Food Chemistry 300 (2019) 125225

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Production and characterization of low-calorie turanose and digestion-resistant starch by an amylosucrase from *Neisseria subflava*

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ARTICLE INFO

Keywords:
Amylosucrase
Turanose
Sucrose
Fructose
Neisseria subflava
Digestion-resistant starch

ABSTRACT

This study was intended to produce turanose and resistant starch (RS) using recombinant amylosucrase from *Neisseria subflava* (*Ns*AS). Turanose production yield maximally reached to 76% of sucrose substrate at 40 °C by *Ns*AS treatment. To evaluate turanose as a low-calorie functional sweetener, its hydrolysis pattern was investigated in continuous artificial digestion system. When turanose was consecutively exposed through small intestinal phase, only 8% of disaccharide was hydrolyzed. Structural modification of gelatinized corn or rice starch was carried out by *Ns*AS with sucrose as a glucosyl donor. Non-digestibility of enzyme-modified starches increased to 47.3% maximally through branch-chain elongation, enough for chain-chain association and recrystallization. Obviously, *Ns*AS-modified starches had higher gelatinization peak temperatures than native counterparts, and their paste viscosity was inversely related to their digestibility due to elongated-chain induced retrogradation. These results suggested that *Ns*AS could be a vital biocatalyst candidate in food industry to produce next generation low-calorie carbohydrate food materials.

1. Introduction

Amylosucrase (ASase; E.C. 2.4.1.4), belonging to glycoside hydrolases family 13, is a remarkable glucosyltransferase (De Montalk, Remaud-Simeon, Willemot, Planchot, & Monsan, 1999; Edward & Hehre, 1949). This enzyme has the specific catalytic capacities to synthesize linear α-(1,4)-linked glucans (amylose-like polymers) using sucrose as a sole substrate and to make extra-long branch chains on pre-existing amylopectin as an acceptor molecule, and release fructose as a byproduct (Albenne et al., 2004; Kim et al., 2013; Skov et al., 2001). The most interesting feature of this enzyme is that it does not require expensive activated nucleotidyl-sugars unlike other α-(1,4)-glucan synthesizing enzymes. The glucan synthesis by ASase has been known to be inhibited when the concentration of sucrose was relatively high in the reaction condition (Wang et al., 2012). The main transglycosylation reaction pathway of ASase clearly shifted from α-(1,4)-glucan synthesis to turanose production by simply increasing sucrose concentration (Wang et al., 2012). Particularly, the concentration of sucrose affected the chain length of the glucan (Albenne et al., 2004). This unique enzymatic property of ASase may provide more feasible applications in the food industry. The resulting elongated polymers displayed a great tendency to have a high content of digestion-resistant starch attributed by starch retrogradation, which was classified to resistant starch type-3 (RS3) (Rolland-Sabaté, Colonna, Potocki-Veronese, Monsan, & Planchot, 2004). Basically, starch is a complex branched glucose polymer whose chain length distribution influences nutritionally important property such as digestion rate. It has two major components, amylose and amylopectin. Amylose is essentially linear glucan chain, while amylopectin is composed of a large number of relatively short branch chains. Starches with higher amylose content or with longer-branched amylopectin have a higher tendency to retrograde, thus slowing down enzymatic degradation. Based on the digestibility, starch can be categorized into rapidly digestible starch (RDS), slowly digestible starch (SDS), and digestion-resistant starch (RS) (Englyst, Kingman, & Cummings, 1992). Therefore, the concept of RS has been developed and industrialized in the food processing field. In the earlier studies (Kim et al., 2013; Ryu et al., 2010), *Np*AS was used in starch processing with different acceptors, such as corn, rice, and barley starches to successfully produce RS3. It was found that the RS contents were significantly increased in the modified starches by *Np*AS compared to those of native starches (Kim et al., 2013; Ryu et al., 2010). In the present study of *Ns*AS, the unique enzymatic property of amylosucrase-type enzyme was utilized to develop an efficient production method of digestion-resistant starches.

Sucrose is a disaccharide that is composed of glucose and fructose and is used in a variety of applications as a sweetener. Sucrose has also

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https://doi.org/10.1016/j.foodchem.2019.125225
Received 23 April 2019; Received in revised form 19 July 2019; Accepted 21 July 2019
Available online 23 July 2019
0308-8146/

been used as a standard for sweetness in the food industry. Rapid hydrolysis of sucrose produced glucose and fructose in human intestines, which was followed by an increase in blood glucose levels. Moreover, undue consumption of sucrose increased proneness to symptoms or diseases like tooth decay, obesity, and diabetes mellitus (Chung et al., 2017; Malik, Schulze, & Hu, 2006), which upturned the necessity for unfermentable low-calorie alternative sweeteners. Turanose, 3-*O*-α-D-glucosyl-D-fructose, is one of the sucrose isomers that naturally exist in honey. The sweetness of turanose is half of sucrose (Potocki-Veronese et al., 2005; Wang et al., 2012), and it is hydrolyzed more slowly than sucrose. Thus, turanose could potentially serve as a low glycemic sweetener similar to palatinose (Rolland-Sabaté et al., 2004), a substitute for sucrose, and mediate beneficial effects for the management of chronic metabolic diseases (Chung, Kim, Kim, & Yoo, 2017). Turanose is a byproduct of the synthesis of linear α-(1,4)-glucan from sucrose, a reaction that is catalyzed by ASase. A recombinant form of this amylosucrase has been generated based on the enzyme in *N. polysaccharea* (*Np*AS) (Kim et al., 2013). Synthesis of sucrose isomers, turanose and trehalulose, and sucrose hydrolysis also occurred as the minor reaction of *Np*AS (Potocki de Montalk et al., 2000; Wang et al., 2012). According to Wang et al. (Wang et al., 2012), turanose is one of the non-cariogenic and low-calorigenic disaccharides. As a structural isomer of sucrose, turanose has a promising potential to be a novel functional sweetener in the food industry. Moreover, until now, chemical synthesis of turanose is too tedious to envisage its application on an industrial scale, unlike commercially launched trehalulose, leucrose, and isomaltulose by enzymatic productions. Consequently, the use of ASase may constitute a novel and very promising way to synthesize this unique turanose. Previous studies proved that the production yield of turanose was highly dependent on the initial sucrose concentration in the ASase bioconversion reaction. According to Shibuya et al. (Shibuya et al., 2004), the production yield of turnaose was maximally 45% (w/w) of solid contents (D-fructose and α-CD) with the cyclomaltodextrin glucanotransferase from *Bacillus stearothermophilus*. Albenne et al. (2004) showed that turanose yield was 14% (w/w) of initial amount of sucrose with *Np*AS at 100 mM sucrose concentration. In 2012, the study of our laboratory showed that the maximal production yield of turanose was 56.2% (w/w) under the reaction condition of 2 M sucrose at 35 °C. Recently, Park et al. (2016) demonstrated that turanose was produced by increasing the amount of extrinsic fructose as a reaction modulator, with 2.0 M sucrose as a sole substrate, which resulted in 73.7% (w/w) of production yield with *Np*AS. Moreover, this enzymatic property of *Np*AS may provide more feasible applications in the food industry, among which the RS could be produced under the reaction of *Np*AS (Ryu et al., 2010). In the present study, we utilized these unique enzymatic properties of the recombinant amylosucrase from *N. subflava* (*Ns*AS) to develop an efficient production method for low-calorie turanose and digestion-resistant starches. The reaction products of the recombinant *Ns*AS were obtained on two commercial starches of different amylose contents and sucrose biomass. Also, its possible applications for RS and turanose productions were evaluated by determining their production yields, and the physical and chemical properties. In addition, biosafety level 1 of the ASase-derived *N. subflava*, unlike *N. polysaccharea*, could be evaluated positively to enhance the feasibility of utilizing this enzyme in the food industry, and thus *N. subflava* strain is unlikely to cause safety problems (Ohta, Hatada, Ito, & Horikoshi, 2005). Therefore, the expected outcome of this study was to find out the possibility of developing the biocatalytical processes for converting high glycemic carbohydrates such as sucrose and starch to low glycemic sugar and carbohydrate alternatives like turanose and digestion resistant starches.

2. Materials and methods

2.1. Chemicals

Glucose, fructose, sucrose, and maltose were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO). Turanose (3-*O*-α-D-glucosyl-D-fructose; CAS No. 547-25-1) was purchased from Carbosynth Ltd (Berkshire, RG20 6NE, UK). Other chemicals used were of analytical grade. Waxy and normal corn starches were gifts from Daesang Co. (Icheon, Korea), and high-amylose corn (amylomaize VII) starch was provided by Ingredion Korea (Icheon, Korea); their apparent amylose contents were < 1%, 25%, and 70%, respectively. Rice starches were isolated in the laboratory from four different cultivars of goamy, dongjin, manmi, and sinsunchal of which the amylose contents were reported as 27.3%, 18.9%, 11.4%, and 3.6%, respectively (Kim et al., 2013).

2.2. Production of NsAS

Construction of an expression vector for active *Ns*AS protein was described in detail previously (Park, Chandrasekaran, & Yoo, 2018). *E. coli* BL21 (DE3) cells containing the recombinant vector were grown in LB broth supplemented with 50 μg/mL of ampicillin at 37 °C until the Δabs_{600} reached 0.6–0.8. The culture was then induced to the expression of amylosucrase by the addition of IPTG to the final concentration of 0.2 mM and incubated at 18 °C for 21 h. The culture was harvested by centrifugation (5000*g*) at 4 °C for 10 min. The cell filtrate was resuspended in 50 mM Tris-HCl buffer (pH 8.0). The suspended cells were disrupted by VibraTM Cell VC 750 disruptor (Sonics & Materials, Inc, Newtown, CT) on the ice and centrifuged at 10,000*g* and 4 °C for 20 min. The supernatants containing *Ns*AS were filtered through a 0.45-μm syringe filter. The process of purification of $(His)_6$-tagged *Ns*AS were conducted in nickel-nitrilotriacetic acid (Ni-NTA) affinity chromatography column (Qiagen, Hilden, Germany). The buffer of eluent was changed to 50 mM Tris-HCl buffer (pH 8.0) and the enzyme solution was concentrated by centrifugation at 2500*g* at 4 °C for 20 min by using 30 K Amicon Ultra-15 centrifugal filters of (Merck Millipore, Carrigtwohill, Ireland).

2.3. Determination of enzyme activity

The *Ns*AS activity was measured by following the procedure of a previous study (Potocki-Veronese et al., 2005) with some modifications. The enzyme assay was carried out in 50 mM Tris-HCl buffer (pH 8.0) containing 0.1 M sucrose at 40 °C for 30 min, and then the reaction was stopped by adding 0.5 mL of DNS (3,5-dinitrosalicylic acid) solution and colorized by heating. One unit of *Ns*AS activity was defined as the amount of enzyme that released 1 μmol of reducing end per min.

2.4. Optimization of the NsAS reaction condition for turanose production

2.4.1. Effect of reaction temperature on turanose synthesis yield

Turanose synthesis was investigated with 2.0 M sucrose as the sole substrate in 50 mM Tris-HCl (pH 8.0) and three different reaction temperatures (35 °C, 40 °C, and 45 °C). The substrate solution was preincubated at three different temperatures for 10 min before the reaction. The enzymatic reaction was carried out with 400 U/L of *Ns*AS for 120 h. At different reaction times, samples of the reaction mixture were collected and inactivated by boiling for 5 min. The yield of turanose was defined to be the amount of turanose produced per the amount of sucrose initially added in the reaction mixture.

2.4.2. Effect of pH on turanose synthesis yield

Turanose synthesis was investigated with 2.0 M sucrose as the sole substrate in three different pH 7.0, 8.0, and 9.0 at 40 °C. The substrate solution was preincubated at 40 °C for 10 min before the reaction. The

enzymatic reaction was carried out with 400 U/L of *Ns*AS for 120 h. At different reaction times, aliquots of the reaction mixture were collected and boiled for 5 min to inactivate the enzyme. The soluble reaction product was analyzed by HPAEC as described in the previous study (Park et al., 2016).

2.4.3. Effect of sucrose and fructose concentration on turanose synthesis yield

Turanose synthesis was investigated by using sucrose as substrate at various concentrations of substrates (1.0, 1.5, and 2.0 M) amendment of various fructose (0.25, 0.5, 0.75, and 1.0 M) in 50 mM Tris-HCl (pH 8.0) at 40 °C. The substrate was pre-incubated at three different temperatures for 10 min. The enzymatic reaction was carried out with 400 U/L of *Ns*AS for 120 h. At different reaction times, samples were collected and inactivated by boiling for 5 min. The soluble fraction of reaction product was analyzed by HPAEC.

2.5. In vitro digestibility of turanose in a continuous simulated digestion fluid system

The degree of turanose hydrolysis in the continuous simulated digestion fluids was carried out by the modified Minekus's method (Minekus et al., 2014). The enzymatic digestion was carried out in three successive steps with artificial digestives at 37 °C, and 130 rpm in a water bath. The simulated salivary fluid (SSF), simulated gastric fluid (SGF), and simulated intestinal fluid (SIF) consisted of electrolyte stock solutions, $CaCl_2$, digestive enzymes, HCl, and water, depending on their own individual physiological conditions (Supplement 1). Maltose and sucrose were used as control sugars at 40 mM level for comparing the digestion degree to turanose. The first digestion step was an enzymatic hydrolysis process in SSF at pH 7.0 for 2 min. In this oral phase, final ratio of sugar sample solution to SSF is targeted to 50:50 (v/v). The composition of 1 mL SSF was as follows: 0.7 mL of SSF electrolyte stock solution, 5 μL of 0.3 M $CaCl_2$, 0.1 mL of human salivary α-amylase solution (1500 U/mL of α-amylase in SSF stock solution), and 195 μL of distilled water. The second step was a gastric phase in SGF at pH 3.0 for 2 h. The 1 mL of SSF was mixed with 1 mL of SGF. The component contents of 1 mL of SGF were as follows: 0.75 mL of SGF electrolyte stock solution, 0.5 μL of 0.3 M $CaCl_2$, 5.6 μL of 1 M HCl, 0.16 mL of porcine pepsin stock solution (25,000 U/mL porcine pepsin) in SGF stock solution, and 83 μL of distilled water. As the final digestion step, artificial intestinal phase was prepared in SIF at pH 7.0 for 4 h. The reaction mixture (1 mL) in the gastric phase was mixed with 1 mL of SIF of which the composition was as follows: 0.25 mL of SIF electrolyte stock solution, 2 μL of 0.3 M $CaCl_2$, 35 μL of 1 M HCl, 0.125 mL of 160 mM of fresh bile, 0.25 mL of pancreatin (0.1 g) solution/1 mL in SIF stock solution, 0.30 mL of intestinal fluid containing 16.7 mg of rat intestinal acetone powder per 1 mL in SIF stock solution, and 38 μL of distilled water. At different reaction time intervals for each digestion step, aliquots (100 μL) were collected, and the degree of hydrolysis was assessed by the amounts of released glucose from the reaction using a glucose diagnosis kit (GOPOD kit, Megazyme International Ireland Limited, Bray, Ireland) (Trinder, 1969).

2.6. Starch modification by NsAS treatment

Enzymatic modification of gelatinized corn and rice starches (3%, w/v) was carried out in 50 mM Tris-HCl buffer (pH 8.0) with 0.3 M of sucrose as described previously (Ryu et al., 2010).

2.7. Determination of RS content of NsAS-treated starches

The freeze-dried non-soluble precipitates were ground with mortar and pestle, and sieved through a 100-mesh screen. RS content was investigated using the Megazyme Resistant Starch Assay Kit (Megazyme International Ireland Limited) following the AACC Method 32-40.01 (McCleary & Monaghan, 2002) that was slightly modified to determine RS content in non-soluble precipitate. The contents of readily digestible starch (RDS), non-soluble resistant starch (NSRS), and total RS content were determined by following the method of (Ryu et al., 2010).

2.8. Branch-chain length distribution analysis by HPAEC of NsAS-treated starches

The branch-chain length distributions of *Ns*AS-treated starch were measured by high-performance anion-exchange chromatography (HPAEC, ICS-5000 SP, Dionex, Sunnyvale, CA) coupled with a pulsed amperometric detector (PAD, ICS-5000 DC, Dionex). Total 50 mg each of normal starch or enzyme-treated starch was completely wetted with 0.5 mL of distilled water for 10 min and then dispersed in 4.5 mL of dimethyl sulfoxide (DMSO). The dispersion in a glass vial was boiled for 1 h with constant stirring, which was additionally stirred for another 12 h at room temperature. The resulting clear solution was mixed with 6 volumes of 99% ethanol, then followed by centrifugation (4500*g*) for 20 min. The precipitates were dissolved in 4 mL of 10 mM sodium acetate buffer (pH 3.5) and then boiled for 1 h with constant stirring. After cooling it, the sample solution was debranched with isoamylase (Megazyme International Ireland Limited) for the hydrolysis of α-(1,6)-D-glucosidic branch linkages at 40 °C for 48 h. After the isoamylolysis of the sample solution, the salts of sodium acetate buffer components were removed for the HPAEC analysis. The reacted samples were mixed with 0.2 g of resin beads (IONAC NM-60H+/OH− form, J. T. Baker, Phillipsburg, NJ) and were shaken to desalt for 1 min. And then, the sample was filtered through a 0.2-μm syringe filter. The filtered sample (25 μL) was injected into a CarboPac™ PA200 analytical column (3 × 250 mm, Dionex). Hydrolyzed products were separated via a linear gradient mode from 150 mM NaOH initially to 600 mM sodium acetate + 150 mM NaOH for 135 min (Jane, Wong, & McPherson, 1997; Lee, Yoo, Ryu, Kim, & Yoo, 2008).

2.9. Thermal analysis of NsAS-treated starches

Thermal properties of enzyme-modified RS products were analyzed by using a differential scanning calorimeter (DSC-200, Netzsch, Germany) (Zeleznak & Hoseney, 1987). The sample (5 mg) was exactly weighed in an aluminum pan, and 15 mg of distilled water was added and sealed. This sample dispersion was equilibrated at ambient temperature for 2 h. The sample was scanned at a heating rate of 5 °C/min from 20 °C to 150 °C, using an empty pan as a reference.

2.10. Pasting profile of NsAS-treated starches

Pasting properties of enzyme-modified RS products were measured by a starch pasting cell attached to a controlled-stress rheometer (AR1500ex, TA Instruments) (Jeong, Kim, & Lee, 2017). The whole sample was added in an aluminum canister with distilled water to prepare a suspension (28 g; 10.7%, w/w) whose viscosity was monitored during the programme heating and cooling cycle. Initially, the starch samples were warmed up to 50 °C and started heating at a rate of 12 °C/min to 95 °C. This temperature was kept for 2.5 min and cooled at a rate of 12 °C/min to 50 °C. Pasting temperature, peak viscosity, final viscosity, breakdown, and setback were determined to explain the pasting properties of *Ns*AS-treated RS products.

2.11. Statistical analysis

ANOVA and Tukey's honestly significant difference tests were performed using Sigmaplot software package 13.0 (Systat Software Inc., San Jose, CA).

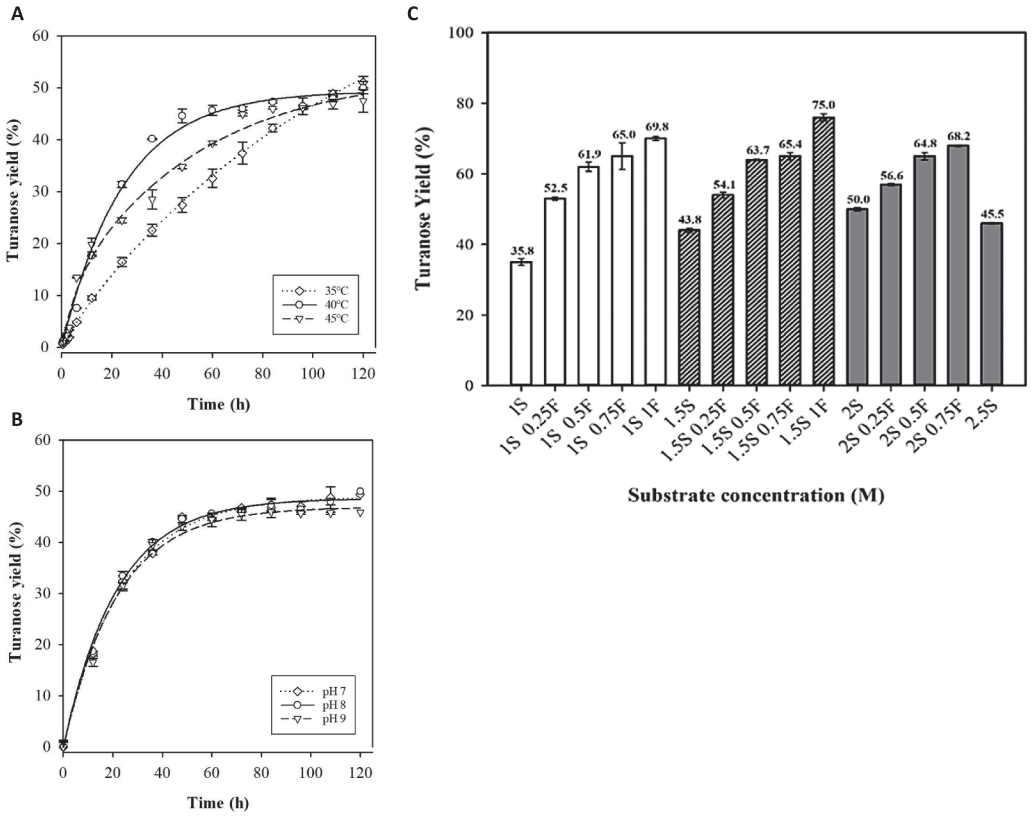

Fig. 1. Effect of reaction operating parameters on turanose yield with 2.0 M sucrose as a substrate. **A**, temperature; **B**, pH; **C**, sucrose and fructose concentrations in 50 mM Tris-HCl buffer (pH 8.0) at 40 °C.

3. Results and discussion

3.1. Optimization of NsAS reaction condition for turanose production

Effect of reaction temperature on turanose yield was performed at 35 °C, 40 °C, and 45 °C with 2.0 M sucrose as a substrate (Fig. 1A). After a 12 h reaction, it was found that *Ns*AS at 45 °C initially produced a greater amount of turanose than those of the other two lower temperatures. However, after 24 h in the middle of the reaction, *Ns*AS at 40 °C produced more turanose than at 45 °C. At the end of the reaction, the final turanose yields were 51.1% at 35 °C, which was the greatest one, and 50.0% at 40 °C and 47.5% at 45 °C, respectively. As a result, the turanose production at a relatively lower temperature was preferred over the α-(1,4)-glucan synthesizing reaction even though the reaction rate at 35 °C was slower than at higher temperatures tested in this study. At 45 °C, a certain fraction of the enzyme might lose its activity by thermal denaturation from a very early time point of the reaction. Thus, it would negatively affect turanose productivity eventually. In our previous study it was also found that *Np*AS decreased turanose yields along with the reaction temperatures (Wang et al., 2012). In order to achieve efficient synthesis of turanose and to avoid inactivation of the enzyme during the reaction conditions, the reaction temperature of 40 °C was applied for further study. If the temperature is lowered too much, it might lead to a decreased catalytic conversion rate of the enzyme in terms of turanose productivity (Wang et al., 2012). In addition, the effect of pH on turanose yield was studied at three different pHs (7.0, 8.0, and 9.0) at 40 °C with 2.0 M sucrose as a substrate (Fig. 1B). No detectable difference was noticed from the initial turanose conversion rate at all the tested pHs. At the end of the reaction equilibrium, turanose yields were 49.4% and 50.0% at pH 7.0 and 8.0, respectively, while it was 45.9% at pH 9.0. Therefore, pH 8.0 was selected as an optimal reaction pH for turanose synthesis of *Ns*AS. The effect of substrate concentration on turanose synthesis yield was investigated at 40 °C with 400 U/L of *Ns*AS (Fig. 1C). The final turanose yield significantly increased after a 120-h enzymatic reaction as the sucrose concentration increased from 1.0 to 2.0 M, which indicated that higher substrate concentration facilitated the turanose production. Our results suggested that turanose yield was highly depending on the initial sucrose concentration, and 1.5 M sucrose +1.0 M fructose was the optimum substrate concentration for the greatest turanose yield (76.0%) in 50 mM Tris-HCl buffer (pH 8.0) at 40 °C for 120 h. According to Wang et al. (2012) with 2.5 M sucrose, the production yield of turanose maximally reached 56.2% at 35 °C after a 120-h reaction of *Np*AS, whereas 73.7% of production yield was obtained by increasing the amount of extrinsic fructose as a reaction modulator for *Np*AS (Park et al., 2016). However, in this *Ns*AS reaction system, turanose yield obviously decreased when substrate concentration of sucrose increased to 2.5 M sucrose. In the reaction mixture with 2.5 M sucrose, *Ns*AS might not completely consume substrate at 40 °C. A previous study reported that the reaction at 35 °C would produce more turanose than that at 40 °C if the sucrose was allowed to be fully consumed by the *Np*AS enzyme with a prolonged reaction time, the higher temperature (40 °C) was still chosen in this study, as the optimal reaction temperature, considering the greater productivity of turanose. It was quite a unique environment of enzyme reaction because the solute concentration in the reaction mixture was enormously high and the solution itself had very high viscosity. In this kind of reaction system, thus, water-deficient environment as well as biochemical property of amylosucrase protein might shift the reaction flow toward the reaction of sucrose isomer production.

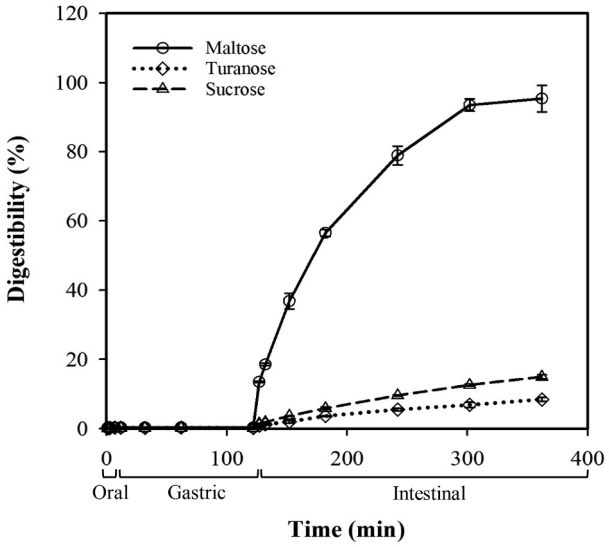

Fig. 2. Turanose hydrolysis pattern in the continuous simulated digestion fluid system.

3.2. *In vitro digestibility of turanose in a continuous simulated digestion system*

To evaluate turanose as a low-calorie functional sweetener, the turanose hydrolysis pattern was investigated in a continuous simulated digestion system (Fig. 2). The degree of hydrolysis in each digestion fluid was determined with the released amount of glucose by the GOPOD method. In the oral phase (simulated salivary fluid; SSF), it was noticed that turanose, sucrose, and maltose were not hydrolyzed at all after 2-min incubation. The SSF contained α-amylase which hydrolyzed dietary starch into oligomers mainly consisting of maltose and maltotriose, and α-limit dextrin. Therefore, little hydrolysis was observed from these disaccharides. After incubation in the simulated gastric fluid (SGF) as the following step, all the sugars tested were not digested at all. In the small intestinal fluid (SIF) phase, approximately only 8% of turanose was hydrolyzed during 4-h incubation. Maltose was completely hydrolyzed up to ≥96% in 4-h reaction, whereas sucrose was hydrolyzed by approximately 15% under the same digestion condition. The rate of glucose released from turanose was significantly slower than those of sucrose and maltose at the SIF phase *in vitro*. Interestingly, the hydrolysis rate of sucrose was very low when compared to other sugar substrates although higher than that of turanose consistently. Previously, there were a few of reports that sucrase-type enzyme showed much greater hydrolyzing activity on α-1,4-glycosidic linkages than

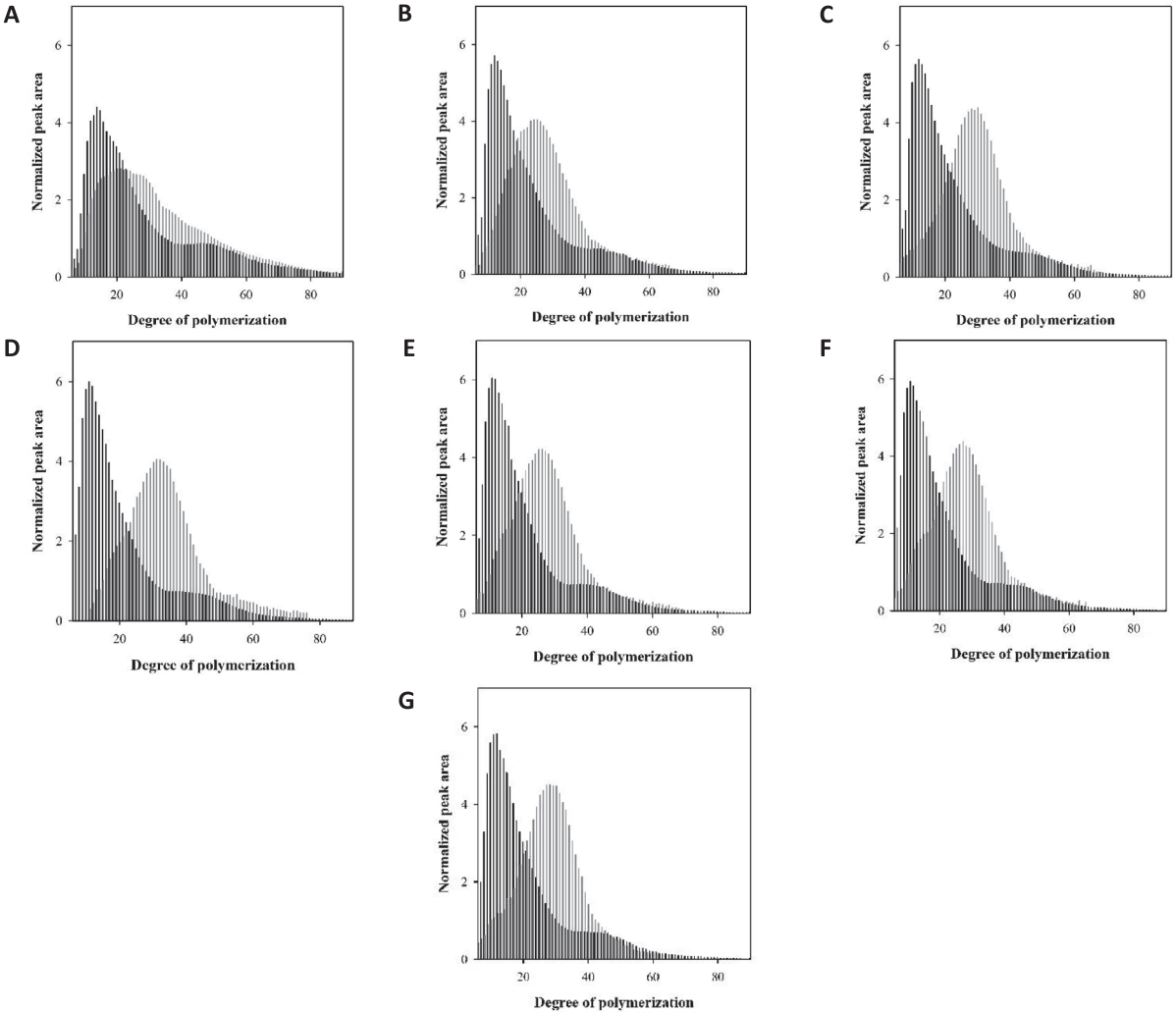

Fig. 3. Branch-chain length distributions of native and *Ns*AS-treated starches. **A**, amylomaize VII; **B**, normal corn starch (NCS); **C**, waxy corn starch (WCS); **D**, goamy rice starch; **E**, dongjin rice starch; **F**, manmi rice starch; **G**, sinsunchal rice starch.

α,β-1,2-linkages of sucrose in mammalian digestive enzyme complexes (Bergoz, Griessen, Infante, de Peyer, & Vallotton, 1981; Lee et al., 2016). Very low pH at gastric fluid environment might more significantly affect the hydrolysis degree of sucrose while no degradation of sucrose was observed at pH 3.0 in this study. It was clearly noticed that turanose had very low digestibility in passing through the whole simulated digestion system. Thus, this result suggested that turanose could be used as a low-calorie sweetener, which will be a new potential substitute for sucrose in the food industry.

3.3. Branch-chain length distribution of enzyme-modified starches

The chemical structures of corn and rice starches were modified by the *Ns*AS treatment with the gelatinized starches (3%, w/v) as acceptor molecules, and 0.3 M sucrose as a glucosyl donor. The branch-chain length distributions of native and *Ns*AS-treated starches were determined by HPAEC analysis. The degree of polymerization (DP) in the isoamylase-treated linear chain was increased after branch chain elongation by the transglycosyl activity of *Ns*AS (Fig. 3). Also, the change in branch-chain length distribution of amylomaizeVII was smaller than the other starches because of the higher proportion of amylose in this starch (Fig. 3A). The glucose moiety from sucrose could be transferred to the non-reducing ends in amylopectin and amylose molecules of native starches, and thus, the glucose-transferring rate to amylopectin was quicker compared to the rate to amylose (Ryu et al., 2010). Whereas, more extended branch chains were observed in the other six starches (Fig. 3B–G). Our results confirmed that the most abundant branch chain with the greatest peak areas increased from 14 to 20 for amylomaize VII, from 12 to 25 for NCS, from 12 to 28 for WCS, from 11 to 26 for goamy, from 11 to 26 for dongjin, from 11 to 27 for manmi, and from 11 to 28 for sinsunchal. A previous study on the influence of amylose chain length on enzyme-resistant starch formation by hydrolyzing potato starch amylose to varying degrees by incubation with barley β-amylase showed that the number of average DP of resistant starch (RS) varied only between 19 and 26 and was independent of the chain length of the amylose (Eerlingen, Decuninck, & Delcour, 1993). In addition, the peak branch-chain length by transferring glucose units seemed to be inversely related to the amylose content of starch (Eerlingen et al., 1993; Ryu et al., 2010). Regardless of starch sources, the final average lengths of *Np*AS-modified amylopectins were within a very narrow range (DP 23–25) (Ryu et al., 2010), suggesting that critical chain length of amylopectin existed for producing insoluble RS products. Consecutively, chemical structure of starches was changed by *Ns*AS treatment, which led to the increase in non-digestibility that was presented by the RS content specifically due to elongation of amylopectin branch chains.

3.4. RS contents of the enzyme modified starches

Total RS contents of the *Ns*AS-modified starch were considerably greater than those of native starch counterparts (Table 1). In *Ns*AS-treated corn starches, such as native NCS and WCS, non-soluble RS (NSRS) contents were increased by 37.7% and 47.3%, respectively. Enzyme-modified amylomaize VII showed relatively less increase in NSRS content (ca. 20.5%) when compared to those of other starches. Among the native starch samples, amylomaize VII did not completely gelatinize, and thus, the *Ns*AS reaction on this starch was not efficient enough to increase in NSRS content significantly. The same pattern of the amylosucrase reaction on amylomaize VII was observed from the previous *Np*AS study (Ryu et al., 2010). In case of rice starches, the increase in NSRS was highest in goamy by 45.7%, which was followed by 45.6%, 44.3%, and 43.1% from dongjin, manmi, and sinsunchal, respectively. Furthermore, RS contents of *Ns*AS-modified starches were higher than the previously reported RS contents of *Np*AS-modified starches (Ryu et al., 2010), suggesting that *Ns*AS treatment was more effective to produce resistant starch than *Np*AS-induced modification. Therefore, the *Ns*AS treatment on starch substantially increased the degree of recrystallization as well as branch-chain length of starch molecules compared to those of *Np*AS, which made the products more resistant to digestive enzymes (Ryu et al., 2010).

3.5. Thermal properties of the enzyme modified starches

Thermal properties of the *Ns*AS-modified starches were analyzed by DSC analysis as shown in an endothermic heat flow profile over heating temperature (Supplement 2). In native corn and rice starch samples, the melting onset (T_o) and peak (T_p) temperatures increased along with their amylose contents (Table 2). Once the gelatinized starches were treated with *Ns*AS, they clearly displayed higher T_p than native starch counterparts; 81.7 → 111.8 °C (ΔT = 30.2 °C), 73.8 → 105.0 °C (31.3 °C), and 74.4 → 104.9 °C (30.5 °C) for amylomaize VII, NCS, and WCS in corn starches, respectively, where the values (ΔT) in parenthesis imply the temperature differences. In the case of the modified rice starches, the T_p increases were much higher than the modified corn starches; 66.8 → 104.9 °C (ΔT = 38.1 °C), 68.7 → 105.3 °C (36.6 °C), 68.7 → 105.1 °C (36.4 °C), and 68.4 → 105.3 °C (36.9 °C), respectively, for goamy, dongjin, manmi, and sinsunchal rice starches. Interestingly, the melting temperatures of all the enzyme-treated corn and rice starches were converged to around 105 °C except for amylomaize VII. Whatever the starting branch-chain length distributions were, that is, the elongated chain lengths by *Ns*AS seemed to be almost identical among them regardless of starch source. In DSC analysis of starch granules, the endothermic peak caused by starch gelatinization has been well known to be attributed from melting of the crystalline region consisting exclusively of amylopectin molecules (Eliasson, 1985). As noticed from branch-chain length distributions of *Ns*AS-treated starches, the elongated peak DP were within a very narrow range of DP25-28 for corn starch and of DP26-28 for rice starch. As described above, the *Ns*AS treatment significantly increased the branch-chain length of native starches, thus making inter-chain association much easier (Ryu et al., 2010). It was known that the starch amylopectin with a larger proportion of long branch-chains displayed higher gelatinization temperatures and enthalpy changes (Sanders, Thompson, & Boyer, 1990; Sasaki & Matsuki, 1998; Shi, Seib, & Bernardin, 1994; Yuan, Thompson, & Boyer, 1993). In our study, the peak branch-chain lengths were almost identical among the starch samples, which was in accordance with the converged peak temperature of melting, although initial gelatinization temperatures were quite different from each other among various starch sources. According to Ryu et al. (2010), a longer linear portion of enzyme-elongated chains in starch amylopectin could make a more perfect crystalline structure by the recrystallization process, which made the products more resistant to digestive enzymes. The findings in this study suggested that rice starch was more than enough to make RS as confirmed by the RS content determination above. Native rice starch gelatinized at a much lower temperature than corn starch, whereas the enzyme-treated ones showed significantly higher T_{gel} that was congregated at the same temperature, around 105 °C. Thus, even low-temperature gelatinized starch might be applicable for generating RS products by utilizing this enzymatic bioprocess technique.

3.6. Pasting properties of the enzyme modified starches

The maximum heating temperature was 95 °C except for dongjin whose heating temperature was increased to 90 °C maximally in the experimental condition because dongjin starch showed tremendously high viscosity and produced significant forming that led to over-flow from the starch pasting cell at 95 °C. Apart from amylomaize VII, all the native starch and RS samples displayed viscosities under the applied temperature gradient profile (Table 3), but there were huge differences in the magnitude of viscosity scale between native and enzyme-modified starches. Peak viscosity temperatures of native NCS, WCS, goamy, dongjin, manmi, and sinsunchal were 89.0, 77.3, 91.1, 90.9, 92.2, and

Table 1
Digestible and resistant starch contents of *Ns*AS-modified products.[1]

Samples		RDS[2] (%)	SRS[3]	NSRS[4]	TRS[5]
Corn starch	Amylomaize VII	67.1 ± 0.6^{d}	12.9 ± 1.9^{fg}	20.0 ± 1.2^{g}	32.9 ± 0.6^{e}
	Modified amylomaize VII	45.0 ± 0.5^{e}	14.6 ± 0.5^{ef}	40.4 ± 0.0^{e}	55.0 ± 0.5^{d}
	NCS[6]	91.2 ± 3.0^{bc}	8.1 ± 2.9^{hi}	0.7 ± 0.1^{h}	8.8 ± 3.0^{fg}
	Modified NCS	39.1 ± 0.8^{f}	22.6 ± 2.6^{b}	38.4 ± 1.8^{f}	60.9 ± 0.8^{c}
	WCS[7]	94.4 ± 0.6^{a}	5.4 ± 0.6^{i}	0.2 ± 0.0^{h}	5.6 ± 0.6^{h}
	Modified WCS	31.3 ± 0.8^{h}	21.1 ± 0.4^{bc}	47.5 ± 0.4^{a}	68.7 ± 0.8^{a}
Rice starch	Goamy	90.7 ± 0.5^{bc}	8.8 ± 0.6^{h}	0.5 ± 0.1^{h}	9.3 ± 0.5^{fg}
	Modified goamy	36.5 ± 1.1^{g}	17.3 ± 0.8^{de}	46.2 ± 0.3^{b}	63.5 ± 1.1^{b}
	Dongjin	90.0 ± 0.6^{bc}	9.6 ± 0.7^{h}	0.5 ± 0.0^{h}	10.0 ± 0.6^{fg}
	Modified dongjin	35.3 ± 1.9^{g}	18.6 ± 1.8^{cd}	46.1 ± 0.0^{b}	64.7 ± 1.9^{b}
	Manmi	89.0 ± 0.4^{c}	10.6 ± 0.3^{gh}	0.4 ± 0.1^{h}	11.0 ± 0.4^{f}
	Modified manmi	35.0 ± 0.5^{g}	20.2 ± 0.9^{bcd}	44.7 ± 0.3^{c}	65.0 ± 0.5^{b}
	Sinsunchal	91.6 ± 0.4^{b}	8.1 ± 0.4^{hi}	0.3 ± 0.0^{h}	8.4 ± 0.4^{g}
	Modified sinsunchal	30.8 ± 0.1^{h}	25.8 ± 0.2^{a}	43.4 ± 0.1^{d}	69.2 ± 0.1^{a}

[1] The data presented are means ± SD of triplicate experiments, and different alphabetic superscripts in the same column are significantly different from each other at $p < 0.05$.
[2] Readily digestible starch (RDS).
[3] Soluble resistant starch (SRS) = 100 − (RDS + NSRS).
[4] Non-soluble resistant starch (NSRS).
[5] Total resistant starch (TRS) = SRS + NSRS.
[6] Normal corn starch (NCS).
[7] Waxy corn starch (WCS).

Table 2
Thermal properties of *Ns*AS-modified starch products.[1]

Samples		T_{onset} (°C)	T_{peak}	T_{end}	ΔH (J/g)
Corn starch	Amylomaize VII	70.9 ± 0.1^{e}	81.7 ± 0.4^{c}	106.0 ± 0.0^{e}	10.6 ± 1.3^{e}
	Modified amylomaize VII	103.2 ± 0.9^{a}	111.8 ± 0.3^{a}	123.9 ± 0.1^{a}	4.2 ± 0.3^{g}
	NCS	69.5 ± 0.1^{f}	73.8 ± 0.1^{e}	79.9 ± 0.1^{f}	14.9 ± 0.1^{bc}
	Modified NCS	90.5 ± 0.6^{b}	105.0 ± 0.3^{b}	118.4 ± 0.4^{d}	8.1 ± 0.9^{f}
	WCS	67.5 ± 0.3^{g}	74.4 ± 0.6^{d}	80.4 ± 0.2^{f}	17.3 ± 0.1^{a}
	Modified WCS	90.1 ± 0.1^{b}	104.9 ± 0.2^{b}	118.6 ± 0.1^{d}	14.4 ± 0.3^{cd}
Rice starch	Goamy	61.1 ± 0.1^{h}	66.8 ± 0.0^{g}	73.9 ± 0.1^{i}	13.3 ± 0.3^{d}
	Modified goamy	89.0 ± 0.1^{c}	104.9 ± 0.1^{b}	119.0 ± 0.3^{cd}	9.5 ± 0.4^{e}
	Dongjin	61.8 ± 0.3^{h}	68.7 ± 0.0^{f}	75.8 ± 0.1^{h}	13.9 ± 0.3^{cd}
	Modified dongjin	87.7 ± 0.1^{d}	105.3 ± 0.1^{b}	119.5 ± 0.5^{c}	9.6 ± 0.3^{e}
	Manmi	62.0 ± 0.1^{h}	68.7 ± 0.3^{f}	75.8 ± 0.3^{h}	13.5 ± 0.8^{d}
	Modified manmi	88.6 ± 0.0^{cd}	105.1 ± 0.1^{b}	120.5 ± 0.7^{b}	10.0 ± 0.3^{e}
	Sinsunchal	61.1 ± 0.4^{h}	68.4 ± 0.6^{f}	77.7 ± 0.3^{g}	16.2 ± 0.1^{ab}
	Modified sinsunchal	89.0 ± 1.1^{c}	105.3 ± 0.4^{b}	119.0 ± 0.1^{cd}	14.0 ± 1.0^{cd}

[1] The data presented are means ± SD of triplicate experiments and alphabetic superscripts are significantly different at $p < 0.05$.

79.0 °C, respectively; peak viscosities of them were 2.7, 1.9, 2.4, 2.6, 2.9, and 3.2 Pa·s. Compared to native starches, < 10% of viscosity was observed from all the enzyme-treated ones. Meanwhile, all the *Ns*AS-modified starches displayed higher pasting temperatures than native starch counterparts; 68.0 → 84.5 °C (ΔT = 16.5 °C), 64.5 → 81.8 °C (17.3 °C), 59.0 → 73.0 °C (14.0 °C), 59.5 → 70.5 °C (11 °C), 60.9 → 80.3 °C (19.4 °C), and 61.3 → 84.4 °C (23.1 °C) for NCS and WCS, and goamy, dongjin, manmi, and sinsunchal rice starches, respectively, where the values in parenthesis imply the increase in pasting temperature by enzyme modification. Thus, the *Ns*AS-modified starches obviously showed substantial increases in peak viscosity temperatures and times, but their peak viscosities drastically decreased (Supplement 3). Once the gelatinized starch was treated by *Ns*AS, its branch chains of amylopectin molecules were significantly elongated by glucosylating reaction of *Ns*AS, and the resulting products were recrystallized resulting in the increase in both gelatinization temperatures and enthalpy changes (Sanders et al., 1990; Sasaki & Matsuki, 1998; Shi et al., 1994; Yuan et al., 1993). The digestion-resistant glucans produced by *Ns*AS treatment on various starch sources did not develop significant pasting viscosities under our experimental condition as shown above, and these RS products would behave differently from native starch counterparts in general cooking and thermal processing. Meanwhile, it has been well known that RS withstands not only hydrolysis by human digestive enzymes but also would remain unchanged under food processing conditions (Lei, Tian, Sun, & Chun, 2008). Thus, this enzymatically-converted RS product can reach a large intestinal environment and improve gut health by selective growth of beneficial microbes even if it has undergone thermal food processes and has passed through the digestive tract.

4. Conclusion

*Ns*AS treatment is a novel biocatalytic method for producing functional food carbohydrate materials. Turanose and RS obtained by *Ns*AS treatment were resistant to the hydrolysis by simulated human digestive enzymes. Biocatalytic technology is one of the most promising ways to develop new functional bio- and food-related materials. The amylosucrase-type enzymes showed excellent potential to be applied industrially in terms of building up carbohydrate architecture. In recent years, two major products using an amylosucrase reaction were

Table 3
Pasting profiles of reaction products by *Ns*AS treatment.[1]

		Pasting temp. (℃)	Peak			Holding strength (Pa·s)	Final visc. (Pa·s)	Breakdown visc. (Pa·s)	Setback visc. (Pa·s)
			Visc. (Pa·s)	Time (min)	Temp (℃)				
Corn starch	Amylomaize VII	ND[1]	ND	ND	ND	ND	ND	ND	ND
	Modified amylomaize VII	ND	ND	ND	ND	ND	ND	ND	ND
	NCS	68.0 ± 0.3^{g}	2.7 ± 0.0^{c}	4.4 ± 0.0^{g}	89.0 ± 0.3^{e}	1.2 ± 0.0^{d}	2.9 ± 0.0^{b}	1.5 ± 0.0^{b}	1.7 ± 0.0^{a}
	Modified NCS	69.8 ± 0.3^{f}	0.3 ± 0.0^{h}	6.3 ± 0.0^{d}	95.6 ± 0.0^{ab}	ND	ND	ND	ND
	WCS	64.5 ± 0.3^{h}	1.9 ± 0.0^{f}	3.4 ± 0.0^{i}	77.3 ± 0.3^{g}	0.6 ± 0.0^{f}	0.7 ± 0.0^{f}	1.3 ± 0.0^{d}	0.1 ± 0.0^{f}
	Modified WCS	81.8 ± 0.2^{b}	0.3 ± 0.0^{i}	7.0 ± 0.0^{a}	95.2 ± 0.0^{b}	ND	ND	ND	ND
Rice starch	Goamy	59.0 ± 0.3^{j}	2.4 ± 0.0^{e}	4.6 ± 0.0^{f}	91.1 ± 0.2^{d}	1.0 ± 0.0^{e}	2.4 ± 0.0^{d}	1.4 ± 0.0^{c}	1.4 ± 0.0^{c}
	Modified goamy	73.0 ± 0.2^{d}	0.3 ± 0.0^{g}	6.3 ± 0.0^{c}	95.7 ± 0.0^{a}	0.3 ± 0.0^{g}	ND	ND	ND
	Dongjin	59.5 ± 0.3^{j}	2.6 ± 0.0^{d}	4.7 ± 0.0^{e}	90.9 ± 0.0^{d}	2.0 ± 0.0^{a}	3.7 ± 0.0^{a}	0.6 ± 0.0^{e}	1.7 ± 0.0^{b}
	Modified dongjin	70.5 ± 0.2^{e}	ND	ND	ND	ND	ND	ND	ND
	Manmi	60.9 ± 0.3^{i}	2.9 ± 0.0^{b}	4.7 ± 0.0^{e}	92.2 ± 0.3^{c}	1.4 ± 0.0^{c}	2.7 ± 0.0^{c}	1.5 ± 0.0^{b}	1.3 ± 0.0^{d}
	Modified manmi	80.3 ± 0.3^{c}	0.1 ± 0.0^{j}	6.9 ± 0.0^{b}	95.3 ± 0.0^{ab}	ND	ND	ND	ND
	Sinsunchal	61.3 ± 0.3^{i}	3.2 ± 0.1^{a}	3.5 ± 0.0^{h}	79.0 ± 0.3^{f}	1.5 ± 0.0^{b}	2.0 ± 0.0^{e}	1.7 ± 0.0^{a}	0.5 ± 0.0^{e}
	Modified sinsunchal	84.4 ± 0.3^{a}	0.1 ± 0.0^{j}	6.9 ± 0.0^{b}	95.3 ± 0.0^{ab}	ND	ND	ND	ND

[2]ND, Not detectable.
[1] The data presented are means ± SD of triplicate experiments and alphabetic superscripts are significantly different at $p < 0.05$.

considered to be possibly commercialized since the reaction optimization conversion yield and productivity were tremendously enhanced. In this study, a newly identified *Ns*AS was utilized to produce turanose and RS, and their outstanding properties were elucidated as well. These biocatalytically converted novel food materials would be promisingly utilized as a functional ingredient in food and other related industries.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (2018R1D1A1B07048087).

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2019.125225.

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