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Impact of Trehalose as a Partial Replacement of Sugar on Physicochemical Properties of Brown Rice Tofu during Storage

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Abstract

Trehalose has been used in the foods, pharmaceuticals, and cosmetics industries due to its multifunctional properties. The aim of this study was to elucidate the effect of trehalose as a partial replacement of sugar on the physicochemical properties of the brown rice tofu during storage. As a result, the *L** values for the brown rice tofu without trehalose addition and with the application of 1% trehalose decreased significantly with the passage of the storage day. In contrast, 10% trehalose replacement provided a marked positive effect on the color retention of the brown rice tofu, especially whiteness index, suggesting the prevention of the browning of the brown rice tofu. The water content of the brown rice tofu with incorporation of 10% trehalose was significantly high when compared with other tested brown rice tofu after 7 days of storage, resulting in suppression of the hardening of the brown rice tofu. In addition, the incorporation of trehalose could be suppressed the microbial growth on the brown rice tofu. This study proved that a partial replacement of sugar with trehalose, especially 10% trehalose, could be beneficial on the prevention of the quality degradation and improvement of the shelf life, such as prevention of the browning and suppression of the hardening and microbial growth of the brown rice tofu during storage.

Keywords: brown rice tofu, trehalose, replacement, color, physicochemical property, shelf life

1. Introduction

Trehalose is a nonreducing disaccharide that exists widely in nature, such as bacteria, fungi, yeasts, algae, plants, and invertebrate animals (Elbein, 1974). It has been used as an ingredient of foods and food related products due to its unique features. For example, trehalose could be retarded the starch retrogradation, such as hardening, dry feeling, and white turbidity, as foods containing the starches were stored at room temperature or low temperature (Takeuchi, 1997). Therefore, it has been widely used for the production of traditional Japanese confectioneries, western confectioneries, and noodles. It is known that starch-based products processed at high temperature such as crisps and cereals contain trace amounts of acrylamide with neurotoxicity. The formation of acrylamide is suppressed by blocking glucose-asparagine reactions, as the mixture of glucose and asparagine is heated in the presence of trehalose (Kubota et al., 2004). Generally, sugars exhibit the stabilizing effects of proteins. Richard and Dexter (2016) investigated the impact of trehalose on the stabilities of egg white proteins containing 5% trehalose during freezing at -20 ^{o}C for 5 days. The proteins containing trehalose did not denature, although those with sucrose, maltose, and sorbitol caused the denaturation in the range of 14-58%. Haque et al. (2015) studied the effects of trehalose (incorporation of 10-40% trehalose) on the stability of whey protein isolate air-dried at 65 and 85 ^{o}C for 500 s. The incorporation of 20% trehalose completely suppressed the denaturation and subsequent coagulation. Thus, trehalose has higher abilities to protect the proteins during freezing and air-drying. Lipid oxidation is one of serious problems that cause the deterioration of food quality (Kanner, 2007). Particularly, the oxidation of unsaturated fatty acids such as linoleic acid and α-linolenic acid yields toxic substances as hydroperoxide, resulting in lost of human's health and accelerated senescence (Domínguez et al., 2019). Oku et al. (2005) investigated the effect of trehalose on 2,2'-azobis 2-amidinopropane dihydrochloride-induced radical oxidation of unsaturated fatty acids. Trehalose remarkably suppressed the formation of hydroperoxides from linoleic acid and α-linolenic acid. In addition, these contents notedly decreased depending on the concentration of trehalose (Oku et al., 2003). Besides, the formation of thiobarbituric acid reactive substance was suppressed with the addition of trehalose when compared with several other saccharides, suggesting that the addition of trehalose was useful for inhibition of lipid oxidation. Moreover, it has been reported the effect of trehalose on the stabilization of colors (Kopjar et al., 2013; Richards & Dexter, 2016), retention of volatile aromas and flavors (Colaço & Roser, 2011; Galmarini et al., 2009, 2011; Komes et al., 2003; Zlatic et al., 2017), and suppression of generation of undesirable odors (Oku et al., 1999) after treatments, such as freezing, freeze-drying, and heated-air drying, of foods and processed foods. Thus, the multifunctionality of trehalose is utilized in foods and food processing applications.

In recent years, we tried to develop brown rice tofu which is a kind of '*kawari tofu*', using *kudzu* flour and non-glutinous rice cultivar *Haenuki* flour produced in Yamagata prefecture, Japan to expand rice consumption for staple food and to create and inherit new food cultures as a side dish in Japan (Tamai et al., 2021). In addition, we tried to improve the preparation conditions and preparation methods to produce high-quality brown rice tofu. As a result, it could produce acceptable high-quality brown rice tofu having smooth and new palate feeling while suppressing adhesiveness and stickiness peculiar to brown rice flour (Tamai et al., 2022a). In contrast, the production of *kudzu* (*Pueraria montana* var. *lobata*) is small and it is expensive. Therefore, we investigated the applicability of starches from various botanical sources as a substitute for *kudzu* flour in the production of brown rice tofu (Tamai et al., 2022b). It was revealed that tapioca starch was the best substitute for *kudzu* flour in brown rice tofu production. However, starch-rich foods, such as brown rice tofu, are predicted to cause the staling of the starches during storage, resulting in the degradation of the product qualities. The objective of the present study was to elucidate the effect of trehalose for a partial replacement of sugar on the physicochemical properties and general hygienic qualities of brown rice tofu during storage.

2. Materials and Methods

2.1 Materials

The brown rice of nonglutinous rice cultivar *Haenuki* (first-class rice) produced in Yamagata prefecture, Japan, sugar (Mitsui Sugar Co., Ltd., Tokyo, Japan), and salt (Kobe Bussan Co., Ltd., Hyogo, Japan) were obtained from local supermarkets, Yamagata, Japan. The *kudzu* flour and trehalose were purchased from Hirohachido, Fukuoka, Japan and Hayashibara Co., Ltd., Okayama, Japan, respectively. The β-amylase (14,100 U/g) from soybean and pullulanase (Amano 3, 3,000 U/g) were obtained from Tokyo Chemical Industry Co., Ltd., Tokyo, Japan and Amano Enzyme Inc., Aichi, Japan, respectively. All other chemicals and reagents were of AR grade.

2.2 Production of Brown Rice Tofu

After washing brown rice with running water and draining with a sieve, these were dried at 30 ^{o}C for 6 h using an oven (WFO-420, Tokyo Rikakikai Co., Ltd., Tokyo, Japan). These rice were roasted at 160-170 ^{o}C for 15 min and then cooled at room temperature for 20 min. After these were milled for 1 min using a high-speed mill (MS-05, Labonect Co., Ltd., Osaka, Japan), these flours were sieved through a sieve with an opening of 212 μm. The flours with a particle size range of < 212 μm were collected, vacuum-packed, and stored in a cool dark place until use. The brown rice tofu was produced as previously described by Tamai et al. (2022a). The recipes for the brown rice tofu formulations were summarized on Table 1. Half the amount of water was added to the *kudzu* flour and mixed well to avoid clumping. The brown rice flour was mixed well with remaining water. The suspension of the brown rice flour, sugar, and salt were added to the suspension of the *kudzu* flour and then mixed well. These were heated at 160 ^{o}C for 14 min to gelatinize the starch and then continued to simmere at the same temperature for 11 min with kneading and mixing. These were immediately poured into dish (59 mm diameter, 35 mm height) and cooled at room temperature for 1 h. In addition, these were poured in parallel into stainless steel Petri dish (ST-40, Yamaden Co., Ltd., Tokyo, Japan) for the texture profile analysis of the brown rice tofu. After solidification, the brown rice tofu was vacuum-packed and then stored in a refrigerator (4 ^{o}C) for 1 day. These were taken out of the refrigerator, left for 1 h at room temperature, and then the colors, gelatinization degrees, physicochemical properties, microbiological analysis, and proximate composition of the brown rice tofu were estimated.

Table 1. Ingredient composition for brown rice tofu produced using trehalose as a partial replacement of sugar

Ingredients	A	B	C	D
Roasted *haenuki* flour (g)	30.0	30.0	30.0	30.0
Kudzu flour (g)	40.0	40.0	40.0	40.0
Sugar (g)	30.0	29.7	28.5	27.0
Trehalose (g)	0	0.3	1.5	3.0
Salt (g)	1.0	1.0	1.0	1.0
Water (g)	450.0	450.0	450.0	450.0

2.3 Color Analysis

The colors (L^*, a^*, and b^*) of the brown rice tofu were determined using a colorimeter (NR-11A, Nippon Denshoku Industries Co., Ltd., Tokyo, Japan) based on the CIELAB space. The instrumental calibration was carried out using white and black standard plates. Metric chroma (C^*), whiteness (W), and color difference (ΔE^*ab) indices were calculated by the following equations, respectively.

$$\text{Metric chroma } (C^*) = (a^{*2} + b^{*2})^{1/2} \quad (1)$$

$$\text{Whiteness (W)} = 100 - [(100 - L^*)^2 + a^{*2} + b^{*2}]^{1/2} \quad (2)$$

$$\Delta E^*ab = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2} \quad (3)$$

2.4 Gelatinization Degree of Starch

The gelatinization degrees of starches on the brown rice tofu were measured according to a previously described by Matsunaga and Kainuma (1981). The gelatinization degrees and decomposition rates of the starches were calculated by the following equations:

$$\text{Gelatinization degree (\%)} = (\text{decomposition rate of sample/decomposition rate of completely gelatinized sample}) \times 100 \quad (4)$$

$$\text{Decomposition rate (\%)} = (\text{produced reducing sugars/total sugars}) \times 100 \quad (5)$$

where, decomposition rate of sample = [(content of reducing sugar on suspension) – (content of reducing sugar on blank)]/2 × (content of total sugar on suspension); decomposition rate of completely gelatinized sample = [(content of reducing sugar on alkaline gelatinized solution) – (content of reducing sugar on blank)]/2 × (content of total sugar on alkaline gelatinized solution). Produced reducing sugar = (content of reducing sugar on suspension) – (content of reducing sugar on blank); total sugar = 2 × (content of total sugar on suspension). The contents of total sugars and reducing sugars were measured by the phenol-sulfuric acid method and the Somogyi-Nelson method, respectively (Aoyagi et al., 2017).

2.5 Moisture Contents

The moisture contents of brown rice tofu were determined at 135 °C for 1 h (Fujita & Yamada, 2011) using a Moisture Determination Balance (FD-600, Kett Electric Laboratory, Tokyo, Japan).

2.6 Texture Profile Analysis

The texture profile analysis of the brown rice tofu was carried out at room temperature using a rheometer (TPU-2, Yamaden Co., Ltd., Tokyo, Japan) equipped with a 20 mm diameter cylindrical plunger No. 56 (8 mm height). The brown rice tofu samples were compressed twice at a compression speed of 10 mm/s to a clearance of 5 mm. The texture characteristics of the brown rice tofu including breaking force, adhesiveness, cohesiveness, and gumminess were displayed based on the force-time curve of the texture profile analysis.

2.7 Microbiological Analysis

The determination of total plate counts and total coliforms of the brown rice tofu were performed using standard methods agar medium and desoxycholate agar medium, respectively. Microbial populations were measured by plating 10-fold serial dilutions of the brown rice tofu. Counts were expressed as colony forming units per gram brown rice tofu (CFU/g).

2.8 Proximate Composition Analysis

The proximate composition analysis of the brown rice tofu was carried out as described by Kagawa (2023). The energies were calculated according to the Atwater's calorie factors (Kagawa, 2023).

2.9 Statistical Analysis

Except for color analysis, each assay was repeated three times independently. These results were expressed as mean ± standard deviation. Significant differences were tested by one-way analysis of variances with the Tukey's test ($p < 0.05$) using Minitab 17 Statistical Software.

3. Results and Discussion

3.1 Instrumental Color Values

Generally, the colors of foods are one of important factors that influence the preference and acceptability of consumers. The instrumental color parameters of the brown rice tofu produced using trehalose as a partial replacement of sugar during the storage are displayed in Table 2. The a^* values (0.20-0.26) for the brown rice tofu with the application of trehalose were statistically comparable to those (0.25-0.27) for the brown rice tofu without incorporation of trehalose during 7 days of storage. Similar trends were also observed for the b^* values of the tested brown rice tofu. That is, the differences in the color parameters, such as redness and yellowness, were not shown among the tested brown rice tofu. The L^* values (66.6-67.0) for the brown rice tofu without incorporation of trehalose and those (66.7-67.3) of the brown rice tofu with the application of 1% trehalose for a partial replacement of sugar did not change for 2 days. However, the L^* values for these brown rice tofu decreased significantly with the passage of the storage day. The L^* values for the brown rice tofu with 5 and 10% trehalose replacement decreased after 3 days of storage, although no statistically significant differences were observed in these values up to 2 days of storage. Then, the L^* values were retained from 3 to 7 days.

Table 2. Color parameters of brown rice tofu produced using trehalose as a partial replacement of sugar during storage

Samples	Days of storage	Color			Whiteness	Color difference	Metric chroma
		L^*	a^*	b^*	(W)	(ΔE^*ab)	(C^*)
A	0	$67.0±0.22^{aA}$	$0.25±0.04^{aA}$	$13.4±0.44^{aA}$	64.4^{aA}	-	13.4^{aA}
	1	$66.6±0.14^{aA}$	$0.26±0.04^{aA}$	$13.2±0.10^{aA}$	64.1^{aA}	Slight	13.2^{aA}
	2	$66.6±0.08^{aA}$	$0.25±0.06^{aA}$	$13.4±0.27^{aA}$	64.0^{aA}	Slight	13.4^{aA}
	3	$65.0±0.87^{bA}$	$0.27±0.06^{aA}$	$13.2±0.07^{aA}$	62.6^{bA}	Noticeable	13.2^{aA}
	7	$64.1±0.53^{cB}$	$0.25±0.07^{aA}$	$12.9±0.15^{aA}$	61.9^{cA}	Noticeable	12.9^{aA}
B	0	$67.3±0.55^{aA}$	$0.20±0.11^{aA}$	$13.3±0.24^{aA}$	64.7^{aA}	-	13.3^{aA}
	1	$66.7±0.29^{aA}$	$0.24±0.06^{aA}$	$13.2±0.19^{aA}$	64.2^{aA}	Slight	13.3^{aA}
	2	$66.7±0.09^{aA}$	$0.25±0.07^{aA}$	$13.5±0.33^{aA}$	64.0^{aA}	Slight	13.5^{aA}
	3	$65.4±0.58^{bA}$	$0.25±0.06^{aA}$	$13.3±0.24^{aA}$	62.9^{bA}	Noticeable	13.3^{aA}
	7	$64.4±0.72^{cB}$	$0.24±0.10^{aA}$	$13.0±0.18^{aA}$	62.1^{cA}	Noticeable	13.0^{aA}
C	0	$67.3±0.75^{aA}$	$0.26±0.03^{aA}$	$13.5±0.25^{aA}$	64.6^{aA}	-	13.5^{aA}
	1	$66.7±0.03^{aA}$	$0.24±0.07^{aA}$	$13.5±0.25^{aA}$	64.1^{aA}	Slight	13.5^{aA}
	2	$66.7±0.07^{aA}$	$0.21±0.04^{aA}$	$13.4±0.20^{aA}$	64.1^{aA}	Slight	13.4^{aA}
	3	$65.4±0.55^{bA}$	$0.23±0.02^{aA}$	$13.2±0.17^{aA}$	63.0^{bA}	Noticeable	13.2^{aA}
	7	$64.7±0.35^{bB}$	$0.22±0.04^{aA}$	$13.0±0.20^{aA}$	62.4^{bA}	Noticeable	13.0^{aA}
D	0	$67.1±0.37^{aA}$	$0.26±0.05^{aA}$	$13.5±0.37^{aA}$	64.5^{aA}	-	13.5^{aA}
	1	$66.7±0.01^{aA}$	$0.24±0.08^{aA}$	$13.5±0.31^{aA}$	64.1^{aA}	Trace	13.5^{aA}
	2	$66.6±0.10^{aA}$	$0.22±0.05^{aA}$	$13.3±0.26^{aA}$	64.1^{aA}	Slight	13.3^{aA}
	3	$65.5±0.71^{bA}$	$0.23±0.04^{aA}$	$13.1±0.16^{aA}$	63.1^{bA}	Noticeable	13.1^{aA}
	7	$65.4±0.48^{bA}$	$0.23±0.07^{aA}$	$13.1±0.14^{aA}$	63.0^{bA}	Noticeable	13.1^{aA}

Note. Sample abbreviations are given in Table 1. Different lowercase letters in the same lane indicate a significant difference for each sample over the days of storage ($p < 0.05$). Different uppercase letters in the same column indicate a significant difference between samples for each day of storage ($p < 0.05$).

Among the brown rice tofu with the application of trehalose, the L^* value (65.4) for the brown rice tofu with 10% trehalose replacement was significantly high when compared with those of other brown rice tofu after 7 days of storage. These results were consistent with trends of whiteness indexes for the tested brown rice tofu.

Next, to confirm the color changes of the brown rice tofu during 7 days of storage, the $\Delta E^{*}ab$ value of each brown rice tofu sample was calculated to the brown rice tofu for 0 day. As a result, these $\Delta E^{*}ab$ values were lower than 3 (data not shown). That is, noticeable differences in the visual perception of the brown rice tofu after 3 and 7 days of storage could be perceived. This indicated that the color differences were observed as the values in L^{*} a^{*} b^{*} color space compared to the brown rice tofu for 0 day, although these could not be detectable by human eye (Fernández-López et al., 2019). In addition, there was little difference on the metric chroma among the tested brown rice tofu without (12.9-13.4) and with trehalose addition (13.0-13.5). It suggested that the incorporation of trehalose, especially 10% trehalose replacement, could be suppressed the declines in lightness of the brown rice tofu. Sugar contains reducing sugars, such as glucose and fructose. Therefore, the nonenzymatic browning reactions, such as Maillard reactions and caramellization reactions cause when foods are cooked with sugars at high temperatures and are storaged at room temperature. In contrast, trehalose, a nonreducing disaccharide, is not involved in a nonenzymatic browning reactions. From these results, it was indicated that the incorporation of trehalose had a trivial influence on the color characteristics of brown rice tofu. Kopjar et al. (2016) investigated the influence of trehalose as a partial replacement (10%) of sucrose on color of orange jelly after preparation and 135 days of storage under the light at room temperature. The L^{*} and b^{*} values for the orange jelly with the application of trehalose after preparation were significantly high when compared with those for the orange jelly without incorporation of trehalose. In addition, the metric chroma of the orange jelly with trehalose addition was significantly high in contrast to that of the orange jelly without trehalose addition. These results indicated that the orange jelly with trehalose addition had vivid color with high degree of yellowness. In contrast, these were no statistically significant differences were observed in these color parameters. The a^{*} values for the orange jellies after storage increased regardless of with and without trehalose addition when compared with those for the orange jellies after preparation. However, the L^{*}, b^{*}, and C^{*} values for the orange jelly after storage decreased significantly regardless of with and without incorporation of trehalose in comparison with those for the orange jelly after preparation. In contrast, the browning index for the orange jelly with trehalose addition after preparation was significantly higher than that for the orange jelly without trehalose addition. Similar behavior was also observed in the orange jelly after storage. The rate constants of nonenzymatic browning reactions for sucrose were from 200 to 2000-fold greater when compared with those for trehalose in freeze-dried model systems (O'Brien, 1996). In our case, the addition of trehalose had a marked positive effect on the color retention of the brown rice tofu.

3.2 Gelatinization Degree of Starch

The gelatinization degrees of the starches on brown rice tofu produced using trehalose as a partial replacement of sugar during the storage are shown in Table 3. The brown rice tofu without incorporation of trehalose gave the highest gelatinization degrees of the starches up to 3 days of storage. However, the degrees decreased significantly after 7 days of storage. In contrast, the application of trehalose resulted in highest gelatinization degrees of the starches during 7 days of storage. Thus, it was suggested that a partial replacement of sugar with 1-10% trehalose on brown rice tofu production could be retarded or suppressed the starch retrogradation.

The decomposition rate indicates the percentage of produced reducing sugars of total sugars contains in brown rice tofu. There were no statistically significant differences in the decomposition rates (59.6-60.6%) of brown rice tofu between samples for each day of storage regardless of replacement rates of trehalose. In contrast, the rates of brown rice tofu without incorporation of trehalose increased after 1 day of storage. As described above, the L^{*} value for the brown rice tofu without incorporation of trehalose were significantly low when compared with those for the brown rice tofu with the application of 5 and 10% trehalose. One reason may be high proportion of produced reducing sugars of total sugars on the brown rice tofu. It was suggested that the production of reducing sugars were responsible for the reduction in whiteness indexes of brown rice tofu without incorporation of trehalose.

3.3 Water Contents

The water contents are associated with the physicochemical properties of foods such as hardness and shelf life and the sensory attributes, which are also related to the direct contacts of consumers. The contents of the brown rice tofu without incorporation of trehalose drastically decreased with the passage of the storage day (Table 4). Similar trends were also observed for the brown rice tofu with the application of 1% trehalose. In contrast, the brown rice tofu incorporated 5% trehalose gave higher water contents up to 3 days of storage (80.4±0.55%).

Table 3. Gelatinization degrees and decomposition rates of brown rice tofu produced using trehalose as a partial replacement of sugar during storage

Samples	Days of storage	Gelatinization degree (%)	Decomposition rate (%)
A	0	99.9±0.14^{aA}	59.7±2.00^{bA}
	1	99.5±2.49^{aA}	61.8±4.46^{aA}
	2	99.4±0.89^{aA}	61.8±0.20^{aA}
	3	99.4±2.50^{aA}	62.1±2.06^{aA}
	7	99.0±1.81^{bA}	62.2±1.73^{aA}
B	0	99.9±3.03^{aA}	59.6±1.64^{aA}
	1	99.8±0.27^{aA}	60.5±1.05^{aB}
	2	99.8±1.07^{aA}	60.6±2.63^{aB}
	3	99.7±1.71^{aA}	60.6±0.70^{aB}
	7	99.4±1.30^{aA}	60.5±0.55^{aB}
C	0	99.9±3.81^{aA}	59.7±4.30^{aA}
	1	99.8±0.65^{aA}	60.1±1.12^{aB}
	2	99.8±0.32^{aA}	60.6±0.63^{aB}
	3	99.8±1.06^{aA}	60.6±1.15^{aB}
	7	99.6±0.72^{aA}	60.5±0.74^{aB}
D	0	99.9±1.52^{aA}	59.6±0.76^{aA}
	1	99.8±0.93^{aA}	60.0±0.82^{aB}
	2	99.8±0.62^{aA}	60.3±0.36^{aB}
	3	99.8±0.15^{aA}	60.4±0.37^{aB}
	7	99.7±0.19^{aA}	60.5±0.27^{aB}

Note. Sample abbreviations are given in Table 1. Different lowerecase letters in the same lane indicate a significant difference for each sample over the days of storage ($p < 0.05$). Different uppercase letters in the same column indicate a significant difference between samples for each day of storage ($p < 0.05$).

However, the contents decreased significantly after 7 days of storage (79.3±0.49%). Additionally, the contents of the brown rice tofu with the application of 10% trehalose were notably high after 3 days of storage (81.0±0.48%). Then, the contents slightly decreased after 7 days of storage (80.5±0.68%). Thus, it was suggested that a partial replacement of sugar with trehalose, especially 10% trehalose, could be enhanced the water holding capacities of the brown rice tofu during the storage when compared with the brown rice tofu without incorporation of trehalose and those with the application of 1% trehalose. Trehalose is known for high hydration activity to water and lowering effect of water activity of foods, resulting in the improvement of shelf life and the suppression of syneresis during freezing and refrigeration (Kubota et al., 2004). In general, the hydration number of sugars is high, when a number of equatorial hydroxyl groups of carbohydrates are high. Trehalose has a lot of equatorial hydroxyl groups when compared with various sugars, such as sucrose and maltose. Therefore, the hydration activity of trehalose is the highest among various sugars (Sakurai & Inoue, 1997). The water holding capacity of trehalose is higher than those of sugars. From these reasons, it was suggested that a partial replacement of sugar with trehalose was useful for the maintenance of water holding capacities on the brown rice tofu during storage.

3.4 Textural Attributes

The textural properties of foods, such as breaking force and adhesiveness, are very important in these quality assessments. In addition, these attributes are reflected in consumer acceptance. In the present study, breaking force, adhesiveness, cohesiveness, and gumminess of the brown rice tofu produced using trehalose as a partial replacement for sugar were investigated. As a result, the breaking forces of the brown rice tohu increased with the passage of the storage day regardless of with and without incorporation of trehalose (Table 4). That is, the brown rice tofu had gradually become hard during 7 days of storage. However, the breaking forces of the brown rice tofu with the application of 5 and 10% trehalose (1.27±1.37 and 1.20±0.95 × 10^2 N/m^2, respectively) were significantly low after 7 days of storage when compared with those of the brown rice tofu without incorporation of trehalose (1.40±1.26 × 10^2 N/m^2) and those of the brown rice tofu with the application of 1% trehalose (1.32±1.01 × 10^2 N/m^2). It was suggested that a partial replacement of sugar with 10% trehalose resulted in the suppression of hardening on the brown rice tofu.

Table 4. Physicochemical properties of brown rice tofu produced using trehalose as a partial replacement of sugar during storage

Samples	Days of storage	Water content (g/100 g)	Breaking force ($\times 10^2$ N/m^2)	Adhesiveness ($\times 10^2$ N/m^2)	Cohesiveness	Gumminess ($\times 10^2$ N/m^2)
A	0	82.2±0.80^{aA}	0.83±4.16^{dA}	0.05±0.39^{bA}	0.80±0.01^{aA}	0.67±2.65^{cA}
	1	81.7±0.65^{aA}	0.99±7.94^{cA}	0.10±0.00^{abA}	0.76±0.04^{abA}	0.75±8.73^{bA}
	2	79.1±0.39^{bB}	1.22±5.29^{bA}	0.14±2.37^{abA}	0.72±0.03^{abA}	0.87±1.56^{bA}
	3	78.3±0.91^{cC}	1.29±2.00^{bA}	0.16±0.46^{aA}	0.70±0.02^{bA}	0.90±3.43^{abA}
	7	77.4±0.67^{dC}	1.40±1.26^{aA}	0.18±0.61^{aA}	0.61±0.05^{cB}	0.96±2.01^{aA}
B	0	82.0±0.81^{aA}	0.79±4.73^{dA}	0.05±1.21^{bA}	0.80±0.04^{aA}	0.63±3.10^{cA}
	1	81.9±0.57^{aA}	1.01±1.15^{cA}	0.10±0.67^{aA}	0.79±0.02^{aA}	0.80±2.81^{bA}
	2	80.5±0.29^{bB}	1.18±3.06^{bA}	0.11±0.90^{aA}	0.76±0.03^{abA}	0.90±5.15^{aA}
	3	79.0±0.74^{cC}	1.24±1.15^{bA}	0.12±0.46^{aA}	0.72±0.02^{bA}	0.89±2.10^{aA}
	7	78.1±0.93^{dC}	1.32±1.01^{aA}	0.14±0.83^{aA}	0.63±0.01^{bB}	0.93±1.33^{aA}
C	0	82.1±0.65^{aA}	0.84±3.06^{cA}	0.04±1.15^{bA}	0.81±0.02^{aA}	0.67±1.15^{cA}
	1	82.0±0.77^{aA}	0.99±1.15^{bA}	0.09±1.74^{aA}	0.80±0.02^{aA}	0.79±2.63^{bA}
	2	81.4±0.18^{aA}	1.20±2.31^{abA}	0.10±1.56^{aA}	0.75±0.02^{abA}	0.90±1.19^{aA}
	3	80.4±0.55^{bB}	1.24±2.00^{aA}	0.12±1.01^{aA}	0.73±0.01^{abA}	0.90±0.47^{aA}
	7	79.3±0.49^{cB}	1.27±1.37^{aAB}	0.13±0.76^{aA}	0.67±0.02^{bB}	0.92±0.91^{aA}
D	0	81.9±0.65^{aA}	0.84±3.46^{cA}	0.04±0.99^{bA}	0.80±0.02^{aA}	0.67±4.10^{cA}
	1	81.8±0.72^{aA}	0.99±2.31^{bA}	0.09±1.22^{aA}	0.80±0.01^{aA}	0.79±2.02^{bA}
	2	81.3±0.59^{aA}	1.13±8.08^{abA}	0.09±0.36^{aA}	0.78±0.03^{aA}	0.88±9.41^{aA}
	3	81.0±0.48^{abA}	1.19±1.15^{aB}	0.10±1.04^{aA}	0.75±0.02^{aA}	0.90±2.28^{aA}
	7	80.5±0.68^{bA}	1.20±0.95^{aB}	0.11±0.79^{aB}	0.74±0.02^{aA}	0.91±1.34^{aA}

Note. Sample abbreviations are given in Table 1. Different lowercase letters in the same lane indicate a significant difference for each sample over the days of storage ($p < 0.05$). Different uppercase letters in the same column indicate a significant difference between samples for each day of storage ($p < 0.05$).

Adhesiveness is defined as the force to need the separation of the surface of foods and material that touches its foods. Regardless of with and without incorporation of trehalose, the adhesiveness of the brown rice tofu increased significantly after 1 day of storage and then did not change till after 7 days of storage (Table 4). Cohesiveness is defined as the internal cohension between components that form the foods. In the case of the brown rice tofu without incorporation of trehalose, the cohesiveness decreased significantly with the passage of the storage day (Table 4). Additionally, the application of 1 and 5% trehalose caused gradual decreases in cohesiveness of the brown rice tofu. In contrast, no statistically significant differences were observed in cohesiveness levels for the brown rice tofu incorporated 10% trehalose during 7 days of storage, suggesting that the brown rice tofu with the application of 10% trehalose had higher internal cohension and elasticity when compared with those without incorporation of trehalose and with the application of 1 and 5% trehalose. Gumminess is the value that is caluculated by hardness × cohesiveness. Regardless of with and without incorporation of trehalose, the gumminess of the brown rice tofu increased significantly with the passage of the storage day (Table 4). No statistically significant differences were found between samples for each day of storage. Hamanishi et al. (2002) prepared the traditional Japanese confection, *kudzumushiyokan*, using *kudzu* and sago starches. In addition, they investigated the effect of trehalose replacement of sucrose on the physical properties of *kudzumushiyokan*. As a result, the firmness immediately after preparation decreased in *kudzumushiyokan* made from *kudzu* starch with the application of 50 and 100% trehalose and that made from sago starch with 100% trehalose replacement when compared with that with the application of sucrose. In addition, the adhesiveness decreased in *kudzumushiyokan* made from *kudzu* starch with the application of 50 and 100% trehalose as substitution of sucrose. In contrast, no differences were observed in cohesiveness of *kudzumushiyokan* made from these starches regardless of with and without incorporation of trehalose. Thus, *kudzumushiyokan* with incorporation of trehalose was the *yokan* with soft texture and low adhesiveness. Next, they investigated the effect of trehalose replacement of sucrose on the physical properties of *kudzumushiyokan* during 7 days of storage at 5 ^{o}C. The trehalose replacement obviously suppressed the hardening of the *kudzumushiyokan*, although the firmness of each *kudzumushiyokan* increased with the passage of the storage day.

In particular, it could be suppressed an increase of firmness on *kudzumushiyokan* made from sago starch with the application of 50% trehalose when compared with that made from *kudzu* starch. In addition, an increase of adhesiveness on *kudzumushiyokan* suppressed with incorporation of trehalose when compared with that prepared using sucrose regardless of the kind of starch. The trehalose replacement of sucrose suppressed the decrease of cohesiveness on *kudzumushiyokan*, especially that made from sago starch with 100% trehalose replacement. Thus, it suggested that the trehalose replacement of sucrose suppressed the hardening and stickiness of the *kudzumushiyokan* during storage at 5 ^{o}C. According to a report described by Kopjar et al. (2016), the gel strength for the orange jelly with the application of trehalose after preparation were significantly low when compared with that for the orange jelly without incorporation of trehalose. Additionally, similar trends were observed for the rapture strength and brittleness of the orange jelly, suggesting lower elasticity of the orange jelly with trehalose addition compared to that of the orange jelly without trehalose addition. In contrast, there were no statistically significant differences on adhesiveness between the orange jellies with and without trehalose addition. After 135 days of storage, the rapture strengths of these jellies increased remarkably in both cases, however, no statistically significant differences were observed for other parameters after preparation and storage. As described above, the water content of the brown rice tofu with the application of 10% trehalose was significantly high when compared with those without and with (1-5%) trehalose addition after 7 days of storage due to high water holding capacity and retardation or suppression of the starch retrogradation of trehalose, resulting in the brown rice tofu with smooth and new palate feeling while suppressing adhesiveness and stickiness peculiar to rice flours during 7 days of storage.

Table 5. Microbiological qualities of brown rice tofu produced using trehalose as a partial replacement of sugar during storage

Samples	Days of storage	Total aerobic plate counts (CFU/g)	Total coliforms (CFU/g)
A	0	Absence	Absence
	1	Absence	Absence
	2	Absence	Absence
	3	2.9×10^2	1.7×10^1
	7	5.8×10^2	4.2×10^1
B	0	Absence	Absence
	1	Absence	Absence
	2	Absence	Absence
	3	1.2×10^2	1.1×10^1
	7	2.6×10^2	2.0×10^1
C	0	Absence	Absence
	1	Absence	Absence
	2	Absence	Absence
	3	Absence	Absence
	7	1.2×10^2	1.3×10^1
D	0	Absence	Absence
	1	Absence	Absence
	2	Absence	Absence
	3	Absence	Absence
	7	Absence	Absence

Note. Sample abbreviations are given in Table 1.

3.5 Microbiological Analysis

Microbiological analysis, such as total aerobic plate counts and total coliforms, is one of important indicators for the general hygienic qualities of foods and processed foods. Total aerobic plate counts of the brown rice tofu without and with incorporation of trehalose were investigated during storage. As a result, the total aerobic plate counts did not detect on the brown rice tofu without trehalose addition and with 1% trehalose addition after 2 days of storage. Then, these counts ranged from 2.9-5.8 × 10^2 CFU/g and 1.2-2.6 × 10^2 CFU/g after 3 and 7 days of storage, respectively. In contrast, the total aerobic plate counts were not detected in the brown rice tofu with

10% trehalose addition during 7 days of storage at all. Next, the total coliforms of the brown rice tofu without and with trehalose addition were measured during 7 days of storage. Similar behavior was observed for the total coliforms of the brown rice tofu. That is, total coliforms did not detect on the brown rice tofu without trehalose addition and with 1% trehalose addition after 2 days of storage. However, the total coliforms were detected after 3 and 7 days of storage ranging from 1.7 to 4.2×10^1 CFU/g and 1.1 to 2.0×10^1 CFU/g, respectively. In contrast, the total coliforms were not detected in the brown rice tofu with incorporation of 10% trehalose during 7 days of storage. Suppression to the total aerobic bacteria and total coliform bacteria tended to increase with increasing of additive rates of trehalose. It suggested that trehalose, especially a partial replacement of sugar with 10% trehalose, possessed superior hydration activity and lowered the water activity of the brown rice tofu, resulting in suppression of microbial growth. From these investigations, a partial replacement of sugar with 10% trehalose was most appropriate for the hygienic quality preservation of the brown rice tofu during storage.

3.6 Proximate Composition

The proximate composition of the brown rice tofu with the application of 10% trehalose was investigated. As a result, the composition was as follows: water (81.9±0.65 g/100 g), crude proteins (0.2±0.15 g/100 g), crude lipids (0.03±0.00 g/100 g), carbohydrates (17.6±0.02 g/100 g), crude ashes (0.23±0.02 g/100 g), and salts (0.20 g/100 g), respectively (Table 6). In addition, the energy was calculated to be 71.5 kcal/100 g. No statistically significant differences in these compositions were observed between the brown rice tofu with the application of 10% trehalose and that without incorporation of trehalose.

Table 6. Proximate compositions of brown rice tofu D produced using trehalose as a partial replacement of sugar

	D	Control*
Energy (kcal/100 g)	71.5^a	70.7^a
Water (g/100 g)	81.9±0.65^a	82.2±0.80^a
Crude proteins (g/100 g)	0.2±0.15^a	0.2±0.43^a
Crude lipids (g/100 g)	0.03±0.00^a	0.03±0.01^a
Carbohydrates (g/100 g)	17.6±0.02^a	17.4±0.83^a
Crude ashes (g/100 g)	0.23±0.02^a	0.21±0.01^a
Salts (g/100 g)	0.20^a	0.21^a

Note. Sample abbreviation is given in Table 1. *Data was quoted from our previous paper (Tamai et al., 2022a). Different lowercase letters in the same row indicate a significant difference ($p < 0.05$).

Trehalose is one of non-toxic dietary carbohydrate and is approved as a food additive in Japan in 1995. The relative sweetness of trehalose is only 45% of sucrose despite the same energy (16.7 kJ/g) as sucrose (Kubota et al., 2004). It is able to reduce sweetness of the final products by replacing sugars with trehalose. In addition, trehalose can improve overall qualities of the products to stabilize the proteins, fats, carbohydrates, colors, and aromatic volatiles. Therefore, trehalose has been used as a functional food material in not only all kinds of processed foods using vegetables, fruits, and seafoods, bakery products, and beverages, but also frozen and refrigerated foods (Richards et al., 2002). In contrast, trehalose is digested slowly in the digestive tract when compared with sucrose, resulting in a lower insulin release and glycaemic index (van Can et al., 2012). Thus, trehalose is an attractive carbohydrate for food related industries and consumers. Hamanishi et al. (2002) evaluated the sensory attributes of *kudzumushiyokan* made from sago starch with incorporation of trehalose after 3 days of storage at 5 ^{o}C. Firmness score for *kudzumushiyokan* with the application of 50% trehalose was the same as that for *kudzumushiyokan* with the application of sucrose, suggesting *kudzumushiyokan* with soft texture. In addition, the overall evaluation score for *kudzumushiyokan* with incorporation of 50% trehalose was significantly high when compared with that for *kudzumushiyokan* incorporated sucrose. Thus, the substitution of sucrose with 50% trehalose suppressed the sago starch retrogradation, resulting in *kudzumushiyokan* with favorable sensory attributes. From the present study, it was revealed that the incorporation of trehalose could be beneficial on the quality of the brown rice tofu during storage. Particularlly, a partial replacement of sugar with 10% trehalose suppressed the browning and hardening, increased the water retention ability, and suppressed the microbial growth of the brown rice tofu during 7 days of storage. Additionally, there were no statistically significant differences in the proximate compositions and sensory attuributes (data not shown) between the brown rice tofu without trehalose addition and that with 10% trehalose addition.

4. Conclusion

In this study, the effect of trehalose as a partial replacement of sugar on the physicochemical properties and general hygienic qualities of brown rice tofu during storage was investigated. The use of trehalose could be prevented the browning of the brown rice tofu and suppressed the hardening of the tofu due to the high water retention ability. In addition, the incorporation of trehalose could be suppressed the microbial growth on the brown rice tofu. This means that a partial replacement of sugar with trehalose, especially 10% trehalose, may be achieved the prevention of the quality degradation and improvement of the shelf life of the brown rice tofu during storage.

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