EFFECTS OF ULTRAVIOLET IRRADIATION ON THE PHYSICOCHEMICAL AND FUNCTIONAL PROPERTIES OF SELECTED FOOD BIOPOLYMERS

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by

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LIST OF ABBREVIATIONS

Abbreviation	Caption
AG	arabinogalactan
AGP	arabinogalactan protein
Ala	alanine
Arg	arginine
Asn	asparagine
Asp	aspartic acid
BSA	bovine serum albumin
C	carbon atom
CH	methane group
cis-	latin preposition *cis* (“on this side of”)
CN	carbon-nitrogen bond
CO	carbonyl group
-COOH	carboxyl group
C-terminus	carboxyl-terminus
Cys	cysteine
DNA	deoxyribonucleic acid
e.g.	latin *exempli gratiā* (“for example”)
et al.	latin *et* (“and”) + *alii* (“others”)
EW	egg white protein
FTIR	Fourier transform infrared
Gln	glutamine
Glu	glutamic acid

Gly	glycine
GP	glycoprotein
GPC	gel permeation chromatography
GPC-MALLS	gel permeation chromatography-multi angle laser-light scattering
H	hydrogen atom
His	histidine
HMW-GS	high molecular weight-glutenin subunits
i.e.	latin *id est* (“that is”)
Ile	isoleucine
IR	infrared
Leu	leucine
LMW-GS	low molecular weight-glutenin subunits
Lys	lysine
Met	methionine
MeV	mega-electron volt
mt	million tons
N	nitrogen atom
NaOH	sodium hydroxide
NH	nitrogen-hydrogen side chain
$-NH_2$	amino group
NZMP	New Zealand Milk Products
O/W	oil-in-water
-OH	hydroxyl group
pH	potential of hydrogen
Phe	phenylalanine

pI	isoelectric point
Pro	proline
Pyl	pyrrolysine
SC	sodium caseinate
SDS-PAGE	sodium dodecyl sulphate polyacrylamide gel electrophoresis
Sec	selenocystein
Ser	serine
SF	soy flour
SPC	soy proteins concentrate
SPH	soy protein hydrolyzate
SPI	soy protein isolate
Tgase	transglutaminase
Thr	threonine
Trp	tryptophan
Tyr	tyrosine
UV	ultraviolet
Val	valine
WG	wheat gluten
WVP	water vapor permeability

LIST OF SYMBOLS

Symbol	Caption
%	percent/ percentage
$\dot{\gamma}$	shear rate
<	less than
>	more than
±	plus-minus sign
℃	degree Celsius
μ	lower case mu, prefix for micro
D[4,3]	volume mean diameter
Da	Dalton
g	gram, unit of mass
K	flow consistency
mL	millilitre
M_n	number average molecular weight
M_w	molecular weight
M_w/M_n	polydispersity index
n	flow behaviour index
nm	nanometer
N_t	total free amino group
V	volume
α	lower case alpha
β	lower case beta
γ	lower case gamma

ε	lower case epsilon
η_a	viscosity
κ	lower case kappa
λ	lower case lambda
τ	lower case tau
ω	lower case omega

LIST OF PUBLICATIONS & CONFERENCES

Conference

1. Kuan, Y. H. & Karim, A. A., 2010. Effects of ultraviolet irradiation on the physicochemical and functional properties of soy protein isolate. *The 10th International Hydrocolloids Conference*. Shanghai, China, 20th – 24th, June, 2010. Oral Presentation.

Publications

1. Kuan, Y. H., Bhat, R., Senan, C., Williams, P. A. & Karim, A. A., 2009. Effects of ultraviolet irradiation on the physicochemical and functional properties of gum arabic. *Journal of Agricultural and Food Chemistry*, 57, pp.9154–9159.

2. Kuan, Y. H. & Karim, A. A., 2011. Effects of ultraviolet irradiation on the physicochemical and functional properties of soy protein isolate. *Food Hydrocolloids*. (Under review).

3. Kuan, Y. H. & Karim, A. A., 2011. Emulsifying and foaming properties of ultraviolet irradiated egg white protein and sodium caseinate. *Journal of Agricultural and Food Chemistry*. (Under review).

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KESAN IRRADIASI ULTRAVIOLET TERHADAP SIFAT FIZIKOKIMIA DAN FUNGSIAN BAGI BIOPOLIMER MAKANAN YANG TERPILIH

ABSTRAK

Aplikasi biopolimer makanan dapat diperkembangkan dengan modifikasi kimia, enzim atau fizikal. Tesis ini mengutarakan tentang penggunaan irradiasi ultraviolet (UV) untuk mengubah-suaikan sifat fungsian bagi biopolimer makanan yang terpilih. Biopolimer makanan tersebut termasuk protein makanan yang diperoleh daripada sumber tumbuhan, khususnya protein soya (SPI) dan gluten gandum (WG) serta protein makanan yang diperoleh daripada sumber haiwan, khususnya protein putih telur (EW) dan natrium kaseinat (SC). Selain itu, biopolimer makanan daripada polisakarida, khususnya gum arabic (GA) juga dipilih. Dalam kajian ini, kesan irradiasi UV terhadap sifat fizikokimia dan fungsian bagi biopolimer makanan yang terpilih juga diselidik, terutamanya terhadap sifat pengemulsian dan pembusaan. Semua sampel telah dipancarkan dengan irradiasi UV selama 30, 60, 90 dan 120 min. Walau bagaimanapun, sampel WG dan sampel SC telah dipancarkan dengan masa irradiasi UV yang dipanjangkan selama 4 dan 6 jam disebabkan tiada perbezaan yang dapat diperhatikan sehingga 120 minit pendedahan irradiasi UV. Sampel GA juga diubahsuai dengan formaldehid untuk tujuan perbandingan.

Bagi protein tumbuhan, sampel SPI dan sampel WG yang telah dipancarkan dengan irradiasi UV menunjukkan penukaran warna yang tidak signifikan ($P > 0.05$) berbanding dengan sampel kawalan. Bagi sampel SPI, analisa daripada jumlah

kumpulan amino bebas, *Sodium Docecyl Sulfate-Polyacrylamide Gel Electrophoresis* (SDS-PAGE) dan *Fourier Transform Infrared Spectroscopy* (FTIR) menunjukkan irradiasi UV dapat mengakibatkan hubung-silang protein; kesan ini menjadi lebih ketara apabila masa pendedahan sampel terhadap irradiasi UV ditingkatkan. Hubung-silang UV ini kemudian menyebabkan peningkatan ($P < 0.05$) pada kelikatan nyata. Semua sampel SPI yang telah dipancarkan dengan irradiasi UV menunjukkan sifat pengemulsian dan pembusaan yang lebih baik daripada sampel kawalan. Sebaliknya, perubahan pada sampel WG tidak dapat dikesan berdasarkan keputusan yang diperolehi daripada jumlah kumpulan amino bebas, SDS-PAGE, kelikatan nyata dan juga sifat pengemulsian dan pembusaan. Akan tetapi, merujuk kepada keputusan yang diperoleh daripada analisa FTIR, perubahan dapat dikesan terhadap pengubahan amida bagi sampel WG yang dipancarkan dengan irradiasi UV pada masa yang dipanjangkan. Oleh itu, adalah dipercayai bahawa dengan pemanjangan masa irradiasi, hubung-silang akan berlaku dan kemudiannya akan meningkatkan sifat pengemulsian dan pembusaan.

Pengukuran warna bagi protein haiwan, iaitu sampel EW dan sampel SC yang telah dipancarkan dengan irradiasi UV menunjukkan warna yang semakin gelap ($P < 0.05$). Analisa daripada jumlah kumpulan amino bebas, SDS-PAGE dan FTIR terhadap sampel EW dan sampel SC yang telah dirawat dengan irradiasi UV menunjukkan hubung-silang telah berlaku apabila masa pendedahan irradiasi UV ditingkatkan. Hubung-silang ini kemudiannya telah membawa kepada peningkatan ($P < 0.05$) pada kelikatan nyata. Tambahan pula, perubahan terhadap struktur protein akibat daripada irradiasi UV juga membawa kepada sifat pengemulsian dan pembusaan yang lebih baik.

Bagi sampel GA, analisa berat molekul dengan menggunakan kromatografi jel penyerapan (GPC) menunjukkan tiada perubahan yang signifikan ($P > 0.05$) berlaku terhadap struktur molekul bagi sampel yang dirawat dengan irradiasi UV. Analisa kumpulan amino bebas pula menunjukkan bahawa irradiasi UV yang sederhana (30 minit) dapat mengakibatkan hubung-silang pada GA; keputusan ini dapat diperbandingkan dengan sampel yang diubahsuai dengan formaldehid. Akan tetapi, penurunan kelikatan telah diperhatikan bagi sampel yang terdedah kepada irradiasi UV untuk masa yang lebih panjang (90 dan 120 minit). Semua sampel yang dirawat dengan irradiasi UV ataupun formaldehid menunjukkan sifat-sifat pengemulsian yang lebih baik daripada sampel kawalan.

Kesimpulannya, semua keputusan yang didapati menunjukkan bahawa sampel SPI, WG, EW, SC dan GA yang telah dipancarkan dengan irradiasi UV dapat digunakan sebagai agen pengemulsian dan agen pembusaan yang baharu untuk dikomersialkan serta diaplikasikan dalam pelbagai sistem makanan.

EFFECTS OF ULTRAVIOLET IRRADIATION ON THE PHYSICOCHEMICAL AND FUNCTIONAL PROPERTIES OF SELECTED FOOD BIOPOLYMERS

ABSTRACT

The application of food biopolymers can be diversified with chemical, enzymatic or physical modifications. This thesis addressed the use of ultraviolet (UV) irradiation to modify the functional properties of selected food biopolymers. These food biopolymers include food proteins derived from plant sources, specifically soy protein isolate (SPI) and wheat gluten (WG) as well as food proteins derived from animal sources, specifically egg white protein (EW) and sodium caseinate (SC). Other than this, food biopolymer from polysaccharides, specifically gum arabic (GA) was also selected. In this study, the effects of UV irradiation on the physicochemical and functional properties of selected food biopolymers were investigated, particularly on the emulsifying and foaming properties. All the samples were treated with UV irradiation for 30, 60, 90 and 120 min. However, the WG and SC samples were subjected to extended UV irradiation for 4 and 6 h as no difference was found on the initial UV exposure time. For GA, the sample was also treated with formaldehyde for comparison.

For plant proteins, UV-irradiated SPI and WG samples exhibited insignificant ($P > 0.05$) colour changes compared with control sample. For SPI samples, total free amino group, Sodium Docecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) and Fourier Transform Infrared Spectroscopy FTIR

analyses indicated that UV irradiation could induce protein cross-linking; this effect was enhanced upon increasing the UV exposure time. UV irradiation induced cross-linking was then given rise to an increased ($P < 0.05$) apparent viscosity. All irradiated SPI samples exhibited better emulsification and foaming properties than un-irradiated samples. On the other hand, for WG samples, changes were not detectable based on results obtained from total free amino group, SDS-PAGE, apparent viscosity profiles as well as emulsification and foaming properties. However, based on the results obtained from FTIR analysis, changes were detected on the alteration of amides in the WG sample treated with extended irradiation time. Therefore, it is believed with prolonged irradiation time, cross-linking would occur on WG samples and subsequently improve the emulsification and foaming properties.

For animal proteins, darkening ($P < 0.05$) was observed on UV-irradiated EW and SC samples for colour measurement. Total free amino group, SDS-PAGE and FTIR analyses on irradiated EW and SC indicated that cross-linking would have occurred upon increasing UV irradiation exposure time. This cross-linking was subsequently brought an increase ($P < 0.05$) on apparent viscosity. Moreover, the changes on protein structures upon UV irradiation were also given rise to improvement on emulsification and foaming properties.

For GA samples, molecular weight analysis using gel permeation chromatography (GPC) indicated that no significant change ($P > 0.05$) occurred on the molecular structure on the samples exposed to UV irradiation. Free amino group analysis indicated that mild UV irradiation (30 min) could induce cross-linking on GA; this result was comparable with that of samples treated with formaldehyde.

However, viscosity break down was observed for samples exposed to UV irradiation for longer times (90 and 120 min). All the UV-irradiated and formaldehyde-treated samples exhibited better emulsification properties than control sample.

Therefore, these results indicate that the UV-irradiated SPI, WG, EW, SC and GA could serve as novel emulsifiers and foaming agents to be exploited commercially and applied in broad food systems.

CHAPTER 1

INTRODUCTION

1.1 Background and Rationale

Biopolymers are macromolecules derived from natural sources; which are also known as biological polymers and produced by living organisms (Khan *et al*., 2007). Examples of biopolymers include polypeptides, polysaccharides, polypeptide/polysaccharide hybrids, polynucleotides, polyhydroxybutyrates (polyesters produced by certain bacterias) and *cis*-1,4-polyisoprene (major component of rubber tree latex) (Tolstoguzov, 2008). Polypeptides are also known as proteins, which are made of amino acids arranged in a linear chain and folded into a globular form. Other than this, there are numerous examples of biopolymers having a polysaccharide and polypeptide in the same molecule, usually with a polysaccharide as a side chain in a polypeptide, or vice versa (Khan *et al*., 2007). Gum arabic is one unique example of biopolymer to represent the hybrid of polypeptides and polysaccharides. In food industry, biopolymers are commonly used to improve the stability and texture of emulsion-based food products (McClements, 2009). A wide variety of food biopolymers can be used as emulsifiers to stabilize emulsion-based food products, including gum arabic, egg albumin, corn zein, soy protein, whey protein and caseinate. Some of the food products naturally contain functional biopolymers (e.g., milk), whereas others contain biopolymers that have been added as ingredients because of their unique functional attributes (e.g., thickening agents, or gelling agents) or they may form part of more complex ingredients (e.g., eggs, milk, or flour) (McClements, 2009).

In the unmodified form, these biopolymers have limited usage in the food industry. Food proteins are often denatured during processing and thus spurring food technologists to manipulate and expand the functionality of food proteins. When the protein undergoes chemical reaction during processing, both the natural function of the molecule, and the properties of the denatured polymeric state may be influenced. The type of chemical reaction that has major consequences on protein functionality in either their native or denatured states is protein cross-linking (Gerrard, 2002). Therefore, it is possible that protein cross-linking could have profound effects on the functional properties of food proteins. Modification of proteins for functionality improvements has been carried out via physical means such as heat treatment (Keerati-u-rai & Corredig, 2009), enzymatic treatment (Wang *et al*., 2007), ultrasonic treatment (Tang *et al*., 2009), elevated pressure treatment (Torrezan *et al*., 2007), or via chemical means such as acidification (Ou *et al*., 2005), application of glyoxal, glutaraldehyde and formaldehyde (Marqui é *et al*., 1995); as well as the Maillard reaction induced cross-linking (Caillard *et al*., 2010).

Obviously, numerous methods have been attempted to induce cross-linking in protein, including chemical treatment, enzymatic treatment, and physical treatment as mentioned above. Among the chemical cross-linking agents, the aldehydes bond very quickly to proteins (Donohue *et al*., 1983), and are especially used in gelatine reticulation in photographic films and in microcapsules produced by coacervation (Thies, 1995). The aldehydes chemically fix the gelatine gel, thus improving its functional properties. However, the application of the chemical cross-linking agents such as glutaraldehyde, formaldehyde and glyoxal are toxic, which limits their application in food systems (Tseng *et al*., 1990). On the other hand, polymerization using enzyme (such as transglutaminase) has been investigated with

various protein sources including casein, soy protein, and gelatin, where different responses in gel strength were dependant on the reaction conditions and on the different protein sources (Sakamoto *et al*., 1994). However, the use of enzyme treatments to induce cross-linking is costly and time-consuming (Sabato *et al*., 2001), thus preventing food processors to expand the application in food industry. Due to the several drawbacks of chemical treatment and enzymatic treatment mentioned above, therefore, a physical method –ultraviolet (UV) irradiation to induce cross-linking –was selected in this study.

The primary advantage of using UV irradiation is that it does not employ radioactive sources, like γ-radiation, thus avoiding environmental issues (Smith & Pillai, 2004). Moreover, UV irradiation is cost effective, non-thermal, and environmental friendly. Due to these reasons, UV irradiation is receiving increasing attention and has been used to improve soy protein films, to cross-link collagen and gelatin films in medical and pharmaceutical research, and to preserve and decontaminate food products (Bintis *et al*., 2000). It is also noteworthy that most of the studies on radiation induced polymers cross-linking are conducted on synthetic polymers, for example polyvinyl alcohol, polystyrene, poly (vinyl chloride), and many others (Chmielewski *et al*., 2005). Similar studies on biopolymer systems are, however, rather sparse. Previous studies were only reporting the effects of UV irradiation and gamma irradiation on the biopolymer films (Gennadios *et al*., 1998; Lee *et al*., 2005a; Lee *et al*., 2005b). However, to our knowledge, no studies have been undertaken towards exploring the impact of UV irradiation on the physicochemical properties of gum arabic (GA), sodium caseinate (SC), soy protein isolate (SPI), egg white protein (EW) and wheat gluten (WG), as well as their functional properties. As evidence of irradiation-induced crosslinking was observed

on protein samples treated with UV and γ-radiation, we hypothesized that UV irradiation would cross-link the protein component in these chosen food biopolymers (SPI, WG, EW, SC and GA) and improve their emulsifying and foaming properties.

It is envisaged that modification of food protein by UV irradiation described in this thesis would provide the basis for further research into the potential applications of food system that requires the use of protein as stabilizer, in order to enhance the emulsifying properties in the emulsion; or in the pharmaceutical industries to produce enhanced properties of gelatin replacer from other sources of food biopolymers. This modification technique would render the protein structure to be more amenable for developing specific application.

1.2 Objectives

The main objective of this study was to investigate the effect of cross-linking treatment involving the use of UV irradiation in selected food biopolymers, specifically on SPI, WG, EW, SC and GA. The effects of UV irradiation on the physicochemical and functional properties of selected biopolymers were studied to provide a basis for further research into the potential application in the food industry. The specific objectives were:

1. To study the effect of UV irradiation on the physicochemical and functional properties on the selected food proteins (SPI, WG, EW and SC) with respect to the colour changes, free amino group, indication of protein cross-linking, structural changes, emulsification properties including emulsifying activity, emulsion stability; and foaming properties including the foaming ability and foaming stability and rheological properties.

2. To study the effect of UV irradiation on the physicochemical and functional properties on GA with respect to the molecular mass, colour changes, free amino group, emulsification properties including emulsifying activity, emulsion stability and rheological properties.

CHAPTER 2

LITERATURE REVIEW

2.1 Proteins in Food

2.1.1 An Introduction

Proteins are the most abundant molecules in cells, making up 50% or more of their dry weight (Vaclavik, 1998). Each protein has a unique structure and conformation, or shape, which enables it to carry out a specific function in a living cell (Chang, 1998). Proteins comprise the complex muscle system and the connective tissue network, and they are important as carriers in the blood system (Vaclavik, 1998). Additionally, enzymes are example of proteins that serve as catalysts for many reactions (both desirable and undesirable) in foods.

Generally, milk, meats (including fish and poultry), eggs, cereals, legumes and oilseeds have been the major sources of food proteins (Damodaran, 1996). Proteins are very important in foods, both nutritionally and as functional ingredients. They play an important role in determining the texture of a food (Gerrard, 2002). They are complex molecules, and it is important to have an understanding of the basics of protein structure to understand the behavior of foods during processing (Vaclavik, 1998). Determining the relationship between the structure of any protein and its function is a challenge that biochemists struggle to meet in many contexts. The correlation of the structure of a food protein with its function, or functionality, within a food system is not easy. For example, the chemical reactions occur in protein during processing would affect the natural function of the molecule, as well as the functional properties (Gerrard, 2002).

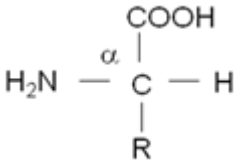
Therefore, the understanding and manipulation of food proteins require knowledge of both protein chemistry and polymer science.

2.1.2 Basic Protein Chemistry

Food proteins are very complex. However, many of them have been purified and characterized (Chang, 1998). Proteins can be classified by their composition, structure, biological function, or solubility properties. All proteins contain carbon, hydrogen, nitrogen, and oxygen. Most proteins contain sulfur, and some contain additional elements; e.g., milk proteins contain phosphorus, and hemoglobin and myoglobin contain iron. Other than this, copper and zinc are also constituents of some proteins (Vaclavik, 1998).

Proteins are made up of amino acids. There are at least 20 different amino acids found in nature which vary in different properties, depending on their structure and composition (Buxbaum, 2007). When these amino acids combined to form a protein, the result is a unique and complex molecule with a characteristic structure and conformation and a specific function in the plant or animal where it belongs (Vaclavik, 1998). Small changes in pH or application of heat in food can cause dramatic changes in protein molecules (Chang, 1998). These changes can always be seen in daily life, e.g., the making of cheese by adding acid to mink or heating and stirring eggs to make scrambled eggs.

Each amino acid contains a central carbon atom, which is attached to a carboxyl group (-COOH), an amino group ($-NH_2$), a hydrogen atom (H), and another group or side chain R specific to the particular amino acid (Buxbaum, 2007). The general formula for an amino acid is



A comprehensive diagram to explain the structure of an amino acid can be found in Figure 2.1. Glycine is the simplest amino acid, with the R group being a hydrogen atom (Vaclavik, 1998). There are more than 20 different amino acids in proteins. Their properties depend on the nature of their side chains or R groups. The 22 amino acids are shown in Figure 2.2.

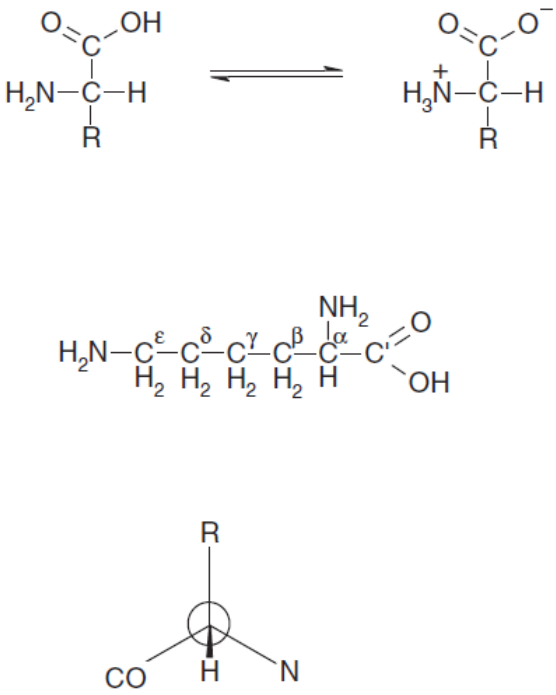


Figure 2.1 TOP: Basic structure of an amino acid. Amino acids can form zwitter-ions. MIDDLE: Nomenclature of carbon atoms, using lysine as example. The Carboxy-carbon is designated C', the following carbon atoms are labeled with the letters of the Greek alphabet. Sometimes the last C-atom is called ω, irrespective of the chain length. BOTTOM: In l-amino acids if the α-carbon is placed on the paper plane, with the hydrogen facing you, the remaining substituents read "CORN". (adapted from: Buxbaum, 2007)

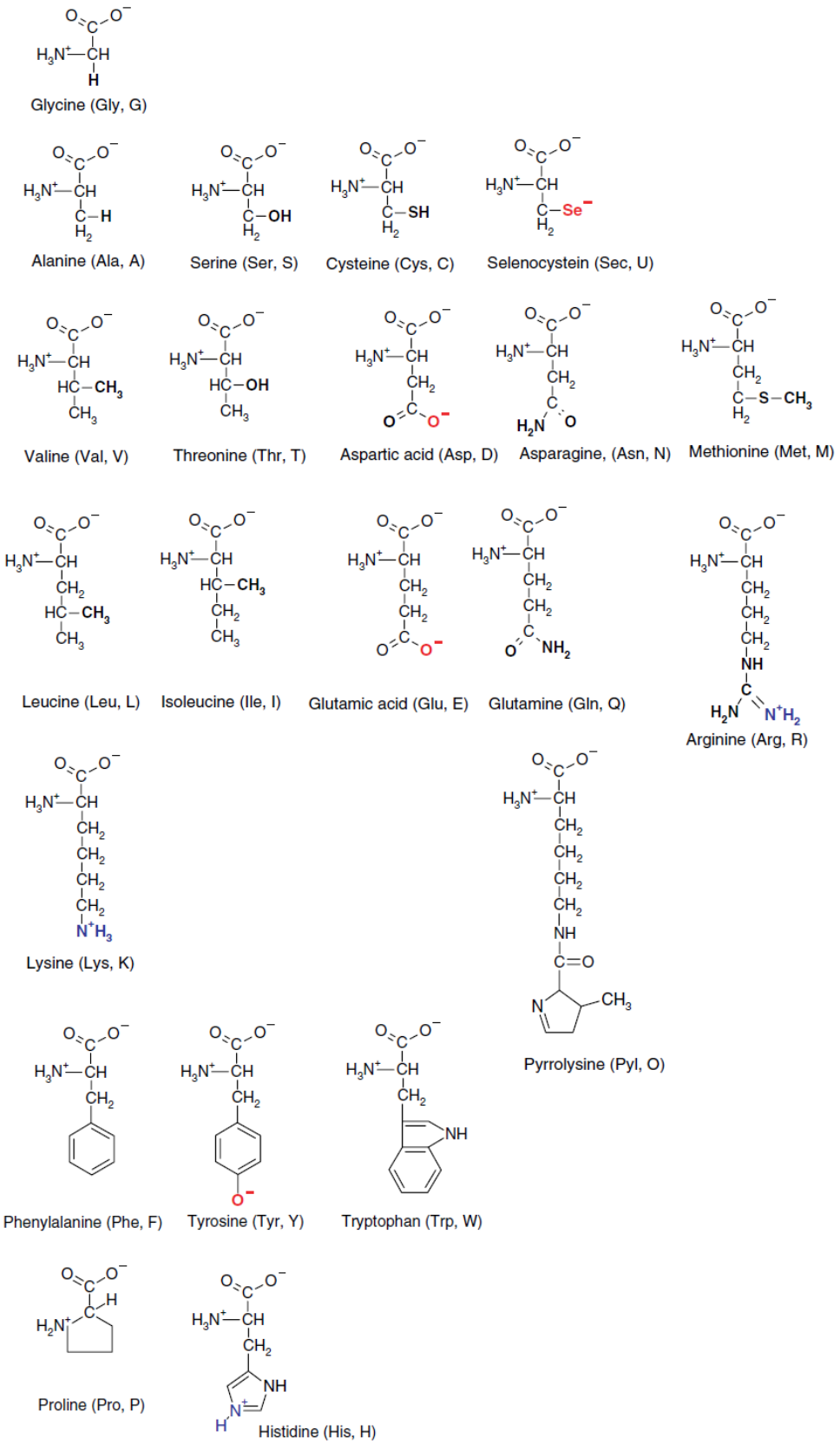
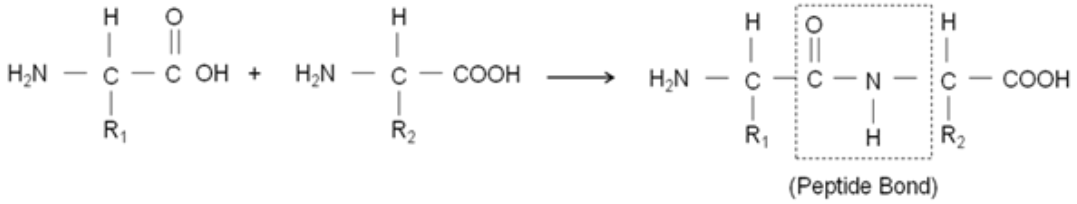


Figure 2.2 The 22 amino acids differ in the chemical nature of the side chain group at the α-carbon atom. Acidic groups marked red, basic groups blue. Note that Thr and Ile have chiral β- in addition to the α-carbon. Pyl has two chiral carbon atoms in the ring. (adapted from: Buxbaum, 2007)

2.1.3 Structure and Organization of Protein

Proteins are made up of many amino acids and joined by peptide bonds (Vaclavik, 1998) as shown below:



Peptide bonds are strong and difficult to disrupt. A dipeptide contains two amino acids joined by a peptide bond. A polypeptide contains several amino acids joined by peptide bonds. Each polypeptide chain has a free amino (N-terminus) and free carboxyl (C-terminus) end (Peterson & Johnson, 1978). Proteins are usually much larger molecules, containing several hundred of amino acids. They can be hydrolyzed and yielding smaller polypeptides, either by enzymes or by acid digestion (Vaclavik, 1998).

According to Vaclavik (1998), each protein has a complex and unique conformation, which is determined by the specific amino acids and the sequence in which they occur along the chain. It is important to understand the basics of protein structure in order to understand the function of proteins in food systems and the changes that occur in proteins during food processing. Proteins are being categorized into four types of structure – primary, secondary, tertiary, and quaternary structure – and these build on each other. The different types of protein structure are outlined in the following context.

2.1.3.1 Primary Structure

The primary structure of a protein is the specific sequence of amino acids polymerized into a linear chain by formation of peptide bonds between successive

amino acid residues (Vaclavik, 1998). This is the simplest structure in protein. However, in reality proteins do not exist as straight chains. The specific sequence of amino acids is responsible for the determination of the form or shape of a particular protein (Damodaran, 1996). Therefore, it is essential to know the primary structure for a detailed understanding on the structure and function of a particular protein.

2.1.3.2 Secondary Structure

The secondary structure of a protein refers to the three-dimensional organization of segments of the polypeptide chain (Peterson & Johnson, 1978). In other words, the protein secondary structure is represented by the coiling of the primary amino acid chain into specific characteristic patterns, usually spiral or helices (ordered structure), beta (β) pleated sheet (ordered structure) and random coil (disordered structure) (Linnaeus, 2007). The common secondary structures in proteins are α-helix and β-pleated sheet. The arrangement of these secondary structures determines the shape of the tertiary structure (Figure 2.3).

Figure 2.3 The secondary structure of a polypeptide chain (α-helix and a strand of β-sheet) and the tertiary structure of a protein. (adapted from: Finkelstein & Ptitsyn, 2002)

The α-helix is a corkscrew structure, with 3.6 amino acids per turn (Linnaeus, 2007). A typical structure of α-helix is shown in Figure 2.4. It is stabilized by intrachain hydrogen bonds; which is referring to the hydrogen bonds occur within a single protein chain, rather between adjacent chains (Vaclavik, 1998). Hydrogen bonds occur between each turn of the helix. The oxygen and hydrogen atoms that comprise the peptide bonds are involved in hydrogen bond formation (Linnaeus, 2007). The α-helix is a stable and organized structure. However, this structure could not be formed with the existence of proline, due to the bulky five-membered ring prevents the formation of the helix (Finkelstein & Ptitsyn, 2002).

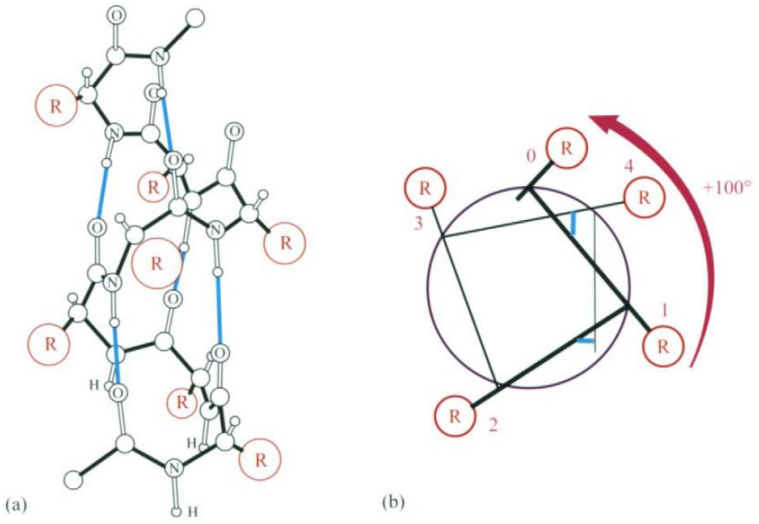


Figure 2.4 The right-handed α-helix. (a) Atomic structure; R = side-chains. Hydrogen bonds are shown as light-blue lines. (b) Axial view of one turn of this α-helix. The arrow shows the turn of the helix (per residue) when it approaches the viewer (the closer to the viewer, the smaller the chain residue number). (adapted from: Finkelstein & Ptitsyn, 2002)

The β-pleated sheet is a more extended conformation than α-helix structure. This β-pleated sheet can be thought of as a zigzag structure rather than a cockscrew (Vaclavik, 1998). A typical structure of β-pleated sheet is shown in Figure 2.5. Several stretched protein chains combine to form β-pleated sheets. These sheets are linked together by interchain hydrogen bonds. The interchain hydrogen bonds

refer to the bonds occur between adjacent sections of the protein chains (Linnaeus, 2007). Again, the hydrogen and oxygen atoms that form the peptide bonds are involved in hydrogen bond formation. Similar with *α*-helix, the *β*-pleated sheet is also an ordered structure (Vaclavik, 1998)

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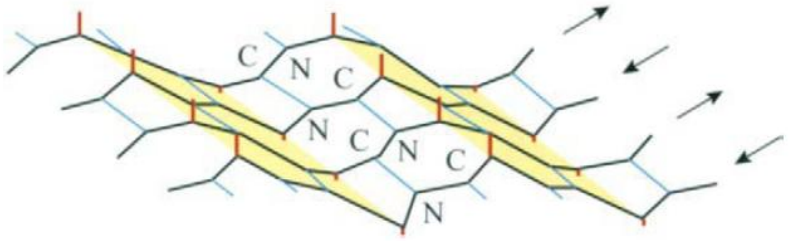

Figure 2.5 The *β*-pleated sheet. The side-chains (shown as short red rods) are at the pleats and directed accordingly. The H-bonds are shown in light-blue. (adapted from: Finkelstein & Ptitsyn, 2002)

The random coil is an ill-defined or disordered secondary structure. This structure formed when amino acid side chains prevent the formation of the *α*-helix or *β*-pleated sheet (Vaclavik, 1998). It is therefore any structure except α-helix and *β*-pleated sheet can be termed random coil (Linnaeus, 2007). The random coil structure occurs if proline is present, and or if there are highly charged regions within the protein which prevents the formation of ordered α-helix and β-pleated sheet structure (Vaclavik, 1998).

2.1.3.3 Tertiary Structure

The protein tertiary structure of a protein refers to the three-dimensional organization of the complete protein chain (Vaclavik, 1998). In other words, this protein tertiary structure refers to the spatial arrangement of a protein chain that contains regions of secondary structures, including *α*-helix, *β*-pleated sheet, and random coil (Damodaran, 1996; Linnaeus, 2007). Therefore, this level of structure is

built on the secondary structure of a specific protein and maintained by various non-covalent interactions, including hydrophobic, electrostatic, van der Waals interactions as well as hydrogen bonding (Peterson & Johnson, 1978; Vaclavik, 1998; Linnaeus, 2007). Generally, there are two types of tertiary structure protein, which are fibrous proteins and globular proteins.

Fibrous proteins refer to the structural proteins such as collagen (connective tissue protein), or actin and myosin which are responsible for muscle contraction (Cohen, 1998). An example of fibrous proteins is shown in Figure 2.6. The protein chains in fibrous proteins are extended, forming rods or fibers. Therefore, a fibrous tertiary structure contains a large amount of ordered secondary structures (e.g., α-helix and β-pleated sheet) (Vaclavik, 1998).

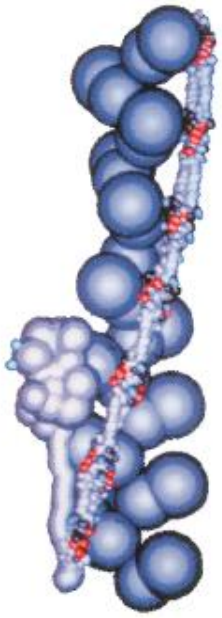

Figure 2.6 Structure of a typical fibrous protein showing tropomyosin and attached troponin complex winding around the actin helix. (adapted from: Cohen, 1998)

On the other hand, globular proteins refer to the protein structure which having a compact molecule and are spherical or elliptical in shape (Figure 2.7). Examples of globular proteins including transport proteins, such as myoglobin

(Peterson & Johnson, 1978), which carries oxygen to the muscle. Other examples may as such whey proteins and the caseins. Globular tertiary structure usually contains proteins with a large number of hydrophobic amino acids residues (Vaclavik, 1998). This hydrophobic property is due to the spherical shape has the least surface area-to-volume ratio, so that more hydrophobic groups can be buried in the protein interior (Damodaran, 1996).

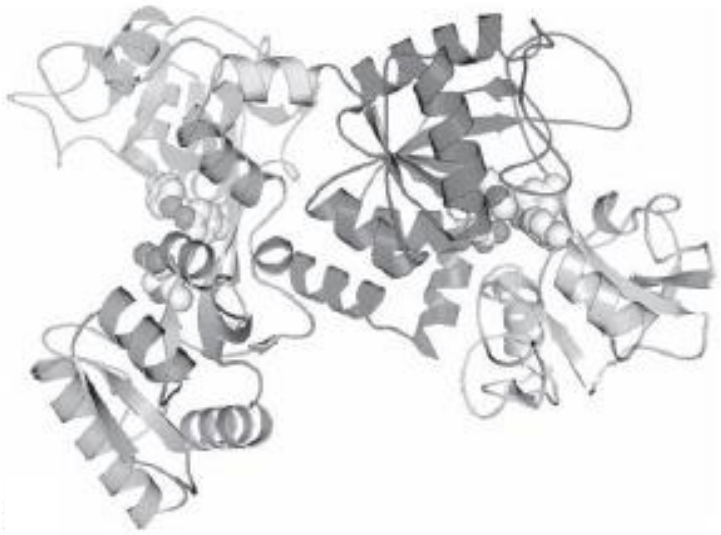

Figure 2.7 Structure of a typical globular protein in a minor component of milk proteins, lactoferrin. (adapted from: Edward *et al*., 2009)

2.1.3.4 Quaternary Structure

The protein quaternary structure refers to spatial arrangement of a protein containing several polypeptide chains, involving the non-covalent association of protein chains (Peterson & Johnson, 1978; Vaclavik, 1998). The protein chains may or may not be identical. Each protein chain is known as a subunit, and the quaternary complex is referred to oligomeric structure (Linnaeus, 2007). In other words, this oligomeric structure describes the way of several polypeptide chains come together to form a single function protein. Examples of quaternary structure include the casein micelles of milk and the actomyosin system of muscle (Vaclavik, 1998).

2.2 Types of Food Proteins

Most food products are multi-component materials with complex structure and texture (Chen & Dickinson, 1999). Proteins are one of the main classes of building blocks in food products after polysaccharides. Food proteins can be defined as those that are easily digestible, nontoxic, nutritionally adequate, functionally useable in food products and available in abundance (Damodaran, 1996). Examples of food proteins can be found in plant and animal sources; including cereals, legumes, oilseeds, milks, meats and eggs. The protein structures are varying in these proteins from plant or animal sources, which determine their functionality in food systems. Therefore, the relationship between the structure and the functionality serves as a challenge for food scientists in order to enhance or improve them to be used in various applications.

Plant proteins are generally less expensive than animal proteins, and yet they still provide beneficial amounts of protein. Legumes, including peas (Valencia *et al*., 2008), mung bean (El-Adawy, 2000), kidney bean (Yin *et al*., 2009) and etc., are examples of plant proteins that have been used as food ingredients in various food systems. Proteins from cereals have also been used in food application, e.g., wheat gluten (Gerrard *et al*., 2003), corn zein (Shukla & Cheryan, 2001), rice protein isolate (Agboola *et al*., 2005), etc. In addition, soy proteins are example of seed proteins that play an important role in wide range of worldwide. The use of soy proteins as functional ingredients is gaining increasing acceptance in food manufacturing from the standpoints of human nutrition and health since the 70's until now (Kinsella, 1979, Belleville, 2002).

Milk proteins are example of animal proteins that have been exploited in abundance. They are part of the milk transport whereby nutrients are passed from

mother to suckling offspring. One of the most useful forms of milk protein ingredients is sodium caseinate, due to its excellent emulsifying and emulsion stabilizing properties (Dickinson, 1999). Other than providing nutrients for growth, the milk proteins are also used as food ingredient (Horne, 2002). Apart from milk proteins, egg proteins and gelatins have been widely used in food system as functional ingredient (Fernandez-Diaz *et al*., 2000; Zhou *et al*., 2006). Gelatins are generally derived from animals or poultries such as cattle, pig, and fish.

2.2.1 Soy Protein Isolate

2.2.1.1 Sources and Utilizations

Soy protein is a commercially available plant source of protein that also is also a by-product derived from soybean oil industry. Despite the low oil content of the seed, soybeans are the largest single source of edible oil and account for approximately 52% of the total oil seed production of the world (Kumar *et al*., 2002). With each ton of crude soybean oil, approximately 4.5 tons of soybean meal (protein content ~ 44%) is produced. Initially, soy bean were planted abundantly in the USA to be used in animal feed (Horan, 1974; Kinsella, 1979). Soy flours, soy concentrates and soy isolated for food application were then produced since 1976, owing to their functionalities such as gelling, emulsifying, and foaming capacity.

Soy protein isolate (SPI) is the soy protein with the highest content of protein which is made from defatted soy meal by removing most of the fats and carbohydrates, yielding a product with 90 percent protein (Yamauchi *et al*., 1991). Table 2.1 shows the typical composition of soy proteins, in which SPI imparts the highest protein content. SPI are traditionally prepared from minimum heat-treated soy flour by dissolving the protein in dilute alkali (pH ~ 8.0), removing the insoluble

materials by centrifugation or filtration, and precipitation of the protein at pH 4.5. The protein curd can be dried or neutralized with alkali and spray dried (Kinsella, 1976). Recent study showed that SPI extraction with aqueous alcohol could remove objectionable flavour and colour-inducing components as well as markedly improved foaming and functional properties (Hua *et al*., 2005).

SPI represents a very important class of technological and functional ingredient that is being used in the food industry for nutritional, sensorial, gelling, hydration, surface and functional purposes to improve quality attributes of foods. It is used in adhesive, plastic, films, coatings, glazing agents and importantly as an emulsifier in foods (Schmidt *et al*., 2005). SPI contains all essential amino acids for growth that is equivalent in quality to the animal proteins in meat, milk, and eggs (Belleville, 2002). Several investigators have suggested that ingesting SPI may reduce the risk of coronary heart disease, regulate appetite/satiety, control weight, enhance immune defence, and prevent osteoporosis, some cancers, and menopausal symptoms (Albertazzi, 2002; Belleville, 2002; Jambrak *et al*., 2009).

Table 2.1 Composition of different soy protein products (dry basis)

Component	Soy Flours (%)	Concentrates (%)	Isolates (%)
Protein (as in)	48.0	64.0	92.0
Fat (min)	0.3	0.3	0.5
Moisture (max)	10.0	10.0	5.0
Fiber (Crude)	3.0	4.5	0.1
Ash	7.0	7.0	4.0
Carbohydrate	31-32	14-15	0.3

(adapted from: Kinsella, 1979; Kumar *et al*., 2002)

2.2.1.2 Physicochemical and Functional Properties

Approximately 90% of the proteins in soybeans are globulins, which exists as dehydrated storage proteins. There are 4 major protein fractions; 2, 7, 11 and 15S (Table 2.2); and these proteins components are classified based on their sedimentation properties. The dynamic functional properties of SPI in food are attributed to their protein structure, predominantly two major protein fractions which are *β*-conglycinin and glycinin (Neilsen, 1985a). Glycinin is a heterogeneous hexameric protein with a high molecular weight (MW) of 300–380 kDa (Neilsen, 1985). Its acidic (MW of 37–42 kDa) A_{1-4} subunits and basic B subunits (MW of 17–20 kDa) are linked by disulfide bridges. This covalent bond contributes to the stability of the molecular structure. In contrast, *β*-conglycinin is a trimetric glycoprotein with a MW of 150–200 kDa; it is composed of three different subunits in various combinations (*α'*, *α*, and *β*) connected by non-covalent interactions (Thanh & Shibasaki, 1979). These *β*-conglycinin and glycinin protein fractions are depicted in SDS-PAGE profile in Figure 2.8. The acidic amino acids (aspartic and glutamic acids) of soy protein, and their corresponding amides (asparagines and glutamins), non-polar amino acids (alanine, valine and leucine), basic amino acids (lysine and arginine), uncharged polar amino acid (glycine) and approximately 1% of cystine; are shown in Table 2.3.

Table 2.2 Approximate distribution of the ultracentrifuge fractions of water extractable soy proteins

Fraction	Content	Principal Components	Molecular Weight
2S	8	Trypsin inhibitors, Cytochrome	8,000 – 21,500 12,000
7S	35	Lipoxygenase, Amylase, Globulins	102,000 61,700 180,000 – 210,000
11S	52	Globulins	350,000
15S	5	Polymers	600,000

(adapted from: Kinsella, 1979)

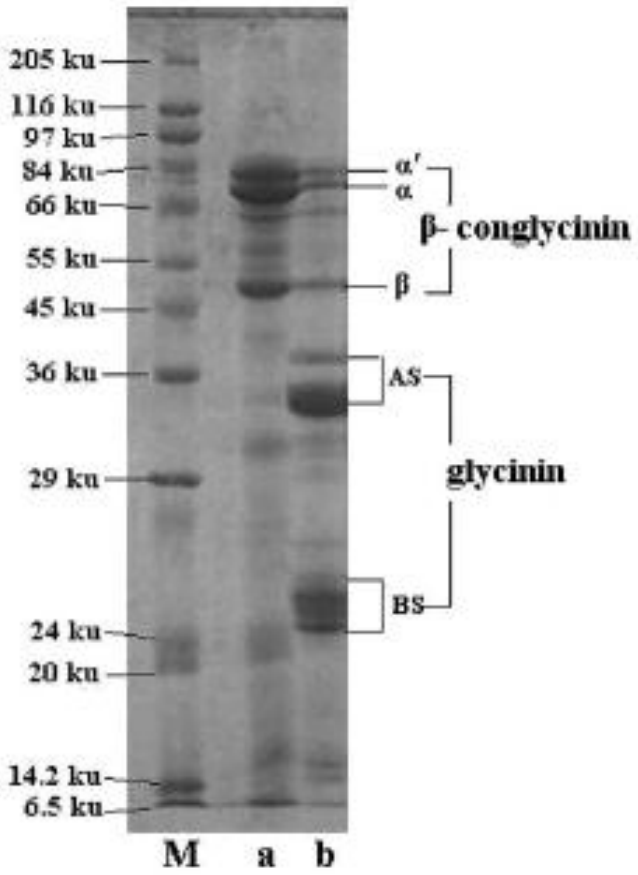

Figure 2.8 The SDS-PAGE patterns of glycinin-rich and *β*-conglycinin-rich SPIs. The lanes a and b indicate the *β*-conglycinin-rich and glycinin-rich SPIs. Lane M indicates the standard protein markers. (adapted from: Tang *et al*., 2006)

Table 2.3 Amino acid composition of soybeans

Amino Acid	Composition g/16 g nitrogen
Isoleucine	4.54
Leucine	7.78
Lysine	6.38
Methionine	1.26
Cystine	1.33
Phenylalanine	4.94
Tyrosine	3.14
Threonine	3.86
Tryptophan	1.28
Valine	4.80
Arginine	7.23
Histidine	2.53
Alanine	4.26
Aspartic acid	11.70
Glutamic acid	18.70
Glycine	4.18
Proline	5.49
Serine	5.12

(adapted from : Berk, 1992)

These protein fractions have two important properties: solubility and hydrodynamic properties. However, glycinin and *β*-conglycinin are easily denatured under some of the extreme conditions that are used during the commercial production of SPI (e.g., high temperature and acid precipitation) (Tang *et al*., 2009). Denatured protein forms aggregates or even precipitates and causes poor solubility, thus limiting the use of SPI in the food industry. Therefore, SPI has become the subject of study of many physical, chemical, and enzymatic modifications to improve the functionalities of this protein source (Chan & Ma, 1999; Babiker, 2000; Molina *et al*., 2001; Jambrak *et al*., 2009; Tang *et al*., 2009).

The expansion on world population places an emphasis on the need for proteins with multiple functional properties. Protein ingredients should have acceptable intrinsic properties like flavour, texture, and colour, good nutritional value

and the requisite functional properties for the variety of intended application (Kinsella 1979). The importance of these properties varies with the different applications, different food systems, and different products. The examples of functional properties of soy proteins are summarized in Table 2.4. However, in some actual applications, the functional properties of soy proteins represent the composite properties of the protein components. Some food products require good solubility of functional ingredient added (Table 2.5), e.g., beverages; while some food products require good emulsification properties, e.g., sausages, bologna, and salad dressings.

Table 2.4 Summary of functional properties of soy proteins in food applications

Properties	**Functional Criteria**
Organoleptic/kinesthetic	Color, flavor, odor, texture, mouthfeel, smoothness, grittiness, turbidity
Hydration	Solubility, wettability, water absorption, swelling, thickening, gelling, syneresis
Surface	Emulsification, foaming (aeration, whipping), protein-lipid, film formation, lipid-binding, flavor binding
Structural Rheological	Elasticity, grittiness, cohesiveness, chewiness, viscosity, adhesion, network-crossbinding, aggregation, stickiness, gelation, dough formation, texturizability, fiber formation, extrudability
Other	Compatibility with additives, enzymatic antioxidant

(adapted from: Kinsella, 1979)

Table 2.5 Functional properties of soy protein products in food

Functional Properties	Functions	Food System	Type of Soy Proteins[a]	Reference
Solubility	Protein solvation, pH dependent	Beverages	SPI, SPH	Achouri & Zhang, 2001; Murray & Mai, 2009
Water Absorption and Binding Capacity	Hydrogen-bonding and entrapment of water	Meats, Pasta	SF, SPC, SPI	Gujral *et al*., 2002; Limroongreungrat & Huang, 2007
Viscosity	Thickening, water binding	Juices, Beverages	SPI	Tiziani & Vodovotz, 2005; Murray & Mai, 2009
Gelation	Protein matrix formation and setting	Meats, tofu curds, pudding	SPC	Gujral *et al*., 2002; Lim & Narsimhan, 2006; Ting *et al*., 2009
Cohesion-adhesion	Acts as adhesive material	Meats, Pasta	SF, SPC, SPI	Gujral *et al*., 2002; Limroongreungrat & Huang, 2007
Elasticity	Disulfide links or cross-linking	Protein film	SPI	Lee *et al*., 2005
Emulsification	Formation and stabilization of fat emulsions	Meats, Salad dressing	SF, SPC, SPI	Gao *et al*., 2005; Chu & McMindes, 2007
Fat Adsorption	Binding of free fatty acids	Salad dressing	SF, SPC, SPI	Gao *et al*., 2005
Flavour-binding	Adsorption, entrapment, release	Simulated meats	SPI	Moon et al., 2007
Foaming	Forms stable films to entrap gas	Whipped Cream	SPC, SPI	Suzuki, 2008
Color Control	Bleaching of lipoxygenase	Breads	SF	Lucas & Riaz, 1995

[a] SF, SPC, SPI, SPH denote soy flour, soy protein concentrate, soy protein isolate, and soy protein hydrolysate; respectively.

2.2.2 Wheat Gluten

2.2.2.1 Sources and Utilizations

Wheat gluten is one of the more recent industrial proteins which is produced on an industrial scale and used for food and non-food application (Maningart *et al*., 1999). Wheat gluten is also an economically important co-product produced during wet processing of wheat flour in the recovery of wheat starch. However, gluten could not be considered as an industrial protein and was not being produced in large scale before 1970s (Hamer, 2003). The discovery of gluten was then led to an intensive study as a key constituent of wheat flour in bread making (Bietz & Lookhart, 1996; Hamer, 2003). According to Hamer (2003), a whole area of cereal research was initially dedicated to investigate the properties of gluten in relation to bread-making quality since there are large differences of protein quality between different wheat varieties. American and Canadian wheat were found consistently performing better than most European-grown wheat varieties. As a consequence, European millers and bakers have to rely on the imported high quality wheat from America and Canada to maintain a consistent quality of their products. The discovery of gluten isolated from European grown wheat could be used as an additive ingredient to be added in the wheat flour produced, and to replace the expensive imported wheat. Since then, gluten was being produced on an industrial scale, and becoming a high-value ingredient for the bakery industry. For this reason, approximately 600,000 tons of gluten are produced annually, with the European Union being the largest gluten producer (60-70% of the total production) of the world (Hamer, 2003).

Gluten is still extracted traditionally from flour by washing out the starch from flour (Hamer, 2003). This underlies that starch is cold-water-soluble while gluten is not; and gluten will bind together strongly during washing. On industrial scale, the separation is done by subjecting slurry of wheat flour with machinery vigorous stirring until the starch dissolves and the gluten consolidates into a mass (Tehtaat, 2007). The product is then collected by centrifugation. Consequently, the water in this wet gluten is removed by means of a screw press, and the residue is sprayed through an atomizing nozzle into a drying chamber. This process is only remained at an elevated temperature for only long enough to evaporate the water without causing protein denature. The end product yielded a flour-like powder with 7% moisture content before sifted and milled. Figure 2.9 shows schematically on the method of gluten isolation from the wheat grain.

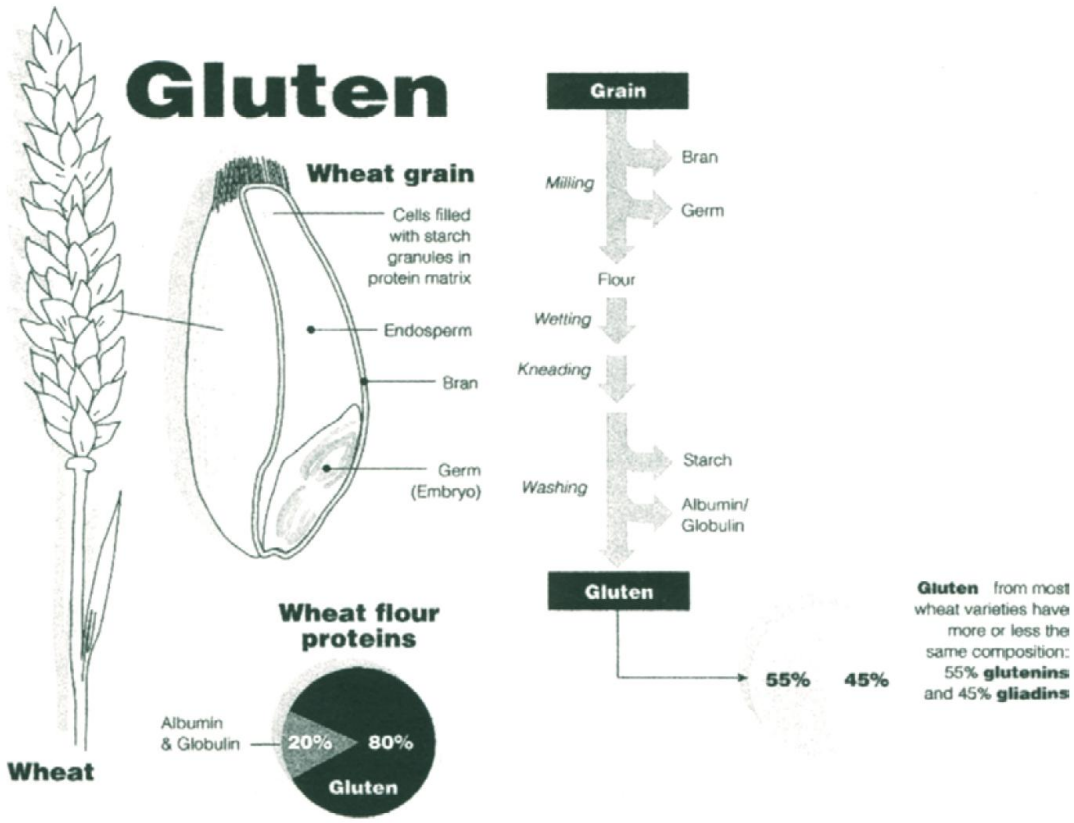

Figure 2.9 The origin and the production of gluten. (adapted from: Hamer, 2003)