PHYSICO-CHEMICAL CHANGES OF GLUTEN MATRIX AS A RESULT OF MAILLARD REACTION WITH GLUCOSE

by

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UNIVERSITI SAINS MALAYSIA

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Dedicated To

My reverend parents

Mohd. Ali Akkas & Mrs. Hamida Begum

And my parents-in-law

Mohd. Shahadat Hossain & Mrs. Anwara Begum

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF SYMBOLS AND ABBREVIATIONS

α	Alpha
β	Beta
γ	Gama
ω	Omega
δ	Delta
ε	Eita
T_d	Denaturation temperature
T_0	Onset temperature
T_{max}	Temperature at peak maximum
%	Percentage
S	Second
min	Minute
h	Hour
rpm	Revolution per minute
g	Gram
mg	Milligram
ml	Milliliter
µg	Microgram
µL	Micro liter
DNA	Deoxyribonucleic acid
LDL	Low density lipoprotein
GDL	Glucono-δ-lactone
DSC	Differential Scanning Calorimetry
MRPs	Maillard reaction products

BSA	Bovine Serum Albumin
ME	β - mercaptoethanol
SDS	Sodium dodecyl sulfate
SDS-PAGE	Sodium dodecyl sulfate – polyacrylamide gel electrophoresis
SPI	Soya protein isolate
G'	Storage modulus
M_r	Molecular weight
M_rs	Molecular weight subunits
Lys	Lysine
pI	Isoelectric point
GRAS	Generally recognized as safe
DHA	Dehydroascorbic acid
Conc.	Concentration
SD	Standard deviation
ANOVA	Analysis of Variance
ARP	Amadori rearrangement product
HMF	hydroxymethylfurfural
HMF^{one}	4-hydroxy-5-methyl-2,3-dihydrofuran-3-one

PERUBAHAAN FISIKO-KIMIA MATKRIKS GLUTEN YANG DISEBABKAN OLEH TINDAKBALAS MAILLARD BERSAMA GLUKOSA

Abstrak

Kajian yang dijalankan ini berobjektif untuk mendapatkan penjelasan tentang kesan interaksi gula-protein terhadap sifat-sifat fisiokimia matriks gluten. Kajian dijalankan dengan menggunakan Kalorimeter Penskanan Perbezaan (DSC), Penganalisa Tekstur, Kolorimeter dan spektrofotometer untuk memerhati kesan-kesan pelbagai gula (glukosa, xilosa dan sukrosa) terhadap protein gluten gandum semasa kejadian tindakbalas Maillard. Kajian 'DSC' menunjukkan bahawa suhu denaturasi termal bagi protein gluten gandum bertambah dengan kehadiran glukosa dan sukrosa dan ini mungkin disebabkan keupayaan gula untuk menstabil gluten gandum asal. Walaubagaimana pun protein gluten gandum yang mengandungi glukosa dan sukrosa tidak menunjukkan transisi pautan silang (eksotermik). Gel protein gluten gandum (WG) yang samada mengandungi gula atau tidak telah disediakan dengan memanaskan serakan ramuan dalam kaleng tertutup, di dalam autoklof berskala makmal pada 121 ^{o}C selarna 30 minit, diikuti dengan pengolahan pada 4^{o}C selarna 18 j. Tindakbalas Maillard menyebabkan pertukaran warna yang signifikan ($p<0.05$), pengurangan pH dan peningkatan pemerangan. Dalam proses ini kehilangan lisina tersedia (~48%) dan kehilangan glukosa (~62%) menunjukkan yang kedua-dua ini terlibat di dalam tindakbalas, sebaliknya tiada kehilangan sukrosa mencadangkan yang sukrosa tidak terlibat dalam tindakbalas. Pembentukan gel gluten gandum

dengan glukosa (WG-G) menunjukkan sineresis yang paling kurang jika dibandingkan dengan gel yang mengandungi sukrosa (WG-S) dan tanpa apa-apa gula (WG). Gel WG-G juga menunjukkan daya pemegangan air (WHC) yang tinggi berbanding gel WG-S dan WG. Sineresis dan WHC yang bertambah baik bagi gel WG-G mungkin disebabkan oleh net cas yang lebih tinggi di molekul protein hasil dari tindakbalas Maillard. Sifat-sifat reologi gel protein gluten gandum dikaji dengan menggunakan 'Texture Analyser'. Ujian-ujian stres-rehat bagi gel dalam mod pemampatan diukur dan respons dianalisa menggunakan persamaan Peleg. Parameter-parameter dalarn persamaan ini, keelastikan gel (K_1 dan K_2) menunjukkan yang ia sangat bergantung kepada system gula. Gel WG-G lebih elastic dari gel-gel WG-S dan WG. Berikutan ini daya pemecahan gel dan modulus residual taksimptotik bagi gel WG-G adalah lebih tinggi dari gel-gel WG-S dan WG. Adalah dicadangkan bahawa gel WG-G yang terbentuk oleh haba (Maillard Gel) bertambah kekuatan gel dan ciri-ciri viskoelastik secara signifikan ($p<0.05$) yang mungkin mengandungi gabungan silang (gabungan silang Maillard) dalam jaringan gel. Kajian keterlarutan protein dalarn larutan pemecah (2% natrium dodesil sulfat + 2% β - mekkapto-etanol) menunjukkan keterlarutan gel WG-G menurun dengan peningkatan kepekatan glukosa, kemungkinan ia disebabkan oleh pembentukan ikatan kovalen dalam jaringannya.

PHYSICO-CHEMICAL CHANGES OF GLUTEN MATRIX AS A RESULT OF MAILLARD REACTION WITH GLUCOSE

ABSTRACT

The present study was undertaken with the objective of elucidating the effect of sugar-protein interactions on the physicochemical properties of gluten matrix. Studies were conducted using Differential Scanning Calorimetry, Texture Analyzer, Colorimeter and Spectrophotometer to observe the effects of various sugars (glucose, xylose and sucrose) on the wheat gluten proteins during the Maillard reaction. The DSC studies revealed that the thermal denaturation temperature of wheat gluten proteins increased in the presence of glucose and sucrose, and this was probably due to their (the sugar) ability to stabilize the native wheat gluten. However, wheat gluten proteins containing glucose and sucrose did not show any cross-linking (exothermic) transition. Wheat gluten protein (WG) gels with or without reducing sugars were prepared by heating the dispersions in sealed cans in a laboratory size autoclave at 121^{o}C for 30 min., followed by curing treatment at 4 ^{o}C for 18 h. The Maillard reaction caused a significant ($p< 0.05$) change in colour, a decrease in pH and increase in browning. In the process the loss of available lysine (~ 48%) and loss of glucose (~ 62%) showed that these were implicated in the reaction, on the other hand there is no loss of sucrose suggesting that sucrose was not implicated in the reaction. Gelling of wheat gluten with glucose (WG-G) showed much less syneresis compared to that with sucrose (WG-S) and without any sugar (WG). The WG-G gels also showed higher water holding capacity (WHC) in

comparison to that of WG-S and WG gels. The improved syneresis and WHC of WG-G gel may be attributed to the higher net charge on the protein molecules as a result of the Maillard reaction. Rheological gel properties of wheat gluten protein gels were studied by Texture Analyzer. The stress relaxation experiments of the gels in compression were measured and the response analyzed using Peleg's equation. The parameters in this equation, the gel elasticity (K_1 & K_2) showed strong dependency on the sugar systems. The WG-G gels were more elastic than the WG-S & WG gels. It follows that, the gel break strength and asymptotic residual modulus of the WG-G gels were higher than those of the WG-S & WG gels. It was suggested that WG-G heat-induced gel (Maillard gel) had a significant ($p< 0.05$) improvement in the gel strength and viscoelastic properties which may contain additional nondisulphide covalent crosslinks ("Maillard cross-links") within the gel network. Studies on protein solubility in disrupting solvent (2% sodium dodecyl sulphate + 2% β-mercapto-ethanol) revealed that the solubility of WG-G gels decreased with increasing glucose concentration, probably due to the formation of additional covalent bonds in their network.

CHAPTER 1

INTRODUCTION

1.1 Background and significant implication of study

The animal proteins (milk and egg) are now being replaced to plant base proteins by manufacturers of food items due to the consumers' attitude as well as the economic reasons. Gluten and soy protein are extensively being used as basic components for vegetarian food products especially in many Asian countries. Wheat gluten protein is an important raw material in the manufacture of foods for breakfast, infant, snack and pasta products. Gluten, which is a mixture of more than 100 heterogeneous polypeptides, is composed of two main storage proteins, namely, Gliadins and glutenins. Glutenins (with molecular mass of 69 to 88 kDa based on SDS-PAGE) (Anderson *et al.,* 1988) are responsible for elastic behavior, whereas gliadins (with molecular mass of 30 to 50 kDa) (Tatham *et al.,* 1990) are responsible for viscous flow properties of the foods.

The most significant aspect of gluten story for the food industry is the importance and the potential of gluten as a commodity, sold for a wide range of uses around the world. 'Vital Wheat Gluten' protein is now a significant ingredient in the food industry and important item of world trade (Krishnakumar & Gordon, 1995; Boland *et al.,* 2005). Its rheological properties are the basis of the functional uses of vital gluten (Day *et al.,* 2006). It is these properties that permit breads, cakes, biscuits and noodles to be made from wheat-flour doughs. Thus, gluten can be considered to be like a dough in which the diluting

effect of starch is no longer present. In the wet state, the protein molecules form a cohesive matrix which, in dough, also holds the starch granules within it. This matrix is also elastic, allowing it to stretch and expand. In aerated doughs, this elasticity permits the expansion of gas bubbles, which produce the texture of bread and cakes (Day *et al.,* 2006).

The nonenzymatic interaction between reducing sugars with amino groups of the lysine residue of proteins, known generally as the Maillard reaction, has proven to be extremely important in food science. Actually it is a group of complex reactions which results in the formation of both large protein aggregates and low molecular weight products that are believed to impart the various flavour, aroma, and colour characteristics of foods (Sun *et al.,* 2004). Over the past few years there has been growing interest in the interaction of reducing sugars and protein to understand structural functionality in compositionally complex food systems (Aoki *et al.,* 1999; Aoki *et al.,* 1997; Morgan *et al.,* 1999). It has been reported that the glycated proteins could improve the functional properties of food, such as thermal stability, emulsifying ability, foaming properties (Kato *et al.,* 1993; Kato *et al.,* 1988; Kato *et al.,* 1995), antioxidative activity (Nakamura *et al.,* 1992; Sun *at al.,* 2004; Benzakul *at al.,* 2005a, 2005b), and gelling properties (Easa *et al.,* 1996b; Matsudomi *at al.,* 2002; Sun *at al.,* 2004; Yamul & Lupano, 2005).

The Maillard reaction may be desirable as in baked, fried or roasted foods or undesirable as in concentrated and dried foods. The Maillard cross-linking in protein can have a profound effect on the structure and function of proteins in

food. Nevertheless, the importance of protein cross-linking in food systems is less well studied, but it is clear that such specific modifications of the properties of a protein are, potentially, of great practical importance in the food industry. The protein gels prepared in the presence of reducing sugar are shown to have changed texture, reduced solubility, and enhanced gelation, due to change in pH, charge on the protein and critical protein concentration that are responsible for gel formation.

Thus it is possible to modify the properties of wheat gluten gel through Maillard reaction in such a way that the "Maillard cross-links" are allowed to form within the gel network. The enhanced physicochemical properties of wheat gluten gel coupled with antioxidative properties will be of sufficiently high commercial values.

1.2 Hypothesis

The Maillard reaction taking place during heating of the wheat gluten dispersion containing reducing sugar may induce changes that are important in the subsequent functional properties of the protein. The induced changes due to the glycation of amino acid (lysine) side chains with glucose allow the generation of brown colouration which coupled with the formation of additional non-disulfide crosslinks within the gel matrix may improve some physicochemical properties of the gluten gel matrix. The enhanced properties of the gel matrix could be exploited for a good use in food processing technology.

1.3 Objectives

The main objective of this project is to investigate the possible use of Maillard reactions to enhance the functional properties of wheat gluten protein. Several specific objectives can be outlined below:

a) To develop a method or protocols for preparation of gluten gels containing reducing sugar that undergo the Maillard reaction during retort heating.

b) To investigate the occurrence of the Maillard reaction in the gluten gel matrices as a function of sugar concentrations.

c) To investigate the contribution of lysine and glucose on the improvement of gluten gel matrix.

d) To investigate the contribution of sucrose and gluten matrix (prepared without any sugar) as control for the comparison of study.

e) To investigate the contribution of the SH groups and SS bonds on the improvement of gluten gel matrix as a function of sugar concentrations.

f) To investigate the occurrence and contribution of the additional "Maillard Cross-links" on the improvement of the gluten gel matrix.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Gels and foams provide traditional and novel structure in many foods. The ability to gel is an important function of proteins in food systems. Many proteins in food have the ability to form gels and provide structure. These include proteins of meat, milk, eggs, and soy which give structure to the products such as yogurt, gelatins, omelets, and surimi. The acceptability of many foods relies on their gel formation capacity that is determined by the protein's ability to bind water (Schnepf, 1989; Alting *et al.,* 2004; Avanza *et al.,* 2005). Protein gels can be formed in several ways but perhaps the most common method occurs by heating the solutions of protein, because that is the most frequently used process in the food industry to obtain the safe products with a prolonged shelf life. Heat is also used to improve the sensory properties of food. However, it may also cause changes that decrease food quality. Many desired as well as undesired effects of heating are due to the Maillard reaction (Martins *et al.,* 2000). The gels formed in such processes are studied and discussed in the present thesis.

2.2 Definition of terms

The terms *denaturation, gelation, coagulation, association, aggregation* and *precipitation* are often used to describe changes to protein upon heating. It is

important to understand and differentiate between these terms. Upon heating, protein molecules may undergo full or partial *denaturation*. The dissociated molecules may then associate and hence form a coagulate*, precipitate,* or *gel* depending upon the conditions of heating.

Denaturation refers to any process which causes a change in the three-dimensional structure of the native protein which does not involve rupture of peptide bonds. Protein-solvent interaction may be involved as well as changes in the physical properties of the protein (Schmidth, 1981).

Aggregation refers to protein-protein interactions which result in the formation of complexes of higher molecular weight (Mulvihill & Kinsella, 1987).

Coagulation is the random aggregation of already denatured protein molecules in which polymer-polymer interactions are formed over polymer-solvent reaction (Mulvihill & Kinsella, 1987).

Protein *gels* are defined as the three-dimensional network in which polymer-polymer and polymer-solvent interactions occur in an ordered manner resulting in the immobilization of large amounts of water by a small proportion of protein (Mulvihill & Kinsella, 1987; Schnepf, 1989). Gelation may be induced by heat or divalent cations.

In gelation, polymer-polymer and polymer-solvent interaction as well as attractive and repulsive forces are balanced (Gossett *et al.,* 1984). *Gelation* differs from *coagulation* and *aggregation* in that a well ordered matrix is formed.

Gelation is thought to proceed by a two step mechanism. First, protein begins to unfold which changes its conformation. This may be followed by *aggregation* of the protein. In the second step, which proceeds more slowly, the denatured protein molecules orient themselves and interact at specific points forming the three-dimensional network (Gossett *et al.,* 1984; Mulvihill & Kinsella, 1987).

The rate of the second step is critical and may determine some of the characteristics of the *gel.* If the second step is slow, the protein polymer will form a fine network. The *gel* will be less opaque, more elastic, and exhibit less syneresis. If step two is fast, a coarser network will be set up and the *gel* will be opaque with more solvent expressed (Gossett *et al.,* 1984).

Many complex interactive forces will determine whether a protein will form an *aggregation, coagulum,* or a *gel.* For a protein to *gel,* there must be a balance between attractive and repulsive forces. *Coagulation* occurs if excessive attractive forces dominate and, likewise, no gel would be formed if excessive repulsive forces are present. The types of forces that hold a gel together include hydrophobic interaction, hydrogen bonding, electrostatic interaction, and disulfide crosslinks or thio-disulfide interchange (Mulvihill & Kinsella, 1987). Protein *association* reactions generally refer to changes occurring at the molecular or subunit level while *aggregation* reactions generally involve the formation of higher molecular weight complexes from *association* reactions. At extreme conditions (i.e. rapid heating or change in pH) protein molecules may not have time to interact sufficiently and these results in less hydrated *aggregates* which *precipitate.*

2.3 Classification of gels

There are two types of gels, the polymer network and the aggregated dispersion.

(a) *Polymer network*

Polymer networks are formed by gelatin and polysaccharides such as agarose and carrageenan. The network is formed by aggregation of disordered chains with regions of local order. These gels are characterised by their low polymer concentrations, fine texture and transparency. They may be formed by a variety of methods including pH adjustment, ion addition and heating or cooling. Gelatin is perhaps the most useful and versatile protein gelling agent in this category (Dickinson & Stainsby, 1982).

(b) *Aggregated dispersion*

Aggregated dispersions are usually formed following heating and denaturation of globular proteins. These gels are characterised by their higher polymer concentration (5-10 %), which is an order of magnitude greater than that of the polymer network gels.

2.4 Gelling systems in this thesis

In this thesis the studies are reported on Maillard gelation of wheat gluten. It is water insoluble globular protein (Mertz, 1967; Belitz & Grosch, 1999; Mehas &

Rodgers, 2002.). In the following paragraphs gelation due to aggregated dispersion is discussed in detail.

2.5 Factors affecting the gelation of globular proteins

The quality of the gels produced by heating a protein solution may depend on features that are associated with the protein, the solvent and the method used to produce the gel and, of course, a combination of these factors. Some of the relevant factors of the said gel are as follows:

A protein network including the tertiary structure of individual polypeptides, is generally formed via non-covalent cross-links such as hydrophobic interactions, hydrogen bonds or electrostatic interactions, and less frequently by covalent interactions such as disulphide bonds. The relative contribution of each type of bond to a gel network varies with the properties of protein and environmental conditions (Smith, 1994). The physical integrity of the gel is maintained by the counter balanced attractive and repulsive forces between the protein molecules. The gelling mechanism is determined by this balance and protein-solvent interactions (Hermansson, 1979; Cheftel *et al.,* 1985; Ziegler & Foegeding, 1990; Kinsella *et al.,* 1994; Matsumura & Mori, 1996; Zayas, 1997).

These protein-protein and protein-solvent interactions are influenced by factors that affect protein gelation, as well as affecting the type and properties of gels (Hermansson, 1979; Kinsella *et al.,* 1994). These factors can be classified (Phillips *et al.,* 1994), as intrinsic and extrinsic, and are listed in Table 2.1.

Table 2.1: Factors affecting the interactions in protein gel formation (Phillips *et al.,* 1994)

Intrinsic factors	**Extrinsic factors**
Electrostatic Interactions	Protein concentration
Disulphide bonds	pH
Molecular weight	Temperature
Amino acid composition	Ionic strength and type of Ion
Hydrophobicity	Pressure

Intrinsic factors are related to the protein *per se*, and are:

a) *Electrostatic interactions:* The net charge of the protein molecule is modified by attractive and repulsive forces, affecting protein-protein and protein-solvent interactions (Phillips *et al.,* 1994). These electrostatic interactions are promoted by changes in ionic strength or pH.

b) *Disulphide bonds and thiol-disulphide interchange:* Covalent disulphide bonds among polypeptide chains involved in protein gelation increase the apparent chain length of the polypeptide, rather than acting as an initial network stabilizer (Clark & Lee-Tufnell, 1986). Disulphide bonds are not essential for gelation of proteins, but their role in gelation is related to their ability to increase the weight-average molecular weight and hence the chain length (Wang & Damodaran, 1990).

c) *Molecular weight:* Variations in the formation *of* a self-supporting gel network, i.e. variations in gel strength, could be related to differences in the weight-average molecular weight and the hydrodynamic size of the

polypeptide species in the gel. The polypeptide critical molecular weight for gel formation is about 23 kDa (Wang & Damodaran, 1990).

d) *Amino acid composition:* Proteins that contain less than 31.5% of hydrophobic residues such as valine, proline, leucine, isoleucine, phenylalanine and tryptophane form a coagulum-type gel, whereas proteins containing above 31.5% hydrophobic residues form a translucent gel (Shimada & Matsushita, 1980).

e) *Hydrophobicity:* Non-polar amino acids are grouped, forming a hydrophobic nucleus surrounded by a polar residue layer in contact with the solvent water, which plays an important role in protein organization and should be taken into account in any protein-folding consideration (Mierovich & Scheraga, 1980).

Because of the propensity of nonpolar amino acid residues to position themselves in the interior of protein molecules in solution, thus avoiding contact with the aqueous surrounding, only a portion of them could be considered as being effective in hydrophobicity. Effective hydrophobicity refers to the value representing the hydrophobicity of protein effectively involved in the interactions between proteins with the surrounding medium (Keshavarz & Nakai, 1979).

The extrinsic factors are the environmental conditions surrounding the proteins. These can be relatively controlled in several ways to achieve a good gel formation:

a) *Protein concentration:* The cross-linking of macromolecules of an arbitrary initial size distribution is required for gelation and is proportional to the protein concentration. There must also be a minimal concentration of the protein itself, below which a continuous three-dimensional structure cannot be formed (Ferry, 1948). Gel strength and deformability is highly dependent upon protein concentration (Samejima *et al.,* 1986; Hongsprabhas & Barbut, 1997).

b) *pH:* The net charge of protein at its isoelectric point is zero but it get charged when away from its isoelectric point. Therefore, the greater the net charge on the protein molecule, the greater the electrostatic repulsion between molecules, preventing the interactions required to form a matrix (Cheftel *et al.,* 1985; Hermansson, 1979; Kinsella *et al.,* 1994; Zayas, 1997).

c) *Temperature:* Temperature is one of the most important factors because it is a driving force to unfold protein domains. When the gelling temperature coefficient is high, the first gelation step (denaturation) is completed faster than second step (aggregation). For a given rate of denaturation, the rate of aggregation is slow if the attractive forces between the denatured protein chains are small, resulting in a fine network and a translucent gel (Ferry, 1948). Consequently increasing temperature will improve a fine network formation because the peptides will aggregate to form the gel network during cooling (Pomeranz, 1991).

d) *Ionic strength:* Ionic strength has a significant effect on water absorption, swelling and solubility of proteins, due to the formation of competitive linkages (Borderias & Montero, 1988). Ionic strength has an effect on the microstructure

of the gel matrix, where at low ionic strengths (< 0. 1M) of monovalent cations a fine-stranded matrix is formed. On the other hand at ionic strengths > 0.1M the matrix becomes mixed (Foegeding *et al.,* 1995).

e) *Type of salt:* Chloride monovalent ions (Li+, K+, Rb+, Cs+) form a fine stranded matrix at ionic strengths less than 0.1 M. The salt concentration required to change gel microstructure depends on the salt's position in the Hofmeister series. Matrix formation also occurs when the protein suspension contains low concentrations *(10-20* mM) of divalent cation (Ca^{2+} , Mg^{2+}, Br^{2+}) chlorides at pH 7.0 (Foegeding *et al.,* 1995).

f) *Pressure:* Pressure affects the sol-gel transition of protein solutions. High pressure modifies the native volume of proteins, which is due to three contributions, (a) volume of constituent atoms (compositional volume), (b) volume of internal cavities, (c) contribution due to solvation. The native structure, that governs the biological activity of proteins, is a delicate balance between stabilizing and destabilizing interactions within the polypeptide chain and with the solvent (Balny & Masson, 1993). Changes in volume caused by pressure will affect these balances (Smith *et al.,* 2000*).*

Gel-induction:

Gelation is a phenomenon, therefore its definition and the gel formed depends on the observer's perspective and the technique(s) used to evaluate it (Ziegler & Foegeding, 1990). A simple definition could be that protein gelation is an

aggregation of denatured molecules with a certain degree of order, resulting in the formation of a continuous network (Wong, 1989). Gelation is basically a two step process: denaturation and aggregation (Kinsella *et al.,* 1994; Matsumura & Mori, 1996). Totosaus *et al.*, (2002) has given a list of the physical and chemical means to induce protein gelation that are listed in Table 2.2**.**

Table 2.2: Physical and chemical means to induce protein gelation (Totosaus *et al.,* 2002)

Physical	Heat	Native protein partially unfolded by heat to form a network. Ordered matrix, by aggregation of the molecules.
	High pressure	Pressure (200-500 MPa) induces hydrophobic interactions and disulphide bonds between protein molecules, resulting in a rearrangement gel structure.
Chemical	Ion	After initial heating and salt addition, electrostatic repulsion or charges are shielded, forming a gel. Disruption of secondary structure induces a hydrophobic effect.
	Urea	Urea promotes intermolecular thiol-disulphide oxidation of thiol groups, resulting in a network formation.
	Acid	Slow pH reduction allows denaturation to form clusters or aggregates. These fractal clusters may be considered as the building blocks of the gel.
	Enzymatic	Enzyme catalyses cross-linking between protein glutamine residues to form a gel structure.

Heat-induced gelation:

Heat induced gelation is the most commonly studied phenomenon in food science, mainly because it is responsible for the structure of many everyday heat-set foods.

Ferry (1948), proposed a two step mechanism for protein gelling: firstly, the unfolding or dissociation of protein molecules provoked by heat, followed by the second step in which the association and aggregation reactions resulted in a gel system, this would only form in the presence of adequate environmental conditions.

In this manner, proteins progressively pass from a native state to a denatured or unfolded transition and then to an aggregated network that forms a sol state, that eventually reaches the final rigid gel state. It is important that the rate of the second step remains lower than the first one, because protein aggregation will then be ordered enough to allow gel formation (Schmidt, 1981; Damodaran, 1989; Kinsella *et al.,* 1994; Aguilera, 1995). Upon heating, a marked increase in the effective hydrophobicity is an indication of protein unfolding, and when too many hydrophobic sites are exposed then interactions are inevitable between the exposed hydrophobic sites causing aggregation of protein molecules (Nakai, 1983). Figure2.1 represents a schematic diagram of some propositions for heat-induced gelation of globular proteins (Shimada & Matsushita, 1980; Foegeding *et al.,* 1986; Damodaran, 1988, 1989; Oakenfull *et al.,* 1997).

Heating rate and/or time of heating affect the unfolding and appear to influence the kind of protein formed (Foegeding *et al.,* 1986). Excessive heating of the protein sol to a degree far higher than needed for denaturation leads to a metasol state which does not set into a gel upon cooling (Damodaran, 1989; Oakenfull *et al.,* 1997). This may be related to β-elimination of disulphide bonds and scission of peptide bonds, which involves aspartate residues at high

temperatures (Damodaran, 1989). During cooling, the unfolded proteins can adopt a refolded conformation. Partial refolding of the protein would decrease the availability of the number of functional groups for intermolecular cross-linking and thus prevent formation of a self-supporting gel network (Damodaran, 1988).

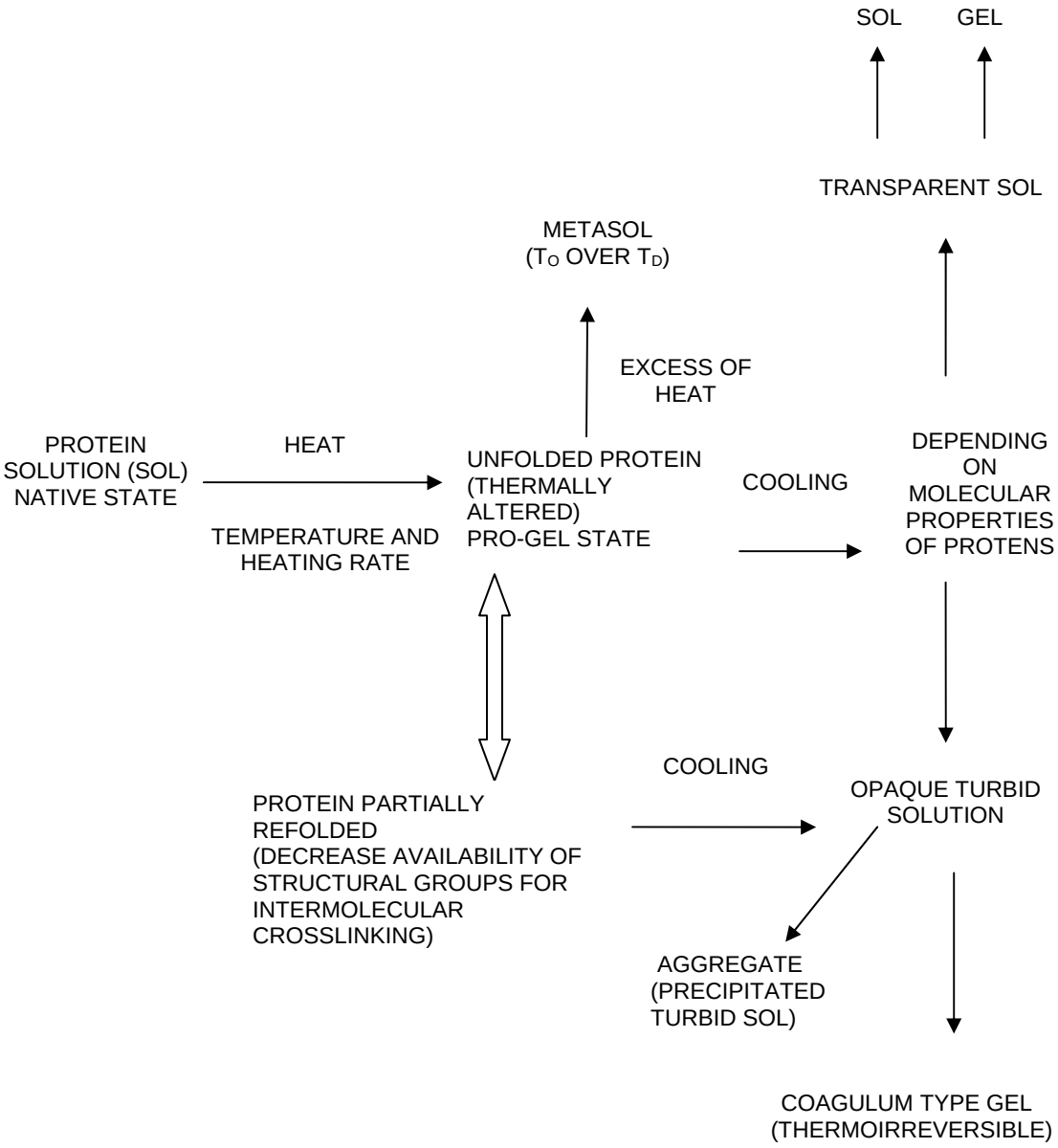

Figure 2.1: Schematization of heat-induced gelation of globular protein.
T_0 = temperature, T_D = denaturation temperature (Totosaus *at al.,*2002).

Depending upon the molecular properties of the protein in the unfolded state, it undergoes either of two types of interactions: proteins that contain high levels of apolar amino acid residues undergo hydrophobic aggregation, resulting in a coagulum type gel. On the other hand proteins that are below a critical level of apolar amino acid residues form soluble aggregates, set into a translucent type gel (Damodaran, 1989). The protein solution becomes opaque if low molecular weight and low protein concentration conditions produce an aggregate, while in conditions of high molecular weight and high protein concentration (because of protein chain entanglement), it forms a coagulum (thermo-irreversible gel). On the other hand, a transparent protein solution remains in the sol state under conditions of low molecular weight and low protein concentration, but forms a gel when cooled if the molecular weight and protein concentration are high (Shimada & Matsushita, 1980).

2.6 Study of the Maillard reaction

The Maillard reaction is important in foods containing reducing sugars and protein. The Maillard reaction has played an important role in improving the appearance and taste of foods during cooking. It has been a central and major challenge in food industry, since the Maillard reaction is related to aroma, taste and colour, particularly in traditional processes such as the roasting of coffee and cocoa beans, the baking of bread and cakes, the toasting of cereals and the cooking of meat. Moreover, during the Maillard reaction a wide range of reaction products are formed with significant importance for the nuttritional value in foods.This can be reduced by decrease of digestibility and possible formation of toxic and mutagenic compounds, but can also be improved by the

formation of antioxidative products (Martins *et al.,* 2000). The Chemistry underlying the Maillard reaction is very complex. It incompasses not one reaction pathway but a whole network of various reactions. The original comprehensive reaction scheme of Hodge (1953) (Fig.2.2) has been developed and elaborated by food technologists ever since, so the understanding of the reaction is advancing steadily.Nevertheless the extent of Maillard reaction is notoriously difficult to control.Various factors involved in food processing influence it and they can be considered as food processing variables.

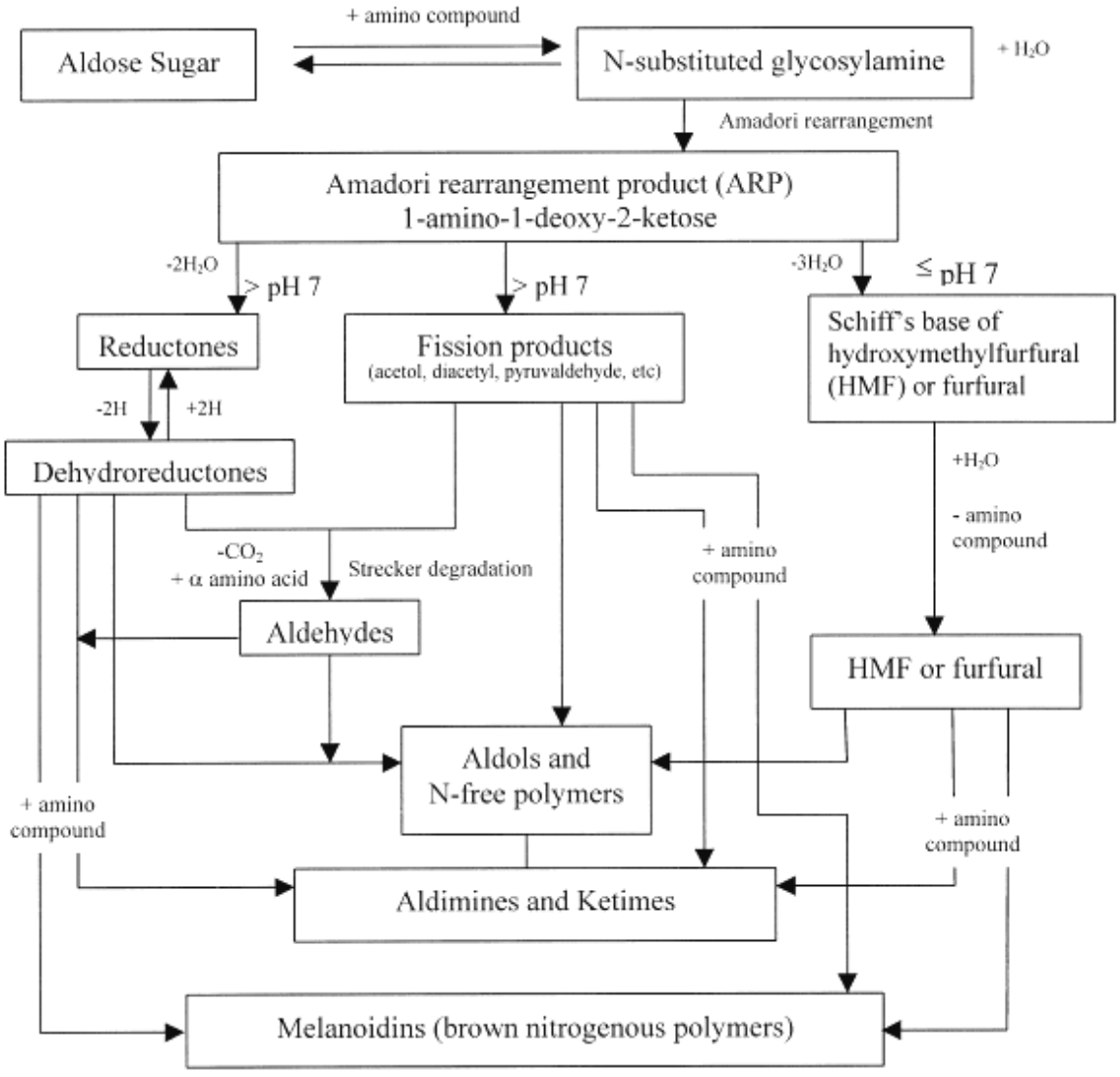

Figure 2.2 : Maillard reaction scheme adapted from Hodge (1953)

The studies are often associated with products of low water activity. Studies to elucidate the mechanisms and the chemical components are normally carried out on a model system consisting of a single amino acid and a reducing sugar(Fig.2.3). However, it is known that even in these simple systems many compounds are being generated via the reactions (Apriyantono & Ames, 1993).Similarly it is expected that many of these compounds can also be generated when a protein, such as wheat gluten is reacted with a reducing sugar(Gerrard *et al.,* 2003a). It might be expected that the compounds formed in the Maillard reaction may then interact with the protein changing thereby the subsequent behaviour of the protein.

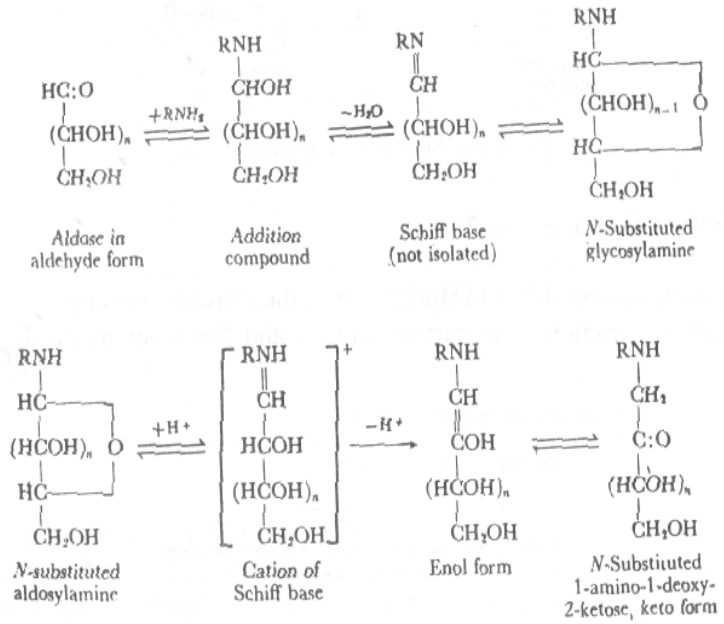

Figure 2.3: Molecular events in the initial stages of the Maillard reaction (Finot *et al.,* 1977; Friedman,1982)

2.6.1 Maillard reaction in wheat gluten

Wheat is unique amongst the cereals in that its flour, when mixed with water, forms viscoelastic dough capable of producing bread upon baking (Bushuk, 2000). The functional properties of dough are largely attributed to the

characteristics of the endosperm storage proteins in the flour, often referred to as gluten proteins (Wrigley *et al.,* 2000). The crosslinking of these glutens has an influence on the properties of the dough and the subsequent baked product (Gerrard *et al.,* 2003a).

Traditionally, only type of protein crosslinking that was considered in the context of wheat dough was disulfide bonding (Kaufman *et al.,* 1986). However, previous work in this laboratory has established that non-disulfide crosslinks, specifically those introduced by the enzyme transglutaminase, can also influence dough properties (Gerrard *et al.,* 1998b; Gerrard *et al.,* 2000; Gerrard *et al.,* 2001). Tilley has also espoused the importance of dityrosine crosslinks in dough (Tilley *et al.,* 2001). Thus it seems that the chemical nature of the protein crosslink itself is not important, and novel methods to introduce crosslinks have the potential to improve cereals processing and quality of cereal food products.

The Maillard reaction is known to result in protein crosslinking, and yet its effects on wheat proteins, and whether these have a functional influence on dough, have received little attention (Gerrard *et al.,* 2003b). Gerrard *et al.,* (2003a) explored the potential of glutaraldehyde, formaldehyde and glyceraldehyde as crosslinking agent for wheat proteins. The crosslinking experiments were performed both in vitro, on purified wheat proteins, and in situ in the dough itself, in order to assess the differential reactivity of the compounds in the test tube and within an actual foodstuff.

The addition of glutaraldehyde and formaldehyde to dough increased relaxation times compared to the controls, containing no additive. This is

indicative of an increase in dough development, presumably due to the introduction of additional crosslinks to the gluten network, via the Maillard reaction (Gerrard *et al.,* 2003b). This corroborates earlier work (Gerrard *et al.,* 1998a; 2000), and contributes to the growing evidence that crosslinks of any chemical nature are extremely important in dough development. The fact that the improving effect of glutaraldehyde on dough development was more substantial than ascorbic acid, suggests that the Maillard reaction, suitably harnessed, could have great potential as a mechanism for dough improvement. Whether sufficiently reactive, food-allowed, molecules, that can undergo protein crosslinking chemistry via Maillard reactions, can be generated in situ remains to be elucidated. However, the results described herein provide proof in principle, that such molecules could be used to manipulate the properties of food (Gerrard *et al*., 2003b).

Use of glutaraldehyde in dough helps in making loaves of smaller size, with good crumb texture and improved crumb strength. It shows that the crosslinks produced by glutaraldehyde, strengthen the gluten network, providing good crumb strength, but reducing the ability of the gas cells to expand during proving and baking (Gerrard *et al*., 2003b).

Breadmaking involves three steps: dough-mixing (flour, water, yeast, and salt), dough fermentation, and baking. During the baking process, the starch is gelatinized and the proteins denatured at an internal temperature of 60-80 °C and then the raw dough is transformed into a light, porous, and readily digestible product (Erbersdobler & Hupe, 1991; Ramirez-Jimenez *et al.*, 2000).

The chemical reactions involved in this process are essentially the Maillard reaction and caramelisation (degradation of sugars). The Maillard reaction is favored in foods with a high protein and carbohydrate and an intermediate moisture content at temperatures above 50 °C and at a pH of 4-7 (Kroh, 1994), producing changes in colour (melanoidins), flavor (aldehydes and ketones), functional properties, and nutritional value (blocking or destruction of lysine) (O'Brien & Morrisey, 1989; Reineccius, 1990). Caramelization needs more drastic conditions (temperatures >120 °C, pH < 3 or pH > 9, and low a_w) (Kroh, 1994). The water content distribution and temperature play an important role in developing the sensory characteristics of these products. During baking, the water content on the surface of the loaf becomes lower than in the middle and this, combined with the high temperature, is one of the factors that makes the crust different from the crumb (Thorvaldsson & Skjöldebrand, 1998).

The early stages of the Maillard reaction can be evaluated by the determination of the furosine (*ε-N*-(furoyl-methyl)-L-lysine) amino acid. The later is formed during acid hydrolysis of the Amadori compounds fructosyl-lysine, lactulosyl-lysine, maltulosyl-lysine produced by reaction of *ε*-amino groups of lysine with glucose, lactose and maltose (Erbersdobler & Hupe, 1991). Furosine determination has been used in cereal to control the processing of pasta (Resmini & Pellegrino, 1994), bakery products (Henle *et al.,* 1995), baby cereals (Guerra-Hernandez *et al.,* 1999), and toasted sliced bread (Ramirez-Jimenez, 1998).

2.7 Chemistry of the Maillard reaction

The Maillard reaction has been named after the French chemist Louis Maillard (1912) who first described it but it was only in 1953 that the first coherent scheme was put forward by Hodge (1953) (Fig.2.2). In essence, it states that in an early stage, a reducing sugar, like glucose, condenses with a compound possessing a free amino group (of an amino acid or in proteins mainly the ε-amino group of lysine, but also the α-amino groups of terminal amino acids) to give a condensation product N-substituted glycosilamine, which rearranges to form the Amadori rearrangement product (ARP). The subsequent degradation of the Amadori product is dependent on the pH of the system (Fig.2.4). At pH 7 or below, it undergoes mainly 1, 2-enolization with the formation of furfural (when pentoses are involved) or hydroxymethylfurfural (HMF) (when hexoses are involved). At pH > 7 the degradation of the Amadori compound is thought to involve mainly 2,3 enolisation where reductones, such as 4-hydroxy-5-methyl-2,3-dihydrofuran-3-one (HMF^{one}), and a variety of fission products, including acetol, pyruvaldehyde and diacetyl are formed.

All these compounds are highly reactive and take part in further reactions. Carbonyl groups can condense with free amino groups, which results in the incorporation of nitrogen into the reaction products. Dicarbonyl compounds will react with amino acids with the formation of aldehydes and α-aminoketones. This reaction is known as the Strecker degradation. Subsequently, in an advanced stage, a range of reactions takes place, including cyclisations, dehydrations, retroaldolisations, rearrangements, isomerisations and further

condensations, which ultimately, in a final stage, lead to the formation of brown nitrogenous polymers and co- polymers, known as melanoidins.

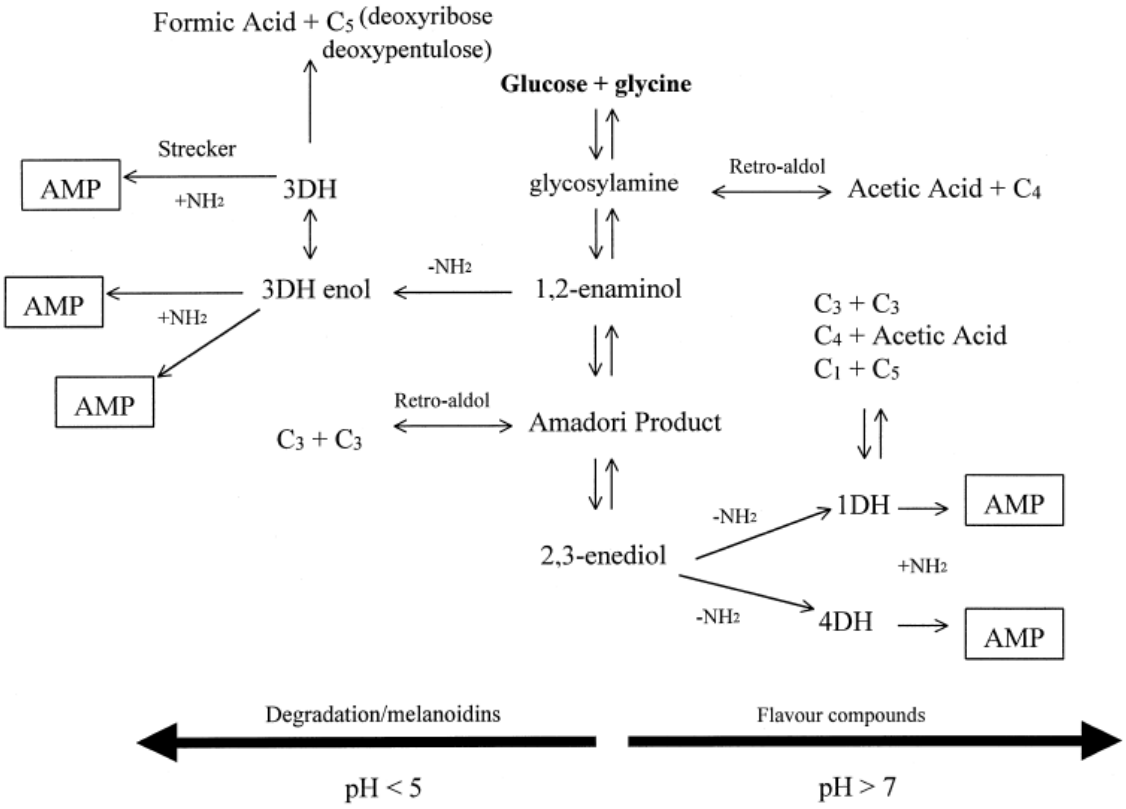

Figure 2.4: Scheme glucose/ glycine Maillard reaction adapted from Tressl *et al.,* (1995). AMP (Advanced Maillard Products);1-DH (1-deoxy-2,3-diketose); 3-DH (3-deoxyaldoketose); 4-DH (4- deoxy-2,3-diketose).

The complexity and the variety of the Maillard reaction products has, throughout the years, raised the interest of scientists in different fields of research (Ericksson,1981; Waller & Feather,1983; Fujimaki *et al.,*1986; Finot, 1990; Labuza *et al.,*1994; Ikan, 1990;). New important pathways, not accounted for by Hodge (1953), have been established. McWeeny *et al.,* (1974) reported that the most important intermediates in colour formation are 3-deoxyosuloses and 3, 4-dideoxyosulos-3-enes, which in the case of glucose is 3-deoxyhexosulose (DH) and 3, 4-dideoxyhexosuloses-3-ene (DDH). Later,