CHAPTER II

LITERATURE REVIEW

2.1. Maillard Reaction Products (MRPs)

Maillard reaction has been well understood as a non-enzymatic reaction between reducing sugars and amino acids to generate the Maillard reaction products (MRPs). The Maillard reaction was first declared in 1912 by Louis-Camille Maillard who has been described that upon gently heating sugars and amino acids in water will generate a yellow-brown color (Bastos et al., 2012). Maillard reaction greatly influence in food attributes such as brown color, flavor, aroma, and also antioxidant. Brown color is the primary characteristic of the MRPs. During the Maillard reaction, carbonyl groups of reducing sugar and amino groups will degraded to form the carbonyl compound. The longer Maillard reaction process, the high in the accumulation the carbonyl compound to form a more stable compound (Martins et al., 2001). The stable compound or called Amadori products are the brown and flavor forming compound on Maillard reaction. It is also known that the food with Maillard reaction process has a higher antioxidant. Alvarenga et al. (2014) reported that brown and flavor compound have the positive correlation with antioxidant activity on food content. One of the preferred intermediate MRPs is Hydroxylmethylfurfural.

5-Hydroxylmethyl-2-furfural (HMF) is a carbonyl compound having two functional groups namely aldehyde (formyl) and alcohol (hydroxyl) bound to position chains 2 and 5 (Surh and Tannenbaum, 1994). Based on O'brien et al.

(1998); Kowalski et al. (2013), HMF formation occurs in three stages, i.e. dehydration of sugar in the C5 chain and the elimination of protons to form enol, hydration of water molecule at the nearest enol to form a carbonyl group in C5, and final formation of HMF due to the accumulation of carbonyl groups. The final compound has high molecular weight (Martins et al., 2001) and may change the pigment formation, and polymerization (O'brien et al., 1998).

2.2. Glycation Reaction

Maillard reaction leads to form glycation between amino group and sugar that provides important role on flavor, aroma, and color. Condensation of amino-carbonyl is the first process which the carbonyl group of the aldose or ketose from reducing sugar reacts with the amino group. This process generates Schiff base, then will exhibit glycosylamine (Labuza et al., 1998). The mechanism is present in Figure 1. Maillard reaction formation is easily applied in alkaline environment.

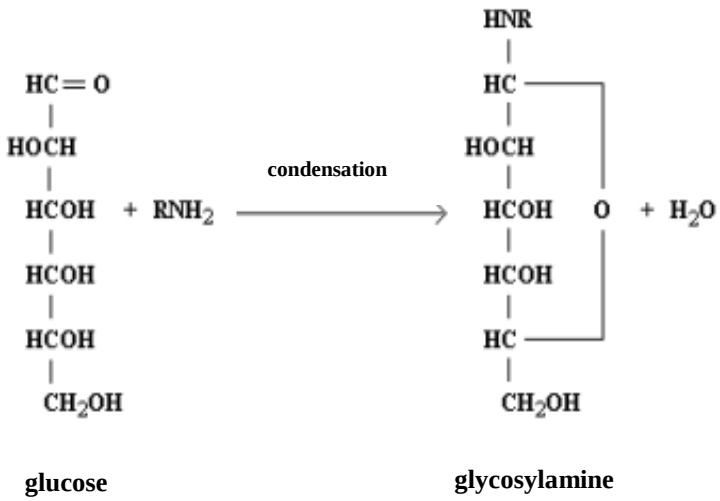


Figure 1. Condensation of Amino Carbonyl

Glycosilamine will be degraded into ketoxyamine (Figure 2), and the name of this process is Amadori rearrangement. Bastoset al. (2012) reported that definition of Amadori Rearrangement means the pathway from N-glycosilamine via 2,3 enolicform to 1-amino-1-deoxy-2-ketose. Amadori rearrangement plays an important role to initiate the brown color. The continuation of reaction is the reduction 4-hydroxy-5-methyl-2,3-dihydrofuran-3-one (HMF) and other aldehyde compound (Martins et al., 2001).

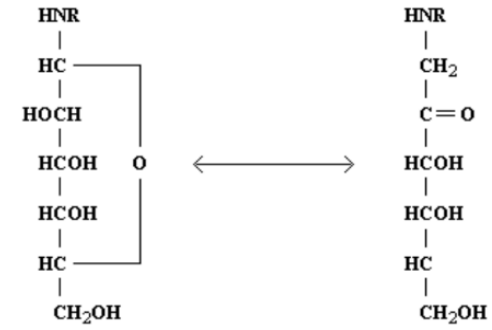


Figure 2. Amadori Rearrangement Mechanism

Degradation of the aldehyde strecker is another process that may occur during Maillard's reaction (Figure 3). This process occurs because of the presence of carbonyl compounds (reducing sugar) and decarboxylated and deaminated amino acids to aldehydes (Figure 4). Strecker degradation of amino acids also play an important role to the formation of aroma compounds and browning intensity during Maillard reaction process.

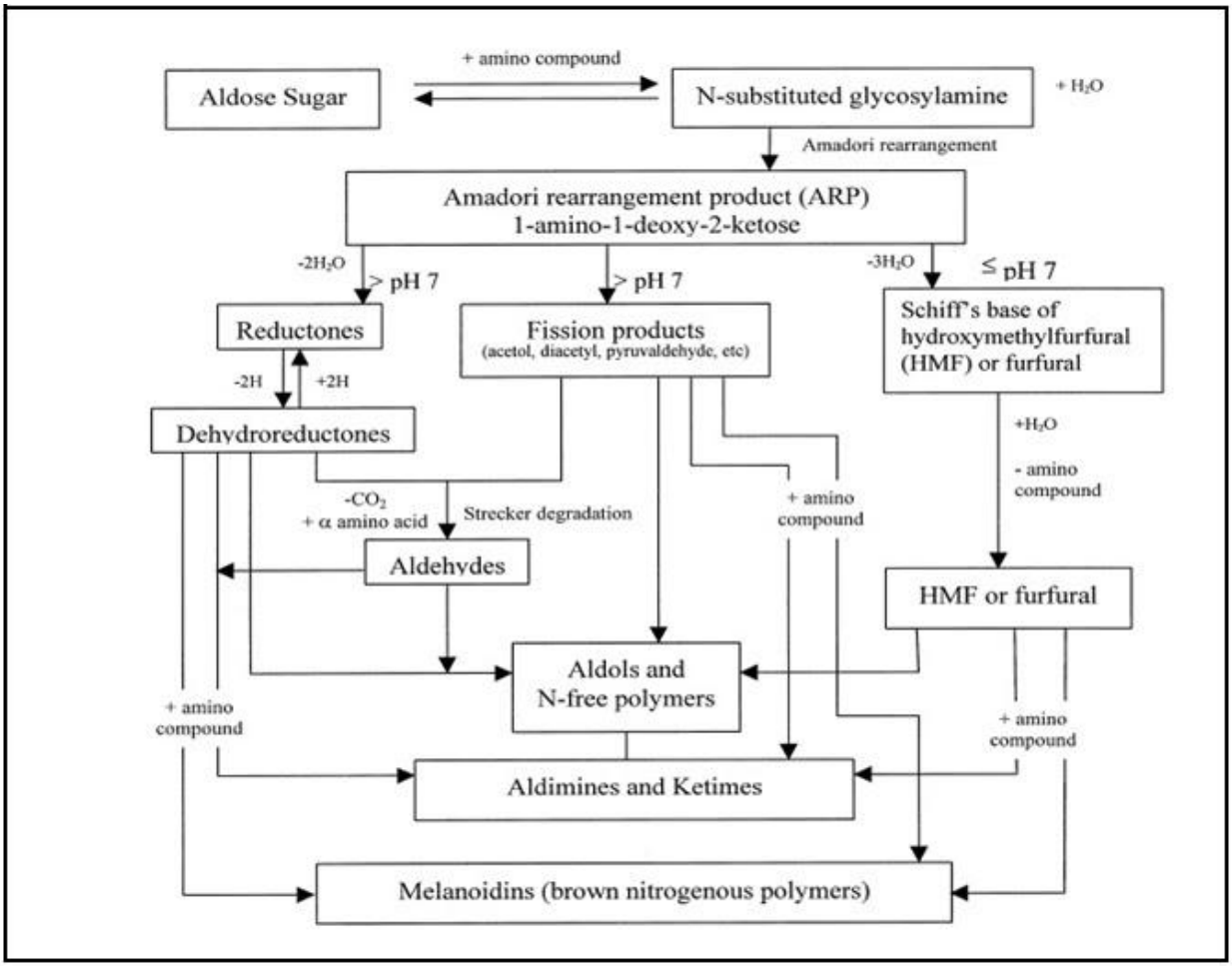


Figure 3. Degradation Strecker Aldehyde

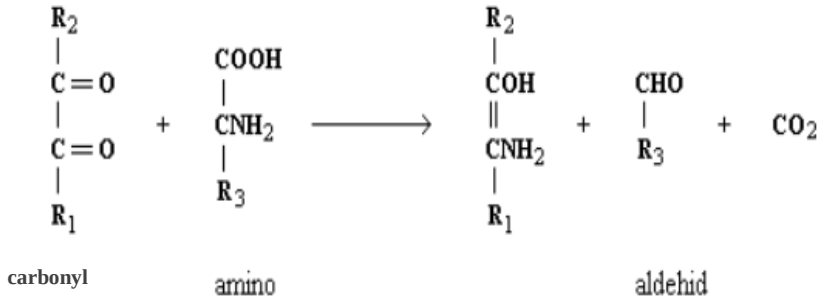


Figure 4. Aldehyde Compound Forming

Carbonyl compounds such as furfural compounds, aldehyde and ketone compounds will accumulate into more stable compound with high molecular weight as melanoidin (Martins et al., 2001).

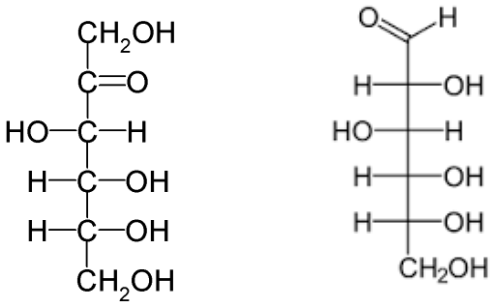
2.3. Lysine - D-Fructose Maillard Reaction

Maillard reaction process depends on the reactivity of reducing sugar and amino acid. Lysine is one of nine essential amino acids in humans required for growth and tissue repair and has been known to be the most reactive amino acid (Fox, 1997). Lysine exists in many foods, mainly red meats, fish, and dairy products.

Lysine has a reactive α-amino and ε-amino groups which play an important role in Maillard reaction formation. The α-amino group plays a role in the degradation deamination process of aldehyde structures, the presence of α-amino groups makes the amino acids degrade faster and form aldehyde compounds. While the highly reactive ε-amino groups make very rapid brown changes in lysine (Namiki et al., 1983).

While, D-Fructose is a natural monosaccharide found mostly in fruit and honey. Based on its chemical structure, D-Fructose has no difference with D-Glucose. Both are reducing sugars with open chain and have C-3 epimer (Sun et al., 2004). The difference between D-fructose and D-Glucose is the constituent group (Figure 5). D-fructose is composed of ketohexose groups while D-glucose is composed of aldohexose groups. D-fructose can also be obtained from D-glucose by isomerase process using aldose isomerase enzyme. Using aldose

isomerase enzyme, aldohexose group on D-glucose can be converted into



D-Fructose D-Glucose

ketohexose group (Granstrom et al., 2004).

Figure 5. Chemical structure of D-Fructose and D-Glucose

In the case of reactants, aldoses have been well studied as the more reactive component with amino acids than ketoses, as well as pentoses were more reactive than hexoses (Hwang et al., 2011; Phisut and Jirapon, 2013). In general, aldoses are considered to be more reactive than ketoses, because aldoses have more electrophilic carbonyl groups. However, the ketose sugar of D-fructose is found to brown faster than the aldose isomer of D-glucose, because it has a high concentration of acyclic forms in aqueous solutions (Jing and Kitts, 2002).

2.4. Browning Intensity

Browning intensity is main character and important indicator of Maillard reaction products and as an indicator of the reaction stage, which was investigated based on the absorbance at 420 nm (Zeng et al. 2011). Formation of browning intensity is in line with duration of heating time, temperature (Jing and Kitts, 2002).

The formation of browning intensity generated by Amadori rearrangement process, when amino acid and carbonyl groups were degraded and dehydration of water. The browning intensity rate depends on several factors, such as reactivity of amino acid and reducing sugar, pH condition, and heating treatment. This statement was supported by Laroque et al. (2008), who have been stated in his previous study that sugar reactivity was assessed as the parameter to determine the browning intensity in Maillard reaction. Browning Intensity are precursor for the brown pigment, aroma, also flavor formation. One of the browning intensity compound that formed during Maillard reaction is Hydroxymehtylfurfural (HMF).

Hydroxymehtylfurfural (HMF) is an intermediate compound formed during Maillard reaction. Reactivity of amino groups has an important role to form carbonyl compound on browning intensity. Amino acid with sulphur side generates a highly intense smell and flavor (Bastos et al. 2011). Special characteristic of amino acid will generate the difference of aroma and flavor on Maillard products or called volatile compound, that contain antioxidant. Food product with Maillard processing such as coffee, popcorn, bread, and cookies have high antioxidant (Martins et al., 2001).

2.5. Browning Index

Browning index or color development is the primary characteristic of the Maillard reaction. Increasing heating time followed by increasing of brown color. The longer heating time will accumulate the Amadori products that generated brown color. Amadori product will accumulate into carbonyl compound with large molecular weight. These compounds contribute particularly to aroma and

color characteristics and are collectively intended to as MRPs (Jing and Kitts, 2004). The longer of the heating process obtained, the browning index increased and the development of color as well, therefore the browning index has shown as a good indicator in color development of the MRPs (Alvarenga et al., 2014). Some pigments in Maillard browning products, which were formed by the presence of sugar during the heating process, contribute numerously to the color change (Sun et al., 2008). Color development in nonenzymatic browning was measured by browning index using the CIE-Lab color system from a digital colorimeter. It was in line with Alvarenga et al. (2014) who explained that color changes or color development were determined using a Minolta colorimeter with diffuse illumination/0 to obtain the CIE L* a* b* values and calculating the browning index using the following equation ;

$$x = \frac{a + 1.75\,(L)}{5.645\,(L) + a - 3.012\,(b)} \qquad (1)$$

$$BI = \frac{100\,(x - 0.31)}{0.172} \qquad (2)$$

L, *a*, and *b* are the values from digital colorimeter, *x* is the value obtained from equation (1), and *BI* is the browning index.

2.6. Spectroscopic Measurements

Maillard reaction consists of both early and advanced Maillard reactions, which can be characterized by the profile of spectra measurement (Jing and Kitts, 2000). According to the study from Wang et al. (2013), during the heating process, the chemical changes in the Maillard reaction model systems would lead to several changes in the spectrum as a result of the consumption of some functional groups and the appearance of others. In addition, Gu et al. (2010)

explained that by the Maillard reaction, functional groups including NH_2 and especially from amino acid may be decreased, whereas the amount of those associated with MRPs, such as the Amadori compound, Schiff base and pyrazines may be increased. These MRPs compound were responsible for the characteristic spectral pattern changes in the spectra measurements. This explanation was in line with Jing and Kitts (2000) who has stated that the prominent differences in the spectral absorbance between sugar-amino acid MRPs model, indicated the differences of chemical components of sugar-amino acid MRP models (Jing and Kitts, 2004; Chen and Kitts, 2008).

2.7. ABTS Radical Scavenging Activity

During the Maillard reaction process, the oxidation may be appeared based on the mechanisms of hydrogen atom transfer and single electron transfer (Morales and Jimenez-Perez, 2001). The antioxidant activity from MRPs break the radical chain by donation of hydrogen, while caramelization products break the radical chain by electron as donors. The antioxidant activity can be analyzed by chemical assays based on the ability of the compound to scavenge model free radicals, such as DPPH (1,1-difenil-2-pikrilhidrazil) and ABTS (2,2'-azino-bis-[3-etilbenzotiazolin sulfonate]) (Hwang et al., 2011; Sun et al., 2006). According to Lee et al. (2009) and Manzocco et al. (2001), there are some advantages of using the ABTS method to determine the antioxidant activity rather than using DPPH method, because it was considered more reactive and had a higher specific rate. Furthermore, ABTS has a specific absorbance at visible wavelengths and can

react in aqueous or organic solution (Shalaby and Shanab, 2012) and providing the blue-green colored solution.

Hwang et al. (2011) explained that the scavenging activity can be calculated using this following equation, $y = \frac{A0-A1}{A0} \times 100$, where A0 is the absorbance with blank and A1 is the absorbance with the sample and calculated by determining the percentage of decolorization of sample at room temperature for certain period of time (Sun et al., 2006).

2.8. Correlation between Browning Intensity and Scavenging Activity

Color changes represents the change on formation of compound during the development of Maillard reaction that may be led to the elevation in antioxidant activity (Mazocco et al., 2001) and shows the high correlation to browning products (Sun et al. 2008). Previous research reported that browning intensity and scavenging activity showed positive lineal correlation (Alvarenga et al., 2014). The significance in the correlation may be concluded from the statistical analysis using GraphPad Prism analysis if P value <0.0001 (Nilsson et al., 2004)