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Comparison of Solid–State Glycation of Whey Proteins through Maillard Reaction between Microwave and Conductive Heating

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The mechanism of microwave heating (MH), which rotates and vibrates the electric dipole of target molecules, is different from conductive heating (CH). In the present study we compared the rate of solid–state glycation of whey proteins (WP) by glucose between MH and CH. During heating for glycation temperature of WP–glucose mixture reached to 60, 75 and 90 °C at the heating time of 10 min in both treatments. No significant difference ($p>0.05$) was observed in the rate of glycation of WP between MH and CH when estimated by molecular mass of glycated WP through SDS–PAGE analysis and by glycation ratio of primary amino groups in β–lactoglobulin using *o*–phthalaldehyde. The results of this study suggest that characteristic heating mechanism of MH does not affect glycation rate of WP in solid–state.

INTRODUCTION

Whey proteins (WP) represent a rich and varied mixture of secreted proteins with wide–ranging chemical, physical and functional properties (Chevalier *et al*., 2001). Due to these beneficial functional properties, WP are used as ingredients in many industrial food products (Chevalier *et al*., 2001; Alomirah and Alli., 2004; Castaño *et al*., 2005). However, β–LG, a dominant protein of WP, is a potent allergen of milk allergy; ~82% of milk allergy patients are sensitive to β–LG (Spies, 1973).

Maillard reaction is the most frequent reaction in food products, because this reaction is catalyzed by just only heating. Maillard reaction is started by the formation of a Schiff base between carbonyl group of reducing sugars and amino groups of amino acids, peptide and protein. The formed Schiff base gives the Amadori product through rearrangements (Mennella, *et al*., 2006). It was confirmed that the glycation of WP through Maillard reaction improved solubility (Castaño *et al*., 2005; Enomoto *et al*., 2007), heat stability (Broersen *et al*., 2004; Chevalier *et al*., 2001), emulsifying properties (Broersen *et al*., 2004; Chevalier *et al*., 2001) and decreased in antigenicity (Enomoto *et al*., 2007; Hattori *et al*., 2004). Therefore modification of WP through Maillard reaction can promote utilization of WP in the food industry.

Microwave heating (MH) is a safe and rapid heating method already used to heating and drying of foods. MH has a different heating mechanism from conductive heating (CH). Microwave rotates and vibrates the electric dipole of target molecules, and that is preferentially absorbed by water molecules. The characteristic heating mechanism of MH can affect the rate of Maillard reaction. Research effort has been directed to determine whether MH has any non–thermal effect on the rate of Maillard reaction. Guan *et al*. (2006) described that MH accelerated the Maillard reaction of soy protein isolate with sugars compared to CH. Shazman *et al*. (2007) described that MH failed to show any significant effect on glycation of glycine by glucose. However Maillard reaction in these studies was performed in water or a buffer solution. It is considered that microwave directly heated water molecules and those water molecules indirectly heated target molecules. To assess the effect of characteristic heating mechanism of microwave on Maillard reaction, MH is required to be done on dry protein powder. In addition, Guan *et al*. (2006) paid no attention to standardize heating profile between MH and CH. Temperature is the most important parameters that affect the Maillard reaction (O'Brien, 1995). This suggests that comparison of Maillard reaction between MH and CH requires standardization of their heating profiles.

In the present study, the rate of solid–state glycation of WP by glucose was compared between MH and CH under standardized heating profiles.

MATERIALS AND METHODS

Glycation of whey proteins by microwave and conductive heating

Whey proteins (WP) were kindly donated by Snow Brand Milk Products (Tokyo, Japan). WP and glucose (Nacalai tesque, Kyoto, Japan) were mixed at a weight ratio of 3.4:1 and dissolved into distilled water, followed by lyophilization. The moisture content of this powder was about 6.6% when estimated by drying at 102 °C under atmospheric pressure. The resultant powder was ground in a mortar and subjected to MH (IMCR–25003 Model, 400W full power, IDX Co., Kanagawa, Japan) or CH (TGradient Thermoblock, Biometra, Germany).

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Temperature change of the sample powder during MH was monitored by a thermocouple directly inserted into the sample powder.

SDS–PAGE

Glycated WP formed after MH or CH was analyzed by SDS–PAGE under reducing condition by the method of Laemmli (1970) using 15% acrylamide gel (BIO CRAFT, Tokyo, Japan). SDS–PAGE was run at a constant current of 30 mA. The gel was stained with coomassie brilliant blue R250.

Measurement of glycation ratio

Separation of β–lactoglobulin

Beta–lactoglobulin (β–LG) was separated from WP by a slightly modified method of Alomirah and Alli (2004). WP was dissolved into distilled water at a concentration of 0.23 mg/ml by mixing at room temperature for 30 min using a rotator. The soluble fraction was obtained after centrifugation at 4 °C, 5000 × g for 30 min and the following filtration through 0.2 μm of membrane filter. Sodium citrate was added to the resultant solution at the final concentration of 150 mM and acidified to pH 3.9 with 6 M citric acid. After the incubation at 35 °C for 45 min, the solution was centrifuged at 4 °C, 5000 × g for 30 min, and the supernatant was carefully transferred to the other tube. Sodium chloride was added to the supernatant to give a concentration of 7% (w/v) and centrifuged at 4 °C, 5000 × g for 30 min, and the same weight of NaCl was added again to the resultant supernatant. After centrifugation at 4 °C, 5000 × g for 30 min, the supernatant was recovered, followed by desalting using Vivaspin filter unit with a 10,000 molecular mass cut–off membrane (Sartorius, Germany). The resulting solution was used as β–LG solution. The separation of β–LG was validated by western blotting. Briefly, the separated β–LG solution was subjected to SDS–PAGE as the same method described above and electroblotted onto PVDF membrane using semi–dry blotter NA–1512 (Nihon Eido, Tokyo, Japan) at a constant current of 200 mA. Primary and secondary antibody used was rabbit anti β–LG polyclonal antibody and avidin–coupled anti rabbit Ig polyclonal antibody, respectively.

Determination of glycation ratio of β–LG

The glycation ratio was determined by measuring glycated primary amino groups in β–LG using *o*–phthaldialdehyde (OPA) by the method of Church *et al.* (1983). A hundred μl of the solution of glycated WP formed by CH or MH was added to 2 ml of OPA reagent, which was prepared just before use by mixing 40 mg of OPA (dissolved in 1 ml of methanol), 25 ml of 100 mM sodium tetraborate, 2.5 ml of 20% (w/w) sodium dodecyl sulfate (SDS) and 100 μl of β–mercaptoethanol, and then diluting to a final volume of 50 ml with distilled water. The solution was mixed by repeated inversion and stood for 2 min at room temperature, and the absorbance was measured at 340 nm in a spectrophotometer (UV–1700, Shimadzu, Kyoto, Japan). Data was shown in the following equation, Glycation ratio (%)={1–(A_{340} of glycated β–LG/A_{340} of unglycated β–LG)} × 100. Significant difference in glycation ratio between CH and MH was determined Student's or Welch's t test after F test.

RESULTS AND DISCUSSION

It is expected that the characteristic heating effect of microwave on Maillard reaction is obtained when the treatment was performed against dry protein powder. In addition, it is considered that heating profile affects the Maillard reaction. Therefore in the present study we compared solid–state glycation through Maillard reaction between MH and CH under similar heating profiles.

First, temperature changes of WP–glucose powder during MH were monitored by a thermocouple directly inserted into the sample powder (Fig. 1). Temperature of the powder was reached to 60, 75 and 90 °C in 10 min, where microwave output power was 210, 240 and 270 W, respectively. To obtain the similar heating profiles in CH, each temperature come–up profile of MH was divided to 4 or 5 parts and linear regression was made for each part (not shown). The CH was performed using a thermoblock, which was programmed to fit each linear regression. WP glycated after MH and CH for 10 min were used to compare the glycation ratio between MH and CH.

It was reported that glycation induced the increase in the molecular weight of protein (Christine *et al.*, 2006). Therefore WP glycated by MH or CH for 10 min were subjected to SDS–PAGE analysis (Fig. 2). Two major bands having presumed molecular weights of 19 and 14 kDa were detected. At 60 and 75 °C, both MH and CH appeared to give no effect on electrophoretic mobility of these bands, suggesting that these treatment conditions were not enough to induce glycation at the detectable level in SDS–PAGE analysis. At 90 °C, both MH and CH increased molecular weight of WP, and the degree of the increase by MH and CH appeared to be similar. These suggest that glycation rate of WP by MH is similar to that by CH.

It is known that primary amino groups show high reactivity in glycation through Maillard reaction. OPA reacts with primary amines in the presence of

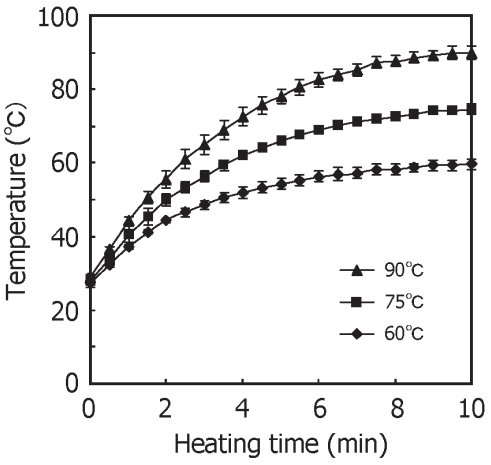

Fig. 1. Heating profiles of lyophilized WP–glucose mixture during MH.

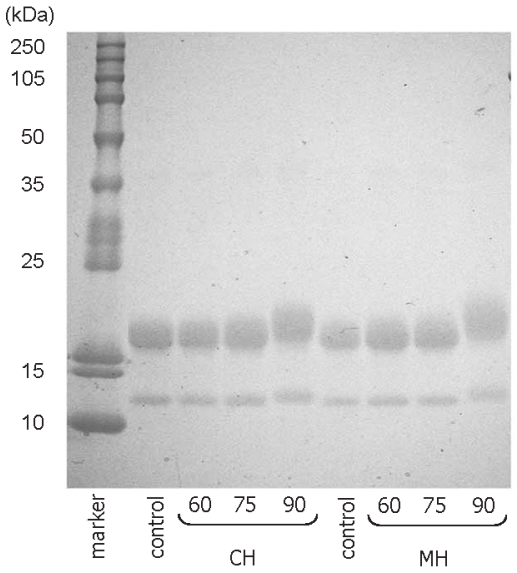

Fig. 2. SDS–PAGE of WP glycated by MH and CH at 60, 75 and 90 °C for 10 min.

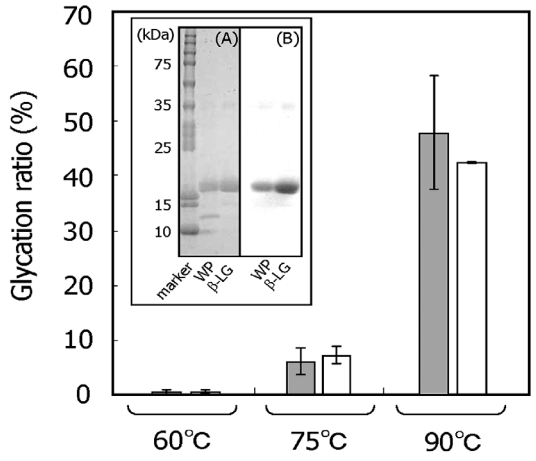

Fig. 3. Glycation ratio of β–LG after MH (gray) or CH (white) for 10 min. SDS–PAGE (A) and western blotting (B) of WP and purified β–LG.

β–mercaptoethanol and SDS (Simons *et al.*, 1976). Beta–LG is the dominant protein of WP and it is largely responsible for the physicochemical characteristics of WP, contributing to solubility, gelation, foaming, emulsification and flavour binding (Castaño *et al.*, 2005). To obtain further quantitative data of glycation levels of WP after MH and CH, we determined glycated primary amino groups of β–LG using OPA. Separation of β–LG from WP was validated by both SDS–PAGE and following western blot analysis using anti β–LG polyclonal antibody as a primary antibody. A band having a molecular weight of about 19 kDa was observed in SDS–PAGE after the separation procedure (Fig. 3A), and that reacted with anti β–LG polyclonal antibody in western blotting (Fig. 3B). This indicated the success of β–LG separation. Fig. 3 shows the glycation ratio of WP after MH and CH at 60, 75 and 90°C for 10 min. Both MH and CH at 60°C induced about 0.5% of glycation ratio. At 75°C, MH and CH induced about 6 and 7% of glycation ratio, respectively. At 90°C, MH and CH caused about 48 and 43% of glycation ratio, respectively. No significant difference ($p>0.05$) was observed between the MH and CH at all temperatures tested. These results suggest that MH has similar effect on glycation of β–LG compared to CH at the tested heating conditions. This corresponded with the results of SDS–PAGE.

In the present study, we compared the effects of MH and CH on the rate of solid–state glycation of WP under same heating profiles. MH has similar effect on glycation rate of WP to CH, suggesting that MH has no non–thermal effect on the rate of solid–state glycation of WP. The possible parameters affecting the rate of solid–state Maillard reaction include the kind of protein and heating rate in addition to the wet or dry heating.

Further accumulation of information is required for determining whether MH has some effect on the glycation.

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