</sup>	23.158
Early	4	70.61	0.18	1.655 <sup>b</sup>	0.613 <sup>abc</sup>	40.506 <sup>a</sup>	23.153
SEM		0.173 <sup>nd</sup>	0.01 <sup>nd</sup>	0.119	0.016	0.224	0.274 <sup>nd</sup>

Data are means and standard errors (SEM) of 6 replicated experiments. SEM's with nd superscripts indicate no significant differences among corresponding means ( $p < 0.05$ ). TBARS=Thiobarbituric acid reactive substances.

## VITA

Dino Rahardiyana was born in Malang, East Java, Indonesia, on June 12, 1975. Dino is the elder of two sons raised by Hari Purnomo and Dina Sri Rahayu. He graduated from Saint Albert Catholic High School in 1994 and began his undergraduate studies majoring in agricultural technology in Brawijaya University, Malang. He worked on wheat gluten protein identifications in Indonesian wheat flour for bread making, at PT. Eloda Mitra with Mr. Willy Bernardi, for his undergraduate research project. After graduating with a bachelor of science degree in agricultural technology (Sarjana Teknologi Pertanian) in 2000, he was employed with Tugu Hotel as public relation specialist. Dino began his research and course work in the Department of Animal Sciences at Louisiana State University in June, 2001. He will graduate with a master of science in animal and dairy sciences in May, 2004.