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Anatomical study of the effect of cooking on differently pigmented rice varieties

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ABSTRACT

In recent years, the consumption of wholemeal rice, and in particular pigmented varieties, has received increased interest because of its content in nutritionally relevant compounds. However, thermal treatment can modify the structure of the matrix, influencing the accessibility and possibly the localization of antioxidants and other compounds. Therefore, in this study the effect of two different cooking methods (i.e. "risotto" and boiling) on the anatomical structure of three differently pigmented wholemeal rice varieties was evaluated. The presence and the localization of tannin inclusions were also analyzed. Cooking caused the formation of voids in the grains and, in particular, black rice presented the highest proportion of voids among the varieties analyzed. After both thermal treatments, a significant increase in the tannin inclusions in endosperm was observed, suggesting a partial resorption of the leached compounds. These observations suggest that an evaluation of the anatomical structure may help to better understand the behavior of cereals during domestic cooking, which in turn, affects their nutritional quality also in terms of compound accessibility.

1. Introduction

Rice (*Oryza sativa* L.) is one of the most important cereal crops for human consumption in the world. Usually rice is consumed in the polished form, in which the outer layers of the caryopsis are removed. However, from the nutritional point of view, the consumption of wholemeal rice is preferable, because a large amount of nutritionally relevant compounds, such as fiber, proteins, vitamins, and minerals, are located in the bran (Fardet, Rock, & Rémésy, 2008). In particular, although not widespread, in recent years pigmented rice varieties have received increased attention because of their antioxidant properties, related to the presence of phenolic compounds located mainly in the outer layer of the caryopsis (Fardet et al., 2008; Finocchiaro, Ferrari, & Gianinetti, 2010).

The thermal treatment can modify the structure of the matrix, influencing the accessibility and probably the localization of antioxidants and other compounds (Parada & Aguilera, 2007). However, analysing the behavior and the localization of individual compounds during cooking is not simple. In fact, the most common techniques for a qualitative and quantitative analysis of phenolic and antioxidant compounds are unable to localize these molecules. Therefore, evaluating the anatomical structure and changes occurring in matrix components during thermal treatment may help to understand the effect of cooking on these compounds. Moreover, observing total tannins may indicate the localization of antioxidant compounds in the caryopsis. The term tannins refers to inclusions that react with the tannin solution (Ruzin, 1999) and the molecules visualized with this technique present red-ox properties and may be comparable to those considered in the antioxidant capacity analysis.

The analysis of food structure is a topical issue and several recent papers have been published on the subject. In previous studies, different techniques, such as microscopy, NMR micro-imaging and spectroscopic methods, have been employed to observe the morphology of cooked rice grains and the effect of thermal treatments on cereal matrix (Briffaz, Mestres, Escoute, Lartaud, & Dornier, 2012; Horigane et al., 1999; Ogawa, Glenn, Orts, & Wood, 2003; Tamura & Ogawa, 2012; Witek et al., 2010). These studies mainly focus on starch gelatinization of milled rice and analyzed the structure of rice in order to evaluate a relationship with the texture and other quality parameters, such as sensorial properties (Leelayuthsoontorn & Thipayarat, 2006; Mestres, Ribeyre, Pons, Fallet, & Matencio, 2011; Rewthong, Soponronnart, Taechapairoj, Tungtrakul, & Prachayawarakorn,

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2011). In particular, the authors observed the formation of cracks and hollows inside the caryopsis (Horigane et al., 1999; Ogawa et al., 2003; Tamura et al., 2014) and the presence of a coated layer on the surface of the rice grain (Tamura & Ogawa, 2012).

However, all these works analyzed milled rice, whereas, to our knowledge, no study has evaluated the anatomical structure of cooked wholemeal rice, in which the presence of pericarp might influence the behavior of rice during cooking. Moreover, in the literature there are no studies observing the presence and localization of tannin inclusions in cereal caryopses.

Therefore, the aim of this study was to evaluate the effect of cooking on the caryopsis rice structure of three differently pigmented wholemeal rice varieties, using two cooking treatments (i.e. boiling and "risotto"). The anatomical changes that occurred during cooking and the formation of internal hollows were observed. The presence and localization of total tannins were also analyzed histochemically.

2. Materials and methods

2.1. Materials

Three commercial varieties of wholemeal rice were analyzed: a white variety (Ribe), a red variety (Ermes), and a black variety (Venere). The grains were purchased at a local market in Parma (Italy).

2.2. Chemicals

All chemicals and solvents were analytical grade. Tolouidine Blue O (TBO) solution was purchased from Carlo Erba (Milan, Italy – C.I. 52040), Periodic Acid Schiff (PAS) staining kit was from Bio-Optica (Milan, Italy). Paraffin for processing and embedding was purchased from Sherwood Medical Co. (St. Luis, MO, USA). Ultrapure water from MilliQ system (Millipore, Bedford, MA, USA) was used throughout the experiments.

2.3. Cooking procedure

Two different domestic cooking techniques were employed: boiling and "risotto". Both cooking methods were designed to achieve the complete starch gelatinization. Briefly, 110–120 mg of rice was weighed into a 10 mL glass tube. The tube was placed in a water bath at 100 °C, after adding a specific amount of boiling water, and covered. The water/rice ratio was 3.6:1 (w/w) for "risotto" and 20:1 (w/w) for boiling. The cooking time was 40 min for all studied varieties and for both cooking procedure. The cooked rice was then cooled in an ice bath for 5 min. Cooking tests were performed in triplicate for each variety.

2.4. Histological and histochemical analyses

Rice samples (uncooked and cooked) were fixed in a formalin: acetic acid: 60%-ethanol solution (2:1:17, v/v; FAA solution) and after at least 2 weeks they were dehydrated with gradual alcohol concentrations. Dehydrated samples were immersed in liquid paraffin for at least 7 days. Samples were embedded in paraffin blocks and the blocks cut into 5 μm thick cross sections with a microtome Leitz 1512 (Wetzlar, Germany). The sections were stained with: (i) TBO (0.1% w/v) and PAS solutions (Ruzin, 1999) to detect changes in anatomical structure during the cooking process for all varieties; (ii) tannin solution (a mixture of 89 mL of water, 0.25 mL acetic acid, 10 mL formalin and 2 g ferrous sulphate) to detect total tannins (Ruzin, 1999). For each staining technique, at least three replicate sections for each variety and treatment were stained. The sections were examined using an optical microscope (Leica DM 4000B, Wetzlar, Germany) equipped with a Leica DC 100 digital camera, which was also used for image capturing. The quantity of tannin inclusions (area) was measured using an image analysis system (QWIN3 Leica Imaging Systems Ltd., Wetzlar, Germany). Features were measured on binary images; feature data included linear dimension, areas and shapes. Data used for the present analysis included only the feature areas in square micrometers. The feature data file was opened in a statistical program for further analysis. Images (1024 × 1536 pixel grayscale) were saved as 300 dpi TIFF files.

2.5. Statistical analysis

The statistical analysis was carried out using SPSS Statistics 21.0 software (SPSS Inc., Chicago, IL, USA). Descriptive statistics were applied to the dataset. All dependent variables were analyzed using two-way ANOVA, with two factors: "Cultivar" and "Cooking treatment". If a statistically significant interaction effect was found, the two factors were evaluated simultaneously by plotting the estimated marginal means for all levels of each factor. For each statistical factor, comparisons of the means were performed using Tukey's post hoc tests. The statistical tests were performed at a 5% significance level.

3. Results and discussion

3.1. Raw samples

Rice samples showed the typical structure of cereal caryopsis; from the outside inwards pericarp, perisperm, aleurone and endosperm were visible (Bechtel & Pomeranz, 1977). The outer layers, or pericarp, consisted of compressed cells and appeared fused together, with a pericarp thickness of 20.6 ± 3.9, 21.8 ± 3.5, 19.1 ± 5.3 μm in the black (Fig. 1A), red (Fig. 1B) and white (Fig. 1C) varieties, respectively. The aleurone consisted of one or more cell layers. In rice this characteristic occurs within a single grain (Evers & Millar, 2002), with single- or multi-cellular layers observed near the embryo. In white rice, aleurone cells were mostly distributed in a single layer, whereas in black rice the aleurone was distribuited in two cells layer; the red variety commonly showed a mono or bi-cellular layer, within a single grain. Aleurone cells, in transversal section, appeared roughly square, with a more elongated form along the tangential diameter in the black variety, whereas in the white variety the radial and tangential diameters were similar. The red rice presented both squared and elongated aleurone cells. The area of the aleurone cells ranged between 370 and 4300 μm^2 in the black rice, between 333 and 3300 μm^2 in the red rice and between 390 and 4150 μm^2 in the white variety. Moreover, the aleurone layer thickness was higher in the white (51.0 ± 11.6 μm) and black (48.8 ± 10.2 μm) varieties with respect to the red one (35.9 ± 5.7 μm). In aleurone cells the cytoplasm was visible, because in raw rice the cells are partially dehydrated due to the maturation process of the caryopsis. Aleurone cells contained some inclusions (Fig. 1A–C): small starch granules (confirmed by PAS staining) and others, likely proteins and other molecules, such as phytates (Krishnan, Ebenezer, & Dayanandan, 2001). In endosperm, the size of the cells gradually increases from small cells in the subaleurone layer to large cells in the inner zone of the endosperm tissue, in accordance with the observations of Briffaz et al. (2012). These cells contain mainly starch inclusions, which were present in the form of granules and clusters of granules (Yu, Zhou, Xiong, & Wang, 2014). In the subaleurone region, starch was in the form of small spherical starch granules, as observed by Bechtel and Pomeranz (1978). Moreover, higher protein and vitamin content was reported in this region compared to the central part of the endosperm (Bechtel & Pomeranz,