CHAPTER II

LITERATURE REVIEW

2.1. Maillard Reaction Products (MRPs)

Maillard reactions also are known as the non-enzymatic reaction between reducing sugar and amino acids with heating treatment. 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 would generated a yellow-brown color (Bastos *et al*., 2011). During this reaction, carbonyl groups of reducing sugar and amino groups will degraded to form the carbonyl compound. During the Maillard reaction takes place the carbonyl compound will accumulated into a new stable compound (Martins *et al*., 2001). The stable compounds were usually called an Amadori product that has forming brown color and flavor on Maillard reaction. The Amadori product known as Maillard reaction products were the results of the desired process also generated the flavor, color, and antioxidant (Phisut and Jirapon, 2013). Applications MRPs on food products may be caused positive impact such an oxidative stability of food products, and preserve food from oxidation and microorganism contamination as well (Norton and Sun, 2008).

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). Glycosilamine will be degraded into ketoxyamine, and the

name of this process is Amadori rearrangement. 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). 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). This reaction fully shows at figure 1.

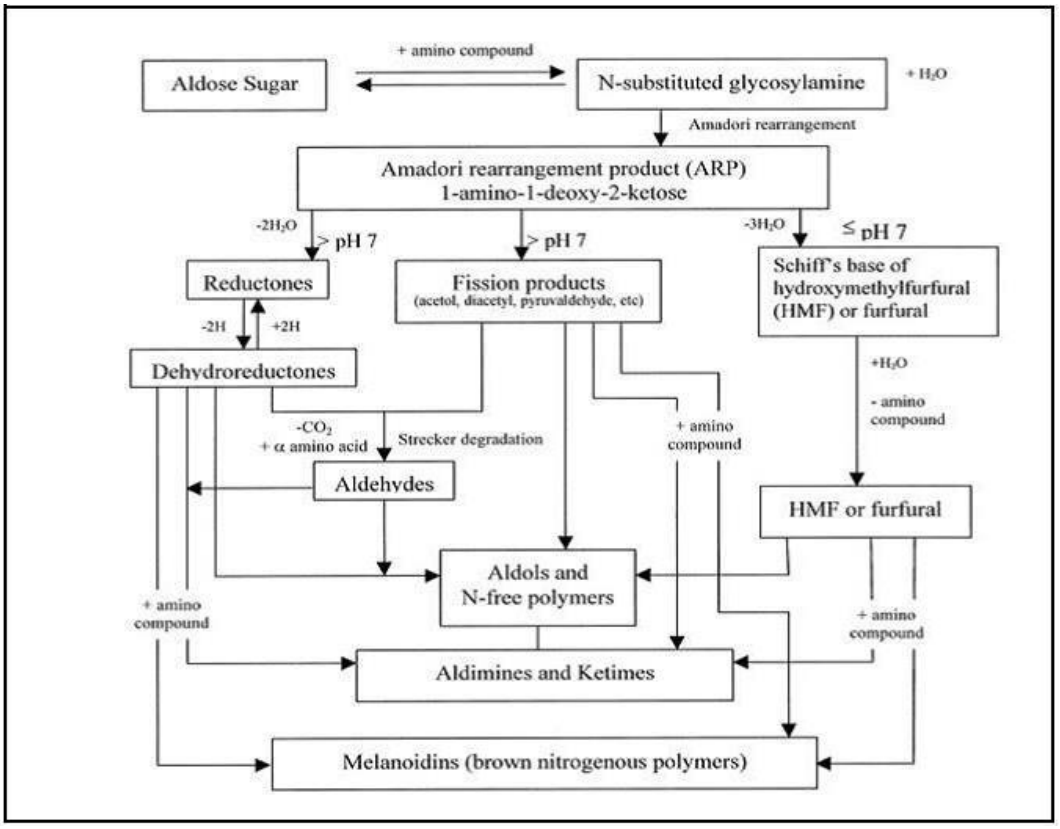


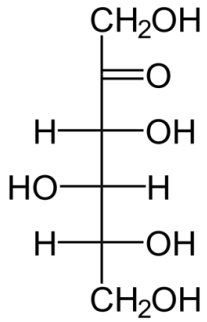
Figure.1 Maillard Reaction Process

2.2. Rare Sugar of D-sorbose

D-sorbose is kind of a rare sugar with ketohexose groups which present in small quantities in nature. D-sorbose can be obtained from the hydrolysis of

sucrose or isomerization of D-tagatose (Sun *et al*., 2004a). D-sorbose is one of ketohexose which may be produced by the enzymatic reaction using D-tagatose 3-epimerase from D-tagatose (Sun *et al*., 2004a; Sun *et al*., 2007; Kim *et al*., 2006).

D-sorbose has been categorized as rare sugars since it is scarcely found in nature, which has equivalent the sweetness with sucrose (Oshima *et al*., 2014; Puangmanee *et al*., 2008; Sun *et al*., 2006). D-sorbose has reactivity higher than natural sugar, especially to attach with amino acid. During Maillard reaction, rare sugar generated MRPs better than natural sugar. In previous research (Zeng *et al*., 2011) reported that Maillard reaction from amino and D-tagatose model system have the high free radical scavenging activity and reducing power than using



D-Sorbose

natural sugar. Chemical structure of D-sorbose presented at **Figure 2.**

Figure 2. Chemical chain of D-sorbose

2.3. Amino Acids Threonine

Amino acids are divided into 2 parts, essentials and non-essential. Threonine is one of the non-essential amino acid, so need intake from outside from the body. This amino acid also can be found in dairy foods, meat, grains and

mushrooms. Threonine has the ability to produce antibodies and support the immune system. Threonine is an amino acid group of Hydroxyl-Aliphatic that has high reactivity, especially in the Maillard reaction. During Maillard reaction takes place, threonine will be degraded by heat and release ammonia. Ammonia that forms during the Maillard reaction called Pyrazine which is one of the Maillard reaction products (Sohn and Ho, 1995). Pyrazine is a volatile compound that has the ability to produce a distinctive aroma and high antioxidant value.

2.4. Browning Intensity

Browning intensity is the main character of Maillard reaction which caused the formation of brown color, flavor and aroma. Browning intensity was measured based on the absorbance 420 nm using spectrophotometer (Zeng *et al*., 2011). During Maillard reaction, the browning intensity value was increased in line with long heating time and also depends on several factors, such as the reactivity of reducing sugar and the reactivity of amino acid (Oshima *et al*., 2014). In previous research reported that the reactivity of reducing sugar very influence on browning intensity. For example the study from Ajandouz *et al*. (2001) who has been described that the browning of fructose aqueous solutions in the presence of amino acids in Maillard reaction model systems was found to take place more quickly than that of glucose, from these researches means that the reactivity of reducing sugar will affect the browning intensity result. Reactivity of reducing sugar plays an important role on brown formation, while the amino groups plays important on flavor and volatile compound formation (Bastos *et al*., 2011). A special characteristic of amino acid will generate the difference of aroma and

flavor on Maillard products or called volatile compound, that contain antioxidant. Food products with Maillard processing such as coffee, popcorn, bread, and cookies have high antioxidant (Martins *et al*., 2001).

2.5. Color Development

Color development is the visible result from Maillard reaction with the development brown color formation. The formation of color development depends on several factors such as reactivity of reducing sugar, amino groups and heating time. Increasing heating time followed by increasing of brown color. Color development formed by condensation of reducing sugar and amino groups to form the carbonyl compound, in line with heating time, carbonyl compound will accumulate into carbonyl compound with high molecular weight or called Amadori Products. Amadori product that generated the brown color. According to Burdurlu and Karadeniz (2003), 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.

In addition, Alvarenga *et al*. (2014) explained that as 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). Browning index could be useful for a good observation of the advanced Maillard reaction stage (Morales and van Boekel, 1999).

2.6. Spectrophotometric

Spectrophotometer measurement is a method that can be used to detect the formation a new compound during Maillard reaction. Spectral pattern formed by absorption absorbance compounds that can be characterized. These patterns leading increase as the duration of reaction (Yen and Hsieh, 1995). According to Morales and Jimenez-Perez (2001) reported that the brown color of a sugar–amino acid mixture lead to increase as the heating time increased and chemical change occurred, it will be followed by the different spectral pattern. During Maillard reaction, reducing sugar and amino groups will degraded and functional groups such as NH_2 may be decrease, whereas the amount of those associated with MRPs, such as the Amadori compound, Schiff base, and pyrazines may be increased (Gu *et al*., 2010). 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 been 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.

The determination method of spectroscopic measurements was explained

by Jing and Kitts (2004) that measured for the spectral pattern of sugar–amino acid MRPs model at an emission spectrum of wavelength 350–550 nm, using a Shimadzu spectrofluorophotometer RF-540 (Kyoto, Japan). This method was in agreement with the study from Chen and Kitts (2008), who described that the absorbance of the heated mixtures was measured after appropriate dilution with phosphate-buffered saline solution (pH 7.4).

2.7. Radical Scavenging Activity

Antioxidant activity is the one of MRPs that formed during reducing sugar and amino groups are degraded into carbonyl compound. Formation of antioxidant compound occurs when carbonyl compound are accumulate or volatile compound that generated from amino groups. The antioxidants of MRPs break the radical chain by donating hydrogen or electron mechanism (Morales and Jimenez-Perez, 2001). 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 sulfonat]) radicals (Hwang *et al*., 2011). According to Thaipong *et al*. (2006), there are some advantages of using the ABTS method to determine the scavenging activity rather than using DPPH method, including that the extract of samples reacted faster which was conducted in 2 h, while using DPPH was in 24 h. Perez-Jimenez and Saura-Calixto (2006) also explained that ABTS is better than DPPH in the interference effect of solvent and food constituents in antioxidant capacity assays test.

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 Scavenging Activity and Browning Intensity

Browning intensity was in line with free radical scavenging activity of the sample. MRPs with high browning intensity assigned a high radical activity (Sun *et al*., 2008). Previous research from Alvarenga *et al*. (2014) reported that browning intensity and antioxidant showed the positive linear correlation. The significance of the correlation may be concluded from the statistical analysis using GraphPad Prism analysis if P value <0.0001 (Nilsson *et al*., 2004).