Controlling Texture in Processed Meat Production

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In Memory of my beloved grandfather

Abstract

The present study showed the effects of a number of hydrocolloids in terms of adding them dry, pre-hydrated or solubilised and gelled, using unmodified wheat starch, 3 types of carrageenan (kappa, iota and lambda), locust bean gum (LBG), and finally citrus fibre on meat stability, textural properties, and microstructure. The study further elucidated the role of heat induced gelation in texture formation of processed meat products. The finding of the study revealed that the maximum hardness related to the dry state of all hydrocolloids when added to a product containing 65% meat (i.e. 7.4% unmodified wheat starch, 1% LBG and 3% citrus fibre, except the carrageenan treatment, which is related to 2% gelled kappa carrageenan). Furthermore, the elasticity or rubbery texture of the sausages increased with 3% pre-hydrated wheat starch, 1 % dry LBG and citrus fibre and finally 1% solubilised kappa carrageenan with 65% meat. However, ameliorating elasticity of LBG and citrus fibre, which are 1 and 3%, respectively, took place with the dry state. The Differential Scanning Calorimetry (DSC) results showed that starch and carrageenan showed clear irreversible and reversible transitions, respectively, on the timescale of the experiment in the region of 60˚C, whereas locust bean gum and citrus fibre showed no transitions. No interaction between carbohydrates and proteins was signalled by the transition temperatures of the unaffected transitions and the addition of enthalpies which was linear. The rheology result indicated that storage modulus (G´) was greater than the loss modulus (G˝) during the test (G´> G˝), which implied predominant elastic behaviour of the sample for the whole range of deformation experimented. Finally, confocal scanning laser microscopy (CSLM) demonstrated that the void space is augmented (visa-a-vis the control sample of 65% meat) after adding dry LBG. However, the size of cavity shrank after adding gelled kappa carrageenan, dry citrus fibre, and dry wheat starch. In all treatments, the fat droplet decreased in mean size in the wake of adding hydrocolloids in the systems. Both of these findings indicate that an increase in batter texture upon the addition of hydrocolloids was sufficient to break up air cells and emulsion droplet, and then had enough strength to maintain the smaller cells droplets. The findings of the study in hand, provide a new approach to meat product manufacturing.

Manuscripts under Preparation*:

- H. Moradiannejad, W. MacNaughtan, DA. Brüggemann, AJ. Rosenthal, T.J. Foster, (2018) "The role of wheat starch in improving the texture of meat reduced sausages". Food Hydrocolloids, in preparation.

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- H. Moradiannejad, W. MacNaughtan, T.J. Foster, The effect of Locust Bean Gum (LBG) on meat batter. 62nd International Congress of Meat Science and Technology (ICoMST).Thailand, Bangkok, 2016 (Poster presentation).
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- 3nd UK Hydrocolloids Symposium on Hydrocolloids Structure Determining Functionality. UK, Sutton Bonington Campus, University of Nottingham, 2017.
- Food Additive and Ingredients Congress. UK, Harper Adams University, 2017.
- Seaweed Conference. Indonesia, Jakarta, 2016.
- Food Safety and Analysis Congress. UK, University of Cambridge, 2016.

Courses and Workshop Attendance:

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- International meeting on beef and lamb carcass grading to underpin consumer satisfaction at INRA (France) 2015.
- Meat course at the University Blaise Pascal, Clermont Ferrand (France) 2015.
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- Level 3 Award in Food Safety Supervision for Manufacturing at the University of Nottingham (UK) 2013.
- Food Flavour course at the University of Nottingham (UK) 2013.
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- Using Posters to Communicate Research at the University of Nottingham (UK) 2013.
- Food Factory Design, an operation course at the University of Nottingham (UK) 2012.
- Industrial and Biomedical Macromolecules course at the University of Nottingham (UK) 2012.

Visiting Industry and Exhibitions:

- PPMA Show (UK) 2017.
- Meatup (UK) 2017.
- Deli Show (UK) 2017.
- Food Matters Live (UK) 2016.
- Cottonii Seaweed Farm (Indonesia) 2016.
- PT Phoenix Food (Indonesia) 2016.
- Seaweed Research Centre (Indonesia) 2016.
- Surya Indoalgas (Indonesia) 2016.
- Indo seaweed (Indonesia) 2016.
- Algalindo (Indonesia) 2016.
- Java Biocolloids (Indonesia) 2016.
- Cahaya Cemerlang (Indonesia) 2016.
- Gracilaria Seaweed Farm (Indonesia) 2016.
- Wahya Putra (Indonesia) 2016.
- Hydrocolloid Indonesia (Indonesia) 2016.
- PT Gab (Indonesia) 2016.
- Gumindo (Indonesia) 2016.
- Agarindo Bogatama (Indonesia) 2016.
- Agar Swallow (Indonesia) 2016.
- Food Ingredients Exhibition (Indonesia) 2016.
- Charoen Pokphand Foods Company (Thailand) 2016.
- IFFA Exhibition (Meat Industry) (Germany) 2016.
- IFE Exhibition (UK) 2015.
- Charal Company (France) 2015.
- Fleischindustrie Company (Germany) 2015.
- Menu Fakturi (Germany) 2015.
- Food Matters Live (UK) 2015.
- Reephos Company (China) 2015.
- Shansong Company (China) 2015.
- Talleres Ramon Company (Spain) 2015.
- Xucla Company (Spain) 2015.

- Grup Vinas Company (Spain) 2015.
- Metalqimia Company (Spain) 2015.
- Quorn Food Company (UK) 2014.
- Foodex (UK) 2014.
- IFFA Exhibition (meat industry) (Germany) 2013.
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CHAPTER 1-INTRODUCTION

Meat products are a complex mixture of biological molecules, formed into a composite structure. Meat proteins are the main structural components and texture is one of the most important quality attributes of meat products, although, appearance, colour, storage-life and flavour are also important. Texture is a result of the underlying microstructural and physical properties of the other components of restructured meat products, such as polysaccharides.

Meat products are divided into three groups. The first is comminuted or meat emulsions, such as sausages, hotdogs and frankfurters. The second is reformed, for instance ham and other whole muscle products. The last one is restructured meat products, such as grill steaks, beef burgers and other minced, chopped or flaked, shaped products.

The three product categories are different from each other in their structural properties, ranging from those where considerable disruption of the native meat muscle structure has occurred, such as sausages, to those where the meat structure remains somewhat intact, for instance ham.

The technology of meat product manufacturing has not changed for many years, and is empirically-rather than scientifically-based. The technology is based on the solubilisation or dispersion of myofibrillar protein to produce either a matrix, which binds fat and water in a meat emulsion, or an exudate, which acts as an adhesive to meat pieces in reformed and restructured products. Processing changes the structure of all meat materials; generally, there are three types of structural rearrangement that occur during processing:

1. Size reduction of tempered or chilled meat, such as breaking, mincing, flaking, dicing or chopping.
2. Mixing or the mechanical treatment of meat with salts such as sodium chlorides and sodium polyphosphate to solubilise or disperse the myofibrillar protein, for instance tumbling, massaging or vertical or horizontal mixing.
3. Heat-induced structural rearrangements, mainly involving meat proteins but also other product components.

The texture of processed meat products depends on the structure of the matrix formed by the protein gel, the solution and the particles entrapped in the gel and the moisture content. Therefore, the matrix formed will depend on factors such as protein-water binding ability, salt, pH, fat content and the addition of non-meat ingredients, such as starch, carrageenan, locust bean gum and fibre.

Polysaccharides play a key role in determining food texture. Thickening and gelling are general hydrocolloid functionalities and the most important, well-recognised and widely used properties of polysaccharides in meat product development. The choice of hydrocolloids for use during meat processing is based on the attributes desired in the finished product and processing capabilities, as well as distribution, holding and reconstitution requirements and finally the formed gel. Understanding the mechanism involved in the interaction between proteins and polysaccharides is important when these components are added to food to improve functional properties.

The purpose of this study was to investigate the interaction and influence between meat proteins and some hydrocolloids, such as unmodified wheat starch and the way in which the structuring can be controlled through competitive processes, such as denaturation and gelatinisation. In order to probe this further the hydrocolloid was added either dry, pre-hydrated or in the state of a gel, three different types of carrageenan (kappa, lambda and iota) Locust bean gum and citrus fibre were also added in the different states to test the effect of the interaction on the structure, textural properties and microstructure, and the role of heat-induced gelation of meat protein on texture formation in processed meat products and its competition for water with the added hydrocolloids.

1.1 Thesis organisation

The results of this work are presented in four separate chapters. A description of the material and methods and a brief explanation of the concepts behind some of the techniques are given in Chapter 3, which follows an introduction to a state-of-the-art-understanding in the area (Chapter 1 and 2).

The work in Chapter 4 is aimed to evaluate the influence of dry, pre-hydrated and gelled unmodified wheat starch addition on meat emulsion stability, textural properties such as hardness, springiness, cohesiveness, chewiness and finally resiliencies in sausage texture formation, as well as microstructure such as location of protein network, starch and fat in cooked sausages. Furthermore, elucidating the role of heat-induced gelation in the texture formation of processed meat products and also involves investigation of the interaction between meat proteins in unmodified wheat starch.

Chapter 5 evaluates the influence of three types of dry, solubilised and gelled carrageenan (Kappa, Lambda and Iota) addition on meat emulsion stability, as described above.

Chapter 6 is based on the study of the influence of dry, solubilised and gelled locust bean gum addition on meat emulsion stability, as described above.

Chapter 7 studies the influence of the dry, and pre-hydrated citrus fibre added on meat emulsion stability, as described above.

The comparisons and conclusions of this work along with suggestions for future studies are presented in Chapter 8.

CHAPTER 2-SURVEY OF THE LITERATURE

The following sections will discuss the key criteria under consideration when contemplating creating meat product textures with the addition of different hydrocolloids.

2.1 Phase separation

Engineered food materials are mainly structured by a complex assembly of various food ingredients, for example proteins, polysaccharides, lipids, sugars, emulsifiers, minerals and water (Gaonkar and McPherson, 2005). Protein-polysaccharide interactions can affect the functional properties in foods where proteins are the major ingredients, such as meat processed products (Tolstoguzov, 1991). During the manufacturing process, intrinsic functional properties of individual components as well as interactions between the different components determine the final structure, texture and stability of food materials. Since texture and stability are the major criteria of food quality, there is keen interest in understanding the fundamental parameters which determine ingredient interactions and structures (Sanchez et al., 1997). Protein and hydrocolloids are known to interact in a variety of ways (Figure 2.1). Firstly, hydrocolloids in general may carry positive or negative charges or be uncharged, such as wheat starch and locust bean gum. It should be pointed out, however, that few hydrocolloids carry positive charges, and most hydrocolloids are either negatively charged (carrageenan are sulphated and pectin in citrus fibre are carbohydrate) or uncharged. Proteins, on the other hand, are positively charged below their isoelectric point (approx. 5.0–5.1) and a negatively charged above their isoelectric point. Upon mixing of hydrocolloids and proteins, a number of different phenomena may, therefore, be observed depending on the chemical nature, such as charge or uncharged, and the concentration of protein and hydrocolloids.

1) Proteins and hydrocolloids may be co-soluble, and in this case, a phase system is formed that contains both polymers simultaneously (co-solubility in Figure 2.1).
2) Protein and hydrocolloids may be thermodynamically incompatible (incompatibility in Figure 2.1). In this case, the two polymer phases separate into phases-one that is rich in hydrocolloids and one that is rich in just proteins.

3) Proteins and hydrocolloids may aggregate and form an insoluble complex (complexation in Figure 2.1). In most cases, the aggregate complex will, over time, precipitate and form a flocculated polymer complex layer at the bottom of the test container.

This modes of interaction may depend upon the way in which the different hydrocolloids are mixed with the meat proteins in the batter. This has been the subject of this study and environmental conditions such as pH, temperature, and ionic strength also influence the interaction.

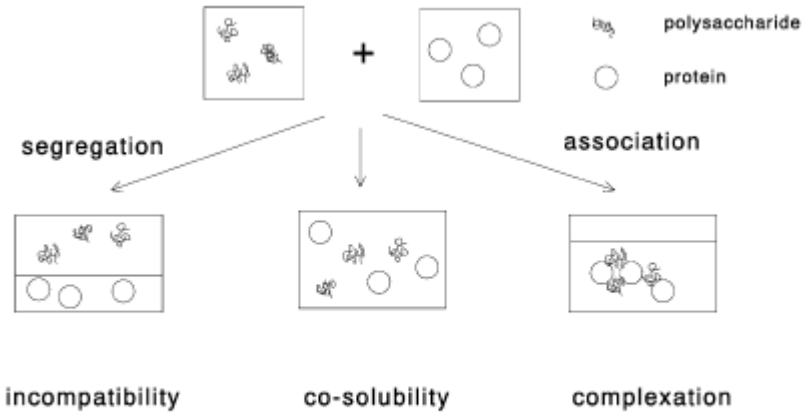


Figure 2.1 Main trends in the behaviour of protein- polysaccharide mixture (de Kruif and Tuinier, 2001).

It is worth spending time introducing the location and properties of the meat proteins used in this study.

2.2 The location and properties of meat proteins in raw meat materials

Meat is defined as the flesh of animals used as food. The chemical composition of various meat cuts varies to a large degree from one cut to another. Meat may contain a range of 45-75% water, 15 -20% proteins, and anywhere from 5-40% fat depending on the cut and trim. Meat contains no carbohydrates (Vaclavik and Christian, 1998).

Proteins are the principal functional and structural component of processed meats (Hermansson et al., 1986). Their functional properties have been defined as physicochemical properties which affect the behaviour of proteins in the food system during preparation, storage, processing and consumption, and contribute to the quality and organoleptic attributes of the food system (Kinsella, 1982). Functionality is an expression of the physicochemical properties of proteins modified by the environmental conditions. Physicochemical properties are derived from a protein's amino acid composition, amino acid sequence, secondary structure, tertiary structure and quaternary structure (Pour-El, 1981). Physicochemical properties include molecular weight, association and dissociation behaviour (Kinsella, 1982, Wilding et al., 1984).

Meat is composed of three major parts: muscle, connective tissue and adipose tissue (fat). There are three types of muscle: (1) skeletal or striated voluntary, (2) cardiac or striated involuntary, and (3) smooth or involuntary muscles.

Striated skeletal muscle adjusts the organism to the external environment, striated cardiac muscles maintains the flow of blood around the body and smooth muscle helps to maintain the internal physiological blanket of the body. Smooth muscle is found in areas such as the viscera, blood vessels, lymphatics, and skin and is usually closely associated with connective tissue layers. The raw materials used in meat production are generally composed of striated skeletal muscle.

In terms of soluble or insoluble muscle protein, this can be divided into three major groups on the basis of solubility characteristics: sarcoplasmic protein (water-soluble), myofibrillar protein (salt-soluble), and stromal protein (insoluble). Of the three groups, myofibrillar proteins play the most important role during meat processing, as they are responsible for the formation of thermally-induced cohesive structures and the firm texture of meat products.

The structural unit of skeletal muscle tissue is the highly specialised cell known as a muscle fibre, myofibre or muscle cell. Muscles fibres are the essential structural unit of all muscles and are arranged and held in place by a series of connective tissue, which acts as a wrapping and divider. Muscle fibres constitute 75-92% of total muscle volume. The remaining volume consists of connective tissue, blood vessels, nerve fibres and extracellular fluid. There are more than 20 different proteins associated with myofibrils. Six of these proteins account for approximately 90% of the total myofibrillar protein. These six in descending

order of relative abundance are myosin, actin, titin, tropomyosin, troponin and nebulin. Actin (20-25%) and myosin (50-55%) constitute the major contractile proteins. Furthermore, there are more than 15 amino acid association with protein (Table 2.1). The term connective tissue covers a wide variety of structures within the body; there are those such as tendons and ligaments that clearly connect and hold tissues together, but there are others that are more intimately associated with specific organs. In muscles, connective tissue provides the functional integrity that is essential for precise muscle action.

Table 2.1 Amino acid composition of Beef (Harfenist, 1953).

Amino acid	Gram amino acid/100 gram protein
Arginine	3.1
Lysine	2.58
Aspartic acid	6.71
Glutamic acid	17.27
Histidine	5.41
Serine	5.28
Threonine	2.1
Tyrosine	12.58
Cystine	12.18
Alanine	4.59
Isoleucine	1.51
Leucine	13.45
Phenylalanine	8.6
Valine	9.65
Proline	2.13
Glycine	5.17

The distribution of proteins in muscles is illustrated in Figure 2.2. An entire muscle is usually surrounded by a heavy connective tissue sheath called the epimysium. Associated with the epimysium is the perimysium, which divides the muscles into groups of fibres called bundles. Very thin extension of the connective tissue surrounds each individual fibre; these thin strands are called the endomysia. Reticulin, a mucoprotein, makes up the finest branched fibre of the endomysium. Below the endomysium lies the muscle cell lipoprotein membrane, the sarcolemma.

Fibres are long, narrow, multinucleated cells, which may stretch from one end of the muscles to the other and may attain a length of 34 cm, but they are usually 1-40 mm long, and only 10-100 micrometer in diameter (Lawrie and Ledward, 2006).

Muscle fibres are connected to tendons, which consist predominantly of closely packed parallel bundles of collagen.

Muscles fibres consist of a series thin cross-striated contractile units known as myofibrils, which are bathed in the cytoplasm of the cell, called the sarcoplasm. Water constitutes about 75-80% of the sarcoplasm. The striation arises from the orderly alignment of the anisotropic and isotropic segments of the myofibrils and resembles the stacking of light and dark segments. The basic unit of myofibrils is shown in Figures 2.2 and is called the sarcomere. The structure responsible for its cross-striated appearance are overlapping arrays of two kinds of filament, for example thin and thick filaments. Thin filaments mainly consist of actin, while thick filaments consist mainly of myosin.

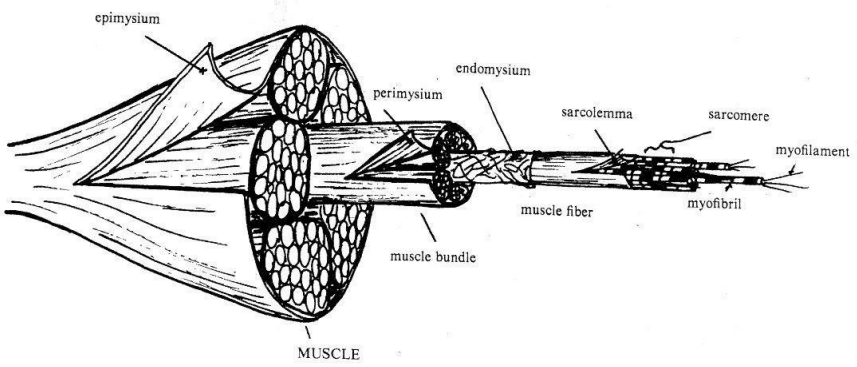


Figure 2.2: Diagram representation of macroscopic and microscopic muscle structure.

As mentioned above, muscles fibres contain bundles of myofibrils. The myofibrils themselves are composed of bundles of protein filaments, as shown in Figure 2.3. These include thin filaments, mostly made of actin, and thick filaments, which contain myosin. They are arranged in a specific pattern within a repeating longitudinal unit called a sarcomere. The thin filaments occur at each end of the sarcomere, and are held in place by Z-lines. This line defines the end of each sarcomere. Thick filaments occur in the centre of the sarcomere, and overlap thin filaments. The extent of overlap depends on whether the muscles are contracted or relaxed. In relaxed muscles, the sarcomeres are extended, and there is not much overlap of thick and thin filaments. However, a contracted

muscle has a lot of overlap since the sarcomeres shorten as part of the contraction process.

Areas of different density are visible within light and dark bands of myofibrils. Since the light band is singly reflective when viewed with polarised light, it is described as being isotropic and is called the I band; in contrast, the broad, dark band is doubly reflective, or anisotropic in polarised light, and is designated the A band. The A band is much denser than the I band, but both bands are bisected by a relatively thin, dense line. The I band is bisected by a dark, thin band called the Z disk or Z line (Figure 2.3); the unit of myofibrils spanning two adjacent Z lines is referred to as a sarcomere. The sarcomere is both an A band and the two half I bands located on either side of the A band. The sarcomere is the repeating structural unit of myofibrils, and is also the basic unit in which muscle contraction and relaxation occur. Sarcomere length is not constant, and its dimensions, as well as those of the I band, depend on the state of contraction at the time at which muscles are examined. In resting mammalian muscle, a sarcomere length of 2.5 μm is fairly typical.

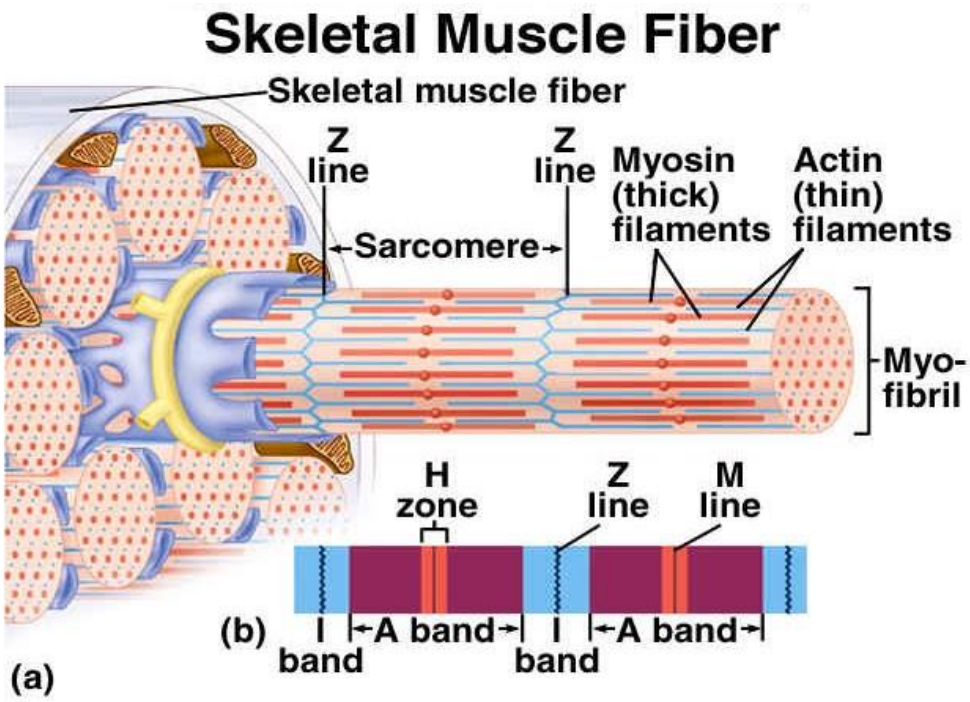


Figure 2.3: Diagram of a skeletal muscle fibre (a) lean muscles (b) sarcomere (from McGraw-Hill Education, 2007).

The structure of muscles and location of proteins have been outlined above; the properties of the most abundant proteins will now be reviewed.

2.2.1 Myosin

Myosin is the most abundant protein in the muscles (approximately 45% of myofibrillar proteins) and has an unusual structure, consisting of two heads at the end of a long tail (Figure 2.4). A myosin molecule is made up of approximately 4,500 amino acid residues. The head region has an amine and the tail region has a carboxylic end. The two globular heads are relatively hydrophobic and are able to bind actin. The rod protein is relatively hydrophilic and is responsible for the assembly of myosin into thick filaments. Each molecules is about 150 nm long and 10 to 13 nm in diameter, with a molecular weight of 500,000 (Asghar et al., 1978; Asghar et al., 1985). Myosin consists of six polypeptide subunits, two large heavy chains with a molecular weight of about 200,000 and four light chains that are located in the two heads of myosin, which are not identical; one is heavier than the other. There are 200-400 molecules of myosin in each thick filament of muscle. About 17% of the amino acid residues in myosin are basic, 18% are acidic and 38% have polar side groups (Asghar and Pearson, 1981). The helical portion of myosin has two hinge points, which make the protein flexible enough to bind with actin during contraction. The isoelectric point of myosin is 5.3; thus, myosin is a negatively charged protein in muscle as well as in meat, whose pH typically ranges from 5.4 to 6.2. Compared with the head portion, which is relatively rich in hydrophobic amino acid residues, the rod portion contains a high portion of charged side chain groups, for instance lysyl residues (Damodaran, 1997). Sodium chloride and some polyphosphates are therefore usually added to meat during manufacture to solubilise or disperse myosin and other salt-soluble myofibrillar proteins. These proteins heat-set to form a gel.

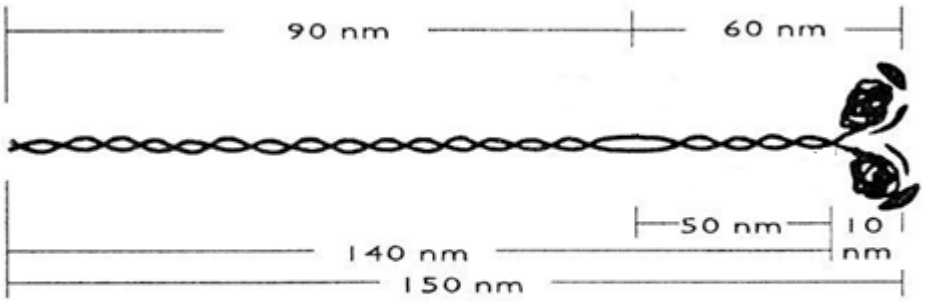


Figure 2.4: Schematic representation of the myosin molecules *(Damodaran, 1997).*

2.2.2 Sarcoplasmic protein

The sarcoplasmic proteins are located within the sarcolemma and between myofibrils. These proteins form about 30-35% of the total protein of muscles after rigor mortis or about 5.5% of the weight of the muscle in mature animals (Asghar et al., 1985). About 100 different proteins are present in the sarcoplasmic fraction, such as myoglobin (MB), Aldolase and Enolose (EN). Asghar and Pearson (1981) reported that there are four classifications of the sarcoplasmic fraction:

1. Nuclear (nuclear protein, RNA, DNA, and lipoporotein),
2. Mitochondrial (mitochondrial),
3. Microsomal (microsomes, sarcoplasmic reticulin),
4. Cytoplasmic (myoglobin and soluble proteins).

These fractions can be separated by using an appropriate centrifugal force and buffer system and each fraction partitioned electrophoretically and characterised by molecular weight. Myoglobin is perhaps the single most important sarcoplasmic protein in meat, since it is primarily responsible for meat colour and, hence, meat quality. Sarcoplasmic proteins are soluble in water or dilute salt solutions. Sarcoplasmic proteins are excluded sterically from the myofibrils; according to Scopes (1970), the concentration of sarcoplasmic proteins in the space between myofibrils is about 25-30%.

2.2.3 Actin

Actin is the second most abundant myofibrillar protein, comprising about 20% of myofibrillar proteins. It is a globular-shaped molecule with a diameter of approximately 5.5 nm. It is the major constituent of the thin myofilament and exists in two forms: globular (G-actin) and fibrous (F-actin). F-actin interacts with myosin filaments to produce mechanical energy for biological movements. Polypeptides of the actin chain are composed of 374 amino acid residues. Actin is the prevailing protein comprising the thin filaments of myofibrils; each thin filament contains approximately 400 actin molecules. In muscle tissue, actin is naturally associated with tropomyosin and the troponin complex. It also contains a myosin-binding site, which allows myosin to form a temporary complex with it during muscle contraction or a permanent myosin-actin complex during rigor mortis development in post-mortem meat (Damodaran, 1997) (Figure 2.5). Actomyosin is not an indigenous protein constituent in the myofibrillar assembly; it is a form of protein complex produces by muscle contraction. Actomyosin is formed by the interaction of actin and myosin in *situ*. In actomyosin, myosin and actin are associated via noncovalent bonds, which can be easily split by high ionic strengths. Actomyosin is soluble at an ionic strength of about 0.5 M, whereas myosin is soluble at about 0.3 M.

Tropomyosin and troponin are the major regulatory proteins. They are located on the thin actin filaments and represent approximately 8% and 5% of the total myofibrillar protein content.

2.2.4 Tropomyosin and Troponin

As the most abundant regulatory protein in the myofibrillar protein family, tropomyosin is a dimeric molecule consisting of two dissimilar subunits designated α- and β-tropomyosin, with molecular weights of about 34,000 and 36,000 Daltons, respectively. The α- and β-subunits can combine to form three possible dimers: ββ, αα and αβ; the distribution of the three dimers varies in different muscles. Tropomyosin is a filamentous molecule composed of a coiled-coil of two α-helices, each approximately 40 nm in length (Figure 2.5). Tropomyosin occupies the peripheral position on the actin double helix filaments, and shields the myosin binding sites on actin filaments in the relaxed state of muscle. During contraction, which involves calcium, tropomyosin

moves to the F-actin groove, finally allowing myosin to bind to the actin filament (Damodaran, 1997).

Troponin is the second most abundant regulatory protein in the myofibrils. It is a complex protein comprised of three subunits designated C, I, and T for their ability to bind calcium, to inhabit contraction, and to bind with tropomyosin, respectively (Figure 2.5).

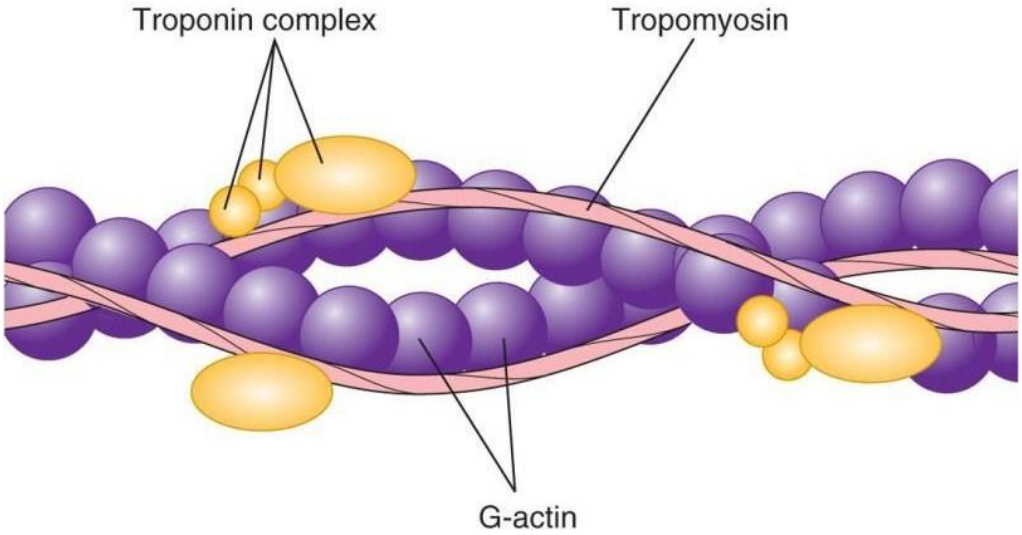


Figure 2.5: Schematic diagram showing actin, tropomyosin and the troponin complex in thin filaments (from McGraw-Hill Education, 2007).

2.2.5 Titin

Titin is the thick and thin filaments of myofibrils, and is the most abundant cytoskeletal protein in muscle, composing about 10% of myofibrillar proteins. Titin is present in both skeletal and cardiac muscle. The protein binds to the outside shaft of thick filaments and the C protein, which constitutes approximately 2% of myofibrillar proteins, encircles and stabilises thick filaments. In myofibrils, it maintains the ordered structure and the integrity of myofibrils within the sarcomere and is thought to be responsible for the resting tension associated with each sarcomere. Locker (1987) indicated that titin is the major constituent of fine filaments located in the gap between actin and myosin filaments, and can be seen in highly stretched muscle fibre. For this reason, these filaments have been referred to as gap filaments. Gap filaments are also called intermediate filaments due to their average diameter compared to actin and myosin filaments. Gap filaments apparently bind to the Z-disk and thick

filaments. For this reason, titin may provide a mechanism to link thick filaments to the Z-disk and interdigitate thick filaments into the sarcomere.

2.2.6 Collagen

Collagens are glycoproteins that exist as a family of related molecules, of which twelve different forms have been identified to date, each with its own unique sequence of amino acids, molecular weight, associated carbohydrate molecules and physicochemical properties (Bailey and Light, 1989). Based on the macromolecular structure, collagen can be divided into three major groups: (a) striated fibre collagen, which includes type I, II and III collagen, (b) non-fibrous collagen, which contains type V and IV or basement membrane collagen, and finally (c) microfibrillar collagen, which encompasses types VI and VII. The molecular organisation of collagen types I to VII is illustrated in Figure 2.6. Collagens have characteristic amino acid compositions, with the most important feature being the presence of the amino acid hydroxyproline at a concentration of about 14%. This amino acid occurs in only a few other proteins, such as elastin (1.6%), certain plant proteins and acetylcholinesterase. Therefore, the presence of hydroxyproline in collagen has been used for many years as a means of estimating the amount of collagen present in meat and meat products. Other features of the amino acid composition of collagens are their high glycine and proline content, which together comprise approximately half of the total amino acid content. The presence of hydroxylysine in collagen is also worthy of note since it is involved in the formation of cross-links to stabilise collagen fibres. Collagens contain only a few aromatic amino acids. The influence of collagen on the nutritional quality of meat has been the basis for the introduction by some countries of regulations requiring minimum contents of non-connective tissue meat protein in meat products (Board et al., 1978). X-ray analysis and electron microscopy have revealed that collagen fibres are composed of rod-like molecules that are about 300 nm long and 1.5 nm in diameter, as shown in Figure 2.6. Each collagen molecule is composed of three polypeptide chains, designated α-chains, which form a triple-helical structure stabilised by H-bonds (Figure 2.7). Each polypeptide contains about 50 amino acids. The helices differ from the typical α-helix due to the abundance of proline and hydroxyproline, which interfere with the α-helical structure (Privalov, 1982). Collagen types I and III

are found aggregate in muscles fibres. Type I is the most prevalent and is the main collagenous component in skin, tendons and bones of meat animals. Type II collagen is the major component in cartilage. Type III collagen has a preponderance in vascular tissue, skin and tendons. Type IV is found in basement membranes, which are unique tissues that have specialised functions, such as providing elastic support. Type V is a minor component of most tissues. The three layers of connective tissue in muscle, endomysium, primysium and epimysium are made up of different types of collagens. Type I is located in the epimysium, both types I and II are present in the primysium in large amounts, type III is primarily found in the primysium and types IV and V in the endomysium (Lawrie and Ledward, 2006). These connective tissues collectively provide toughness to meat. Collagen from older animals is less soluble than that from immature animals, and this decreasing solubility is accompanied by an increase in the toughness of the fibre. Collagen fibres undergo thermal contraction when heated to 65˚C, through collapse of the tertiary structure and conversion of the highly organised triple helix to a random coil configuration (Lawrie and Ledward, 2006).

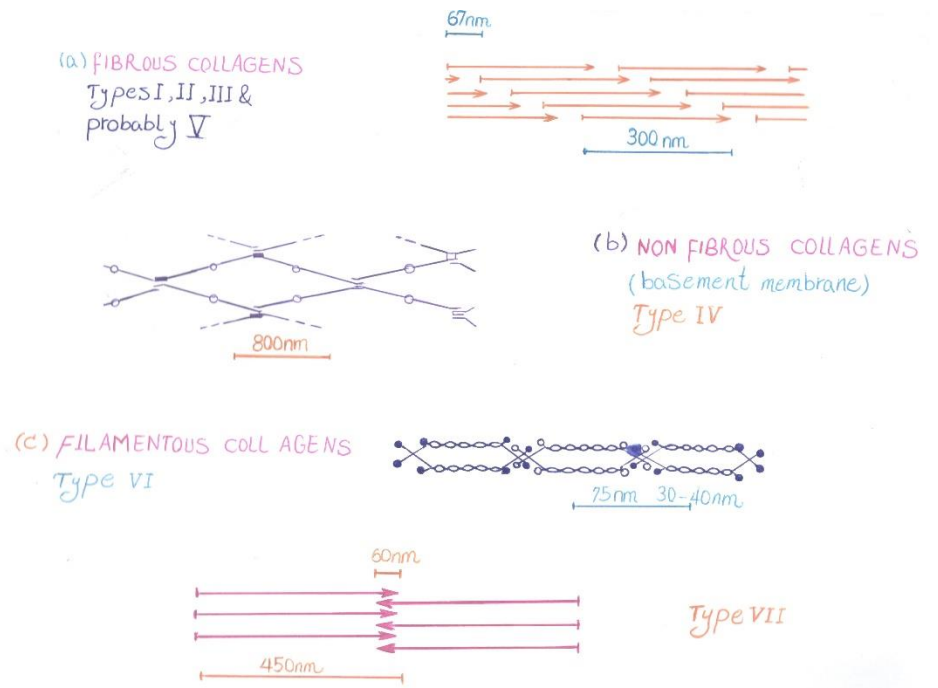


Figure 2.6: Molecular organisation of the different types of collagen molecules in (a) fibrous collagen, (b) non-fibrous collagen and (c) filamentous collagen.

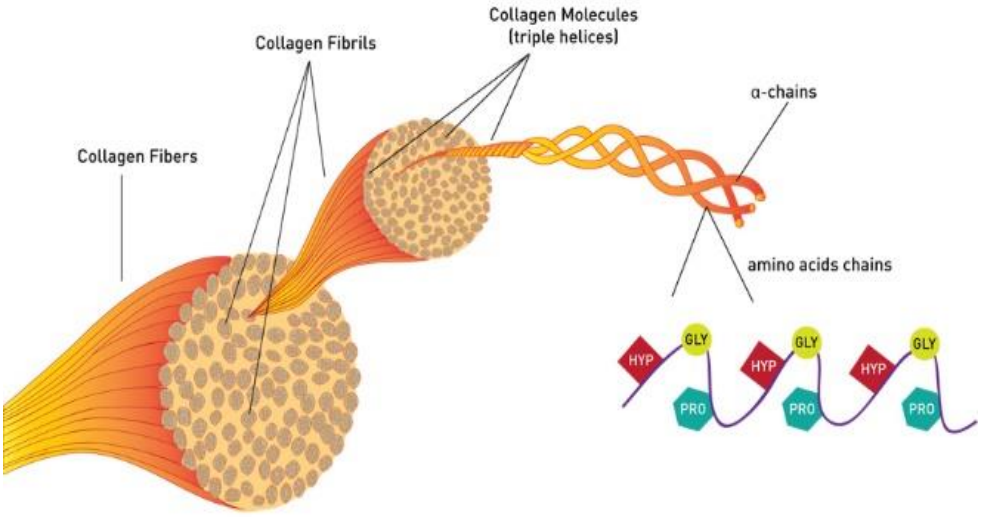


Figure 2.7: Schematic representation of collagen (from https://world.gold-collagen.com/skincare-science-and-research/).

Now that the location and properties of meat proteins have been discussed, it is worth spending time introducing the polysaccharides used in this study.

2.3 Hydrocolloids

The terms hydrocolloids or gums are commonly used to describe a group of water-soluble naturally occurring polymers found abundantly in nature (Phillips and Williams, 2009). Hydrocolloids seldom constitute an entire finished product, but are most often used as additives to improve or control the properties of a food product. Their key function in food products is to control the texture and organoleptic properties, mainly by enhancing the viscosity and gel characteristics. As a result of the increasing consumer demand for convenience foods and the modern trend towards healthier high-fibre and low-fat diets, hydrocolloids are finding ever–increasing applications in food products to improve product texture and quality. These materials are found in exudates from trees or bushes such as gum Arabic, extracts from plants such as pectin, or from seaweed, for example carrageenan and alginate, gums from seeds such as locust bean gum and guar or starches from cereals such as wheat and maize, and from fermentation processes like xanthan (Glicksman and Glicksman, 1982).

2.3.1 Starch

Starch is a major storage polysaccharide of higher plants, where it occurs in organs such as seeds, tubers, or roots and also in smaller quantities in stems and leaves. It exists as water insoluble, roughly spherical granules whose shape, size and size distribution are characteristic of the particular plant species. Starch finds application in a wide range of products, such as in the food industry as a thickener, and it is very important for human diets. Corn, potato, wheat, tapioca and rice starch are the most common sources. The primary functionality of starch is to bind water, provide various degrees of freeze/thaw stability and contribute to texture.

2.3.2 Starch composition

The major components of starch are the two polysaccharides amylose and amylopectin. Normal wheat starch is comprised of 25% amylose and 75% amylopectin; the amylose is mainly linear and composed of glucopyranosyl monomers linked by α1→4 glycosidic bonds. Amylopectin has the same composition but is also branched by α1→6 links (Figure 2.8). Amylose is a mixture of linear and lightly branched molecules (0.2-0.8% linkages), with an average of degree of polymerisation (DP, which is number of monomeric units in a macromolecule or polymer or oligomer molecule) of ~1000-5000; on the other hand, amylopectin is monodisperse and highly branched (4-6% of linkages) with a DP of ~10000 or more (French, 1972). In aqueous solutions, amylose forms an ordered crystalline structure much more rapidly than amylopectin (Miles et al., 1985) (Figure 2.9). The starch granules contain minor amounts of lipids, proteins, and phosphorous and trace amounts of inorganic compounds.

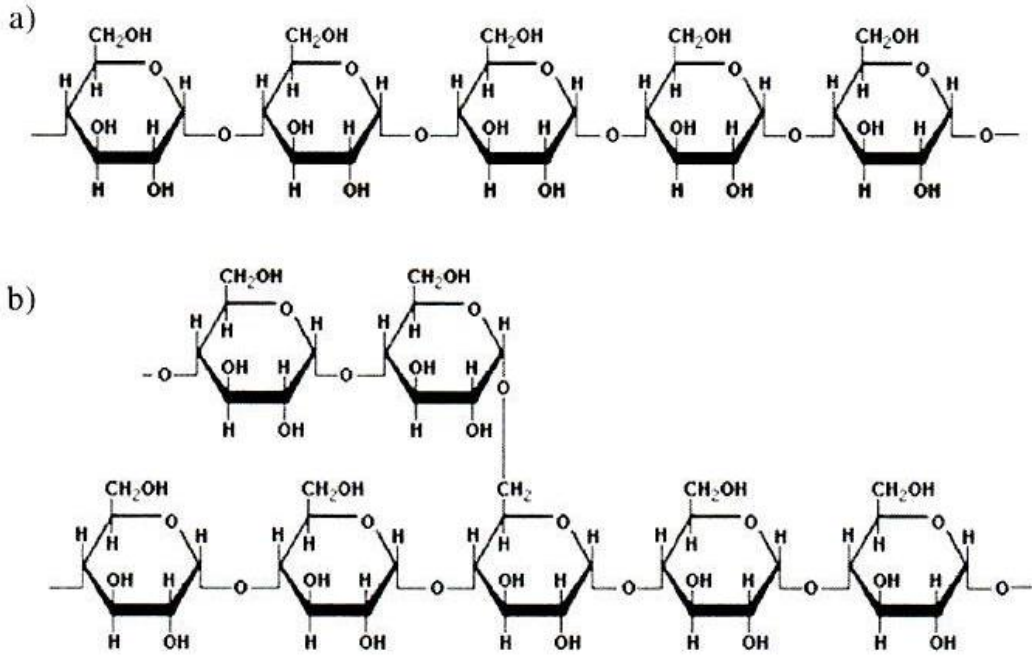


Figure 2.8: Type of linkage present in (a) amylose and (b) amylopectin (from http://ibhumanbiochemistry.wikispaces.com/C.3.4).

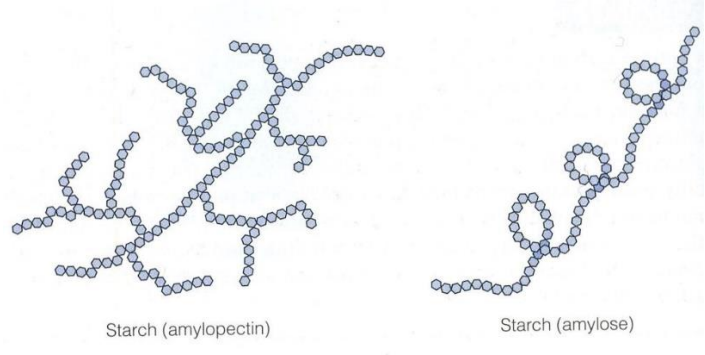


Figure 2.9: Schematic model of a starch molecules in branched chains (amylopectin) or unbranched chains (amylose) (from https://thescienceofnutrition.wordpress.com/tag/amylopectin/).

The most generally accepted model for the structure of amylopectin is the cluster model (French, 1972), which consists of alternating and amorphous regions (Figure 2.10). There is one single C-chain in the structure of amylopectin which contains the only reducing end group. The A-chain is linked to the main structure at only one position. B-chains carry at least one A-chain and also form the link between the A- and C-chains.

a) b) c)

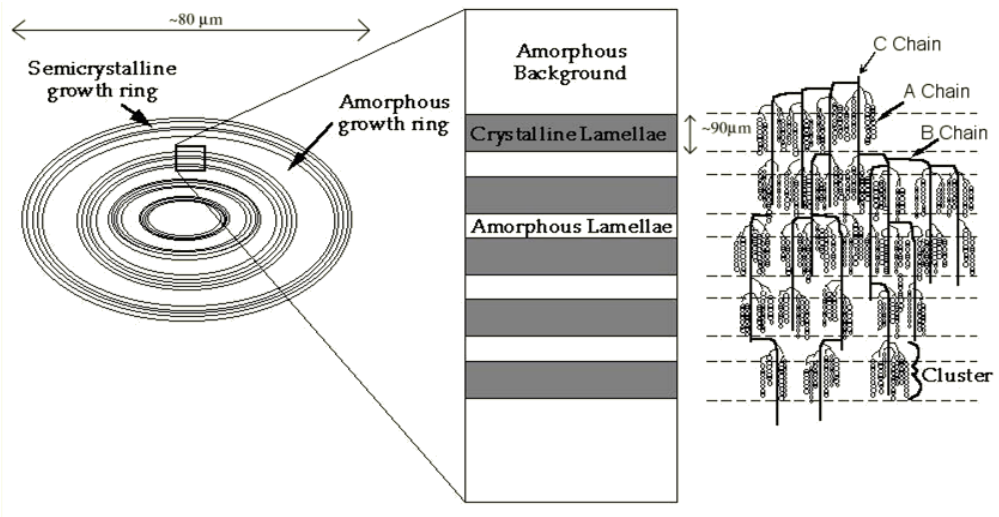


Figure 2.10: Schematic representing of the molecular organisation of starch granules: (a) A single granule, comprised of concentric rings of alternating amorphous and semi-crystalline composition. The semi-crystalline growth rings contain stacks of amorphous and crystalline lamellae. (b) Prediction of scattering for a model structure which consists of a paracrystalline lamellar stack, embedded in an amorphous background medium. (C) The currently accepted amylopectin cluster structure within the semi-crystalline growth ring. A-chain sections of amylopectin from double helices, which are regularly packed into crystalline lamella. B-chain of amylopectin provides inter-cluster connections (Jenkins et al., 1994).

2.3.3 Pre-hydrated unmodified wheat starch

In many applications and in almost all food applications, starches are heated before use. Starch granules are insoluble in water but they swell and burst on heating, releasing amylose and forming a viscous paste. When starch granules are heated in excess water to a progressively higher temperature, a point is reached where the granular crystalline structures starts to disappear and the granules begin to swell irreversibly. These phenomena associate with the disrupted granule structure, called gelatinisation. Gelatinisation can be defined as the collapse of molecular order, such as breaking of H-bonds within granules, along with all concomitant and irreversible changes in properties, for instance water uptake, granular swelling, crystallite melting, starch solubilisation and the development of viscosity. The viscosity begins in excess water at about 65˚C when amylopectin crystals begin to melt. During heating, the crystalline structure melts and part of the starch solubilises. As the sample is heated further, viscosity rapidly increases as starch granules swell. The swelling of starch granules is also accompanied by the leaching of starch molecules from the granules. This material is largely amylose, although amylopectin may also

solubilise depending on the natural of the starch (Eliasson and Gudmundsson, 2006) (Figure 2.11). The peak viscosity occurs when swelling and shear are balanced. Leaching of amylose out of granules during heating has been studied by a number of groups such as Eliasson (1986) and Doublier (1981). The rate is relatively slow during the first stage of swelling and increases drastically at temperatures above 95˚C. The molecular weight of the leached starch increases with increasing temperature (Ghiasi and Hoseney, 1982; Hizukuri, 1991). Solubilisation of amylose starts in the amorphous centre of the granule; this solubilisation and swelling from the centre of the granule take place in the first stage, when the degree of amylose leaching out of the granule is low. With increasing temperature, more amylose leaches out of the granules. Wheat starch amylopectin is less soluble than amylose and phase separation takes place due to the solubilisation and diffusion of amylose out of the swollen granules.

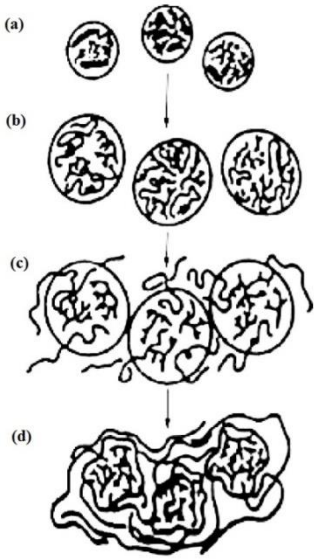


Figure 2.11: Schematic representation of pre-hydrated starch. (a) Raw starch granules made up of amylose, helix, and branched amylopectin. (b) The addition of water breaks up amylose crystallinity and disrupts helixes, so that granules swell. (c) The addition of heat and more water causes increased swelling; amylose begins to diffuse out of the granules. (d) Granules, now containing mostly amylopectin, collapse and are held in a matrix of amylose, forming a gel (from http://processplant.info/the-role-of-ingredients-in-extrusion-processingby-dennis-forte-1195).

2.3.4 Gelation of unmodified wheat starch

Gels are a form of matter intermediate between a solid and a liquid. They consist of polymeric molecules cross-link to a form a tangled, interconnected molecular network (Figure 2.12) immersed in a liquid medium. In the food system this liquid is water. The properties of the gel are the net result of the complex interactions between these two components. The water influences the nature and magnitude of the intermolecular forces which maintain the integrity of the polymer network; the polymer network holds the water, preventing it from flowing away. Several events take place during the heating of starch granules in water that bring about large changes in behaviour and are responsible for the establishment of a gel network structure. These include the swelling of granules, crystallite melting, demixing of amylose and amylopectin, changes in the extent of molecular entanglements and rearrangements in the granules and exuded material and fragmentation of the swollen granules. Gelation can be characterized as the process by which dispersed starch molecules in an aqueous medium begin to reassociate and form a three-dimensional network structure. Gelation occurs when starch granules suspended in water are heated above a certain critical temperature (the gelatinisation temperature) and then cooled. Irreversible swelling of the granules then occurs, accompanied by solubilisation of amylose and the rate of aggregation is dependent on amylose chain length (Figure 2.12). Very little amylopectin is released. The granules fragments increase the hardness of the amylose matrix (Miles et al., 1985). Upon cooling, the solution eventually associates over a long period of time to form an opaque thermoreversible gel. Aqueous solutions of amylose are quite unstable and upon cooling to room temperature, show turbidity, before forming a precipitate or gel depending on the concentration and molecular size of the polymer chain. A minimum amylose concentration for gelation between 0.8-1.1% has been reported to be independent of the molecular size of the amylose (Hayashi et al., 1983). The branched polysaccharide amylopectin will also gel (Leloup et al., 1992). Amylopectin gelation is a much slower process than that observed for amylose and occurs at substantially higher polysaccharide concentrations (more than 10%). The microstructure and viscoelastic properties of starch gel are influenced by the thermodynamic incompatibility of the starch polysaccharides (Hansen et al., 1991). Amylose and amylopectin in aqueous solutions have been

shown to be immiscible at moderate concentrations, for example 6%, leading to phase separation of the two polymers; one phase is enriched in amylose, while the other mostly contains amylopectin. When phase separation occurs, the effective concentration of both polymers in their respective microphase is increased, which may have a profound influence on the properties of the composite system. The polymer composition of the continuous and dispersed phases is dependent on the ratio of amylose:amylopectin. Structural characteristics of gels formed with amylose and amylopectin at different ratios have been studied at a fixed total polysaccharide concentration of 8% (Leloup et al., 1992). When a hot amylose solution is cooled, gelation occurs if the concentration is above a critical value (below this concentration there is precipitation rather than gelation). This changes on cooling have been terms retrogradation. The rate of retrogradation increases with increasing molecular weight. Upon cooling, the sample forms turbid viscoelastic pastes or, at a sufficiently high polysaccharide concentration of more than 6% w/w, an opaque thermoirreversible elastic gel. Also, the viscosity breakdown after viscosity peak and this is happened when the paste reached to 50˚C. The mechanical properties of concentrated starch gels are greatly affected by storage, as the starch molecules are prone to retrogradation, forming aggregates and crystallites which stiffen the structure. During the storage process, the stiffness of the starch gel slowly increases; otherwise, the stiffness of an equivalent amylose gel remains unchanged. Heating to over 95˚C reduced the stiffness of amylose.

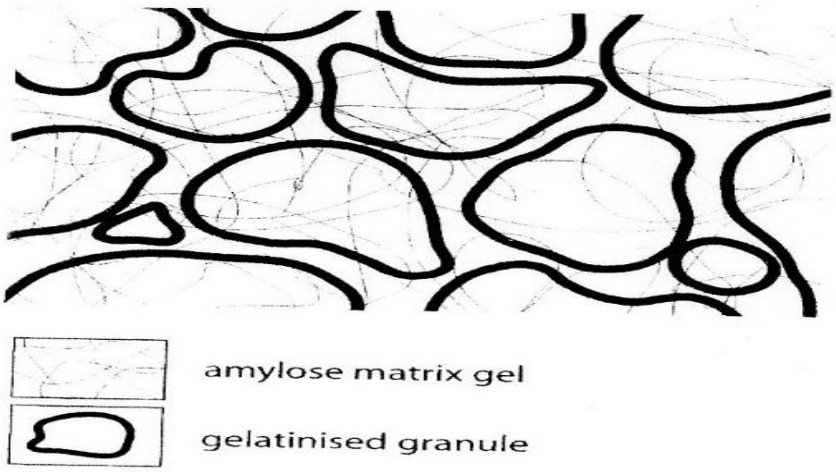


Figure 2.12: Schematic model of a composition starch gel showing gelatinised granules filling an amylose gel network (Sair and Fetzer,1944).

2.3.5 Carrageenan

Carrageenans, as defined in the Food Chemicals Codex, are hydrocolloids consisting of sulphated linear polysaccharide units (Bater et al., 1992b, Bernal et al., 1987). Such polysaccharides, generally referred to as gums, can be solubilised or dispersed in water, and provide either thickening or gelling functions in food products (Bater Et Al., 1992a; Bodeaubellion, 1983). Carrageenan has been described as sulphated linear polysaccharides of D-galactose and 3,6-anhydro-D-galactose. On the other hand, carrageenans differ from one another in their content of 3,6-anhydro-D-galactose and the number and position of the ester sulphate group. Carrageenans are classified into three main types: kappa, iota and lambda (Igoe, 1982). The best known and main source of carrageenans is *Chondrus cripspus*, which is also known as Irish moss. Irish moss was first used in foods, medicine and as a fertiliser by the Irish people of Carraghen country more than 600 years ago (Towle, 1973). The Food and Drug administration recognise carrageenans as hydrocolloids extracted from the following members of the families Gigartinaceae and Solieriaceae of the class Rhodophyceae: *Chondrus crispus, Chondrus ocellatus, Eucheuma cottonii, Eucheuma spinosum, Gigartina acicularis, Gigartina pistillata, Gigartina radula*, and *Gigartina stellata* (FDA, 1992). Different types of carrageenan confer a wide spectrum of texture, and act as a viscous thickener (for lambda carrageenan) to form a thermally-reversible gel, which ranges in texture from soft and elastic (for iota) to firm and brittle (for kappa carrageenan and furcellaran). Lambda carrageenans do not gel and are primarily used for the suspension of particulates in sauces and dressings. Kappa carrageenans form a firm gel with potassium ions. Iota carrageenan interacts with calcium ions to give soft, elastic gels, but salts have no effect on the properties of lambda carrageenans (Phillips and Williams, 2009). Food grade carrageenans have average molecular weights of 100,000 g/mol or higher (Wogan, 1976).

Red seaweed is commonly harvested by traditional manual methods. Also, red seaweed is collected from the shore after storms; however, in this case, considerable exogenous materials are harvested along with the algae. Before being shipped to the processing plant, seaweed is normally dried to reduce the total weight. Once in the processing plant and after washing, samples are macerated in a hot alkaline solution to achieve extraction. Alkaline treatments

have two advantages. The first advantage is the extraction of carrageenan from plants and the other is catalysis of the formation of 3,6-anhydro-D-galactose, which is essential to increase gel strength (Towle, 1973). The quantity of alkali, and the time and temperature of the heat treatment will determine the amount of 3,6-anhydro-D-galactose present in the polymer. After extraction, the seaweeds can be processed in three different ways, which are shown in Figure 2.13. Washing and drying or filtration steps are followed by either drum drying or alcohol precipitation. The best quality carrageenans are obtained via the alcohol precipitation method (B).

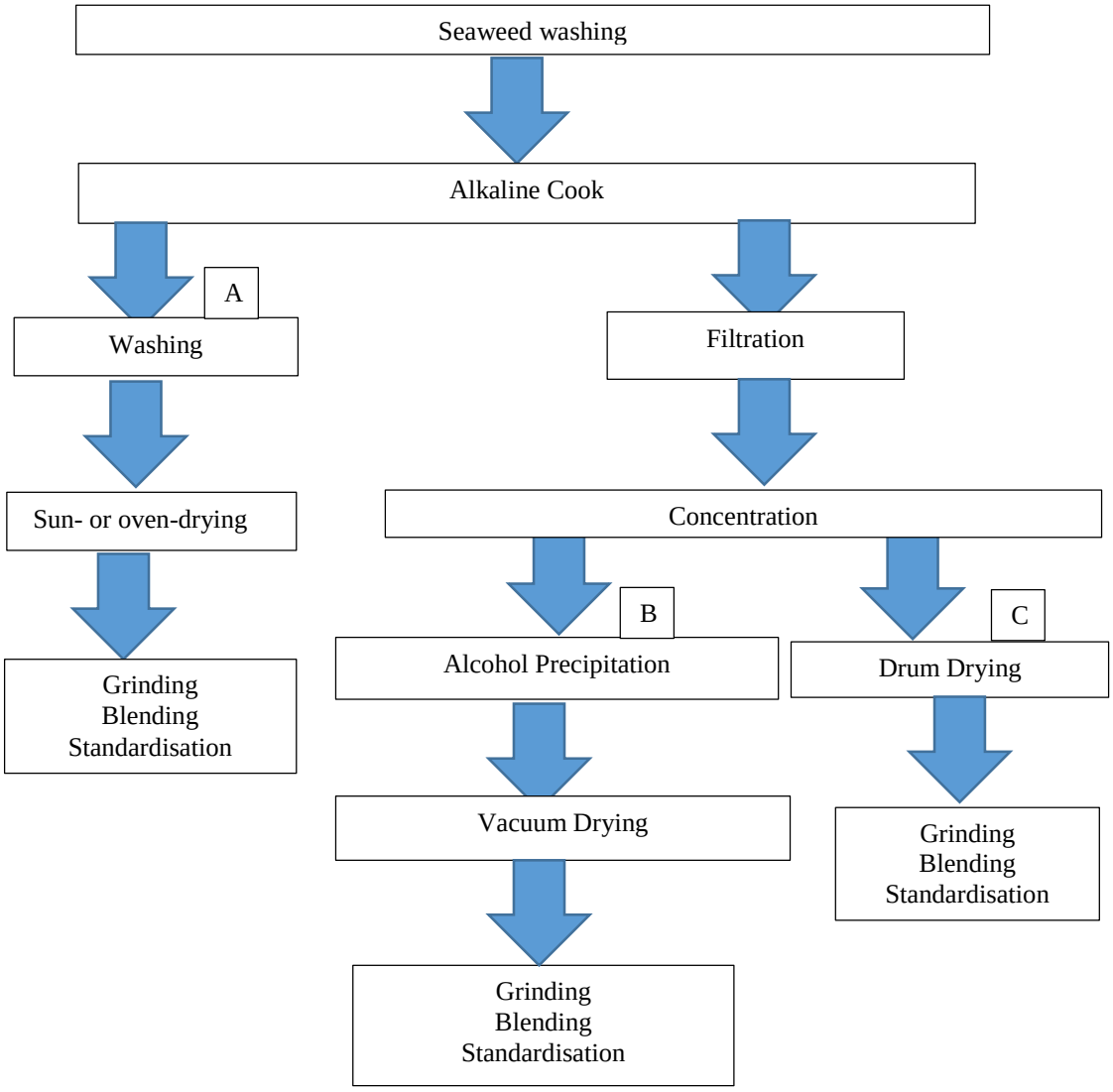


Figure 2.13: Manufacturing processes for carrageenan.

2.3.6 Kappa carrageenan

Kappa carrageenan is formed of alternating 1,3-linked D-galactose-4-sulphate and 1,4-linked 3,6-anhydro-D-galactose units (Figure 2.14a). Kappa carrageenan exhibits gelling capacity and greater sensitivity to potassium than calcium, which means that potassium is present and strong gels are formed (Glicksman and Glicksman, 1982). Potassium cations, because of their small size, can fit between the carrageenan helix and neutralise the negative sulphate group of the carrageenan (Rees, 1968). For kappa carrageenan, the potassium ion promotes helix-helix aggregations. The aggregation of kappa carrageenan is induced by potassium ions and produces strong and turbid samples that show syneresis which is the contraction of a gel accompanied by the separating out of liquid.

2.3.7 Iota carrageenan

Iota carrageenan is formed of alternating 1,3-linked D-galactose-4-sulphate and 1,4-linked 3,6-anhydro-D-galactose-2–sulphate (figure 2.14b). In the presence of potassium cations, this carrageenan forms gels that are much more elastic than kappa carrageenans. When calcium cations are present, iota carrageenans form relatively strong gels in contrast to kappa carrageenan, which forms weak gels (Rees, 1968). Once the gel is formed, however, hydrolysis is no longer a problem, even at low pH levels, and the gel is stable (Glicksman and Glicksman, 1982).

2.3.8 Lambda carrageenan

Lambda carrageenan is a non-gelling carrageenan and is formed of units of 1,3 linked D-galactose-2-sulphate and 1,4-linked D-galactose-2,6-disulphate (Figure 2.14c). Carrageenans are quite stable at pH levels of seven or higher, but chemical stability decreases below pH 7, particularly at high temperatures. At pH 5 to 7, changes are small, but as the pH is further lowered, the hydrolysis of carrageenan occurs, which induces the loss of viscosity and gelling ability.

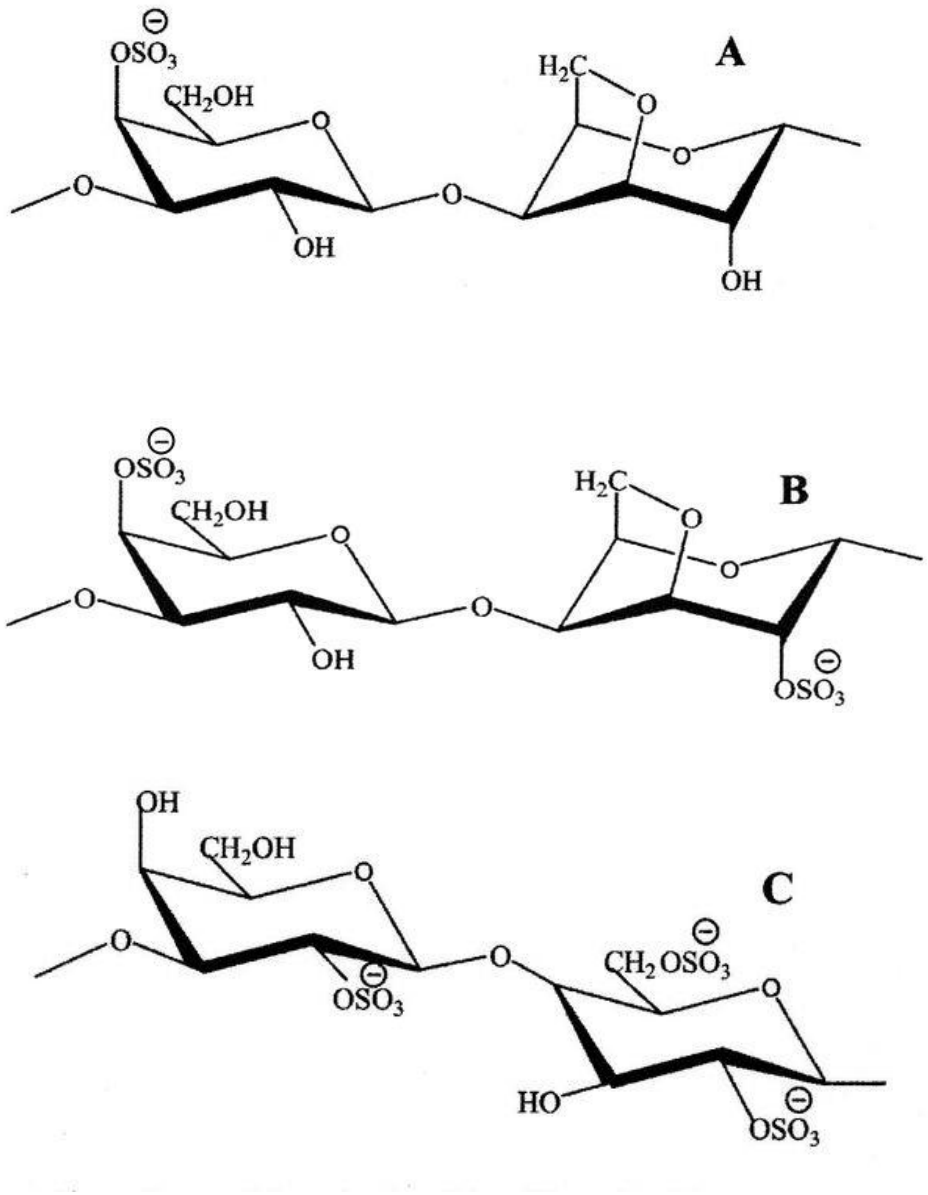


Figure 2.14: Structural formula of (a) kappa, (b) iota and (c) lambda (from https://www.cermav.cnrs.fr/lessons/modeling/).

2.3.9 Carrageenan gels

Carrageenans that form aqueous gels do so because of association of the molecular chain into double helices. Gels are an intermediate between a liquid and a solid state and can be rheologically considered viscoelastic solids (Oakenfull and Glicksman, 1987). The gel is the most common state for polysaccharides, both in biological and artificial systems. The polymer chains form an interconnected network, entrapping the solvent. Polysaccharide gels have many biological functions, but also have widespread commercial uses in

the food, cosmetic, paper and textile industries (Rees, 1968). Kappa and iota carrageenans are known as gelling carrageenans due to their ability to form thermoreversible gels. The anhydro-D-galactose units, the natural sugar anhydride present in both carrageenans, is essential for the gelation process (Rinaudo, 1993). A recently advanced model for gelation in these system postulates that, on cooling, the polymer chains associate into double helices, interrupted by irregularities (1,4-linked galactose 6-sulphate or 2,6-disulphate units), which interlink to form relatively small domains. This occur regardless of the counter-ions present, and dose not directly lead to gelation. For gelation to occur, the domains must aggregate to form a three-dimensional network (Figure 2.15). In the case of iota and kappa carrageenan, only when potassium or the gel pomading cations are present will the domains aggregate into a three-dimensional network. A gel is formed when enough of these helices have been formed to provide cross-links (Rees, 1972). During cooling, kappa carrageenan forms a helical structure composing to polymer chains in such a way as to focus the 4-sulphate groups toward each other, and charges are then neutralised by potassium cations. Once stabilisation occurs, hydrogen bonding allows the formation of a double helix. Further cooling allows the formation helical aggregates; this process can be intensified by the addition of divalent cations, such as calcium or magnesium (Towle, 1973). For iota carrageenans, the gelation process is the same, except that there is no additional hydrogen bonding or aggregation of carrageenan helices since the 2-sulphate present in this carrageenan projects outwards from the double helix. These sulphate groups interact with divalent cations, commonly calcium, through the formation of ionic bridges between helices. This peculiarity allows the formation of a gel with special properties of flexibility and good water binding capacity (Modliszewski, 1984). In the sol state (above the helix melting temperature), the chains are present in a random coil conformation and on cooling develop into a helix. Polysaccharide molecules can be held together by several weak intermolecular forces such as hydrogen bonds and electrostatic interactions. The cross-links are extensive interactions between the molecules and are not limited to small regions. These associated regions are called junction zones (Rees, 1968, Oakenfull and Glicksman, 1987). The gelation process is basically the formation of junction zones, and the gel becomes progressively more cross-linked as

further helices form or as helices associate to form super junctions (Figure 2.15). The last stage of gelation is known as syneresis and results when the formation of helices or aggregations becomes so extensive that the gel contracts, releasing liquid (Rees, 1972). The presence of sulphate esters tends to maintain the carrageenan chains in the soluble or random coil state, which is more difficult to convert into a gel. For this reason, iota carrageenan, which is more sulphated than kappa, forms gels that do not show syneresis and that are more elastic than those formed by kappa carrageenan. The increase in sulphate group is accompanied by an increase in the polymer concentration necessary for gelation as well as a decrease in the syneresis (Rees, 1972). This difference in gelation mechanism of kappa and iota carrageenan creates a wide range of melting/gelation temperatures depending on cation concentration, and show thermal hysteresis, where aggregate bundles need to see higher temperatures before the helices within them can begin to melt. In kappa carrageenan gels, junction zones are aggregates of up to six double helices, but in iota carrageenan gels, junction zones involve two or three helices. The junction zones in kappa carrageenan gels also present larger molecular weight aggregates and greater thermodynamic stability (Oakenfull and Scott, 1988). The sol-gel transition temperatures of carrageenans typically exhibit hysteresis, the melting temperature being higher than the gelling temperature. Also, the transition temperature of carrageenans depend primarily on the concentration of gelling cations present.

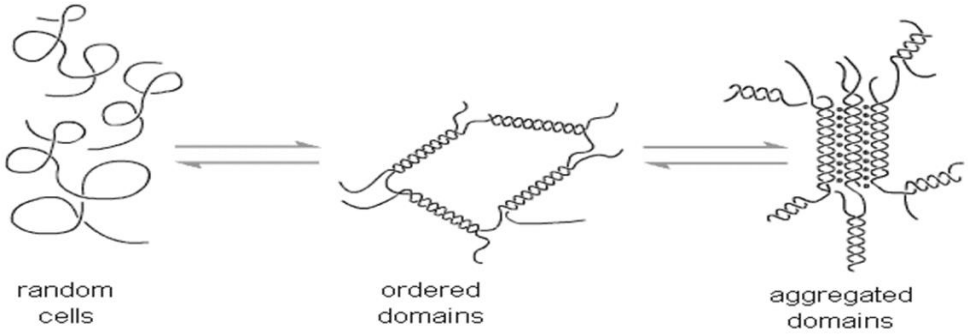


Figure 2.15: Mechanism of gel formation of carrageenan (stanley, 1990).

2.3.10 Locust bean gum

Locust bean gum (LBG) (also called carob bean gum and carubin) is a refined endosperm of the seed of the carob tree, an evergreen of the legume family, which is known botanically as *Ceretonia silique*. It is a food reserve for the seeds

and helps to retain water under arid conditions. The tree grows most extensively in Spain but is also cultivated in significant quantities in Italy, Cyprus and other Mediterranean countries. The structure of LBG is galactomannan with a β-(1→4)-linked D-mannose backbone to which a single unit of α-D-Glactopyranosyl residue is attached at 0-6 (see Figure 2.16). The ratio of mannose-galactose depends on the source; the value of 3.5 is typical for LBG. This gum is a neutral polysaccharide with a molecular weight reported to be between 319 and 1,380 g/mol (Dea and Morrison, 1975). The typical composition of a high purity LBG is 10-13% moisture, 5% protein, 1% ash, 1% fat, 1% fibre, and 80-85% galactomannan. LBG forms a thermally irreversible weak gel due to the association of galactose-deficient regions as a result of freezing and thawing (Richardson et al., 1998). The gum finds use in paper marketing, texture sizing, and other industrial applications; its major current use however is in food.

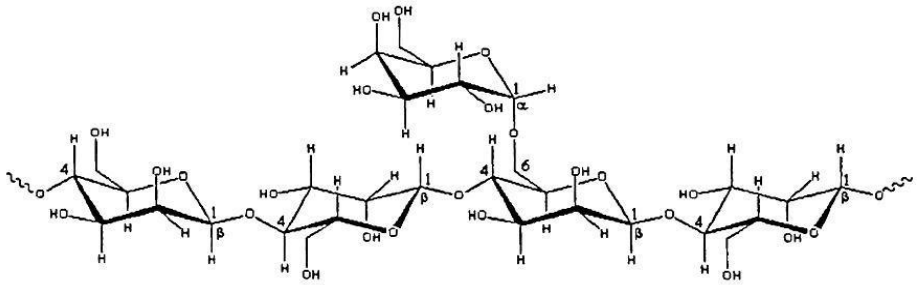


Figure 2.16: Structure of locust bean gum (from http://www.cybercolloids.net/information/technical-articles/lbg-structure).

2.3.11 Solubilised of Locust bean gum

LBG is only slightly soluble in cold water and dispersions must be heated to about 85˚C to achieve full viscosity potential. The pH of a 1% solution is typically between 5.4 and 6.5 and viscosity is relatively unaffected in the pH range from 3 to 11 (Dea et al., 1986).

2.3.12 Locust bean gum gels

Locust bean gum specifically retards ice crystal growth by forming structured gels at the solid/liquid interface; this particularly occurs on freeze-thaw cycling, which encourages the association of mannose where the weight-average mannan block length is greater than six monomer units (Dea et al., 1986). The gelation rate initially increased with decreasing temperature until a maximum gelation

rate was found close to -5˚C; at -5˚C, the gelation rate is at a maximum (Richardson and Norton, 1998).

2.3.13 Fibre

Dietary fibre (DF) is defined as the remnants of the edible part of the plant and analogous carbohydrates that are resistant to digestion and absorption in the human small intensive with complete or partial fermentation in the human large intestine (Prosky, 1999). Joint FAO/WHO (2009) reported that dietary fibre consists of carbohydrate polymers with ten or more monomeric units, which are not hydrolysed by the endogenous enzymes in the small intestine of humans and belong to the following categories: the first one, edible carbohydrate polymers, naturally occur in the food as it is consumed; the second is carbohydrate polymers, which are obtained from food raw materials and have physiological effects to health; the final form is synthetic carbohydrate polymers, which have also been shown to have a physiological effect of benefits to health. For several decades, interest in the role of DFs in health and nutrition has promoted a wide range of research and received considerable public attention. Various studies have shown that a fibre–rich diet has a number of beneficial effects such as reducing the risk of coronary heart disease, diabetes, obesity and some forms of cancer; and helps to regulate constipation (Verma and Banerjee, 2010; WHO and Consultation, 2003). Furthermore, DF possesses technological properties that are useful for food processing, especially for meat products. Since they can be recovered from the industrial processing residue, their use in value-added products provides a means for upgrading co-products. DF ingredients from seaweed (red and brown algae), tubers (potato, konjac), roots (carrot, sugar beet) legumes (soy, peas), cereals (oat, rice, wheat), and finally fruits (apple, lemon, orange, citrus) have been used as additives or ingredients in the manufacture of meat products, particularly for technological purposes. Residues of citrus juice production are composed principally of water, soluble sugar, fibre, organic acids, amino acids and proteins, minerals, and oils and lipids; they also contain flavonoids and vitamins. All of these components are found in different amounts depending on the fraction of the fruit, such as juice, albedo, flavedo, pulp and seeds (Braddock, 1995). Furthermore, the DF proportion in citrus juice residue depends on the juice extraction system used (Marın et al., 2002). Classic fruit

fibres are by-products of the fruit juice industry. They are the de-juiced part of the fruit which are carefully dried and ground to the required particle size. These proverbial fibres consist to 60-80% of cell wall material, which is a complex matrix of the cell wall polymers cellulose, hemicellulose and pectin, glucose (67.8%), xylose (9.5%), mannose (8.4%), arabinose (2.3%) and galactose (12.1%) as well as some minor components, including cell wall proteins, colourants and flavours. The citrus fibres have a high water binding capacity (Fischer, 2000). Pectin is a hydrocolloid consisting mainly of polygalacturonic acid and its methyl ester (Figure 2.17). Pectin has been used as thickener, emulsifier, and stabiliser and gelling agent in variety of food products. Cellulose the principal structural component of plant is the most abundant source of complex carbohydrate in the world. Cellulose is a linear polymer of β-(1, 4)-D-glucose, as shown in Figure 2.18. Glucose comes from the cellulose and hemicellulose whereas xylose and mannose are the main monosaccharide in the hemicellulose of the primary cell walls in the majority of plants (Jarvis, 2011). Arabinose and galactose can also be found in the hemicellulose and pectin (Harris and Smith, 2006).

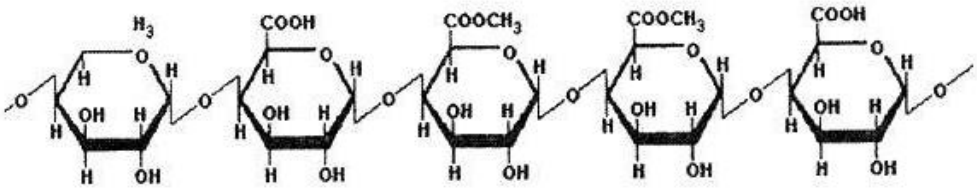


Figure 2.17*:* Structure of pectin (Imeson, 1997).

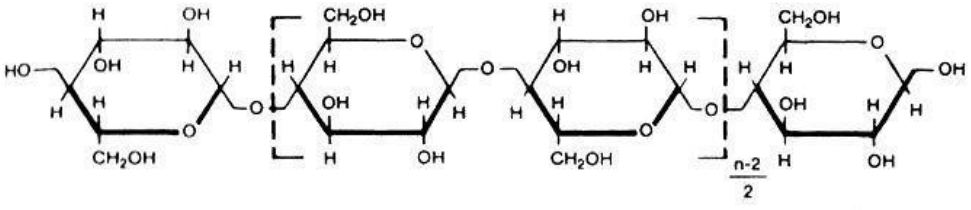


Figure 2.18: Structure of cellulose (Phillips and Williams, 2009).

DF is traditionally divided into 2 major groups: soluble and insoluble fibre. Soluble fibre is generally present at high levels in fruit, oat, beans and vegetables (Anderson et al., 1994). Whole grains are a major food source for insoluble fibre

(Welsh et al., 1994, Slavin et al., 1997). The main advantage of DF from citrus fruit when compared to alternative sources of fibre such as cereals is its higher proportion of soluble dietary fibre with about 33% in citrus fruit, while only 7% is present in wheat bran (Gorinstein et al., 2001; Grigelmo-Miguel and Martín-Belloso, 1999; Prosky et al., 1987). Moreover, fibres from citrus fruits have an additional advantage over dietary fibre from other sources due to the presence of associated bioactive components such as vitamin C and flavonoids.

2.3.14 Pre-hydrated of citrus fibre

The pre-hydration of fibres is related to the chemical structure of the polysaccharide components and other factors, for instance porosity, particle size, pH, temperature, and ion strength (Elleuch et al., 2011). Depending on type and conditions of use, fibre can bind considerable amount of water (swelling particles). The ability of fibre to bind water has been used in the manufacture of processed meat products (Grigelmo-Miguel et al., 1999). These properties contribute not only to the final cooking yield and drip loss in chilling, storage and purging, but also to the final quality of the products such appearance, texture, colour, juiciness and flavour.

2.4 The composition of meat products and interactions between selected components

Most meat products are a very complex mixture of meat proteins, fat and water, and for the purposes of final product quality may also contain non-meat ingredients such as plant protein and hydrocolloids.

Therefore, it is worth spending time introducing the emulsification of meat products and the functional properties of cooked products.

2.4.1 Emulsification of Meat product

Emulsified meat products are generally defined according to their appearance; meat particles are so fine that they are not visually distinguishable on the smooth product surface. The term meat emulsion has been used as a general terms to describe finely chopped meat mixtures (Heinz and Hautzinger, 2009). Comminution is defined as the process of reducing a material to a fine particular state.

2.4.2 Characteristics of Meat Emulsion

Finely chopped or comminuted meat in the presence of fat are often regarded as emulsion-type products. In a meat the fat globules are dispersed and stabilised in an aqueous matrix system consisting of salt-soluble myofibrillar proteins, segments of muscle fibres and myofibrils, connective tissue fibres, collagen and various other ingredients (Figure 2.19). For this reason, a meat emulsion is commonly referred to as a meat batter to reflect its multiphasic, multicomponent nature. The functional behaviour of myofibrillar proteins is manifested by their ability to produce three-dimensional, viscoelastic gel matrices via protein-protein interactions, to bind water, and to form cohesive and strong membranes on the surface of fat globules in an emulsion system or flexible films around the air/water interface. These functional properties are the major factors contributing to palatability or sensory perception, such as tenderness, juiciness and mouthfeel of processed meat products (Jensen et al., 2004).

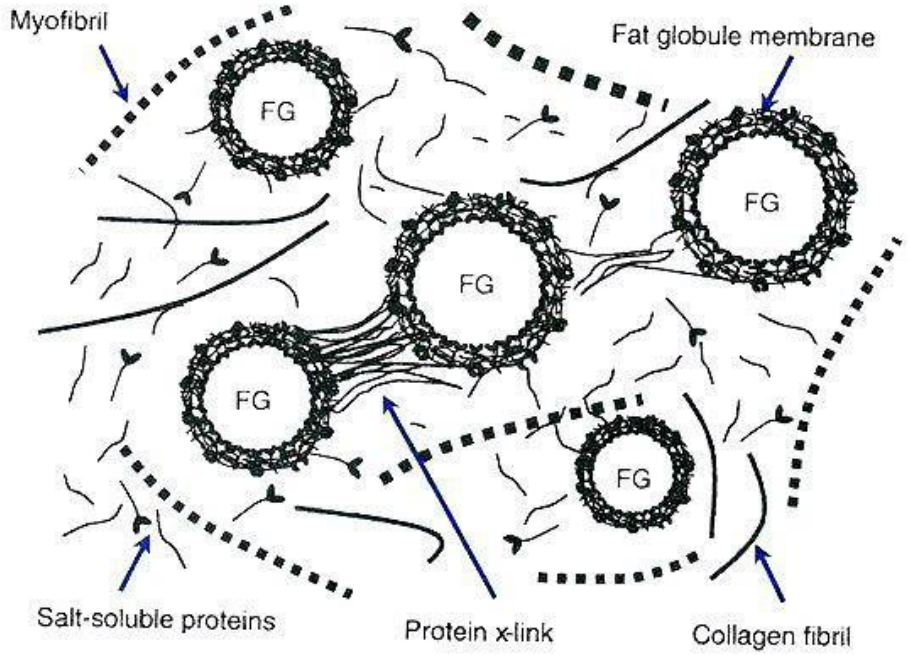


Figure 2.19: Schematic representation of a typical meat emulsion (batter). FG, Fat globule (Jensen et al., 2004).

Sausages are made by chopping meat with the addition of water and salt into a fine homogenate (batter), in which the fat is usually further dispersed and emulsified. Through comminution, the muscle cells are broken down and the

proteins are extracted. This processing permits molecular interactions to occur between the added components. The solubilisation of muscle proteins by interactions with salt solution is an important physicochemical process that follows the comminution and blending of meat. The salt-soluble proteins then form a surrounding layer at the surface of the released fat and a stable emulsion is formed (Figure 2.19). Muscle proteins are amphoteric molecules possessing both polar and nonpolar group. Hence, the input of mechanical energy through the shearing process is known as emulsification; here, proteins can adsorb at the fat-water interface where the hydrophobic group will anchor into the fat and the hydrophilic group will extend into the aqueous phase.

The ability of muscle proteins to form a viscoelastic and flexible membrane as a coating around the fat globule is a critical factor for the formation and stabilisation of the emulsion. The contribution of individual muscle proteins to meat emulsion stability follows the order: myosin > actomyosin > sarcoplasmic protein > actin > collagen.

The mechanism by which hydrocolloids interact with these proteins is not yet known and there is not any theories yet. After the solubilisation of muscles, the hydrocolloids may interact with proteins or may not; this depends on the charge of polysaccharides, pH, ionic environment and the type of polysaccharides used. Carr (1993) reported that interactions between carrageenan molecules and proteins within the meat system are thought to have a significant impact on the entire system. Carrageenan molecules may interact with negatively charged carboxyl groups on the protein through cation bridging or may interact directly with the positively charged amino groups of the protein (Figure 2.20). It is clear that pH, specific ions, and proteins present in the meat system will play important roles in these carrageenan–protein interactions.

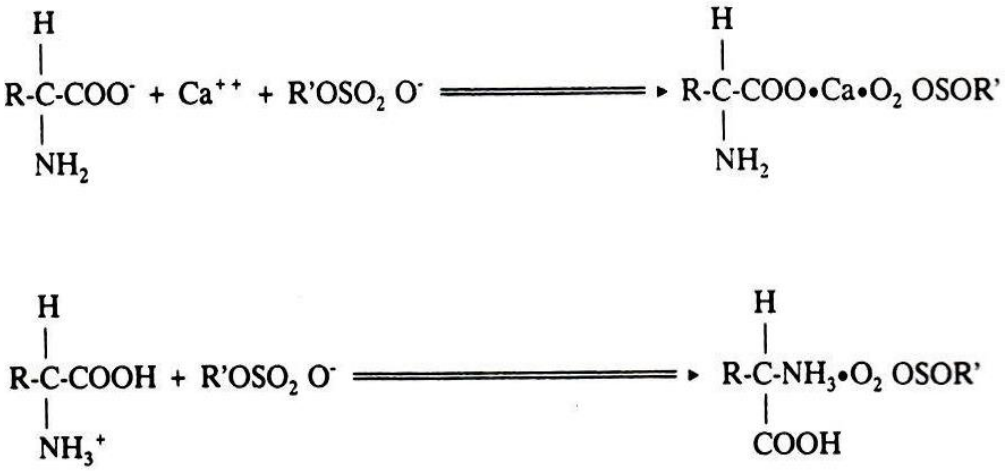


Figure 2.20: Mechanism of carrageenan-protein interactions (Carr, 1993).

Pectin is a negatively charged hydrocolloid and as such interacts with positively charged polymers in solution. The interaction with proteins below their isoelectric point (approx.5.0-5.1) has been studied (Glahn, 1982). Information about myofibrillar protein interactions with pectin is scarce.

2.4.3 Functional properties in cooked meat products

The cooking step is the final key process in the development of the matrix, with the type of matrix formed being dependent on the relatively dispersed or aggregated state of the protein, prior to gelation. The ability to form a gel is one of the most important physicochemical features of muscle proteins occurring in meat processing. Gelation is a result of unfolding and the subsequent association of extracted (soluble) protein molecules to form aggregates and strands, typically initiated by heating. The gel formed acts as an adhesive to bind comminuted meat particles and offer a three-dimensional network to stabilise emulsified fat globules and entrap flavour components and other meat ingredients such as hydrocolloids, as well as a complex capacity system to immobilise moisture. For this reason, protein gelation is responsible for an array of texture related quality in products, such as sausages and frankfurters. Microstructure and rheological characteristics of the gel formed are largely responsible for the texture, appearance and cooked yield of comminuted products. Myofibrillar proteins are largely responsible for the gelation that takes place in processed muscle food. In comparison, sarcoplasmic proteins exhibit a poor gelling ability. Collagen and connective tissue in meat have a minimal role in gelation. The triple α-helix of

collagen molecules does not unfold until approximately 65˚C (approaching the final cooking temperature) (Damodaran, 1997).

The most important gel from myofibrillar proteins is myosin gel. Using extracted myosin as a model protein, the following sequential changes have been established for the heat-induced myosin gelation (pH 6.0-6.5) (Figure 2.21). Unfolding of the myosin head (s1) commences at 35˚C and leads to the formation of dimers and oligomers via heat-heat interactions. As the temperature increases to 40-45˚C, a globular mass is formed that consists of a tightly associated heat clump with tails radiating outwards, resembling the shape of the Black Widow Spider. At above 45˚C, oligomers coexist with the aggregation formed by the coalescence of two or more oligomers. From 55˚C, these oligomers aggregate further, apparently involving tail-tail (rod) cross-linking to form particles that make up the strands of the gel network (Smith, 1988). Protein-water interaction is the most important phenomenon in the formation of heat set protein gels and at a low degree of interaction, aggregation or precipitation of the protein is obtained. The water binding capacity of proteins in gels is a limiting factor in the production of textured foods in which water becomes a structurally integral component. The interaction between protein proteins and water plays an important role in gel formation, particularly during the sol→gel phase transition. In the three-dimensional network of protein gels, a large amount of water is physically entrapped, in some gels 10 g water per gram protein. Gel properties are determined by protein-water interaction and water may be bonded by hydrogen bonds to polar groups, by dipolar interactions with ionic groups or retained as structured water. Hydrogen bonds are necessarily very weak because water is itself a hydrogen bond donor and acceptor. Water can be held back in the pores of the protein network through capillary mechanism. The diameter of the pores in the microstructure is the determining factor for moisture retention. In gel with pore size in the range of 1-100 nm, water is strongly held by capillary forces and thus, water is separated during gel shrinkage, when the gel is heated at temperature above gelation. However, large pore size (100-200 nm) are also important for water binding. Change in the pore size 100-200 nm cause changes in moisture loss from 10 to 50%. Shrinkage of gel will occur if protein-protein interactions are uniform in all the gel sample. As a result shrinkage, water will be removed from the gel. It is interestingly to note that, the temperature of heating and the level of protein denaturation influence the water holding capacity

of gels and the degree of phase separation. Increasing the heating temperature above the gelation point will cause a loss of moisture.

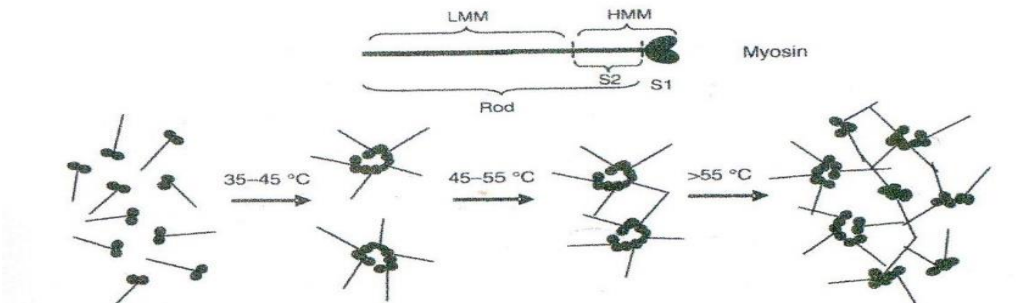


Figure 2.21: Schematic representation of heat-induced aggregation of myosin during gelation at a pH of 6.0-6.5. (Light meromyosin, LMM; heavy memoyosin, HMM; road; subfragment 1, s1; and subfragment 2, S2) (Jensen et al., 2004).

There are three types of non-meat ingredients using in meat processing:

1. Non-meat ingredients, which have a direct action on the solubility or dispersion of myofibrillar proteins such as salt and phosphate.

2. Non-meat ingredients such as soya and milk protein that may interact with solubilised or dispersed myofibrillar proteins to modify the structure of the salt-soluble meat protein gel network (Lin and Ito, 1985).

3. Non-meat ingredients that compete with meat proteins in the system, including polysaccharides (Foegeding and Ramsey, 1987).

In terms of the first category, there are three reasons that are generally quoted in the literature for the addition of salt to meat products (Rankin, 1984, Ruusunen and Puolanne, 2005):

1) Taste
2) Inhibition of bacterial growth
3) Solubilisation of myofibrillar proteins

The effect of NaCl on different proteins in meat is very complex, and the complexity increases if different concentrations and combinations of NaCl and phosphate are acting simultaneously (Offer and Knight, 1988, Hamm, 1972). The effect of NaCl on the solubility of myofibrillar proteins is to improve the water binding and adhesiveness properties of meat, each of which has a major influence on the structure and texture of meat products (Ruusunen and Puolanne, 2005). The solubility of myosin increases as the NaCl concentration rises from 0.04 to 0.5 M. The swelling of myofibrils starts at 0.5 M without any added

phosphate and at 0.4 M with added phosphate, where an extensive extraction of myosin starts to take place (Offer et al., 1989). In this study, we used 0.46 M (2.7%) NaCl for the formulation of sausages. Phosphates are often added to meat products with salt to achieve an enhanced effect on water binding and adhesion than the salt only effect (Trout and Schmidt, 1983). Phosphate also improves the scores of saltiness, juiciness, binding, firmness and cooking yield (Matlock et al., 1984). One of the main actions of phosphate on meat structure is the pH buffering effect and ionic strength, increasing the solubility of meat proteins (Lewis et al., 1986).

Regarding the second group of non-meat ingredients, non-meat proteins such as egg protein, soya and milk have been used in meat products for many years and have been the subject of many investigations (Hermansson and Åkesson, 1975; Shiga and Kushida, 1983; Bianchi et al., 1987). Lin and Ito (1985) postulated that soya binds to the actin-binding site of myosin.

Finally, the last non-meat ingredients are hydrocolloids, which were described earlier.

The next section reviews the influence of hydrocolloids used in this study and meat proteins.

Starch is the most widely used carbohydrate in the meat industry on a global basis. Both cost and functionality are primary reasons for its wide use. The advantage of using starch in meat production is the ability of starch to gelatinise when heated in a water–containing medium, thereby binding relatively large amounts of water (Fennema et al., 1975). Gelatinisation causes the swelling of starch granules and may result in gels when starch concentrations are optimal. Prior to heating, starch exerts only a minimum water-holding capacity.

A single study observed interactions between meat and starch. Li and Yeh (2002) reported that there is no interaction between modified wheat starch and meat proteins in ham.

In emulsified meat products, for instance sausages of any kind, cooked starches are especially useful when lower quality meats or high water levels are used. The starch is added at the beginning of the initial chopping process and binds the freely available water during the cooking process of the sausages. Another possibility is to add starches as fat replacers, for instance in low-fat frankfurters. Yang et al. (1995) were able to improve the texture with modified potato starch.

Modified potato starch also was used by Claus and Hunt (1991) for texture improvement in low fat bologna. Kao and Lin (2006) incorporated gels made of mixed konjac and potato starch into reduced–fat frankfurters and reported that they had a similar juiciness and firmness as the high-fat products. Also, Skrede (1989) used 5 types of starch in meat sausages and reported that potato flour proved to be well suited to add to sausages. In addition, he pointed out that wheat starch was also suited for use in sausages.

Starch is also using for tumbling in meat production. Halden et al. (1992) patented the classical industrial method of pickling or marinating meat with starch at 0.5-5% of the weight of meat, with the success of injection and tumbling. The addition of starch has also been shown to affect the flavour intensity of products, with Hughes et al. (1998) showing this in products containing 12% fat.

The effect of carrageenan addition on the functional properties of meat products has been the subject of numerous studies.

Carrageenan particles bind water during the thermal processing of meat products and gels on cooling. The temperature of swelling and gelling depends on the carrageenan type and the ionic environment. In a solution containing 2.2% salt (NaCl) and 0.5% phosphate (normal conditions in a processed meat system), kappa carrageenan begins to solubilise and swell at 46˚C, completely solubilises at 66˚C, and starts to gel at 44˚C subsequently when cooled. When sodium chloride and phosphate concentration decrease, kappa carrageenan begins to solubilise and gel at lower temperatures. Likewise, if salt and phosphate concentrations are increased, solubilisation and gelation temperatures increase. Therefore, when ionic concentration increases, the dispersion of the carrageenan will decrease since the meat protein begins to coagulate at 52˚C and is completely gelled at 67˚C. The same occurs with iota carrageenan; however, lower temperatures are needed for its complete solubilisation since it is more readily soluble due to its higher degree of sulphation (Bater et al., 1992a).

Carrageenan helps processed meat products to retain moisture, and also improves the sliceabilty of meat products, especially for products destined for high speed slicers (Laaman, 2010). Kappa and iota are the carrageenans showing the greatest benefit in meat applications, as water managers and texture modifiers.

A single study has observed interactions between meat and carrageenans. Verbeken et al. (2005) reported that dry carrageenan does not seem to interact with meat protein to participate in network formulations.

In terms of texture, Hsu and Chung (2001) reported increasing hardness, cooking yield which is weighing the meat product before and after cooking and is expressed as percentage cooking yield (cooked weight divided by raw weight × 100) and other textural profile analysis parameters when adding up to 2% kappa carrageenan to low-fat emulsified meatballs. Xiong et al. (1999) observed both kappa and iota carrageenan were found to increase the cooking yield, hardness and binding strength of low-fat sausages, although these effects were less pronounced at high salt concentrations.

Kappa carrageenan was also found to increase the hardness of the meat batter when replacing fat with a water-gum solution, whereas iota carrageenan importantly increased the water holding ability (Barbut and Mittal, 1992; Foegeding and Ramsey, 1986). Furthermore, Bater et al. (1992b) found that kappa carrageenan caused an increase in yield, slicebility and rigidity and a decrease in expressible juice in roast turkey breasts. In cooked slice meat, carrageenan is used to improve moisture retention, slice properties, mouthfeel, cooking yield and juiciness (Trius et al., 1996; Phillips and Williams, 2009). In this product, the application of carrageenan is based on its low viscosity when dispersed in the brine to be injected in the meat, its hydration during cooking of the ham and its gelation upon cooling (Phillips and Williams, 2009; Pietrasik and Duda, 2000; Therkelsen, 1993; Trius et al., 1996). Also, DeFreitas et al. (1997) reported increased gel strength and water retention when adding kappa carrageenan to a salt-soluble meat protein gel. In addition, carrageenan is used as a gelling agent in canned meats and pet foods and allows an important reduction in fat content in meat products like frankfurters (Xiong et al., 1999; Stanley, 1990; Pietrasik and Duda, 2000; Bernal et al., 1987).

In the meat industry, LBG acts as a binding and stabilising agent in processed comminuted meats, for instance sausages, to improve the homogeneity and texture of products. It has a lubricating effect on the meat mix, resulting in easier extrusion and stuffing. Increasing water binding results in an improved yield and longer shelf life.

Ramırez et al. (2002) reported that locust bean gum is not appropriated to be employed as a surimi additive; however, these gums presented a profitable effect when used with xanthan via ratio of 0.25/0.75. García-García and Totosaus (2008) and Damásio et al. (1994) reported that if locust bean gum and kappa carrageenan are added to the meat batter, it can cause improved texture and water retention, with only a minor effect on sausage colour. A strong synergetic effect between locust bean gum and kappa carrageenan has been reported (Chen et al., 2001, Lundin and Hermansson, 1997, Stading and Hermansson, 1993). Luruena-Martınez et al. (2004) reported that the addition of locust bean/xanthan did not affect the textural properties and overall acceptability of a meat product.

Dietary fibre has been incorporated into meat products as non-caloronic bulking agents, enhancers of water and in oil retention, to help to improve texture or emulsion stability and help to overcome the effects produced by composition change; for instance, the fat reduction process, on the characteristics of meat products. Different types of fibre have been successfully used in the processing of meat products: beet, oat, wheat, peas, and carrots and among fruit fibres: peach, pear, lemon, apple and citrus fibre are used to increase the cooking yield due to their water and fat–binding properties and to improve texture and reduce formulation costs (Cofrades et al., 2000; Claus and Hunt, 1991; Cofrades et al., 1995; Casimir and Akoh, 1998; Goldberg and Williams, 1991; Colmenero, 1996; Keeton, 1994; Aleson-Carbonell et al., 2003; Viuda-Martos et al., 2009; Verma and Banerjee, 2010).

Citrus fibre (orange, lemon, and grapefruit) has better quality than other fibres due to the presence of associated bioactive compounds such as flavonoids, polyphenols and carotenes with antioxidant properties, which may exert a higher health-promoting effect than the dietary fibre itself (Nagy and Attaway, 1993; Marin et al., 2002). Also, the water absorption of citrus fibre is actually extremely high, probably based on the high soluble fibre content. An important consideration when formulating citrus fibres is the taste profile. Furthermore, it may be used as a tool for improving texture, managing moisture in the replacement of fat, adding colour, reducing formulation cost and finally as a natural antioxidant. Citrus fibre may be incorporated into a broad range of products, particularly meat products (Fernández-López et al., 2007; Aleson-Carbonell et al., 2005; Fernández-López et al., 2004; Fernández-Ginés et al.,

2003). The addition of citrus fibre to dry-cured sausages has been reported to reduce residual nitrite (Viuda-Martos et al., 2009).

The source of fibre is also important because different plant structures can affect fibre properties. Grigelmo-Miguel et al. (1999) reported that the addition of 1% and 2% of peach dietary fibre suspension to Frankfurters increased viscosity and decreased pH without influencing the cooking loss. High levels of oat bran were associated with decreased moisture and increased shear stress in low-fat chicken frankfurters (Chang and Carpenter, 1997).

From this background, it is clear that future studies of meat protein-polysaccharide systems are necessary to enable meat manufacturers and ingredient suppliers to obtain maximum benefits from these ingredients.

In this study the physical attributes of different polysaccharide systems are compared. Different materials are used with respect to whether they are soluble or particulate; whether they are gel forming or not; and with relation to how they hydrate and compete for water during the heat induced meat protein gel function. The physical attributes mentioned above are compared with in individual polysaccharide types and across different polysaccharides.

2.5 United Kingdom, EU and Iran legislation of meat and meat products

Commission Directive 2001/101/EC as amended by Commission Directive 2002/86/EC provides European definition of meat used as an ingredient in compound foods for labelling purpose. This directive is implemented in England by the Food Labelling Regulations 1996 (as amended by the Food Labelling (Amendment) Regulations 2003). Detailed guidance notes for the labelling and composition of meat products have also been produced by the Food Standard Agency (FSA guidance notes, 2003). According to the Iranian National Standardization Organization definition of meat is the same as the UK definition (Table 2.2) however, pigs, forbidden in Iran according to religion rules. The EU definition of meat states that in mammalian products, other than poultry, rabbit and pig, the maximum limits are 25% fat and 25% connective tissue. For pigs, the corresponding figures are 30% fat and 25 % connective tissue, whilst for birds and rabbit they are 15% fat and 10% connective tissue. Fat or connective tissue present above these levels must be separately declared as for example pork fat or pork rind.

Table 2.2 Definition of meat

Generic name	Ingredients	Condition of use of generic name
Meat and the name of the animal species from which it comes, or a word which described the meat by reference to the animal spices from which it comes	Any skeletal muscle, including the diaphragm and the masseters, of a mammalian or bird species recognised as fit for human consumption which any naturally included or adherent tissue, but excluding 1) the heart 2) the tongue 3) the muscle of the head (other than the masseters) 4) the muscle of the carpus 5) the tarsus 6) the tail 7) mechanically recovered meat	The total fat and connective tissue content must not exceed the limits specified in part II of this Schedule of the Food Labelling Regulation (1996) and the meat must constitute an ingredient of another food. If such a limit is exceeded, but the ingredient falls within the description in column 2 of this entry, any reference to the meat content must be adjusted downwards accordingly and the list of ingredients must also mention the presence of fat or connective tissue, as appropriate.

The Meat Product (England) Regulations (MPR) 2014 compositional standards linked to reserved descriptions for meat products in England. Similar controls apply elsewhere in the UK, however there is no similar parent EU legislation. Reserved descriptions include burgers, hamburgers, corned meat, pies, chopped meat, luncheon meat and sausages. Taking beef sausages as an example, the reserved description state that it must contain not less than 42% meat however, according to the Iranian National Standardization Organization (INSO) 2007 and 2017, minimum meat contents for products using the reserved meat in not less

than 40% meat and the maximum is 90% meat. During the development of low fat meat products for which reserved descriptions are laid down, any meat that is removed to reduced fat is replaced with an appropriate amount of leaner meat to ensure the compositional standards are met. It is interesting to note that according to INSO 2017, sausages specification and test methods, people in Iran likes hard sausages and too Flavour is intensive. Also, according to standard of Meat Product Regulations (MPR) 2014 and Iranian National Standardization Organization (INSO) 2007, Ham specification and test method, Phosphates are allowed in certain meat preparations: breakfast sausages (UK sausages), burgers and in meat products up to a maximum of 0.6% (6000 mg/kg) expressed as P_2O_5 . Also, the maximum sodium chloride in meat production is 2.7%. Nitrites are allowed to a maximum of 150 mg/kg (ingoing) in non-traditional raw or cooked meat products. Nitrates are allowed to a maximum of 150 mg/kg (ingoing) in non-traditional raw meat products, but not in cooked meat products. For traditional dry cure and immersion cure products, there is a maximum residual level for each, which varies from product to product. However, according to the Iranian National Standardization Organization (INSO) 2017 the maximum nitrite is 120 mg/kg (ingoing) in final meat production and finally, according to (INSO) 2017 the maximum added water in meat production is 35%.

CHAPTER 3 MATERIALS AND METHODS

This chapter describes the materials and methods used in this study and provides a description of some of the basic principles of the main techniques used to analyse the results.

3.1 Materials

3.1.1 Sausage processing and formulation

The fat trimmed from topside meat was obtained from a local butcher (Meat4u, Beeston, Nottingham, United Kingdom) 24 h after slaughtering. The topside (Figure 3.1) was minced on a 5 mm plate, using a Manica mincer PT-82/22 (Ashford, United Kingdom). The meats were pre-weighed following the formulation shown in Tables 3.1-3.28, vacuum-packed separately and frozen at -20˚C until use. The ingredients used in the homogenous formulation included unmodified wheat starch (Cargill, Weybridge, United Kingdom), citrus fibre (Herbafood Ingredients GmbH, Werder, Germany), locust bean gum (CP Kelco, Atlanta, Georgia, United States), refined kappa carrageenan extract from *Eucheuma Cottonii* species (CP Kelco, Atlanta, Georgia, United States), refined iota carrageenan extract from *Eucheuma Spinosum* species (CP Kelco, Atlanta, Georgia, United States), refined lambda carrageenan extract from *Gigartina* species (CP Kelco, Atlanta, Georgia, United States), sodium chloride (Saxa, Spalding, United Kingdom), and sodium triphosphate (Kilo, Dudley, United Kingdom).

Pre-hydrated starch and solubilised carrageenan (except lambda), LBG and pre-hydrated fibre were prepared from aqueous suspensions according to the relevant formulas (Tables 3.1-3.28). Hydrocolloids were mixed with reverse-osmosis water for 1 hour in a mixer at moderate speed and by heating to 95˚C for 1 hour with constant stirring. After cooling at ≈40˚C, the solutions were added to the meat batter. For the addition of gels these hydrocolloids, except LBG, the hot solution was poured into plastic cylinders (38.5×31.8 mm) and kept for 12 h in a cool room (5˚C); after that, the gels were added to the meat batter. For the gelled LBG, the hot solution was placed in the freezer for 12 h; after defrosting, the solid LBG was mixed into the meat batter. For lambda carrageenan, solubilisation occurred in cold water; for this reason, the lambda carrageenan was mixed with cold reverse osmosis water (RO) (Figure 3.2).

Before the meat was processed, it was stored at 4˚C for 24 hours (pH 5.67). It is worth to mention that topside typically used in sausages. Sausage formulations

(Tables 3.1-3.28) were prepared. The mincemeat was chopped with sodium chloride (NaCl) and sodium triphosphate ($Na_5P_3O_{10}$) and half of the ice was added using the high speed setting for 1 minute; mixtures were kept in the bowl chopper for 5 minutes to allow enough time for protein extraction under high salt concentration (pH 5.87). After 6 minutes, hydrocolloids (dry, pre-hydrated or solubilised and gelled) were added to the batter. The bowl chopper was set to operate at high speed (knife speed: 2800 rpm, bowl speed: 24 rpm) for 60 seconds and the remaining ice was added to the mix (the amount varied to accommodate the various levels of hydration of the added hydrocolloids). This was then processed for an additional 3 minutes (knife speed: 2800 rpm, bowl speed 24 rpm); the temperature of the mix did not exceed 13˚C (pH 5.99). Generally, the higher quality the meat product, the less the particle size must be reduced. Immediately after processing, the prepared sausage mixture was stuffed into 26 mm-diameter cellulose casing (Tehran Navid, Tehran, Iran). After the sausages were mechanically linked to a standard size (10 cm in length and 70-80 g in weight each), they were cooked in a hot water bath (Ban Mari, UK) at 100˚C for 1 hour, which was sufficient for the interior of the products to reach 72˚C. A thermocouple probe (Omega Engineering,Inc., Stamford,Conn, USA) positioned in the geometric centre of the sausage was used to monitor product temperature. The sausages were then rinsed in cold water for 15 minutes, after which the water was wiped off the products with a clean towel and they were stored in a refrigerator (0-4˚C) for periodic measurements (Figure 3.3). All samples were produced in triplicate.

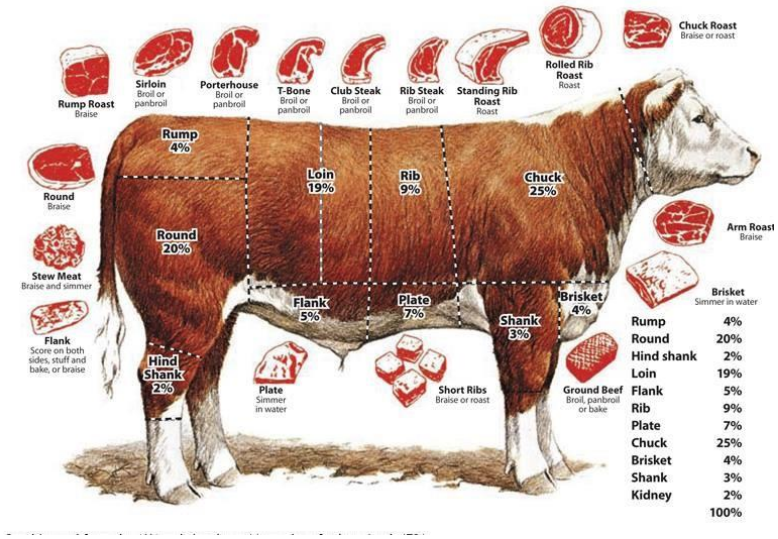


Figure 3.1*:* Cut of beef (From: https://en.wikipedia.org/wiki/Cut_of_beef).

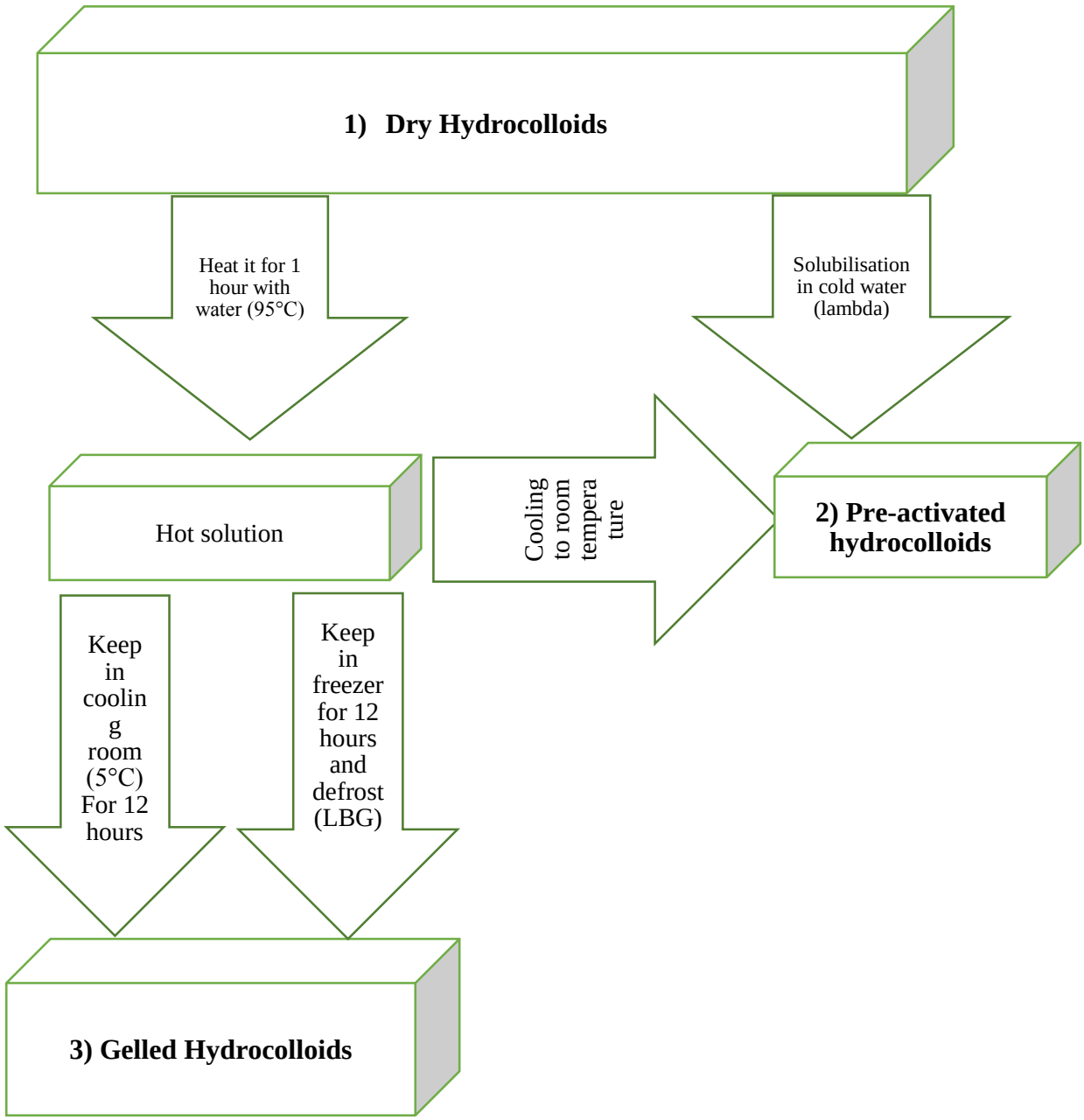


Figure 3.2*:* Schematic of pre-activated and gelled hydrocolloids.

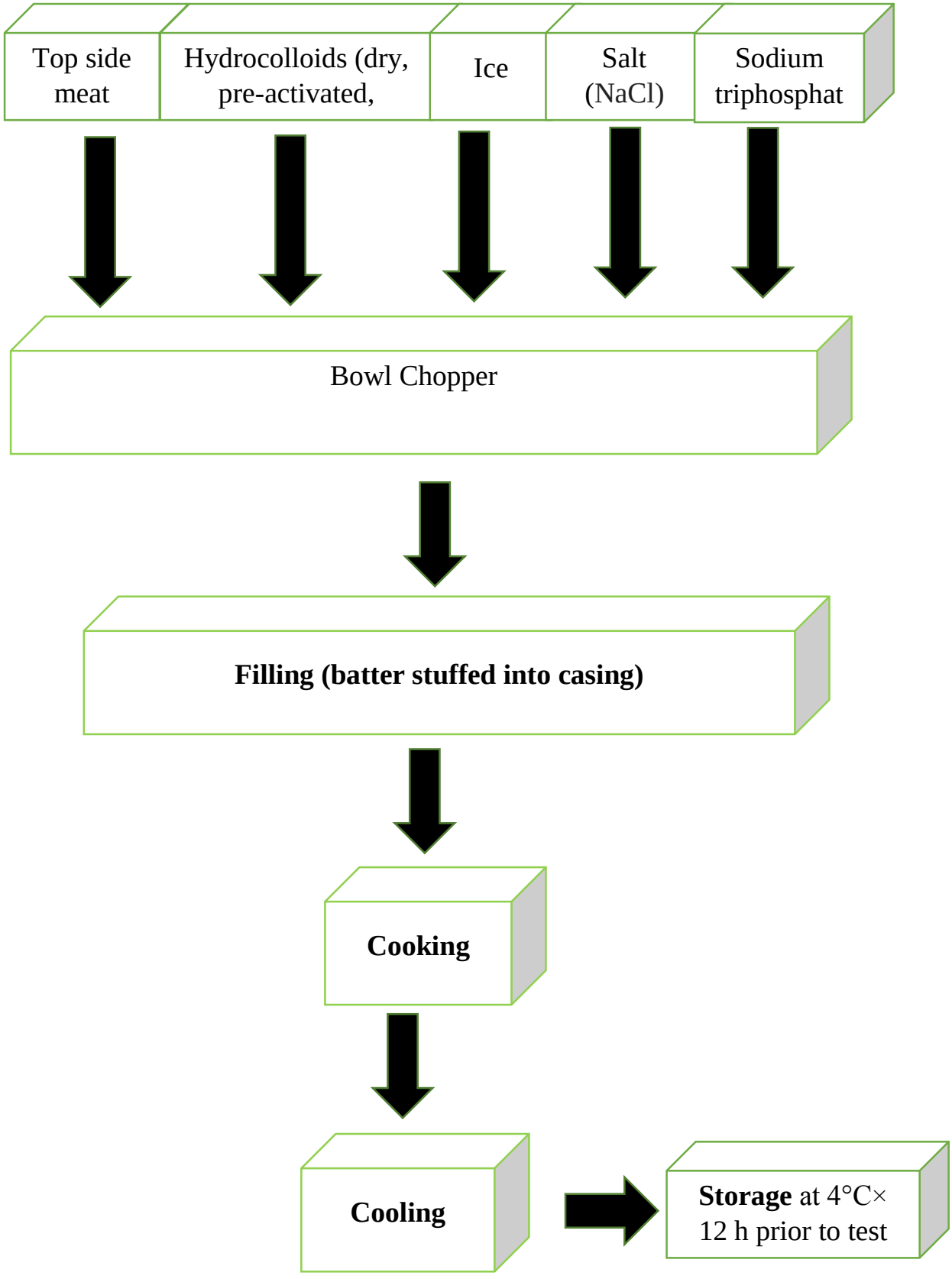


Figure 3.3*:* Schematic of sausage manufacturing process.

Control sausages Formula

Table 3.1: Sausage formula stated as Reference control.

Ingredient	Composition (%)
Top side meat	85
Hydrocolloids	0
Ice	11.7
Sodium chloride	2.7
Sodium triphosphate	0.6
Total	**100**

Table 3.2: Sausage formula stated as 65% meat control.

Ingredient	Composition (%)
Top side meat	65
Hydrocolloids	0
Ice	31.7
Sodium chloride	2.7
Sodium triphosphate	0.6
Total	**100**

Sausage formula with unmodified wheat starch

Table 3.3: Sausage formula of 3% dry starch with 65% meat.

Ingredient	Composition (%)
Top side meat	65
Dry starch	3
Ice	28.7
Sodium chloride	2.7
Sodium triphosphate	0.6
Total	**100**

Table 3.4: Sausage formula of 3% pre-hydrated starch with 65% meat.

Ingredient	Composition (%)
Top side meat	65
Pre-hydrated starch (include water)	31.7
Sodium chloride	2.7
Sodium triphosphate	0.6
Total	**100**

Table 3.5. Sausage formula of 3% gelled starch with 65%.

Ingredient	Composition (%)
Top side meat	65
Gelled starch	31.7
Sodium chloride	2.7
Sodium triphosphate	0.6
Total	**100**

Table 3.6: Sausage formula of 7.4% dry starch with 65% meat.

Ingredient	Composition (%)
Top side meat	65
Dry starch	7.4
Ice	24.3
Sodium chloride	2.7
Sodium triphosphate	0.6
Total	**100**

Table 3.7: Sausage formula of 7.4% pre-hydrated starch with 65% meat.

Ingredient	Composition (%)
Top side meat	65
Pre-hydrated starch (include water)	31.7
Sodium chloride	2.7
Sodium triphosphate	0.6
Total	**100**

Table 3.8: Sausage formula of 7.4% gelled starch with 65% meat.

Ingredient	Composition (%)
Top side meat	65
Gelled starch	31.7
Sodium chloride	2.7
Sodium triphosphate	0.6
Total	**100**

Sausage formula with 3 types of carrageenan (kappa, lambda, iota)

Table 3.9: Sausage formula of 0.5% dry carrageenan with 65% meat.

Ingredient	Composition (%)
Top side meat	65
Dry carrageenan	0.5
Ice	31.2
Sodium chloride	2.7
Sodium triphosphate	0.6
Total	**100**

Table 3.10: Sausage formula of 0.5% solubilised carrageenan with 65% meat.

Ingredient	Composition (%)
Top side meat	65
Solubilised Carrageenan	31.7
Sodium chloride	2.7
Sodium triphosphate	0.6
Total	**100**

Table 3.11: Sausage formula of 0.5% gelled carrageenan with 65% meat.

Ingredient	Composition (%)
Top side meat	65
Gelled carrageenan	31.7
Sodium chloride	2.7
Sodium triphosphate	0.6
Total	**100**

Table 3.12: Sausage formula of 1% dry carrageenan with 65% meat.

Ingredient	Composition (%)
Top side meat	65
Dry carrageenan	1
Ice	30.7
Sodium chloride	2.7
Sodium triphosphate	0.6
Total	**100**

Table 3.13: Sausage formula of 1% solubilised carrageenan with 65% meat.

Ingredient	Composition (%)
Top side meat	65
Solubilised carrageenan	31.7
Sodium chloride	2.7
Sodium triphosphate	0.6
Total	**100**

Table 3.14: Sausage formula of 1% gelled carrageenan with 65% meat.

Ingredient	Composition (%)
Top side meat	65
Gelled carrageenan	31.7
Sodium chloride	2.7
Sodium triphosphate	0.6
Total	**100**

Table 3.15: Sausage formula of 2% dry carrageenan with 65% meat.

Ingredient	Composition (%)
Top side meat	65
Dry carrageenan	2
Ice	29.7
Sodium chloride	2.7
Sodium triphosphate	0.6
Total	**100**

Table 3.16: Sausage formula of 2% carrageenan with 65% meat.

Ingredient	Composition (%)
Top side meat	65
Solubilised carrageenan	31.7
Sodium chloride	2.7
Sodium triphosphate	0.6
Total	**100**

Table 3.17: Sausage formula of 2% gelled carrageenan with 65% meat.

Ingredient	Composition (%)
Top side meat	65
Gelled carrageenan	31.7
Sodium chloride	2.7
Sodium triphosphate	0.6
Total	**100**

Sausage formula with citrus fibre

Table 3.18: Sausage formula of 3% dry citrus fibre with 65% meat.

Ingredient	Composition (%)
Top side meat	65
Dry citrus fibre	3
Ice	28.7
Sodium chloride	2.7
Sodium triphosphate	0.6
Total	**100**

Table 3.19: Sausage formula of 3% pre-hydrated citrus fibre with 65% meat.

Ingredient	Composition (%)
Top side meat	65
Pre-hydrated citrus fibre	31.7
Sodium chloride	2.7
Sodium triphosphate	0.6
Total	**100**

Sausage formula with locust bean gum

Table 3.20: Sausage formula of 0.5% dry LBG with 65% meat.

Ingredient	Composition (%)
Top side meat	65
Dry LBG	0.5
Ice	31.2
Sodium chloride	2.7
Sodium triphosphate	0.6
Total	**100**

Table 3.21: Sausage formula of+ 0.5% solubilised LBG with 65% meat.

Ingredient	Composition (%)
Top side meat	65
Solubilised LBG	31.7
Sodium chloride	2.7
Sodium triphosphate	0.6
Total	**100**

Table 3.22: Sausage formula of 0.5% gelled LBG with 65% meat.

Ingredient	Composition (%)
Top side meat	65
Gelled LBG	31.7
Sodium chloride	2.7
Sodium triphosphate	0.6
Total	**100**

Table 3.23: Sausage formula of 1% dry LBG with 65% meat.

Ingredient	Composition (%)
Top side meat	65
Dry LBG	1
Ice	30.7
Sodium chloride	2.7
Sodium triphosphate	0.6
Total	**100**

Table 3.24: Sausage formula of 1% solubilised LBG with 65% meat.

Ingredient	Composition (%)
Top side meat	65
Solubilised LBG	31.7
Sodium chloride	2.7
Sodium triphosphate	0.6
Total	**100**

Table 3.25: Sausage formula of 1% gelled LBG with 65% meat.

Ingredient	Composition (%)
Top side meat	65
Gelled LBG	31.7
Sodium chloride	2.7
Sodium triphosphate	0.6
Total	**100**

Table 3.26: Sausage formula of 2% dry LBG with 65% meat.

Ingredient	Composition (%)
Top side meat	65
Dry LBG	2
Ice	29.7
Sodium chloride	2.7
Sodium triphosphate	0.6
Total	**100**

Table 3.27: Sausage formula of 2% solubilised LBG with 65% meat.

Ingredient	Composition (%)
Top side meat	65
Solubilised LBG	31.7
Sodium chloride	2.7
Sodium triphosphate	0.6
Total	**100**

Table 3.28: Sausage formula of 2% gelled LBG with 65% meat.

Ingredient	Composition (%)
Top side meat	65
Gelled LBG	31.7
Sodium chloride	2.7
Sodium triphosphate	0.6
Total	**100**

It is interesting to note that in this study, we chose polymers, which were unmodified wheat starch, with the level of 3% for lower boundary and 7.4% for higher boundary; carrageenan and LBG with the level of 0.5% for lower boundary, 1% and 2% for higher boundary; citrus fibre with level of 3% with 65% meat for processability of the batter since less than this amount, the texture would be too watery (Figure 3.4) and above this amount 'tiger stripes' would occur, which means that the particles would not be evenly dispersed within the meat structure, and in some cases the filler would be broken. In terms of the starch for instance, we produced the level of 4% unmodified wheat starch with 65% meat, but it was not significantly different from 3%, and also we produced 6% unmodified wheat starch with 65% meat, but it was not significantly different from 7.4% furthemore, 7.5% was too thick to extrude throught the filler for this reason, 7.4% was as high and 3% as low as practically possible. Interestingly, with a reduction in the level of polymer, we increased the level of water to blance the formulation of sausage (Tables 3.1-3.28). Also, it worth to note that in this study the sausages contain 85% meat is called Reference control and this is related to industry perspective.



Figure 3.4*:* A watery sausage which is made from 2% unmodified wheat starch to a 65 % meat.

3.2 Methods

3.2.1 Texture analyser

Texture is a quality attribute that is closely related to the structural and mechanical properties of a food. Civille and Szczesniak (1973) reported that texture is the sensory manifestation of the structure or internal make-up of foods. It is perceived in the sense of the skin (tactile) and muscles (kinaesthetic).

Instrumental methods for texture measurement have been divided into three classes: the first is fundamental tests, which measure properties that are familiar to engineers, such as ultimate strength and various moduli such as Young´s modulus which is a measure of the ability of a material to withstand changes in length when under lengthwise tension or compression, shear modulus is defined as the ratio of shear stress to the shear strain and bulk modulus which is the relative change in the volume of a body produced by a unit compressive or tensile stress acting uniformly over its surface. Fundamental tests usually correlate poorly with sensory evaluations of the textural properties of food. The next class is empirical tests, such as puncture, shear, extrusion and so on; although poorly defined, practical experience has shown these to be correlated with texture quality. The final class is imitative tests, which are those tests that attempt to use instruments to imitate the conditions to which the food is subjected in the mouth or on the palate. This is the area into which texture profile analysis falls (Szczesniak, 1963). One of the most highly-valued instrumental techniques for the simultaneous measurement of various texture parameters is instrumental texture profile analysis (TPA) (Rosenthal, 2010). TPA is an objective method of sensory analysis that was pioneered by Szczesniak (1963), who defined the textural parameters first used in this method of analysis. Later, Bourne (1978) adapted the instron to perform TPA by compressing standard- sized samples of food twice. As described by Brandt et al. (1963), the texture profile is the analysis of the texture of a food in terms of its mechanical characteristics, geometric characteristics and "other characteristics" such as fat and moisture characteristics. Mechanical characteristics are manifested by the reaction of the food to stress. They are measured organoleptically by pressure exerted on the teeth, tongue, and roof of the mouth during eating. Geometrical characteristics refer to the arrangement of the constituents of the food, and are reflected mainly in the appearance of the food product. They are mostly sensed visually. However, these characteristics are often sufficiently pronounced to produce an oral sensation through the sense of touch and pressure. In the category of "other characteristic" are included mouthfeel factors that cannot be easily resolved on the basis mechanical and geometrical properties.

3.2.1.1 TPA Terminology

According to Szczesniak (1963) the mechanical characterise are probably the most important in determining the manner in which the food handles and behaves in the mouth. They can be divided into the following four basic parameters (primary characterise) which are hardness, cohesiveness, elasticity and adhesiveness. The first three characterise are related to forces of attraction acting between particles of food and opposing disintegration, whereas adhesiveness is related to surface properties. Furthermore, to the above four primary parameters, it appears to be desirable to characterise food texture in terms of three additional secondary parameters in order to make the characterisation as meaningful as possible to induvial accustomed to popular terminology, while at the same time keeping it in agreement with basic rheological principles. These secondary parameters are: brittleness, chewiness and gumminess. In terms of the geometrical characterise, they are two groups of qualities. 1) Those related to size and shape of the particles and 2) those related to shape and orientation. Finally, in terms of the "other characterise", this group comprises mouthfeel qualities related to the perception of the moisture and fat content of the food such as moisture content and fat content (oiliness and greasiness). It worth to mention that, these qualities may also be considered to be concerned with the lubrication properties of the product. The popular terms used to describe the moisture content of a food reflect not only the total amount of moisture perceived but its rate and manner of release. With fat content, the total amount fat and its melting point, as related to mouth-coating properties, become important. Table 3.29 shows the relations between the popular nomenclature of food texture and the development classification of texture characteristics. It will thus be seen that that the terms soft, firm and hard are actually degree of the characteristics of hardness, and the terms crumbly, crunchy and brittle are degree of the characteristic of cohesiveness. It is worth spending time to describe of the TPA parameters.

Table 3.29 Relations between textural parameters and popular nomenclature. Adapted from Szczesniak (1963).

Primary Characteristics	
Hardeness	Soft→Firm→Hard
Cohesivness	Crumbly→Crunchy→Brittle
Adhesivness	Sticky→Tacky→Gooey
Springiness (Elasticity)	Plastic→Elastic
Secondary Characteristics	
Fracturability (Brittleness)	Crumbly →Crunchy→Brittle
Chewiness	Tender→Chewy→Tough
Gumminess	Short→Mealy→Pasty→Gummy

3.2.1.2 Hardness

This parameter was originally defined by Szczesniak (1963) as the force needed to attain a given deformation. With the use of a general food Texturometer, Friedman et al. (1963) quantified it as the height of the first chew without specifying what happens if the maximum force attained during the first compression corresponds to the rupture point of the sample instead of to the value attained at the selected deformation. Sanderson et al. (1988) determined instron TPA parameters to investigate the influence of gellan gum and calcium ion concentration on the textural properties of gellan gum gel, defining hardness as the maximum force that occurs at any time during the first compression cycle. Hardness is defined as the maximum peak force during the first compression cycle (first bite) and has often been substituted by the term firmness. It is worth to mention that it can be to calculate the modulus (stress/strain) to find the hardness of samples. Furthermore, in terms of sensory techniques for the evaluation of mechanical texture characteristics, hardness is assessed by placing a sample between molar teeth and biting down evenly, before evaluating the force required to compress the food (Civille and Szczesniak, 1973).

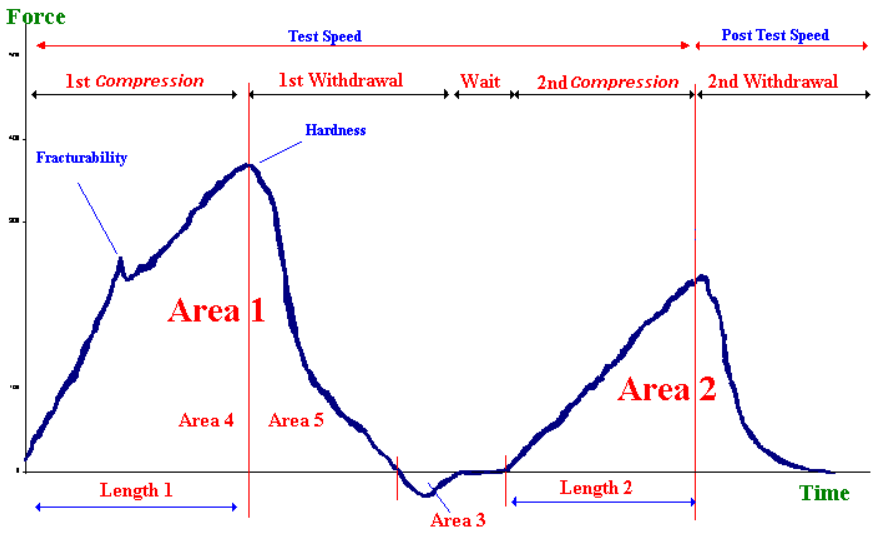


Figure 3.5: Texture profile analysis for texture export software (from the advance texture analyser short course, micro system, United Kingdom).

3.2.1.3 Cohesiveness

Szczesniak (1963) defined this parameter as the strength of the internal bonds making up the body of the product. It is measured as the area of work during the second compression divided by the area of work during the first compression (Refer to Area 2/Area 1 in Figure 3.5). Cohesiveness was first recognised as cohesiveness of the mass by the panellists (Civille and Liska, 1975), and is defined as the ratio of the positive force area during the second compression to that during the first compression. Cohesiveness may be measured as the rate at which the material disintegrates under mechanical action. Cohesiveness is usually tested in terms of the secondary parameters brittleness, chewiness and gumminess (Breene, 1975). Moreover, in terms of sensory techniques for the evaluation mechanical texture characteristics, cohesiveness is assessed by placing the sample between the molar teeth, and then compressing it and evaluating the amount of deformation before rupture occurs (Civille and Szczesniak, 1973).

3.2.1.4 Adhesiveness

Szczesniak (1963) defined this term as the work that is necessary to overcome the attractive force between the surface of the food and the surface of other materials with which the food comes in contact such as teeth, tongue, and the palate. Therefore, adhesiveness is related to surface properties. Adhesiveness is defined as the negative force area for the first bite and representing the work required to overcome the attractive force between the surface of a food and the surface of other materials with which the food comes into contact,i.e. the total force necessary to pull the compression plunger away from the sample (Area 3 in Figure 3.5). Moreover, in terms of sensory techniques for the evaluation of mechanical texture characteristics, adhesiveness is assessed by placing the sample on the tongue, pressing it against the palate, and evaluating the force required to remove it with the tongue (Civille and Szczesniak, 1973).

3.2.1.5 Springiness

This parameter was originally named elasticity by Szczesniak (1963) and is how well a product physically springs back after it has been deformed during the first compression. The springback is measured at the downstroke of the second compression, so the wait time between two strokes can be relatively important. In some cases an excessively long wait time will allow a product to springback more than it might under the condition being researched (eg you would not wait 60 seconds between chews). Springiness is measured several ways, but most typically, by the distance of the detected height of the product on the second compression (length 2 in Figure 3.5), as divided by the original compression distance (Length1). The original definition of springiness used the length 2 only, and the units were in mm or other units of distance. We do not subscribe to that original description of springiness since the springiness value can only be compared among product which are identical in their original shape and height. Also, in terms of sensory techniques for the evaluation of mechanical texture characteristics, springiness is assessed by placing the sample either between molar teeth (if it is solid) or between the tongue and the palate (if it is semi-solid) and compressing it partially; when the force is removed, the degree and speed of recoveryis evaluated (Civille and Szczesniak, 1973).

3.2.1.6 Resilience

Resilience is a measurement of how the sample recovers from deformation, both in terms of speed and the force derived. The calculation is the area during the withdrawal of the first compression, divided by the area of the first compression (Area 5/Area 4 in Figure 3.5). It is interesting to note that this parameter is from the original texture profile analysis work, but has developed from looking more closely at the elastic recovery of the sample.

3.2.1.7 Fracturability

Szczesniak (1963) originally named this parameter brittleness and defined it as the force with which the material fractures, related to the primary parameters hardness and cohesiveness. Not all products fracture; but when they do fracture the fracturability point occurs where the plot has its first significant peak (where the force falls off) during the probe´s first compression of the product (Friedman et al., 1963). Moreover, in terms of sensory techniques for the evaluation of mechanical texture characteristics, fracturability is assessed by placing the sample between molar teeth and biting down evenly until the food crumbles, cracks or shatters, evaluating the force with which the food moves away from the teeth (Civille and Szczesniak, 1973).

3.2.1.8 Chewiness

Szczesniak (1963) stated that chewiness is the energy required to masticate solid food product to a state ready for swallowing and is related to the primary parameters of hardness, cohesiveness and elasticity. Chewiness is calculated as the product of hardness x cohesiveness ×springiness (which is Length 1/Length 2 in Figure 3.5). Chewiness is mutually exclusive with gumminess since a product would not be both a solid and a semi-solid at the same time. Chewiness, tenderness and toughness are measured in terms of the energy required to masticate solid food. They are the characteristics that are most difficult to measure precisely, since mastication involves compressing, shearing, piercing, grinding, tearing and cutting, along with adequate lubrication by saliva at body temperature. In addition, in terms of sensory techniques for the evaluation of mechanical texture characteristics, chewiness is assessed by placing the sample in the mouth and masticating at a rate of one chew per second at a force that is equal to that required to penetrate a gum drop in a second, and finally the number of chews required to reduce the sample to a state that is ready to swallow (Civille and Szczesniak, 1973)

3.2.1.9 Gumminess

Szczesniak (1963) stated that gumminess is the energy required to disintegrate a semisolid food product to a state ready for swallowing, and is related to the primary parameters of hardness and cohesiveness. Gumminess is defined as the product of hardness×cohesiveness (which is Area 2/Area 1 in Figure 3.5), and is a characteristic of semisolid foods with a low degree of hardness and a high degree of cohesiveness. Moreover, in terms of sensory techniques for the evaluation of mechanical texture characteristics, gumminess is assessed by placing the sample in the mouth and manipulating it with the tongue against the palate, determining the amount of manipulation necessary before the food disintegrates (Civille and Szczesniak, 1973).

3.2.1.10 TPA details

For the extraction of the textural properties of sausages, assessment with the cold storage texture analyser TA.XT (Stable Micro System, Godalming, UK) with a load cell of 30 kg was used to measuring the force-time curve. All samples were cut into a cylinder that is 2 cm in height and 1 cm in diameter, and were then compressed twice with 50% deformation with a stainless compression probe of 74.9 cm in diameter. Parameters for measurement were as follows: pre-test speed, 2.0 mm/s; test speed, 5.0 mm/s; post-test speed, 5.0 mm/s; time between two compressions, 5.0 s; trigger type, auto-20 g; and data acquisition rate, 200 point/s. The TPA parameters were obtained from the texture expert software exponent V.1.22 attached to the texture analyser (Stable Micro System, Godalming, UK). They were determined from force-time curves as described by Bourne (1978), as discussed above. All measurements were repeated 8 times.

3.2.2 Micro-DSC

Differential scanning calorimetry (DSC) is a widely used thermal technique that measures the energy change associated with material transitions which occur as a sample is heated, cooled or held isothermally. Micro-DSC is distinguished from "conventional" DSC by the increased sensitivity of the machine, the larger sample volumes and the slower scan rates; normally, a maximum of about 1˚C min^{-1} is used. An additional benefit is therefore the higher resolution and noise-free traces. In this work the micro-calorimetry was carried out with a Micro DSC III (Setaram, Caluire, France) using cells made from hastalloy, which is capable

of holding approximately 0.8 ml, and sealed using hastalloy screw tops with viton "O" rings. The Micro DSC III is a heat flux instrument. The rate of temperature increase in these experiments was 1˚C min^{-1}, which is close to the maximum rate that the machine can achieve. The increased sensitivity is attained by a design optimised to collect the heat radiating from the cells and the larger sample mass.

The definitions of the samples are as follows. Firstly the so called dry sample is prepared as follows. The powder is simply added to water, mixed by constant stirring at room temperature and then placed in the DSC pan. The pre-activated hydrocolloid is prepared by adding the powder to the water in a beaker and then boiled for 1 hour. This is then placed in the DSC pan. The gelled sample is prepared in the same way as the pre-activated sample but is then allowed to cool and is put in a refrigerator for 24 hours. The sausage batter sample was placed in the cell and initially cooled to a starting temperature of 5˚C and held for a 10 minute period of equilibration. Samples were then scanned at 1˚C min^{-1} from 5-95˚C, cooled, and rerun, recording all steps. Normally, 2 complete heating and cooling cycles were run. The heating rate was selected as a compromise between the need to maintain equilibrium (heating rate as low as possible) and an acceptable signal/noise ratio (heating rate not too low).

The reference cell was filled with ultrapure water and matched for heat capacity with the sample. This ensured that the calorimeter was balanced and that the heat flow signal was centred at approximately the zero level which gave the most sensitive result. Enthalpy values were calculated using Setaram software with a linear extrapolated baseline, based on an extension of the trace before and after the thermal event. Heat and temperate calibrations are set in the machine but can be checked using the transition in naphthalene. The overall enthalpy of transition, i.e. the area under the transition peak, was calculated using Setaram bespoke software. A linear rather than any form of curved baseline was used. The transition parameters determined for all the samples were T0 = onset temperature, Te = end set temperature, Tm = peak temperature and ΔH = enthalpy of transition. The onset and end set temperatures were calculated from the intersections of best fit lines to either the leading or trailing edges of the peak and the baseline for comparison in the literature. It can be necessary to normalise the enthalpy values for multicomponent systems in order to enable meaningful comparisons with values for transitions of single component materials. The enthalpy of transition can be normalised to the wet weight of the pure ingredient.

For example, the meat samples used here contain a substantial proportion of water and fat. Therefore, the overall enthalpy value for a typical 65% meat-containing system can be divided by 0.65 to obtain the normalised enthalpy per g of pure meat. This will be enable enabled comparisons with the transition enthalpy for a reference sample. Similarly, for starch (7.4%) the enthalpy value can be divided by 0.074, despite the fact that starch powders can contain upwards of 10% moisture in give value more consistent with the literature. Therefore, comparisons are made with the wet weights of materials. The y-axes of the DSC graphs are described in terms of a non-normalised heat flow. This means that the heat flow in milliWatts (mJoules/sec) has not been divided (normalised) by the weight of the sample. However in all calculations of enthalpy of transition, the weight of the sample (normally the complete solution) is used. All measurement were repeated in triplicate.

3.2.2.1 The origin of the DSC signal

Proteins are made up of amino acids. Aside from the obvious nutritional aspects, one of the most important properties of proteins, from a food processing point of view, is the ability to form a gel. Protein gelation is a subtle combination of denaturation and aggregation, for which many different models have been proposed.

Native protein → denatured protein → aggregated protein

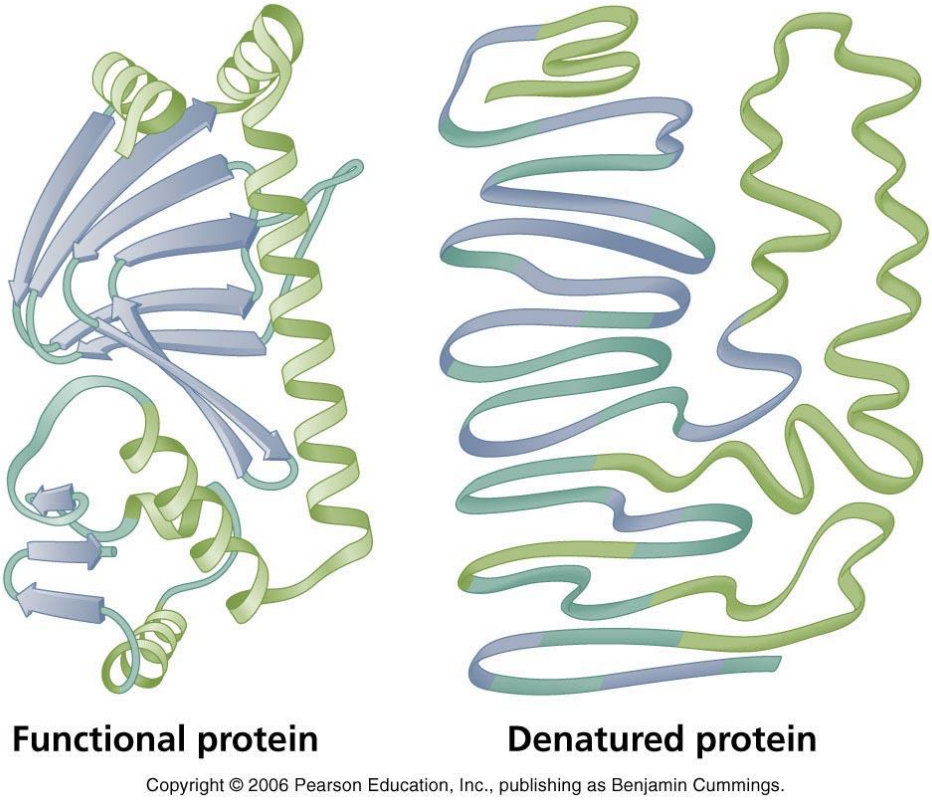


Figure 3.6: Schematic of native and denatured protein (from Max Rubner Institute publishing as Benjamin Cumming).

Changes in the secondary, tertiary or quaternary structure of protein molecules are usually referred to as denaturation (Figure 3.6). There is no change in the primary structure, and denaturation does not involve breaking peptide bonds. The protein unfolds, but there is no change in its amino acid sequence. For food proteins, including plant proteins, these changes will probably affect the functionality and thus their applicability in food systems. Factors such as heat, acid, alkali, organic solvent, detergent, urea and guanidine hydrochloride are often responsible for denaturation and the effects of these on plant protein have been reviewed by Wu and Inglett (1974). Any of these factors may cause breaking of hydrogen bonds. As a result, the protein will unfold and side chains that were buried in the centre of the molecule will become exposed. This reaction is irreversible. Denatured proteins normally lose their functional properties, meaning that they are unable to perform their normal function in foods. The denaturation process uses very little energy, but can be detected by different sensitive scanning calorimetry instruments. Furthermore, denaturation is an

endothermic event and often an irreversible process. The aggregation of protein results from the macroscopic association of protein molecules. Association of myofibrillar proteins probably begins prior to the heating process. Aggregation is generally considered to be an exothermic and irreversible process; however, the overall heat change is a sum of these plus other processes. DSC has been employed to elucidate the role of heat-induced gelation in the texture formation of processed meat products. Changes in the sample that are associated with the absorption or evolution of heat can cause a change in the differential heat flow between the sample and the reference in a heatflux instrument, which is then recorded as a peak. The area under the peak is directly proportion to the enthalpic change and its direction indicates whether the thermal event is endothermic or exothermic.

A protein molecule in its natural state usually consists of a chain of amino acids folded together. The native structure of a protein is held together by internal molecular forces that in most cases are weakened as the temperature increases, leading to thermal denaturation above a certain temperature. In the process of the cooking of meat, time and temperature have a strong influence on the final toughness as well as other quality factors of the meat, such as juiciness, colour and taste. The amino acid chain is more or less unfolded, whereby part of the interior of the previously native molecule is exposed at the molecular surface, changing the molecule's affinity to other molecules. If this affinity increases, the degree of aggregation between protein molecules will increase. If the affinity decreases, solubilisation of the native aggregate may occur instead.

3.2.3 Rheology

Rheology is the study of the deformation and flow of matter. Food rheology is the study of the deformation and flow of raw materials, intermediate products, and the final products of the food industry. Rheology is important to the food technologist because it has many applications in the three major categories of food acceptability:

1) Appearance
2) Flavour
3) Touch

There is small component of rheology in appearance, since certain structural and mechanical properties of foods can be determined by appearance. In terms of flavour, rheology has no direct role; however, the manner of food breakdown in

the mouth can affect the rate of release of flavour compounds. Finally, in terms of touch, rheological properties are a major factor in the evacuation of food quality by the sense of touch.

3.2.3.1 Rheology Method

The simplest model to illustrate rheological properties is the parallel plate (Figure 3.7). The top plate, which has a surface area A (m^2), is moved by a force ($N=kgm/s^2$) at a speed V (m/s). The bottom plate remains static. The distance between the plates to which the material adheres is defined by h (mm) (shear rate in equation 3.1). The shear stress (τ) (equation 3.2) is described as the force required to produce the deformation, divided by the surface area (A) of the sample, giving the units of pressure (i.e. Pa), and the strain (γ) (equation 3.3) is defined as the distance moved divided by the original length of the material, which is dimensionless. The rate at which the deformation occurs is the shear rate (γ).

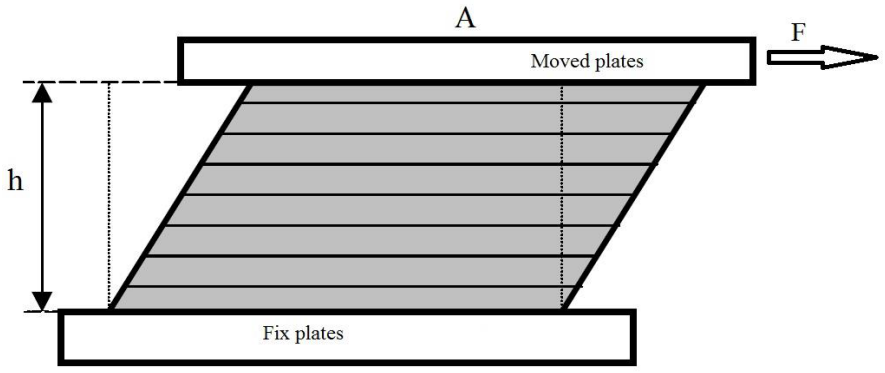


Figure 3.7: Parallel plate model.

Shear rate $\dot{\gamma}$ = V/h (3.1)

Shear stress τ = F/A *(3.2)*

Shear strain = dx/h *(3.3)*

Based on rheological properties, materials can shows viscous, elastic, and viscoelastic behaviours. For an elastic solid material, the energy used for the deformation process is stored as potential recoverable energy; by removing of deforming force, the object will resume its original shape.

In the case of a viscous (liquid) material, the input energy contributing to flow is transformed and dissipated as heat; no recovery occurs when the force is removed.

The ideal elastic or viscous materials represent 'extremes' of rheological behaviour; however, most materials show both elastic and viscous properties together, giving rise to the term 'viscoelasticity'.

3.2.3.2 Oscillation rheometry

This mode of testing is also referred to as dynamic mechanical analysis (DMA). Oscillatory tests are used to examine all kinds of viscoelastic material, from low-viscosity liquids to polymer solutions and melts, dispersions (suspensions, emulsions and foams), pastes, gels, elastomers, and even rigid solids. Oscillatory rheometry has been frequently used to measure the elastic and viscoelastic properties of meat batter.

The sample is subjected to a time-dependent strain–wave of the form (equation 3.4):

$$\gamma = \gamma_0 \sin \omega t \qquad (3.4)$$

Where γ is the time-dependent strain, γ_0 is the maximum strain amplitude and ω is the angular frequency (Figure 3.8).

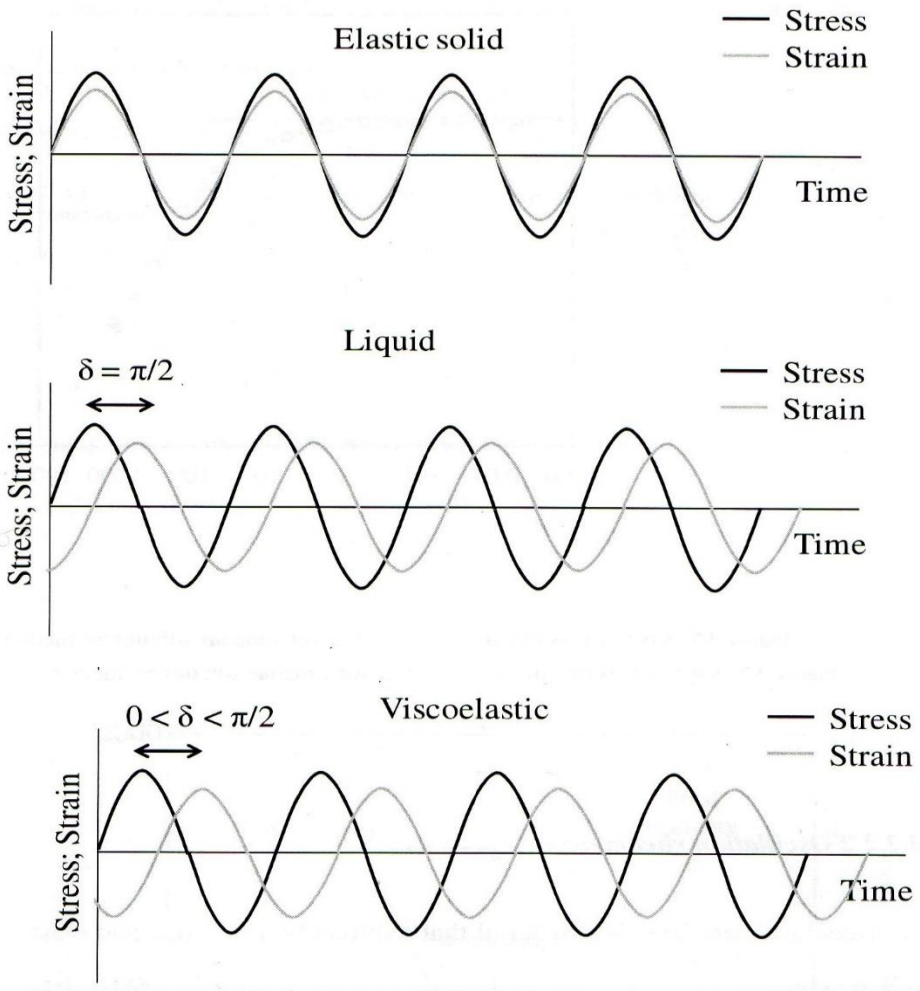


Figure 3.8: Resultant stresses due to a sinusoidal drive strain (Mezger, 2006**).**

The resulting shear-stress wave will also be a sine wave, but will differ in amplitude and phase (equation 3.5):

$$\sigma = \sigma_0 \sin(\omega t + \delta) \quad (3.5)$$

Where σ is the time-dependent stress, σ_0 is the maximum stress amplitude and δ is the phase angle difference between the two waves.

For ideal elastic (solid) materials, the strain and stress waves will be in-phase, i.e. all of the input energy applied to the sample is stored and immediately returned on relaxation; therefore, the maximum stress is when the slope of the strain wave is at a minimum (Figure 3.8). However, for a viscous liquid, the strain is $\pi/2$ radians out-phase with the stress, i.e. the maximum stress will occur when the slope of the strain wave is at a maximum, i.e. at the zero cross-over points. A viscoelastic sample stands somewhere between, i.e. it has contributions from both the in- phase and out-of-phase components (Figure 3.8).

Assigning different symbols for the in-phase and out-phase components, the stress wave function may be written in the form of equation 3.6:

$\sigma = \gamma_0 (G' \sin \omega t + G'' \cos \omega t)$ (3.6)

Where G´ is the in-phase, storage modulus and G″ is the out-of-phase, loss modulus. Re-writing equation (3.7) as:

$\sigma = \sigma_0 (\cos \delta \sin \omega t + \sin \delta \cos \omega t)$ (3.7)

and substituting in equation (3.8):

$G' = (\sigma_0 / \gamma_0) \cos \delta$ (3.9)

$G'' = (\sigma_0 / \gamma_0) \sin \delta$ (3.10)

An additional frequency-dependent parameter can be obtained from oscillatory measurements. The tangent of the phase angle – the ratio of viscous modulus (G") to elastic modulus (G') and a useful quantifier of the presence and extent of elasticity in a fluid. Tan delta values of less than unity indicate elastic-dominant (i.e. solid-like) behaviour and values greater than unity indicate viscous-dominant (i.e. liquid-like) behaviour. The tangent of the phase angle δ indicates the solid or the viscous like character of the material (equation 3.11):

$G''/ G' = \text{Delta tan}$ (3.11)

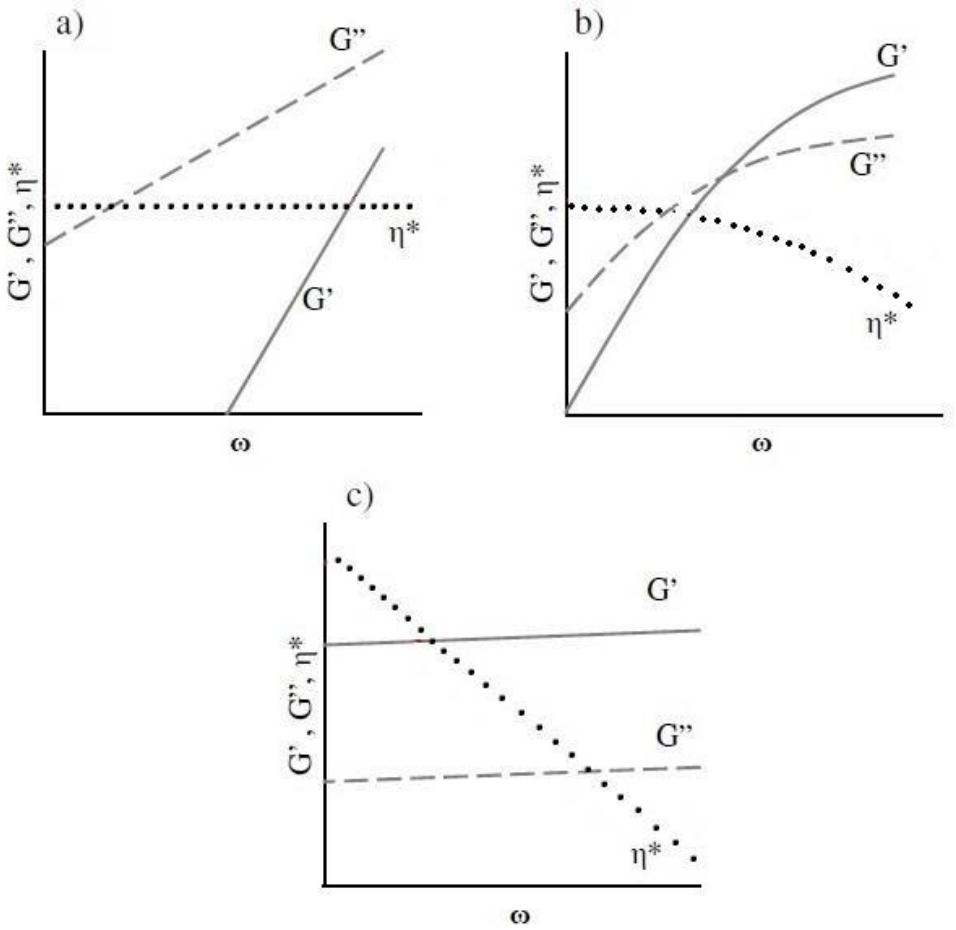


Figure 3.9: Three main categories of mechanical spectra (a) dilute solution (b) entangled network and (c) physical gel network (Mezger, 2006).

The complex modulus is related to the storage and loss moduli by equation 2.8, and the complex viscosity (η*) is defined as the complex modulus divided by the frequency of oscillation ω; this is shown in equation 3.13:

$$G^* = [(G')^2 + (G'')^2]^{1/2} \quad (3.12)$$

$$|\eta^*| = |G^*| / \omega \quad (3.13)$$

Figure 3.9 shows some examples of mechanical spectra of different materials, i.e. the storage modulus (G´, which is a measure of the energy that is stored elastically in the structure during periodic applications of stress), loss modulus (G″ which is a measure of the energy dissipated or the viscous response), and dynamic viscosity (η*), plotted as a function of angular frequency (ω). The moduli in the dilute solution are strongly frequency-dependent. However, at all frequencies, G″ is greater than G´, indicating viscous-dominated properties (Figure 3.9a). In the gel, G´ is higher than G″ at all frequencies and the moduli are generally frequency-independent (Figure 3.9c). The mechanical spectrum of a concentrated solution (Figure 3.9b) is between these two extremes. There is a noticeable frequency-dependency; at lower frequency ranges, G″ is higher than G´ (indicating viscous properties), whereas at high frequencies, the G´ modulus is dominant (Morris and Foster, 1994; Ross-Murphy et al., 1983).

3.2.3.3 Dynamic Temperate Ramp Test

Using this kind of oscillatory testing, both the frequency and the amplitude are kept at a constant in each test interval, the only variable is the temperature. Therefore, besides the constant dynamic-mechanical shear conditions, a profile of the measuring temperature is present. This kind of testing is also referred to as dynamic-mechanical thermoanalysis or dynamic thermomechanical analysis (DTMA). This kind of testing is used to investigate temperature-dependent behaviour of material showing hardness of product during the measurements.

3.2.3.4 Rheology experimental details

The viscoelastic properties of the prepared fresh meat batters were measured during heat-induced gelation with a rotational rheometer (RDII, Hamburg, Germany) operated with RSI orchestrator software immediately after the fresh batter was prepared. Measurements were conducted with a strain of 1% and a constant frequency of 10 rad/s. Samples were heated at a rate of 1˚C/min from

30-90˚C between 25 mm parallel plates (gap=2 mm). The exposed surface of the protein samples was coated with mineral oil to prevent evaporation. All treatments were tested in triplicate.

3.2.4 Confocal Scanning Laser Microscopy

Confocal scanning laser microscopy (CSLM) is widely used for the study of food microstructure. CLSM is a recently developed microscopy technique which permits the observation of selected levels within thick samples. This microscope improves the resolution along the viewing axis and permits optical sectioning of the sample. Furthermore, CLSM greatly enhances the ability to obtain sharp, well resolved images from the selected level within relatively thick, three-dimensional objects (Hepler and Gunning, 1998). In confocal scanning laser microscopy, the sample is scanned by a focused spot of light; the light emitted through the interaction of the scanning light with focused areas can be recorded using a non-imaging photodetection device such as a photomultiplier (Figure 3.10). An excitation beam from the laser is reflected by the dichroic mirror (a chromatic beam splitter) and focused by the objective lens to a limited spot or micro-volume in the specimen. Fluorescent light is emitted from the irradiated spot, passing through the dichroic mirror and an adjustable imaging aperture or pinhole which is placed confocally with respect to the illumination aperture, and is collected by a photomultiplier. The instantaneous response of the light detector at all points of this scan must be displayed with the equivalent spatial position and relative brightness on a monitor (Wilson, 1990; Shotton, 1989).

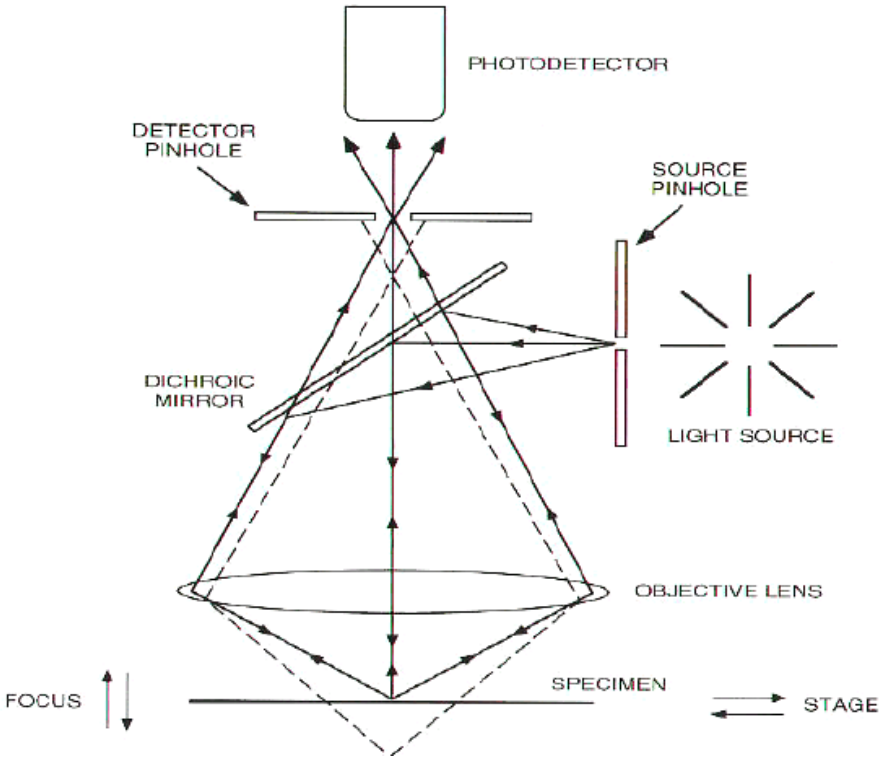


Figure 3.10: Confocal schematic. Light from the laser is reflected by the dichroic mirror into the objective lens. The lens focuses the light beam at a spot within the specimen. The florescent light, because of its longer wavelength, passes through the dichroic mirror and comes into focus at the plane of the detector pinhole (From Royal Society Light Microscopy).

3.2.4.1 Confocal experimental details

Structural alterations in the sausage matrix were assessed by CSLM. Rhodamine B (rhodamine) for the protein, Safranin O (safranin) for starch, Acridine orange for citrus fibre and BODIPY493/503 for fat were purchased from Sigma. All of these fluorophores were received in the form of powder, and dissolved in solvents. Rhodamine B, Acridine orange and Safranin O were dissolved in Phosphate-buffered saline (PBS) (a phosphate pH 7.4) buffer, and BODIPY493/503 was dissolved in Dimethyl sulfoxide (DMSO) at a concentration of 1 mg/ml, which was also used to stain the samples. For the samples, small 1 cm^3 cubes were cut and frozen in liquid nitrogen-cooled isopentane and stored at -80˚C until further use. Then, 50 µm sections were cut from the cubes using a Leica cryostat. Staining was performed for 30 min and then the staining liquid was poured off; the sections were washed 3-5 times or as many times as necessary. Indication of sufficient washing is transparent washing water. Then, the excess liquid was removed from the sections, and they were mounted with mounting medium and covered with a coverslip. Sections

were kept at +5˚C until use. Samples for CSLM analysis were randomly taken from different sausages of the same batch, at different points throughout the sausages. Ten micrographs per slide were recorded and imaged with a Nikon D Eclipse C1 (Nikon GmbhMicroskope, Dusseldorf, Germany), attached to a Nikon EclipsTiinvertes microscope (30 µm pinhole). Images were recorded at the magnification of 20× (apochromat 20×/0.75/1.0 mm) and analysed with the Ez-c1 (gold version 3.90) program of Nikon. It is worth noting that for DSC, rheometer, and CSLM, we chose the highest mean value of hardness parameter for analysing and comparing them to control samples because according to the Iranian National Standardization Organization (INSO) 2017, people in Iran likes hard sausages.

3.2.5 Statistical Package for the Social Sciences (SPSS)

3.2.5.1 One-Way Analysis of Variance (ANOVA)

ANOVA is used to compare differences between means when there are more than 2 sets of scores. This is achieved by looking at variation in the data and finding where exactly that variation is. Specifically, ANOVA compares the amount of variation between groups with the amount of variation within groups. Mathematically, ANOVA can be written as (equation 3.14):

$$x_{ij} = \mu_i + \varepsilon_{ij} i=1, 2 ,..., k \; j=1,2,...,n \qquad (3.14)$$

where x denotes the individual data points (i and j denote the group and the individual observation, respectively), ε is the unexplained variation and the parameters of the model (μ) are the population means of each group. Thus, each data point (x_{ij}) is its group mean plus error.

3.2.5.2 Hypothesis Testing

Like other classical statistical tests, we used ANOVA to calculate a test statistic (the F-ratio) with which we can obtain the probability (the P-value) of obtaining the data assuming the null hypothesis. A significant P-value (usually taken as $P<0.05$) suggests that at least one group mean is significantly different from the others and when $P>0.05$ all population are the same.

Null hypothesis: all population means are equal or same.

Alternative hypothesis: at least one population mean is different from the rest.

3.2.5.3 Calculation of the F ratio

ANOVA separates the variation in the dataset into 2 parts: between-group and within-group. These variations are called the sum of squares, which can be seen in the equations below.

Step 1) Variation between groups

The between-group variation (or between-group sums of squares, BSS) is calculated by comparing the mean of each group with the overall mean of the data (equation 3.15).

Specifically, this is:

$$Between\ SS = n_1(\bar{x}_1 - \bar{x})^2 + n_2(\bar{x}_2 - \bar{x})^2 + \cdots + n_k(\bar{x}_k - \bar{x})^2 \quad (3.15)$$

i.e. by adding the square of the differences between each group mean $\bar{x}_i$ and the overall population mean $\bar{x}$, multiplied by sample size n_i, and assuming that three groups are being compared (i = 1, 2,...,k).

The BSS is then divided by the number of degrees of freedom [this is like sample size, except it is n-1, because the deviations must sum to zero, and once you know n-1, the last one is also known] to get an estimate of the mean variation between groups.

Step 2) Variation within groups

The within-group variation (or the within-group sums of squares) is the variation of each observation from its group mean (equation 3.16).

$$Whithin\ SS = s_1^2(n_1 - 1) + s_2^2(n_2 - 1) + \cdots + s_k^2(n_k - 1) \quad (3.16)$$

i.e. by adding the variance of each group time by the degrees of freedom of each group. Note, the total SS (sum of square) might also be identified. Within SS is the Total SS minus Between SS.

As before, this is then divided by the total degrees of freedom to get the mean variation within groups.

Step 3) The F ratio

The F ratio is then calculated as (equation 3.17):

$$\frac{Mean\ Between\ group\ SS}{Mean\ Whithin\ group\ SS} = \frac{MS_{Between}}{MS_{Whithin}} \quad (3.17)$$

If the average difference between groups is similar to that within groups, the F ratio is about 1 and we can conclude that all groups are the same. As the average difference between groups becomes greater than that within groups, the F ratio

becomes larger than 1, so, at least one group mean is significantly different from the others.

To obtain a P-value, it can be tested against the F-distribution of a random variable with the degrees of freedom associated with the numerator and denominator of the ratio. The P-value is the probably of achieving that F ratio or a greater one. Larger F-ratios gives smaller P-values. A significant P-value (usually taken as $P<0.05$) suggests that at least one group mean is significantly different from the others and if $P>0.05$ then all groups are the same. However, it does not tell you which specific groups differed; post hoc tests do this. Because post hoc tests are run to confirm where the differences occur between groups, they should only be run when an overall significant difference is shown in group means. Post hoc analysis is the examination of differences between pairs of groups after the global analysis. The Scheffe test computes a new critical value for an F test conducted when comparing two groups from the larger ANOVA. A significant P-value (usually taken as $P<0.05$) implies that two group means are significantly different from each other.

3.2.5.4 Scheffe's Test

When the decision from the One-Way Analysis of Variance (ANOVA) is to reject the null hypothesis, it means that at least one of the means is not the same as the other means. What we need is a way to figure out where the differences lie, not just that there is a difference.

This is where the Scheffe' test come into play. It is worth to note that the Tukey test is only usable when the sample sizes are the same but The Scheffe' test is customarily used with unequal sample sizes, although it could be used with equal sample sizes. In the process of my experiment, data in each ID-Test may be missed and all Id-Test don't have a same sample size so we decide to use Scheffe' test. This helps to analyse pairs of means to see if there is a difference much like the difference of two means covered earlier. This test is set up to test if pairs of means are different. The formulas refer to mean m and mean n. The values of a and b vary, and the total number of tests will be equal to $k(k-1)/2$, where k is the number of samples.

Null hypothesis: Two population means are similar.

Alternative hypothesis: Two population mean are different.

The critical value for the Scheffe' test is (equation 3.18):

$$\mathrm{CV} = (\mathrm{k}-1)\mathrm{F}(\mathrm{k}-1, \mathrm{N}-\mathrm{k}, 0.05) \quad (3.18)$$

The test statistic is a little bit harder to compute (equation 3.19)

$$F_s = \frac{(\bar{x}_a - \bar{x}_b)^2}{s_w^2(\frac{1}{n_a}+\frac{1}{n_b})} \quad (3.19)$$

Reject H_0 if the test statistic is greater than the critical value. If there is no difference between the means, the numerator will be close to zero, and the P-value is greater than 0.05and we can conclude that two groups are the same.

3.2.5.5 Confidence Interval (CI)

In statistics, a confidence interval (CI) is a type of interval estimate of a mean parameter. It is an observed interval (i.e., it is calculated from the observations); this observed interval contains the parameter determined by the confidence level or confidence coefficient. More specifically, the meaning of the term "confidence level" is that, if CI are constructed across many separate data analyses of replicated (and possibly different) experiments; the proportion of such intervals that contain the true value of the parameter will match the given confidence level. Whereas two-sided confidence limits form a confidence interval, their one-sided counterparts are referred to as lower/upper confidence bounds (or limits).

Confidence intervals consist of a range of values (interval) that act as good estimates of the unknown population parameter; however, the interval computed from a particular sample does not necessarily include the true value of the parameter. When we say, "we are 95% confident that the true value of the parameter is in our confidence interval", we express that 95% of the hypothetically observed confidence intervals will hold the true value of the parameter.

CHAPTER 4 STARCH ADDITION TO MEAT BATTER: DOMINATED BY GELATINISATION OR GELATION?

4.1 Introduction

Protein–polysaccharide interaction plays an important role in the structure and stability of different kinds of food processes. Functional properties of food protein, such as solubility, gel forming and emulsifying capacity are significantly affected by their interaction with polysaccharides (PS). In the case of formulated and cooked meat products, myofibrillar proteins (PR) play a key role during processing because of their ability to product three-dimensional gels upon heating and subsequent cooling, which significantly affects sensorial and textural properties of the processed product (Smith, 1988). In the literature, there is substantial evidence for PS–PR interaction in non-meat products (e.g. milk, egg, and similar products) (Tarté, 2009). Nevertheless, more studies are required to understand the mixing of meat protein with polysaccharide in order to achieve desirable characteristics in formulated meat products. Starches are multifunctional food ingredients. In many countries, starch has traditionally been used in meat products to improve quality product. In many applications and in almost all food applications, starches are heated during processing. In emulsified meat products, for instance sausages of any kind, starches are especially useful when lower quality meats or high water levels are used. The advantage of using starch in meat production is the ability of starch to gelatinise when heated in a water–containing medium, thereby binding relatively large amounts of water (Fennema et al., 1975). The starch is added at the beginning of the initial chopping process and binds the free available water during the cooking process of the sausages. The formation of protein-starch gels is a complicated phenomenon in terms of composition, structure and key physicochemical properties. A plethora of studies have scrutinised the effects of the addition of dry starch on the properties of formulated meat products and several types of starch are available for use in meat production, as discussed in the introduction. In spite of these published studies, meat processing, particularly beef meat processors, need more scientific data to deliver formulated products that are able to meet the special requirement of consumers. Examples of such requirements may be an improvement in chewiness and cohesiveness of the final product, linking such properties to the product's microstructure. The present work consequently seeks to evaluate the influence of the addition of dry, pre-hydrated and gelled unmodified wheat starch in meat emulsion stability, textural

properties–such as hardness, springiness, cohesiveness, chewiness and finally resilience in sausage texture formation– and microstructure, such as the location of the protein network, starch and fat in cooked sausages. No reports have been published regarding the quality characteristics of sausage batter when comparing the addition of dry, pre-hydrated and gelled formation of unmodified wheat starch. Furthermore, this study aims to elucidate the role of heat-induced gelation in the texture formation of processed meat products and also involves the investigation of the interaction between meat protein and unmodified wheat starches because an understanding of the formation of starch/meat composites would be helpful in the development of new products.

4.2 Materials and Methods

Materials and methods used for this study are described in Chapter 3.

4.3 Results and Discussion

4.3.1 Texture properties

Gelling of myofibrillar proteins with unmodified wheat starch makes the meat pastes change from a viscous semi-liquid state to a rigid semi-solid state. Protein interactions during heating induce the formulation of a three-dimensional matrix which traps the water and starch granules and the same time the starch granules are competing for water during their gelatinisation process. Adding starches which have already been gelatinised (pre-hydrated) or gelatinised and gelled; locking up different amount of water may affect the properties of the system and show different levels of hardness, cohesiveness, springiness, resilience and chewiness. Its worthy to note that in this study, we don't have a fracturability and gumminess parameters because fracturability parameter was measured as a first significant break sausages in the first peak and we did not see and record any sausage break in the first peak. Also, Szczesniak (1996) reported that gumminess was defined as the energy required to disintegrate a semisolid food product to a state of readiness for swallowing however, the product in this study (sausages) is not semisolid material.

Table 4.1: Scheffe's test results (p-values) for the hardness of controls (65% meat and reference) with 65% meat containing dry, pre-hydrated and gelled unmodified wheat starch (**<0.01 and *<0.05).

	Reference control	3% Dry starch with 65% meat	3% Pre-hydrated starch with 65% meat	3% Gelled starch with 65% meat	7.4% Dry starch with 65% meat	7.4% Pre-hydrated starch with 65% meat	7.4% Gelled starch with 65% meat
3% Dry starch with 65% meat	0.00*						
3% Pre-hydrated starch with 65% meat	0.00**	0.02*					
3% Gelled starch with 65% meat	0.00**	0.44	0.92				
7.4% Dry starch with 65% meat	0.43	0.00**	0.00**	0.00**			
7.4% Pre-hydrated starch with 65% meat	0.00**	0.85	0.57	0.99	0.00**		
7.4% Gelled starch with 65% meat	0.00**	0.99	0.02*	0.41	0.00**	0.82	
65% Meat	0.00**	0.07	0.99	0.91	0.00**	0.62	0.06

Table 4.2: Scheffe's test results (p-values) for the springiness of controls (65% meat and reference) with 65% meat containing dry, pre-hydrated and gelled unmodified wheat starch (**<0.01 and *<0.05).

	Reference control	3% Dry starch with 65% meat	3% Pre-hydrated starch with 65% meat	3% Gelled starch with 65% meat	7.4% Dry starch with 65% meat	7.4% Pre-hydrated starch with 65% meat	7.4% Gelled starch with 65% meat
3% Dry starch with 65% meat	0.89						
3% Gelled starch with 65% meat	0.99	0.99	0.99				
7.4% Dry starch with 65% meat	0.99	0.99	0.99	0.99			
7.4% Pre-hydrated starch with 65% meat	0.98	0.99	0.94	0.98	0.99		
7.4% Gelled starch with 65% meat	0.99	0.99	0.98	0.99	0.99	0.99	
65% Meat	0.99	0.99	0.99	0.99	0.99	0.99	0.99

Table 4.3: Scheffe's test results (p-values) for the cohesiveness of controls (65% meat and reference) with 65% meat containing dry, pre-hydrated and gelled unmodified wheat starch (**<0.01 and *<0.05).

	Reference control	3% Dry starch with 65% meat	3% Pre-hydrated starch with 65% meat	3% Gelled starch with 65% meat	7.4% Dry starch with 65% meat	7.4% Pre-hydrated starch with 65% meat	7.4% Gelled starch with 65% meat
3% Dry starch with 65% meat	0.99						
3% Pre-hydrated starch with 65% meat	0.00**	0.00**					
3% Gelled starch with 65% meat	0.57	0.82	0.19				
7.4% Dry starch with 65% meat	0.02*	0.03*	0.99	0.65			
7.4% Pre-hydrated starch with 65% meat	0.00**	0.00**	0.8	0.00**	0.32		
7.4% Gelled starch with 65% meat	0.00**	0.00**	0.99	0.35	0.99	0.62	
65% Meat	0.99	0.99	0.04*	0.96	0.19	0.00**	0.08

Table 4.4: Scheffe's test results (p-values) for the chewiness of controls (65% meat and reference) with 65% meat containing dry, pre-hydrated and gelled unmodified wheat starch (**<0.01 and *<0.05).

	Reference control	3% Dry starch with 65% meat	3% Pre-hydrated starch with 65% meat	3% Gelled starch with 65% meat	7.4% Dry starch with 65% meat	7.4% Pre-hydrated starch with 65% meat	7.4% Gelled starch with 65% meat
3% Dry starch with 65% meat	0.00**						
3% Pre-hydrated starch with 65% meat	0.00**	0.00**					
3% Gelled starch with 65% meat	0.00**	0.19	0.29				
7.4% Dry starch with 65% meat	0.13	0.00**	0.00**	0.00**			
7.4% Pre-hydrated starch with 65% meat	0.00**	0.00**	0.99	0.66	0.00**		
7.4% Gelled starch with 65% meat	0.00**	0.69	0.1	0.99	0.00**	0.33	
65% Meat	0.00**	0.12	0.93	0.99	0.00**	0.99	0.94

Table 4.5: Scheffe's test results (p-values) for the resilience of controls (65% meat and reference) with 65% meat containing dry, pre-hydrated and gelled unmodified wheat starch (**<0.01 and *<0.05).

	Reference control	3% Dry starch with 65% meat	3% Pre-hydrated starch with 65% meat	3% Gelled starch with 65% meat	7.4% Dry starch with 65% meat	7.4% Pre-hydrated starch with 65% meat	7.4% Gelled starch with 65% meat
3% Dry starch with 65% meat	0.12						
3% Pre-hydrated starch with 65% meat	0.00**	0.00**					
3% Gelled starch with 65% meat	0.00**	0.25	0.32				
7.4% Dry starch with 65% meat	0.00**	0.01*	0.96	0.93			
7.4% Pre-hydrated starch with 65% meat	0.00**	0.00**	0.3	0.00**	0.02*		
7.4% Gelled starch with 65% meat	0.00**	0.00**	0.99	0.06	0.64	0.76	
65% Meat	0.97	0.78	0.00**	0.01*	0.00**	0.00**	0.00**

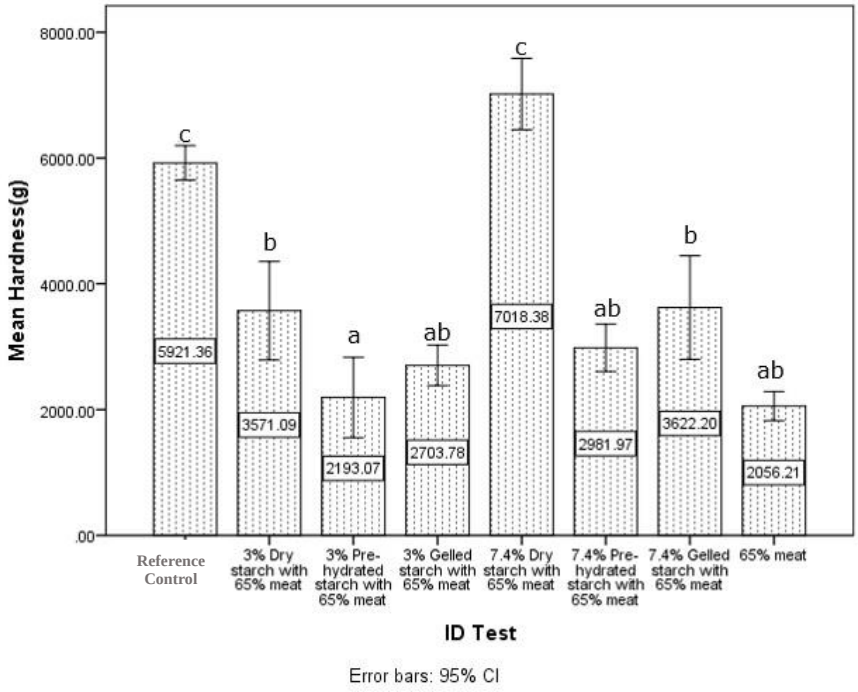


Figure 4.1: Hardness of adding dry, pre-hydrated and gelled unmodified wheat starch to a 65% meat, compared to control samples (65% meat and reference).

The general linear model ANOVA was utilized to test for differences between treatments (ID-Test) in the hardness. Analysis of variance (ANOVA) showed that at least one ID-Test mean is different from the others. So, Scheffe's test was applied in order to compare the means of the hardness ID-Test.

In Tables 4.1, 4.2, 4.3, 4.4 and 4.5 p-values less than 0.05 are considered to be significant, so, the terms marked as an asterisk (*), if P-value<0.05, means that difference of two ID-Test are statistically significant at 95% confidence level. The terms marked by two asterisks (**), if P-value<0.01, means that difference of two ID-Test statistically significant at 99% and without asterisk means that the ID-Tests do not differ statistically.

In Figure 4.1, the ID-Tests having the same letters do not differ statistically because the p-value was higher than 0.05 and ID-Tests with different letters differ statistically because p-value less than 0.05.

First of all, in terms of the hardness a comparison of the control samples, which are 65% and reference, shows that they are significantly different to one another. Now comparing the control 65% meat product to the other treatments (except the reference) shows that there is a statistically significant difference with the sample with the addition of 7.4% dry starch. Indeed, the addition of 7.4% dry starch to a 65% meat product is statistically not different to the reference.

Focusing on the sausages with 3% concentration of added starch and comparing them with each other, there is only a statistical difference between the addition of dry starch and when the starch is pre-hydrated. Furthermore, when the starch concentration is increased to 7.4%, dry starch is significantly different to both gelled and pre-hydrated starch. Moreover, by comparing the two different starch concentrations there is a significant difference when the starch is added dry. Also, the addition of 3% pre-hydrated starch only differs statistically from 7.4% dry starch and 7.4% gelled starch, whereas 3% gelled starch only differs statistically from 7.4% dry starch.

These results indicate that the addition of 7.4% dry unmodified wheat starch allows a 35% reduction in meat while retaining the same hardness of a reference. Therefore, the gelatinisation of starch during the heating process reinforces the meat protein gel and that lower starch levels (3% and 0%: 65% meat alone) are insufficient. The addition of pre-hydrated or gelled starch at 7.4% is also not sufficient to provide the required hardness values, indicating the importance of the required amount of water for protein gelation. The granules of starch are trapped in the protein matrix and stabilise the meat texture (see section 4.3.4). Tarté (2009) reported a typical starch dosage level would be 2–3% of the final product in a meat batter, but higher levels are not uncommon, especially when native starches are used and maybe starch pockets occur. However, in this study, 7.4% of unmodified wheat starches were used but there was no occurrence of starch pockets (tiger stripes), which can occur when too much starch is used in meat products and the starch is not evenly distributed within the meat structure. Dexter et al. (1993) reported a reduction in cooking and purge loss due to the addition of potato starch during the chopping process of turkey bologna, although they did not observe an increase in hardness of product. However, Carballo et al. (1996) reported that a more compact and strong heat-induced protein network resulted when starches were added to a meat emulsion. Previous studies conducted showed that the addition of dry of starch in meat batter causes increased hardness of processed sausages (García-García and Totosaus, 2008; Pietrasik, 1999) and the results of this study agree with previous findings, while indicating that the state in which the starch is added is of equal importance.

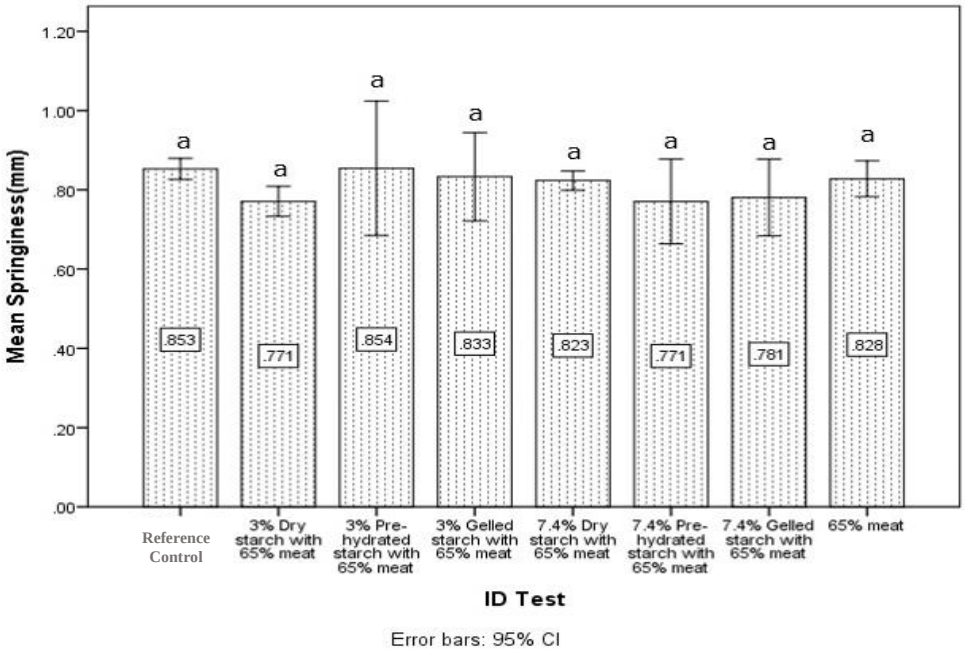


Figure 4.2: Springiness of adding dry, pre-hydrated and gelled unmodified wheat starch to a 65% meat, compared to control samples (65% meat and reference).

Springiness (sometimes also referred to as 'elasticity') is a perception of gel 'rubberiness' in the mouth, and is a measure of how much the sausage is broken down by the initial compression (Sanderson, 1990). Analysis of variance (ANOVA) showed that springiness doesn't differ significantly by ID-Test types (Table 4.2). So we do not have a difference between every ID Test. What is evident here therefore is that the springiness parameters of meat products containing starch are independent of the state of starch, and more surprisingly that reference control and 65% meat sausages are not significantly different. Therefore, this parameter cannot differentiate between tests samples in the way in which the hardness parameter does, in further studies the consumer response during eating of the products would provide a clearer indication of which texture parameter are unimportant.

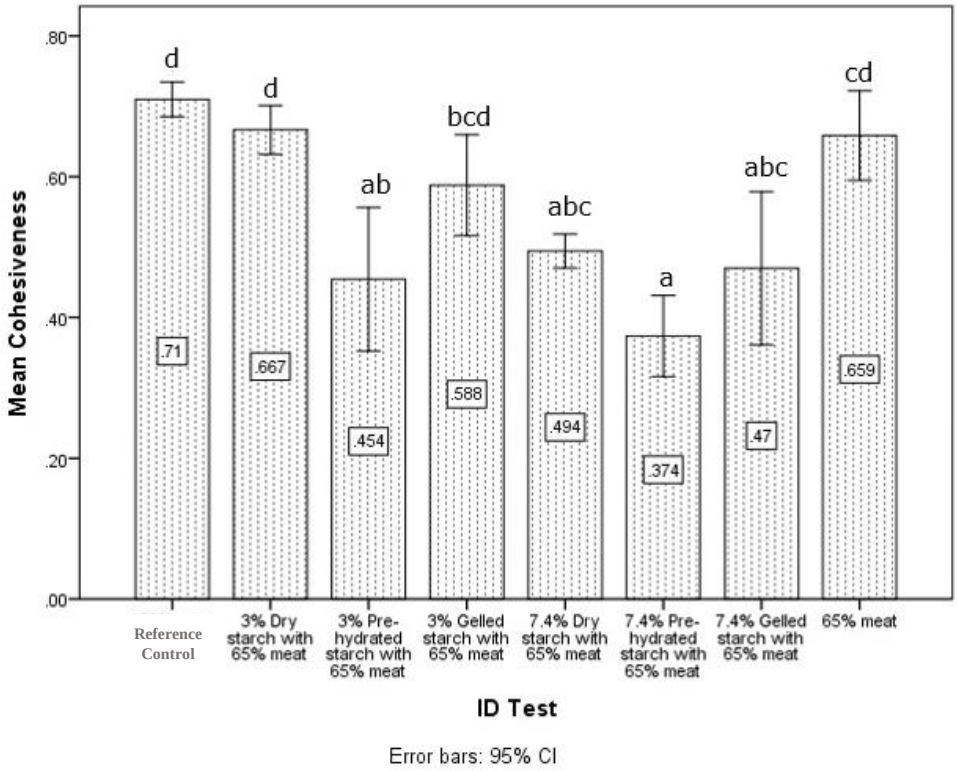


Figure 4.3: Cohesiveness of adding dry, pre-hydrated and gelled unmodified wheat starch to a 65% meat, compared to control samples (65% meat and reference).

Analysis of variance (ANOVA) showed that cohesiveness differed significantly by ID-Test type. So, Scheffe's test was applied in order to compare the means of the cohesiveness ID-Test (Table 4.3).

First of all, when comparing the control samples (65% and reference) they do not have a statistical significant difference form one other. Comparing the 65% meat control to the other treatments shows statistically significant difference with 3% and 7.4% pre-hydrated starch. Also, by focusing on the 3% starch concentration there is only a difference between 3% dry starch and 3% pre-hydrated starch. Furthermore, at the 7.4% addition level there is no difference between any of the samples. Moreover, by comparing 3% concentration with 7.4% there is a statistical difference between 3% dry starch with each of the starch treatments. Also, 3% pre-hydrated starch doesn't differ statistically from 7.4% dry, pre-hydrated and gelled starch. Furthermore, 3% gelled starch only differs statistically from 7.4% pre-hydrated starch with 65%.

Therefore, when investigating cohesiveness, Figure 4.3 shows that while reference control has the largest mean, there is no statistical difference between this control and samples with 35% reduction in meat content with samples with

either no added starch or when starch is added at the 3% level in either the dry or gelled state. Adding unmodified wheat starch to a meat batter caused a near complete breakdown of the continuous network and consequently a decrease in the level of cohesiveness in the meat system.

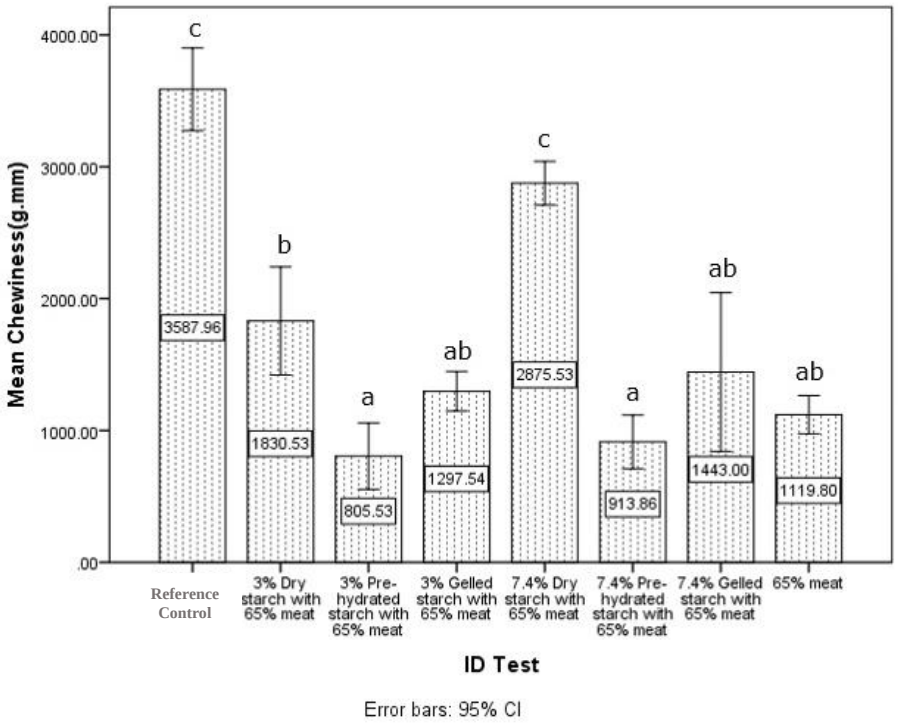


Figure 4.4: Chewiness of adding dry, pre-hydrated and gelled unmodified wheat starch to a 65% meat; compared to control samples (65% meat and reference).

Analysis of variance (ANOVA) showed that chewiness differed significantly by ID-Test type. So, Scheffe's test was applied in order to compare the means of the chewiness ID-Test (Table 4.4).

First of all, it can be seen that the control samples are statistically different from are another. At a meat control of 65% the only starch treatment to be statistically different is the addition of 7.4% dry starch, which is seen to be statistically not different to the reference control. There is a statistical difference between 3% dry starch and 3% pre-hydrated starch. Furthermore, at the 7.4% starch level, the addition of dry starch is statistically different to the other two treatments. Moreover, there is a statistical difference between 3% and 7.4% dry starch. The lowest mean chewiness results are for the pre-hydrated treatment, which is significantly different at comparative addition level to the addition of dry starch, but not when adding gelled starch, or indeed the 65% meat control.

A previous study showed that the addition of starch was not able to reduce the chewiness of a product (Pietrasik, 1999), but in this study the chewiness is measured by the state of starch addition with dry (3 and 7.4%) starch being higher than that of pre-hydrated and gelled (3 and 7.4%) starch. Also, the addition of 3% dry starch shows that 35% meat can be removed with no significant difference seen in chewiness. This may be because of small aggregates of partially gelatinised starch granules during the cooking of meat batter, consequently improving chewiness in the final sausage product. Pre-hydrating or gelling starch does not have this effect at such reduced meat levels.

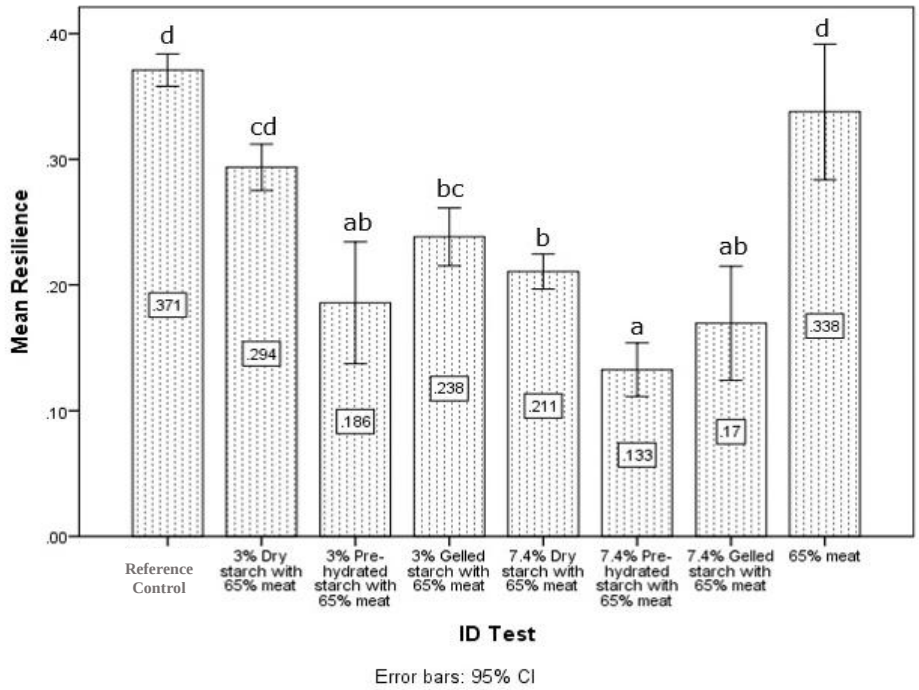


Figure 4.5: Resilience of adding dry, pre-hydrated and gelled unmodified wheat starch to a 65% meat; compared to control samples (65% meat and reference).

Analysis of variance (ANOVA) showed that resilience differed significantly by ID-Test type. So, Scheffe's test was applied in order to compare the means of the resilience ID-Test (Table 4.5).

First of all, in terms of resilience the control samples are not statistically significantly different. When comparing the 65% samples the 3% dry starch sample is statistically not different to the control, which the other treatments being different to the control. The 3% dry starch sample is statistically not different to when 3% is gelled but different to when the starch is pre-hydrated. The same is seen at the 7.4% starch addition level, with gelled being similar and

pre-hydrated being different. The reference control samples have the highest resilience, which is a measurement of how the sausage sample recovers from deformation both in terms of speed and the force derived. Therefore the addition of 3% dry starch allows similar performance, but more dry starch or pre-hydrated starch changes the way in which the sausage samples recover.

4.3.2 Differential scanning calorimetry

In order to understand what the different starch treatments were having during heating the samples were studied using DSC. In this study the functions of dry unmodified wheat starch (7.4% with 65% meat) on the formulation of sausage batter composite during heating were investigated. It is worth to mention that the meaning of "without processing" in Figure 4.6 is the meat without adding salt and phosphate and also without chopper process and it is clear that the meaning of " with processing" is the meat adding salt and phosphate with chopper process. According to Foegeding (1988), proteins are the main constituents forming the structure in meat products, affecting their texture. Muscle proteins are acknowledged to be primarily responsible for the texturisation of the final sausage product. The change in the protein structural stability when adding starch should, be important to the texturising mechanism, and the differential scanning calorimetry technique seems useful for characterising that change. Myosin, one such protein, is one of the major factors in thermal aggregation needed for structure formation, especially in finely ground processed meat products (Egelandsdal et al., 1986). In addition, other non-meat components normally found in meat emulsion, such as starch, also affect product properties during cooking. In sausages, starch has been recognized as filler to increase the firmness of products (Verrez-Bagnis et al., 1993; Wu et al., 1985) and to enhance the gel strength (Kim and Lee, 1987). In order to achieve this, gelatinisation of starch in sausages is necessary (Chung and Lee, 1991). The composite reinforcing effect of starch may be due to the swelling of starch granules embedded in the protein gel, which compresses the matrix, and because the protein matrix loses moisture and becomes firmer (Kim and Lee, 1987). Furthermore, the gelatinisation of starch is of primary importance in the food industry because of its impact on the texture of starch-based foods (Biliaderis, 1983).

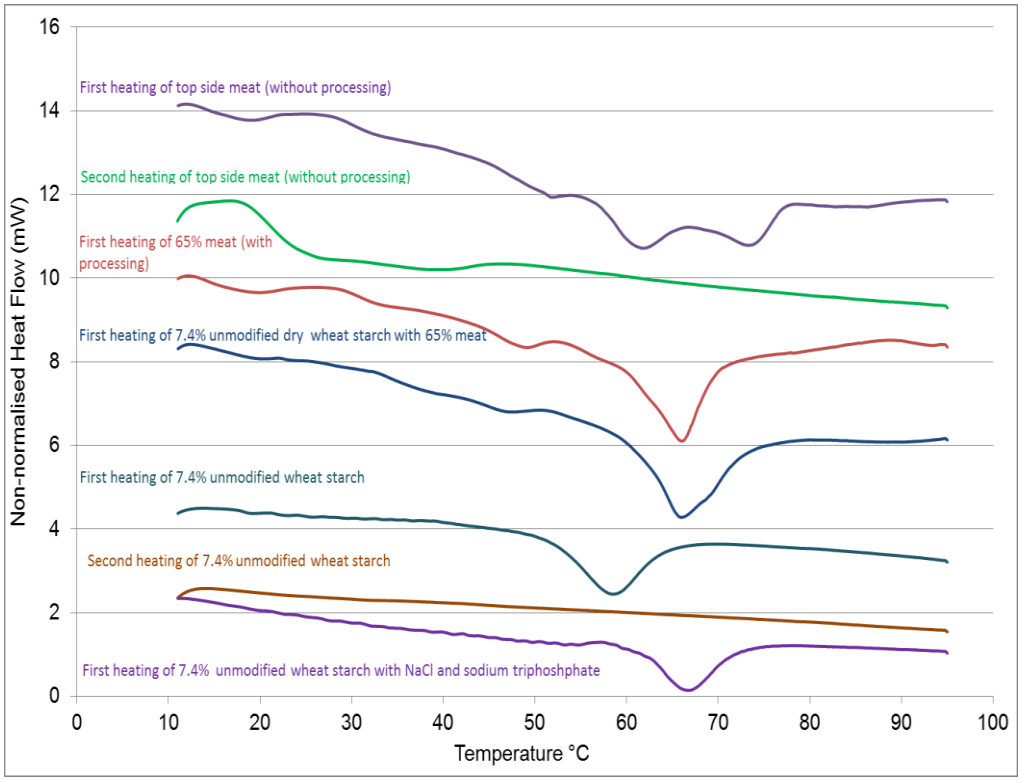


Figure 4.6: DSC thermograms for first heating of top side meat (without processing); second heating of top side meat (without processing); first heating of 65% meat (with processing); first heating of 7.4% dry unmodified wheat starch with 65% meat; first heating of 7.4% unmodified wheat starch; second heating of 7.4% unmodified wheat starch, and finally first heating of 7.4% unmodified wheat starch with NaCl and sodium triphosphate (details of sample preparation can be found in method and material chapter).

Table 4.6: Endothermic heating transition parameters for first heating topside of meat (without processing); first heating of 65% meat (with processing); first heating of the 7.4% unmodified wheat starch with 65% meat; first heat of 7.4% unmodified wheat starch; first heating of 7.4% unmodified wheat starch with NaCl and sodium triphosphate . T_0 = onset temperature; T_e= end set temperature; T_m = peak temperature and ΔH = non-normalised enthalpy of transition.

samples	T_0	T_e	T_m	ΔH (J/g)
First heating of topside meat (without processing)	55.4	77.7	61.7	2.0
First heating of 65% meat (with processing)	59.2	67	66.1	2.1
First heating of 7.4% dry unmodified wheat starch with 65% meat	60.9	73.7	66.4	2.4
First heating of 7.4% unmodified wheat starch	51.9	64	58.5	0.8
First heating of 7.4% unmodified wheat starch with NaCl and sodium triphosphate	62	71.6	66.7	0.6

The curve of endothermic topside meat (without processing) shows meat fat to be completely melted at 35˚C, also reported by Quinn et al. (1980). The most obvious features when scanning the topside of meat are the denaturation peaks,

which are three endotherms, which are thought to reflect the principal components: myosin (51.6˚C), sarcoplasmic protein (61.7˚C) and actin (73.6˚C). The assignment of protein denaturation peaks has, been carried out by Wright et al. (1977) on rabbit muscle. These authors found three endothermic peaks in a thermogram of whole muscle, a major peak at 60˚C, a minor peak at 67˚C, and another major peak at 80˚C. Isolation studies showed these to result from the thermal denaturation transition of myosin, sarcoplasmic protein and actin, respectively. Also earlier studies yielded the assignment of muscle protein components to the three transitions as follows. The first transition displays its maximum between 54 and 58˚C and has been attributed to myosin (Martens and Vold, 1976). The second transition, which occurs between 65 and 67˚C, was assigned to collagen (Stabursvik and Martens, 1980) and to the sarcoplasmic proteins. The third transition has been consistently assigned to actin and is found between 80 and 83˚C (Wright and Wilding, 1984). Differences in thermal curves between studies may have resulted from variations in meat parts, sample preparation, the age of the animals, composition and the pH of meat. In this study the major myofibrillar peak related to the sarcoplasmic protein of topside; however, Ensor et al. (1991) reported that the major myofibrillar peak probably represented the thermal denaturation of myosin in beef muscles, especially the flank. The differences between the myofibrillar peaks was related to the different parts of the meat. Martens et al. (1982) reported that the firmness dramatically increased in temperatures of ≈53˚C and between 61 and 73˚C. Both these temperature ranges correspond to myofibrillar protein denaturation. These authors also reported that juiciness in the muscles fell slightly from 45 to 63˚C, drastically between 63 and 73˚C and again slightly between 73 and 79˚C. Firmness and juiciness are the main sensory properties that were needed to describe the main texture changes observed in the meat.

The reheating of topside meat curve showed no response, which indicated the protein is irreversibly denatured during the initial heat treatment. Karmas and DiMarco (1970) published similar curves regarding meat tissue.

Comparing the curve of topside meat (without processing) and 65% meat (with processing), it is obvious that the salt and phosphate affected the structural stability of muscle proteins, changing their thermal responses. During the preparation of meat processing, 2.7% salt was added to solubilise protein. When salt is added to the sausage batter, the pH should increase (Puolanne et al., 2001).

Salt (NaCl) is added to meat products to improve their binding and water-holding properties. Furthermore, salt is the most commonly used ingredient for extracting muscle protein (mainly actin) (Tornberg, 2005a). With the addition of salt and sodium triphosphate to the meat batter the first transition is less affected and shifted to a lower temperature (46˚C). However, the second and third transitions disappeared and shifted to the higher temperature. The small peak represents myosin and the large peak represents the denaturation of sarcoplasmic protein, connective tissue and actin (66.1˚C). The effect of sodium chloride on meat proteins is most probably caused by the fact that chloride ion is more strongly bound to the protein than sodium ion (Ruusunen and Puolanne, 2005). In the presence of NaCl at pH >5, the charge on both the thick and thin filaments is negative and there will be a repulsive force between the filaments tending to enlarge the myofibrillar lattice. Increasing the pH raises the charge on the filament, causing the myofibrillar to swell. Hamm (1972) claimed that this repulsion between the myofibrillar proteins (myofilaments), results in a swelling of the myofibrils or even a partial solubilisation of filaments, the latter being due to the repulsion of individual molecules. The cross-bridge between the filaments curtails the unlimited swelling of the myofibrils. The degree of swelling depends on the pH (Millman, 1998; Offer et al., 1989). Offer and Knight (1988) claimed that there was a repulsion between the filaments, but this was between the molecules of myosin filaments breaking down the shaft of the filaments. If phosphate is not used, the S1 until of heavy meromyosin in myosin are still attached to actin filament. If salt and phosphates are added myosin becomes less soluble and for this reason in this study we can see the small peak in DSC transition; the mechanism of this is as yet unknown.

Salt diffuses through the ultrastructure of meat and finally reaches the sarcomeres (Puolanne et al., 2001). The chloride ion (Cl^-) has been suggested to be bound to myofilaments, making them mainly negatively charged, which will lead to the repulsion of myosin molecules, the swelling of myosin filaments and, consequently, all of the myofibrils (Regini and Elliott, 2001). The sodium ion (Na^+) would interact both with actin and myosin filaments while the effect of Cl^- would concern only myosin filaments. In the absence of phosphate, swelling is accompanied by extraction of the middle of the A-band however, in the presence of phosphate the A-band is completely extracted (Offer and Trinick, 1983).

These authors also claimed that Cl^- bind to the filaments and increase the electrostatic repulsive force between them.

NaCl increases the protein solubility of meat products, which means that it decreases the protein–protein interaction (Zayas, 1997). The effect of NaCl on different proteins in meat is very complex, and this complexity increases if different concentrations of added NaCl and phosphates act simultaneously (Offer and Knight, 1988). Briefly, the solubility of myosin increases as the NaCl concentration increases from 0.04 to 0.5 M. After the initial aggregation and formation of filaments, the structure starts to dissociate at salt contents higher than 0.25 M. The swelling of myofibrils begins at 0.5 M (2.9%), without added phosphates and 0.4 M (2.3%) with added phosphates, when extensive extraction of myosin also occurs (Offer and Knight, 1988). It is mention to note that in this study the concentration of salt for processing is 0.46 M. Myofibrils swelled most in 1 M (5.8%) NaCl and hardly at all in 5 M (29%) NaCl (Knight and Parsons, 1988). At high concentrations of salt, salt dissolves the myosin filaments; in other words, myosin filaments are more or less disrupted when salts are added.

Comparing the curves of top side meat and the 65% meat with 7.4% dry starch addition it is clear that the process affected the structural stability of muscle proteins, changing their thermal responses. It was seen that when adding starch, salt and phosphate in to the batter caused a disappearance of the three peaks and the appearance of two peaks, representing myosin (46˚C) and a mix of denaturation protein (sarcoplasmic, actin) and gelatinisation of starch. The second endothermic peak had an onset temperature of 66.4˚C and this corresponds to the remaining components of the sausage matrix, namely, proteins and carbohydrates. The small gelatinisation peak maybe occurs when the temperature is in the region of 66.4˚C, when starch swells upon hydration, an increase in chain motions in the amorphous parts of the starch granules destabillises the system and causes the slow melting of starch crystallites (Donovan et al., 1983).

According to the literature, the endothermic peak of DSC-starch transition (gelatinisation and fusion of the most stable crystallites) should reach a peak temperature at about 60˚C, although as the water content decreases, the peak shifts to a higher temperature (León et al., 2003). In this study the peak gelatinisation of 7.4% unmodified wheat starch with 92. 6% water occurs at a temperature in the region of 58.5˚C. This is in accordance with previous studies

(Alamri et al., 2013; Zaidul et al., 2008), which reported wheat starch gelatinisation occurred between 58 and 62.6˚C.

The second scan of 7.4% wheat starch with water shows that complete gelatinisation was achieved during the first heating treatment and that no significant re-crystallization in the gel took place over the time of experiments. This means that the second scan of starch gelatinisation is an irreversible process.

The thermograms of the endothermic peak of unmodified wheat starch (7.4%) with water and salt and sodium phosphate shows only one large peak occurring at 66.7˚C. Salt and phosphate affect the starch gelatinisation. Salt (NaCl) inhibited the opening of crystalline regions in the starch granules and as a result of it shifted to the high transition (Ganz, 1965). Phosphate prevents amylose and amylopectin degradation on heating the starch granule. Also, all kinds of starch are affected by low levels of sulphate (0.01%) (Paterson et al., 1994).

Table 4.6 shows the maximum onset related to samples of starch, water, salt and phosphate, which is 62˚C, and the maximum offset related to topside meat, which is 77.7˚C. Within the error of measurement and baseline fitting, enthalpies were linearly additive, suggesting no interaction between meat and unmodified wheat starch. This study supports the previous study (Li and Yeh, 2002), which showed that there is no interaction between meat and modified starch.

Figure 4.7 shows the effect of the form of the starch (dry, pre-hydrated or gelled) on the thermograms of 7.4% starch/65% meat containing samples. The arrowed shoulder on the top trace is thought to represent the gelatinisation of the “dry” starch sample in the presence of high concentrations of salt and triphosphate. This shoulder is not seen on the first heating runs of the other samples as the starch in these samples was previously gelatinised. This observation was examined in detail in the Figure 4.8.

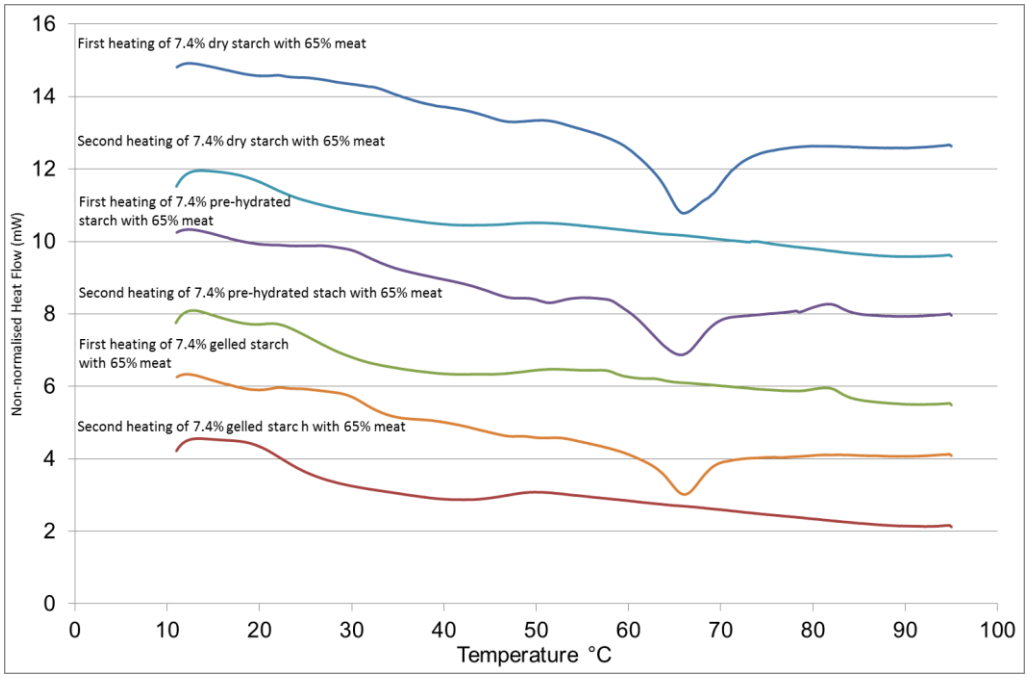


Figure 4.7 DSC thermograms for first and second heats of 7.4% dry starch with 65% meat; 7.4% pre-hydrated starch with 65% meat; 7.4% gelled starch with 65% meat.

Figure 4.8 shows the result of fitting the joint meat starch trace using linear combinations of the individual starch and meat traces. It was necessary to shift the starch trace upwards by approximately 2.5˚C in order to achieve the best fit. Rather than proposing any interaction between the meat protein and the starch it was felt that at these very high concentrations of meat, starch, salt and phosphate and consequent highly non-ideal environment, the salt and water availability was probably changed. It has already been seen that the presence of salt and phosphate increased the starch transition by 8˚C from 58.5 to 66.5˚C. However in a system containing a high level of meat components the effective concentration of salt and phosphate available to the starch may be higher perhaps due to imitate availability of free water pushing the transition temperature up further. This is an indirect rather than a direct interaction between components. In addition the optimum fit required a slight reduction in the enthalpies of transition of that the starch and meat by a factor of 0.8. However the shape and width of the transitions appear identical suggesting that a linear combination of the component transitions is a good match with the transition of the joint materials further suggesting minimal direct interaction.

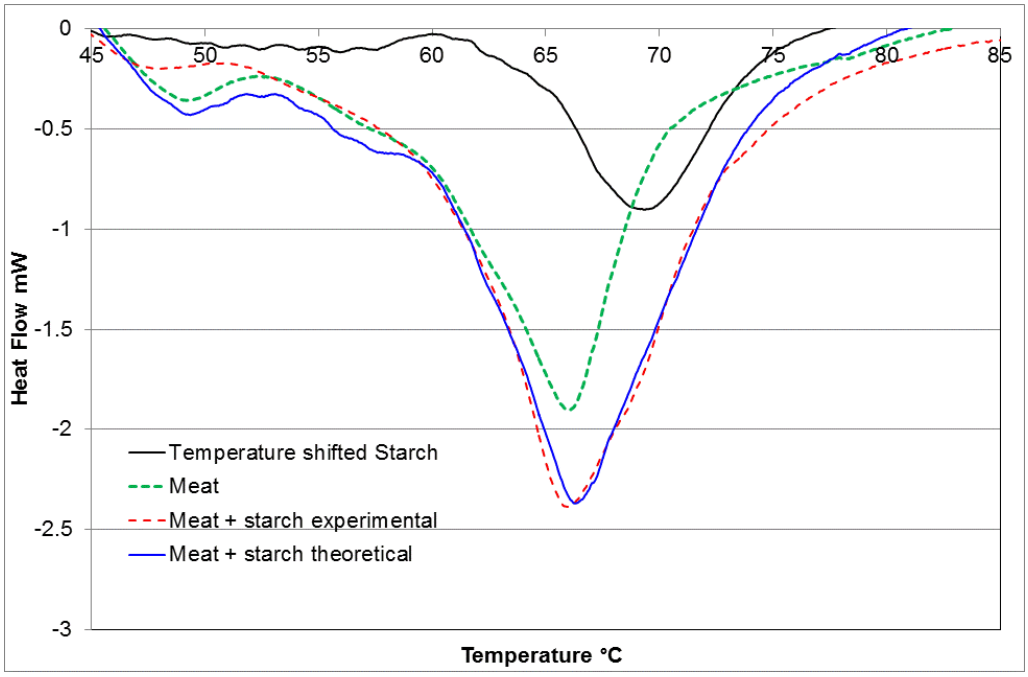


Figure 4.8 A test for protein carbohydrate interaction. *The optimal fit to experimental data for a combined 7.4% starch 65% meat system using a linear combination of individual endotherms for starch and meat. It was necessary to shift the temperature of the starch endotherm by approximately 2˚C for reasons outlined in the text.*

4.3.3 Small deformation mechanical spectroscopy

Temperature sweeps showed the thermal behaviour of samples in terms of their storage and loss moduli and phase angle (G´, G˝ and tan δ) as a function of temperature (30–90˚C) (Figure 4.9). G´ (Pa) represents the elastic element, whereas G˝ (Pa) is the viscous element of the material. Tan δ (=G˝/G´) indicates the change in elasticity and viscosity during thermal gelation. Differences in thermal rheological properties were observed between the different formulations due to their different composition. During meat batter cooking, through heating, a great number of reactions take place and several mechanisms are involved, including gelation of myofibrillar proteins and starch gelatinisation. In all samples the storage modulus (G´) was greater than the loss modulus (G˝) during the test, (G´> G˝), which indicates a predominantly elastic behaviour of the samples within the entire range of the temperatures. In the physical entrapment theory, myofibrillar proteins in the continuous phase of uncooked meat batter (emulsion) exist in a sol form; they aggregate during cooking to form a gel (Egelandsdal et al., 1986; Wu et al., 2011). The onset of increase in G´ is consistent with the temperature of the myosin transition seen in Figure 4.9. One

hypothesis is that, initially, a weak gel forms from 'native' (i.e. consisting of non-denatured molecules) synthetic myosin filaments by interfilamental interaction between myosin heads (S-1), sub-fragment 2 (S-2) and/or heavy meromyosin (HMM) as a whole (Rodgers and Harrington, 1982; Waller and Lowey, 1983). It has been widely recognised that myosin is the main component involved in the meat emulsion gelation mechanism (Ikeuchi et al., 1994; Samejima et al., 1985; Tornberg, 2005b;). Furthermore, most of the myosin molecules may have been partially aggregated (Visessanguan et al., 2000). Denaturation of HMM occurs between 40 and 50˚C (Egelandsdal et al., 1986).

Tan δ in both Figures (the control 65% meat sample and 7.4% dry unmodified wheat starch with 65% meat) decreased rapidly at 47˚C, indicating sol-to-gel transition. This also, indicates that the natural actomyosin become elastic. This suggests that conformational changes and interaction of the natural actomyosin molecules occur (Sano et al., 1988). The DSC thermogram (Figure 4.6) shows an endothermic region in this range, peaking at 45˚C. After 60˚C, tan δ decreased at a slower rate and reached equilibrium, suggesting gel development was complete. Gelation of protein is defined by an increase in storage modulus and a decrease in tan δ. The low tan δ and high G´ at 50˚C indicate the development of protein gel elasticity.

Conformational changes in the muscle protein have been observed taking place within different temperature ranges, such as 51–58˚C for myosin, 61–67˚C for collagen and sarcoplasmic protein, and 73–83˚C for titin and actin (Markowski et al., 2004; Tornberg, 2005b).

The result of G´ in 7.4% unmodified wheat starch with 65% meat and control samples (65% meat) showed that there are two peaks, which represent the unfolding of myosin molecules: irreversible interfilamental interaction between myosin heads (45–50˚C) (Egelandsdal et al., 1986, Sano et al., 1988) and another shoulder may be further explained by the denaturation and precipitation/coagulation of myosin (Ikeuchi et al., 1994;Samejima et al., 1981). In another hypothesis in the second development stage of G´, whereby the area of the peak/shoulder for the 7.4% dry unmodified wheat starch with 65% meat occurs at a temperature of around 51˚C, the increase in G´ was referred to as 'gel setting', in which the loose gel structure was formed and this is the changed conformation of the myosin molecule. It is interesting to note that the two peaks in the 65% meat merge together and this is due to the fast interaction of the

myosin heads or the interaction of myosin with other components, such as tropomyosin (Sano et al., 1988).

During isothermal heating, the G´ in both figures increased up to 48˚C but decreased at 60˚C and the same result was reported by Foegeding et al. (1986). The initial decrease in the temperature of 56˚C in G´ values in both figures shows that it could be related to the breaking hydrogen bonds of myosin as the temperature increases. Another hypothesis for this range suggests that light meromyosin (LMM) denaturation can be seen as a weakening of the interactions between the myosin molecules, leading to a less strong gel (Egelandsdal et al., 1986; Tornberg, 2005b).

As can be seen in both figures, the protein gel's starting point as evidenced by the sudden increase in G´ was 58˚C and 60˚C, respectively, when a heating rate of 1˚C/min was employed. Future increases in the temperature of G´ (60–70˚C), after the point were gelation begins, have been attributed to a network formed by the aggregation and entanglement of unfolded protein molecules through non-specific hydrophobic and sulphydryl-disulphide interaction, due to their more reactive condition towards neighbouring molecules (Aguilera and Rojas, 1997; McSwiney et al., 1994). The DSC thermogram (Figure 4.6) shows a broad endothermic region at 55–70˚C, suggesting confirmation of the rheology results. Some scientists, such as Rolee and LeMeste (1999) indicate that wheat starch gelatinisation, for instance, which is a well-known phenomenon occurring when there is an excess of water, is expected to increase the rigidity of the gel in the temperature range of 50–70˚C. In this study the increase between 60 and 70˚C in G´ and G˝ of starch/meat composite appeared to be associated with the small aggregates of partially gelatinised starch granules. The increase in rigidity of the actomyosin–starch combination is caused by the small swelling of the starch granules during gelatinisation (Niva et al., 1988) and the increase in the hardness of sausage is caused by starch (Carballo et al., 1995). Furthermore, as the temperature increased to between 60 and 70˚C, the connective tissue and the muscle fibres contract longitudinally, and such a contraction increases with temperature (Siripurapu et al., 1987). Also, collagen fibres can contract to a quarter of their original length under heating at 60–70˚C and if they are unrestricted (Tornberg, 2005b), as would be expected in a high moisture content environment, such as the meat batter considered here. A slight increase G´ in

both figures during heating between the ranges of 70 and 80˚C is related to titin denaturation.

Towards the end of the heating stage (at around 90˚C–the finishing temperature of the sausages in the present study), a slight decrease in G´ can be seen in both figures, which may be attributed to the connective tissue and collagen softening at this stage, as observed by Siripurapu et al. (1987) in meat emulsion products. Comparing the two tan δ curves there is a slight shoulder in the starch containing samples at 70˚C, consistent with the gelatinisation temperature of starch.

Curves depicted in these figures show a typical behaviour of thermosetting gelation (protein) and gelatinisation (starch) in the food system similar to that considered in this work (Bruno and Moresi, 2004). The results of the present study support the previous study by Li and Yeh (2001).

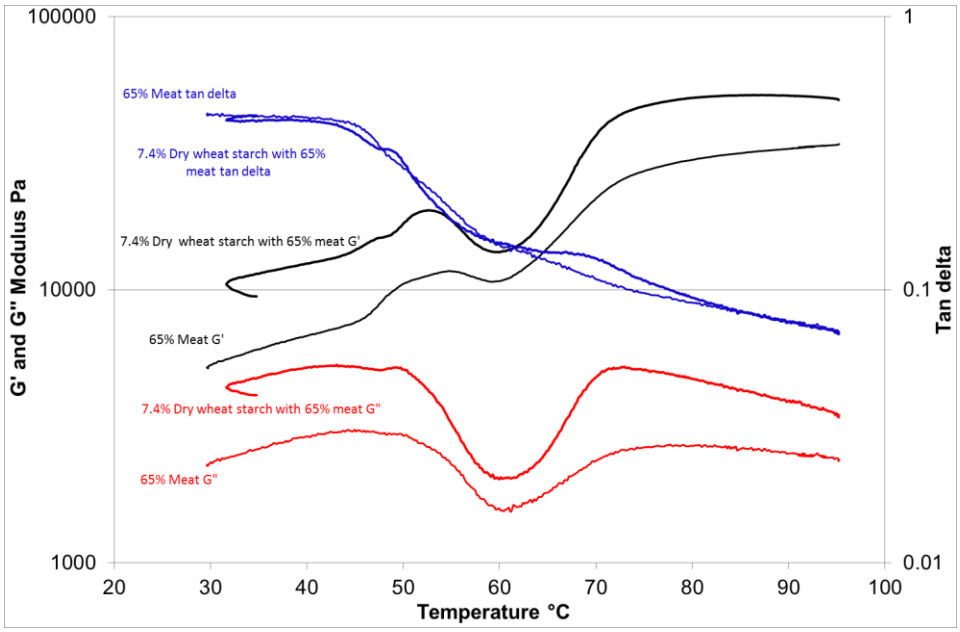


Figure 4.9: A comparison of the storage, loss moduli and phase angle (G´, G˝ and tan δ) when adding 7.4% dry unmodified wheat starch to a 65% meat and control sample (65% meat) as a function of heating. The curve represents the average of three replicate.

4.3.4 Structure of heated meat batters

Confocal scanning laser microscopy (CSLM) is widely used for the study of food microstructure (Blonk and Van Aalst, 1993; Brooker, 1991; Heertje et al., 1987;Vodovotz et al., 1996). Applying CSLM, the microstructure of control samples of beef sausages (65% meat and reference) and 7.4% dry unmodified wheat starch with 65% meat were visualised. In addition, in the food industry, starch is used as a key ingredient in many products for the control of texture. For

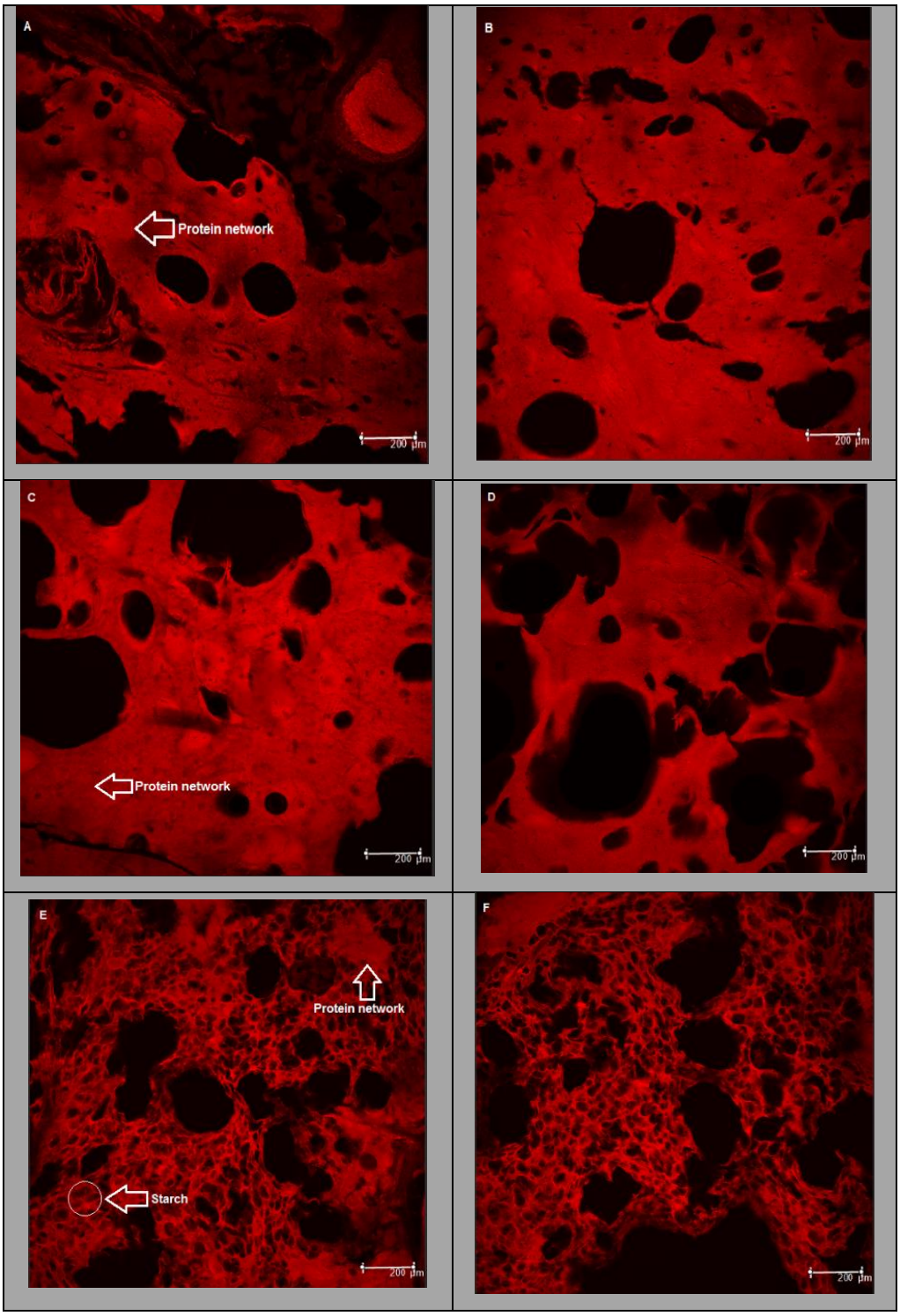
a better understanding of the microstructure is of a meat product, visualisation of this microstate is of great importance. Confocal scanning laser microscopy images were recorded to assess whether changes to the structure of the heated meat products occurred when starches were added. The goal of the visualisation was to determine whether changes occurred in the structure of the protein matrix when adding hydrocolloids or not. Confocal scanning laser microscopy is an effective method to visualise compartmentalisation in a multicomponent system. For instance, CSLM images of the gelatin–maltodextrin mixed system have previously shown phase separation of the PS–PR matrix when salt was added or the pH and temperature altered (Lorén et al., 1999). Figure 4.10 shows CSLM images of the sausages with three different fluorescent colours: BODIPY493/503 is used for fat, safranin O for localised starch and finally Rhodamine B is used for protein in beef sausages.

The black voids in the image are mostly air. Reference control sausage images of (A and B) and 65% meat (C and D) shows that the protein matrix or muscle fibre (red colour) is completely distributed in the system (Figure 4.10). Because of the formulation of the control samples the size of the cavities in the 65% meat (500–550 µm) is larger than those in the reference control (200–300 µm).

Microscopic observations (E and F) clearly show that phase separation between protein aggregation and starch granules takes place. Sausage images (E and F) of samples containing 7.4% dry unmodified wheat starch with 65% meat show that the protein matrix (continuous area) is gradually being decreased, with the addition of 7.4% dry wheat starch in batter creating a protein gel filled with starch granules (Figure 4.10). The size of the cavities decreases when adding 7.4% dry wheat starch (200–250 µm) compared to the control samples of 65% meat (500–550 µm). Van de Velde et al. (2002) reported that the granules of wheat starch in confocal microscopy are between 5 and 45 µm and have a round shape; however, in this study the granules of starch in the protein network are between 40 and 55 µm and have an ovate shape. It seems that small aggregates of partially gelatinised starch granules were dispersed within the protein matrix. The gelatinised starch (round area), was either coated on the peripheral surface of the protein network or was trapped in the protein matrix, because when starch is added in the dry state, the amount of water in the batter is retained mainly by the myofibrillar protein and hence the starch is only entrapped in the protein network (Montero et al., 2000) and for this reason the size of the cavity

decreased. Also, another hypothesis mentioned that during thermal gelation of proteins, water will be entrapped in the protein gel network such that the availability of water for starch gelatinisation could be limited (Appelqvist and Debet, 1997).

The BODIPY493/503 stain was used to visualise the location of the dispersed fat particles. Figure 4.10, images G and H, which are the control samples (65% meat) and the 7.4% dry wheat starch with 65% meat, show that fat coated by proteins continuous phase forming the typical sausage emulsion-like structure. Also, fat is fairly evenly distributed in the protein network throughout the structure. It is interesting to note that the fat droplets are decreased in mean size when dry starch is added to the system. The size of the fat droplet is 50 µm or less; however the fat droplet of control samples are between 60 and 100 µm. Chattong et al., (2007) reported similar results for fat droplets when adding hydrocolloids to the meat system.


A
Protein network
200 µm
B
200 µm
C
Protein network
200 µm
D
200 µm
E
Protein network
Starch
200 µm
F
200 µm

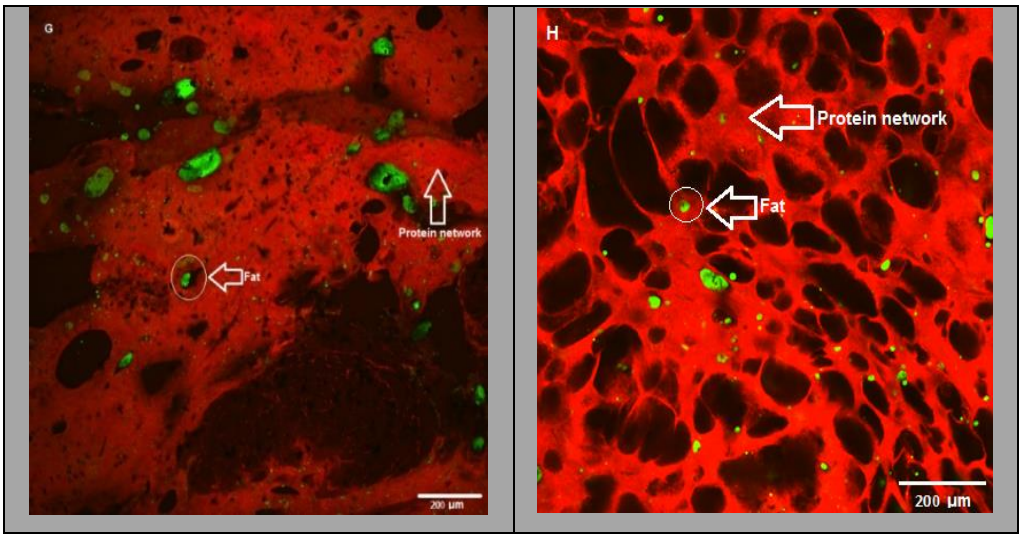


Figure 4.10: CSLM images of sausage containing 7.4% dry unmodified wheat starch to a 65% meat, compared to control samples (65% meat, reference). Images A and B are reference control samples stained for protein matrix; images C and D are the control samples (65% meat) stained for protein matrix; E and F images are related to the 7.4% dry unmodified wheat starch with 65% meat stained for protein matrix and starch; image G is the control sample (65% meat) stained for fat and protein matrix, and finally image H is the 7.4% unmodified dry wheat starch with 65% meat stained for fat and protein matrix.

4.3.5 Visual appearance of sausages

Figure 4.11 shows the visualisation of the 4 samples of the same sausages of the sample made of 7.4% dry unmodified wheat starch with 65% meat (A), compared to 4 same sausages of the control sample, 65% meat (B). The sample made of 7.4% dry starch is hard and acceptable for people in Iran when compared to the control sample (65% meat) because according to the Iranian National Standardization Organization (INSO) 2017, people in Iran like hard sausages. Also, there is a statistically significant difference when comparing the control 65% meat to 7.4% dry starch; however, this similar to the reference control. Furthermore, according to the TPA, the rate of chewiness is high in 7.4% dry starch sausage. The firmness (hardness) of a meat product is important for sliceability and unmodified wheat starch improves the sliceability of meat products.



Figure 4.11: Visual appearance of adding sausage containing 7.4% dry unmodified wheat starch to a 65% meat (A), compared to control samples, 65% meat (B).

4.4 Conclusion

Texture analyser, calorimetric, rheological, and microscopy approaches were applied as a means to better elucidate the underlying mechanisms governing the physico-chemical responses in sausages formulation. The maximum hardness of sausage in all treatments related to 7.4% dry unmodified wheat starch with 65% meat (p-value<0.01). From CSLM it is seem that the starch granules are fillers in the protein matrix, and that they gelatinise during cooking (from DSC and rheology). A reduction in 35% meat (control samples) shows statistically significant differences for the parameters of the hardness and chewiness however, no difference in terms of springiness, cohesiveness and resilience. The addition of 7.4% dry starch to a 35% reduced meat product corrects the differences for hardness and chewiness but in turn creates a difference in cohesiveness and resilience. The state in which the starch is added is also important since pre-hydrated or gelled 7.4% starch retains differences in hardness and chewiness. When the level of added starch is reduced to 3% similarity to the reference control cannot be noticed, other than for cohesiveness

when added dry or gelled. Therefore, the way in which the starch takes up water during gelatinisation in the presence of and surrounded by a protein matrix appears to be critical in affecting the physical structure of a reduced meat protein. The DSC analysis and heat ramps for rheology were correlated, thereby showing complementary protein denaturation and a small aggregation of granules of starch profile and increasing elastic behaviour (G´) (gel hardening). Wheat starch is a non-ionic polymer. Since it does not carry a charge also the pH of meat proteins strongly affected the meat batter, i.e. they carry an overall positive charge below their isoelectric point (approx. 5.0–5.1) and a negative charge above their isoelectronic point. Thus, one can expect that there is not an overall electronic interaction between starch and meat proteins. The DSC for meat/starch samples confirms this idea, which is that in the meat/starch sample a comparison of the enthalpies of transition shows that within the error of measurement, the enthalpies of starch gelatinisation and protein denaturation are linearly additive, hence there is no enthalpic interaction. In comparing the G´ profile between the control sample (65% meat) and the 7.4% unmodified wheat starch test demonstrates the complexity of the multicomponent gel system in terms of structure, and the influence that various ingredient combinations can have on the potential gelling properties of re-formed meats. Furthermore, the data indicate that the G´ profile of the 7.4% dry unmodified wheat starch with 65% meat is higher than that of the control samples with 65% meat. Confocal scanning laser microscopy (CSLM) is a powerful tool for the visualisation of the protein matrix, fat and starch through their final application. The CSLM images showed the phase separation between protein aggregation and starch granules, and the micrographs show that the small aggregates of partially gelatinised starch granules were dispersed within the protein matrix. These results, particularly those of the texture analyser, have important implications for both meat scientists and the meat industry because previous results (BeMiller, 2011) claimed that unmodified starch does not possess the ideal properties for the preparation of food products, especially meat products. However, in this study it has been shown that, depending on the state (dry, pre-hydrated and gelled) and also the concentration of added starch to the meat batter, unmodified wheat starch improves the structure of meat production, making it harder.

Referring to the chapter title, is it clear that gelation did not improve the texture and micro structure of the sausages because as it was mentioned above, the maximum hardness of sausage in all treatments related to 7.4% dry unmodified wheat starch with 65% meat because starch takes up water during gelatinisation and is surrounded by a protein matrix; this kind of sausage, according to the Iranian National Standardization Organization (INSO) 2017, is accepted.

CHAPTER 5 CARRAGEENAN ADDITION TO MEAT BATTER: DOMINATED BY HYDRATION OR GELATION?

5.1 Introduction

In the current developments in food ingredients technology, the availability of functional non-meat proteins (dairy, vegetable and cereal proteins) as well as polysaccharides (starches, carrageenan and gums) for the manufacture of processed meat products has received much attention. This is due primarily to the latter's ability to act as potential binding, extending, and texture modifying and/or nutritional-enhancing adjuncts. When hydrocolloids are used in a meat formulation, a certain amount of water needs to be added. Water addition provides an environment for a desirable texture by interaction with hydrocolloids (Candogan and Kolsarici, 2003). Carrageenans are water-soluble polysaccharides of the hydrocolloid group extracted from red seaweed. The functionality of carrageenan in meat products is related to its thermally reversible gelation properties (Pietrasik, 2003). Carrageenan dissolves throughout the meat during thermal processing and gels on cooling. It improves water retention, consistency and texture of comminuted meat products (Trius et al., 1996). Three fractions of carrageenan exist: kappa (κ-), iota (ι-) and lambda (λ-) differing in sulphate ester and 3, 6 anhydrogalactose content. Kappa and iota are most commonly used in the meat processing industry because of their thickening, gelling and water-binding properties, since lambda is non-gelling (Modliszewski, 1984). Lambda carrageenan could increase the water-holding capacity of meat batter, however it may decrease the gel strength of the cooked product (Hsu and Chung, 2001). Also, lambda may show benefits for retention of moisture, but it is detrimental to the texture of the meat (Foegeding and Ramsey, 1986). The effect of kappa carrageenan and gums on low-fat meat batters have been evaluated and found to improve cooked product hardness, while iota did not. Commercial carrageenans are available in the sodium, potassium and calcium salt forms or most commonly as a mixture of these (Stanley, 1990). Kappa carrageenans form strong and brittle gels that are thermally reversible. Iota carrageenans form weak and elastic gels that are also thermally reversible. Lambda carrageenans thicken but do not form gels. The effect of the addition of carrageenan on the functional properties of meat products has been the subject of numerous studies. Carrageenan is used to improve moisture retention, slicing properties, mouth-feel, cooking yield and juiciness (Phillips and Williams, 2009; Trius et al., 1996 Kappa carrageenan was also found to increase the hardness of meat batter when replacing fat with water-gum solution, whereas iota carrageenan substantially increase the batter's

water holding ability (Barbut and Mittal, 1992; Foegeding and Ramsey, 1986). Furthermore, Bater et al. (1992b) found that kappa carrageenan caused an increase in yield, sliceability and rigidity and a decrease in expressible juice in roasted turkey breasts. Carrageenan is used as a gelling agent in canned meats and pet foods and allows an important reduction in fat content in comminuted meat products like frankfurters (Bernal et al., 1987; Pietrasik and Duda, 2000; Stanley, 1990; Xiong et al., 1999). Despite these published studies, meat processing–particularly beef meat processors–need more scientific data to deliver formulated products that are able to meet the special requirements of consumers. Examples of such requirements may be an improvement in chewiness and cohesiveness of the final product, linking such properties to the product's microstructure. The aim of this study is the influence of the dry, solubilised and gelled of kappa, iota, and lambda carrageenan on meat emulsion, textural properties–such as hardness, springiness, cohesiveness, chewiness and resiliencies in sausage texture formation and microstructure such as location of protein network, carrageenan and fat in cooked sausages. To the best of our knowledge no results are reported regarding the quality characteristics of sausage batter when comparing the addition of dry, solubilised and gelled properties of carrageenan. Furthermore, this study aims to elucidate the role of heat-induced gelation in the texture formation of processed meat products and also involves the investigation of the interaction between meat protein and carrageenan, because understanding the formation of carrageenan/meat composite would be helpful in the development of new products.

5.2 Materials and Methods

Materials and methods used for this study are described in Chapter 3.

5.3 Results and Discussion

5.3.1 Textural properties

Results obtained from the statistical analyses of the collected data are presented in Table 5.1, 5.2, 5.3, 5.4, 5.5 and 5.6.

Table 5.1: Scheffe's test results (p-values) for the hardness of controls (65% meat and reference) with 65% meat containing dry, solubilised and gelled kappa, lambda and iota carrageenan (**<0.01 and *<0.05).

	Reference control	0.5% Dry Kappa with 65% meat	0.5% Solubilised Kappa with 65% meat	0.5% Gelled Kappa with 65% meat	0.5% Dry Iota with 65% meat	0.5% Solubilised Iota with 65% meat	0.5% Gelled Iota with 65% meat	0.5% Dry Lambda with 65% meat	0.5% Solubilised Lambda with 65% meat	1% Dry Kappa with 65% meat	1% Solubilised Kappa with 65% meat	1% Gelled Kappa with 65% meat	1% Dry Iota with 65% meat	1% Solubilised Iota with 65% meat	1% Gelled Iota with 65% meat	1% Dry Lambda with 65% meat	1% Solubilised Lambda with 65% meat	2% Dry Kappa with 65% meat	2% Solubilised Kappa with 65% meat	2% Gelled Kappa with 65% meat	2% Dry Iota with 65% meat	2% Solubilised Iota with 65% meat	2% Gelled Iota with 65% meat	2% Dry Lambda with 65% meat	2% Solubilised Lambda with 65% meat
0.5% Dry Kappa with 65% meat	0.13																								
0.5% Solubilised Kappa with 65% meat	0.01**	0.99																							
0.5% Gelled Kappa with 65% meat	0.99	0.06	0.01**																						
0.5% Dry Iota with 65% meat	0.01**	0.99	0.99	0.01**																					
0.5% Solubilised Iota with 65% meat	0.01**	0.99	0.99	0.01**	0.99																				
0.5% Gelled Iota with 65% meat	0.00**	0.99	0.99	0.01**	0.99	0.99																			
0.5% Dry Lambda with 65% meat	0.31	0.99	0.99	0.22	0.99	0.99	0.99																		
0.5% Solubilised Lambda with 65% meat	0.01**	0.83	0.99	0.01**	0.99	0.99	0.99	0.69																	
1% Dry Kappa with 65% meat	0.99	0.96	0.16	0.99	0.44	0.1	0.37	0.99	0.01**																
1% Solubilised Kappa with 65% meat	0.1	0.99	0.99	0.05	0.99	0.99	0.99	0.99	0.94	0.93															
1% Gelled Kappa with 65% meat	0.99	0.01**	0.01**	0.99	0.01**	0.01**	0.01**	0.01**	0.01**	0.52	0.01**														
1% Dry Iota with 65% meat	0.99	0.87	0.07	0.99	0.24	0.05	0.18	0.98	0.01**	0.99	0.81	0.75													
1% Solubilised Iota with 65% meat	0.45	0.99	0.99	0.36	0.99	0.99	0.99	0.99	0.34	0.99	0.99	0.01**	0.99												
1% Gelled Iota with 65% meat	0.24	0.99	0.99	0.14	0.99	0.99	0.99	0.99	0.63	0.99	0.99	0.01**	0.96	0.99											
1% Dry Lambda with 65% meat	0.17	0.99	0.99	0.08	0.99	0.99	0.99	0.99	0.75	0.98	0.99	0.01**	0.92	0.99	0.99										
1% Solubilised Lambda with 65% meat	0.01**	0.59	0.99	0.01**	0.98	0.99	0.99	0.42	0.99	0.01**	0.81	0.01**	0.01**	0.14	0.36	0.49									
2% Dry Kappa with 65% meat	0.99	0.01**	0.01**	0.99	0.01**	0.01**	0.01**	0.01**	0.01**	0.11	0.01**	0.99	0.26	0.01**	0.01**	0.01**	0.01**								
2% Solubilised Kappa with 65% meat	0.87	0.99	0.97	0.9	0.99	0.93	0.99	0.99	0.18	0.99	0.99	0.06	0.99	0.99	0.99	0.99	0.06	0.00**							
2% Gelled Kappa with 65% meat	0.91	0.01**	0.01**	0.13	0.01**	0.01**	0.01**	0.01**	0.01**	0.01**	0.01**	0.98	0.01**	0.01**	0.01**	0.01**	0.01**	0.99	0.01**						
2% Dry Iota with 65% meat	0.99	0.05	0.01**	0.99	0.01**	0.01**	0.01**	0.16	0.01**	0.99	0.05	0.99	0.99	0.28	0.1	0.06	0.01**	0.99	0.85	0.19					
2% Solubilised Iota with 65% meat	0.01**	0.99	0.99	0.01**	0.99	0.99	0.99	0.99	0.99	0.11	0.99	0.01**	0.05	0.99	0.99	0.99	0.99	0.01**	0.95	0.01**	0.01**				
2% Gelled Iota with 65% meat	0.1	0.99	0.99	0.05	0.99	0.99	0.99	0.99	0.87	0.94	0.99	0.01**	0.83	0.99	0.99	0.99	0.65	0.01**	0.99	0.01**	0.05	0.99			
2% Dry Lambda with 65% meat	0.46	0.99	0.99	0.35	0.99	0.99	0.99	0.99	0.49	0.99	0.99	0.00**	0.99	0.99	0.99	0.99	0.25	0.01**	0.99	0.01**	0.31	0.99	0.99		
2% Solubilised Lambda with 65% meat	0.01**	0.69	0.99	0.01**	0.99	0.99	0.99	0.53	0.99	0.01**	0.87	0.01**	0.01**	0.21	0.46	0.6	0.99	0.01**	0.1	0.01**	0.01**	0.99	0.75	0.34	
65% meat	0.00**	0.99	0.99	0.01**	0.99	0.99	0.99	0.99	0.99	0.2	0.99	0.01**	0.1	0.98	0.99	0.99	0.99	0.01**	0.93	0.01**	0.01**	0.99	0.99	0.99	0.99

Table 5.2: Scheffe's test results (p-values) for the adhesiveness of controls (65% meat and reference) with 65% meat containing dry, solubilised and gelled kappa, lambda and iota carrageenan (**<0.01 and *<0.05).

	Reference control	0.5% Dry Kappa with 65% meat	0.5% Solubilised Kappa with 65% meat	0.5% Gelled Kappa with 65% meat	0.5% Dry Iota with 65% meat	0.5% Solubilised Iota with 65% meat	0.5% Gelled Iota with 65% meat	0.5% Dry Lambda with 65% meat	0.5% Solubilised Lambda with 65% meat	1% Dry Kappa with 65% meat	1% Solubilised Kappa with 65% meat	1% Gelled Kappa with 65% meat	1% Dry Iota with 65% meat	1% Solubilised Iota with 65% meat	1% Gelled Iota with 65% meat	1% Dry Lambda with 65% meat	1% Solubilised Lambda with 65% meat	2% Dry Kappa with 65% meat	2% Solubilised Kappa with 65% meat	2% Gelled Kappa with 65% meat	2% Dry Iota with 65% meat	2% Solubilised Iota with 65% meat	2% Gelled Iota with 65% meat	2% Dry Lambda with 65% meat	2% Solubilised Lambda with 65% meat
0.5% Dry Kappa with 65% meat	0.99																								
0.5% Solubilised Kappa with 65% meat	0.99	0.99																							
0.5% Gelled Kappa with 65% meat	0.99	0.99	0.99																						
0.5% Dry Iota with 65% meat	0.99	0.99	0.99	0.99																					
0.5% Solubilised Iota with 65% meat	0.99	0.99	0.99	0.99	0.94																				
0.5% Gelled Iota with 65% meat	0.99	0.99	0.99	0.99	0.98	0.99																			
0.5% Dry Lambda with 65% meat	0.99	0.99	0.99	0.99	0.94	0.99	0.99																		
0.5% Solubilised Lambda with 65% meat	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99																	
1% Dry Kappa with 65% meat	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99																
1% Solubilised Kappa with 65% meat	0.99	0.99	0.99	0.99	0.99	0.91	0.97	0.91	0.99	0.99															
1% Gelled Kappa with 65% meat	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99														
1% Dry Iota with 65% meat	0.99	0.99	0.99	0.99	0.99	0.97	0.99	0.97	0.99	0.99	0.99	0.99													
1% Solubilised Iota with 65% meat	0.99	0.99	0.99	0.99	0.94	0.99	0.99	0.99	0.99	0.99	0.91	0.99	0.96												
1% Gelled Iota with 65% meat	0.99	0.99	0.99	0.99	0.93	0.99	0.99	0.99	0.99	0.99	0.9	0.99	0.96	0.99											
1% Dry Lambda with 65% meat	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99										
1% Solubilised Lambda with 65% meat	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99									
2% Dry Kappa with 65% meat	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99								
2% Solubilised Kappa with 65% meat	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99							
2% Gelled Kappa with 65% meat	0.99	0.99	0.99	0.99	0.95	0.99	0.99	0.99	0.99	0.99	0.92	0.99	0.97	0.99	0.99	0.99	0.99	0.99	0.99						
2% Dry Iota with 65% meat	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99					
2% Solubilised Iota with 65% meat	0.99	0.99	0.99	0.99	0.98	0.99	0.99	0.99	0.99	0.99	0.04*	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99				
2% Gelled Iota with 65% meat	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99			
2% Dry Lambda with 65% meat	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99		
2% Solubilised Lambda with 65% meat	0.99	0.99	0.99	0.99	0.99	0.97	0.99	0.97	0.99	0.99	0.99	0.99	0.99	0.97	0.96	0.99	0.99	0.99	0.99	0.97	0.99	0.99	0.99	0.99	
65% meat	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99

Table 5.3: Scheffe's test results (p-values) for the springiness of controls (65% meat and reference) with 65% meat containing dry, solubilised and gelled kappa, lambda and iota carrageenan (**<0.01 and *<0.05).

	Reference control	0.5% Dry Kappa with 65% meat	0.5% Solubilised Kappa with 65% meat	0.5% Gelled Kappa with 65% meat	0.5% Dry Iota with 65% meat	0.5% Solubilised Iota with 65% meat	0.5% Gelled Iota with 65% meat	0.5% Dry Lambda with 65% meat	0.5% Solubilised Lambda with 65% meat	1% Dry Kappa with 65% meat	1% Solubilised Kappa with 65% meat	1% Gelled Kappa with 65% meat	1% Dry Iota with 65% meat	1% Solubilised Iota with 65% meat	1% Gelled Iota with 65% meat	1% Dry Lambda with 65% meat	1% Solubilised Lambda with 65% meat	2% Dry Kappa with 65% meat	2% Solubilised Kappa with 65% meat	2% Gelled Kappa with 65% meat	2% Dry Iota with 65% meat	2% Solubilised Iota with 65% meat	2% Gelled Iota with 65% meat	2% Dry Lambda with 65% meat	2% Solubilised Lambda with 65% meat
0.5% Dry Kappa with 65% meat	0.99																								
0.5% Solubilised Kappa with 65% meat	0.99	0.99																							
0.5% Gelled Kappa with 65% meat	0.99	0.99	0.99																						
0.5% Dry Iota with 65% meat	0.99	0.99	0.99	0.99																					
0.5% Solubilised Iota with 65% meat	0.99	0.99	0.99	0.99	0.99																				
0.5% Gelled Iota with 65% meat	0.99	0.99	0.99	0.99	0.99	0.99																			
0.5% Dry Lambda with 65% meat	0.99	0.99	0.99	0.99	0.99	0.99	0.99																		
0.5% Solubilised Lambda with 65% meat	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99																	
1% Dry Kappa with 65% meat	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99																
1% Solubilised Kappa with 65% meat	0.99	0.99	0.04*	0.99	0.99	0.99	0.99	0.99	0.83	0.99															
1% Gelled Kappa with 65% meat	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.98														
1% Dry Iota with 65% meat	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99													
1% Solubilised Iota with 65% meat	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99												
1% Gelled Iota with 65% meat	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99											
1% Dry Lambda with 65% meat	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99										
1% Solubilised Lambda with 65% meat	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99									
2% Dry Kappa with 65% meat	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99								
2% Solubilised Kappa with 65% meat	0.99	0.99	0.71	0.99	0.99	0.99	0.99	0.99	0.94	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99							
2% Gelled Kappa with 65% meat	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99						
2% Dry Iota with 65% meat	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99					
2% Solubilised Iota with 65% meat	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.84	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.95	0.99	0.99				
2% Gelled Iota with 65% meat	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99			
2% Dry Lambda with 65% meat	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99		
2% Solubilised Lambda with 65% meat	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.8	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.93	0.99	0.99	0.99	0.99	0.99	
65% meat	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99

Table 5.4: Scheffe's test results (p-values) for the cohesiveness of controls (65% meat and reference) with 65% meat containing dry, solubilised and gelled kappa, lambda and iota carrageenan (**<0.01 and *<0.05)

	Reference control	0.5% Dry Kappa with 65% meat	0.5% Solubilised Kappa with 65% meat	0.5% Gelled Kappa with 65% meat	0.5% Dry Iota with 65% meat	0.5% Solubilised Iota with 65% meat	0.5% Gelled Iota with 65% meat	0.5% Dry Lambda with 65% meat	0.5% Solubilised Lambda with 65% meat	1% Dry Kappa with 65% meat	1% Solubilised Kappa with 65% meat	1% Gelled Kappa with 65% meat	1% Dry Iota with 65% meat	1% Solubilised Iota with 65% meat	1% Gelled Iota with 65% meat	1% Dry Lambda with 65% meat	1% Solubilised Lambda with 65% meat	2% Dry Kappa with 65% meat	2% Solubilised Kappa with 65% meat	2% Gelled Kappa with 65% meat	2% Dry Iota with 65% meat	2% Solubilised Iota with 65% meat	2% Gelled Iota with 65% meat	2% Dry Lambda with 65% meat	2% Solubilised Lambda with 65% meat
0.5% Dry Kappa with 65% meat	0.99																								
0.5% Solubilised Kappa with 65% meat	0.99	0.99																							
0.5% Gelled Kappa with 65% meat	0.98	0.99	0.35																						
0.5% Dry Iota with 65% meat	0.99	0.99	0.99	0.69																					
0.5% Solubilised Iota with 65% meat	0.02*	0.12	0.01**	0.92	0.01**																				
0.5% Gelled Iota with 65% meat	0.95	0.99	0.21	0.99	0.52	0.97																			
0.5% Dry Lambda with 65% meat	0.99	0.99	0.99	0.99	0.99	0.02*	0.99																		
0.5% Solubilised Lambda with 65% mea	0.99	0.98	0.99	0.29	0.99	0.01**	0.16	0.99																	
1% Dry Kappa with 65% meat	0.99	0.99	0.67	0.99	0.91	0.73	0.99	0.99	0.59																
1% Solubilised Kappa with 65% meat	0.99	0.99	0.99	0.45	0.99	0.01**	0.29	0.99	0.99	0.75															
1% Gelled Kappa with 65% meat	0.99	0.99	0.95	0.99	0.99	0.33	0.99	0.99	0.91	0.99	0.96														
1% Dry Iota with 65% meat	0.99	0.99	0.99	0.62	0.99	0.01**	0.45	0.99	0.99	0.88	0.99	0.99													
1% Solubilised Iota with 65% meat	0.01**	0.01**	0.01**	0.16	0.01**	0.99	0.28	0.01**	0.01**	0.06	0.01**	0.00**	0.01**												
1% Gelled Iota with 65% meat	0.23	0.73	0.01**	0.99	0.00**	0.99	0.99	0.33	0.01**	0.99	0.00**	0.93	0.00**	0.99											
1% Dry Lambda with 65% meat	0.99	0.99	0.99	0.99	0.99	0.02*	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.01**	0.33										
1% Solubilised Lambda with 65% meat	0.99	0.91	0.99	0.08	0.99	0.01**	0.05	0.99	0.99	0.27	0.99	0.67	0.99	0.01**	0.01**	0.99									
2% Dry Kappa with 65% meat	0.99	0.99	0.904	0.99	0.98	0.58	0.99	0.99	0.86	0.99	0.93	0.99	0.98	0.02*	0.98	0.99	0.58								
2% Solubilised Kappa with 65% meat	0.99	0.99	0.99	0.65	0.99	0.01**	0.47	0.99	0.99	0.89	0.99	0.99	0.99	0.01**	0.00**	0.99	0.99	0.98							
2% Gelled Kappa with 65% meat	0.99	0.99	0.71	0.99	0.93	0.69	0.99	0.99	0.64	0.99	0.78	0.99	0.9	0.03*	0.99	0.99	0.3	0.99	0.91						
2% Dry Iota with 65% meat	0.99	0.99	0.99	0.65	0.99	0.01**	0.47	0.99	0.99	0.89	0.99	0.99	0.99	0.01**	0.00**	0.99	0.99	0.98	0.99	0.91					
2% Solubilised Iota with 65% meat	0.84	0.99	0.08	0.99	0.25	0.99	0.99	0.96	0.06	0.99	0.11	0.99	0.2	0.55	0.99	0.96	0.05	0.99	0.22	0.99	0.22				
2% Gelled Iota with 65% meat	0.01**	0.00**	0.01**	0.36	0.01**	0.99	0.54	0.01**	0.01**	0.13	0.01**	0.02*	0.01**	0.99	0.99	0.01**	0.01**	0.07	0.01**	0.11	0.01**	0.8			
2% Dry Lambda with 65% meat	0.99	0.99	0.99	0.99	0.99	0.04*	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.01**	0.46	0.99	0.98	0.99	0.99	0.99	0.99	0.98	0.01**		
2% Solubilised Lambda with 65% meat	0.99	0.99	0.99	0.37	0.99	0.01**	0.23	0.99	0.99	0.69	0.99	0.95	0.99	0.01**	0.01**	0.99	0.99	0.91	0.99	0.73	0.99	0.08	0.01**	0.99	
65% meat	0.99	0.99	0.99	0.99	0.99	0.25	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.00**	0.81	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.02*	0.99	0.99

Table 5.5: Scheffe's test results (p-values) for the chewiness of controls (65% meat and reference) with 65% meat containing dry, solubilised and gelled kappa, lambda and iota carrageenan (**<0.01 and *<0.05).

	Reference control	0.5% Dry Kappa with 65% meat	0.5% Solubilised Kappa with 65% meat	0.5% Gelled Kappa with 65% meat	0.5% Dry Iota with 65% meat	0.5% Solubilised Iota with 65% meat	0.5% Gelled Iota with 65% meat	0.5% Dry Lambda with 65% meat	0.5% Solubilised Lambda with 65% meat	1% Dry Kappa with 65% meat	1% Solubilised Kappa with 65% meat	1% Gelled Kappa with 65% meat	1% Dry Iota with 65% meat	1% Solubilised Iota with 65% meat	1% Gelled Iota with 65% meat	1% Dry Lambda with 65% meat	1% Solubilised Lambda with 65% meat	2% Dry Kappa with 65% meat	2% Solubilised Kappa with 65% meat	2% Gelled Kappa with 65% meat	2% Dry Iota with 65% meat	2% Solubilised Iota with 65% meat	2% Gelled Iota with 65% meat	2% Dry Lambda with 65% meat	2% Solubilised Lambda with 65% meat
0.5% Dry Kappa with 65% meat	0.56																								
0.5% Solubilised Kappa with 65% meat	0.12	0.99																							
0.5% Gelled Kappa with 65% meat	0.99	0.99	0.94																						
0.5% Dry Iota with 65% meat	0.49	0.99	0.99	0.99																					
0.5% Solubilised Iota with 65% meat	0.02*	0.99	0.99	0.55	0.99																				
0.5% Gelled Iota with 65% meat	0.15	0.99	0.99	0.97	0.99	0.99																			
0.5% Dry Lambda with 65% meat	0.85	0.99	0.99	0.99	0.99	0.99	0.99																		
0.5% Solubilised Lambda with 65% meat	0.03*	0.99	0.99	0.7	0.99	0.99	0.99	0.99																	
1% Dry Kappa with 65% meat	0.97	0.99	0.99	0.99	0.99	0.93	0.99	0.99	0.97																
1% Solubilised Kappa with 65% meat	0.99	0.99	0.91	0.99	0.99	0.45	0.94	0.99	0.6	0.99															
1% Gelled Kappa with 65% meat	0.99	0.94	0.4	0.99	0.91	0.06	0.48	0.99	0.11	0.99	0.99														
1% Dry Iota with 65% meat	0.99	0.96	0.51	0.99	0.95	0.1	0.59	0.99	0.17	0.99	0.99	0.99													
1% Solubilised Iota with 65% meat	0.07	0.99	0.99	0.89	0.99	0.99	0.99	0.99	0.99	0.99	0.84	0.28	0.38												
1% Gelled Iota with 65% meat	0.25	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.98	0.67	0.77	0.99											
1% Dry Lambda with 65% meat	0.67	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.97	0.98	0.99	0.99										
1% Solubilised Lambda with 65% meat	0.05	0.99	0.99	0.85	0.99	0.99	0.99	0.99	0.99	0.99	0.77	0.22	0.3	0.99	0.99	0.99									
2% Dry Kappa with 65% meat	0.99	0.71	0.13	0.99	0.63	0.01**	0.17	0.95	0.02*	0.99	0.99	0.99	0.99	0.07	0.32	0.82	0.06								
2% Solubilised Kappa with 65% meat	0.99	0.14	0.00**	0.99	0.1	0.01**	0.00**	0.5	0.01**	0.81	0.99	0.99	0.99	0.00**	0.05	0.23	0.01**	0.99							
2% Gelled Kappa with 65% meat	0.99	0.26	0.02*	0.99	0.21	0.01**	0.02*	0.67	0.01**	0.92	0.99	0.99	0.99	0.00**	0.06	0.39	0.00**	0.99	0.99						
2% Dry Iota with 65% meat	0.99	0.28	0.02*	0.99	0.22	0.01**	0.02*	0.69	0.00**	0.93	0.99	0.99	0.99	0.00**	0.06	0.41	0.00**	0.99	0.99	0.99					
2% Solubilised Iota with 65% meat	0.01**	0.99	0.99	0.55	0.99	0.99	0.99	0.99	0.99	0.93	0.45	0.06	0.09	0.99	0.99	0.99	0.99	0.01**	0.01**	0.01**	0.01**				
2% Gelled Iota with 65% meat	0.05	0.99	0.99	0.83	0.99	0.99	0.99	0.99	0.99	0.99	0.76	0.22	0.3	0.99	0.99	0.99	0.99	0.06	0.00**	0.00**	0.00**	0.99			
2% Dry Lambda with 65% meat	0.77	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.91	0.38	0.55	0.58	0.99	0.99		
2% Solubilised Lambda with 65% meat	0.02*	0.99	0.99	0.57	0.99	0.99	0.99	0.99	0.99	0.93	0.47	0.07	0.11	0.99	0.99	0.99	0.99	0.01**	0.01**	0.01**	0.01**	0.99	0.99	0.99	
65% meat	0.41	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.84	0.89	0.99	0.99	0.99	0.99	0.59	0.14	0.23	0.25	0.99	0.99	0.99	0.99

Table 5.6: Scheffe's test results (p-values) for the resilience of controls (65% meat and reference) with 65% meat containing dry, solubilised and gelled kappa, lambda and iota carrageenan (**<0.01 and *<0.05).

	Reference control	0.5% Dry Kappa with 65% meat	0.5% Solubilised Kappa with 65% meat	0.5% Gelled Kappa with 65% meat	0.5% Dry Iota with 65% meat	0.5% Solubilised Iota with 65% meat	0.5% Gelled Iota with 65% meat	0.5% Dry Lambda with 65% meat	0.5% Solubilised Lambda with 65% meat	1% Dry Kappa with 65% meat	1% Solubilised Kappa with 65% meat	1% Gelled Kappa with 65% meat	1% Dry Iota with 65% meat	1% Solubilised Iota with 65% meat	1% Gelled Iota with 65% meat	1% Dry Lambda with 65% meat	1% Solubilised Lambda with 65% meat	2% Dry Kappa with 65% meat	2% Solubilised Kappa with 65% meat	2% Gelled Kappa with 65% meat	2% Dry Iota with 65% meat	2% Solubilised Iota with 65% meat	2% Gelled Iota with 65% meat	2% Dry Lambda with 65% meat	2% Solubilised Lambda with 65% meat
0.5% Dry Kappa with 65% meat	0.99																								
0.5% Solubilised Kappa with 65% meat	0.99	0.99																							
0.5% Gelled Kappa with 65% meat	0.79	0.99	0.58																						
0.5% Dry Iota with 65% meat	0.99	0.99	0.99	0.16																					
0.5% Solubilised Iota with 65% meat	0.00**	0.08	0.01**	0.97	0.01**																				
0.5% Gelled Iota with 65% meat	0.89	0.99	0.76	0.99	0.29	0.92																			
0.5% Dry Lambda with 65% meat	0.99	0.99	0.99	0.89	0.99	0.01**	0.96																		
0.5% Solubilised Lambda with 65% mea	0.99	0.92	0.99	0.06	0.99	0.01**	0.12	0.99																	
1% Dry Kappa with 65% meat	0.98	0.99	0.94	0.99	0.61	0.7	0.99	0.99	0.31																
1% Solubilised Kappa with 65% meat	0.99	0.99	0.99	0.99	0.99	0.02*	0.99	0.99	0.99	0.99															
1% Gelled Kappa with 65% meat	0.95	0.99	0.87	0.99	0.45	0.83	0.99	0.98	0.2	0.99	0.99														
1% Dry Iota with 65% meat	0.99	0.87	0.99	0.05	0.99	0.01**	0.05	0.99	0.99	0.17	0.99	0.09													
1% Solubilised Iota with 65% meat	0.01**	0.01**	0.01**	0.37	0.01**	0.99	0.22	0.01**	0.01**	0.06	0.01**	0.12	0.01**												
1% Gelled Iota with 65% meat	0.22	0.9	0.05	0.99	0.00**	0.99	0.99	0.24	0.00**	0.99	0.63	0.99	0.01**	0.94											
1% Dry Lambda with 65% meat	0.99	0.99	0.99	0.82	0.99	0.01**	0.92	0.99	0.99	0.99	0.99	0.97	0.99	0.01**	0.16										
1% Solubilised Lambda with 65% meat	0.99	0.71	0.99	0.00**	0.99	0.01**	0.02*	0.99	0.99	0.07	0.97	0.05	0.99	0.01**	0.01**	0.99									
2% Dry Kappa with 65% meat	0.99	0.99	0.98	0.99	0.79	0.67	0.99	0.99	0.47	0.99	0.99	0.99	0.32	0.06	0.99	0.99	0.17								
2% Solubilised Kappa with 65% meat	0.99	0.99	0.99	0.99	0.99	0.11	0.99	0.99	0.88	0.99	0.99	0.99	0.81	0.01**	0.94	0.99	0.62	0.99							
2% Gelled Kappa with 65% meat	0.64	0.99	0.39	0.99	0.04*	0.99	0.99	0.75	0.02*	0.99	0.96	0.99	0.00**	0.56	0.99	0.65	0.01**	0.99	0.99						
2% Dry Iota with 65% meat	0.99	0.93	0.99	0.05	0.99	0.01**	0.07	0.99	0.99	0.24	0.99	0.14	0.99	0.01**	0.01**	0.99	0.99	0.42	0.88	0.01**					
2% Solubilised Iota with 65% meat	0.7	0.99	0.45	0.99	0.04*	0.99	0.99	0.81	0.03*	0.99	0.97	0.99	0.00**	0.49	0.99	0.71	0.00**	0.99	0.99	0.99	0.02*				
2% Gelled Iota with 65% meat	0.01**	0.00**	0.01**	0.62	0.01**	0.99	0.43	0.01**	0.01**	0.17	0.01**	0.28	0.01**	0.99	0.99	0.01**	0.01**	0.16	0.00**	0.8	0.01**	0.74			
2% Dry Lambda with 65% meat	0.99	0.99	0.99	0.94	0.99	0.00**	0.98	0.99	0.99	0.99	0.99	0.99	0.99	0.01**	0.33	0.99	0.99	0.99	0.99	0.84	0.99	0.88	0.01**		
2% Solubilised Lambda with 65% meat	0.99	0.99	0.99	0.39	0.99	0.01**	0.56	0.99	0.99	0.83	0.99	0.71	0.99	0.01**	0.05	0.99	0.99	0.92	0.99	0.23	0.99	0.28	0.01**	0.99	
65% meat	0.99	0.99	0.99	0.99	0.99	0.04*	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.00**	0.79	0.99	0.99	0.99	0.99	0.98	0.99	0.98	0.00**	0.99	0.99

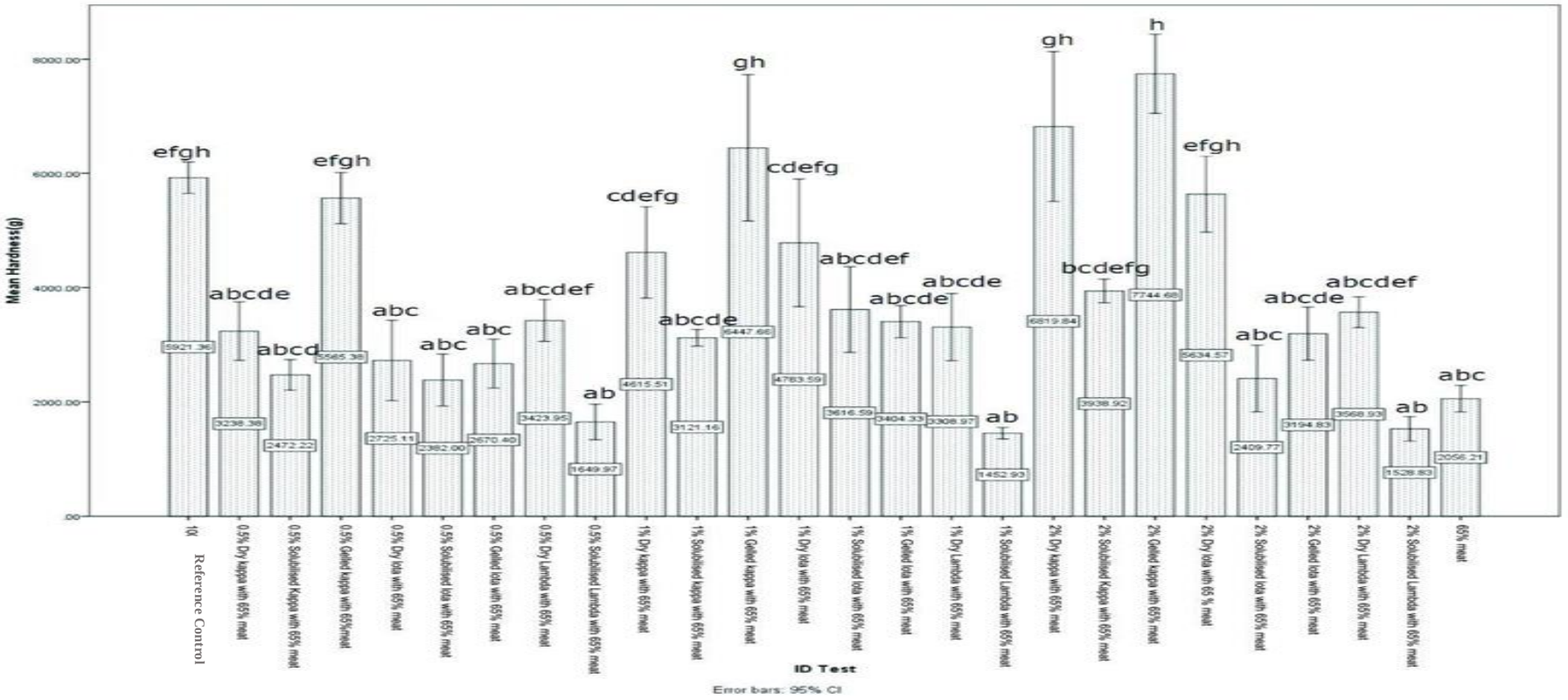


Figure 5.1: Hardness of adding dry, solubilised, and gelled carrageenan (kappa, lambda and iota) to a 65% meat, compared to control samples (65% meat and reference).

The general linear model ANOVA was utilized to test for differences between treatments (ID-Test) in the hardness. Analysis of variance (ANOVA) showed that at least one ID-Test mean was different from the others. Hence, Scheffe's test (Table 5.1) was applied in order to compare the means of the hardness ID-Test in a pair-wise fashion.

First of all, in terms of the hardness, it was shown in chapter 4 that the reference control and 65% meat are statistically different. After that, we compared the 65% meat control to the other treatments, and the result showed that 65% meat was significantly different from samples with gelled kappa added at all concentrations used and upon addition of both kappa and iota when added dry at concentration of 2%. All of these samples were not significantly different to the reference control. In addition, by choosing the 0.5% concentration and comparing them with each other, it was found that there was a significant difference between 0.5% gelled kappa and the other treatments except 0.5% dry lambda. At the 1% addition level gelled kappa was not significantly different to dry kappa and dry iota. These three treatment were also not significantly different when the concentration was increased to 2%. Solubilising all carrageenan types doesn't seem to enhance hardness values at all concentrations used, with all samples being statistically not different to the 65% meat control. According to the literature (Llanto et al., 1990), it is reported that the increased hardness of surimi, which occurs when dry carrageenan is added, is due to the interaction of the carrageenan's sulphate groups with the myofibrillar protein. It is worth mentioning that during processing and extraction of carrageenan, most of the factories add potassium to better the extraction. Foegeding and Ramsey (1987) also found that dry kappa carrageenan increased the hardness of beef gel more effectively than did iota carrageenan. Ayadi et al., (2009) reported that dry carrageenan caused an increase in the hardness and cohesiveness of the formulated sausage samples. However, in this study we showed that the second hardness of sausages is related to dry and the first and third hardness happened when gelled is added which are 2 and 1%. The highest level of hardness was measured for 2% gelled kappa carrageenan because in this range 'tiger stripes' occur (see section 6.3.5), which means that the particles of gel are not evenly dispersed within the meat structure and this is very clear to see in the final

product and it is for this reason that the meat is harder. The hardness value measured here are similar to those of 7.4% dry starch (discussed in previous chapter) and the gelled carrageenan pieces are analogous to the gelatinising starch granules. This is the case between 0.5-2%, and also when dry kappa and iota are added they may hydrate and swell upon cooking in a similar way to starch. 1% gelled kappa is third hardness of product and this is maybe due to the due to kappa carrageenan gel, caused kappa to drape with the water network, and when added in the meat system, the water is not competed with meat and caused water holding capacity (WHC), which is the ability of meat product to hold on to its own water, of beef meat sausages improved. Softer products are created when the carrageenans are solubilised, indicating a more uniform distribution of the carrageenan occurs within the batter. In previous findings, Barbut and Mittal (1992) reported that kappa carrageenan improves the tenderness of beef sausages and Trius et al. (1995) also showed that the softest texture occurred when iota carrageenan was added to the meat batter; however, in this study we showed that the solubilised of lambda carrageenan improves tenderness; since lambda carrageenan is not a gelling gum, but a thickener, it probably caused the formation of a weak matrix when the meat protein was gelled. Trius et al. (1994a) also observed that the addition of lambda carrageenan produced the softest texture.

Ayadi et al., (2009) found that variations of carrageenan concentration from 0% to 0.5% had no significant effect on sausage hardness and it is for this reason that in this study 0.5% was chosen the lowest boundary concentration of carrageenan in the batter. Also, Ayadi et al. (2009) claimed that when dry kappa carrageenan reaches 0.8% and 1.5%, there is a significant increase in sausage hardness. However, in this study it is shown that when dry kappa carrageenan is added at 0.5 to 2% there is significant increase in sausage hardness. Xiong et al. (1999) reported that adding 1% dry kappa carrageenan caused an increase in the hardness of the sausages. Additionally, in this study it is shown that adding 2% dry kappa also increase the hardness of sausage. Furthermore, Hsu and Chung (2001) reported that the addition of dry kappa carrageenan at levels of less than 2% significantly affected hardness, adhesiveness, chewiness, and gumminess. Also, Bloukas et al. (1997) reported that by increasing the level of kappa

carrageenan from 0 to 1%, the hardness of sausages increases and this is the same line with this study. Moreover, Cierach et al. (2009) reported that adding 0.41% and 0.5% dry kappa carrageenan in meat batter causes an increase in hardness, gumminess and chewiness however, in this study showed that less than 0.5% the texture is too watery (see section 3.1.2). In addition, Pietrasik (2003) observed that using 0.5% carrageenan caused increased hardness and fracturability of beef gels, but was unable to improve springiness or cohesiveness; and finally, García-García and Totosaus (2008) reported that kappa carrageenan improved textural properties such as hardness and cohesiveness and this is again same line with this study.

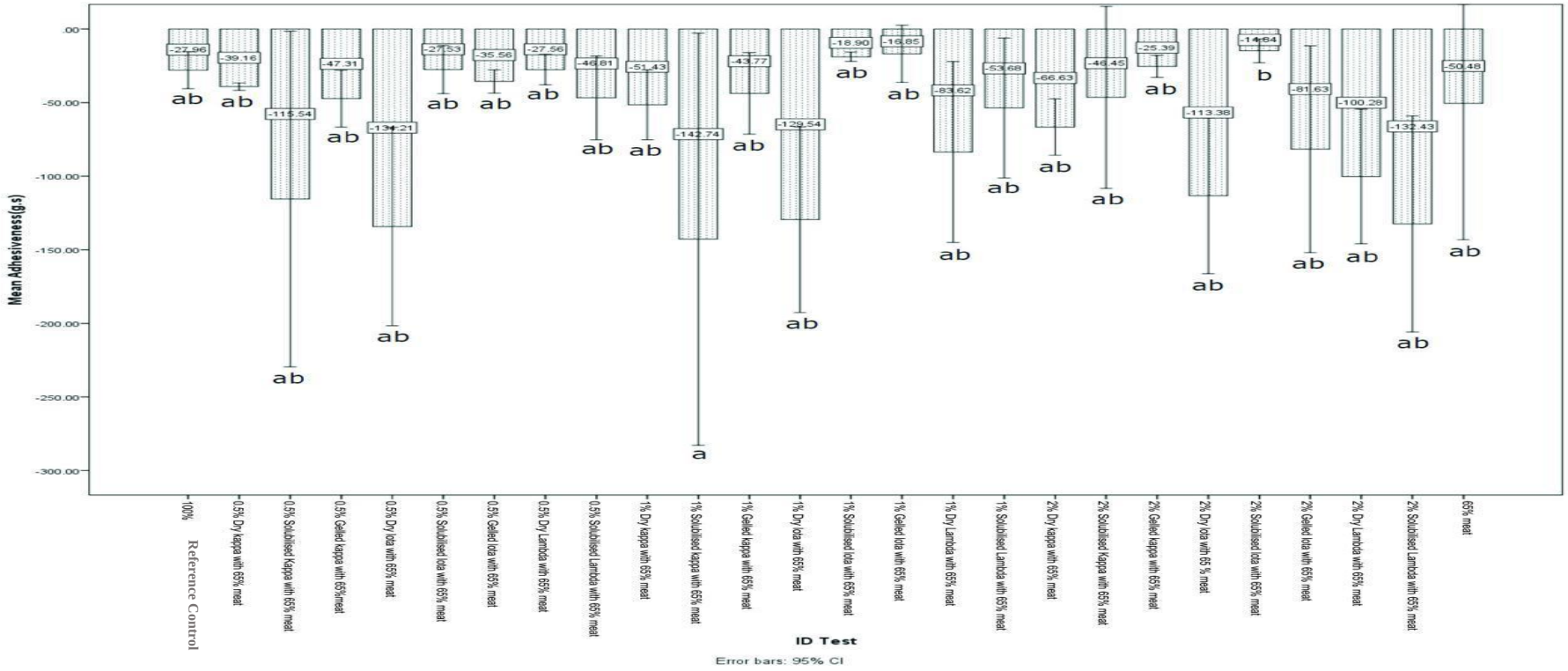


Figure 5.2: Adhesiveness of adding dry, solubilised, and gelled carrageenan (kappa, lambda and iota) to a 65% meat, compared to control samples (65% meat and reference).

Analysis of variance (ANOVA) showed that adhesiveness differed significantly by ID-Test types. Thus, Scheffe's test was applied in order to compare the means of the ID-Test of adhesiveness pair-wise (Table 5.2).

Adhesiveness is more of a surface characteristic and depends on an effect of cohesiveness force, as well as other characteristics, including viscosity and viscoelasticity (Adhikari et al., 2001). First of all, in terms of the adhesiveness, when comparing the control sample, although the error bar for the 65% meat product were the large the result show that the products are not significantly different. Indeed all samples were not seen to be different, due to high error in the measurement of adhesiveness. However, samples which stand out with higher means are solubilised kappa at 0.5 and 1% and dry iota at three concentrations. Solubilised lambda also gave a high mean adhesiveness. These results indicate that adhesiveness is not a good parameter to use to differentiate between samples and that a more uniform distribution of carrageenan in the batter initiates a more adhesive sausage. Ulu (2006) reported that with the addition of 1% dry carrageenan, adhesiveness is increased. However, in this study we showed that adhesiveness improved with the level of the dry kappa carrageenan. Eiji Niwa et al. (1988) reported high adhesiveness occurred in surimi when dry kappa carrageenan was added, but in this study the adhesiveness of sausage increased in the wake of adding solubilised of kappa carrageenan with a concentration of 1%, since upon heating the kappa carrageenan and added it to meat batter, kappa carrageenan bound with large amount of water and consequently the water-holding capacity of sausages increased, which resulted in the adhesiveness of the sausages being increased.

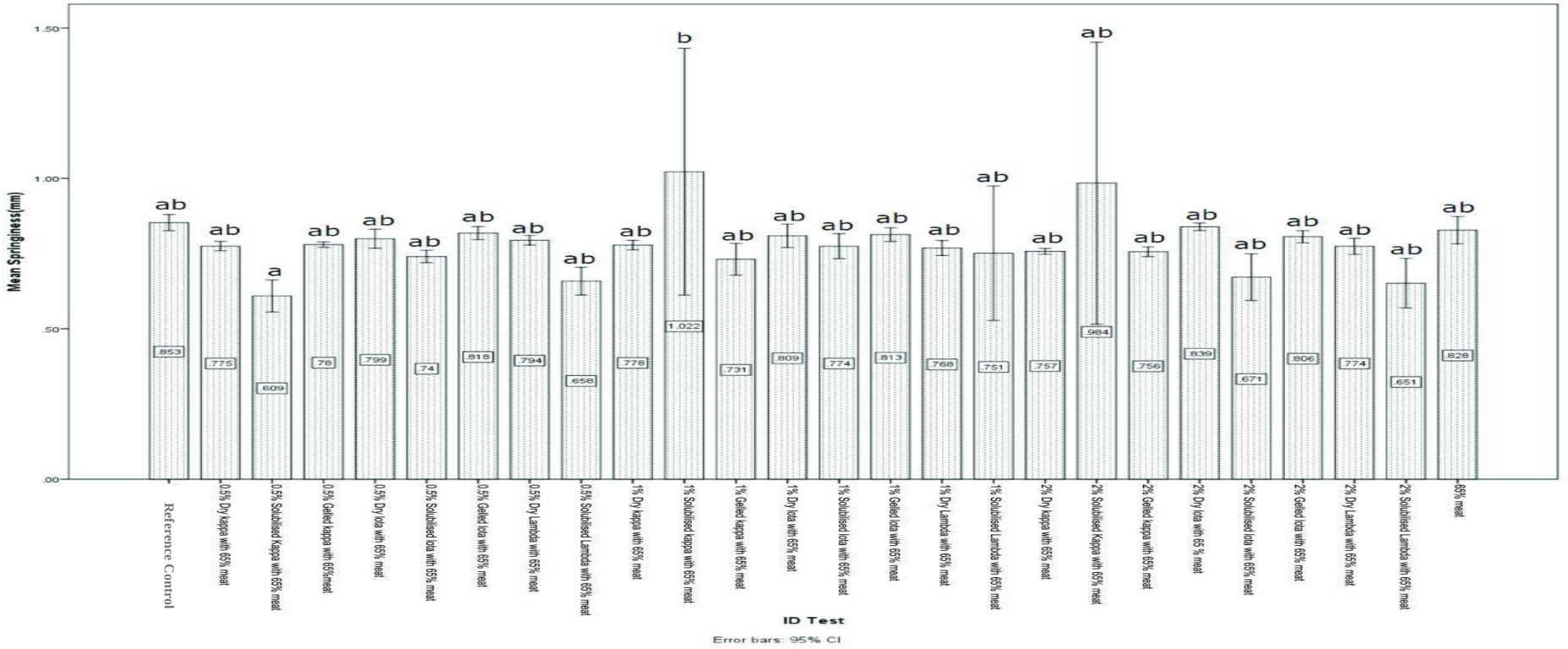


Figure 5.3: Springiness of adding dry, solubilised, and gelled carrageenan (kappa, lambda and iota) to a 65% meat, compared to control samples (65% meat and reference).

From Figure 5.3 it can be seen that, as for starch treatments, there is no significant in springiness across all carrageenan treatments. Barbut and Mittal (1992) reported that adding dry kappa carrageenan to beef meat batter does not improve the springiness of the product and this is same line with this study. In some factories, during the processing of meat, carrageenan is first dispersed in a hot brine solution (solubilised), then introduced into the meat by injecting and massaging, and then dissolved and distributed throughout the meat by thermal processing (Bater et al., 1993). During the cooling of cooked products, the carrageenan solution together with the meat exudate form a firm, cohesive gel structure (Trudso, 1985). However, this study has shown that independent of carrageenan type, usage level and mode of addition to the product springiness value, compared to reference control and a 35% reduced meat products are not significantly different.

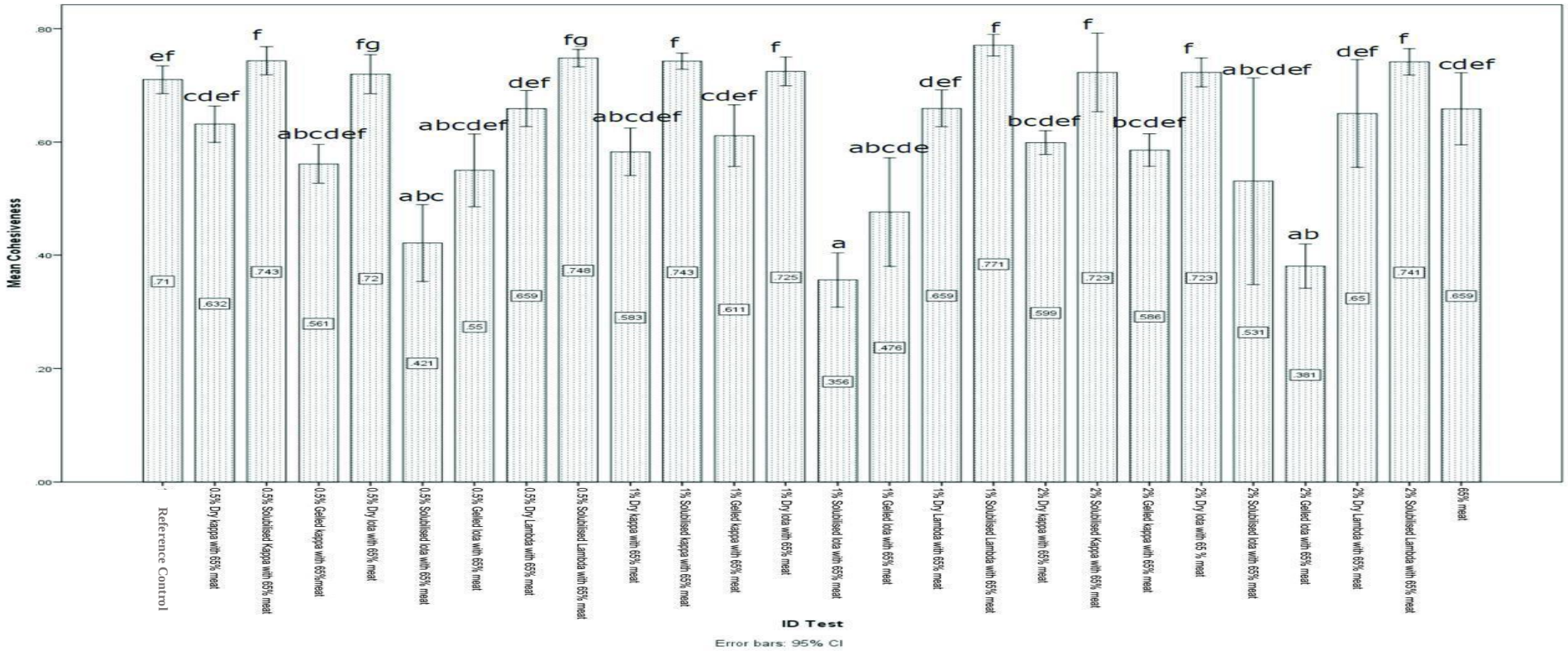


Figure 5.4: Cohesiveness of adding dry, solubilised, and gelled carrageenan (kappa, lambda and iota) to a 65% meat, compared to control samples (65% meat and reference).

Analysis of variance (ANOVA) showed that cohesiveness differed significantly by ID-Test types. Thus, Scheffe's test was applied in order to compare the means of the ID-Test of cohesiveness pair-wise (Table 5.4).

In terms of the cohesiveness, when comparing the control samples, the results showed that reference control and 65% were not significantly different. All samples show no significant difference from the controls except for 0.5% and 1% solubilised iota and 1 and 2% gelled iota and also showing lower mean values of cohesiveness were the solubilised and gelled samples. Ayadi et al. (2009) found that cohesiveness increased continually with increases in the addition of dry carrageenan concentration, except at the concentration of 0.5% and 1%, when cohesiveness decreased slightly. However, it is interesting to note that in this study, cohesiveness decreased with solubilised of iota carrageenan, while not significantly different to the controls the higher mean cohesiveness values were obtained for solubilised kappa (all concentrations), dry iota (all concentrations) and solubilised lambda (all concentrations). In general, the increase in cohesiveness and firmness normally associated with fat reduction in processed meat products and can be offset by increasing the amount of added water (Claus et al., 1990). Again the parameter of cohesiveness seems to be dominated by a well dispersed/uniform distribution of carrageenan in the meat batter.

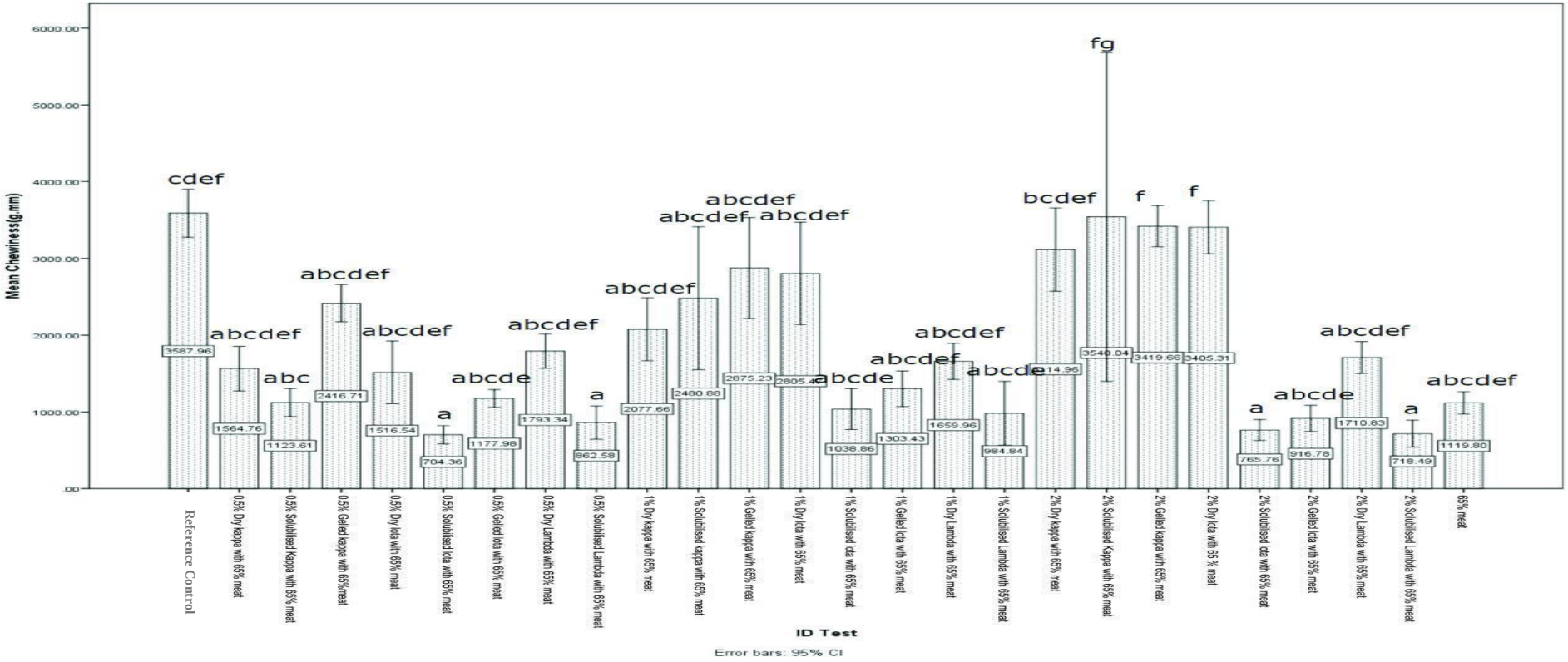


Figure 5.5: Chewiness of adding dry, solubilised, and gelled carrageenan (kappa, lambda and iota) to a 65% meat, compared to control samples (65% meat and reference).

Analysis of variance (ANOVA) showed that chewiness differed significantly by ID-Test types. So, Scheffe's post hoc test was conducted in order to compare the means of the ID-Tests of chewiness in a pair-wise manner (Table 5.5).

In terms of the chewiness, when comparing the control samples, the result show that they are not significantly different. The treatment at 35% reduction in meat control that were all statistically not different to the other treatments. The addition of solubilised kappa, lambda and iota in concentration of 0.5 and 2 % was significantly different to reference. Indeed, the reference control is the highest mean value of all treatments. In this study, the maximum chewiness related to the reference control sample. Ayadi et al. (2009) reported that chewiness increased with kappa carrageenan concentration and the results from this study confirm that kappa carrageenan (2% solubilised, gelled and dry with 65% meat), after the reference control sample, has the highest chewiness, except for 2% dry of iota with 65% meat, compared to the other types of carrageenan and concentrations.

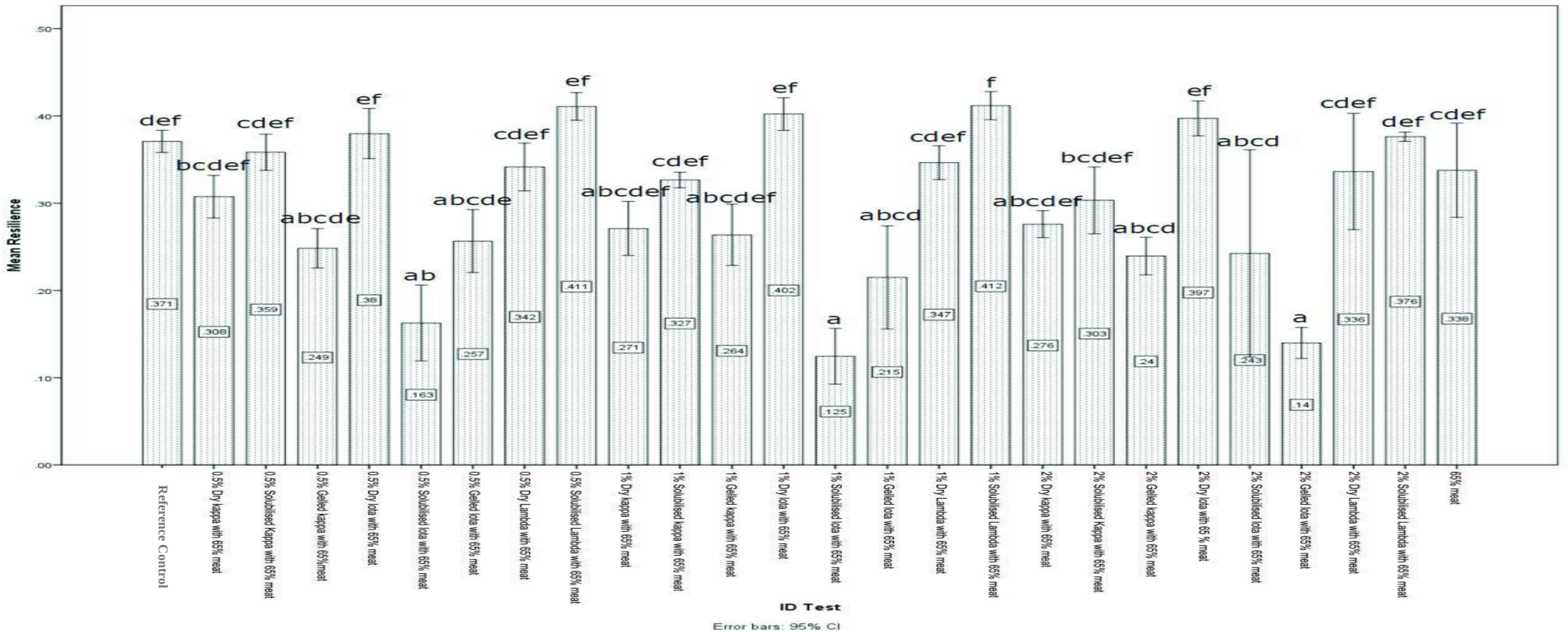


Figure 5.6: Resilience of adding dry, solubilised, and gelled carrageenan (kappa, lambda and iota) to a 65% meat, compared to control samples (65 % meat and reference).

Analysis of variance (ANOVA) showed that resilience differed significantly by ID-Test types; hence, Scheffe's post hoc test was run in order to come up with pair-wise comparisons of the means of the ID-Tests of resilience (Table 5.6).
In terms of the resilience, when comparing the control samples to each other the result shows that they are not significantly different. After that, we compared 65% meat to the other treatments, and the results showed that the control is statistically significant difference to 0.5 and 1% solubilised iota and 2% gelled iota. The higher concentration (2%) of solubilised iota had large error bars, but wasn't significantly different, however it did have a low mean resilience, similar to the lower concentrations. All other treatments were statistically not different to the controls. However, with respect to trends the dry iota (at all concentrations) and the solubilised lambda at 0.5 and 1% had the highest mean score for resilience.

5.3.2 Differential scanning calorimetry

In this section the functions of gelled kappa carrageenan (2% with 65% meat) on the formulation of sausage batter composite during heating were investigated and as a model to try to explain how the other treatments, described in the previous section, are functioning. Changes in the thermal denaturation of meat protein that occur upon the addition of non-meat ingredients may indicate shifts in the physical state or interaction between meat proteins and non-meat ingredients. The most important heat-induced process in a meat system is thermal denaturation of protein because it initiates other molecule processes, such as protein–protein aggregation and gelation. Figure 5.7 illustrated the study of DSC initial scanning of sausage treatments. As previously described in chapter 4, the transition temperature for muscle protein can range from 43 to 67˚C for myosin, 67 to 69˚C for sarcoplasmic protein and 71 to 83˚C for actin; the exact temperatures are subject to pH and salt conditions (Findlay et al., 1990; Smyth et al., 1996; Wright et al., 1977; Xiong et al., 1987). It is also seen that a second heating of this sample shows there to be no protein related transitions, as expected and indeed upon first cooling only the treatments related to fat are evident (Figure 5.8). While, as previously shown, the protein transitions of a processed 65% meat control are shifted to merge the actin and myosin peaks

because NaCl plays an important role in the binding priorities of meat, and in addition solubilisation and extraction of the meat protein is usually achieved through the addition of salt (Blanshard and Mitchell, 1988; Ruusunen and Puolanne, 2005). Also, the addition of salt (NaCl) was found to reduce the stability of meat protein (DeFreitas et al., 1997b; Ensor et al., 1991; Shiga et al., 1988). Furthermore, phosphate enhances the effect of salt (Knipe et al., 1985). A cooling of this material also confirms that only fat transitions are seen for processed meat. Unstable forms can form on cooling such as α- and β′- forms of the fat (Macnaughtan et al., 2006). The α-form is formed on rapid cooling; however, there is a higher activation energy barrier to the formation of the β and β′ forms, therefore these do not crystallise at the same time. Initial scanning of 35% reduced meat sample with 2% gelled kappa carrageenan indicates that there are two peaks that can occur, which the actin and myosin peaks similar to the processed control. At temperatures higher than the proteins transitions a very noisy baseline might indicate of the helix-coil transition of carrageenan. When kappa carrageenan is cooled, the kappa carrageenan molecules in the random coil form transform to a double helical configuration (Ikeda and Kumagai, 1998; Nilsson and Piculell, 1991). Upon first cooling the coil-helix transition is clearly evident (Figure 5.8) with clear helix melting upon second heating. Nishinari et al. (1990) reported a similar trace for kappa carrageenan. Figure 5.9 and 5.10 showed the transitions observed in the meat protein (reference control) and dry carbohydrate (1% iota; 0.5 and 2 % lambda carrageenan) which showed greatest similarity with the reference control. As can be seen in Figure 5.9 the top trace shows the transitions occurring in reference control. It would be expected that the 2nd trace from the top which shows the results for 65% meat (and 1% dry iota carrageenan) would have a lower value for the enthalpy of transition of the proteins assuming no interaction and a linear reduction according to content. The helix to coil transitions in carrageenan are dependent on the sulphate content. Kappa carrageenan has the lowest content of sulphate and this results in an intense transition in the region of 84.4˚C at a salt content of 2.7%. Dry iota carrageenan shows a transition in the region of 90˚C (here at a salt composition and content of sodium triphosphate and sodium chloride at 2.7% and 0.6% respectively). Lambda carrageenan does not show a transition. All the transitions

are reversible unlike the protein denaturation transitions. In the second from top trace where the meat sample contains 1% dry iota carrageenan, an endothermic transition occurs at between 85 and 90˚C as shown by the arrow. The first cooling trace on Figure 5.10 shows an exothermic transition occurring at around 85˚C. A second reheat (6^{th} curve down on Figure 5.9) shows the presence of the high temperature transition due to the carbohydrate but a complete absence of the irreversible denaturation due to the protein transition. Similarly in Figure 5.10, the first and 4^{th} curves from the top show reversible exothermic transition of iota carrageenan on cooling. The meat sample with lambda carrageenan shows no additional transitions to the denaturation peak of the protein. Table 5.7 and 5.8 show that the carrageenan shows a thermal hysteresis of ~20˚C, indicating that in the meat batter/cooked batter environment the carrageenan double helices are forming aggregates. Interestingly, when the gel that is added to the meat is scanned on its own the thermal transitions are ~30˚C higher when in the meat, also showing a much smaller thermal hysteresis (~12˚C). Clearly, in the presence of meat protein, the processing salt are also present, so their effects on the thermal transition of kappa carrageenan were investigated. Their presence increases the helix-coil transition by ~19˚C, within a thermal hysteresis of ~17˚C. This is in line with previous findings (Viebke et al., 1998). Also, Watase et al. (1990), reported that the endothermic peak temperatures accompanying gel-to-sol transition shifted to a higher temperature with the added concentration of salt. This has been attributed to the shielding of the electrostatic repulsion of sulphate groups in kappa carrageenan molecules by ions, as in the case of the addition of sodium chloride. It has been reported that when using sodium chloride in kappa carrageenan gel, the number of junction zones increases, and because of this the melting point shifted to the higher temperature (del Carmen Núñez-Santiago and Tecante, 2007). Also, the addition of salts such as NaCl, KCl and $CaCl_2$, appears to improve the gel or elastic modulus of kappa carrageenan gels through enhancing conformation ordering and subsequent aggregation (Braudo, 1992). Bater et al. (1992a); Shand et al. (1994) concluded that the peak transition temperatures of kappa carrageenan treatments were increased by the inclusion of salt and sodium triphosphate. From these results it becomes evident that 1) the addition of gelled kappa carrageenan in to the meat

'process' affects the carrageenan gel added, causing a noisy first DSC heating baseline, an indication of heterogeneous gel structures, and 2) the presence of protein and processing salts elevates the transition temperature beyond that of the starting gel structure and beyond that of the influence of just the salts alone. When heating and cooling DSC curves are compared, the exothermic peak due to sol–gel transition was narrow and the endothermic peak broad. Quantitative determination of heat of transition (ΔH) is a useful tool for studying protein denaturation and also for the study of the interaction between protein–polysaccharide (Findlay and Stanley, 1984a, 1984b; Findlay et al., 1986; Wagner and Anon, 1985a, 1985b). Temperature profile and transition enthalpies (Table 5.7) indicate that the sample of reheating 2% gelled Kappa carrageenan with 65% meat has the largest endothermic heating parameter (ΔH). Shand et al. (1994) reported that when added to meat batter, dry kappa carrageenan had a very small effect on the thermal transition temperature of beef; however, in this study it has been shown that the addition of gelled kappa carrageenan to meat batter resulted in the occurrence of a broad thermal transition. DeFreitas et al. (1997b) reported that kappa carrageenan did not affect the thermal denaturation of myofibrillar meat proteins in the presence of salt; however, in this study it seems that in the presence of salt, carrageenan will not swell completely until after the proteins have begun gelling, isolating the carrageenan particle within the protein gel matrix. This is supported in previous findings (Norton et al., 1984), because salt concentration, which causes an increase in ionic strength, may delay the solubilised kappa carrageenan in the meat system. Furthermore, Trius (1996) recommended that carrageenan be added to meat batter after the dissociation of salt and phosphate. Under these ionic conditions, the hydration of carrageenan is inhibited and therefore meat batter viscosity is kept to a minimum (Tye, 1988). Within the error of measurement it seems that a true interaction between carrageenan and myofibril protein in the formulation of a gel network was unlikely to occur. However it is very difficult to say definitely whether there is any interaction between carrageenan and the protein, and this is supported from a previous study (Bernal et al., 1987). Also, Verbeken et al. (2005) reported that dry carrageenan does not seem to interact with meat protein in the formulation of a network.

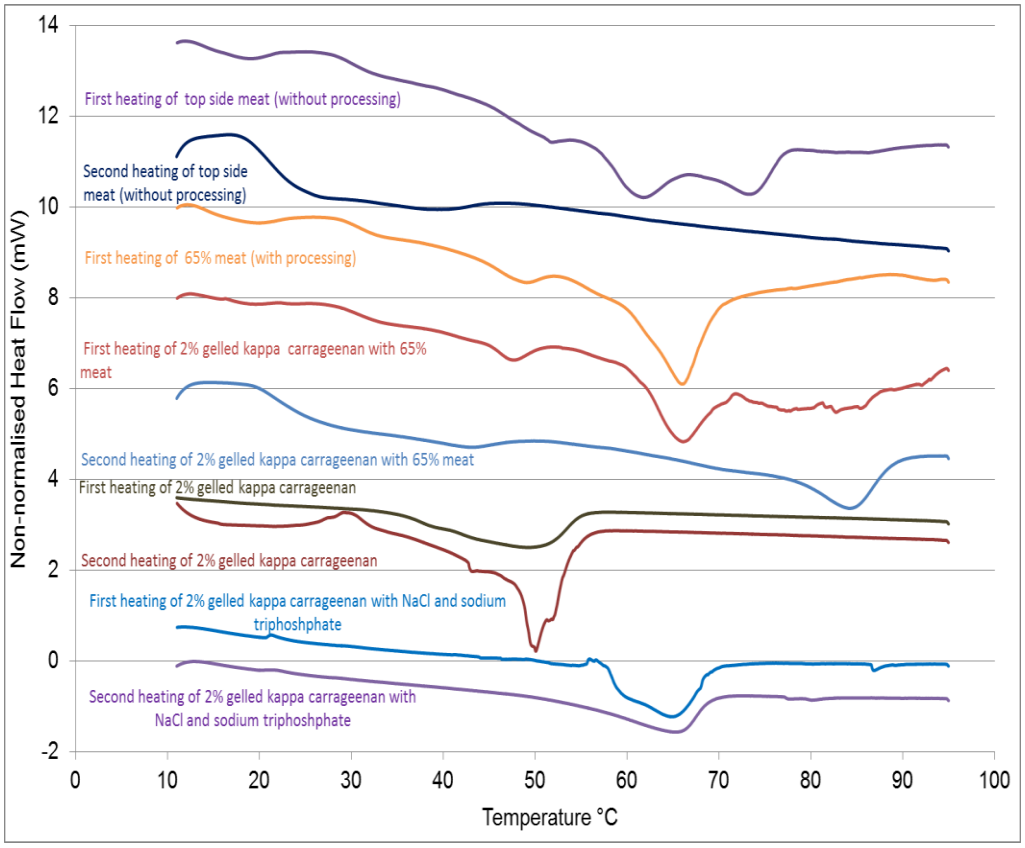


Figure 5.7: Endothermic DSC thermograms for first heating of top side meat (without processing); second heating of top side meat (without processing);first heating of 65 % meat (with processing); first heating of 2% gelled kappa carrageenan with 65% meat; second heating of 2% gelled kappa carrageenan with 65% meat; first heating of 2% gelled kappa carrageenan; second heating of 2% gelled kappa carrageenan heat ; first heating of 2% gelled kappa carrageenan with NaCl and sodium triphosphate; second heating of 2% gelled kappa carrageenan with NaCl and sodium triphosphate triphosphate (details of sample preparation can be found in method and material chapter).

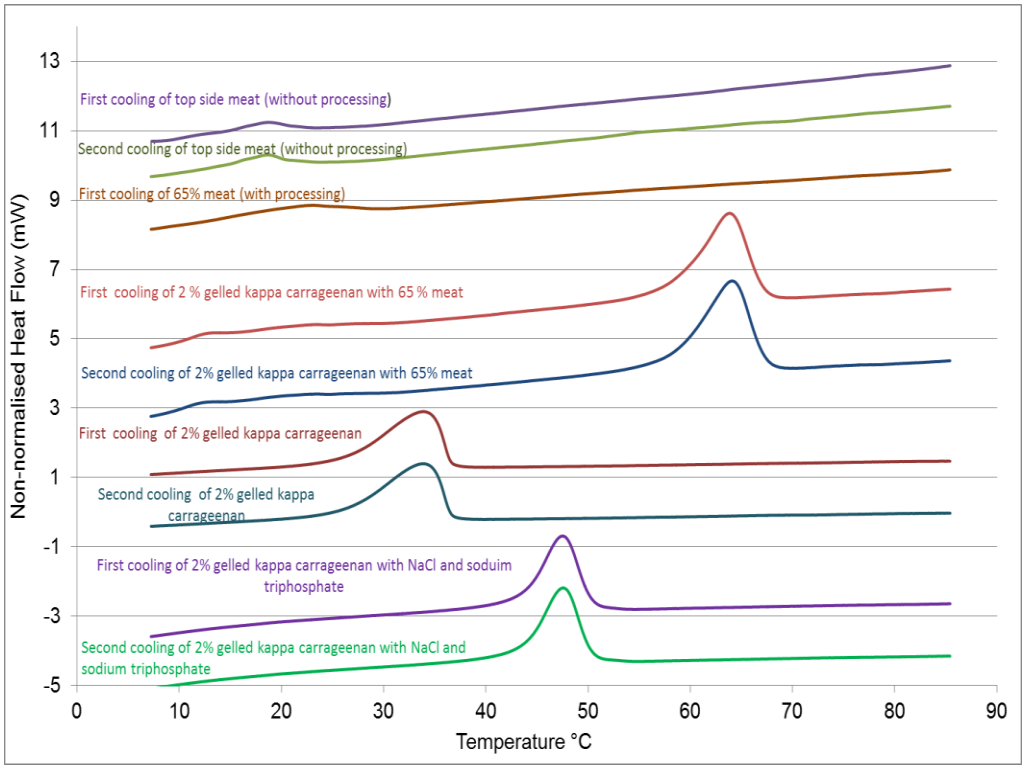


Figure 5.8: Exothermic cooling DSC for first cooling top side meat (without processing); second cooling of top side meat without processing);first cooling of 65% meat(with processing); first cooling of 2% gelled kappa carrageenan with 65% meat; second cooling of 2% gelled kappa carrageenan with 65% meat; first cooling of 2% gelled kappa carrageenan; second cooling of 2% gelled kappa carrageenan heat ; first cooling of 2% gelled kappa carrageenan with NaCl and sodium triphosphate; second cooling of 2% gelled kappa carrageenan with NaCl and sodium triphosphate.

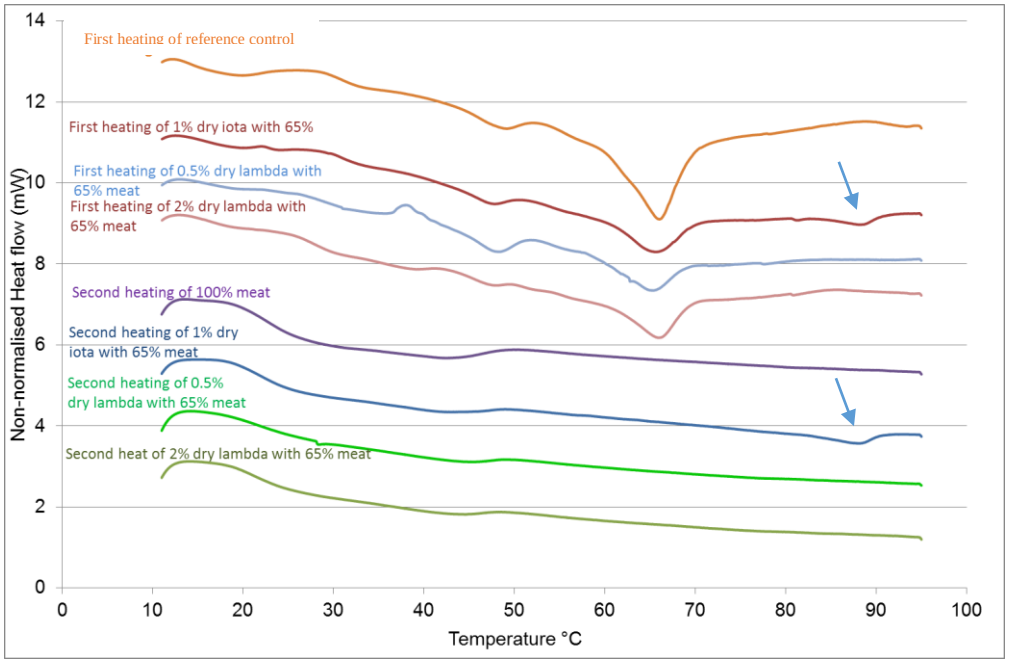


Figure 5.9: Endothermic DSC thermograms for first heating of reference control (with processing); first heating of 1% dry iota carrageenan with 65 % meat; first heating of 0.5% dry lambda carrageenan with 65% meat; first heating of 2% dry lambda carrageenan with 65% meat; second heating of reference; second heating of 1% dry iota carrageenan with 65% meat; second heating of 0.5% dry lambda carrageenan with 65% meat; second heating of 2% dry lambda carrageenan with 65% meat (details of sample preparation can be found in method and material chapter).

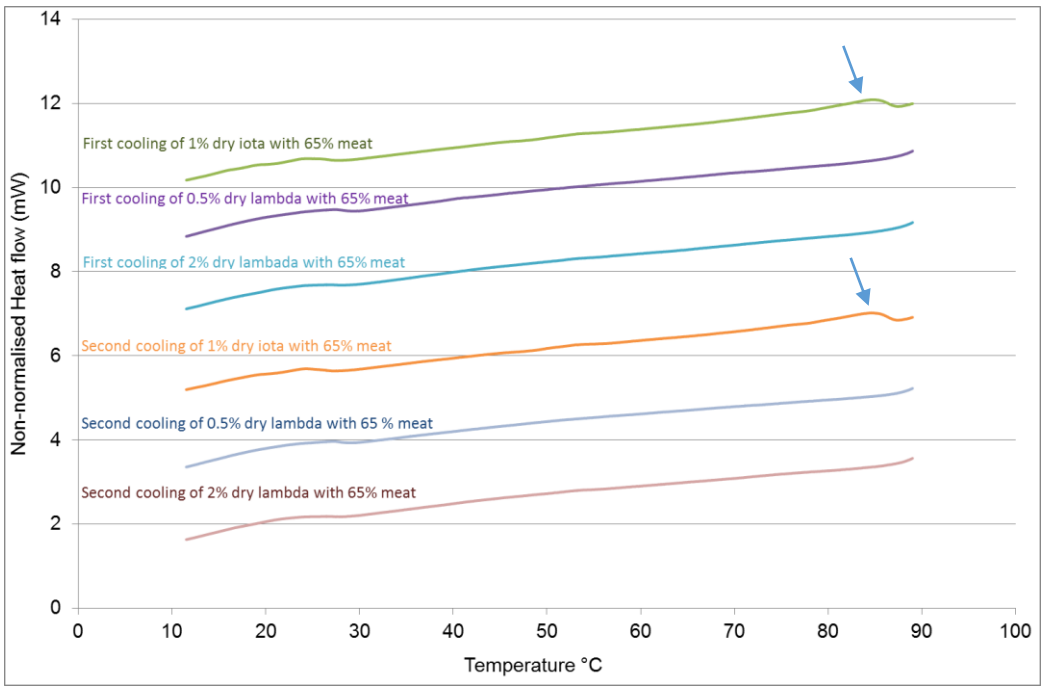


Figure 5.10: Exothermic cooling DSC for first cooling of 1% dry iota carrageenan with 65% meat; first cooling of 0.5% dry lambda carrageenan with 65% meat; first cooling of 2% dry lambda carrageenan with 65% meat; second cooling of 1% dry iota carrageenan with 65% meat; second cooling of 0.5% dry lambda carrageenan with 65% meat; second cooling of 2% dry lambda carrageenan with 65% meat.

Table 5.7: Endothermic transition heating parameters for 65% meat; 2% gelled kappa carrageenan with 65% meat (first heating); 2% gelled kappa carrageenan with 65% meat (second heating); 2% gelled kappa carrageenan (first heating); 2% gelled kappa carrageenan (second heating); 2% gelled kappa carrageenan with NaCl and sodium triphosphate (first heating); 2% gelled kappa carrageenan with NaCl and sodium triphosphate (second heating). T_0 = onset temperature; T_e = end set temperature; T_m = melting peak temperature and ΔH = non-normalised enthalpy of transition.

samples	T_0	T_e	T_m	ΔH (J/g)
Topside meat (without processing)	55.4	77.7	61.7	2.0
65% meat (with processing)	59.2	67	66.1	2.1
2% gelled kappa carrageenan with 65% meat (first heating)	61.4	73.6	66.1	2.1
2% gelled kappa carrageenan with 65% meat (second heating)	78.49	88.4	84.46	0.5
2% gelled kappa carrageenan (first heating)	48.3	51.47	45.23	0.7
2% gelled kappa carrageenan (second heating)	45.8	48	47.5	0.8
2% gelled kappa carrageenan with NaCl and sodium triphosphate (first heating)	57.8	68.6	64.8	0.7
2% gelled kappa carrageenan with NaCl and sodium triphosphate (second heating)	55.9	68.8	65.3	0.6

Table 5.8: Exothermic cooling transition parameters for 2% gelled kappa carrageenan with 65% meat (first cooling); 2% gelled kappa carrageenan with 65% meat (second cooling); 2% gelled kappa carrageenan (first cooling); 2% gelled kappa carrageenan (second cooling); 2% gelled kappa carrageenan with NaCl and sodium triphosphate (first cooling); 2% gelled kappa carrageenan with NaCl and sodium triphosphate (second cooling)

T_0 = onset temperature; T_e = end set temperature; T_g = gel peak temperature and ΔH = non-normalised enthalpy of transition.

samples	T_0	T_e	T_g	ΔH (J/g)
2% gelled kappa carrageenan with 65% meat (first cooling)	67	58	63.8	-1.0
2% gelled kappa carrageenan with 65% meat (second cooling)	67.2	58.4	64.1	-1.0
2% gelled kappa carrageenan (first cooling)	35.8	25.8	33.1	-0.8
2% gelled kappa carrageenan (second cooling)	35.8	25.3	33.1	-0.9
2% gelled kappa carrageenan with Nacl and sodium triphosphate (first cooling)	50.3	43.59	47.48	-0.7
2% gelled kappa carrageenan with Nacl and sodium triphosphate (second cooling)	50.3	43.6	47.57	-0.8

5.3.3 Small deformation mechanical spectroscopy

Rheological transitions determined non-destructively are reported in Figure 5.11. The control treatments (65% meat) show the effect of 2% gelled kappa carrageenan on the batter. Temperature sweeps showed the thermal behaviour of samples in terms of their G´ (elastic component), G˝ (viscous component) and phase angle (tan δ) as a function of temperature (30–90˚C). It can be clearly seen that the addition of carrageenan resulted in higher G´ values and, more surprisingly, that G´ values were already higher from the beginning of the structure build-up during the heating stage. This potentially supports the DSC findings in that the presence of the meat effects the carrageenan gel, and in turn such effects are seen in the dispersion of carrageenan in the batter, affecting it overall rheology. Indeed, a sharp decrease in tan δ occurred in control sample (65% meat) upon heating , representative of the protein gel formation, but when 2 % gelled carrageenan is added the initial tan δ, before heating , is lower and upon heating the decrease in tan δ is much shallower. Changes in G´ of a control sample, which is 65% meat during heating from 30–90˚C, could be classified into four general temperature ranges: phase I gel point development (30–45˚C); phase II (45–54˚C); phase III (54–60˚C), and phase IV (60–90˚C).

Gel point, defined as the increase in G´ (Pa) and decrease in δ value (Hermansson, 1989), was recorded at 38˚C. Also, in this phase (I) denaturation of the head proteins of myosin and subsequent aggregation are responsible for

the initial increase in G´ taking place (Verbeken et al., 2005) since myosin is the major factor in the heat-induced gelation of meat proteins (Ikeuchi et al., 1994). The DSC analyses (Figure 5.7) shows that myosin had a small endothermic region at 45˚C, which is confirmation that the myosin molecules changed in this temperature range.

In phase II, the increase in G´, which is indicated in just one shoulder at around 54˚C, maybe explained by the unfolding of myosin (denaturation) molecules and irreversible interfilamental interaction between myosin heads (Egelandsdal et al., 1986; Sano et al., 1988).

Initial decreasing in G´ in control samples in phase III may be due to the breaking hydrogen bonds of myosin as the temperature increases. Also, in this phase denaturation and precipitation/coagulation of myosin heads takes place (Ikeuchi et al., 1994; Samejima et al., 1981). Furthermore, dissociation of the actin–myosin complex has also been suggested to contribute to the decrease in G´ between 54 and 60˚C (Verbeken et al., 2005).

The G´ value increases in phase IV and this is due to protein gel starting point when a heating rate of 1˚C/min was employed. Also, it is during this final thermal phase that significant gelation of myosin is achieved (Ishioroshi et al., 1979, Ziegler and Acton, 1984). Increases in G´, after the point where gelation starts, have been attributed to a network formation by the aggregation and entanglement of unfolded protein molecules (Aguilera and Rojas, 1997; McSwiney et al., 1994).

The addition of selected non-meat ingredients resulted in smoother G´ profiles. A 2% gelled kappa carrageenan resulted in a higher G´ value in meat batter. Thermal transition recorded during phase I was similar to those observed in the control sample. The elastic component increased in both curves; however, in the control sample a sharp increase in G´ was observed.

The changes in G´ recorded for control samples in phases II and III did not occur on the addition of carrageenan blends (Foegeding and Ramsey, 1987). Storage modulus G´ values for the 2% gelled kappa were shown to exhibit three transition phases. Phase I (44–59˚C) occurred between decreases in G´ value and an increase in temperature; phase II (60–75˚C) with an increase in G´ at 72˚C

and finally phase III (75–90˚C) with a decrease in G´ as the temperature increased.

In phase I dissociation of the actin–myosin complex has also been suggested to contribute to the decrease in G´ around a temperature of 54 to 60˚C. Also, a decrease observed in G´ when the temperature was close to 60˚C may be caused by the melting of weaker junction zones of non-aggregated kappa carrageenan helices.

In phase II, carrageenan treatment was slightly increased at 60˚C and there was an unlimited increase in 70˚C. Foegeding and Ramsey (1987), reported that carrageenan treatments increased rigidity above 58˚C and increased ultimate rigidity at 70˚C. The peak formations occur in phase II and this may be due to the melting of gelled of kappa carrageenan (helix → coil). The increase in G´ observed by the addition of gelled kappa carrageenan in this phase was not only due to the melting of kappa carrageenans but also to their ability to form a thermally reversible gel. In addition, in this phase the protein starts to gel and gelation of myosin is achieved. In this phase, between the ranges of 61 and 67˚C, the denaturation of sarcoplasmic protein and collagen also takes place. The DSC thermogram (Figure 5.7) shows a broad endothermic region at a peak of 66.1˚C, confirming the rheology results.

Phase III resulted in a final decrease in G´ values between 76 and 90˚C and this may be induced by the complete melting of the stronger junction zone formed by the aggregated helices in temperatures around 85˚C (helix → coil). Before 75˚C, aggregation and entanglement of unfolded protein molecules occurs while in the range of 70 and 80˚C titin denaturation also takes place. It seems that carrageenan treatment will not swell completely until after the proteins have begun gelling, isolating the carrageenan particle within the protein gel matrix, and this supports the results from the DSC of this study as well as from previous findings (Norton et al., 1984).

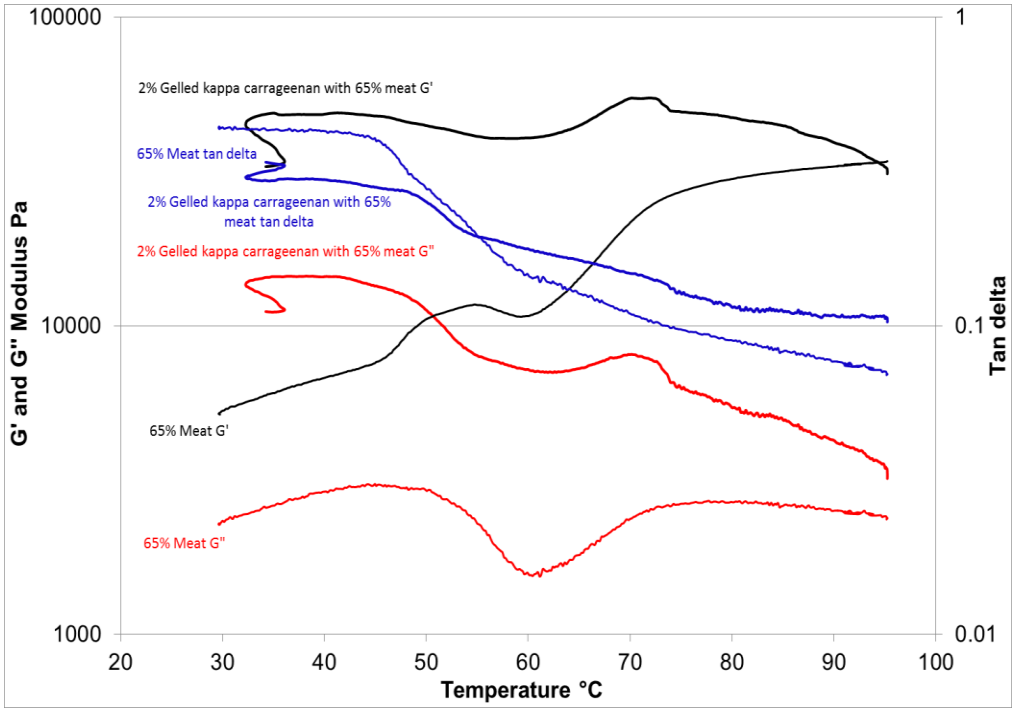


Figure 5.11: A comparison of changes of the storage, loss moduli and phase angle (G′, G″ and tan δ) when adding 2% gelled kappa carrageenan to a 65% meat and control sample (65% meat) as a function of heating. The curve represents the average of the three replicate.

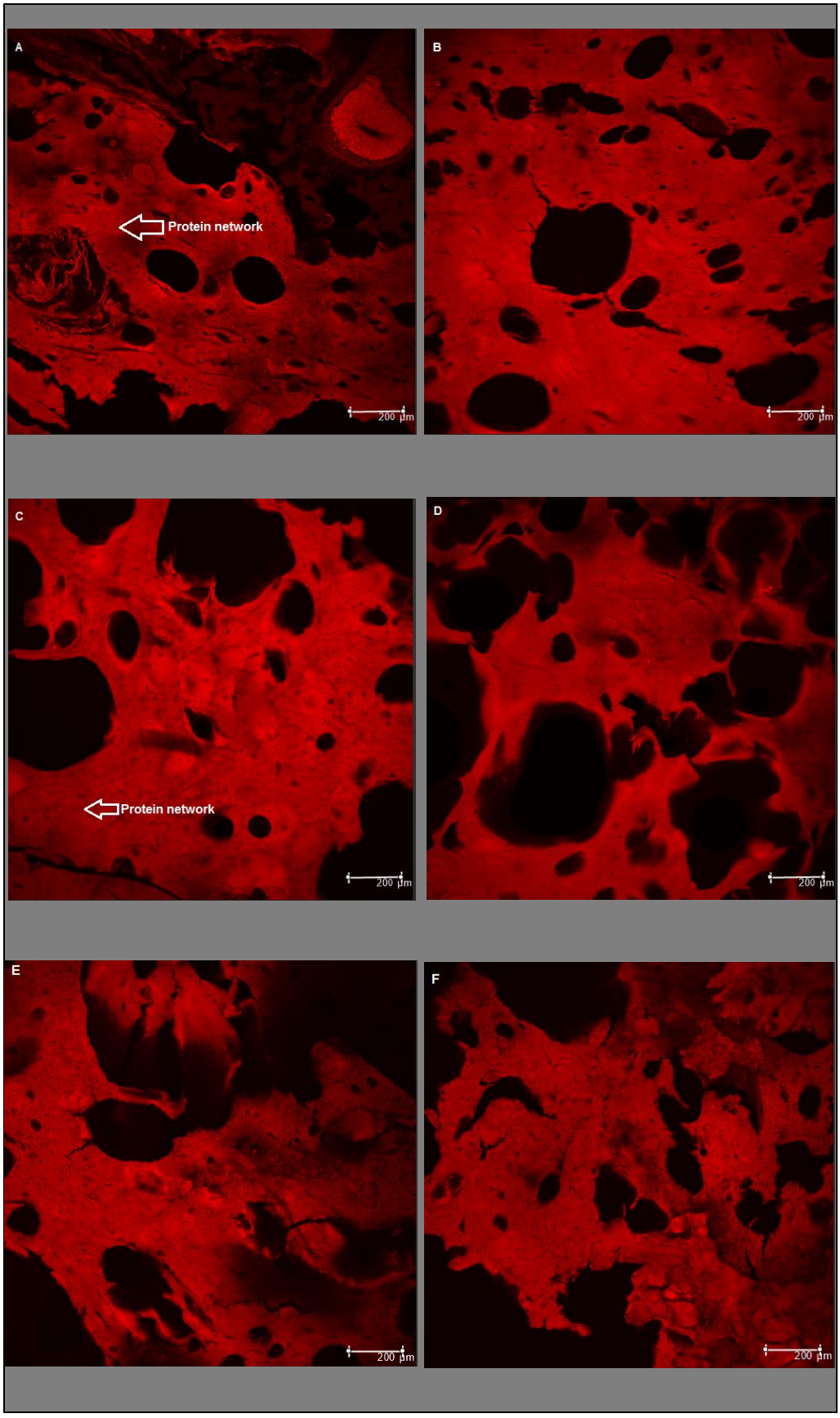
5.3.4 Structure of heated meat batters

Applying CSLM, the microstructure of beef sausage control samples (65% meat and reference) and 2% gelled kappa carrageenan with 65% meat were visualised. The goal of the visualisation was to determine the change in the structure of the protein matrix when adding hydrocolloids or not. Figure 5.12 shows CLSM images of the sausages with two different fluorescent colours (BODIPY493/503) has been used for fat, and meat proteins were labelled using rhodamine B, which selectively adsorbs on the protein fraction of foods (Tromp et al., 2001; van de Velde et al., 2002; van de Velde et al., 2003). Also, the carrageenan like protein was labelled with rhodamine B according to the procedure described by Tromp et al. (2001) because carrageenans are sulphated polysaccharides, and therefore the sulphur can be used to identify the distribution of the carrageenan in the gel. The control sausage images (A, B, C and D) are already described in chapter 4. Sausage images (E and F) of samples containing 2% gelled kappa carrageenan show that the protein matrix (continuous area) is gradually being disrupted and the gelled kappa carrageenan network is evenly distributed in system. It seems that the size of the cavity is decreased when adding gelled kappa carrageenan

(100-150 μm) probably due to the increase in texture of the meat batter containing carrageenan. This is evidenced by an increase in G´ and a subsequent decrease in tan δ, indicating that during batter processing smaller air cells are created and stabilised. However, the protein network and gelled kappa carrageenan were not distinguishable from each other under the CLSM view and this could be due to the rhodamine B stain indicating both the carrageenan and protein network so can't see the carrageenan network.

Previous work visualising dry kappa carrageenan in sausage–for instance, Prabhu and Sebranek (1997)–found that dry kappa carrageenan particles were dispersed through a ham product without evidence of gel forming. Also, this author reported that dry carrageenan was dispersed randomly in voids that could be air or aqueous regions and in spaces where the binding of meat pieces took place. This could be due to the fact that dry kappa carrageenan gels at above 80˚C, where myosin has already gelled and therefore would melt and solubilised above this temperture, preventing uniform distribution of carrageenan throughout the meat matrix. This means that proteins gel before carrageenan becomes fully soluble and therefore the carrageenan gets trapped within the protein gel structure. Recent optical microscopy observation by Montero et al. (2000) shows dry kappa carrageenan creates large, elongated cavities; however, dry iota carrageenan have small, round cavities in the protein matrix.

Confocal microscopy suggests that the samples consist of a general protein matrix (continuous area) with fat material which is green (round area), which are evenly distributed in protein network throughout the structure. Also, fat (round area) is coated by protein continuous the phase, forming the typical sausage emulsion-like structure. Visualising the control image (65% meat, image G) as a comparison, these droplets can be seen to decrease in mean size when adding gelled kappa carrageenan (image H). The size of the fat droplets is 10 μm or less; however the fat droplets of control samples are between 60 and 100 μm. Chattong et al. (2007) reported similar results when adding hydrocolloids.


A
Protein network
200 µm
B
200 µm
C
Protein network
200 µm
D
200 µm
E
200 µm
F
200 µm

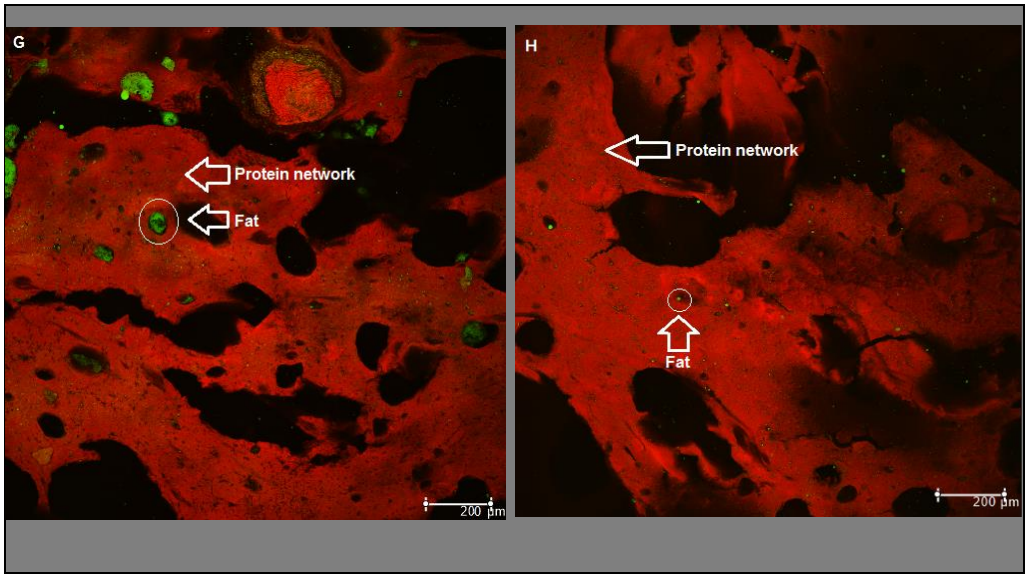


Figure 5.12: CLSM images of sausage containing gelled kappa carrageenan to a 65% meat, compared to control samples (65% meat and reference). Images A and B are reference control samples stained for protein matrix; images C and D are the control samples (65% meat) stained for protein matrix; E and F images relate to 2% gelled kappa carrageenan with 65% meat stained for protein matrix and carrageenan; image G is control sample (65% meat) stained for fat and protein matrix and finally image H is 2% gelled kappa carrageenan with 65% meat stained for fat and protein matrix.

5.3.5 Visual appearance of sausages

Figure 5.13 shows the visualisation of the 4 same sample of the 35% reduced meat product containing 2% gelled kappa carrageenan (A), compared to 4 same sausages of the control sample, 65% meat (B). When compared with the samples A and B, we found that the sample made of 2% gelled kappa carrageenan shows that particles of gelled kappa carrageenan are visible in the final product. The presence of the large carrageenan particles might explain the increase in G´ observed in the rheology experiments, but DSC also indicates that the protein itself also affects the properties of the carrageenan gel with in the protein matrix which has a consequent effect on the texture parameters described previously. In addition, according to Texture Profile Analyser, the control sample (65% meat) was significantly different from 2% gelled kappa carrageenan. Additionally, firmness, which is one of the descriptions of hardness of a meat product, is important for sliceability and carrageenan may therefore improve the sliceability of meat products.

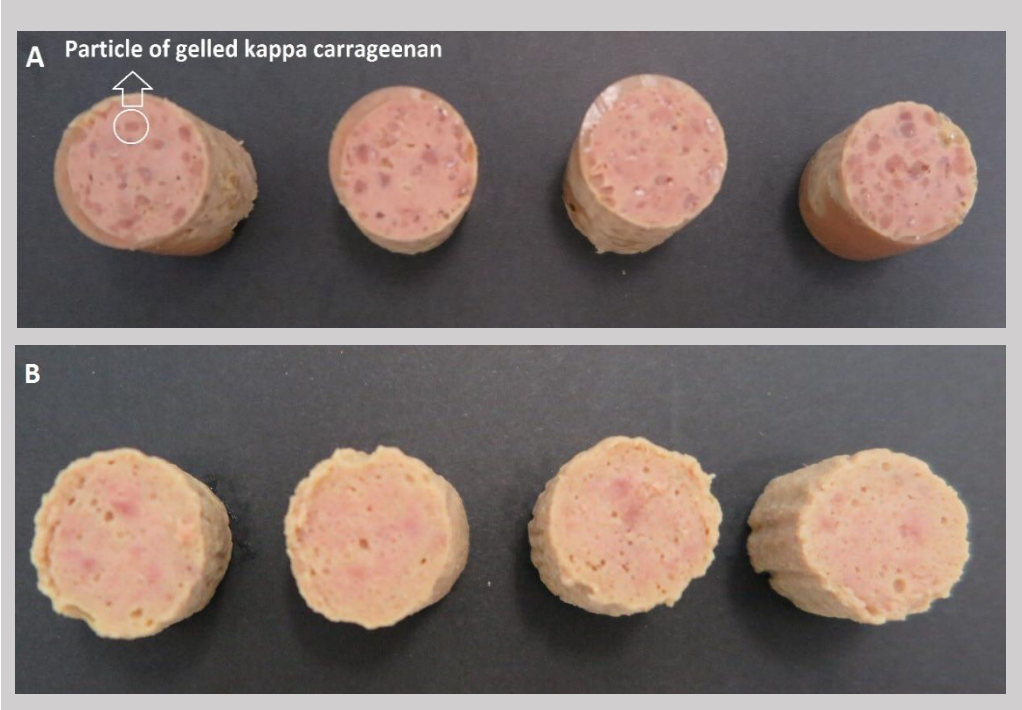


Figure 5.13: Visual appearance of adding 2% gelled kappa carrageenan to 65% meat *(A), compared to control samples, 65% meat (B).*

5.4 Conclusion

The results obtained from the texture analysis show that 2% gelled kappa carrageenan added as a component of the batter causes increased hardness (P-value less than 0.01), but was not able to improve cohesiveness and resilience. The addition of 1% solubilised lambda carrageenan to meat batter, improves the cohesiveness and resilience. Also, in this range the tenderness which is one of the descriptions of chewiness of sausage occurs. By the addition of 1% solubilised kappa carrageenan, an increase in springiness and adhesiveness is observed. The DSC studies of myofibrillar protein (MP) and gelled kappa carrageenan revealed carrageenan treatment will not melt completely until after the proteins have begun gelling, isolating the carrageenan particle within the protein gel matrix, and the rheology results confirm this data. Furthermore, there was only a slight effect on the meat protein with the addition of gelled kappa carrageenan indicating a possible notable polysaccharide-protein interaction. However, it is very difficult to say whether there is a molecular interaction between the meat and the kappa carrageenan gel. Greater changes in the transition temperature of meat proteins were observed on the addition of salt.

The protein and salt used in the process affect the thermal transition of the carrageenan gel, and the particles of gel added are still visible in the final product. Three peaks, characterised by myosin, collagen, or sarcoplasmic protein and actin, were observed in myofibrillar protein (MP); 2.7% sodium chloride caused an effect on the second and third transition peak. With the addition to the meat batter of salt and sodium triphosphate the first transition is less affected (myosin). However, the second and third transition disappeared and shifted to the higher temperature (sarcoplasmic protein, connective tissue and actin). The addition of 2% gelled kappa carrageenan resulted in higher G´ values compared to the control sample (65 % meat) and, more interestingly, the G ´ values were already higher from the beginning of the structure build-up during the heating stage. The difference in the G´ profile between the control sample (65% meat) and the 2% gelled kappa carrageenan test demonstrates the complexity of the multicomponent gel system in terms of structure, and the influence that various ingredient combinations can have on the potential gelling properties of re-formed meats. Microstructure observation shows that adding 2% gelled kappa carrageenan to the sausage formulation leads to the appearance of the carrageenan gel network; however, the protein network and the gelled kappa carrageenan were not distinguishable from each other. Furthermore, the droplets of fat and air cells decrease in mean size when adding gelled kappa carrageenan into the system because change in both pore and particle size and matrix homogeneity can influence water holding and texture properties of meat batter or gels.

With regard to the chapter title, is it obvious that hydration did not improve the texture of the sausages; on the other hand, gelation improved the hardness and microstructure of the sausages and according to the Iranian National Standardization Organization (INSO) 2017, this kind of sausages is satisfactory; however, particles of gelled kappa carrageenan are visible in the final product. The results, especially those of the texture analysis, have important significance for meat scientists and the meat industry. Depending on the concentration of the added carrageenan (κ, ι and λ) in the meat batter, and also using dry, solubilised and gelled of carrageenan in the system, may lead to unexpected changes in the product, which affect a whole host of food quality attributes.

CHAPTER 6 LOCUST BEAN GUM ADDITION TO MEAT BATTER: DOMINATED BY HYDRATION OR GELATION?

6.1 Introduction

The functionality of comminuted meat products depends on numerous factors, such as the water-holding capacity of the meat, non-meat ingredients, ionic strength, pH and temperature and the heating profile (Acton et al., 1982; Asghar et al., 1985; Gordon et al., 1992; Smith, 1988). Non-meat ingredients are useful in comminuted meat products because of their functional properties, including emulsification, water-and fat-binding capacity and improving of texture and appearance (Hoogenkamp, 1992; Shand et al., 1990). Non-muscle proteins and polysaccharides are often used to enhance the texture and binding properties of comminuted meat products (Whiting, 1988). The protein–polysaccharide (Pro-Poly) interaction plays a significant role in the structure and stability of many processed foods. Functional properties of food protein, such as gel-forming ability, conformation stability, solubility and emulsifying and foaming properties are effected by their interactions with polysaccharides. Pro–Poly interactions are well documented for non-meat proteins. However, more studies are required regarding the interactions of meat proteins with hydrocolloids. Locust bean gum (LBG) is obtained from carob bean, harvested from *Ceratoniasilique*, a Mediterranean tree. Carob gum or locust bean gum (LBG) is a galactomannan containing (1→4)-β-D-mannopyranosyl backbone with attachment of (1→6)-α-D galactose units (Dey, 1978). The ratio of mannose to galactose varies between three and five for LBG, depending on the source and preparation method. It is mention to note that the ratio of the mannose and galactose is unknown in this study. Furthermore, LBG reduces syneresis and helps to produce gels of equivalent strength (Therkelsen, 1993). Synergistic effects appear when it is mixed with other hydrocolloids (Dea, 1979). Widely used as a thickener, LBG is well known to form a gel after a freeze/thaw cycle (Dea et al., 1977). It dissolves in hot water and forms a viscous solution (Doublier and Launay, 1981). There are few papers to be found in the literature that deal with the effects of LBG addition on the properties of formulation meat products. Among these, Ramırez et al. (2002) reported that LBG is not appropriate to be employed as a surimi additive; however, these gums presented a profitable effect when used with xanthan in a ratio of 0.25/0.75. Also, Damásio et al. (1994) and García-García and Totosaus (2008) reported that if LBG and

kappa carrageenan are added to the meat batter it causes an improvement in texture and water retention, with only a minor effect on sausage colour. In spite of these few published studies, the meat processing sector, particularly beef meat processors, need more scientific data to deliver formulated products that are able to meet the special requirements of consumers. Examples of such requirements may be an improvement in the cohesiveness and chewiness of the final product, linking such properties to product microstructure. The present work consequently seeks to evaluate the influence of adding dry, solubilised and gelled LBG on meat emulsion stability, textural properties such as hardness, springiness, cohesiveness, chewiness and finally, resilience in sausage texture formation. In addition, the study investigates the microstructure of meat products, for example the location of protein networks, LBG and fat in cooked sausages, since no research has been conducted comparing dry, solubilised and gelled LBG in meat products. Furthermore, the study aims to elucidate the role of heat-induced gelation in texture formation of processed meat products and how it is affected when LBG is added in different states.

6.2 Materials and Methods

Materials and methods used for this study are described in Chapter 3.

6.3 Results and Discussion

6.3.1 Texture properties

Results obtained from the statistical analyses of the collected data are presented in Table 6.1, 6.2, 6.3, 6.4, 6.5 and 6.6.

Table 6.1: Scheffe's test results (p-values) for the hardness of controls (65% meat and reference) with 65% meat containing dry and solubilised LBG (**<0.01 and *<0.05).

	Reference control	0.5% Dry LBG with 65% meat	0.5% Solubilised LBG with 65% meat	0.5% Gelled LBG with 65% meat	1% Dry LBG with 65% meat	1% Solubilised LBG with 65% meat	1% Gelled LBG with 65% meat	2% Dry LBG with 65% meat	2% Solubilised LBG with 65% meat	2% Gelled LBG with 65% meat
0.5% Dry LBG with 65% meat	0.00**									
0.5% Solubilised LBG with 65% meat	0.00**	1.00								
0.5% Gelled LBG with 65% meat	0.00**	0.91	0.99							
1% Dry LBG with 65% meat	0.00**	0.99	0.97	0.48						
1% Solubilised LBG with 65% meat	0.00**	1.00	1.00	0.97	0.99					
1% Gelled LBG with 65% meat	0.00**	1.00	1.00	0.81	1.00	1.00				
2% Dry LBG with 65% meat	0.00**	1.00	1.00	0.94	0.99	1.00	1.00			
2% Solubilised LBG with 65% meat	0.00**	0.82	0.98	1.00	0.36	0.92	0.68	0.86		
2% Gelled LBG with 65% meat	0.00**	1.00	0.98	0.51	1.00	0.99	1.00	0.99	0.37	
65% Meat	0.00**	1.00	1.00	0.99	0.99	1.00	1.00	1.00	0.98	0.99

Table 6.2: Scheffe's test results (p-values) for the adhesiveness of controls (65% meat and reference) with 65% meat containing dry and solubilised LBG (**<0.01 and *<0.05).

	Reference control	0.5% Dry LBG with 65% meat	0.5% Solubilised LBG with 65% meat	0.5% Gelled LBG with 65% meat	1% Dry LBG with 65% meat	1% Solubilised LBG with 65% meat	1% Gelled LBG with 65% meat	2% Dry LBG with 65% meat	2% Solubilised LBG with 65% meat	2% Gelled LBG with 65% meat
0.5% Dry LBG with 65% meat	0.92									
0.5% Solubilised LBG with 65% meat	1.00	0.99								
0.5% Gelled LBG with 65% meat	0.98	1.00	1.00							
1% Dry LBG with 65% meat	0.47	0.99	0.76	0.97						
1% Solubilised LBG with 65% meat	0.35	0.99	0.62	0.92	1.00					
1% Gelled LBG with 65% meat	0.15	0.91	0.29	0.66	1.00	1.00				
2% Dry LBG with 65% meat	0.97	1.00	1.00	1.00	0.98	0.94	0.71			
2% Solubilised LBG with 65% meat	0.00**	0.00**	0.00**	0.00**	0.00**	0.00**	0.02*	0.00**		
2% Gelled LBG with 65% meat	0.98	1.00	1.00	1.00	0.97	0.93	0.70	1.00	0.00**	
65% Meat	1.00	0.93	1.00	0.98	0.53	0.42	0.21	0.97	0.00**	0.98

Table 6.3: Scheffe's test results (p-values) for the springiness of controls (65% meat and reference) with 65% meat containing dry and solubilised LBG (**<0.01 and *<0.05).

	Reference control	0.5% Dry LBG with 65% meat	0.5% Solubilised LBG with 65% meat	0.5% Gelled LBG with 65% meat	1% Dry LBG with 65% meat	1% Solubilised LBG with 65% meat	1% Gelled LBG with 65% meat	2% Dry LBG with 65% meat	2% Solubilised LBG with 65% meat	2% Gelled LBG with 65% meat
0.5% Dry LBG with 65% meat	0.98									
0.5% Solubilised LBG with 65% meat	0.24	0.85								
0.5% Gelled LBG with 65% meat	0.95	1.00	0.93							
1% Dry LBG with 65% meat	0.99	1.00	0.82	1.00						
1% Solubilised LBG with 65% meat	0.30	0.90	1.00	0.96	0.88					
1% Gelled LBG with 65% meat	0.90	1.00	0.98	1.00	1.00	0.99				
2% Dry LBG with 65% meat	0.99	1.00	0.76	1.00	1.00	0.83	1.00			
2% Solubilised LBG with 65% meat	0.21	0.82	1.00	0.91	0.79	1.00	0.97	0.72		
2% Gelled LBG with 65% meat	0.04*	0.45	1.00	0.59	0.44	1.00	0.80	0.33	1.00	
65% Meat	1.00	1.00	0.50	0.99	1.00	0.58	0.98	1.00	0.46	0.19

Table 6.4: Scheffe's test results (p-values) for the cohesiveness of controls (65% meat and reference) with 65% meat containing dry and solubilised LBG (**<0.01 and *<0.05).

	Reference control	0.5% Dry LBG with 65% meat	0.5% Solubilised LBG with 65% meat	0.5% Gelled LBG with 65% meat	1% Dry LBG with 65% meat	1% Solubilised LBG with 65% meat	1% Gelled LBG with 65% meat	2% Dry LBG with 65% meat	2% Solubilised LBG with 65% meat	2% Gelled LBG with 65% meat
0.5% Dry LBG with 65% meat	1.00									
0.5% Solubilised LBG with 65% meat	1.00	1.00								
0.5% Gelled LBG with 65% meat	1.00	1.00	1.00							
1% Dry LBG with 65% meat	1.00	1.00	1.00	1.00						
1% Solubilised LBG with 65% meat	0.10	0.05	0.07	0.07	0.09					
1% Gelled LBG with 65% meat	1.00	1.00	0.99	0.99	0.99	0.00**				
2% Dry LBG with 65% meat	0.92	0.89	0.97	0.95	0.97	0.85	0.53			
2% Solubilised LBG with 65% meat	0.03*	0.01**	0.01**	0.01**	0.01**	1.00	0.00**	0.50		
2% Gelled LBG with 65% meat	0.99	0.99	0.99	0.99	1.00	0.45	0.83	1.00	0.14	
65% Meat	1.00	1.00	1.00	1.00	1.00	0.47	0.99	0.99	0.20	1.00

Table 6.5: Scheffe's test results (p-values) for the chewiness of controls (65% meat and reference) with 65% meat containing dry and solubilised LBG (**<0.01 and *<0.05).

	Reference control	0.5% Dry LBG with 65% meat	0.5% Solubilised LBG with 65% meat	0.5% Gelled LBG with 65% meat	1% Dry LBG with 65% meat	1% Solubilised LBG with 65% meat	1% Gelled LBG with 65% meat	2% Dry LBG with 65% meat	2% Solubilised LBG with 65% meat	2% Gelled LBG with 65% meat
0.5% Dry LBG with 65% meat	0.00**									
0.5% Solubilised LBG with 65% meat	0.00**	0.99								
0.5% Gelled LBG with 65% meat	0.00**	0.93	1.00							
1% Dry LBG with 65% meat	0.00**	1.00	0.82	0.56						
1% Solubilised LBG with 65% meat	0.00**	0.91	1.00	1.00	0.52					
1% Gelled LBG with 65% meat	0.00**	1.00	0.79	0.48	1.00	0.45				
2% Dry LBG with 65% meat	0.00**	1.00	1.00	0.99	0.98	0.99	0.98			
2% Solubilised LBG with 65% meat	0.00**	0.16	0.79	0.95	0.03*	0.98	0.01*	0.40		
2% Gelled LBG with 65% meat	0.00**	1.00	1.00	0.99	0.97	0.99	0.97	1.00	0.55	
65% Meat	0.00**	1.00	1.00	0.99	0.99	0.99	0.99	1.00	0.57	1.00

Table 6.6: Scheffe's test results (p-values) for the resilience of controls (65% meat and reference) with 65% meat containing dry and solubilised LBG (**<0.01 and *<0.05).

	Reference control	0.5% Dry LBG with 65% meat	0.5% Solubilised LBG with 65% meat	0.5% Gelled LBG with 65% meat	1% Dry LBG with 65% meat	1% Solubilised LBG with 65% meat	1% Gelled LBG with 65% meat	2% Dry LBG with 65% meat	2% Solubilised LBG with 65% meat	2% Gelled LBG with 65% meat
0.5% Dry LBG with 65% meat	1.00									
0.5% Solubilised LBG with 65% meat	1.00	1.00								
0.5% Gelled LBG with 65% meat	0.99	0.99	1.00							
1% Dry LBG with 65% meat	1.00	1.00	1.00	0.99						
1% Solubilised LBG with 65% meat	0.04*	0.01*	0.07	0.19	0.01*					
1% Gelled LBG with 65% meat	1.00	1.00	0.98	0.91	1.00	0.00**				
2% Dry LBG with 65% meat	0.92	0.83	0.99	1.00	0.89	0.63	0.51			
2% Solubilised LBG with 65% meat	0.00**	0.00**	0.01*	0.01*	0.00**	0.99	0.00**	0.11		
2% Gelled LBG with 65% meat	0.98	0.94	1.00	1.00	0.97	0.39	0.74	1.00	0.05	
65% Meat	1.00	1.00	1.00	1.00	1.00	0.31	0.99	0.99	0.04*	1.00

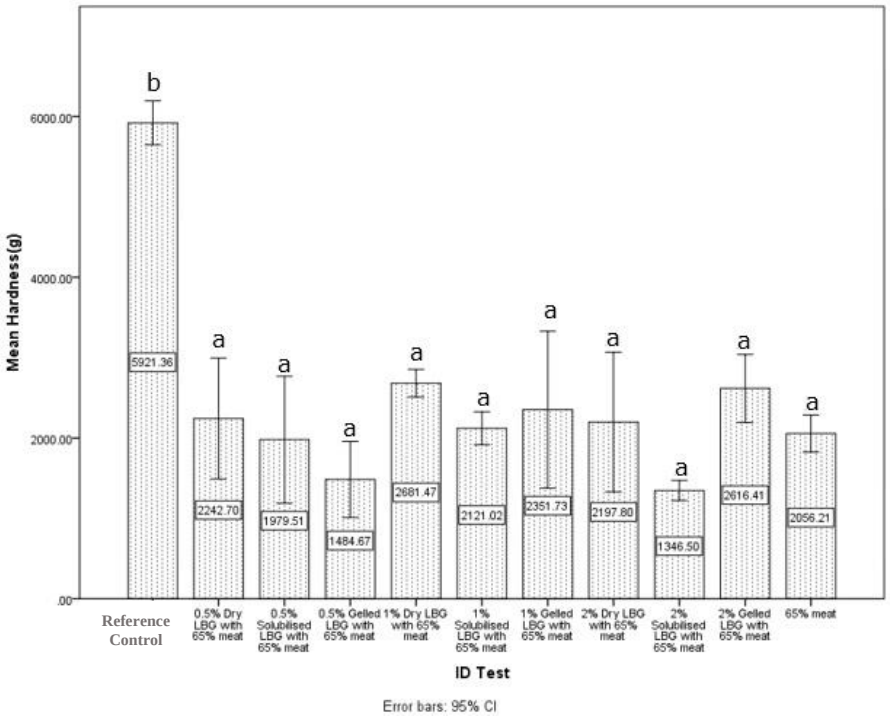


Figure 6.1: Hardness of adding dry, solubilised and gelled LBG to a 65% meat, compared to control samples (65% meat and reference).

The general linear model ANOVA was utilized to test for differences between the treatments (ID-Test) in relation to hardness. Analysis of variance (ANOVA) showed that at least one ID-Test mean was different from the others. Hence, Scheffe's post hoc test was applied in order to compare the means of the hardness ID-Test in a pair-wise fashion (Table 6.1).

In terms of hardness, it was shown in chapter 4 that the control 65% meat and reference control are statistically different. Now, comparing the 65% meat control to the other treatments the result indicated that there was not a statistically significant difference between 65% meat and other ID-Tests. By comparing the 0.5% LBG concentration, it was found that there was not a considerable difference between states in which LBG is added. The same result was also obtained at the higher LBG concentrations. Additionally, by comparing the 0.5%, 1% and 2% LBG concentrations, it was revealed that there was not a statistically significant difference for any treatment. This indicate that the protein matrix in meat batter, which is aggregated during heating, is not affected by the addition of LBG in any state. These results lend support to the results of Luruena-Martınez et al. (2004), who reported that there was no significant change in hardness when adding 0.5% LBG into meat batter.

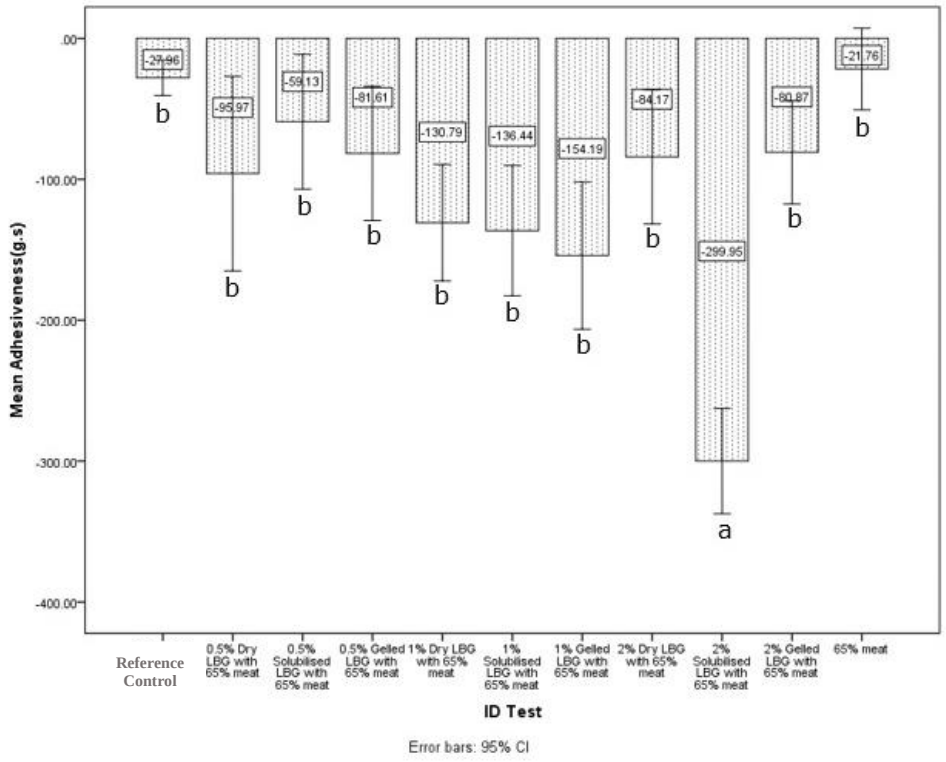


Figure 6.2: Adhesiveness of adding dry, solubilised and gelled LBG to a 65% meat, compared to control samples (65% meat and reference).

The general linear model ANOVA was also utilized to test for differences between treatments (ID-Test) in terms of adhesiveness. Analysis of variance (ANOVA) showed that at least one ID-Test mean was different from the others. Consequently, Scheffe's post hoc test was applied in order to pinpoint the exact locations of the differences between the means of the adhesiveness ID-Tests (Table 6.2).

First of all, in terms of the adhesiveness, we compared the control samples which are 65% and reference control to each other, and the result showed that there was not a statistically significant difference between reference control and 65% meat. Afterwards, we compared 65% meat to the other treatments, and the result showed that 65% meat was significantly different from 2% solubilised LBG only. All other treatments showed no statistically differences, indicating that the concentration of LBG and the state in which it is added to the meat batter does not affect adhesiveness. The 2% solubilised of LBG with 65% meat was shown to possess the highest adhesiveness in this study since, when heated in water, LBG was bound with a large amount of water, and consequently, after that, when added into the meat batter, the water-holding capacity of the sausages increased, and as a result of this, the adhesiveness of sausages increased.

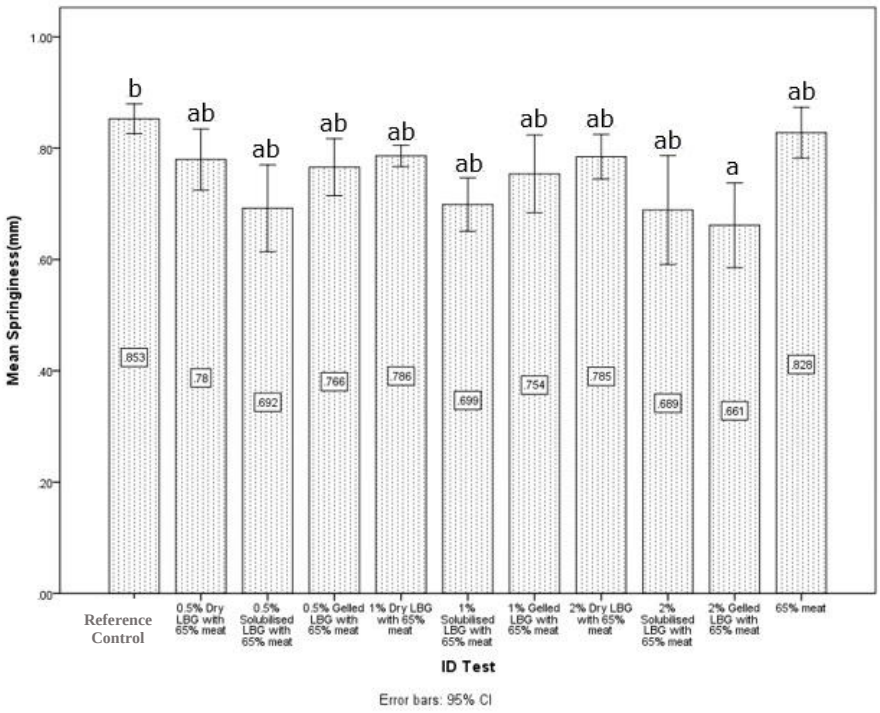


Figure 6.3: Springiness of adding dry, solubilised and gelled LBG to a 65% meat, compared to control samples (65% meat and reference).

The general linear model ANOVA was also utilized to test for differences between treatments (ID-Test) in regard to springiness. Analysis of variance (ANOVA) showed that at least one ID-Test mean was different from the others. Accordingly, Scheffe's post hoc test was applied in order to come up with the pair-wise comparisons of the means of the springiness ID-Tests (Table 6.3).

Firstly, in terms of the springiness, we compared the control samples which are 65% and reference control to each other and the result showed that reference control and 65% meat were not significantly different form each other. Secondly, we compared 65% meat to the other treatments except the reference control and the result showed that there was not a statistically significant difference between 65% meat and other ID-Tests. The only significantly different result in this comparison was when 2% gelled LBG was added to a 35% reduced meat product when compared to the reference control. Therefore, adding water bound up in a 2% LBG gel decrease springiness. All other treatments were not significantly different to a reference control.

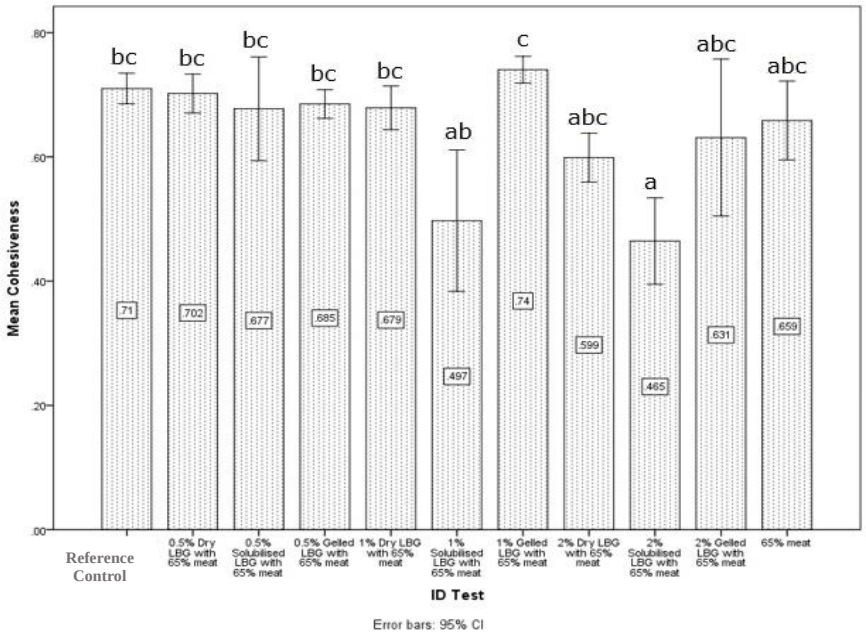


Figure 6.4: Cohesiveness of adding dry, solubilised and gelled LBG to a 65% meat, compared to control samples (65% meat and reference).

The general linear model ANOVA was utilized again, but this time to test for differences between treatments (ID-Test) in the cohesiveness. Analysis of variance (ANOVA) showed that at least one ID-Test mean was different from the others. That is why Scheffe's post hoc test was conducted in order to find the exact locations where the differences lay between the means of the cohesiveness ID-Tests (Table 6.4).

First and foremost, the control samples (i.e. 65% and reference) were compared to each other, and the result showed that reference control and 65% meat did not differ significantly from each other. Then, the 65% meat control was compared to the other treatments, and the results showed that 65% meat did not differ significantly with other ID-Tests as well. A closer examination of the results shows that again 2% solubilised LBG in a 35% reduced meat product is significantly different to the reference control however, 65% meat products with 0.5% LBG in any state and when 1% LBG is added dry or in gel state is statistically not different. This 2% solubilised LBG sample is not significantly different to the 65% meat control, and when 2% LBG is added in different states and the 1% solubilised LBG. It is interesting to note that the sample with 1% gelled LBG added had a highest mean cohesiveness however, 1 and 2% solubilised LBG decreases cohesiveness. This may be due to the LBG gel, after

freeze-thaw cycling, retaining the water away from the meat matrix causing an improvement in water holding capacity.

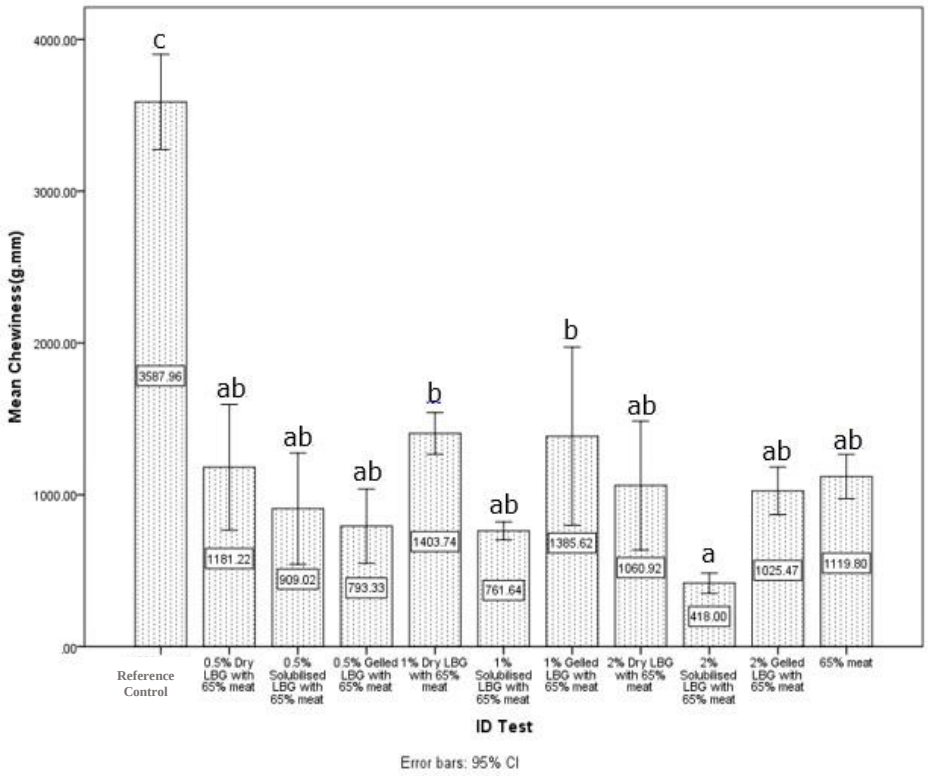


Figure 6.5: Chewiness of adding dry, solubilised and gelled LBG to a 65% meat, compared to control samples (65% meat and reference).

The general linear model ANOVA was also utilized to test for differences between treatments (ID-Test) in the chewiness. Analysis of variance (ANOVA) showed that at least one ID-Test mean was different from the others. Thus, Scheffe's post hoc test was applied in order to compare the means of the chewiness ID-Tests in a pair-wise fashion (Table 6.5).

With chewiness being the result of gumminess multiplied by springiness it is worth reminding that there was little significantly difference between all springiness scores, therefore chewiness scores follow those for gumminess, where the 2% solubilised LBG sample is significantly different to 1% dry and gelled samples, respectively. All other samples are not significantly different but all significantly different to the reference control.

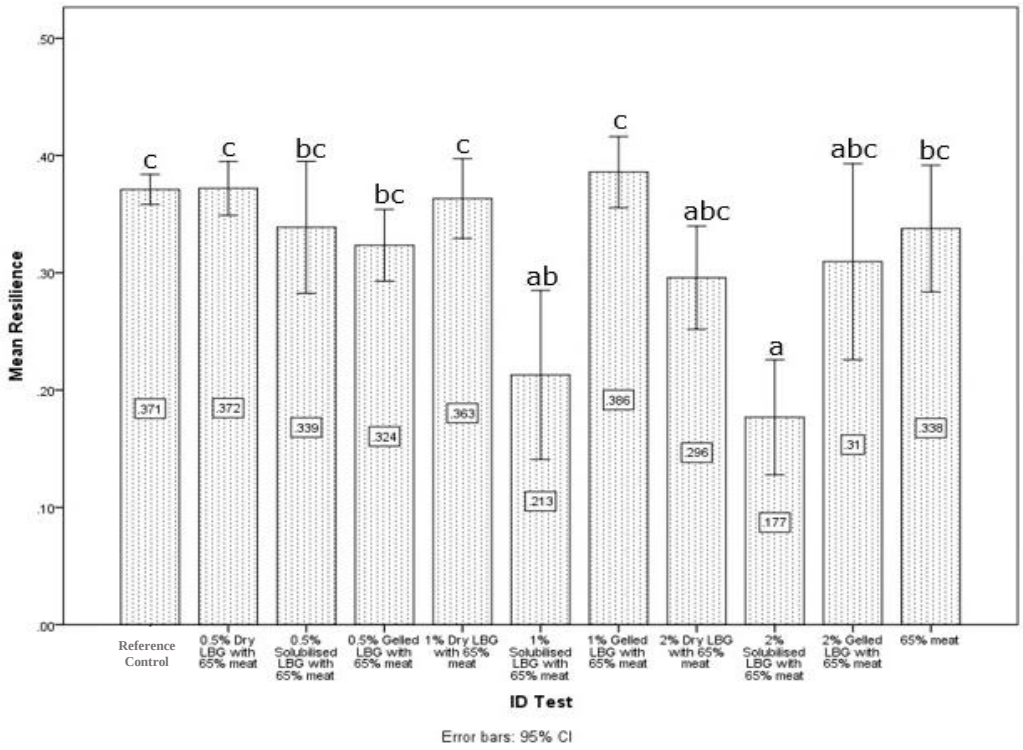


Figure 6.6: Resilience of adding dry, solubilised and gelled LBG to a 65% meat, compared to control samples (65% meat and reference).

Resilience, which is dimensionless, refers to the measurement of how the sausage sample recovers from deformation both in terms of speed and force derived, and in the investigation, it was found that 1% gelled LBG had the highest level of recovery from deformation and was not significantly different to both control samples and when LBG was added to 0.5% (in any format) and when LBG was added dry at 1 and 2%, and when 2% gelled LBG was added. Again this shows that when a higher concentration of LBG (1 and 2%) were added in the solubilised form 35% reduced meat samples were formed with significantly lower resilience, and again that this seems to affect the protein network. García-García and Totosaus (2008) reported that when 1% dry LBG is added to the meat batter, the resilience value is high; however, in this study, we found that 1% gelled LBG improved the resilience of the product and adding lower levels of LBG (0.5) in any forms of addition dose not impact an resilience.

6.3.2 Differential scanning calorimetry

In this study the function of 1% dry LBG in a 65% meat formulation was investigated (Figure 6.7). The first heat and second heat of top side meat without processing and also the first heating of control sample (65% meat) are already described in previous chapters.

It is surprising that the initial scanning of 1% dry LBG with 65% meat indicates a wide transition with an endothermic peak of 65.4˚C. It seems that two peaks– myosin denaturation and sarcoplasmic and actin denaturation– merge together and it is not possible to distinguish them from each other. The loss of the myosin peak and shift to lower temperature of the higher denaturation peak might indicate that LBG and affecting how the meat proteins mix. The curve of the second heating scan of the 1% LBG meat sample shows no response and indicates an irreversible transition from the LBG and proteins. To investigate the effect of the 1% dry LBG in reverse osmosis water was measured it can be concluded that there are no reactions during the first and second heating of LBG. Table 6.7 shows the onset and offset and peak temperatures for the endothermic peaks observed on heating the individual components or meat/LBG mixtures. The raw heat change data has been corrected for the weight of the specified component in the mixture. The table 6.7 shows the maximum onset related to 65% meat, which is 59.2˚C, and maximum offset related to the first heating of the 1% LBG with 65% meat, which is 85.6˚C. Within the error of measurement, baseline fitting and enthalpies it seems that any interaction between LBG and meat is unlikely to occur.

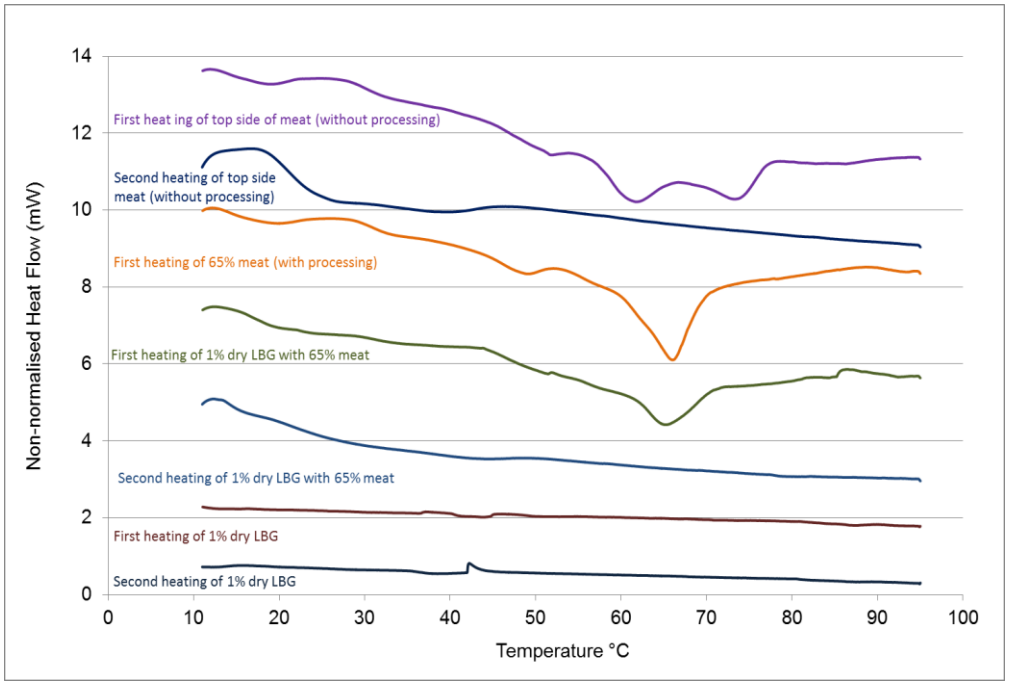


Figure 6.7: DSC thermograms for first heating of top side meat (without processing); second heating of top side meat (without processing); first heating of 65% meat; first heating of 1% dry LBG with 65% meat; second heating of 1% dry LBG with 65% meat; first heating of 1% dry LBG; second heating of 1% dry LBG triphosphate (details of sample preparation can be found in method and material chapter).

Table 6.7: Endothermic heating transition parametersfor top side meat (first heating); 65% meat (first heating); 1% dry LBG with 65% meat (first heating); 1% dry LBG with NaCl and sodium triphosphate (first heating).T_0 = onset temperature; T_e= end set temperature; T_m = peak temperature and ΔH = non-normalised enthalpy of transition.

samples	T_0	T_e	T_m	ΔH (J/g)
First heating of top side meat (without processing)	55.4	77.7	61.7	2.0
First heating of 65% meat (with processing)	59.2	67	66.1	2.1
First heating of 1% dry LBG with 65% meat	58.6	85.6	65.1	2.0

6.3.3 Small deformation mechanical spectroscopy

The 1% dry LBG with 65% meat samples were analysed for storage (G´) and loss (G˝) moduli and tan δ by dynamic oscillatory test, and compared to the 65% meat control as a function of temperature (30–90˚C) with rate of 1˚C/min (Figure 6.8).

Between the temperatures of 30 until 44˚C the structure build-up during the heating stage which is discussed in previous chapter.

Between the temperature of 44-54˚C the result of G´ in 1% dry LBG showed that there are two peaks, which represent the unfolding of myosin molecules; the irreversible interfilamental interaction between the myosin heads (45–50˚C)

(Egelandsdal et al., 1986; Sano et al., 1988); and another shoulder that maybe further explained by the denaturation and precipitation/coagulation of myosin (Ikeuchi et al., 1994; Samejima et al., 1981). Another hypothesis regarding the second development stage of G´ in 1% dry LBG with 65% meat was referred to as 'gel setting', in which the loose gel structure was formed and this is the conformation of the myosin molecules having changed. It is interesting to note that the two peaks in 65% meat are merging together and this is because of the fast interaction of the myosin heads or the interaction of myosin with other components, such as tropomyosin (Sano et al., 1988). The storage modulus increases gradually as the temperature increases, and this is due to the viscosity of the LBG treatments (Richardson and Norton, 1998). The storage modulus at above 48˚C, shows an initial decrease followed by a gradual increase maybe due to the solubilisation of LBG, and this is reflected by an initial shoulder for tan delta (tan δ=G˝/G´) which then decays with increasing temperatures. After 50˚C, tan δ of both samples decreased sharply. After 60˚C this decreases at a slower rate and reaches an equilibrium, suggesting gel development was complete. Also, at a temperature around 52˚C, denaturation of the myosin tails takes place, which leads to an increase in the protein network. The dissociation of the actin–myosin complex has also been suggested to contribute to the decrease in G´ between 54 and 60˚C.

Between the temperatures of 60–90˚C protein gelation take place. It is also during this temperature range that significant gelation of myosin is achieved (Ishioroshi et al., 1979; Ziegler and Acton, 1984). Further increases in G´ (60–70˚C), after the point where gelation starts, have been attributed to a network formation by the aggregation and entanglement of unfolded protein molecules. The contribution from the connective tissue proteins in increasing G´ during heating (Figure 6.8) must also be acknowledged, as collagen fibre contraction can occur up to the one-quarter of their original length under heating at 60–70˚C and if they are unrestricted (Tornberg, 2005), as would be expected in a high moisture content environment, such as the meat batter considered here. As the temperature reaches between 60 and 70˚C, the connective tissue and the muscle fibres are longitudinally contracted, and such a contraction increases with the temperature (Siripurapu et al., 1987). The slight increase in G´ in both figures during heating between the ranges of 70 to 80˚C is related to titin denaturation. Towards the end of the heating stage (at around 90˚C), a slight decrease in G´ can be seen in both figures, which may be attributed to the connected tissue and

collagen softening at this stage, as observed by Siripurapu et al. (1987) in meat emulsion products. In all samples the storage modulus (G´) was greater than the loss modulus (G˝) during the test (G´>G˝), which indicates the predominantly elastic behaviour of the sample for the whole range of deformation tested.

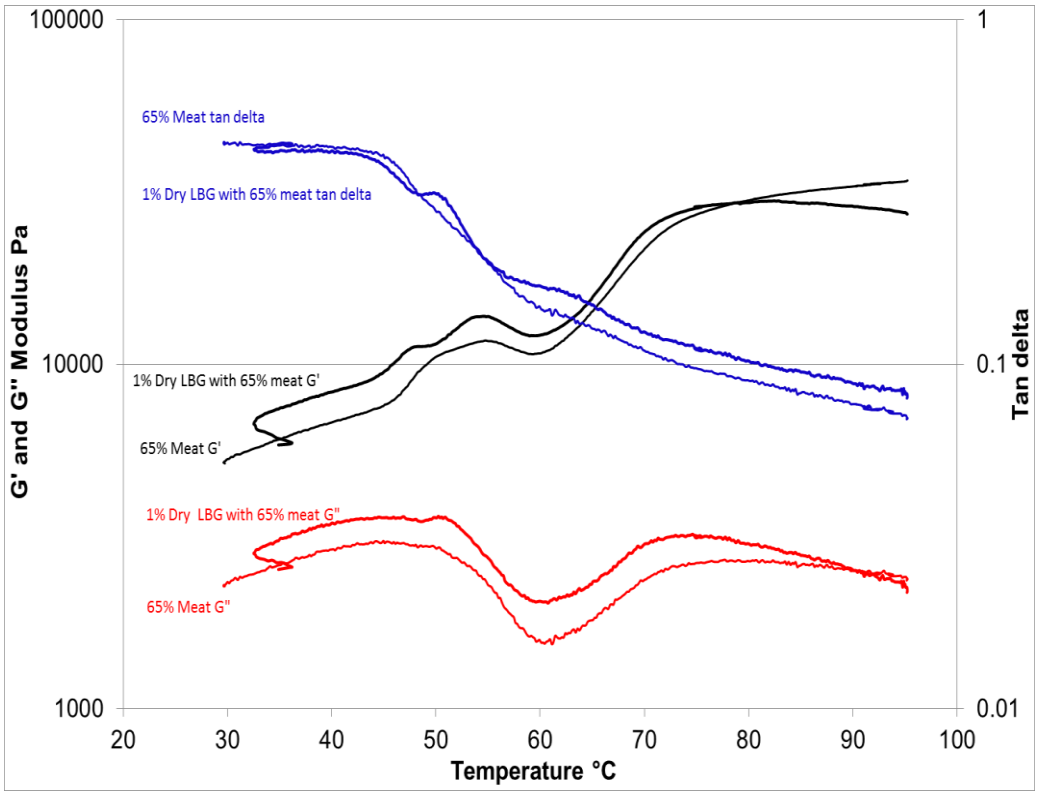


Figure 6.8: A comparison of the storage, loss moduli and phase angle (G´, G˝ and tan δ) when adding 1% dry LBG to a 65% meat and control sample (65% meat) as a function of heating. The curve represents the average of three replicate.

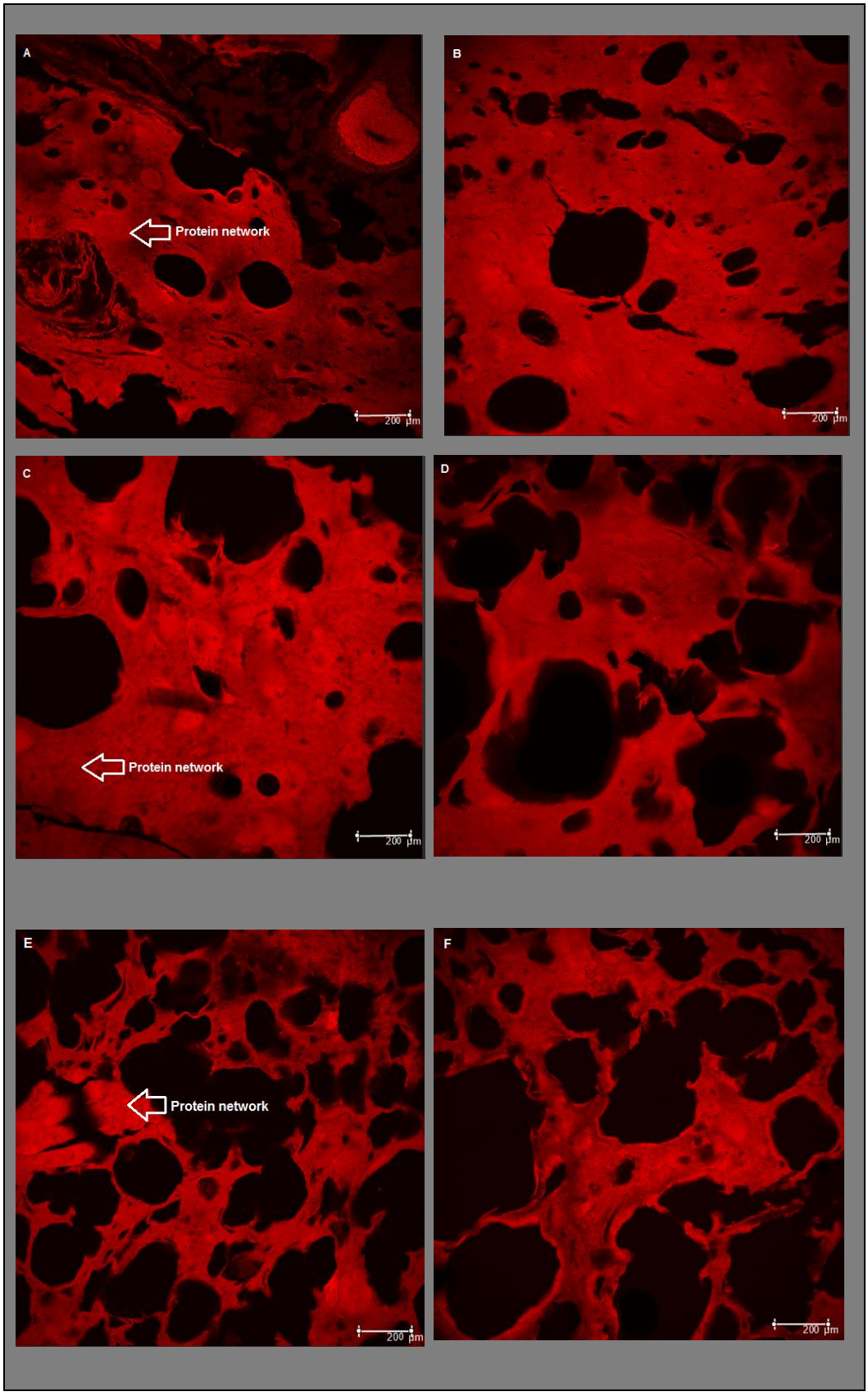
6.3.4 Structure of heated meat batters

Applying CLSM, the microstructure of beef sausage control samples (65% meat and reference) and 1% dry LBG with 65% meat was visualised (Figure 6.9). Sausage reference control images (A and B) and images (C and D) of 65% meat are already described in chapter 4.

Adding dry LBG to batter causes a change in the sausage microstructure with an apparent decrease in protein phase volume (E and F). Also, the size of the cavity is increased when adding 1% dry LBG (650–700 µm) when compared to control samples, which are 65% meat (500–550 µm), because the protein network in not evenly distributed in this system. The confocal results are inconclusive because in this study we did not succeed in staining LBG with fluorescein isothiocyanate

(FITC, 0.02% w/v in water/ethanol) in the protein matrix and it was not possible to definitively locate LBG, however, it is believe that LBG phase separates from the meat proteins in the meat batter product.

Confocal scanning laser microscopy has been used to see the location of fat in the system. Labelling the fat with BODIPY493/503 allowed the fat material, which is green (round area), to be identified as being fairly evenly distributed in the protein network (continuous area) throughout the structure. Control samples (65% meat) and 1% dry LBG with 65% meat show that fat coated by protein continuous phase forming the typical sausage emulsion-like structure. Furthermore, the fat droplets decrease in mean size when adding dry LBG to the system. The size of the fat droplets is 60 μm or less; however, the fat droplets of control samples are between 60 and 100 μm because the LBG hydrates, phase separates and increases the viscosity of the batter, providing a more efficient matrix for fat droplet break-up (Glicksman, 1969).


A
Protein network
200 µm
B
200 µm
C
Protein network
200 µm
D
200 µm
E
Protein network
200 µm
F
200 µm

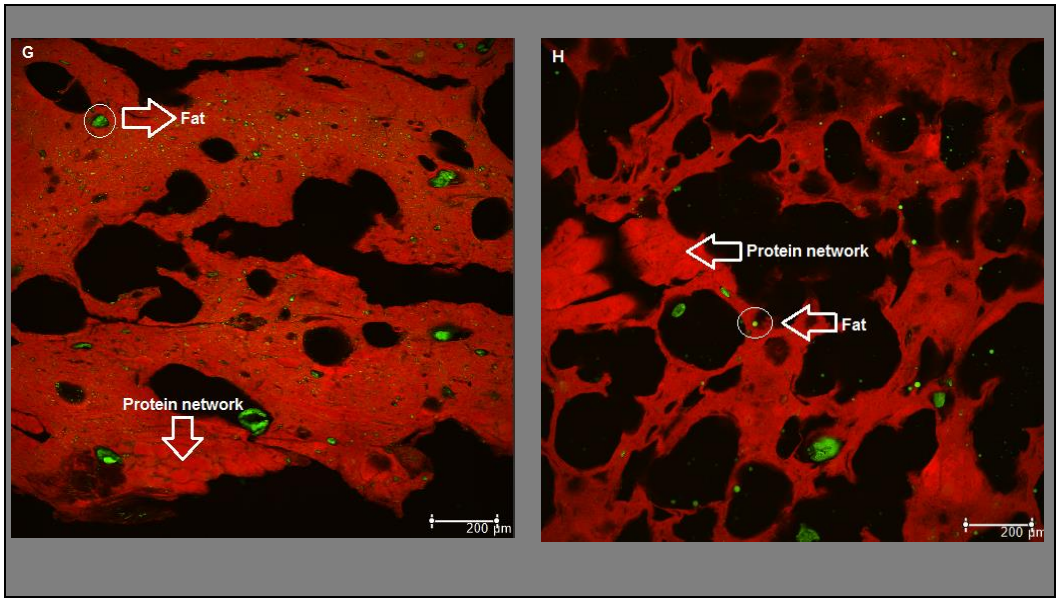


Figure 6.9: CLSM images of sausage containing dry LBG to a 65% meat, compared to control samples (65% meat) and reference. Images A and B are reference control samples stained for protein matrix; image C and D are the control samples (65% meat) stained for protein matrix; E and F images related to 1% dry LBG with 65% meat stained for protein matrix; image G is a control sample (65% meat) stained for fat and protein matrix, and finally image H is 1% dry LBG with 65% meat stained for fat and protein matrix.

6.3.5 Visual appearance of sausage

Figure 6.10 shows the visualisation of the 4 same sausages made by adding 1% dry LBG with 65% meat (A), compared to 4 same sausages of the control sample, 65% meat (B). There are similarities to the 65% meat control samples when compared to 1% dry LBG. In addition, according to the TPA, the rate of springiness and chewiness is high in this sausage.



Figure 6.10: Visual appearance of adding 1% dry LBG to 65 % meat *(A), compared to control samples, 65% meat (B).*

6.4 Conclusion

Locust bean gum, like other non-meat ingredients, can be viewed as being a 'filler' ingredient. 'Filler' ingredients can act to influence the formulation of the continuous gel matrix, modifying the physiochemical properties of the aqueous phase and influencing the final product. In terms of hardness, chewiness, springiness and cohesiveness, the texture analyser results indicated that 65% meat was not statistically different from 65% meat with added LBG. However, with regard to adhesiveness and resilience 65% meat was significantly different to 65% meat containing 2% solubilised LBG. Differential scanning calorimetry studies of myofibrillar protein and dry LBG revealed a slight effect on the meat protein with the addition of 1% dry LBG to meat. The rheology results show

changes corresponding to the differences seen in the DSC thermograms, with an overall reduction in elastic modulus and increase in tan δ. CLSM images which show that the phase separation between protein and LBG also takes place, and that the meat matrix is weakend because the protein network in not evenly distributed in this system. According to the chapter title, LBG (gelation) did not improve the hardness of the sausages; however, when dry LBG was added to this system, it improved the hardness of the sausages.

CHAPTER 7 CITRUS FIBRE ADDITION TO MEAT BATTER: DOMINATED BY SWELLING PARTICLES?

7.1 introduction

Interactions between polysaccharides and proteins occurs widely in the biological systems. Their interaction in food systems often play a role in determining the functional properties of these systems (Stainsby, 1980). Understanding the mechanisms involved in the interactions between proteins and polysaccharides and the way in which these interactions are affected during processing is important when these components are added into foods to improve their functional properties (Ledward, 1979). One of the healthy ingredients that has received more scientific attention is dietary fibre, whose consumption is highly recommended as it reduces the risk of several diseases and is perceived as being healthy (Verma and Banerjee, 2010). Dietary fibre is a group of food components that are resistant to hydrolysis by human digestive enzymes and are necessary for promoting good health. Dietary fibres are some of the ingredients that are used to provide meat products with low-fat and high fibre, and can also use in emulsified meat products (Grigelmo-Miguel et al., 1999). Different types of fibre have been successfully used in the processing of meat products: beet, oat, wheat, peas and carrots, and among fruit fibres, peach, pear, lemon, apple and citrus fibre are used to increase the cooking yield, due to their water and fat–binding properties and to improve texture and reducing production costs (Aleson-Carbonell et al., 2003; Casimir and Akoh, 1998; Claus and Hunt, 1991; Cofrades et al., 1995; Cofrades et al., 2000; Colmenero, 1996; Goldberg and Williams, 1991; Keeton, 1994; Verma and Banerjee, 2010; Viuda-Martos et al., 2009). Fibres are not only desirable for functional and technological properties, because they can be used as agriculture products and by-products for use as food ingredients, but they are also desirable for their nutritional properties (Thebaudin et al., 1997). The source of fibre is also important because different plant structures can affect different fibre properties. The composition of the citrus fibre was described in chapter 2. Grigelmo-Miguel et al. (1999) reported that the addition of 1% and 2% of peach dietary fibre suspension to frankfurters increased viscosity and decreased pH without influencing cooking loss. High levels of oat bran were associated with decreased expression of moisture and increased shear stress in low-fat chicken frankfurters (Chang and Carpenter, 1997). The water absorption of a citrus fibre is actually extremely high, likely to be based on its high soluble fibre content. Additionally the insoluble fibre is known to swell and contribute to water binding capacity (Fischer, 2000). An important consideration when formulating with citrus fibres is the taste profile.

Furthermore, citrus fibre may be used as a tool for improving texture, managing moisture in the replacement of fat, adding colour, reducing formulation costs and finally as a natural antioxidant. Citrus fibre may be incorporated into a broad range of products, particularly meat products (Aleson-Carbonell et al., 2005; Fernández-Ginés et al., 2003; Fernández-López et al., 2004; Fernández-López et al., 2007). Moreover, the addition of citrus fibre to dry-cured sausages has been reported to reduce residual nitrite (Viuda-Martos et al., 2009). However, there are few reports on the use of citrus fibre in beef meat sausages, and meat scientists need more scientific data to deliver formulated products that are able to meet the special requirements of consumers. Examples of such requirements may be an improvement in the chewiness and cohesiveness of the final product, linking such properties to product microstructure. The aims of this study were to evaluate a comparison of the influence of dry and pre-hydrated of citrus fibre on meat emulsion stability, the textural properties such as hardness, springiness, cohesiveness, chewiness and finally resiliencie in sausage texture formation; to evaluate the microstructure, such as location of protein network, fibre and fat in cooked sausages and furthermore, to elucidate the role of heat-induced gelation in the texture formation of processed meat products in the presence of citrus fibre.

7.2 Materials and Methods

Materials and methods used for this study are described in Chapter 3.

7.3 Results and Discussion

7.3.1 Texture properties

Results obtained from the statistical analyses of the collected data are presented in Table 7.1, 7.2, 7.3, 7.4, 7.5 and 7.6.

Table.7.1: Scheffe's test results (p-values) for the hardness of controls (65% meat and reference) with 65% meat containing dry and pre-hydrated citrus fibre (**<0.01 and *<0.05).

	Reference control	3% Dry citrus fibre with 65% meat	3% Pre-hydrated citrus fibre with 65% meat
3% Dry citrus fibre with 65% meat	0.05		
3% Pre-hydrated citrus fibre with 65% meat	0.00**	0.14	
65% Meat	0.00**	0.00**	0.01*

Table.7.2: Scheffe's test results (p-values) for the adhesiveness of controls (65% meat and reference) with 65% meat containing dry and pre-hydrated citrus fibre (**<0.01 and *<0.05).

	Reference control	3% Dry citrus fibre with 65% meat	3% Pre-hydrated citrus fibre with 65% meat
3% Dry citrus fibre with 65% meat	0.78		
3% Pre-hydrated citrus fibre with 65% meat	0.66	0.99	
65% Meat	0.98	0.61	0.49

Table.7.3: Scheffe's test results (p-values) for the springiness of controls (65% meat and reference) with 65% meat containing dry and pre-hydrated citrus fibre (**<0.01 and *<0.05).

	Reference control	3% Dry citrus fibre with 65% meat	3% Pre-hydrated citrus fibre with 65% meat
3% Dry citrus fibre with 65% meat	0.06		
3% Pre-hydrated citrus fibre with 65% meat	0.04*	0.99	
65% Meat	0.96	0.17	0.13

Table.7.4: Scheffe's test results (p-values) for the cohesiveness of controls (65% meat and reference) with 65% meat containing dry and pre-hydrated citrus fibre (**<0.01 and *<0.05).

	Reference control	3% Dry citrus fibre with 65% meat	3% Pre-hydrated citrus fibre with 65% meat
3% Dry citrus fibre with 65% meat	0.99		
3% Pre-hydrated citrus fibre with 65% meat	0.00**	0.00**	
65% Meat	0.67	0.47	0.01*

Table.7.5: Scheffe's test results (p-values) for the chewiness of controls (65% meat and reference) with 65% meat containing dry and pre-hydrated citrus fibre (**<0.01 and *<0.05).

	Reference control	3% Dry citrus fibre with 65% meat	3% Pre-hydrated citrus fibre with 65% meat
3% Dry citrus fibre with 65% meat	0.00**		
3% Pre-hydrated citrus fibre with 65% meat	0.00**	0.00**	
65% Meat	0.00**	0.00**	0.93

Table.7.6: Scheffe's test results (p-values) for the resilience of controls (65% meat and reference) with 65% meat containing dry and pre-hydrated citrus fibre (**<0.01 and *<0.05).

	Reference control	3% Dry citrus fibre with 65% meat	3% Pre-hydrated citrus fibre with 65% meat
3% Dry citrus fibre with 65% meat	0.98		
3% Pre-hydrated citrus fibre with 65% meat	0.00**	0.00**	
65% Meat	0.76	0.87	0.00**

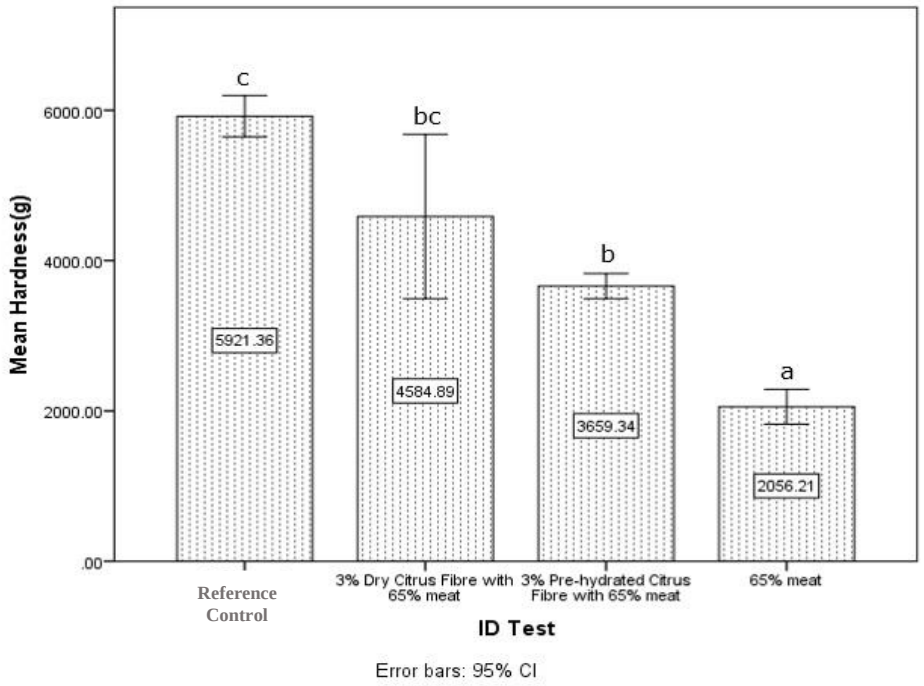


Figure 7.1: Hardness of adding dry and pre-hydrated citrus fibre to a 65% meat, compared to control samples (65% meat and reference).

Analysis of variance (ANOVA) showed that hardness differed significantly by ID-Test type. Hence, Scheffe's test was applied in order to compare the means of the cohesiveness ID-Test (Table 7.1).

As previously observed the control samples were statistically different. What can be seen is that there is a significantly difference between the 65% meat control and both samples with added citrus fibre. Also, there is a significant difference in the hardness when comparing the pre-hydrated citrus fibre sample with the reference. However, although lower in mean hardness values the dry citrus fibre 65% meat control sample is statistically not different to the reference. This is in agreement with previous findings which is reported that the hardness of frankfurters was increased by the addition of 0–2% dry citrus fibre (Petridis et al., 2014). Furthermore, these authors showed that the greater the amount of citrus fibre added, the more hardness increased. What this present study has shown is that the state of citrus fibre in meat batter production is also important. Also, in a similar study, Fernández-Ginés et al. (2003), found that adding dry citrus fibre to bologna-type sausages increased the hardness of the products in all the examined concentrations (0.5, 1, 1.5 and 2%). Water-holding capacity (WHC) is the ability of meat to hold on to its own or added water during

processing (Talukder, 2015). Good WHC is essential as it gives desirable characteristics to meat products, and citrus fibre is suitable for being added to meat products to increase the WHC (Cofrades et al., 2000). For this reason, citrus fibre improves the hardness of beef sausages. However, 3% pre-hydrated citrus fibre with 65% meat has a lower mean hardness value, because the meat structure is unable to complete efficiently for the water once the citrus fibre is hydrated.

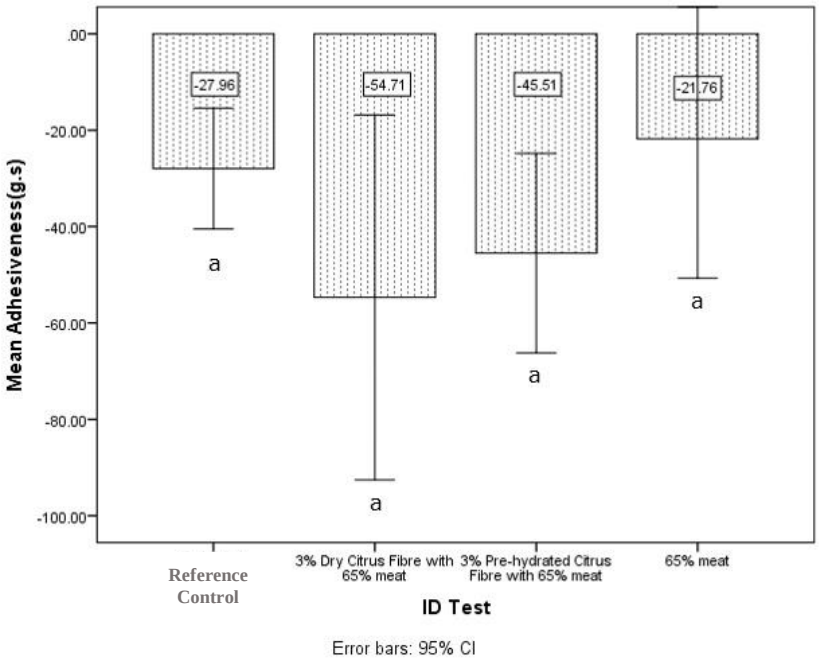


Figure 7.2: Adhesiveness of adding dry and pre-hydrated citrus fibre to a 65% meat, compared to control samples (65% meat and reference).

Analysis of variance (ANOVA) showed that adhesiveness did not differ significantly by ID-Test types (Table 7.2). Therefore, there was no statistically significant difference between ID-Tests, as the error bar are too large. Petridis et al. (2014) reported that with the addition of 2% dry citrus fibre, the adhesiveness of the product increased. However, in the current study a difference in state of a slightly higher concentration of citrus fibre has no impact on product adhesiveness.

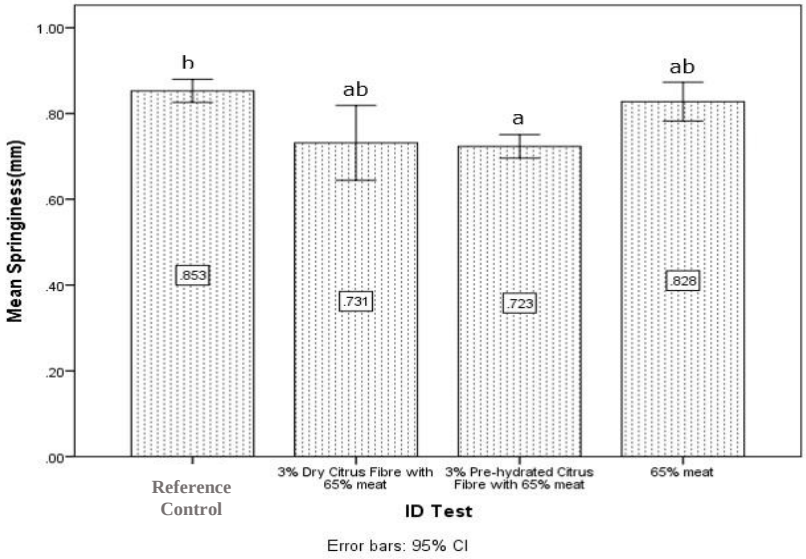


Figure 7.3: Springiness of adding dry and pre-hydrated citrus fibre to a 65% meat, compared to control samples (65% meat and reference).

Analysis of variance (ANOVA) showed that springiness differed significantly by ID-Test types. Thus, Scheffe's test was applied in order to compare the means of the springiness ID-Test (Table 7.3).

As before, the control samples are not significantly different in springiness values. Additionally, the addition of 3% dry citrus fibre to a 65% meat product shows springiness values not significantly different to the reference control. However, when 3% pre-hydrated citrus fibre is added the sample is significantly different to the reference control. Fernández-Ginés et al., (2003) reported that springiness increased with a level of 2% dry citrus fibre, however, in this study the mean value decreased for both dry and pre-hydrated citrus fibre at the 3% addition level.

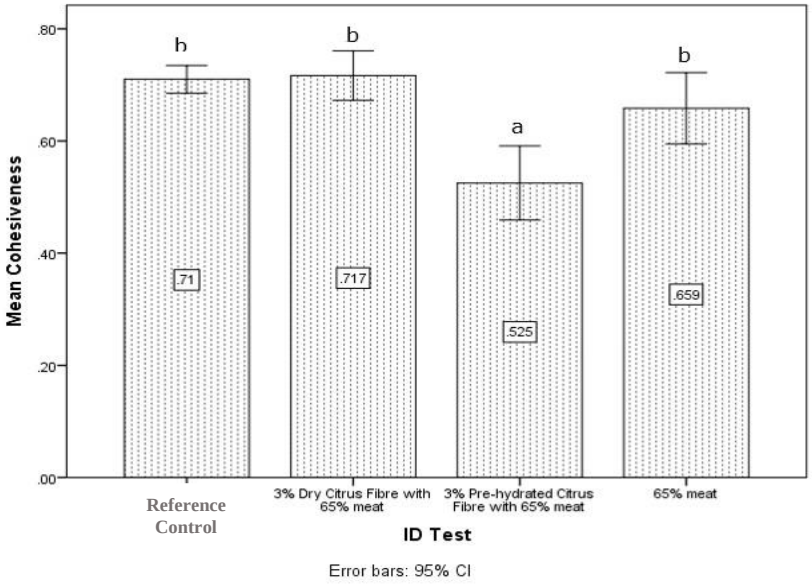


Figure 7.4: Cohesiveness of dry and pre-hydrated citrus fibre to a 65% meat, compared to control samples (65% meat and reference).

Analysis of variance (ANOVA) showed that cohesiveness differed significantly by ID-Test type (Table 7.4). Consequently, Scheffe's test was applied in order to compare the means of the cohesiveness ID-Tests. The only one of the samples to be significantly different was the 3% pre-hydrated citrus fibre samples. It is interestingly to note that such an addition of material with high WHC is sufficient to be different (with the lower mean value) to be 65% control, but also when that material is added as a dry material then values not significantly different to the reference control are achievable. Fernández-Ginés et al. (2003) reported that cohesiveness was affected only when dry citrus fibre was added at 2%, while in this study we showed that 3% dry citrus fibre improved cohesiveness (increased mean values) but if added at that in concentration in a pre-hydrated state the cohesiveness is decreased.

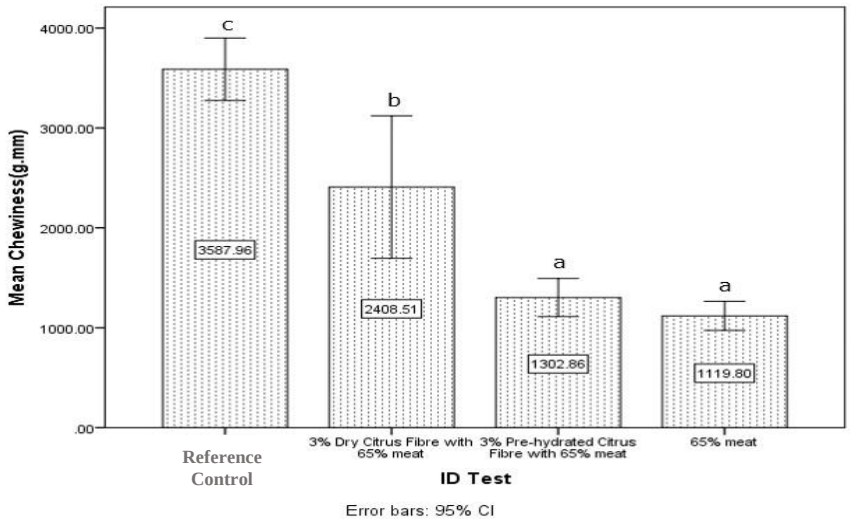


Figure 7.5: Chewiness of adding dry and pre-hydrated citrus fibre to a 65% meat, compared to control samples (65% meat and reference).

The statistical analysis for chewiness of the samples matched that found for gumminess where reference control>3% dry citrus fibre>3% pre-hydrated citrus fibre = 65% meat control.

Fernández-Ginés et al. (2003) showed that chewiness like springiness increased with the addition of 2% dry citrus fibre and significantly improved the meat batter, and in this study we showed that the addition of 3% dry and pre-hydrated citrus fibre caused reduction chewiness and this is because of again meat structure is unable to complete efficiently for the water once the citrus fibre is hydrated.

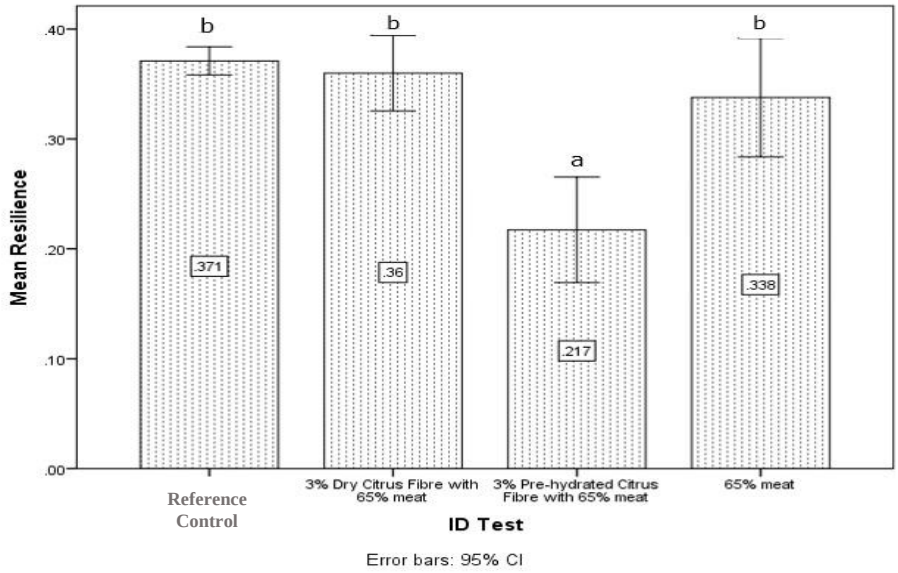


Figure 7.6: Resilience of adding dry and pre-hydrated citrus fibre to a 65% meat, compared to control samples (65% meat and reference).

Analysis of variance (ANOVA) showed that resilience differed significantly by ID-Test type. Hence, Scheffe's test was conducted in order to compare the means of the resilience ID-Tests (Table 7.6). The resilience results shows that there is no significant difference between the control samples or the 3% dry citrus fibre sample. However, the 3% pre-hydrated citrus fibre sample has a lower mean resilience value and is significantly different from the other samples. Resilience, is a measurement of how the sausage sample recovers from deformation both in terms of speed and force derived and therefore, 3% dry citrus fibre addition enable a 35% meat reduction with a matched resilience to the reference control. The 65% meat control, while being not significantly different to the reference control has a lower mean resilience value and large error bar. The attractiveness of the addition of dry citrus fibre is heightened when the pre-hydrated citrus fibre does not produce samples that which the reference control at the lower meat content. Again this shows that the state of added citrus fibre is important in providing stable meat textures because citrus fibre too efficient in WHC and creating too much heterogeneity in the product and therefore affecting the protein matrix and is in agreement with previous results (Lundberg, 2005; Verma and Banerjee, 2010).

7.3.2 Differential scanning calorimetry

In this study the functions of dry citrus fibre (3% with 65% meat) on the formulation of sausage batter composite during heating were investigated. Studies of 3% dry citrus fibre with 65% meat indicated that there were two transitions. The first transition (I) was due to myosin (45˚C) and the second transition peak (II) at 65.47˚C, which is broad, is due to the formation of a gel structure, therefore the differences with the control are due to the effect that the hydration citrus fibre has on the meat protein structure and the denaturation of sarcoplasmic protein, connective tissue and actin. It seems that when adding dry citrus fibre to batter, the protein network and the citrus fibre compete for the water and during cooking treatment myofibrillar proteins entrap the partially hydrated fibre and as a consequence improves the WHC of the final product. This is in agreement with previous findings (Cofrades et al., 2000) because during heating without citrus fibre loss of water from the structure takes place, which has an inverse relationship to the juiciness of products. No major transitions were detected in the second heating thermograms of the 3% dry citrus fibre with 65% meat, which indicates the protein and also citrus fibre undergo an irreversible transition during the heat treatment. To confirm this result we studied the first heating of 3% citrus fibre in reverse osmosis water and this indicated that no transition was found when the citrus fibre samples were scanned up to 90˚C under conditions of the DSC experiment; this is confirmed from a previous finding from Aguilera et al. (1998) who reported that for apple fibre one wide endothermic peak between 124 and 136˚C occurs, which indicates that there is no transition below 90˚C for any kind of fibre. Furthermore, Bertran and Dale (1986) reported the thermal behaviour of a cellulose sample in which a broad endothermic peak appears between 110 until 160˚C, this being due to the loss of water bonding. To investigate the effect of sodium chloride and sodium triphosphate on citrus fibre the first heating of 3% citrus fibre with 2.7% NaCl, and 0.6% sodium triphosphate in reverse osmosis water was measured it can be concluded that there are no reactions during the heating of citrus fibre. Table 7.7 shows the onset and offset and peak temperatures for the endothermic peaks observed on heating the individual components or meat/citrus fibre mixtures. The data has been corrected for the weight of the specified component in the mixture. The table shows that the maximum onset is related to the control sample (65% meat) which is 59.2˚C, and the maximum offset is related to topside, meat which is 77.7˚C. Citrus fibre contains 0.22 grams of pectin/g of

the sample. Pectin is an anionic hydrocolloid with different degrees of methoxylation, therefore, contaning a different levels of ionic charge depending on the type of pectin used or present in the sample. Barrera et al. (2002) reported that there is no interaction between low methoxyl (LM) pectin and surimi gels and this is because the covalent bonded surami gel masked or inhibited the protein–calcium–pectin interactions, or because this kind of interaction has no beneficial effect on the mechanical properties of surami gels. This author also reported that high methoxyl (HM) pectin interacts with proteins above the isoelectric point (IP); however, Glahn (1982) reported that the interaction between pectin with proteins below their isoelectric point (approx.5.0–5.1) occurred. In this study within the error of measurement and baseline fitting, enthalpies were linearly additive, suggesting no interaction between meat and dry citrus fibre. According to the DSC results in this study it seems that pectin in citrus fibre does not interact with proteins because, the pH of batter after adding sodium chloride and sodium triphosphate was 5.8 and an interaction was unlikely to happen.

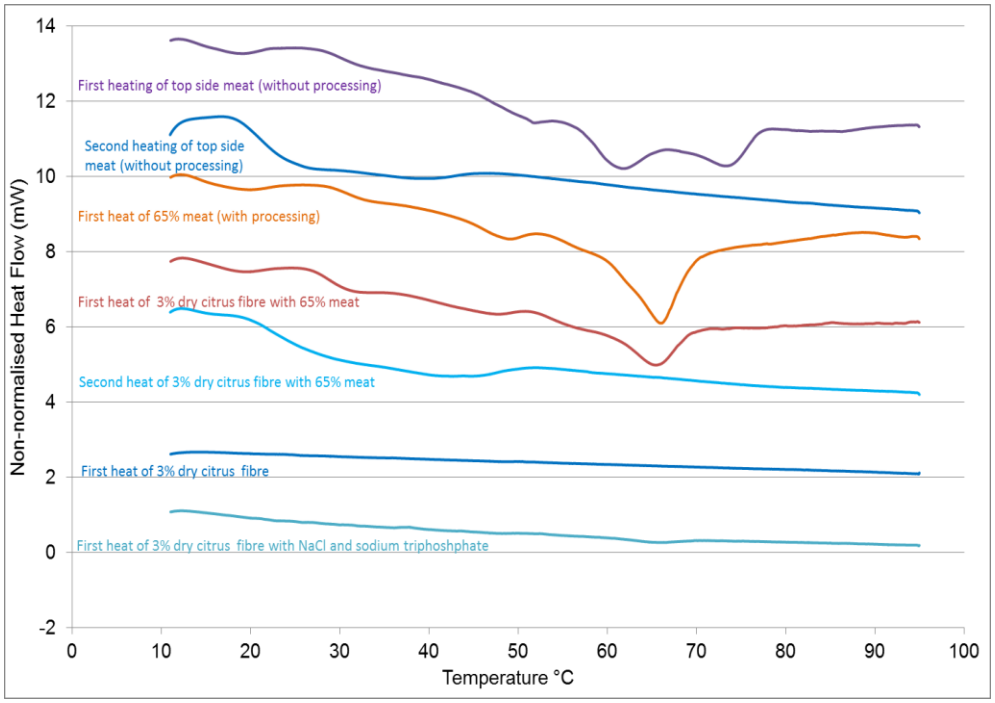


Figure 7.7: Endothermic DSC Thermograms for first heating of top side meat (without processing); second heating of top side meat (without processing); first heating of 65% meat (with processing); first heating of 3% dry citrus fibre with 65% meat; second heating of 3% dry citrus fibre with 65% meat; first heating of 3% dry citrus fibre; first heating of 3% dry citrus fibre with NaCl and sodium triphosphate triphosphate (details of sample preparation can be found in method and material chapter).

Table 7.7: Endothermic heating transition parameters for Topside meat; 65% meat; 3% citrus fibre with 65% meat (first heating) *T0 = onset temperature; Te= end set temperature; Tm = melting peak temperature and ΔH = non-normalised enthalpy of transition.*

Samples	T_0	T_e	T_m	ΔH (J/g)
Topside meat (without processing)	55.4	77.7	61.7	2.0
65% meat (with processing)	59.2	67	66.1	2.1
3% citrus fibre with 65% meat (first heating)	58.6	70	65.4	2.0

7.3.3 Small deformation mechanical spectroscopy

Rheological characteristics of the 3% dry citrus fibre with 65% meat and the 65% meat control sample, during heating from 30˚C to 90˚C are described in figure 7.8. Tangent δ for both samples decreased slightly with a rise in temperature to around 47˚C, and then decreased sharply to around 90˚C, consistent with the protein denaturation events evidenced by DSC. In terms of the citrus fibre sample tan δ showed small peak at around 50˚C which was different to the control, indicating that the presence of citrus fibre in the batter production affect the protein structuring. The change in storage modulus of both treatments can be divided into four characteristic temperature ranges: 30–45˚C; 45–54˚C; 54–60˚C and 60–90˚C. In the 30–45˚C range, the storage modulus (G´) increased slightly with the rise of temperature and the tan δ decreased slightly, indicating that the formation of gel structure was occurring. Also, in this range the denaturation of the head proteins of myosin and subsequent aggregation responsible for the initial increase in G´ occurs (Verbeken et al., 2005). The DSC analysis (Figure 7.7) shows that myosin had a small endothermic region at 45˚C, suggesting that the conformation of myosin molecules changed in this temperature range. Formation of an elastic gel network (an increase in G´) from 45 to 54˚C probably result from an aggregation of myosin due to the unfolding of the head proteins. The 3% dry citrus fibre with 65% meat indicating that two peaks, which representing the unfolding of the myosin molecules; the irreversible interfilamental interaction between myosin heads (45–50˚C) (Egelandsdal et al., 1986; Sano et al., 1988) and another peak may be further explained by the denaturation and precipitation/coagulation of myosin (Ikeuchi et al., 1994; Samejima et al., 1981). The increase in G´ at around 51˚C was referred to as ‘gel setting’, in which the structure was formed, and this is the changed conformation of the myosin molecules. It is important to note that the

two peaks in the 65% control sample, merge together and this is due to the fast interaction of the myosin heads or the interaction of myosin with other components, such as tropomyosin (Sano et al., 1988). From 55 to 60˚C, G´ decreased, reaching a minimum at 60˚C, and this is due to the breaking hydrogen bonds of myosin. Also in this phase, denaturation and precipitation/coagulation of myosin heads occurs (Ikeuchi et al., 1994; Samejima et al., 1981). Hence, gel elasticity decreases. In the last development stage of modulus in the 60–90˚C range, the increase in the storage modulus happened with the increasing temperature, consistent with the major thermal transitions reported in Figure 7.7. Furthermore, during this final thermal phase, significant gelation of myosin is achieved (Ishioroshi et al., 1979; Ziegler and Acton, 1984). Future increases in G´ with temperature, after the point where gelation starts, have been attributed to a network formation by the aggregation and entanglement of unfolded protein molecules excluded from the citrus fibre. Titin denaturation can occur in the range of 70 to 80˚C and this is shown by a slight increase in G´ in both figures during heating. The differences between the rhology of batters contaning citrus fibre and other treatments is the high starting elastic modulus at 30˚C, and lower tan δ. This indicate that citrus fibre is very efficient at binding water and provides evidence that pre-hydration is probably too efficient, not allowing sufficient protein network formatted for producing sausages.

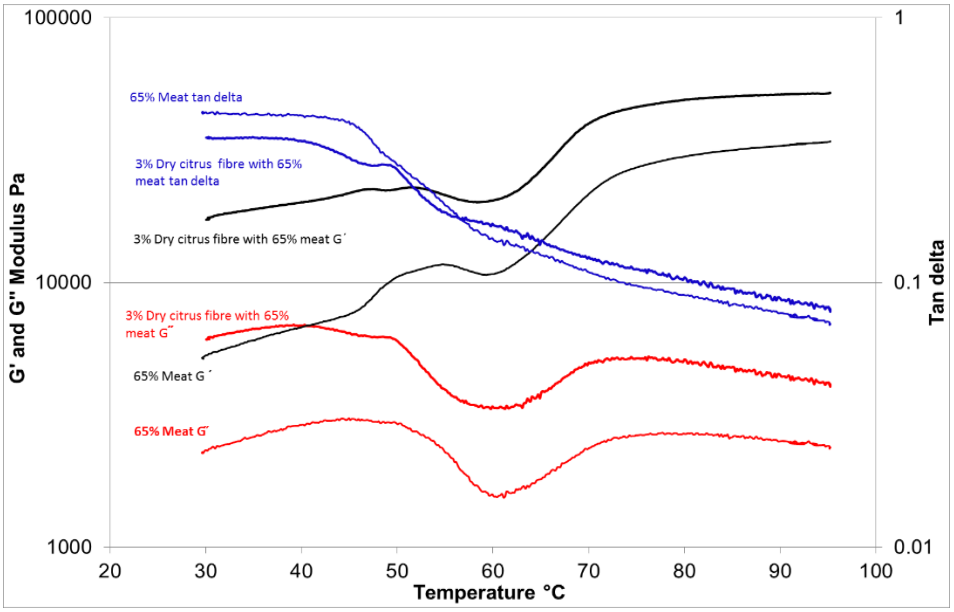
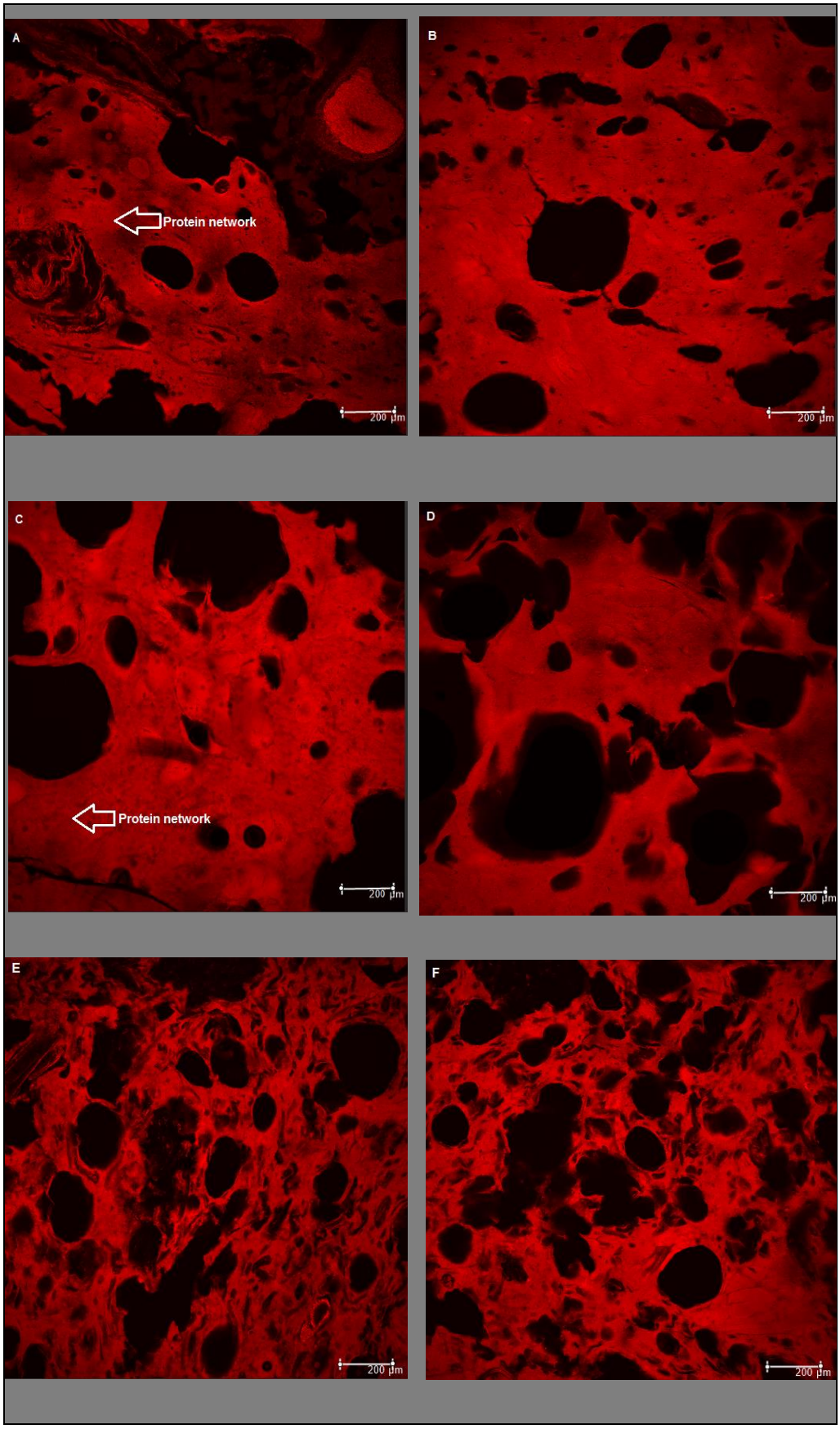


Figure 7.8: A comparison of change of the storage, loss moduli and phase angle (G´, G˝ and tan δ) when adding 3% dry citrus fibre to a 65% meat and control sample (65% meat) by the effect of heating. The curve represents the average of three replicate.

7.3.4 Structure of heated meat batters

Confocal scanning laser microscopy was again used to visualise the microstructure of beef sausage control samples (65% meat and reference) and 3% dry citrus fibre with 65% meat. The aim of the visualisation was to determine changes in the micro structure of the protein matrix when adding citrus fibre or not. Reference control images (A and B) and 65% meat images (C and D) are shown in Figure 7.9. The protein matrix or muscle fibre (red colour) is completely distributed throughout the system. The large black cavities are believed to be air cells and the size is decreased with increased amount of meat protein. The citrus fibre seem to be entrapped in the protein network and the void size decreased (image E and F). Presumably by the increase in viscosity of the batter, which was also evidenced in the higher modulus of the citrus fibre containing batter in Figure 7.8, the citrus fibre would enhance the structuring of the protein network. The presence of citrus fibre (image G and H) could be imaged with Acridine orange 0.01% (w/v), and the citrus fibre were visible evenly distributed, and also evidence as large pieces of plant fibre irregularly distributed in the sausages (200-220 µm length and 8-13 µm width). It worth to mentioning that in this study, we did not succeed to stain protein and carbohydrate simultaneously.

Labelling the fat with BODIPY493/503 allowed the fat material, which is green (round area), to be identified as being evenly distributed in the protein network (continuous area) throughout the structure. Control sample, (image I) and 3% dry citrus fibre with 65% meat (image J) shows that fat coated with protein continuous phase forming the typical sausage emulsion-like structure. Furthermore, the fat droplets appears to decrease in size when 3% dry citrus fibre is added to the system, again a consequence of an increase in the batter viscosity. The size of the fat droplets is between 20 and 50 µm for the citrus fibre sample, but between 60 and 100 µm for the control. The decrease of both air cell size and fat droplet size is thought to be a function of the increased viscosity/texture of the meat batter during comminution.


A
Protein network
200 µm
B
200 µm
C
Protein network
200 µm
D
200 µm
E
200 µm
F
200 µm

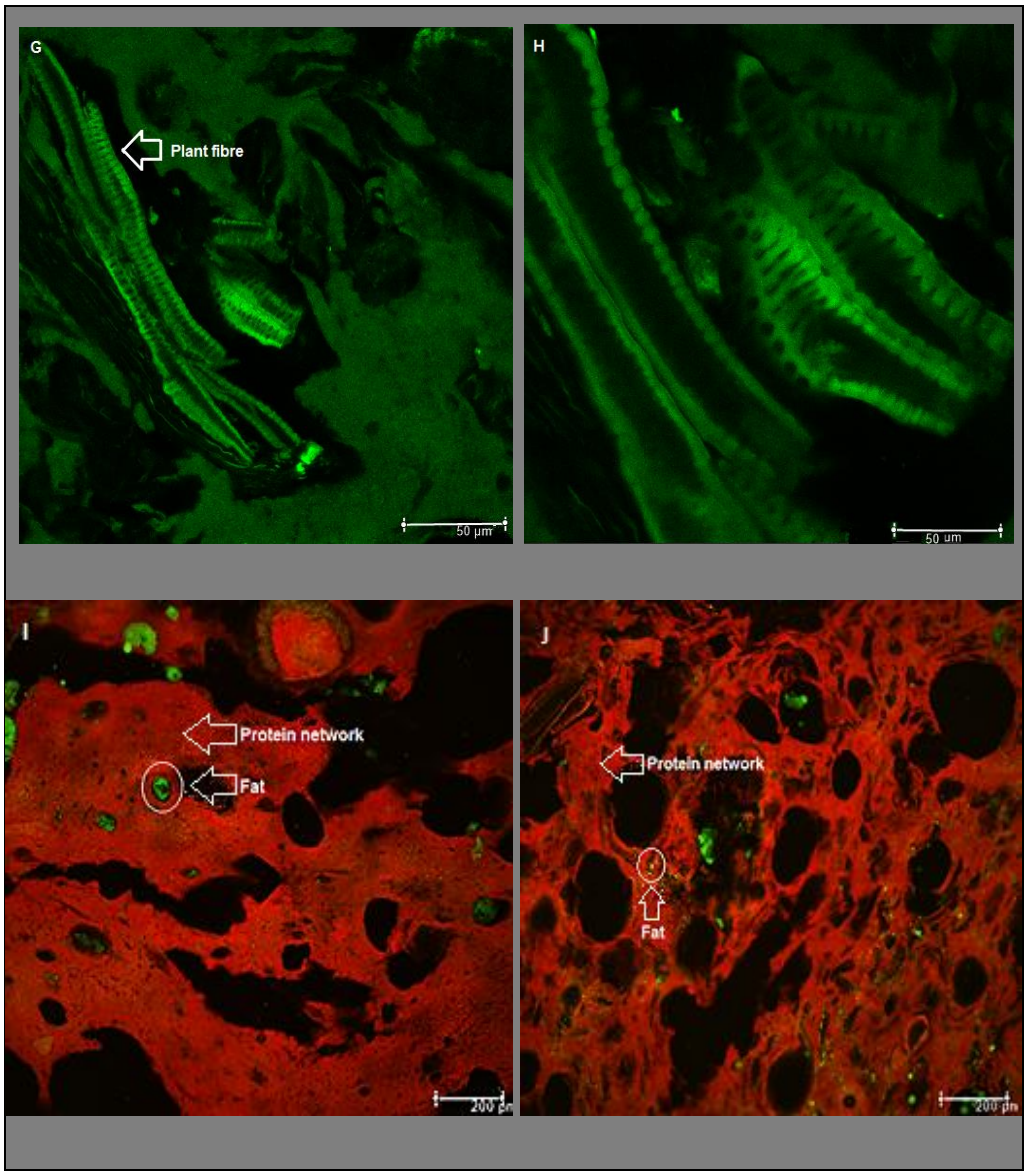


Figure 7.9: CLSM images of sausage containing dry citrus fibre to a 65% meat, compared to control samples (65% meat) and reference. Images A and B are reference control samples stained for protein matrix; images C and D are the control samples (65% meat) stained for protein matrix; images E and F are 3% dry citrus fibre with 65% meat stained for protein matrix; image G and H are 3 % dry citrus fibre with 65 % meat stain for citrus fibre; image I is the control sample (65% meat) stained for fat and protein matrix; and finally image J is 3% dry citrus fibre with 65% meat stained for fat and protein matrix.

7.3.5 Visual appearance of sausage

Figure 7.10 shows the visualisation of the same sausage of 3% dry citrus fibre with 65% meat (A), compared to 4 same sausages of control sample, 65% meat (B). In terms of the hardness and chewiness, there are statistically significant differences when comparing the control sample (65% meat). However, with regards to adhesiveness, springiness, cohesiveness and resilience, there are similarities to the 65% meat control. Also, the highest mean value of

adhesiveness and cohesiveness of the beef samples are related to 3% dry citrus fibre with 65% meat.



Figure 7.10: Visual appearance of adding 3% dry citrus fibre to a 65% meat *(A), compared to control samples, 65% meat (B).*

7.4 Conclusion

The results obtained from the texture analysis show that the p-value of 3% dry citrus fibre in all of parameters are higher than 3% pre-hydrated and can be concluded that adding dry citrus fibre is better than adding it in a pre-hydrated. Also, 3% dry citrus fibre is statistically different from reference control sample in terms of chewiness. However, 3% pre-hydrated citrus fibre is statistically different with reference control samples in all of parameters except adhesiveness. The finding of the present work unveils a significant change in the texture of beef sausage when adding citrus fibre to meat batter. This study reports, for the first time, the differential scanning calorimetry (DSC) and rheology results, showing the effect of citrus fibre on the thermal stability of myofibrillar proteins in meat batter. The DSC results suggested there is no intraction between proteins and polysaccharides. The rheology results confirmed

the DSC results and also show an increase in elastisity of materials. Furthermore, the presence of citrus fibre affects the protein structure during heating because the protein network and the citrus fibre compete for the water. Confocal scanning laser microscopy (CLSM) showed that the size of cavities in the meat matrix decreased on the addition of 3% dry citrus fibre. The mean diameters of the fat droplets also decreased in a 3% dry citrus fibre/65% meat system. Also, the elongated citrus fibre are effective at incresing the modulus of the protein network and they are evenly distributed thoughout the protein gel and that the citrus fibre is very effective hydrating agent, producing swollen particles within the meat matrix.

Refering to the chapter title, it could be concluded that adding dry citrus fibre during processing improved the texture parameters of final product such as hardness becuse fibre swells during processing and for this resaon 3% dry citrus fibre in all of parameters is higher than 3% pre-hydrated. It is proposed that this type of fundamental knowledge will help improve meat processing technology.

CHAPTER 8 COMPARISONS AND GENERAL CONCLUSIONS

8.1 Comparisons

In this chapter the materials are compared in the different modes of application. The rheological parameters of hardness, springiness, cohesiveness and resilience have been chosen for the comparison due to them being direct measurements of material properties (primary characteristics in Table 3.9) and not compared measures.

8.1.1 Comparisons of texture properties when the ingredients were added dry

Results obtained from the statistical analyses of the collected data are presented in table 8.1, 8.2, 8.3 and 8.4.

Table 8.1: Scheffe's test results (p-values) for the hardness of adding dry LBG, kappa, iota, lambda, citrus fibre and starch to a 65% meat, compared to control samples (65% meat and reference). (**<0.1 and *<0.5).

	65% Meat	3% Dry citrus fibre with 65% meat	3% Dry starch with 65% meat	7.4% Dry starch with 65% meat	0.5% Dry LBG with 65% meat	0.5% Dry kappa with 65% meat	0.5% Dry iota with 65% meat	0.5% Dry lambda with 65% meat	1% Dry LBG with 65% meat	1% Dry kappa with 65% meat	1% Dry iota with 65% meat	1% Dry lambda with 65% meat	2% Dry LBG with 65% meat	2% Dry kappa with 65% meat	2% Dry iota with 65% meat	2% Dry lambda with 65% meat
3% Dry citrus fibre with 65% meat	0.22															
3% Dry starch with 65% meat	0.95	0.99														
7.4% Dry starch with 65% meat	0.00**	0.07	0.00**													
0.5% Dry LBG with 65% meat	1.00	0.08	0.91	0.00**												
0.5% Dry kappa with 65% meat	0.99	0.92	1.00	0.00**	0.99											
0.5% Dry iota with 65% meat	1.00	0.44	0.99	0.00**	1.00	1.00										
0.5% Dry lambda with 65% meat	0.98	0.98	1.00	0.00**	0.97	1.00	1.00									
1% Dry LBG with 65% meat	1.00	0.54	0.99	0.00**	1.00	1.00	1.00	1.00								
1% Dry kappa with 65% meat	0.16	1.00	0.99	0.05	0.04*	0.88	0.34	0.97	0.45							
1% Dry iota with 65% meat	0.09	1.00	0.95	0.12	0.01*	0.73	0.19	0.91	0.28	1.00						
1% Dry lambda with 65% meat	0.99	0.95	1.00	0.00**	0.98	1.00	1.00	1.00	1.00	0.92	0.80					
2% Dry LBG with 65% meat	1.00	0.06	0.88	0.00**	1.00	0.99	1.00	0.96	1.00	0.03*	0.01*	0.98				
2% Dry kappa with 65% meat	0.00**	0.12	0.00**	1.00	0.00**	0.00**	0.00**	0.00**	0.00**	0.10	0.20	0.00**	0.00**			
2% Dry iota with 65% meat	0.00**	0.99	0.18	0.90	0.00**	0.03*	0.00**	0.13	0.00	0.99	0.99	0.05	0.00**	0.96		
2% Dry lambda with 65% meat	0.96	0.99	1.00	0.00**	0.93	1.00	1.00	1.00	1.00	0.99	0.97	1.00	0.91	0.00**	0.24	
Reference control	0.00**	0.98	0.31	0.99	0.00**	0.10	0.01*	0.24	0.02*	0.98	0.99	0.14	0.00**	1.00	1.00	0.35

Table 8.2: Scheffe's test results (p-values) for the springiness of adding dry LBG, kappa, iota, lambda, citrus fibre and starch to a 65% meat, compared to control samples (65% meat and reference). (**<0.1 and *<0.5).

	65% Meat	3% Dry citrus fibre with 65% meat	3% Dry starch with 65% meat	7.4% Dry starch with 65% meat	0.5% Dry LBG with 65% meat	0.5% Dry kappa with 65% meat	0.5% Dry iota with 65% meat	0.5% Dry lambda with 65% meat	1% Dry LBG with 65% meat	1% Dry kappa with 65% meat	1% Dry iota with 65% meat	1% Dry lambda with 65% meat	2% Dry LBG with 65% meat	2% Dry kappa with 65% meat	2% Dry iota with 65% meat	2% Dry lambda with 65% meat
3% Dry citrus fibre with 65% meat	0.59															
3% Dry starch with 65% meat	0.99	0.99														
7.4% Dry starch with 65% meat	1.00	0.28	0.98													
0.5% Dry LBG with 65% meat	1.00	0.99	1.00	0.99												
0.5% Dry kappa with 65% meat	0.99	0.99	1.00	0.99	1.00											
0.5% Dry iota with 65% meat	1.00	0.87	1.00	1.00	1.00	1.00										
0.5% Dry lambda with 65% meat	1.00	0.90	1.00	1.00	1.00	1.00	1.00									
1% Dry LBG with 65% meat	1.00	0.98	1.00	1.00	1.00	1.00	1.00	1.00								
1% Dry kappa with 65% meat	0.99	0.99	1.00	0.99	1.00	1.00	1.00	1.00	1.00							
1% Dry iota with 65% meat	1.00	0.62	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00						
1% Dry lambda with 65% meat	0.99	1.00	1.00	0.97	1.00	1.00	1.00	1.00	1.00	1.00	0.99					
2% Dry LBG with 65% meat	1.00	0.97	1.00	0.99	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00				
2% Dry kappa with 65% meat	0.96	1.00	1.00	0.89	1.00	1.00	0.99	1.00	1.00	1.00	0.98	1.00	1.00			
2% Dry iota with 65% meat	1.00	0.06	0.82	1.00	0.95	0.91	0.99	0.99	0.99	0.94	1.00	0.77	0.97	0.58		
2% Dry lambda with 65% meat	1.00	0.99	1.00	0.99	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.90	
Reference control	1.00	0.15	0.84	1.00	0.95	0.91	0.99	0.99	0.98	0.94	1.00	0.81	0.96	0.65	1.00	0.90

Table 8.3: Scheffe's test results (p-values) for the cohesiveness of controls (65% meat and reference) with 65% meat containing dry LBG, kappa, iota, lambda, citrus fibre and starch (**<0.01 and *<0.05).

	65% Meat	3% Dry citrus fibre with 65% meat	3% Dry starch with 65% meat	7.4% Dry starch with 65% meat	0.5% Dry LBG with 65% meat	0.5% Dry kappa with 65% meat	0.5% Dry iota with 65% meat	0.5% Dry lambda with 65% meat	1% Dry LBG with 65% meat	1% Dry kappa with 65% meat	1% Dry iota with 65% meat	1% Dry lambda with 65% meat	2% Dry LBG with 65% meat	2% Dry kappa with 65% meat	2% Dry iota with 65% meat	2% Dry lambda with 65% meat
3% Dry citrus fibre with 65% meat	0.99															
3% Dry starch with 65% meat	1.00	0.99														
7.4% Dry starch with 65% meat	0.01*	0.00**	0.00**													
0.5% Dry LBG with 65% meat	1.00	1.00	1.00	0.00**												
0.5% Dry kappa with 65% meat	1.00	0.65	1.00	0.01*	0.95											
0.5% Dry iota with 65% meat	0.99	1.00	0.99	0.00**	1.00	0.59										
0.5% Dry lambda with 65% meat	1.00	0.98	1.00	0.00**	1.00	1.00	0.97									
1% Dry LBG with 65% meat	1.00	1.00	1.00	0.00**	1.00	0.99	1.00	1.00								
1% Dry kappa with 65% meat	0.97	0.01*	0.68	0.59	0.17	0.99	0.01*	0.82	0.48							
1% Dry iota with 65% meat	0.99	1.00	0.98	0.00**	1.00	0.48	1.00	0.94	0.99	0.00**						
1% Dry lambda with 65% meat	1.00	0.98	1.00	0.00**	1.00	1.00	0.97	1.00	1.00	0.82	0.95					
2% Dry LBG with 65% meat	0.99	0.12	0.94	0.32	0.48	1.00	0.09	0.98	0.84	1.00	0.06	0.98				
2% Dry kappa with 65% meat	0.99	0.12	0.95	0.31	0.49	1.00	0.10	0.98	0.85	1.00	0.06	0.98	1.00			
2% Dry iota with 65% meat	0.99	1.00	0.98	0.00**	1.00	0.52	1.00	0.96	1.00	0.00**	1.00	0.96	0.07	0.07		
2% Dry lambda with 65% meat	1.00	0.94	1.00	0.00**	0.99	1.00	0.91	1.00	1.00	0.93	0.85	1.00	0.99	0.99	0.88	
Reference control	1.00	1.00	1.00	0.00**	1.00	0.95	1.00	1.00	0.48	0.26	1.00	1.00	0.58	0.58	1.00	0.99

Table 8.4: Scheffe's test results (p-values) for the resilience of controls (65% meat and reference) with 65% meat containing dry LBG, kappa, iota, lambda, citrus fibre and starch (**<0.01 and *<0.05).

	65% Meat	3% Dry citrus fibre with 65% meat	3% Dry starch with 65% meat	7.4% Dry starch with 65% meat	0.5% Dry LBG with 65% meat	0.5% Dry kappa with 65% meat	0.5% Dry iota with 65% meat	0.5% Dry lambda with 65% meat	1% Dry LBG with 65% meat	1% Dry kappa with 65% meat	1% Dry iota with 65% meat	1% Dry lambda with 65% meat	2% Dry LBG with 65% meat	2% Dry kappa with 65% meat	2% Dry iota with 65% meat	2% Dry lambda with 65% meat
3% Dry citrus fibre with 65% meat	1.00															
3% Dry starch with 65% meat	0.99	0.63														
7.4% Dry starch with 65% meat	0.01*	0.00**	0.19													
0.5% Dry LBG with 65% meat	1.00	1.00	0.45	0.00**												
0.5% Dry kappa with 65% meat	1.00	0.92	1.00	0.04*	0.06											
0.5% Dry iota with 65% meat	0.99	1.00	0.14	0.00**	1.00	0.45										
0.5% Dry lambda with 65% meat	1.00	1.00	0.96	0.00**	1.00	0.99	0.99									
1% Dry LBG with 65% meat	1.00	1.00	0.60	0.00**	0.51	0.90	0.17	1.00								
1% Dry kappa with 65% meat	0.90	0.10	1.00	0.78	0.14	0.99	0.02*	0.51	0.10							
1% Dry iota with 65% meat	0.92	0.99	0.00	0.00**	1.00	0.05	1.00	0.78	0.99	0.00**						
1% Dry lambda with 65% meat	1.00	1.00	0.92	0.00**	1.00	0.99	0.98	1.00	1.00	0.36	0.88					
2% Dry LBG with 65% meat	0.99	0.69	1.00	0.15	1.00	1.00	1.00	0.97	0.66	1.00	0.01*	0.94				
2% Dry kappa with 65% meat	0.95	0.23	1.00	0.71	0.06	1.00	0.99	0.71	0.22	1.00	0.00**	0.58	1.00			
2% Dry iota with 65% meat	0.96	0.99	0.01*0	0.00**	1.00	0.09	0.17	0.88	1.00	0.00**	1.00	0.94	0.02*	0.00		
2% Dry lambda with 65% meat	1.00	1.00	0.99	0.00**	1.00	1.00	0.02*	1.00	1.00	0.66	0.64	1.00	0.99	0.83	0.77	
Reference control	1.00	1.00	0.71	0.00**	0.51	0.95	1.00	1.00	0.22	1.00	1.00	0.76	0.36	1.00	1.00	

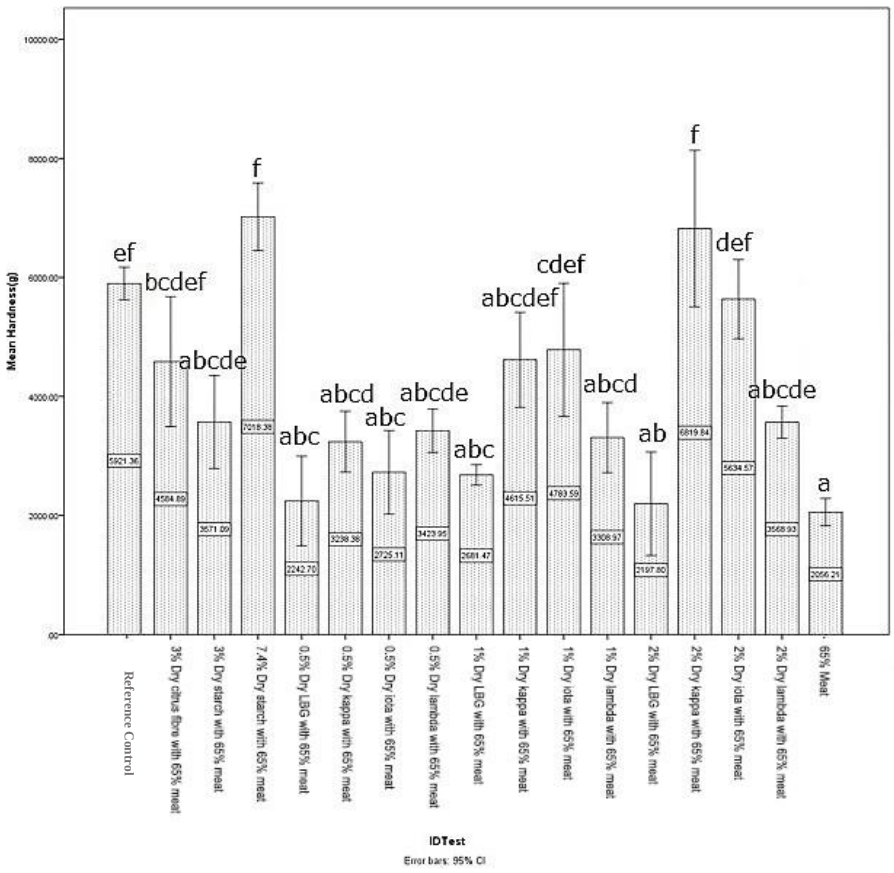


Figure 8.1: Hardness of adding dry LBG, kappa, iota, lambda, citrus fibre and starch to a 65% meat, compared to control samples (65% meat and reference).

The general linear model ANOVA was utilized to test the differences between the treatments (ID-Test) in relation to hardness. Analysis of variance (ANOVA) showed that at least one ID-Test mean was different from the others. Hence, Scheffe's post hoc test was applied in order to compare the means of the hardness ID-Test in a pair-wise fashion (Table 8.1). This comparison identifies that the hardness of a reference control can be matched when dry starch, citrus fibre, kappa carrageenan and iota carrageenan are added to the 35% reduced meat. The concentration of each is critical in that starch matches the hardness value at 7.4% addition level, but not at 3%. However, 3% citrus fibre is sufficient. Both carrageenan (kappa and iota) are significantly different to the reference control when added at 0.5%, but at 1% and 2% addition levels both match the reference control. Regardless, of addition level (up to 2%) the non-gelling LBG and lambda carrageenan are significantly different

to the reference control. Therefore, in terms of hardness, it appears that a material which can swell and take up water in the batter and then retain that water in the form of gel (starch, kappa and iota carrageenan) or hierarchically structured material (citrus fibre) can give a firm, 35% reduced meat product. These materials would behave differently upon reheating, so a follow-on study might look at mechanical properties upon reheating the sausages. This does, however, appear to provide some ingredient flexibility and, depending on individual ingredients costs, may provide cost saving. Other additional pieces of work that could be done in future studies would be to assess the efficacy of dry blending these types of materials. Also, using the carrageenan and citrus fibre allows the inclusion of more dietary fibre in the human diet.

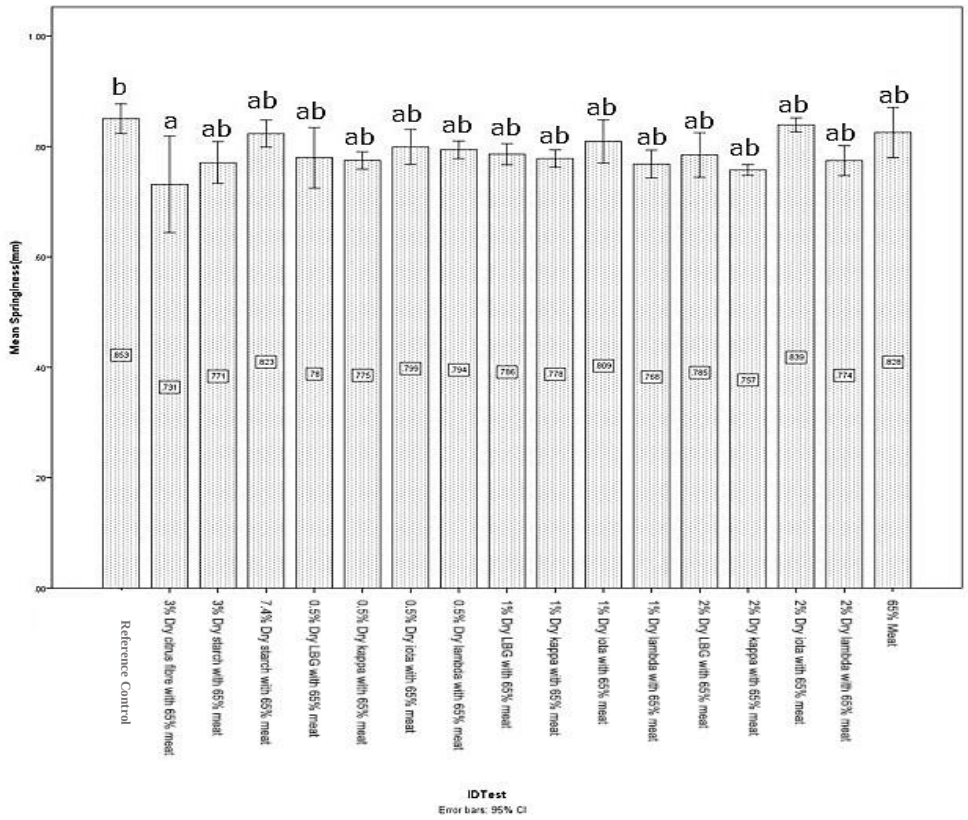


Figure 8.2: Springiness of adding dry LBG, kappa, iota, lambda, citrus fibre and starch to a 65% meat, compared to control samples (65% meat and reference).

Analysis of variance (ANOVA) showed that springiness differed significantly by ID-Test type. Hence, Scheffe's test was applied in order to compare the means of the springiness ID-Test (Table 8.2).

The springiness of dry showed that there was not a statistically significant difference among the parameters except, for 3% dry citrus fibre, which was statistically different from reference control. This indicates that the citrus fibre affects the protein network to a greater extent than the other materials, and that lower amounts of citrus fibre in combination with other ingredients might be able to provide ideal properties. Furthermore, the maximum p-value was that of 2% dry iota with 65% meat. As discussed in previous chapter, the springiness parameter cannot differentiate between the test samples in which the hardness parameter does.

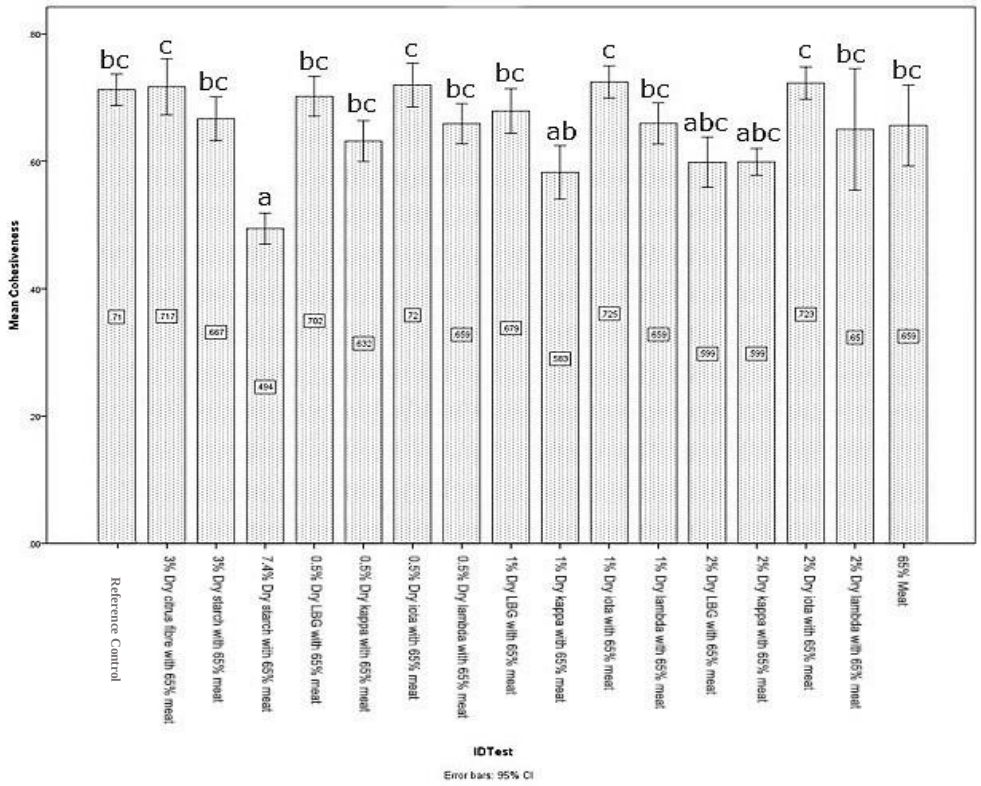


Figure 8.3: Cohesiveness of adding dry LBG, kappa, iota, lambda, citrus fibre and starch to a 65% meat, compared to control samples (65% meat and reference).

Analysis of variance (ANOVA) showed that cohesiveness differed significantly by ID-Test type. Hence, Scheffe's test was applied in order to compare the means of the cohesiveness ID-Test (Table 8.3).

The cohesiveness results showed that only 7.4% dry starch was statistically different from the reference control. These results showed that, unlike hardness, now the non-gelling lambda carrageenan and LBG match the cohesiveness value of the reference control. Another interesting set of data in Figure 8.3 shows that iota carrageenan in all states (0.5, 1 and 2%) was statistically different from 7.4% starch, and 1% dry kappa carrageenan. This could indicate that the 7.4% starch sample is too efficient at binding the water in the sausage structure, but the other materials can 'release' the water in the cohesiveness measurement-providing a 'sticky' structure, and that the effectiveness of WHC of citrus fibre does not prevent cohesive structures in this mode of experimentation.

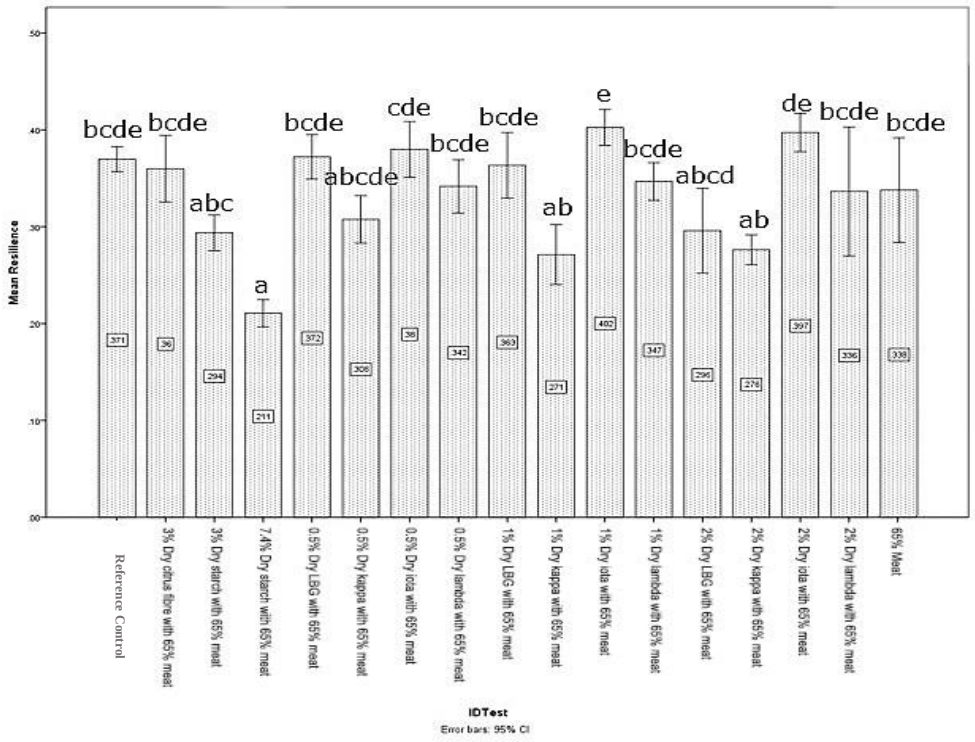


Figure 8.4: Resilience of adding dry LBG, kappa, iota, lambda, citrus fibre and starch to a 65% meat, compared to control samples (65% meat and reference).

Analysis of variance (ANOVA) showed that resilience differed significantly by ID-Test type. Hence, Scheffe's test was applied in order to compare the means of the cohesiveness ID-Test (Table 8.4).

In terms of the resilience, the 7.4% dry starch is only sample statistically different to the reference control. Other samples with lower mean resilience values are the dry kappa carrageenan samples (at all addition level). It therefore appears that the harder types of gels effect the resilience measurements. It is known that iota gels are more elastic than kappa gels and therefore resilience seems to be better developed using softer, more elastic gels or non-gelling materials. This again points towards either using combination of materials to match properties at lowest formulation cost, or just iota carrageenan, which matches the reference control in all measured parameters.

8.1.2 Comparisons of solubilised or pre-hydrated materials

In this section, the texture properties are compared when LBG, kappa, iota, lambda, citrus fibre, and starch are solubilised or pre-hydrated in a 65% meat product, and

also the control samples which are 65% and reference control presented in table 8.5, 8.6, 8.7 and 8.8.

Table 8.5: Scheffe's test results (p-values) for the hardness of controls (65% meat and reference) with 65% meat containing solubilised or pre-hydrated LBG, kappa, iota, lambda, citrus fibre and starch (**<0.01 and *<0.05).

	65% Meat	3% Pre-hydrated citrus fibre with 65% meat	3% Pre-hydrated starch with 65% meat	7.4% Pre-hydrated starch with 65% meat	0.5% Solubilised LBG with 65% meat	0.5% Solubilised kappa with 65% meat	0.5% Solubilised iota with 65% meat	0.5% Solubilised lambda with 65% meat	1% Solubilised LBG with 65% meat	1% Solubilised kappa with 65% meat	1% Solubilised iota with 65% meat	1% Solubilised lambda with 65% meat	2% Solubilised LBG with 65% meat	2% Solubilised kappa with 65% meat	2% Solubilised iota with 65% meat	2% Solubilised lambda with 65% meat
3% Pre-hydrated citrus fibre with 65% meat	0.06															
3% Pre-hydrated starch with 65% meat	1.00	0.01*														
7.4% Pre-hydrated starch with 65% meat	0.89	0.97	0.85													
0.5% Solubilised LBG with 65% meat	1.00	0.00**	1.00	0.45												
0.5% Solubilised kappa with 65% meat	1.00	0.19	1.00	0.99	0.99											
0.5% Solubilised iota with 65% meat	1.00	0.10	1.00	0.98	1.00	1.00										
0.5% Solubilised lambda with 65% meat	1.00	0.00**	0.99	0.04*	1.00	0.80	0.92									
1% Solubilised LBG with 65% meat	1.00	0.00**	1.00	0.74	1.00	1.00	1.00	0.99								
1% Solubilised kappa with 65% meat	0.76	0.99	0.67	1.00	0.26	0.98	0.93	0.01*	0.52							
1% Solubilised iota with 65% meat	0.07	1.00	0.01*	0.97	0.00**	0.20	0.10	0.00**	0.00**	0.99						
1% Solubilised lambda with 65% meat	0.99	0.00**	0.91	0.00**	0.99	0.41	0.60	1.00	0.96	0.00**	0.00**					
2% Solubilised LBG with 65% meat	0.99	0.00**	0.81	0.00**	0.98	0.28	0.45	1.00	0.90	0.00**	0.00**	1.00				
2% Solubilised kappa with 65% meat	0.01*	1.00	0.00**	0.69	0.00**	0.03*	0.01*	0.00**	0.00**	0.91	1.00	0.00**	0.00**			
2% Solubilised iota with 65% meat	1.00	0.12	1.00	0.99	1.00	1.00	1.00	0.89	1.00	0.95	0.12	0.54	0.40	0.01*		
2% Solubilised lambda with 65% meat	1.00	0.00**	0.96	0.01*	1.00	0.57	0.75	1.00	0.99	0.00**	0.00**	1.00	1.00	0.00**	0.70	
Reference control	0.00**	0.00**	0.00**	0.00**	0.00**	0.00**	0.00**	0.00**	0.00**	0.00**	0.00**	0.00**	0.00**	0.00**	0.00**	0.00**

Table 8.6: Scheffe's test results (p-values) for the springiness of controls (65% meat and reference) with 65% meat containing solubilised or pre-hydrated LBG, kappa, iota, lambda, citrus fibre and starch (**<0.01 and *<0.05).

	65% Meat	3% Pre-hydrated citrus fibre with 65% meat	3% Pre-hydrated starch with 65% meat	7.4% Pre-hydrated starch with 65% meat	0.5% Solubilised LBG with 65% meat	0.5% Solubilised kappa with 65% meat	0.5% Solubilised iota with 65% meat	0.5% Solubilised lambda with 65% meat	1% Solubilised LBG with 65% meat	1% Solubilised kappa with 65% meat	1% Solubilised iota with 65% meat	1% Solubilised lambda with 65% meat	2% Solubilised LBG with 65% meat	2% Solubilised kappa with 65% meat	2% Solubilised iota with 65% meat	2% Solubilised lambda with 65% meat
3% Pre-hydrated citrus fibre with 65% meat	1.00															
3% Pre-hydrated starch with 65% meat	1.00	1.00														
7.4% Pre-hydrated starch with 65% meat	1.00	1.00	1.00													
0.5% Solubilised LBG with 65% meat	1.00	1.00	1.00	1.00												
0.5% Solubilised kappa with 65% meat	1.00	1.00	0.99	1.00	1.00											
0.5% Solubilised iota with 65% meat	1.00	1.00	1.00	1.00	1.00	1.00										
0.5% Solubilised lambda with 65% meat	1.00	1.00	1.00	1.00	1.00	1.00	1.00									
1% Solubilised LBG with 65% meat	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00								
1% Solubilised kappa with 65% meat	1.00	0.96	1.00	0.99	0.89	0.57	0.98	0.83	0.91							
1% Solubilised iota with 65% meat	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.99						
1% Solubilised lambda with 65% meat	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.98	1.00					
2% Solubilised LBG with 65% meat	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.89	1.00	1.00				
2% Solubilised kappa with 65% meat	1.00	0.99	1.00	0.99	0.96	0.75	0.99	0.93	0.97	1.00	0.99	0.99	0.96			
2% Solubilised iota with 65% meat	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.84	1.00	1.00	1.00	0.93		
2% Solubilised lambda with 65% meat	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.81	1.00	1.00	1.00	0.91	1.00	
Reference control	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00

Table 8.7: Scheffe's test results (p-values) for the cohesiveness of controls (65% meat and reference) with 65% meat containing solubilised or pre-hydrated LBG, kappa, iota, lambda, citrus fibre and starch (**<0.01 and *<0.05).

	65% Meat	3% Pre-hydrated citrus fibre with 65% meat	3% Pre-hydrated starch with 65% meat	7.4% Pre-hydrated starch with 65% meat	0.5% Solubilised LBG with 65% meat	0.5% Solubilised kappa with 65% meat	0.5% Solubilised iota with 65% meat	0.5% Solubilised lambda with 65% meat	1% Solubilised LBG with 65% meat	1% Solubilised kappa with 65% meat	1% Solubilised iota with 65% meat	1% Solubilised lambda with 65% meat	2% Solubilised LBG with 65% meat	2% Solubilised kappa with 65% meat	2% Solubilised iota with 65% meat	2% Solubilised lambda with 65% meat
3% Pre-hydrated citrus fibre with 65% meat	0.98															
3% Pre-hydrated starch with 65% meat	0.62	1.00														
7.4% Pre-hydrated starch with 65% meat	0.06	0.78	1.00													
0.5% Solubilised LBG with 65% meat	1.00	0.78	0.11	0.00**												
0.5% Solubilised kappa with 65% meat	1.00	0.13	0.00**	0.00**	1.00											
0.5% Solubilised iota with 65% meat	0.37	0.99	1.00	1.00	0.03*	0.00**										
0.5% Solubilised lambda with 65% meat	1.00	0.10	0.00**	0.00**	1.00	1.00	0.00**									
1% Solubilised LBG with 65% meat	0.92	1.00	1.00	0.95	0.47	0.03*	1.00	0.02*								
1% Solubilised kappa with 65% meat	1.00	0.18	0.00**	0.00**	1.00	1.00	0.00**	1.00	0.05							
1% Solubilised iota with 65% meat	0.04*	0.67	0.99	1.00	0.00**	0.00**	1.00	0.00**	0.90	0.00**						
1% Solubilised lambda with 65% meat	0.99	0.03*	0.00**	0.00**	0.99	1.00	0.00**	1.00	0.00**	1.00	0.00**					
2% Solubilised LBG with 65% meat	0.72	1.00	1.00	0.99	0.16	0.00**	1.00	0.00**	1.00	0.00**	0.99	0.00**				
2% Solubilised kappa with 65% meat	1.00	0.28	0.00**	0.00**	1.00	1.00	0.00**	1.00	0.09	1.00	0.00**	1.00	0.01*			
2% Solubilised iota with 65% meat	0.99	1.00	1.00	0.73	0.82	0.16	0.99	0.13	1.00	0.22	0.60	0.05	1.00	0.34		
2% Solubilised lambda with 65% meat	1.00	0.14	0.00**	0.00**	1.00	1.00	0.00**	1.00	0.03*	1.00	0.00**	1.00	0.00**	1.00	0.17	
Reference control	1.00	0.79	0.19	0.00**	1.00	1.00	0.07	1.00	0.54	1.00	0.00**	1.00	0.26	1.00	0.83	1.00

Table 8.8: Scheffe's test results (p-values) for the resilience of controls (65% meat and reference) with 65% meat containing solubilised or pre-hydrated LBG, kappa, iota, lambda, citrus fibre and starch (**<0.01 and *<0.05).

	65% Meat	3% Pre-hydrated citrus fibre with 65% meat	3% Pre-hydrated starch with 65% meat	7.4% Pre-hydrated starch with 65% meat	0.5% Solubilised LBG with 65% meat	0.5% Solubilised kappa with 65% meat	0.5% Solubilised iota with 65% meat	0.5% Solubilised lambda with 65% meat	1% Solubilised LBG with 65% meat	1% Solubilised kappa with 65% meat	1% Solubilised iota with 65% meat	1% Solubilised lambda with 65% meat	2% Solubilised LBG with 65% meat	2% Solubilised kappa with 65% meat	2% Solubilised iota with 65% meat	2% Solubilised lambda with 65% meat
3% Pre-hydrated citrus fibre with 65% meat	0.77															
3% Pre-hydrated starch with 65% meat	0.32	1.00														
7.4% Pre-hydrated starch with 65% meat	0.01*	0.92	1.00													
0.5% Solubilised LBG with 65% meat	1.00	0.36	0.05	0.00**												
0.5% Solubilised kappa with 65% meat	1.00	0.12	0.00**	0.00**	1.00											
0.5% Solubilised iota with 65% meat	0.13	1.00	1.00	1.00	0.01*	0.00**										
0.5% Solubilised lambda with 65% meat	0.99	0.01*	0.00**	0.00**	0.99	1.00	0.00**									
1% Solubilised LBG with 65% meat	0.71	1.00	1.00	0.95	0.30	0.09	1.00	0.00**								
1% Solubilised kappa with 65% meat	1.00	0.65	0.16	0.00**	1.00	1.00	0.05	0.98	0.57							
1% Solubilised iota with 65% meat	0.01*	0.88	0.99	1.00	0.00**	0.00**	1.00	0.00**	0.92	0.00**						
1% Solubilised lambda with 65% meat	0.99	0.00**	0.00**	0.00**	0.98	1.00	0.00**	1.00	0.00**	0.94	0.00**					
2% Solubilised LBG with 65% meat	0.22	1.00	1.00	1.00	0.02*	0.00**	1.00	0.00**	1.00	0.09	1.00	0.00**				
2% Solubilised kappa with 65% meat	1.00	0.91	0.43	0.01*	1.00	0.99	0.17	0.82	0.87	1.00	0.00**	0.60	0.29			
2% Solubilised iota with 65% meat	0.96	1.00	0.99	0.57	0.80	0.46	0.96	0.07	1.00	0.94	0.50	0.01*	0.99	0.99		
2% Solubilised lambda with 65% meat	1.00	0.07	0.00**	0.00**	1.00	1.00	0.00**	1.00	0.05	1.00	0.00**	1.00	0.00**	0.99	0.33	
Reference control	1.00	0.30	0.05	0.00**	1.00	1.00	0.01*	1.00	0.25	1.00	0.00**	1.00	0.03*	0.99	0.66	1.00

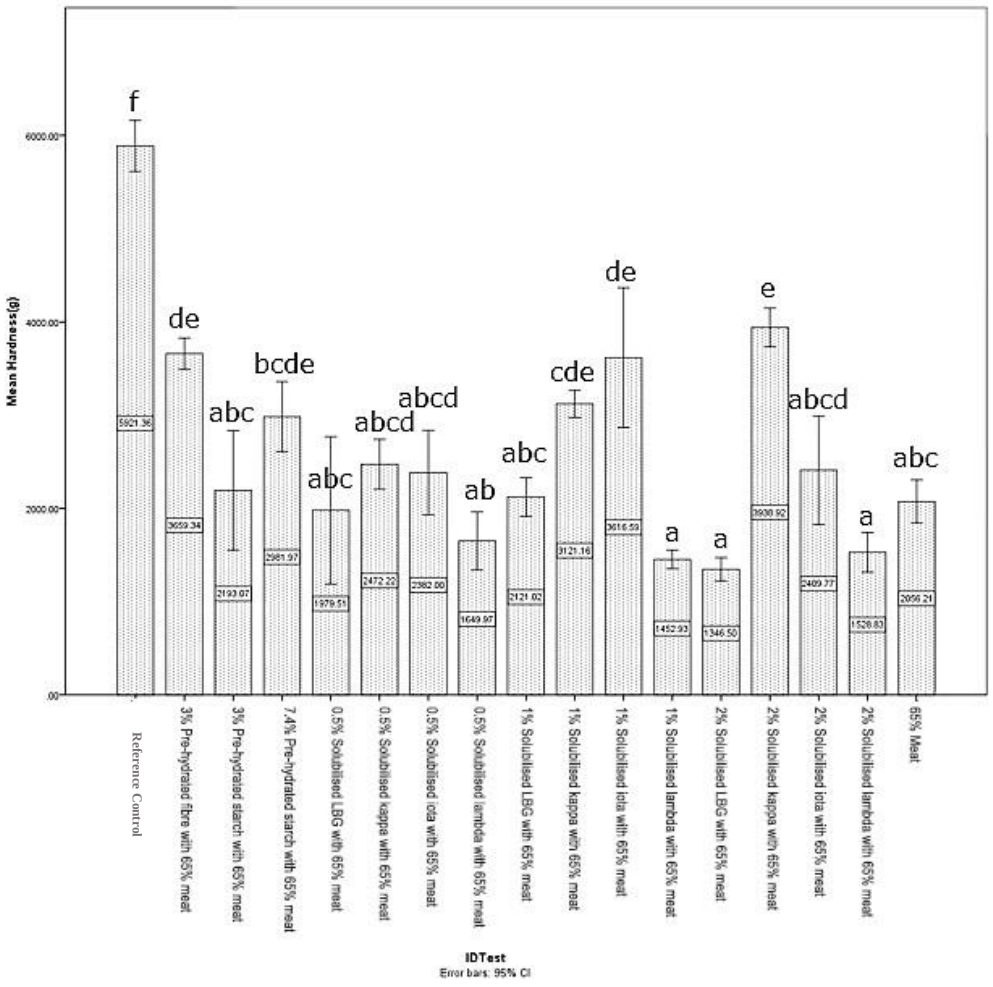


Figure 8.5: Hardness of adding solubilised or pre-hydrated LBG, kappa, iota, lambda, citrus fibre and starch to a 65% meat, compared to control samples (65% meat and reference).

The general linear model ANOVA was also utilized to test the differences between treatments (ID-Test) in terms of hardness. Analysis of variance showed that at least one ID-Test mean was different from the others. Consequently, Scheffe's post hoc test was applied in order to pinpoint the exact locations of the differences between the means of the hardness ID-Tests (Table 8.5). What can be seen is that there was a significant difference between the reference control and all samples. Therefore, pre-hydrated does not benefit the inclusion of any of these different ingredients. When samples with the highest mean values are compared there is statistical similarity between the citrus fibre sample, 7.4% starch, 1% iota and 2% kappa carrageenan. The citrus fibre would swell and thicken the water, and the carrageenan would also swell, whereas starch would only swell during cooking. However, the

swelling and gelling in the batter, competing with the meat protein during cooking appears to be more effective way of ingredient inclusion into a batter, rather than allowing the polymer to experience excess water before addition to the batter.

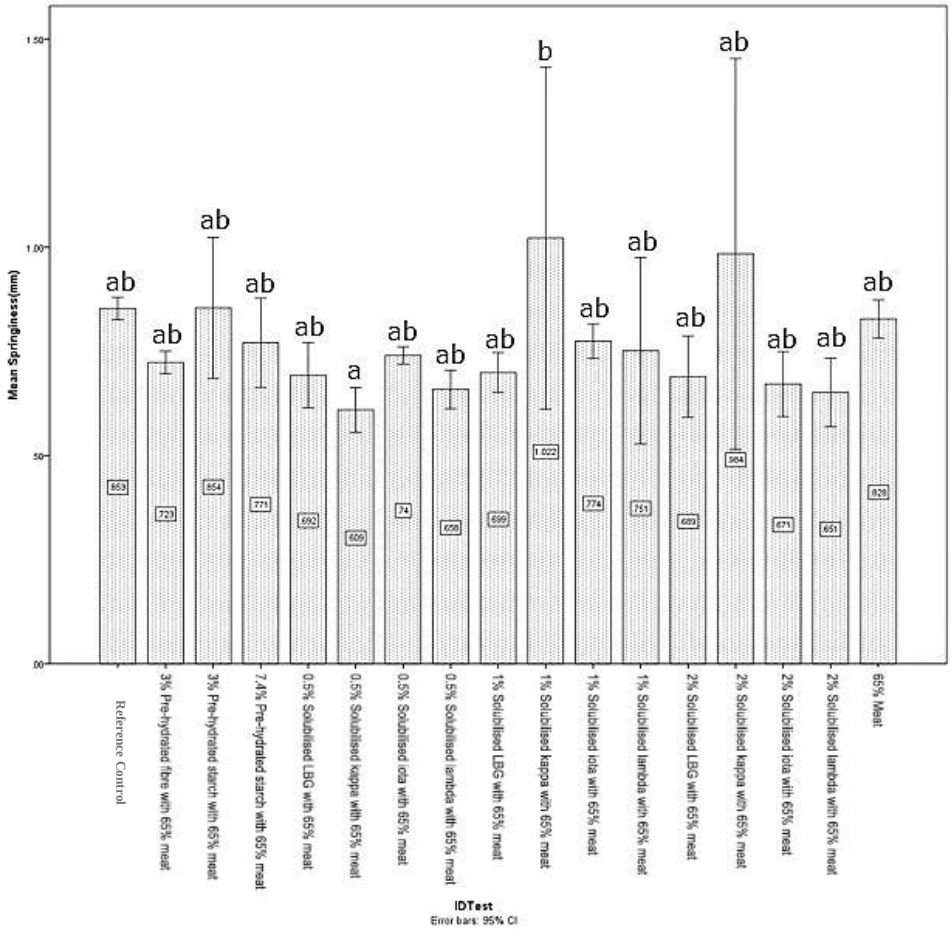


Figure 8.6: Springiness of adding solubilised or pre-hydrated LBG, kappa, iota, lambda, citrus fibre and starch to a 65% meat, compared to control samples (65% meat and reference).

Analysis of variance (ANOVA) showed that springiness differed significantly by ID-Test types. Thus, Scheffe's test was applied in order to compare the means of the springiness ID-Test (Table 8.6). Again, the reference control was not different form all of the treatments; however, 1 and 0.5% solubilised kappa were statistically different from each other. The highest mean springiness values were for the 1% and 2% kappa carrageenan samples, and the lowest for the non-gelled LBG (0.5%) and lambda carrageenan (2%). Therefore, while there is no significant difference in springiness the choice of polymer type may have an overall indicative role in achieving the desired textural attributes of a reference control when reducing the

meat content. When kappa carrageenan was heated and added to meat batter, kappa carrageenan bound with a large amount of water, and consequently the water-holding capacity of sausages increased, which resulted in an increase in the springiness of the sausages.

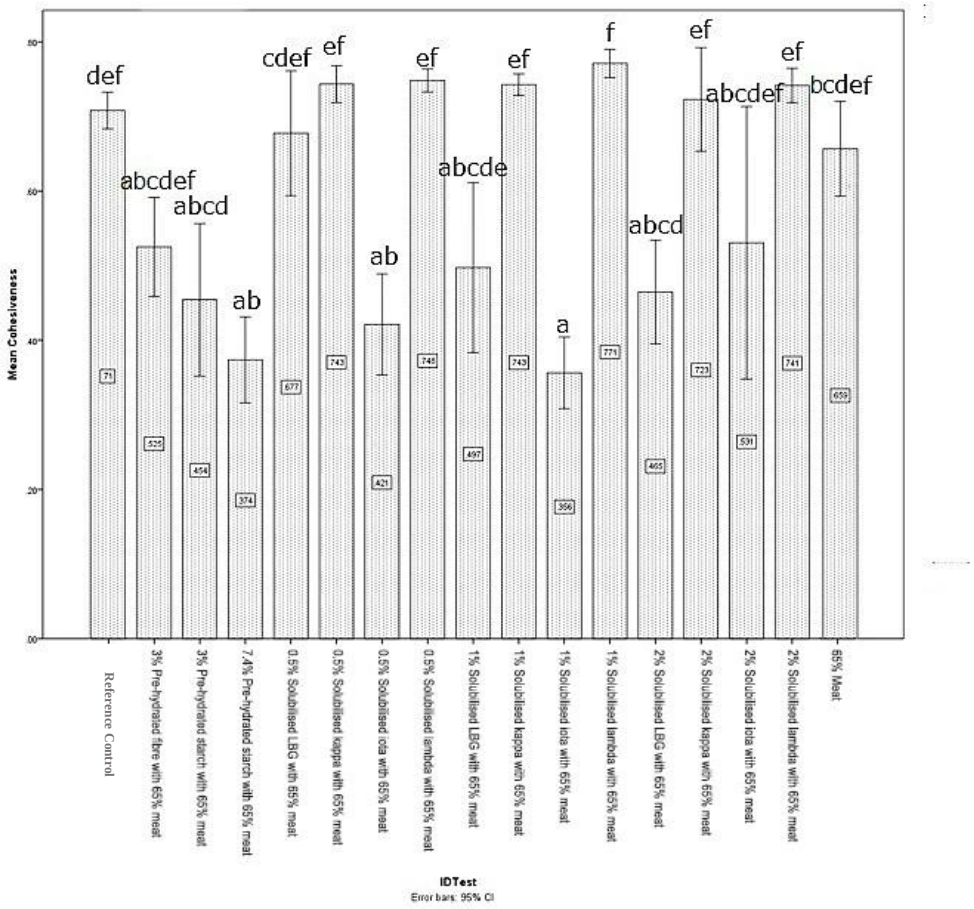


Figure 8.7: Cohesiveness of adding solubilised or pre-hydrated LBG, kappa, iota, lambda, citrus fibre and starch to a 65% meat, compared to control samples (65% meat and reference).

Analysis of variance (ANOVA) showed that cohesiveness differed significantly by ID-Test types. Thus, Scheffe's test was applied in order to compare the means of the springiness ID-Test (Table 8.7). As for adding 7.4% starch dry, pre-hydrated is also shows a significant difference from the reference. Upon pre-hydrtion 1% iota carrageenan are now also significantly different from the reference. This indicates that the iota now is effective at reducing the cohesiveness of the product, thereby providing effective water binding. The lambda carrageenan samples show the highest mean cohesiveness scores, while the other non-gelling polymer, LBG, does

not have such an effect. This may be due to the different viscosities of the pre-hydrated solutions, and would be a subject for future research.

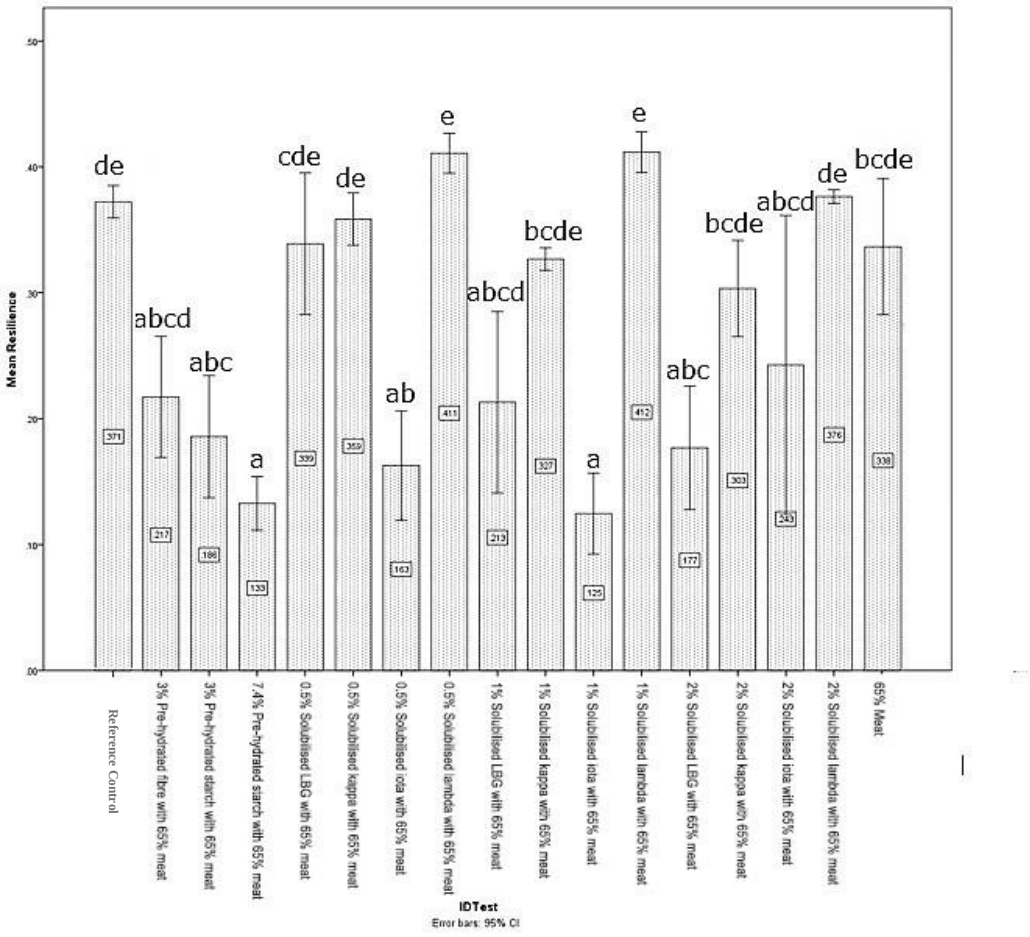


Figure 8.8: Resilience of adding solubilised or pre-hydrated LBG, kappa, iota, lambda, citrus fibre and starch to a 65% meat, compared to control samples (65% meat and reference).

Analysis of variance (ANOVA) showed that resilience differed significantly by ID-Test types. Thus, Scheffe's test was applied in order to compare the means of the resilience ID-Test (Table 8.8). The resilience result showed that reference control was statistically different from pre-hydrated starch in all states (3 and 7.4%), 0.5 and 1% solubilised iota, and 2% solubilised LBG. This is therefore an indication that these samples is not recover once the maximum force is reached. The change in material properties of the product as a result of pre-hydration of these materials affect the overall composite properties of the meat protein structure, probably as a result of the polymers being more effective in forming their own structural networks.

The maximum P-value, like in cohesiveness, was related to 1% solubilised lambda carrageenan.

8.1.3 Comparisons of gelled texture properties

In this section a comparison of the texture properties of 65% meat with added gel materials is made also with the control samples of 65 and reference control presented in table 8.9, 8.10, 8.11 and 8.12.

Table 8.9: Scheffe's test results (p-values) for the hardness of controls (65% meat and reference) with 65% meat containing gelled LBG, kappa, iota and starch (**<0.01 and *<0.05).

	65% Meat	3% Gelled starch with 65% meat	7.4% Gelled starch with 65% meat	0.5% Gelled LBG with 65% meat	0.5% Gelled kappa with 65% meat	0.5% Gelled iota with 65% meat	1% Gelled LBG with 65% meat	1% Gelled kappa with 65% meat	1% Gelled iota with 65% meat	2% Gelled LBG with 65% meat	2% Gelled kappa with 65% meat	2% Gelled iota with 65% meat
3% Gelled starch with 65% meat	0.99											
7.4% Gelled starch with 65% meat	0.55	0.93										
0.5% Gelled LBG with 65% meat	1.00	0.59	0.00**									
0.5% Gelled kappa with 65% meat	0.00**	0.00**	0.03*0*	0.00**								
0.5% Gelled iota with 65% meat	1.00	1.00	0.91	0.64	0.00**							
1% Gelled LBG with 65% meat	1.00	1.00	0.58	0.94	0.00**	1.00						
1% Gelled kappa with 65% meat	0.00**	0.00**	0.00**	0.00**	0.93	0.00**	0.00**					
1% Gelled iota with 65% meat	0.75	0.99	1.00	.02*	0.00**	0.98	0.80	0.00**				
2% Gelled LBG with 65% meat	1.00	1.00	0.90	0.76	0.00**	1.00	1.00	0.00**	0.98			
2% Gelled kappa with 65% meat	0.00**	0.00**	0.00**	0.00**	0.00**	0.00**	0.00**	0.48	0.00**	0.00**		
2% Gelled iota with 65% meat	0.91	1.00	1.00	0.08	0.00**	0.99	0.95	0.00**	1.00	0.99	0.00**	
Reference control	0.00**	0.00**	0.04*	0.00**	1.00	0.00**	0.00**	1.00	0.01*	0.00**	0.25	0.00**

Table 8.10: Scheffe's test results (p-values) for the springiness of controls (65% meat and reference) with 65% meat containing gelled LBG, kappa, iota and starch (**<0.01 and *<0.05).

	65% Meat	3% Gelled starch with 65% meat	7.4% Gelled starch with 65% meat	0.5% Gelled LBG with 65% meat	0.5% Gelled kappa with 65% meat	0.5% Gelled iota with 65% meat	1% Gelled LBG with 65% meat	1% Gelled kappa with 65% meat	1% Gelled iota with 65% meat	2% Gelled LBG with 65% meat	2% Gelled kappa with 65% meat	2% Gelled iota with 65% meat
3% Gelled starch with 65% meat	1.00											
7.4% Gelled starch with 65% meat	1.00	0.99										
0.5% Gelled LBG with 65% meat	0.99	0.97	1.00									
0.5% Gelled kappa with 65% meat	1.00	0.99	1.00	1.00								
0.5% Gelled iota with 65% meat	1.00	1.00	1.00	0.99	1.00							
1% Gelled LBG with 65% meat	0.99	0.93	1.00	1.00	1.00	0.98						
1% Gelled kappa with 65% meat	0.91	0.63	0.99	1.00	0.99	0.84	1.00					
1% Gelled iota with 65% meat	1.00	1.00	1.00	0.99	1.00	1.00	0.99	0.89				
2% Gelled LBG with 65% meat	0.16	0.01*	0.49	0.59	0.43	0.04*	0.81	0.96	0.06			
2% Gelled kappa with 65% meat	0.99	0.92	1.00	1.00	1.00	0.98	1.00	1.00	0.99	0.74		
2% Gelled iota with 65% meat	1.00	1.00	1.00	1.00	1.00	1.00	0.99	0.94	1.00	0.09	0.99	
Reference control	1.00	1.00	0.99	0.96	0.99	1.00	0.91	0.67	1.00	0.04*	0.91	1.00

Table 8.11: Scheffe's test results (p-values) for the cohesiveness of controls (65% meat and reference) with 65% meat containing gelled LBG, kappa, iota and starch (**<0.01 and *<0.05).

	65% Meat	3% Gelled starch with 65% meat	7.4% Gelled starch with 65% meat	0.5% Gelled LBG with 65% meat	0.5% Gelled kappa with 65% meat	0.5% Gelled iota with 65% meat	1% Gelled LBG with 65% meat	1% Gelled kappa with 65% meat	1% Gelled iota with 65% meat	2% Gelled LBG with 65% meat	2% Gelled kappa with 65% meat	2% Gelled iota with 65% meat
3% Gelled starch with 65% meat	0.99											
7.4% Gelled starch with 65% meat	0.31	0.75										
0.5% Gelled LBG with 65% meat	1.00	0.94	0.02*									
0.5% Gelled kappa with 65% meat	0.98	1.00	0.95	0.74								
0.5% Gelled iota with 65% meat	0.96	1.00	0.98	0.60	1.00							
1% Gelled LBG with 65% meat	0.99	0.47	0.00**	1.00	0.20	0.12						
1% Gelled kappa with 65% meat	1.00	1.00	0.47	0.99	1.00	0.99	0.74					
1% Gelled iota with 65% meat	0.37	0.82	1.00	0.03*	0.97	0.99	0.00**	0.55				
2% Gelled LBG with 65% meat	1.00	1.00	0.24	1.00	0.99	0.98	0.90	1.00	0.30			
2% Gelled kappa with 65% meat	0.99	1.00	0.78	0.93	1.00	1.00	0.44	1.00	0.84	1.00		
2% Gelled iota with 65% meat	0.00**	0.02*	0.96	0.00**	0.10	0.17	0.00**	0.00**	0.93	0.00**	0.02*	
Reference control	1.00	0.91	0.04*	1.00	0.72	0.60	1.00	0.98	0.06	0.99	0.90	0.00**

Table 8.12: Scheffe's test results (p-values) for the resilience of controls (65% meat and reference) with 65% meat containing gelled LBG, kappa, iota and starch (**<0.01 and *<0.05).

	65% Meat	3% Gelled starch with 65% meat	7.4% Gelled starch with 65% meat	0.5% Gelled LBG with 65% meat	0.5% Gelled kappa with 65% meat	0.5% Gelled iota with 65% meat	1% Gelled LBG with 65% meat	1% Gelled kappa with 65% meat	1% Gelled iota with 65% meat	2% Gelled LBG with 65% meat	2% Gelled kappa with 65% meat	2% Gelled iota with 65% meat
3% Gelled starch with 65% meat	0.49											
7.4% Gelled starch with 65% meat	0.00**	0.76										
0.5% Gelled LBG with 65% meat	1.00	0.41	0.00**									
0.5% Gelled kappa with 65% meat	0.68	1.00	0.54	0.63								
0.5% Gelled iota with 65% meat	0.80	1.00	0.37	0.79	1.00							
1% Gelled LBG with 65% meat	0.99	0.00**	0.00**	0.86	0.00**	0.01*						
1% Gelled kappa with 65% meat	0.89	1.00	0.24	0.90	1.00	1.00	0.02*					
1% Gelled iota with 65% meat	0.16	1.00	0.98	0.08	0.99	0.99	0.00**	0.97				
2% Gelled LBG with 65% meat	1.00	0.71	0.00**	1.00	0.88	0.95	0.60	0.98	0.24			
2% Gelled kappa with 65% meat	0.52	1.00	0.73	0.44	1.00	1.00	0.00**	1.00	1.00	0.74		
2% Gelled iota with 65% meat	0.00**	0.18	1.00	0.00**	0.08	0.03*	0.00**	0.01*	0.63	0.00**	0.16	
Reference control	1.00	0.08	0.00**	0.99	0.16	0.25	1.00	0.36	0.01*	0.97	0.09	0.00**

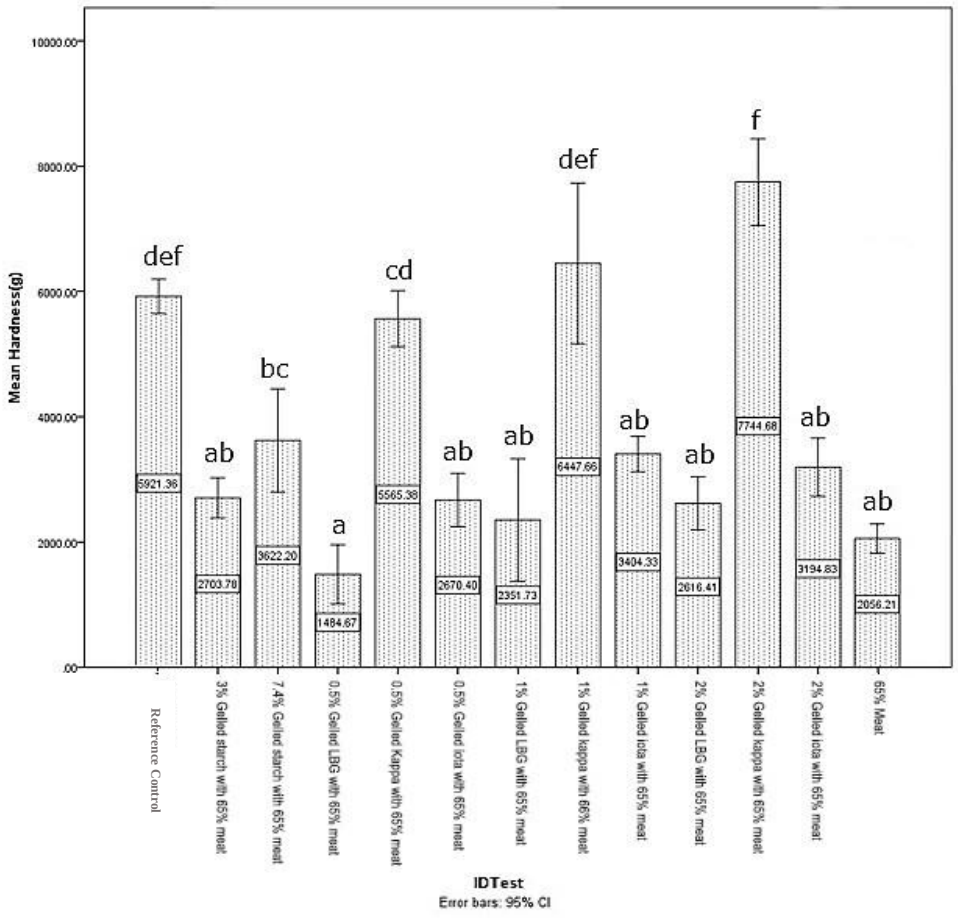


Figure 8.9: Hardness of adding gelled LBG, kappa, iota and starch to a 65% meat, compared to control samples (65% meat and reference).

Analysis of variance (ANOVA) showed that hardness differed significantly by ID-Test types. Thus, Scheffe's test was applied in order to compare the means of the hardness ID-Test (Table 8.9). The hardness results showed that reference control was not statistically different from when gelled kappa carrageenan was added at all addition levels; also the maximum P-value was that of 2% gelled kappa. All other gel formats showed significant difference to the reference control. It is known from previous literature that kappa carrageenan gels from hard/firm gels. The addition of gelled materials removes any chance of water redistribution in to the meat network. Therefore, following the polymer blending laws introduced by Morris (1990) that hard filler would increase the mechanical properties of a composite (iso-strain) and soft filler (in this case iota, LBG and starch-iso-stress).

The DSC data shown in Figure 5.7 also indicated that the ionic environment of the meat batter altered the gel melting and setting temperature of kappa carrageenan, and therefore the gel properties would be altered during the processing, and is a subject for future work.

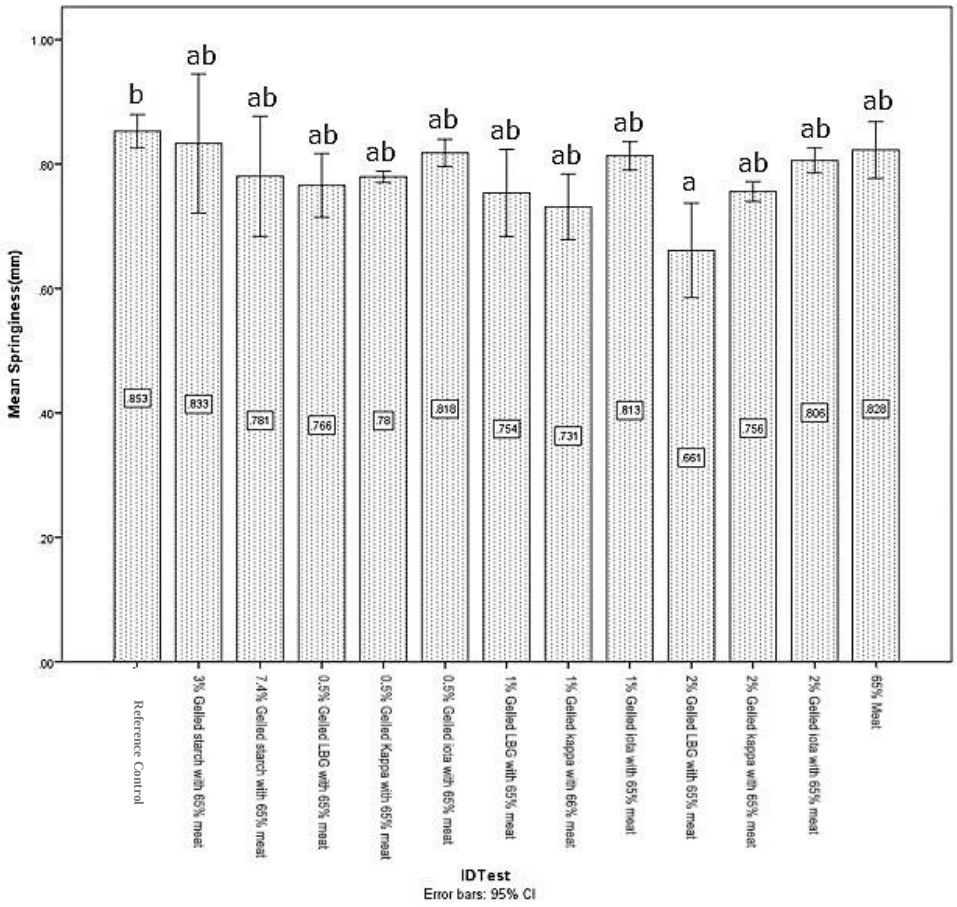


Figure 8.10: Springiness of adding gelled LBG, kappa, iota and starch to a 65% meat, compared to control samples (65% meat and reference).

Analysis of variance (ANOVA) showed that springiness differed significantly by ID-Test types. Thus, Scheffe's test was applied in order to compare the means of the springiness ID-Test (Table 8.10)**.** What can be seen is that there was a significant difference between the reference control and 2% gelled LBG; however, other treatments were not statistically different from each other. This indicates, as has been found for most springiness measurements, that polymer type, addition level and mode of addition do not affect springiness, and it is the same for adding gelled samples. LBG gels added at 2% might be different due to how the gel breaks up in

the bowl chopper during batter processing. This, however, hasn't been checked and could be topic further experimentation in the future.

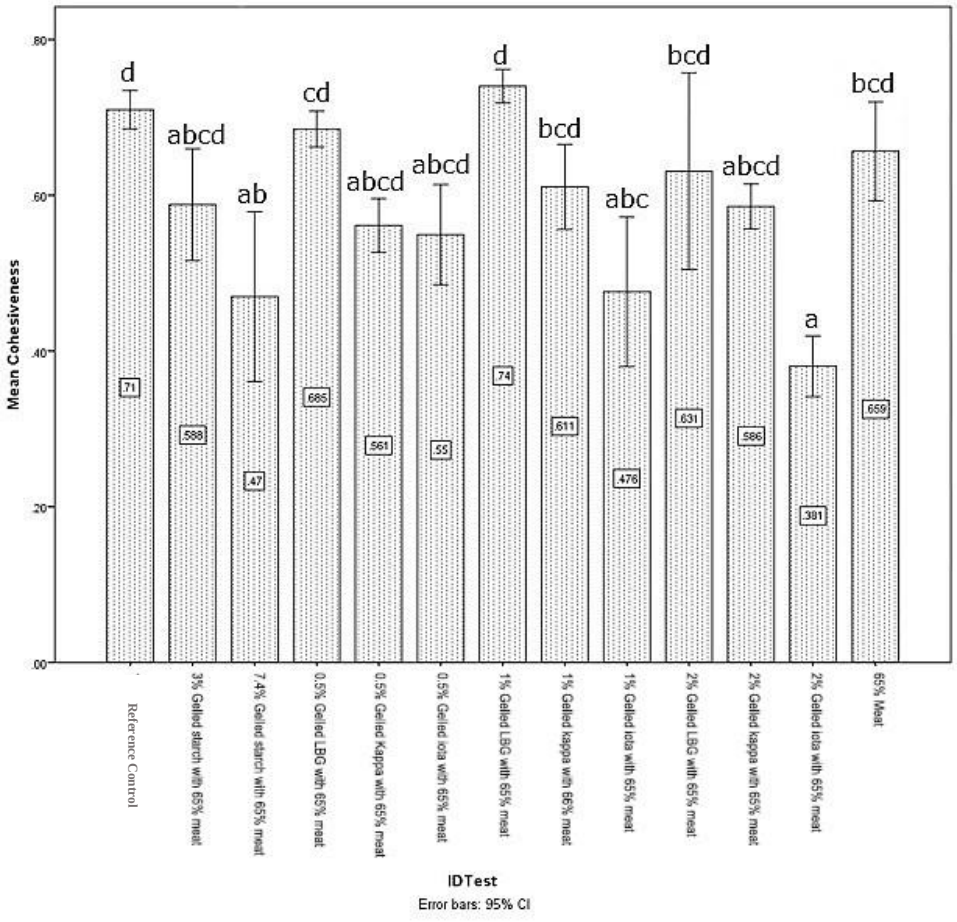


Figure 8.11: Cohesiveness of adding gelled LBG, kappa, iota and starch to a 65% meat, compared to control samples (65% meat and reference).

Analysis of variance (ANOVA) showed that cohesiveness differed significantly by ID-Test types. Thus, Scheffe's test was applied in order to compare the means of the cohesiveness ID-Test (Table 8.11). The results show that reference control was statistically different from 7.4% gelled starch and 1% and 2% gelled iota. These results indicates that the addition of these gels decreased the area under the cure on the second compression, and that these 'softer' gels have strength under first compression, and the kappa carrageenan and LBG gels retain coherence to be stronger in the second compression.

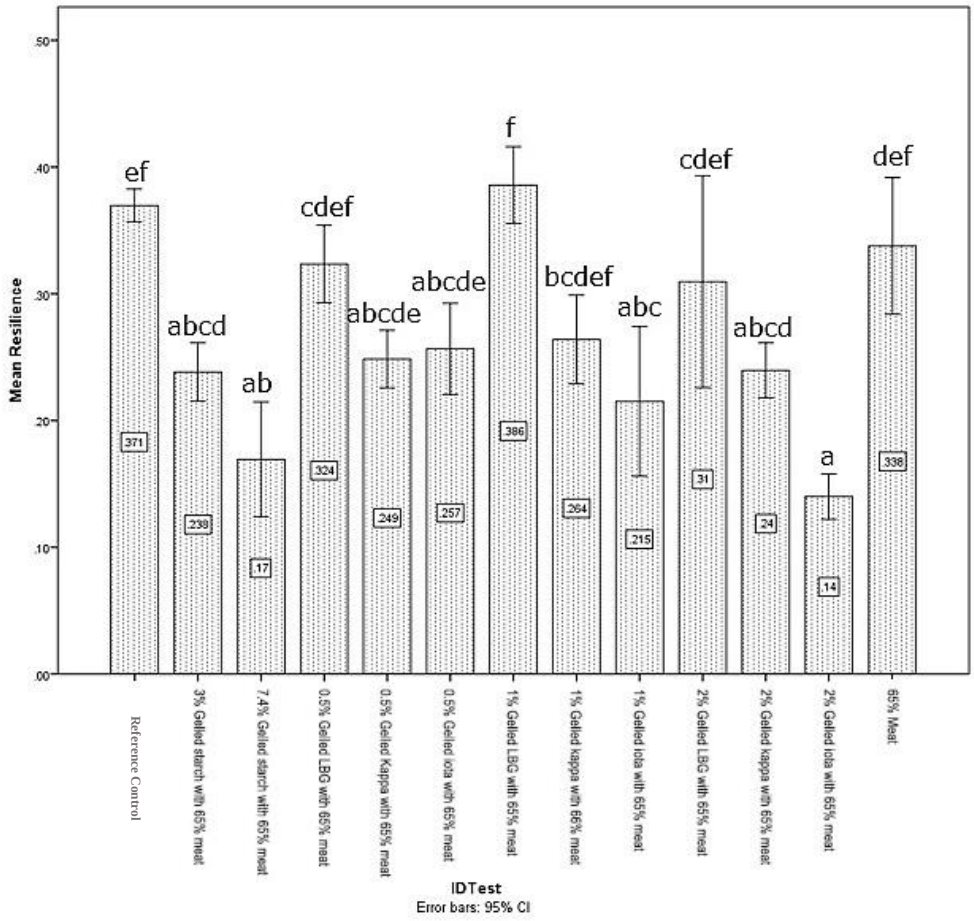


Figure 8.12: Resilience of adding gelled LBG, kappa, iota and starch to a 65% meat, compared to control samples (65% meat and reference).

Analysis of variance (ANOVA) showed that resilience differed significantly by ID-Test types. Thus, Scheffe's test was applied in order to compare the means of the resilience ID-Test (Table 8.12). The results showed that reference control was statistically different from gelled starch at both addition level (3 and 7.4%), gelled iota when added at 1 and 2% and gelled kappa carrageenan at 2%. This again indicates that the softer gels also break up more easily during first compression and once at maximum force the product´s composite structure fails more readily. This is with the exception of LBG, whose gel structure enables a retention of composite properties after maximum force has been reached, so as a gel showing similar failure properties to the reference control, and for the harder kappa carrageenan gel. Like the cohesiveness parameter, the maximum p-value was related to 1% gelled LBG.

8.2 General conclusions

This investigation has explored how the properties of myofibrillar protein can be modified using different additives, which can influence the formation of the continuous matrix of the muscle during the formation of the paste batter or during heat-induced gelation. Some additives (carrageenan and citrus fibre) may interact with proteins to form a more structured system, while others act as fillers (unmodified wheat starch and LBG) modifying the viscosity of the system. Protein-polysaccharide interactions can affect the functional properties in foods where proteins are the major ingredients, such as meat processed products. In general, the interactions can result in three equilibrium state: co-solubility (miscibility), association (complex) and segregation (incompatibility) (Zhang et al., 1994). In this study, the mixture of polysaccharides and proteins show thermodynamic incompatibility. In this case, a two phase phenomena associative phase separation and segregative phase separation can be observed. The most common form of interaction between polymers is the thermodynamic incompatibility induced segregative phase separation (Foster et al., 1996). For the dry material addition, in an effort to look for evidence of interactions between the carbohydrate and the meat protein, attention has been focused on the temperatures (onset, endset and peak) and the enthalpy of the major transitions in the respective materials. The enthalpies presented in Tables 4.6, 5.7, 6.7 and 7.7 are non-normalised, which means they are taken straight from the DSC traces after calculation by Setaram software. Starch, caarageenan (kappa and iota) show clear irreversible and reversible transitions, respectively, on the timescale of the experiment in the region of 66.4˚C for starch, 84.4˚C for kappa and 90˚C for iota caarageenan , whereas locust bean gum and citrus fibre show no transitions. No interaction between carbohydrates and proteins would be signalled by the transition temperatures of the unaffected transitions and the addition of enthalpies being linear.

It is important to carry out the DSC experiments under identical conditions, which means that unmodified wheat starch and kappa carrageenan have been made up in solutions of salt and triphosphate, closely approximating the conditions for the meat proteins. However, the presence of high solids contents in the meat means that the true concentration of salt appropriate for the carbohydrate is uncertain and should probably be higher. It appears that the

presence of salt and triphosphate increases the temperatures of transition and decreases the enthalpy (see Tables 4.6 and 5.7) for both starch and carrageenan. These effects are documented in the literature (Paterson et al., 1994; Ganz, 1965; Watase et al., 1990).

There is considerable error in placing temperature limits and the type of baseline used for the calculation of transition enthalpies, particularly for the spread transitions observed here. This can easily give errors on the enthalpy of up to +/- 0.3 J/g. Similarly, any distortions in the peaks themselves can make the interpolation software generate errors at the onset and endset temperatures. The peak temperature is, therefore, probably the most reliable parameter to within +/- a few degrees.

In the case of locust bean gum and citrus fibre, there appears to be no significant effect of the carbohydrate from either the temperatures or the enthalpy of the protein transition (Tables 6.7 and 7.7).

In the case of starch, the temperatures are probably the same within error. Similarly, although the enthalpy of the mixture at first seems reduced compared with the addition of the individual components (2.4 as opposed to 2.1 + 0.6 = 2.7 J/g), this is probably within error and furthermore, the result of the fitting the join meat starch trace (Figure 4.8) indicated that the presence of salt and phosphate increased the starch transition by 8˚C from 58.5 to 66.5˚C. Also, the shape and width of the transitions appear identical suggesting that a linear combination of the component transitions is a good match with the transition of the joint materials hence, no interaction would be suggested.

The situation with carrageenan is more complex. At first it appears that there is a major discrepancy between the joint enthalpy (2.1 J/g) and the sum of the individual components (2.1 + 0.7 = 2.8 J/g). However, a close inspection of Figure 5.7 and Table 5.7 shows a major effect of salt on this transition, which is perhaps to be expected for a charged carbohydrate polymer. This effect is so large that with the increased solids content of the meat probably increasing the effective concentration of salt experienced by the carrageenan, it appears to have separated the transitions completely and broadened the carrageenan transition to the point where it becomes difficult to measure. The 2.1 J/g for the joint transition is, therefore, probably due to the protein fraction only. It becomes very difficult to say whether there is any definite evidence of an interaction between

carrageenan and the protein. The rheology result from Figures 4.9, 5.11, 6.8 and 7.8 shows storage modulus (G´) was greater than the loss modulus (G˝) during the test, (G´>G˝) which indicates predominant elastic behaviour of the sample for the whole range of deformation experimented. Also, in all of the treatments after the temperature of 60˚C there was major increase in G´, which is due to a network formation by the aggregation and entanglement of unfolded protein molecules with hydrocolloids.

The DSC thermograms (Figures 4.6, 5.7, 6.7 and 7.7) showed a broad endothermic region (after temperature of 60˚C), suggesting a confirmation of the rheology result. The rheology results of LBG treatments (Figure 6.8) weak viscoelastic behaviour observed by small strain oscillatory rheology since of G´ of LBG treatment is very similar and near to control samples (65% meat) and this is observed by parameter of hardness in the texture analyser (Figure 6.1). However, the rheology of kappa carrageenan treatments (Figure 5.11) observed a strong gel because the addition of kappa carrageenan resulted in higher G´ values. Also, the G´ of the kappa carrageenan treatment is too far from the control samples, which is 65% meat. Confocal scanning laser microscopy (CLSM) showed that the size of the cavity is increased when adding dry LBG (650 to 700 µm) (Figure 6.9, images E and F) compared to control samples, which is 65% meat (500–550 µm) (Figure 6.9, images C and D). This result is confirmed by DSC and rheology of the LBG treatments. However, the size of cavity is decreased when adding gelled kappa carrageenan (Figure 5.12, images E and F), dry citrus fibre (Figure 7.9, images E and F) and dry wheat starch (Figure 4.10, images E and F), which is 100–150, 150–200, and 200–250 µm, respectively. This is because myosin has already gelled, preventing uniform distribution of these hydrocolloids throughout the meat matrix and, therefore, these hydrocolloids are trapped within protein gel structure. As a consequence, the size of the cavity is decreased.

In all treatments, the fat droplet decreases in mean size when adding hydrocolloids in the systems. The firmness and elasticity of beef meat sausages are important parameters for consumers. Table 8.13 shows a nice comparison of all treatments to a statistical match to a reference control. The treatments highlighted in grey are those which appear to provide a 35% reduction in meat while retaining the physical parameters of the control. With regard to the Iranian

National Standardization Organization (INSO) 2017, Iranian people like hard sausages and it is seen that the maximum hardness related to the dry state of all hydrocolloids with 65% meat, which are 7.4% unmodified wheat starch (p-value< 0.01), 1% LBG and 3% citrus fibre (Figures 4.2, 6.1 and 7.1), except the carrageenan treatment (Figure 5.1), which is related to 2% gelled kappa carrageenan with 65% meat (p-value <0.01) because in this range 'tiger stripes' occur. Furthermore, the elasticity or rubbery texture of the sausages increased with 3% pre-hydrated wheat starch (Figure 4.3) and 1% solubilised kappa carrageenan (Figure 5.3) with 65% (p-value< 0.05) meat. This is because when heating these hydrocolloids, hydrocolloids bind with large amounts of water, and consequently, increase the water-holding capacity of sausages; therefore, the sausages are more rubbery. However, improving elasticity of LBG and citrus fibre, which are 1 and 3%, respectively, takes place with the dry state (Figures 6.3 and 7.3). Furthermore, the result of hardness of dry, pre-hydrated and gelled of all of the polymers indicate that instead of using too much polymer such as 7.4% starch we can used another polymer with low concentration and get a same hardness .

Considering the above findings and objectives of this work, the present study has important implications for meat scientists and the meat industry. The addition of hydrocolloids in different ways clearly affect the physical properties of the meat product. Depending on which parameters are required/ favoured by the consumer, this work provides direction as to how the parameters can be changed, and which materials should be used, and in what state they should be added. Table 8.13 shows some unexpected results if all parameters are to be matched. Such success can be attained by adding 2% dry iota carrageenan. The most surprising result is that full success can be achieved adding 0.5% and 2% dry lambda carrageenan because this polymer is a non-gelling polymer as indicated in the DSC result (Figure 5.9 and 5.10) because the meat sample with lambda carrageenan shows no additional transitions to the denaturation peak of the protein and this polymer out performs LBG when also added dry. This may be due the fact that lambda carrageenan is highly charged and thereby might hydrate to a greater extent than LBG. What has also been indicated is the fact that proprietary blends of these materials may provide all of the textural attributes of a reference control product when reducing the meat content of future

meat containing products. This would be a subject of future studies and potentially very interesting for industry, and to meet for future generations when meat supplies are predicated to be scare/insufficient. An added benefit is also the inclusion of more fibre in the diet, while providing consumer acceptable products. Therefore, future studies should also indicate sensory analysis of these type of products.

Table 8.13: Comparison of all treatments to a statistical match to a reference.
(Match means all of the lower case letters, in the texture figures are the same; Mid-range means one lower case letter is the same, and no match means there are no similar lower case letters in the texture figures)

Polymer	Mode of Addition	Statistical match to the 100% Meat Control			
		Hardness	Springness	Cohesivness	Resilience
3% Starch	Dry				
7.4% Starch					
3% Citrus Fibre					
0.5% kappa carrageenan					
1% kappa carrageenan					
2% kappa carrageenan					
0.5% iota carrageenan					
1% iota carrageenan					
2% iota carrageenan					
0.5% lambda carrageena					
1% lambda carrageena					
2% lambda carrageena					
0.5% LBG					
1% LBG					
2% LBG					
3% Starch	Pre-hydrated				
7.4% Starch					
3% Citrus Fibre					
0.5% kappa carrageenan					
1% kappa carrageenan					
2% kappa carrageenan					
0.5% iota carrageenan					
1% iota carrageenan					
2% iota carrageenan					
0.5% lambda carrageena					
1% lambda carrageena					
2% lambda carrageena					
0.5% LBG					
1% LBG					
2% LBG					
3% Starch	Gelled				
7.4% Starch					
0.5% kappa carrageenan					
1% kappa carrageenan					
2% kappa carrageenan					
0.5% iota carrageenan					
1% iota carrageenan					
2% iota carrageenan					
0.5% LBG					
1% LBG					
2% LBG					
Match					
Mid-range					
No Match					

8.3 Future experiments and work

Aside from the usual issue of conservatism in the food industry in general, a common problem in the meat industry is that any changes in food composition generate large differences in texture and the sensation of eating. Textural properties of meat are particularly sensitive to changes such as this. Meat scientists have attempted to improve the texture and achieve a higher quality of meat by adding high quality non-meat ingredients such as hydrocolloids.

The purpose of the work presented here has been to attempt to quantify these changes as well as to understand the fundamental changes that occur in properties of the various components. Chief among the effects that are examined, is the possibility of interactions or nonlinear additions of properties. If there are no interactions, then the resultant properties of the composite should be much more easily predictable. For example, the simple linear addition of gel strengths might be predicted in a system exhibiting no interactions, which is comparatively easily managed. It is, therefore, crucial to examine the possibility for interaction between components.

It can be seen from the work presented here that the determination of interaction, whilst appearing superficially simple, is actually very complex. For example, when looking at interactions between carrageenan and meat, it was noted that the presence of the meat has the possibility of changing the salt concentration in the carrageenan phase. In any future works, these changes will have to be investigated more thoroughly. This could be done in two ways. Firstly, a more rigorous experimental design could be used in an attempt to define these effects. Systematic variations in salt and meat levels would make it possible to approach this problem. In addition, the ‘meat’ fraction would have to be defined much more clearly in terms of water, fat and salt content, and variations from batch to batch more closely monitored.

Further experimentation could be carried out, for example, blending unmodified wheat starch, carrageenan, LBG or citrus fibre with meat and examining how these changes in food composition affected the texture of beef meat sausages. However, there appears to be no definite, unequivocal evidence of interactions between hydrocolloids and the proteins in meat. This author could find only one

study related to the effect of potato starch, kappa carrageenan and locust bean gum in meat batter (García-García & Totosaus, 2008).

Future research should also focus on the interaction between protein-polysaccharide with other methods such as NMR spectrum. For instance, if we dissolved starch and protein at low concentrations in an appropriate solvent, then we would think by far the best way to look for interactions would be to examine peaks for shifts in an NMR spectrum because any peaks shift in NMR relative to the individual materials in the solvent alone would reflect a conformational change as a result of the interaction. Furthermore, future research can focus on developing more effective methods of staining the carrageenan's and LBG in meat beef sausage in order to visualise these components more successfully in confocal microscopy, and thus developing a better feel for the type of microstructure present. In addition, flavour must be considered for this kind of sausages for sensory studies, and additional research should be carried out to check whether the meats are palatable. However, they might even be preferred because texture is a direct relationship between hardness, tenderness and juiciness of the final product.

CHAPTER 9 REFERENCES

Acton, J. (1972) The effect of meat particle size on extractable protein, cooking loss and binding strength in chicken loaves. *Journal of Food Science*, 37, 240–243.

Acton, J. C., Ziegler, G. R., Burge Jr, D. L. & Froning, G. W. (1982) Functionality of muscle constituents in the processing of comminuted meat products. *Critical Reviews in Food Science & Nutrition*, 18, 99–121.

Adhikari, B., Howes, T., Bhandari, B. & Truong, V. (2001) Stickiness in foods: A review of mechanisms and test methods. *International Journal of Food Properties*, 4, 1–33.

Aguilera, J. M. & Rojas, G. V. (1997) Determination of kinetics of gelation of whey protein and cassava starch by oscillatory rheometry. *Food Research International*, 30, 349–357.

Aguilera, J., Cuadros, T. & Del Valle, J. (1998) Differential scanning calorimetry of low-moisture apple products. *Carbohydrate Polymers*, 37, 79–86.

Aguilera, J. M. & Rojas, G. V. (1997) Determination of kinetics of gelation of whey protein and cassava starch by oscillatory rheometry. *Food Research International*, 30**,** 349–357.

Alamri, M. S., Mohamed, A. A. & Hussain, S. (2013) Effects of alkaline-soluble okra gum on rheological and thermal properties of systems with wheat or corn starch. *Food Hydrocolloids*, 30**,** 541–551.

Aleson-Carbonell, L., Fernández-López, J., Perez-Alvarez, J. & Kuri, V. (2005) Functional and sensory effects of fibre-rich ingredients on breakfast fresh sausages manufacture. *Food Science and Technology International*, 11, 89–97.

Aleson-Carbonell, L., Fernández-López, J., Sayas-Barberá, E., Sendra, E. & Pérez-Alvarez, J. (2003) Utilization of lemon albedo in dry-cured sausages. *Journal of Food Science*, 68, 1826–1830.

Amako, D. E. & Xiong, Y. L. (2001) Effects of carrageenan on thermal stability of proteins from chicken thigh and breast muscles. *Food Research International*, 34, 247–253.

Anderson, J. W., Smith, B. M. & Gustafson, N. J. (1994) Health benefits and practical aspects of high-fibre diets. *The American Journal of Clinical Nutrition*, 59, 1242S-1247S.

Ang, J. & Miller, W. (1991) Multiple functions of powdered cellulose as a food ingredient. *Cereal Foods World*, 36, 558–564.

Appelqvist, I. A. & Debet, M. R. (1997) Starch-biopolymer interactions: A review. *Food Reviews International*, 13**,** 163–224.

Asghar, A., Samejima, K., Yasui, T. & Henrickson, R. L. (1985) Functionality of muscle proteins in gelation mechanisms of structured meat products. *Critical Reviews in Food Science & Nutrition*, 22, 27–106.

Asghar, A. & Pearson, A. (1981) Influence of ante-and post-mofitem treatments upon muscle composition and meat quality. *Advances in Food Research*, 26, 53.

Asghar, A., Yeates, N. & Hannan, R. (1978) The mechanism for the promotion of tenderness in meat during the post-mortem process: A review. *Critical Reviews in Food Science & Nutrition*, 10, 115-145.

Ayadi, M., Kechaou, A., Makni, I. & Attia, H. (2009) Influence of carrageenan addition on turkey meat sausages properties. *Journal of Food Engineering*, 93, 278–283.

Barbut, S. & Mittal, G. (1992) Use of carrageenans and xanthan gum in reduced fat breakfast sausages. *Lebensmittel-Wissenschaft+ Technologie*, 25, 509–513.

Barrera, A., Ramırez, J., González-Cabriales, J. & Vázquez, M. (2002) Effect of pectins on the gelling properties of surimi from silver carp. *Food Hydrocolloids*, 16, 441–447.

Bater, B., Descamps, O. & Maurer, A. J. (1992a) Kappa-carrageenan effects on the gelation properties of simulated oven-roasted turkey breast juice. *Journal of Food Science*, 57, 845–847.

Bater, B., Descamps, O. & Maurer, A. J. (1992b) Quality characteristics of hydrocolloid-added oven-roasted turkey breasts. *Journal of Food Science*, 57, 1068–1070.

Bater, B., Descamps, O. & Maurer, A. (1993) Quality characteristics of cured turkey thigh meat with added hydrocolloids. *Poultry Science*, 72, 349–354.

Bailey, A. J. & Light, N. D. (1989) *Connective tissue in meat and meat products*, Elsevier Applied Science.

Bemiller, J. N. (2011) Pasting, paste, and gel properties of starch: Hydrocolloid combinations. *Carbohydrate Polymers*, 86**,** 386–423.

Bertran, M. S. & Dale, B. E. (1986) Determination of cellulose accessibility by differential scanning calorimetry. *Journal of Applied Polymer Science*, 32, 4241–4253.

Bernal, V., Smajda, C., Smith, J. & Stanley, D. (1987) Interactions in protein/polysaccharide/calcium gels. *Journal of Food Science,* 52, 1121–1125.

Bianchi, M., Pilosof, A. & Bartholomai, G. (1987) Influence of soy protein and NaCl on the rheological properties of beef, pork or chicken muscle homogenates. *Journal of Food Science,* 52, 890-892.

Biliaderis, C. G. (1983) Differential scanning calorimetry in food research: A review. *Food Chemistry*, 10**,** 239–265.

Blanshard, J. & Mitchell, J. (1988) *Food Structure: Its Creation and Evaluation*, Oxford,Elsevier.

Blonk, J. & Van Aalst, H. (1993) Confocal scanning light microscopy in food research. *Food Research International*, 26**,** 297–311.

Bloukas, J., Paneras, E. & Papadima, S. (1997) Effect of carrageenan on processing and quality characteristics of low-fat frankfurters. *Journal of Muscle Foods*, 8, 63–83.

Board, P. W., Montgomery, W. A. & Rutledge, P. J. (1978) Collagen content of some Australian canned meats. *Journal of the Science of Food and Agriculture*, 29, 569-573.

Bodeaubellion, C. (1983) Analysis of carrageenan structure. *Physiologie Vegetale,* 21, 785-793.

Bourne, M. C. (1978) Texture profile analysis [Food acceptability]. *Food Technology*,7,50-60.

Braddock, R. (1995) By-products of citrus fruit. *Food Technology,* 49, 74-77.

Brandt, M. A., Skinner, E. Z. & Coleman, J. A. (1963) Texture profile method. *Journal of Food Science,* 28, 404-409.

Braudo, E. (1992) Mechanism of galactan gelation. *Food Hydrocolloids,* 6, 25–43.

Breene, W. M. (1975) Application of texture profile analysis to instrumental food texture evaluation. *Journal of Texture Studies,* 6, 53-82.

Brooker, B. (1991) The study of food systems using confocal laser scanning microscopy. *Microscopy and Analysis*, 27**,** 13–15.

Bruno, M. & Moresi, M. (2004) Viscoelastic properties of Bologna sausages by dynamic methods. *Journal of Food Engineering*, 63**,** 291–298.

Bungenberg De Jong, H. & Kruyt, H. (1949) Colloid science. *Kruyt, H, Ed*, 335-432.

Carr, J. (1993) Use of carrageenans in cured meat products. *Cured Meat Short Course, Ames, IA, Jan*, 12.

Casimir, C. A. & Akoh, P. (1998) Fat replacers. *Food Technology*, 52, 47–53.

Candogan, K. & Kolsarici, N. (2003a) Storage stability of low-fat beef frankfurters formulated with carrageenan or carrageenan with pectin. *Meat Science*, 64, 207–214.

Candogan, K. & Kolsarici, N. (2003b) The effects of carrageenan and pectin on some quality characteristics of low-fat beef frankfurters. *Meat Science*, 64**,** 199–206.

Carballo, J., Barreto, G. & Colmenero, F. J. (1995) Starch and egg white lnfluence on properties of bologna sausage as related to fat content. *Journal of Food Science*, 60**,** 673–677.

Carballo, J., Fernandez, P., Barreto, G., Solas, M. T. & Colmenero, F. J. (1996) Morphology and texture of bologna sausage as related to content of fat, starch and egg white. *Journal of Food Science*, 61**,** 652–665.

Chattong, U., Apichartsrangkoon, A. & Bell, A. E. (2007) Effects of hydrocolloid addition and high pressure processing on the rheological properties and microstructure of a commercial ostrich meat product 'Yor' (Thai sausage). *Meat Science*, 76, 548–554.

Chang, H. C. & Carpenter, J. A. (1997) Optimizing quality of frankfurters containing oat bran and added water. *Journal of Food Science*, 62, 194–197.

Chen, Y., Liao, M.-L., Boger, D. & Dunstan, D. (2001) Rheological characterisation of κ-carrageenan/locust bean gum mixtures. *Carbohydrate Polymers*, 46, 117–124.

Chung, K. & Lee, C. (1991) Water binding and ingredient dispersion pattern effects on surimi gel texture. *Journal of Food Science*, 56**,** 1263–1266.

Cierach, M., Modzelewska-Kapituła, M. & Szaciło, K. (2009) The influence of carrageenan on the properties of low-fat frankfurters. *Meat Science*, 82, 295–299.

Civille, G. V. & Szczesniak, A. S. (1973) Guidelines to training a texture profile panel. *Journal of Texture Studies,* 4, 204-223.

Civille, G. V. & Liska, I. H. (1975) Modifications and applications to foods of the General Foods sensory texture profile technique. *Journal of Texture Studies,* 6, 19-31.

Claus, J., Hunt, M. & Kastner, C. (1990) Effects of substituting added water for fat on the textural, sensory, and processing characteristics of bologna1. *Journal of Muscle Foods*, 1, 1–21.

Claus, J. & Hunt, M. (1991) Low-fat, high added-water bologna formulated with texture-modifying ingredients. *Journal of Food Science*, 56, 643–647.

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

Cofrades, S., Troy, D. & Hughes, E. (1995) The effect of fat level on textural characteristics of low-fat emulsion type meat products. *Annual International Congress of Meat Science and Technology, 1995*, pp, 66–67.

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

Colmenero, F. J. (1996) Technologies for developing low-fat meat products. *Trends in Food Science & Technology*, 7, 41–48.

Damásio, M., Costell, E., Izquierdo, L. & Durán, L. (1994) Influence of composition on non-oral texture of kappa-carrageenan – locust bean gum – guar gum mixed gels. *Food Quality and Preference*, 5, 195–201.

Damodaran, S. (1997) *Food proteins and their applications*, CRC Press.

Dea, I. C., Morris, E. R., Rees, D. A., Welsh, E. J., Barnes, H. A. & Price, J. (1977) Associations of like and unlike polysaccharides: mechanism and specificity in galactomannans, interacting bacterial polysaccharides, and related systems. *Carbohydrate Research*, 57, 249–272.

Dea, I. (1979) Interactions of ordered polysaccharide structures: Synergism and freeze–thaw phenomena. *Journal of food and agriculture organisation of the united state*, 27, 229-247.

Dea, I. C., Clark, A. H. & Mccleary, B. V. (1986) Effect of the molecular fine structure of galactomannans on their interaction properties: The role of unsubstituted sides. *Food Hydrocolloids*, 1, 129–140.

Defreitas, Z., Sebranek, J., Olson, D. & Carr, J. (1997a) Carrageenan effects on salt-soluble meat proteins in model systems. *Journal of Food Science*, 62, 539–543.

Defreitas, Z., Sebranek, J., Olson, D. & Carr, J. (1997b) Carrageenan effects on thermal stability of meat proteins. *Journal of Food Science*, 62, 544–547.

Del Carmen Núñez-Santiago, M. & Tecante, A. (2007) Rheological and calorimetric study of the sol–gel transition of κ-carrageenan. *Carbohydrate Polymers*, 69, 763–773.

Dey, P. M. (1978) Biochemistry of plant galactomannans. *Advances in Carbohydrate Chemistry and Biochemistry*, 35, 341–376.

De Kruif, C. & Tuinie, R. 2001. Polysaccharide protein interactions. *Food hydrocolloids,* 15**,** 555-563.

Dexter, D., Sofos, J. & Schmidt, G. (1993) Quality characteristics of turkey bologna formulated with carrageenan, starch, milk and soy protein. *Journal of Muscle Foods*, 4**,** 207–223.

Donovan, J., Lorenz, K. & Kulp, K. (1983) Differential scanning calorimetry of heat-moisture treated wheat and potato starches. *Cereal Chemistry (USA)*, 60,5-20.

Doublier, J. L. (1981) Rheological studies on starch—Flow behaviour of wheat starch pastes. *Starch-Stärke,* 33, 415-420.

Doublier, J. & Launay, B. (1981) Rheology of galactomannan solutions: Comparative study of guar gum and locust bean gum. *Journal of Texture Studies*, 12, 151–172.

Dušek, K. & Prins, W. (1969) *Structure and Elasticity of Non-crystalline Polymer Networks*. Berlin. Springer.

Egelandsdal, B., Fretheim, K. & Samejima, K. (1986) Dynamic rheological measurements on heat-induced myosin gels: Effect of ionic strength, protein

concentration and addition of adenosine triphosphate or pyrophosphate. *Journal of the Science of Food and Agriculture*, 37, 915–926.

Eiji Niwa, T.-T. W., Satoshi Kanoh, Teruo Nakayama (1988) Contribution of gelling substance to muscular protein network structure within kamaboko. *Nippon Suisan Gakkaishi*, 54, 989–992.

Elleuch, M., Bedigian, D., Roiseux, O., Besbes, S., Blecker, C. & Attia, H. (2011) Dietary fibre and fibre-rich by-products of food processing: Characterisation, technological functionality and commercial applications: A review. *Food Chemistry*, 124, 411-421.

Eliasson, A. C. (1986) Viscoelastic behaviour during the gelatinisation of starch I. comparison of wheat, maize, potato and waxy-barley starches. *Journal of Texture Studies*, 17, 253-265.

Eliasson, A.-C. & Gudmundsson, M. (2006) 10 Starch. *Carbohydrates in Food*, 159, 391.

Ensor, S. A., Sofos, J. N. & Schmidt, G. R. (1991) Differential scanning calorimetric studies of meat protein–aginate mixtures. *Journal of Food Science*, 56, 175–179.

Fda (1992) Chonddrus Extract *In:* 21 CFR 182 (ed.).

Fennema, O. R., Karel, M. & Lund, D. B. (1975) *Principles of Food Science*. New York, Dekker.

Fernández-Ginés, J., Fernández-López, J., Sayas-Barberá, E., Sendra, E. & Pérez-Alvarez, J. (2003) Effect of storage conditions on quality characteristics of bologna sausages made with citrus fiber. *Journal of Food Science*, 68, 710–714.

Fernández-López, J., Fernández-Ginés, J., Aleson-Carbonell, L., Sendra, E., Sayas-Barberá, E. & Pérez-Alvarez, J. (2004) Application of functional citrus by-products to meat products. *Trends in Food Science & Technology*, 15, 176–185.

Fernández-López, J., Viuda-Martos, M., Sendra, E., Sayas-Barberá, E., Navarro, C. & Pérez-Alvarez, J. A. (2007) Orange fibre as potential functional ingredient for dry-cured sausages. *European Food Research and Technology*, 226, 1–6.

Findlay, C., Barbut, S. & Harwalkar, H. (1990) Thermal analysis of meat. *Thermal Analysis of Foods*, 55 ,92–98.

Findlay, C. & Stanley, D. (1984a) Differential scanning calorimetry of beef muscle: Influence of postmortem conditioning. *Journal of Food Science*, 49**,** 1513–1516.

Findlay, C. & Stanley, D. (1984b) Differential scanning calorimetry of beef muscle: Influence of sarcomere length. *Journal of Food Science*, 49, 1529–1531.

Findlay, C., Parkin, K. & Stanley, D. (1986) Differential scanning calorimetry can determine kinetics of thermal denaturation of beef muscle proteins. *Journal of Food Biochemistry*, 10**,** 1–15.

Fischer, J. (2000) Functional properties of Herbacel AQ Plus fruit fibres. Poster presented at 1st International Conference on Dietary Fiber, Dublin, Ireland.

Foegeding, E. & Ramsey, S. (1986) Effect of gums on low-fat meat batters. *Journal of Food Science*, 51, 33–36.

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

Foegeding, E. & Ramsey, S. (1987) Rheological and water-holding properties of gelled meat batters containing iota carrageenan, kappa carrageenan or xanthan gum. *Journal of Food Science*, 52, 549–553.

Foegeding, E. (1988) Thermally induced changes in muscle proteins. *Food Technology*, 42**,** 58–64.

Foster, T.J., Underdown, J., Brown, C.R.T., Ferdinando, D.P. and Norton. I.T. (1996). Emulsions hebaviour of non-gelled biopolymer mixtures, in Food Proteins, Lipids and Polysaccharides. Eds, E. Dickinson and B. Bergenstahl, Royal Society of Chemistry, Cambridge, UK, 346-355.

French, D. (1972) Fine structure of starch and its relationship to the organization of starch granules. , 19, 8-25.

Friedmaan, H. H., Whitney, J. E. & Szczesniak, A. S. (1963) the texturometer—a new instrument for objective texture measurement. *Journal of Food Science*, 28, 390-396.

Gaonkar, A. G. & Mcpherson, A. (2005) *Ingredient interactions: effects on food quality*, CRC Press.

García-García, E. & Totosaus, A. (2008) Low-fat sodium-reduced sausages: Effect of the interaction between locust bean gum, potato starch and κ-carrageenan by a mixture design approach. *Meat Science*, 78, 406–413.

Ganz, A. (1965) *Effect of Sodium Chloride on Pasting of Wheat Starch Granules*. American Association of Cereal Chemists, 340, 55121-2097.

García-García, E. & Totosaus, A. (2008) Low-fat sodium-reduced sausages: Effect of the interaction between locust bean gum, potato starch and κ-carrageenan by a mixture design approach. *Meat Science*, 78, 406-413.

Ghiasi, K. & Hoseney, R. (1982) Gelatinisation of wheat starch. II. Starch-surfactant interaction. *Cereal Chemistry*, 75, 220-235.

Glahn, P. (1982) Hydrocolloid stabilization of protein suspensions at low pH. *Progress in food and nutrition science*, 6, 171-177.

Glicksman, M. (1969) Gum technology in the food industry, 590.

Glicksman, M. & Glicksman, M. 1982. *Food hydrocolloids*, CRC Press: Boca Raton, FL.

Goncalves, M., Gomes, C., Langdon, M., Viebke, C. & Williams, P. (1997) Studies on κ-carrageenan/locust bean gum mixtures in the presence of sodium chloride and sodium iodide. *Biopolymers*, 41, 657–671.

Gordon, A., Barbut, S. & Schmidt, G. (1992) Mechanisms of meat batter stabilization: A review. *Critical Reviews in Food Science & Nutrition*, 32, 299–332.

Gorinstein, S., Martín-Belloso, O., Park, Y.-S., Haruenkit, R., Lojek, A., Ĉíž, M., Caspi, A., Libman, I. & Trakhtenberg, S. (2001) Comparison of some biochemical characteristics of different citrus fruits. *Food Chemistry*, 74, 309-315.

Goldberg, I. & Williams, R. (1991) *Biotechnology and Food Ingredients*. Berlin, Springer Science.

Goldberg, I. & Williams, R. (1991) *Biotechnology and food ingredients*, Springer Science & Business Media.

Grigelmo-Miguel, N. & Martín-Belloso, O. (1999) Comparison of dietary fibre from by-products of processing fruits and greens and from cereals. *LWT-Food Science and Technology*, 32, 503-508.

Grigelmo-Miguel, N., Abadías-Serós, M. A. I. & Martín-Belloso, O. (1999) Characterisation of low-fat high-dietary fibre frankfurters. *Meat Science*, 52, 247–256.

Halden, J. P., Mathiesen, C.-H. & Proctor, M. S. (1992) Marinating or pickling of meat. US Patents, 5, 158,221.

Hamm, R. (1972) Colloid chemistry of meat; the water-binding capacity of muscle protein in theory and practice. *Journal of Food and Agriculture Organization Of the United Nations.* 7, 275-280.

Hansen, L., Hoseney, R. & Faubion, J. (1991) Oscillatory rheometry of starch-water systems: Effect of starch concentration and temperature. *Cereal Chemistry,* 68, 347-351.

Harris, P. J. & Smith, B. G. (2006) Plant cell walls and cell-wall polysaccharides: structures, properties and uses in food products. *International Journal of Food science & Technology,* 41**,** 129-143.

Harfenist, E. J. 1953. The amino acid compositions of insulins isolated from beef, pork and sheep glands. *Journal of the American Chemical Society,* 75**,** 5528-5533.

Hayashi, A., Kinoshita, K., Miyake, Y. & Cho, C.-H. (1983) Gelation and its related changes in amylose solution. *Agricultural and Biological Chemistry,* 47, 1699-1704.

Heertje, I., Blonk, J., Hendrickx, H. & Brakenhoff, G. (1987) Confocal scanning laser microscopy in food research: Some observations. *Food Microstructure*, 6, 2-10.

Heinz, G. & Hautzinger, P. (2009) Meat processing technology for small to medium scale producers. *RAP Publication (FAO)*.

Hepler, P. K. & Gunning, B. E. (1998) confocal fluorescence microscopy of plant cells. *Protoplasma,* 201, 121-157.

Hermansson, A. M. & Åkesson, C. (1975) Functional properties of added proteins correlated with properties of meat systems. Effect of concentration and temperature on water-binding properties of model meat systems. *Journal of Food Science*, 40, 595-602.

Hermansson, A. M., Harbitz, O. & Langton, M. (1986) Formation of two types of gels from bovine myosin. *Journal of the Science of Food and Agriculture,* 37, 69-84.

Hermansson, A.-M. (1989) Rheological and microstructural evidence for transient states during gelation of kappa-carrageenan in the presence of potassium. *Carbohydrate Polymers*, 10, 163–181.

Hizukuri, S. (1991) Properties of hot-water-extractable amylose. *Carbohydrate Research,* 217, 251-253.

Hoogenkamp, H. W. (1992) Update: Functionality of non meat ingredients. *Reciprocal Meat Conference Proceedings*, 1992, 81–84.

Hsu, S. & Chung, H.-Y. (2001) Effects of κ-carrageenan, salt, phosphates and fat on qualities of low fat emulsified meatballs. *Journal of Food Engineering,* 47, 115–121.

Hughes, E., Mullen, A. & Troy, D. (1998) Effects of fat level, tapioca starch and whey protein on frankfurters formulated with 5% and 12% fat. *Meat Science*, 48, 169–180.

Hughes, J., Oiseth, S., Purslow, P. & Warner, R. (2014) A structural approach to understanding the interactions between colour, water-holding capacity and tenderness. *Meat Science*, 98, 520–532.

Hutchings, J. B. & Lillford, P. (1988) The perception of food texture: The philosophy of the breakdown path. *Journal of Texture Studies,* 19, 103–115.

Igoe, R. (1982) Hydrocolloid interactions useful in food systems. *Food Technology (USA),* 36,4.

Iijima, M., Hatakeyama, T., Takahashi, M. & Hatakeyama, H. (2007) Effect of thermal history on kappa-carrageenan hydrogelation by differential scanning calorimetry. *Thermochimica Acta*, 452, 53–58.

Ikeda, S. & Kumagai, H. (1998) Dielectric analysis of sol-gel transition of κ-carrageenan with scaling concept. *Journal of Agricultural and Food Chemistry*, 46, 3687–3693.

Ikeuchi, Y., Tanji, H., Kakimoto, T. & Suzuki, A. (1994) Dynamic rheological behaviour and biochemical properties of rabbit skeletal actomyosin during storage at 0° C. *Journal of the Science of Food and Agriculture*, 65, 77–84.

Imeson, A. (1997) *Thickening and Gelling Agents for Food,* Springer.

Ishioroshi, M., Jima, K. S. & Yasui, T. (1979) Heat-induced gelation of myosin: Factors of pH and salt concentrations. *Journal of Food Science*, 44, 1280–1284.

INSO (2017), Sausage specifications and test methods, Amd. No 2, 2303.

INSO (2007), Ham specification and test methods 5753.

Jenkins, P., Comerson, R., Donald, A., Bras, W., Derbyshire, G., Mant, G. & Ryan, A. (1994) In situ simultaneous small and wide angle x-ray scattering: A new technique to study starch gelatinization. *Journal of Polymer Science Part B: Polymer Physics,* 32, 1579-1583.

Jarvis, M. C. (2011) Plant cell walls: supramolecular assemblies. *Food Hydrocolloids,* 25**,** 257-262.

Jensen, W. K., Devine, C. & Dikeman, M. (2004) *Encyclopaedia of meat sciences*, Elsevier Academic Press.

Joint FAO/WHO (2009) Foods derived from modern biotechnology. Second edition, Codex Alimentarius, Rome, ISBN 978-92-5-105914-2.

Kao, W. & Lin, K. (2006) Quality of reduced-fat frankfurter modified by konjac–starch mixed gels. *Journal of Food Science*, 71, S326–S332.

Karmas, E. & Dimarco, G. R. (1970) Denaturation thermoprofiles of some proteins. *Journal of Food Science*, 35, 725–727.

Keeton, J. (1994) Low-fat meat products: Technological problems with processing. *Meat Science*, 36, 261–276.

Kim, J. & Lee, C. (1987) Effect of starch of textural properties of surimi gel. *Journal of Food Science*, 52, 722–725.

Kinsella, J. E. (1982) Protein structure and functional properties: Emulsification and flavour binding effects. ACS Symposium series-American Chemical Society (USA).

Knight, P. & Parsons, N. (1988) Action of NaCl and polyphosphates in meat processing: Responses of myofibrils to concentrated salt solutions. *Meat Science*, 24, 275–300.

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

Laaman, T. R. (2010) *Hydrocolloids in Food Processing*, Wiley-Blackwell.

Ledward, D. (1979) Protein-polysaccharide interactions. *Canadian Institute of Food Science and Technology Journal*, 12, A16.

Leloup, V., Colonna, P., Ring, S. G., Roberts, K. & Wells, B. (1992) Microstructure of amylose gels. *Carbohydrate Polymers*, 18, 189-197.

León, A., Rosell, C. M. & De Barber, C. B. (2003) A differential scanning calorimetry study of wheat proteins. *European Food Research and Technology*, 217**,** 13–16.

Lewis, D., Groves, K. & Holgate, J. (1986) Action of polyphosphates in meat products. *Food Microstructure (USA)*, 5, 1-7.

Llanto, M., Bullens, C., Modliszewski, J. & Bushway, A. (1990) Effect of carrageenan on gelling potential of surimi prepared from Atlantic pollack. *Advances in Fisheries, Technology and Biotechnology for Increased Profitability, 34, 301-322.*

Li, J.-Y. & Yeh, A.-I. (2001) Relationships between thermal, rheological characteristics and swelling power for various starches. *Journal of Food Engineering*, 50, 141–148.

Li, J. Y. & Yeh, A. I. (2002) Functions of starch in formation of starch/meat composite during heating. *Journal of Texture Studies*, 33, 341–366.

Lin, L. C. & Ito, T. (1985) Rheological property of myosin B fibre and the fibre of the mixture of myosin B and soya protein; and the interaction between myosin B and soya protein. *International Journal of Food Science & Technology*, 20, 219-235.

Locker, R. H. (1987) The non-sliding filaments of the sarcomere. *Meat Science*, 20, 217-236.

Lorén, N., Langton, M. & Hermansson, A.-M. (1999) Confocal laser scanning microscopy and image analysis of kinetically trapped phase-separated gelatin/maltodextrin gels. *Food Hydrocolloids*, 13**,** 185–198.

Lundberg, B. (2005) Using highly expanded citrus fiber to improve the quality and nutritional properties of foods. *Cereal Foods World*, 50, 248–252.

Lundin, L. & Hermansson, A.-M. (1997) Rheology and microstructure of Ca-and Na-K-carrageenan and locust bean gum gels. *Carbohydrate Polymers*, 34, 365–375.

Luruena-Martınez, M., Vivar-quintana, a. & Revilla, I. (2004) Effect of locust bean/xanthan gum addition and replacement of pork fat with olive oil on the quality characteristics of low-fat frankfurters. *Meat Science*, 68, 383–389.

Macnaughtan, W., Farhat, I., Himawan, C., Starov, V. & Stapley, A. (2006) A differential scanning calorimetry study of the crystallization kinetics of tristearin-tripalmitin mixtures. *Journal of the American Oil Chemists' Society*, 83, 1–9.

Maier, H., Anderson, M., Karl, C., Magnuson, K. & Whistler, R. (1993) Guar, locust bean, tara, and fenugreek gums. *Industrial Gums: Polysaccharides and their Derivatives*, 3, 215–218.

Marin, F., Frutos, M., Pérez-Alvarez, J., Martinez-Sanchez, F. & Del Rio, J. (2002) Flavonoids as nutraceuticals: Structural-related antioxidant properties and their role on ascorbic acid preservation. *Studies in Natural Products Chemistry*, 26, 741–778.

Marın, F., Martinez, M., Uribesalgo, T., Castillo, S. & Frutos, M. (2002) Changes in nutraceutical composition of lemon juices according to different industrial extraction systems. *Food Chemistry,* 78, 319-324.

Markowski, M., Bialobrzewski, I., Cierach, M. & Paulo, A. (2004) Determination of thermal diffusivity of Lyoner type sausages during water bath cooking and cooling. *Journal of Food Engineering*, 65, 591–598.

Martens, H., Stabursvik, E. & Martens, M. (1982). Texture and colour changes in meat during cooking related to thermal denaturation of muscle proteins. *Journal of Texture Studies,* 13, 291-309.

Martens, H. & Vold, E. (1976) DSC studies of muscle tissue protein denaturation. *Congress Documentation. Proceedings of the European Meeting of Meat Research Workers*, 1976.

Matlock, R., Terrell, R., Savell, J., Rhee, K. & Dutson, T. (1984) Factors Affecting Properties of Raw-Frozen Pork Sausage Patties Made with Various NaCl/Phosphate Combinations. *Journal of Food Science,* 49, 1363-1366.

Mcswiney, M., Singh, H. & Campanella, O. H. (1994) Thermal aggregation and gelation of bovine β-lactoglobulin. *Food Hydrocolloids*, 8, 441–453.

Mezger, T. G. (2006). *The rheology handbook: for users of rotational and oscillatory rheometers*, Vincentz Network GmbH & Co KG.

Miles, M. J., Morris, V. J., Orford, P. D. & Ring, S. G. (1985) The roles of amylose and amylopectin in the gelation and retrogradation of starch. *Carbohydrate Research*, 135, 271-281.

Micklander, E., Peshlov, B., Purslow, P. P. & Engelsen, S. B. (2002) NMR-cooking: Monitoring the changes in meat during cooking by low-field 1 H-NMR. *Trends in Food Science & Technology*, 13, 341–346.

Millman, B. M. (1998) The filament lattice of striated muscle. *Physiological Reviews*, 78, 359–391.

Modliszewski, J. (1984) Carrageenan. *New York State Agricultural Experiment Station Special Report*, 25–31.

Montero, P., Hurtado, J. L. & Pérez-Mateos, M. (2000) Microstructural behaviour and gelling characteristics of myosystem protein gels interacting with hydrocolloids. *Food Hydrocolloids*, 14, 455–461.

Morris, E. R., Rees, D. A. & Robinson, G. (1980) Cation-specific aggregation of carrageenan helices: Domain model of polymer gel structure. *Journal of Molecular Biology*, 138, 349–362.

Morris, V., Mitchell, J. & Ledward, D. (1986) Functional properties of food macromolecules. *Norwich: JR Mitchell and DA Ledward*, 121–8.

Morris, E. R. 1990. Mixed polymer gels. *Food gels*. Springer.

Morris, E. (1995) Polysaccharide rheology and in-mouth perception. *Food Science and Technology-New York-Marcel Dekker-*, 517-517.

Morris, E. & Foster, T. (1994) Role of conformation in synergistic interactions of xanthan. *Carbohydrate Polymers*, 23, 133-135.

MPR (2014), technical guide for meat production.

Nagy, S. & Attaway, J. A. (1993) Anticarcinogenic activity of phytochemicals in citrus fruit and their juice products. *Florida State Horticultural Society. Meeting (USA)*, 1993.

Nagy, S. & Attaway, J. A. (1993) Anti-carcinogenic activity of phytochemicals in citrus fruit and their juice products. Florida State Horticultural Society. Meeting (USA).

Nilsson, S. & Piculell, L. (1991) Helix-coil transitions of ionic polysaccharides analyzed within the Poisson-Boltzmann cell model. 4. Effects of site-specific counterion binding. *Macromolecules*, 24, 3804–3811.

Nishinari, K., Watase, M., Williams, P. A. & Phillips, G. O. (1990) Kappa-carrageenan gels: Effect of sucrose, glucose, urea, and guanidine hydrochloride on the rheological and thermal properties. *Journal of Agricultural and Food Chemistry*, 38, 1188–1193.

Niva & Al., E. (1988) Contribution of gelling substance to muscular protein network structure within kamaboko. *Nippon Suisan Gakkaishi*, 54, 989–992.

Norton, I. T., Morris, E. R. & Rees, D. A. (1984) Lyotropic effects of simple anions on the conformation and interactions of kappa-carrageenan. *Carbohydrate Research*, 134, 89–101.

Núñez-Santiago, M., Tecante, A., Garnier, C. & Doublier, J. L. (2011) Rheology and microstructure of κ-carrageenan under different conformations induced by several concentrations of potassium ion. *Food Hydrocolloids*, 25, 32–41.

Oakenfull, D. & Glicksman, M. (1987) Gelling agents. *Critical Reviews in Food Science & Nutrition*, 26, 1-25.

Oakenfull, D. G. & Scott, A. (1988) Size and stability of the junction zones in gels of iota and kappa carrageenan. *Journal of Food and Agriculture Organisation Of the United Nations, 8, 32-43.*

Offer, G. & Knight, P. (1988) Structural basis of water-holding in meat. 2. Drip losses. *Developments in Meat Science*, 4, 173-243.

Offer, G., Knight, P., Jeacocke, R., Almond, R., Cousins, T., Elsey, J., Parsons, N., Sharp, A., Starr, R. & Purslow, P. (1989) The structural basis of the water-holding, appearance and toughness of meat and meat products. *Food Microstructure (USA)*,8,120-142.

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

Offer, G. & Knight, P. (1988) Structural basis of water-holding in meat. 2. Drip losses. *Developments in meat science*.

Offer, G., Knight, P., Jeacocke, R., Almond, R., Cousins, T., Elsey, J., Parsons, N., Sharp, A., Starr, R. & Purslow, P. (1989) The structural basis of the water-holding, appearance and toughness of meat and meat products. *Food microstructure (USA)*, 8, 17-30.

Paterson, L., Hashim, D. M., Hill, S., Mitchell, J., & Blanshard, J. (1994) The effect of sulphite on the integrity of the starch granule. Food Hydrocolloids, 8, 259-263.

Paulsen, M. T., Nys, A., Kvarberg, R. & Hersleth, M. (2014) Effects of NaCl substitution on the sensory properties of sausages: Temporal aspects. *Meat Science*, 98, 164–170.

Petridis, D., Raizi, P. & Ritzoulis, C. (2014) Influence of citrus fiber, rice bran and collagen on the texture and organoleptic properties of low-fat frankfurters. *Journal of Food Processing and Preservation*, 38, 1759–1771.

Phillips, G. O. & Williams, P. A. (2009) *Handbook of Hydrocolloids*.Second Edition,Cambridge, Woodhead publishing Ltd.

Piculell, L. & Lindman, B. (1992) Association and segregation in aqueous polymer/polymer, polymer/surfactant, and surfactant/surfactant mixtures: similarities and differences. *Advances in Colloid and Interface Science*, 41, 149-178.

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

Pietrasik, Z. (1999) Effect of content of protein, fat and modified starch on binding textural characteristics, and colour of comminuted scalded sausages. *Meat Science*, 51, 17–25.

Pietrasik, Z. & Duda, Z. (2000) Effect of fat content and soy protein/carrageenan mix on the quality characteristics of comminuted, scalded sausages. *Meat Science*, 56, 181–188.

Pike, O. (2003) Fat characterization. *Food Analysis*, 3, 237–240.

Prabhu, G. & Sebranek, J. (1997) Quality characteristics of ham formulated with modified corn starch and kappa-carrageenan. *Journal of Food Science*, 62, 198–202.

Pour-El, A. (1981) Protein functionality: classification, definition, and methodology. *Journal of Food and Agriculture Organization of the United Nations*, 5, 60-73.

Privalov, P. (1982) Proteins which do not present a single cooperative system. *Advances in Protein Chemistry,* 35, 1-104.

Prosky, L., Asp, N.-G., Schweizer, T., Devries, J. & Furda, I. (1987) Determination of insoluble, soluble, and total dietary fibre in foods and food products: inter-laboratory study. *Journal-Association of Official Analytical Chemists,* 71, 1017-1023.

Prosky, L. (1999) What is fibre? Current controversies. *Trends in Food Science & Technology,* 10, 271-275.

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

Quinn, J., Raymond, D. & Harwalkar, V. (1980) Differential scanning calorimetry of meat proteins as affected by processing treatment. *Journal of Food Science*, 45, 1146–1149.

Ramırez, J., Barrera, M., Morales, O. & Vázquez, M. (2002) Effect of xanthan and locust bean gums on the gelling properties of myofibrillar protein. *Food Hydrocolloids*, 16, 11–16.

Rankin, M. D. (1984) *Notes on Meat Products*, Leatherhead Food RA.

Rees, D. (1972) Polysaccharide gels. A molecular view. *Chem. Ind,* 19, 630-636.

Rees, D. A. (1968) Structure, conformation, and mechanism in the formation of polysaccharide gels and networks. *Advances in carbohydrate chemistry and biochemistry,* 24, 267-332.

Regini, J. W. & Elliott, G. (2001) The effect of temperature on the Donnan potentials in biological polyelectrolyte gels: Cornea and striated muscle. *International Journal of Biological Macromolecules*, 28, 245–254.

Richardson, P. & Norton, I. (1998) Gelation behavior of concentrated locust bean gum solutions. *Macromolecules*, 31, 1575–1583.

Richardson, P. H., Willmer, J. & Foster, T. J. (1998) Dilute solution properties of guar and locust bean gum in sucrose solutions. *Food Hydrocolloids,* 12**,** 339-348.

Richardson, P., Clark, A., Russell, A., Aymard, P. & Norton, I. (1999) Galactomannan gelation: A thermal and rheological investigation analyzed using the cascade model. *Macromolecules*, 32, 1519–1527.

Rinaudo, M. (1993) Gelation of polysaccharides. *Journal of Intelligent Material Systems and Structures,* 4, 210-215.

Rodgers, M. & Harrington, W. (1982) Evidence for self-association of heavy-meromyosin. *Biophysical Journal*,. Biophysical Society 9650 Rockville Pike, Bethesda, MD 20814-3998, A41-A41.

Rolee, A. & Lemeste, M. (1999) Effect of moisture content on thermomechanical behavior of concentrated wheat starch-water preparations. *Cereal Chemistry*, 76, 452–458.

Rosenthal, A. J. 2010. Texture profile analysis–how important are the parameters? *Journal of texture studies,* 41**,** 672-684.

Ross-Murphy, S. B., Morris, V. J. & Morris, E. R. (1983) Molecular viscoelasticity of xanthan polysaccharide. *Faraday Symposia of the Chemical Society,* Royal Society of Chemistry, 115-129.

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

Sair, L. & Fetzer, W. (1944) Water Sorption By Starches. *Industrial & Engineering Chemistry*, 36, 205-208.

Samejima, K., Ishioroshi, M. & Yasui, T. (1981) Relative roles of the head and tail portions of the molecule in heat-induced gelation of myosin. *Journal of Food Science*, 46, 1412–1418.

Samejima, K., Egelandsdal, B. & Fretheim, K. (1985) Heat gelation properties and protein extractability of beef myofibrils. *Journal of Food Science*, 50, 1540–1543.

Sanchez, C., Schmitt, C., Babak, V. & Hardy, J. (1997) Rheology of whey protein isolate-xanthan mixed solutions and gels. Effect of pH and xanthan concentration. *Food/Nahrung*, 41, 336-343.

Sanderson, G. (1990) Gellan gum. *Food Gels*. Springer.

Sanderson, G., Bell, V., Clark, R. & Ortega, D. (1988) The texture of gellan gum gels. *Journal of Food and Agriculture Organisation of the United Nation*, 22, 47-53.

Sano, T., Noguchi, S. F., Tsuchiya, T. & Matsumoto, J. J. (1988) Dynamic viscoelastic behavior of natural actomyosin and myosin during thermal gelation. *Journal of Food Science*, 53, 924–928.

Sano, T., Noguchi, S. F., Tsuchiya, T. & Matsumoto, J. J. (1988) Dynamic viscoelastic behavior of natural actomyosin and myosin during thermal gelation. *Journal of Food Science*, 53, 924–928.

Scopes, R. (1970) Characterisation and study of sarcoplasmic proteins. *Briskey, Physiol and Biochem of Muscle as a Food*, 5, 471-492.

Shand, P. J., Schmidt, G. R., Mandigo, R. W. & Claus, J. (1990) New technology for low-fat meat products. *Proceedings-Annual Reciprocal Meat Conference of the American Meat Science Association (USA)*, 1990.

Shand, P., Sofos, J. & Schmidt, G. (1994) Differential scanning calorimetry of beef/kappa-carrageenan mixtures. *Journal of Food Science*, 59, 711–715.

Shiga, K., Kami, T. & Fujii, M. (1988) Relation between gelation behavior of ground chicken muscle and soybean proteins and their differential scanning calorimetric studies. *Journal of Food Science*, 53, 1076–1080.

Shiga, K. & Kushida, Y. (1983) Physical properties of meat products containing soybean proteins. *Journal of Japanese Society of Food Science and Technology (Japan)*, 7, 30-50.

Shotton, D. M. (1989) Confocal scanning optical microscopy and its applications for biological specimens. *Journal of Cell Science*, 94, 175-206.

Siripurapu, S., Mittal, G. & Blaisdell, J. (1987) Textural and viscoelastic characteristics of meat emulsion products during cooking. *Lebensmittel-Wissenschaft+ Technologie*, 20, 68–73.

Skrede, G. (1989) Comparison of various types of starch when used in meat sausages. *Meat Science*, 25**,** 21–36.

Stainsby, G. (1980) Proteinaceous gelling systems and their complexes with polysaccharides. *Food Chemistry*, 6, 3–14.

Slavin, J., Jacobs, D. & Marquart, L. (1997) Whole-grain consumption and chronic disease: protective mechanisms. *Nutrition and Cancer*, 27, 14-21.

Smith, D. (1988) Meat proteins-functional-properties in comminuted meat products. *Food Technology,* 42, 116–121.

Smyth, A. B., Smith, D. M., Vega-Warner, V. & O'neill, E. (1996) Thermal denaturation and aggregation of chicken breast muscle myosin and subfragments. *Journal of Agricultural and Food Chemistry*, 44, 1005–1010.

Stabursvik, E. & Martens, H. (1980) Thermal denaturation of proteins in post rigor muscle tissue as studied by differential scanning calorimetry. *Journal of the Science of Food and Agriculture*, 31, 1034–1042.

Stading, M. & Hermansson, A.-M. (1993) Rheological behaviour of mixed gels of κ-carrageenan-locust bean gum. *Carbohydrate Polymers*, 22, 49–56.

Stanley, N. F. (1990) Carrageenans. *Food Gels*. Netherlands, Springer, pp.79-119.

Stevens, D. & Elton, G. (1971) Thermal properties of the starch/water system part I. Measurement of heat of gelatinisation by differential scanning calorimetry. *Starch-Stärke,* 23, 8-11.

Syrbe, A., Bauer, W. J., & Klostermeyer, N. (1998). Polymer science concepts in dairy systems - An overview of milk protein and food hydrocolloid interaction. *International Dairy Journal*, 8, 179-193.

Szczesniak, A. S. (1963). Classification of textural characteristics. *J. Food Sci,* 28**,** 385-389.

Szczesniak, A. (1996). Texture profile analysis-Methodology interpretation clarified (Reprinted from Journal of Food Science, vol 60 pg vii, 1995). *Journal of Texture Studies,* 27**,** R6-R7.

Talukder, S. (2015) Effect of dietary fiber on properties and acceptance of meat products: A review. *Critical Reviews in Food Science and Nutrition*, 55, 1005–1011.

Tarté, R. (2009) Ingredients in meat products. *Ingredients in Meat Products: Properties, Functionality and Applications*. New York, Springer-Verlag. ISBN 978-0-387-71326-7. New York, Springer-Verlag.

Thebaudin, J., Lefebvre, A., Harrington, M. & Bourgeois, C. (1997) Dietary fibres: Nutritional and technological interest. *Trends in Food Science & Technology*, 8, 41–48.

Therkelsen, G. H. (1993) *Carrageenan. Industrial Gums, Polysaccharides and their Derivatives*. New York, Academic Press, 145–180.

Timasheff, S. N. (1998) Control of protein stability and reactions by weakly interacting cosolvents: The simplicity of the complicated. *Adv. Protein Chemistry*, 51, 355–432, Cambridge Academic Press.

Tolstoguzov, V. (1991) Functional properties of food proteins and role of protein-polysaccharide interaction. *Food Hydrocolloids*, 4, 429-468.

Tolstoguzov, V. (1998) Functional properties of protein-polysaccharide. *Functional Properties of Food Macromolecules*, 252.

Tornberg, E. (2005) Effects of heat on meat proteins: Implications on structure and quality of meat products. *Meat Science*, 70, 493–508.

Towle, G. A. (1973) Carrageenan. *Industrial Gums: Polysaccharides and Their Derivatives (Whistler RL, ed.). New York, Academic Press*, 84-109.

Trius, A., Sebranek, J. & Lanier, T. (1996) Carrageenans and their use in meat products. *Critical Reviews in Food Science & Nutrition*, 36, 69–85.

Trius, A., Sebranek, J., Rust, R. & Carr, J. (1994a) Low-fat bologna and beaker sausage: Effects of carrageenans and chloride salts. *Journal of Food Science*, 59, 941–945.

Trius, A., Sebranek, J., Rust, R. & Carr, J. (1994b) Carrageenans in beaker sausage as affected by pH and sodium tripolyphosphate. *Journal of Food Science*, 59, 946–951.

Trius, A., Sebranek, J., Rust, R. & Carr, J. (1995) Ionic strength and chloride salt effects on the performance of carrageenans in a model system sausage. *Journal of Muscle Foods*, 6, 227–242.

Trius, A., Sebranek, J. & Lanier, T. (1985) Carageenan in processed meat products *The Copenhagen Pectin Factory*,45, 21–24.

Tromp, R. H., Van De Velde, F., Van Riel, J. & Paques, M. (2001) Confocal scanning light microscopy (CSLM) on mixtures of gelatine and polysaccharides. *Food Research International*, 34, 931–938.

Trout, G. & Schmidt, G. (1983) Utilisation of phosphates in meat products. *Meat Processing*, 9, 48-50.

Trudso, J. (1985) Increasing yields with carrageenan. *Meat Processing*, 24, 37.

Tye, R. J. (1988) The hydration of carageenans in mixed electrolytes. *Food Hydrocolloids*, 2, 69–82.

Ueda, K., Itoh, M., Matsuzaki, Y., Ochiai, H. & Imamura, A. (1998) Observation of the molecular weight change during the helix-coil transition of κ-carrageenan measured by the SEC-LALLS method. *Macromolecules*, 31, 675–680.

Ulu, H. (2006) Effects of carrageenam and guar gum on the cooking and textural properties of low-fat meatballs. *Food Chemistry*, 95, 600–605.

Van De Velde, F., Van Riel, J. & Tromp, R. H. (2002) Visualisation of starch granule morphologies using confocal scanning laser microscopy (CSLM). *Journal of the Science of Food and Agriculture*, 82, 1528–1536.

Van De Velde, F., Weinbreck, F., Edelman, M. W., Van Der Linden, E. & Tromp, R. H. (2003) Visualisation of biopolymer mixtures using confocal scanning laser microscopy (CSLM) and covalent labelling techniques. *Colloids and Surfaces B: Biointerfaces*, 31, 159–168.

Viebke, C., Borgström, J., Carlsson, I., Piculell, L. & Williams, P. 1998. A differential scanning calorimetry study of κ-carrageenan in the NaCl/NaI/CsI/CsCl systems and analysis by Poisson-Boltzmann calculations. *Macromolecules*, 31, 1833-1841.

Vega-Warner, V., Merkel, R. & Smith, D. (1999.)Composition, solubility and gel properties of salt soluble proteins from two bovine muscle types. *Meat Science*, 51, 197–203.

Velinov, P., Cassens, R., Greaser, M. & Fritz, J. (1990) Confocal scanning optical microscopy of meat products. *Journal of Food Science*, 55, 1751–1752.

Verbeken, D., Neirinck, N., Van Der Meeren, P. & Dewettinck, K. (2005) Influence of κ-carrageenan on the thermal gelation of salt-soluble meat proteins. *Meat Science*, 70, 161–166.

Verma, A. K. & Banerjee, R. (2010) Dietary fibre as functional ingredient in meat products: A novel approach for healthy living: A review. *Journal of Food Science and Technology*, 47, 247–257.

Verrez-Bagnis, V., Bouchet, B. & Gallant, D. (1993) Relationship between the starch granule structure and the textural properties of heat-induced surimi gels. *Food Structure*, 12, 309–320.

*Essentials of Food Science, (*1998) Directed by vickie a. Vaclavik & Christian, e. W. United States.

Viuda-Martos, M., Fernández-López, J., Sayas-Barbera, E., Sendra, E., Navarro, C. & Pérez-Álvarez, J. (2009) Citrus co-products as technological strategy to reduce residual nitrite content in meat products. *Journal of Food Science*, 74, R93–R100.

Visessanguan, W., Ogawa, M., Nakai, S. & An, H. (2000) Physicochemical changes and mechanism of heat-induced gelation of arrowtooth flounder myosin. *Journal of Agricultural and Food Chemistry*, 48, 1016–1023.

Vodovotz, Y., Vittadini, E., Coupland, J., Mcclements, D. & Chinachoti, P. (1996) Bridging the gap: Use of confocal microscopy in food research. *Food Technology (USA)*, 50, 6.

Vodovotz, Y., Vittadini, E., Coupland, J., Mcclements, D. & Chinachoti, P. (1996) Bridging the gap: Use of confocal microscopy in food research. *Journal of Food and Agriculture Organisation Of the United Nation*, 4, 51-62.

Wagner, J. & Anon, M. (1985a) Denaturation kinetics of myofibrillar proteins in bovine muscle. *Journal of Food Science*, 50, 1547–1550.

Wagner, J. & Anon, M. (1985b) Effect of freezing rate on the denaturation of myofibrillar proteins. *International Journal of Food Science & Technology*, 20, 735–744.

Waller, G. & Lowey, S. (1983) Are myosin subunits in dynamic equilibrium? *Biophysical Journal*,. Biophysical Society 9650 Rockville Pike, Bethesda, MD 20814-3998, A299-A299.

Watase, M., Nishinari, K., Williams, P. & Phillips, G. (1990) Effect of ammonium salts on rheological and thermal properties of kappa-carrageenan gels. *Food Hydrocolloids*, 4, 227–237.

Wang, S., Smith, D. & Steffe, J. (1990) Effect of pH on the dynamic rheological properties of chicken breast salt-soluble proteins during heat-induced gelation. *Poultry Science*, 69**,** 2220–2227.

Wang, S. & Smith, D. (1994) Poultry muscle proteins and heat-induced gelation. *Poultry Science Reviews (United Kingdom)*, 5, 3.

Welsh, S., Shaw, A. & Davis, C. (1994) Achieving dietary recommendations: Whole-grain foods in the food guide pyramid. *Critical Reviews in Food Science & Nutrition*, 34, 441-451.

Whiting, R. (1988) Ingredients and processing factors that control muscle protein functionality. *Food Technology*, 42, 104–114.

Who, J. & Consultation, F. E. (2003) Diet, nutrition and the prevention of chronic diseases. *World Health Organisation Technical Report Series*, 916.

Wilding, P., Lillford, P. J. & Regenstein, J. M. (1984) Functional properties of proteins in foods. *Journal of Chemical Technology and Biotechnology. Biotechnology*, 34, 182-189.

Wilson, T. 1990. Confocal microscopy. *Academic Press: London*, 426, 1-64.

Wright, D. J., Leach, I. B. & Wilding, P. (1977) Differential scanning calorimetric studies of muscle and its constituent proteins. *Journal of the Science of Food and Agriculture*, 28, 557–564.

Wright, D. J. & Wilding, P. (1984) Differential scanning calorimetric study of muscle and its proteins: Myosin and its subfragments. *Journal of the Science of Food and Agriculture*, 35**,** 357–372.

Wogan, G. (1976) Undesirable or potentially undesirable constituents of food. *Principles of Food Science*. 73, 515-537.

Wu, M., Lanier, T. & Hamann, D. (1985) Thermal transitions of admixed starch/fish protein systems during heating. *Journal of Food Science*, 50**,** 20–25.

Wu, M., Xiong, Y. L. & Chen, J. (2011) Rheology and microstructure of myofibrillar protein – plant lipid composite gels: Effect of emulsion droplet size and membrane type. *Journal of Food Engineering*, 106**,** 318–324.

Wu, Y. V. & Inglett, G. E. (1974) Denaturation of plant proteins related to functionality and food applications. A review. *Journal of Food Science*, 39, 218-225.

Yang, H., Yan, R., Chen, H., Lee, D. H. & Zheng, C. (2007) Characteristics of hemicellulose, cellulose and lignin pyrolysis. *Fuel*, 86, 1781–1788.

Yang, A., Trout, G. & Shay, B. (1995) Evaluation of carrageenan, isolated soy protein and a modified starch in low-fat frankfurters. *Annual International Congress of Meat Science and Technology, 4*, 435–436.

Xiong, Y. (1997) Structure-function relationships of muscle proteins. *Food Science and Technology*, New York-Marcel Dekker, 21, 341–392.

Xiong, Y., Brekke, C. & Leung, H. (1987) Thermal denaturation of muscle proteins from different species and muscle types as studied by differential scanning calorimetry. *Canadian Institute of Food Science and Technology Journal*, 20, 357–362.

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

Yang, A., Trout, G. & Shay, B. (1995) Evaluation of carrageenan, isolated soy protein and a modified starch in low-fat frankfurters. Annual International Congress of Meat Science and Technology, 435-436.

Zaidul, I., Absar, N., Kim, S.-J., Suzuki, T., Karim, A., Yamauchi, H. & Noda, T. (2008) DSC study of mixtures of wheat flour and potato, sweet potato, cassava, and yam starches. *Journal of Food Engineering*, 86, 68–73.

Zayas, J. F. (1997) *Functionality of Protein in Food*. Berlin, Springer, 30, 1-5.

Zhang, K., Karlstroem, G. & Lindman, B. (1994) Ternary aqueous mixtures of a non-ionic polymer with a surfactant or a second polymer. A theoretical and experimental investigation of the phase behaviour. *The Journal of Physical Chemistry*, 98, 4411-4421

Zhang, J. & Wang, Z.-W. (2012) Thermal analysis and physiological behavior of cellulose/pectin complex from Canna edulis Ker by-product. *Carbohydrate Polymers*, 87, 1153–1158.

Ziegler, G. & Acton, J. (1984) Mechanisms of gel formation by proteins of muscle tissue. *Journal of Food and Agricalture Organisation Of the United Nation*, 95, 71-86.

Ziegler, G. R. & Foegeding, E. A. (1990) The gelation of proteins. *Advances in Food and Nutrition Research*, 34, 203–298.