# AGRICULTURAL AND FOOD CHEMISTRY

# Influence of a Rare Sugar, D-Psicose, on the Physicochemical and Functional Properties of an Aerated Food System Containing Egg Albumen

Yuanxia Sun,\* Shigeru Hayakawa, Masahiro Ogawa, Kazuhiro Fukada, and Ken Izumori

Department of Biochemistry and Food Science, Kagawa University, 2393 Ikenobe, Miki, Kagawa 761-0795, Japan

p-Psicose (Psi) might be an ideal sucrose (Suc) substitute for food products due to its sweet taste, easy processing, and functional properties (noncaloric and low glycemic response). In the present study, the effects of Psi on foaming properties of egg white (EW) protein and the quality of butter cookies were analyzed to find a better use of Psi in aerated food systems. The results showed that Psi could improve the foaming properties of EW protein with increasing whipping time in comparison to Suc and p-fructose (Fru). The addition of Psi to butter cookies, as partial replacement of Suc, had no influence on the cook loss while significantly contributing to a color change of the cookie crust through a nonenzymatic browning reaction. Furthermore, Psi-containing cookies possessed the highest antioxidant capacity in all tested cookies using two assays of radical scavenging activity and ferric reducing power. It was found that there was a close correlation between the crust color and the antioxidant activity of the cookie. The results suggest that the addition of Psi enhanced the browning reaction during cookie processing and, consequently, produced a strong antioxidant activity.

KEYWORDS: D-Psicose; egg white; foaming property; antioxidant activity; butter cookie

## INTRODUCTION

Hen eggs provide many desirable attributes as an ingredient in food products. They contribute not only a high nutritional quality of their proteins but also multifunctional properties, such as gelling, foaming, emulsification, color, and flavor (1). One of the typical functional properties is foaming, and egg white (EW) proteins with high foaming capacity are extensively used in aerated food systems to provide a range of unique textures that are associated with many foods including cake, nougat, whipped cream, and chocolate mousse (2).

Sucrose (Suc) is a principal ingredient in aerated foods. Besides its contribution to product flavor, Suc also contributes to foaming properties. During the making of foam, the presence of sugar enhances the foam stability by increasing the viscosity of lamellar water and thereby retarding liquid drainage (3). In food products such as sponge cake, Suc contributes to bulk and volume, and the reduction of its levels in a cake system affects structural and sensory properties (4). However, the excessive consumption of Suc can be ill-advised because of the high calorie content and a high glycemic response. On the other hand, artificial intense sweeteners (such as aspartame, sucralose, saccharin, and cyclamate) are almost calorie-free, but their function is only to sweeten and inherently lack the bulk of Suc. Food formulators generally need to blend them with sugar to obtain successful end products. Hence, Suc cannot be substituted by only intense sweeteners.

As expected, an ideal Suc substitute would retain Suc's clean taste and valuable functional properties. D-Psicose (Psi), a noncalorie ketohexose rare sugar with a lower glycemic response (5, 6), could help food processors meet these requirements. Psi has 70% of the sweetness of Suc and has a higher solubility that makes it easy to use for food processing. It has been reported that the addition of Psi in food products improved the gelling behavior and produced good flavor and high antioxidative substances, namely, Maillard reaction products (MRPs) (7, 8). Furthermore, food products containing Psi maintained a high level of antioxidant effect over a long period of storage, which was able to delay the onset of lipid autoxidation and extend the food storage time (8). In summary, Psi could give proper sweetness, smooth texture, desirable mouthfeel, and great shelf stability to food products. All of these characteristics would qualify Psi as an invaluable complement to Suc and artificial intense sweeteners.

In our previous studies, it was reported that the addition of rare sugars in food products formed a considerable amount of antioxidant substances (MRPs), which showed strong radical scavenging activity and reducing power (7, 9). These MRP functional compounds are currently gaining a lot of attention. Many studies have reported that there was a high antioxidant capacity of MRPs in model foods, such as coffee (10) and bakery products (11). It was indicated that the MRPs may offer

<sup>\*</sup> Corresponding author (fax +81-87-891-3021; e-mail syx0430@ hotmail.com).

substantial health-promoting activity as they can act as reducing agents, metal chelators, and radical scavengers (12-14). Recently, we have also focused on one way to achieve a healthy food product by replacing Suc with Psi in food processing (8). A functional food with a low calorie content and high antioxidant properties would offset the degenerative changes of aging and may help prevent diseases related to lifestyle (15).

At present, however, it is not clear whether the addition of Psi as a Suc substitute would affect the foaming properties of protein and the quality of aerated food products. In this study, the objective was to investigate the flow behavior and foaming properties of EW solutions in the presence of Psi in comparison to Suc and Fru. Furthermore, the effects of Psi, as a partial substitute for Suc, on the color of cookie crust, cook loss, and antioxidant activity were investigated in a model butter cookie, as a function of baking time, at the two temperatures of 130 and 150 °C. The relationship between the color change of the crusts of cookies and their antioxidant activity was also analyzed to give insight into the functional ingredient changes taking place in food processing.

#### MATERIALS AND METHODS

**Chemicals.** D-Psicose was provided by Kagawa Rare Sugar Research Center (Takamatsu, Japan). Potassium ferricyanide  $[K_3Fe(SCN)_6]$  was purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan). 1,1-Diphenyl-2-picrylhydrazyl (DPPH) was purchased from Nacalai Tesque, Inc. (Kyoto, Japan). Fresh egg, butter, wheat flour, Fru, and Suc were obtained from a local supermarket. All reagents used were of analytical reagent grade.

**Determination of Viscosity.** The EW solution was separated from the egg yolk, and the chalazae were removed. The EW solution was gently homogenized and freeze-dried. The viscosity of the EW solution (5 wt % of EW powder in distilled water) containing different sugars was determined at  $25 \pm 0.1$  °C using a rotational viscometer (TV-20, Toki Sangyo Co., Tokyo, Japan), which was based on torque measurements when a cone plate (1°  $34' \times R 24$  mm) rotates in a container. Viscosity ( $\tau$ ) was recorded at a rotational speed ( $\gamma$ ) ranging from 0.3 to 60 rpm. The measurement errors were within 5%. A power law model [ $\tau = K(\gamma)^{-n}$ ] was used to analyze the flow curves, where *K* and *n* are fluid consistency and flow behavior index, respectively.

**Determination of Foaming Properties.** The EW powder (5 wt %) and sugar (15 wt %) were dissolved in distilled water, and the mixed solutions were allowed to stand for about 2 h at room temperature to ensure dissolution of constituents. After that, the sugar-EW solution (200 mL) was whipped at ambient temperature using a KitchenAid mixer (KSM5, FMI Co., Osaka, Japan) in a bowl with rotating beaters at rotational speed setting 8 (230 rpm).

Foaming capacity was characterized using the increase in the volume of foam, foam overrun, according to the method described by Phillips et al. (*16*, *17*). Foam was gently scooped from the bowl with a rubber spatula, and a preweighed polypropylene vessel (110 mL) was carefully filled with the foam. This phase of the procedure was limited to <2 min. The overrun was calculated by the equation

overrun (%) = 
$$[(W_s - W_f)/W_f] \times 100$$
 (1)

where  $W_s$  is the weight (g) of the unwhipped protein solution and  $W_f$  is the weight (g) of the whipped protein foam.

To measure foam stability, a hole ( $\varphi = 3 \text{ mm}$ ) was drilled in the bottom edge of the polypropylene vessel (110 mL), and the hole was sealed with sticky tape before the vessel was filled with foam sample. This modified vessel was carefully filled with foam sample and quickly weighed, and then the vessel was placed above a plastic container at about a 30° angle. The hole was always the lowest point. The tape was removed, and the liquid generated from the foam sample was collected in the container. The liquid in the container was weighed at regular intervals during the time course of the experiment. Foam stability was evaluated using the rate of liquid drainage rate (percent) for a specified amount of time according to the modified method of Phillips et al. (*16, 17*).

drainage (%) = 
$$(W_d/W_f) \times 100$$
 (2)

 $W_d$  is the weight (g) of the drained liquid, and  $W_f$  is the weight (g) of the whipped protein foam.

**Preparation of Model Butter Cookie and Its Extract.** The control butter cookie (Ct-cookie) was prepared according to the following recipe: 20 g of wheat flour, 10 g of butter, 55 g of egg solution, and 15 g of Suc. The sample butter cookies (Psi- and Fru-cookies) were prepared by replacing 20% of Suc with Psi and Fru, respectively. The fresh egg solution and sugar were weighed directly into a mixing bowl and whipped to form the well-emulsion foam. The wheat flour was added and mixed, and then the premelted butter was added. Portions of 15 g of the pastry were rolled out to cake ware (diameter 8 cm) after which trays of these wares were baked in an electric baking oven (Nichiwa Co.) at 130 and 150 °C, respectively. Cookies were produced from each recipe by baking for 10-30 at 5 min intervals.

For the determination of the antioxidant activity of the samples, 15 g of pastry and a cookie baked from 15 g of pastry were suspended in 30 mL of 75% ethanol and homogenized using a Polytron (PT10-35, Kinematica AG). The slurry sample obtained was then cooled at 4 °C for 1 h to ensure good phase separation and then centrifuged at 12000 rpm for 10 min at 4 °C. Finally, the ethanolic cookie extract was filtered through a paper filter. The obtained extracts were stored at 4 °C for not more than 15 h in hermetically sealed containers. Storage exceeding 24 h led to a significant loss of antioxidant activity.

Antioxidant Activity Determination. Scavenging Activity of the DPPH Free Radical. Each of the 2 mL samples was mixed with 0.5 mL of a 1 mM DPPH radical solution in 99.5% methanol. The mixture was shaken vigorously and allowed to stand at room temperature for 30 min, and then the absorbance was measured at 517 nm (18). For all experiments, a 75% ethanol solution instead of sample solution was used as a control. The DPPH radical scavenging effect was calculated as

scavenging effect (%) =

$$[(OD_{517\text{control}} - OD_{517\text{sample}})/(OD_{517\text{control}})] \times 100 \quad (3)$$

*Ferric Reducing Antioxidative Power.* The reducing power of sample was determined according to the Oyaizu method (*19*). The sample (1 mL) was mixed with a sodium phosphate buffer (1 mL, 0.2 M, pH 6.6) and potassium ferricyanide (1 mL, 1.0%). The mixed solution was incubated at 50 °C for 20 min. After trichloroacetic acid (1 mL, 10%) was added, the mixed solution was centrifuged at 10000 rpm for 5 min. The resulting supernatant (2.5 mL) was mixed with distilled water (2.5 mL) and ferric chloride (0.5 mL, 0.1%), and the absorbance was measured at 700 nm.

**Color and Cook Loss Measurement.** Color measurements were carried out using a colorimeter (ND-300A, Nippon Denshoku Ind. Co., Japan) as defined by the Commission Internationale de l'Eclairage (20). The colorimeter was standardized with a white plate, and the values of  $L^*$  (luminosity) and  $a^*$  and  $b^*$  (chromaticity coordinates) were recorded (21). For each treatment, three cookies were measured, and four readings were taken on each test with rotation of 90°. Color change ( $\Delta E^*$ ) was calculated for the samples in comparison to the control cookie using the following equation (22):

$$\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$$
(4)

Cook loss of cookies was defined by the percent weight loss after baking, and the percent weight loss was then calculated using the equation

cook loss (%) = 
$$[(W_p - W_c)/W_p] \times 100$$
 (5)

where  $W_p$  is the pastry weight (g) and  $W_c$  is the cookie weight (g) after baking for different times.

**Statistical Analysis.** Data from this study are reported as the mean and standard deviation for at least three independent replicates for each sample. Differences between samples were statistically evaluated by means of Student's *t* test. A two-tailed *p* value lower than 0.05 was considered to be significant. Correlation was expressed with the  $R^2$  coefficient of the correlation.



Figure 1. Effect of D-psicose concentration (wt %) on flow curves of viscosity versus rotational speed at 25 °C for 5 wt % EW solution.

**Table 1.** Effect of Different Sugars on the Consistency (K) and Flow

 Behavior Index (n) of EW Solutions at the Different Sugar Contents

|        | sugar content (%) | K <sup>a</sup>                       | п     | R <sup>2</sup> |
|--------|-------------------|--------------------------------------|-------|----------------|
| Ct-EW  | 0                 | $304.59 \pm 10.32a$                  | 0.952 | 0.999          |
| Suc-EW | 15                | $318.52\pm4.94\mathrm{b}$            | 0.924 | 0.999          |
| Fru-EW | 15                | $317.02 \pm 2.56b$                   | 0.919 | 0.999          |
| Psi-EW | 15                | $314.72 \pm \mathbf{4.74b}$          | 0.916 | 0.998          |
| Suc-EW | 30                | $337.78\pm5.08\mathrm{c}$            | 0.914 | 0.999          |
| Fru-EW | 30                | $343.61 \pm 6.95 c$                  | 0.915 | 0.999          |
| Psi-EW | 30                | $\textbf{324.67} \pm \textbf{4.35d}$ | 0.906 | 0.999          |
| Suc-EW | 45                | $378.13 \pm 4.11e$                   | 0.892 | 0.998          |
| Fru-EW | 45                | $\textbf{383.60} \pm \textbf{9.21e}$ | 0.904 | 0.999          |
| Psi-EW | 45                | $\textbf{355.14} \pm \textbf{5.82f}$ | 0.875 | 0.999          |
|        |                   |                                      |       |                |

<sup>*a*</sup> K values followed by different letters are significantly different (P < 0.05).

# RESULTS

Viscosity Measurement. The flow behavior of sugar-EW solution influences the foaming capacity and the quality of the final products. First, as an example, the flow curves of Psicontaining EW solutions are depicted at 15, 30, and 45 wt % of Psi concentration as shown in Figure 1. This variation in viscosity was found to be exponential in both EW alone and sugar-EW solutions and exhibited a shear-thinning behavior. Furthermore, the good fits in power law model ( $R^2 > 0.99$ ) for the shear rate dependence of viscosity are shown in **Figure 1**, and the values of fluid consistency (K) and flow behavior index (n) are listed in **Table 1**. The n values of sugar-EW solutions were lower than those of Ct-EW solution, which indicated a decreasing trend with increasing sugar concentration, suggesting that the shear-thinning behavior became less pronounced with the addition of sugar. As expected, the increase in K values with increasing sugar concentration was observed in all sugar-EW solutions tested. At 15 wt % of sugar content, the viscosity of Psi-EW was similar to that of Suc- and Fru-EW solutions. but the former was lower than the latter at 30 and 45 wt %, suggesting that the effect of sugars on the fluid consistency of protein solution had a concentration dependency.

**Foaming Properties.** The EW protein foams are the basis for the production of a variety of flour confectionery and other aerated foods. For all of these applications, the protein foam must first obtain a desired level of air phase volume (foaming ability) and then maintain foam stability when subjected to a variety of processes. In this study, the EW solutions were subjected to whipping for 5, 10, and 15 min in the presence of different sugars. Compared to the foams from EW solution alone



**Figure 2.** Overrun (%) versus whipping time for 5 wt % EW alone and with three added sugars (Suc, sucrose; Fru, D-fructose; Psi, D-psicose) at 15 wt % of concentration.



Figure 3. Drainage rate patterns of EW protein dispersions (5 wt %) at different whipping times.

(Ct-EW), the foams from all sugar-EW solutions were smooth, showing smaller bubble size and less sensitivity to foam collapse.

Moreover, their foaming ability against overrun was investigated and is shown in Figure 2. The overrun values of foams from all sugar-EW solutions were higher than those of from Ct-EW solution, indicating that sugar is very important to foaming capacity. On the other hand, the addition of different sugars also affected the foaming capacity of EW proteins, and this effect was strongly dependent on whipping times. The foaming ability for Psi-EW solution kept increasing as the whipping time was extended to 15 min; by contrast, for Suc-EW solution, foaming ability reached a maximum at 10 min of whipping, after which time the overrun significantly decreased (p < 0.05). As for the Fru-EW solution, the overrun did not show a significant change between 10 and 15 min of whipping. In summary, the longer whipping times had a negative impact on foaming ability for Ct-EW and Suc-EW solutions, whereas the Fru-EW appeared to be fairly unaffected. Conversely, the addition of Psi to EW solution significantly improved foaming ability beyond 10 min of whipping (p < 0.05).

The foam stability of the Ct-EW solution as a model was measured using the rates of liquid drainage (percent) at an interval of 2.5 min for 60 min (**Figure 3**). The results showed that the drainage (percent) for Ct-EW foam significantly increased with the increase of whipping time, indicating that the EW foam easily collapsed due to longer whipping time.



Figure 4. Effect of three sugars on foam stability of EW solution (5 wt %) following 10 min of drainage.

There was a linear relationship of drainage against standing time in the range of 0-15 min, and then the drainage increased slowly beyond 20 min and finally reached a plateau. From this result, a specified amount of standing time (10 min) was selected as a function of time point for measuring the drainage (percent) to compare foam stabilities of different sugar-EW solutions.

**Figure 4** shows the effect of sugar on foam stability of EW solution. The results indicate that the sugar-EW samples were far more stable compared to the EW solution, especially for reducing sugar-containing EW solutions. In the sugar-EW solutions, Suc- and Fru-EW samples reached the maximum foam stability at 10 min of whipping and the longer whipping times resulted in a stability decline. The Psi-EW sample was found to have a foam stability similar to that of Fru-EW at 10 min of whipping, but the Psi-EW sample remained a very stable foam at 15 min of whipping. These results indicated that the addition of Psi could either keep good foam stability or significantly improve foaming ability of EW protein solution by controlling the whipping time.

**Color Properties and Cook Loss.** The Ct-cookie (100% Suc) and Fru- and Psi-cookies (Fru and Psi replaced 20% of the Suc) were prepared at 130 and 150 °C, respectively. To evaluate the color change of the cookie crust, the colorimetric values of  $L^*$ (black-white component, luminosity),  $a^*$  (+, red; -, green component), and  $b^*$  (+, yellow; -, blue component) were measured, and the results are shown in Figure 5. The instrumental analysis of color showed a notable decrease in the  $L^*$ value with the increase of baking time for all cookies, and the L\* value of cookies prepared at 150 °C was lower than that of those prepared at 130 °C (Figure 5a,b). As baking time increased, the L\* values of the Fru- and Psi-cookies decreased away from those of the Ct-cookies. Similarly, the cookies containing reducing sugars (Fru and Psi) showed lower b\* values under all baking conditions (Figure 5c,d). These results expose a remarkable loss in lightness and yellowness; that is, the Psiand Fru-cookies are more "brown" in overall color. Although the  $L^*$  and  $b^*$  values showed a drastic decrease in the cookie containing reducing sugars, Fru- and Psi-cookies were not very different from each other in luminosity and yellowness, and the color of both cookies was on an acceptable level in appearance under the tested conditions.

Conversely, the  $a^*$  value tended to increase with baking time, although this increase was less pronounced beyond 20 min of baking time (**Figure 5e,f**). This might be explained by the fact that the color of the cookies was browner as the baking time increased, and the prominent brown in cookie masked the red component. Furthermore, by comparison of the three cookies, the Psi-cookie showed the highest redness as shown in the  $a^*$  values, and it was exhibited in the following order: Psi- > Fru- > Ct-cookies. It was inferred that differences detected in the color of cookie crust could be related to the fact that the reducing sugars, especially Psi, were able to significantly promote browning reactions during heat treatment.

As moisture is lost during cooking, cookie quality, such as flavor, tenderness, and texture, can be negatively affected. **Figure 6** shows the changes of cook loss for different cookies prepared at two baking temperatures. The results note that raising the baking temperature and prolonging the baking time significantly increased the cook loss. However, no difference in cook loss was found among three cookies under each baking condition tested. In addition, there were no differences in the thickness and diameter of each cookie (data not shown). Therefore, the partial addition of Psi as substitute of Suc did not influence the cook loss and the volume of cookies.

Antioxidative Activity. Many studies have demonstrated that the browning reaction products possessed very high antioxidant activity (23). In this study, each pastry (15 g) and cookie prepared from the same pastry at different baking times and temperatures were extracted, and their antioxidant activities were measured. The results indicated that the antioxidant activity of the pastries containing different sugars showed no differences and was very low (data not shown). In the cases of short baking times (10 and 15 min), similar but low antioxidant activities were observed in cookies prepared at 130 °C (Figure 7a,c).

Furthermore, the cookies, especially Fru- and Psi-cookies, exhibited stronger antiradical activity and reducing power with baking time. As shown in panels **a** and **b** of **Figure 7**, when the baking time increased to 30 min, the radical scavenging activity of the Psi-cookie extract remarkably increased to 25.7 and 69.0%, whereas that of the Fru-cookie extract increased to 12.8 and 46.3% at 130 and 150 °C, respectively. However, the antiradical capacity of the extract from Ct-cookies showed a very low value (about 5%) at 130 °C and almost no increase with an increase of baking time. In the case of baking at 150 °C, there was a slight increase in the antiradical activity of Ct-cookies with an increase in the duration of baking, reaching 17.1% when baked for 30 min.

Similar patterns were found in the reducing power of cookies (**Figure 7c,d**). In the case of baking at 130 °C for 30 min, the reducing power values of Ct-, Fru-, and Psi-cookies were 0.32, 0.66, and 1.1, respectively. At 150 °C for 30 min, their values were 0.53, 1.39, and 2.13, respectively. The results also suggested that the antioxidant activity was much more pronounced at high baking temperature, and the degree of antioxidant activity significantly depended on the type of sugar. An explanation for the findings could be that reducing sugars, especially Psi, reacted favorably with free amino group through the MR; however, in the case of Suc, a certain heating and reaction time were needed to break the bond between Glc and Fru, releasing the two reducing sugars, which would then cause the MR.

#### DISCUSSION

Several studies have shown that Suc improves the foam stability of protein solutions (3, 24). This was partly explained as being due to the increased viscosity of the air bulk phase and the delayed thinning of lamellae around the bubbles. Our results indicate that sugar-EW solutions showed a higher consistency index of viscosity than the EW solution alone. This may be one of the reasons the foam properties of EW protein



Figure 5. Variation of color parameters for L\* value (a, b), a\* value (c, d), and b\* value (e, f) of cookie crust: left, baking at 130 °C; right, baking at 150 °C.



Figure 6. Variation of cook loss versus baking time for butter cookies prepared at 130 °C (solid symbols) and 150 °C (open symbols), respectively.

solution were significantly improved by the addition of sugars. However, it was found that both foaming ability and foam stability of the Suc-EW solution were curtailed compared to the Psi-EW solution. The Psi-EW solution, unlike the Suc-EW solution, did not show a remarkable decrease in foaming ability and foam stability with an increase in whipping time (**Figures 2** and **4**). These results showed that enhancement of foaming properties was not simply accompanied by the differences in viscosity among three foam-forming solutions at 15 wt % of sugar content, implying that viscosity was not a determining factor in the increased foam capacity of EW solutions containing different sugars.

The above results did not show a clear mechanism by which addition of Psi induced the high foaming property of EW protein, but confirmed that the addition of Psi was much more effective than the addition of Suc, especially in the case of a longer whipping. In general, egg white proteins act as amphiphilic emulsifiers between the air and the aqueous phase to stabilize the foam. The quality of protein-based foams depends largely on the conformational characteristics of proteins. In a previous paper (7), it was inferred that, first, Psi might reduce the water activity and make water-protein interactions less effective, to easily cause the partial unfolding of protein; and, second, Psi might interact directly with the protein through hydrogen bonding (H-bonding) and lead to a change in the protein surface hydrophobicity. Consequently, the presence of Psi could enhance hydrophobic interaction on the surface of protein, and such surface hydrophobicity might be increased further due to the input of higher amounts of mechanical energy with increasing whipping time. The protein structure with both hydrophobic and hydrophilic groups on the surface and good flexibility is needed for a protein to generate good foams. Hence, the partial unfolding of these globular proteins in the presence of Psi could expose more hydrophobic groups on the surface and increase their amphiphilic nature and flexibility, so that their foaming properties could be improved.

Lopez de la Paz and others (25) have used NMR and IR to study how the orientation of hydroxyl (OH) groups in carbohydrate derivatives influences H-bond cooperativity in the molecule. It was reported that for the six-carbon D-sugars, the different isomeric forms have different water H-bonding networks using a computational approach (26). Furthermore, Furuki (27) investigated the effect of stereochemistry on the antifreeze characteristics of aqueous solutions of hexoses and pentoses. Of these monosaccharides, a rare sugar, Psi, presented a higher unfrozen water value as compared to other ketohexoses. It has been supposed that more water molecules unfrozen in the aqueous solutions of carbohydrates indicate poorer compatibility



Figure 7. Variation of the DPPH radical scavenging activity ( $\mathbf{a}$ ,  $\mathbf{b}$ ) and reducing power ( $\mathbf{c}$ ,  $\mathbf{d}$ ) as a function of baking time for ethanolic cookie extracts: left, baking at 130 °C; right, baking at 150 °C.



Figure 8. Relationships between between radical scavenging activity and color change  $\Delta E^*$  (**a**, **b**) and between reducing power and color change  $\Delta E^*$  (**c**, **d**) generated from Ct-, Fru-, and Psi-cookie model systems: left, baking at 130 °C; right, baking at 150 °C.

with the three-dimensional H-bonding network of ice, suggesting reduced water activity in the Psi solution. These studies proved that although the sugar molecules had the same chemical formula and the same functional groups, the arrangement of water molecules is quite different around the different isomeric forms of sugars. Comparison of the effects of Psi to thsoe of Fru, in which two ketohexoses differ only in the position of the OH group on the third carbon, on the flow behavior and foaming properties of EW solution showed that the effects were very different. Hence, the isomeric-dependent water activities could be an important reason for the difference of functional properties in sugar—protein solutions.

Baking is a complex process that brings about a series of physical, chemical, and biochemical changes in a product such as volume expansion, evaporation of water, formation of a porous structure, crust formation, and browning reaction. Our study above revealed that the addition of Psi in a butter cookie as partial substitute for Suc did not influence the cook loss and volume while significantly enhancing nonenzymatic browning reaction. Although the crust color of the Psi-cookie showed less luminosity than that of the Ct-cookie, its color change was similar to that of the Fru-cookie, and their crust color could be visually acceptable. These results suggested that Psi might be an ideal replacement for Suc because it can reduce the calorie content of the final products without affecting the quality and it is suitable for diabetics and weight-conscious people.

We have previously analyzed the relationship between browning and antioxidant activity of the MRPs generated from the glycated EW with different reducing sugars (28). Here, we extended the study to butter cookies including sugar and egg protein as a model food product. The above results showed that as the baking time was increased, the  $L^*$  and  $b^*$  values decreased and the  $a^*$  value increased. These color changes ( $\Delta E^*$ ) are further calculated from the three parameters based on eq 5. The

#### Functional Properties of Psi-cookie

results showed the  $\Delta E^*$  values also varied largely, ranging from 0 to 20, for butter cookies baked from 10 to 30 min under two baking temperatures (Figure 8). There was a linear relationship between the  $\Delta E^*$  value and antioxidant activity; the coefficients of correlation were 0.87 and 0.93 for radical scavenging activity and 0.80 and 0.89 for reducing power when baked at 130 and 150 °C, respectively. Some pigments in Maillard browning products, which were formed during the heating phase in the presence of sugar, contribute substantially to the color change of cookies. These browning products possessed a high antiradical activity and reducing power. Consequently, the color change of the cookie showed a strong influence on the antioxidant activity of the cookies. This is in agreement with the earlier studies by Woffenden et al. (29), who reported a positive correlation between color and antioxidant properties in foods in which the formation of antioxidant MRPs is the prevalent event during processing.

In this study, we used two assays to measure the antioxidant activity of butter cookies, and the Psi-containing cookie showed the strongest antioxidant activity in all tested cookies. However, Psi-containing pastry, like other pastries, failed to show any antioxidant activity, suggesting that the strong antioxidant activity of Psi-cookie was produced during baking rather than addition of Psi itself. In our previous study, an excellent antioxidant activity could be obtained in custard pudding gels generated from milk/egg protein and Psi by heating in the range from 80 to 95 °C (7). Sumaya-Martinez et al. (30) pointed out on a ribose-tuna stomach hydrolysate model system that sugar caramelization could also contribute to the antiradical activity measured by DPPH test and browning reactions at high temperatures. There is substantial evidence suggesting that antioxidative food components suppress oxidative stress and have potential health benefits in preventing aging and diseases related to lifestyle (14, 31). In addition, the antioxidant activity of MRPs can result in retarding lipid autoxidation and prevent fat spoilage and consequently increase the stability and shelf life of food products (32, 33). Further work on the characterization of antioxidant compounds in Psi-added bakery products is in progress to elucidate their physiological effects.

In conclusion, Psi-EW solution in flow consistency was similar to Fru- and Suc-EW solutions at 15% of sugar content, and its fluid consistency had a decreasing tendency with increasing sugar content. When the samples were subjected to longer whipping time, the Psi-EW solution was found to be better in foaming capacity compared to Suc- and Fru-EW solutions. Furthermore, the addition of Psi as partial replacement of Suc did not affect the quality of cookies. Conversely, it dramatically increased antioxidant substances produced through the MR during heat processing. The antiradical activity and reducing power of the cookies are strongly related to the color change of the cookie crust. Thus, Psi could be used as a sweetener to develop a functional food with a high antioxidant activity and a low calorie content by controlling the color change of the final products.

### ACKNOWLEDGMENT

We are very grateful to H. Oshima, Food Research Branch of Kagawa Industrial Technology Center, Japan, for performing the color measurement and to Peter G. Lutes, Kagawa University, for helpful discussions.

# LITERATURE CITED

- Mine, Y. Recent advances in the understanding of egg white protein functionality. <u>*Trends Food Sci. Technol.*</u> 1995, 6, 225– 232.
- (2) Campbell, G. M.; Mougeot, E. Creation and characterization of aerated food products. <u>*Trends Food Sci. Technol.*</u> 1999, 10, 283– 296.
- (3) Lau, C. K.; Dickinson, E. Instability and structural change in an aerated system containing egg albumen and invert sugar. *Food Hydrocolloids* 2005, 19, 111–121.
- (4) Frye, A. M.; Setser, C. S. Optimizing texture of reduced-calorie sponge cakes. *Cereal Chem.* 1991, 69, 338–343.
- (5) Matsuo, T.; Suzuki, H.; Hashiguchi, M.; Izumori, K. D-Psicose is a rare sugar that provides no energy to growing rats. <u>J. Nutr. Sci.</u> <u>Vitaminol.</u> 2002, 48, 77–80.
- (6) Matsuo, T. Inhibitory effect of D-psicose on glycemic responses after oral carbohydrate tolerance test in rats. J. Jpn Soc. Nutr. Food Sci. 2006, 59, 191–121.
- (7) Sun, Y.; Hayakawa, S.; Jiang, H.; Ogawa, M.; Izumori, K. Rheological characteristics of heat-induced custard pudding gels with high antioxidative activity. *Biosci., Biotechnol., Biochem.* 2006, 70, 2859–2867.
- (8) Sun, Y.; Hayakawa, S.; Ogawa, M.; Izumori, K. Antioxidant properties of custard pudding dessert containing rare hexose, D-psicose. *Food Control* 2007, *18*, 220–227.
- (9) Sun, Y.; Hayakawa, S.; Puangmanee, S.; Izumori, K. Chemical properties and antioxidative activity of glycated α-lactalbumin with a rare sugar, D-allose, by Maillard reaction. *Food Chem.* 2006, 95, 509–517.
- (10) Del Castillo, M. D.; Ames, J. M.; Gordon, M. H. Effect of roasting on the antioxidant activity of coffee brews. <u>J. Agric. Food Chem</u>. 2002, 50, 3698–3703.
- (11) Borrelli, R. C.; Mennella, C.; Barba, F.; Russo, G. L.; Krome, K.; Erbersdobler, H. F.; Faist, V.; Fogliano, V. Characterization of coloured compounds obtained by enzymatic extraction of bakery products. *Food Chem. Toxicol.* **2003**, *41*, 1367–1374.
- (12) Hayase, F.; Shibuya, T.; Sato, J.; Yamamoto, M. Effects of oxygen and transition metals on the advanced Maillard reaction of proteins with glucose. <u>*Biosci., Biotechnol., Biochem.*</u> **1996**, *60*, 1820–1825.
- (13) Wijewickreme, A. N.; Kitts, D. D. Metal chelating and antioxidant activity of model Maillard reaction products. <u>Adv. Exp. Med. Biol</u>. 1998, 434, 245–254.
- (14) Monti, S. M.; Ritieni, A.; Graziani, G.; Randazzo, G.; Mannina, L.; Segre, A. L.; Fogliano, V. LC/MS analysis and antioxidative efficiency of Maillard reaction products from a lactose-lysine model system. *J. Agric. Food Chem.* **1999**, *47*, 1506–1513.
- (15) Harman, D. Free radical involvement in aging, pathophysiology and therapeutic implications. <u>*Drugs Aging*</u> 1993, *3*, 60–80.
- (16) Phillips, L. G.; Haque, Z.; Kinsella, J. E. A method for the measurement of foam formation and stability. <u>J. Food Sci.</u> 1987, 52, 1074–1077.
- (17) Phillips, L. G.; German, J. B.; O'Neill, T. E.; Foegeding, E. A.; Harwalkar, V. R.; Kilara, A.; Lewis, B. A.; Mangino, M. E.; Morr, S. V.; Regenstein, J. M.; Smith, D. M.; Kinsella, J. E. A standardized procedure for measuring foaming properties of three proteins. <u>J. Food Sci.</u> 1990, 55, 1441–1444.
- (18) Shimada, K.; Fujikawa, K.; Yahara, K.; Nakamura, T. Antioxidative properties of xanthan on the antioxidation of soybean oil in cyclodextrin emulsion. <u>J. Agric. Food Chem</u>. **1992**, 40, 945– 948.
- (19) Oyaizu, M. Studies on products of browning reactions: antioxidative activities of products of browning reaction prepared from glucosamine. *Jpn. J. Nutr.* **1986**, *44*, 307–315.
- (20) *CIE Publication 15.2. Colorimetry*, 2nd ed.; CIE Central Bureau Kegelgasse: Vienna, Austria, 1986; p 27.
- (21) Felipe, A.; Artigas, J. M. Limitations, anomalies and particularities of the CIELAB uniform color system. <u>Opt. Pura Apl.</u> 1986, 19, 173–178.
- (22) Hutchings, J. B. *Food Coloring and Appearance*; Blackie Academic and Professional: Glasgow, U.K., 1994; pp 199-237.

- (23) Nicoli, M. C.; Anese, M.; Parpinel, M. T.; Franceschi, S.; Lerici, C. R. Loss and /or formation of antioxidants during food processing and storage. <u>*Cancer Lett.*</u> **1997**, *114*, 71–74.
- (24) Halling, P. J. Protein-stabilized foam and emulsions. <u>CRC Crit.</u> <u>Rev. Food Sci. Nutr</u>, 1981, 15, 155–203.
- (25) Lopez de la Paz, M.; Ellis, G.; Perez, M.; Perkins, J.; Jiminez-Barbero, J.; Vicent, C. Carbohydrate H-bonding cooperativityintramolecular hydrogen bonds and their cooperative effect on intermolecular processes-binding to hydrogen-bond acceptor molecule. *Eur. J. Org. Chem.* **2002**, 845–855.
- (26) Dashnau, J. L.; Vanderkooi, J. M. Computational approaches to investigate how biological macromolecules can be protected in extreme conditions. *J. Food Sci.* 2007, 72, R1–R10.
- (27) Furuki, T. Effect of stereochemistry on the anti-freeze characteristics of carbohydrates. A thermal study of aqueous monosaccharides at subzero temperatures. <u>*Carbohydr. Res.*</u> 2000, 323, 185–191.
- (28) Sun, Y.; Hayakawa, S.; Izumori, K. Antioxidative activity and gelling rheological properties of dried egg white glycated with a rare ketohexose through the Maillard reaction. *J. Food Sci.* 2004, 69, 427–434.
- (29) Woffenden, H. M.; Ames, J. M.; Chandra, S. Relationships between antioxidant activity, color, and flavor compounds of crystal malt extracts. *J. Agric. Food Chem.* **2001**, *49*, 5524–5530.

- (30) Sumaya-Martinez, M. T.; Thomas, S.; Linard, B.; Binet, A.; Guerard, F. Effect of Maillard reaction conditions on browning and antiradical activity of sugar-tuna stomach hydrolysate model system. *Food Res. Int.* 2005, *38*, 1045–1050.
- (31) Ames, B.; Shigenaga, M.; Hagen, T. Oxidants, antioxidants and the degenerative diseases of aging. *Proc. Natl. Acad. Sci. U.S.A.* 1993, 90, 7915–7922.
- (32) Manzocco, L.; Calligaris, S.; Mastrocola, D.; Nicoli, M. C.; Lerici, C. R. Review of non-enzymatic browning and antioxidant capacity in processed food. <u>*Trends Food Sci. Technol.*</u> 2001, 11, 340–346.
- (33) Bressa, F.; Tesson, N.; Dalla Rosa, M.; Sensidoni, A.; Tubaro, F. Antioxidant effect of Maillard reaction products: application to a butter cookie of a competitive kinetics analysis. *J. Agric. Food Chem.* 1996, 44, 692–695.

Received for review January 8, 2008. Revised manuscript received April 5, 2008. Accepted April 16, 2008. This project was financially supported by the "intellectual cluster" project from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

JF800050D