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RESEARCH ARTICLE

Feasibility and characterization of the cycloamylose production from high amylose corn starch

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Abstract

Background and objectives: Cycloamylose (CA), a promising encapsulating agent, was efficiently produced from high amylose corn starch (HACS, HYLON® VII) by sequentially combined enzyme treatment of isoamylase and *Thermus aquaticus* 4-α-glucanotransferase (TAαGT). The CA production performance of HACS was compared with that of rice starch (RS), especially from the viewpoint of differences in their molecular characteristics.

Findings: The maximum conversion yield of CA from HACS reached 76.35%, which was 2.4 times higher than that of RS (31.36%). The degree of polymerization (DP) of CA produced from HACS ranged from 7 to 41, where a major portion lied in DP 23–41, with DP 26 showing the highest yield. In contrast, CA produced from RS showed a relatively larger amount of smaller cyclic glucans (DP 6–16).

Conclusions: The significantly high production yield and DP profile of CA were attributed to high apparent amylose content and long average amylopectin branch chain length of HACS compared to those RS.

Significance and novelty: This study can provide a better understanding of CA production depending on starch molecular characteristics and attract industrial consideration in utilizing HACS for CA production.

KEYWORDS
4-α-glucanotransferase, cycloamylose, high amylose corn starch, rice starch

1 | INTRODUCTION

Cycloamylose (CA), a cyclic α-1,4-linked glucose polymer, is produced by the action of 4-α-glucanotransferase (4αGTase; EC 2.4.1.25) on linear α-1,4-glucans, such as amylose from starch (Bhuiyan, Kitaoka, & Hayashi, 2003; Kim et al., 2011; Tachibana, Takaha, Fujiwara, Takagi, & Imanaka, 2000; Takaha & Smith, 1999; Terada, Fujii, Takaha, & Okada, 1999; Yanase et al., 2002). The degree of polymerization (DP) of CA produced from amylose ranges from 17 to several hundred (Takaha, Yanase, Takata, Okada, & Smith, 1996), and conformations of CA in an aqueous environment have been proposed to be diverse and heavily dependent on its DP (Kitamura et al., 1997; Shimada, Handa, Kaneko, & Takada, 1996). CA can form a hydrophobic cavity in an aqueous environment and thus, it is capable of encapsulating insoluble or volatile guest molecules to form inclusion complexes (Baek et al., 2011, 2012). CA has higher solubility and stability in water than cyclodextrin (CD) that is the cyclic α-1,4-glucan of smaller DP (6, 7, and 8) (Taira, Nagase, Endo, & Ueda, 2006). CA has been used to deliver various drugs and biological molecules such as a gene and siRNA (Toita, Morimoto, & Akiyoshi, 2010; Toita, Soma, Morimoto, & Akiyoshi, 2009; Tomono et al., 2002) and are also commercially available as an efficient chaperone for protein refolding (Machida et al., 2000). CA produced by 4αGTase is regarded as enzymatically modified starch without

known toxicity since it is composed of simple glucose polymer (Toita et al., 2010). Tafazoli et al. (2010) reported that in a 13-week subchronic toxicity study in rats, oral administration of 4αGTase did not produce compound-related clinical signs or toxicity, supporting the safety of 4αGTase in food production. In fact, there exists 4αGTase-modified starch under the trade name of Etenia™ as a food material on the market (Euverink & Binnema, 1998). Therefore, CA can be a highly promising complexing agent or stabilizer for food and pharmaceutical industries (Kuttiyawong, Saehu, Ito, & Pongsawasdi, 2015; Mun, Rho, & Kim, 2009; Rho et al., 2017).

Generally, CA is produced by treating 4αGTase with synthetic amylose or amylose isolated from potato starch (Kim et al., 2011; Takaha et al., 1996). Because the enzyme catalyzes the transfer of α-1,4-glucan to another molecule or glucose, amylose, which is composed of α-1,4 linkages, serves as the ultimate substrate for producing CA (Takaha & Smith, 1999). However, such amylose is not suitable for high-volume production of CA because it is expensive.

Several recent studies have examined the economic production of CA using starches and have found that the yield and DP of CA depend on the type of starch, enzyme source, and enzymatic treatment process (Chu et al., 2016; Lee, Oh, & Yoo, 2009; Xu et al., 2014). In a study that used corn starch, the yield of CA produced by 4αGTase from *Synechocystis* sp. PCC 6803 was 5.4%–30.3% depending on the type of corn starch, and the DP range was 22–33 (Lee et al., 2009). The yield of CA produced by treating 4αGTase from *Thermus aquaticus* (TAαGT) on amylomaize V (ca. 50% amylose content) and debranched amylomaize V was 24.55% and 45.58%, respectively, and the DP ranged from 5 to 40 (Xu et al., 2014). In a study that used sweet potato starch, the maximum yield of CA produced by sequential treatment with isoamylase and TAαGT was 48.56%, and the DP range was 5–40 (Chu et al., 2016).

The maximum yield of CA produced from starch is approximately one-half of that produced from synthetic amylose (approximately 84%) (Terada et al., 1999). Therefore, to increase the yield of CA as much as synthetic amylose using less-expensive starches, we used high amylose corn starch (HACS, HYLON® VII) and optimized the enzyme treatment conditions. HACS is a promising starch because it has the highest amylose content (approximately 70%) among starches and has a high percentage of amylopectin long branch chains. In this study, we improved the production yield of CA from HACS and investigated the factors affecting the yield and DP profile of CA by comparing the characteristics of CA produced from HACS and normal rice starch (RS). Among cereal starches, RS is typically characterized as a low amylose content and a high proportion of short amylopectin branch chains (Jane et al., 1999). Therefore, in this study, RS was selected to compare with HACS that has a high amylose content and a high proportion of long amylopectin branch chains in terms of the yield and DP profile of CA produced using TAαGT.

2 | MATERIALS AND METHODS

2.1 | Materials

High amylose corn starch (HACS, HYLON® VII) was products of the National Starch and Chemical Company (Bridgewater, NJ, USA) and rice "Ilpumbyeo" (National Institute of Crop Science, Rural Development Administration, Jeonju, Korea) starch was obtained by alkaline extraction method (Yang, Lai, & Lii, 1984). Isoamylase (EC 3.2.1.68) and glucoamylase (EC 3.2.1.3) were purchased from Megazyme International Ireland Ltd. (Wicklow, Ireland). Commercial cycloamylose as CA standard was purchased from Ezaki Glico Co., Ltd. (Osaka, Japan). Other chemicals, analytical grade, were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA) and Duksan Pure Chemical Co., Ltd (Ansan, Korea). All other reagents were of analytical-reagent grade and were used as received.

2.2 | Preparation of TAαGT

The 4-α-glucanotransferase gene derived from *T. aquaticus* was cloned and transformed in *Escherichia coli* MC1061 [F^- *araD139 recA13 Δ(araABC-leu)7696 galU galK ΔlacX74 rpsL thi hsdR2 mcrB*] according to Park et al. (2007). The transformant cells were inoculated on LB (Luria-Bertani) medium containing ampicillin (100 μg/ml) and cultured at 37°C for 12 hr. The precipitated cells were suspended in a lysis solution (50 mM Tris-HCl [pH 7.5], 300 mM NaCl, and 10 mM imidazole) and disrupted by ultrasonication (VCX 750; Sonics & Materials, INC., Newtown, CT, USA). The TAαGT was purified using Ni-NTA affinity chromatography (HisTrap™ HP; GE Healthcare, Uppsala, Sweden). TAαGT activity was determined by measuring absorbance change in iodine-staining during the conversion of amylose to CA according to Liebl, Feil, Gabelsberger, Kellermann, and Schleifer (1992).

2.3 | Optimization of enzymatic reaction conditions

The reaction conditions were optimized for each enzyme (isoamylase, TAαGT, and glucoamylase) used in the preparation of CA from starches. About 5 U/g (starch weight basis) of isoamylase was used to hydrolyze α-1,6-glucans of gelatinized starch. The isoamylase treatment time was optimized according to debranching degree of starch by

measuring reducing sugar using the DNS (3,5-dinitrosalicylic acid) method (Miller, 1959). The appropriate concentration and reaction time of TAαGT was determined by measuring the amount of cyclic glucans produced by TAαGT with high-performance size-exclusion chromatography (HPSEC). In order to remove the remaining non-cyclic glucans, the reaction conditions for glucoamylase were optimized. The optimized enzymatic reaction conditions for each substrate were the basis for producing CA as described in next section.

2.4 | Production of CA from high amylose corn and RS

High amylose corn starch and RS (1%, dry wt.) were dissolved in 50 mM sodium acetate buffer (pH 4.5) and autoclaved (121°C, 15 min) for gelatinization. Then, the isoamylase (5 U/g, starch weight basis) was added for debranching of starches. After 8 hr of reaction, the substrate was boiled for 10 min for enzyme inactivation and precipitated by adding five volumes of 95% ethanol using the centrifugation at 14,000 *g* for 10 min. For the production of cyclic glucans, TAαGT was reacted on the debranched starches. First of all, the debranched starches (1%, dry wt.) were completely dissolved in 6 ml of 90% (*v*/*v*) DMSO solution at 95°C for 30 min and then added 24 ml of 50 mM Tris-HCl buffer (pH 7.5) for reaction with TAαGT. Subsequently, the debranched HACS and RS solution were reacted with 10 U/g (starch weight basis) of TAαGT at 75°C for 14 and 12 hr, respectively, and then terminated by boiling for 10 min. After precipitation by 5 volumes of 95% ethanol, the precipitate of HACS and RS solution was dissolved in 50 mM sodium acetate buffer (pH 4.5) and incubated with 50 U/g glucoamylase for 10 and 6 hr, respectively. After boiling for 10 min, the supernatant was collected by centrifugation (14,000 *g*, 10 min) and washed twice with 85% ethanol and 80% ethanol successively. The precipitants (final CA products) were dissolved in distilled water and lyophilized to obtain powder formulations for analysis.

2.5 | High-performance size-exclusion chromatography analysis

High-performance size-exclusion chromatography (ProStar 210, ProStar 355 RI [refractive index] Detector; Varian Inc., Palo Alto, CA, USA) was used to analyze the molecular weight distribution of the starches. The dried starch (0.5%, *w*/*v*) was dissolved in hot water and boiled for 30 min. The dispersed solutions were filtered with 5.0 μm PVDF (polyvinylidene fluoride) membrane syringe filter (Millex®-SV; Merck Millipore Ltd., Tullagreen Carrigtwohill, Co Cork, Ireland) and injected with 100 μl into HPSEC. Two SEC columns (OHpak SB-806 HQ and OHpak SB-804 HQ; Showa Denko K.K, Tokyo, Japan) were used and the mobile phase was eluted with HPLC water at a rate of 0.4 ml/min. For the analysis of produced CA, the CA product (0.5%, *w*/*v*) was dissolved in hot water and filtered with 0.45 μm cellulose acetate syringe filter (Minisart®-Plus; Sartorius Stedim Biotech, Goettingen, Germany). The supernatant was injected into the HPSEC system with two columns connected (OHpak SB-804 HQ and OHpak SB-802.5 HQ; Showa Denko K.K) and eluted by HPLC water at a flow rate of 0.8 ml/min.

2.6 | High-performance anion-exchange chromatography (HPAEC) analysis

The branch chain length distribution of native starches and the changed profile of enzyme treated starches were analyzed using HPAEC system (Dionex Dx-300 with a pulsed amperometric detector [ED40, Dionex]; Dionex Co., Sunnyvale, CA, USA). The debranched starch (1% [*w*/*v*] in 50 mM sodium acetate buffer [pH 4.5]) and CA solutions (1% [*w*/*v*] in distilled water) were filtered with 0.45 μm cellulose acetate filter before injection into the HPAEC. The injected sample (20 μl) was passed through a CarboPac™ PA-1 column (250 mm × 4.0, Dionex Co.) and eluted by flowing mobile phase (150 mM NaOH and 600 mM sodium acetate in 150 mM NaOH) with a gradient of 0%–100% at a flow rate of 1.0 ml/min. DP was determined by the maltooligosaccharide standard (DP 1–7; CARBOEXPERT Inc., Daejeon, Korea).

2.7 | Matrix-assisted desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) analysis

Qualitative analysis of the produced CA was conducted by obtaining the mass spectrum using MALDI-TOF MS system (Voyager-DE™ STR Biospectrometry workstation; PE Biosystems, Framingham, MA, USA). It was equipped with a 337 nm nitrogen laser and a 2 m flight tube. The CA sample was mixed with matrix solution (2,5-Dihydroxybenzoic acid) in equal volume, and 1 μl of the mixture was spotted on a stainless steel plate. Mass spectra were obtained in the positive ion mode and accelerating voltage was used 20 kV.

3 | RESULTS AND DISCUSSION

3.1 | Amylose content and amylopectin branch chain length distribution

The apparent amylose content of starch was determined according to McGrance, Cornell, and Rix (1998). The apparent amylose content of HACS was measured to be 70.76%,

significantly higher than that of RS (16.14%). The amylopectin branch chain length distribution in HACS was measured by HPAEC after debranching with isoamylase, as shown in Figure 1 and Table 1 in comparison with that in RS. Observably, the amylopectin branch chain length distribution profile varied with the type of starch. The profile of RS displayed a first peak at DP 12 and a second at DP 42 (Figure 1a), respectively, whereas that of HACS manifested peaks at longer branch chains (first peak at DP 15 and second peak at DP 47) than RS. Comparing the relative chain length distributions within DP 6–65, it was found that branch chains shorter than DP 17 were more abundant in RS, while branch chains longer than DP 17 were prevalent in HACS (Figure 1b). The DP grouping of the branch chain length distribution revealed that HACS known as B-type starch had a higher proportion of long chains (40.01% for DP >25) than RS belonging to A-type starch (24.24% for DP >25; Table 1), which was similar to the result of Jane et al. (1999). Therefore, the results of CA production and comparison of DP profile of CA between these two starches of different amylose content and amylopectin branch chain length distribution were compared in sections 3.4 and 3.5.

TABLE 1 Branch chain length distributions of starches

Type of starch	Distribution (%)[a] DP 6–12	DP 13–24	DP 25–36	DP ≥37	Average CL
RS	28.21	47.55	13.18	11.06	20.08
HACS	10.21	49.78	21.13	18.88	25.40

DP: degree of polymerization; HACS: high amylose corn starch; RS: rice starch.

[a]Grouping of degree of polymerization (DP) numbers followed that of Hanashiro, Abe, and Hizukuri (1996).

3.2 | Molecular weight distribution of native and isoamylase-treated starches

For producing CA from starch, the hydrolysis of α-1,6-branched chains is essential to increase the amount of linear chains, which affects the yield of CA and to ensure that the product composed solely of α-1,4-glucan (Yanase et al., 2002). Therefore, the molecular weight distributions of starches before and after debranching were measured by HPSEC to compare the debranched chain profiles of HACS and RS. As shown in Figure 2a, the amylose peak (third peak, R_T 32–50 min) in the HACS profile (solid line) was greater than the amylopectin peak (first peak, R_T 20–25 min). After the debranching, the amylopectin portion of HACS (broken line) significantly decreased, while the amylose portion decreased only slightly. Furthermore, a large peak (fourth peak, R_T 45–50 min) was newly generated and was attributed to the debranched linear short chains of glucan, mainly from amylopectin. In comparison, the distribution profile of RS was mainly composed of amylopectin (first peak) rather than amylose (third peak). Moreover,

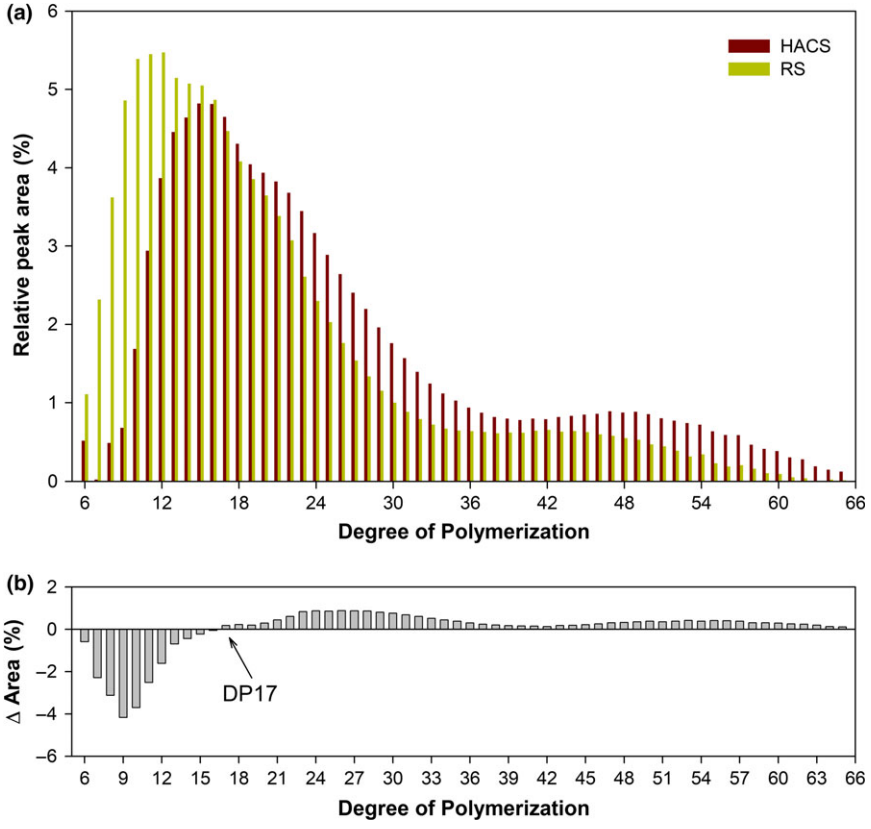

FIGURE 1 (a) High-performance anion-exchange chromatography (HPAEC) analysis of branch chain length distribution of high amylose corn starch (HACS) and rice starch (RS), the *Y*-axis represents the relative peak area (%) from degree of polymerization (DP) 6–65. (b) The difference of branch chain length distribution between HACS and RS, Δ Area (%) = $\text{Area}_{\text{HACS}} - \text{Area}_{\text{RS}}$ [Color figure can be viewed at wileyonlinelibrary.com]

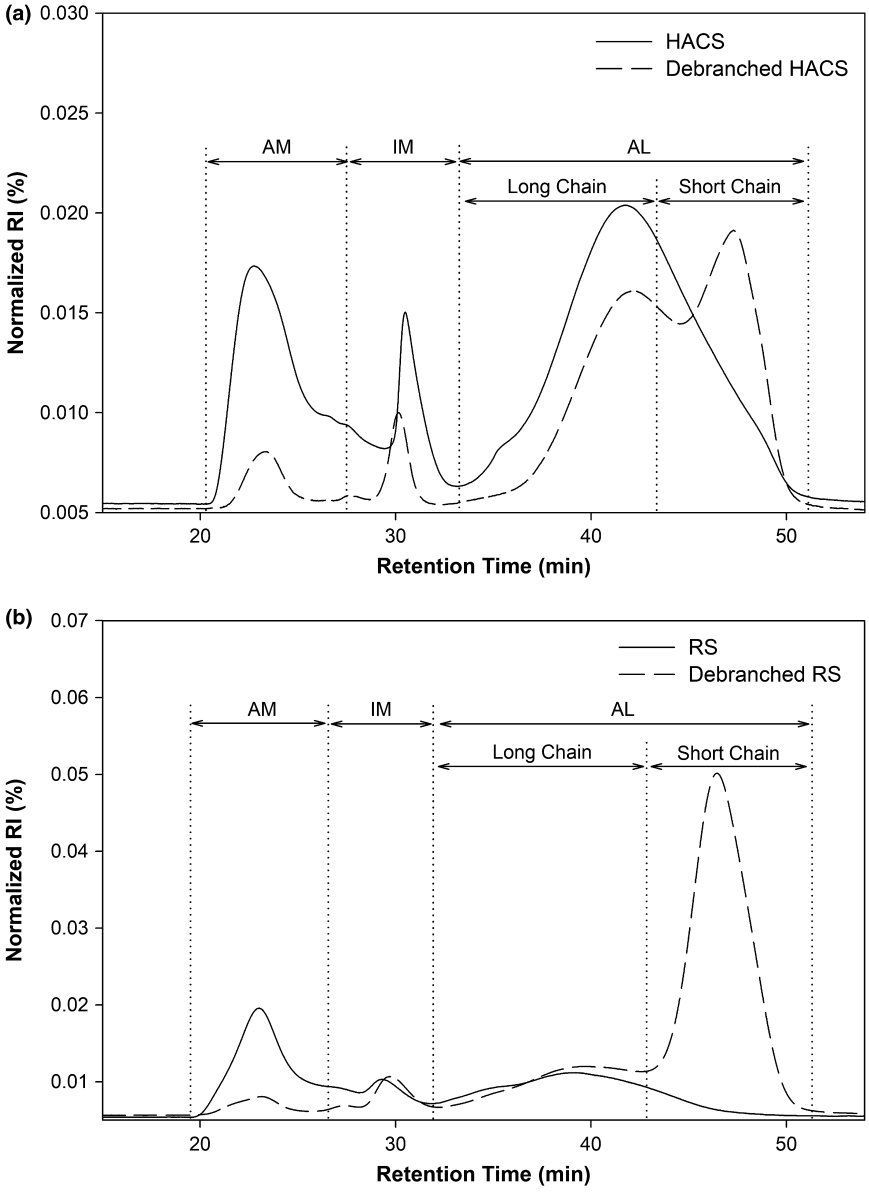

FIGURE 2 High-performance size-exclusion chromatography (HPSEC) analysis of molecular weight distribution of starch and isoamylase-treated starch. (a) High amylose corn starch (HACS) and debranched HACS, (b) Rice starch (RS) and debranched RS, the *Y*-axis represents the relative RI response (%) from 15–54 min of retention time. AL: amylose; AP: amylopectin; IM: intermediate

after debranching, the portion of amylopectin (first peak) decreased, while the peak of short chains of glucan (fourth peak) remarkably increased at R_T 42–50 min (Figure 2b). Therefore, it was confirmed that a greater amount of long linear chains of glucan from amylose and amylopectin was produced from HACS than from RS after debranching of both.

3.3 | Optimization of reaction conditions for CA production

Cycloamylose was generated from HACS by the sequential reaction of isoamylase, TAαGT, and glucoamylase. A recent study reported that the maximum conversion yield of CA from debranched starch was 1.9 times higher than that from starch without debranching (Xu et al., 2014). It was also revealed that the debranching process with starch as a substrate is important because the α-1,4-linked glucans from debranched starch promote the production of CA. In this study, the α-1,6-linkage of HACS was hydrolyzed by adding 5 U/g of isoamylase and by optimizing the reaction time through the measurement of the accumulated linear α-1,4-linked glucans. The maximum amount of linear α-1,4-linked glucan was obtained 8 hr after the reaction of isoamylase and the amount continuously decreased as the reaction proceeded further (data not shown). This result was consistent with that reported by Chu et al. (2016), who attributed it to the aggregation and precipitation of increase in the number of debranched linear chains. Therefore, the reaction time of isoamylase should be optimized in order to make linear glucans suitable as a substrate for TAαGT.

The optimum reaction time of TAαGT was determined by monitoring the amount of CA produced with

debranched HACS as a substrate (Figure 3). The reaction with 10 U/g of TAαGT for 24 hr afforded the maximum CA production yield at 14 hr. It steadily increased for 14 hr and decreased thereafter, indicating the influence of the reaction time of TAαGT on the production yield of CA. This agrees with other studies, confirming that the maximum production yield of CA depends on the reaction time of TAαGT and it decreases with prolonged reaction time (Chu et al., 2016; Xu et al., 2014). Previous studies attributed this phenomenon to the reversibility of coupling and cyclization reactions of TAαGT, which could convert a large molecular weight CA to a linear glucan and a small molecular weight CA, resulting in its yield reduction. The hydrolytic activity of TAαGT also contributed to the reduction in CA production yield (Fujii et al., 2005; Takaha et al., 1996). The reaction condition of TAαGT influences the maximum production yield of CA. Hence, the CA production conditions from HACS was optimized by the sequential treatment of isoamylase and TAαGT for 8 and 14 hr under the enzyme unit and buffer conditions described in the Method section.

Amylose complexed with lipids may slow the action of enzymes, especially for high amylose starch (Lauro, Poutanen, & Forssell, 2000). Despite the high lipid content of HACS, CA production yield reached higher than 70% in this study. Previous studies have shown that the CA production yield of wild-type TAαGT from synthetic amylose ranged from about 60% to 90% depending on the reaction time (Fujii et al., 2005). Considering the fact that CA production yield from HACS, the amylose content of which is ~70% (apparent amylose content measured was ~80%), was ~76%, TAαGT seemed sufficiently act on HACS at optimized reaction conditions used in this study to produce CA without significant influence of amylose-lipid complex in HACS.

3.4 | Comparison of CA produced from HACS and RS

The conversion yield of CA under optimum conditions was determined by comparing the peak area measured by HPSEC with the peak area determined from the standard curve equation within the concentration range (0.1%–1%, *w*/*v*) of standard CA. The production yield of CA from HACS was calculated to be 76.35%, significantly higher than those from amylomaize (45.58%) and sweet potato (48.56%) (Chu et al., 2016; Xu et al., 2014). Surprisingly, this result was the highest yield reported so far for CA production from native starches (Table 2). Furthermore, the production yield of CA from RS under optimal reaction conditions of enzymes was 31.36%, which was significantly lower than that from HACS. These results suggested that the production yield of CA depended on the type of starch because of the differences in their structural characteristics such as amylose content and branch chain length distribution of amylopectin. Previous studies have reported that starch substrates with a high amylose content afforded high CA production yield (Cho et al., 2009; Vongpichayapaiboon, Pongsawasdi, & Krusong, 2016). Our study also showed that HACS with a higher amylose content than RS also afforded a higher CA production yield.

As shown in Table 2, despite the high amylose content of HACS, its CA yield without amylopectin debranching was only about a half of that with debranching. This phenomenon applies to other starch (Vongpichayapaiboon et al., 2016; Xu et al., 2014). It is known that linear long chain α-1,4-glucans such as amylose molecules are the most effective substrate of 4αGTase to produce CA. However, the above results imply that the presence of amylopectin may interfere with the production of CA from amylose. The action modes of 4αGTase on α-1,4-glucans

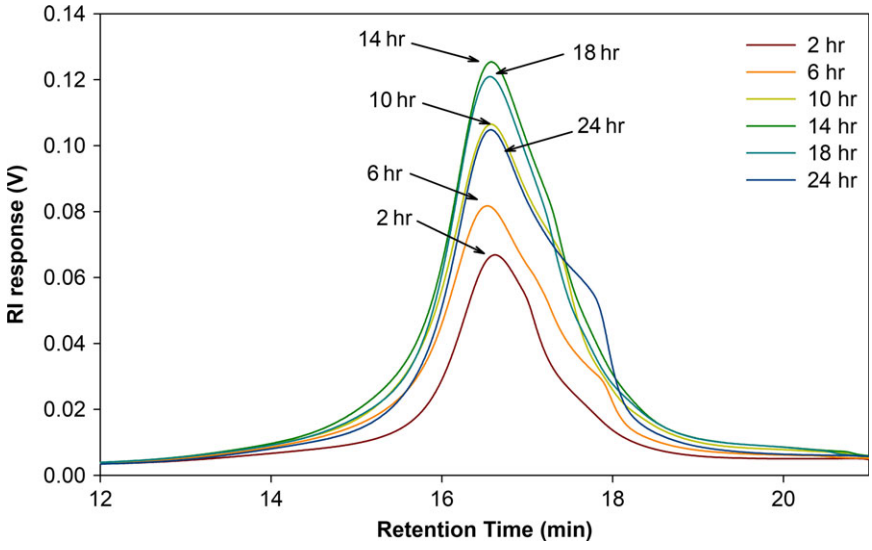

FIGURE 3 The production yield of cycloamylose (CA) depending on the reaction time of TAαGT (10 U/g) [Color figure can be viewed at wileyonline library.com]

TABLE 2 Comparison of production yields of cycloamylose (CA) from different type of starches

Type of starch	Apparent amylose content (%)	Debranched with isoamylase	Production yield of CA (%)	Reference
RS (Ilpumbyeo)	16.14 ± 0.27	Debranched	31.36	Present study
HACS (Hylon VII)	70.76 ± 6.05	Debranched	76.35	
HACS (Hylon VII)	68–70	Not debranched	30.3	Lee et al., 2009
HACS (Hylon V)	52–55	Not debranched	24.55	Xu et al., 2014
		Debranched	45.58	
SPS (Sweet potato starch)	21.07 ± 0.79	Debranched	48.56	Chu et al., 2016
Rice starch	15.2 ± 0.5	Not debranched	4.1 ± 0.5	Vongpichayapaiboon et al., 2016
		Debranched	13.7 ± 1.3	

HACS: high amylose corn starch; RS: rice starch.

are mainly characterized as a disproportionation, cleaving α-1,4-linkage and transferring the newly formed reducing end either to the nonreducing end of other acceptor glucans (intermolecular transglycosylation) or to its own nonreducing end (intramolecular transglycosylation) to form cyclic glucans (Takaha et al., 1996). By this reaction, glucans from amylose can be transferred to amylopectin branch chains (Park et al., 2007), since a large number of nonreducing ends in amylopectin could play a role of active acceptors of disproportionation reaction. For an amylopectin substrate without debranching, 4αGTase mainly produces either cyclic or non-cyclic clustered dextrin with a highly branched structure, and only a small amount of cyclic α-1,4-glucans are formed (Takaha, Yanase, Takata, Okada, & Smith, 1998). Therefore, debranching of amylopectin is highly important to increase the CA yield from starch not only by increasing the amount of linear molecules but also by eliminating linear glucan loss to branched amylopectin.

Cycloamylose produced from starch contains low molecular weight CA (DP 5–DP 16). This is different from CA produced from amylose with DP 22 and higher. Low molecular weight CA can be attributed to the supply of branch chain of amylopectin. Thus, the branch chain of branched amylopectin also contributes to the production yield of CA.

This study showed that the length of linear α-1,4-glucan produced after debranching of amylopectin is also an important factor in CA production. Endo, Zheng, and Zimmermann (2002) reported that the production yield of CA significantly increased with the increasing molecular weight of linear α-1,4-glucan. Therefore, in starch, amylose plays a major role in CA production because it has a large molecular weight. Similarly, the long branch chain of amylopectin can act as a desirable substrate for CA production as compared to the short branch chain. This was found by the difference in CA profile between HACS and RS (Figure 5). HACS with relatively long branch chain generated the lower distribution of small CA than RS with shorter branch chain, indicating that the long branch chain contributes to the formation of large CA. The distribution of large CAs is mainly due to amylose, but it can be also affected by the long branch chain of amylopectin. The degree to which the long branch of amylopectin contributes to the yield of CA production is not obvious, but it is assumed that this may be related to the action of 4αGTase on the substrate. The action of 4αGTase on synthetic amylose (AS320) produces initially large CAs and is subsequently converted to small CAs (Takaha et al., 1996). In contrast, the action of 4αGTase on amylose or amylopectin of starch produces initially small CAs and later increased large CAs (Xu et al., 2014). These suggest that the equilibrium of the cyclization and linearization reaction of 4αGTase tends toward the formation of CA of the suitable size. Therefore, a supply of the preferred length substrate for the stable cyclization reaction of 4αGTase can be effective for CA production. However, further research will be required to reveal the correlation between 4αGTase action and CA yield on the branch chain length of amylopectin.

Overall, the factors affecting the production yield of CA when using starch as a substrate include: (a) the amylose content of starch, (b) the debranching of amylopectin, and (b) the branch chain length of the debranched amylopectin. Therefore, HACS with a high amylose content and abundant long branch chains are desirable substrates for CA production.

3.5 | CA profile analysis by HPAEC and MALDI-TOF

The DP profiles of the CA synthesized from HACS and RS were analyzed by HPAEC and compared with that

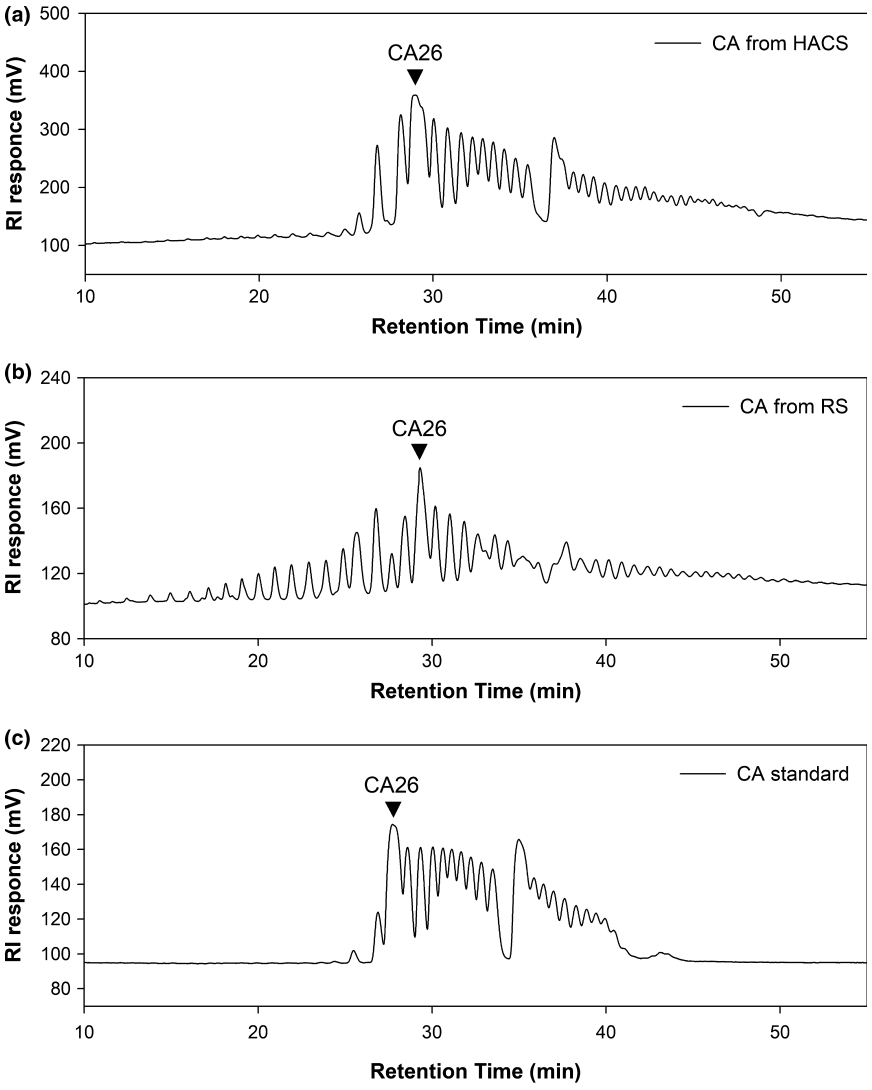

FIGURE 4 High-performance anion-exchange chromatography (HPAEC) analysis of degree of polymerization (DP) profile of cycloamyloses (CAs) produced from starch. (a) CAs from high amylose corn starch (HACS), (b) CAs from rice starch (RS), (c) CA standard

of the standard CA (Figure 4). The HPAEC results of CA from HACS (Figure 4a) showed various DPs ranging from 24 to 45 min and exhibited a profile resembling that of standard CA (Figure 4c). Moreover, the HPAEC results of CA from RS showed a peak over a wide range from about 15 to 45 min (Figure 4b) with significantly more peaks at lower DP region. Previous studies suggested that CA with high DP forms when linear α-1,4-glucans with a high molecular weight were used. Therefore, this result was ascribed to shorter branch chains of RS than those of HACS, suggesting that the branch chain length of amylopectin affects the DP profile of CA.

The molecular weight of CA was determined by MALDI-TOF to prove that the enzymatically produced CA comprised pure cyclic α-1,4-linked glucans. MALDI-TOF has been reported to be a suitable tool for determining the carbohydrate residues by measuring their molecular weight. The theoretical molecular weight of non-cyclic glucans was calculated as $162.1436n + 22.9898 + 18.01534$ Da, whereas that of cyclic glucan was calculated as $162.1436n + 22.9898$ Da, which is reduced by the molecular weight of one H_2O molecule (Koizumi, Sanbe, Kubota, Terada, & Takaha, 1999). Most of the calculated peaks were assigned to cyclic glucans with a few linear glucans (Figure 5a). This result confirmed that the products obtained from HACS were predominantly pure CA.

The DP profiles of CA produced from both starches were divided into two groups, small and large DP distributions at DP 23 (Figure 5). Comparing the relative signals of their MALDI-TOF MS measurements, DP profiles categorized into large DP distribution was observed to be higher in CA produced from HACS than from RS. Furthermore, CA produced from RS was mainly distributed below DP 23. These results supported HPAEC analysis. Therefore, the effect of the type of starch on the CA profile was attributed to the amylose content and branch chain distribution of amylopectin in it.

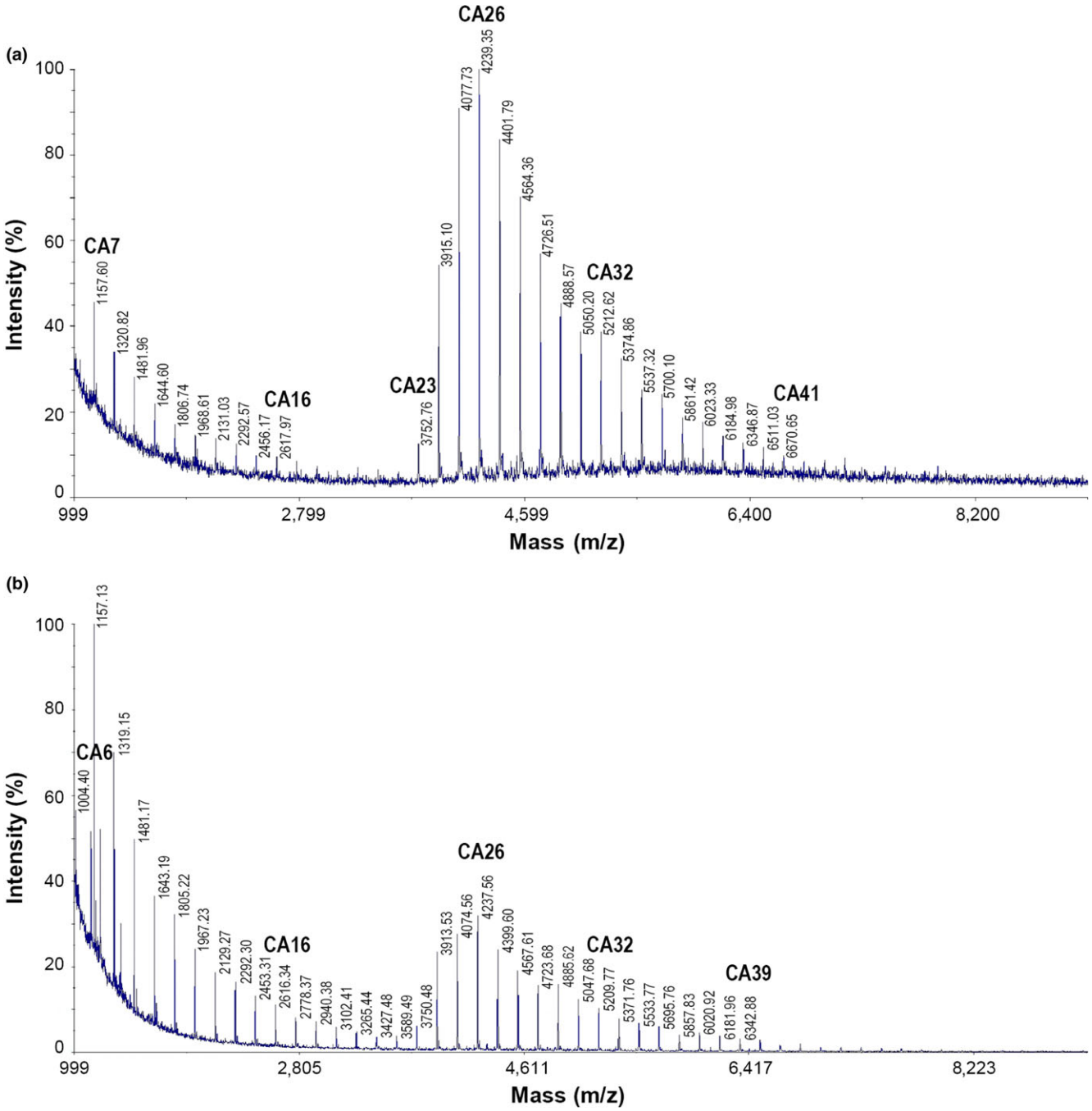

FIGURE 5 Matrix-assisted desorption ionization-time of flight (MALDI-TOF) analysis of molecular weight distribution of cycloamyloses (CAs) produced from high amylose corn starch (a) and CAs produced from rice starch (b) [Color figure can be viewed at wileyonlinelibrary.com]

4 | CONCLUSIONS

Synthesis of CA from HACS resulted in extremely high production yield (76.35%) than that reported in previous studies so far, proposing the application of HACS as an industrial material for CA production. The amylose content was found to affect the production yield of CA, as evidenced by the yield of CA produced from RS, having less amylose content, being half of that from HACS, which has a greater amylose content. In addition, the process of debranching the amylopectin moiety of starch with isoamylase to increase the amount of linear α-1,4-glucan as a substrate of TAαGT improved the production yield of CA. Therefore, this enzymatic process of substrate preparation for CA production is important for increasing the production yield of CA. However, further research is needed to determine the cause of the different reaction conditions of enzymes, depending on the type of starch. The starch structure was found to affect both the CA yield and its DP profile, i.e., the size distribution of the produced CA relative

to the length of the starch branch chain. These results can be used as a basis for future studies to investigate the effect of the size distribution of CA on its ability to capture food materials and its solubility in water.

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