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Demonstration of Scavenging Activity and Physical Phenomenas from D-psicose and Methionine Maillard Reaction Products

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Abstract

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Maillard reaction has been well understood as a non-enzymatic reaction between reducing sugars and amino acids to generate the Maillard reaction products (MRPs). This study is aimed to demonstrate the browning intensity, color development, spectra measurement, scavenging activity, and the correlation between browning index (BI) and scavenging activity (AO) of the MRPs generated from D-Psicose (Psi) and Methionine (Met) at 50°C. The browning intensity of MRPs was investigated based on the absorbance using spectrophotometer at 420 nm, the color development was observed using digital colorimeter to gained browning index value, the spectra was analyzed using spectrophotometer at 190 - 750 nm, and the scavenging activity was determined with ABTS method using spectrophotometer at 734 nm. The browning intensity, color development, and scavenging activity were improved along with the increase in heating process. Based on spectra analysis, MRPs from Psi-Met was initially detected at 21st h of heating treatment. The correlation between browning index and scavenging activity were assigned as a positive linear correlation. This finding may provide beneficial information to the food industries which applies MRPs in their products.

Keywords : Maillard reaction products, scavenging activity, browning, D-psicose, methionine.

1. Introduction

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Maillard reaction has been well understood as a non-enzymatic reaction between reducing sugars and amino acids to generate the Maillard reaction products (MRPs). A temperatur 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°C (Alvarenga et al., 2014), thus resulting the conclution 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). In several food industries, maillard reaction products were desirable process to generate the flavour, colour, and antioxidant activity

(Phisut & Jirapon, 2013; Hwang et al., 2011). Therefore 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. In the case of reactants, aldoses has been well studied as more reactive component with amino acids than ketoses, as well as pentoses was more reactive than hexoses (Hwang et al., 2011; Phisut & Jirapon, 2013).

D-psicose (Psi) is one of ketohexose which may be produced by the enzymatic reaction using D-tagatose 3-epimerase from D-fructose (Fru) (Kim et al., 2006; Sun et al., 2014). Psi has been categorized as rare sugars since it is scarcely found in nature. Though D-psicose has 70% of the sweetness of sucrose, the reactivity to proteins may produce foods with excellent antioxidant activity and good rheological properties (Oshima et al., 2014; Puangmanee et al., 2008; Sun et al., 2006). While, methionine is an essential amino acid that usually used in the food industry to produce aroma compounds such as cooked potatoes, coffee, or roasted meat (SITASI). Methionine may contribute to produce MRPs when it has interactions with reducing sugars through thermal condition (Pfeifer and Kroh, 2010). The previous study from Pfeifer and Kroh (2010) also stated that methionine has great effect on the formation of specific R-dicarbonyl compounds in maillard reaction.

The non-enzymatic browning reaction derived from methionine and D-glucose has been evaluated previously (SITASI), but based on our knowledge, few document was found on maillard reaction products from methionine and D-psicose. The previous research studied browning color intensity of the D-psicose and non-polar amino acids mixtures at high temperature but none was found when the mixture was applied in minimum/low temperature. Therefore, the objectives of this present study is to demonstrate the MRPs generated from Psi and Met at low temperature. We investigated the browning intensity, color development, spectra measurement, ABTS radical scavenging activity, and the correlation between browning index (BI) and scavenging activity (AO) of MRPs produced by heating process.

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2. Materials and Methods

2.1. Materials

Rare sugar of D-psicose was produced from Kagawa Rare Sugar Research Center, Japan. Methionine 99% was obtained from PT. Cheil Jedang Indonesia. Carbonate buffer (pH 9) solution, phosphate buffer (pH 7,4) solution, ethanol 90%, potassium persulfate and ABTS solution.

2.2. Preparation of model MRP systems

The D-psicose (Psi) as a control and 1:1 D-psicose Methionine (Psi-Met) were dissolved in 500 ml of carbonate buffer (pH 9) solution and taken 200 µm to put in a

microtube for 16 model samples. Samples were heated at 50 °C for 48 hours and were analyzed every 3 hours after diluted with phosphate buffer (pH 7) solution.

2.3. Physical and chemical analysis

2.3.1. Browning intensity

The browning intensity of the MRPs were measured according to the method of Ajandouz et al., (2001). The MRPs samples of Psi and Psi-Met after heating were diluted with phosphate buffer until 200 µm in microtube. Browning intensity of the samples were recorded by the MRPs absorbance at 420 nm on a spectrophotometer (UV-1280; Shimadzu, Kyoto, Japan) using a 1 cm path length cell after dilute with distilled water.

2.3.2. Color Development

The MRPs samples of Psi and Psi-Met after heating were diluted with phosphate buffer until 200 µm in microtube. The color changes of MRPs were determined using a digital colorimeter TES-135 with diffuse illumination/0° to obtain the CIE L* a* b* values and then calculate the browning index. The instrument was calibrated with a standard white tile (Y = 88.2, x = 0.309, y = 0.316) before measurement. Then calculated using the formula as below (Alvarenga et al., 2014) ;

$$x = \frac{a + 1.75(L)}{5.645(L) + a - 3.012(b)} \quad (1); \quad BI = \frac{100(x-0.31)}{0.172} \quad (2)$$

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

2.3.3. Spectroscopic measurements

The MRPs samples of Psi and Psi-Met after heating were diluted with phosphate buffer until 200 µm in microtube. The samples were measured for emission spectrum (190–750 nm) using spectrum spectrophotometer (UV-1280; Shimadzu, Kyoto, Japan).

2.3.4. ABTS radical scavenging activity

The antioxidant activity of the MRPs were detected using ABTS procedure according to the method of Hwang et al. (2011), with modifications. The 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 mixture 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 (UV-1280; Shimadzu, Kyoto, Japan). Then, 1 : 9 of MRPs samples mixed with ethanol 90% and ABTS solution were analyzed using a spectrophotometer. According to Hwang et al. (2011), the percentage inhibition of the MRPs scavenging activity was calculated as below ;

$$y = \frac{A_0 - A_1}{A_0} \times 100 \quad (3)$$

A0 is the absorbance with blanko and A1 is the absorbance with the sample.

2.3.5. The correlation between browning index and scavenging activity

The browning index and the scavenging activity results were compared using linear graphic to determine their correlation when producing MRPs from Psi and Psi-Met.

1 3. Results and discussion

3.1. Browning Intensity

The browning of the Maillard reaction model systems was investigated based on the absorbance at 420 nm, which in Maillard reaction stage was often used as an indicator (Morales and Jimenez-Perez, 2001). The browning intensity of MRPs on Psi-Met and Psi as its control are shown in Fig. 4, respectively. The Psi-Met mixture showed an increased result due the heating process, and Psi as well. It was same with the study from Oshima et al., (2014) that the brown color intensity increased as the reaction heating process was raised.

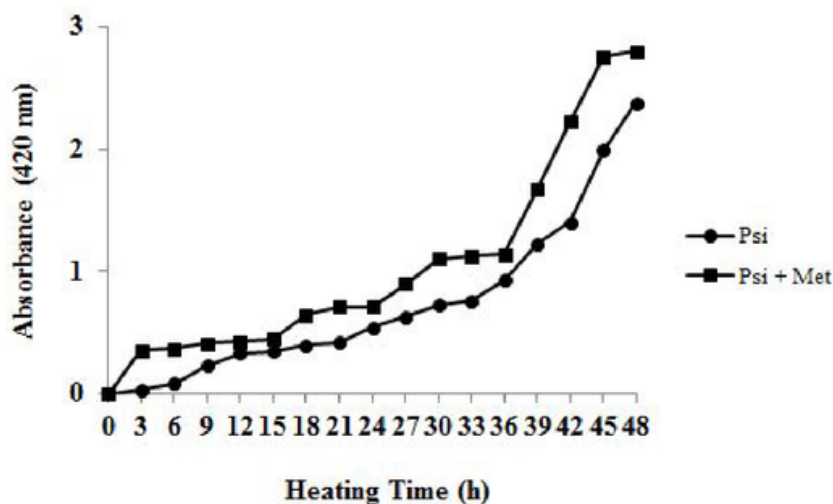


Figure 4. Browning intensity of Psi and Psi-Met.

The browning intensity of Psi-Met is higher than Psi, start from their early reaction until their final reaction. It was caused of browning intensity is significantly influenced by the type of reducing sugar and amino acid involved in the reaction. D-psicose is a ketohexose which has good reactivity when react with amino acids in heating condition. Brands et al., (2000) stated that ketose was browned more quickly than aldose when heated up to 60 min. While, Methionine is an amino acid that was found to be the least reactive

essential amino acid. It showed conspicuous effects when react with reducing sugar at pH 8.0-10.0 (Ajandouz and Puigserver, 1999). The reaction between D-psicose and Methionine showed a greater increase and effective role in browning than those from D-psicose, especially entering the advanced Maillard reaction stage at 36 h. Thus, it was in agreement with the study from Benjakul et al., (2005), who reported that MRPs derived from an amino acid–sugar complex could be formed more easily.

3.2. Color Development

Color development of the MRPs is also become an indirect measurement of the development of Maillard reaction. The browning index (BI) can be known from the digital colorimeter TES-135 to be calculated using the formula (Fig.1 & 2). The browning index shown to be a good indicator of the color changes of MRPs (Alvarenga et al., 2014). The browning index of Psi and psi-Met is shown in Table 1, respectively.

Table 1. Browning index obtained from Psi and Psi-Met

Heating Time (h)	Browning Index	
	Psi	Psi + Met
0	N/A	N/A
3	N/A	N/A
6	N/A	N/A
9	N/A	N/A
12	N/A	N/A
15	N/A	N/A
18	N/A	N/A
21	59.33114	30.26567
24	68.83892	54.23013
27	68.53976	59.39821
30	81.91095	62.99235
33	102.96824	63.40400
36	124.10767	64.21321
39	138.03722	65.67719
42	160.70650	66.15904
45	210.25845	68.04152
48	212.55945	72.43385

According to Table 1, it shown that index browning at 21 h gave a significant value from the previous hours and it reached the highest value at 48 hours. It suggest that as the longer of the heating process gained, the browning index increased and the development of color as well. This result is very 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. However, the browning index of Psi is entirely higher than Psi-Met. According to Alvarenga et al. (2014), it was caused by caramelization effect in Psi.

3.3. Spectroscopic characteristics of Psi and Psi-Met MRPs

The MRPs spectrum measurements of Psi and Psi-Met were using a spectrum spectrophotometer with the wavelength ranging from 190 to 750 nm. The result of spectrum measurements is shown in Fig. 5 and 6, respectively. The MRPs of Psi-Met model was produced characteristically different spectral patterns during the heating process than Psi model. These spectra measurements of MRPs solutions created by the heating process, allowed the detection of changes related to subsequent stages of the Maillard reaction (Jing and Kitts, 2004).

Figure 5. Development of emission spectra (190–750 nm) of Psi MRPs model

Figure 6. Development of emission spectra (190–750 nm) of Psi-Met MRPs model

The peak obtained from Psi-Met model start to changed significantly at 21 h. While, the peak obtained from Psi start to changed significantly at 24 h. It defined that the MRPs derived from Psi-Met was produced faster than MRPs obtained from Psi. Yet fully, as all the reaction continued to increase, the spectrum change develops as well and reached the highest point at 48 h.

1 3.4. ABTS Radical Scavenging Activity

The scavenging activity was observed using ABTS method based on Hwang et al. (2011) at 734 nm spectrophotometer. The scavenging activity can be known from the spectrophotometer then calculated using the formula (Fig.7).The scavenging activity of MRPs of Psi and Psi-Met are shown in Fig. 6, respectively. The MRPs from Psi-Met was entirely higher than Psi during the whole heating process. It was caused by reducing sugar that react more easily with amino acid in heating condition and produce antioxidant activity. It is in the same agreement with the previous study from Benjakul et al., (2005) who was stated that an amino acid with reducing sugar could be formed more easily. It was further indicated that the potential effect of Psi-Met is better than Psi in the antioxidant activity of the Maillard reaction products. In the previous study also noted that the proteins with D-psicose created the remarkable antioxidant capacity compared with alimentary sugars (Sun et al., 2004).

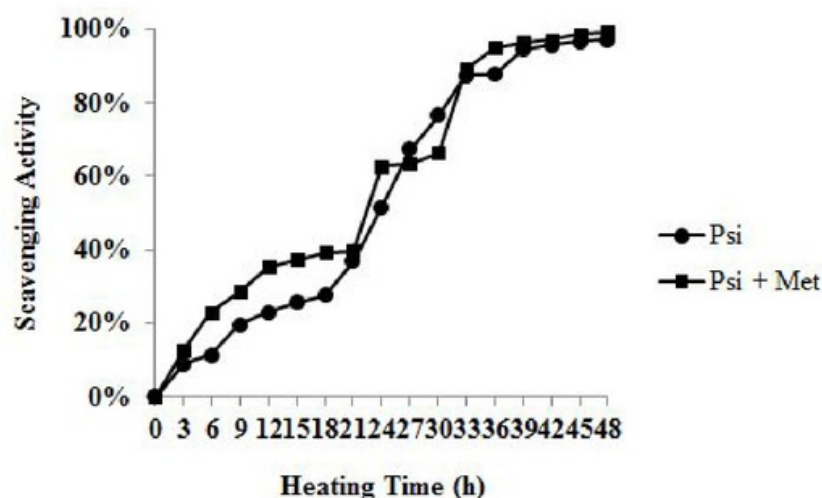
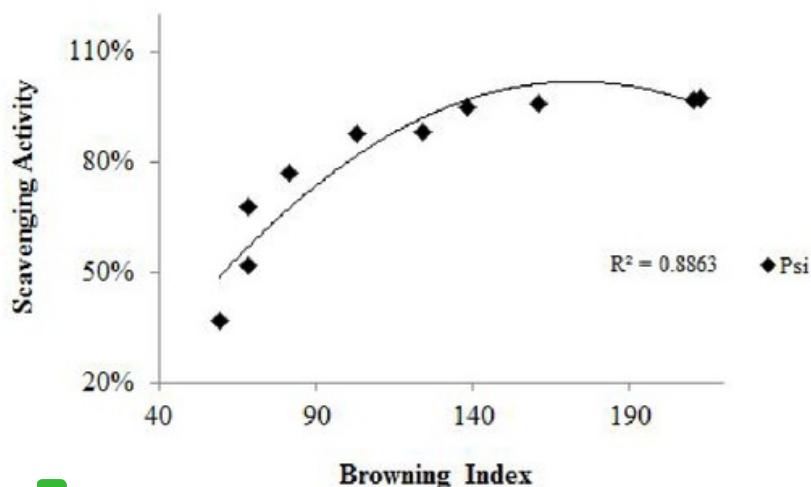


Figure 7. The scavenging activity of Psi and Psi-Met

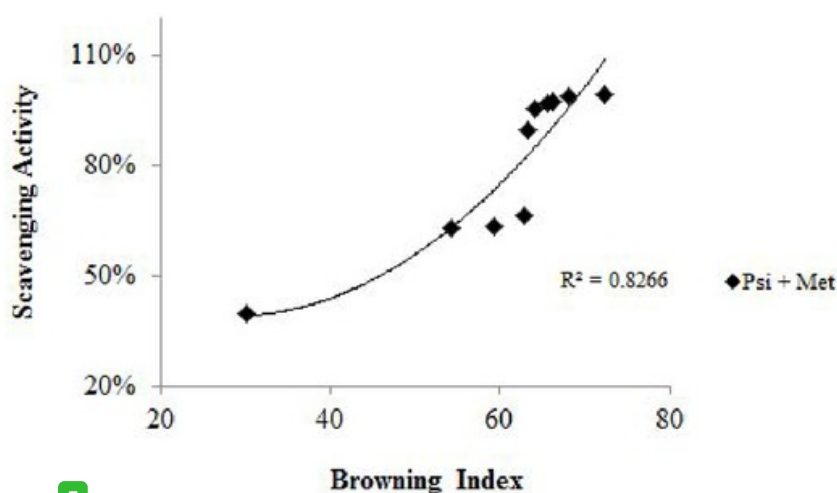
These following series of further reactions shown that the MRPs were easily formed in the present of Psi. Therefore, it is assumed that the strong antioxidant potency of the Psi-Met was attributed to MRPs formed during the heating process. Furthermore, the scavenging activity start to increase significantly at 21 h. It shows that at the applicative heating time before 24 h were already produced a result. However, at 27 h and 30 h, the scavenging activity of Psi-Met was lower than the scavenging activity of Psi. It might caused by due to the Maillard reaction step reach under the necessary conditions or caused by caramelization effect (Alvarenga et al., 2014).

3.5. The correlation between browning index and scavenging activity

The browning index and scavenging activity were compared to investigated their correlation. These correlation is shown in Fig. 8 and Fig. 9. Both of these correlation shown that the browning index and scavenging activity increased as the time increased. A positive linear correlation between browning index and scavenging activity was found at value, Psi;0.8863 and Psi-Met;0.8266. But, leading to the end of heating process, the graph in Psi model were decreased.



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Figure 8. A positive correlation between browning index and scavenging activity of Psi



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Figure 9. A positive correlation between browning index and scavenging activity of Psi-Met

4. Conclusions

During the Maillard process to derive MRPs, Psi-Met showing the better reaction than Psi. The browning intensity, color development, and scavenging activity were improved according to the heating process increased. The MRPs derived from Psi-Met (21 h) is faster to produce than MRPs derived from Psi (24 h) proven by spectrum measurement. The correlation between browning index and scavenging activity were

assigned a positive non-linear correlation (Psi;0.8863 & Psi-Met;0.8266). Essentially, the MRPs derived from Psi-Met has better scavenging activity and physical phenomenas than MRPs derived from Psi.

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