Evaluation of digestibility of cooked rice grain using in vitro digestion technique

M. Tamura[1], J. Singh[2], L. Kaur[2], Y. Ogawa[3*]

(1. *Faculty of Agriculture, Utsunomiya University, Utsunomioya, Japan;*
2. *Riddet Institute, Massey University, Palmerston North, New Zealand;*
3. *Graduate School of Horticulture, Chiba University, Matsudo, Japan.*)

Abstract: Impact of postharvest and/or food processing (such as flour milling to agricultural and food-resource materials) on the digestibility of foodstuff was investigated through a simulated gastro-intestinal in vitro digestion technique. The differences among the starch digestibility of cooked rice grain and slurry were examined. In vitro digestion techniques are commonly applied to starch-based milled materials such as flours used for bread making. However, rice is not usually milled to flour but cooked as a whole for consumption. Therefore, maintaining an intact structure of cooked grain during in vitro digestion is very important to evaluate its starch digestibility. Two hundred grams of polished rice grain were cooked using electric rice cooker with 300 ml RO water after soaking for 30 min at 30 ℃. A part of cooked grain was grinded using a household blender to produce cooked rice slurry. The starch hydrolysis of the rice sample was measured and calculated during in vitro digestion process and regarded as sample digestibility (%). Changes in grain tissue structure during in vitro digestion process were also measured and evaluated. The digestion percentage at 90 min of in vitro small intestine digestion for the cooked rice slurry and grain was approximately 88% and 58%, respectively. Furthermore, approximately 76% of digestion percentage for cooked rice slurry was measured within 5 min of small intestinal digestion, whereas the cooked rice grain digestibility ranged at 24% at the same time period. These results indicated that the digestion rate of grain was different from the slurry. To examine the differences in in vitro digestion rate, microstructural changes in the grain during in vitro digestion process were observed using fluorescent microscopic techniques. As a result, the aleurone and the sub-aleurone layers of endosperm appeared as thin-film like materials during in vitro digestion, therefore they may be regarded as less digestive materials during digestion. These results can be useful in predicting the glucose release and other glycaemic properties of whole grain-based, starch-based slurries and milled starchy foods during human digestion.

Keywords: Grain, slurry, tissue structure, cell wall, starch, in vitro digestion, Japan

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1 Introduction

Rice (*Oliza sativa* L.) is usually consumed as cooked grain. Although grain structures of the cooked rice are partly damaged during cooking (Ogawa et al., 2003), almost grain shape and inner cell matrix, which enveloped starchy substances, can be maintained (Tamura & Ogawa, 2012, Tamura et al., 2014). The grain structures are destructed by mastication when they are ingested and masticated at the human oral cavity. However, cell scale structures tend to be maintained even if the grain is compressed by simulated mastication using mechanical compression test (Ogawa et al., 2006). Bornhorst et al. (2013a; 2013b) reported a tissue structure of cooked rice grain was partially maintained when the grain was grinded in stomach by in vivo gastric digestion studies. Usual procedures for in vitro digestive studies of starchy foodstuffs, samples are prepared as powder or slurry state due to reduction of experimental time (Englyst et al., 1992). However, Muir and O'dea (1993) and Woolnough et al. (2008) discussed it was necessary to establish a standard method for physical destruction of digestive samples concerning mastication effect with structural change.

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***Corresponding author: Yukiharu Ogawa,** Graduate School of Horticulture, Chiba University, 648, Matsudo, Matsudo 271-8510, Japan. Email: ogwy@faculty.chiba-u.jp

Arai et al. (2010) reported that cooked rice grain digestibility was involved not only properties of apparent amylose and chain length of amylopectin but also indigestible cell wall in the starch endosperm. However, less study can be found about digestibility of starchy foodstuffs maintaining their tissue or grain scale structures. In this study, the digestibility of grain of cooked rice, which maintained its histochemical tissue structures, was examined using simulated in vitro digestive method and microscopy approaches.

2 Materials and methods

2.1 Materials

Polished rice grain (Sunrice, Australian medium grain, white rice) was purchased at local store in Palmerston North, New Zealand and stored in a refrigerator at 4℃ before experiment.

2.2 Sample cooking and preparation

Two hundred grams of sample rice grain was soaked into 300 ml RO water for 30 min at room temperature and then cooked using a rice cooker (TK-RC12, Eupa, Tokyo, Japan). The heating time was approximately 16 min, with a 10 min interval to ripen the cooked grain in the rice cooker, and then the cooked grain was cooled down to room temperature for 30 min in the switched off rice cooker. To obtain powdered materials for measurement of total starch content of the cooked grain, a small amount of sample grain after cooling was freeze dried using freeze dryer (FDU1100, Eyela, Tokyo, Japan), ground and sieved using 0.5 mm meshed sieve. The total starch content of the sample powders was determined using a total starch assay kit (K-TSTA 07/11, Megazyme International, Ireland).

To apply in vitro digestion process for the grain sample, a part of cooked grain was placed into a commercial use net-type polyethylene bag, in which grain samples can avoid directly contact with rotated magnetic stirrer bar and can be easily contacted with simulated digestive buffer. Contrary, another part of cooked grain was homogenized using household blender (HB605, Kenwood, Havant, UK) for 2 min to obtain cooked rice slurry, which was usually used to determine the food digestibility (Frei et al., 2003; Li et al., 2014). The slurry can be simply agitated using magnetic stirrer bar during digestion process.

A simulated gastric fluid (SGF) and a simulated small intestinal fluid (SIF) were prepared in accordance with the US pharmacopeia (2000). SGF consisted of 0.12 g pepsin (porcine gastric mucosa, 800-2500 units/mg protein, Sigma-Aldrich Ltd., USA) in 25 ml gastric fluid buffer (2 g NaCl and 7 ml 12M HCl adjusted in 1 L of RO water, pH 1.2) and SIF consisted of 0.1 g pancreatin (hog pancreas, 4× USP, Sigma-Aldrich Ltd), 0.0075 g invertase (Invertase, grade VII from baker's yeast, 401 U/mg solid, Sigma-Aldrich Ltd) and 2 ml amyloglucosidase (3260 U/ml, Megazyme International) in 23 ml intestinal fluid buffer (6.8 g KH2PO4, 77 ml 0.2 N NaOH adjusted in 1 L of RO water, pH 6.8).

2.3 In vitro digestion

A two-stage gastro-intestinal in vitro digestion model (Dartois et al., 2010) was arranged and employed in this study. The prepared cooked rice grain or slurry samples were put into beakers respectively, and added appropriate amount of RO water to reach 4% concentration of their total starch content. One hundred seventy grams of the prepared 4% concentration solutions involving grains or slurry were moved into the identical 500 ml jacketed glass reactors, respectively, and continuously agitated by magnetic stirrer (C-MAG MS 4, Ikamag, Staufen, Germany) in the reactor. The reactor can be maintained its temperature at 37℃ during simulated gastric and intestinal digestive reactions by 37℃ of circulatory water.

The pH of the prepared solutions was measured using pH meter (FE20; Mettler Toledo, Greifensee, Switzerland). The solution pH was adjusted to 1.20 ± 0.01, when the simulated gastric digestion process was regarded to start. The pH was continuously maintained during the process by addition of small amount and several concentrations of HCl solution. After 30 min of

gastric digestive reaction, pepsin was inactivated according to the pH change to approximately 6.0 by addition of appropriate amount and concentration of NaOH solution. To continue the simulated small intestine digestion process, SIF was added to the simulated gastric reaction mixture. And then, the pH was adjusted to 6.80 ± 0.01 using appropriate amount and concentration of NaOH or HCl solutions.

Supernatants (0.5 ml) were collected to analyze the glucose content during the process at 5min and 30 min for simulated gastric digestion, 45 min, 60 min and 90 min for simulated small intestinal digestion. The supernatants were mixed with 95% ethanol (3 ml) to stop the enzyme reaction. The ethanol mixed solutions were centrifuged at 1800 g for 10 min and then incubated for 10 min at 37℃ with amyloglucosidase and invertase (Monro et al., 2009).

The glucose concentration of the incubated mixture was measured using D-glucose assay kit (GOPOD Format K-GLUK 07/11, Megazyme International) and spectrophotometer (GENESYS 10uv, Thermo Fisher Scientific, Waltham, MA). Results were represented as percentage of starch hydrolysis for sample digestibility using below Equation (1).

$$\%SH = Sh / Si$$
$$= 0.9 \times Gp / Si \quad (1)$$

where, *%SH* is a percentage of starch hydrolysis, *Sh* is an amount of hydrolyzed starch, *Si* is an initial amount of starch, and *Gp* is an amount of produced glucose. A conversion factor of 0.9 which is generally calculated from the molecular weight of starch monomer/molecular of glucose (162 / 180 = 0.9) was used (Goñi et al., 1997).

2.4 Microscopy

The cooked rice grains and slurries were collected during in vitro digestion process at 0 min (cooked) and 60 min from the glass reactor. The collected samples were soaked in mildly basic or mildly acidic solution to stop gastric or small intestinal enzyme reaction, respectively at 10℃ until observed. To visualize compound distributions of the grain and slurry, the fluorescently-stain techniques were applied. The sample grain was carefully and thinly sliced using blade knife (Feather S35 type, Feather, Osaka, Japan) by hand, double-stained with 0.01% acridine orange (Wako Pure Chemicals) and 0.2% rhodamine B (Wako Pure Chemicals) in acetone, and covered with cover glass. A drop of sample slurry was also spread on the glass slide, then stained with 0.01% acridine orange (Wako Pure Chemicals, Osaka, Japan) and covered with cover glass. The slide glass with samples was observed using simple fluorescent observation mode of confocal laser scanning microscope (LSM510, Carl Zeiss, Oberkochen, Germany) equipped with GFP filter.

3 Results and discussion

Figure 1 shows kinetics of starch digestibility for the grain and slurry of cooked rice during in vitro digestion. In the simulated gastric digestion process at pH 1.20, almost zero percent of digestibility was depicted both slurry and grain samples. The digestibility increased during simulated small intestinal digestion process after 30 min, in which approximately 76% of digestibility for the slurry was shown within 45 min. The digestibility of the slurry continuously increased during simulated small intestinal digestion process to approximately 90% at 90 min, while the digestibility of grain of cooked rice showed 35% at 45 min and 60% at 90 min.

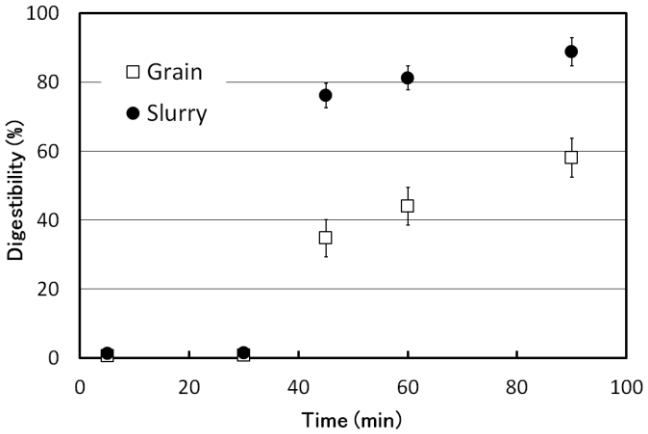

Figure 1 Changes in digestibility of grain and slurry of cooked rice during in vitro digestion. Error bars represent standard deviation (n = 3).

Figure 2 shows structural changes of the cooked rice grain (Figure 2A, C) and slurry (Figure 2B, D) samples during in vitro digestion. Figure 2A, B indicates the sample structures before digestion (0 min, just cooked samples). The sample structures at 60 min were also depicted at Figure 2 C, D. In the images for grains (Figure 2A, C), proteins and a part of cell wall materials, which contained membrane proteins, were presented as red or orange by rhodamine B (Likitwattanasade and Hongsprabhas, 2010) and carbohydrate starchy materials were presented as yellow or green by acridine orange (Vidal et al., 2007), respectively. In the slurry images (Figure 2B, D), starch granules and cell wall fragments were represented as green and yellow, respectively.

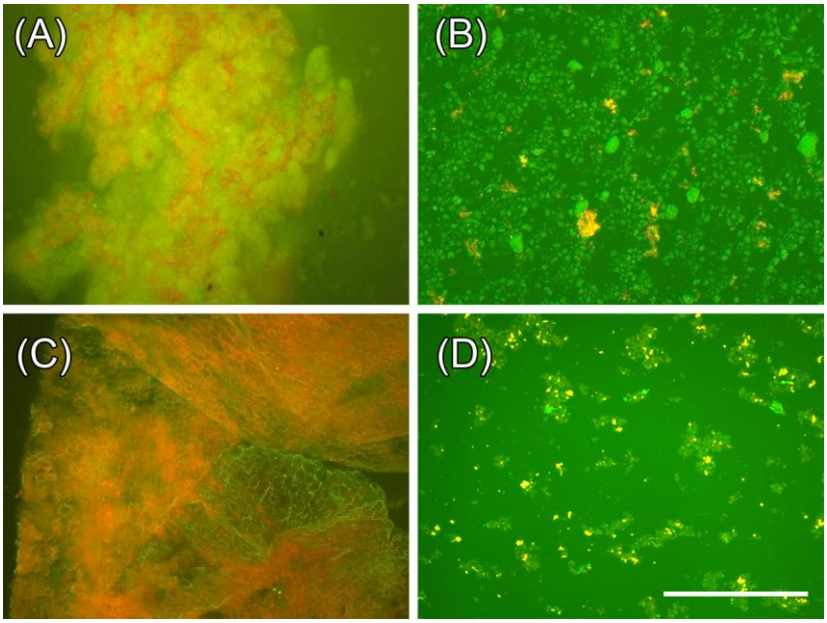

Figure 2 Histochemical structures of the grain (A, C) and slurry (B, D) of cooked rice at before digestion (A, B) and 60 min (C, D). Scale bars show 500 μm

As shown in Figure 2A, honeycomb-like structures, dyed in red and the size was approximately 15~20 μm, indicated cell wall distributions, because the distributions corresponded with autofluorescent images using DAPI filter, which showed cell wall materials (Ogawa et al., 2003). The honeycomb-like structures tended to be shrunk with the progression of digestion process, which would relate to grain size reduction (Figure 2C). The non-starchy materials shown as red in the Figure 2C would be aleurone cells (Ogawa et al., 2001). A protein and lipid were mostly filled in small cells at the aleurone layer (Champagne et al., 2006), which would inhibit SIF penetration. Contrary, as shown in the Figure 2B, D, the starch particles in the slurry were bared and should easily be accessible to the digestion fluid. The size of starch granules observed in the slurry at 60 min was several micrometers, while the slurry of before digestion contained over 10 μm size starch granules. The cell wall fragments, however, almost maintain its size during in vitro digestion process. These results confirmed starch granules in the slurry were mainly degraded between 30 min and 45 min in which the simulated small intestine digestion was started by addition of SIF.

The grain surface of cooked rice, therefore, should be a kind of resistant materials against digestion fluid and prevented starchy materials in its endosperm at the early stage of small intestine digestion. Contrary, the cooked rice slurry had no resistant structures against SIF and also had large surface area compared with the grain. In the simulated gastric digestion process, there was little starch hydrolysis reaction because of no amylolysis enzyme in the SGF. However, low pH conditions allow decrease

of grain firmness with moisture content increasing of cooked rice (Kong et al., 2011). These phenomena suggested that the enzyme accessibility to rice starch enveloped into starch-stored cells in the endosperm would make increase due to physicochemical property changes of structural cell attributes by low pH, in which dehydration at starch-protein interactions must be occurred (Würsch et al., 1986). Therefore, these structural characteristics would relate to the digestibility changes during simulated small intestinal digestion process.

4 Conclusions

The grain structures would inhibit penetration of digestive fluid to grain inside, which impacted on the starch digestive rate during digestion. Our results indicated that the degree of chewing would be influenced in the degree of rise in blood sugar in human body when the cooked rice grain was ingested.

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