STUDY ON SOUS-VIDE COOKING ON PHYSICAL CHARACTERISTICS OF THAI LOCAL BEEF

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ABSTRACT

Thai local beef (*Bos indicus*) is preferred for many kinds of Thai food. However, its texture is tough and requires a long cooking time to make it palatable. Sous-vide cooking process has been investigated in order to characterize physical properties the quality of Thai local beef. Round and sirloin samples were sealed in low density polyethylene bags and cooked at 60, 70 or 80°C for 0-36 hours. Determination of color, sous-vide cooking loss, re-heating loss and shear force were carried out. All cooked round and sirloin samples were significantly ($p \leq 0.05$) lighter (L^*) and higher in yellowness (b^*), with less redness (a^*) compared to the raw sample. However, lightness of cooked round and sirloin samples was not significantly ($p > 0.05$) different after undergoing various cooking temperatures and times, but yellowness and redness were depended on cooking temperatures and times. Increasing cooking temperature resulted in increasing shear force, while increasing the cooking time resulted in significantly ($p \leq 0.05$) decreasing shear force. These can be indicated that, within the range used in this experiment, the lower temperature and shorter cooking time showed more effective in softening the Thai local beef. Cooking at higher temperatures for longer times significantly ($p \leq 0.05$) increased the sous-vide cooking loss and decreased the re-heating loss. Image analysis of the surface texture showed that the muscle fiber was at first clear and firm then it shrank and layering was clearly visible during cooking. Surface texture images of samples after different sous-vide cooking conditions were calculated into percentage of extra fiber bundle area. It was found to correlate with their physical properties in terms of Pearson's correlation and multiple linear regressions (MLR). The percentage of extra fiber bundle area displayed the highest correlation with re-heating loss ($R = -0.68$) of sirloin sample and shear force ($R = 0.87$) of round muscle, respectively. The multiple linear regressions fitting R^2 for calibration and prediction of the texture surface analysis of cooked round sample and its physical properties of lightness, sous-vide cooking loss, re-heating loss and shear force measurements with a regression correlations determination (R^2) of 0.72-0.98 for calibration and 0.70-0.94 for validation. Light microscopy was allowed to study histological structure of round sample during sous-vide cooking. An *in situ* infrared microspectroscopy approach was developed to detect the effects of sous-vide conditions on protein structure. Histological structure changes of round sample during sous-vide cooking at 70 and 80°C induced a lower fiber lateral contraction than that of 60°C ($p \leq 0.05$). In addition, up to 6 hours cooking time, 60°C round cooked sample gave the more tender meat with an optimum after 24 hours of cooking in our experimental conditions. Protein secondary structure was investigated by mid infrared

microspectroscopy in muscle fibers. Spectral variability originated from the transformation and protonation of protein caused by heat and time treatments. In the amide I region (1700-1600 cm^{-1}), a higher band intensity at 1624 cm^{-1} relative to 1655 cm^{-1} was observed in samples. The 1655 cm^{-1} peak, assigned to alpha helix of the amide I band of proteins, decreased with increasing temperature while the 1624 cm^{-1} peak assigned to aggregated beta sheets structure increase, reflecting protein denaturation.

Keywords: Histological structural, Image processing, Physical properties, Sous-vide cooking and Thai local beef.

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CHAPTER 1

INTRODUCTION

1.1 Statement of the problems

Sous-vide cooking is a favorite traditional French preparation method for food (Tiampo, 2006). It can be defined as cooking of raw material under controlled conditions of temperature and time inside heat-stable vacuumized pouches or containers. After heating, the products are rapidly cooled to 0-3°C at least 2 hours because of safety considerations from microorganisms (Pulgar et al., 2012; Komoltri, 2012; Roldán et al., 2013). During sous-vide cooking, heat from water is transferred slowly to the vacuum packed food under controlled temperature and time to induce heat stability to the food. Therefore, cooking time tends to be longer than other conventional cooking methods. Benefits of sous-vide cooked food are improving retention of nutrient, preserving color, controlling of microbial contamination, for example. Sous-vide cooking is used in restaurants, catering and industrial processing because of its ease of use and appropriateness for the management of food stuff preparation. It also provides a suitable preparation method for ready cooked food with no risk of microbial contamination (Pulgar et al., 2012; Roldán et al., 2013) and giving the food increased shelf-life (Roldán et al., 2013). Researchers have studied and reviewed the effects of sous-vide processing on qualities and nutrients retention of difference foods. Creed (1995) reviewed the retention of vitamins A, B1, B2, B6, B12, biotin and pantothenic acid content of beef, veal, lamb, pork and salmon after sous-vide processing andSchellekens (1996) also reviewed the retention of vitamins

A, B6 and folate in broccoli after sous-vide processing with higher than those of other traditional cooking methods (steaming and boiling). In addition, temperature and cooking time had a major effect on eating quality of meat (Roldán et al., 2013) and changes in physicochemical characteristics of the muscle proteins (García-Segovia et al., 2007; Christensen et al., 2013). The physicochemical characteristics of muscle proteins control toughness of beef mainly the myofibrillar proteins (mostly actin and myosin) and connective tissue proteins (mostly collagen and elastin) (García-Segovia et al., 2007). Nui et al. (2007) reported that heating induces denaturation of proteins, fiber shrinkage, myofibrillar and sarcoplasmic protein aggregate and gel formation and finally connective tissue shrinkage and solubilization. These changes could affect meat toughness that increase into two separate phases when using of higher temperature. The first increase is with cooking from 40-50°C, that can be described by denaturation and shrinkage of myofibrillar proteins, especially myosin. The second increase is with cooking from 60-80°C where denaturation of intramuscular collagen or myofibrillar changes in structure (Christensen et al., 2000; García-Segovia et al., 2007).

Thai local beef had been existed in Thailand for a long time (Jaturasitha et al., 2009). It had been used as material in many kinds of Thai foods because of its lower price than those of imported beef. However, its texture was tough and induced spending long time for cooking. Since texture of meat related its structure, researchers attempted to improve texture of Thai local beef. Kongpeam et al. (2015) applied sous-vide process to improve flank steak quality of Thai local beef. They found that sous-vide processing affected physical properties of flank steak and induced lower toughness of cooked flank steak compared with traditional cooked flank steak.

During comparatively low temperature and long-time sous-vide cooking, heat is transferred slowly to the beef resulting in changes in its physical, chemical and structural properties. Studying the kinetic change of beef during sous-vide cooking could help to understand and relate these properties and finally correlate relationships between structural, physical and chemical changes of beef during sous-vide cooking. The objectives of this study were to determine the influence of sous-vide cooking with non-additives on physical properties changes of Thai local beef, as well as to allow a better understanding of the relationships between physical properties and changes in structural mechanisms during sous-vide cooking. Moreover, macromolecular structure and morphology of beef changes in protein structure during cooking process were assessed using mid-infrared microspectroscopy and light microscopy, respectively.

1.2 Objectives

1.2.1 To study effects of temperatures and cooking times on physical properties and surface texture of beef during sous-vide cooking

1.2.2 To correlate relationships between physical properties and surface texture changes of beef during sous-vide cooking using Pearson's correlation and regression analysis

1.2.3 To study effect of temperatures and cooking times on histological structural changes of beef during sous-vide cooking

1.2.4 To investigate effect of various sous-vide cooking parameters on the macromolecular changes of proteins

1.3 Scopes of the research

The physical properties (color, sous-vide cooking loss, re-heating loss, shear force and surface texture) of sirloin and round samples from Thai local beef were determined at cooking temperatures of 60, 70 and 80°C for 0-36 hours. The surface texture of the round and sirloin samples in RGB format was converted to black and white format (BW) then the numerical data of surface texture (extra fiber bundle area and muscle fiber bundle area) were determined. The relationships between percentage of muscle fiber bundles and their physical properties were displayed using Pearson's correlation and regression analysis. Samples were also observed the histological structural changes during cooking at temperatures of 60, 70 and 80°C for 0-36 hours using light microscopy. Finally, the effect of various sous-vide cooking parameters on the macromolecular changes of myofibrillar proteins and connective tissue were investigated. The macromolecular structure of proteins was assessed in situ, using mid infrared microspectroscopy.

1.4 Expected benefit

Characteristics of Thai local beef during sous-vide cooking could be defined for further application of this cooking technique to improve the texture of Thai local beef.

CHAPTER 2

LITERATURE REVIEW

2.1 Thai local beef

2.1.1 Thai local beef varieties

Beef cattle in Thailand can be classified into three groups according to their genetic types. The main group is Thai local beef which up to 61% of the population, although they have been decreasing in number by 0.7% annually. The second group constists of Brahman and Brahman crossbreeds (35% of the population) and the last group is fattening beef (Bunmee et al., 2018). Thai local beef, a *Bos indicus* (Zebu) genotype, are also called humped cattle because they have a hump on their shoulders. They also have small ears and dewlap (Bureau of Animal Husbandry and Genetic Improvement, 2015). They originated from India but have subsequently spread across Southeast Asia including Thailand where they have been farmed for many years (Jaturasitha et al., 2009). The cattle are typically quite light with adult males and females usually weighing of 300-350 kg and 200-270 kg, respectively (Jaturasitha et al., 2009).

There are four native breeds, originated from different regions of Thailand that have been officially recognized by the Department of Livestock, Ministry of Agriculture, Thailand: DLD (Wangkumhang et al., 2015). These are Kho-Khaolumpoon (Northern Thailand), Kho-Isaan (Northeastern Thailand), Kho-Lan (Central Thailand) and Kho-Chon (Southern Thailand) (Figure 2.1).

Other physical characteristics are in a variety between breeds, such as color of its body. Kho-Khaolumpoon is white, Kho-Isaan is deep reddish-brown (some of them are also dark, red, brown, white or beige), Kho-Lan is light-brown, dark-brown and red and Kho-Chon is dark, red, light-brown, or white (Bureau of Animal Husbandry and Genetic Improvement, 2015).

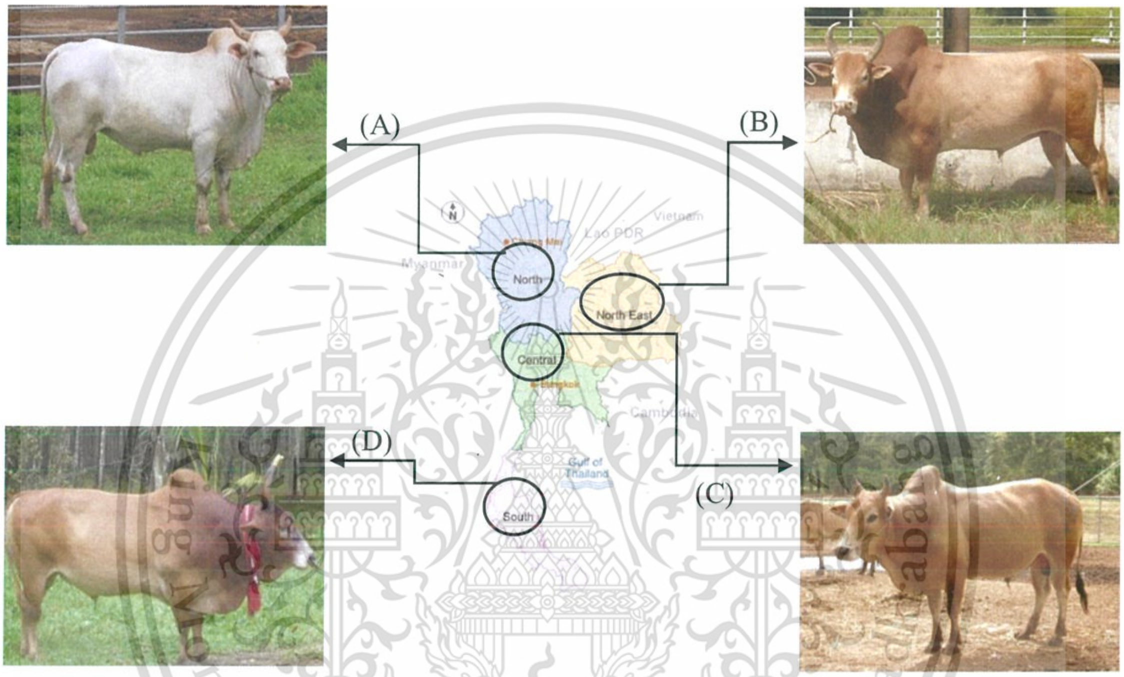


Figure 2.1 Thai local beef; Kho-Khaolumpoon (A), Kho-Isaan (B), Kho-Lan (C), and Kho-Chon (D)

Source: adapted from Bureau of Animal Husbandry and Genetic Improvement (2017)

2.1.2 Consumption of Thai local beef

The beef cattle population in Thailand is currently about 4.9 million head. About 1.0 million heads of beef cattle are slaughtered annually. Beef cattle are mainly in the North-east for48%, with 16% in Northern area and 12% in Southern area. The greatest proportionate increase has occurred in the Southern and Northern regions, where cattle numbers have increased by 11.5% and 7.7%, respectively

(Bunmee et al., 2018). Their meat has been used in many kinds of Thai food such as “Chim-Chum” (hot pot with clear soups) or grill (without marbling) (Sethakul and Sivapirunthep, 2009) since it tends to be cheaper than imported beef and meat from other types of cattle produced in Thailand.

2.2 Selected characteristics of beef

Characteristics of beef depend on a number of factors including genetic predisposition, age, gender and sex, level of nutrition, and exercise (Lawrie, 1991). Selected characteristics of beef such as meat tenderness, color and marbling (intramuscular fat quantity) are important factors which establish meat quality perceived by consumers (Aberle et al., 2001; Guzek et al., 2013), and as a consequence shape market demand. Moreover, other beef characteristics such as water holding capacity related to juiciness and ability of beef after reheating are important to establish its quality perceived by instruments.

2.2.1 Color

Color is one of the most important factors with respect to initial selection of beef by consummers (Varnam and Sutheland, 1995; Calnan et al., 2014). In red meat, a bright red color is a positive determinant of quality. The main coloring matter of meat is myoglobin. The content of myoglobin in muscles and the color of the meat, varies considerably according to species, breed and age, with concentration increasing with age. Meat from male animals usually contains more myoglobin than that from females. The function of myoglobin is oxygen storage and levels are higher in muscles with higher activity (Varnam and Sutheland, 1995).

2.2.2 Lipid and marbling

There are three distinct types of lipids in meat consisting of subcutaneous, intermuscular and intramuscular. Lean mutton, beef and pork usually contain 5-10% fat, while chicken contains 4% fat. Fat generally begins to develop just below the skin while fat in muscle is the last to be laid down. Accumulation of fat inside the muscle (intramuscular fat) is known as marbling and is often considered indicative of good eating quality and a factor often used by people in their choice of meat (Varnam and Sutberland, 1995). Marbling refers to the intramuscular fat which can be defined as the amount and spatial distribution of the visible fat, which appears as fine flecks in the muscle giving it an appearance similar to marbles (Sharma, 1999; Aberle et al., 2001; Muñoz et al., 2015). Marbling is one of the characteristics influencing the acceptability of meat and meat products (Muñoz et al., 2015; Konarska et al., 2017). Moreover, it affects consumer preference and acceptance of beef steaks (Konarska et al., 2017), with increases marbling levels improving the palatability and acceptability of beef by affecting the meat tenderness and taste (Tong et al., 2015).

2.2.3 Water holding capacity

The capacity of meat to retain its water during the application of physical forces is known as water holding capacity (WHC). This property has special significance because it contributes to the juiciness of cooked meat including its texture and color (Sharma, 1999). WHC of meat is important during cooking, processing and storage since it affected quality attributes, particularly of red meat, strongly influencing consumer preferences (Sharma, 1999; Kamruzzaman et al., 2016).

2.2.4 Tenderness

Tenderness and its variability in beef is an important characteristic for sensory acceptance by consumers (Varnam and Sutherland, 1995; Listrat et al., 2016). Beef has much higher basic toughness and lower tenderization than pork or poultry, therefore aging is essential for improving beef tenderness. In cattle, the relationships between fiber characteristics and tenderness are complex and vary according to muscle, sex, age and breed (Listrat et al., 2016). The degree of tenderness is directly related to quality and the method and extent of cooking strongly affect consumer's perceptions of quality (Varnam and Sutherland, 1995). From a sensory point of view, one of the most important quality trait is tenderness. Ellies-Oury et al. (2013) reported that increasing tenderness of foods appears to increase the enjoyment during eating.

2.2.5 Juiciness

Juiciness is extremely important in defining the liking of meat. It is possible to draw a distinction between an immediate component, given by the moisture sensation during the first chew that is due to the fast release of fluids by the meat, and an extended component that is mainly due to the stimulus of salivation given by the meat fats. This explains why meat from young animals can initially give a juiciness sensation, and then be perceived as dry, due to its relatively low amount of intramuscular fat (Lawrie, 1991).

2.2.6 Cooking loss and re-heating loss

Meat loses volume and weight during cooking process by expulsion of fluid. This change in fluid content brings about modifications in the textural qualities of meat which are in addition to the heat-induced changes in protein and fat (Purslow et al., 2016). Both temperature and time are important factors in the processes of heat and mass transfer, protein denaturation and, in some cases, protein solubilisation that occur on cooking. The percentage of weight lost from meat gradually increases with cooking temperature. The muscle (sarcomere) length, pH and salt content also affect the weight loss on cooking (Tornberg, 2005).

2.3 Low temperature long time and sous-vide cooking

The meaning of low temperature cooking is to use a lower temperature than normal than those normally used in traditional cooking. Heat treatment at lower temperatures, between 50°C and 60°C, for prolonged times (LTLT) increases tenderness of beef (Baldwin, 2012). In LTLT cooking, the cooking time can be varying from hours to days, creating a spectrum of manipulation possibilities of food preparation (Solvig, 2014).

Sous-vide is French for "under vacuum" and sous-vide cooking is defined as raw materials or raw materials with intermediate foods that are cooked under controlled conditions of temperature and time inside heat-stable vacuumized pouches (Figure 2.2) (Schellekens, 1996; Baldwin, 2012). This technique differs in two fundamental ways from traditional cooking. The first way is the raw food is vacuum sealed in heat-stable pouches, which it allows almost-perfect reproducibility and greater control over cooking than traditional cooking methods. Also, food can be pasteurized and made safe at lower temperatures (Baldwin, 2012).

Sous-vide pouches remain safe and palatable for three to four weeks when there are held at below 3.3°C (Church, 1998). Vacuum-sealing has benefits since it allows heat to be efficiently transferred from the water (or steam) to the food. This gives extended shelf-life to the food by eliminating the risk of microbial contamination during storage.

(A) (B) (C)

Figure 2.2 Sous-vide cooking technique including; (A) vacuum packed beef, (B) temperature controlled water bath and (C) sous-vide cooked beef

In addition, off-flavors from oxidation are inhibited since it prevents evaporative losses of flavor volatiles, nutrients and moisture during cooking (Church and Parsons, 2000; Baldwin, 2012). The taste profile of food is also improved with improved tenderness and juiciness (Schafheitle, 1990; Church and Parsons, 2000). Cooking in vacuum packs at temperatures below 100°C has also been shown to affect texture (smoothness), juiciness and a less weight loss (5-10%) compared to traditional cooking methods (25-40%) (Baldwin, 2012). For example, the difference between steak cooking by the traditional methods (oven) and steak from sous-vide cooking as showed in Figure 2.3.was found that beef steak after oven cooking is overcooked at the surface, while inside is undercooked whereas beef steak from sous-vide cooking is evenly from surface to inside without over cooking the outside and maintaining moisture and this is expect.

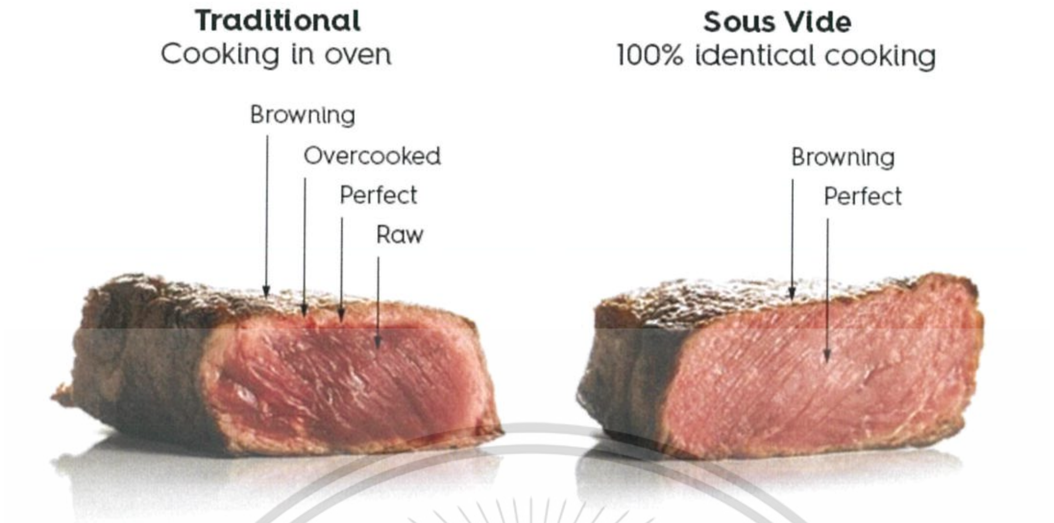


Figure 2.3 Sous-vide cooking and traditional cooking (cooking in oven) in beef steak

Source: adapted from OBH Nordica (2014)

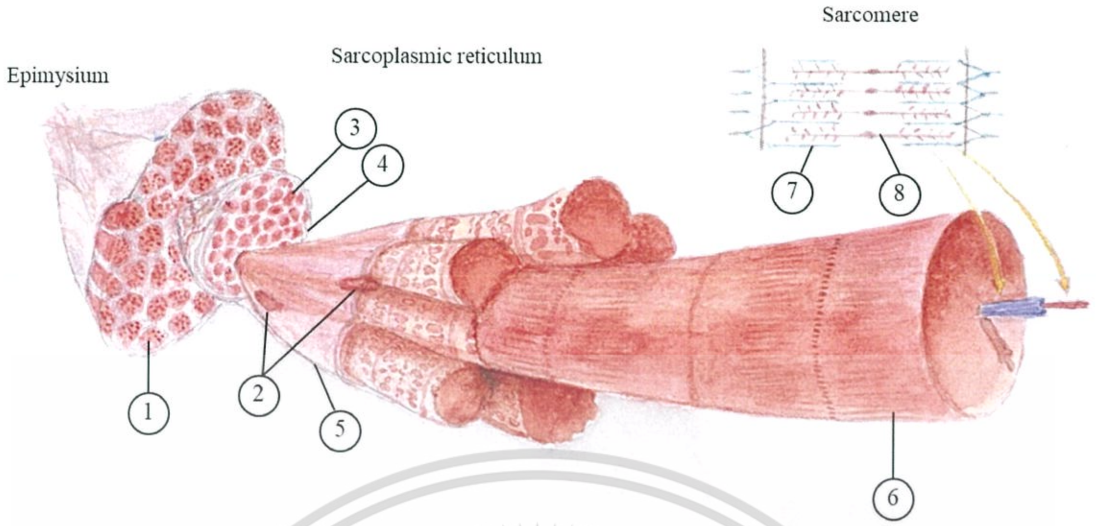
2.4 Effect of heating on selected properties of meat

2.4.1 Muscle meat

Skeletal muscles are directly attached to the bones via ligaments, fascia, cartilage or skin (Sharma, 1999; Aberle et al., 2001). There are more than 600 muscles in the animal body with vary in shape, size and action. Specific characteristics of a given muscle are dictated by its function (Aberle et al., 2001). Many of above features of muscle are showed in Figure 2.4. Each skeletal muscle fiber is a single cylindrical muscle cell. Each muscle is surrounded by a connective tissue sheath called the epimysium (Sharma, 1999; Listrat et al., 2016). From the inner surface of epimysium, a septum of connective tissue penetrates into muscle and surrounds the bundles of muscle fibers or fasciculi. This connective tissue is called perimysium. Perimysium consists of major blood vessels and nerves. Muscle fibers or specialized muscle cells are the structure unit of the skeletal muscle tissue. Each individual muscle cell, called a muscle fiber, is surrounded by connective tissue called the endomysium, beneath which is delicate sarcolemma or muscle cell membrane. It transmits nervous signals along the surface of muscle fiber (Sharma, 1999).

Meat contains 75% water, 20% protein, 2.5-5% fat and other substances (Lawrie, 1991; Baldwin, 2012). Water is principal constitute of extracellular fluid and numerous chemical constituents are dissolved or suspended in it. Because of this, it serves as the medium for transport of substances between the vascular bed and muscle fibers (Aberle et al., 2001).

Proteins of meat are composed of myofibrillar (50-55%), sarcoplasmic (30-34%), and stroma or connective tissue (10-15%) (Sharma, 1999; Baldwin, 2012). The myofibrillar proteins (mostly myosin and actin) and the connective tissue proteins (mostly collagen) contract when heated, while the sarcoplasmic proteins expand (Baldwin, 2012). During heating, the muscle fibers shrink transversely and longitudinally, the sarcoplasmic proteins aggregate, form a gel, and then connective tissues shrink and solubilize (Garcı´a-Segovia et al., 2007; Christensen et al., 2011; Baldwin, 2012). Muscle fibers begin to shrink at 35-40°C and shrinkage increases almost linearly with temperature up to 80°C. The aggregation and gelation of sarcoplasmic proteins begin around 40°C and finish at around 60°C. Connective tissues start to shrink around 60°C and contracts more intensely over 65°C. These slow changes mainly increase tenderness by dissolving collagen into gelatin and reducing inter-fiber adhesion (Baldwin, 2012).



Where

1 is Perimysium	5 is Endomysium
2 is Nucleus	6 is Myofibril
3 is Fiber	7 is Actin
4 is Fiber bundle	8 is Myosin

Figure 2.4 Structure of skeletal muscle

Source: adapted from Listrat et al. (2016)

2.4.1.1 Myofibrillar proteins

There are twenty different myofibrillar proteins, with 65-70% being myosin or actin (Baldwin, 2012; Nishimura, 2015). Thick filaments from a protein called myosin which has important properties of thermal gel formation in fish and land animal meat while thin filaments mainly contain actin (Nishimura, 2015). During heating to 35-40°C, the muscle fibers start to shrink and increase shrinking up to 80°C. Shrinking and swelling of myofibrils in meat affect the water-holding capacity (WHC) of whole muscle meat.

Palka and Daun (1999) suggested that the structure of myofibrillar protein changed during heating due to myosin was denatured and led to water retention during the first part of shrinkage. Subsequently, the actomyosin complex was denatured which induces dehydration of the protein. In addition, the muscle fiber shrank both transversely and longitudinally causing the sarcoplasmic protein to aggregate and form a gel and finally connective tissue shrank and solubilized (Garcı´a-Segovia et al., 2007; Christensen et al., 2011).

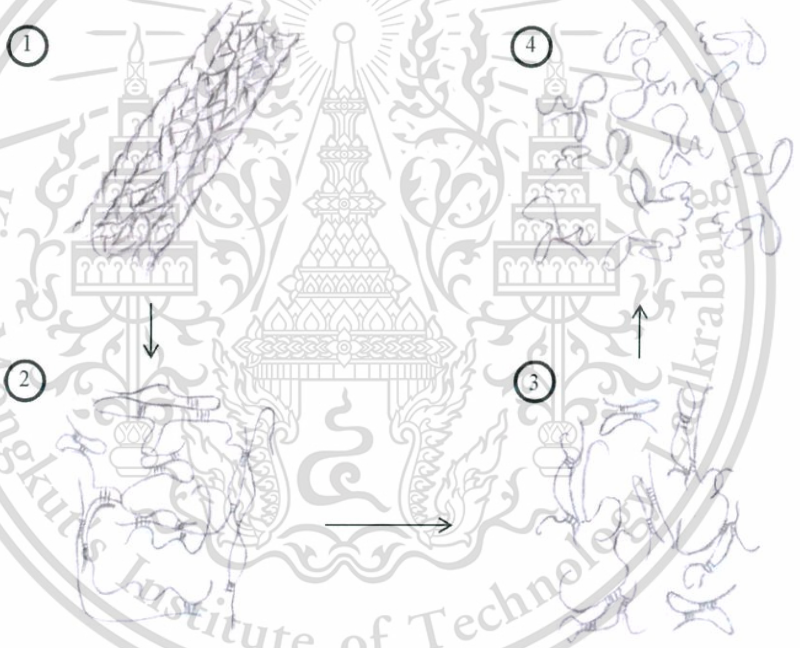
2.4.1.2 Sarcoplasmic proteins

Sarcoplasmic proteins or water soluble proteins consist of myoglobin, hemoglobin, cytochrome proteins and a wide variety of endogenous enzymes but mostly myoglobin (Baldwin, 2012; Hemung and Chin, 2013). Denaturation of sarcoplasmic proteins has an impact on meat quality parameters including color and water holding capacity (Marcos et al., 2010). The aggregation and gelation of sarcoplasmic proteins begin around 40°C and finishe at around 60°C. Before these enzymes are denatured, they significantly induce the tenderness of the meat (Baldwin, 2012).

Marcos et al. (2010) studied the combined effect of pressure and moderate temperature treatments on bovine sarcoplasmic proteins and quality parameters. M. *longissimus dorsi* samples were pressurized in a range of pressures (200-600 MPa) and temperatures (10-30°C) and found that there was a significant modification (p<0.001) of meat color and a reduction of its water holding capacity (WHC) at pressure higher than 200 MPa. The changes were induced by high pressure processing on sarcoplasmic proteins and confirm a relationship between modification of the sarcoplasmic protein fraction and alteration of meat quality characteristics.

2.4.1.3 Connective tissue

Connective tissue (or insoluble proteins), which holds the muscle fibers, bones, and fat in place, surrounds individual muscle fibers (endomysium) and bundles of these fibers (perimysium) and bundles of these bundles (epimysium) (Figure 2.4). Connective tissue consists of collagen and elastin fibers embedded in amorphous intercellular substances (Baldwin, 2012). Collagen is the most abundant protein in animals.



Where

1 is Fibrous collagen tissue

2 is Heat denaturation and solubilization

3 is Cooling and gel formation

4 is Gelatin

Figure 2.5 Thermal denaturation of collagen in to gelatin

Source: adapted from Solvig (2014)

This fibrous, structural protein comprises a right-handed bundle of three parallel, had polyproline II-type helices (Baldwin, 2012; Roy et al., 2015). Solvig (2014) and Baldwin (2012) reported that when cooking meat at temperature up to 64°C the triple helical structure stayed stable, while when increasing the temperature further the helix started to denature. When collagen fiber in meat denatures it induced contraction and shrinking to the fibers, but with continued heating above 70°C the collagen could be seen to solubilize resulting in a gelatin formation (Solvig, 2014) (Figure 2.5).

2.4.2 Color

Myoglobin and oxymyoglobin (blooming) had the capacity to lose an electron (called oxidation) which turns the pigment to a brown color and yields metmyoglobin. Thus, myoglobin can be changed from a dark purple color to a bright red color simply from oxygenation or to a brown color by losing electrons. The pigments myoglobin, oxymyoglobin and metmyoglobin can be changed from one to the others, depending on the conditions in which the meat is stored. After cooking, a brown pigment called denatured metmyoglobin can be formed, which normally could not be changed to form another pigment (Lawrie, 1991; Varnam and Sutheland, 1995). This reaction was reversible and dependent on the availability of oxygen, active enzymes and reducing compounds in the muscle (Aberle et al., 2001).

2.4.3 Tenderness

Tenderloin is well known as the most expensive cut of beef for steak. When chewing, meat is deformed and fractured. Changes of meat tenderness during cooking are associated with heat-induced alteration of myofibrillar proteins and connective tissue, since heat solubilizes the connective tissue which leads to tenderization, while denaturation of myofibrillar proteins caused meat toughening (Roldán et al., 2013). Most studies apply a Warner-Bratzler (W-B) shear test perpendicular to the muscle fibers to measure tenderness, which seems to correlate well with sensory taste tests.

2.4.4 Water loss and re-heating loss

The extent of water or fluid cooking losses depended mainly on the cooking temperature and to a smaller degree on the cooking time. Ranken (2000) showed that there was a large increased in water loss during cooking at 50-60°C with 80-100% of the total loss had occurring by the time the sample had reached 80°C. Water losses from the muscle tissue during heating also contributes to meat toughening, but the change from a viscoelastic to an elastic material also influence the changes in texture during heating (Roldán et al., 2013).

2.4.5 Microbial zone

At the temperature between 10°C and 63°C (50-145°F), most of the microbes which cause spoilage and all these which cause food poisoning can grow rapidly. This temperature range must be avoided as far as practicable. The UK Food Hygiene Regulation requires food to be held for the minimum practicable time at temperature between 10°C (50°F) and 62.7°C (145°F) and stages that they should be rapidly heated to, or cooled to, a higher or lower temperature (Ranken, 2000).

2.4.6 Sensory and nutrition qualities

Creed (1999) reported that the sous-vide cooking method produces a food with a better flavor, color, texture and nutrient retention that conventionally cooked foods. Schellekens (1996) reported the effects of sous-vide processing, steaming and traditional boiling of broccoli florets on the retention of ascorbic acid, vitamin B and folate. They found that the retention of all three vitamins examined was lowest for boiling and highest for sous-vide processing and a little lower for steaming. With sous-vide processing, ascorbic acid retention was highly dependent on the degree of vacuum in the package. However, the retention of vitamin B was independent of the degree of vacuum. Sensory evaluation was also revealed that fresh prepared sous-vide cooked and steamed broccoli florets generally had higher acceptability than boiled sample. They also reported the retention of the B vitamins in beef bourguignon, roast veal, roast lamb, roast fillet of pork, salmon and cod. Their results are summarized in Table 2.1. Comparisons were made with retention values from the literature and it was concluded from this that the sous-vide cooking preserved vitamins liable to oxidation better than traditional cooking.

Table 2.1 Percentage retention of vitamins in sous-vide processed meat

Vitamin	Beef	Veal	Pork	Salmon	Cod
Vitamin B1	70	91	90	90	85
Vitamin B2	100	52	100	100	63
Vitamin B6	100	100	85	85	100
Vitamin B12	87	100	92	92	72
Biotin	100	100	100	95	95
Vitamin A	-	-	-	78	67

Source: applied from Creed (1995)

2.5 Surface characteristic of food

Recent advances in the area of computer vision, which consists of associating a camera for image acquisition, with a computer for image processing and analysis, have created alternative ways to monitor quality in the food industry. Computer vision has a potential for evaluating meat quality as image analysis techniques can quantitatively and consistently characterize complex geometric, color and textural properties. Application of computer vision techniques in meat quality evaluation has been reviewed (Chandraratne et al., 2006).

It is now possible to acquire digital images of the meat surface and to quantify some morphological or physical changes that can be assigned to several food properties. Meat tenderness can be predicted by analyzing the surface texture of meat (Li et al., 1999; Li et al., 2001; Chandraratne et al., 2006). Color, marbling and texture features were also extracted from the beef images. Statistical and neural network analyses were performed to relate the image features to sensory tenderness scores. Image texture features were found to be useful indicators of beef tenderness. Partial least squares and neural network models were able to predict beef tenderness from color, marbling and image texture features to R^2 values up to 0.70 (Li et al., 1999). Li et al. (2001) used texture features images to classify beef samples into tender and tough categories in terms of cooked beef tenderness. The texture feature data for 90 sample images were used to train and test sample classifiers. A correct classification rate of 83.3% was obtained in cross validations. Chandraratne et al. (2006) investigated the prediction of lamp cooked meat tenderness using image and texture analyses together. The highest R^2 of prediction was 0.75. Supaphon et al. (2013) applied technique of image processing to change surface images of sirloin steak with different grades into numerical data. Then it was correlated with their physical properties using Pearson's correlation comparing to multiple linear regressions (MLR) technique. It was found that correlations between surface images and physical properties of sirloin steak analyzed using MLR determined from regression analysis had higher R^2 compared with in the case of R determined from Pearson's correlation in all cases.

Previous studies have shown that image texture features of food materials can be observed from surface images. Translation of the surface images into numerical data and their interpretation could be more useful to relate the physical properties of foods non-destructively after various cooking conditions.

2.6 Optical microscopy

Optical microscopy or light microscopy offers the simplest way to obtain magnified images of biological tissues. This field covers a large range of techniques that have been used for years to characterize meat and meat product structures. Samples must be prepared in thin cuts using very early phase contrast measurement (Ranvier, 1889). Several studies have been conducted on the application of optical microscopy for observing meat tissues. For instance, Ichinoseki et al. (2006) studied the effect of high pressure on intramuscular collagen fibrils in bovine connective tissue, while Rusman et al. (2007) reported the effects of high pressure and heat on histological characteristics of bovine muscle, such as inter-myofiber space, Z line degradation, sarcomere length decreases and endomysium structure. In addition, Astruc et al. (2012) successfully evaluated fiber shape, fiber area (density, cross-section area, perimeter, maximum diameter, minimum diameter, extracellular space area and extracellular spaces), actomyosin ATPase activity, dehydrogenase activity

(SDH activity), reaction for glycogen characterization and laminin proteins of rhea limb muscles using optical microscopy application.

2.7 Infrared spectroscopy

The infrared portion of the electromagnetic spectrum can be splited into three regions: near infrared spectroscopy, mid-infrared spectroscopy and far-infrared spectroscopy that are named for their relation to the visible spectrum (Da-Wen, 2010). Infrared spectroscopy (IR) is typically employed in pharmaceutical applications, medical diagnostics, food and agrochemical quality control and combustion research. Infrared spectroscopy is based on the principle that the chemical bonds in organic molecules absorb or emit infrared light when their vibrational state changes. In the near infrared area spectrum, there are major changes in vibrational state. A major challenge in meat science applications for near infrared spectroscopy is sample presentation. Transmission is the most powerful method that is well-suited to liquids and gases but is inappropriate for undiluted solids (Damez and Clerjon, 2008).

IR spectroscopy has been applied to meat and muscle studies particularly means of indirectly measuring meat structure. Damez and Clerjon (2008) reported that IR spectroscopy was useful for the determination of chemical constituents and for categorizing meat into quality classes since IR spectroscopy gives direct molecular-level information. They reported that IR can be successfully used to determine macroscopic structural changes associated with meat or muscle structure. Motoyama et al. (2008) used IR spectroscopy to evaluate effects of acid and heat treatments on protein in meat during gastric digestion after cooking. The application of IR spectroscopy has been taken into consideration of meat quality by De Marchi et al. (2013) who applied visible-near infrared (Vis-NIR) reflectance spectroscopy to predict pH, color indexes (L^*, a^*, b^*, H, and SI), cooking loss, and Warner-Bratzler shear force. Meullenet et al. (2004) showed that NIR spectroscopy could be used to predict the texture of cooked poultry meat and to classify muscles according to tenderness levels as measured instrumentally. IR spectroscopy has also been used to demonstrate microstructure changes in salted pork (Bocker et al., 2007). For frozen products, IR spectroscopy has also made it possible to separate between fresh and frozen-thawed products from broiler breast meat (Lyon et al., 2001) or fish (Uddin et al., 2005), which is of high interest for the control of fraudulent freezing-thawing cycle. In summary, IR spectroscopy is considered useful for the determination of meat quality and meat structural changes during processing.

2.8 Mathematical modeling of food processing

Mathematical modeling of food processing operations has been widely used for many reasons (Jun and Irudayaraj, 2009; Alasalvar et al., 2011) starting with Teixeira et al. (1969) who applied finite different numerical methodology to optimize nutrient retention of foods in thermal processing. Models have become significant for design and optimization purposes (Yanniotis et al., 2013). By using mathematical modeling, experimental methodologies can be carried out with the advantage of obtaining results for difference conditions by easier, quicker and more economical ways. Mathematical modeling of food processing operations is based on physical fundamental mechanisms governing a given process and has the benefit of providing a basic understanding of the process. With the given fundamentals, processes can be described with a set of differential equations (Jun and Irudayaraj, 2009; Yanniotis et al., 2013). Modeling then involves solutions to these equations for heat transfer to determine temperature distribution, for mass transfer to determine variation in concentration of certain component and momentum transfer for variations in velocity and momentum distributions of a fluid. In addition, reaction kinetics also play a significant role in simulation studies if destruction of a certain nutrients, or variations, for example, in textural or sensory attributes or the formation of certain food safety problems as a result of applied processing technology is the objective to determine (Jun and Irudayaraj, 2009; Alasalvar et al., 2011). Therefore. food process modeling is accepted as an interdisciplinary approach that involves engineering with chemistry, reaction kinetics and predictive microbiology (Yanniotis et al., 2013).

Dym (2004) and Yanniotis et al. (2013) described the construction of mathematical modeling principles starting from Step 1 tostate the problem with its rationale and defining any physical, chemical or biological changes. Then step 2 is to develop mathematical basis of the process with appropriate assumptions. Step 3 is to convert the model into a numerical scheme if necessary. Step 4 is to conduct experiments or using literature data for required properties of the product. Steps 5 and 6 are to validate and test the model.

Researchers have applied mathematical modeling to food processing. For example, Hii et al. (2009) applied a new model for air drying of cocoa beans with overnight tempering at ambient temperature. The new model was a combination of the page and two-term drying model and they found that this new model described the drying process better under the conditions tested. Movagharnejad and Nikzad (2007) successfully studied drying of tomatoes in a tray dryer using a model covering different variables like power of heater and air flow velocity.

2.9 Related researches

The temperatures and cooking times have a large effect on eating quality of meat (García-Segovia, Andrés-Bello and Martínez-Monzó, 2007; Roldán et al., 2013). Botinestean et al. (2016) also found that sous-vide cooking at 60°C for 270 min on *M. semitendinosus* beef steaks significantly reduced the Warner-Bratzler shear force values from 32.97 N (control) to 27.80 N.

Pulgar et al. (2012) studied influence of different factors affecting quality of sous-vide cooked pork. Pork cheeks were cooked at 60 or 80°C for 5 or 12 hours and then packed either in vacuum or air. They found that weight losses were lower and moisture content was higher in samples cooked for a shorter time and a lower temperature. Moreover, samples cooked at 60°C showed higher lightness (L^*) and redness (a^*) than those cooked at 80°C.

Roldán et al. (2013) studied effects of different temperatures and times (60, 70 and 80°C for 6, 12 or 24 hours) on physicochemical, microbiological and textural structural features of sous-vide cooked lamb loins. They found that increased cooking temperatures resulted samples with higher weight losses and lower moisture contents, whereas the effect of cooking time on these variables was limited. Samples cooked at 60°C were found to have the highest lightness and redness, while increasing cooking temperature and cooking time resulted in higher yellowness values. Most of textural variables tested in a texture profile analysis of sample showed a marked interaction between cooking temperature and time. Samples cooked for 24 hours had significantly lower values for most of the studied textural parameters at all the temperatures.

Christensen et al. (2013) studied the relationships between meat toughness and some properties of connective tissue of meat from cows and young bulls that had been heat treated at low temperatures for prolonged times. Measurements of toughness, collagen solubility, cathepsin activity and protein denaturation of beef *semitendinosus* heated at temperatures between 53°C and 63°C for up to 19.5 hours were conducted. The results revealed that slightly higher temperatures and prolonged heating times were required to reduce toughness of *semitendinosus* from cows to the same level as that from young bulls. They advised for reduced toughness of *semitendinosus* to use low temperature for prolonged times, which would result from weakening of the connective tissue that was caused partly by denaturation or conformational changes of the proteins and/or by solubilization of collagen.

In part of these qualities, improvement comes from changes in physicochemical characteristics of the muscle proteins that happens during heating treatment (Christensen et al., 2013; García-Segovia et al., 2007). Palka and Daun (1999) suggested that myofibrillar protein was changed in its structure during heating. Then, myosin was denatured and led water retention at the first part of shrinkage. After that, actomyosin complex was denatured and induced dehydration to protein. In addition, the muscle fiber shrank both transversely and longitudinally, then sarcoplasmic protein aggregated and formed gel and finally connective tissue were shrank and solubilized (Garcı´a-Segovia et al., 2007; Christensen et al., 2011)

Astruc et al. (2010) studied the microstructural changes in bovine *musculus rectus abdominis* muscle after heating. Pieces of *rectus abdominis* muscle were heated at 100°C for varying times from 15 to 60 min and at 270°C for 1 min. Samples were then prepared for optical and transmission electron microscopy. It was found that heating of meat led to structural changes, depending on heating temperature and duration of cooking. The overall structural changes stabilize when the muscle temperature reached at 100°C for several minutes. Maintaining temperature for 60 min did not significantly modify the structure of the muscle. Simulating quick panfrying of the sample changed the kinetic evolution of the tissue, decreasing the fiber area less, but significantly increasing the size of the subsarcolemmal spaces more compared to longer periods of heating at 100°C.

Image analysis, it is now possible to acquire digital images of the meat surface and to quantify some morphological or physical changes that can be assigned to several food properties and meat tenderness can be predicted by analyzing the surface texture of meat (Chandraratne et al., 2006; Li et al., 2001). For example, Shiranita et al. (2000) studied the implementation of a meat-quality grading system using marbling score and image processing. Data was correlated using neural network techniques and multiple regression analysis. Results were found that marbling score evaluated from sensory had high correlation to meat quality system achieved from images. Supaphon et al. (2014) applied image processing to changes in the surface of different grades of sirloin steak. Numerical data collected from image analysis was correlated with their physical properties using Pearson's correlation and multiple linear regressions (MLR). They found that the correlations between data of surface images and physical properties of sirloin steak analyzed using MLR determined by regression analysis had a higher relationships compared with the R determined from Pearson's correlation in all cases tested.

Shiranita et al. (2000) studied the implementation of a meat-quality grading system using marbling score and image processing. Data was correlated using neural network technique and multiple regression analysis. Results were found that marbling score evaluated from sensory had high correlation to meat quality system achieved from images.

CHAPTER 3

METHODOLOGY

3.1 Materials

Round (Figure 3.1A) and sirloin (Figure 3.1B) samples from Thai local beef that had been reared in Kanchanaburi province (18 months old, weighting 200-250 kgs, 24 hours post slaughter), Thailand, were used as materials. Each muscle was purchased from Huatakhe market in Bangkok province, Thailand. The rest of the sample was stored at 4°C until cooking treatments (not over than 2 hours).

(A) **(B)**

Figure 3.1 Round (A) and sirloin (B) beef samples

3.2 Chemicals

3.2.1 Acetone	Sigma, Germany
3.2.2 Faramount aqueous base-Mounting medium	Sigma, Germany

3.2.3 Eosin Y 0.5% w/v	Sigma, USA
3.2.4 Ethanol	Sigma, Germany
3.2.5 Haematoxyin harris	Leica, USA
3.2.6 HCl	Anapure, Hong Kong
3.2.7 Hydrochloric 37%	VWR, USA
3.2.8 Isopentane (2-methylbutane)	Honeywell, USA
3.2.9 KCI	Sigma, USA
3.2.10 KH_2PO_4	Ajax Finechem, Australia
3.2.11 Liquid nitrogen	
3.2.12 Methylcyclohexane	Sigma, Germany
3.2.13 NaCl	Sigma, USA
3.2.14 Na_2HPO_4	Ajax Finechem, Australia
3.2.15 NaOH	Sigma, USA
3.2.16 OCT Embedding Matrix	CellPath, UK
3.2.17 Paraformaldehyde powder	Sigma, USA
3.2.18 Saffron powder	VWR, USA

3.3 Equipments

3.3.1 Colorimeter	Minolta CR-400 Konica, Japan
3.3.2 Cryostat	CM1950 Leica Microsystems, Germany
3.3.3 Digital acquisition kit	Olympus DP 71, Japan
3.3.4 Fujifilm camera	XT-10, Japan

3.3.5 Infrared microscope	Nicolet iN10, Thermo Fisher, USA
3.3.6 Spectromicroscope	Olympus BX 61, Japan
3.3.7 Texture analyzer	TA-XT plus, England
3.3.8 Vacuum machine	DZQ-500B, China

3.4 Methodology

3.4.1 Studying effects of temperatures and cooking times on physical properties and surface texture of sous-vide cooked beef

3.4.1.1 Preparation of sample

Round and sirloin beef samples from section 3.1 were used in this section. The exudate from the samples was blotted dry with tissue paper, rather than washing that could affect moisture content of sample. Fat and connective tissue were then trimmed and samples were cut into 7×7×7 cm cubes (in parallel to muscle fiber cross-section of sample).

3.4.1.2 Sous-vide cooking process

Round and sirloin beef samples from section 3.4.1.1 were vacuum packed in laminated low density polyethylene (LLDPE) film bags with a size of 15×23 cm using a vacuum machine. The sous-vide cooking conditions were tested using a water bath and maintained at 60, 70 or 80°C for 0, 6, 12, 18, 24, 30 or 36 hours. After cooking, cooked round and sirloin samples were taken out and cooled in iced water for 30 mins (or until the core temperature reached at 20°C) and then stored at room temperature (about 25°C). Then color, surface texture, sous-vide cooking loss, re-heating loss and shear force of samples were determined for each condition in triplicate.

3.4.1.3 Color

Color was measured across the cut surfaces of the round and sirloin cooked samples at room temperature (25°C). Lightness value (L^*), redness/greenness value ($+a^*/-a^*$) and yellowness/blueness value ($+b^*/-b^*$) were measured using a Minolta colorimeter, which was calibrated using a white ceramic tile before measurement. The readings were taken at three points on each side of round and sirloin cooked samples for each sample (6 measurements for each sample) and combined to give an overall mean value. The measurements were performed in three replicates for each time/temperature condition.

3.4.1.4 Sous-vide cooking loss

Sous-vide cooking loss of round and sirloin samples was determined on three muscle pieces by weighing each sample before and after sous-vide cooking and calculating weight loss using following equation (3.1). Analyses were performed in three replicates for each time/temperature condition (measurement on 9 different pieces of muscle).

$$\text{\% sous-vide cooking loss} = \frac{(w_2 - w_1)}{w_2} \times 100 \qquad (3.1)$$

Where w_1 was weight of sample after sous-vide cooking (g)

w_2 was weight of sample before sous-vide cooking (g)

3.4.1.5 Re-heating loss

Re-heating loss of cooked round and sirloin samples was determined by weighing each sample before and after reheating following Kim and Lee (2003) and calculating the difference on 3 muscle pieces. Samples were cut into 3×1×1 cm pieces, packed in polyethylene film bags and heated at 75°C for 30 mins in a water bath. After heating, samples were removed from the water bath and cooled at room temperature (25°C) for 30 mins or until the core temperature reached about 25°C. Three replications were analyzed in each time/temperature condition. Percentage of re-heating loss was calculated using equation (3.2).

$$\%\ \text{Re-heating loss} = \frac{(w_2-w_1)}{w_2} \times 100 \qquad (3.2)$$

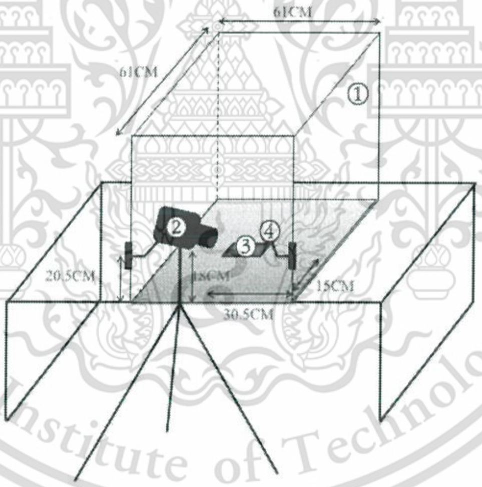
Where w_1 was weight of sample after cooking (g)
w_2 was weight of sample before cooking (g)

3.4.1.6 Shear force

Shear force of sous-vide cooked samples was measured using a texture analyzer with a Warner-Bratzler blade (3×1×1 cm) (Roldán et al., 2013). The operating parameters consisted of a speed of 1 $\text{mm}\cdot\text{s}^{-1}$ and a 50 kg load cell. Shear force was measured perpendicularly to the muscle fibers in 7 block samples. The maximum force (N) required to shear the cooked round and sirloin samples was measured in three points for each time/temperature sous-vide cooking condition.

3.4.1.7 Surface texture

The set-up for image analysis was presented in Figure 3.2. Round and sirloin samples after sous-vide cooking were cut in parallel to the muscle fiber. Then surface texture image from each side was acquired using a Fujifilm camera with image analysis set-up as described by Supaphon et al. (2014). Cooked round and sirloin samples were placed in a black box (61×61×61 cm). The height of the camera tripod was 18 cm and distance between camera and surface of the beef was 15 cm. Two LED (Light-Emitting Diode) lamps, each with a 5watt bulb, size of 70×116 mm, were placed approximately 20.5 cm in front of adaptor as a light source. Images were captured in RGB (Red-Green-Blue color) format and kept in .bmp with an image size of 4,896×3,264 pixels.



Where 1 was black box

2 was digital camera

3 was sample after cut in parallel to muscle fiber

4 was two LED (Light-Emitting Diode) lamps

Figure 3.2 Image analysis set-up

The surface texture of the sample images was analyzed using ImageJ software (Version 1.51n, National Institutes of Health, USA). RGB images were converted to gray-scale image (0-255). It was changed into black and white (BW) selecting threshold value to separate the image into two parts; the background and foreground (extra fiber bundle area and muscle fiber bundle area, respectively) and found the threshold average mean values of experiments. In our study, the threshold at 170 could divide the image into two parts consisting of extra fiber bundle area (170-255) and muscle fiber bundle area (0-169) which clearly observed.

Dark pixels displayed extra fiber bundle area including connective tissue, whereas white pixels displayed muscle fiber bundle area. The total numbers of each pixel were calculated from black and white images and presented as percentage of the layer of muscle fiber bundle shrinkage area (Figure 3.3).


(A) (B)

Figure 3.3 Image analysis from RGB (A) to BW (B) of the cut surface of beef (0.5× magnification)

3.4.1.8 Correlation determination

The effects of different cooking times and cooking temperatures on physical properties of samples during sous-vide cooking were analyzed using SPSS software (Version 21, IBM, Chicago, USA) by a one-way analysis of variance (7 cooking times × 3 cooking temperatures) together with their interaction using general linear mode (GLM) produce. The Tukey's test used at the 5% probability level to make comparisons between sample means when pertinent.

3.4.2 Determination relationships between physical properties and surface texture of sample during sous-vide cooking

Relationships between physical properties and surface texture of beef samples during sous-vide cooking were analyzed using Pearson's correlation and regression analysis as described below.

3.4.2.2 Pearson's correlations

Pearson's correlation analysis was conducted to evaluate relationships between percentage of muscle fiber bundle area and extra fiber bundle area and properties of samples (color, sous-vide cooking loss, re-heating loss and shear force). Results were presented in term of R (correlation coefficient) where the value of R is such that -1 < R < +1. The + and – signs were used for positive linear correlations and negative linear correlations, respectively. In this study a high correlation coefficient above 0.6 was considered acceptable according to a rule of Thumb (Asuero et al., 2006) (Table 3.1).

Table 3.1 Strength of correlation (R)

Value of R	Interpretation
0.90 - 1.00	Very high correlation
0.70 - 1.89	High correlation
0.50 - 0.69	Moderate correlation
0.30 - 0.49	Low correlation
0.00 - 0.29	Little if any correlation

Source: Asuero et al. (2006)

3.4.2.3 Regression analysis

Regression analysis was used to identify and quantify the relationships between image determination and determination of physical properties by SPSS software (Version 21, IBM, Chicago, USA). Samples were divided into 2 groups used for the calibration set (70%) and the prediction set (30%) (Lin et al., 2011). The calibration set was used to create the model and the prediction set was used to the test model. Results were presented in terms of percentages of extra fiber bundle area (X_1) and percentages of muscle fiber bundle area (X_2). The performance of the final model was evaluated according to the correlation coefficient of calibration (R^2_C) and validation (R^2_{CV}), along with the standard error of calibration (SEE_C) and validation (SEE_{CV}) also being reported. Acceptable models should have low SEE_C and SEE_{CV}, with high R^2.

3.4.3 The effects of temperatures and cooking times on histological structure of sample during sous-vide cooking

3.4.3.1 Sample preparation

Samples (achieved from section 3.1) were sliced into 7×7×7 cm cubes, vacuum packed and sous-vide cooked at 60, 70 or 80°C for 0-36 hours. The sample size of 1×1×1.5 cm blocks was taken from the core of each sous-vide cooked meat piece and immersed in a solution of 4% formaldehyde in a phosphate buffer (pH 7.2) until required for use (Figure 3.4). Each sample block was cryo-fixed in cooled isopentane (-160°C) with liquid nitrogen (-196°C) from which the histological structure and macromolecular changes of proteins during sous-vide cooking were measured (Figure 3.5) (Sheehan and Hrapchak, 1980).

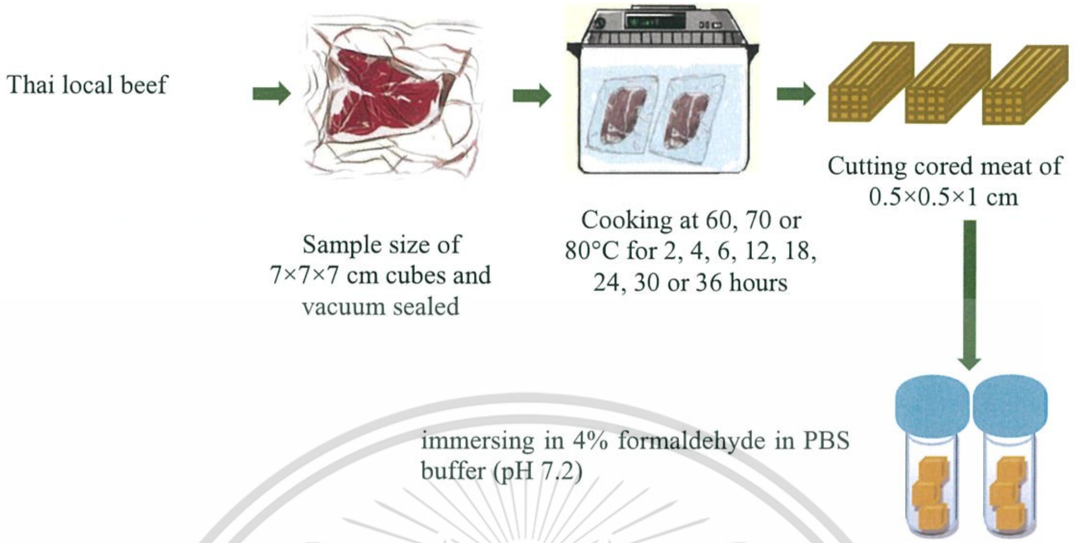


Figure 3.4 Steps of sample preparation

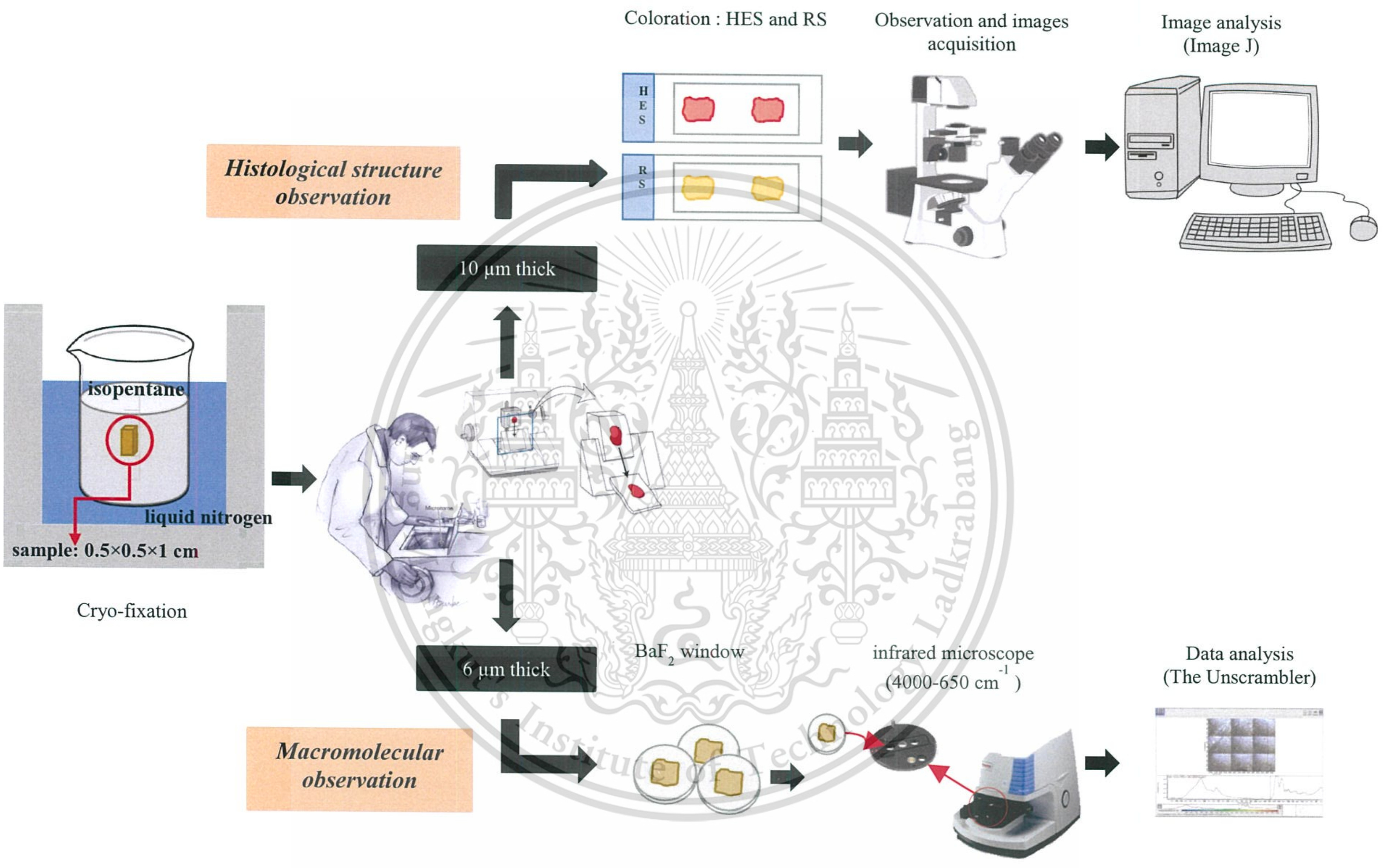
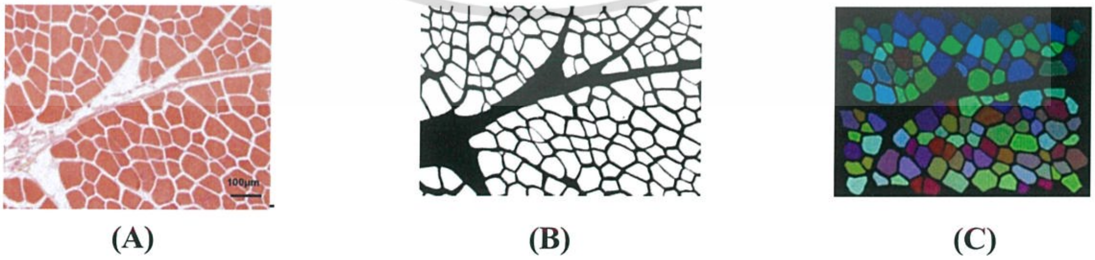


Figure 3.5 Steps of histological structure and macromolecular observation

3.4.3.2 Histochemistry observation

Cross sections samples (from 3.4.3.1) of 10 µm thick were sliced using a cryostat at -20°C and mounted on glass slides. Sections were then air-dried at room temperature (20°C) and stained with Hematoxylin Eosin Safran and Sirius Red in order to clearly observe structures. The observations were performed in triplicate for each time/temperature sous-vide cooking condition.

Following the sample staining using Hematoxylin Eosin Safran (HES) and Picro Sirius Red (RS) according to the method of Sheehan and Hrapchak (1980) and Astruc et al. (2012), steps of the staining were shown in Table 3.2. After staining, each slide of sample was viewed in an Olympus BX 61 microscope coupled to a high resolution digital camera using Cell Sens software. Six images were taken by image analysis (ImageJ software) to observe morphological changes during cooking viz. percentage of extracellular space area (ECS) and fiber cross sectional area (CSA). Quantification of percentage changes of ECS and CSA were analyzed using image analysis software (ImageJ software). First, the transversal section of the beef sample that had been stained (Figure 3.6A) was converted to a black and white format (Figure 3.5B) by thresholding in order to observe extracellular space area. After that, the fiber cross sectional area was measured (Figure 3.6C).



(A) (B) (C)

Figure 3.6 Transversal section of beef sample and stained with haematoxylin eosin safran solution (A), BW format by image analysis (B), fiber cross section area observed by image analysis (C)

Table 3.2 Steps of Hematoxylin Eosin Safran and Sirius Red staining

Hematoxylin Eosin Safran		**Sirius Red**	
Steps	Duration	Steps	Duration
1. Place the slides with the section in a metal staining rack.	-	1. Place the slides with the section in a metal staining rack.	-
2. Immerse sections in the filtered Harris Hematoxylin	5 mins	2. Immerse sections in acetone	60 mins
3. Rinse with H_2O (2 baths)	1 min	3. Immerse sections in Picro-Formalin	10 mins
4. Immerse sections in Eosin stain	15 sec	4. Rinse with 90% ethanol	1 min
5. Rinse with H_2O (2 baths)	1 min	5. Rinse with H_2O	10 mins
6. Dehydrate in ascending alcohol solutions		6. Immerse sections in Rouge Picro-Sirius	60 mins
- 80%	2 mins		
- 95%	5 mins		
- 100%	5 mins		
7. Immerse sections in Safran bath	8 mins	7. Rinse with 0.01M HCL	5 mins
8. Rinse with 100 % ethanol (2 baths)	-	8. Rinse with H_2O	1 min
9. Rinse with Methylcyclohexane bath (2 baths)	-	9. Dehydrate in ascending alcohol solutions	
		- 95%	-
		- 100%	
		- 100%	
10. Place coverslips on slides using Eukitt mounting medium	-	10. Rinse with Methylcyclohexane bath (2 baths)	-
		11. Place coverslips on slides using Eukitt mounting medium	-

3.4.3.2 Statistical analysis

Quantification of changes percentage of extracellular space area (ECS) and fiber cross sectional area (CSA) were analyzed under the statistical analysis by one-way. The results were presented in terms of percentage of ECS and CSA and analysis of variance (ANOVA) using the general linear model procedure was carried out. The Tukey's test used at the 5% probability level to make comparisons between sample means when pertinent.

3.4.4 The effect of various sous-vide cooking parameters on the macromolecular changes of proteins

For Infrared spectroscopy, 6 μm thick cross cryo-section, using a Cryostat, was mounted on BaF_2 windows and air-dried at room temperature. Spectra acquisition was performed using an infrared microscope. For each time/temperature condition, IR spectra was collected point-by-point in a 4000-650 cm^{-1} range and recorded at a spectral resolution of 4 cm^{-1}. The amount of 20 spectra was acquired from twenty muscle fibers (one acquisition by muscle fiber) with a scanning area of 30×30 μm (accumulation of 64 scans), while perimysium 10 spectra were collected along a line in a selected area (spatial resolution of 10×10 μm, 64 scans). Spectra had undergone an extended multiplicative signal correction and a second derivative. The 1500-1700 cm^{-1} part of the spectra, most assigned to the protein signal, was selected for principal component analysis (PCA). PCA was performed using The Unscrambler software version 9.8 (CAMO Software AS).

3.5 Experimental Places

3.5.1 Faculty of Agro-industry, King Mongkut's Institute of Technology Ladkrabang, Chalongkrung Road, Ladkrabang, Bangkok, 10520, Thailand

3.5.2 INRA (Institut National de la Recherche Agronomique), UR0370 QuaPA, 63122 Saint-Genès-Champanelle, France

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Effects of temperatures and cooking times on physical properties and surface texture of sous-vide beef

During sous-vide cooking of beef, the physical properties changes in terms of color, sous-vide cooking loss, re-heating loss, shear force and surface texture were determined. Sous-vide cooking treatments affected these properties and discussed below.

4.1.1Color

L^* (lightness), a^* (redness) and b^* (yellowness) parameters of cooked samples at different temperatures and times are shown in Table 4.1. Compared to the raw control, cooked samples were lighter and more yellow (higher b^*), whereas less red than raw sample ($p \leq 0.05$). These results followed the same trend as those previously reported by García-Segovia et al. (2007) and Nikmaram et al. (2011).

The lightness is important in term of consumer acceptance (García-Segovia et al., 2007). The increase in L^* of cooked samples (compared to raw sample) is probably linked to the muscle fiber shrinkage that led to a decrease in light penetration in the meat and thus producing a paler meat (Nikmaram et al., 2011; Roldán et al., 2013). In addition, cooking led to higher denaturation and aggregation of sarcoplasmic and myofibrillar proteins, which would increase light scattering

(Christensen et al., 2011; Roldán et al., 2013; Wattanachant et al., 2005). In addition, samples cooked at 60°C represented slightly higher L^* values than those cooked at 70°C and 80°C, which could be assigned to a better water binding. Indeed, low cooking temperature limit the cooking loss resulting in more free water both at surface and inside the meat. This would increase light scattering reflected by higher lightness value (Roldán et al., 2013).

Compared to control samples, redness in cooked sample is mostly related to the degree of denatured myoglobin, which starts at 60°C. The redness of samples tended to decrease during cooking at all the times and temperature tested. This indicated a higher myoglobin degradation as cooking temperature and cooking time increased. The compound largely responsible for the brown-gray color is globin hemichrome (Fe^{3+}), which result of the globin (the protein part of myoglobin) denaturation during heating (Wattanachant et al., 2005). The globin denatures at around 60°C depending on its oxidation status before cooking (Bejerholm et al., 2014). This loss of redness with increasing cooking temperature is in accordance with the results obtained by García-Segovia et al. (2007) who cooked beef samples at 60-80°C for 15-60 mins and Roldán et al. (2013) who cooked lamb loins at 60-80°C for 6-24 hours.

The higher b^* values as consequence of increasing both cooking temperature and time is most likely due to the formation of metmyoglobin and further heat denaturation of this protein, giving rise to a brownish color (Roldán et al., 2013) and to the heme conversion into nicotinamide hemichrome that increases the brown hue (Suman and Joseph, 2013). Indeed, most of myoglobin was denatured after 6 hours of cooking for all cooking temperature tested. The increase of b^* could also be due to Maillard reactions which happen during cooking (Hamm, 1977; Shahidi et al., 2014). The slight increase in yellowness (b^*) along cooking duration was not statistically significant ($p>0.05$), excepted from 30 hours at 80°C and 36 hours at 80°C or cooked round and sirloin samples, respectively ($p\leq0.05$). Overall, color results agreed with previous study (Christensen et al., 2011; Nikmaram et al., 2011). The most aggressive condition of cooking (30-36 hours of cooking at 80°C) could promote larger denaturation of myoglobin and Maillard reaction compared to other cooking temperatures.

Table 4.1 Color parameters of sous-vide cooked round and sirloin samples at different temperatures and times

Temperature (°C)	**Cooking Time (hours)**	**Round**			**Sirloin**		
		L^*	a^*	b^*	L^*	a^*	b^*
60	control	40.33 ± 3.20^{a}	19.38 ± 1.42^{f}	1.52 ± 1.12^{a}	41.28 ± 2.95^{a}	19.15 ± 2.31^{e}	1.64 ± 1.76^{a}
	6	58.96 ± 1.60^{b}	16.80 ± 0.52^{e}	7.90 ± 0.71^{bcd}	60.38 ± 1.14^{cdef}	17.21 ± 1.08^{d}	8.70 ± 0.53^{bcd}
	12	61.92 ± 0.40^{b}	14.43 ± 0.25^{abcd}	6.02 ± 0.19^{b}	61.30 ± 3.55^{cdefg}	15.51 ± 0.62^{c}	7.55 ± 0.71^{bcd}
	18	63.64 ± 1.54^{b}	13.20 ± 0.85^{abcd}	5.94 ± 0.47^{b}	63.26 ± 1.01^{gh}	13.39 ± 0.34^{ab}	7.31 ± 0.79^{bcd}
	24	58.78 ± 4.99^{b}	13.23 ± 0.40^{abcd}	6.55 ± 0.46^{b}	64.16 ± 2.42^{h}	14.40 ± 1.05^{bc}	6.73 ± 0.62^{bc}
	30	61.49 ± 2.06^{b}	13.00 ± 0.49^{abcd}	7.15 ± 0.55^{bcd}	62.92 ± 2.48^{fgh}	12.06 ± 0.37^{a}	6.87 ± 0.30^{bc}
	36	63.32 ± 1.30^{b}	11.81 ± 0.83^{a}	7.20 ± 0.66^{bcd}	63.51 ± 0.60^{gh}	12.14 ± 0.16^{a}	7.14 ± 0.21^{bcd}
70	control	40.33 ± 3.20^{a}	19.38 ± 1.42^{f}	1.52 ± 1.12^{a}	41.28 ± 2.95^{a}	19.15 ± 2.31^{e}	1.64 ± 1.76^{a}
	6	60.73 ± 4.25^{b}	11.95 ± 0.36^{ab}	6.27 ± 0.74^{b}	61.47 ± 1.25^{defgh}	12.49 ± 0.33^{a}	6.43 ± 0.37^{b}
	12	61.84 ± 1.84^{b}	12.90 ± 0.61^{abcd}	5.91 ± 0.48^{b}	62.69 ± 1.88^{efgh}	12.11 ± 0.34^{a}	6.79 ± 0.32^{bc}
	18	60.70 ± 2.81^{b}	12.88 ± 0.43^{abcd}	6.05 ± 0.65^{bc}	59.35 ± 1.67^{cde}	12.57 ± 0.32^{a}	8.00 ± 0.52^{bcd}
	24	61.34 ± 2.24^{b}	12.66 ± 0.46^{abc}	6.83 ± 0.91^{b}	62.99 ± 1.02^{fgh}	12.35 ± 0.41^{a}	6.91 ± 1.39^{bc}
	30	60.60 ± 3.78^{b}	12.58 ± 0.69^{abc}	8.10 ± 1.17^{bcd}	61.68 ± 2.25^{defgh}	12.32 ± 0.56^{a}	7.53 ± 0.73^{bcd}
	36	60.07 ± 2.94^{b}	12.56 ± 0.69^{abc}	8.68 ± 1.24^{bcd}	61.36 ± 1.15^{cdefgh}	11.96 ± 0.56^{a}	8.07 ± 0.41^{bcd}
80	control	40.33 ± 3.20^{a}	19.38 ± 1.42^{f}	1.52 ± 1.12^{a}	41.28 ± 2.95^{a}	19.15 ± 2.31^{e}	1.64 ± 1.76^{a}
	6	60.47 ± 4.69^{b}	12.12 ± 0.59^{ab}	6.35 ± 0.48^{b}	57.86 ± 1.63^{bc}	12.11 ± 0.77^{a}	7.86 ± 0.66^{bcd}
	12	61.62 ± 4.08^{b}	13.00 ± 0.46^{abcd}	6.89 ± 1.09^{bc}	59.58 ± 2.52^{cdef}	12.41 ± 0.33^{ab}	8.51 ± 1.42^{cd}
	18	59.43 ± 2.60^{b}	13.45 ± 0.08^{bcd}	7.59 ± 1.80^{b}	59.45 ± 3.09^{cdef}	13.16 ± 1.84^{a}	11.29 ± 0.91^{e}
	24	57.11 ± 3.29^{b}	13.49 ± 0.49^{bcd}	9.83 ± 2.32^{dc}	58.43 ± 1.90^{cd}	13.09 ± 0.55^{ab}	12.34 ± 2.65^{e}
	30	59.08 ± 5.26^{b}	14.35 ± 0.75^{d}	9.36 ± 1.92^{cde}	57.96 ± 5.04^{bc}	13.13 ± 0.64^{ab}	12.10 ± 2.35^{e}
	36	57.21 ± 1.90^{b}	14.02 ± 0.75^{cd}	11.36 ± 3.8^{e}	55.01 ± 4.12^{b}	14.81 ± 0.86^{c}	15.45 ± 1.58^{f}

Note: Data are presented as means ± SD

Different letters within the same column mean significant differences between the different times ($p \leq 0.05$)

4.1.2 Sous-vide cooking loss

The raw muscle from Thai local beef was composed of 76±0.3% water content. During sous-vide cooking, juice loss increased significantly (*p*≤0.05) and was affected by temperature and time (Figures 4.1A and 4.1B). Overall, increasing the cooking temperature lead to an increase of cooking loss (*p*≤0.05) according to previous studies (Hamm, 1977; Bouton and Harris, 1972; Tornberg, 2005; Bouhrara et al., 2011). Indeed, cooking generally lead to myofibrillar mass and collagen contraction that expel water in the extracellular space and outside the piece of meat (Tornberg, 2005; Astruc et al., 2010). On the other hand, our results showed that for a given cooking temperature, increasing cooking time did not increase the cooking loss (*p*>0.05). These results are also consistent with the scientific literature which showed at first an increase in water losses during heating and then a stabilization of water losses (Laakkonen et al., 1970; Locker and Daines, 1974). In our experiment, the water losses occurred in the first 6 hours of heating and our results showed the results only after the period of water loss.

The results of cooked round sample were presented in Figure 4.1A. For 6- 36 hours the percentage of cooking loss significantly increased from 60°C to 70°C (*p*≤0.05) but increasing the temperature from 70°C to 80°C did not increase the water loss (*p*>0.05). As for sirloin, increasing cooking time did not change significantly the water loss from 6 to 36 hours (*p*>0.05).

Cooked sirloin sample (Figure 4.1B) was found that cooking loss remained stable during process from 6 to 36 hours at 60°C (*p*>0.05). At 70°C and 80°C, cooking loss decreased from 6 to 12 hours then increased (70°C) and decreased (80°C) slowly at 18 hours and became stable for 18 hours (*p*>0.05) until 36 hours.

These slight variations are surprising and could be due to differences in the composition of the pieces of muscle.

In conventional cooking (not sous-vide), cooking loss increases with cooking temperature in the first phase of heating (Bouton and Harris, 1972; Christensen et al., 2011; Davey and Gilbert, 1974; Tornberg, 2005). After several times of cooking (depending on the size of meat pieces), increasing cooking time does not increase the cooking loss (Locker and Daines, 1974). In this last study (Locker and Daines, 1974), beef meat cooked at 80°C released water during 90 mins, but increasing the cooking time above 90 mins did not increase the water loss. In the present study, cooking loss increases significantly from 60 to 70 or 80°C but not from 70°C to 80°C.

4.1.3 Re-heating loss

The re-heating loss of round and sirloin samples after sous-vide cooking were measured. It was found that the temperature and time of sous-vide cooking significantly ($p \leq 0.05$) affected cooking loss of sample (Figures 4.2A and 4.2B). As mentioned earlier that cooked samples at 60°C had lowest percentage sous-vide loss values and hence retained the highest water content. Sous-vide cooked sample at 60°C had higher percentage of re-heating loss compared to 70 or 80°C, while increasing the time significantly ($p \leq 0.05$) decreased cooking loss. Results were supported by Kongpeam et al. (2015) who found that sous-vide cooked flank steak which had higher sous-vide loss values and displayed lower cooking losses. This might be because increasing temperature caused changes in the protein structure, particularly causing it to be denatured and to shrink and released water from the muscle.

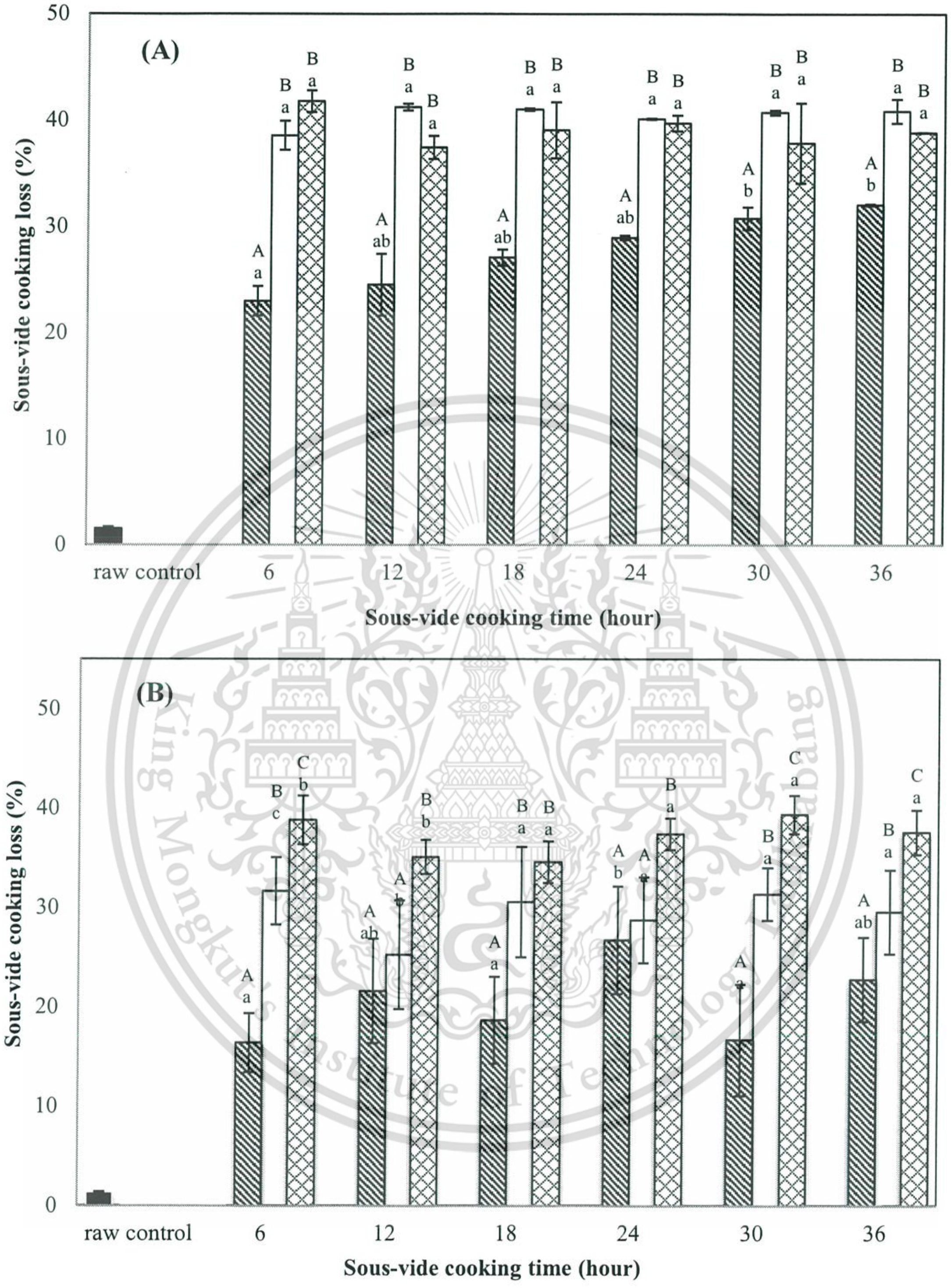


Figure 4.1 Percentage of sous-vide cooking loss of cooked round (A) and sirloin (B) sample at 60, 70 or 80°C for 0-36 hours. Uppercase indicate significant difference ($p≤0.05$) in cooking temperature (same cooking time) and lowercase indicate significant difference ($p≤0.05$) in cooking time (same cooking temperature)

■ raw control ▨ 60°C □ 70°C ⊠ 80°C

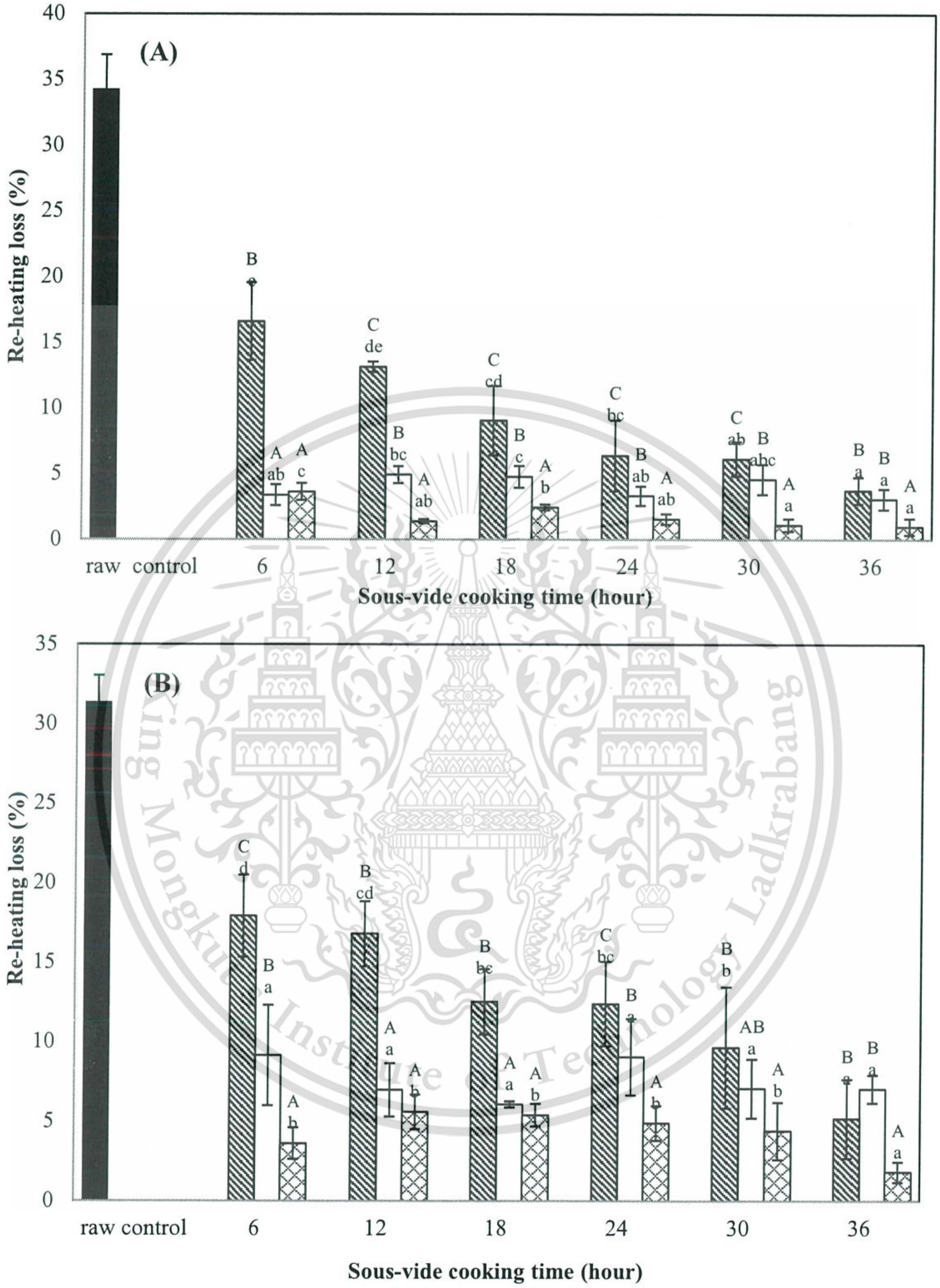


Figure 4.2 Percentage of sous-vide re-heating loss of cooked round (A) and sirloin (B) sample at 60, 70 or 80°C for 0-36 hours. Uppercase indicate significant difference ($p \leq 0.05$) in cooking temperature (same cooking time) and lowercase indicate significant difference ($p \leq 0.05$) in cooking time (same cooking temperature)

■ raw control ▨ 60°C ☐ 70°C ⊠ 80°C

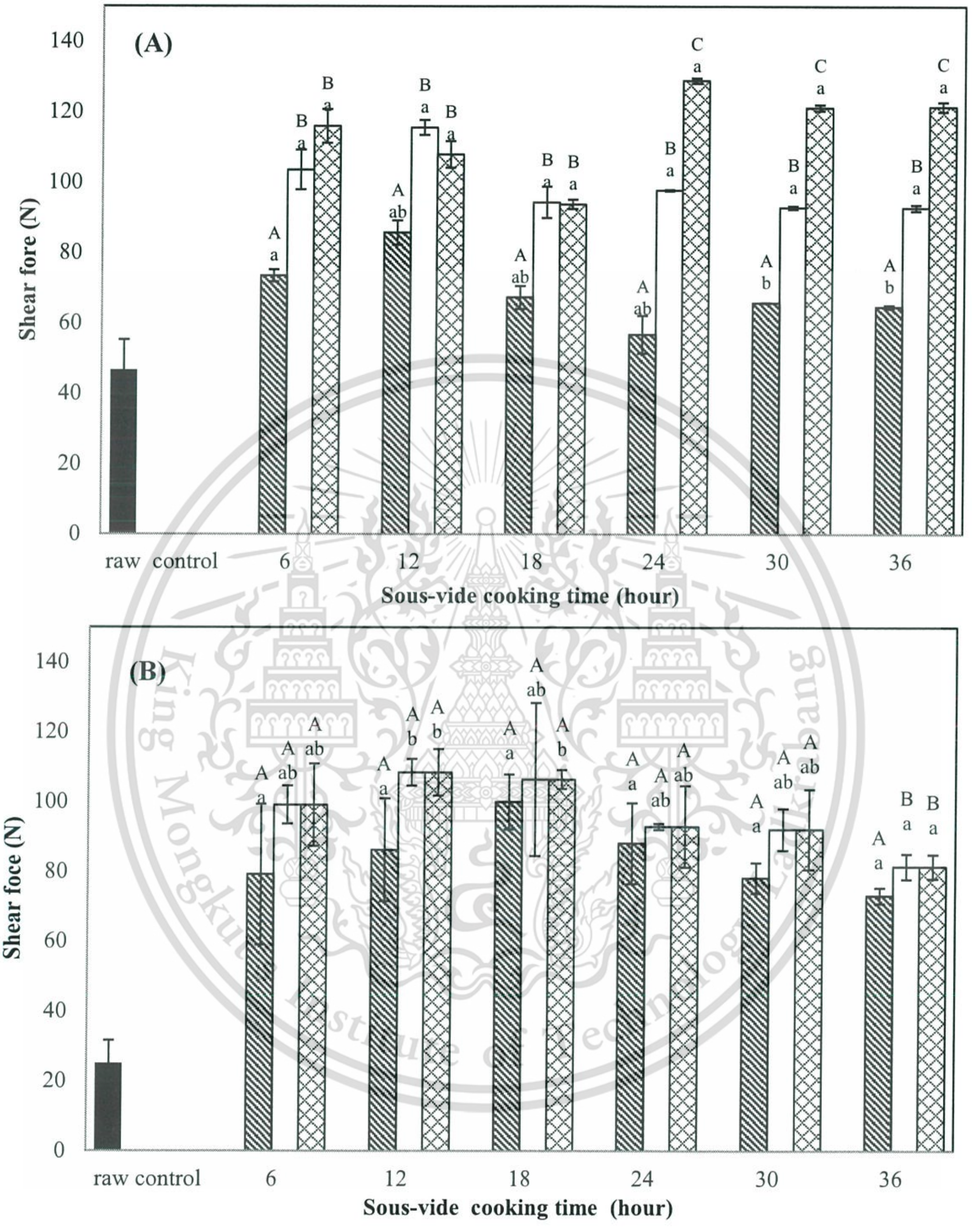


Figure 4.3 shear force value of cooked round (A) and sirloin (B) sample at 60, 70 or 80°C for 0-36 hours. Uppercase indicate significant difference (*p≤0.05*) in cooking temperature (same cooking time) and lowercase indicate significant difference (*p*≤0.05) in cooking time (same cooking temperature)

■ raw control ▨ 60°C □ 70°C ⊠ 80°C

4.1.4 Shear force

The effects of cooking temperature (70, 60 or 80°C) and cooking time (6, 12, 18, 24, 30 or 36 hours) on shear force values of cooked round and sirloin samples were shown in Figures 4.3A and 4.3B, respectively. The raw samples were characterized by a peak shear force of about 29.94 N (sirloin muscle) and 46.52 N (round muscle). All samples presented higher shear force value, using Warner-Bratzler blade, compared to the raw sample, which indicated that there was viscous flow in the fluid-filled channels between fibers and fiber bundles as reported by Roldán et al. (2013). Shear force values increased with cooking temperature from 60 to 80°C, which was the same trend reported by García-Segovia et al. (2007). Kongpeam et al. (2015) studied physical properties of flank steak of local Thai beef affected by the sous-vide cooking. They found that increasing of cooking temperature from 55°C to 65°C resulted in increases in their firmness and toughness. This increase can be explained by the way that myofibrillar protein structure changes during cooking (Palka and Daun, 1999). Moreover, Rinaldi et al. (2014) suggested that using longer cooking times caused the denaturation of myofibrillar proteins and caused meat toughening at low temperature and longer times in sous-vide cooked beef. Heating up to 65°C increased tenderness since the sarcoplasmic proteins aggregated and gelled. This rendered the meat easier to fracture. However, after heating between 65°C to 80°C, the meat became tougher again because the elastic modulus increased and required larger tensile stress to extend fractures (Baldwin, 2012). Tornberg et al. (1997) characterized the evolution of muscle fibers and connective tissue of bovine muscle biceps femoris. These authors observed a lateral and longitudinal shrinkage of muscle fibers from 40°C, but this shrinkage increased with temperature until more than 80°C. Shrinkage of connective tissue started at 60°C and increased significantly up to 80°C. These phenomena were generally associated with water loss and increased hardness (Davey and Gilbert, 1974; Hamm, 1977) according to the result decreased with solubilization of collagen into gelatin. This solubilization depended on the rate of crosslink in the collagen. In older animals, the high crosslink rate required a temperature above 80°C to properly solubilize the collagen (Rochdi et al., 2000). Moreover, Rochdi et al. (2000) evidenced that the collagen solubilization was fast during the first hour of cooking at 90°C and slower for longer cooking times.

In our study, the cooking temperature did not exceed 80°C and the meat was derived from a 18-month-old rustic breed of cattle, probably with an already high collagen crosslinking rate which could explain that shear force did not change a lot from 6 hours cooking. Rinaldi et al. (2014) suggested that using long sous vide cooking time at low temperature caused the denaturation of myofibrillar proteins and meat toughening.

4.1.5 Surface texture imaging

The effects of sous-vide temperatures and times on surface texture imaging cooked of round and sirloin samples were shown in Figures 4.4 and 4.5, respectively. It was found that using of higher temperature and longer time tended to change surface of round and sirloin cooked muscles after sous-vide cooking and exhibited two distinct characteristics. In the first characteristic, muscles fiber bundles and inter bundles spaces of muscle fiber were clear and firm. The onset of these changes differed in time based on cooking temperatures after which the muscle fiber layer was clearly observed.

In the present study, sample muscle was slightly changed the surface texture then muscle fiber bundles were clear and firm up to 6-18 hours, after that the muscle fiber layer was clearly observed from 24 hours at 60°C cooking time. Similar trends were obtained for cooking at 70 or 80°C where muscle fibers were clear and firm after 12 and 6 hours cooking time, respectively and the muscle fiber layers were then started to appear from 18 and 12 hours, for 70 and 80°C, respectively.

Images showed the appearance of gaps in between the muscle fiber bundles depending on temperature and time. For 60°C, the number of gaps and their size increased significantly from 24 hours. Gaps appeared at 18 hours for 70°C cooked samples and at 6 hours for 80°C cooked samples with larger hole size from 24 to 36 hours cooking. These results reflected muscle fiber bundle lateral shrinkage that is moderate for 60°C cooking and increase at 70°C and 80°C.

Connective tissue of the perimysium was seen (when zooming on the images) for 60°C and 70°C cooked samples whatever the cooking time, and for 6-18 hours 80°C cooked samples. Collagen contraction probably largely participated to the fibers bundle shrinkage. Connective tissue was less visible on 80°C cooked samples from 24 to 36 hours cooking showing more degraded muscle fiber bundles. This decrease in perimysium density suggested a partial solubilization of collagen for long time cooking at 80°C, that weaken the links between fiber bundles. Moreover, from 24 hours cooking at 80°C, the color of meat became brown, which could be due to changes in myoglobin molecule and/or to Maillard reaction products.

The appearance of fiber layering at the onset of second phase was due to surface muscle fibers shrinkage and layering due to water loss after cooking. These results were consistent with sous-vide cooking loss. Water loss after sous-vide cooking was about 34% and these losses tended to be from the muscle fiber bundles, which occurred slowly at 60°C, but quicker at 70°C and 80°C. Astruc et al. (2010), Astruc et al. (2012) and Pulgar et al. (2012) reported that during heat processing, some of the proteins are denatured and then it becomes complex and aggregated and the muscle fibers shrink and become layered. This could be an indication of high compression due to the collagen shrinking and water being released from the muscle fiber (Modzelewska-Kapitułaet al., 2015).

This compression effect might be due to shrinkage of the endomysial collagen, which gelatinized at 80°C (Baldwin, 2012; Supaphon et al., 2016). Control and cooked meat display changes in their structure when their water content was reduced, cell membrane damaged, muscular fibers shrunk both transversal and longitudinal, sarcoplasmic proteins aggregated and gelled, and connective tissues shrank and solubilized. Modzelewska-Kapitułaet al. (2015) reported that these effects collectively resulted in the appearance of granular fibers.

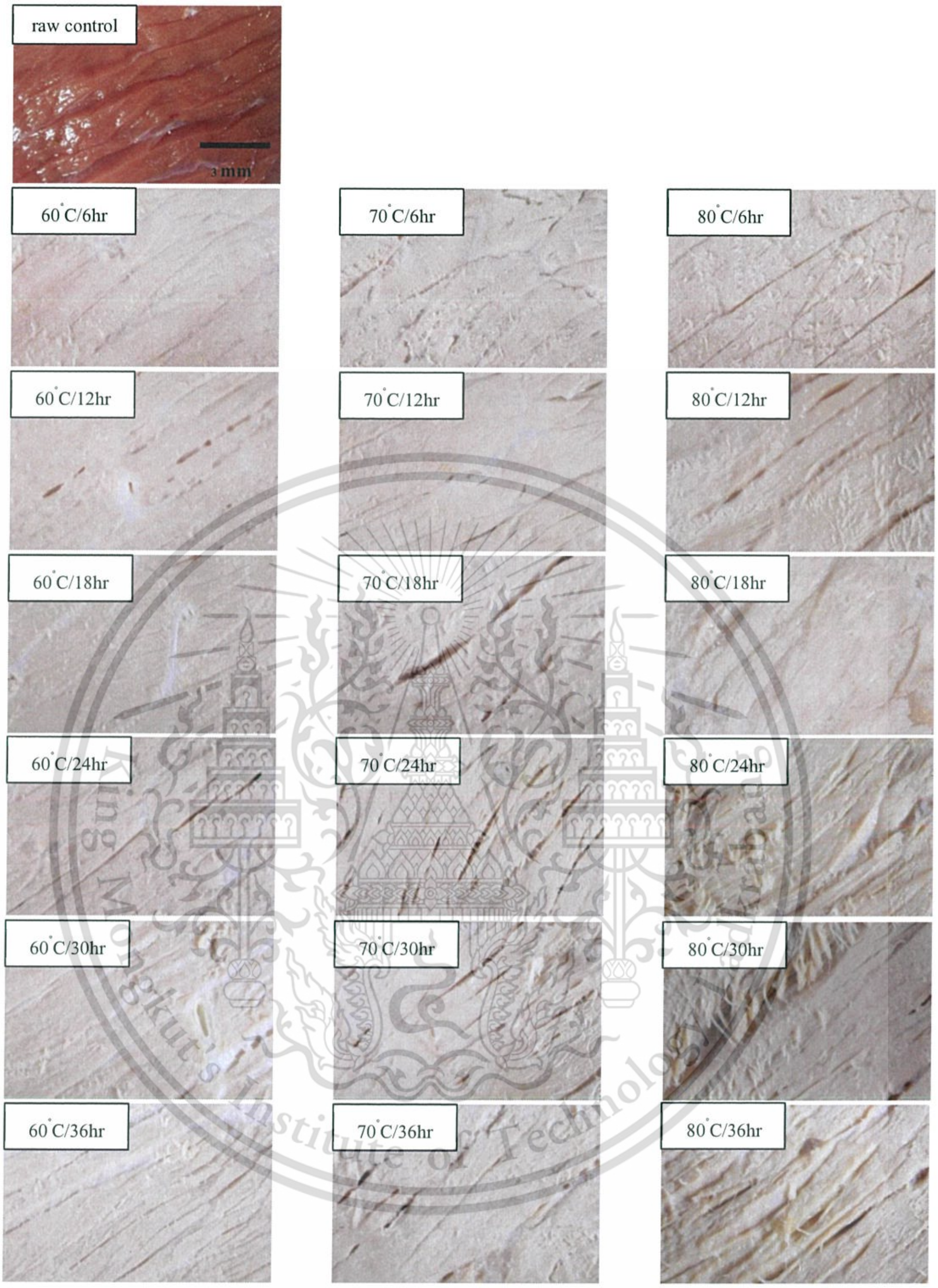


Figure. 4.4 Image analysis of the cut surface of cooked round sample before and after sous-vide cooking at 60, 70 or 80°C for 6, 12, 18, 24, 30 or 36 hours

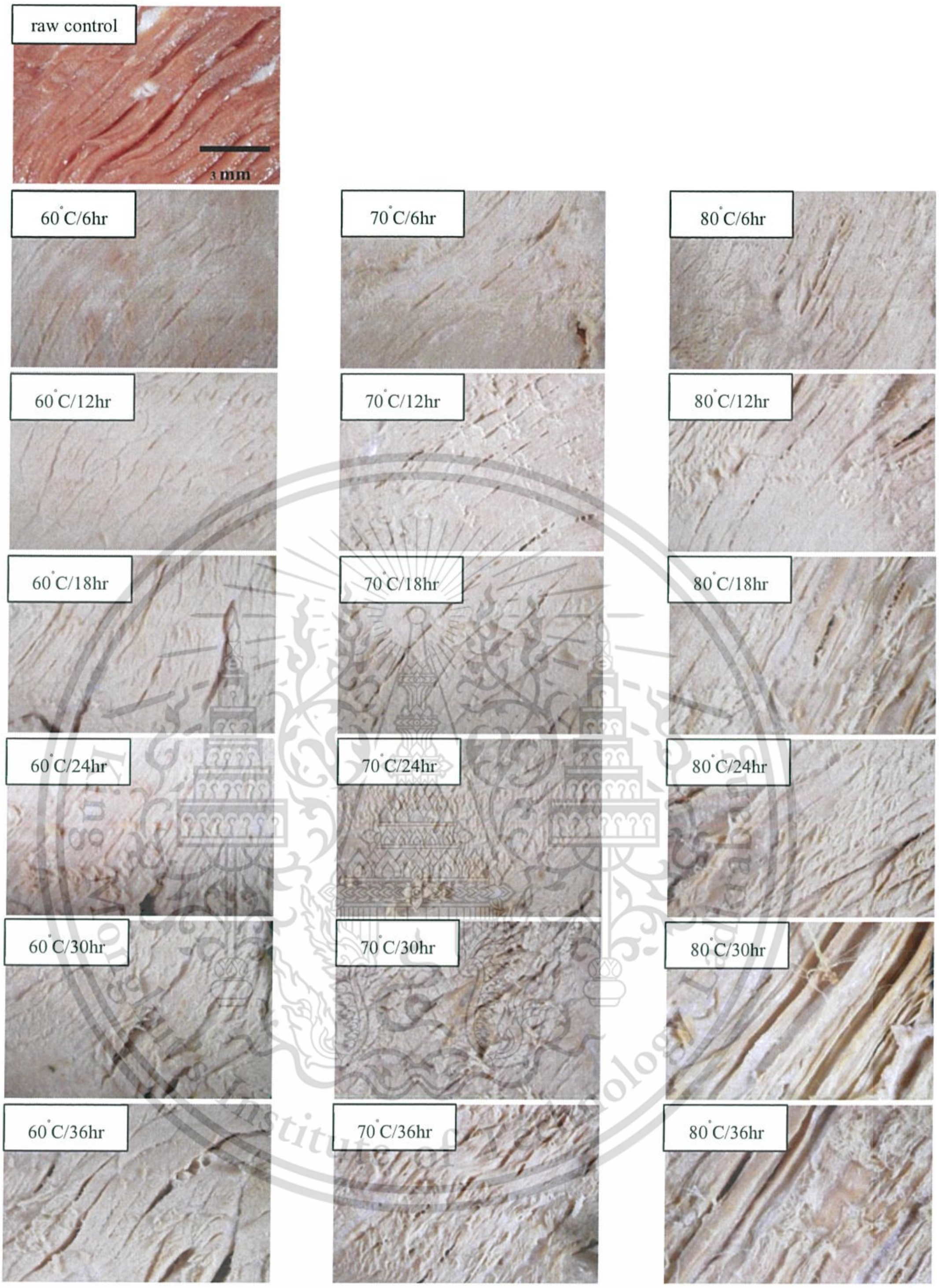


Figure. 4.5 Image analysis of the cut surface of cooked sirloin sample before and after sous-vide cooking at 60, 70 or 80°C for 6, 12, 18, 24, 30 or 36 hours

4.2 The relationships between physical properties and surface texture of beef during sous-vide cooking

4.2.1 Pearson's correlation

Pearson's correlations between numerical data analyzed by percentage of muscle fiber bundles and physical properties of sous-vide cooked sirloin and round beef were presented in Tables 4.2 and 4.3, respectively. Percentage of extra fiber bundle area showed the highest positive correlation with yellowness, *b** (R= 0.66) and shear force (R= 0.87) of sirloin and round muscle, respectively. However, highest percentage of extra fiber bundle area represented negative correlation with percentage of re-heating loss (R= -0.68) and percentage of sous-vide cooking loss (R= -0.79) of sirloin and round muscle, respectively. Moreover, in case of round muscle, the correlation between selected physical properties such as lightness, *L** had high correlation with sous-vide cooking loss (R= -0.90), redness, *a** with re-heating loss (R= 0.90) and yellowness, *b** with muscle fiber bundles (R= 0.86). From the results it was found that percentage of muscle fiber bundles from round image trended to correlate with physical properties of sample, while the correlations between percentage of muscle fiber bundles from sirloin image and its physical properties seems to be low. This might be because each beef cut consisted of different composition and might need different techniques to correlate the dat. For that reason, numerical data analyzed of percentage of extra fiber bundle area from round image was further used to identify and quantify the relationships of image determination and physical properties determination.

4.2.2 Mathematical modeling

Multiple regression was applied to develop the correlation model. Correlation of determination of calibration set and validation set (R^2_C and R^2_{CV}) and standard error of calibration set and cross validation set (SEE_C and SEE_{CV}) of numerical data analyzed from round images and physical properties using multiple regression were represented in Table 4.4. Data analyzed from round images consisting of percentages of extra fiber bundle area (X_1) and percentages of muscle fiber bundle area from image analysis (X_2) had highest correlation with percentages of sous-vide cooking loss with R^2_C of 0.98 and SEE_C of 3.61. In addition, the model had highest validation correlation (R^2_{cv}) of 0.94 and standard error of estimate (SEE_{CV}) of 5.78 for percentages of sous-vide cooking loss. The results of multiple regression gave validation results with R^2_{cv} of 0.81, 0.78, 0.81, 0.94, 0.85 and 0.70 for L^*, a^*, b^*, percentages of sous-vide cooking loss, percentages of re-heating loss and shear force, respectively. The results demonstrated that imaging system was a potential technique for non-destructive prediction of beef quality attributes in term of round sample, thus facilitating identification and classification of beef meat in a simple and fast way.

Table 4.2 Pearson's correlation analysis of cooked sirloin sample

Values	**Extra fiber bundle area (%)**	***L****	***a****	***b****	**Sous-vide cooking loss (%)**	**Re-heating loss (%)**	**Shear force (N)**
Extra fiber bundle area (%)	1						
L*	-0.62	1					
a*	-0.21	-0.14	1				
b*	0.66	-0.77	0.44	1			
Sous-vide cooking loss (%)	0.60	-0.74	-0.33	-0.46	1		
Re-heating loss (%)	-0.68	0.63	0.58	0.40	-0.84	1	
Shear force (N)	-0.19	0.14	-0.26	-0.13	0.47	-0.30	1

Note: Correlation is significant at the 0.01 level (2-tailed)

Table 4.3 Pearson's correlation analysis of cooked round sample

Values	**Extra fiber bundle area (%)**	***L****	***a****	***b****	**Sous-vide cooking loss (%)**	**Re-heating loss (%)**	**Shear force (N)**
Extra fiber bundle area (%)	1						
L*	0.59	1					
a*	-0.62	-0.88	1				
b*	0.86	0.64	-0.58	1			
Sous-vide cooking loss (%)	-0.53	-0.90	0.78	-0.67	1		
Re-heating loss (%)	-0.79	-0.87	0.90	-0.81	0.83	1	
Shear force (N)	0.87	0.60	-0.61	0.73	-0.49	-0.79	1

Note: Correlation is significant at the 0.01 level (2-tailed)

Table 4.4 Multiple regression models between surface image characteristics and physical properties of cooked round sample (n=756)

Variable	**Equation**	**Calibration (n=529)**		**Validation (n=171)**	
		R_C^2	SEE_C	R_{cv}^2	SEE_{CV}
L**	$42.090 + 6.584X_1 + 0.294\ X_1^2 - 5.021\times10^{-5}(X_1X_2)^2 + 3.268\times10^{-8}(X_1X_2)^3$	0.90	2.41	0.81	2.01
a**	$19.151 - 1.342X_1 + 0.047\ X_1^2 + 7.533\times10^{-6}(X_1X_2)^2 + 4.338\times10^{-9}(X_1X_2)^3$	0.79	1.22	0.78	1.58
b**	$1.673 + 0.744X_1 + 0.112\ X_1^2 - 9.082\times10^{-6}(X_1X_2)^2 - 7.494\times10^{-9}(X_1X_2)^3$	0.85	1.32	0.81	1.27
Sous-vide cooking loss (%)	$99.820 - 27.606X_1 + 1.145X_1^2 + 2.420\times10^{-4}(X_1X_2)^2 - 1.432\times10^{-7}(X_1X_2)^3$	0.98	3.61	0.94	5.78
Re-heating loss (%)	$34.232 - 5.631X_1 + 0.084X_1^2 + 3.118\times10^{-5}(X_1X_2)^2 - 8.487\times10^{-9}(X_1X_2)^3$	0.92	3.66	0.85	1.54
Shear force (N)	$215.803 - 3.282\times10^{-5}(X_1X_2)^2 + 1.708\times10^{-6}(X_1^5) - 1.982\times10^{-8}(X_2)^5 + 1.000\times10^{-3}(X_1X_2)^6$	0.72	15.23	0.70	7.06

Note: Result of multiple linear regressions showing the R^2 values and the SEE values

R^2_C and R^2_{CV} = correlation coefficient of calibration set and cross validation set

SEE_C and SEE_{CV} = standard error of calibration set and cross validation set

$p \leq 0.05$

X_1 = percentages of extra fiber bundle area from image analysis

X_2 = percentages of muscle fiber bundle area from image analysis

4.3 Effects of temperatures and cooking times on histological structure of sample during sous-vide cooking

Localisation of tissue components by histochemistry were represented in Figures 4.6 and 4.7. Haematoxylin Eosin Safran (HES) coloration presented the muscle cells in red-pink color, fat cells in white color whereas Sirius Red (RS) coloration presented muscle cells in yellow-orange color and connective tissue in pink-red color (Sheehan and Hrapchak, 1980 and Astruc et al., 2012).

Percentage of extracellular space area (ECS) and percentage of fiber cross sectional area (CSA) of round sample at different temperatures and times were shown in Figure 4.8. Cooking temperature and time significantly ($p \leq 0.05$) affected the extracellular space area and fiber cross sectional area (Figure 4.8). It was found that condition of 60°C cooking temperature tended to decrease percentage of extracellular space area of sample whereas percentage of fiber cross sectional area increased. Compared to 60°C samples, 70°C samples did not show significant difference in CSA and ECS data (Figure 4.8). Increasing temperature from 60-70°C to 80°C resulted to a significant increase in CSA (2, 4, 6 and 30 hours of heating) and a significant decrease in ECS (4, 6 and 30 hours of heating).

Shear force did not vary significantly during the first 4 hours of cooking. From 6 hours of cooking, shear force was significantly ($p \leq 0.05$) lower for 60°C cooked samples than for 70-80°C cooked samples. From 24 hours of cooking, the shear force increased with cooking temperature (Figure 4.3A). The lowest shear force was reach after 18 hours cooking at 60°C. Conventional cooking generally causes lateral and longitudinal contraction of fibers, which is associated with an increase in meat toughness (Tornberg, 2005). The lateral contraction of muscle fibers increases with temperature in the first phase of cooking (Tornberg, 2005, Hostetler and

Landmann, 1968) then stabilizes (Hostetler and Landmann, 1968). However, our results was found, on the opposite: a swelling of the muscle fibers after cooking which increased with cooking temperature. These results are in agreement with those of Wattanachant et al. (2005) on sous-vide cooking of Thai indigenous chicken. Roldan et al. (2013) also evidenced an increase in muscle fiber size when increasing sous vide cooking temperature. Compared to conventional cooking, this difference in the behavior of muscle fibers change during sous vide cooking could be related to partial solubilization of the collagen that makes up the endomysium. The lateral contraction of muscle fibers during cooking results from the contraction of the myofibrillar material and collagen (Tornberg, 2005). Long time cooking results in collagen solubilization to gelatin (Vasanthi et al., 2007).

The slightest compression of the muscle fibers would allow their lateral expansion. This expansion was probably not linked to a water transfer from the extracellular space to the intracellular space of muscle fibers since the cooking loss increased after cooking (results not shown). This increase in muscle fiber volume was not associate with a decrease in shear force (Figure 4.3A). This phenomenon could due to the fact that it was rather the perimysium which was responsible for the hardness of the meat and not the endomysium. Perimysium, denser than the endomysium, was also more difficult to solubilize than the endomysium located around the muscle fibers.

However, the effects of temperatures and cooking times on histological structural changes of Thai local beef during sous-vide cooking are still unclear such as the increase of fiber cross sectional area (CSA) and decrease of extracellular space area (ECS) undergoing sous-vide cooking and the larger size of muscle fiber changed during sous-vide cooking at 70-80°C compared to meat cooked at 60°C. Further investigations are needed to verify this hypothesis, and Electron microscopy analysis should give information that will help the understanding of this phenomenon.

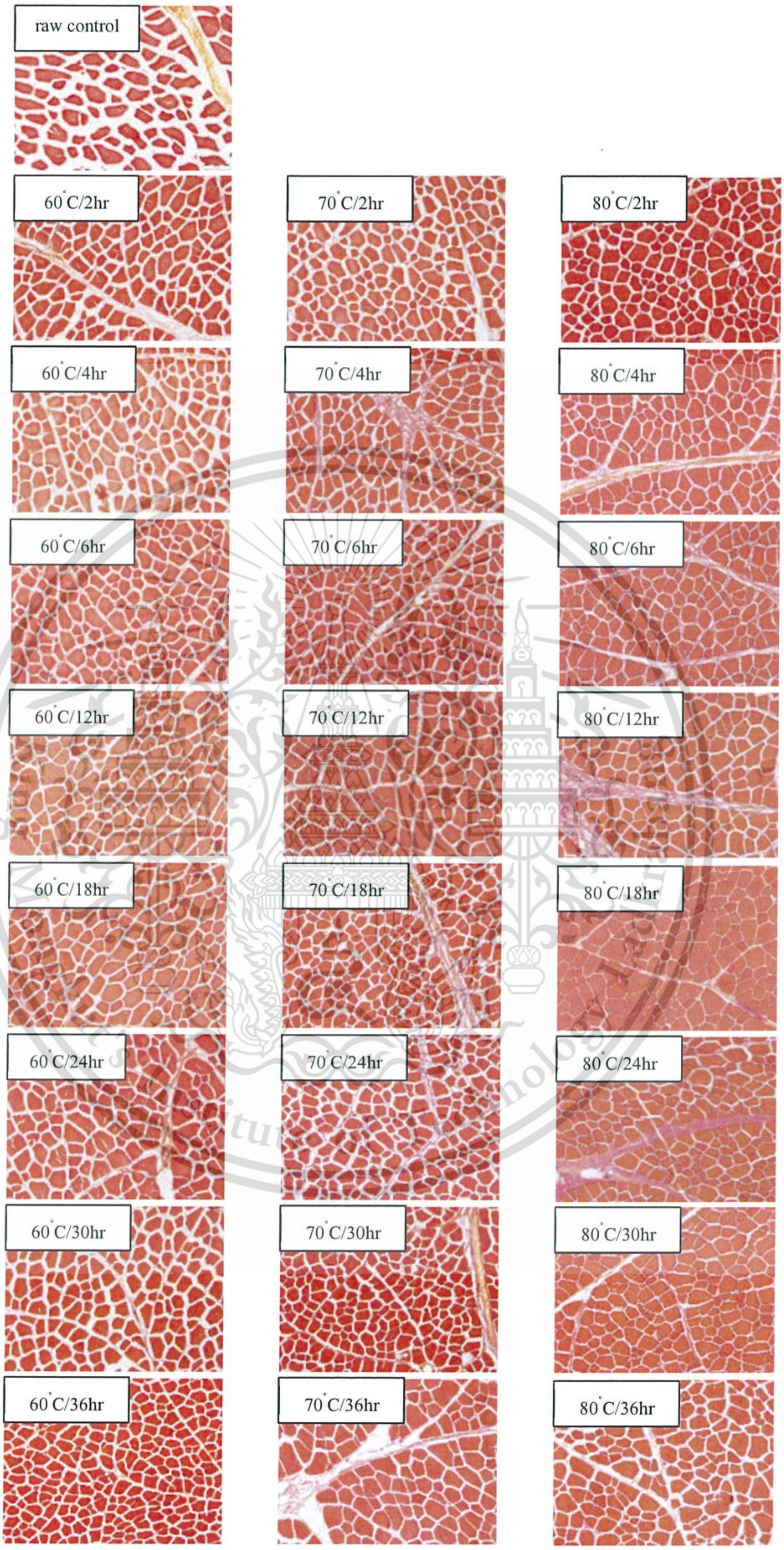


Figure 4.6 Histological cross-sections stained with haematoxylin eosin safran (HES)

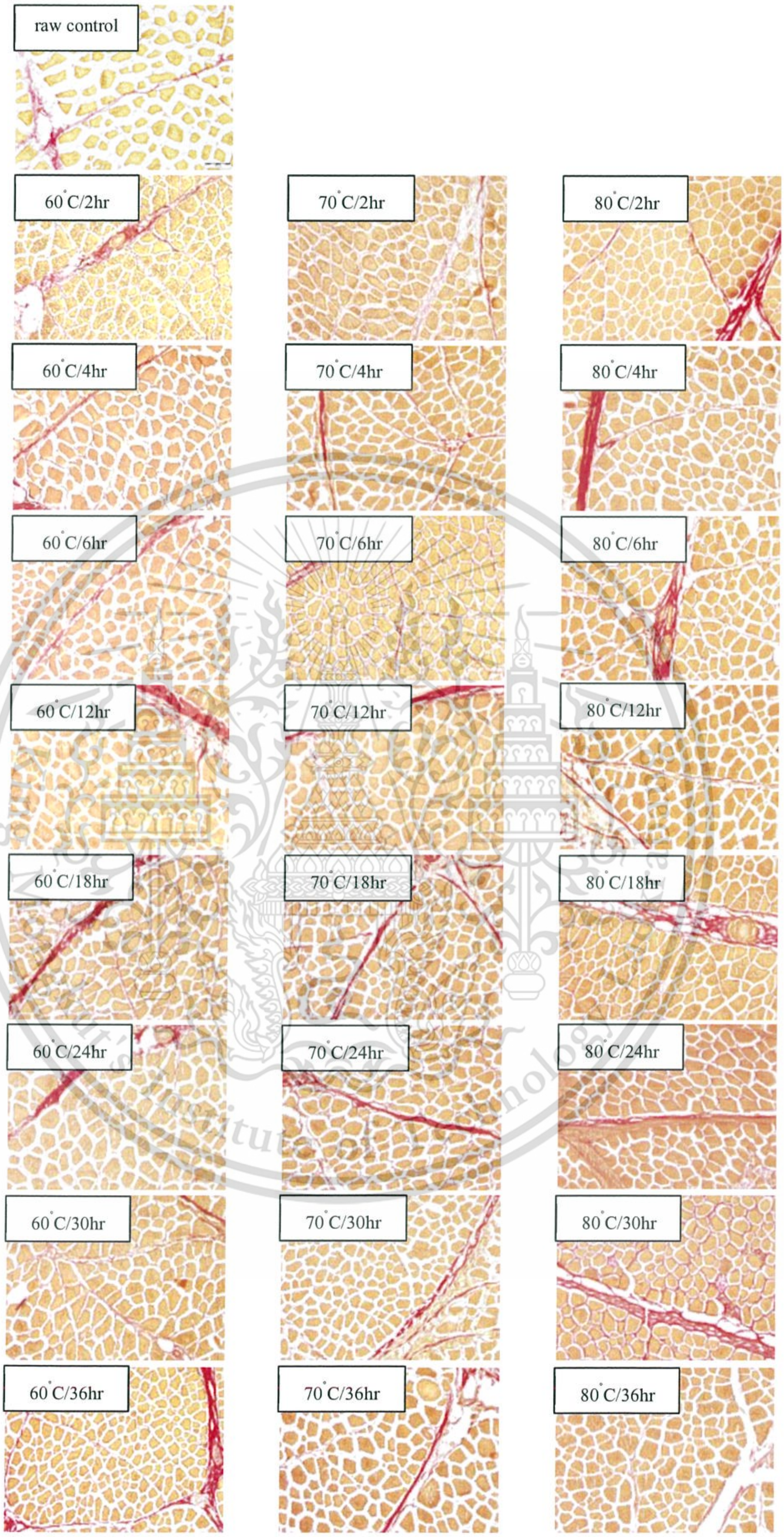


Figure 4.7 Histological cross-sections stained with sirius red (RS)

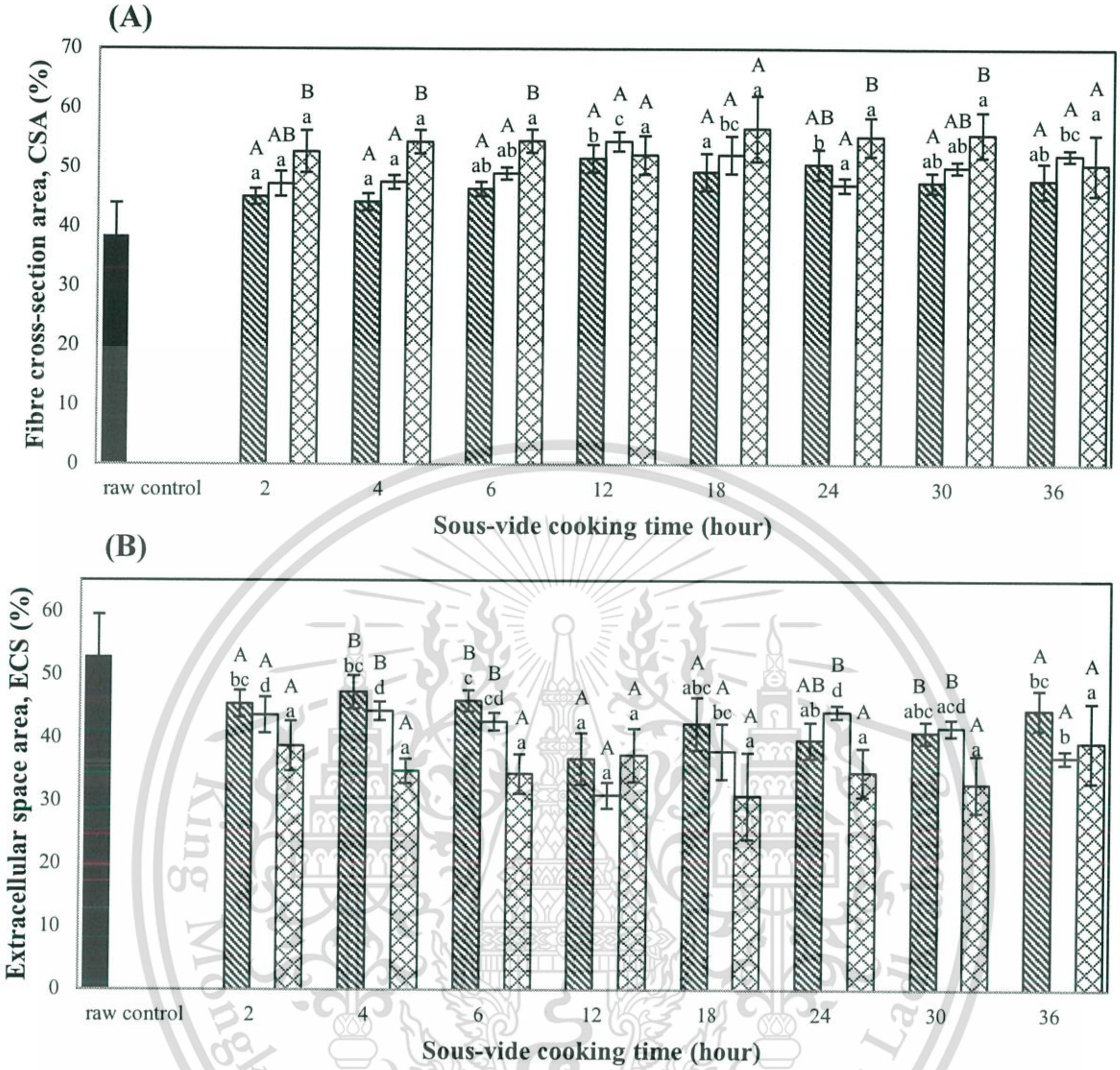


Figure 4.8 Percentage of fiber cross section area (A) and extracellular space area (B) of cooked round sample at 60, 70 and 80°C for 2-36 hour. The letters A and B were used to indicate significant difference ($p \leq 0.05$) in cooking temperature (same cooking time) and the letters a,b,c and d were used to indicate significant difference ($p \leq 0.05$) in cooking time (same cooking temperature)

■ raw control ▧60°C □70°C ⊠80°C

4.4 Effect of various sous-vide cooking parameters on the macromolecular changes of proteins

The amide I band situated around 1650 cm^{-1} is the most useful for analysis of secondary protein structure due to its sensitivity to hydrogen-bonding pattern, dipole-dipole interaction and geometry of the polypeptide backbone. Indeed, the amide I band informs on components such as α-helix, β-sheets, turns and irregular structures (Barth, 2007; Jackson and Mantsch, 1995). Second derivatives of spectra were calculated to enhance the spectral resolution of the infrared band. Component Analysis or PCA is powerful chemometric method to reveal variances or combination of variables among multivariate data. The PCA score plot and loading of muscle fibers are shown in Figures 4.9 and 4.10, respectively.

From Figure 4.8, PCA including, variables (control and three round cooked samples), component 1(PC1) and component 2 (PC2) were explained variance. For example, at 2 hours cooking time, 82% of the variance was explained by PC1 separated the variance by y-axis whereas PC2 separated the variance by x-axis. Whatever the cooking time, the sample from 3 cooking temperatures and raw sample were clearly separated on PC1 the segregation between 3 cooking temperature seen on PC2. For cooking time at 2 and 4 hours were clearly separated in 4 groups on the PC1. Then sample was more variability where cooking at 60°C for 6 hours then became more homogeneous for 12 and 18 hours.

For longer cooking times, at 24 and 30 hours, they were not clear to separate the variance because round samples had more variability in round cooked sample, whereas 36 hours the samples from 3 cooking temperatures and raw sample were clearly separated on 83% by PC1.

Mean spectra of the muscle fibers from round sample wase shown in Figure 4.10. Spectral variability originated from the transformation and protonation of protein caused by heat and time treatments. In the amide I region (1700-1600 cm^{-1}), a higher band intensity at 1624 cm^{-1} relative to 1655 cm^{-1} was observed in samples. The 1655 cm^{-1} peak, assigned to alpha helix of the amide I band of proteins, decreased with increasing temperature, while the 1624 cm^{-1} peak assigned to aggregated beta sheets structure increase, reflecting protein denaturation (Astruc et al., 2012; Motoyama et al., 2018). Moreover, aggregated β-sheet structure also gave a shoulder band at around 1695 cm^{-1}, which was a splitting band of the amide I mode (Motoyama et al., 2018). The band shape of amide II (1600-1500 cm^{-1}) also changed after the treatments. For example, the highest peak around 1540 cm^{-1} and its shoulder peak around 1520 cm^{-1} became broader and the band feature gradually disappeared. Amide II is sensitive to protein main chain structure, and hardly affected by side chain vibrations similarly to amide I mode (Barth, 2007; Motoyama et al., 2018).

This loss of α-helical structure and more β-sheet structure with increasing cooking temperature was in accordance with the results were obtained Kirschneret al. (2004), major changes in response to heat treatment occur in the amide I region, which exhibits two well defined peaks at 1628 cm^{-1} and 1655 cm^{-1}. The band at 1655 cm^{-1}, which was assigned to α-helical structure, decreased with temperature, while the band at 1628 cm cm^{-1}, which was assigned to aggregated β-sheet structure (Barth, 2007; Jackson and Mantsch, 1995), increased.

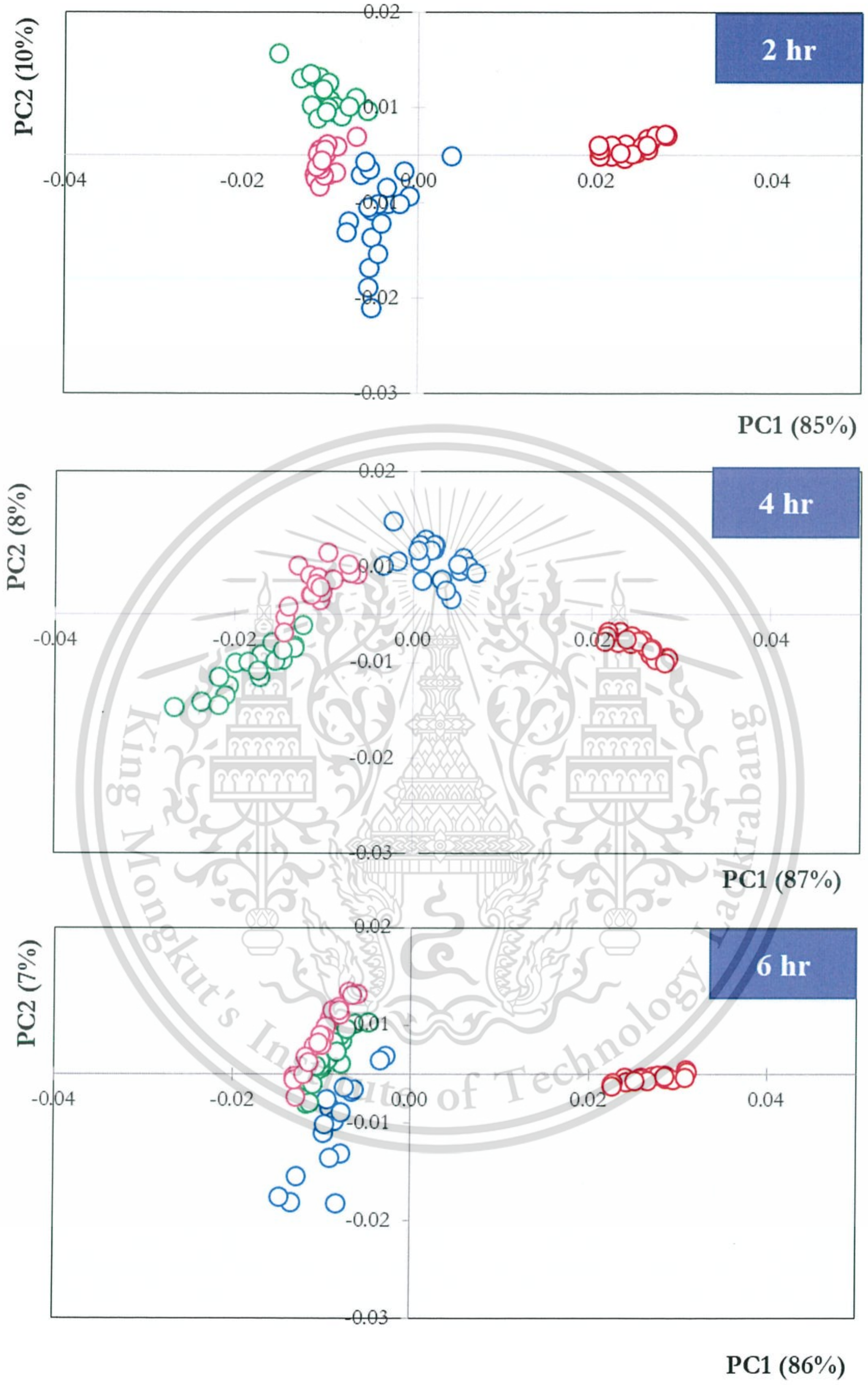


Figure 4.9 PCA score plot of the IR spectra obtained from myofibers (1700-1500 cm^{-1}) of control and round cooked samples

○ control ○ 60°C ○ 70°C ○ 80°C

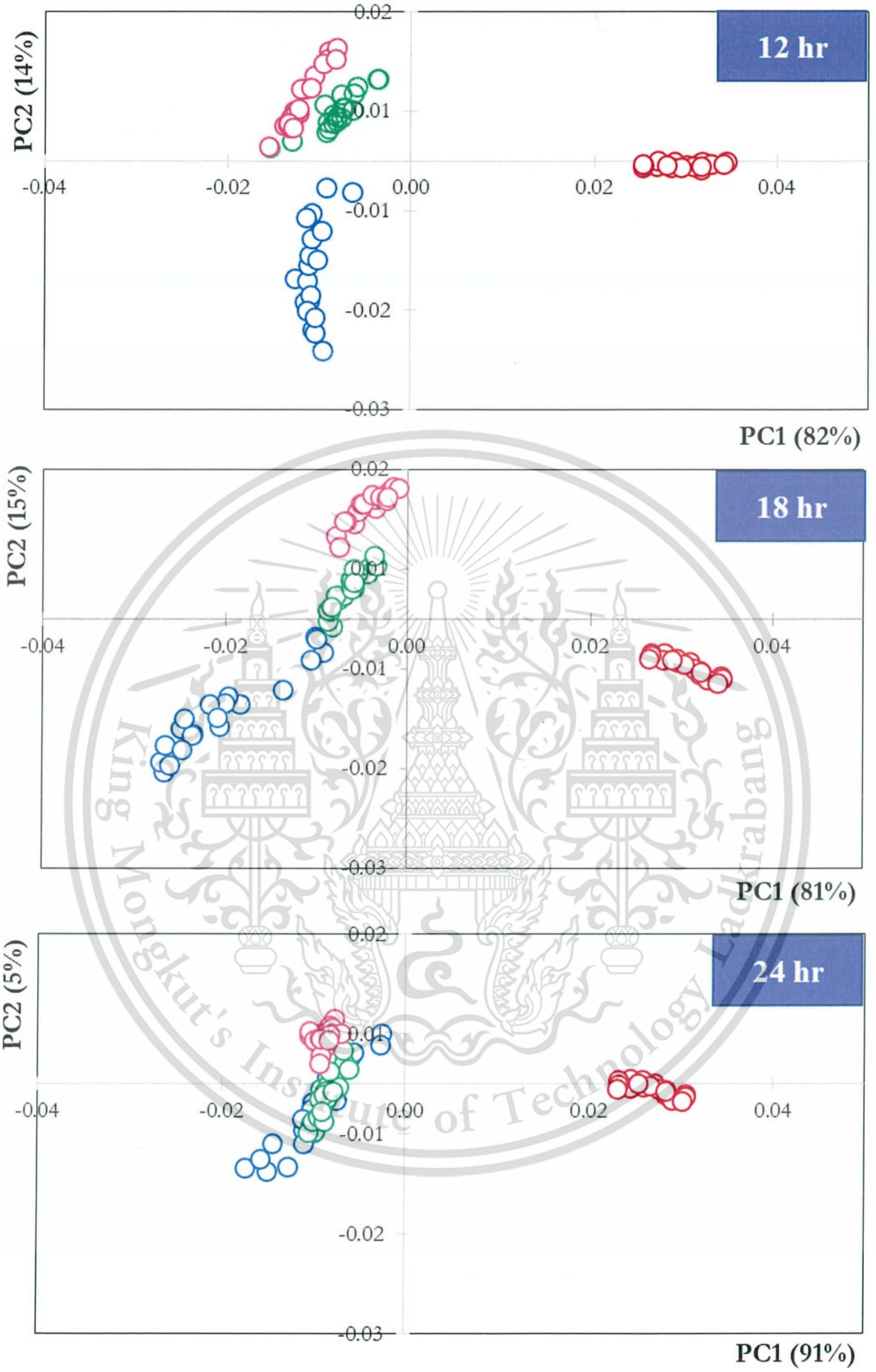


Figure 4.9 (con't) PCA score plot of the IR spectra obtained from myofibers ($1700\text{-}1500\ cm^{-1}$) of control and round cooked samples

○ control ○ 60°C ○ 70°C ○ 80°C

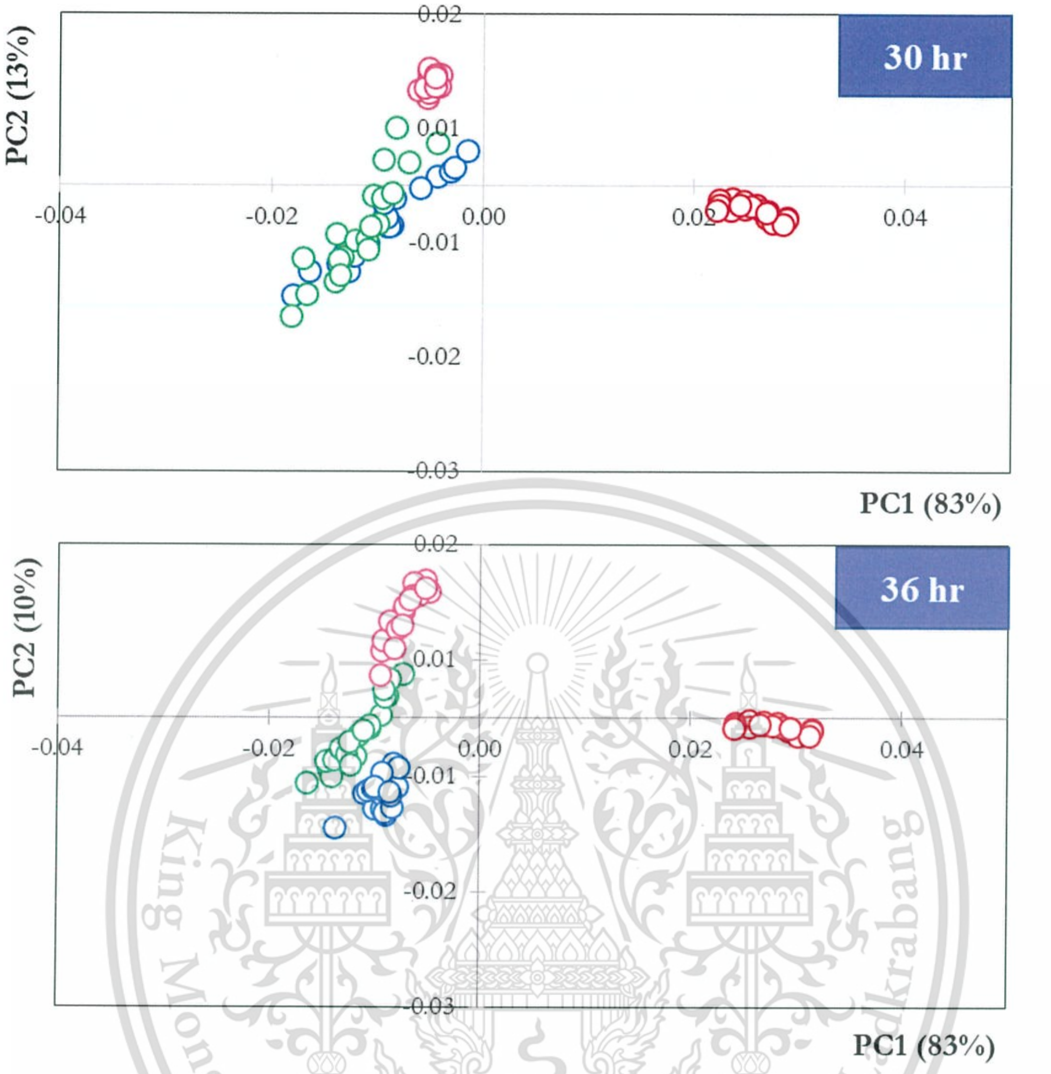


Figure 4.9 (con't) PCA score plot of the IR spectra obtained from myofibers (1700-1500 cm^{-1}) of control and round cooked samples

○control ○60°C ○70°C ○80°C

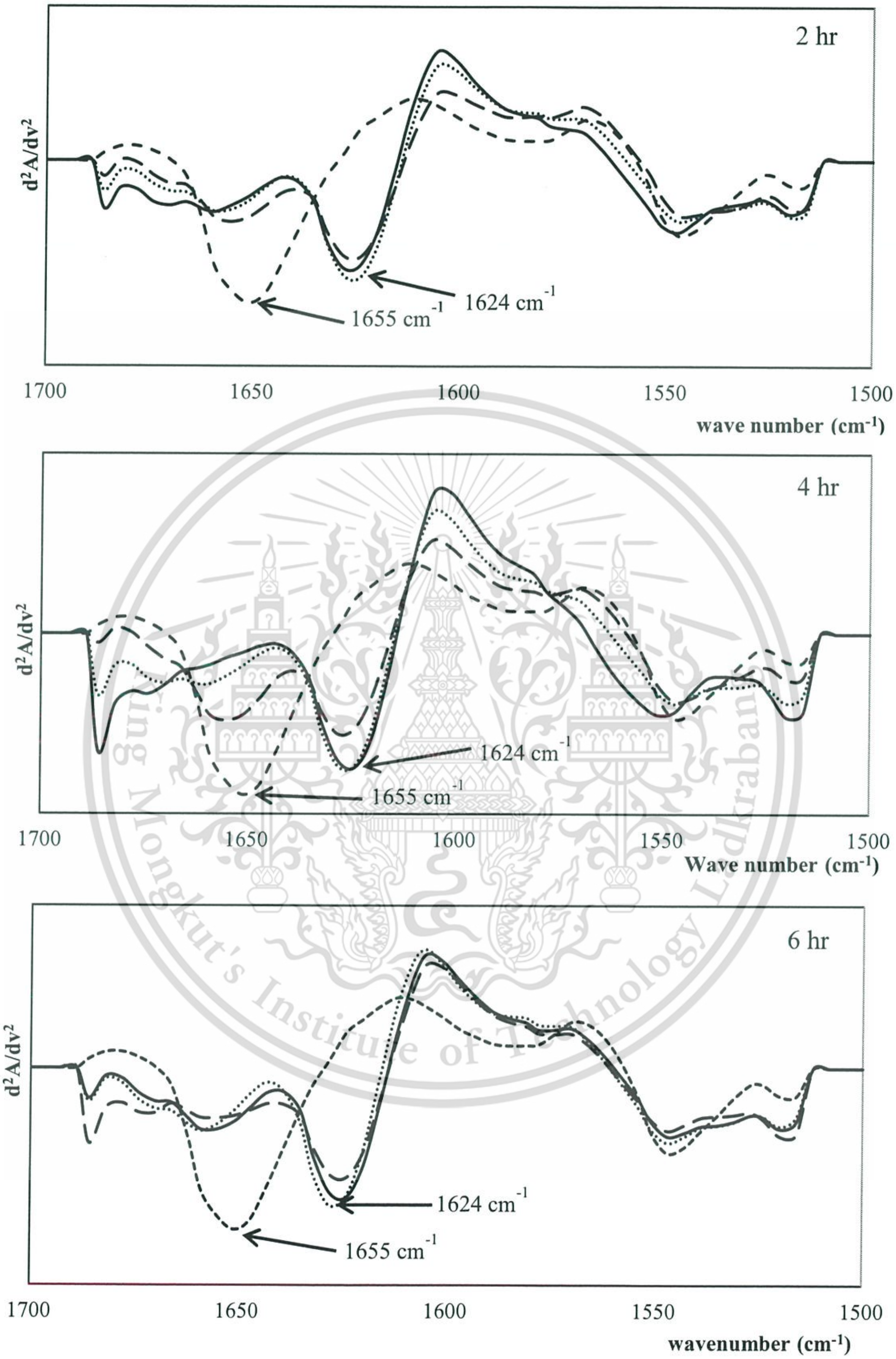


Figure 4.10 The second derivative of the IR spectra (range 1700-1500 cm^{-1}) obtained from myofibers of control and round cooked samples

-------- control — — — 60°C ——— 70°C ············ 80°C

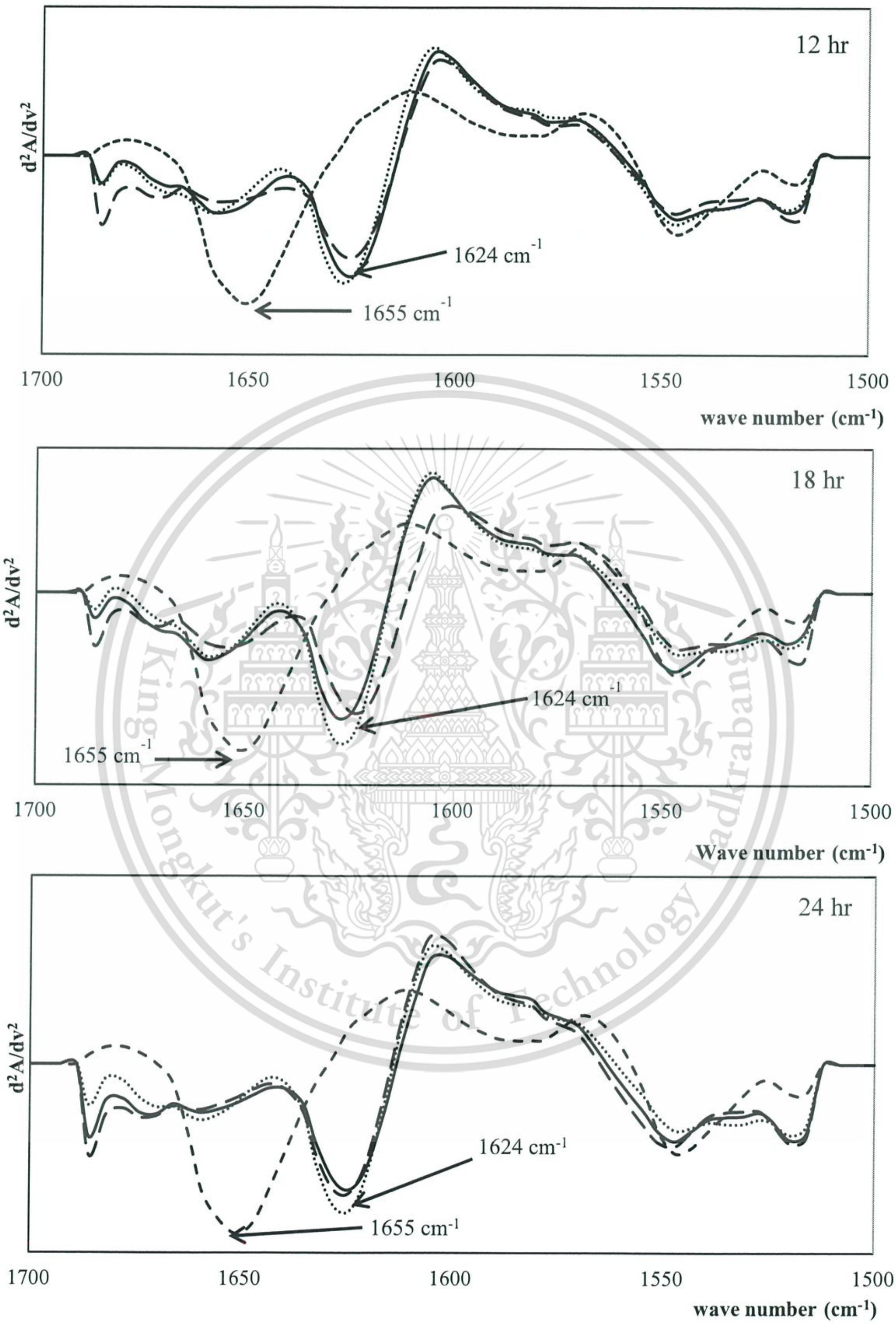


Figure 4.10 (con't) The second derivative of the IR spectra (range 1700-1500 cm^{-1}) obtained from myofibers of control and round cooked samples

-------- control – – – 60°C —— 70°C ········ 80°C

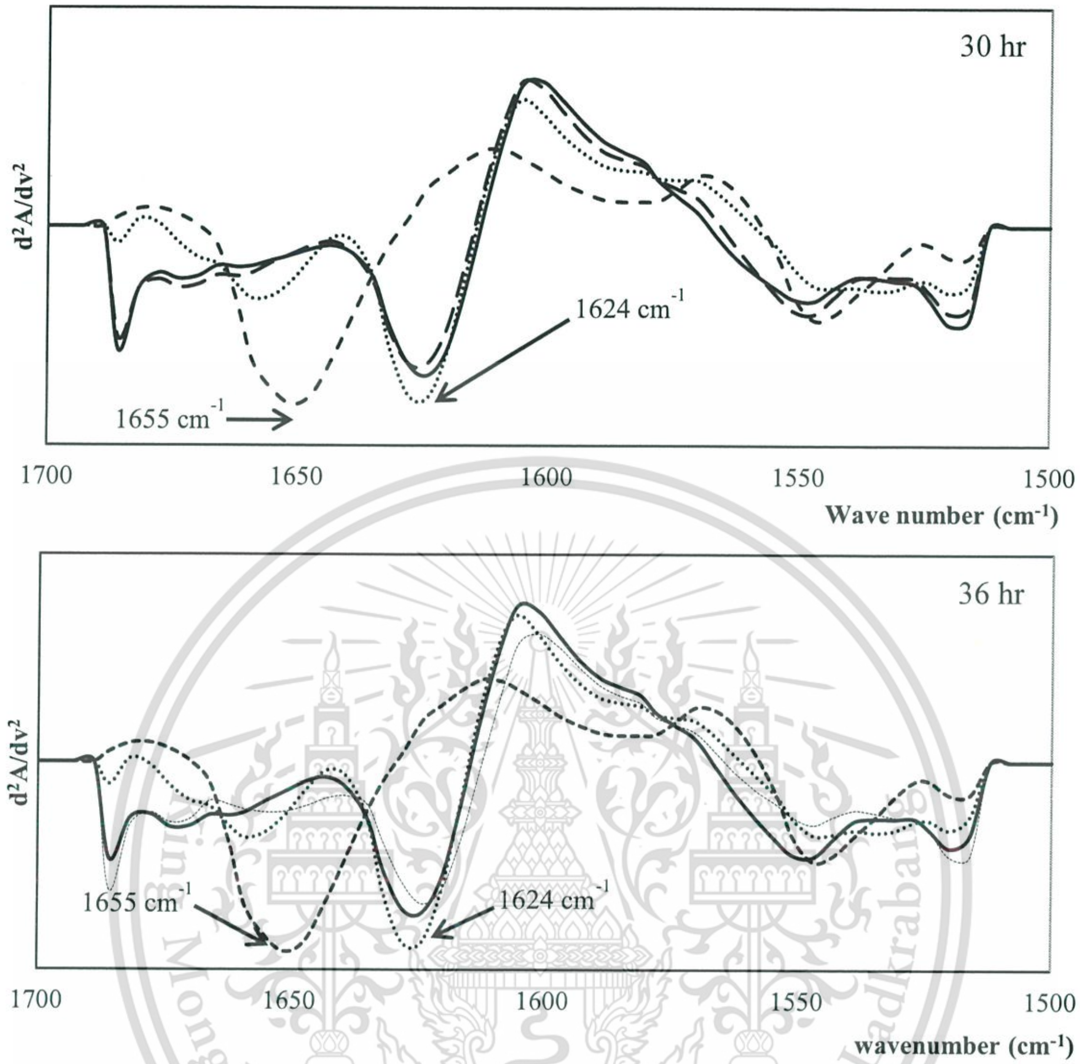


Figure 4.10 (con't) The second derivative of the IR spectra (range 1700-1500 cm^{-1}) obtained from myofibers of control and round cooked samples

-------- control –·–·– 60°C ——— 70°C ·········· 80°C

CHAPTER 5

CONCLUSIONS

5.1 Conclusions

Effects of sous-vide cooking conditions on physical properties and surface changes of Thai local sirloin and round beef were determined. It was found that sirloin and round cooked samples were generally lighter (L^*) and more yellowness (b^*), whereas less redness (a^*) than raw beef. Cooking at higher temperatures for longer times trended to increase percentage of sous-vide cooking loss and decreased percentage of re-heating loss. In addition, cooking at the lowest temperature tested (60°C) gave the lowest percentage of sous-vide cooking loss and shear force. Percentage of muscle fiber bundles of round cooked meat correlated well with physical properties and had high correlation to some physical properties were between R=0.60-0.87. For regression correlation, percentage of muscle fiber bundles and percentage of extra fiber bundles from round cooked images were found to have high correlation to the physical properties of round beef with R^2 values higher than 0.7 in all cases. Besides, in this research, percentage of muscle fiber bundles and percentage of extra fiber bundles had high R^2 values with percentage of sous-vide cooking loss of round beef with R^2 of 0.94.

Effects of sous-vide cooking conditions on structure feature changes of Thai local round beef were determined. It was found that sous-vide cooking of meat led to structural changes, which depended on the cooking temperature and duration of cooking. At 60°C cooking temperature tended to decrease percentage of extracellular space area of sample while percentage of fiber cross sectional area increased.

Cooking at 60°C to 70°C did not significantly modify the structure of the round muscle. Increasing temperature from 60 to 80°C resulted to a significantly increase in fiber cross sectional area (2, 4, 6 and 30 hours of heating) and a significantly decrease in extracellular space area (4, 6 and 30 hours). Mid infrared microspectroscopy was used to allow the structural changes of muscle fiber proteins in round beef during sous-vide cooking. This research presented potential mid infrared microspectroscopy indices that allowed the separate detection of raw control and cook treatments. It was found that increasing temperature resulted in round beef sample with decreased α-helix structures and increased β-sheet structures. Moreover, proteins from 80°C cooked samples seemed to be highly denatured after 6 hours of heating and perhaps did not evolve when increasing the cooking time. However, denaturation increased with time for samples cooked at 60 and 70°C until 36 hours of heating.

5.2 Suggestions

5.1 The images used to calculate physical properties this study were two dimensional if more sophisticated may better to represent microstructural images in the future work.

5.2 The percentages of extra fiber bundle area, percentages of muscle fiber bundle area and other physical properties changes of meat product can also be studied. The correlations between those additional physical properties and microstructural changes should be studied in the further.

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APPENDICES

APPENDIX A

A.1 Sample preparation for fixation

Samples after sous-vide cooking were cut into 1×1×1.5 cm with fiber direction in the larger length of sample (Figure A.1). Then samples were put in a plastic tube (Figure A.2), which contained 4% formaldehyde in phosphate buffer saline (PBS: pH7.2). Samples were kept at room temperature (30°C) until use.

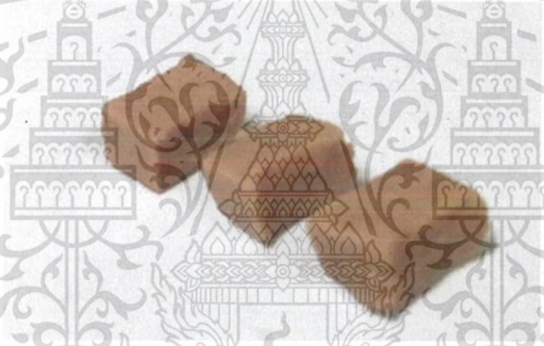

Figure A.1 Sample for fixation

Figure A.2 Sample in plastic tube

A.2 Preparation of 4% Paraformaldehyde in PBS buffer (pH 7.2)

A.2.1 PBS buffer (1X, pH 7.2) preparation (room temperature)

1) Poured 800 mL of H_2O in a glass beaker

2) Added 8 g of NaCl, 200 mg of KCl, 1.44 g of Na_2HPO_4 and 240 mg of KH_2PO_4 to the solution

3) Adjusted solution to desired pH (typically pH $\approx$ 7.2) and adjust the final volume of the solution to 1 L with H_2O

A.2.2 4% Formaldehyde Solution in PBS (1L)

1) Poured 800 mL of 1X PBS (achieved from A.2.1) in a glass beaker on a stir plate in a ventilated hood andheated (to approximately 60°C) and stirred until mixed well

2) Added 40 g of paraformaldehyde powder to the heated PBS solution (add1 N of NaOH drop until the solution clears)

3) Once the paraformaldehyde was dissolved, the solution should be cooled and filtered

4) Adjusted the final volume of the solution to 1 L with 1X PBS

APPENDIX B

B.1 Sample preparation for cryofixation

Muscles were cut about 2 to 3 cubes/condition; the size of the samples was 0.5×0.5×1 cm block taken in the core of the meat piece. Then cork platelets were cut with size larger than the muscle sample (about 2 cm cube). Then little coating medium (OCT) was put on the cork board. Muscles were stuck on the cork board (Figure B.1)

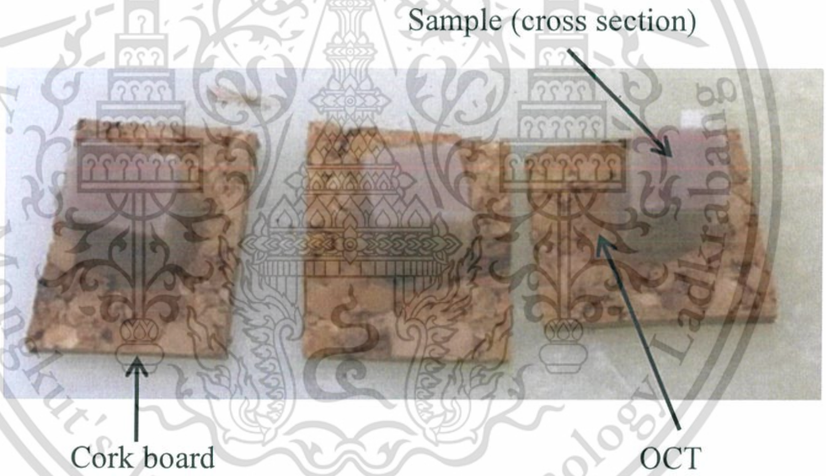

Figure B.1 The sample fixed on cork board

B.2 Isopantance cryofixation

Samples (achieved from APPENDIX B.1) were immersed in isopentane (Figure B.2) for 40 seconds by rotating and avoiding to slam with the isopentane crystal at the bottom of the container. Then sample was put in small holes in bags, and placed the container in freezer at -80°C.

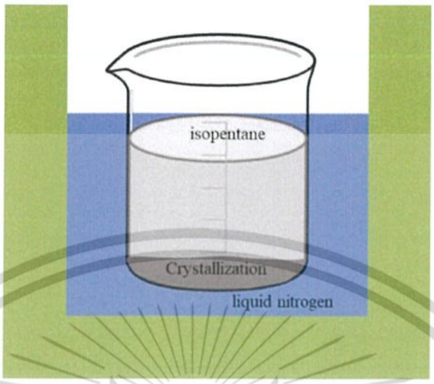

Figure B.2 Cooled isopentane (-160°C) by liquid nitrogen (-196°C)

APPENDIX C

C. Surface texture in black and white format

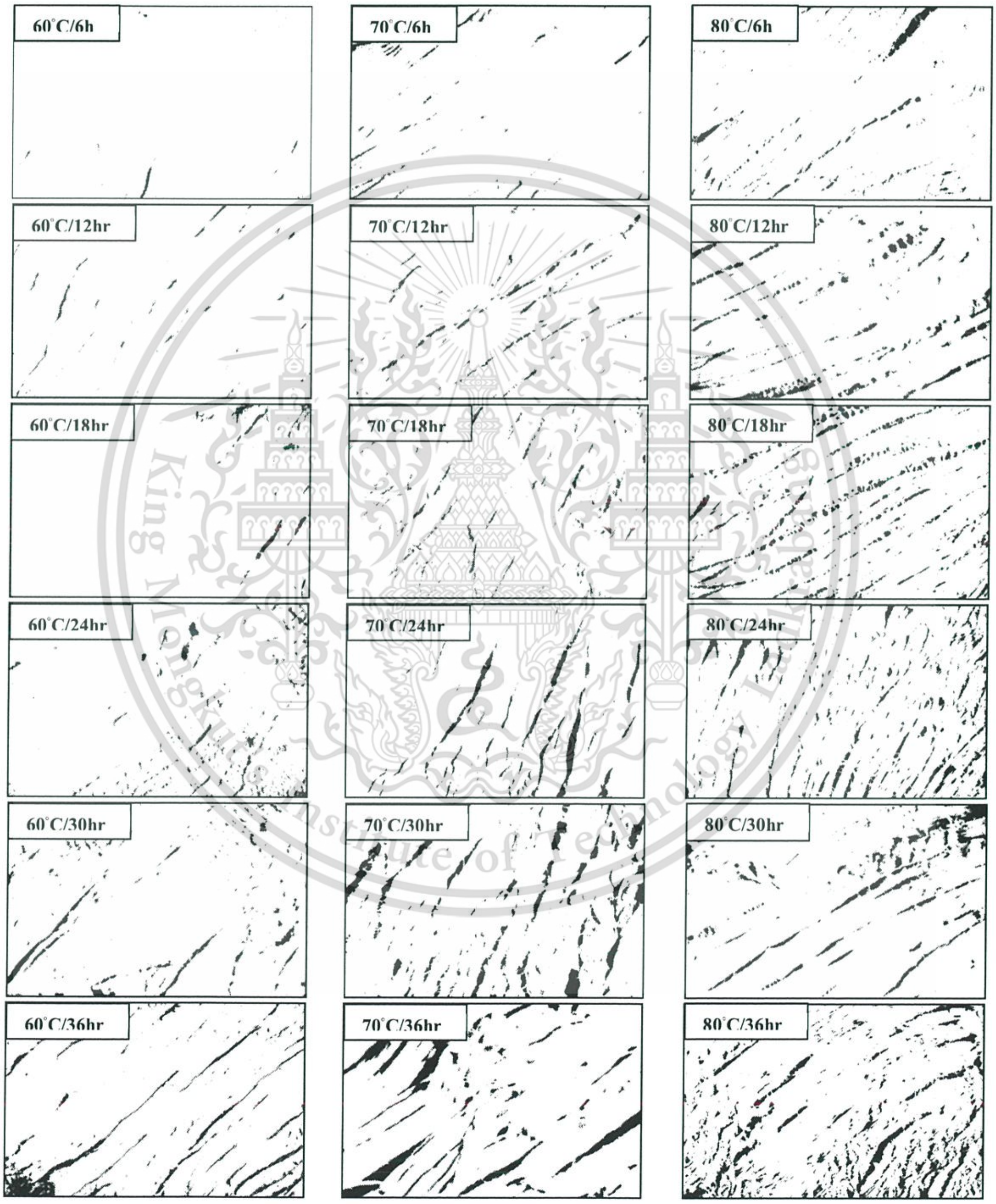

Figure C.1 Images of the surface texture (BW format) of beef after sous-vide cooking at 60, 70 or 80°C for 6, 12, 18, 24, 30 or 36 hours

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2012 - 2014	M.Sc. (Food Science), Faculty of Agro-Industry, King Mongkut's Institute of Technology Ladkrabang, Thailand. **Thesis:** Detection of physicochemical properties for sirloin beef steak by image analysis technique using artificial neural network and multiple regression analysis
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PUBLICATION

Supaphon, P., Astruc, T. and Kerdpiboon, S. (in press). Physical characteristics and surface physical properties relationship of local beef during sous-vide processing. The Agriculture and Natural Resources.

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- Training course "Good Manufacturing Practice (GMP) and Hazard Analysis Critical Control Point (HACCP) for Food Industry", Kasetsart University, Thailand.
- Training course "The British Retail Consortium (BRC) and ISO 9001", Kasetsart University, Thailand.