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Slovak Journal of Food Sciences

Potravinarstvo Slovak Journal of Food Sciences
vol. 11, 2017, no. 1, p. 417-424
doi: https://dx.doi.org/10.5219/776
Received: 7 March 2017. Accepted: 17 May 2017.
Available online: 29 June 2017 at www.potravinarstvo.com

ISSN 1337-0960 (online)

DEMONSTRATION OF PHYSICAL PHENOMENAS AND SCAVENGING ACTIVITY FROM D-PSICOSE AND METHIONINE MAILLARD REACTION PRODUCTS

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ABSTRACT

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 measurements, scavenging activity, and the correlation between browning intensity and scavenging activity of the MRPs generated from D-Psicose and Methionine (Psi-Met) at 50℃. 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 L*a*b* value then calculated as browning index, the spectra development 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 21 h and Psi at 24 h of heating treatment, which indicating that Psi-Met have faster and better reaction than Psi during heating process. Positive non-linear and significant correlation between browning intensity and scavenging activity were assigned. This finding may provide beneficial information of D-psicose and MRPs to the next scientific research and to the food industries which applies MRPs in their products.

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

INTRODUCTION

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 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 °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)**. In several food industries, Maillard reaction products were desirable process to generate the flavour, colour, and antioxidant activity **(Phisut and 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 have 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 and 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., 2004)**. 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, that may lead to contribute to produce 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 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 **(Ajandouz and Puigserver, 1999)**, 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 and our scientific hypothesis is the MRPs of Psi-Met will might have better scavenging activity and physical phenomenas than heated product of Psi. We investigated the browning intensity, color development, spectra measurements, ABTS radical scavenging activity, and the correlation between browning intensity and scavenging activity of MRPs produced by heating process.

MATERIAL AND METHODOLOGY

Materials

Rare sugar of D-psicose and D-fructose were obtained from Kagawa Rare Sugar Research Center, Japan. Methionine (with the purity index 99%) was obtained from Cheil Jedang Indonesia, Co. Ltd. ABTS or 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) was purchased from AppliChem, Germany (Lot No.2X001714). Unless otherwise specified, all other chemicals were reagent grade.

Methodology

Preparation of MRPs model

The preparation method of MRPs model has been adopted from **Yu et al. (2012)** with some modifications. The D-psicose (Psi) as a control and 1 : 1 D-psicose-Methionine (Psi-Met) were dissolved in 500 mL of 10 mM carbonate buffer (pH 9) solution. Two hundred microliter was transferred to 1.5 mL microtube prior to application for heat treatment. The sample was subjected to 48 h heating process at 50 ±1.0 °C RH 60% using the controlled dry oven. Measurements were performed every three hour in duplicate with three individual replicates of sample on hour 0 up to 24. The applied heating process was 50 °C for 48 hours using controlled dry oven. After heating treatment, the dried sample were cooled immediately in the air for 1 min, and then kept at 4 °C. Prior to measurement, samples were diluted with 200 µL of 10 mM phosphate buffer (pH 7).

Browning intensity

The browning intensity of the MRPs was measured according to the method of **Ajandouz et al. (2001)**. The MRPs samples of Psi-Met and heated product of Psi after heating process were diluted with phosphate buffer until 200 µL 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 diluted with distilled water.

Color development

The MRPs samples of Psi-Met and heated product of Psi after heating were diluted with phosphate buffer until 200 µL in microtube. The color changes of MRPs were determined using a digital colorimeter (TES-135A; USA) to obtain the L*a*b* values. The instrument was calibrated with a standard white before measurement, then the browning index was calculated using the equation (2) **(Alvarenga et al., 2014)**.

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

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

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

Spectroscopic measurements

The MRPs samples of Psi-Met and heated product of Psi after heating were diluted with phosphate buffer until 200 µL in microtube. The samples were measured for emission spectrum (190 – 750 nm) using spectrophotometer (UV-1280; Shimadzu, Kyoto, Japan). This spectroscopic measurement method has been adopted from **Jing and Kitts (2004)**.

ABTS radical scavenging activity

The antioxidant activity of the MRPs was detected using ABTS procedure according to the method of **Hwang et al. (2011)**, with minor 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 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. Then, 1 : 9 of MRPs samples mixed with 90% ethanol and ABTS stock solution were loaded in spectrophotometer (UV-1280; Shimadzu, Kyoto, Japan). The percentage inhibition of the MRPs scavenging activity from **Hwang et al. (2011)** was calculated in equation (3).

$$y = \frac{A0 - A1}{A0} \times 100 \quad (3)$$

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

The correlation between browning intensity and scavenging activity

The browning intensity and the scavenging activity were analyzed for R^2 value and linearity by regression analysis. Values of R^2 should be in a range 0 to 1 and it is close to 1, the positive correlation between browning intensity and scavenging activity should be occurred. This correlation analysis method has been adopted from **Alvarenga et al. (2014)**.

Data analysis

The results were reported in figures. The physical phenomenas and scavenging activity of MRPs were analyzed using descriptive analysis, and the significance of correlation between browning intensity and scavenging activity was analyzed using Graphpad Prism version 6.0. This study was adopted from **Nilsson et al. (2004)** who stated that the significant correlation should be if p value <0.0001.

RESULTS AND DISCUSSION

Browning intensity

The browning of the Maillard reaction model systems was investigated based on the absorbance at 420 nm as a common indicator of MRPs generation **(Morales and Jimenzez-Perez, 2001)**. The browning intensity of MRPs on Psi-Met and heated product of Psi as its control are shown in Figure 1. The heating process increased the absorbance of all samples. The previous work on browning conducted by **Oshima et al. (2014)** showed that the increase in browning intensity was linear with the reaction heating process.

The browning intensity of Psi-Met was higher than Psi, starting from early reaction until final reaction process. This may be explained by the influence of the type of reducing sugar and amino acid involved in the reaction **(Laroque et al., 2008)**. D-psicose has been known as a ketohexose which has good reactivity with amino acids **(Brands et al., 2000)**, while methionine was found to be a reactive essential amino acid **(Ajandouz and Puigserver, 1999)**. Maillard reaction involving amino acid and sugar resulted in the formation of new compounds, as indicated by browning intensity in our study. A newly formed compound shifted the color into brown **(Oh et al., 2016)**.

It has been understood that pH is an important factor influencing MRPs formation in the model system and it showed conspicuous effects when the model system was applied at pH 8.0 – 10.0 **(Ajandouz and Puigserver, 1999)**. Therefore, the reaction between Psi-Met was applied on pH 9.0 were resulting a greater increase in browning than those from Psi, especially entering the advanced Maillard reaction stage at 36 h. The applied pH at 7.0 in model system showed the negligible differences between Psi-Met and Psi (data not shown). This finding 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 in basic pH.

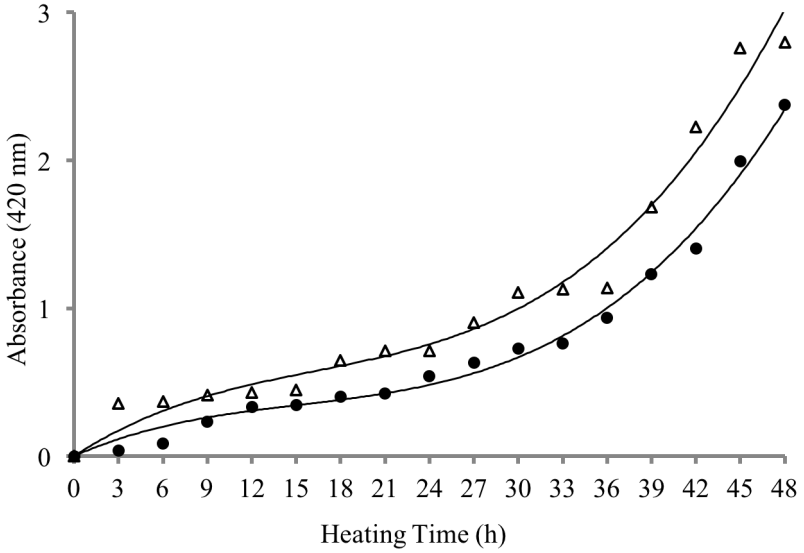

Figure 1 Browning intensity of rare sugar Psi (•) and Psi-Met (Δ) model systems when was heated at 50 °C. The absorbance at 420 nm were measured every 3 h for 48 h.

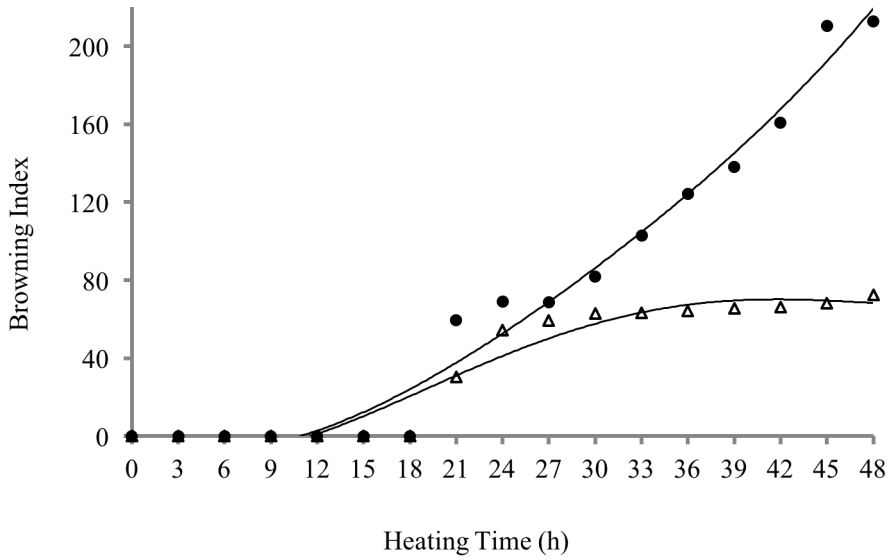

Figure 2 Browning index of rare sugar Psi (•) and Psi-Met (Δ) model systems when was heated at 50 °C. Data were obtained from the equation (2) using L*a*b* values from digital colorimeter.

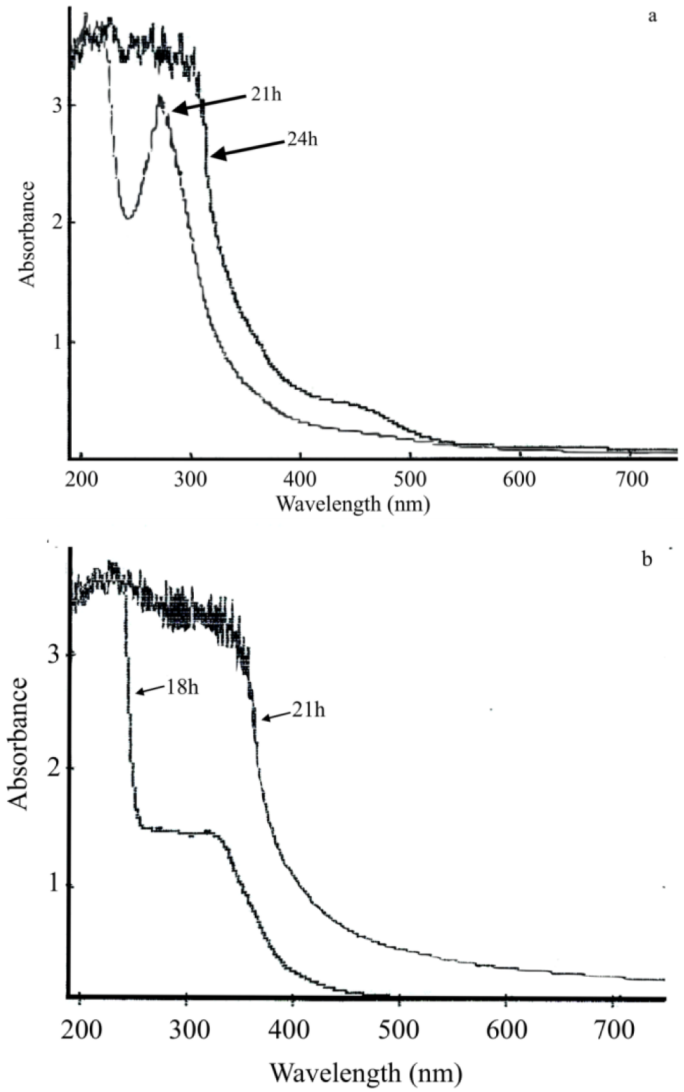

Figure 3 Development of emission spectra 190 – 750 nm of Psi (a) and Psi-Met (b) when was heated at 50 °C for 48 h. The curves for Psi was generated from the heating time 21 and 24th while the curve of Psi-Met was generated from the heating time 18 and 21st.

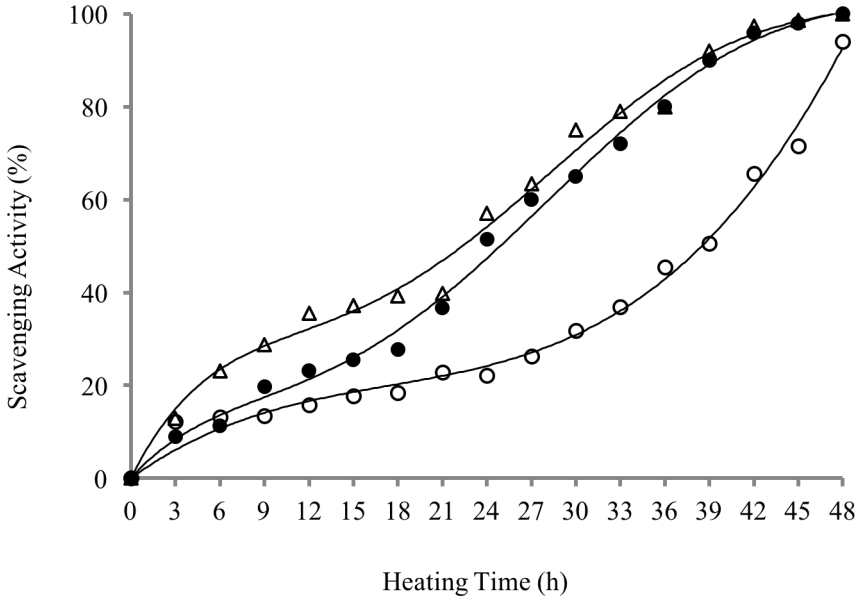

Figure 4 The scavenging activity of Psi (•), Psi-Met (Δ) and Fru-Met (o) model systems when was heated at 50 °C. The scavenging activity were analyzed every 3 h for 48 h by using ABTS method with the wavelength set at 734 nm.

Color development

L*a*b* value is known color development parameter to assess the progress of the Maillard reaction using colorimetry **(Morales and van Boekel, 1999; Alvarenga et al., 2014)**. L* value has been known to indicate the darkness (–) and lightness (+) of the solutions; while a* determines the greenness (–) and the redness (+) of samples; and b* shows blue (on its negative) and yellow (on its positive values). Based on L*a*b* value, the browning index could be determined. Browning index values are the elaborated those tristimulus value using calculation at equation (1) and equation (2). The browning index of Psi and Psi-Met is shown in Figure 2.

As described in Figure 2, the rate of browning index values incline over time indicating that browning index was influenced by duration of heating. The incline of browning index was continued up to 48 h. Nonetheless, Psi had a larger influence on the browning index than Psi-Met. Samples of Psi-Met incubated at 48 h were equally in browning index as compared to the Psi samples at 27 h.

Browning index gave a remarkable value at 21 h and reached the highest value at 48 h. It suggests that as the longer of the heating process gained, 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 to 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. In addition, the browning index of Psi is entirely two-three times higher than Psi-Met. According to **Alvarenga et al. (2014)**, it was caused by caramelization effect in Psi. **Laroque et al. (2008)** revealed that browning index cannot be attributed to the sugar caramelization because sugars heated alone failed to generate compounds absorbing at 420 nm.

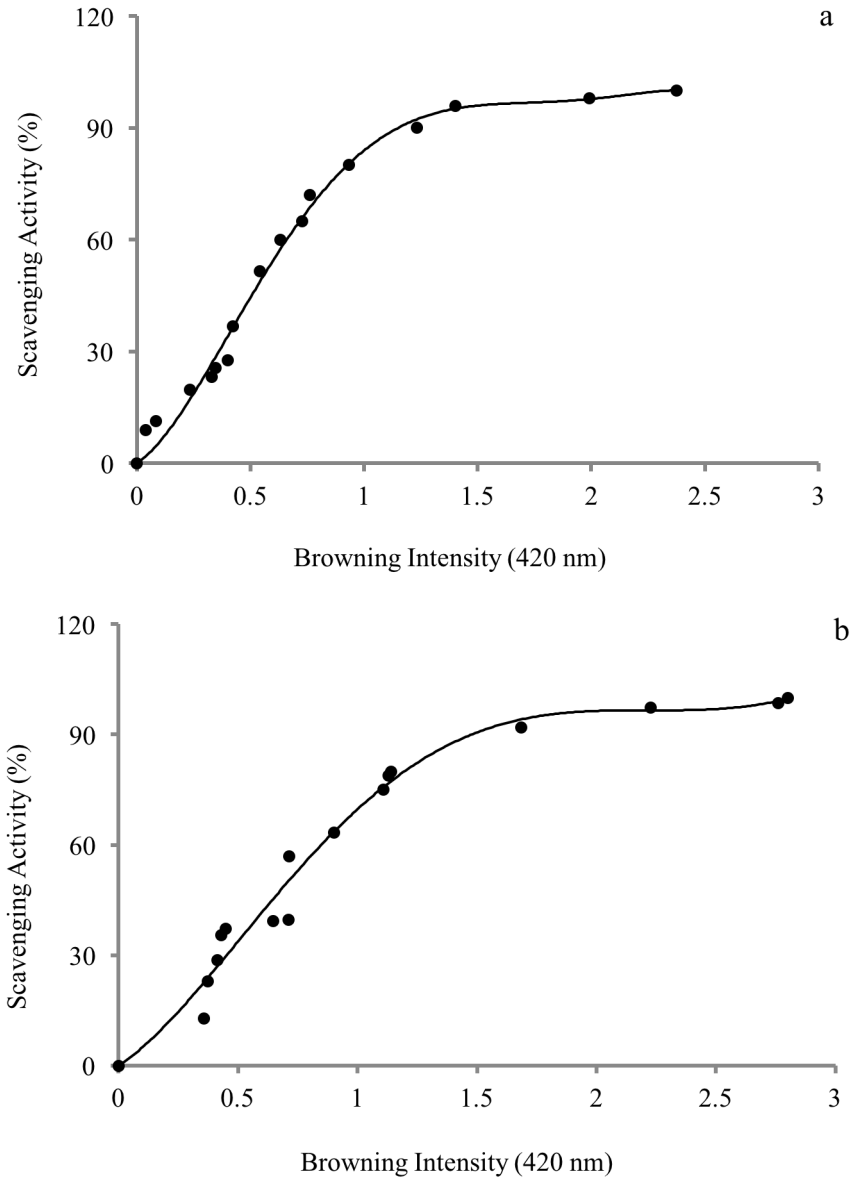

Figure 5 Positive non-linear and significant correlation between browning intensity and scavenging activity of Psi (a) whereas R2 value at 0.9464, *p*-value at <0.0001, and Psi-Met (b) whereas R2 value at 0.9475, *p*-value at <0.0001. Analysis was conducted using GraphPad Prism analysis.

Spectroscopic characteristics of Psi and Psi-Met MRPs

The absorbance range (190 to 750 nm) to investigate the spectroscopic characteristics of Psi and Psi-Met has been associated from the early study **(Jing and Kitts, 2004),** who observed a similar spectral pattern with a strong absorbance between 250 and 700 nm from sugar–Lys MRPs. The result of spectrum measurement is shown in Figure 3. 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)**.

The spectral analysis was done at every three hour (data not presented). The peak obtained from Psi-Met model (Figure 3b) start to changed remarkably at 21 h, while the peak obtained from Psi (Figure 3a) start to change remarkably at 24 h. It defined that the MRPs derived from Psi-Met was produced faster than heated product 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.

ABTS radical scavenging activity

Antioxidants are specific substances that oxidize themselves and protect other sensitive bioactive food components from destruction. It also restricts and stabilizes the activity of free radicals become less active forms **(Bajcan et al., 2016)**. This antioxidant potency can be analyzed by the scavenging activity. The scavenging activity of this research 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 equation (3). Regarding to the previous result of browning intensity, the development of browning intensity may led the antioxidant activity of MRPs increased as well **(Yu et al., 2012; Chen and Kitts, 2008)**. The scavenging activity of Psi-Met and Psi are shown in Figure 4. 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 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)**.

The result also showed that the scavenging activity of Psi was comparable to Psi-Met indicating the strong antioxidant potency of the Psi during the heating process. It might be explained by caramelization effect **(Alvarenga et al., 2014)**. Further study about Maillard reaction on D-fructose and methionine (Fru-Met) showed a slow increase during heating process indicating Psi generated higher antioxidant activity than MRPs product of Fru-Met. This result in line with **Zeng et al. (2011)** revealed that the MRPs from D-psicose have better performance than MRPs from D-fructose in the radical-scavenging activity of ABTS. At the advanced stage of Maillard reaction, D- psicose have higher reactivity than D-fructose, since D-fructose is characterised by a much less accessible carbonyl function, which is become the main explanation of its less reactivity **(Laroque et al., 2008)**.

The Correlation between browning intensity and scavenging activity

In this research, we attempted to study the relationship between browning intensity and scavenging activity of Psi and Psi-Met. This correlation analysis has been adopted from **Alvarenga et al. (2014)**, who found a positive lineal correlation (R^2 = 0.743) between available amino groups and browning index of glycoprotein samples in Maillard reaction condition. The correlation results of Psi and Psi-Met are shown in Figure 5a and 5b. Both of these correlations shown that the browning intensity and scavenging activity increased as the time increased. A positive non-linear correlation between browning intensity and scavenging activity was found at R^2 value of Psi and Psi-Met: 0.9464 and 0.9475, respectively.

The significance correlation between browning intensity and scavenging activity of Psi and Psi-Met were analyzed as well using GraphPad Prism version 6.0. Both of these results suggested that browning intensity was significantly correlated to scavenging activity (p <0.0001).

CONCLUSION

During the Maillard process to derive MRPs, Psi-Met were showing the better reaction than Psi. The browning intensity, color development, and scavenging activity were improved according to the heating process increased. The MRPs product derived from Psi-Met was able to produce at 21 h proven by spectra measurement. The correlation between browning intensity and scavenging activity were assigned significantly positive non-linear correlation and significant correlation. Essentially, the MRPs derived from Psi-Met have better scavenging activity and physical phenomenas than heated product of Psi. This finding may provide beneficial information of D-psicose to the food industries which applies MRPs in their products.

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Acknowledgments:

The authors wish to thank Ministry of Research, Technology, and Higher Education of Indonesia for entirely supporting this research. The authors also would like to thank Shigeru Hayakawa and Masahiro Ogawa for providing rare sugar D-psicose and D-fructose.

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