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Characteristics and antioxidant activity of Maillard reaction products derived from chitosan-sugar solution

[1]*Phisut, N. and [2]Jiraporn, B.

[1]Faculty of Agricultural Product Innovation and Technology, Srinakharinwirot University, Sukhumvit 23, Bangkok, 10110, Thailand
[2]National Bureau of Agricultural Commodity and Food Standards 50 Phaholyothin Rd Chatuchak, Bangkok 10900, Thailand

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Abstract

The Maillard reaction occurring between amino group and carbonyl group produces neo-formed compounds having certain levels of antioxidant activity depending on the reaction conditions and the type of reactants. In this study, maillard reaction products (MRPs) derived from four model systems including chitosan-glucose (CG), chitosan-fructose (CF), chitosan-maltose (CM) and chitosan-lactose (CL) solutions were prepared by heating at 100 °C for various times (0, 1, 2, 3, 4, 5, 6 and 7 h) and their characteristics and antioxidant activity were investigated. Browning intensity and intermediate products, as monitored by A_{420} and $A_{UV\lambda max}$, increased with heating time. The increase in browning intensity and formation of intermediate products were concomitant with the decreases in free amino group and reducing sugar contents. DPPH radical scavenging activity, FRAP, reducing power, hydroxyl radical scavenging activity and inhibition of lipid peroxidation of all MRPs derived from chitosan-sugar model systems increased with the increase in browning intensity and intermediate formation. Among all MRPs tested, those derived from CG system rendered the highest browning intensity, intermediate products and antioxidant activity as evidenced by the greatest scavenging effect, reducing power, hydroxyl radical scavenging activity and inhibition of lipid peroxidation. Thus, MRPs derived from chitosan-sugar model system can be promoted as a novel antioxidant to prevent lipid oxidation in food system.

Introduction

Maillard reaction is a non-enzymatic interaction between reducing sugar and amino acid, peptide or protein, resulting in a variety of by-products, intermediates and brown products (melanoidins), which contribute markedly to the aroma, taste and colour, as well as to the antioxidant potential of stored and processed foods (Manzocco *et al*., 2001). It is one of the major reactions taking place during thermal processing, cooking, and storage of foods. The Maillard reaction, produced from an amino acid–sugar model system, has been known to be associated with the formation of compounds with pronounced antioxidant activity. The development of antioxidant molecules is one of the desirable effects of the Maillard reaction. Thus, the antioxidative properties of Maillard reaction products (MRPs) produced by heat treatment of amino acid–sugar and protein-sugar have been studied in model systems (Yeboah *et al*., 1999; Jing and Kitts, 2002a; Jing and Kitts, 2002b; Jaythilakan and Sharma, 2006).

Chitosan is a deacetylated derivative of chitin extracted from the exoskeletons of shrimp and shellfish. It is an abundant by product from seafood industry and has been found to be non-toxic, biodegradable, biofunctional and biocompatible. In addition, chitosan is widely used for a natural renewable resource in the field of medicine, food, pharmaceuticals, nutrition, and agriculture. Given the above prominent properties of chitosan, it becomes more interesting as functional ingredients in various applications (Harish and Tharanathan, 2007). Chitosan has amino groups which can react with the carbonyl group of reducing sugar leading to formation of MRPs. Therefore, the presence of amino group makes chitosan as a candidate to react with the carbonyl group of a reducing sugar, and allows it to be a participant in the Maillard reaction (Lin and Chou, 2004; Kanatt *et al*., 2008; Chang *et al*., 2011; Rao *et al*., 2011).

Reducing sugars are essential ingredients in

*Corresponding author.
Email: *phisut@swu.ac.th*

Maillard reaction, as they provide the carbonyl groups to interact with the free amino groups of amino acids, peptides and proteins. The reactivity of the reducing sugar to form MRPs is different. Sugar type affects not only spectroscopic characteristics, such as UV-absorbance and browning development among various sugar-generated MRPs, but also possibly associated biological properties. Some investigators have tried to associate different antioxidant activities of MRPs, such as DPPH radical scavenging activity, with characteristically different MRPs produced from various sugar–amino acid Maillard reaction model systems (Yeboah *et al*., 1999; Benjakul *et al*., 2005; Chawla *et al*., 2009; Gu *et al*., 2009; Kim and Lee, 2009; Jing and Kitt, 2002a; Jing and Kitts, 2002b; Rao *et al*., 2011; Zeng *et al*., 2011; Chen and Kitts, 2011). Moreover, the different sources of amino group may influence the reactivity of the reducing sugar to form MRPs. Rao *et al*. (2011) reported that the MRPs produced by the irradiation of chitosan-glucose solution showed the antioxidant activity. However, no information on the heating process of chitosan with different sugar solutions to form MRPs and resultant effects on antioxidant activity is available. The aim of this study was to investigate the characteristics and antioxidant activity of chitosan-sugar during heating.

Materials and Methods

Materials and reagents

Chitosan powder (degree of deacetylation of 90%, moisture content less than 10%) was provided by Seafresh Ltd. (Thailand). D-glucose, D-fructose, α-Lactose monohydrate and maltose were purchased from Fluka (Messerchmittstr, Switzerland). Methanol, acetic acid and hydrochloric acid were obtained from Prolabo (Paris, France). Sodium hydroxide, copper sulfate pentahydrate, trichloroacetic acid (TCA), potassium ferricyanide, sodium acetate trihydrate, ferric chloride hexahydrate and sodium sulfite were obtained from Merck (Darmstadt, Germany). Potassium sodium tartrate, lead acetate and potassium oxalate were obtained from Riedel-deHaen (Seelze, Germany). 2,4,6-Trinitrobenzenesulfonic acid (TNBS), hydroxymethylfurfural, thiobarbituric acid, 2,2-diphenyl-2-picryl-hydrazil (DPPH), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), thaiobarbituric acid (TBA) and 2,4,6-Tripyridyl-s-triazine (TPTZ) were purchased from Sigma-Aldrich (St.Louis. MO, USA).

Preparation of Maillard reaction products (MRPs)

Chitosan-sugar model systems were prepared as reported by Rao *et al*. (2011) with some modifications. Each model system was prepared by mixing chitosan with different reducing sugars (glucose, fructose, maltose and lactose) to obtain chitosan-sugar solutions that contained 1% of chitosan and 0.05 M of reducing sugar. Each chitosan-sugar solution was then transferred to screw-sealed tubes, tightly capped and heated in water bath at 100°C. The samples were taken after heating for 0, 1, 2, 3, 4, 5, 6 and 7 h. The heated samples were cooled immediately in iced water. Each MRPs sample obtained was kept at 4 °C until analysed. All model systems were prepared in triplicate.

Measurement of colourless browning intermediate and browning intensity

The colourless browning intermediate was estimated by the absorbance at $UV_{\lambda max}$. In this study, the peak absorption wavelength (λ_{max}) of sample differed depending on types of sugars. The browning intensity was determined by monitoring the absorbance at 420 nm. The absorbance was measured by using a spectrophotometer (Shimadzu, Kyoto, Japan), as described by Tsai *et al*. (2009).

Determination of free amino group content

Free amino group content was determined according to the method of Benjakul and Morrissey (1997). Sample was diluted through appropriate dilution. Then 125 µL of the sample was collected and mixed with 2.0 mL of 0.21 M phosphate buffer, pH 8.2, and 1.0 mL of 0.01% TNBS solution. The solutions were mixed thoroughly and placed in a temperature-controlled water bath (Memmert, Bavaris, Germany) at 50°C for 30 min in dark. The reaction was terminated by adding 2.0 mL of 0.1 M sodium sulfite. The mixture was cooled at room temperature for 15 min. The absorbance was measured at 420 nm by using a spectrophotometer (Shimadzu, Kyoto, Japan). The blank was prepared as same condition as the samples except that the distilled water was used instead of 0.01% TNBS.

Determination of reducing sugar

Reducing sugar content was determined according to the method of Chaplin and Kennedy (1994). One mL of MRP samples was mixed with 1.0 mL of Somogyi copper reagent in screw-caped tubes and heated at 100°C for 15 min and then cooled with tap water. One mL of Nelson arsenomolybdate reagent was added and mixed well. Finally, 3.0 mL of deionized water was added to the mixtures. The absorbance was measured at 520 nm by using a spectrophotometer (Shimadzu, Kyoto, Japan).

Determination of DPPH radical scavenging activity

DPPH radical-scavenging activity was determined by DPPH assay, as described by Binsan *et al*. (2008) with a slight modification. Sample (1.5 mL) was added to 1.5 mL of 0.15 mM 2,2-diphenyl-1-picrylhydrazyl (DPPH) in methanol. The mixture was mixed vigorously and allowed to stand at room temperature in the dark for 60 min. The absorbance was measured at 517 nm using a spectrophotometer (Shimadzu, Kyoto, Japan). The blank was prepared in the same manner, except that distilled water was used instead of the sample. A standard curve was prepared using Trolox. The activity was expressed as μmol trolox equivalents (TE) per 1 mL of MRPs.

Determination of ferric reducing antioxidant power (FRAP)

Ferric reducing antioxidant power (FRAP) was assayed according to the method of Benzie and Strain (1996). Stock solutions included 300 mM acetate buffer (pH 3.6), 10 mM TPTZ solution in 40 mM HCl, and 20 mM $FeCl_3.6H_2O$. A working solution was prepared by mixing 25 mL of acetate buffer, 2.5 mL of TPTZ solution and 2.5 mL of $FeCl_3.6H_2O$ solution. The mixed solution was incubated at 37°C for 30 min and was referred to as FRAP solution. A sample (150 μL) was mixed with 2850 μL of FRAP solution and kept for 30 min in the dark. The ferrous tripyridyltriazine complex (colored product) was measured by reading the absorbance at 593 nm. A standard curve was prepared using Trolox. The activity was expressed as μmol trolox equivalents (TE) per 1 mL of MRPs.

Determination of reducing power

Reducing power of MRPs sample was measured as described by Matmaroh *et al*. (2006) with some modifications. One mL of each sample (appropriate dilution) was mixed with 1.0 mL of 0.2 M sodium phosphate buffer, pH 6.6 and 1.0 mL of 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 min, followed by addition of 1 ml of 10% trichloroacetic acid. The mixtures were then centrifuged at 3000 rpm 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.

Determination of hydroxyl radical-scavenging activity

Hydroxyl radical-scavenging activity of MRPs sample was determined according to the modified method of Halliwell *et al*. (1987). 1.0 mL of the appropriately diluted sample, 1.0 mL phosphate buffer (0.1 M, pH 7.4) containing 1 mM ferric chloride, 1 mM EDTA, 1 mM ascorbic acid, 30 mM deoxyribose, and 20 mM hydrogen peroxide were added. After incubation at 37 °C for 90 min, 2.0 mL of 2% TCA and 2.0 mL of 1% TBA were added. The mixture was heated in a boiling water bath for 15 min. The absorbance of the pink colour was measured at 532 nm using a spectrophotometer. The percentage of hydroxyl radical-scavenging activity was calculated as:

$$\% \text{ inhibition} = 100 \times [(A_{control}-A_{sample})/A_{control}]$$

where $A_{control}$ is the absorbance of the control and A_{sample} is the absorbance of the sample.

Inhibition of lipid peroxidation induced by iron

Lecithin was suspended in phosphate buffered saline (0.01 M, pH 7.4) at a concentration of 10.0 mg/mL and stirred with magnetic stirrer (Yi *et al*., 1997; Gu *et al*., 2009). The solution was labeled as LLS. Fifteen gram of trichloroacetic acid (TCA), 0.37 g of thiobarbituric acid (TBA) and 2.0 mL of concentrated hydrochloric acid (HCl) were mixed to deionized water, and the volume was adjusted to 100 mL with deionized water. The solution was labeled as TCA–TBA–HCl. One mL of LLS, 1.0 mL of 400 μM $FeCl_3$, 1.0 mL of 400 μM ascorbic acid and 1.0 mL of sample solution were mixed and incubated in water bath at 37°C in dark for 60 min, and then 2.0 mL of TCA–TBA–HCl was added. The system was cooled down with ice after heating at 100°C for 15 min in water bath. The pink solution was centrifuged at 3500 rpm for 10 min, and the absorbance of the supernatant was measured at 532 nm, and called as As. The blank was made with 1.0 mL of deionized water substitution for 1.0 mL of sample, named as Ac. The inhibition percentage (%) was calculated as formula;

$$\text{Inhibition percentage (\%)} = (Ac-As)/Ac \times 100$$

Statistical analysis

All analysis and measurements were performed in triplicates. The experimental design was a completely randomized design (CRD). Data was subjected to analysis of variance (ANOVA). Comparison of means was carried out by Duncan's multiple-range test. Analysis was performed using a SPSS package (SPSS Inc, Chicago, IL).

Results and Discussion

Changes in UV $_{\lambda max}$ ($A_{UV\ \lambda max}$) and browning intensity (A_{420})

The $A_{UV\ \lambda\ max}$ and browning intensity (A_{420}) are indicators of colourless intermediate compounds and final browning compounds, respectively (Ajandouz *et al*., 2011). A small change in $A_{UV\ \lambda max}$ of all MRPs samples was found with increasing heating time up to 2 h ($P<0.05$). Thereafter, a sharp increase in $A_{UV\ \lambda max}$ was observed as the heating time of all MRPs derived from chitosan-sugar model systems increased up to 7 h ($P<0.05$) as depicted in Figure 1a. The MRPs derived from CG showed the highest increase in $A_{UV\ \lambda max}$, followed by those derived from CL, CM and CF, respectively. The increase in $A_{UV\ \lambda max}$ with heating time was in agreement with Lerici *et al*. (1990) who found that heat treatment of a glucose-glycine mixture caused the increment of absorbance at 294 nm. Moreover, Benjakul *et al*. (2005) also reported that the continuous increase in absorbance at 294 nm was observed with heating time of porcine plasma protein-reducing sugar model system. Maillard reaction is associated with development of UV-absorbing intermediate compounds, prior to generation of brown pigments. The result suggested that intermediate products were produced to a great extent. It appears that some intermediate products might undergo conversion to the final brown compounds, while some intermediates are still being generated by both reactants as evidenced by the increase in browning intensity during heating. Browning intensity (A_{420}) of all MRPs, except MRPs derived from CG, slightly increased up to 3 h of heating ($P<0.05$). Thereafter, A_{420} increased sharply up to 7 h of heating ($P<0.05$) (Figure 1b). For MRPs derived from CG, A_{420} increased markedly after 1 h of heating. The MRPs samples derived from CG model system showed the highest increase in A_{420}, followed by those derived from CL, CM and CF model systems, respectively. The increase in browning depended on sugar types. In the final stage, the intermediates polymerise and coloured polymers are formed. It is clearly known that heat treatment leads to Maillard reaction. This reaction consists of the condensation of amino compound and sugar fragments into polymerised protein and the brown pigment melanoidin (Van Boekel, 1998). Moreover, Caramelisation could take place during heating of each model system. The increase in brown pigment development was coincidental with the increase in colourless intermediate formation, as evidenced by the increase in $A_{UV\ \lambda max}$. This suggested that brown pigments were formed proportionally with the generation of intermediate products.

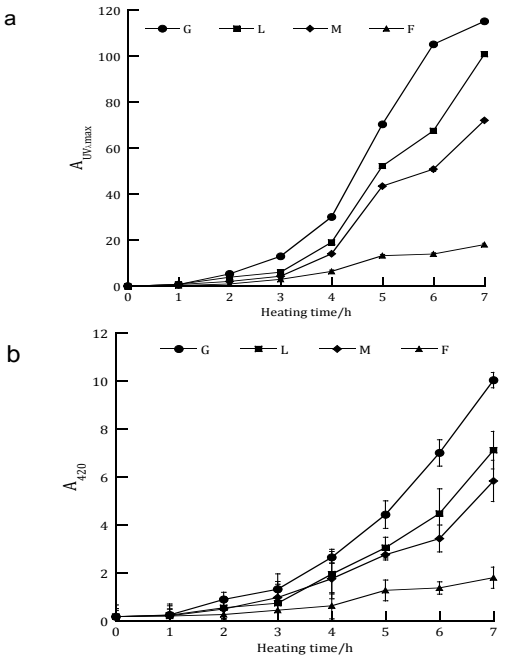

Figure 1. Changes in $A_{UV\ \lambda max}$ and A_{420} of MRPs derived from chitosan-sugar model systems during heating for various times

Browning rate is influenced by the types of reducing sugar involved in the reaction. The initial kinetics of glycation depend on the proportion of the reducing sugar existing in the acyclic or active form under the reaction condition and on the electrophilicity of the sugar carbonyl group. The reactivity of reducing sugar was reported to decrease in the following order: aldopentoses > aldohexoses > ketohexoses > disaccharides (Spark, 1969). Based on browning development, glucose was the most reactive in the chitosan–sugar system, which might be explained by the more reactive nature of aldehyde carbonyl group of glucose compared with the ketone group of fructose (Yeboah *et al*., 1999). From the result, lactose and maltose presented a greater increase in $A_{UV\ \lambda max}$ and A_{420} than that from fructose. This probably due to the hydrolysis of lactose and maltose took place during heating in acid medium. Upon hydrolysis of lactose, aldose including glucose and galactose are formed. Benjakul *et al*. (2005) reported that MRPs derived from porcine plasma protein-galactose model system showed a greater increase in browning intensity than those from glucose and fructose. Thus, the formation of galactose during heating of chitosan-lactose model systems could enhance the Maillard reaction. Moreover, glucose was formed during hydrolysis of maltose, resulting in the promotion of the Maillard reaction. However, some researchers reported that MRPs derived from fructose showed more browning intensity than glucose, presumably because fructose had a higher proportion of open chain form than glucose. Thus, an amino acid–sugar complex could be formed more easily (Naranjo *et al*., 1998; Benjakul

Table 1. Changes in absorbance ratio ($A_{UV\ \lambda max}/A_{420}$) of MRPs derived from chitosan-sugar model systems during heating for various times

Time (h)	Glucose	Lactose	Maltose	Fructose
0	0.41	0.38	0.39	0.39
1	2.96	4.31	3.33	1.40
2	8.24	7.04	4.24	3.76
3	9.87	8.18	4.51	6.71
4	11.35	9.18	7.99	10.21
5	15.88	17.10	15.75	10.47
6	13.29	15.09	14.81	10.27
7	11.47	14.17	12.36	10.04

et al., 2005). The differences among different studies were possibly due to the diversity source of amino groups and carbonyl groups, as well as conditions used to prepare MRPs.

The $A_{UV\ \lambda max}$ to A_{420} ratio is a useful indicator of the extent of polymerization of products that contribute to browning (Ajandouz *et al*., 2011). The absorbance ratios ($A_{UV\ \lambda max}/A_{420}$) of all MRPs derived from chitosan-sugar model systems with different times are shown in Table 1. All absorbance ratios of all MRPs model systems, except CF, increased up to 5 h of heating times with the development of browning. Subsequently, a decrease was observed up to 7 h. The absorbance ratios of MRPs derived from chitosan-fructose model system reached a maximum within 4 h and then tended to be constant until the end of heating time. The increase of absorbance ratios suggested that the intermediates were generated to a larger extent with lower transformation to brown polymers. The subsequent decrease in absorbance ratios suggested the formation of brown polymers from the intermediate. Thus, the formation of brown polymers from different intermediates varied with the type of sugar (Benjakul *et al*., 2005).

Changes in free amino group content

Changes in reactive amino groups of all MRPs derived from chitosan-sugar model systems are depicted in Figure 2a. Initially, few reactive amino groups were detected in all chitosan–sugar solutions as chitosan is a polymer with extensive hydrogen bonding. Upon heating the hydrogen bonds are broken and amino groups are exposed (Rao *et al*., 2011). A continuous decrease in the amino group content of all MRP samples was noticed as the heating time increased ($P<0.05$). This result suggested that amino group of chitosan covalently attached to sugar to form glycated product to a greater extent, particularly when the heating time increased. The first glycation product, or Schiff base, rearranges to a more stable ketoamine or 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). The result was in agreement with Wahyuni *et al*. (1999) who reported that free amino groups of

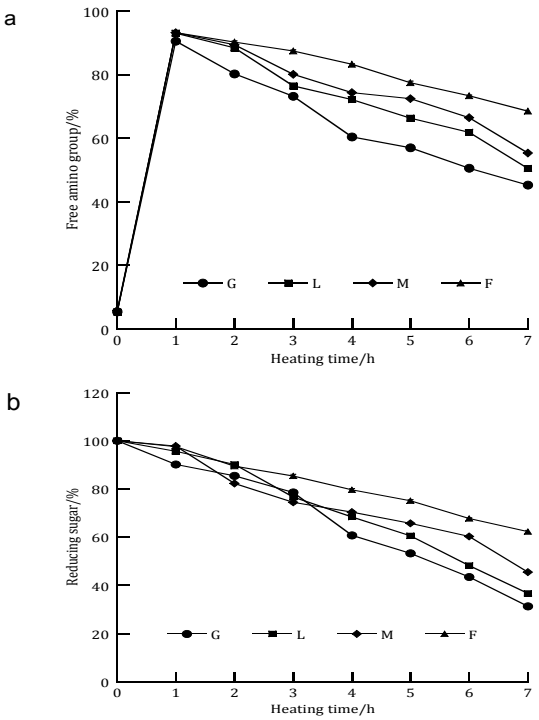

Figure 2. Changes in free amino group and reducing sugar content of MRPs derived from chitosan-sugar model systems during heating for various times

fish water-soluble protein decreased gradually via Maillard reaction with glucose-6-phosphate. The loss of lysine was observed in a casein–sugar system during extended heating (Naranjo *et al*., 1998). The free amino group was lost during heating of porcine plasma protein-sugar model system as reported by Benjakul *et al*. (2005).

Changes in reducing sugar content

Changes in reducing sugar content of all MRPs derived from chitosan–sugar solutions, as a function of the heating time, are shown in Figure 2b. A gradual decrease in reducing sugar content was found when heated up to 7 h ($P<0.05$). The greatest decrease in reducing sugar content was observed in MRPs derived from CG model system. This result indicated that the glucose was used as a reactant in the Maillard reaction and the reaction rate was faster when compared to other sugars. The reduction in reducing sugar content during heat-induced Maillard reaction in casein-glucose, fructose-lysine, and irradiated chitosan-glucose induced Maillard reaction has been reported (Gu *et al*., 2009; Rao *et al*., 2011; Zeng *et al*., 2011). These results indicated the involvement of the amino group and carbonyl group of sugar in the formation of MRPs derived from chitosan-sugar during heat treatment, as substantiated by the lower free amino groups and reducing sugars remaining upon heat treatment.

From the results, the decrease in free amino group was in accordance with the increase of browning

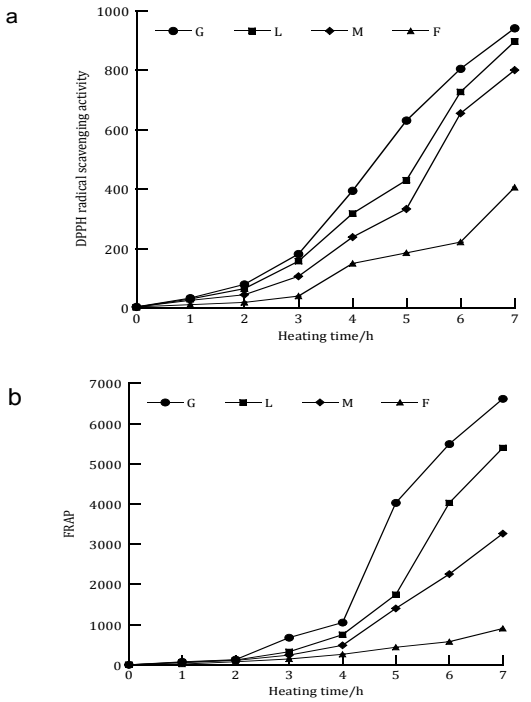

Figure 3. Changes in DPPH radical scavenging activity and FRAP of MRPs derived from chitosan-sugar model systems during heating for various times. Data expressed as μmol trolox equivalents (TE) per 1 mL of MRPs

intensity (A_{420}) and $A_{UV\ \lambda max}$. This indicated that extended heating catalysed the interaction between amino groups, such as -NH2 groups of chitosan, and reducing sugar via glycation process. The intermediate products were formed and further converted to brown pigments, as observed by the increment in browning intensity. As a result, glucose was more reactive to form the glycated chitosan than other sugars, as shown by the greatest decrease of free amino groups with the concomitant increase of 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 (Naranjo *et al*., 1998; Bunn and Higgins, 1981).

Changes in DPPH radical-scavenging activity

DPPH is a chromogen-radical-containing compound that can directly react with antioxidants. When the DPPH radical is scavenged by antioxidants through the donation of hydrogen to form a stable DPPH-H molecule, the colour is changed from purple to yellow (Shon *et al*., 2003). Stable radical DPPH has been widely used for the determination of primary antioxidant activity, that is, the free radical scavenging activities of pure antioxidant compounds, plant and fruit extracts, and food materials (Shih *et al*., 2006). The changes in the DPPH radical scavenging activity of MRPs derived from all chitosan-sugar model systems as a function of heating time are shown in Figure 3a. A small increase in the DPPH radical scavenging activity was observed as the heating time of all MRPs model systems increased up to 2 h ($P<0.05$). Subsequently, the DPPH radical scavenging activity was markedly increased as a function of heating time ($P<0.05$). The MRPs derived from CG exerted greater DPPH radical scavenging activity compared to MRPs derived from other sugars. DPPH radical scavenging activity was in the descending order: glucose > lactose > maltose > fructose. This result could be explained by the highest MRPs was formed during heating of CG solution. It is recognised that DPPH radicals can be scavenged by MRPs through donation of hydrogen to form stable DPPH-H (Gu, 2009). The present results were in agreement with previous observations where porcine plasma MRPs of protein-reducing sugars (Benjakul *et al*., 2005) and ultra-filtered MRPs from a casein-glucose model system showed strong DPPH radical-scavenging activity (Gu *et al*., 2009). This result indicated that MRPs derived from the chitosan–sugar model system were free radical inhibitors, which can work as the primary antioxidant. Moreover, Siripatrawan and Harte (2010) reported that the scavenging mechanism of chitosan is related to the fact that free radical can react with the residual free amino (NH_2) groups to form stable macromolecule radicals, and the amino groups can form ammonium (NH^{3+}) groups by absorbing a hydrogen ion from the solution. In addition, Caramelisation products (CPs) had an antioxidant activity. The intermediate products at the earlier stage of Caramelisation reaction that can be taken place during heating of chitosan-sugar model system had an antioxidant activity (Manzocco *et al*., 2011).

Changes in ferric reducing antioxidant power (FRAP)

Antioxidants can be explained as reductants, and inactivation of oxidants by reductants can be described as redox reactions in which one reaction species (oxidant) is reduced at the expense of the oxidation of the other. The FRAP assay measures the antioxidant effect of any substance in the reaction medium as reducing ability. Antioxidant potential of all MRPS derived from chitosan-sugar model systems was estimated from their ability to reduce TPTZ–Fe (III) complex to TPTZ–Fe (II) complex. This assay is also commonly used for the routine analysis of single antioxidant or total antioxidant (Kim and Lee, 2009; Morales *et al*., 2009). The changes in the FRAP of MRPs derived from all chitosan-sugar model systems as a function of heating time are presented in Figure 3b. A small increase in the FRAP was observed as the heating time of all MRPs model systems

increased up to 2 h ($P<0.05$). The FRAP of MRPs derived from the CG and CL model systems was markedly increased after 4 h of heating time while the FRAP of MRPs derived from the CM and CF model systems was slowly increased during heating for 7 h. The FRAP of MRPs derived from CG model system was the highest as a function of heating time, followed by those derived from the CL, CM and CF model systems, respectively. These results are in agreement with those obtained from the antioxidant activity determined by the DPPH radical scavenging assay. Kim and Lee (2009) and Rufian-Henares and Morales (2007) pointed out that the ferric reducing ability of melanoidins was in parallel with the data from DPPH method. Compounds responsible for reducing activity are formed during the thermolysis of Amadori products in the primary phase of Maillard reaction (Hwang *et al*., 2001) or they could be formed by heterocyclic compounds of Maillard reaction or Caramelisation of sugars (Charurin *et al*., 2005). The results revealed that the MRPs could function as electron doners. The hydroxyl groups of MRPs play an important role in reducing activity (Yoshimura *et al*., 1997). Additionally, the intermediate reductone compounds of MRPs were reported to break the radical chain by donation of hydrogen atom (Kim and Lee, 2009; Manzocco, 2011). As mentioned previously, Caramelisation also took place during heating, resulting in the formation of CPs. The hydroxyl group of CPs could function as electron donors.

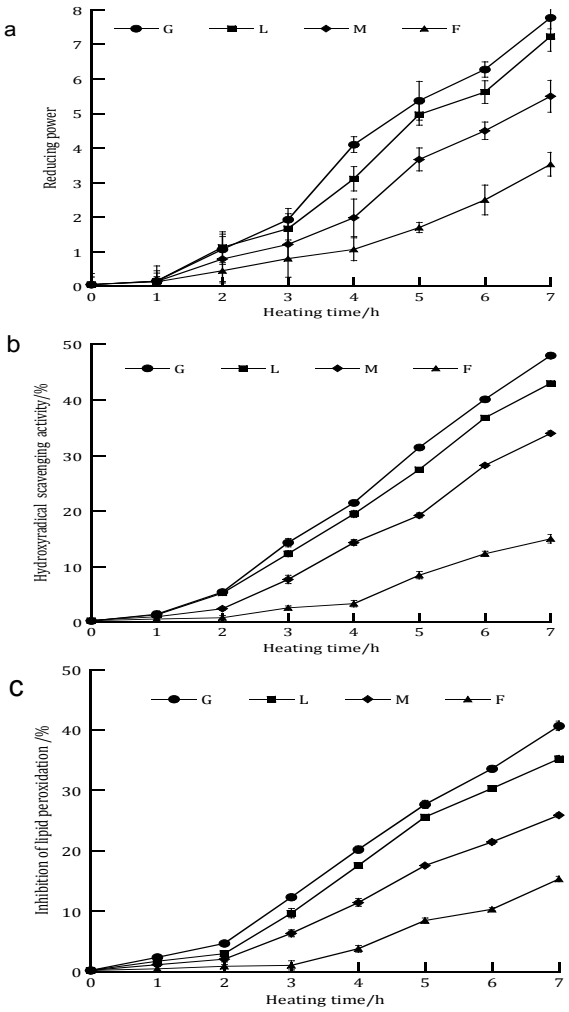

Figure 4. Changes in reducing power, hydroxyl radical-scavenging activity and inhibition of lipid peroxidation of MRPs derived from chitosan-sugar model system during heating for various times

Changes in reducing power

Reducing power assay has also been used to evaluate the stability of antioxidants to donate electrons. During the reducing power assay, the presence of reductants in the samples results in reducing Fe^{3+}/ferricyanide complex to the ferrous form (Fe^{2+}). The Fe^{2+} can be monitored by measuring the formation of Perl's Prussian blue at 700 nm (Yoshimura *et al*., 1997). Changes in reducing power of all MRPs chitosan-sugar model systems were shown in Figure 4a. A slow increase in reducing power in the first hour of heating for all MRPs derived from chitosan-sugar model systems was found. Thereafter, a sharp increase in reducing power was observed until the end of heating time. Yoshimura *et al*. (1997) reported that the reducing power increased with increasing heating time of glucose-glycine mixture. The MRPs derived from xylose-lysine (Yen and Hsieh, 1995) and glucose-lysine (Yoshimura *et al*., 1997) model systems also possessed reducing power. The results demonstrated that MRPs derived from chitosan-sugar model systems were electron donors to free radical to terminate radical chain reaction. The intermediate reductone compounds of MRPs were reported to break the radical chain by donation of a hydrogen atom. Moreover, hydroxyl groups of advanced MRPs may play a role in reducing activity.

Changes in hydroxyl radical-scavenging activity

The hydroxyl radical is the most reactive of species and induces most severe damage to adjacent biomolecules, resulting in lipid peroxidation in biological systems. In the present study the Fenton reaction system was used in the deoxyribose degradation by generating hydroxyl radicals. The treatment of deoxyribose with Fenton reaction reagent resulted in a high rate of deoxyribose degradation. Hydroxyl radical-scavenging activity of chitosan-sugar model systems was investigated (Figure 4b). Hydroxyl radical-scavenging activity of all MRPs model systems slowly increased in the first hour. Then the hydroxyl radical-scavenging activity increased at a higher rate with increasing of heating times. Among all MRPs model systems, those derived

from CG model system showed the highest hydroxyl radical-scavenging activity. From the result, the increment of hydroxyl radical-scavenging activity was in accordance with the formation of intermediate products and browning pigment. These findings revealed that compounds formed upon heat treatment of chitosan-sugar solution have the potential of being antioxidants in biological systems. Development of compounds capable of scavenging hydroxyl radicals and the utility of this test in studies to demonstrate in vitro hydroxyl radical-scavenging activity of heat-induced MRPs have been reported (Jing and Kitts, 2002a).

Inhibition of lipid peroxidation

Since lipid peroxidation is concerned, free radicals abstract hydrogen from a fatty acid double bond to produce fatty acid free radicals, and then react with oxygen to produce fatty acid hydroperoxide. The hydroperoxide is unstable and degraded readily to shorter chain hydrocarbons such as aldehydes. The intermediate products can be determined as thiobarbituric acid reactive substances (TBARS) assay. Figure 4c shows the inhibition percentage of MRPs to lecithin oxidation, inhibition percentage increased evidently with increased heating time of all MRPs derived from chitosan-sugar model systems. From the result, the MRPs derived from CG model system had greater lecithin antioxidant inhibition percentage than others. In the presence of MRPs, the propagation step might be inhibited and lead to lower oxidation (Gu *et al*., 2009).

DPPH radical scavenging activity, FRAP, reducing power, hydroxyl radical scavenging activity and inhibition of lipid peroxidation of all MRPs derived from chitosan-sugar model systems were correlated to the changes in $A_{UV\ \lambda max}$ and A_{420}. This indicated that brown pigments and colourless intermediate compounds had the hydrogen-donating properties, which varied with type of sugar. From the result, glucose was more reactive in forming the glycated chitosan than were maltose, lactose and fructose, as shown by the highest antioxidant activity.

Conclusions

MRPs derived from chitosan-sugar model systems were good in antioxidant properties. These antioxidant properties may vary with the types of reducing sugar used. Among sugar tested, glucose is the best reactant for participating in Maillard reaction with primary amino groups of chitosan as evidenced by the highest formation of browning pigment and antioxidant activity. In addition, the prolonged heating resulted in MRPs derived from chitosan-sugar with more effective antioxidant properties. On the basis of the results obtained, MRPs derived from chitosan-sugar with presumed antioxidant properties may be used as a source of antioxidants, as a possible food supplement or ingredient or in the pharmaceutical industry.

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