

Chapter 1

INTRODUCTION

The oxidation reaction directly affects food quality and is commonly associated with the changes of flavor and texture. Therefore, prevention of lipid oxidation has been of concern in the food industry. The use of synthetic antioxidants is an old practice, however, their safety could be questioned by the consumer. The alternative natural compounds with the efficient antioxidative activity have been paid increasing attention. Non-enzymatic interaction between reducing sugar and amino acids, peptides or proteins has been known as the Maillard reaction. Glycosylation or glycation is an important reaction which induces the covalent attachment of sugars to α - or ϵ -NH₂ groups of amino acids and protein to form glycated proteins (Friedman, 1996). The Maillard reaction produces a variety of intermediate products and finally brown pigments (melanoidins) are formed (Van Boekel, 1998). The Maillard reaction is influenced by many factors including reactant concentration, temperature, time, initial pH and water activity (Baxter, 1995; Ashoor and Zent, 1984; Naranjo *et al.*, 1998; Jing and Kitts, 2002; Wijewickreme and Kitts, 1997; Tanaka *et al.*, 1994; Mastrocola and Munari, 2000; Alaiz *et al.*, 1999). The Maillard reaction products (MRPs) from amino acid-sugar model system have been known to exhibit strong antioxidant activity (Yoshimura *et al.*, 1997; Tanaka *et al.*, 1990; Yen and Hsieh, 1995). Antioxidant activity of MRPs derived from protein-sugar system has also been studied (Jing and Kitts, 2002; Yeboah *et al.*, 1999). However, Lingnert and Eriksson (1980a) found a lower antioxidative activity of MRPs derived from protein-sugar

model systems than amino acid-sugar model system. MRPs were used to prevent lipid oxidation in many products. MRPs exhibited an antioxidative activity in meat products (Alfawaz *et al.*, 1994; Bedinghaus and Ockerman, 1995). Furthermore, MRPs derived from fructose-tryptophan system could prevent the oxidation of sardine lipid (Chiu *et al.*, 1991).

Generally, MRPs are mainly produced from commercial amino acid and reducing sugar. As a result, the MRPs have the high cost for production. Therefore, the use of new sources of protein with the reactive amino groups could be a promising means to lower the cost. Porcine blood is an abundant by-product in the slaughtering process in Thailand with an estimated amount of 30,000 metric tons per year (Benjakul *et al.*, 2001a). Blood plasma contains a variety of bioactive compounds including proteinase inhibitor and plasma transglutaminase (Benjakul *et al.*, 2001a; b). The addition of porcine plasma protein could increase the breaking force and deformation of bigeye snapper surimi gels (Benjakul *et al.*, 2001a). Apart from utilization as a surimi gel enhancer, porcine plasma can be used as potential inexpensive source of proteins or peptides for Maillard reaction in the presence of appropriate reactants and condition. MRPs from porcine plasma-sugar system with high antioxidative activity can be produced and used as a novel antioxidant to prevent the lipid oxidation in the food systems.

Literature Review

1. Blood and plasma

1.1 Composition of the blood

Blood is a complex tissue that consists of blood cells and cell-like components suspended in a clear, straw-colored liquid called plasma (Table 1) (Brum *et al.*, 1994). The pig can yield 10-12 L of blood (Wismer-Pedersen, 1979), about 55% of which is plasma. Blood transports dissolved nutrients, gases, hormones, and water through the body. Approximately two-thirds of the weight of blood is a plasma composed of 90% water and 7% protein (Chan *et al.*, 2001). Pig blood consists of 79.2% water, 18.5% protein, and 0.1% fat (FAO, 1996). Blood constituents vary depending on animal species (Table 2) (Lenzell, 1974).

Table 1 Blood components and their functions.

Component	Percent	Functions
Plasma	55	Suspends blood cells so they flow. Contains substances that stabilize pH and osmotic pressure, promote clotting, and resist foreign invasion. Transports nutrients, wastes, gases, and other substances.
White blood cells	<0.1	Allow phagocytosis of foreign cells and debris. Acts as mediators of immune response.
Platelets	<0.01	Seal leak in blood vessels.
Red blood cells	45	Transport oxygen and carbon dioxide.

Source : Brum *et al.* (1994)

Table 2 Concentrations (mg/ml) of some blood constituents in the goat, cow and pig.

	Goat	Cow	Pig
Blood			
Glucose	460	500	560
Acetate	90	90	20
Lactate	70	80	130
Plasma			
3-Hydroxybutyrate	60	50	10
Acetoacetate	3	6	6
Triacylglycerols	220	90	320
Free fatty acids	90	80	4
Phospholipids	1600	800	550
Cholesterol	370	230	170
Cholesterol esters	1000	1830	240
Free glycerol	3.4	not determined	not determined
Methionine	3	3	8
Phenylalanine	7	7	21
Leucine	21	22	43
Threonine	10	10	27
Lysine	21	12	42
Arginine	25	13	41
Isoleucine	18	17	30
Histidine	10	10	24
Valine	28	31	49
Glutamate	19	9	62
Tyrosine	10	7	26
Asparagine	9	4	6
Proline	26	8	45
Ornithine	11	9	19
Aspartate	3	2	4
Alanine	17	16	37
Glutamine	37	26	64
Glycine	69	18	51
Citruline	19	12	not determined
Serine	14	9	17
Total amino acids	380	250	630

Source : Lenzell (1974)

1.2 Blood plasma

Blood plasma is a straw-colored liquid made up of water and dissolved substances. These soluble constituents can be grouped into three categories (Alters, 1996):

1. Nutrients, hormones, respiratory gases, and wastes. These substances move from one place to another in the body and are used or produced by the metabolism of cells. These substances include glucose, lipoproteins (a soluble form of lipids), amino acids, vitamins, hormones, and the respiratory gases.

2. Salts and ions. Plasma is a dilute salt solution. In water, salts dissociate into their component ions. The chief plasma ions are sodium (Na^+), chloride (Cl^-), and bicarbonate (HCO_3^-). In addition, there are trace amounts of other ions, such as calcium (Ca^{2+}), magnesium (Mg^{2+}), copper (Cu^{2+}), potassium (K^+), and zinc (Zn^{2+}). In living systems, these ions are called electrolytes, which serve general functions in the body.

3. Proteins. Three major plasma proteins are (1) albumins, which help maintain the blood's osmotic pressure; (2) globulins, which transport nutrients and play a role in the immune system; and (3) fibrinogen, which is important in blood clotting. (Audesirk *et al.*, 2002; Donnelly and Delaney, 1977). Plasma is an important source of protein (Table 3) (Howell and Lawrie, 1983).

Table 3 Compositional analysis of plasma protein sample

Parameter	Source of plasma	
	Porcine plasma	Freeze dried porcine plasma
Protein % (w/w) (Nx6.25)	6.8 (± 0.1)	68.0 (± 0.1)
Moisture % (w/w)	91.0 (± 0.1)	9.2 (± 0.1)
Ash % (w/w)	1.1 (± 0.1)	11.5 (± 0.1)
Sodium % (w/w)	0.5 (± 0.1)	5.2 (± 0.1)
Citrate % (w/w)	0.44 (± 0.1)	4.6 (± 0.1)
Lipid % (w/w)	0.15 (± 0.1)	2.0 (± 0.1)
pH of 6% (w/w) protein solution	8.1 (± 0.1)	8.1 (± 0.1)
Total bacterial count at 37°C	390/ml to 4×10^3 /ml	3.8×10^3 /g

Source : Howell and Lawrie (1983)

1.3 Blood processing

Blood is the first by-product obtained in slaughtering process. Approximately 4% of the animal live weight (Chan *et al.*, 2001) or 50% of the total animal's blood volume can be collected during bleeding in the slaughter operation and the remaining portion is retained in the capillary system throughout the carcass (Ranken, 1980; Wismer-Pedersen, 1988; Ockerman and Hansen, 2000). In general, blood is collected hygienically and then an anticoagulant, usually trisodium citrate (0.2% with or without water), is added. Processing of blood includes centrifugation to separate light plasma (52 to 70%) from heavy erythrocytes and then chilling to 2°C, if not previously done, to minimize bacterial growth (Ockerman and Hansen, 2000).

Among the most commonly used anticoagulants, sodium citrate and a number of phosphates at different concentrations are used as follows (FAO, 1996).

Sodium citrate solution (10%)	30 ml/L blood
Sodium pyrophosphate solution (10%)	30 ml/L blood
Sodium tripolyphosphate solution (10%)	20 ml/L blood
Solution containing 4.5% sodium citrate and 5% sodium chloride	90 ml/L blood

1.4 Utilization of blood and plasma

Blood or plasma has been used as a nutritional supplement in the bakery products such as cake (Myhara and Kruker, 1998). Bovine plasma, a by-product of animal slaughtering, has received attention as an egg substitute in cake (Lee *et al.*, 1991) bread and biscuit (Johnson *et al.*, 1979). The use of blood protein in meat products is becoming increasingly popular as an important dietary iron source, as well as protein source, and as a meat substitute due to its lower cost compared to meat (Chan *et al.*, 2001).

Blood is used in many countries in the manufacture of traditional products, such as blood sausage and meat loaf. It can be added at low concentrations (0.5-2.0%) in order to improve the color of meat products (FAO, 1996). The utilization in large proportions is limited by its effect on the sensory properties of the products, particularly the color and strong metallic flavor (Howell and Lawrie, 1983). This problem can be overcome by using plasma instead of whole blood. Generally, plasma and red blood cells can be separated by centrifuging the whole blood at 1000xg for 30 min at 4°C (Benjakul *et al.*, 2001a).

Blood plasma consists of various proteins, mainly albumin and globulins, which act as efficient emulsifiers in the sausages (Cofrades *et al.*, 2000; Chan *et al.*, 2001). Animal plasma proteins have a good emulsifier properties (Saito and Taira, 1987) and have been used as fat replacement in bologna sausage (Cofrades *et al.*, 2000). However, losses of their emulsifying properties were found after thermal process. In general, 60% of the emulsifying properties of plasma proteins was lost after heat treatment at 80°C for 30 min (Saito *et al.*, 1988). So, the utilization of plasma protein has been limited. Emulsifying properties and heat stability of plasma protein could be improved by glycosylation process. Maillard reaction between plasma protein and galactomannan could form plasma protein-galactomannan complex with higher emulsifying activity and emulsion stability than those of plasma protein (Yamamoto *et al.*, 1990; Kim *et al.*, 2003; Matsudomi *et al.*, 1995). Moreover, the emulsifying properties of bovine serum albumin could be improved by the formation of bovine serum albumin-galactomannan conjugates (Kim *et al.*, 2003).

Blood plasma could improve the properties of surimi gel (Lee *et al.*, 2000) and increased the water holding capacity in meat products (Dill, 1976; Caldironi and Ockerman, 1982). Gordon (1971) and Tybor *et al.* (1973) reported that plasma protein isolates were excellent emulsifiers and binders in cook meat products. Lee *et al.* (2000) purified the L-kininogen, a cysteine proteinase inhibitor, from porcine plasma. L-kininogen derived from porcine plasma exhibited the inhibitory activities on many cysteine proteinase in fish muscles, such as μ -calpain, m-calpains, cathepsin B, L and L-like as well as papain, which could cause the thermal degradation of mackerel surimi gels (Jiang *et al.*, 1997). In addition, the bovine plasma protein could

effectively inhibit the surimi degradation caused by endogenous proteinases (Morrissey *et al.*, 1993).

Chilled or frozen plasma can be used at levels up to 10% substituting for either part of the ice or 2-3% of the meat in emulsified meat products. Liquid plasma utilization is limited by its high water and low protein content, and drying it to a powder with a water content below 10% is very costly (FAO, 1996).

2. Lipid oxidation

In food systems, the spontaneous oxidative reactions result in the deterioration of lipids. This direct reaction of a lipid molecule with a molecule of oxygen, termed autoxidation, is a free-radical chain reaction. The mechanism of autoxidation can be distinguished in three distinct steps: initiation, propagation and termination (Jadhav *et al.*, 1996).

2.1 Initiation

The autoxidation of fat is thought to be initiated with the formation of free radicals. The formation of lipid radical (R^{\bullet}) is usually mediated by trace metals, irradiation, light or heat (Eq. 1). Initiation reactions take place either by the abstraction of hydrogen radical from an allylic methylene group of an unsaturated fatty acid or by the addition of a radical to a double bond. The rearrangement of the double bonds results in the formation of conjugated diene (-CH=CH-CH=CH-), showing a characteristic UV absorption at 232-234 nm (Nakayama *et al.*, 1994).



Also, lipid hydroperoxide, which exists in trace quantities prior to the oxidation, breaks down to yield radicals as shown by Eqs. (2) and (3). Lipid hydroperoxides are formed by various pathways including the reaction of singlet oxygen with unsaturated lipids or the lipoxygenase-catalyzed oxidation of polyunsaturated fatty acids (Jadhav *et al.*, 1996).

2.2 Propagation

In propagation reaction, free radicals are converted into other radicals. Propagation of free-radical oxidation processes occurs by chain reactions that consume oxygen and yield new free-radical species (peroxy radicals, ROO^\bullet) or by the formation of peroxides (ROOH) as in Eqs. (4) and (5) (Jadhav *et al.*, 1996).



The product R^\bullet and ROO^\bullet can further propagate free-radical reactions.

Lipid peroxy radicals (ROO^\bullet) initiate a chain reaction with other molecules, resulting in the formation of lipid hydroperoxides and lipid free radicals. This reaction, when repeated many times, produces an accumulation of hydroperoxides. The propagation reaction becomes a continuous process as long as unsaturated lipid or fatty acid molecules are available. Since lipid radicals are highly reactive, they can easily undergo propagation reactions by two mechanisms: by reaction with oxygen

molecule in the triplet ground state (Eq. 4) or by removal of a hydrogen atom (Eq. 5). In essence, this reaction leads to the formation of a peroxy radical (ROO[•]) whose concentration becomes greater than that of R[•] in most food systems containing oxygen (Jadhav *et al.*, 1996).

2.3 Termination

A free radical has been defined as a molecular entity possessing an unpaired electron. Free radicals are electrically neutral, and solvation effects are generally very small. Owing to the bonding-deficiency and structural instability, radicals therefore tend to react whenever possible to restore normal bonding. When there is a reduction in the amount of unsaturated lipids (or fatty acids) present, radicals bond to one another, forming a stable nonradical compound (Eqs. 6, 7 and 8). Thus the termination reactions lead to interruption of the repeating sequence of propagating steps of the chain reaction (Jadhav *et al.*, 1996).



3. Antioxidants

Antioxidant in food is defined as any substance which is capable of delaying, retarding or preventing the development of rancidity or other flavor deterioration due to oxidation (Gordon, 2001). In general, antioxidants function by reducing the rate of initiation reaction in the free-radical chain reactions and are

functional at very low concentrations, 0.01% or less (Rajalakshmi and Narasimhan, 1996).

The use of antioxidants in food products is governed by laws and regulations of the country or by international standards. Even though many natural and synthetic compounds have antioxidant properties, only a few of them have been accepted as “generally recognized as safe” (GRAS) substances for use in food products by international bodies such as the Joint FAO/WHO Expert Committee for Food Additives (JECFA) and the European Community’s Scientific Committee for Food (SCF).

3.1 Classification of food antioxidants

3.1.1 Primary antioxidants

Primary antioxidants terminate the free-radical chain reaction by donating hydrogen or electron to free radicals and converting them to more stable products. They may also function by addition in reactions with the lipid radicals, forming lipid-antioxidant complexes. Many of the naturally occurring phenolic compounds like flavonoids, eugenol, vanilin and rosemary antioxidant also have chain-breaking properties (Rajalakshmi and Narasimhan, 1996). The Maillard reaction products derived from glucose-lysine, xylose-lysine, fructose-lysine, and casein-sugar model system also possess the primary antioxidative activity (Yen and Hsieh, 1995; Yoshimura *et al.*, 1997; Ajandouz *et al.*, 2001). Primary antioxidants are effective at very low concentrations but at higher levels they may become prooxidants (Rajalakshmi and Narasimhan, 1996).

3.1.2 Secondary antioxidants

Secondary or preventive antioxidants such as thiopropionic acid and dilauryl thiodipropionate function by decomposing the lipid peroxides into stable end products (Rajalakshmi and Narasimhan, 1996). Synergistic antioxidants can be broadly classified as oxygen scavengers and chelators. However, they may act as hydrogen donors to the phenoxy radical, thereby regenerating the primary antioxidant. Hence phenolic antioxidants can be used at lower levels if a synergist is added simultaneously to the food product. Oxygen scavengers such as ascorbic acid, ascorbyl palmitate, sulfites and erythorbates react with free oxygen and remove it in a closed system (Rajalakshmi and Narasimhan, 1996). The synergistic effect of citric acid is attributed to metal chelation (Frankel, 1998). Other polyvalent acids such as tartaric, malic, gluconic, oxalic, succinic and dehydroglutaric acids, as well as sodium triphosphate and pyrophosphate also possess synergistic properties similar to those of citric acid (Yanishlieva-Maslarova, 2001). Moreover, ascorbic acid can act as a synergist with tocopherols by regenerating or restoring their antioxidant properties (Niki, 1987). Ascorbic acid and its derivatives may also function as oxygen scavengers (Yanishlieva-Maslarova, 2001).

Flavonoids and related compounds and amino acids function as both primary antioxidants and synergists. Nitrites and nitrates, which are used mainly in meat curing, probably function as antioxidants by converting heme proteins to inactive nitric oxide forms and by chelating the metal ions, especially nonheme iron, copper and cobalt that are present in meat. β -carotene and related carotenoids are effective quenchers of singlet oxygen and also prevent the formation of hydroperoxides. The

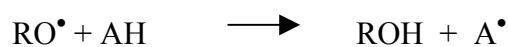
Maillard reaction prepared from glucose-glycine, glucose-lysine and fructose-lysine exhibited the metal chelators (Yoshimura *et al.*, 1997; Wijewickreme *et al.*, 1997)

3.2 Mode of action of antioxidants in food

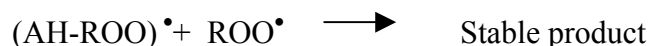
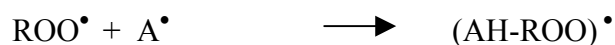
3.2.1 Radical scavenger

Antioxidants can delay or inhibit lipid oxidation by inactivating or scavenging free radicals, thus inhibiting initiation and propagation reactions. Free radical scavengers or chain-breaking antioxidants are capable of accepting a radical from oxidizing lipids species such as peroxy (ROO^\bullet) and alkoxy (RO^\bullet) radicals to form stable end products (Decker, 1998; Akoh and Min, 1998). Two different mechanisms as radical scavengers have been proposed for antioxidant; either as hydrogen donors or as electron donor that form charge-transfer complexes (Namiki, 1990; Osawa, 1994).

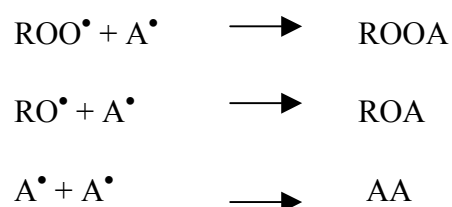
Hydrogen donor



Electron donor



The free antioxidant radicals (A^\bullet) may undergo additional reactions that remove radical from the system. Termination reactions of antioxidant with other free antioxidant radicals or lipid radicals can form nonradical species.



3.2.2 Peroxide decomposer

Some phenols, amine, dithiopropionic acid and thiopropionic acid function by decomposing the lipid peroxide into stable end products such as alcohol, ketone and aldehyde (Dziezak, 1986; Namiki, 1990).

3.2.3 Singlet oxygen quenchers

Singlet oxygen is generated from the triplet state oxygen. The mechanism of converting triplet oxygen to singlet oxygen is initiated by the transfer of the photosensitizer to its electronically excited state due to the absorption of light in the visible or near-UV region. Subsequently, the photosensitizer is able to transfer its excess energy to an oxygen molecules, giving rise to singlet oxygen (Shahidi and Wanasundara, 1992). Thus, the singlet oxygen can react with a lipid molecule to yield a hydroperoxide. Singlet oxygen reacts about 1,000-10,000 times as fast as normal oxygen with methyl linoleates (Jadhav *et al.*, 1996). Lipid oxidation initiated by xanthine oxidase can be inhibited by β -carotene because of its ability to quence singlet oxygen (Rajalakshmi and Narasimhan, 1996; Namiki, 1990). The Maillard reaction derived from xylose-lysine, tryptophan-glucose and glucose-

glycine model systems had a high scavenging effect on active oxygen (Tanaka *et al.*, 1992; Yen and Hsieh, 1995; Yoshimura *et al.*, 1997).

3.2.4 Lipoxygenase inhibitor

Lipoxygenase is a non-heme iron-containing enzyme that catalyzes the oxygenation of the 1,4-pentadiene sequence of polyunsaturated fatty acid to produce their corresponding hydroperoxide (Salas *et al.*, 1999). Free-radical intermediates occur during lipoxygenase catalysis, and these can lead to cooxidation of easily oxidized compounds, e.g. carotenoids and polyphenols (Rajalakshmi and Narasimhan, 1996).

3.2.5 Synergists

A. Chelating agents

Chelating agents are not antioxidants, however, they play a valuable role in stabilizing foods. Chelating agents that improve the shelf life of lipid-containing food are EDTA, citric acid and phosphoric acid derivatives (Jadhav *et al.*, 1996). Chelating agents form stable complexes with prooxidant metals such as iron and copper. Chelating agents bind metal ions and forms sigma bonds with a metal. It is considered as an effective secondary antioxidant because of the stabilized oxidation form of the metal ion. An unshared pair electrons in their molecule structure promotes the chelating action (Dziezak, 1986; Rajalakshmi and Narasimhan, 1996; Jadhav *et al.*, 1996).

B. Reducing agents or oxygen scavengers

Reducing agents or oxygen scavengers function by various mechanisms. They may act as hydrogen donors to the phenoxy radical, thereby regenerating the primary antioxidant or react with free oxygen and remove it in a closed system. (Giese, 1996; Rajalakshmi and Narasimhan, 1996). Ascorbic acid is a strong reducing agent, readily losing H^+ to become dehydroascorbic acid, which also has vitamin C activity. However, vitamin C activity is lost, when the lactone ring of dehydroascorbic acid is hydrolyzed to yield diketogluconic acid (Gordon, 2001).

4. Maillard reaction

The formation of brown pigments and melanoidins of heated glucose-lysine solution was first observed in 1912 by the French chemist Louis Camille Maillard (Martins *et al.*, 2001). This reaction was subsequently referred to as the Maillard reaction which covers all reactions involving compounds with amino groups and carbonyl groups present in foods (Figure 1). Amines, amino acids, and proteins generally interact with sugars, aldehydes, and ketones, as well as with products of lipid oxidation (Jing and Kitts, 2002; Van Bokel, 1998; Wijewickreme *et al.*, 1997). Maillard reaction is one of the most important phenomena occurring in food during processing and storage (Manzocco *et al.*, 1999; Olano and Martinez, 1996). Maillard reaction in heated food also lead to creation of a large number of volatile products (Wu and Pan, 1997). These volatile Maillard products contribute greatly to the processed flavor of many heat-treated foods, including boiled, roasted, grilled and fried meats and fish (Wu and Pan, 1997; Lindenmeier *et al.*, 2002; Sikorski, 2001).

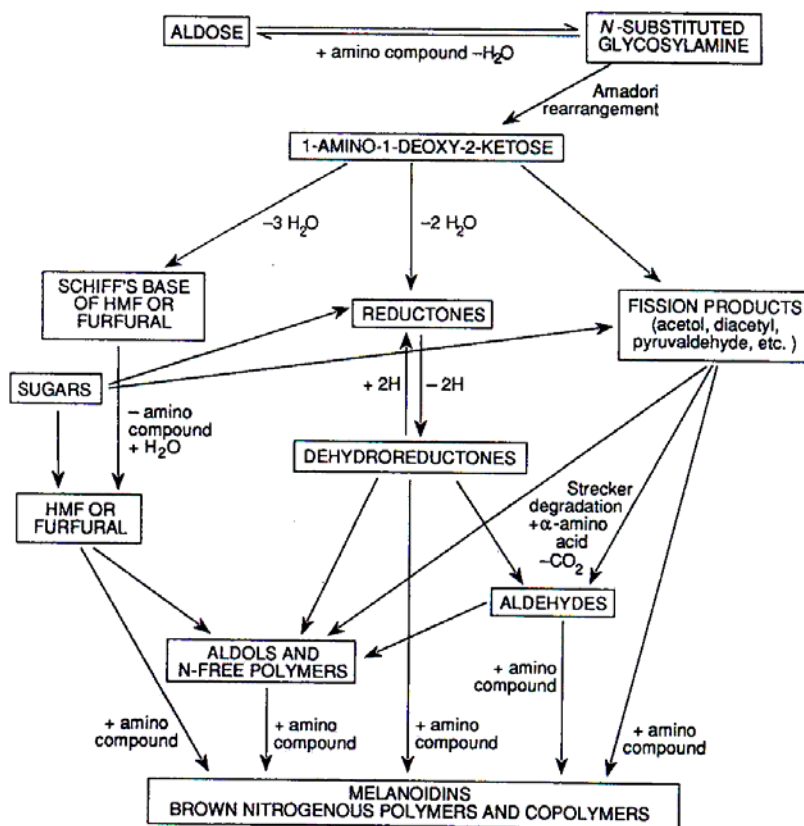


Figure 1 Nonenzymatic browning

Sources : Hodge (1953)

4.1 Stages of Maillard reaction

The Maillard reaction is divided into three stages as follows:

4.1.1 The early stage of Maillard reaction

The first step in the Maillard reaction involves condensation between the α -amino groups of amino acids or proteins (Eskin, 1990) as well as ϵ -amino groups of lysine and arginine (Wijewickreme *et al.*, 1997; Jing and Kitts, 2002) and the carbonyl groups of reducing sugars, defined as “carbonylamino” reaction. The protein molecules are composed of many amino acids joined covalently by peptide bonds, in

which the amino acids are presumably unavailable for interaction (Brans and Van Boekel, 2002). The initial product rapidly loses water to form a Schiff base followed by cyclization to the corresponding N-substituted glycosylamine (Figure 2) (Van Boekel, 1998). The formation of N-substitute glycosylamine easily occurs when the pH of the aqueous model system is above the isoelectric point of the amino groups (Eskin, 1990). The unstable N-substitute glycosylamine can be transformed by Amadori rearrangement (Moreno *et al.*, 2003) via oxidation, degradation rearrangement reaction (Kislinger *et al.*, 2003) and isomerization (Brans and Van Boekel, 2002).

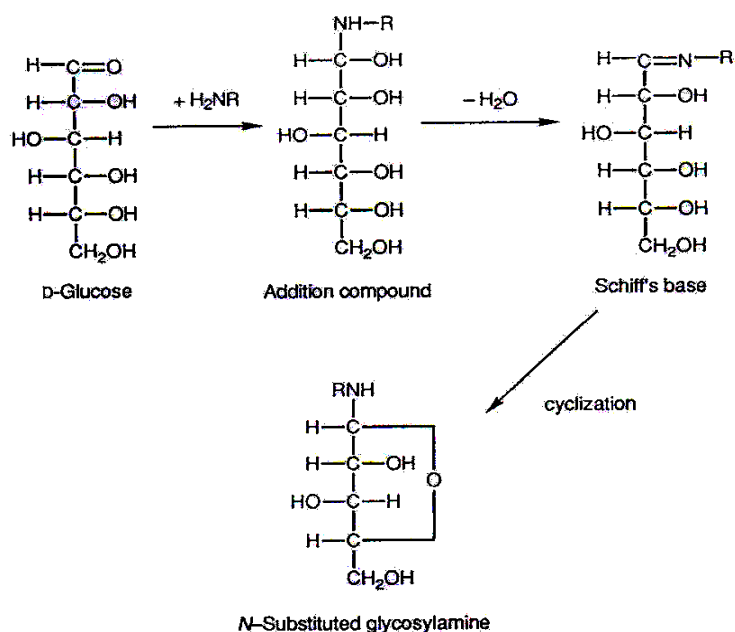


Figure 2 Early stage of Maillard reaction

Source : Eskin (1990)

4.1.2. The advanced stage of Maillard reaction

In the advanced stage, the Amadori product is subjected to breakdown (Van Boekel, 1998) and the various amino-sugar compounds are formed, including reductone intermediate and furfural (Moreno *et al.*, 2003). The breakdown of Amadori product occurs in three different pathways, which give different types of reaction products (Figure 3) (Van Boekel, 1998).

1) The 3-deoxyosone-pathway leads to products such as hydroxymethylfurfural and pyrrole. This route is not specific for disaccharides and occurs at acid condition. In this pathway, the loss of the amine from C-1 to form 3-deoxyosone is accomplished (O' Brien, 1995).

2) The 1-deoxyosone-pathway is the most important pathway at neutral pH. The degradation of the Amadori compound would be via the 2,3 enolization route, in which a hydroxyl group is lost at C-3, followed by deamination at C-1, to form 1-deoxyosone (O' Brien, 1995).

3) The 4-deoxyosone pathway is significant for disaccharides under alkaline conditions. The two products formed, 4-deoxyaminoreductone and 5,6-dihydroxypyridone, are further degraded again upon prolonged heating (Pischetsrieder and Severin, 1996).

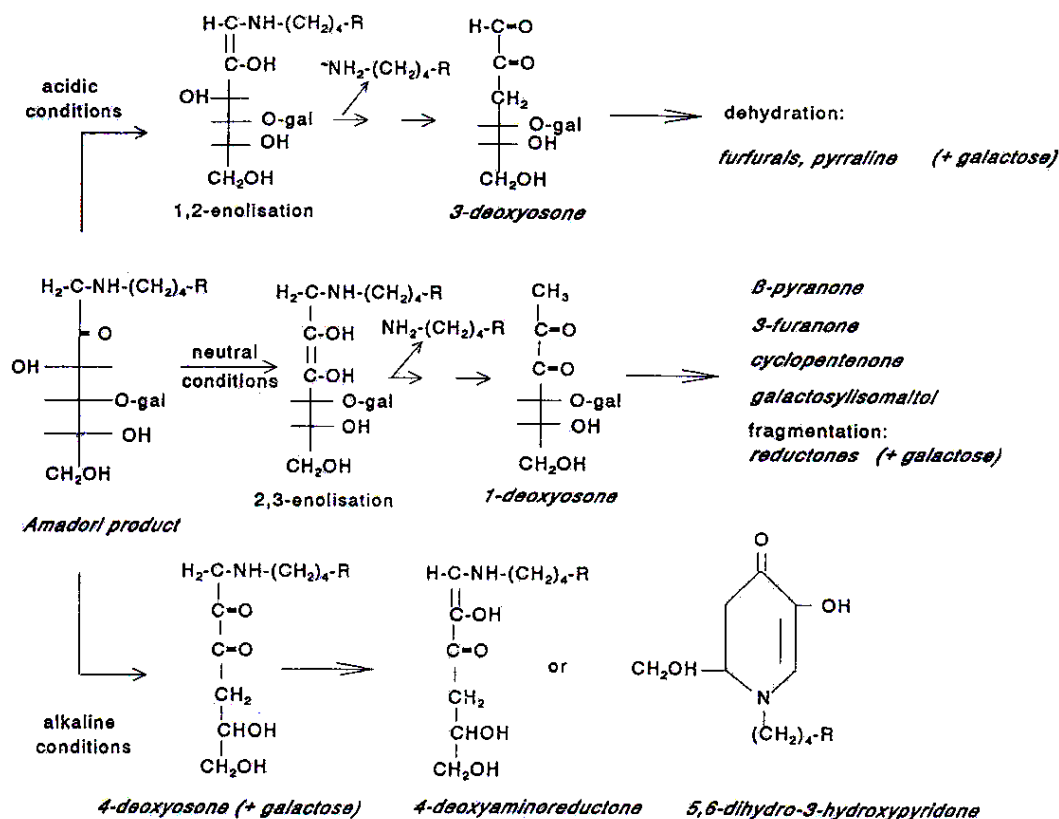


Figure 3 Degradation of Amadori compound

Sources : Van Boekle (1998)

The formation of Amadori compounds represents a pathway requiring less energy for sugar degradation as compared to caramelization (Davidek *et al.*, 2002). The intermediate products occurring in the advanced stage have the UV-absorbance at 294 nm. Therefore, the UV-absorbance can be used as the method to detect Maillard intermediate products (Ajandouz *et al.*, 2001). The intermediate reductone compound of MRPs were reported to break the radical chain by donation of a hydrogen atom

(Eichner, 1981). However, some intermediate products in Maillard reaction have a prooxidant activity (Munari *et al.*, 1995).

Fluorescent compounds are also generated in the advanced stage of Maillard reaction prior to the generation of brown pigment (Jing and Kitts, 2002; 2004; Morales *et al.*, 1996). The fluorescent intermediates are possibly reactive in formation of brown product (Benjakul *et al.*, 2004; Morales *et al.*, 1996). Nevertheless, the generation of fluorescent compounds are not correlated with the radical scavenging property of MRPs (Morales and Van Boekel, 1997). Jing and Kitts (2002) reported that the types of sugar influence the generation of fluorescent compounds in heated casein-sugar model system at 55°C, pH 7 for 28 days. The compound with chromophoric group (-N=CH-CH=CH-NH) showed fluorescent characteristics (Chio and Tappel, 1961). Additionally, Park and Kim (1983) found that some antioxidant compounds were generated prior to the formation of browning. These antioxidants product may be the Amadori compound and the low-molecular-weight products (Park and Kim, 1983)

4.1.3. The final stage of Maillard reaction

This is the stage in which brown pigments (melanoidins) are formed from reactive compound generated in the advanced stage of Maillard reaction. The reaction consists of the condensation of amino compound and sugar fragment into polymerized protein and brown pigment (Van Boekel, 1998). This brown product can be observed by measuring the absorbance at 420 nm (Ajandouz *et al.*, 2001). The brown melanoidins are a heterogenous mixture of high-molecular-weight compounds (up to about 100,000 Dal) (O'Brien and Morrissey, 1989; Van Boekel, 1998). Apart from high-molecular-weight melanoidin, browning can also be due to low-molecular-

weight colored compounds, sometimes referred to as low-molecular-weight melanoidin (Leong and Wedzicha, 2000; Rizzi, 1997). The development of low and high molecular weight brown products have been found in the amino acid-sugar model system (Morales and Jimenez-Perez, 2001). Brown substances generated from casein-sugar model systems are due mainly to the formation of protein oligomers that are mediated by chromophoric substructures derived from carbohydrates (Hofmann, 1998). Hofmann *et al.* (1999a; b) reported that melanoidin development in bread-crust, roasted cocoa and coffee beans, was derived from the condensation between 1,4-bis(5-amino-5-carboxy-1-pentyl) pyrazinium radical cation and protein. Hofmann (2001) and Hofmann *et al.* (2002) found that melanoprotein were formed at the side chain of lysine.

Benzing-Purdie *et al.* (1985) examined the effect of temperature on the structure of the melanoidins formed in model systems composed of D-xylose and glycine. In the presence of equimolar amounts of the reactants, an increase in temperature (22, 68 and 100°C) was accompanied by an increase in the aromatic nature of both low- and high-molecular-weight melanoidin products. The considerable differences in the nature between the melanoidins produced at 22°C and those formed at the higher temperatures, with different types of aliphatic carbons and fewer unsaturated carbons, were observed.

4.2. Factors affecting Maillard reaction

The Maillard reaction is the complex reaction, in which the reaction rates depend on many factors such as pH, buffer, temperature, A_w , metal ions, heating time as well as the types and concentration of reactant in the systems. (Nursten, 1986;

Lingnert, 1990; Wijewickreme *et al.*, 1997; Van Boekel, 2001; Rizzi, 2004; Kwak and Lim, 2004).

4.2.1 pH

The carbonylamino reaction can develop in acidic or alkaline media, although it is favored under alkaline conditions. In alkaline condition, amine groups of the amino acids, peptides, and proteins are in the basic form, the active form of amino reactant (Van Boekel, 2001). Increasing the pH also ensures that sugars are in the open chain or reducing form (Burton and McWeeney, 1963; Van Boekel, 2001). Ajandouz *et al.* (2001) reported that the increase in Maillard reaction rate was observed as the initial pH of heated fructose-lysine model system at 100°C increased. The decrease in pH of sugar-amino system after heating, might be due to the formation of organic acids. Formic and acetic acids were generated from the degradation of 1,2-enediol and 2,3-enediol, the intermediate products from the heated casein-sugar model system (Van Boekel, 1996; Brands and Van Boekel, 2002). Generally, Maillard reaction will be inhibited in low pH food (Namiki and Hayashi, 1982; Ashoor and Zent, 1984).

Sucrose, a nonreducing sugar, will only participate in Maillard reaction when the glycosidic bond is hydrolyzed and the reducing monosaccharide constituents are released. Hydrolysis of the glycosidic bond in sucrose is facilitated by a low pH and high moisture levels, resulting in an increase in the Maillard reaction rate in protein-sucrose systems (Hurrell and Carpenter, 1974).

4.2.2 Temperature

Generally, the rate of chemical reactions increases with increasing temperature. Since the Maillard reaction consists of several reaction steps, each with a possibly different temperature sensitivity, it strongly depends on temperature (Brands and Van Boekel, 2002). At low temperature (20-60°C), the reaction rate is lower than that of high temperature (100-150°C). Furthermore, temperature affects the activities of the reactants. The active form of the sugar is considered to be the open chain, which is formed markedly with increasing temperature (Van Boekel, 2001). The percentage of fructose in its acyclic form at neutral pH is about 0.7% at room temperature and 13.1% at 80°C (Yaylayan *et al.*, 1993). The Maillard reaction rate increased four-fold for each 10°C rise in temperature (Eskin, 1990). Brands and Van Boekel (2002) reported that an increase of heating temperature in monosaccharide-casein model system leads to a higher loss of the reactants and an increased formation of the reaction products.

Depending on the intensity of the thermal treatment applied, prooxidant or antioxidant molecules are expected to be produced (Nicoli *et al.*, 1999). Heat treatment of milk can promote an increase in its prooxidant activity, probably as a consequence of both the loss of natural antioxidants and the formation of novel oxidative molecules in the early stage of Maillard reaction (Brands and Van Boekel, 2002).

4.2.3 Water activity

Water activity is frequently mentioned as an important factor determining Maillard reaction. In liquid systems, the rate of browning decreases continuously with increasing water activity (Buera *et al.*, 1987; Bell *et al.*, 1998a; b). A maximal browning rate was observed at water activity between 0.5 and 0.8 (Eichner and Karel,

1972; Karmas and Karel, 1994). However, high water activity had an insignificant effect on the rate of browning (Petriella *et al.*, 1985). With low water activity, the diffusion of reactant was unlikely to occur, due to reactant immobility (Karmas *et al.*, 1992; Karmas and Karel, 1994; Buera and Karel, 1995; Bell *et al.*, 1998a). The reactant concentration also affects browning rate in low and intermediate moisture solids (Bell, 1995)

Generally, the Maillard reaction proceeds rapidly in solution, although complete dehydration or excessive moisture levels inhibit this process (Eskin, 1990). Tanaka *et al.* (1994) reported that the Maillard reaction between freeze-dried squid meat and sugar occurred rapidly at the water activity between 0.4 and 0.7.

4.2.4 Sugars

Reducing sugars are essential ingredients in these reactions, as they provide the carbonyl groups for interaction with the free amino groups of amino acids, peptides, and proteins. The initial kinetics of glycation are dependent on the proportion of the reducing sugar existing in the acyclic or active form under the reaction condition (Yaylayan *et al.*, 1993; Labuza and Baisier, 1992) and on the electrophilicity of the sugar carbonyl group (Bunn and Higgins, 1981). The reactivity of reducing sugars was reported to decrease in the following order: aldopentoses > aldohexoses > aldoketoses > disaccharides (Spark, 1969). Among hexoses examined, the reactivity decreased in the order of D-galactose > D-mannose > D-glucose, corresponding to the decreasing rate of ring opening (Eskin, 1990). Brands and Van Boekel (2002) revealed large differences between the reaction behavior of glucose and fructose when heated in the presence of a protein. These differences are mainly due to the differences in the reaction mechanism between aldose and ketose sugars.

Aldehyde group of the acyclic form of aldoses was more electrophilic than the keto group of the acyclic form of ketose (Yeboah *et al.*, 1999). With increasing pH of the system, the concentration of the open chain form of the reducing sugar increased (Brands and Van Boekel, 2002). Harward and Angyal (1977) reported that the percentage of galactose in its acyclic form was about 10 times higher than that of glucose at neutral pH at room temperature, which has been generally believed to be the reason for a higher reactivity of galactose than glucose. Apart from the changes of sugar to acyclic form, aldose sugar and ketose sugar can isomerize into each other via the Lobry de bruin-Alberda van Ekenstein transformation (Martins and Van Boekel, 2005). Farag *et al.* (1982) found that pentose produced browning much faster than aldohexoses. Moreover, the higher browning intensity was noticeable with the straight-chain amino acid, lysine, compared to the branch-chain amino acids, leucine and valine.

4.2.5 Amino acid and Protein

The α -amino acid (glycine, lysine or glutamic acid) participating in the generation of melanoidins is more influence on the melanoidin formation than that of the carbonyl compound (glucose or L-(+)-ascorbic acid) (Eskin, 1990). The reactivity of the amino acids to form Maillard reaction products is different. The reactivity decreased in the order of lysine > glycine > alanine (Morales and Jimenez-Perez, 2001). Additionally, the increase in reaction rate was observed with increasing amino acid concentration (Toribio and Lozano, 1986). Kwak and Lim (2004) found that the reactivity of lysine to form MRPs was 2-3 times higher than other amino acid. The high reactivity of lysine is attributed to the two α - and ϵ -amino groups (Miller *et al.*, 1984). Cysteine was found to have the lowest contributory effect to browning. It is

known that sulfur amino acids and peptides such as cysteine and glutathione are generally effective for inhibiting non-enzymatic browning (Kwak and Lim, 2004).

4.2.6 Metal

The formation of metal complexes with amino acids can influence the Maillard reaction. The Maillard reaction is catalyzed by copper and iron, while manganese and tin can inhibit this reaction (Ellis, 1959; Markuze, 1963). Kato *et al.* (1981) found that Cu^{2+} and Fe^{3+} increased the reaction rate more effectively than Fe^{2+} , while Na^+ showed no effect on the reaction rate. Kwak and Lim (2004) reported that the effect of metal ions on browning was dependent on the type of amino acid, heating time, as well as the type of metal ions. Cu^{2+} and Fe^{2+} ions enhanced browning. However, Fallico and Ames (1999) reported that there was only small effect of iron on the Maillard reaction in model system.

4.2.7 Reaction time

The Maillard reaction time has influence on browning, fluorescent development and also Maillard products generation. Jing and Kitts (2002) found that an increase in heating time of casein-sugar model system at 55°C and pH 7 enhanced the generation of fluorescent compound and brown products. Additionally, antioxidant activity of MRPs was determined by the reaction time. Friedman (1996) reported that glucose-tryptophan MRPs prepared at various temperatures and heating times have different antioxidant activity. Therefore, the Maillard reaction products derived from appropriate condition and reaction time exhibit the high antioxidant activity. Antony *et al.* (2000) reported that antioxidant activity of MRPs prepared by refluxing honey-lysine increased when the heating time increase. Ajandouz *et al.*

(2001) reported that no significant loss of lysine in fructose-lysine model system was found at the later heating period, probably due to some limitation in the reaction or in the release of amino group at advanced stage of the Maillard reaction. A no-loss period was observed in glycine-glucose model system stored at 37°C (Baisier and Labuza, 1992), as well as in glucose-lysine, glucose-methionine and glucose-threonine model system when heated to 100°C (Ajandouz and Puigserver, 1999).

4.2.8 Other factors

The role of buffers in nonenzymatic reactions has been shown to determine the rate of browning for sugar-amino acid systems as a result of their influence on the ionic environment in which the reaction takes place (Eskin, 1990; Van Boekel, 2001; Bell, 1997). Phosphate buffer promotes the browning development and the loss of glycine. No effect of citrate buffer on Maillard reaction were observed. Nevertheless, the rate of browning development was decreased with increasing citrate concentration. The bifunctional catalytic ability of the phosphate anion was proposed as an explanation of differing effects of buffers (Bell *et al.*, 1998a). Shen and Wu (2004) reported that Maillard browning in ethanolic solutions of 0.2 M glucose-0.2 M glycine system was more pronounced with an increase in ethanol concentration (0% to 50%, v/v). Furthermore, Moreno *et al.* (2003) reported that the high pressure affected Maillard reaction mainly due to pressure-induced changes in the pH of the system.

4.3 The antioxidative activity of Maillard reaction products

Maillard reaction can produce many MRPs with different antioxidant activity. In particular, the antioxidant properties of MRPs have been reported to be strongly affected by the physico-chemical properties of the system and by the processing

conditions (Lingnert and Eriksson, 1981; Homma *et al.*, 1997; Manzocco *et al.*, 1999). MRPs are well known to exhibit antioxidant activities in both model lipid (McGookin and Augustin, 1991; Wejewishkreme and Kitts, 1997) and food system (Bedinghaus and Ockerman, 1995; Wejewishkreme and Kitts, 1998a; b). The antioxidant activity of MRPs is summarized in Table 4. Some intermediate Maillard products, such as reductones, have high antioxidant activity in aqueous solutions or emulsions (Serevini and Lericci, 1995). The heterocyclic derivatives produced in oxidized lipid-amino acid MRPs promote the antioxidant in vegetable oils (Alaiz *et al.*, 1995; 1997). Eiserich and Shibamoto (1994) showed that alkylthiophenes, 2-thiophenethiol, 2-methyl-3-furanthiol and furfuryl mercaptan have antioxidant properties. The antioxidant activity of these compounds depended on the degree of unsaturation in the heterocyclic ring, as well as substituent type. Moreover, Lingnert and Waller (1983) examined the effect of time, initial pH and molar ratio of arginine to xylose on antioxidant activity. The pH of 5.0 appeared to be optimal for antioxidant activity and molar ratio of 1:1 exhibited the maximal effect. Lingnert and Eriksson (1980b) reported that neutral or slightly basic conditions favored the formation of antioxidant products from histidine-glucose model system. Dialysis treatment influence on the loss of antioxidant activity suggested that the treatment caused the loss of low-molecular-weight antioxidants (Kawashima *et al.*, 1977). MRPs retarded lipid oxidation in preheated model system containing pregelatinized starch, glucose, lysine and soybean oil during storage at 25°C (Mastrocola and Munari, 2000). In addition, several nitrogen-and/or sulfur-containing heterocyclic compounds, which are major flavor compounds formed by the Maillard reaction exhibited antioxidative activity (Shibamoto, 1983; Eiserich and Shibomoto, 1994).

Table 4 Antioxidant activity of Maillard reaction products.

Type of compounds	Precursors	Effect on food stability
Imines (Schiff bases)	Sugars, amino acids	Hydroperoxide reduction
Amino deoxy sugars	Schiff bases	Hydroperoxide reduction
Amadori, Heyns products	Amino deoxy sugars	Hydroperoxide reduction
Melanoidins	Premelanoidins	Metal chelation
Dihydrocyclic derivatives	Strecker compounds	Hydroperoxide reduction
Reductones	Dideoxytriose	Free radical scavenging

Source: Gordon (2001)

4.3.1 Radical scavenging activity

Maillard reaction products possess the radical scavenging activity (Jing and Kitts, 2002; Murakami *et al.*, 2002; Morales and Jimenez-Perez, 2001; Yen and Hsieh, 1995; Yoshimura *et al.*, 1997; Benjakul *et al.*, 2004). MRPs produced by heating the glucose-glycine mixture had an scavenging effect toward hydroxyl radical (OH^\bullet) (Yoshimura *et al.*, 1997). Additionally, MRPs derived from glucose, fructose and ribose-lysine (Wijewickreme *et al.*, 1999) casein-sugar (Jing and Kitts, 2002) as well as xylose-lysine (Yen and Hsieh, 1995) also exhibited the hydroxyl radical scavenging activity in the deoxyribose assay. The relative OH^\bullet scavenging activities of MRPs varied considerably with source of the reducing sugar used (Wijewickreme *et al.*, 1999).

Morales and Jimenez-Perez (2001), Jing and Kitts (2002), Yen and Hsieh (1995) and Benjakul *et al.* (2004) evaluated the free radical scavenging activity of the MRPs towards DPPH radical in polar organic solvent. They found that MRPs had an DPPH radical scavenging activity. The browning is not directly related to the free

radical scavenging properties of MRPs formed at prolonged heating condition. However, the fluorescence intensity of heated sugar-amino system is more effective than browning to follow the formation of MRPs with free radical scavenging activities (Morales and Jimenez-Perez, 2001). In general, MRPs scavenge the DPPH radical by donation of hydrogen atom to form a stable DPPH-H molecule (Matthaus, 2002; Yen and Hsieh, 1995; Benjakul *et al.*, 2004).

Efficiency of MRPs to scavenge the radical depends on the nature of radicals. Jing and Kitts (2002) found that the hydrophilic radicals (OH^\bullet) are more efficient to be quenched by casein-sugar MRPs than hydrophobic radicals (DPPH^\bullet). Yoo *et al.* (2004) evaluated the antioxidant activity of fractionated brown substances, formed during roasting of sesame seeds, using the order of chloroform, ethyl acetate, butanol and methanol. DPPH radical scavenging activity was highest in chloroform fraction. However, the greatest hydroxyl radical scavenging activity was observed in methanol fraction. Jing and Kitts (2004) reported that the high molecular weight sugar-lysine MRPs fraction have a higher antioxidant activity to scavenge hydroxyl and DPPH radicals than low molecular weight sugar-lysine MRPs fraction.

4.3.2 Metal chelating activity

Maillard products also possess the chelating activity towards heavy metal ions or active complexes (Kajimoto and Yoshida, 1975). Johnson *et al.* (1983) reported that melanoidin had a high metal binding capacity, presumably owing to the reductone moiety of MRPs. Yoshimura *et al.* (1997) also suggested that the high-molecular-weight fraction obtained from glucose-glycine MRPs had a stronger metal chelating capacity than the low-molecular-weight fraction. An antimicrobial activity of MRPs may be partially explained by the chelation of metal ions essential for

microorganisms (Einarsson *et al.*, 1988). Additionally, Brun-Merimee *et al.* (2004) found that MRPs from aqueous equimolar (0.25M) glucose or fructose with glutathione model, heated at 90°C for 15-39 h, could inhibit apple polyphenoloxidase (PPO), suggesting that MRPs have a chelating effect on copper ion at the active site of PPO.

Melanoidins behave as chelating agent for polyvalent metal cations and thereby may influence the bioavailability of these ions (Rendleman, 1987). Morales *et al.* (2005) reported that melanoidins from model system containing glucose had significantly strong iron-binding properties than melanoidins from system having lactose. Wijewickreme *et al.* (1997) revealed that the copper chelating activity of crude and fractionated MRPs (from glucose-lysine and fructose-lysine) was greatly influenced by the type of reactant sugar and reaction condition used. Morales *et al.* (2005) classified the melanoidins into three groups according to their number of coordinating site (n) for iron; these being low ($n \times 10^3 < 50$), medium ($50 < n \times 10^3 < 100$) and high iron chelating activity ($n \times 10^3 > 100$) melanoidins. Gomyo and Hirikoshi (1976) reported that the melanoidins behave as anionic hydrophilic polymers, which can form stable complexes with metal cations. At pH values close to those found in most foods, melanoidins have a negative net charge and are able to bind metallic ions (Migo *et al.*, 1997). O'Brien and Morrissey (1997) reported the binding of Zn^{2+} , Cu^{2+} , Mg^{2+} and Ca^{2+} by the glucose-glutamine MRPs. The strength of binding occurred in the order of $Mg^{2+} > Cu^{2+} = Ca^{2+} > Zn^{2+}$.

4.3.3 Scavenging of active oxygen

MRPs can scavenge the active oxygen (Tanaka *et al.*, 1992). Melanoidins, the final Maillard products, have an active oxygen scavenging properties (Hayese *et al.*,

1989). The MRPs derived from xylose-lysine (Yen and Hsieh, 1995) and tryptophan-glucose model systems (Tanaka *et al.*, 1992) possess a high scavenging effect on active oxygen. However, scavenging effect of MRPs on superoxide was markedly decreased after decolorization with Sep-Pak Cartridge C18 (Yen and Hsieh, 1995)

Volatile products in Maillard reaction, such as dihydrofuran, dihydropyridine or dihydropyrazine derivatives, are further oxidized into substituted furan, pyridines and pyrazines, respectively. Oxygen present in the system is consumed by MRPs and the oxidation of lipid or antioxidants is prevented (Pokorny, 2001). Yoshimura *et al.* (1997) reported that the glucose-glycine MRPs heated over boiling water bath for 1 h could scavenge more than 90% of active oxygen species. The reducing materials produced from Maillard reaction have a scavenging activity towards active oxygen (Lingnert and Eriksson, 1981; Kato, 1992; Kato and Hayase, 1989).

4.4 The antioxidative stability of MRPs

The stability of antioxidative activity of MRPs has been reported to depend on many factors. Lingnert and Waller (1983) examined the antioxidant activity of product generated from histidine-glucose system. The loss of antioxidant activity was evident in the presence of air compared to storage at 25°C under nitrogen. Moreover, the loss of antioxidant activity was less at low pH (2.0) compared to high pH (8-10).

Mastrocola and Munari (2000) studied the antioxidant activity of MRPs derived from model systems containing 50% pregelatinized starch, 30% water, 16% glucose and 4% lysine heated at 100°C for 90 min and found that the antioxidant activity of MRPs, stored at 25°C for 180 days, decreased after 40 days of storage.

5. Application of Maillard reaction products

The Maillard reaction products are important not only for their antioxidant activity but also for the development of color and flavor of food (Tanaka *et al.*, 1992). The antioxidant activity of MRPs has been noticeable in many food systems, such as beer (Woffenden *et al.*, 2001), coffee (Nicoli *et al.*, 1997; Del Castillo *et al.*, 2002), biscuits (Borrelli *et al.*, 2003), milk (Calligaris *et al.*, 2004), pasta (Anese *et al.*, 1999a), tomato (Anese *et al.*, 1999b), cookies (Bressa *et al.*, 1996) and meat products (Antony *et al.*, 2002; Smith and Alfawaz, 1995; Alfawaz *et al.*, 1994; Bedinghaus and Ockerman, 1995).

MRPs derived from fructose-tryptophan system possessed an antioxidant effect to prevent oxidation in the sardine lipid during storage at 5°C for 2 weeks (Chiu *et al.*, 1991). Furthermore, fructose-tryptophan MRPs had a synergistic effect with α -tocopherol. Antony *et al.* (2002) reported that antioxidative effect of honey-lysine MRPs in meat was in a concentration-dependent manner. Bedinghaus and Ockerman (1995) reported that MRPs prepared by refluxing 0.2M of three individual reducing sugars (glucose, xylose and dihydroxyacetone) with 0.2M of five free amino acids (arginine, histidine, leucine, lysine and tryptophan) inhibited lipid oxidation in ground pork patties effectively. The most effective antioxidative activities were found in xylose-lysine, xylose-tryptophan, dihydroxyacetone-histidine and hydroxyacetone-tryptophan MRPs. Bressa *et al.* (1996) evaluated the antioxidative effect of MRPs in butter cookies. To prepared the MRPs, glucose and lysine were added in butter cookie formulation before cooking at 150°C for 0, 10, 20, 30, or 40 min. They found that

MRPs had an antioxidative effect in the butter cookies, when cooking time increased up to 30 min.

Alfawaz *et al.* (1994) evaluated the antioxidative activity of MRPs, obtained by autoclaving glucose with acid or enzymatic protein hydrolysates of egg albumin or soy protein isolate, in cooked ground beef during refrigerated storage for 8 days. The antioxidative of MRPs was found to be influenced by the heating time of protein hydrolysates-glucose mixture and by the MRPs level added. Lingnert and Lundgren (1980) reported that MRPs from enzymatic hemoglobin hydrolysate and glucose were effective antioxidants in sausage during frozen storage. Bailey *et al.* (1987) also found that the addition of MRPs from histidine and glucose was effective in retarding rancid flavor in cooked ground beef during refrigerated storage. Smith and Alfawaz (1995) also found that MRPs obtained by autoclaving egg albumin acid hydrolysate and glucose for 1 h rendered the antioxidative activity in cooked ground beef stored at 4° C for 8 days.

However, protein hydrolysate itself also exhibits the antioxidative activity. Wu *et al.* (2003) reported that mackerel hydrolysates, prepared by an autolytic process and accelerated hydrolysis with a commercial enzyme (Protease N), had an antioxidant properties. The relative antioxidant activity of Protease N hydrolysate was much higher than that of autolysis hydrolysate. The hydrolysate with a higher free amino acid and peptide level possessed stronger antioxidant activity. Additionally, the peptide from mackerel protein hydrolysate with molecular weight of approximately 1400 daltons possessed a stronger antioxidant activity than that of the 900 and 200 daltons peptides. Sathivel *et al.* (2003) reported that herring byproduct hydrolysate showed antioxidative properties. The herring byproduct hydrolysate obtained from

whole herring hydrolysate (18.3% DH) had the highest antioxidative activity, followed by the hydrolysate from body (13% DH), gonad (13% DH) and head (10.1% DH). Jao and Ko (2002) found that protein hydrolysates from tuna cooking juice had the DPPH radical scavenging effect. The antioxidant activity was observed in the peptide sequences comprising four to eight amino acid residues, including Val, Ser, Pro, His, Ala, Asp, Lys, Glu, Gly, or Tyr. Je *et al.* (2005) also found that protein hydrolysate from Alaska pollack had the antioxidative effect. The hydrolysate fraction with molecular weight of 1-3 kDa exhibited the higher antioxidant activity than other fractions with greater molecular weight.

Objectives

1. To study some parameters affecting the formation of Maillard reaction and antioxidant activity of PPP-sugar model system.
2. To study the effects of decolorization and fractionation treatments on the characteristics and antioxidative activity of MRPs.
3. To investigate the uses of MRPs to retard lipid oxidation of sardine mince and sardine emulsion sausage during storage.