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# Starch retrogradation studied by thermogravimetric analysis (TGA)

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ABSTRACT

Thermogravimetric analysis (TGA) as an analytical method to follow retrogradation properties of rice starch was investigated in this study. Our results showed that the bound water content ($M_b$) of retrograded starch samples was significantly increased with the increasing of storage time. Based on the measurement of bound water, degree of starch retrogradation was determined, and it was consistent with the data obtained from differential scanning calorimetry (DSC). The degree of starch retrogradation determined from the TGA was further employed to study starch recrystallization kinetics, indicating that the recrystallization data were well suited to Avrami theory ($R > 0.98$). The results also found that the absorbed water content ($M_a$) was significantly reduced for the retrograded starch ($P \leq 0.05$). These findings suggest that the TGA as well as the DSC is able to provide potential data for evaluating starch retrogradation.



## 1. Introduction

Starch retrogradation is an unavoidable phenomenon for the texture and digestibility of many ready-meals or precooked chilled foods (Miles, Morris, Orford, & Ring, 1985; Rendon-Villalobos, Bello-Pérez, Osorio-Díaz, Tovar, & Paredes-López, 2002). Retrograded starch gels show altered properties, such as lower solubility, lower susceptibility to acid and enzyme hydrolysis, and lower ability to form complex with iodine ions (Karim, Norziah, & Seow, 2000; Leloup, Colonna, Ring, Roberts, & Wells, 1992; Tian et al., 2011). There have been a number of useful techniques developed for evaluating retrogradation and other thermal properties of retrograded starches during food storage. These techniques include, but are not limited to, pulsed nuclear magnetic resonance (PNMR), X-ray diffraction (XRD), near-infrared spectroscopy (NIRS), far-infrared spectroscopy (FIRS), size-exclusion high performance liquid chromatography (SE-HPLC), atomic force microscopy (AFM), differential scanning calorimetry (DSC), turbidimetric assay, and enzymatic susceptibility (Bao, Shen, & Jin, 2007; Karim et al., 2000; Tian, Li, et al., 2010; Tian, Yang, et al., 2010; Yao & Ding, 2002).

TGA is widely employed to evaluate the stability of starch and starch/other component blends because of their simplicity and effective information provided by a simple thermogram. Aggarwal and Dollimore (1997) and Teramoto, Motoyama, Yosomiya, and Shibata (2003) used the TGA method to investigate the thermal stability of the main starch components and found that there was difference in thermal resistance between amylose and amylopectin in corn starch. Principally based upon the different thermal resistance of the two main components, a calibrated TGA technique has been developed for estimating the contents of amylose and amylopectin in potato starches (Stawski, 2008). Tomassetti, Campanella, and Aureli (1989) and Liu, Yi, and Feng (1999) also employed the TGA to determine the contents of bound water in maize and wheat starches via the analysis of mass loss in the first decomposition and concluded that TGA was an effective and accurate method for the measurement of bound water. Nevertheless, the relationship between the bound water and the storage time of starch gels has not yet been explored.

The objective of this study therefore was to follow retrogradation by measuring the bound water and evaluate the absorbed moisture content ($M_a$) of the retrograded starch. The retrogradation degree determined from the TGA was also compared to that evaluated from the DSC.

## 2. Experimental

### 2.1. Materials

Normal rice starch I (SI), normal rice starch II (SII), and waxy rice starch III (SIII) were isolated from rice grains of different lines (Shandong Mei-Jing Rice Inc., China) according to the protocols described by Sodhi and Singh (2003). They contained 29.3%, 13.5%, and 1.9% amylose, respectively. All other chemicals and solvents were of analytical grade unless otherwise noted.

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### 2.2. Preparation of retrograded starch samples

Three grams of SI were mixed with 6 mL of distilled water in a beaker with a cover and heated in boiling water for 30 min. The gelatinized gel (around 60% water content, w/w) was stored at 4 °C in a sealed container for 0, 1, 3, 7, 14, 21, and 35 days to perform the aging study. These gels were dried at 32 °C for 6 h in a vacuum oven. The dry samples were then milled to pass through a 100-mesh sieve and equilibrated in a sealed vessel with saturated sodium chloride for one week. Other gelatinized and retrograded samples were prepared from SII and SIII under the same treatment.

### 2.3. Thermogravimetric analysis (TGA)

Thermogravimetric analysis was conducted with a Mettler-Toledo TGA/SDTA851E. The operation was performed from 30 °C to 500 °C at a heating rate of 10 °C/min under nitrogen. A sample mass of 6 mg was kept throughout the test. The mass loss at the first decomposition stage (30–190 °C) was analyzed by a level leap tool of the machine. The bound water content ($M_b$) and the moisture content ($M_a$) absorbed during the operation were directly converted from the mass loss.

### 2.4. Differential scanning calorimetry (DSC)

Thermal analysis was performed using a Pris 1 DSC (Pekin-Elmer Inc., USA) under ultrahigh-purity nitrogen atmosphere. Three milligrams of each prepared sample and 6 μL of distilled water were mixed together and sealed into aluminum pan. The resultant suspension was equilibrated at 25 °C for 6 h and then scanned from 25 °C to 100 °C at a constant rate of 8 °C/min to collect the enthalpy change ($\Delta H$) in the phase transition. The degree of retrogradation was calculated as Eq. (1) described by Tian, Li, et al. (2010).

$$DR(t) = \frac{\Delta H_t - \Delta H_0}{\Delta H_\infty - \Delta H_0} \quad (1)$$

where $DR(t)$ indicates the crystalline fraction (%) developed at storage time $t$, $\Delta H_0$ is the enthalpy change (J/g) at storage time zero (the freshly gelatinized sample), $\Delta H_t$ is the enthalpy change at storage time $t$, and $\Delta H_\infty$ is the enthalpy change of the 35-day retrograded starch.

### 2.5. Statistical analysis

Statistical analysis was performed using ORIGIN 8.0 program (OriginLab Inc., USA). Data were expressed as means ± standard deviations of at least three determinations on one starch for each time period and analyzed by a one-way analysis of variance (ANOVA).

## 3. Results and discussion

### 3.1. Mass loss versus temperature curves

The mass loss at the first decomposition stage was 10.7% (w/w, wet basis) for the gelatinized starch prepared from normal rice starch with high-level amylose, while it was significantly increased to 14.5% (w/w, wet basis) for the 35-day retrograded sample (Fig. 1a). For the other two starches (SII and SIII), there was also higher mass loss observed for the retrograded starch (Fig. 1b and c). Liu et al. (1999) reported that the mass loss was ascribed to the decomposition of bound water in the starch samples. Therefore these results indicated that the higher bound water was presented for the retrograded starch than the gelatinized one. Furthermore, it is evident that the bound water was positively correlated with the retrogradation time of the tested samples (Fig. 1). This correlation

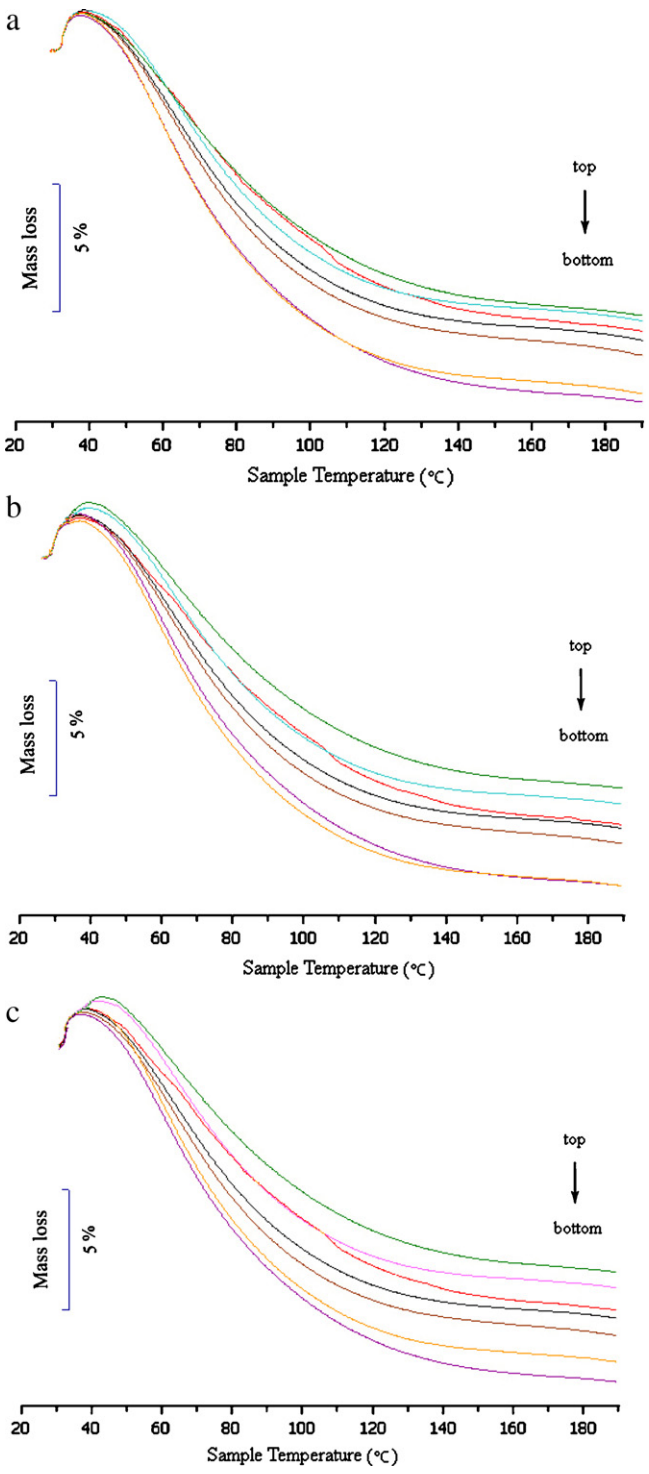


**Fig. 1.** Curves of sample mass loss plotted against heating temperature for different samples, (a) normal rice starch I with high-level amylose, (b) normal rice starch II with medium-level amylose, and (c) waxy rice starch with low-level amylose. Curves from top to bottom representing samples with storage time of 0, 1, 3, 7, 14, 21, and 35 days.

suggests that the measurement of bound water might be developed as a method to follow starch retrogradation.

The potential retrogradation degree ($DRp$) of starch determined by the TGA was preliminarily defined as Eq. (2) in this study.

$$DRp(t) = \frac{Mb_t - Mb_0}{Mb_\infty - Mb_0} \times 100\% \quad (2)$$

where the $DRp(t)$ represents the crystallinity of the $t$-day retrograded starch, $Mb_t$ is the bound water for the $t$-day retrograded starch, $Mb_0$ is the bound water for the freshly gelatinized starch, and $Mb_\infty$ is the bound water of the 35-day retrograded starch.

### 3.2. Comparison of the retrogradation degree determined by the TGA and the DSC

The retrogradation degree of all tested samples determined by the TGA and the DSC is illustrated in Fig. 2. The results showed that the retrogradation degree obtained from the TGA was higher than that from the DSC for each sample prepared from normal rice starch with high-level amylose (Fig. 2a). The higher value was attributed to the total retrogradation of both amylose and amylopectin determined from the TGA, while only amylopection retrogradation that could be examined by the DSC (Russell, 1987). For each sample prepared from rice starches with medium-level and low-level amylose (SII and SIII), there was no significant difference observed in retrogradation degree, although the value determined by the TGA was slightly higher than that by the DSC (Fig. 2b and c). This phenomenon could be partly interpreted by the fact that the content of amylose crystallite could be omitted since the formed crystallite was frequently considered as a nucleus for amylopectin retrogradation under the lower amylose condition (Gudmundsson, 1994). These results suggest that the TGA as well as the DSC was suitably employed to evaluate the retrogradation degree of starch samples.

### 3.3. Recrystallization kinetic parameters obtained from the TGA and the DSC

Many researchers have employed the Avrami theory to investigate the kinetics of starch recrystallization (Jouppila, Kansikas, & Roos, 1998; Mua & Jackson, 1998). The Avrami equation and its rearranged terms in this work are expressed as follows:

$$1 - \theta = \exp(-kt^n) \tag{3}$$

$$\theta = DRp(t) \quad \text{or} \quad DR(t) \tag{4}$$

$$\ln[-\ln(1 - DRp(t))] = \ln k + n \ln t \quad \text{or}$$

$$\ln[-\ln(1 - DR(t))] = \ln k + n \ln t \tag{5}$$

$$\tau_{1/2} = \left(\frac{-\ln 0.5}{k}\right)^{1/n} \tag{6}$$

where $DRp(t)$ and $DR(t)$ represent the retrogradation degree of the $t$-day retrograded samples determined by the TGA and the DSC, respectively; $n$ is the Avrami exponent obtained from the slope of $\ln[-\ln(1 - DRp(t))]$ or $\ln[-\ln(1 - DR(t))]$ versus $\ln t$ plot; $k$ is the rate constant evaluated from the intercept of the plot; and $\tau_{1/2}$, half-time for crystallization, is considered as the time required to achieve 50% of the leveling-off extent of crystallinity.

The results showed that recrystallization kinetics data, obtained from the TGA and the DSC at the required temperature (4 °C), were well fitted to the Avrami equation as all regression coefficient ($R$) values were higher than 0.98 (Table 1). The values of the Avrami exponent ($n$) obtained from the TGA varied from 1.09 to 1.38 and were higher for the samples prepared from waxy rice starch with lower amylose. This result was consistent with the earlier suggestions of Mua and Jackson (1998), who reported that the $n$ values of some starches with lower amylose were higher than 1 and the nucleation type of starch recrystallization was close to rod-like growth of sporadic nuclei. Furthermore, it was found that the rates of recrystallization ($k$) obtained from the TGA and the DSC for the three tested starches were arranged in the order as follows: SI > SII > SIII, while the half-time ($\tau_{1/2}$) for the crystallization was shown in the following order: SI < SII < SIII. These results suggest that the recrystallization data obtained from the TGA were able to evaluate recrystallization kinetics of rice starch.

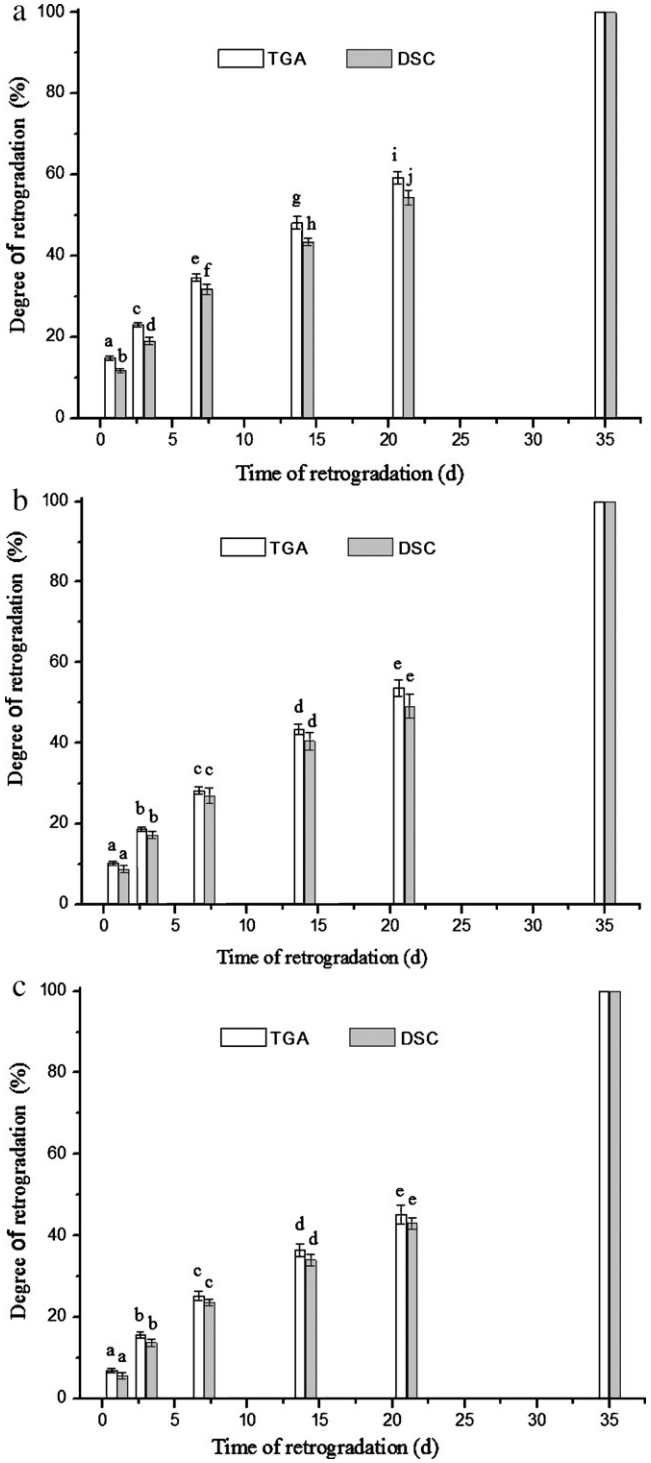


**Fig. 2.** Comparison of $DR$ values (%) determined by the TGA and the DSC, (a) normal rice starch I with high-level amylose, (b) normal rice starch II with medium-level amylose, and (c) waxy rice starch III with low-level amylose. Sample means with different lower case letters are significantly different at $P \leq 0.05$.

### 3.4. Effect of storage time on the absorbed water content

The results showed that the absorbed moisture content ($M_a$) of the tested samples was significantly decreased with the increasing

**Table 1**
Recrystallization kinetic parameters obtained from the TGA and the DSC at 4 °C for different rice starch samples.

| Samples | Methods | Kinetic parameters[b] | | | $R$ |
|---|---|---|---|---|---|
| | | $n$ | $k$ (day$^{-1}$) | $\tau_{1/2}$ (days) | |
| SI[a] | TGA | 1.09 ± 0.03a | 0.036 ± 0.003a | 15.1 ± 0.6a | 0.9804 |
| | DSC | 1.11 ± 0.02a | 0.032 ± 0.002a | 15.9 ± 0.4a | 0.9836 |
| SII | TGA | 1.22 ± 0.04b | 0.020 ± 0.002b | 18.3 ± 0.4b | 0.9903 |
| | DSC | 1.25 ± 0.03b | 0.018 ± 0.003b | 18.6 ± 0.2b | 0.9921 |
| SIII | TGA | 1.33 ± 0.04c | 0.013 ± 0.001c | 19.9 ± 0.3c | 0.9845 |
| | DSC | 1.38 ± 0.05c | 0.011 ± 0.002c | 20.1 ± 0.5c | 0.9877 |

[a] SI, normal rice starch I with high-level amylose; SII, normal rice starch II with medium-level amylose; SIII, waxy rice starch III with low-level amylose.
[b] Sample means with different lower case letters in the same column are significantly different at $P \leq 0.05$.

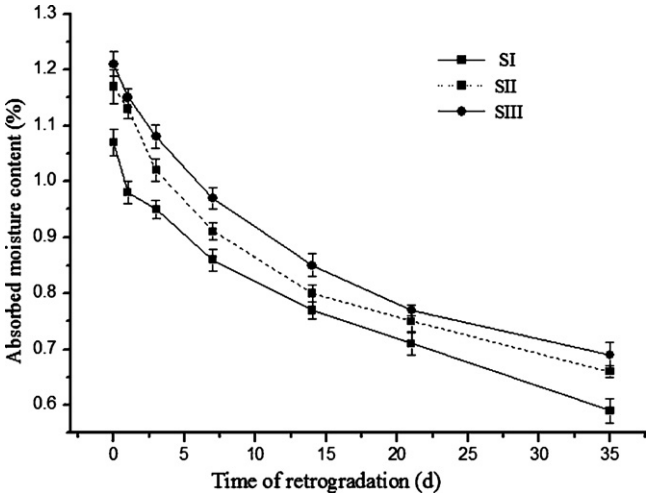


**Fig. 3.** Effect of storage time on the moisture content absorbed during the TGA operation.

of retrogradation time (Fig. 3). This indicated that the retrograded starch prevented the free water molecules from incorporating. The prevention in retrograded samples was attributed to the formed crystalline area that had lower capability to accommodate water molecules (Leloup et al., 1992). On the other hand, the crystallinity of the retrograded starch could obtain a maximum value in a designated starch/water environment (Slade & Levine, 1991). This indicated that the channel of the crystalline area might become more hydrophobic than that of the amorphous area. The hydrophobic channel could reduce the ability to hold water molecules and even make them separated.

## 4. Conclusions

This study made it clear that the TGA was suitably used to determine the total starch retrogradation of both amylose and amylopectin by measuring the bound water and reflected that the recrystallization data obtained from the TGA were well suited to the Avrami equation, as also were the data analyzed from the DSC. These results suggest that the TGA is applicable as a simple and accurate analytical method for evaluating starch retrogradation in food industry.

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## References

Aggarwal, P., & Dollimore, D. (1997). The combustion of starch, cellulose and cationically modified products of these compounds investigated using thermal analysis. *Thermochimica Acta*, *291*, 65–72.
Bao, J., Shen, Y., & Jin, L. (2007). Determination of thermal and retrogradation properties of rice starch using near-infrared spectroscopy. *Journal of Cereal Science*, *46*, 75–81.
Gudmundsson, M. (1994). Retrogradation of starch and the role of its components. *Thermochimica Acta*, *246*, 329–341.
Jouppila, K., Kansikas, J., & Roos, Y. H. (1998). Factors affecting crystallization and crystallization kinetics in amorphous corn starch. *Carbohydrate Polymers*, *36*, 143–149.
Karim, A. A., Norziah, M. H., & Seow, C. C. (2000). Methods for the study of starch retrogradation. *Food Chemistry*, *71*, 9–36.
Leloup, V. M., Colonna, P., Ring, S. G., Roberts, K., & Wells, B. (1992). Microstructure of amylose gels. *Carbohydrate Polymers*, *18*, 189–197.
Liu, Z., Yi, X., & Feng, Y. (1999). Effect of bound water on thermal behaviors of native starch, amylose and amylopectin. *Starch/Stärke*, *51*, 406–410.
Miles, M. J., Morris, V. J., Orford, P. D., & Ring, S. G. (1985). The roles of amylose and amylopectin in the gelation and retrogradation of starch. *Carbohydrate Research*, *135*, 271–281.
Mua, J. P., & Jackson, D. S. (1998). Retrogradation and gel textural attributes of corn starch amylose and amylopectin fractions. *Journal of Cereal Science*, *27*, 157–166.
Rendon-Villalobos, R., Bello-Pérez, L. R., Osorio-Díaz, P., Tovar, J., & Paredes-López, O. (2002). Effect of storage time on in vitro digestibility and resistant starch content of nixtamal, masa, and tortilla. *Cereal Chemistry*, *79*, 340–344.
Russell, P. L. (1987). The ageing of gels from starches of different amylose/amylopectin content studied by differential scanning calorimetry. *Journal of Cereal Science*, *6*, 147–158.
Slade, L., & Levine, H. (1991). Beyond water activity: Recent advances based on an alternative approach to the assessment of food quality and safety. *Critical Reviews in Food Science and Nutrition*, *30*, 115–360.
Sodhi, N. S., & Singh, N. (2003). Morphological, thermal and rheological properties of starches separated from rice cultivars grown in India. *Food Chemistry*, *80*, 99–108.
Stawski, D. (2008). New determination method of amylose content in potato starch. *Food Chemistry*, *110*, 777–781.
Teramoto, N., Motoyama, T., Yosomiya, R., & Shibata, M. (2003). Synthesis, thermal properties, and biodegradability of propyl-etherified starch. *European Polymer Journal*, *39*, 255–261.
Tian, Y., Bai, Y., Li, Y., Xu, X., Xie, Z., & Jin, Z. (2011). Use of the resistance effect between retrograded starch and iodine for evaluating retrogradation properties of rice starch. *Food Chemistry*, *125*, 1291–1293.
Tian, Y., Li, Y., Xu, X., Jin, Z., Jiao, A., Wang, J., & Yu, B. (2010). A novel size-exclusion high performance liquid chromatography (SE-HPLC) method for measuring degree of amylose retrogradation in rice starch. *Food Chemistry*, *118*, 445–448.
Tian, Y., Yang, N., Li, Y., Xu, X., Zhan, J., & Jin, Z. (2010). Potential interaction between β-cyclodextrin and amylose–lipid complex in retrograded rice starch. *Carbohydrate Polymers*, *80*, 581–584.
Tomassetti, M., Campanella, L., & Aureli, T. (1989). Thermogravimetric analysis of some spices and commercial food products. Comparison with other analytical methods for moisture content determination (Part 3). *Thermochimica Acta*, *143*, 15–26.
Yao, Y., & Ding, X. (2002). Pulsed nuclear magnetic resonance (PNMR) study of rice starch retrogradation. *Cereal Chemistry*, *79*, 751–756.