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学位論文全文に代わる要約
Extended Summary in Lieu of Dissertation

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学位論文題目：
Title of Dissertation
D-Psicose Improves the Functional Properties of Processed Food Containing Egg White
(D-プシコースによる卵加工食品の高機能化)

学位論文要約：
Dissertation Summary

Sugars, *e.g.* sucrose (Suc) and isomerized sugar, are important ingredients in food processing because they function as sweeteners, preservatives, anti-freezing agents, *etc.* However, excessive consumption of sugars leads to a risk of lifestyle-related diseases such as obesity, type-2 diabetes, and stroke. The substitution of sugars with a low-calorie sweetener may reduce the risk of lifestyle-related diseases. Low-calorie sweetener is divided into 2 groups; natural and artificial sweeteners. Consumers prefer natural sweetener to artificial sweetener because they are skeptical about the safety of artificial sweetener and dislike unpleasant aftertaste of some artificial sweeteners. To date, there is an increased demand for using natural sweetener.

Recently, rare sugar D-psicose (Psi) has attracted a great deal of public attention because it has much lower calorie count (approximately 0.39 kcal/g) than D-glucose (Glc) and Suc (approximately 3.8 kcal/g) despite monosaccharide. In addition, Psi shows biological functions, *e.g.* suppressing the elevation of postprandial blood glucose level and reducing body fat accumulation. Psi was approved as generally recognized as safe (GRAS) by the US Food and Drug Administration. Due to the low-calorie count and the biological functions, Psi has high potential to apply to food products. However, the practical application of Psi to food products is not widespread in the world's nations except Japan owing to a low degree of recognition by less information on the benefits of Psi in food

processing properties. The objective of this study was to investigate the effects of Psi on the functional properties of processed foods containing egg white (EW): thermal and non-thermal processed foods. Moreover, the effects of Maillard reaction with Psi on food properties of egg white protein (EWP) were investigated to provide more information on food processing properties of Psi.

In Chapter 2, Psi was applied to thermal processed foods. In this study, baked meringue containing a large amount of Suc (50% (w/w) of total weight) was employed as a thermal processed food. Thirty percent of Suc in meringue was replaced with D-ketohexoses (D-fructose (Fru), Psi, D-tagatose (Tag), and D-sorbose (Sor)). Meringue was prepared by whipping EW (50 g) 2min and gradually adding mixed sugar (sugar containing 30% D-ketohexoses) (50 g) during whipping for 13 min. Then, meringue was baked at 93 °C for 2 h. The effects of Psi on the processing characteristics of baked meringue were investigated.

All the D-ketohexoses increased the antioxidant activity of baked meringue determined by ABTS and DPPH methods. The antioxidant activity of baked meringue was possibly due to melanoidins produced by Maillard reaction (MR) during heat processing. Furthermore, D-ketohexose replacement influenced physical properties of baked meringue.

All the D-ketohexoses increased foaming capacity (percent overrun) of EW. The percent overruns of meringues replaced with Psi (P30), Tag (T30), and Sor (S30) were significantly higher than that of Fru (F30), suggesting that Psi, Tag, and Sor are superior to Fru in term of lowering the interfacial tension of EW foam. 30% Psi (P30)-, 30% Tag (T30)-, and 30% Sor (S30)-meringues had higher specific volume (SV) than 30% Fru (F30)-meringue and Ct-meringue. These results show that Psi, Tag, and Sor have a higher expansion ratio of a gas in the foam network of meringue than Fru and Suc. The difference in the gas expansion ratio may reflect the difference in the extent of protein-sugar interactions.

Peak temperature (T_d) values were increased by the addition of sugars by 9.8 to 11.2°C and 11.9 to 13.0°C for ovotransferrin and ovalbumin, respectively. The results imply that each sugar stabilized ovotransferrin and ovalbumin. The stabilizing effect of sugar for the two proteins was approximately 1°C smaller in P30- and T30-EWs than in Ct-, F30-, and S30-EWs, suggesting that P30- and T30-meringues, compared to Ct-meringue, induce protein denaturation at ca. 1°C lower temperature in the baking process. This may be related to the high SV of P30- and T30-meringues.

The solid structure of baked meringue was observed using scanning electron microscope (SEM). Baked Ct-meringue had thin and rough meringue matrix and large-sized air bubbles. In contrast, baked D-ketohexose meringues (F30, P30, T30, and S30) had thick and smooth meringue matrix and small-sized air bubbles. The small-sized air bubbles indicate the coexistence of D-ketohexose protects the foamy structure from the over-expansion of gas or expedites the solidification of meringue.

The rheological properties of baked D-ketohexose meringues were determined as breaking stress and strain. The breaking stress and strain indicate hardness and retard the deformation of baked meringue, respectively. The baked D-ketohexose meringues (F30, P30, T30, and S30) had 30-72% higher breaking stress than the baked Ct-meringue. Particularly, the breaking stress of P30-meringue was outstandingly high, showing that the addition of Psi hardened the baked meringue. The high breaking stress of D-ketohexose meringues could be closely related to the thick meringue matrix seen by SEM observation. It is reasonable to conclude that a thicker meringue matrix confers the hard texture. The size of the air bubbles might also affect the hardness of the baked meringue.

The air bubble size of the baked meringues was estimated from SEM images. The degree of average air bubble size of meringue was Ct-meringue (500 μm)> F30-meringue (250 μm)> S30-meringue (200-250 μm)> T30-meringue (125-250 μm)> P30-meringue (125 μm). Thus, the formation of small-sized air bubbles results in creating a hard meringue. A structure of baked meringue with large-size air bubble could fracture more easily than that with small-size air bubble when a compressive force is applied to meringue.

For breaking strain, P30-meringue showed a higher breaking strain than Ct-, F30-, T30-, and S30-meringues, suggesting that Psi, compared to the other sugars including Suc, makes meringue harder to crumble. These results probably lead to the crunchiest texture of baked Psi-meringue. Overall results indicate that Psi contribute high antioxidant activity and crunchy texture to baked meringue. Thus, Psi is helpful for modifying functional properties of baked meringue. Psi may also be useful for quality improvement of other thermal processed foods.

As the results of Chapter 2, D-ketohexoses, especially Psi, were found to improve the food properties of egg white proteins (EWP), for example, increasing the foaming capacity and antioxidant activity, and lowering the temperature to form rigid structure. The improvement of food properties of EWP by Psi is possibly related to MR with EWP. In Chapter 3, the progress of the MR between EWP and the four D-ketohexoses (Fru, Psi, Tag, and Sor) and the effects of their Maillard reaction products (MRPs) on the food properties of EWP were investigated.

Glc free EW was mixed with D-ketohexoses (Fru, Psi, Tag, and Sor) at the weight ratio of 100:8 of protein to sugar and pH value of 7. Then, the mixtures were lyophilized and incubated at 50 °C and 55% relative humidity (RH) for 48 h. the control EWP sample without sugar (Ct-EWP) was also prepared in the same manner.

The SDS-PAGE pattern of EWP incubated with sugar shows that the bands of EWP incubated with Fru (Fru-EWP), Psi (Psi-EWP), Tag (Tag-EWP), and Sor (Sor-EWP) were shifted towards lower mobility compared to native EWP and Ct-EWP. This indicates sugar attachment of EWP. In addition, smear bands appeared in the range of above 100 kDa in sugar-EWPs, especially Psi-EWP, Tag-EWP, and Sor-EWP. These smear bands are probably due to protein aggregation caused in the final stage of the MR.

MR is divided into 3 stages; the early, intermediate, and final stages. The early stage of the MR is a condensation reaction of primary amino groups of protein with reducing sugar, which is determined by measuring the content of the free amino groups of EWP (OPA method). The intermediate stage of the MR was determined by measuring the absorbance at 294 nm. The final stage was determined by measuring the browning intensity at the absorbance of 420 nm and the fluorescence intensity. The results of OPA method, absorbance of 294 and 420 nm, and fluorescence index showed that the MR progressed faster in Psi, Tag, and Sor than in Fru. The results also suggest that the reaction rates to the intermediate and final stages of MR are not dependent on the rate of condensation reaction in the initial stage.

MR begins when the carbonyl group of an acyclic sugar is condensed with the primary amino groups of a protein. Thus, the concentration of the acyclic structure of each sugar should have an effect on the reactivity between sugar and protein. This study found that the MR degree among the four sugars appears not to be directly related to the difference in the abundance of the acyclic form.

The antioxidant activity of Psi- and Tag-EWPs was higher than Ct-, Fru-, and Sor-EWPs. These results demonstrate that Psi- and Tag-EWPs act as more potent antioxidants compared with Ct-, Fru-, and Sor-EWPs. The enhancement of antioxidant activity by MR may be due to formation of some melanoidins formed by Psi- and Tag-EWPs. Thus, we assume that MR with Psi and Tag improves the antioxidant activity of EWP.

Gelling properties of EWP were determined as water holding capacity (WHC), breaking and strain of EWP gel. Gels prepared from the sugar-EWPs showed significantly higher WHC than the Ct-EWP gel. This suggests that MR with the four sugars improve the ability to hold water of an EWP gel. The breaking stress of gel was higher in Psi-, Tag-, and Sor-EWP gels than in Fru-EWP gel. Thus, Psi-EWP, Tag-EWP, and Sor-EWP made harder gels than Fru-EWP and Ct-EWP. On the other hand, the breaking strain was higher in Psi- and Tag-EWPs than Fru-EWP. This result demonstrates that Psi-EWP and Tag-EWP gels are more difficult to deform than Fru-EWP gel. The attachment of sugar onto the proteins through MR contributes to partial unfolding of globular proteins. Unfolding of the proteins results in the exposure of buried hydrophobic portions to the protein surface. The exposed hydrophobic groups generally cause strong hydrophobic interactions and concomitant formation of intermolecular disulfide bonds. Such conformational changes of the proteins caused by the MR probably resulted in the enhancement of gel network formation of EWPs reacted with the four sugars.

Emulsifying properties of sugar-EWPs were determined as emulsifying capacity (measuring the absorbance of 500 nm), emulsion stability (evaluating creaming rate using LUmiFuge 116 machine) and oil droplet size of emulsion (measuring particle size of oil droplet using laser diffraction particle analyzer). Emulsifying capacity of sugar-EWPs was 128-162% higher than that of Ct-EWP. Sor- and Psi-EWPs had higher emulsifying activities than Fru-EWP. The emulsion stability of sugar-EWPs was had 62-75% lower than Ct-EWP. In addition, Sor- and Psi-EWPs had lower creaming rate than Tag- and Fru-EWPs. According to the creaming rate of sugar-EWP, the MR of EWP with Sor and Psi is the most effective to stabilize emulsions. The droplet size of the sugar-EWP emulsions was 26-38% smaller than that of Ct-EWP. Thus, the MR of EWP with Psi and Sor is effective for developing a functional emulsifier.

Foaming properties of sugar-EWPs were determined as foaming capacity (evaluated by percent overrun) and foam stability (determined by percent drainage after the foam has been left standing for a period of time). Psi- and Sor-EWPs at a 25 minute-whipping time showed an extremely high overrun of 800% compared with Ct-, Fru-, and Tag-EWPs, which were 651, 687, and 698%, respectively. Ct-EWP showed a decrease in overrun in whipping times of more than 15 min. This is due to the overbeating of EW. The proteins are partially unfolded owing to sugar binding, leading to the enhancement of hydrophobic interaction on the protein surface. The hydrophobic interaction may improve the foam formation. On the other hand, the foams of sugar-EWPs were stabilized by increasing the whipping time, while Ct-EWP foams were not stabilized. The result indicates that sugar-EWPs may be able to form stronger continuous viscoelastic membrane-like film around air cells than Ct-EWP. For this reason, Psi- and Sor-EWPs showing extremely high overrun values significantly improved the foam stability of EWP. Sugar-EWP foams were also observed by confocal scanning laser microscopy. The Ct-EWP foam consisted of large-size air bubbles (average air bubble size >400 μm). Air bubbles from sugar-EWPs were smaller (≤400 μm). The degree of average sizes of air bubbles was Fru-EWP > Tag-EWP >Psi-EWP > Sor-EWP. The difference in air bubble size is possibly responsible for the differences in foam stability.

The antioxidant activity and gelling properties were found to be effectively enhanced by MR with Psi and Tag that have hydroxyl (OH) groups onto C-3 and C-4 oriented in the same direction in Fischer projection formulas. On the other hand, the emulsifying and foaming properties were found to be effectively enhanced by Psi and Sor that have the OH group onto C-3 oriented to the right side. All of the results suggested that the enhancement of food properties of EWP may not be directly related to the degree of MR between EWP and D-ketohexose. It may relate to the relative position (configuration) of the OH groups of sugar. Overall, Psi showed the greatest improvement of food properties of EWP.

The current study indicates that Psi is useful for improving physical properties, such as texture, of protein-based food products. In addition, Psi can be used in the form of protein reacted with Psi (such as Psi-EWP) as an ingredient to improve the products' quality. Consequently, this study provides to support the application of Psi to thermal processed food products.

In Chapter 4, Psi was applied to non-thermal processed food. The concept of non-thermal processing is the processing below the temperature used for thermal pasteurization. Thus, loss of essential nutrients and flavor compounds during the processing are minimally suppressed. However, non-thermal processed foods have shorter shelf-life than thermal processed foods, because of the proliferation of bacteria and fungi and the deterioration in the quality of foods. In this study, the effects of Psi on the quality and shelf-life of non-thermal processed foods were investigated using awayuki; a Japanese traditional unbaked sweet. Awayuki is a perishable food because it contains a large amount of food components which proliferates microbes. The effects of Psi on shelf-life of awayuki were investigated and were compared to other D-ketohexoses.

Awayuki was prepared using 50 g sucrose, 35 g egg white, 2 g agar, and 125 mL water. Fifty percent of Suc in awayuki was replaced with D-ketohexoses. All the D-ketohexoses lowered syneresis (water separated from awayuki) of awayuki. The shelf-life (the durability of texture and the microbial growth) of awayuki was investigated at 8 and 25 °C.

Psi and Sor stabilized the texture of awayuki during the storage. Microbial growth of awayuki inoculated with *Micrococcus luteus* was investigated. Psi, Tag, and Sor slightly suppressed the growth of *M. luteus*. The suppressive effect may be because *M. luteus* cannot utilize Psi and Sor as energy source when the bacteria grow up. Furthermore, the results of microbial count also suggest that Psi, Tag, and Sor possibly act as competitive inhibitor of sugar-metabolic enzymes in *M luteus*. Psi, Tag, and Sor, instead of Suc, may bind to the active site of an enzyme. Thus Suc cannot be utilized transiently, resulting in the delay of the microbial growth. Psi and Sor effectively act as competitive inhibitor than Tag does. Thus, the microbial growth rate in awayuki containing Psi and Sor is lower than that in awayuki containing Tag. Overall results indicate that Psi and Sor are useful for controlling the microbial growth as well as maintaining the desirable texture of awayuki for longer storage time.

The studies in Chapters 2-4 suggested that Psi has potential utility for applying to processed foods containing EW. Psi, compared to other D-ketohexoses including Fru, helps improve overall quality of the products. Once, one eats EW-based food products containing Psi, Psi contained in the products is absorbed into human body via small intestine and is excreted in the urine without being metabolized. In this process, Psi performs several biological functions in the bloodstream such as suppression of the elevation of postprandial blood glucose level. However, the absorption route of Psi in small intestine is unknown.

In Chapter 5, the trans-cellular transport pathway of Psi in human intestine was investigated using Caco-2 cell monolayer. The study showed that the transport route of Psi is the same as that of Fru. Psi is incorporated from the intestinal lumen into the enterocytes via GLUT5 and possibly via GLUT7 located in the apical epithelial membrane and is released to the lamina propria via GLUT2 located in the basolateral membrane. Moreover, the permeability of Psi is similar to that of Fru. It is considered that the similarity in behavior of Psi to Fru is because the transporters GLUT5 and GLUT2 recognize the two sugar molecules as similar molecules.

The application of Psi to food products is not widespread in the world's nations except Japan, even though Psi shows high potential utility for applying to food products. This is due to less information about the effects of Psi on processing properties of food products. Thus this study was conducted to provide more information on food processing properties of Psi. The present study revealed that Psi improves overall quality of egg white-based processed foods; both thermal and non-thermal processed foods. For thermal processed foods, the improvement of product's quality relates to the MR between Psi and EWP. For non-thermal processed foods, the improvement of product's quality may relate to the structure of Psi. In addition, EWP reacted with Psi (Psi-EWP) showed the greatest overall improvement of the food properties. Thus, Psi-EWP may be used as an ingredient to improve the products' quality. Therefore, these findings may powerfully encourage the practical application of Psi to food products.