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Influence of germination conditions on malting potential of low and normal amylose paddy and changes in enzymatic activity and physico chemical properties

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ABSTRACT

Malting potential of two rice cultivar i.e. low (12.5%) (LR) and normal (20.2%) amylose paddy (NR) were evaluated under different germination conditions (25–40 °C for 120 h). Based on malting potential, 30 and 35 °C germination temperatures were found suitable. Enzymes (amylolytic and α-amylase) activity increased at both temperatures for LR and NR whereas NR exhibited higher activity. NR malted rice showed higher starch degradation (64 and 74 times) when compared to LR (58 and 62 times) at 30 and 35 °C respectively leading to formation of reducing sugar and lowering of viscosity. During germination of LR and NR significant morphological and structural changes were observed as evident from pinholes and eroded surface in micrographs, decreased % crystallinity and FTIR peaks. Outcome of present study concluded that the amylose amylopectin ratio played an important role during germination and NR paddy was found more suitable for malting at 30 and 35 °C.

1. Introduction

Rice belongs to the *Gramineae* family and has two cultivated species, *Oryza sativa* L. (Asian cultivated species) and *Oryza glaberrima* (species found in Western Africa). Between these Oryza sativa is widely cultivated and consumed worldwide. Rice is reported to be rich in carbohydrates, vitamins, minerals but contains less protein and dietary fibre compared to other cereals such as wheat and maize (Saman, Vazquez, & Pandiella, 2008). The nutritional composition of rice can be improved by the traditional and economical method of processing known as malting (Quadir, Wani, Bhat, Wani, & Quraazah, 2012). Paddy verities known for poor milling characteristics may be used for preparation of malted rice powder which may be employed as a modified starch in some formulations.

Malting mainly involves the process of germination of the cereal grains during which the seed absorbs water and the embryo develops and leads to the production of phytohormones specially gibberellic acid (GA) which diffuses to the aleurone layer via the scutellum. Eventually the aleuron layer cells are induced to produce and secrete hydrolytic enzymes (α and β amylase) which otherwise remains dormant into the endosperm before malting. The endosperm consisting of starch degrades to simple sugars (maltose, glucose and dextrin) and protein to peptides and amino acids (Ayernor & Ocloo, 2007).

Published literature reveal that malting apart from generation of bio-functional components also improves the organoleptic properties due to release of flavoring compounds and softening of texture (Wu et al., 2013). Thereby malted rice flour can also be potentially used in various novel functional food products such as weaning foods, gluten free beer, tortillas, beverages, puddings, salad dressings, gluten free confectionaries.

Starch is one of the major components of rice and is composed of amylose and amylopectin. Amylose is a linear glucose polymer with α,1-4 linkages whereas amylopectin is branched structure in which linear chains of α-1,4 glucose residues are interlinked by α-1,6 linkages. The amylases produced during germination specifically cleaves these glyosidic linkages in starch. Based on the amylose content rice can be classified as waxy (0–2% amylose), very low (5–12% amylose), low (12–20% amylose), intermediate/normal (20–25% amylose) and high amylose (25–30% amylose) rice (Juliano et al., 1981).

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The variation in amylose amylopectin ratio in different rice cultivars may influence the malting potential and physico chemical properties of malted rice under different germination conditions. Although malting of paddy from various cultivars is reported from different parts of the world (Ayernor & Ocloo, 2007; Olugbile, Obadina, Atanda, Omemu, & Olatope, 2013; Wu et al., 2013) but the role of amylose and amylopectin ratio during the process of germination is scarcely reported. Therefore, the objective of the present study was planned to investigate the effect of amylose amylopectin ratio on malting potential of two paddy (low amylose and normal amylose) under different germination conditions. Further, the change in enzyme activity and physiochemical properties of malted rice will be estimated. This study will help us to get an insight into the germination induced changes in rice. The rice with modified functional properties may be used as an ingredient in brewing and weaning foods.

2. Materials and method

2.1. Materials

The ratio of amylose and amylopectin of paddy was taken into consideration for the present study. Two recently harvested (in 2016) locally available paddies viz. "Kola Chokuwa" as low amylose rice (LR) (12.5 ± 0.13%) and Aijong rice as normal amylose rice (NR) (20.2 ± 0.8%) were procured from the cultivars of Nagaon, Assam, India. The moisture content of the LR and NR paddy was observed to be 12 and 12.3% respectively after harvesting. The paddy was washed to remove husk and infested grain and only healthy grains were taken for experiment. The chemicals used for analysis in this study were obtained from Merck and Himedia.

2.2. Preparation of germinated rice

Malted rice was prepared by steeping, kilning, dehusking and grinding of germinated paddy. Germination of paddy was performed according to the method described by Quadir et al. (2012). Clean and healthy paddies were soaked (stepping) separately in potable water (grain to water ratio 1:3 w/v) at ambient temperature (28 °C) for 24 h. The drained paddies were placed between layers of wet muslin clothes after rinsing with 70% ethanol to avoid microbial growth. The trays were wrapped with aluminum foil to avoid moisture loss and germinated at 30 and 35 °C for 120 h. First sample was drawn after 24 h and subsequent samples were drawn at an interval of 12 h up to 120 h. Each sample was kilned (dried in a hot air oven) at 50 °C for 24 h. Kilned paddy was dehusked using laboratory dehusker followed by grinding (80 μm sieve) using lab grain mill (Pulverisette 14, Fritsch, Germany. It was stored under refrigerated condition for further analysis. The unmalted rice was taken as the native rice.

2.3. Malting potential of germinated paddy

Both paddy were tested for viability by evaluating the malting potential which is described by Germination Rate (GR), Dormancy Rate (DR), and Germination Capacity (GC) (Olugbile et al., 2013). Healthy paddy were soaked in water for 24 h at 28 °C. The soaked grains (100 Nos each) were placed in wet muslin cloth bag and germinated at four different temperatures (25, 30, 35 and 40 °C) separately for 120 h. Germination status of each bag was examined after first 24 h and thereafter at an interval of 12 h up to 120 h by counting germinated and ungerminated grains. Percentage *GR*, *DR* and *GC* of germinated paddy were calculated using the Eqs. (1), (2) and (3) and malting potential was evaluated.

$$GR\ (\%) = \frac{Number\ of\ viable\ grains}{Total\ number\ of\ grains} \times 100 \quad (1)$$

$$DR\ (\%) = \frac{Number\ of\ unviable\ grains}{Total\ number\ of\ grains} \times 100 \quad (2)$$

$$GC\ (\%) = GR - DR \quad (3)$$

2.4. Malting loss and yield of malted brown rice

The germinated paddy at 30 and 35 °C was analyzed for its physical properties malting loss and yield of each sample that were calculated by the method given by Ayernor and Ocloo (2007). Rice kernel (100 Nos) were weighed before and after malting. The plumule and radicle (roots) of malted grains were removed by hand before weighing. The malting loss and yield was expressed as percentage (g/100 g of dry matter) by Eq. (4) and Eq. (5) respectively.

$$\text{Malting loss}\ (\%) = \frac{Weight\ of\ unmalted\ grain - Weight\ of\ malted\ grain}{Weight\ of\ unmalted\ grain} \times 100 \quad (4)$$

$$\text{Malting yield}\ (\%) = \frac{Weight\ of\ malted\ grain}{Weight\ of\ unmalted\ grain} \times 100 \quad (5)$$

2.5. Enzyme activity determination

2.5.1. Enzyme extraction

One gram of malted rice was mixed with 10 ml of ice cold 10 mM $CaCl_2$ solution and mixture was incubated at 4 °C overnight for enzyme extraction. It was centrifuged at 3000 rpm for 20 min in a refrigerated centrifuge (4 °C) and the supernatant obtained was used as enzyme extract for analysis of total amylolytic and α-amylase activity.

2.5.2. Amylolytic and α-amylase activity

The Amylolytic activity was determined by measuring the amount of reducing sugar produced by hydrolysis of starch using DNSA method (Asante, Adjaottor, & Woode, 2013) with slight modification. Enzyme extract (1 ml) and 1% starch solution was mixed in 1:1 ratio and incubated at 27 °C for 15 min. DNSA reagent (3 ml) was added to stop the hydrolysis of starch. The solution was heated in boiling water for 5 min and developed colour was measured at 560 nm in a UV–Vis spectrophotometer (CECIL, CE7400). Maltose (200 μg/ml) was used as standard for estimation of reducing sugar. The alpha amylase activity was determined similarly by the DNSA method except that the extracted enzyme was incubated at 70 °C for 15 min to inactivate β-amylase and debranching enzyme (Tian et al., 2010).

One unit of enzyme activity (U) was defined as the amount of micromoles of maltose produced per minute under the test condition and calculated using the formula given by Asante et al. (2013).

$$Activities\ (U/ml) = \left(\frac{(mg/ml\ in\ terms\ of\ maltose) \times 10^3}{Molecular\ weight\ of\ maltose \times Time\ (\text{min})} \right) \times 2 \quad (6)$$

2.6. Determination of chemical composition

2.6.1. Total starch and reducing sugar

The starch content of the both rice were determined by the method described in AOAC (1990). The total reducing sugar was measured by the dinitrosalicylic acid as described by Saqib and