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Visualization of the coated layer at the surface of rice grain cooked with varying amounts of cooking water

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ABSTRACT

A coated layer at the surface of cooked rice grain was visualized using a simple sectioning method, fluorescence microscopy, and digital image processing. Polished rice grain (150 g) was added to 150, 225, or 300 ml of water, which was 1.0×, 1.5×, or 2.0× (w/w) the weight of the grain, respectively. The rice was cooked and then examined. The sections of whole size grain obtained by the simple sectioning method were captured using a stereomicroscopy with transmission and fluorescent modes. The actual grain section including the coated layer was observed in the transmission image. In contrast, the cell morphology in the grain, which showed the exact size of the sectioned grain, was visualized in the fluorescence image. The composite image of both the fluorescent and transmission images captured at the same position can show extra portions of the actual section, which can be distinguished from the morphological tissue area and regarded as the coated layer. The specific layer thicknesses estimated from the composite images shown in this report were approximately 1–7 μm, which increased with increasing amount of cooking water. Differences in the layer thickness at the dorsal and ventral sides were also observed.

1. Introduction

Rice is usually consumed as a boiled or steamed grain. The Japanese rice cooking process simply consists of adding water to the grain and heating until the water is absorbed within a grain and/or evaporated. Hence, starch contained within a grain is gelatinized during cooking, producing the mechanical grain properties such as hardness and stickiness, which are correlated with textural properties (Tani et al., 1969). In general, starch gelatinization is influenced by the heating conditions and amount of cooking water, and hence control of these conditions is important for cooked rice qualities (Kainuma and Ema, 1987; Mabashi et al., 2007).

When the grain contacts boiled water, starch stored within a cell swells with gelatinization during boiling, the cell wall is disrupted, and stored starch, cell wall fragments, and other materials are eluted into the water. The eluted materials are condensed and coated onto the surface of cooked grain as a coated layer when the water is finally absorbed or evaporated. The condition of coated layers is also related to cooked rice qualities such as stickiness and appearance. A grain compression test measuring stickiness and hardness showed that a cooked grain compressed to 25% of grain thickness correlated to eating quality, which is one of the important grain quality indices (Okadome et al., 1999). The amount of coated layer was correlated with the stickiness of cooked rice (Ikeda, 2001), and a thick coated layer was correlated with a smooth surface of high quality cooked grain (Okuda et al., 2009). The layer appearance is also related to physicochemical properties such as the *L* value for chroma and the luster, which are related to the sensory quality of cooked rice (Konishi et al., 1996). However, Kainuma (1992) reported that the materials eluted during cooking were insensitive to the physical property of the coated layer of cooked grain.

Because the coated layer is a physical structure of the cooked rice grain, microscopic techniques have been applied to analyze the layer or the grain surface conditions. A scanning electron microscope (SEM) analysis of different varieties of grains showed two distinct surface layers of the cooked grains, which were related to the different rice varieties (Sasahara et al., 1980). Rewthong et al. (2011) also used SEM for investigation of the morphological properties of freshly cooked and dried cooked grains with different cooking and drying methods, and showed a higher number of voids or less compacted starch clusters at the surface of cooked grain. However, these studies are insufficient to understand the properties of coated layers, especially the formation process and thickness, because few techniques have been developed to distinguish the

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boundary between morphological and actual tissue areas of the cooked grain.

The tissue structures of whole size rice grain were successfully visualized using a simple sectioning technique with a paraffin embedding protocol for light microscopy and the autofluorescent properties of cell walls exposed to UV light (Ogawa et al., 2003a). The technique could also apply to observing the cooked grain tissues (Ogawa et al., 2003b). In this study, a modified technique was developed for visualization of the tissue structure and coated layer of cooked rice grain. The layer thickness was examined using digital composites of transmission and autofluorescent images.

2. Materials and methods

2.1. Materials

Brown, nonwaxy, japonica rice grains (*Oryza sativa* L., cv. Koshihikari) harvested in 2010 in Niigata, Japan, were purchased at a local rice store in Sagamihara, stored in a refrigerator at 4 °C, and polished by a household rice polisher (BT-AE05; Zojirushi, Osaka, Japan) before examination. The polished grains were 90% of the original brown grain weight, and bran on the surface of the polished grain was removed using a thin paper cloth.

2.2. Rice cooking

One hundred and fifty grams of polished rice grain with 1.0× (150 ml), 1.5× (225 ml), or 2.0× (300 ml) added distilled water was cooked using an electric rice cooker (SR-L10L; National, Osaka, Japan). The samples were soaked in the water for 30 min at 30 °C and then boiled until the water evaporated. The cooked rice grain was cooled to 25 °C after cooking, and used for physical measurement and microscopic observation. Changes in the water temperature during cooking were measured by T-type thermocouple devices connected with a data logger (GL220; Graphtec, Yokohama, Japan).

2.3. Moisture content

To measure the moisture content of the cooked rice grains, 10 g of sample grains was dried (105 °C, 24 h) by a dryer (WFO-400; EYELA, Tokyo, Japan). The moisture content on a wet basis (w.b.) was calculated as the percentage of water weight divided by the original sample weight.

2.4. Measurement of coated layer amount

A simplified quantity index of an adhesive coated layer developed by Kainuma (1992) was applied to measure the coated layer quantity. Fifty grams of cooked rice grain were removed from the rice cooker when the cooking was finished, placed into a 200 ml beaker, and then 100 g of distilled water was added. The samples were stirred 20 times per 10 s using sticks to separate the layer from the grain surface during rinsing, and then the rinsed grains were removed from the liquid-coated layer mixture using a mesh strainer. The liquid mixture was dried (105 °C, 24 h) using a dryer, and the dry matter weight of the coated layer was determined.

2.5. Measurement of adhesiveness

Adhesiveness of cooled cooked rice samples was measured by a creep meter (RE2-3305S; Yamaden, Tokyo, Japan). Individual grain samples were compressed twice with the instrument plunger, and then two positive and two negative curves for the texture profile were obtained. The mechanical properties of the surface of cooked rice grains were estimated by the load for 25% compression of the grains, which were mostly employed for the surface texture of cooked rice (Okadome et al., 1996). A planar plunger (Φ30 mm) with a 1 mm/s compression ratio was employed.

2.6. Sectioning

The cooked rice cooled to ambient temperature was prepared for microscopic observation using a simple sectioning method (Ogawa et al., 2003b). The sample grains were soaked in 10% (v/v) formaldehyde for 5 min for physically hardening of the surface portion of the coated layer, and then carefully dehydrated in a graded ethanol series (40%, 50%, 60%, 70%, 80%, 90%, 95% × 2, 99.5% × 2) for 5 min per step, soaked in 99.5% (v/v) ethanol overnight, and then soaked in Lemosol reagent (Wako Pure Chemical Industries, Tokyo, Japan), which is an alternative of xylene, for three changes of 1 h each at room temperature. Note that the fixation and dehydration time were set for shorter than usual procedures to maintain the morphological structures of the cooked grain because it had shrinkable, fragile and separable properties for the dehydration process. The dehydrated sample was soaked in liquid paraffin for 1 h at 70 °C and chilled to harden the paraffin in a refrigerator at 4 °C for embedding in a paraffin block.

The dehydrated sample in the paraffin block was sectioned at ambient temperature using a microtome (SM2000R; Leica, Wetzlar, Germany) equipped with disposable blades (Feather S35 type; Feather, Osaka, Japan). The sections were collected from a center portion of the cooked rice using adhesive tape (Toshiba Machine, Numazu, Japan). Cross or longitudinal sections were taken according to the dorsal–ventral axis of a grain (Fig. 1). The section thickness was 20 μm. The sectioned specimen adhered on the tape was faced up and placed onto a glass slide. The surrounding paraffin was removed by heating at 70 °C and dissolving into the Lemosol

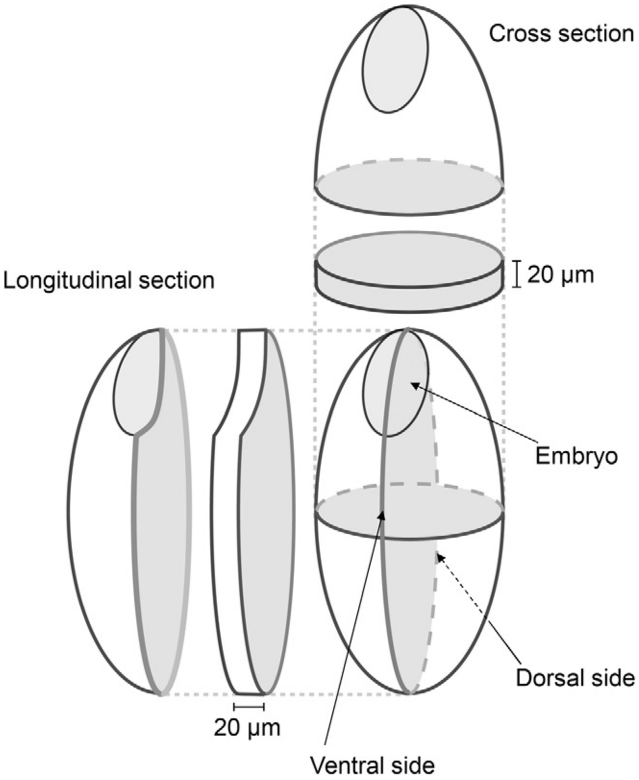

Fig. 1. Schematic diagram of the section positions within a grain.