FUNCTIONAL PROPERTIES AND STORAGE STABILITY OF ALKALI SOLUBLE CHICKEN MEAT PROTEIN AND MODIFIED WAXY CORN STARCH BLEND SYSTEM

by

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My beloved parents, Mr. Zhang Shiyan, Mrs. Bai Zilan, my husband Cheng Cheng and my son Cheng Qingxiao.

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LIST OF ABBREVIATIONS

Abbreviation	Caption
σ	Shear stress
γ	Shear rate
η	Coefficient viscosity
σ_0	Yield stress
γ_0	The maximum shear stress or deformation
$\Delta_{gresidue}$	the hydrophobicity of each amino acid side chain
AECP	Alkali-extracted chicken protein
CBMP	Chicken breast meat powder
CCD	A central composite design
CE	Coefficients
CFU	Colony forming unit
CMB	Chicken breast meat powder and modified waxy corn starch blends
CMBC	CMB with the condiments
CMC	Carboxyl methyl cellulose
DSC	Differential Scanning Calorimetry
E.coli	*Escherichia coli*
G'	Storage modulus
G''	Loss modulus
K	Consistency index
K_H	The consistency index
MWCS	Modified waxy corn starch
n	The flow behavior index
n	the total number of residues in the protein
n^-	the total number of negatively charge residues
n^+	the total number of positively charge residues
NG	No growth
n_H	The flow behavior index

PCA	Plate Count Agar
PDA	Potato Dextrose Agar
pI	isoelectric point
Rf	The rate of the distance migrated by protein to the distance migrated by dye
RSM	response surface methodology
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
SEM	Scaning electron microscopy
SFU	Spore forming unit
Tan δ	Loss tangent
T_c	conclusion temperature
T_m	melting temperature
T_o	Onset temperature
TPC	Total plate counts
WHC	Water holding capacity
XRD	X-ray diffractometry
δ	Phase angle

FUNCTIONAL PROPERTIES AND STORAGE STABILITY OF ALKALI SOLUBLE CHICKEN MEAT PROTEIN AND MODIFIED WAXY CORN STARCH BLEND SYSTEM

ABSTRACT

The functional properties of alkali soluble chicken meat protein/starch blend were studied in three phases. In the first phase chicken breast meat powder was solubilized with alkali in a 2 × 2 factorial experiment. The results from microscopic appearance, microscopy, SEM, consistency, solubility, X-ray diffractometry, SDS-Page showed changes in microstructural, textural and molecular weight of protein profile which were significantly affected ($P \leq 0.05$) by heating temperature and 1% alkali, thus indicating involvement of molecular changes affecting the physical characteristics. In the second phase of the project, the study focused on characterizing the physicochemical characteristics of chicken meat protein and crosslinked acid modified waxy corn starch blends (CMB). Prior to this, consistency, rheological and freeze thaw stability of starches, potato, tapioca and crosslinked acid modified waxy corn starch (MWCS) were compared. As a result, MWCS was selected to be used in the protein/starch blends for its higher gel strength and better freeze thaw stability. The subsequent study was carried out involving a 4 × 3 factorial experiment which found the strongest synergism to be between 3% chicken breast meat powder with 0.75% alkali at 80°C for 15 minutes and 4% MWCS at pH 6, changing the physicochemical properties of chicken breast meat powder substantially, with enhanced homogeneous, smooth texture and true gel like behaviour as indicated by G' > G'' and better freeze thaw stability. A central

composite design using 2 × 3 factorial plan which were conducted on the influence of alkali treatment (temperature, time, alkali volume) on the flow behaviour of the blend confirmed that the functional properties of CMB were contributed by protein molecular weight and solubility. A study on the effect of preservation treatment on the storage life of the blends was also conducted in the third stage. The effect of three treatments: cycle heating, single heating, and use of preservative (sorbic acid) over a storage period of 6 months showed that cycle heating gave a very stable product with no growth of microorganisms, stable colour, water holding capacity and rheological behaviour. Thus, these results suggest the possibility of development of new fabricated or restructured products based on such CMB system.

CIRI-CIRI BERFUNGSI DAN KESTABILAN PENSTORAN SISTEM CAMPURAN PROTEIN DAGING AYAM TERLARUT ALKALI DAN KANJI JAGUNG BERLILIN TERUBAH SUAI

ABSTRAK

Ciri-ciri berfungsi campuran protein/ kanji telah dikaji dalam 3 fasa. Dalam fasa pertama, serbuk daging dada ayam telah dilarutkan dengan alkali dan dikaji. Keputusan kajian kemudian dianalisis dengan eksperimen faktorial 2x2. Keputusan daripada mikrostruktur, mikroskopi, SEM, konsistensi, keterlarutan, X-ray difraktometri, SDS Page menunjukkan terdapat perubahan berlaku dalam mikrostruktur, tekstur dan profil berat molekul protein yang telah dipengaruhi secara signifikan ($P \leq 0.05$) oleh suhu pemanasan dan 1% alkali, maka menunjukkan perubahan molekular telah berlaku dan memberi kesan terhadap ciri-ciri fizikal. Dalam fasa kedua projek ini, kajian telah difokuskan pada pencirian ciri-ciri fizikokimia campuran protein daging dada ayam dan kanji jagung 'waxy' terubahsuai (CMB). Sebelum pencirian ini dilakukan, konsistensi, reologikal dan kestabilan 'freeze thaw' kanji-kanji ubi kentang, ubi kayu dan jagung 'waxy' terubahsuai (MWCS) telah dibandingkan. Akhirnya, MWCS telah dipilih untuk digunakan dalam campuran protein/ kanji disebabkan keteguhan gel yang lebih kuat dan kestabilan 'freeze thaw' yang lebih baik. Kajian seterusnya telah dijalankan dengan menggunakan eksperimen faktorial 4 x 3 dan didapati terdapat hubungan sinergistik yang paling kuat pada 3% serbuk daging dada ayam yang mengandungi 0.75% alkali pada 80°C selama 15

minutes dan 4% MWCS pada pH 6.0 yang telah mendatangkan perubahan yang besar terhadap ciri-ciri fizikokimia serbuk daging dada ayam, dengan peningkatkan ciri-ciri kehomogenan, kelicinan tekstur dan kecerahan gel seperti yang ditunjukkan oleh G' > G'' dan juga kestabilan 'freeze thaw' yang lebih baik. Rekabentuk 'central composite' dengan pelan faktorial 2 x 3 yang dilakukan terhadap kesan perawatan alkali (suhu, masa, isipadu alkali) terhadap sifat aliran campuran mengesahkan bahawa ciri-ciri berfungsi CMB disumbangkan oleh saiz protein dan keterlarutan. Suatu kajian terhadap kesan pengawetan perawatan terhadap jangka hayat campuran juga dilakukan pada tahap ketiga. Kesan ketiga-tiga perawatan: pemanasan berulang, pemanasan tunggal dan penggunaan pengawet (asid sorbik) dalam jangka hayat 6 bulan telah menunjukkan pemanasan berulang menghasilkan produk yang sangat stabil dengan tiada pertumbuhan mikroorganisma dan juga stabil terhadap warna, keupayaan memegang air dan ciri-ciri reologikal. Maka, keputusan ini mengesahkan kemungkinan perkembangan produk rekaan baru atas dasar sistem CMB ini.

1 INTRODUCTION

1.1 Background and rationale

Chicken meat is a rich source of nutritionally valuable proteins. In comparison to many plant foods, chicken meat proteins contain all the essential amino acids in the balance proportion required by our human body for optimum growth of lean, calorie burning tissue (Noss & Rady, 1999). The trend of chicken breeding and production is increasing nowadays, but processed chicken food contributes to less than 1% of the total output in developing countries and this figure is far lower than the average of 20% reported in developed countries (Zhou *et al.,* 2005). For chicken-based food, it is commonly prepared in the form of cooked, cooked–chilled, cooked-frozen and fried meat. Therefore, developments of extensively processed chicken products such as ready to serve paste are desirable.

In addition to high nutritional value, functional properties of proteins have made it widely used in food applications as a water retention agent, stabilizer, gelling agent, adhesive, and so on (Yasui *et al.*, 1980; Jelen *et al*., 1982; Colmenero, 2002; Comfort & Howell, 2002). Nevertheless, these behaviors in turn varied with type of proteins, its molecular weight, ionic strength, pH of the environment, and the presence of coexisting substances (Hattori *et al*., 1995).

In recent years, studies on the interactions between proteins and polysaccharides have received much attention (Muhrbeck & Eliasson, 1991;

Aguilera & Rojas, 1996; Okechukwu & Rao, 1997; Bongkosh *et al*., 2001; Shim & Mulvaney, 2001) because the synergistic interaction between both biopolymers was found to improve their gelation, emulsification and water holding capacity properties. The use of protein-starch mixture in food product increased the quality of the food products in terms of flow, stability, texture and mouth-feel characteristics (Ravindra *et al*., 2004). The general interaction of protein and polysaccharides has been described in detailed by many authors. Hermansson (1979a) described a specific interaction between starch and casein, resulting in the formation of a complex with altered rheological properties. An electrostatic interaction between α-casein and acidified potato starch have been reviewed by Takeushi (1969). Sperling (1977) claimed that in a mixed system based on two gel-forming synthetic polymers, the one that polymerized first will form the continuous network. It is reported that the entanglement between protein and starch molecules is responsible for changes in the viscosity rather than specific protein effects on the swelling of the starch granules (Ling, 1984).

In general, the gelation behavior of protein-polysaccharide mixtures generally falls into three patterns: formation of covalent bonds between two polymers; polyanion-polycation electrostatic interactions; and formation of composite gel due to mutual exclusion of each component (Morris, 1990). It is anticipated that alkali-treated chicken proteins interact differently with MWSC under different conditions. Both may experience co-precipitation when pH of the solution is adjusted to the isoelectric point of the chicken protein. However, when

the pH is far from the isoelectric point, a homogeneous and stable mixed system could be produced, due to the formation of a continuous network. Nevertheless, of extremely with alkali or acidic condition, both proteins and starch molecules would experience hydrolysis and may result in short fragments of molecular chain that may fail to interact and form a gelled network. However, the interaction between chicken meat protein and starch has not been discussed elsewhere, thus the present study is expected to provide an insight towards interaction between these two biopolymers.

Owing to the inherent structural limitation, chicken meat proteins lack the required physicochemical properties to meet the needs of various food and industrial uses. For example, chicken powder prepared by mechanical grinding when directly processed into chicken paste will result in a rough, heterogeneous and grainy texture due to the presence of fragmented muscle bundles. Therefore, chicken meat powder needs to be treated before being processed into final products. Modification of structural and conformational properties, to obtain optimum size, surface charge, hydrophobic/hydrophilic ratio, and molecular flexibility are useful approaches for improving desirable functional properties (Damodaran, 1996).

Chemical modification such as alkali treatment (Rhee, 1989) has been shown to improve functional properties of proteins. Alkali-extracted chicken protein (AECP) followed by acid precipitation have been widely studied in poultry meat processing (Jelen *et al*., 1982; Kijowski *et al*., 1985) and other animal meat

processing (Shahidi & Synowiecki, 1996). Studies showed that when AECP from bone residue is used as replacement in all-chicken luncheon meats and sausage, very small differences in texture and flavor as compared to ordinary luncheon meats and sausage was observed. The nutritional properties (Protein Efficiency Ratios, Body Weight Gain, Net Protein Utilization, and True Digestion) of alkali-extracted, acid precipitated chicken protein from mechanically separated poultry residues showed little difference in comparison to mechanically separated chicken paste (Ozimek *et al*., 1986).

Most studies have concentrated on the non-meat portions of chicken as valuable ingredients in various comminuted meat products and much less on chicken meat itself. In fact, alkalizing agents play an important role in the development of desirable product quality characteristics such as color and texture in the preparation of many traditional starch-based and protein-based food products (e.g., Chinese wheat noodles, waxy rice dumplings, tofu and preserved egg). Alkali treated soy protein has improved adhesive and hydrophobic properties (Hettiarachchy & Kalapathy, 1998). Thus, chicken meat powder was treated with alkali and mixed it with starch in order to improve meat protein functionality. In this study, modified waxy corn starch (MWCS) was used in the protein/starch blends, as it provides a greater stability to heat, pH, shear and freeze-thaw cycles compared to native starches (Langan, 1986). However, before such a system can be developed and commercialized, it is important to know the behavior of the mixtures at different factors, such as alkali

concentration, amount of starch, processing conditions(e.g. heating temperature and time), presence of other ingredients (e.g. salt and sugar), pH of the medium and storage conditions.

The objective of the present work was to study the effects of molecular and physicochemical properties of chicken meat proteins after alkali treatment on its morphology, rheological behavior and water holding capacity. This would help to better understand alkali treated chicken meat protein functional properties and to predict potential applications of alkali treated chicken meat proteins in food system.

1.2 Project objectives

1.2.1 General objective

The general objective of the project was to develop and characterize chicken breast meat protein and modified waxy corn starch blends (CMB) in order to better understand the functional properties and to predict the potential application of chicken meat proteins in food systems.

1.2.2 Specific objective

Four specific objectives of this study were listed as follows:

(a) To investigate the influence of alkali conditions for chicken breast meat powder pretreatment on the physicochemical properties of chicken proteins.

(b) To conduct a systematic and thorough evaluation and characterizations of physicochemical properties of the protein/starch blends system (CMB).

(c) To identify the optimal processing parameters (alkali concentration, heating temperature, heating time, pH and so on) for CMB preparation.

(d) To observe the effects of different treatments on physicochemical properties of CMB during 6-month storage.

1.3 Project Protocol

This project was mainly divided into two sections: literature review and experimental work. In the first section, a systematic review of the literature on the various aspects of protein and starch such as physical, chemical and functional properties was carried out. The subsequent review was conducted involving a thorough review of the measurement methods of chicken protein and modified waxy corn starch blend gel. Based on these reviews, a clear picture of past achievements and current status in this research field could be obtained, assisting in choosing appropriate research methodologies.

The experimental work of the Project included three main parts: the preparation and characterization of chicken breast meat powder, functional properties of chicken protein/starch blend (CMB) and storage stability of CMB.

In the first phase, the characteristics of alkaline soluble proteins from

chicken meat powder were investigated under various conditions. The samples were analyzed in terms of solubility, texture, structure and molecular weight changes. The preliminary study, as described in Chapter 4.1, was aimed at identifying the relationship of the molecular and solubility properties of chicken meat proteins after alkali treatment on its texture, structural, and morphology properties. A back extrusion test, light microscopy, scanning electron microscopy, X-ray diffractometry and SDS-Pages were used in this study.

In the second phase of the Project, the study focused on characterizing the physicochemical characteristics of chicken meat protein and modified waxy corn starch blend (CMB). Prior to this, consistency, rheological and freeze thaw stability properties of starches, potato, tapioca and modified waxy corn starch were compared. As a result, modified waxy corn starch was selected to be used in CMB preparation due to its consistency and freeze thaw stability, as described in Chapter 4.2. The subsequent practical aspects of the Project commenced with several studies on the functional properties of CMB at different conditions, in terms of morphology observation, consistency, freeze thaw stability and rheological measurement. Results of the study are presented in 4.3.1.

Following that, CMB rheological behavior was studied under different conditions. A central composite design using 2×3 factorial plan was carried out to investigate the influence of alkali treatment conditions, pH and additive on flow behavior of CMB, which was described in Chapter 4.3.2. Chapter 4.3.3

discusses the rheological properties of CMB on heating and cooling upon pretreatment at various conditions, adjustment of pH and addition of additives.

In Chapter 4.3.4, influence of pH on rheological behavior, microstructural properties and freeze thaw stability of CMB was elucidated in greater detailed.

The final part of the Project focused on the storage stability of CMB. The effects of different treatments (single heating, cycle heating and sorbic acid) on the survival of microorganisms in CMB were investigated. The study was concentrated on microbial observation and physicochemical properties measurement. The results were presented in Chapter 4.4.

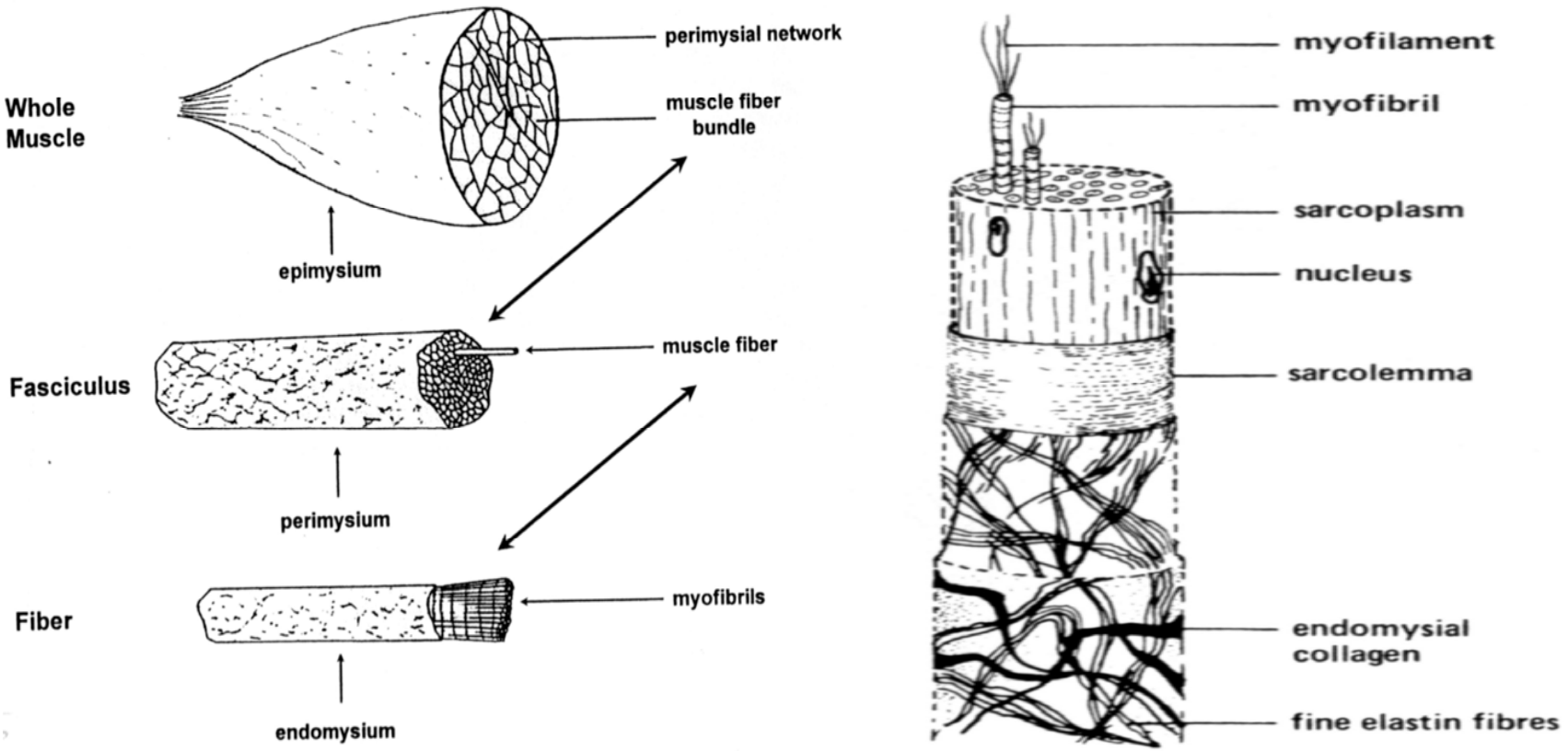
2 LITERATURE REVIEW

2.1 Muscle and proteins in general

Numerous studies have contributed to our understanding of the relationship between muscle proteins and meat quality; however, much of this knowledge comes from research on beef and pork. While the basic principles of growth, development, and structure between mammalian and poultry muscle are similar, there are several significant physical and biochemical differences among these species. Poultry meat is different from beef or pork in color, chemical composition, and functional characteristics (Nakai & Modler, 2000a). Thus, the properties of poultry muscle proteins and the characteristics of those proteins responsible for their function as food are reviewed in this section.

2.1.1 Composition and structure of poultry muscle tissue

The gross structure of poultry muscle tissue resembles that of most other species (Figure 2.1(A)) (Purslow, 1987). In skeletal muscle, the whole muscle is surrounded by a heavy layer of connective tissue called the epimysium. Within the epimysium are bundles of muscle fibers or cells (fascicule), each of which is encased by another layer of connective tissue sheet, the perimysium that is an extension of the inner surface of the epimysium. Individual muscle fibers within a fasciculus are enclosed in a sheath of small collagen fibers called the endomysium (Young & Northcutt, 2000). The essential unit of muscle is the



(A) (B)

Figure 2.1(A) Gross structure of the poultry muscle (Purslow, 1987); (B) Illustration of a muscle fiber (Hermansson, 1986).

fiber. Muscle fibers are multi-nucleated long cells which may attain a length of over 30 cm, although they can have a diameter of only 100 μm. Each individual fibre is composed of a number of myofibrils. Surrounding each fibre is a membrane, the sarcolemma, and a sheath of endomysial collagen network (Hermansson, 1986a). A sketch of a muscle fibre is shown in Figure 2.1(B). Within the fiber are the myofibrils, the smallest functional units of the muscle. Together, these myofibrils, fibers, and connective tissue membranes serve to secure the muscle to the skeleton and to transmit the force of the muscle contractile elements to the tendons and bones.

In most of the literatures (Acton *et al.* 1983; 1985; Greaser, 1986), muscle proteins are classified according to their physiological functions and solubility into three groups: approximately 30% water soluble protein, 30% salt-soluble protein, 30% insoluble proteins (connective tissue), it is readily apparent that the soluble muscle proteins are largely responsible for the distinctive characteristics of a wiener . Breast meat has a higher percentage of protein, a lower percentage of fat, and more unsaturated fat than thigh meat.

Proteins are macromolecules with different levels of structural organization, including four types: the primary structure, secondary structure, tertiary and quaternary structures (Figure 2.2) (http://www.proteomesoftware.com. 2007a). The primary structure of proteins relates to the peptide bonds between component amino acids and also to the amino acid sequence in the molecule. The secondary structures of proteins involve folding the primary structure.

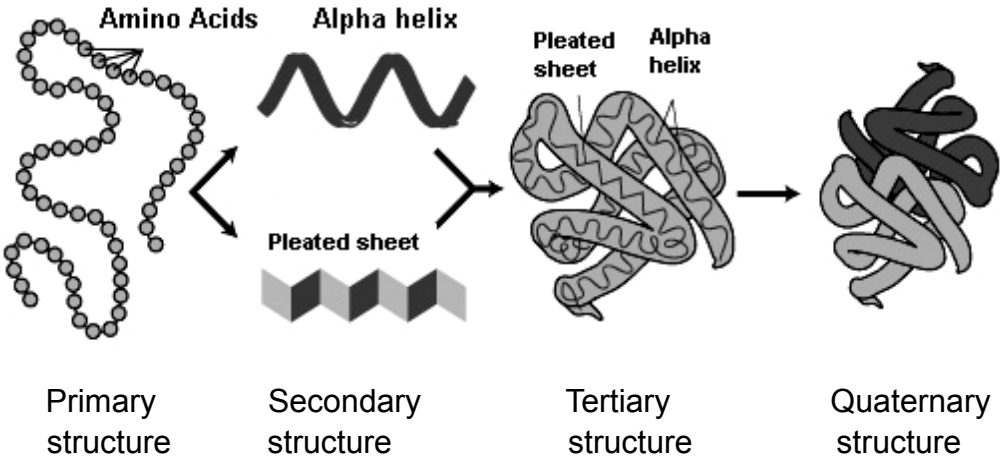


Figure 2.2 The different type of structure of proteins in solution. Available from http://www.proteomesoftware.com. (2007a).

Hydrogen bonds between amide nitrogen and carbonyl oxygen are the major stabilizing force. These bonds may be formed between different areas of the same polypeptide chain or between adjacent chains. In aqueous media, the hydrogen bonds may be less significant, and van der Waals forces and hydrophobic interaction between apolar side chains may contribute to the stability of the secondary structure. The secondary structure may be either the α-helix or the β-pleated sheet or random coil. The helical structures are stabilized by intramolecular hydrogen bonds, the sheet structures by intermolecular hydrogen bonds. Tertiary and quaternary structures of proteins are the denomination of the three-dimensional structure and the association of protein entities in solution, respectively. The stabilization of these structures of a given protein system is dependent mostly on non-covalent forces, such as

hydrogen bonding, van der Waal's forces, electrostatic and hydrophobic interactions, and also on interactions involving binding of prosthetic groups/ligands. The relative contributions of these various interactions to stability depend on the type of proteins and their functions in biological systems (Damodaran & Paraf, 1997). A schematic overview of the different types of interactions in proteins is given in Figure 2.3 (http://www.kvhs.nbed.nb.ca. 2007b). The structural form of proteins in meat is the fibrous form. Actin, myosin and collagen in meat are typical fibrous proteins. These proteins are built up from three main structures, namely the α-helix, the antiparallel β-pleated sheet and the triple helix (the last one only collagen). Myosin is α-helical, whereas collagen uses the triple helix. To stabilize these structures hydrogen bonding is important (Dickerson & Geis, 1969).

For the fibrous proteins, the large amount of hydrogen bonds and electrostatic interactions that keep the stretched molecules in register in the large building blocks, where the fibrous proteins take part, are broken on heating. This results in the molecules having a greater freedom to form any random configuration, as driven by entropy. Since the proteins are relatively stretched in the fibrous form, fibrous proteins contract on heating.

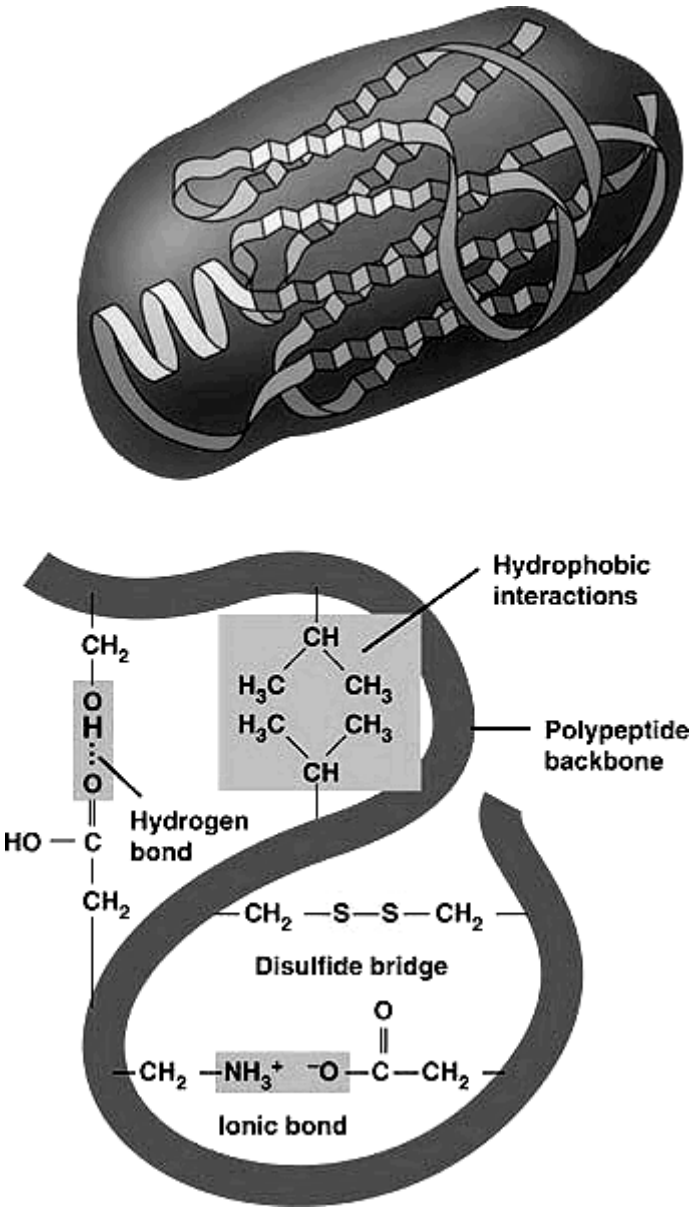


Figure 2.3 Schematic presentation of the stabilizing interactions (hydrophobic interactions, hydrogen bond, polypeptide backbone, disulfide bridge and ionic bond) in proteins. Available from http://www.kvhs.nbed.nb.ca (2007b).

2.1.2 Physical and chemical properties

Total protein content in striated muscle varies between 18-20%. It can be divided into three groups: sarcoplasmic proteins, soluble in low ionic strength solutions; myofibrillar or structural proteins, soluble in high ionic

strength solutions and connective tissue proteins, insoluble in salt solutions. Myofibrillar proteins are mainly actin, myosin and actomyosin, their isoelectric point is between 4.5 and 5.5. Sarcoplasmic proteins: myoglobin, myogenin, hemoglobin and albumin among others, are mostly related to metabolic activity, with isoelectric point between 6 and 7 (Ranken, 1985; Totosaua *et al.,* 1997). Muscle proteins participate in three kinds of interactions: protein-water, protein-lipid and protein-protein, these basic interactions are characterized by water-binding, fat-binding and gelification properties, respectively (Whiting, 1988; Totosaus *et al.,* 1998).

Protein solubility

Solubility of proteins is of a primary importance for the manufacture of processed muscle foods, including comminuted, restructured, and formed meats. This is because most functional properties of muscle proteins are related to protein solubility. Solubility can be defined as total percentage of protein remaining in solution under moderated centrifugal forces (Morrissey *et al.,* 1987; Totosaua *et al.,* 1998). In this way solubility represents saturation at equilibrium between a solute (protein) and a solvent (water or salt solution). Several functional properties, such as thickening, foaming, emulsification, and gelation of proteins are affected by protein solubility. Solubility of a protein is fundamentally related to its hydrophilicity / hydrophobicity balance (Damodaran, 1997). Thus, the amino acid composition of a protein inherently affects its

solubility characteristics. The more hydrophobicity of amino acid residues and charge frequency, the higher the solubility (Bigelow, 1967). The amount and characteristics of the extracted proteins depend on previous conditions, such as final pH of the muscle and ionic strength of the extracting solution (Lan *et al.,* 1993). Environment conditions, as pH, temperature, ionic strength and interactions with other meat constituents, have also great influence on solubility (Totosaua *et al.,* 1998). Protein solubility in aqueous solutions is dependent on pH. At pH values above and below the isoelectric pH, proteins carry a net charge, electrostatic repulsion and ionic hydration promote solubilization of the protein (Bernal & Jelen, 1984).

Protein denaturation, aggregation and coagulum

Many complex interactive forces will determine whether a protein will form an aggregation, coagulum, or a gel. In general, denaturation refers to any process which causes a change in the three-dimensional structure of the native protein which does not play a role in the rupture of peptide bonds. Aggregation refers to protein-protein interactions which result in the formation of complexes of higher molecular weight. Coagulations are the random aggregation of already denatured protein molecules in which polymer-polymer interactions are formed over polymer-solvent reaction (Schnepf, 1989).

Denaturation of food proteins has been defined as a process (or sequence of processes) in which the spatial arrangement of polypeptide chains

within the molecule is changed from that typical of the native protein to a disordered arrangement (Boye *et al.,* 1997).The native structure of a protein is the net result of various attractive and repulsive interactions emanating from various intramolecular forces as well as interaction of various protein groups with surrounding solvent water. However, native structure is largely the product of the protein's environment. The native state (of a single protein molecule) is thermodynamically the most stable with lowest feasible free energy at physiological conditions. Any change in its environment, such as pH, ionic strength, temperature, and solvent composition etc., will force the molecule to assume a new equilibrium structure such as physical or intramolecular rearrangement without hydrolysis of the chemical bonds linking the constituent amino acids of the proteins' polypeptide chain.

One of the most common methods of denaturing proteins is to heat them in solution. Heat treatment of globular proteins in water or solvent increases their thermal motion, leading to the rupture of various intermolecular and intramolecular bonds stabilizing the native protein structure. This results in reorganization of both the secondary and tertiary configuration where previously inward-oriented "hydrophobic" amino acid residues become exposed to solvent. This process normally results in the formation of new short-lived intermediary conformations (Boye *et al.,* 1997). Thermal coagulation is the random interaction of protein molecules by heat treatment, leading to formation of aggregates that could be either soluble or insoluble (precipitates). Thermal

gelation is the formation of a three-dimensional network exhibiting certain degree of order (Hermansson, 1979b). On the other hand, unfolded protein molecules associate through intermolecular interaction to form aggregates of irreversibly denatured molecules, which may lead to precipitation, coagulation, or gelation. Figure 2.4 (Nakamura & Doi, 2000) shows model of heat denaturation and aggregation of ovalbumin. The interactions involved in determining native protein structure are therefore similarly involved in protein coagulation and gelation. The formation of a thermally induced gel matrix or coagulum from proteins involves the following three sequential events (Edwards *et al.,* 1987):

denaturation ⟶ aggregation ⟶ cross-linking

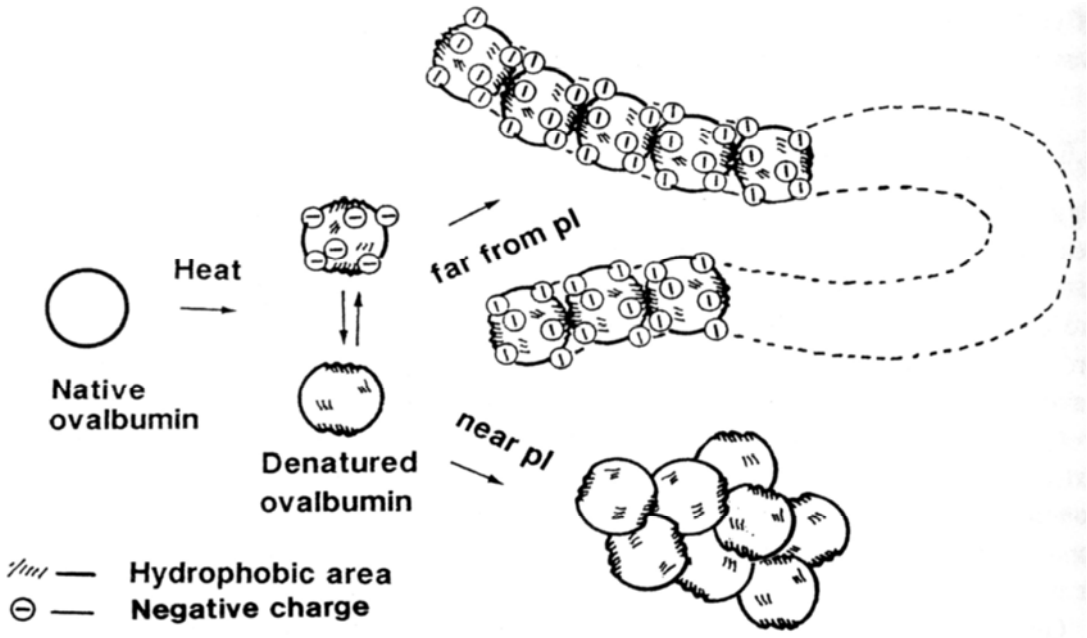


Figure 2.4 Model for the heat denaturation and formation of aggregates of ovalbumin (Nakamura & Doi, 2000).

Chemical modification

Chemical modification such as alkali treatment, acylation, alkylation, phosphorylation, and deamidation has been shown to improve functional properties of proteins. Acylation, phosphorylation and alkylation can modify amino acid side chains in proteins and lead to desirable structural and functional characteristics. Alkali modification has improved adhesive and hydrophobic properties of soy protein (Hettiarachchy & Kalapathy, 1998). Alkali treatment also results in protein unfolding, disulfide cleavage and dissociation. High pH treatments often lead to more severe denaturation than those caused by thermal treatments (Das & Kinsella, 1990). A combined treatment of soy proteins at about 65°C and a pH of 10-11 resulted in an optimum exposure of hydrophobic groups to exhibit higher emulsifying capacity (Petruccelli & Anon, 1996). The 11 S fraction is more susceptible to alkali denaturation than the 7 S fraction due to their high disulfide content (about 18-20 disulfide bonds per mole) (Hettiarachchy & Kalapathy, 1998).

2.2 Functional properties of proteins

Proteins, the most important functional components in muscle, confer many of the desirable physicochemical and sensory attributes of muscle foods. The functional properties of proteins in foods are related to their structural and other physicochemical characteristics, which include size, shape, amino acid composition and sequence, charge, and charge distribution. The properties of food proteins that affect functionality include hydrophilicity / hydrophobicity

ratio, secondary structure content and their distribution (e.g., α-helix, β-sheet, and aperiodic structures), tertiary and quaternary arrangements of the polypeptide segments, inter- and intrasubunit cross-links (e.g., disulfide bonds), the rigidity / flexibility of the protein in response to external conditions (Damodaran, 1989). A fundamental understanding of physical, chemical, and functional properties of proteins and the changes these properties undergo during processing is essential if the performance of proteins in foods is to be improved and if underutilized proteins are to be increasingly used in traditional and processed food products. Most functional properties affect the textural qualities of a food and play an important role in determining the physical behavior of a food during preparation, processing, and storage.

Increasing emphasis is being placed on isolating proteins from various sources and using them as food ingredients. In many applications functional properties are of great importance. Functional properties have been defined as those physical and chemical properties that affect the behavior of proteins in food systems during processing, storage, preparation, and consumption (Kinsella, 1982). When small amounts of protein ingredients are added to food, they may significantly influence some of the physical properties of food such as firmness (Hermansson, 1973). Beside this, proteins have many other functions such as solvation, thickening, water holding ability, gelation, emulsification and so on. A summary of these properties is given in Table 2.1 and Table 2.2. The role of muscle proteins in processed foods may be grouped

into the following functions: water-binding, fat emulsification, color, flavor, texture, and nutrition. Depending upon the particular food product, one or more of these properties of muscle protein will contribute to the products' characterization.

Table 2.1 Functional properties of food proteins

General property	Functional Criteria
Organoleptic	Color, flavor, odor
Kinesthetic	Texture, mouth feel, smoothness, grittiness, turbidity
Hydration	Solubility, wettability, water absorption, swelling, thickening, gelling, syneresis, viscosity
Surface	Emulsification, foaming (aeration, whipping), film formation
Binding	Lipid-binding, flavor-binding
Structural	Elasticity, cohesiveness, chewiness, adhesion, network, cross-binding, aggregation, dough formation, texturiz ability, fiber formation, extrudability
Rheological	Viscosity, gelation
Enzymatic	Coagulation (rennet), tenderzation (papain), mellowing ("proteinnases")
"Blendability"	Complementarity(wheat-soy,gluten-casein)
Antioxidant	Off-flavor prevention (fluid emusions)

Source: From Kinsella (1982)

Table 2.2 Functional properties of proteins in food systems.

Functional property	Mode of action	Food system
Solubility	Protein solvation	Beverages
Water absorption and binding	Hydrogen bonding of water; entrapment of water(no drip)	Meat, sausages, bread, cakes
Viscosity	Thickening; water binding	Soups, gravies
Gelation	Protein acts as	Meat, sausages, baked goods, pasta products
Elasticity	adhesive material Hydrophobic bonding	Meats, bakery product
Emulsification	gluten; disulfide links in gels	Sausages,bologna,soup Cakes Meat,sausages,doughnut
Fat absorption Flavor-binding	Formation and stabilization of emulsion Binding of free fat	Simulated meats,bakery Products, etc.
Foaming	Absorption, entrapment, release Forms stable films to entrap gas	Whipped toppings,chiffon Desserts,angel cakes

Source: From Kinsella (1982).

Solubility

The functional properties of proteins are often affected by protein solubility and those most affected are thickening, foaming, emulsifying, and gelling.

Bigelow (1967) proposed that the solubility of a protein is fundamentally related to the average hydrophobicity of the amino acid residues and the charge frequency. The average hydrophobicity is defined as

$$\Delta_g = \Sigma\Delta_{gresidue} / n \qquad (1)$$

Where $\Delta_{gresidue}$ is the hydrophobicity of each amino acid side chain obtained from the free energy change for transfer from ethanol to water, and *n* is the total number of residues in the protein. The charge frequency is defined as

$$\sigma = (n^+ + n^-)/ n \qquad (2)$$

Where n^+ and n^- are the total number of positively and negatively charge residues, respectively, and *n* is the total number of residues. According to Bigelow (1967), the smaller the average hydrophobicity and the larger the charge frequency, the greater will be solubility of the protein. Based on solubility characteristics, proteins are classified into four categories. *Albumins* are those that are soluble in water at pH 6.6 (e.g., serum albumin, ovalbumin, and α-lactalbumin). *Globulins* are those that are soluble in dilute salt solutions at pH 7.0 (e.g., glycinin, phaseolin, and β-lactoglobulin), *glutelins* are those that are soluble only in acid (pH2) and alkaline (pH12) solutions (e.g., wheat glutelins), and prolamines are those soluble in 70% ethanol (e.g., zein and gliadins). In addition to these, solubility is influenced by several solution conditions, such as pH, ionic strength, temperature, and the presence of organic solvents.

Addition of organic solvents, such as ethanol or acetone, lowers the permittivity of an aqueous medium. This increase intra- and intermolecular

electrostatic forces, both repulsive as well as attractive. The repulsive intramolecular electrostatic interactions cause unfolding of the protein molecule. In the unfolded state, the permittivity promotes intermolecular hydrogen bonding between the exposed peptide groups and attractive intermolecular electrostatic interactions between oppositely charged groups. These intermolecular polar interactions lead to precipitation of the protein in organic solvents or reduced solubility in an aqueous medium. The role of hydrophobic interactions in causing precipitation in organic solvents is minimal because of the solubilizing effect of organic solvents on nonpolar residues (Damodaran, 1996).

Gelation

Gel formation is an important property of proteins. Proteins can form gels by acid coagulation, action of enzymes, heat, and storage. A gel is a protein network that immobilizes a large amount of water. The network is formed by protein-protein interactions. Gels are characterized by having relatively high non-Newtonian viscosity, elasticity, and plasticity. Gelation has been described as a two stage process (Pomeranz, 1991). The first stage is a denaturation of the native protein into unfolded polypeptides, and the second stage is a gradual association to form the gel matrix. The type of association and, therefore, the nature of the gel depend on a variety of covalent and noncovalent interactions involving disulfide bonds, hydrogen bonds, ionic and hydrophobic interactions, or combinations of these. Protein gels can be divided into two types: aggregated