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Structure and antioxidant activity of Maillard reaction products from α-lactalbumin and β-lactoglobulin with ribose in aqueous model system

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Abstract

Maillard reaction products (MRPs) were prepared from aqueous model mixtures containing 3% (w/w) ribose and 3% (w/w) α-lactalbumin (α-LA) or β-lactoglobulin (β-LG), heated at 95°C, for up to 5 hours. The pH of MRPs decreased significantly during heat treatment of α-LA-Ribose and β-LG-Ribose mixtures from 8.4 to 5.3. The amino group content in MRPs derived from the α-LA-Ribose and β-LG-Ribose model system was decreased noticeably during the first hour and did not change thereafter. The loss of free ribose in MRPs was higher for β-LG-Ribose than for α-LA-Ribose. During the Maillard Reaction, the concentration of native and non-native α-LA or β-LG decreased and the formation of aggregates was observed. Fluorescence intensity of the β-LG-Ribose MRPs reached the maximum within 1 hour, compared to 2 hours for α-LA-Ribose MRPs. Meanwhile, modification of the UV/vis absorption spectra for α-LA and β-LG was mainly due to a condensation reaction with ribose. Dynamic light scattering showed a significant increase in the particle size of the MRPs. Size exclusion chromatography of MRPs revealed the production of both high and low molecular weight material. Electrophoresis of MRPs indicated polymerization of α-LA and β-LG monomers via inter-molecular disulfide bridge but also via other covelant bonds. MRPs from α-LA-Ribose and β-LG-Ribose exhibited increased antioxidant activities, therefore theses MRPs may be used as natural antioxidants in food products.

Key words: Maillard reaction, α-Lactalbumin, β-Lactoglobulin, Ribose, Antioxidant Activity

Introduction

Maillard reaction is one of the most important chemical reactions during food preparation (James P, 1986; Jennifer M, 1990; Werner, 1982). Maillard reaction involves a series of reactions steps involving the condensation between carbonyl groups and amino groups, resulting in a large number of the Maillard reaction products (MRPs), such as volatile compounds, non-volatile intermediates and dark-brown melanoidins (Ledl, Beck, Sengl, Osiander, Estendorfer, Severin, et al., 1989; Van Boekel, 1998). Although Maillard reaction is of a great importance in production of aroma, taste and colour, it can result a loss of nutritional value, digestive incompatibility and potential toxicity. Characterization of the MRPs could help controlling the formation of advanced Maillard reaction products (noxious effects in diabetes and in age-related cardiovascular diseases). However, controlled reactions can also modify protein functionality (Hiller & Lorenzen, 2010; Katayama & Saeki, 2004; Li, Lu, Luo, Chen, Mao, Shoemaker, et al., 2009; Oliver, Melton, & Stanley, 2006; Qi, Yang, & Liao, 2009; Wahyuni, Ishizaki, & Tanaka, 1998; Wooster & Augustin, 2006).

Whey proteins are extensively utilized in the food processing. α-lactalbumin (α-LA) and β-lactoglobulin (β-LG) are two protein major components in whey and play important roles in the functional properties and physiological function of whey proteins (Chatterton, Smithers, Roupas, & Brodkorb, 2006; Croguennec, Renault, Bouhallab, & Pezennec, 2006; Rojas, Goff, Senaratne, Dalgleish, & Flores, 1997). During recent years, increasing attention has been directed towards the utilization of Maillard reaction to improve functional properties of α-LA or β-LG including emulsifying properties, foaming properties and heat stability (Aoki, Kitahata, Fukumoto, Sugimoto, Ibrahim, Kimura, et al., 1997; Chevalier, Chobert, Popineau, Nicolas, & Haertlé, 2001; Medrano, Abirached, Panizzolo, Moyna, & Añón, 2009; Wooster & Augustin, 2006). Some reports focused on mechanism and chemical properties of Maillard reaction from α-LA or β-LG and sugars. For example, characteristics of conjugates between β-LG and acacia gum (Schmitt, Bovay, & Frossard, 2005) or chitosan (Miralles, Martínez-Rodríguez, Santiago, van de Lagemaat, & Heras, 2007) were investigated upon for dry -state model systems. Some structural characterization of MRPs of glycated β-LG with galactose and tagatose has been carried out under dry heat condition (Corzo-Martinez, Moreno, Olano, & Villamiel, 2008). Above-mentioned studies on MRPs of α-LA or β-LG were only based on dry heating and relatively mild reaction condition. However, during the food processing, Maillard reaction generally happens in hydrated or aqueous system under high temperature conditions. However, only

few published examples are available to date. Meanwhile, few studies were carried out on the antioxidant properties of Maillard reaction products of α-LA or β-LG. Conjugates of α-LA with three monosaccharides produced in the dry state showed antioxidant activities (Sun, Hayakawa, Puangmanee, & Izumori, 2006). However, the MRPs were not fully characterised, the objectives of this study were to describe the structural changes and antioxidant activity during Maillard reaction of α-LA and β-LG with ribose in an aqueous system. Therefore conditions were chosen to promote Maillard reaction i.e. high heat and pH as well as the choice of ribose as the one of the most reactive monosaccharides.

2. Materials and methods

2.1. Materials

Commercial α-lactalbumin (95% protein content, approximately 3.0% β-lactoglobulin) and β-lactoglobulin (94% protein content, approximately 3.5% α-lactalbumin) were purchased from Davisco Foods International, Inc. (Eden Prairie, MN, USA). D-Ribose, OPA (o-phthaldialdehyde), L-leucine, Potassium ferricyanide(III), 2,2-diphenyl-1-picryl-hydrazyl (DPPH), sodium dodecyl sulphate (SDS), β-mercaptoethanol, L-leucine, and trifluoroacetic acid (TFA) and other reagents were purchased from Sigma Aldrich Co. (St. Louis, MO, USA) unless stated otherwise. All chemicals used in this study were of analytical grade.

2.2. Preparation of α-LA-Ribose and β-LG-Ribose MRPs

α-LA and β-LG were dissolved in Milli-Q® water at a protein concentration of 30 g/L with the ribose (30 g/L), respectively. The initial pH of the mixtures was adjusted to 8.4 using pH meter (pH 340i, WTW, 82362, Germany). The mixtures were then transferred to sealed screw-top test tubes, and heated in water bath at 95°C. Samples were taken after heating for 0, 0.5, 1, 2, 3, 4, and 5 hours and immediately submerged in ice water. Control experiments were also carried out with no added ribose.

2.3. Determination of free amino groups

The quantity of free amino groups was determined by the OPA method (Church, Swaisgood, Porter, & Catignani, 1983). MRPs (35μL, 12-fold dilution) were mixed with 1 mL of OPA reagent. After vortexing and a minimal 5 min delay in the dark at room temperature, the absorbance was recorded at 340 nm. Absorbance readings were converted into free amino contents using a calibration curve obtained with L-leucine (0 to 0.5 g/L) as a standard. The blank was determined in the same manner, except that MilliQ® water was used instead of sample. Data were finally expressed as relative concentrations (%) in comparison with the content of unheated samples.

106 2.4. Determination of ribose in MRPs

107 The free ribose in MRPs samples were determined by HPLC (waters 2695 Alliance, Waters Inc., USA)
108 equipped with a WATO44355 carbohydrate column (4.6×250 mm I.D., 4 μm particle size, Waters,
109 Ireland). 50μL of sample were injected and eluted at a flow rate of 0.5mL/min using a mobile phase of
110 9mM H_2SO_4. The column temperature was set at 60°C. A refractive index detector (2414 Waters Inc.,
111 USA) was used for sample detection. Data analysis and quantification were performed using
112 Empower® Chemstation software. Changes in ribose were expressed as relative concentrations (%) in
113 comparison with the original ribose content in non-heated samples.

114 2.5. Size exclusion chromatography (SEC)

115 The MRP samples were separated by size exclusion chromatography using a series connection column
116 with TSK G 3000SW XL (7.8mm ID×30cm L, 5μm) and TSK G 2000SW XL (7.8mm ID×30cm L,
117 5μm, TosoHaas, Montogomeryville, PA, USA) fitted to a TSK guard column (7.5mm×7.5cm).
118 Samples (20 μL of 2.5 g/L protein) were eluted with 50 mM Tris-HCl buffer (pH 7.0) was used as the
119 eluent at a flow rate of 0.6 mL/min. The absorbance was monitored at 280nm. 5mL of the MRP
120 samples were added 5mL of 0.01M acetate buffer pH 4.6. The mixtures were centrifuged for 20 min at
121 5,000g in order to get the supernatant. The native protein concentration in MRPs was determined by
122 adding 0.01M acetate buffer pH 4.6 analysis of the supernatant by SEC. The method was calibrated
123 using α-LA and β-LG standards.

124 2.6. Determination of Native, Non-native and Aggregated α-LA and β-LG concentrations in MRPs

125 The concentration of native protein was determined as described above. Monomeric protein (including
126 both native and non-native) were directly obtained using SEC. The concentrations of non-native
127 monomeric α-LA and β-LG were calculated by subtraction of the level of native protein from that of
128 monomeric protein. The amount of aggregation was calculated by subtraction of the amount of
129 monomer (SEC) from the initial protein concentration.

130 2.7. Absorption and fluorescence spectroscopy

131 UV/vis absorption spectra were recorded using a CARY 1 UV-visible spectrophotometer (Varian, USA)
132 with the wavelength ranging from 200 to 800 nm. The α-LA-Ribose and β-LG-Ribose model MRPs
133 were diluted (1:120, v/v) with Milli-Q® water for UV/vis analysis.

134 Fluorescence spectra of α-LA-Ribose and β-LG-Ribose MRPs were determined using a temperature
135 controlled Cary Eclipse Fluorescence Spectrometer (Varian, USA). Samples were diluted (1:120 v/v)

136 with MilliQ® water. For fluorescence excitation spectra, the emission wavelength was to 420nm and
137 the excitation was scanned from 300 to 400 nm. For fluorescence spectra, the excitation wavelength
138 was 337 nm, and the emission was scanned from 370 to 570 nm. Both excitation and emission slits
139 were set to 10 nm, the temperature was 20 °C, and the scan rate was 120 nm/min.

140 2.8. Dynamic Light Scattering (DLS)

141 The hydrodynamic diameter of α-LA-Ribose and β-LG-Ribose MRPs were determined using a
142 Zetasizer Nano system (Malvern Instruments Inc., Worcester, UK). The measurements were carried out
143 at 25 °C using a scattering angle of 12°and the viscosity of the solvent was assumed to be the same as
144 water.

145 2.9. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE)

146 SDS-PAGE was performed according to a modified method of Laemmli (1970), using 4% acrylamide
147 stacking gel and 15% acrylamide separating gel with a vertical gel electrophoresis cell (Bio-Rad,
148 Richmond, CA, USA). MRP samples were prepared under both reducing (with β-mercaptoethanol) and
149 non-reducing conditions. Gels were stained with 0.5% Coomassie Brilliant Blue R-250. Destaining was
150 conducted with a solution of isopropanol and acetic acid. Molecular weight markers (10 to 250 kDa)
151 (Bio-Rad Laboratories, CA, USA) were used for M_w estimation of samples.

152 2.10. Determination of reducing power

153 The reducing power of samples of α-LA-Ribose and β-LG-Ribose MRPs was determined according to
154 the method of Chawla, Chander and Sharma (2009) with some modifications. 0.1mL of MRPs sample
155 (12-fold dilution) was mixed with 0.5 mL of 0.2 M sodium phosphate buffer (pH 6.6) and 0.5 mL of
156 1% potassium ferricyanide. The reaction mixtures were incubated in a water bath at 50°C for 20 min,
157 followed by addition of 0.5 mL of 10% trichloroacetic acid. The mixtures were then centrifuged at
158 5,000g using a Eppendorf 5417R centrifuge (Eppendorf AG, Hamburg, Germany) for 10 min at 25 °C.
159 The supernatant (0.5 mL) was treated with 0.5 mL of Milli-Q® water and 0.1mL of 0.1% $FeCl_3$. After
160 the reaction, the mixture was left for 10 min, the absorbance was measured at 700 nm. Higher
161 absorbance of the reaction mixture indicated greater reducing power.

162 2.11. Radical scavenging activity using the 2,2-Diphenyl-1-picryl-hydrazyl (DPPH)

163 DPPH radical scavenging activity of the MRPs was estimated according to a modified method of Yen
164 and Hsieh (1995). An aliquot of MRP sample (250 μL) was added to 1 mL of a solution of DPPH,
165 prepared freshly, at a concentration of 0.1mM in ethanol. The reaction solution was then mixed

vigorously and allowed to stand at room temperature for 30 min in the dark. The mixture was centrifuged for 10 min at 4,000g. The absorbance of the supernatant was measured at 517 nm using a CARY 1 UV-visible spectrophotometer. The percentage of DPPH radical scavenging activity was calculated as follows:

$$\text{DPPH radical scavenging activity (\%)} = \left(1 - \frac{Ai - Aj}{Ac}\right) \times 100\%$$

Where A_i is the absorbance of sample, A_j is the absorbance of ethanol added instead of 0.1 mM DPPH and Ac is the absorbance of ethanol added instead of MRP samples.

2.12 Statistical analysis

Analyses including the determination of pH, free amino group, ribose, reducing power and DPPH radical scavenging activity were run in at least triplicate**.** Results were expressed as means with the standard deviation. Analysis of variance (ANOVA) was performed and significant differences among means from triplicate or more by the Duncan's multiple range tests using the SPSS system software 13.0. For the DLS analysis, the experiments were carried out in duplicates and each measurement was made in triplicate. SEC measurements were carried out in duplicates.

3. Results and Discussion

3.1. Changes in pH

The changes in the pH of MRPs derived from aqueous α-LA-Ribose and β-LG-Ribose systems as a function of heating time were shown in Fig.1A. The pH of α-LA-Ribose and β-LG-Ribose MRPs decreased significantly as the heating time increased up to 5 h ($P < 0.05$). During the first 2 hours, the pH decreased at a higher rate, and the pH of β-LG-Ribose MRPs decreased more quickly than α-LA-Ribose MRPs ($P<0.05$). After 2 hours, the pH of α-LA-Ribose and β-LG-Ribose MRPs slightly decreased up to 5 hours and there was no obvious difference in the change of pH between two systems. The result was in accordance with Benjakul, Lertittikul and Bauer (2005) and Gu, Kim, Hayat, Xia, Feng and Zhang (2009) who found that the pH of protein-sugar MRPs decreased with the heating time and levelled off with prolonged heating. The reduction in pH is generally attributed to the formation of organic acids, such as acetic acid and formic acid in the intermediate MRPs (Brands & van Boekel, 2002; Chen, Jin, & Chen, 2005; Rufian-Henares, Delgado-Andrade, & Morales, 2006). In addition, the consumption of amino groups and the formation of acidic compounds could also contribute to the decrease in pH. In contrast to this, the pH in α-LA and β-LG heated alone (95°C), only slightly

195 decreased during heating, possibly due to the thermal protein degradation (Lan, Liu, Xia, Jia, Mukunzi,
196 Zhang, et al., 2010). Therefore, it was assumed that formation of organic acids from Maillard reaction
197 was a key factor in the pH decrease of MRPs.

198 3.2. Changes in the protein amino groups

199 During Maillard reaction, available amino groups of the proteins contain terminal α-amino groups of
200 protein and ε-amino groups of lysine residues. To assay amino group availability, the quantities of
201 amino groups in α-LA and β-LG heated in the presence and absence (control sample) of ribose for up
202 to 5 h were measured using the OPA method and results were shown in the Fig.1B. When α-LA and β-
203 LG was heated without ribose at pH 8.4, the free amino groups did not significantly change during the
204 whole heating time ($P>0.05$). Similar results were reported previously (Sun, Hayakawa, Puangmanee,
205 & Izumori, 2006), where free amino groups of heated control α-LA did not significantly change during
206 the 48h incubation. However, compared to the initial α-LA-Ribose and β-LG-Ribose MRPs, a
207 significant decrease ($P<0.05$) in free amino group content, 32.52±0.81% and 28.32±1.11% respectively,
208 was observed in the α-LA-Ribose and β-LG-Ribose MRPs when heated for one hour. Then the amino
209 group loss did not changed significantly with the prolonged heating time to 5h. This indicates that
210 heating quickly induced interaction of free amino groups, such as α-NH_2 and ε-NH_2 of lysine residues,
211 with ribose by Maillard reaction within 1 hour. The pH decrease of protein-sugar systems and decrease
212 in reactivity of the protein resulted in only a small change in the free amino group consumption during
213 prolonged heating.

214 3.3. Changes of ribose and protein in MRPs

215 The loss of ribose and protein in the α-LA-Ribose and β-LG-Ribose MRPs, were given in Fig.1C and
216 D as a function of the heating time. A sharp decrease in ribose concentration in both MRP model
217 systems was observed for up to 5 h of the heating time ($P<0.05$). Within the first 3 hour, there was no
218 statistical significance in the loss of ribose between the α-LA-Ribose and the β-LG-Ribose ($P>0.05$).
219 However, from 4 h to 5h of heating, the loss of ribose was higher in β-LG-Ribose than in α-LA-Ribose.
220 Sugar consumption is a critical property to evaluate the reactivity of sugars in the Maillard reaction. It
221 was also noted that during the Maillard reaction, ribose was the most active among five tested sugars
222 (Xylose, Arabinose, Glucose and Fructose) (Laroque, Inisan, Berger, Vouland, Dufossé, & Guérard,
223 2008). In the present study, ribose consumption from the α-LA-Ribose and the β-LG-Ribose was
224 35.74±1.51% and 39.26±0.25% at 5 h, respectively, compared to the initial α-LA-Ribose and the β-

LG-Ribose. 12 hours of heating led to 30% of remaining glucose when reacted with different amino acids (Kwak & Lim, 2004). Kim and Lee (2009) also reported that the loss of glucose in MRPs derived from the Glucose-Glycine, Glucose-Diglycine and Glucose-Triglycine model system was a function of heating time. The distribution of native, non-native and aggregated proteins in α-LA-Ribose and β-LG-Ribose reaction mixtures is shown in the Fig.1C and Fig.1D. Prior to heating, α-LA-Ribose MRPs model system contained 73.73% native protein and 26.27% non-native protein, but β-LG-Ribose MRPs contained 55.98 % native protein, 15.36% non-native protein and 28.66% aggregates, which is within acceptable limits for commercial α-LA and β-LG, probably due to slow denaturation and aggregation in the powders. Native protein concentration in the α-LA-Ribose and β-LG-Ribose MRPs decreased gradually and whereas aggregate increased quickly with increase heating time up to 5 h. After 0.5h of heat-treatment, the concentrations of non-native protein, which probably included those previously described (Croguennec, Bouhallab, Molle, O'Kennedy, & Mehra, 2003; Kehoe, Wang, Morris, & Brodkorb, 2011), were highest and decreased thereafter. The above results suggested that Maillard reaction of α-LA and β-LG with ribose resulted in substantial amounts of aggregates.

3.4. Spectroscopic analysis

UV/vis absorption, fluorescence excitation and emission spectra of α-LA-Ribose and β-LG-Ribose model system are shown in the Fig.2. The appearance of new compounds with an excitation maximum of 337±1nm and an emission maximum of 420±1nm, was observed within the first 1 to 2 hours (faster for β-LG-Ribose than α-LA-Ribose) and followed by a rapid decline. The corresponding fluorescence reached a maximum value within 2 hours for α-LA-Ribose MRPs, compared to 1 hours for β-LG-Ribose MRPs. The fluorescence is associated with the early stage of the Maillard reaction and the development of fluorescent compounds, which are considered to be precursors of brown pigments formed during Maillard reaction (Morales, Romero, & JimenezPerez, 1996). The development of fluorescent compounds was found to increase with longer heating times of the rice protein and glucose (Li, Lu, Luo, Chen, Mao, Shoemaker, et al., 2009). In contrast, fluorescence was not found to increase linearly with heating time in ribose-casein systems (Jing & Kitts, 2004).

The changes in the UV/vis absorption spectra of α-LA-Ribose and β-LG-Ribose MRPs as a function of the heating time are shown in Fig. 2A' and 2B'. The absorbance of MRP samples increased significantly as the heating time increased for up to 5 h. This results are in accordance with previous observations (Kim & Lee, 2009).

255 3.5. Dynamic Light Scattering (DLS)

256 Particle size of α-LA-Ribose and β-LG-Ribose (Fig.3.) increased as a function of heating time, β-LG-
257 Ribose Particles being larger than those of α-LA-Ribose. However, under the experimental condition
258 (pH 8.4), particle sizes of heated α-LA and β-LG exhibited only marginal changes.

259 3.6. SDS-PAGE

260 All of α-LA-Ribose and β-LG-Ribose MRPs samples were analyzed by both non-reducing and
261 reducing SDS-PAGE. The noncovalent interactions within aggregates are generally disrupted in the
262 presence of SDS (non-reducing conditions) whereas addition of β-mercaptoethanol cleaves all
263 disulphide bonds (reducing conditions).

264 *α-LA-ribose:* Both non-reducing and reducing SDS-PAGE (Fig. 4A and 4B, respectively), the amount
265 of aggregates increased with the prolonged heat treatment. Comparing the amounts of aggregate on
266 non-reducing and reducing SDS-PAGE, it was estimated that some aggregates were linked by disulfide
267 bonds. However, reducing conditions failed to dissociate aggregates, suggesting other covalent, inter-
268 molecular bonds, possibly conjugates of proteins and ribose. A gradual increase in conjugate size, from
269 monomer to dimers and trimers etc. could be detected. Several bands between approximately 26 to
270 34kDa are clearly visible under non-reducing conditions, suggesting that in addition to homo-
271 conjugates of α-LA with α-LA (M_w of 28kDa), hetero-conjugates such as α-LA with β-LG (M_w of
272 32kDa) could be produced. After 5 hours heat-treatment, no remaining monomeric α-LA was detected.

273 *β-LG-ribose*: Similar observations were made for β-LG-Ribose (Fig. 4 C and D). However,
274 polymerisation appeared to be faster than for α-LA-ribose. After 4 hours heat-treatment, practically no
275 remaining monomeric β-LG was detected. Under non-reducing condition monomers, dimers and
276 trimers of β-LG were observed prior heating, which indicated that initial β-LG contained disulphide-
277 linked aggregates, which was also confirmed by the above-mentioned structural characterisation
278 (Fig.1D).

279 3.7. Size exclusion chromatography (SEC)

280 Molecular rearrangements in α-LA-Ribose and β-LG-Ribose MRPs could be observed by size
281 exclusion chromatography (Fig.5.). It showed a decrease in the amount of monomer and an increase in
282 aggregated α-LA and β-LG. The appearance of a new peak corresponded to small molecules. The
283 amount of both aggregates and small molecular weight (M_w) material increased with increasing heating
284 time; accordingly, the amount of monomer decreased. Meanwhile, the production rate and

285 concentrations of smaller compounds of MRPs derived from β-LG were quicker and higher than those
286 from α-LA. α-LA and β-LG were probably covalently attached to ribose or ribose degradation
287 products, which resulted in high M_w MRPs (melanoproteins). Meanwhile, caramelisation of ribose
288 heated at high pH or degradation products of Maillard reaction may induce the formation of low and
289 medium Mw compounds.

290 3.8. Antioxidant properties

291 3.8.1 DPPH radical scavenging activity

292 DPPH radical-scavenging activity of α-LA-Ribose and β-LG-Ribose MRPs increased significantly ($P<$
293 0.05) for the first two hours of heating but levelled off at 4 to 5 hours of heating, as shown in the
294 Fig. 5A. There was no significant difference in DPPH radical-scavenging activity between α-LA-
295 Ribose and β-LG-Ribose as the heating time increased ($P>0.05$). In contrast to this, heated α-LA and
296 β-LG lacked the DPPH radical-scavenging activity of its corresponding MRPs. It is recognised that
297 DPPH radicals can be scavenged by MRPs through donation of hydrogen to form stable DPPH-H
298 (Matthaus, 2002). The present results were in agreement with previous observations where porcine
299 plasma MRPs of protein-reducing sugars (Benjakul, Lertittikul, & Bauer, 2005) and ultra-filtered
300 MRPs from a casein-glucose model system showed strong DPPH radical-scavenging activity (Gu, Kim,
301 Hayat, Xia, Feng, & Zhang, 2009).

302 3.8.2 Reducing power of MRPs

303 Reducing power of α-LA-Ribose and β-LG-Ribose MRPs increased significantly as the heating time
304 increased ($P< 0.05$), as shown by an increase in absorbance at 700 nm (Fig. 6B). Reducing power of
305 two model systems also increased notably within the first 2 hours but levelled off at after 5 hours of
306 heating. Reducing power of β-LG-Ribose MRPs was significantly higher than that of α-LA-Ribose
307 MRPs ($P<0.05$). However, it was also shown that there was a somewhat smaller reducing power of
308 heated α-LA and β-LG. Hydroxyl groups and pyrrole groups of MRPs may play a drinving role in
309 reducing activity (Yanagimoto, Lee, Ochi, & Shibamoto, 2002; Yoshimura, Iijima, Watanabe, &
310 Nakazawa, 1997). Heat-induced MRPs from casein/glucose (Gu, Abbas, & Zhang, 2009; Gu, Kim,
311 Abbas, Zhang, Xia, & Chen, 2010) and porcine plasma protein/glucose models (Benjakul, Lertittikul,
312 & Bauer, 2005) also exhibited reducing power.

313 **4. Conclusion**

MRPs of α-LA and β-LG with ribose were prepared under high temperature and alkali pH conditions. Free amino groups, pH and free ribose decreased considerably with increasing heat. Characteristics of Maillard reaction of α-LA-Ribose and β-LG-Ribose model system indicated production of new smaller molecules, molecular rearrangements and polymer production occurred with heat treatment increased. Based on the reducing power and radical-scavenging activity of the α-LA-Ribose and β-LG-Ribose MRPs, they may be considered as possible antioxidants for food products.

Acknowledgements

The studies were supported by Program for New Century Excellent Talents in Heilongjiang Province University (1251-NCET-009) and the Innovative Research Team of Higher Education of Heilongjiang Province (2010td11). The studies were also supported by the food program of the Teagasc Food Research Centre, Moorepark.

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443 (white) and aggregate (grey) α-LA (C) and β-LG (D) in protein-ribose mode systems during heat
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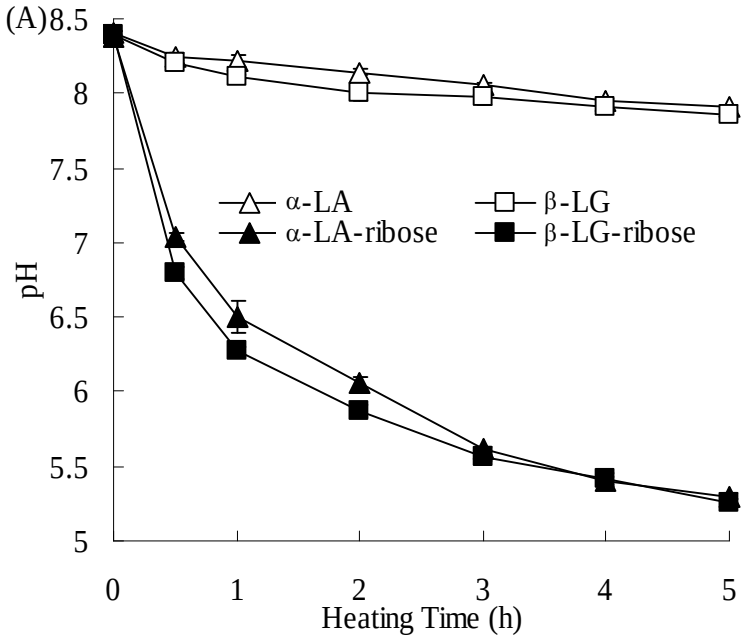
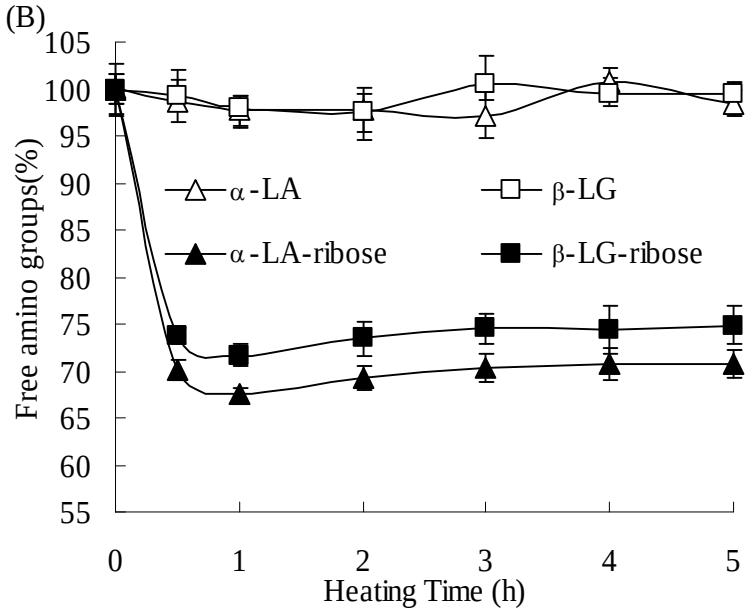
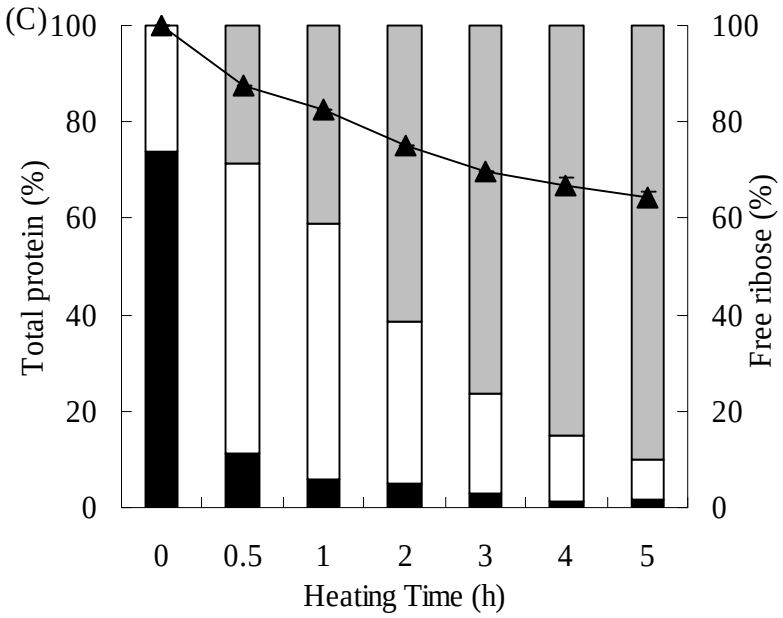

(A)
8.5
8
7.5
7
6.5
6
5.5
5
pH
α-LA
β-LG
α-LA-ribose
β-LG-ribose
0
1
2
3
4
5
Heating Time (h)

465


(B)
105
100
95
90
85
80
75
70
65
60
55
Free amino groups(%)
α-LA
β-LG
α-LA-ribose
β-LG-ribose
0
1
2
3
4
5
Heating Time (h)

466



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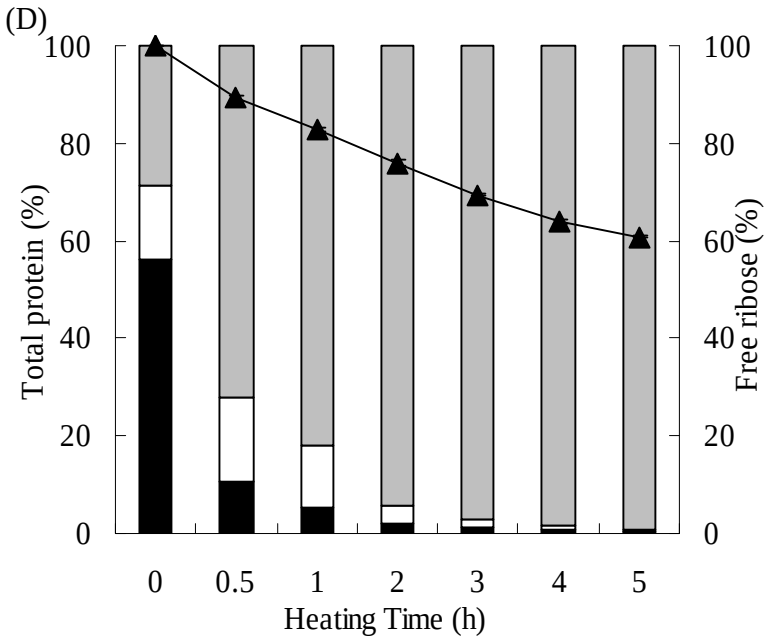

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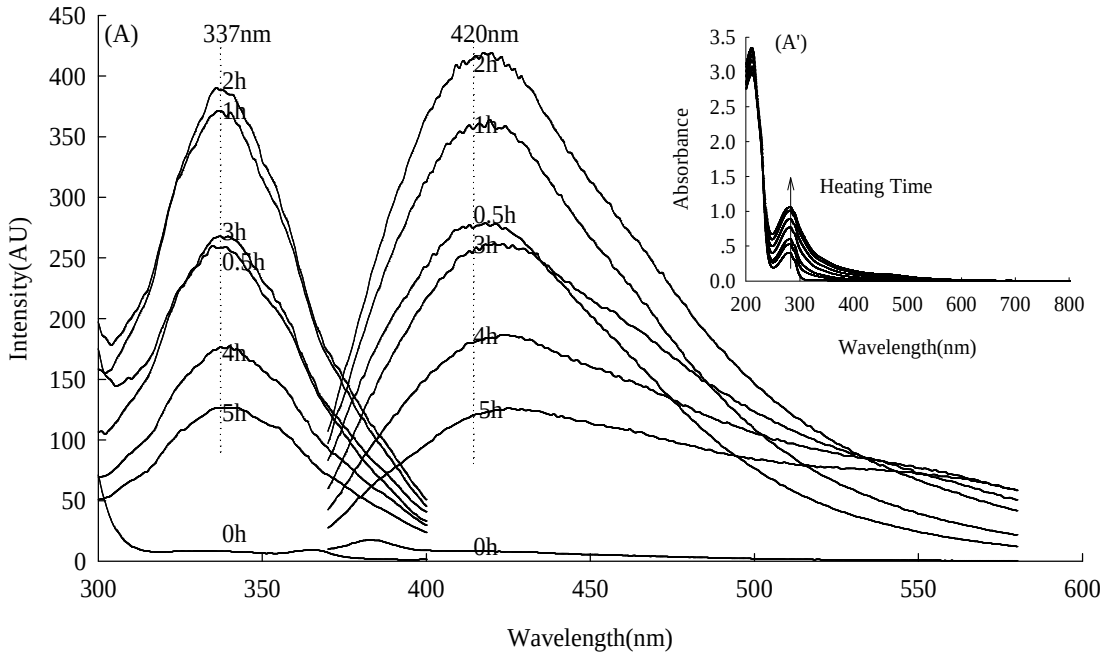

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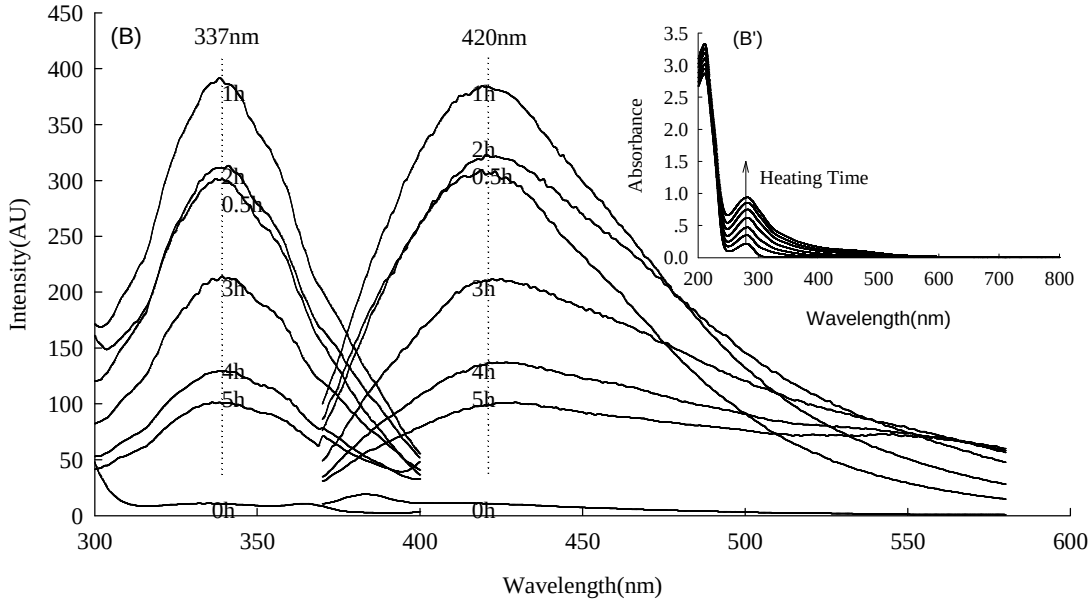

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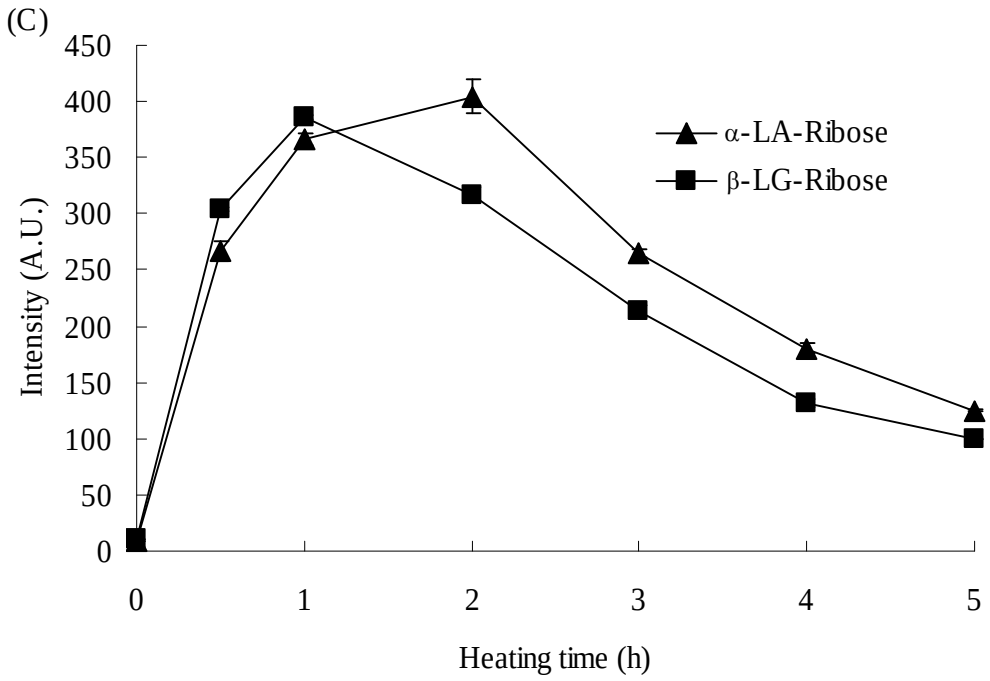

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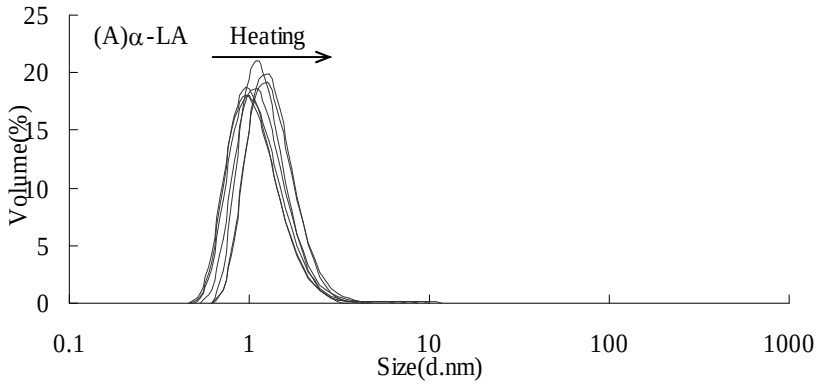

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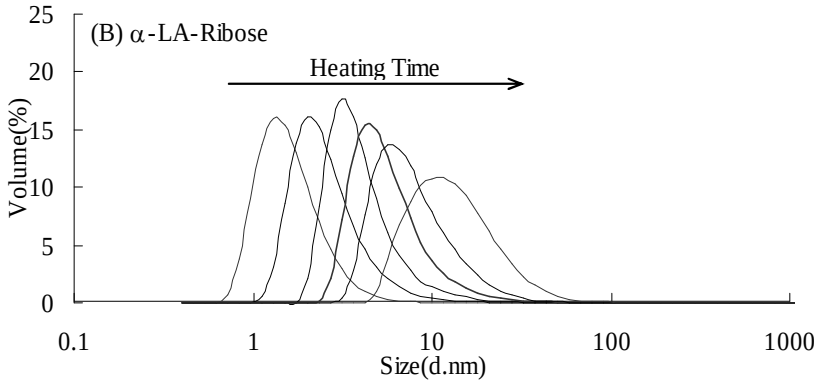

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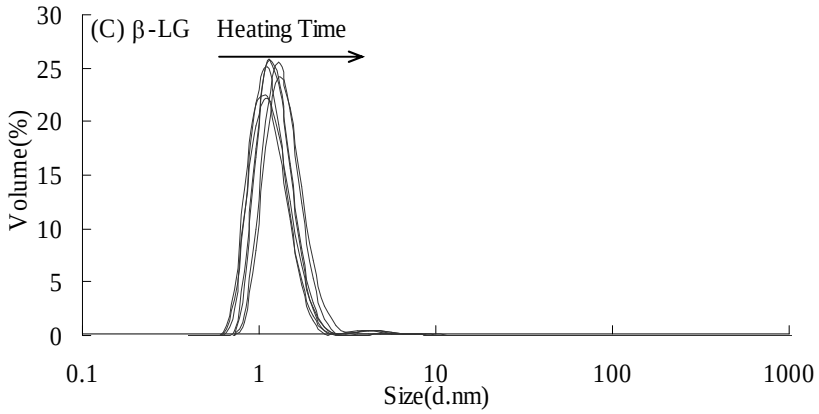

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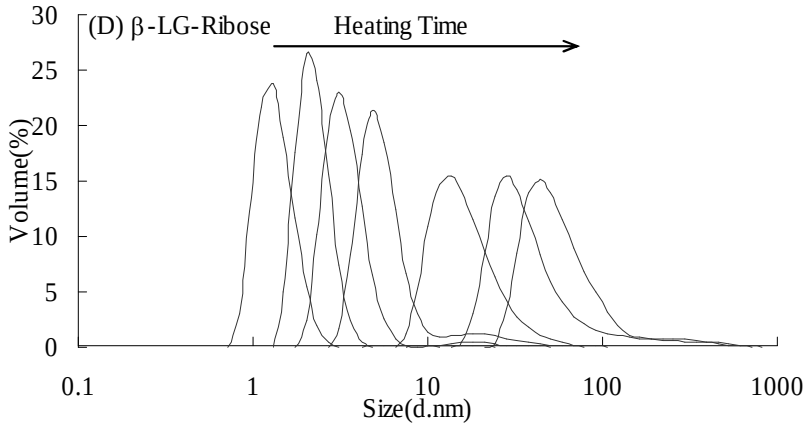

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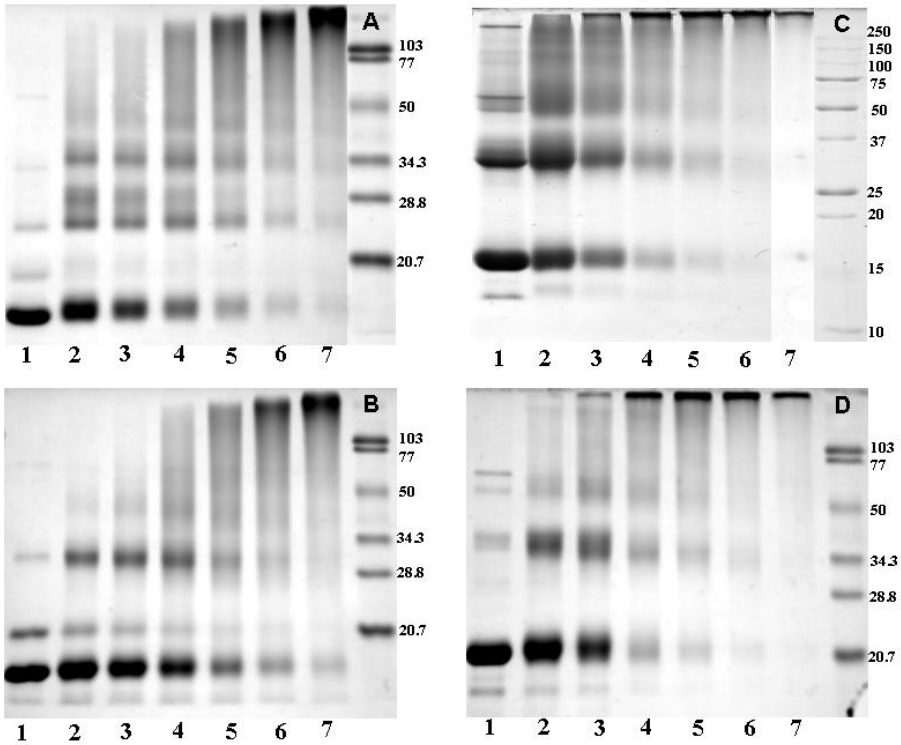

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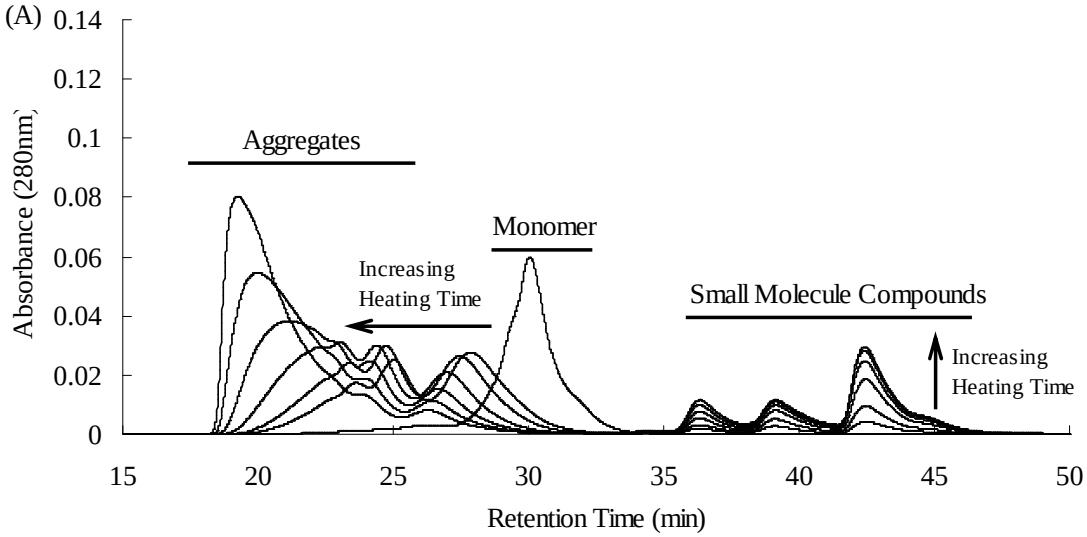

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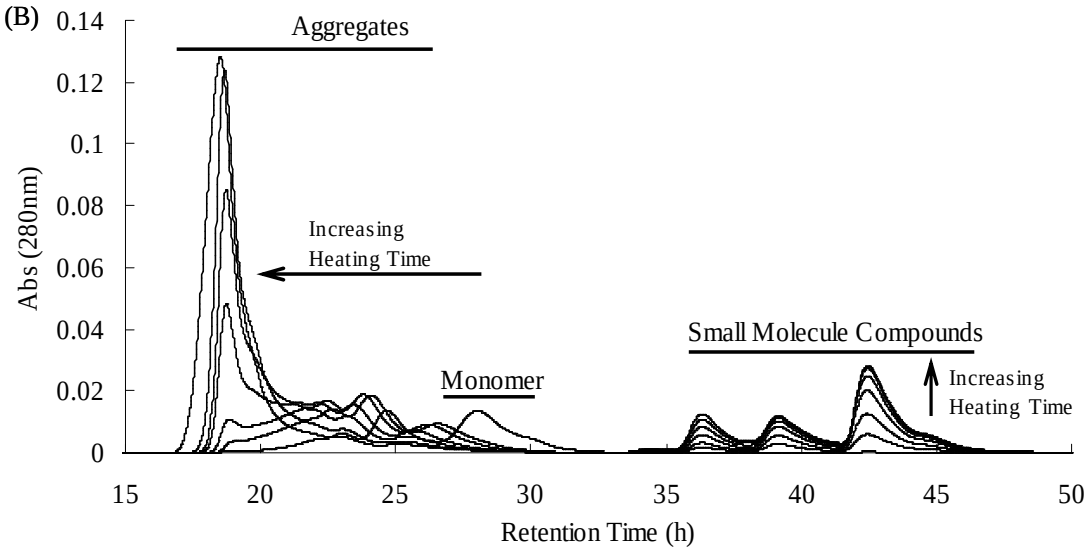

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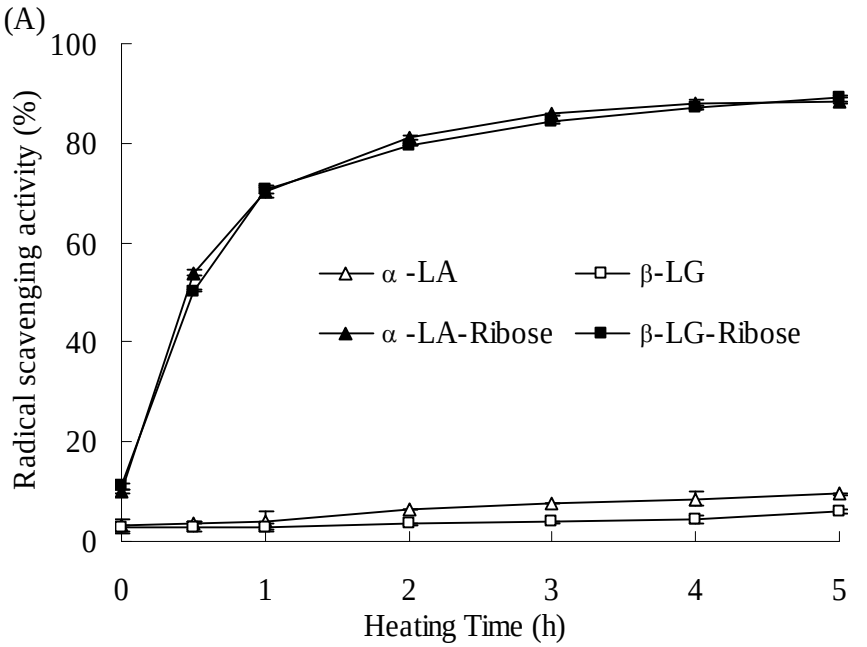

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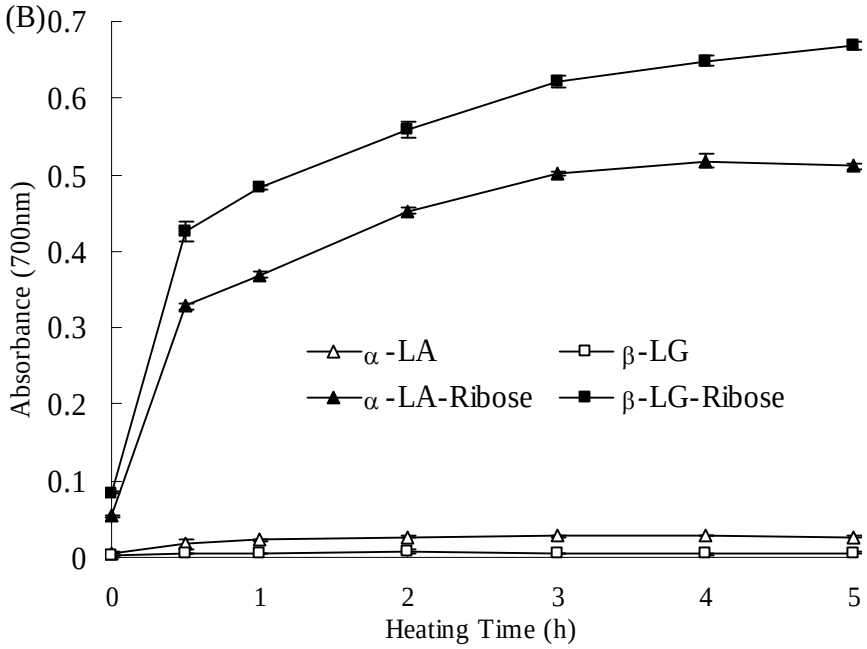

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