

KKU Res. J. 2012; 17(5):794-799 http://resjournal.kku.ac.th

Prebiotic isomalto-oligosaccharide production from economic crops of Thailand

Premsuda Saman*, Achara Chaiongkarn, Somporn Moonmangmee and Suparp Artjariyasripong

Bioscience Department, Thailand Institute of Scientific and Technological Research 35 M3 Technopolis, Khlong 5, Khlong Luang, Pathum Thani. 12120, Thailand *Correspondent author: premsuda@tistr.or.th

Abstract

The production of Isomalto-oligosaccharide by fungal fermentation using economic crops such as rice and cassava as substrates was investigated. Solid-state fermentation with *Aspergillus oryzae* TISTR 3102 was studied to maximise isomalto-oligosaccharides yield. Results show that the fermentation of rice with *A. oryzae* TISTR 3102 produced the highest concentrations of total reducing sugar (753 mg/g) and free amino nitrogen (33.6 mg/g) together with the highest levels of amylolytic activity (136.4 U/g), Ω -amylase (52.5 U/g) and Ω -glucosidase (2.4 U/g). After appropriate fermentation time at the 5-day, both fermented rice and cassava slurries were experimented on further in mashing for the production of syrup. The rice syrup obtained contained the highest amounts of isomaltose, panose and isomaltotriose which were prebiotic isomalto-oligosaccharides.

Keywords: solid-state fermentation, rice, isomalto-oligosaccharides

1. Introduction

Solid-state fermentation (SSF) has been applied in the production of many fermented foods (1). Various fungi have been used in order to produce amylolytic enzymes for starch degradation. Many fungal amylolytic enzymes are used to advantage in prebiotic oligosaccharide production. Isomaltooligosaccharides which are known as prebiotic branched-oligosaccharides have been synthesized from starch (2,3). The specific amylolytic enzyme, Ω -glucosidase, has been found to possess the activity of transglucosylation. This enzyme can catalyse both the hydrolysis of Ω -D-glucooligosaccharides and transfer of the glucosyl group to 6-OH of other glucosyl residues resulting in the synthesis of isomalto-oligosaccharides (4).

Isomalto-oligosaccharides have a great potential to improve the physiochemical quality of many foods as anti-fading agent for food pigments, as food antioxidant and as a sweetener. In addition, these oligosaccharides have physiological functions such as the improvement of intestinal microflora based on the selective proliferation of bifidobacteria stimulation (5,6). They are also associated with a lower risk of infections and diarrhea, and an improvement of the immune system response (7). This study was designed to investigate the potential use of economic crops of Thailand to produce prebiotic isomalto-oligosaccharides using the process of fungal fermentation. The effects of agricultural substrate and fungal strain were studied to maximise the isomaltooligosaccharides yield.

2. Materials and methods

2.1 Preparation of fungal spore inoculum

Spore suspensions of *Aspergillus oryzae* TISTR 3102 were prepared from a fully sporulated (7 days old) PDA slant culture using 10 ml of 0.85% NaCl solution. This spore suspension was used as the master suspension, which was appropriately diluted to required density. Spore concentration in the inoculum was estimated by a haemacytometer.

2.2 Substrate preparation

Two economic crops consisting of rice (*Oryza* sativa L.) and cassava (*Manihot esculenta*) were used in the experiment. The 400 g (on a dry basis) of each substrate was weighed separately into a 2-1 Erlenmeyer flasks and distilled water containing 10% (v/v) of supplementing salt solution (30 ppm of CaCl₂) was added and adjusted to 60% moisture level (8). The contents of the flasks were mixed thoroughly and autoclaved at 121°C for 15 minutes.

2.3 Solid-state fermentation

The sterilised solid substrate was inoculated with one ml of the prepared inoculum. The contents were mixed thoroughly and incubated at 30°C for 7 days. Samples of triplicate flasks were withdrawn after desired incubation.

2.4 Mashing

The fermented mass was mixed into water to form the slurry of 30% w/v (on a dry basis). One litre of the slurry was added with 0.03 g of CaCl₂ and adjusted

to pH 6 by using 0.1 M lactic acid. Mashing was carried out by following the method of Okafor and Iwouno (9). 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 elatinized 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.

2.5 Measure of total reducing sugar (TRS) and free amino nitrogen (FAN)

The samples of fermented mass were diluted with distilled water and analysed for TRS and FAN following the methods of Miller (10) and Lie (11) respectively.

2.6 Enzyme activity

Crude enzyme from the fermented mass was extracted by simple extraction. A fermented mass of 10 g was mixed thoroughly with distilled water to a total extract volume of 100 ml. Contents were mixed by shaking for one hour at 30°C on a 150 rpm shaker. At the end of the extraction, the suspension was centrifuged at 7,000 rpm for 10 min. The extracted solution was measured for amylolytic activity, α -amylase activity and α -glucosidase activity.

2.7 Determination of amylolytic enzyme and Q-amylase

The amylolytic activity was assayed using the Terashima method (12) 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 (10). One unit of the enzyme activity (U) is defined as the amount of enzyme required to liberate 1 μ mol 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 (13). One unit of α -Amylase activity (U) is then defined as the amount of enzymes required to liberate 1 µmol of maltose per min.

2.8 Determination of α-glucosidase

The α -glucosidase activity was determined using a modified method of McCue and Shetty (14). 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.

2.9 Determination of sugars by high performance liquid chromatography (HPLC)

The samples of sugars and oligosaccharides were diluted and analyzed by HPLC using a Inertsil NH2 column (5 μ m, 250×4.6 mm, Shimadzu, Japan) maintained at 40°C. The injection volume was 20 μ l, and the flow rate 1.2 ml/min. The elution of sugars was carried out with 75% acetronitrile with detection with a differential refractometer (RID-10A, Shimadzu, Japan)

3. Results

3.1 Solid-state fermentation with *Aspergillus* oryzae

SSF of rice and cassava with *Aspergillus oryzae* were demonstrated to optimise the time-course of incubation. Initial moisture content of each substrate was 60%. All SSF were inoculated with 1% of the prepared inoculum having 10⁸ spores/ml and maintained at 30°C. Results in Figure 1 show that the highest concentrations of TRS (753 mg/g) and FAN (33.6 mg/g) were obtained from SSF of rice. Similar trends of pH value were found in SSF of cassava and rice. The initial pH decreased from 5.3 to 4.3 in both SSF through a 7-day period.

Upon penetration of the kernel the fungal hyphae will come across a variety of polysaccharides in the kernel coat. The rice grain is hydrolysed by fungal amylolytic enzymes and protease resulting in increases in TRS and FAN content. The decrease of FAN and TRS after the 5-day fermentation could be due to the consumption by the synthesis of fungal biomass. The other reason of TRS decrease may be apparently due to the decrease of amylolytic enzyme (Figure 2). Yanfang et al. (15) reported that amylase decreased after 5 days of SSF due to enzyme denaturation. This could affect amount of TRS. A decrease in pH level was also observed. This could be due to the production of fungal metabolism.



Figure 1. Evolution of TRS, FAN and pH during solid-state fermentation of rice (•) and cassava (0) at 30°C for 7 days

This study presented the potential of using SSF to enhance the prebiotic components of fermented food. SSF not only served as a process for fungal growth but also produced many hydrolytic enzymes for starch degradation. These enzymes broke down starches into sugars and oligosaccharides, and proteins into peptides and amino acids (16). In the experiment, SSF was used as a source of enzymes for starch hydrolysis instead of direct amylolytic enzyme application. It is important to reduce the cost of production of prebiotic from the viewpoint of economically viable industrial applications. During fermentation starch was degraded by amylolytic enzymes produced from fungi to release smaller sugars and oligosaccharides. Comparing between two substrates, rice and cassava, significant difference in the levels of amylolytic enzyme and α -glucosidase were observed after fermentation. Figure 2 shows the highest concentrations of amylolytic activity, α -amylase and α -glucosidase were obtained in SSF of rice with the value of 136.4 U/g, 52.5 U/g and 2.4 U/g respectively.

Focusing on amylose content between two substrates, rice contains amylose 0.1 to 0.3% whereas cassava contains amylose 17 to 24%. These might be affect amylolytic activity of fungal enzyme. As shown in Figure 1 and Figure 2, the concentration of TRS in SSF using rice is higher than that in SSF using cassava as a substrate together with the amylolytic activity and the activities of Ω -amylase and Ω -glucosidase.

The results show similar trends in amylolytic activity and the activities of α -amylase and α -glucosidase in both SSF of rice and cassava. Fogarty (17) reported that the main amylolytic enzymes in Aspergillus spp. were α -amylase, amyloglucosidase, and α -glucosidase. α -Amylase is the key enzyme in starch degradation. α -Amylase hydrolyses α -(1,4)-glucosidic linkages in amylose and amylopectin and release malto-oligosaccharides of varying chain lengths while amyloglucosidase is an exo-acting starch-degrading enzyme that produces glucose from the non-reducing chain ends of the amylose and amylopectin, α -Glucosidase catalyses liberation of glucose from non-reducing ends of oligosaccharides and polysaccharides. This enzyme is able to transfer sugar moieties or groups of sugar residues from one compound to another with the formation of a similar or a distinct type of linkage. Thus, an Ω -(1,4) link in a chain might be broken and the separated end could be joined to the same or different chain via either and α -(1,4) or α -(1,6) link to produce molecules of maltose, isomaltose, panose, isomaltose or long chain of oligosaccharides.



Figure 2. Evolution of amylolytic activity, α-amylase and α-glucosidase during solid-state fermentation of rice
(•) and cassava (o) at 30°C for 7 days

3.2 Production of the rice syrup

After 5 days of fermentation, starch molecules of rice and cassava were hydrolysed by fungal amylolytic enzyme to produce small molecules of sugars and oligosaccharides. Figure 3 shows that rice slurry contain high concentration of glucose (7.8 g/l) and small amounts of maltose, isomaltose, maltotriose and panose whereas cassava slurry contains small amounts of glucose, maltose, maltotriose, panose and isomaltotriose

To avoid dissolution, the fermented slurry was used directly in mashing. The appropriate control conditions such as pH and temperature was applied in the process in order to stimulate the action of enzymes. During mashing the amylolytic and proteolytic enzymes can move freely in the liquid medium and have enough time for full starch and protein hydrolysis. The amylolytic enzymes continue to hydrolyse the remaining starch and release large amounts of fermentable sugars, in particular maltose and glucose. An increase in the levels of isomalto-oligosaccharides (isomaltose, panose and isomaltotriose) was observed after mashing (Figure 4). This could be due to the transglucosidase activity generated by *A. oryzae*. Comparing between two syrups, rice syrup contained the largest amounts of fermentable sugars, especially glucose and maltose. The highest levels of isomalto-oligosaccharides (isomaltose, panose and isomaltotriose) were also observed in rice syrup.



Figure 3. The concentration of sugars and oligosaccharides in rice and cassava slurries after 5 days fermentation with *Aspergillus oryzae*



Figure 4. The concentration of sugars and oligosaccharides in rice and cassava syrups after mashing

4. References

- Couto, S.R. and Sanroman, M.A. Application of solid-state fermentation to food industry-A review. Journal of Food Engineering. 2006; 76:291-302.
- (2) Kuriki, T., Yanase, M., Takata, H., Takesada, Y., Imanaka, T. and Okada, S. A new way of (10) producing isomalto-oligosaccharide syrup by using the transglycosylation reaction of neopullulanase. Applied and Environmental Microbiology. 1993; (11) 59: 953-9.
- (3) Pan, Y.C. and Lee, W.C. 'Production of high-purity isomalto-oligosaccharides syrup by the enzymatic conversion of transglucosidase and fermentation of yeast cells. Biotechnology and Bioengineering. 2005; 89:797-804.
- (4) Nakakuki, T. Development of functional oligosaccharides in Japan. Trends in Glycoscience (13) and Glycotechnology. 2003; 15:57-64.
- (5) Kaneko, T., Kohmoto, T., Kikuchi, H., Shiota, M., Iino, H. and Mitsuoka, T. Effects of isomaltooligosaccharides with different degrees of polymerization on human fecal bifidobacteria. (14) Bioscience Biotechnology and Biochemistry. 1994; 58: 2288–90.
- (6) Thitaram, S.N., Chung, C.H., Day, D.F., Hinton, A., Bailey, J.S. and Siragusa, G.R. Isomaltooligosaccharide increases cecal (15) Bifidobacterium population in young broiler chickens. Poultry Science. 2005; 84:998-1003.
- (7) Mizubuchi, H., Yajima, T., Aoi, N., Tomita, T. and Yoshikai, Y. Isomalto-oligosaccharides polarize Th1-like responses in intestinal and systemic immunity in mice. Journal of Nutrition. 2005; 135:2857-2861.
- (8) Anto, H., Trivedi, U.B. and Patel, K.C. (17) Glucoamylase production by solid-state fermentation using rice flake manufacturing waste products as substrate. Bioresource

Technology. 2006; 97:1161-6.

- (9) Okafor, N. and 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 Journal of Microbiology and Biotechnology. 1990; 6: 187-94.
- (10) Miller, G.L. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Analytical Chemistry. 1959; 31:426-8.
- Lie, S. 'Nihydrin method for determination of free α-amino nitrogen. Journal of the Institute of Brewing. 1973; 79:37-41.
- (12) Terashima, M., Kubo, A. and Suzawa, M. The roles of the N-linked carbohydrate chain of rice α-amylase in thermostability and enzyme kinetics. European Journal of Biochemistry. 1994; 226:249-54.
- (13) Sun, Z. and Henson, C.A. A quantitative assessment of the importance of barley seed α-amylase, debranching enzyme, and α-glucosidase in starch degradation. Archives of Biochemistry and Biophysics. 1991; 1284:298-305.
- (14) McCue, P. and Shetty, K. Role of carbohydratecleaving enzymes in phenolic antioxidant mobilization from whole soybean fermented with *Rhizopus oligosporus*. Food Biotechnology. 2003; 17:27-37.
- (15) Yanfang, Z., lijuan, W. and Wenyi, T. Biochemical changes in low-salt fermentation of solidstate soy sauce. African Journal of Biotechnology. 2009; 8:7028-34.
- (16) Abe, K., Gomi, K., Hasegawa, F. and Machida, M. Impact of *Aspergillus oryzae* genomics on industrial production of metabolites. Mycopathologia. 2006; 162:143-53.
- Fogarty, W.M. Enzyme of Genus Aspergillus.
 In: Biotechnoloy handbook : Aspergillus. New York: Plenum Press. 1994.