Controlled Germination to Enhance the Functional Properties of Rice

Premsuda Saman[1], José Antonio Vázquez[1,2] and Severino S. Pandiella[1]*

1 Chemical Engineering and Analytical Science
The University of Manchester
PO Box 88, Sackville Street, Manchester, M60 1QD, UK.

2 Grupo de Reciclado y Valorización de Materiales Residuales
Instituto de Investigacións Mariñas (CSIC)
r/ Eduardo Cabello, 6. Vigo-36208. Galicia – Spain

* **Corresponding author:** Dr Severino S Pandiella,
Fax +44(0)161 306 4399, Email: s.pandiella@manchester.ac.uk

Abstract

The production of prebiotic oligosaccharides during germination of rice has been investigated. Germination of waxy (RD6) and non-waxy (RD17) rice was compared by evaluating the total reducing sugars, free amino nitrogen, pH and enzyme activities over a 7-day period. An increment of amylolytic enzymes and chemical changes were observed in both varieties. RD6 showed higher levels of total reducing sugars and also higher amylolytic activities including α-amylase and α-glucosidase, which reached maximum values at the third day of germination. However, the amount of free amino nitrogen in RD6 was lower than in RD17. Sugar analysis indicate that RD6 produces higher concentration of sugars and oligosaccharides during germination. Based on these results, germinated RD6 at different times was used to produce malted rice syrup through mashing. After saccharification, the malted rice syrup contained different concentrations of sugars and oligosaccharides, particularly isomaltose, panose and isomaltotriose, which have been reported to have prebiotic properties.

Keywords rice, germination, saccharification, prebiotic, malto-oligosaccharides, isomalto-oligosaccharides

Introduction

Cereal foods in various forms are an essential component of the daily diet. Rice is one of the most consumed cereals worldwide. Nutritionally it is an important source of carbohydrates, vitamin B6, zinc and copper but it contains the lowest protein and dietary fibre content among cereals [1].

In principle, rice can be modified by germination to improve its functionality. Starch represents the main reserve compound in the rice grains [2]. This polysaccharide is stored mainly in the endosperm where it is hydrolyzed during germination to provide soluble sugars to the germinating seedling [3]. Starch degradation is a complex biochemical process, which is modulated by both hormonal and metabolic regulation [4]. A set of enzymes are needed to carry out the starch breakdown: α-amylase, β-amylase, debranching enzyme, and α–glucosidase [5]. Both α-glucosidase and α-amylase are able to degrade native starch granules, but the later enzyme plays the main role in this process and it is the key enzyme in starch hydrolysis.

During the saccharification process, other enzymes contained in malted rice are also activated to break down starch and release more sugars and oligosaccharides, especially isomalto-oligosaccharides which are potentially prebiotic [5,6].

Starch oligosaccharides, which represent the fragments of the original polysaccharide, are composed of α-D-glucopyranosyl units linked by α-1,4 and/or α-1,6 bonds.

Oligosaccharides containing only α-1,4 glucosidic linkages are called malto-oligosaccharides, while those containing both α-1,4 and α-1,6 glucosidic linkages are called branched-oligosaccharides or isomalto-oligosaccharides [8].

Prebiotic oligosaccharides can be used as functional food ingredients to provide useful modifications to the physiochemical properties of foods. It has been reported that these oligosaccharides have various physiological functions and improve the intestinal microflora by selective proliferation of bifidobacteria [7]. They also stimulate mineral absorption, have anticancerogenic potential, and reduce both plasma cholesterol and blood glucose levels [9]. Among these sugars, isomalto-oligosaccharides (IMO) has received a considerable interest as prebiotic in recent years. IMO have demonstrated their useful in normalizing bowel movement, increasing stool bulk, colon microbial activity, as stimulate growth of *Bifidobacterium* and *Lactobacillus* and systemic immunity [10-12].

In the present work, controlled germinations of two varieties of rice were performed to monitor the different enzyme activities and the chemical changes. The modified malted rice was also used to produce rice syrup with different sugar and oligosaccharide contents.

Materials and methods

Malted rice preparation

Two cultivars of Thai rice (*Oryza sativa* L.), RD6 (waxy rice) and RD17 (non-waxy rice), were used in these experiments. These two varieties (RD6 and RD7) were obtained from Seed Center in Phatumthani province and Srisaket province, Thailand, respectively. RD6, which is a glutinous rice, widely grown in the northeast of Thailand. It is a commercial rice cultivar represented in the Thai rice export market [12]. It contains amylose 0.1-0.3% [13-16]. RD17 contains amylose 28-30% and it is widely grown in central part of Thailand [17]. It is also one of Thai commercial rice cultivar [18]. Both varieties were washed and steeped separately in distilled water for 24 h. After soaking, 500 g of seeds were placed in Petri dishes containing filter paper moistened with sterile water and maintained at 30°C under aerobic conditions [19]. 2.5 ml of water were added to the Petri dishes every day to avoid drying and maintain the moisture content. Samples were taken every day over a 7-day period.

Preparation of malted rice powder

After germination, the seeds were dried in an oven (LTE, UK at 50°C for 24 h. Then, the roots and shoots were removed and the remaining portion of the paddy was dehusked using a laboratory dehusker (THU35A, Satake, Japan). The malted rice was then ground using a hammer mill (Type 120, Falling number, Sweden) and sieved through a 355 µm screen.

Preparation of malted rice syrup

The mashing process was modified by following the method of Okafor and Iwouno (1990) as well as Ayernor and Ocloo (2007). 200 g (dry weight) of ground malted rice were mixed with water contained 30 ppm Ca2+ (CaCl2 dissolved in water) and adjusted to 2 l. The mixture was adjusted to pH 6 by using lactic acid. The slurry was initially mashed at 50°C and allowed to stand for 30 min. The supernatant was decanted and the remained flour was heated until it gelatinized at 88°C. The supernatant was returned to the cooled and gelatinized slurry, giving an overall temperature of 62°C. The mash was held at this temperature for 60 min. The pH of the mash was tested and adjusted to 5.6 by adding a few drops of lactic acid. One-half of the mash was withdrawn, boiled and returned to the main mash and the temperature increased to between 69 and 71°C. The mixture was held at this temperature for 60 min. The mash was cooled and filtered using funnel and folded Whatman No. 1 filter paper. The filtered solution was finally boiled for 60 min to yield the malt rice syrup.

Measure of Total Reducing Sugar (TRS) and Free Amino Nitrogen (FAN)

The samples of malted rice and rice syrup were diluted with distilled water and analyzed for TRS and FAN following the methods of Miller [21] and Lie [22] respectively.

Enzyme extracted solution

One gram of malted rice was suspended in 10 ml of 0.2 % calcium chloride solution, mixed in vortex mixer for 1 min, and centrifuged at 3000 rpm (Minifuge T, Heraeus, Germany) for 10 min. The supernatant was used to measure the enzyme activity.

Determination of amylolytic enzyme and α-amylase

The amylolytic activity was assayed using the Terashima method [23] after crude extraction of malted rice. 0.5 ml of the supernatant was added to 0.5 ml of a 1% soluble starch solution in 0.05 M acetate buffer. The sample was incubated at 60°C for 5 min and the increase of reducing sugars was measured [21]. One unit of the enzyme activity (U) is defined as the amount of enzyme required to liberate 1 µmol of maltose per min.

The α-Amylase activity was measured following the increase of reducing sugars with time. 0.5 ml of the supernatant solution was added to 0.5 ml of a 1% soluble starch solution in 0.05 M acetate buffer. The mixture was incubated at 70°C for 15 min in order to inactivate β-amylase, debranching enzyme, and α-glucosidase [24]. One unit of α-Amylase activity (U) is then defined as the amount of enzymes required to liberate 1 µmol of maltose per min.

Determination of α-glucosidase

The α-glucosidase activity was determined using a modified method of McCue and Shetty [25]. A standard reaction solution is prepared by mixing 0.1 ml of 9 mM p-nitrophenol α-D-glucopyranoside and 0.8 ml of 200 mM sodium of acetate buffer at pH 4.6 in a glass tube. The tubes were pre-incubated at 50°C for 5 min before addition of 0.1 ml of the enzyme extract. The reaction tubes were then incubated for a further 30 min. The enzymatic hydrolysis was stopped by addition of 1 ml of 100 mM sodium

carbonate, and the samples were clarified by centrifugation at 13,500 rpm at room temperature for 5 min. The released p-nitrophenol in each sample was determined by measuring the absorbance at 400 nm compared with the blank. A standard curve was established using pure p-nitrophenol dissolved in sodium acetate buffer. One unit of α-glucosidase activity is defined as the amount of enzyme that releases 1 µmol of p-nitrophenol per min at pH 4.6 and 50°C under assay conditions.

Determination of sugars by High Performance Liquid Chromatography (HPLC)

The samples of sugars and oligosaccharides were diluted and analyzed by HPLC. The system has a two solvent delivery module (model 210 Varian, UK), an auto sampler (model 410 Varian, UK) and an evaporative light scattering detector (ELSD) (model PL-ELS 2100 Simadzu, UK). The column was a spherisorb 5 µm NH_2 (200x4.6 mm, Phenomenex, UK). The injection volume was 20 µl, and the flow rate 1.2 ml/min. For complete separation of the sugars, a mobile phase A (acetronitrile) and a mobile phase B (deionised water) were used in gradient system. The gradient system was 80% of A initially, decreased to 50% in 30 min, increased again to 80% in 5 min and then maintained at 80% for 5 min (the total cycle time was 40 min). The ELSD was set to measure at the evaporator temperature of 90°C, nebulizer temperature of 50°C and gas flow rate of 1.6 ml/min.

Results

Chemical changes during germination of non-waxy rice RD17 and waxy rice RD6

This experiment was performed to investigate the chemical changes during the germination of rice varieties RD17 and RD6. These results are shown in Figure 1.

Additions of 5 ml of water per kg of rice were done every day in order to compensate for evaporation and to maintain the moisture between 30 and 70%. During the germination of RD17 it was found that the pH decreased slightly from 6.4 to 5.9. TRS and FAN concentrations increased and reached a maximum at day 4 and 5 respectively to then slightly decrease. The amylolytic activity reached a peak value of 72.7 U/g at the fifth day, whereas α-amylase and α-glucosidase reached a maximum of 26.8 and 14.3 U/g respectively at the third day of germination.

During the germination of waxy rice RD6 the pH decreased slightly from 6.6 to 5.6. The amount of TRS and FAN increased and reached maximum values of 64.8 and 0.3mg/g respectively at the third day of germination. α-Amylase and α-glucosidase activities were maxima at day 3, while the highest amylolytic activity was observed at day 5.

HPLC was used to analyze the composition of sugars and oligosaccharides in the malted rice over a 7-day period (figure 2). The amount of sugars and oligosaccharides increases from the beginning of germination for both varieties but in a different way.

Concentrations of glucose, maltose and maltotriose reached a maximum in the third day of germination in both cases (58.9, 25.4 and 1.8 mg/g for RD6, and 47.3, 18.2 and 0.23 mg/g for RD17, respectively).

The rest of the sugars exhibit a maximum at different days depending of the variety. For RD6 the maximum concentrations of maltotetraose, maltopentaose, maltohexaose and maltoheptaose are always obtained later in the germination process and at 5, 6, 6 and 5 days respectively. Maxima for the same sugars in RD17 were obtained at 2, 4, 3 and 4 days respectively.

Chemical composition of malted rice syrups

Since results in figures 1 and 2 show that malted waxy rice RD6 contains higher levels of sugars, oligosaccharides and amylolytic enzymes (including α-amylase and α-glucosidase), this variety was used to produce a malted rice syrup through mashing. In order to stop the germination process, samples of rice were dried at 50°C for 24 h. The final moisture content was 10-11%. The dried malted RD6 was then milled and mashed with water for 3 h to reactivate the enzymes and continue the starch hydrolysis. Starch is further degraded into sugars and oligosaccharides during mashing, and saccharification produces a sweet malted rice syrup.

The concentration of TRS and FAN obtained from syrups produced with mated rice at different stages of germination is shown in figure 3. The maximum TRS concentration

(108.2 g/l) is obtained from the sample of day 3, while the maximum FAN concentration (18.9 mg/l) is obtain from the sample of day 1.

Figure 4 shows the sugar concentration in the RD6 syrups produced from malted rice with different degrees of germination. The maximum glucose and maltose extraction takes place in samples of day 3 and remains more or less stationary from them on. The maximum concentrations of the prebiotic oligosaccharides isomaltose, isomaltotriose and panose are observed from samples of day 5 and 6. The concentration of the rest of oligosaccharides measured in the syrup (maltotriose, maltotetraose, maltopentaose, maltohexaose and maltoheptaose) also increased with the germination time though in a different manner.

Discussion

To activate germination, rice was initially soaked in water to increase kernel moisture. Takahashi [26] reported that the water requirement for germination was dependent on the cultivar and the dormancy period. Hence, both varieties, RD17 and RD6, were soaked for 24 h to obtain a similar moisture content of approximately 27%. The functions of the steep water include initiation of cell elongation, respiration, secretory activity of the embryo and activation of enzymes [27]. Generally, malting must provide enough water to allow germination, but not too much. The grains will actually show a reduction in germination vigour if exposed to an excess of water. For this reason, a

small amount of water was added every day (0.5 ml/100 g seeds) over the 7-day germination period in order to maintain moisture and prevent dehydration. The moisture content was kept between 30-70%.

In this experiment, aerobic conditions were maintained throughout germination. Although it has been reported that rice seeds can germinate and grow at much lower oxygen concentrations than many other plants, gaseous concentrations below 0.3% retard germination, decrease growth, and reduce the root/shoot ratio [28].

Temperature is one of the main factors affecting germination and could have had an important role in the development of sugars [29]. For temperatures between 27 and 37 $^{\circ}$C, the majority of germination (90-97%) takes place during the first 48 h. The germination rate drops sharply for lower temperatures [30]. As correspondingly reported by Cruz and Milach [31], temperatures below 15°C prevent or reduce rice germination at the early stage.

It has been reported that during the germination of rice the protease activity increases within the first 2-3 days and decreases from them on [32]. The FAN profile shown in figure 1 reflects this fact. FAN inceases during the first days of germination due to the proteolitic activity to then decrease when rootlets and shoots in the grain begin to grow. Changes in FAN and metabolic activities could also be related to the change in pH as reported by Magalhfies and Huber [33]. Another possible cause for this decrease in that the presence of phenolic acids [32] and/or phytic acid and tannins [33,34] may act as inhibitors of the enzymatic activity in the germination process.

The differences observed between RD6 and RD17 could be due to their different protein content and protease activity [37,38].

The amylose:amylopectin ratio in rice starch not only affects its chemical and physical characteristics but also the enzymatic hydrolysis developed during the germination stages. Amylose consists of unbranded chains of poly-[(1→4)-α-D-glucopyranose] and is strongly associated with many polar substances, including some lipids, to form crystalline complexes. In Amylopectin the α-(1→4)-linked chains are extensively branched through α-(1→6)-linkages and the macromolecule has a ramified structure [39]. While amylose molecules have a single reducing and non-reducing glucose end, amylopectin has a reducing end with numerous non-reducing glucose residues in its branches.

α-amylase is the main enzyme responsible for the starch hydrolysis while α-glucosidase is involved in transglucosylation reactions for the production of isomalto-oligosaccharices. The activity of these enzymes increases during the first 3 days of germination to then decrease steadily. This is also reflected in the TRS profile where after three days the concentration in the grain also decreases due to the formation of the new plant. As a whole the amylolytic actitivy increases till day 5. The differences observed between waxy (RD6) and non-waxy rice (RD17) could be due to the different amylose/amylopectin ratio.

Briggs et al [39] reported that during germination α-amylase attacks α-(1→4) linkages at

293 random locations within the starch chain. The hydrolysis slows down near the chain
294 ends and stops at α-(1→6) branches. This enzyme acting on its own is able to degrade
295 starch into a complex mixture of sugars including glucose, maltose, maltotriose and a
296 wide range of dextrins, some of which containing α-(1→6) link branches. α-Glucosidase
297 is able to hydrolyse α-(1→4) or α-(1→6) linkages, and release molecules of glucose
298 from the non-reducing end. This enzyme is also able to transfer sugar moieties or
299 groups of sugar residues from one compound to another with the formation of a similar
300 or a distinct type of linkage. Thus, a α-(1→4) link in a chain might be broken and the
301 separated end could be joined to the same or a different chain via either an α-(1→4) or
302 α-(1→6) link. The product of this hydrolysis could be of maltose, isomaltose, panose,
303 isomaltose or long chains of oligosaccharides.
304
305 Figure 2 shows the evolution of all the sugars measured in the grain during the
306 germination of the two rice varieties. The evolution of the glucose and maltose
307 concentrations are very similar in both varieties and the maxima reached after three
308 days of germination are of the same order of magnitude. After three days the
309 concentration decreases, which suggests these sugars are used in the formation roots
310 and shoots.
311
312 The profiles for the other oligosaccarides is complex, which reflects the complexity of the
313 enzymatic paths taking place. A major difference between the two varieties is the order
314 of magnitude of the oligosaccharides produced. The waxy variety (RD6) produces
315 oligosaccharide concentrations approximately 10-fold when compared to RD17, which is
316 probably due to the higher levels of amylopectin. Most of these oligosaccharides reach

317 a maximum concentration later on in the germination process, but in some cases (e.g.
318 maltotriose and isomaltotriose in RD17) a maximum concentration is maintain, which
319 indicates that these sugars are not used for plant formation in this particular variety.
320
321 The enzymatic activity generated by germination is maintained during the drying and
322 milling of the grains. These amylolytic enzymes continue to act during mashing resulting
323 in an increase of total reducing sugars in the syrup [40]. At temperatures of
324 approximately 50°C the proteolytic enzymes also get activated causing the breakdown
325 of proteins into aminoacids [41], which is clearly observed in figure 3. Clear differences
326 in FAN and TRS concentrations were observed in the germinating grain. During
327 mashing the amylolytic and protelytic enzymes can move freely in the liquid medium and
328 have enough time for full starch and protein hydrolysis. The decrease of FAN in the
329 syrups after day 3 could be due aminoacid utilisation in root and shoot development.
330
331 Figure 4 shows that the malted rice syrups obtained from day 3 of germination contain
332 the highest concentrations of maltose and glucose. No significant changes in these
333 sugars are observer in syrups produced from rice with higher degrees of germination. It
334 could be suggested that after three days the existing amylolytic enzymes are able to
335 hydrolyse the gelatinised starch to produce the maximum glucose and maltose
336 concentration.
337
338 As RD6 waxy rice starch contains higher levels of amylopectin, the branched chains can
339 be hydrolysed by amylolytic enzymes to produce molecues of malto-oligosaccharides
340 and isomalto-oligosaccharides (branched chain oligosaccharides). Transglucosylation

by α-glucosidase might also take place during mashing [42]. α-glucosidase could catalyse both the hydrolysis of α-D-gluco-oligosaccharides and transfer the glucosyl group to 6-OH of glucose. The transfer of glucosyl to D-glucose yields isomaltose, and to maltose yields panose.

The oligosaccharides produced in the syrup will depend on the composition of the rice (amylose:amylopectin ratio), the types of enzymes present and the temperature strategy during mashing. These interactions are complex and make it difficult to justify the development of oligosaccharides during mashing. What seems clear is that higher oligosaccharides are produced from syrups of malted rice with higher degrees of germination. It should also be noted that the prebiotic oligosaccharides (panose, isomaltose and isomaltotriose) are produced from syrups from highly germinated rice (though in small amounts).

Traditionally, malting and mashing strategies have been developed to maximise sugar production for later fermentation like in the brewing process. Results in this work suggest that different malting strategies, maybe with longer germination times, could be designed to maximise the production of prebiotic oligosaccharides in the grain or in the syrup. These naturally developed prebiotics could then be used as functional food ingredients.

In addition to the functional properties of non-digestible carbohydrate like prebiotics, the phenolic acids content and α-1-6 amylase activity should also be studied. The rice-

based product could then be tested *in vitro* using batch fermentation vessels inoculated with faecal slurries. The use of animal models and human trials [43] could also be considered.

Acknowledgements

This research was carried out with the financial support of The Royal Thai Government Scholarship.

References

[1] Zhou Z, Robards K, Helliwell S, Blanchrad C. Composition and functional properties of rice. International Journal of Food Science and Technology 2002;37:849-868.

[2] Ayernor GS, Ocloo FCK. Physico-chemical changes and diastatic activity associated with germinating paddy rice (PSB.Rc 34). Afr J Food Sci 2007;1:37-41.

[3] Sun Z, Henson CA. A quantitative assessment of the importance of barley seed α-amylase, debranching enzyme, and α-glucosidase in starch degradation. Arch Biochem Biophys 1991;1284:298-305.

[4] Perata P, Guglielminetti L, Alpi A. Mobilization of Endosperm Reserves in Cereal Seeds under Anoxia Ann Bot 1997;79A:49-56.

[5] Dunn G. A model for starch breakdown in higher plants. Phytochemistry 1974;13:1341-1346.

[6] Mizubuchi H, Yajima T, Aoi N, Tomita T. Isomalto-Oligosaccharides Polarize Th1-Like Responses in Intestinal and Systemic Immunity in Mice. Nutr Immunol 2005;135:2857-2861.

[7] Parracho H, McCartney AL, Gibson GR. Probiotics and prebiotics in infant nutrition." Proc Nutr Soc 2007;66:405-411.

[8] Barreteau H, Delattre C, Michaud P. Production of oligosaccharides as promising new food additive generation. Oligosaccharides as Food Additives. Food Technol Biotechnol 2006;44:323-333.

[9] Crittenden R, Playne MJ. Production, properties and applications of food-grade oligosaccharides. Trends Food Sci Techn 1996;71:353-361.

[10] Chung CH, Day DF. Efficacy of *Leuconostoc mesenteroides* (ATCC 13146) isomaltooligosaccharides as a poultry prebiotic. Poultry Sci 2004;83:1302-1306.

[11] Chen HL, Lu HO, Lin JJ, Ko LY. Effects of isomalto-oligosaccharides on bowel functions and indicators of nutritional status in constipated elderly men. J Amer Coll Nutr 2001;20:44-49.

411

412 [12] Hirayama M. Novel physiological functions of oligosaccharides. Pure Appl Chem
413 2002;74:1271-1279.

414

415 [13] Pipatpanukul T, Bunnag S, Theerakulpisut P, Kosittrakul M. Transformation of
416 indica rice (*Oryza sativa L.*) cv. RD6 mediated by *Agrobacterium tumefaciens*.
417 Songklanakarin J Sci Technol 2004;26:1-13.

418

419 [14] Tester RF, Morrison WR. Swelling and gelatinization of cereal starches: II. Waxy
420 rice starches. Cereal Chem 1990;67:558-563.

421

422 [15] Tershima M, Kubo A, Suzawa M. The roles of the N-linked carbohydrate chain of
423 rice α-amylase in thermostability and enzyme kinetics. Eur J Biochem 1994;226:249-
424 254.

425

426 [16] Qi X, Tester RF, Snape CE, Ansell R. Molecular basis of the gelatinisation and
427 swelling characteristics of waxy rice starches grown in the same location during the
428 same season. J Cereal Sci 2003;37:363-376.

429

430 [17] Papademetriou MK, Dent FJ, Edward MH. Bridging the rice yield gap in the Asia-
431 Pacific region. Bangkok, Food and Agriculture Organization of the United Nations,
432 Regional Office for Asia and the Pacific 2000;61.

433

[18] Chitrakon S. Rice variety in Thailand advance in rice technology. Annual Report. Thailand National Center of Genetic Engineering and Biotechnology 2002.

[19] Loretil E, Yamaguchi J, Alpi A, Perata P. Gibberellins are not required for rice germination under anoxia. Plant Soil 2003;253:137-143.

[20] Okafor N, Iwouno J. Malting and brewing qualities of some Nigerian rice (*Oryza sativa* L.) varieties and some thoughts on the assessment of malts from tropical cereals. World J Microb Biotech 1990;6:187-194.

[21] Miller GL. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal Chem 1959;31:426-428.

[22] Lie S. Nihydrin method for determination of free α-amino nitrogen. J Inst Brew 1973;79:37-41.

[23] Terashima M, Kubo A, Suzawa M. The roles of the N-linked carbohydrate chain of rice α-amylase in thermostability and enzyme kinetics. Eur J Biochem 1994;226:249-254.

[24] Antranikian G, Zablowski P, Gottschalk G. Conditions for the overproduction and excretion of thermostable α-amylase and pullulanase from *Clostridium thermohydrosulfuricum* DSM 567. Appl Microbiol Biotechnol 1987;27:75-81.

[25] McCue P, Shetty K. Role of carbohydrate-cleaving enzymes in phenolic antioxidant mobilization from whole soybean fermented with *Rhizopus oligosporus*. Food Biotech 2003;17:27–37.

[26] Takahashi N. Seed germination and seedling growth. In: Tsunods S, Takahashi N editors. Developments in Crop Science vol. 7. New York: Elsevier Science; 1984. p. 71-88.

[27] Pyler RE, Thomas DA. Malted cereals: Their production and use. In: Kulp K, Ponte JG editors. Handbook of Cereal Science and Technology vol 2. New York: Marcel Dekker Inc; 2000. p. 685-696.

[28] Baker AM. Calcium peroxide as a seed coating material for padi rice: I. Requirement for and provision of oxygen. Plant Soil 1987;99:357-363.

[29] Aniche NG, Okafor N. Studies on the effect of germination time and temperature on malting of rice. J Inst Brewing 1989;95:165-167.

[30] Karen AK, Julia H. Rice morphology and development. In: Smith CW, Dilday RH editors. Rice: Origin, History, Technology, and Production vol. 2. New York: John Wiley& Sons; 2003:103-127.

[31] Cruz RP, Milach SCK. Cold tolerance at the germination stage of rice: Methods of

481 the evaluation and characterization of genotypes. Sci. Agric. (Piracicaba, Braz.)
482 2004;61:1-8.
483
484 [32] Palmiano EP, Juliano BO. Biochemical changes in the rice grain during germination.
485 Plant Physiol 1972;49:751-756.
486
487 [33] Magalhfies JR, Huber DM. Ammonium assimilation in different plant species as
488 affected by nitrogen form and pH control in solution culture. Fertilizer Res 1989;21:1-6.
489
490 [34] Rao RSP, Muralikrishna G. Non-starch polysaccharide-phenolic acid complexes
491 from native and germinated cereals and millet. Food Chem 2004;84:527-531.
492
493 [35] Osman MA. Changes in sorghum enzyme inhibitors, phytic acid, tannins and in vitro
494 protein digestibility occurring during Khamir (local bread) fermentation. Food Chem
495 2004;88:129-134.
496
497 [36] Dicko MH, Gruppen H, Zouzouho OC, Traor AS, van Berkel WJH, Voragen AGJ.
498 Effects of germination on the activities of amylases and phenolic enzymes in sorghum
499 varieties grouped according to food end-use properties. J Sci Food Agric 2006;86:953-
500 963.
501
502 [37] Dendy DAV. Rice In: Dendy DAV, Dobraszczyk, BJ editors. Cereals and cereal
503 products: chemistry and technology. Maryland: Aspen Publishers; 2001:276-313.
504

505 [38] Lasztity R, editor. The importance and general characterization of cereal proteins.
506 The Chemistry of Cereal Proteins. New York: CRC Press; 1996;2:3-17.
507
508 [39] Briggs DE, Hough JS, Stevens R, Young TW, editors. Malting and Brewing Science
509 Volume I Malt and sweet wort. London: Chapman and Hall; 1981:390.
510
511 [40] Tse KL, Boswell CD, Nienow AW, Fryer PJ. Assessment of the effects of agitation
512 on mashing for beer production in a small vessel. Trans IChemE 2003;81:3-12.
513
514 [41] Reilly DI, Cleirigh CO, Walsh PK. Laboratory-scale production of high-gravity wort
515 suitable for a Broad Variety of Research Applications. J Am Soc Brew Chem 2004;62:
516 23-28.
517
518 [42] Pan YC, Lee WC. Production of high-purity isoaltooligosaccharides syrup by the
519 enzymatic conversion of transglucosidase and fermentation of yeast cell. Biotech Bioeng
520 2005;89:797-804.
521
522 [43] Hughes SA, Shewry PR, Li L, Gibson GR, Sanz ML, Rastall RA. *In vitro*
523 fermentation by human faecal microflora of wheat arabinoxylans. J Agric Food Chem
524 2007;55:4589-4595.
525
526
527

FIGURE CAPTIONS

Figure 1. Evolution of pH, FAN, TRS, amylolytic activity (AA), α-amylase (Am) and α-glucosidase (GI) in RD17 (●) and RD6 (○) during germination at 30°C. Error bars are the confidence intervals (α=0.05, n=3).

Figure 2. Evolution of glucose, maltose, maltotriose, isomaltotriose, maltotetraose, maltopentaose, maltohexaose and maltoheptaose in malted RD17 (left) and RD6 (right) during germination. Error bars are the confidence intervals (α=0.05, n=3).

Figure 3. Concentrations of TRS (●) and FAN (○) in RD6 malted rice syrup with different degrees of germination. Error bars are the confidence intervals (α=0.05, n=3).

Figure 4. Concentrations of glucose, maltose, isomaltose, panose, isomaltotriose, maltotriose, maltotetraose, maltopentaose, maltohexaose and maltoheptaose in RD6 malted rice syrup after mashing. Error bars are the confidence intervals (α=0.05, n=3).

551

FIGURE 1

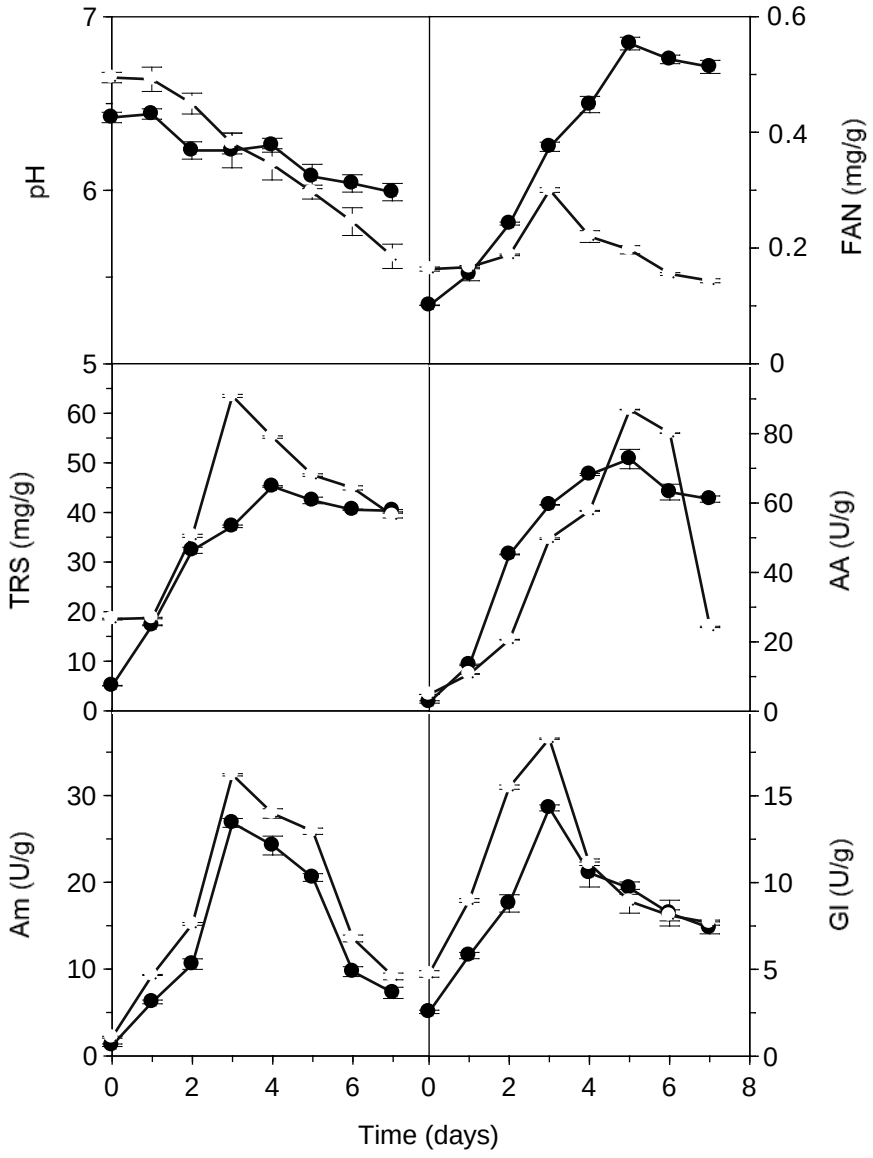


FIGURE 2

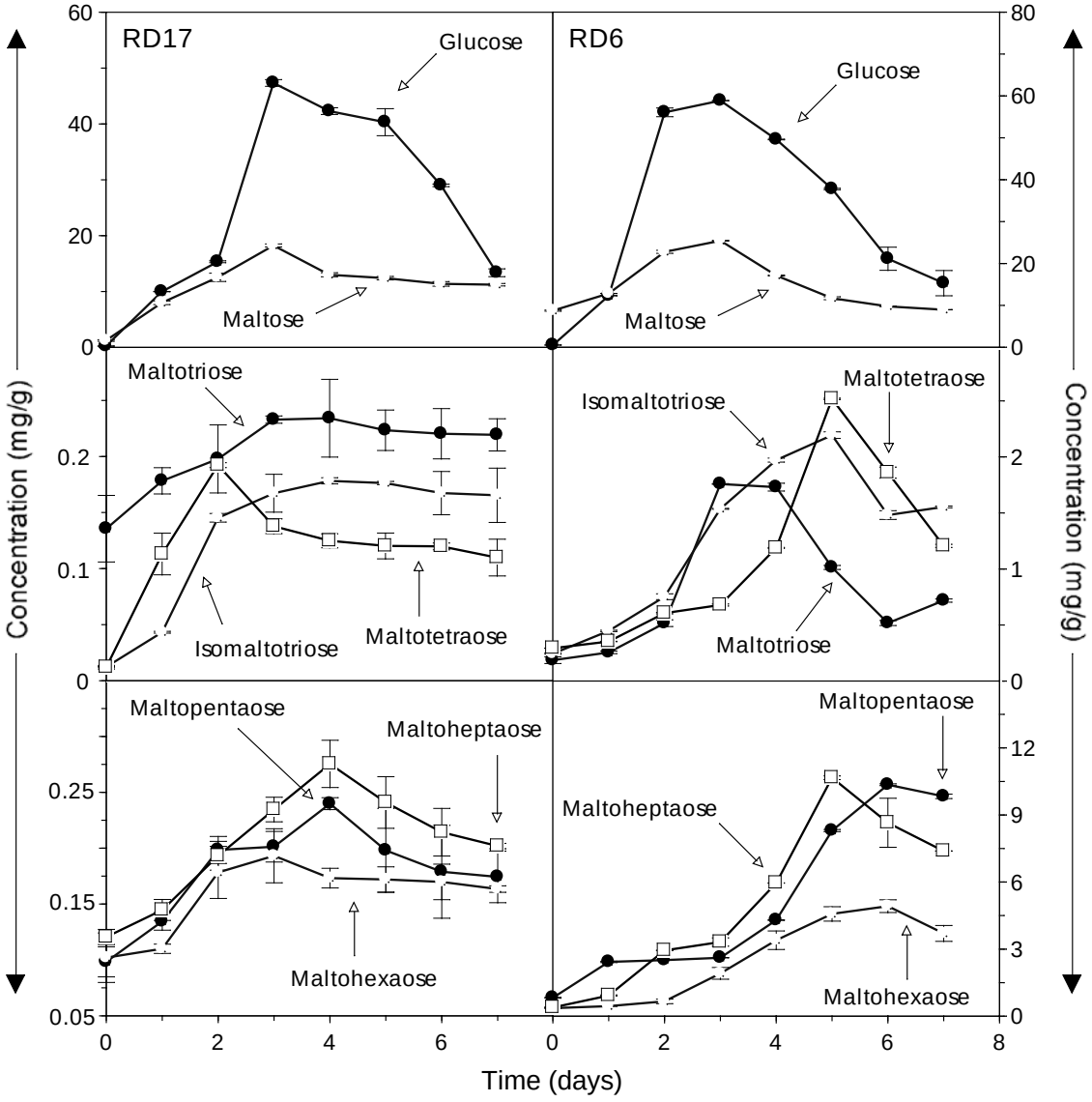

RD17
Glucose
Maltose
Maltotriose
Isomaltotriose
Maltotetraose
Maltopentaose
Maltoheptaose
Maltohexaose
RD6
Glucose
Maltose
Isomaltotriose
Maltotetraose
Maltotriose
Maltopentaose
Maltoheptaose
Maltohexaose
Concentration (mg/g)
Concentration (mg/g)
Time (days)

FIGURE 3

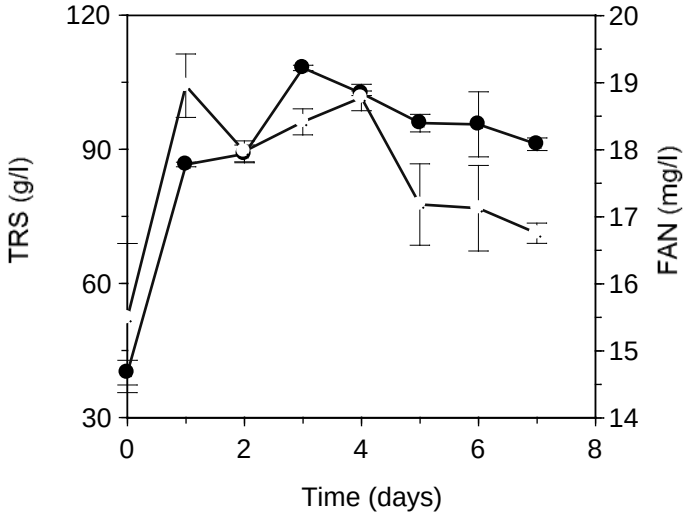
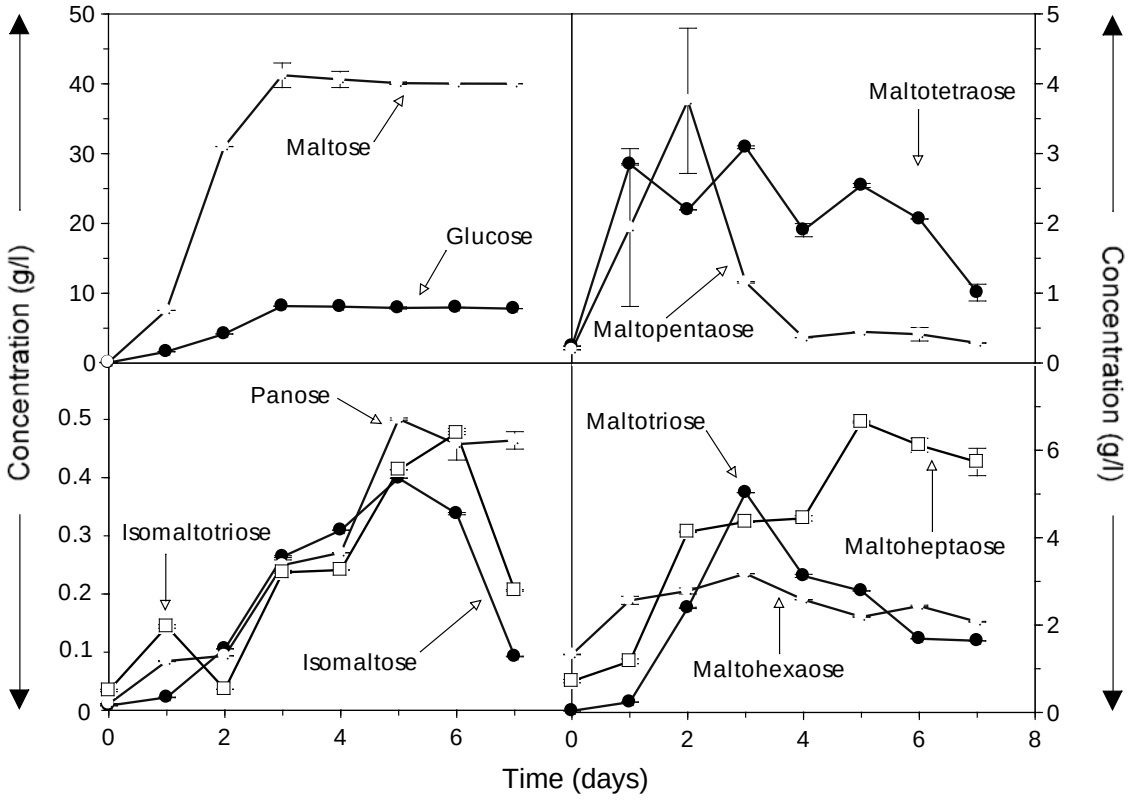


FIGURE 4


Concentration (g/l)
Concentration (g/l)
Time (days)
Maltose
Glucose
Maltotetraose
Maltopentaose
Panose
Isomaltotriose
Isomaltose
Maltotriose
Maltoheptaose
Maltohexaose