Effects of Frozen Storage and Thawing Conditions on Physical Properties of Glutinous Rice: (Part 1) Ice Crystal and Color Measurement

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Summary

The effect of the freezing process and frozen storage periods on the microstructure of cooked glutinous rice has been investigated. Cooked glutinous rice (cv. Koganemochi) was frozen and stored in a household freezer (about -22 °C) during 1, 5, 10, 30 and 60-day storage periods. Its microstructure was observed by using fluorescence staining method and the morphology of ice crystals including equivalent diameter and number was analyzed. Colorimetric measurements were conducted after natural and steaming thawing on samples of each storage period. It was found that average equivalent diameter and number of ice crystals increased with storage period from 5 days, and recrystallization occurred in temperature fluctuation during freezing process and storage affected microstructure of glutinous rice. Moreover, by analyzing the distribution of ice crystals, it was found that most of them are under 10µm in each storage period. Frozen stored glutinous rice after 60 days showed darker and more unpleasant color than freshly cooked one by both thawing methods. Since the tendency of color change during the whole frozen storage was unstable, it could be assumed that the control of thawing process should not be neglected.

Keywords: Freezing, Frozen storage, Glutinous Rice, Ice crystal, Thawing

1. Introduction

Glutinous rice, which is also called waxy rice or sticky rice, is a type of rice grown mainly in Southeast and East Asia. In China, glutinous rice is a major raw material used to make a variety of traditional festival foods such as rice dumplings (Zongzi), desserts and rice cakes. Besides, glutinous rice is also widely used in traditional Chinese medicine to enhance physical power, to invigorate the spleen, and to prevent fatigue. Zongzi is a typical traditional Chinese food made of glutinous rice stuffed with different fillings and wrapped in bamboo, reed, or other large flat leaves, before being steamed or boiled. With the improvement of manufacturing technology and the change of consumption concept in people, glutinous rice food like Zongzi is gradually turning from traditional food to leisure food.

Nevertheless, there still remains a low level of standardized production and the manufacture of glutinous rice food mostly follows traditional processing technique, such as handwork, which not only shorten the shelf life but also bring about low production efficiency and instable product quality.

Long stewing and sterilizing time leads to energy dissipation, the increase of production cost, and even affects the taste.

As the living standard among Chinese people grows, the demand of quality as well as convenience of traditional food becomes higher and higher. Frozen ready-to-eat food develops fastest in the market and glutinous rice products have become available among the glutinous rice food industry. On the other hand, with the popularization of household freezers, more and more people also start to care about the influence of cooking methods, storage periods, etc., on the taste of home-made glutinous rice food.

Normally, the shelf-life of frozen glutinous rice products is between half and 1 year in China while the freezing conditions and influence of the storage periods on the palatability are usually neglected.

Considering these problems, packaging during freezing and storage is also an important factor to consider while producing foods. However, a severe heat treatment is required in the production of the aseptic packaged cooked rice as all micro-organisms are killed†. It was found that frozen cooked rice may come closer to the sensory characteristics of home-
made rice than the aseptic packaged cooked rice due to its similar manufacturing process\(^3\).

A previous study\(^3\) pointed out that the equivalent diameter of the ice crystals in frozen cooked rice stored at \(-5\) to \(-45^\circ C\) during 1 to 90 days varied inversely with the palatability of rice while hardness scores in sensory evaluation increased. Moreover, it was demonstrated that comparing to air thawing at room temperature, microwave heating had a superior function recovering the deteriorated quality of frozen cooked rice after storage. However, the previous studies did not mention the influence of change in numbers of ice crystals during frozen storage, which could also lead to the destruction of the microstructure.

The objective of this work is to determine the effect of the freezing process and frozen storage periods on the microstructure of cooked glutinous rice.

2. Materials and Methods

2.1 Sample preparation

Glutinous rice (Koganemochi, produced in Niigata Prefecture, Japan; harvested in 2015) was kept in a thermostatic room controlled at 4°C until the experiment. A 242 g of glutinous rice was soaked within 200 ml water of tap water under room temperature (25°C) for 1.0 h. Since glutinous rice has a high ability of water absorption, it was added another 100 ml water for cooking. Soaked glutinous rice was cooked in a household rice cooker (NP-NC 10, Zojirushi Co., Japan) for 70 min. The cooking mode of rice cooker was set as for cooking glutinous rice. After cooking, every 150 g cooked glutinous rice was removed from the rice cooker to a sheet of foil which was designed for cooking and absorbing extra moisture from cooked rice. The surface of rice piled on the sheet was flattened for cooling until it reached room temperature (25°C). The samples were made into discs of which the size was 150 mm in diameter and 10 mm in height and then be put into bags designed for freezing.

2.2 Temperature measurement

T-Type thermocouple probes (HYP-1, OMEGA, Tokyo, Japan) and a data logger (midi LOGGER GL220, GRAPHTEC, Yokohama, Japan) were used to measure the temperature change during cooking and cooling. Cooking temperatures were measured at the central part of the rice-water mixture and the bottom part of the rice kettle. The temperature change in the central part of a sample was monitored during freezing and thawing. The temperatures were recorded every 10 s.

2.3 Weight measurement

The weight of freshly cooked rice was measured 3 times after cooling period (about 90 min), and the average weight was 481.61±3.83g.

2.4 Freezing and thawing

A freezer (L595xW330xH575; RRS-102CNF, REMACOM Corp., Mishima, Japan) was used in this experiment as the one specialized for home and business use, equipped with quick freezing function, which could be as low as \(-27^\circ C\).

The samples were sealed in Freezer Preservation Bags (Ziploc, AsahiKASEI, Tokyo, Japan). Then the quick freeze function was enabled for 1 h and temperature data with time were recorded. The final temperature of the samples was \(-23^\circ C\). Afterwards the samples were stored at \(-20^\circ C\) in the freezer for 1, 5, 10, 30 and 60 days.

Two methods of thawing were used after each period of storage: heating by a household steamer (SERIE S02, T-fal Co., Tokyo, Japan) and natural thawing at room temperature (25°C). In the steaming method, the steamer was preheated up to 100°C in advance.

2.5 Colorimetric measurement

Colorimetric measurements were conducted after thawing on samples of each storage period and repeated 4 times. The measurement was performed using handy spectrophotometer NF-333 (Nippon Denshoku, Co., Tokyo, Japan) and the color parameters of glutinous rice were recorded.

2.6 Ice crystal measurement

A fluorescence staining method\(^3\) was used for the measurement of ice crystals grown in central part of the samples. During the preparation of samples, the glutinous rice was soaked with aqueous solution of 0.1 wt% fluorescent Rhodamine B (C\(_{28}\)H\(_{36}\)ClN\(_{5}\)O\(_{3}\)) in concentration at room temperature (25°C) for 1.0 h. The stained glutinous rice was then cooked and frozen with the method as mentioned above. After each period of storage, the samples were collected from the central part for avoiding the edge effects of heat flow. Then the samples were first embedded within Optical Cutting Temperature (O.C.T) compound in a low-temperature cryostat which was set at \(-20^\circ C\). The embedded samples were trimmed transversally until the desired portion was exposed with the cross section of the central part in approximately 7.8 mm long and 3.2 mm wide. Samples were sliced into 3 μm-thick sections with a freezing rotary microtome (CM1510 cryostat, Leica, Germany) equipped with disposable blades. The sliced samples were placed on the cryostage (LK-600PM, Linkam, UK) of a microscope (BX-50, Olympus, Tokyo, Japan) equipped with a mercury arc lamp and the WIG filter cube (Olympus, Tokyo, Japan) for fluorescence. The WIG filter cube was composed of an excitation filter (BP520-550, Olympus, Japan), an absorption filter (BA580IF, Olympus, Tokyo, Japan) and a dichroic mirror (DM565, Olympus, Tokyo, Japan). The cross-sections of frozen block-shaped glutinous rice
samples were observed and photographed under the microscope whose cryostage was maintained at -20°C during observation. Fluorescence images were captured by a commercially available digital camera (DP-20, Olympus, Tokyo, Japan) which was mounted on the microscope. The morphology of ice crystals was analyzed by WinROOF (Mitani Corp., Tokyo, Japan), a commercial image-analysis software. Captured images were binarized and modified by means of inversion. The equivalent diameters of ice crystals were calculated from the images and the numbers of ice crystal particles were counted. All image analyses of every storage period were done for more than 5 times.

3. Results and Discussion

3.1 Cooking, freezing and thawing processes

Temperature changes of glutinous rice during cooking process was recorded by thermocouples and the data logger, which indicated 3 stages during cooking process of glutinous rice (Fig.1). The water absorption period lasted about 20 minutes at the temperature interval of 40~60°C. The cooking period at 100°C continued about 15 min, followed by the steaming period ultimately.

Fig.2 shows the temperature change of freshly cooked rice during cooling process. The water absorption rate of glutinous rice during cooking process was calculated as 99.14%, which showed a high swelling capacity. A previous study has indicated that greater swelling capacity is an indication of weaker binding forces in the starch granules\(^4\), which demonstrated that a large amount of amylopectin in glutinous rice hydrated under the cooking process. The low amylose content in glutinous rice, which associates with the strength of the micellar network in starch granules, led to higher swelling power\(^5\). Upon increasing temperature in the presence of water, starch molecules mobility was increased thereby weakening the binding forces\(^6\).

Fig.3 shows the temperature-time plot of both glutinous rice sample and ambient temperature in the freezer. There was an initial rapid decrease in environmental temperature at the start of freezing because quick thawing function was enabled. It reached -27.5°C in 1 h as the lowest point of temperature during the freezing period. The total freezing time for the cooked glutinous rice sample was about 8 h, including more than 2 h spent in the maximum ice crystal formation zone due to the limited efficiency of conductive heat transfer. Since this freezing time reflected temperature change at the central part, it was assumed to be much slower than other parts of the glutinous rice sample.

On the whole, there were 3 distinct periods during the freezing process, including pre-freezing, phase change and post-freezing. During the pre-freezing period, the temperature of the glutinous rice sample decreased to its freezing point with the removal of sensible heat above the freezing point. The temperature plot showed a brief period of supercooling, which is below 0°C. Once nucleation occurred and ice crystals began to form, the freezing point increased to 0°C. The temperature remained at the freezing point until a complete phase change occurred as latent heat of fusion was removed from liquid water to convert it into solid ice in glutinous rice. The remaining water became more concentrated with solutes and depressed the freezing point, which was shown as the valley before the freezing plateau in the figure. This gradual change in temperature with the additional removal of latent heat continued until the glutinous rice was largely a mix of the initial solid components and ice. Finally, during the post-freezing period, further cooling led to the desired subfreezing temperature, with the removal of sensible heat below the freezing point.

The freezing rate (°C/h) at the central part of the glutinous rice is about 5.75°C/h, which is defined as the difference between the initial (23.6~24.0°C) and the final temperature (-22.3~ -23.0°C) divided by the freezing time (8.0 h)\(^7\). It should be noted that the factor with the most significant influence on this freezing time is the convective heat-transfer coefficient, which could influence freezing time through the equipment design.
After a freezing period of almost 8 hours, the temperature in glutinous rice sample reached an equilibrium and the storage period started, which continued on with a considerable temperature fluctuation between -18.5 to -26.2°C. This temperature fluctuation might be one of the reasons to deteriorate the quality and shorten the shelf-life of the frozen product.

Temperature change in central part of glutinous rice during natural thawing and steaming thawing was shown in Fig. 4. In steaming thawing, frozen glutinous rice samples were heated by the steamer over 100°C within 5 min and the samples were taken out from the steamer to be cooled up to room temperature. The sample reached room temperature in approximately 70 min, which was a little faster than cooling of freshly cooked ones. The samples thawed by natural thawing reached room temperature within 3.5 h.

### 3.2 Color

Color parameters of 60-day period samples showed significantly (p < 0.05) lower values than the freshly cooked glutinous rice (L*: 66.54±2.33, a*: -0.36±0.72, b*: 5.69±1.11) by both the two thawing methods (Table 1), which suggested a deterioration of long-time stored glutinous rice. When it comes to other periods, samples thawed by steaming method showed barely any significant differences between freshly cooked ones. This might be due to the non-enzymatic browning reaction called Maillard browning that induced by the heat-moisture treatment, which has been reported to contribute to the color change (larger a* and b*) in parboiled rice. The formation of Maillard reaction products was reported to increase with increasing severity of hydrothermal treatment. Since Maillard browning mainly occurs during the steaming step, which both existed in cooking and steaming thawing process, the few differences in color parameters between them could be explained. In addition, a previous work confirmed that the carotenoids present in the epidermal layers got reduced to trace levels after steam parboiling and hence do not contribute to the final color of parboiled rice.

Besides, the color parameters were found to show a significant (p < 0.05) decrease that started from 5-day storage period in naturally thawed samples, where the values of b* began its decrease from the first day of frozen storage. This indicated that dehydration phenomenon occurred during natural thawing, possibly due to the melting of ice crystals generated in the samples, might reduce the lightness (L*) of glutinous rice, and the absence of Maillard browning during it led to lower redness (a*) and yellowness (b*).

With regards to the increase in 10 and 30-day color parameters, especially in L*, the thawing conditions could be considered as influential factors since colorimetric measurements were conducted soon after thawing processes. The time of steaming had already been proved to have significant effects on color parameters of rice. However, linear regression results showed barely any correlations with the steaming speed and time spent on passing the maximum ice crystal formation zone during natural thawing. This indicated that except the above factors, other periods during the whole thawing process, such as the time needed to reach room temperature in both methods might also be taken into account, which were not precisely controlled this time.

The standard deviation of a* and b* shown in Table 1 might be caused by the errors of CIELAB method, which provided the calculation of color differences as vector distances but proved not a suitable way of describing tiny differences in color. This also indicated that the color change during frozen storage of glutinous rice in redness and yellowness could be regarded as almost imperceptible to naked eye.
Table 1: Color parameters of freeze-thawed glutinous rice during frozen storage

<table>
<thead>
<tr>
<th>Thawing methods</th>
<th>Color parameters</th>
<th>Storage periods (d)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>L*</td>
<td>1</td>
<td>5</td>
<td>10</td>
<td>30</td>
</tr>
<tr>
<td>Steaming</td>
<td>70.91 ±1.26 *</td>
<td>70.84 ±2.42 *</td>
<td></td>
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<tr>
<td></td>
<td>65.08 ±3.88</td>
<td>68.64 ±1.26</td>
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</tr>
<tr>
<td></td>
<td>68.64 ±1.26</td>
<td>61.82 ±2.73 *</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Natural</td>
<td>68.23 ±3.11</td>
<td>64.61 ±2.75</td>
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</tr>
<tr>
<td></td>
<td>61.64 ±1.31 *</td>
<td>68.62 ±0.61</td>
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</tr>
<tr>
<td></td>
<td>64.61 ±2.75</td>
<td>60.86 ±4.82</td>
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</tr>
<tr>
<td></td>
<td>66.54 ±2.33; a*: -0.36±0.72; b*: 5.69±1.11</td>
<td></td>
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</tbody>
</table>

Values are given as means ± standard deviations.
* Significant at P<.05 against freshly cooked glutinous rice (L*: 66.54±2.33; a*: -0.36±0.72; b*: 5.69±1.11)

3.3 Mean equivalent diameters and numbers of ice crystals

Fig. 5 shows micrographs of frozen glutinous rice samples obtained by the fluorescence method, which allowed the cryo-scanning electron microscopic observation of ice crystal evolution during the frozen storage. Since ice crystals within food are formed by eliminating solute and impurities during freezing, they could be observed as an achromatic figure by staining the main ingredients of foods such as starch and proteins. Thus, ice crystals were visualized as black parts in the following images.
Fig. 6 shows average numbers and equivalent diameter changes of ice crystal during frozen storage. While the average numbers of ice crystals increased with increasing the days of storage period, the average equivalent diameters did not significantly change regardless of the days of storage period. Kono et al. (2015) represented that the mean equivalent diameters of frozen cooked rice stored at -30°C for 0 to 90 days were in the range of 13.2 to 14.6 μm, which corresponded to almost the same range obtained in this study.

Fig.6 Average numbers and equivalent diameter changes during frozen storage

4. Conclusion

The temperature fluctuation could probably influence the growth of ice crystals inside glutinous rice during the frozen storage in a household freezer. The size of ice crystals would increase significantly in 1 day and 30-day points, which could be due to the maximum ice crystal formation zone and recrystallization, respectively. Moreover, by analyzing the distribution of ice crystals, it was found that most of them are under 10μm in each storage period, which indicates that numbers of small ice crystals should be considered as the major reason for the structure and texture changes. Frozen stored glutinous rice after 60 days shows darker and more unpleasant color than freshly cooked one by both thawing methods, which indicates the deterioration on the surface, while natural thawed glutinous rice tends to show dark and unpleasant color by a relatively short time storage. Since the tendency of color change during the whole frozen storage was unstable, it could be assumed that the control of thawing process should not be neglected.

References

7) International Institute of Refrigeration (IIR), Recommendations for the processing and handling of frozen foods, (3rd Ed) 1996, Paris: IIR.