Artificial saliva-induced structural breakdown of rice flour gels under simulated chewing conditions

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Abstract Rice flour gels with different amylose contents were subjected to instrumental compression cycles under artificial saliva-spraying conditions to simulate oral mastication and their structural breakdowns were evaluated in terms of rheological and tomographic characteristics. Both mechanical disruption of the gel structure by successive compressions and enzymatic degradation by artificial saliva featured in the simulated chewing process. Highly linear correlations ($R^2 > 0.95$) were observed in the log plots of peak chewing force and time. The rice flour gels containing higher levels of amylose required high forces to be deformed by compression. Micro-CT analysis demonstrated that the rice flour gels with a weak cohesive texture were fragmented into smaller pieces by chewing, consequently providing channels for artificial saliva to penetrate inside the gel samples. The cohesive nature of foods appeared thus to play an important role in their disruption rate during chewing, probably influencing the masticatory performance with saliva and subsequent swallowing.

Keywords Artificial saliva · Structural breakdown · Rice flour gels · Texture · Simulated chewing

Introduction

The digestion of multiple types of food has received considerable scientific attention since it plays important roles in their absorption and bioavailability. Although human studies provide direct and accurate information on digestion in a body, *in vivo* studies are not readily carried out due to several limitations such as ethics, regulation, and expenses (Minekus et al., 2014). Therefore, *in vitro* methods with a wide variety of enzymes have been extensively applied as a useful alternative to assess the digestion of food (Hur et al., 2011; Woolnough et al., 2008). The primary sites of digestion in the human digestive system are the oral cavity, stomach, and intestine. Hence, the *in vitro* simulated digestion testing generally consists of the oral, gastric, and intestinal steps. However, most of these digestion studies do not encompass chewing behaviors in the oral phase due to their complexities (Low et al., 2015). Therefore, the inclusion of the chewing process may contribute to enhancing the study of digestion by closely simulating the actual digestion process.

Based on preceding studies (Minekus et al., 2014), food mastication is simulated by incorporating the size reduction process, followed by incubation with human saliva α -amylase. The methods for size reduction included actual chewing by volunteers (Hoebler et al., 2000) and mechanical treatments such as cutting (Tudorica et al., 2002) and mincing (Mandalari et al., 2013). In addition, several masticatory devices have been developed by simulating human jaw movements with the assistance of mechanical devices (Ng et al., 2017; Salles et al., 2007; Xu et al., 2008). Although these methods can provide a valuable insight for better understanding of oral processing, they do not fully simulate the actual oral digestion process where both disruption by mastication (chewing) and

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wetting/lubrication by salivation take place simultaneously, forming a bolus for swallowing. Furthermore, most of the studies reported in the literature were carried out in the field of clinical science and could not be favorably compared due to methodological variations (Jalabert-Malbos et al., 2007; Woda et al., 2006). Therefore, there is a need to develop food industry-friendly approaches to simulate the actual chewing process for practical food applications.

In this study, the chewing process was simulated by incorporating artificial saliva-food interaction into instrumental texture measurements. Specifically, rice flour gels were prepared with three rice varieties with different amylose contents and their disintegration patterns during instrument texture measurement with artificial saliva were characterized in terms of rheological and tomographic properties.

Materials and methods

Materials

Three different varieties of rice flours (Backjinju, Hanareum, and Milyang harvested in 2014) were provided from the National Institute of Crop Science (Rural Development Administration, Suwon, Korea). The rice flours were obtained by dry-milling with an air-jet miller (Air-Classification Mill, ACM185, Hankook Crusher Co., Korea) and sieving (100–150 mesh). They had different amylose contents that were determined to be 10.2, 17.7, and 25.5%, respectively (Jeong et al., 2016). The rice flour samples were respectively designated as low, intermediate, or high amylose rice flour depending on their amylose contents.

Preparation of rice flour gels and artificial saliva

Rice flour gels were prepared by mixing each variety of rice flour with distilled water at 30% (w/w) and heating them in a boiling water bath with agitation for 20 min. The hot paste was then poured into a cylindrical mold (diameter 2.6 cm, height 2 cm) and cooled in a refrigerator for 24 h to prepare the gels. Based on the method of van Ruth et al. (2001), artificial saliva was composed of NaHCO_3 (5.208 g), $\text{K}_2\text{HPO}_4 \cdot 3\text{H}_2\text{O}$ (1.369 g), NaCl (0.877 g), KCl (0.477 g), $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ (0.441 g), mucin (2.160 g), and α -amylase (200,000 Unit, hog pancreas α -amylase, Sigma-Aldrich, St. Louis, Missouri, USA) in 1 L of distilled water. The salivary pH was adjusted to 7.0 with 2 M HCl.

Rheological measurement during in vitro simulated oral digestion

Each rice flour was mixed with distilled water (12%, w/w) in an aluminum canister and heated by increasing the temperature to 90 °C with a rapid visco-analyzer (RVA4, Newport Scientific, Sydney, Australia). When the rice flour sample was cooled down to 37 °C, the artificial saliva (250 units (α -amylase)/g sample) was added in the canister and the viscosity was then monitored at 37 °C for 60 s, based on the method of Jeong et al. (2016).

Instrumental setup for simulated chewing experiments

An instrumental system was developed to establish a simulated chewing environment. Figure 1A illustrates the experimental set-up used for the instrumental masticatory measurements. It was composed of an artificial saliva-spraying box and texture analyzer (TA-XT Plus, Stable Micro-Systems, Surrey, UK). The artificial saliva box (W: 70 mm, L: 70 mm, H: 40 mm) was custom-made of Plexiglas plates with two ultrasonic oscillators (Likeme, Inc., Seoul, Korea) on opposite sides. Artificial saliva was homogeneously nebulized onto the gel surface from the ultrasonic oscillators connected with a beaker containing artificial saliva by a pump (BT 100-1 F. Baoding Long Peristaltic Pump Co., Ltd., Hebei Province, China).

Texture measurement during simulated chewing process

Each rice flour gel (diameter 2.6 cm, height 2 cm) was loaded in the artificial saliva box placed on a texture analyzer (TA-XT plus, Stable Micro- Systems) with a cylindrical probe (5 cm diameter). The gel sample was subjected to 20 successive compressions where the texture profiles of the artificial saliva-treated and untreated samples became distinctly distinguishable from the preliminary experiments. During the texture measurement, the artificial saliva was sprayed onto the gels at a rate of 4.5 mL/min, determined from the study of Jo (2016). The crosshead speed and strain used were 100 mm/min and 60%, respectively. The force and time values were collected at the rate of 250 Hz. Figure 1B exhibits the plots of peak force versus time of a rice gel sample that were converted into a linear form on a log scale. The slope values from the logarithmic plots were obtained as degradation rate.

X-ray micro-computed tomographic (CT) analysis

The structural changes of rice flour gel samples were characterized using the desktop X-ray micro-CT system

Fig. 1 Instrumental simulation of (A) oral breakdown and (B) texture profile of a rice flour gel

(Skyscan 1174, Bruker, Kontich, Belgium). After the instrumental mastication, the samples were placed on a plate of the micro-CT and then rotated 180° at a rotation step of 1° . The operating conditions were a voltage of 50 kV, current of $800 \mu\text{A}$, and 7500 ms exposure time. The tomograms (1024×1024 pixels) were reconstructed and analyzed using the Skyscan software package.

Statistical analysis

All the tests were run in triplicate and the experimental results were statically analyzed with SAS software (SAS Institute, Cary, NC, USA). An analysis of variance (ANOVA) was applied, followed by Duncan's multiple range tests for mean comparisons at a confidence level of 95%.

Results and discussion

Mechanical treatments such as mincing and grinding have conventionally been applied to simulate the breakdown of foods for *in vitro* chewing experiments (Hoebler et al., 2000; Woolnough et al., 2008). The oral digestion of rice

flour gels was thus imitated by addition of artificial saliva with mechanical agitation. Figure 2 exhibits the viscosities of the rice samples after treatment with the artificial saliva. All of them exhibited similar viscosity patterns, showing reduced viscosity over time through enzymatic hydrolysis. The highest viscosity was observed in the high amylose rice samples, followed by the intermediate and low amylose samples. These trends were in good agreement with

Fig. 2 Changes in the viscosity of rice flour gels with different amylose contents during simulated oral digestion

the results of several preceding studies that reported the enzymatic resistance of high amylose starches (Htoon et al., 2009; Shrestha et al., 2010).

Figure 3 exhibits the plots of peak compressive force versus time of rice flour gels with different amylose contents. As shown in Fig. 3, the peak force had a tendency to decrease with increasing times. Specifically, the peak forces were distinctly reduced at the early chewing stage and then became constant or gradually decreased during the

Fig. 3 Force responses of rice flour gels with different amylose contents during simulated chewing [(A) low amylose, (B) intermediate amylose, and (C) high amylose]

further chewing cycles. They could be explained by the features of the simulated chewing process of this study that were both mechanical disruption of the food structure by successive compressions and enzymatic degradation by artificial saliva. It was interesting to note that the decreasing patterns of the peak force seemed to vary depending on both amylose content and the use of artificial saliva. Overall, the rice gel samples containing high levels of amylose showed higher force values. Thus, the rice gels containing higher levels of amylose required higher force to be deformed by the compressions. These results could be explained by the fact that amylose positively contributes to the firmer structural formation of the gels (Sandhu and Singh, 2007). In addition, the artificial saliva-treated samples exhibited significantly lower values of peak force that also continued to be reduced during the compressions. These results clearly indicated that the artificial saliva-treated samples became softer during the compression cycles.

Table 1 presents the degradation rate of the artificial saliva-untreated and treated gel samples made from rice flours with different amylose contents. When the gel samples were treated with artificial saliva, the absolute values of their degradation rate were significantly higher compared to the artificial saliva-untreated samples. Since the slope values could provide a good approximation to the degradation response of the gel samples by the simulate chewing, it suggested that the gel samples with artificial saliva became much softer during the compression cycles. It was interesting, however, that the degradation rate varied depending on the amylose content. The highest degradation rate was observed in the high amylose rice gels while the intermediate amylose samples exhibited the lowest degradation rate, rather than the low amylose gels. Thus, the structural degradation of the rice gel samples was not consistent with their amylose levels and gel hardness.

Table 1 Degradation rate of rice flour gels with different amylose via simulated chewing (means with different letters in the same column differ significantly at $p < 0.05$)

Sample	Degradation rate (N/s)	R^2
Low amylose		
Without artificial saliva	-0.1125 ± 0.0003^c	0.9986
With artificial saliva	-0.1748 ± 0.0051^d	0.9915
Intermediate amylose		
Without artificial saliva	-0.0404 ± 0.0033^a	0.9531
With artificial saliva	-0.1038 ± 0.0081^c	0.9953
High amylose		
Without artificial saliva	-0.0911 ± 0.0118^b	0.9838
With artificial saliva	-0.2724 ± 0.0009^e	0.9500

These results were unexpected since digestibility is recognized to be retarded by high amylose content due to lower susceptibility to enzymatic hydrolysis (Jeong et al., 2016; Sasaki et al., 2009). Therefore, further tomographic studies were carried in order to explain these phenomena.

The inner structures of the rice gels before and after the simulated chewing were non-destructively visualized by using X-ray microcomputed tomography. When X-rays are irradiated into a sample, their attenuations are highly dependent on the density of the sample. Therefore, the sum of all local attenuations along the X-ray beam generates tomographic images where highly dense areas become brighter (Kim et al., 2017). Figure 4 exhibits the two-dimensional images of the rice gel samples before and after the simulated chewing where void spaces were visible as darker areas and solid regions were brighter. As can be seen in Fig. 4, a number of dark regions which correspond to voids were detected in the high amylose rice gel samples after chewing, followed by low and intermediate amylose gels samples. These results suggested that the high amylose rice gels were favorably fragmented into smaller pieces by the simulated chewing. As a result, the interstitial spaces between the disrupted fragments of the high amylose gel samples appeared to provide channels for artificial saliva to penetrate inside the gel samples. On the other hand, the samples containing lower levels of amylose consisted mostly of bright areas (solid parts). Few open spaces were available in the intermediate amylose gels, indicating that they were laterally deformed with minimal structural fragmentation by the simulated chewing, compared to the other two samples.

The compressive methods used in this study belong to large deformation tests that imitate the destructive process occurring in the human mouth. Under large deformation conditions, samples experience a great deal of stress,

consequently being deformed in different ways depending on their mechanical properties. In particular, cohesiveness is one of the important mechanical parameters to maintain structural integrity against compressions (Dabour et al., 2006). Thus, the cohesive nature of the rice gel samples was obtained by the peak area ratio of the first and final compressions although cohesiveness is generally calculated as the ratio of positive peak area during the second compression to that during the first compression (Bourne, 2002). As shown in Fig. 5, the intermediate amylose gel samples exhibited the most cohesive texture while the high amylose ones had the lowest value of cohesiveness. This indicated that it would not take much chewing to break down the high amylose gel samples into smaller pieces, while the intermediate amylose samples seemed to be more tolerant of the compressive deformation and maintain a lumpy structure. The structural degradation of the gels by artificial saliva involves the reactions between the enzymes in the artificial saliva and the solid surface of the gels (Jung et al., 2013). Therefore, it seemed that the gel fragments derived from the compressive deformations were more vulnerable to enzymatic hydrolysis due to the presence of pores and crevices in the structure. These observations could be clearly detected from the micro-CT images as already shown in Fig. 4 where the high amylose rice samples with a weak cohesive texture had a number of black areas while the intermediate amylose samples with strong cohesion showed brighter images. Although the relationship between chewed food particle size and digestibility have hardly been tested on human subjects, Heaton et al. (1988) reported the faster *in vitro* hydrolysis of wheat, maize, and oat starches by pancreatic amylase with decreasing particle size. A preceding animal study (Kononoff et al., 2003) also showed that the reduction of corn silage particle size was effective in increasing starch

Fig. 4 Micro-CT images of rice gels with different amylose contents before and after simulated chewing

Fig. 5 Changes in the cohesive property of rice flour gels with different amylose contents after simulated chewing (means with different letters on the bars differ significantly at $p < 0.05$)

digestibility. Therefore, the cohesive nature of food may influence the degree of disruption into smaller fragments during chewing, possibly playing an important role in the masticatory performance with saliva and subsequent swallowing.

The structural breakdown of rice flour gels with different amylose contents was evaluated under artificial saliva-spraying conditions by instrumental texture measurements. Highly linear lines ($R^2 > 0.95$) were well fitted to the logarithmic plots of peak chewing force and time. Degradation rate of the rice flour gels was more influenced by cohesiveness rather than their hardness and amylose contents. These results were confirmed by the micro-computed tomographic analysis that showed the interstitial spaces between the disrupted fragments of the rice flour gel samples with a weak cohesive nature. The results of this study thus contributed to understanding the changes in food structure by considering the saliva-food interactions under simulated chewing conditions. In further studies, it will be worthwhile to extend this simulated chewing system to a wider variety of food products for the perception and appreciation of their quality attributes such as flavor and texture.

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