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Structural, Textural, and Thermal Properties of Freeze-thawed Quick-frozen Cooked Rice PSB Rc 18 (*Oryza sativa* L.)

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The study characterized the quality properties of freeze-thawed quick-frozen intermediate amylose cooked rice PSB Rc 18 (*Oryza sativa* L.). Scanning electron microscopy (SEM), texture profile analysis (TPA), and differential scanning calorimetry (DSC) were employed to monitor structural, textural, and thermal properties including retrogradation in quick-frozen rice as subjected to 1, 3, and 5 freeze-thaw cycles (FTCs) (–18 °C for 16 h; 10 °C for 8 h). Data from SEM, TPA, and DSC explained progressive deteriorative quality changes with increasing FTC. The SEM micrographs revealed the formation of honeycomb structures. The TPA results showed significant ($p < 0.05$) increase in hardness, cohesiveness, chewiness, springiness; as well as a decrease in adhesiveness. The DSC analysis demonstrated the decreasing trend of thermal transition temperatures. Based on the obtained results, it is recommended that quick-frozen cooked rice should not be exposed to more than 3 FTCs.

Keywords: freeze-thaw cycle, frozen rice, intermediate amylose, *Oryza sativa* L., retrogradation

INTRODUCTION

FTCs have established negative effects in the overall quality of frozen commodities that alter functional, structural, textural, physical, and chemical properties of food products – particularly those that are carbohydrate-based (Srichuwong *et al.* 2012; Tao *et al.* 2015; Wu *et al.* 2017; Wang *et al.* 2018). Recent advances in rice processing technologies now encompass freezing. In fact, frozen cooked rice – as a convenience food – is already being marketed in Japan, Australia (Yu *et al.* 2016), Korea (Kwak *et al.* 2015), and Thailand (Trithavisup and Charoenrein 2018) using their own specific rice varieties. Several studies reported that quick freezing technology has a lot more potential to efficiently retain the desirable qualities of which rice consumers prefer (Yu *et al.* 2010a; Kwak *et al.* 2013, 2015). Unfortunately, cooked rice – as a convenience food-to-go traded under a cook-freeze-thaw scheme – is subject to the negative effects of FTC during conveyance and distribution.

Definitively, exposure to FTC may nullify whatever positive effects of quick freezing to frozen products. Studies reported a loss of textural, structural, sensorial and decrease in shelflife of frozen rice due to retrogradation (Wu *et al.* 2017; Wang *et al.* 2018) upon subject to 5 FTC. Moreover, the *fake rice* incident in the Philippines is a possible example of a severe occurrence of retrogradation in cooked rice during FTC (Perez 2015). The rice involved in the reported *fake rice* incident was described to have *styrofoam* appearance with a chalky texture after being subjected apparently to a number of FTC, as reported by

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Ranada (2015). The properties of the so-called *fake rice* deviated from the preferred characteristics of the freshly cooked rice that is soft and fluffy (Cuevas *et al.* 2016). This study aimed to evaluate the quality parameters of the quick-frozen cooked rice that have been subjected to 5 FTC.

MATERIALS AND METHODS

Collection and Preparation Rice Samples

The intermediate amylose (20–24%) test Philippine rice variety used in the study was Philippine Seed Board Rice cultivar 18 (PSB Rc 18). Rice sample was harvested during the wet season (October 2017) and obtained from a reputable rice miller (JD Aguilar Rice Mill, San Leonardo, Nueva Ecija). It was washed-drained using a heavy-duty mixer (5K5SS, 315W, 220V, Kitchen Aid, USA) with a 1:3 rice-tap water ratio at 60 rpm for 10 s (four times), admixed with 1:2 grain-water ratio, cooked in an automatic rice cooker (KW-2042, 1L, 400 W, 220 V, 60Hz, Kyowa, Japan), quick-frozen using a laboratory-scale freezing conveyor system (–35 ± 5 °C, 5 m), packed in a CPET tray, and stored at –18 ± 1 °C.

FTC

The FTC applied in the study was storing quick-frozen cooked rice samples at –18 ± 1 °C for 16 h then thawing at 10 ± 1 °C in a chiller for 8 h. This FTC was repeated up to 5 cycles. Freeze-thawed quick-frozen cooked rice samples (FTC 1, FTC 3, and FTC 5) were subjected to SEM, DSC, and TPA analyses. Raw, freshly cooked, and quick-frozen cooked rice samples were also included in the analyses.

Scanning Electron Microscope (SEM)

The micrographs of the surface and cross-section of samples were viewed using an SEM (FEI Helios Nanolab 600i, Dual Beam System, USA). Thawed rice samples were freeze-dried and sputter-coated with gold using an ion sputter (Jeol JFC-1001E Sputter Coater, Nikon, USA). For cross-sectional observation, samples were cut with a razor blade prior to sputter-coating. Observations were made at magnifications of 100x and 500x with an accelerating voltage of 5 kV. For surface observation, magnifications of 500x and 1000x with an accelerating voltage of 2 kV were used (Teng *et al.* 2013). Representative micrographs from all the samples were presented to compare structural changes of all rice samples. The analysis was done in triplicates.

TPA

The TPA of samples was performed using a texture analyzer (TA XT.plus, Texture Technologies Corp., UK) with a 50 kg load cell using a two-cycle compression. A 7.5-mm diameter compression plate was used to compress three equidistant grains with a speed of 1.0 mm/s (Ma and Sun 2009). The analysis was done in triplicates.

DSC

Samples' thermal properties were analyzed using a Perkin Elmer DSC 4000 differential scanning calorimeter (Perkin Elmer, USA). Rice samples (≈ 3 mg) were weighed accurately into an aluminum pan and held isothermally at 20 °C for 1 min before being heated from 20 to 100 °C at 10 °C/min (Ma and Sun 2009). For raw grain samples, distilled water (≈ 6 mg) was added prior isothermal holding process. Then, DSC was calibrated with indium (melting point = 156.6 °C, ΔH_f = 28.6 J/g) and an empty pan was used as a reference. Rice samples were scanned using the heating profiles describe for rice gelatinization. The onset (To), peak (Tp), and conclusion (Tc) temperatures plus enthalpy (ΔH) of gelatinization/retrogradation were obtained by Pyris Software, version 11 (Perkin Elmer, USA). The degree of retrogradation (%DR) was calculated using the formula: $\%DR = \Delta H_R/\Delta H_G \times 100\%$. The analysis was conducted in triplicates.

Statistical Analysis

Data obtained were reported as mean values and standards deviations. Analysis of variance (ANOVA) by Scheffe's test ($p < 0.05$) were conducted using SAS 9.4 (SAS Institute, Inc., USA).

RESULTS

SEM

Figure 1 shows SEM cross-section (A–E) and surface (F–J) images of raw, newly quick-frozen, and freeze-thawed quick-frozen cooked rice samples at FTC 1, 3, and 5, respectively. Raw grain images (Figures 1A and 1F) were observed to have a starch granular appearance at the cross-section (Figure 1A), with fissures and cracks on the surface (Figure 1F). Quick-frozen rice images, on the other hand, did not show the previously described granular appearance, fissures, and cracks. Only images showing some level of porosity were observed both in the surface and cross-section (Figure 1B and 1G). With increasing FTC, rice samples showed prominent reappearance of fissures and cracks on cross-section (Figure 1C–E) and increasing level of porosity on the surface (Figures 1H–J).

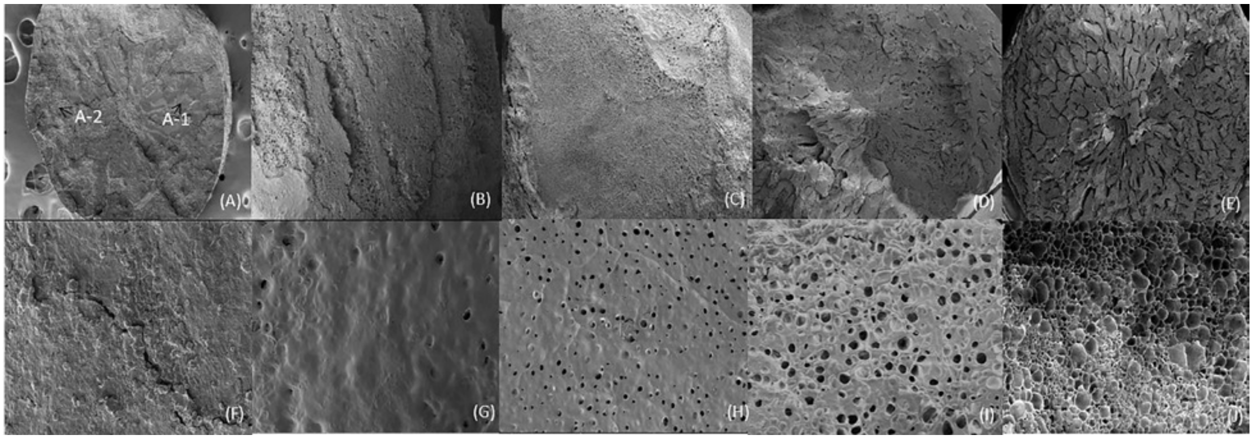

Figure 1. SEM images of cross-section A–E (100x) and surface F–J (1000x) of rice samples.
Legends: raw grain (A and F), quick-frozen cooked rice (B and G), FTC 1 (C and H), FTC 3 (D and I), FTC 5 (E and J), smooth area (A-1), and rough area (A-2).

Table 1. Texture profile of rice samples (PSB Rc18).

	Hardness (kgf)	Cohesiveness	Springiness (mm)	Gumminess (kgf)	Chewiness (kgf.mm)	Adhesiveness (kgf.mm)	Stiffness (kgf.mm)
Freshly cooked	5.11 ± 0.15[a]	0.04 ± 0.01[a]	0.94 ± 0.08[a]	0.18 ± 0.05[a]	0.19 ± 0.01[a]	0.02 ± 0.01[a]	10.08 ± 1.68[a]
Freeze-Thawed Cycle 1	5.12 ± 0.14[a]	0.07 ± 0.02[a]	0.99 ± 0.14[a]	0.33 ± 0.15[a]	0.22 ± 0.02[a]	0.0001 ± 0.0000[a]	8.76 ± 1.22[a]
Freeze-Thawed Cycle 3	5.66 ± 0.11[b]	0.14 ± 0.04[b]	1.94 ± 0.23[b]	0.69 ± 0.18[b]	1.29 ± 0.12[b]	–0.0011 ± 0.0001[b]	9.65 ± 0.71[a]

[a,b] Mean values (n= 30) with the same letter within the same column are not significantly different ($p \geq 0.05$) using Scheffe grouping

TPA

The TPA results of freshly cooked (C) and freeze-thawed cooked rice (FTC 1 and FTC 3) samples were shown in Table 1. Raw grain, quick-frozen cooked rice, and 5th cycle freeze-thawed quick-frozen cooked rice could not be subjected to the TPA analysis since the sample hardness was beyond the maximum instrument measurement capacity of 5.00 ± 0.50 kgf.

The results showed that hardness, cohesiveness, springiness, gumminess, and chewiness significantly ($p < 0.05$) increased after FTC 3. Conversely, the adhesiveness value significantly ($p < 0.05$) decreased after samples were exposed to FTC 3. Results on stiffness values were not conclusive.

DSC

The DSC results shown in Figure 1 illustrated that rice samples exhibited endothermic reactions except for the freshly cooked and quick-frozen cooked rice, which remarkably had no peaks depicted in the same temperature range.

The raw rice grain curve was found to have a broad endotherm and the peak was noticeable between 80–90 °C. Moreover, it was observed that there was a notable shifting (decreasing) of endotherms as FTC is increased.

DISCUSSION

SEM

Structurally, starch granular appearance with two distinct areas, smooth (Figure 1A–1) and rough (Figure 1A–2) were observed in the SEM image cross-section (Figure 1A) of raw rice grain. These two distinct areas were hypothesized by Buggenhout *et al.* (2013) as starch granules of different orientations. While the observed fissures and cracks in the surface of raw grain (Figure 1F) were attributed to the loss of water through the course of maturation of the grain (Ogawa *et al.* 2003) and will eventually function as water passages during cooking (Jung *et al.* 2017). Moreover, the appearance of pores on both cross-section (Figure 1B) and surface (Figure 1G) images of quick-frozen cooked rice was reported to

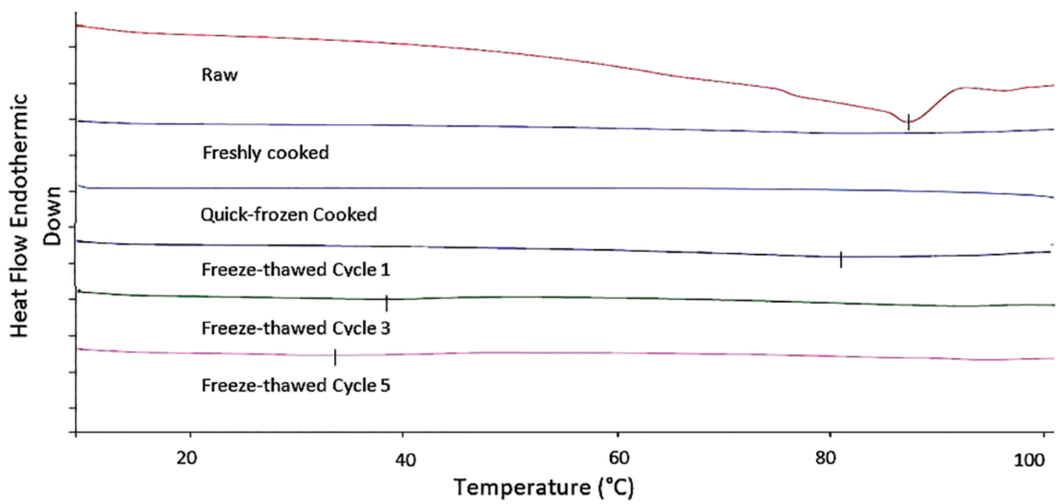

Figure 2. Endothermic heat flow diagram of raw, freshly cooked, quick-frozen cooked, and FTC 1, 3, 5 rice samples.

represent the emptied starch granule periphery resulted from the leaching of starch into the water during cooking (Yang *et al.* 2016; Jung *et al.* 2017). These dissolved starches then form films on the surface producing a smooth appearance (Ogawa *et al.* 2003; Jung *et al.* 2017).

On the other hand, samples subjected to continuous freezing and thawing resulted in the prominent reappearance of fissures and cracks on the cross-section (Figure 1C–E) and increasing level of porosity on the surface (Figure 1H–J). It is assumed that the prominent reappearance of fissures and cracks on the cross-section (Figure 1C–E) were attributed to the result of moisture redistribution and starch molecules association during retrogradation (Jung *et al.* 2017), and the increase in porosity and formation of honeycomb structures are results of surface film removal during successive thawing through syneresis. Similarly, the same findings of honeycombing were also obtained by Ye *et al.* (2016). This honeycomb structure may be responsible for the spongy-like texture of severely retrograded cooked rice.

TPA

As shown in Table 1, results in all the parameters were found to have high standard deviations and, therefore, indicate a highly dispersed data sets. This high variability in results may be attributed to the heterogeneity and varying starch composition of each rice kernel samples. However, despite the variability, statistical differentiation was obtained.

The results showed all parameters, except for adhesiveness, increased with repetitive FTC. The notable increase in the 5 TPA parameters could possibly be related to the reassociation of starch molecules during the cycles; on the other hand, the decrease in adhesiveness may be attributed to the removal of leached starches through syneresis during thawing. The observed textural changes may indicate the starch structural damage due to retrogradation during FTCs (Perdon *et al.* 1999; Yu *et al.* 2010b; Wang *et al.* 2013; Katekhong and Charoenrein 2014; Zambrano *et al.* 2016; Trithavisup and Charoenrein 2018).

DSC

The DSC results showed that all rice samples exhibited an endothermic heat flow (Figure 2) except for the freshly cooked and quick-frozen cooked rice. Broad endotherm was observed in the raw grain sample. The DSC parameter setting used was not able to capture the complete curve of the raw grain sample. Apparently, To and Tp were only obtained. Tc and ΔH, which are needed for the calculation of %DR of all other test samples, were not detected. The broadness of the endotherm may be attributed to the intact physical structure of raw grain, where compartments such as subaleurone and endosperm layers are present and may serve as natural barriers that retard heat and water penetration (Normand and Marshall 1989) during the analysis.

Freshly cooked and quick-frozen cooked rice samples, on the other hand, showed no peaks. The complete gelatinization of starch granules during cooking (Nakazawa *et al.* 2014) and hindered the movement of starch chains as a result of quick freezing (Yu *et al.* 2010; Charoenrein and Udomrati 2013) may have caused the absence of endothermic heat flow curve.

Furthermore, with repetitive FTC, notable shallowing and shifting of endotherms towards decreasing temperature with respect to the To and Tp were observed. This may indicate instability and non-uniformity of the crystallites due to the several irreversible structural and functional transformations during freezing and thawing (Tao *et al.* 2015). Also, several studies reported that there is a disruption of crystalline and molecular order of starch that causes the decrease of transition temperatures upon retrogradation (Zambrano *et al.* 2016; Jung *et al.* 2017; Zhu *et al.* 2017).

CONCLUSION

The study evaluated the changes in textural, structural, and thermal properties of the quick-frozen cooked rice. This study strongly established that repeated FTC caused substantial deteriorative changes in the quality of the quick-frozen cooked rice. Significant deteriorative changes in structure, texture, and thermal properties were prominently noted after the 3rd FTC. Therefore, quick-frozen cooked rice should be subjected to less than 3 FTC to achieve quality cooked rice. Moreover, the three test parameters (SEM, TPA, and DSC) were good indices for determining the degree of structural, textural, and thermal quality changes of the rice samples.

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