CHAPTER II
LITERATURE REVIEW

2.1. Maillard Reaction Products

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., 2011). Maillard reaction has been well understood as a non-enzymatic reaction between reducing sugars and amino acids to generate the Maillard reaction products (MRPs). Maillard reaction products were desirable process to generate the flavor, color, and antioxidant activity (Phisut and Jirapon, 2013; Hwang et al., 2011). The application of food processing practices to generate MRPs can improve the oxidative stability of food products, and preserve food from oxidation and microorganism contamination as well (Sun et al., 2004b). Moreover, the typical characteristics of MRPs' flavor and antioxidative properties, denote another major category of volatile components (Imafidon and Spanier, 1994).

A temperature has been stated as important key for producing MRPs. It has been recognized that significant increase of MRPs were obtained after an increase of temperature from 50 to 60^{o}C (Alvarenga et al., 2014), thus resulting the conclusion that MRPs was temperature-dependent products. In the other hand, Maillard reaction was relied on the pH of medium. It was stated that increase in pH medium might enhance the reaction of Maillard (Ajandouz and Puigserver, 1999). Several factors in the reaction, which are reactants type and concentration, temperature, heating time, pH, and humidity (Lamberts et al., 2008; Hwang et al.,

2011) could not be disregarded. It is generally concluded that reactants and reaction conditions truly affect the result of final Maillard reaction products (Delgado-Andrade et al., 2004).

2.2. Rare Sugar of D-Psicose

Rare sugars are defined as monosaccharide and their derivatives that are rare in nature. D-psicose is kind of a rare ketohexose which present in small quantities in commercial mixtures of D-glucose and D-fructose obtained from the hydrolysis of sucrose or isomerization of D-glucose (Sun et al., 2004a). D-psicose is one of ketohexose which may be produced by the enzymatic reaction using D-tagatose 3-epimerase from D-fructose (Sun et al., 2004a; Sun et al., 2007; Kim et al., 2006). D-Psicose is a ketohexose monosaccharide sweetener, which is a C-3 epimer of D-fructose and is rarely found in nature (Mu et al., 2012).

D-psicose has been categorized as rare sugars since it is scarcely found in nature, which has 70% of the sweetness of sucrose, and the reactivity to proteins may produce foods with excellent antioxidant activity and good rheological properties (Oshima et al., 2014a; Puangmanee et al., 2008; Sun et al., 2006). This statement is in line with Mu et al., (2012) who describe that D-psicose has 70 % relative sweetness but 0.3 % energy of sucrose, and it suggested as an ideal substitute of sucrose for food products. D-psicose shows important physiological functions, such as blood glucose suppressive effect, reactive oxygen species scavenging activity, and neuroprotective effect. It also improves the gelling behavior and produces good flavor during food process. This matter also

explained by Matsuo et al. (2003) who stated D-psicose exists in small quantities of commercial carbohydrate or agricultural products and difficult to be chemically produced, which have role of absorbed poorly in the digestive system, zero energy or zero calorie for growth, and it can be a useful low-calorie sweetener. Moreover, it can be used as an intestinal α-glycosidase for reducing body fat accumulation and inhibitor of hepatic lipogenic enzyme as well.

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). Since D-psicose categorized as ketose sugar, higher reaction temperatures and higher initial pH will generate higher levels of D-psicose degradation, higher browning intensity during heating process, and higher decrease in final pH with longer heating times. This D-psicose concentration decreased after heating process at high temperature for a long period (Oshima et al., 2014b). In addition, Sun et al. (2008) has been studied that the presence of D-psicose were able to significantly enhance the browning reaction during heat treatment in cookie processing and, as consequent as produced a strong antioxidant activity.

2.3. Amino Acid of Methionine

Methionine is an essential amino acid that contained in many different foods, particularly it can be found in fish, meat, vegetables, egg, whole-grain bread, and rice. It usually used in the food industry to produce aroma compounds such as cooked potatoes, coffee, or roasted meat, that may lead to contribute in producing MRPs when it has interactions with reducing sugars through thermal conditions (Pfeifer and Kroh, 2010). The previous study from Pfeifer and Kroh (2010) also stated that methionine has a great effect on the formation of specific R-dicarbonyl compounds in Maillard reaction.

The previous study from Yaylayan and Keyhani (2001) showed that D-glucose and methionine become the precursors of important compounds in meat aroma, such as thiofurans, thiopyrroles, and thiopyrazines. Delgado-Andrade et al. (2004) added that although methionine is less reactive than lysine, methionine is also degraded by the Maillard reaction during heating process, generating volatile compounds and losing availability as well. Weight loss in the glucose–methionine mixtures was increased as the heating time increased. In addition, Delgado-Andrade et al. (2005) also stated that as the heating time was increased, free residual methionine will be decreased, while the browning rate will be enhanced.

2.4. Physical and Chemical Properties of MRPs

The physical and chemical properties of Maillard Reaction Products (MRPs) determined by some parameters, which are browning intensity, color development, spectroscopic measurements, ABTS radical scavenging activity.

Then, the correlation between browning intensity and scavenging activity was studied.

2.4.1. Browning Intensity

Browning intensity is mostly become the important indicator result of Maillard reaction. This statement is in line with Zeng et al. (2011); Morales and Jimenez-Perez (2001), who explained that the browning of Maillard reaction model system was often used as an indicator of the reaction stage, which was investigated based on the absorbance at 420 nm using a spectrophotometer. In addition, Zeng et al. (2011) and Oshima et al. (2014b) also explained that browning intensity increased significantly as the reaction time increased. This explanation was in agreement with Jing and Kitts (2002) who has been stated that their study about browning intensity of glucose and fructose–casein Maillard reaction model systems were increased as the result of increasing heating time.

The browning intensity result of Maillard reaction also depends on several factors, such as the reactivity of reducing sugar and the reactivity of amino acid. There have been many studies discussed about this matter previously, 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, by that means the reactivity of reducing sugar will affect the browning intensity result. 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. Moreover, Morales and Jimenez-Perez (2001) also explained that browning intensity showed a slight induction period, depending on the reactivity of amino acid and the reactivity of sugar.

The determination method of browning intensity based on the study from Ajandouz et al. (2001) described that the browning intensity determination of the aqueous solutions containing fructose or fructose and lysine were measured at room temperature at 420 nm, using a Beckman model DU 640 spectrophotometer. Appropriate dilutions were made if necessary to obtain an optical density of less than 1.5. This determination method of browning intensity was in line with Brands and van Boekel (2001) who has been stated that the browning intensity of the heated reaction mixtures of Maillard reaction was determined by measuring the absorbance at 420 nm with a spectrophotometer.

2.4.2. Color Development

The Maillard reaction starts with the condensation of carbonyl groups from reducing sugars and amino groups of amino acids, and it develops into a complex set of reactions that derives generous products of early volatile compounds, intermediate products, and large molecular weight polymers. These compounds contribute particularly to aroma and color characteristics and are collectively intended to as MRPs (Jing and Kitts, 2004). Some pigments in Maillard browning products, which were formed by the presence of sugar during the heating process, contribute numerously to the color change of cookies (Sun et al., 2008). 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). This result is related with the study from Bosch et al. (2007) who reported that the browning index increased as the time rose, and at 48 h of heating process were generated the highest browning index value. Browning index could be useful for a good observation of the advanced Maillard reaction stage (Morales and van Boekel, 1999).

2.4.3. Spectroscopic Measurements

Maillard reaction consists of both early and advanced Maillard reactions, which can be characterized by absorption spectra measurement (Jing and Kitts, 2000). These spectra indicated that Maillard reaction products had scavenging activity on the hydroxyl radical, and lead to increase as the duration of reaction

increase (Yen and Hsieh, 1995). This statement was in line with Jing and Kitts (2002); Morales and Jimenez-Perez (2001), who have been explained that the browning of a sugar–amino acid mixture lead to increase as the heating time increase, and it will be followed by the different spectra pattern that reaches a maximum phase, before plateauing off during the heating time.

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 NH2 and especially from amino acid may be decreased, whereas the amount of those associated with MRPs, such as the Amadori compound (C=O), Schiff base (C=N) and pyrazines (C–N) 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 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. These differences reflected the variety in chemical and biochemical activities to both antioxidative and cytotoxic activities.

The determination method of spectroscopic measurements was explained by Jing and Kitts (2004), who described from their study that the heated solutions of sugar–amino acid MRPs model were diluted with phosphate-buffered saline (PBS, 50 mM, pH 7.4), and were measured for the spectra 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). The fluorescence spectra of the heated mixtures were measured with an emission wavelength from 350 to 550 nm using a spectrophotometer.

2.4.4. ABTS Radical Scavenging Activity

The antioxidants perform their roles principally based on the mechanisms of hydrogen atom transfer and single electron transfer (Morales and Jimenez-Perez, 2001). 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 sulfonat]) radicals (Hwang et al., 2011). These kind of chemical characteristics of MRPs were performed as important factors for evaluating the antioxidant activity (Sun et al., 2006). 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.

The free radical scavenging activity of Maillard reaction products (MRPs) produced by heating sugar-amino acids (Morales and Jimenez-Perez, 2001). This result is in agreement with Jing and Kitts (2002) who described that MRPs have free radical scavenging affinities in different model systems, and the antioxidant activities of MRPs derived from peptide–protein–sugar systems was greatly increased when protein was heated with reducing sugar. In addition, there are several factors that will correspond to the scavenging analysis result. MRPs have different free radical scavenging activities according to the heating time and peptide chain length. The ABTS radical scavenging activity of all MRP samples was increased as the heating time increased (Kim and Lee, 2009). Besides that, MRPs have different antioxidant activities according to the type of reducing sugar and amino acid involved in the Maillard reaction (Hwang et al., 2011). According to Zeng et al. (2011), during the whole reaction stage, the ABTS radical scavenging activity of the Maillard reaction products from psicose-amino acid increased faster than that from fructose-amino acid.

The determination of ABTS radical scavenging activity has been explained by Sun et al. (2006), who have been stated that the ABTS method gives a measure of MRPs antioxidant activity by analyzed the reduction of the radical cation as the percentage inhibition. The ABTS radical cation method was used to evaluate the free radical scavenging effect of MRPs, and the ABTS+ cation was formed by adding $K_2S_2O_8$ to ABTS. The ABTS+ stock solution was diluted with 10 mM phosphate buffer (pH 7.4) to a final absorbance of the control of 0.7 ± 0.02 at 734 nm using a spectrophotometer. This ABTS radical scavenging activity method

was in agreement with Hwang et al. (2011), who explained that 7 mM ABTS was diluted with 10 mM phosphate buffer (pH 7.4). These 5 mL of ABTS solution was added with 88 µL of 140 mM potassium persulfate. These mixtures were incubated for 16 hours in the dark condition at room temperature, to reach a final absorbance of 0.7±0.02 at 734 nm using a spectrophotometer.

Moreover, Hwang et al. (2011) explained that the percentage inhibition of the MRPs 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. In addition, scavenging activity of MRPs was calculated by determining the percentage of decolorization at room temperature exactly 20 s after the initial mixing and up to 6 min. Since the gradual decrease in absorbance of the working ABTS+ solution without the sample added, an appropriate solvent blanks were used in every measurements (Sun et al., 2006).

2.4.5. Correlation between Browning Intensity and Scavenging Activity

Browning intensity was parallel to the appearance of free radical scavenging activity (Zamora et al., 2011). This is in line with Sun et al. (2008) that browning products assigned a high antiradical activity. Based on the study from Hwang et al. (2011), ABTS radical scavenging activity was higher in the MRPs of the fructose-amino acid model than in those of the glucose–amino acid model, and this result was well correlated with UV absorbance and browning intensity.

The earlier study from Woffenden et al. (2001), reported a positive correlation between color and antioxidant properties in foods. The positive lineal correlation (R^2 = 0.743) between available amino groups and browning index of glycoprotein samples in Maillard reaction condition also has been studied by Alvarenga et al. (2014). The significant correlation between two parameters is obtained, if P value <0.0001 using GraphPad Prism analysis (Nilsson et al., 2004; Aon and Colaneri, 2001).