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The importance of an oral digestion step in evaluating simulated *in vitro* digestibility of starch from cooked rice grain

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ABSTRACT

To examine the effect of oral digestion step in a simulated *in vitro* starch digestion model, the digestibility of intact, homogenized and actual chewed cooked rice grains was investigated and analyzed. The kinetics of starch digestibility were calculated from changes in the hydrolysis percent of starch that were achieved during simulated small intestinal digestion stage. Morphological and histological microscopic tissue structures were also examined. Compared with the trend of starch hydrolysis changes of the actual chewed grain, 1.3 U/ml of salivary α-amylase concentration treated for 60 min was regarded as a mimicked condition to the simulate *in vitro* oral digestion step in this study. The results showed that the equilibrium percent of starch hydrolysis for all of the samples ranged from 84.2% to 95.9% with no significant differences observed regardless of whether the oral digestion step was included ($p > 0.05$). In contrast, the kinetic constant, which is one of the measure of starch digestion rate during small intestinal stage, significantly increased with the degree of grain homogenization increased: 120 s > actual chewed ≥1 s > intact, for both the gastrointestinal and oral plus gastrointestinal processes. These results indicated that the kinetic constant was influenced by the change of cooked rice grain structure in oral digestion step that would be related to increase in enzyme accessibility to rice starch. Thus, rice grain digestibility was affected by grain-scale structural changes, including grain tissue damages which were normally observed during the oral digestion step.

1. Introduction

A rapid postprandial increase in blood glucose levels is normally observed following the rapid ingestion of carbohydrate-rich foods. When this type of response is consistently induced, there is an increased risk of acquiring life-style related diseases such as type 2 diabetes, hyperlipidemia, and cardiovascular disease (Jenkins et al., 2002; Lehmann & Robin, 2007). In contrast, a slow rate of ingestion of starchy foods may avoid potential problems and may have health benefits. Digestibility of the starches associated with carbohydrate-rich foods is also a relevant consideration.

To investigate the digestibility of starchy foods, both *in vivo* animal models and *in vitro* simulated models have been developed to represent the gastrointestinal digestion system. Recently, the ease of using an *in vitro* simulated digestion process was demonstrated, as well as its flexibility regarding experimental conditions and its capacity for observing changes in food attributes (Tamura, Singh, Kaur, & Ogawa, 2016a, 2016b). Generally *in vitro* approaches consist of a two-stage simulated digestion system in which a gastric stage is established with a low pH and pepsin, and a subsequent small intestinal stage is established with a neutral pH and intestinal enzymes (Dartois, Singh, Kaur, & Singh, 2010). However, an oral digestion step, which is an intrinsic system for the human body, has typically not been considered for *in vitro* studies of starchy food digestibility because the food samples are often prepared as a powder or a slurry in order to reduce the experimental time (Englyst, Kingman, & Cummings, 1992).

Oral digestion as the first step of food digestion has two simultaneous actions: chewing to provide mechanical grinding and enzymatic starch hydrolysis that is mediated by the saliva. After food is crushed into small pieces and mixed with saliva with chewing, then amylase that is present in the saliva is able to act on the starch in the food. Hoebler et al. (1998) reported that approximately 25% of the starch in pasta and 50% of the starch in bread are hydrolyzed during oral starch digestion, thereby making a portion of the starch available for further enzymatic digestion in the gastrointestinal process. Bornhorst and Singh (2013) also demonstrated that α-amylase from saliva plays an important role in the breakdown kinetics of bread boluses in *in vitro* models. Meanwhile, Woolnough, Bird, Monro, and Brennan (2010) reported that prolonged exposure to saliva during the chewing of starchy foods by volunteers did not contribute to the hydrolysis of starch during the gastrointestinal digestion process. Strahler, Mueller, Rosenloecher,

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Kirschbaum, and Rohleder (2010) further reported that the concentration of salivary α-amylase in humans varies with age, psychosocial stress, and other factors, all of which were regarded as highly subjective according to individual conditions.

Rice (*Oryza sativa* L.) is a major source of carbohydrates, especially in Asian countries. Since rice is cooked and consumed as a whole grain, the structural attributes of cooked rice grain influence the digestibility of its starch components (Tamura et al., 2016a, 2016b). However, oral digestion approaches have not been considered and applied to the simulated digestion model for cooked rice grain. In this study, the effects of mimicked oral digestion conditions, including varying degrees of sample homogenization and varying concentrations of α-amylase in simulated salivary fluid, on a simulated *in vitro* starch digestion model were investigated and analyzed in relation to the digestibility of cooked rice starch.

2. Materials and methods

2.1. Materials

Precooked steamed rice packaged as a ready-to-eat meal for the microwave oven (Wooke, Toyama, Japan) was purchased. The rice cultivar used in the product was "Koshihikari" and it was harvested in Nyuzen, Toyama, Japan in 2013. Alpha-amylase (porcine pancreas, type VI-B, ≥ 10 U/mg, solid, A3176-500KU), pepsin (P7000, porcine gastric mucosa, ≥ 250 U/mg, solid), pancreatin (hog pancreas, 4× USP), and invertase (from baker's yeast, grade VII, ≥ 300 U/mg, solid) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Amyloglucosidase (3260 U/ml) was purchased from Megazyme International Ireland (Wicklow, Ireland).

2.2. Sample preparation

To prepare cooked grain samples, 60 g of pre-cooked steamed rice was put in a Ziplock bag and heated in a microwave oven (RE-SX10-W, Sharp, Osaka, Japan) at 500 W for 50 s. The heated sample was then cooled in an incubator (A0601-2 V, Keepit, Nagano, Japan) at 30 °C for 30 min.

To obtain mechanically crushed grain samples as an imitative chewed model, the cooked grain samples were homogenized using a household blender (THM310, Tescom, Tokyo, Japan) for 1 s or for 120 s. In addition, an actual chewed grain model was prepared as previously described (Akerberg, Liljeberg, Granfeldt, Drews, & Bjorck, 1998; Beer, Wood, Weisz, & Fillion, 1997; Germaine et al., 2008; Woolnough et al., 2010). Two volunteers, who were early twenties and thirties and have normal dentition, were employed in this study. They brushed their teeth before chewing. Briefly, 20 g of a grain sample (approximately 6.8 g of total starch content equivalent) was bitten 15 times in 15 s by each volunteer for two replicates. During actual chewing, grain sample was crushed and mixed with salivary α-amylase. The sample was then expectorated and left to sit as chewed boluses.

A small portion of the cooked grain samples were freeze-dried (FDU-1100, Eyela, Tokyo, Japan), then ground using a household mixer (MR-280, Yamazen, Tokyo, Japan) and sieved using a 0.5 mm meshed sieve. The powdered material obtained was used to measure total starch (TS) and resistant starch (RS) contents using a resistant starch kit (K-RSTAR 08/11, Megazyme International).

2.3. Moisture, amylose, and protein content

The cooked grain samples were dried using a hot air oven (WFO-400, Eyela) at 135 °C, and after 24 h, the moisture content in wet basis (w.b.) was calculated. The sample grain was ground using a household mixer (MR-280, Yamazen) and was passed through a 0.5 mm sieve to produce a sample powder. The apparent amylose content of the powder was then measured as previously described (Tamura et al., 2014b). Nitrogen content of the powder samples were measured by using a CN Corder (MT-700, Yanaco, Kyoto, Japan). Crude protein content was calculated from the measured nitrogen content with a nitrogen-protein conversion factor of 5.95. Hippuric acid (200-37032, Kishida Chemical, Osaka, Japan) was used as a standard for the nitrogen measurements.

2.4. Microscopy

Grain shape was observed with a digital microscope (VH-Z-05 & VH-8000, Keyence, Osaka, Japan). Projected area was calculated from a number of pixels that represented grain portion after image binarization. Approximately 500 grains or grain fragments were randomly selected from the cooked grain sample and were manually arranged so that they did not touch each other on the observation stage of the microscope.

To visualize the histological tissue structures of whole size rice grain, the autofluorescent imaging technique was applied (Ogawa, Orts, Glenn, & Wood, 2003a; Tamura & Ogawa, 2012). To obtain morphological and histological images of the grains, a fluorescent stereomicroscope (MZ-FLIII, Leica, Wetzlar, Germany) equipped with a digital camera (DS-5 M, Nikon, Tokyo, Japan) was used. The microscope had a 100 W mercury arc lamp (ebq 100 dc, Leica), an ultraviolet fluorescence filter set (360/40 nm excitation filter, 420 nm barrier filter, Leica) for histological tissue observation, and a halogen lamp for morphological tissue examination. All imaging parameters, including focusing, lighting, and shuttering, were standardized for each sample. The captured images were processed and analyzed with Photoshop CC2014 software (Adobe, San Jose, CA, USA).

2.5. Particle size

Particle size distribution for the grain samples that were homogenized for 120 s was determined using a laser diffraction particle size analyzer (SALD-3100, Shimadzu, Kyoto, Japan). The applied relative refractive index was 1.70.

2.6. Simulated digestive fluid

Simulated salivary fluid (SSF) was prepared as previously described (Beer et al., 1997; Tamura, Kumagai, & Ogawa, 2013) and examined preliminarily. Briefly, 5 mg of α-amylase was dissolved in 1 ml of 0.036 mol/l calcium chlorite as a standard SSF in which the final α-amylase concentration was 0.3 U/ml. Two additional SSF standards were generated with 10 mg and 20 mg of α-amylase, resulting in final α-amylase concentrations of 0.7 U/ml and 1.3 U/ml, respectively. Simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) were prepared according to the method of Dartois et al. (2010). The SGF contained pepcine. The SIF contained pancreatine, invertase, and amyloglucosidase.

2.7. Simulated in vitro gastrointestinal digestion

A simulated *in vitro* gastrointestinal digestion model was established as previously described (Dartois et al., 2010). Briefly, 170 g of rice sample mixture with distilled water, which was prepared to contain 4% of total starch, was added into a 500 ml jacketed glass reactor and continuously stirred with a magnetic stirrer (Color Squid, Ika, Staufen, Germany) at 350 rpm. When intact grain samples or grain samples homogenized for 1 s were placed in the reactor, they were placed in a polyethylene mesh to prevent direct contact between the samples and the magnetic bar stirring in the reactor. The reactor was connected to a circulatory water bath (NTT-20S, Tokyo Rikakikai, Tokyo, Japan) to maintain a temperature of 37 °C during simulated digestion. A pH meter (AS800, As One, Osaka, Japan) was used to adjust the pH of the liquid mixture to 1.20 ± 0.01. Addition of SGF started the simulated gastric stage and the pH during this stage was continuously maintained with the addition of 3 mol/l HCl solution. After 30 min, the pH was adjusted to 6.00 ± 0.01 with the addition of