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Antioxidant activity of Maillard reaction products from a porcine plasma protein–sugar model system

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Abstract

Maillard reaction products (MRPs) were prepared by heating 2% porcine plasma protein (PPP) and reducing sugars (glucose, fructose and galactose) at the levels of 1% or 2% at 100 °C up to 5 h without pH control. Browning and intermediate products, as monitored by absorbance at 420 nm and absorbance at 294 nm, increased as heating time increased ($P < 0.05$). However, fluorescence (Ex 347 and Em 415 nm) sharply increased within 1 h and subsequently decreased when heating time increased ($P < 0.05$). Increase in browning and formation of intermediate products was observed with a concomitant decrease in free amino groups. Among sugars and concentrations used, galactose at 2% rendered the highest browning and intermediate products. MRPs derived from galactose, especially at a level of 2% possessed greater reducing power and DPPH radical-scavenging activity than those prepared from fructose and glucose. MRPs derived from fructose or galactose at the level of 2% showed the increase in reducing power and DPPH radical-scavenging activity in a concentration-dependent manner. In general, antioxidative activity of PPP–sugar MRPs was coincidental with the browning development and the formation of intermediate products.

Keywords: Maillard reaction; Browning; Antioxidant; Radical scavenging; Reducing power; Porcine plasma protein

1. Introduction

Non-enzymatic interaction between a reducing sugar and an amino acid, peptide or protein has been known as the Maillard reaction. Glycosylation or glycation is an important reaction, which induces the covalent attachment of sugars to α- or ε-NH_2 groups of amino acids and protein to form glycated proteins (Friedman, 1996). The Maillard reaction produces a variety of intermediate products and finally brown pigments (melanoidins) are formed (Van Boekel, 1998). The Maillard reaction is influenced by many factors, including reactant concentration, temperature, time, initial pH and water activity (Baxter, 1995; Ashoor & Zent, 1984; Naranjo, Malec, & Vigo, 1998; Tanaka, Chiba, Ishizaki, Takai, & Taguchi, 1994; Wijewickreme & Kitts, 1997).

The Maillard reaction produced from an amino acid–sugar model system has been associated with the formation of compounds with strong antioxidant activity (Tanaka, Chui, Nagashima, & Taguchi, 1990; Yen & Hsieh, 1995; Yoshimura, Iijima, Watanabe, & Nakazawa, 1997). Antioxidant activity of MRPs derived from a protein–sugar system has been also studied (Jing & Kitts, 2002; Yeboah, Alli, & Yaylayan, 1999). However, Lingnert & Eriksson (1980) found a lower antioxidative activity of MRPs derived from protein–sugar model systems than amino acid–sugar model systems. Additionally, antioxidative activities of MRPs are affected by

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pH and temperature used (Alaiz, Hidalgo, & Zamora, 1999; Mastrocola & Munari, 2000). MRPs have been used to prevent lipid oxidation in many products. MRPs exhibit an antioxidative activity in meat products (Alfawaz, Smith, & Jeon, 1994; Bedinghaus & Ockerman, 1995). Furthermore, MRPs derived from a fructose–tryptophan system also prevent the oxidation of sardine lipid (Chiu, Tanaka, Nagashima, & Tagushi, 1991).

Porcine blood is an abundant by-product in the slaughtering process in Thailand with an estimated amount of 30,000 metric tons per year (Benjakul, Visessanguan, & Srivilai, 2001a). Blood plasma contains a variety of bioactive compounds, including a proteinase inhibitor and plasma transglutaminase (Benjakul et al., 2001a, Benjakul, Visessanguan, & Srivilai, 2001b). This could increase the breaking force and deformation of bigeye snapper surimi gels (Benjakul et al., 2001a). Apart from utilization as a surimi gel enhancer, porcine plasma can be used as the potential source of proteins or peptides for Maillard reactions in the presence of an appropriate sugar. However, no information regarding the MRPs prepared from porcine plasma protein on its antioxidative activity has been reported. The objectives of this investigation were to study the Maillard reaction products derived from porcine plasma protein and different reducing sugars and to determine the antioxidative activity of MRPs obtained under different conditions.

2. Materials and methods

2.1. Chemicals

2,4,6-Trinitrobenzenesulfonic acid (TNBS), L-leucine, 1,1-diphenyl-2-picryl-hydrazyl (DPPH) and potassium ferricyanide were purchased from Sigma–Aldrich (St. Louis, MO, USA). Trichloroacetic acid was obtained from Riedel-deHaen (Seelze, Germany). Ferric chloride, sodium sulfite, trisodium citrate and glucose were purchased from Merck (Damstadt, Germany). Fructose and galactose were obtained from Fluka (Messerchmittstr, Switzerland).

2.2. Preparation of porcine plasma protein

Porcine blood was collected from a slaughter house in Hat Yai, Thailand. The blood was mixed with 3.8% trisodium citrate at a ratio of 1:9 (v/v) to prevent blood coagulation and transported to the Department of Food Technology, Prince of Songkla University, Hat Yai. The blood was then centrifuged at 1000*g* for 30 min at 4 °C using a Sorvall Model RC-5B Plus centrifuge (Newtown, CT, USA) to remove red blood cells. The resultant supernatant was dialysed with 10 volumes of distilled water at 4 °C for three times. Dialysed plasma protein was freeze-dried and kept at 4 °C until used. The dry powder was referred to as porcine plasma protein (PPP).

2.3. Effect of sugars and heating time on the characteristics and antioxidative activity of Maillard reaction products (MRPs)

PPP (2 g) was mixed with different reducing sugars (glucose, fructose and galactose) at two different levels (1 and 2 g). Distilled water was added to dissolve the mixture and the volume was adjusted to 100 ml. The mixture was then transferred to screw-sealed tubes, tightly capped and heated in oil bath (Buchi labortechnik AG, Flawil, Switzerland) at 100 °C. The samples were taken after heating for 0, 1, 2, 3, 4, and 5 h. The heated samples were cooled immediately in iced water. MRPs samples obtained were kept at 4 °C until analysed.

2.4. Effect of MRP amount on the antioxidative activity

Sugars of the type and level exhibiting the highest antioxidative activity were used to prepare MRP. PPP–sugar mixtures were heated at 100 °C for 5 h. MRPs were then cooled in iced water. Different amounts of MRPs were determined for reducing power (0, 25, 50, 100, 150 and 200 μl) and DPPH radical-scavenging activity (0, 10, 20, 40, 60 and 80 μl).

2.5. Analyses

2.5.1. Measurement of UV-absorbance and browning

The UV-absorbance and browning of MRP samples were measured according to the method of Ajandouz, Tchiakpe, Ore, Benajiba, & Puigserver (2001). Appropriate dilution was made using distilled water and the absorbance was measured at 294 and 420 nm using a UV-1601 spectrophotometer (Shimadzu, Kyoto, Japan) for determining UV-absorbance and browning intensity, respectively.

2.5.2. Measurement of fluorescence

Fluorescence of MRP samples with an appropriate dilution was determined as described by Morales & Jimenez-Perez (2001) with a slight modification. The fluorescence intensity was measured at an excitation wavelength of 347 nm and emission wavelength of 415 nm using a RF-1501 Fluorescence spectrophotometer (Shimadzu, Kyoto, Japan).

2.5.3. Determination of free amino group content

Free amino group content was determined according to the method of Benjakul & Morrissey (1997). MRP samples (5-fold dilution) (125 μl) were mixed with 2.0 ml of 0.2125 M phosphate buffer, pH 8.2 and 1.0 ml

of 0.01% TNBS solution was added. The solutions were mixed thoroughly and placed in a temperature-controlled water bath (Memmert, Bavaria, Germany) at 50 °C for 30 min in the dark. The reaction was terminated by adding 2.0 ml of 0.1 M sodium sulfite. The mixtures were cooled at room temperature for 15 min. The absorbance was measured at 420 nm. Free amino acid was expressed in term of L-leucine.

2.5.4. Determination of reducing power

The reducing power of MRP samples was determined according to the method of Oyaizu (1986) with a slight modification. One milliliter of MRPs sample (5-fold dilution) was mixed with 1 ml of 0.2 M sodium phosphate buffer (pH 6.6) and 1 ml of 1% potassium ferricyanide ($K_3Fe(CN_6)$). The reaction mixtures were incubated in a temperature-controlled water bath at 50 °C for 20 min, followed by addition of 1 ml of 10% trichloroacetic acid. The mixtures were then centrifuged at 750*g* using a Mikro 20 centrifuge (Hettick zentrifugen, Tuttlingen, Germany) for 10 min at 25 °C. The supernatant obtained (1 ml) was treated with 1 ml of distilled water and 200 μl of 0.1% $FeCl_3$. The absorbance of the reaction mixture was measured at 700 nm. An increase in absorbance was used as the measure of reducing power.

2.5.5. Determination DPPH radical-scavenging activity

DPPH radical-scavenging activity was determined according to the method of Yen & Hsieh (1995) with a slight modification. An aliquot of MRP sample (80 μl) was treated with 320 μl of distilled water and 2 ml of 0.12 mM DPPH in methanol was added. The solution was then mixed vigorously and allowed to stand at room temperature in the dark for 30 min. The absorbance of mixtures was measured at 517 nm using a UV-1601 spectrophotometer. The control was prepared in the same manner, except that distilled water was used instead of MRP samples. For the blank, the assay was conducted in the same manner but methanol was added instead of DPPH solution. The percentage of DPPH radical-scavenging activity was calculated as follows (Singh & Rajini, 2004):

$$\text{Radical scavenging activity } (\%) = (1 - (A_{\text{sample}\,(517\,\text{nm})}/A_{\text{control}\,(517\,\text{nm})})) \times 100,$$

where $A_{\text{sample}\,(517\,\text{nm})}$ is the absorbance of sample and $A_{\text{control}\,(517\,\text{nm})}$ is the absorbance of the control.

2.5.6. Statistical analysis

All analyses were run in triplicate. Analysis of variance (ANOVA) was performed and mean comparisons were done by Duncan's multiple range test (Steel & Torrie, 1980). Analysis was performed using a SPSS package (SPSS 8.0 for windows, SPSS Inc, Chicago, IL).

3. Results and discussion

3.1. Effect of sugars and heating time on the characteristics and antioxidative activity of Maillard reaction products (MRPs)

3.1.1. Changes in pH

The pH of all PPP–sugar MRPs decreased gradually as the heating time increased up to 5 h (Fig. 1). Different sugars and concentrations resulted in different pH values of MRPs. Among sugars tested, glucose rendered a higher pH of MRPs, than did fructose and galactose. Sugar, at a concentration of 2%, caused a greater decrease in pH of MRPs than did 1% sugar. From this result, the pH of PPP–sugar MRPs was decreased from approximately 7.57–7.73 to 5.96–6.59 and varied with type and concentration of sugar used. The result was in accordance with Morales & Jimenez-Perez (2001) who found that sugar–amino MRPs had decreased pH as the heating time increased. The decrease in pH of MRPs was dependent on protein or amino acid. MRPs derived from lysine showed a significant drop of pH, compared with other amino acids (Morales & Jimenez-Perez, 2001).

3.1.2. Changes in fluorescence and absorbance at 294 nm

Fluorescence intensity of all MRPs, except MRPs derived from 1% glucose, reached a maximum at a heating time of 1 h ($P < 0.05$). Thereafter, fluorescence intensity decreased sharply up to 5 h of heating (Fig. 2(a)). For the MRPs derived from 1% glucose, fluorescence intensity decreased markedly after 2 h. Decrease in fluorescence was observed with increasing heating time. Development of fluorescent compounds occurs in the Maillard reaction prior to the generation of brown pigments (Jing & Kitts, 2002; Morales, Romero, & Jimenez-Perez, 1996). Jing & Kitts (2002) found that fluorescence of a ribose–casein mixture heated at 55 °C reached maximum within 4 days before decreasing.

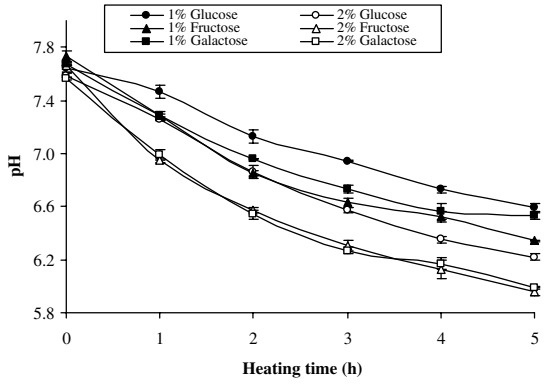

Fig. 1. Changes in pH of various PPP–sugar MRPs during heating for different times. Bars indicate the standard deviation from triplicate determinations.

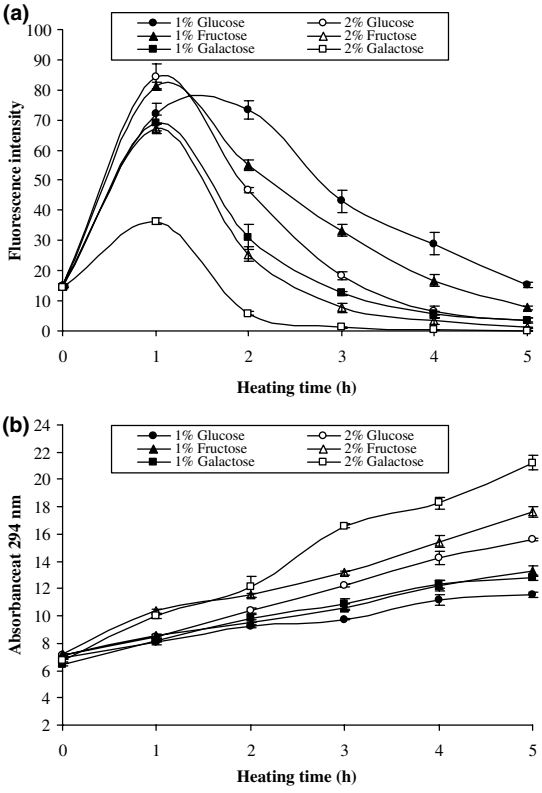

Fig. 2. Changes in fluorescence (a) and absorbance at 294 nm (b) of various PPP–sugar MRPs during heating for different times. Bars indicate the standard deviation from triplicate determinations.

Florescent compounds are possible precursors of brown pigments (Labuza & Baisier, 1992). Therefore, the decrease in fluorescence intensity, as heating time increased, was presumably due to the decrease in precursor, which was associated with the development of browning.

Continuous increase in absorbance at 294 nm was observed as the heating time of all PPP–reducing sugar mixture increased up to 5 h ($P < 0.05$) (Fig. 2(b)). MRPs derived from galactose showed the highest increase in absorbance at 294 nm, followed by fructose and glucose, respectively. MRPs derived from 2% sugar had a greater absorbance at 294 nm than did those with 1% sugar. Absorbance at 294 nm was used to determine the intermediate compounds of the Maillard reaction (Ajandouz et al., 2001; Lerici, Barbanti, Manzano, & Cherubin, 1990). From the result, the increase in absorbance at 294 nm suggested the formation of an uncoloured compound, which could be the precursor of the Maillard reaction (Ajandouz et al., 2001; Lerici et al., 1990). This result was in agreement with Lerici et al. (1990) who found that heat treatment of a glucose–glycine mixture caused marked increase in absorbance at 294 nm.

The difference in pattern between fluorescence and absorbance (at 294 nm) of MRPs suggested that different types of intermediate products, either fluorescent or non-fluorescent compounds, were formed and underwent the final stage of reaction at different rates. However, the florescent intermediate was possibly more reactive in formation of brown colour than non-fluorescent compounds, as shown by the decrease in fluorescence intensity as heating time increased. Therefore, the formation of intermediate compounds varied, depending on the type of sugar and heating time. For the PPP–sugar system, fructose was more reactive than galactose in forming the fluorescent intermediate compounds, whereas galactose was more reactive than fructose, generating non-fluorescent intermediates. For the system derived from glucose, fluorescent compounds were formed to the highest extent but non-fluorescent intermediates were generated at a lower level than with those from galactose and fructose.

3.1.3. Changes in browning intensity

An increase in browning of PPP–sugar MRPs, as measured by absorbance at 420 nm, was observed as the heating time increased ($P < 0.05$). MRP samples derived from 2% galactose showed a greater increase in browning intensity than those from 2% fructose on 2% glucose. From this result, MRPs derived from glucose showed the lowest browning ($P < 0.05$). The increase in browning was found to depend on sugar concentration. The higher concentration of sugar used, the higher the increase in browning was found. The increase in absorbance at 420 nm was used as an indicator for browning development in the final stage of the browning reaction (Ajandouz et al., 2001; Morales & Jimenez-Perez, 2001). Browning rate is influenced by the type of reducing sugar involved in the reaction The reactivity of reducing sugar was reported to decrease in the following order: aldopentoses > aldohexoses > ketohexoses > disaccharides (Spark, 1969). Based on browning development, galactose was most reactive in the PPP–sugar system. Yeboah et al. (1999) found that the aldehyde group of the acyclic form of aldoses was more electrophilic than the keto group of the acyclic form of ketose. However, MRPs derived from fructose showed more browning intensity than those with glucose, presumably because fructose had a higher proportion of open chain form than glucose (Naranjo et al., 1998). Thus, an amino acid–sugar complex could be formed more easily. The differences among different studies were possibly due to the diversity of sugars, protein and amino acids, as well as conditions used to prepare MRPs. Apart from the Maillard reaction, caramelisation of sugar could occur, leading to browning of the mixture (Ajandouz et al., 2001; Benjakul, Visessanguan, Phongkanpai, & Tanaka, 2005). Fructose, a ketose sugar, was browned more quickly than glucose, an aldose sugar, when heated at 120 °C for up to 60 min (Brands, Alink, van Boekel, & Jongen, 2000).

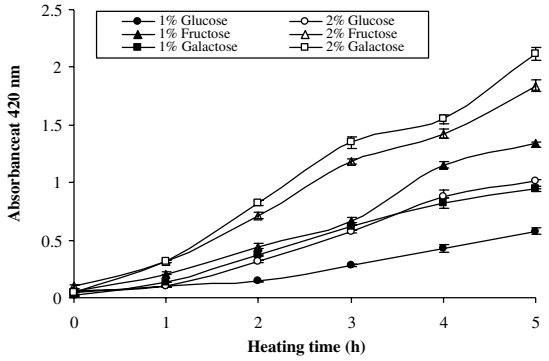

Fig. 3. Changes in browning of various PPP–sugar MRPs during heating for different times. Bars indicate the standard deviation from triplicate determinations.

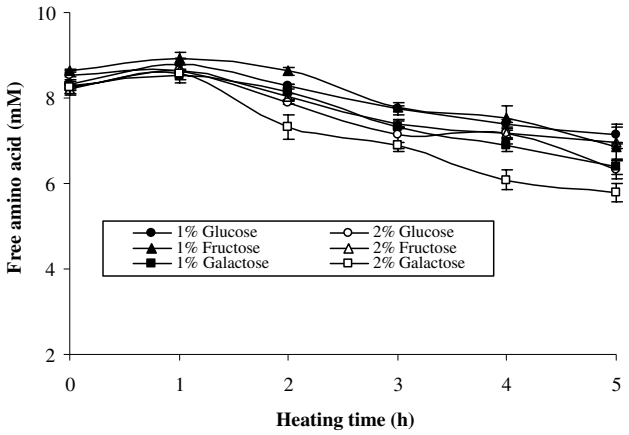

Fig. 4. Changes in free amino groups of various PPP–sugar MRPs during heating for different times. Bars indicate the standard deviation from triplicate determinations.

3.1.4. Changes in free amino group content

Changes in free amino group content of MRPs during heating are depicted in Fig. 4. No changes in free amino group content of MRPs were observed when heating time was 1 h ($P > 0.05$). However, a continuous decrease in amino acid group content of all MRP samples was noticeable when the heating time increased ($P < 0.05$). This result suggested that an α- or ε-NH_2 group of amino acids or proteins covalently attached to a sugar to form glycated proteins to a greater extent, particularly when the heating time increased. The first glycation product, or Schiff base, rearranges to a more stable ketoamine or

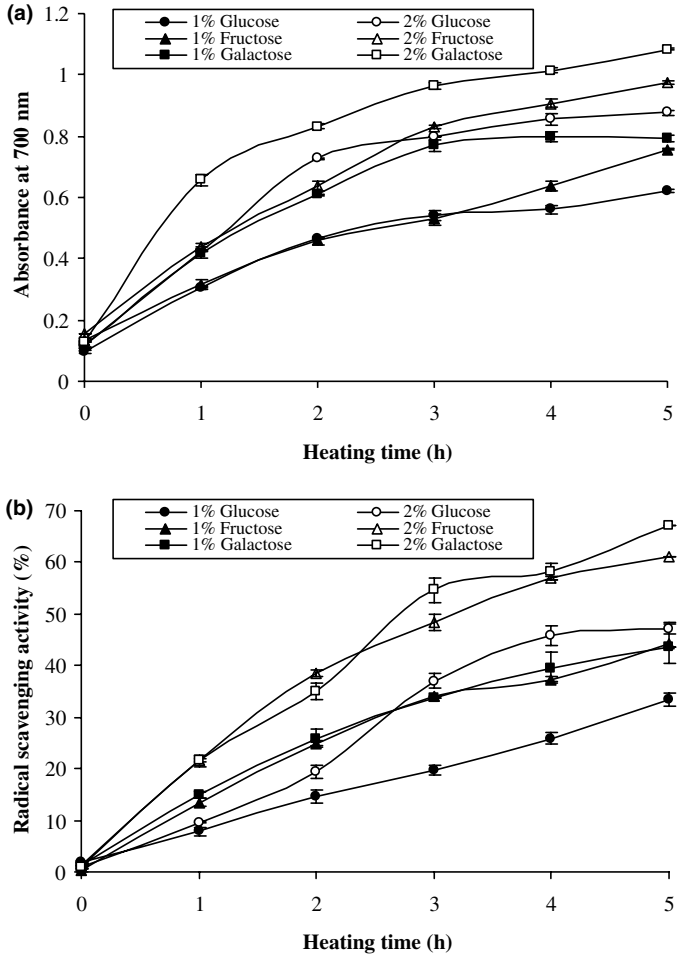

Fig. 5. Reducing power (a) and DPPH radical-scavenging activities (b) of various PPP–sugar MRPs during heating for different times. Bars indicate the standard deviation from triplicate determinations.

Amadori product. The Amadori products can then form cross-links between adjacent proteins or with other amino groups, resulting in polymeric aggregates called advanced glycation end-products (Friedman, 1996). Our result was in agreement with Wahyuni, Ishizaki, & Tanaka (1999) who reported that free amino groups of fish water-soluble protein decreased gradually via Maillard reaction with glucose-6-phosphate. Available lysine losses were observed in a casein–sugar system during extended heating (Naranjo et al., 1998).

From the results, the decreases in free amino group were in accordance with the increase in browning (Fig. 3) and absorbance at 294 nm (Fig. 2(b)) and the decrease in fluorescence intensity (Fig. 2(a)). This indicated that extended heating catalysed the interaction between free amino groups, such as ε-NH_2 groups of lysine, and sugar via glycation process. As a result, intermediate products were formed and further converted to brown pigments, as observed by the increased absorbance at 420 nm. In general, galactose was more reactive in forming the glycated PPP than were fructose or glucose, as shown by the greatest decrease in free amino groups with the concomitant increase in browning. The reaction rate of glycation between casein and sugars depended on the percentage of the acyclic form and the electrophilicity of the carbonyl groups (Bunn & Higgins, 1981; Naranjo et al., 1998). Naranjo et al. (1998) found that glucose, which is more electrophilic, could react faster than fructose in a casein–sugar system. The difference in reaction rate of sugar observed in many studies was possibly due to the different compositions of amino acid and conformation of protein, as well as the conditions used in different studies.

3.1.5. Changes in reducing power

Reducing power of MRPs increased as the heating time increased, as shown by an increase in absorbance at 700 nm ($P < 0.05$) (Fig. 5(a)). MRP samples prepared with galactose showed the greatest reducing power. MRPs derived from glucose exhibited the lowest reducing power, especially with increasing heating time. MRP samples containing a higher level of sugar had a greater reducing power than those with a lower level. The result revealed that MRPs could function as electron donors. This was in accordance with Yoshimura et al. (1997) who reported that MRPs from a glucose–glycine mixture had a higher reducing power, especially when the heating time increased. Hydroxyl groups of MRPs play a role in reducing activity (Yoshimura et al., 1997). From the results, reducing power correlated well with browning intensity and absorbance at 294 nm, but not fluorescence intensity.

3.1.6. Changes in DPPH radical-scavenging activity

DPPH radical-scavenging activity of MRPs increased as the heating time increased ($P < 0.05$) (Fig. 5(b)). The DPPH radical was scavenged by MRPs by donation of hydrogen to form a stable DPPH-H molecule (Matthaus, 2002).The DPPH radical had an absorbance at 515–520 nm. The colour changed from purple to yellow by acceptance of a hydrogen radical from MRPs and it became a stable diamagnetic molecule. From the results, the decrease in DPPH radical indicated the radical-scavenging activity of MRP samples. At the same concentration used, MRP samples derived from glucose showed the lowest radical-scavenging activity ($P < 0.05$). MRPs derived from galactose and fructose had similar activities. However, a slightly greater activity was found with MRP derived from 2% galactose with a heating time of 3–5 h. Yen & Hsieh (1995) also found the DPPH radical-scavenging activity of xylose–lysine MRPs. Therefore, PPP–sugar MRPs possessed hydrogen-donating ability, suggesting potency to react with free radicals.

DPPH radical-scavenging activity of the heated sugar–amino acid mixture was correlated to the changes in fluorescence (Morales & Jimenez-Perez, 2001). The present study showed no correlation between DPPH radical-scavenging activity and fluorescence intensity. However, radical-scavenging activity correlated well with browning intensity and absorbance at 294 nm.

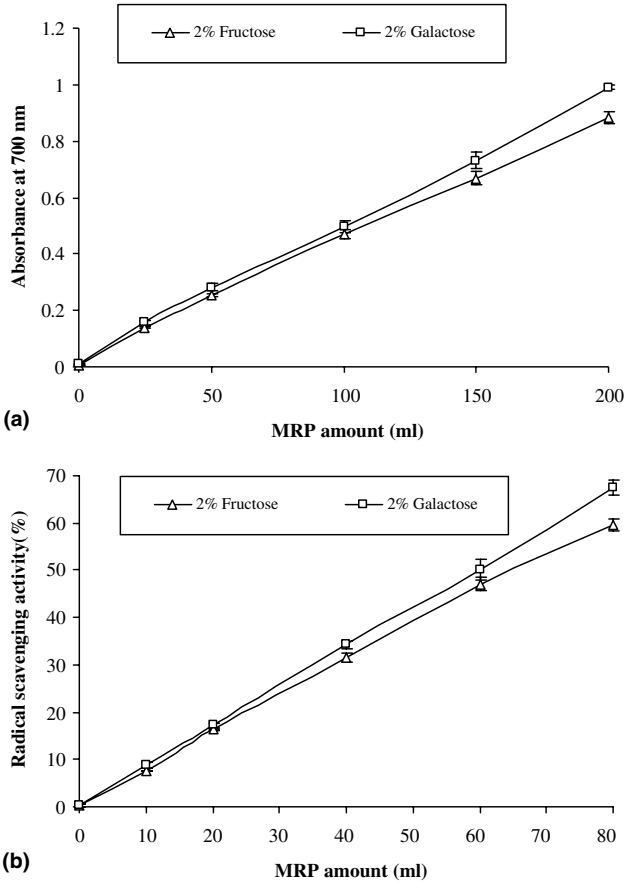

Fig. 6. Reducing power (a) and DPPH radical-scavenging activity (b) of various PPP–sugar MRPs with different amounts. Bars indicate the standard deviation from triplicate determinations.

Due to the reducing power and radical-scavenging activity of MRPs from the PPP–sugar system, it can be used as an antioxidant to prevent lipid oxidation in food products.

3.2. Effect of MRP amounts on antioxidative activity

PPP–sugar MRPs were prepared by heating 2% fructose or 2% galactose with 2% PPP at 100°C for 5 h. Different amounts of MRPs from both sugars showed different antioxidative activities (Fig. 6). From the results, reducing power and DPPH radical-scavenging activity increased linearly with the amount of MRPs. It is suggested that MRPs in increasing amounts could donate hydrogen more effectively to the free radicals. In general, MRPs derived from 2% galactose exhibited a slightly greater reducing power and radical scavenging-activity than did those from 2% fructose. The results indicated that the PPP–galactose system gave a greater antioxidative activity than the PPP–fructose system. Chiu et al. (1991) reported that the antioxidative effect of MRP against sardine lipid oxidation during storage at 37°C was increased with the increase of MRP amount.

4. Conclusion

MRPs derived from a PPP–sugar system exhibited antioxidant activity via electron donating and radical-scavenging. MRPs prepared by heating 2% PPP with 2% galactose showed the highest antioxidant activity. Antioxidative activity was coincidental with the increase in browning and absorbance at 294 nm and the decrease in free amino groups. Generally, MRP showed increased antioxidative activity with increasing concenration.

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