Carbohydrate Polymers 87 (2012) 2455–2463

Contents lists available at SciVerse ScienceDirect

Carbohydrate Polymers

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

Structural and physicochemical properties of starch gels prepared from partially modified starches using *Thermus aquaticus* 4-α-glucanotransferase

Ha V. Do[a], Eun-Jung Lee[b], Jin-Hee Park[c], Kwan-Hwa Park[d], Jae-Yong Shim[e], Saehun Mun[a], Yong-Ro Kim[a,*]

[a] Department of Biosystems & Biomaterials Science and Engineering, Center for Agricultural Biomaterials, College of Agriculture and Life Sciences, Seoul National University, 599 Gwanak-ro Gwanak-gu, Seoul 151-921, Republic of Korea
[b] R&D center, Samsung Fine Chemicals Corporation, 696-3, Gojan-dong, Namdong-gu, Incheon 405-819, Republic of Korea
[c] CJ Foods R&D, CJ Cheiljedang Corporation, 636, Guro-dong, Guro-gu, Seoul 152-051, Republic of Korea
[d] Department of Foodservice Management and Nutrition, College of Natural Sciences, Sangmyung University, 7 Hongji-dong, Jongno-gu, Seoul 110-743, Republic of Korea
[e] Department of Food & Biotechnology, Food and Bio-industrial Research Center, Hankyong National University, Ansung 456-749, Republic of Korea

ARTICLE INFO

Article history:
Received 23 February 2011
Received in revised form 27 October 2011
Accepted 7 November 2011
Available online 17 November 2011

Keywords:
4-α-Glucanotransferase (4αGT)
Modified starch
Starch gel
Microstructure
Physicochemical properties

ABSTRACT

The modified starch gels prepared from partial enzyme treatments (1, 3, and 6 U/g starch; 2-h incubation) of the corn and rice starch pastes using *Thermus aquaticus* 4-α-glucanotransferase (TAαGT) were investigated for their molecular characteristics, microstructures, and physicochemical properties. Unlike the native and partially modified normal starches, the native and partially modified waxy starches could not form gels strong enough for textural analysis after 24 h for gel setting. Features of the partially modified normal starches were the specific apparent amylose contents and maximum iodine absorption wavelength (λ_{max}, ~567 nm), as well as the tri-modal molecular weight profiles and flatter side-chain distributions. Also, the partially modified normal starch gels possessed fractured surfaces with discontinuous crystalline fibrous assembly that differed from the native starch gels' porous continuous network, which resulted in more brittle, rigid, and resilient gels compared with the native gels.

1. Introduction

Starches have been used as functional ingredients in food because they can yield highly viscous pastes or gels when added in sufficient concentrations (Durrani & Donald, 1995; Ortega-Ojeda, Larsson, & Eliasson, 2004; Rosalina & Bhattacharya, 2002). Two main components of starch, amylose and amylopectin, are responsible for the unique physicochemical characteristics that set it apart from other carbohydrates. Amylose is responsible for the short-term gelation and retrogradation changes whereas amylopectin is responsible for all long-term rheological and structural changes (Gudmundsson, 1994). These changes profoundly affect the starch gel's texture (Miles, Morris, Oxford, & Ring, 1985) and network formation (Durrani & Donald, 1995; Leloup, Colonna, Ring, Roberts, & Wells, 1992). The network formation affects the final properties of the gel, and it can be modified by a number of additives or emulsifiers (Richardson, Kidman, Langton, & Hermansson, 2004; Richardson, Laugton, Bark, & Hermansson, 2003; Richardson, Sun, Langton, & Hermansson, 2004). Furthermore, a change in the relative concentrations of amylose and amylopectin can have a substantial effect as the network formation in an amylose–amylopectin mixture markedly differs from that in a pure amylose gel (Deffenbaugh, 1997; Leloup et al., 1992; Richardson et al., 2003). However, the network formation or the microstructure of a starch gel, and how it affects the starch gel's physicochemical properties, are still poorly understood.

Since the use of unmodified starch in foods is limited by its physical and chemical properties, the physicochemical properties of starches have been modified by physical, chemical, or enzymatic means (Choi & Kerr, 2003; Hoover & Vasanthan, 1994; Ma, Cai, Wang, & Sun, 2006; Morikawa & Nishinari, 2000; Oh, Choi, Lee, Kim, & Moon, 2008). Therefore, correlations and/or relationships between the physicochemical properties and structural characteristics of starch gel need to be identified and better understood to facilitate applications in food processing. Recently, food manufacturers have been pressured to produce more natural food products, avoiding chemical treatments as much as possible. One possible solution for this trend is to use enzymes as starch modifiers.

The enzyme 4-α-glucanotransferase (4αGT), also called amylomaltase or D-enzyme, which is involved in starch metabolism in microorganisms and plants, has multiple action modes (disproportionation, cyclization, coupling, and hydrolysis) (Fujii et al., 2005). Although 4αGT is known for the ability to produce cycloamyloses from various sources (Cho, Auh, Kim, et al., 2009; Cho, Auh,

* Corresponding author. Tel.: +82 2 880 4607; fax: +82 2 873 2049.
E-mail address: yongro@snu.ac.kr (Y.-R. Kim).

0144-8617/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2011.11.021

Ryu, et al., 2009; Lee et al., 2009; Park et al., 2007; Takaha et al., 1993, 1998; Terada et al., 1999), it can also be used in starch processing. 4αGTs have been shown to produce unique gelling products, i.e. the 4αGT from *Thermus scotoductus* can produce a freeze–thaw stable and thermoreversible rice starch gel (Lee et al., 2006), and 4αGT from *Thermus thermophilus* or *Pyrobaculum aerophilum* can produce a highly thermoreversible starch gel that is similar to gelatin (Hansen et al., 2009; Kaper et al., 2004, 2005; van der Maarel et al., 2005). However, a limited amount of work has focused on the effects of these enzyme actions on the structural and physicochemical properties of partially modified starch gels. The aim of this study was to modify starches from different botanical sources with 4αGT and to characterize the enzyme-treated starch gels in terms of their structural and physicochemical properties, in order to facilitate applications in food and/or other related industries.

2. Materials and methods

2.1. Materials

Waxy corn (WC) and normal corn (NC) starches were provided by Samyang Genex Co. (Seoul, South Korea). Waxy rice (WR) and native rice (RS) starches were imported from Bangkok Interfood Co. (Bangkok, Thailand). Commercial amylose (AM) and amylopectin (AP) from potato starch were purchased from Sigma–Aldrich (St. Louis, MO, USA). The chemicals used were of analytical grade. Recombinant *Thermus aquaticus* 4-α-glucanotransferase (TAαGT) was cloned and expressed in *Escherichia coli* and purified as described by Park et al. (2007). Isoamylase (EC 3.2.1.68, from *Pseudomonas amyloderamosa*, 106,000,000 U/mg) was purchased from Sigma–Aldrich. Pullulan standards (Shodex Standards, Japan) were used to set the starch Mw-calibrated standard curve.

2.2. Enzyme activity

TAαGT activity was determined by measuring the optical change in iodine-staining during the conversion of amylose by the enzyme (Liebl et al., 1992). The enzyme reaction mixture contained 250 μL of 0.2% (w/v) amylose, 50 μL of 1% (w/v) maltose, 600 μL of 50 mM Tris–HCl buffer, pH 7.5, and 100 μL of enzyme solution. The mixture was incubated at 70 °C for 10 min. The reaction was terminated by boiling for 10 min. Aliquots (0.1 mL) were mixed with 1 mL of iodine solution (0.02% I_2 and 0.2% KI), and the absorbance at 620 nm was measured immediately using an UltraSpec 4000 UV/visible spectrophotometer (Amersham Bioscience, UK). One unit of TAαGT was defined as the amount of enzyme that degrades 0.5 mg/mL of amylose per min under the assay conditions used. The protein concentration was determined according to the method of Bradford (1976) using bovine serum albumin as the standard.

2.3. Starch gel preparation

Starch–water suspensions (10% dm) were gelatinized at 95 °C for 30 min in a water bath equipped with a mechanical stirrer. The starch pastes were cooled and incubated at 75 °C with appropriate amounts of TAαGT (1, 3, and 6 U/g starch d.b.) for 2 h. The enzyme reactions were terminated by boiling for 30 min. Then, the obtained pastes (or solutions) were poured into Petri dishes (35 mm diameter × 10 mm height), which were taped around the edge to accommodate excess pouring levels, and cooled at room temperature for 1 h. The pastes (or solutions) were kept at 4 °C for gel-setting and storage.

2.4. High-performance size-exclusion chromatography (HPSEC) analysis

Freeze-dried starch gels were gently ground into fine powders using mortar and pestle and Mw distributions of starch gels' powders were analyzed using size-exclusion chromatography. Weighed sample powders (5 mg) were dissolved in 1 N NaOH (200 μL) at 75 °C for 15–25 min, diluted with 50 mM $NaNO_3$ (mobile phase), and neutralized with 1 N HCl, resulting in 0.5% (w/v) solutions. The sample solutions were filtered using 5.0-μm disposable membrane filters and injected into an HPSEC system. The HPSEC system consisted of a solvent delivery module (Prostar 210, Varian Inc., California, USA), an injection valve with a 100-μL sample loop (Rheodyne 7725i, Cotati, CA, USA), a differential refractive index detector (Prostar 355, Varian Inc., California, USA), and two size-exclusion chromatography (SEC) columns in combination (G5000 PW, 7.5 mm × 600 mm and G3000 PW, 7.8 mm × 300 mm, Tosoh Co., Tokyo, Japan). The columns were kept at room temperature. The flow rate of the mobile phase was constant at 0.4 mL/min.

2.5. High-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) analysis

Freeze-dried starch gels were dispersed in a 90% (v/v) solution of dimethyl sulfoxide (DMSO), and a two-fold volume of ethanol was added to precipitate and separate the starch components from small oligosaccharides following drying at 40 °C for 6–8 h. The weighed sample powders (10 mg) were dissolved in 100 mM sodium acetate–HCl buffer (900 μL, pH 3.5) and further treated with isoamylase (100 μL, 100×) at 52 °C for at least 48 h. The enzyme reactions were then terminated by boiling for 15 min, and the reaction mixtures were filtered using 0.45-μm disposable membrane filters. The composition of the starch branched chains was analyzed using an HPAEC-PAD (Dionex DX500 Sunnyvale, CA, USA) system. The filtrate (200 μL) was injected and analyzed using a CarboPac PA1 analytical column (4 mm × 250 mm). The system was equipped with a pulsed amperometric detector (ED40, Dionex). Two eluents, A and B, were 150 mM sodium hydroxide and 150 mM sodium hydroxide in 600 mM sodium acetate solutions, respectively. The eluent gradients were operated at a flow rate of 1 mL/min. The branch chain-length distribution was calculated based on relative peak areas up to a degree of polymerization (DP) of 78.

2.6. Determination of amylose content (AC) and maximum absorption wavelength (λ_{max})

The AC of starch was determined using the method of Chrastil (1987) with a slight modification. Weighed amounts of starch (20 mg) were solubilized in 6 mL of alkaline solution (mixture of 1:2 volume ratio of 1 N NaOH solution to water) in a 15-mL screw-cap tube. The tube was heated in a water bath at 95 °C for 30 min with intermittent mixing. The tube was cooled to room temperature, and the contents (0.05 mL) were added to 5 mL of 0.5% trichloroacetic acid (TCA) in a separate tube. The solution was mixed, and 0.05 mL of iodine solution (1.27 g I_2 + 3 g KI per L) was added and mixed immediately. The developed color was read after 30 min at 680 nm (for AC determination) or wave-scanning from 400 to 800 nm (for spectrum pattern and λ_{max} determination). Potato amylose and waxy corn starch were used to obtain a calibration curve from 0 to 100% amylose for corn starch-derived samples, while waxy rice starch was used for the calibration curve for rice starch-derived samples.

2.7. Texture profile analysis (TPA)

The starch gels were characterized by measuring how the samples behaved when compressed to a large degree of deformation. TPA was performed using a texture analyzer (TA-XT2i, Stable Microsystems, Surrey, UK), fitted with a 50-mm diameter cylinder aluminum probe. Cylindrical gel samples (35 mm diameter × 10 mm height) were compressed twice at a constant speed of 1 mm/s to 90% of the initial height of the samples. From the resulting force/deformation curves, textural parameters were obtained.

2.8. Scanning electron microscopy (SEM)

The microstructures of freeze-dried starch gels were observed using SEM. The starch gel samples were refrigerated at 4 °C for 1 week before freeze-drying for 3 days. The freeze-dried starch gels were then cut into small, thin rectangular layers (6 mm × 6 mm × 2 mm) at horizontal and vertical sides and mounted on copper sample holders. Finally, they were sputter-coated with gold and examined using a scanning electron microscope (JSM-5410LV, Jeol, Tokyo, Japan) operated at an acceleration voltage of 15 kV. Micrographs of each gel were taken at 50× (surface observations), 35×, and 350× magnifications (inner structure observations).

2.9. Statistical analysis

Textural parameters were calculated from TPA graphs using the Texture Expert software. Experimental data were analyzed by one-way analysis of variance (ANOVA) and Turkey's test. Least significant differences were computed at $p < 0.05$.

3. Results and discussion

3.1. Starch fine structures

The native (waxy, normal) corn and rice starches, as well as their corresponding TAαGT-modified starches, differed noticeably in Mw distributions upon enzyme digestion, as revealed by HPSEC (Fig. 1, Table 1). The 4αGT has been noted for its action of degrading starch components into smaller molecules with a range of poly-modal distributions (Cho, Auh, Kim, et al., 2009; Cho, Auh, Ryu, et al., 2009; Lee et al., 2009; Mun et al., 2009; Park et al., 2007). Fig. 1 showed the poly-modal Mw distribution profiles of the TAαGT-modified starches during enzymatic degradation, which were categorized into fractions I (elution time 30–38 min, Mw $\geq 1 \times 10^6$), II (38–53 min, $1 \times 10^5 \leq$ Mw $\leq 1 \times 10^6$), and III (53–70 min, $1 \times 10^3 \leq$ Mw $\leq 1 \times 10^5$). As enzyme concentration increased, fraction I gradually decreased, whereas fractions II and III increased. Fraction I consisted of high-Mw glucans, primarily amylopectin. Fractions II and III consisted of intermediate- and low-Mw glucans, which could be TAαGT-modified amylopectin and/or amylose such as smaller amylopectin clusters and cyclic glucans (Park et al., 2007; Takaha et al., 1996, 1998). It appeared that the decreased portion of amylopectin in fraction I moved to fraction II and then to fraction III, in turn, because fraction II developed prior to fraction III. Amylopectin branch chain length (CL) distributions of TAαGT-modified starches greatly differed from those of native starches (Fig. 2). Although the Mw distributions of starches differed considerably, depending on the extent of enzyme treatment, the amylopectin branch chain length distributions were almost identical among TAαGT-modified starches. The amylopectin branch chain length distribution reached equilibrium rapidly (the distribution did not change significantly after treatment for longer times; data not shown), whereas Mw decreased more slowly. Hansen et al. (2008) also reported that extended amylomaltase treatments

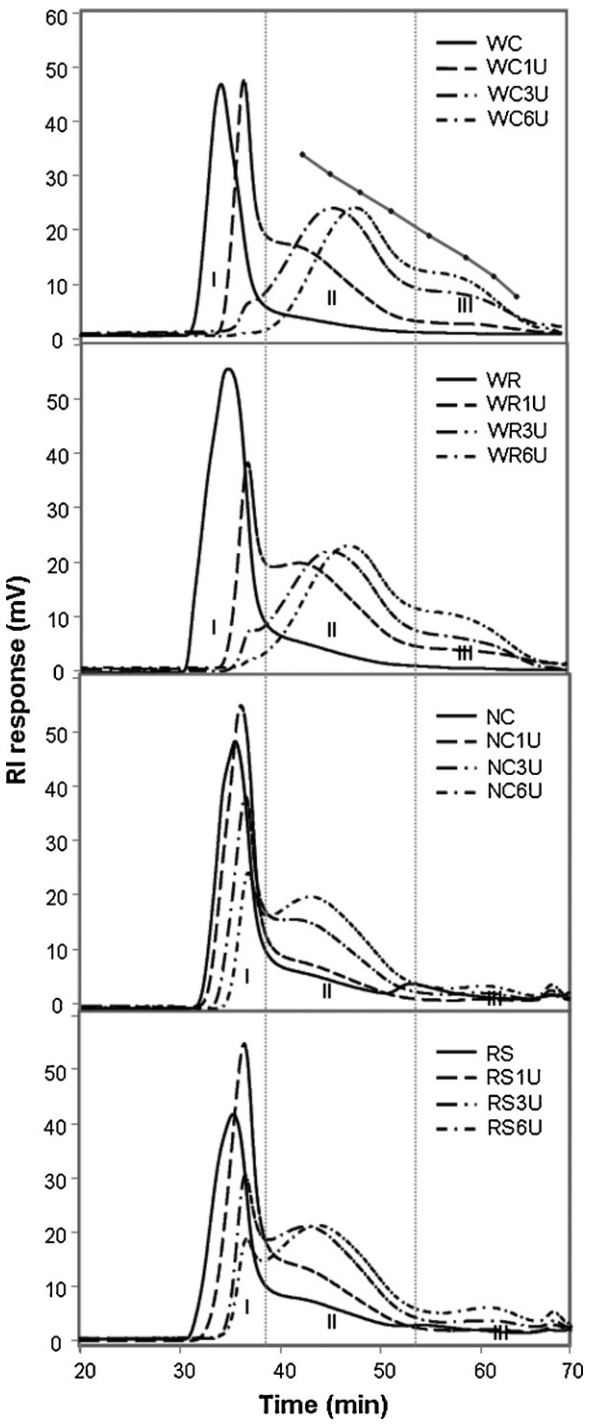

Fig. 1. HPSEC chromatograms of native and TAαGT-modified starches: I: high-Mw fraction; II: intermediate-Mw fraction; III: low-Mw fraction. Points of the calibration curve stand for Mw (Da) of 7.88×10^5, 4.04×10^5, 2.12×10^5, 1.12×10^5, 4.73×10^4, 2.28×10^4, 1.18×10^4, 5.9×10^3 from left to right, respectively [WC: waxy corn starch, WR: waxy rice starch, NC: normal corn starch, RS: normal rice starch, 1, 3, or 6U: different enzyme concentrations (1, 3, or 6 U/g starch, respectively) used for modification].

reduced the apparent molecular weight and the gel texture without changing the amylopectin chain length distribution. The branch chain length distribution of native starches was classified (Fig. 2) based on previously reported studies (Bertoft, 1991; Bertoft et al., 2010; Hizukuri, 1986). The most dramatic change upon enzyme treatment was that the relative portion of intermediate chains (IC, DP 9–24) decreased, while those of short chains (SC, DP <9) and long

Table 1
Molecular fine structures of native and TAαGT-modified starches.

Samples	Fraction I (%)	Fraction II (%)	Fraction III (%)	Mean CL	SC (DP <9)	IC (DP 9–24)	LC (DP >24)
NC[a]	58.81	27.28	13.91	18.07	3.98	79.26	16.76
NC1U[a]	58.35	31.92	9.73	19.73	12.54	59.97	27.49
NC3U[a]	32.98	53.87	13.15	20.17	14.60	55.62	29.78
NC6U[a]	17.51	65.30	17.19	20.43	14.59	54.69	30.72
RS[b]	58.48	30.30	11.22	18.50	3.82	78.60	17.58
RS1U[b]	47.40	43.40	9.20	20.69	12.13	57.02	30.85
RS3U[b]	20.99	64.02	14.99	20.94	13.60	53.88	32.52
RS6U[b]	13.17	65.01	21.82	21.02	12.67	54.83	32.50

[a] Native and TAαGT-modified corn starches at different enzyme concentrations (1, 3, and 6 U/g starch d.b.).
[b] Native and TAαGT-modified rice starches at different enzyme concentrations (1, 3, and 6 U/g starch d.b.).

chains (LC, DP >24) increased, resulting in flatter distributions. The decreased portion of the distribution consisted of A and short B chains (B1a, DP 4–24), which were major fractions of amylopectin branch chains (Bertoft, 1991). Thus, those external chains were highly susceptible to the disproportionation reaction of TAαGT, through which their chain lengths were rapidly redistributed more evenly. The modification of external branch chains of amylopectin did not significantly contribute to the reduction of Mw. According to the accepted model for the amylopectin structure (Bertoft et al., 2010; Hizukuri, 1986), long B2 chains (DP >36) are involved in the interconnection of clusters of short chains. Cleaving these internal chains would effectively reduce the amylopectin Mw. Reduction of amylopectin Mw is possible by both hydrolysis and transglycosylation. Actually, in the case of potato D-enzyme reported to exhibit essentially zero hydrolytic activity, effectively decreased Mw of waxy corn starch down to 3000–30,000 Da by means of transglycosylation (Takaha et al., 1998). Even though TAαGT is known to demonstrate weak hydrolytic activity, its major activity is transglycosylation (Fujii et al., 2005; Kuriki et al., 2006). Therefore, relatively slow rate of Mw decrease compared to rapid branch chain length re-distribution of amylopectin was probably due to the steric hindrance in attacking internal B2 chains interconnecting amylopectin clusters and/or the nature of disproportionation reaction: reduction of amylopectin Mw by disproportionation would be possible only when smaller amylopectin clusters are separated to form cyclic clusters by intramolecular transglycosylation and/or separated amylopectin clusters are transferred to smaller glucans by intermolecular transglycosylation (Takaha et al., 1996, 1998). Fig. 2 also shows that the proportions of LC (DP >24) were higher in normal starches compared with those of waxy starches after TAαGT treatment, indicating that glucans from amylose molecules were transferred to amylopectin branch chains, as reported previously (Kaper et al., 2004; Park et al., 2007).

In short, the above molecular fine structures of the native and partially modified starches revealed that TAαGT readily modified the amylopectin branch chains to make steady distribution and reduced molecular weight at a lower rate, which eventually resulted in liquefaction of the native starch pastes. By controlling the degree of enzyme treatment (i.e. concentration and/or incubation time), TAαGT-modified starches with different molecular fine structures were obtained, resulting in diverse mechanical properties, as discussed later.

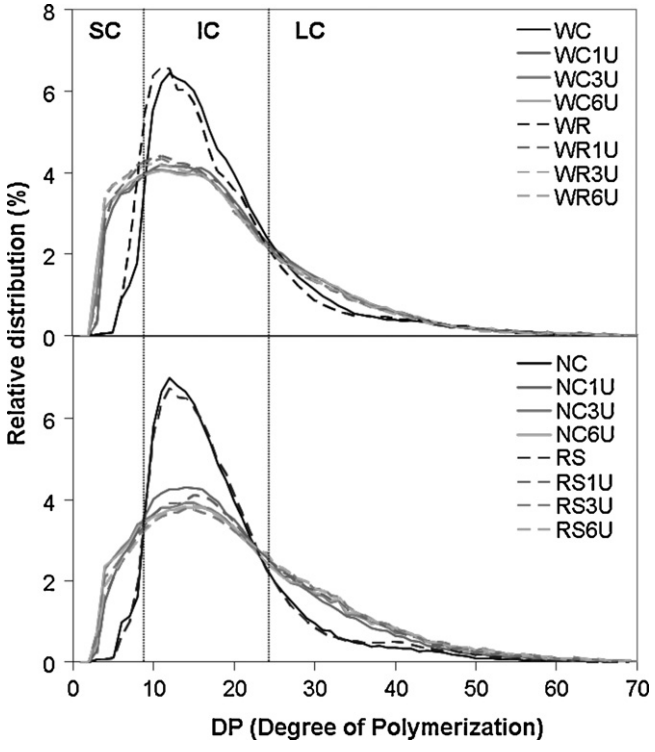

Fig. 2. Chain length distributions of isoamylase-debranched native and TAαGT-modified starches determined by HPAEC-PAD. This figure was sectioned into three parts: short chains (SC, DP <9), intermediate chains (IC, DP 9–24), and long chains (LC, DP >24) [WC: waxy corn starch, WR: waxy rice starch, NC: normal corn starch, RS: normal rice starch, 1, 3, or 6U: different enzyme concentrations (1, 3, or 6 U/g starch, respectively) used for modification].

3.2. Iodine absorption spectra and amylose contents

Fig. 3 shows the characteristic absorption spectra of amylose and amylopectin from potato starch, corn, and rice starches as well as their TAαGT-modified starches after iodine-binding. Estimated values for AC and λ_{max} are also presented in Table 2. Native starches

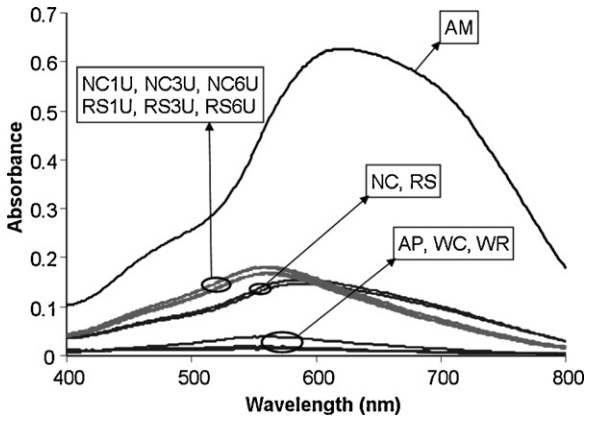

Fig. 3. Iodine absorption spectra of starch samples. AM: potato amylose, AP: potato amylopectin, WC: waxy corn starch, WR: waxy rice starch, NC: normal corn starch, RS: normal rice starch, 1, 3, or 6U: different enzyme concentration (1, 3, or 6 U/g starch, respectively) used for modification.

Table 2
Amylose contents (AC) and maximum absorption wavelength (λ_{max}) of the iodine-binding starches.

Samples	AC (%)	λ_{max} (nm)
AM[a]	–	620.5
AP[a]	–	567.0
WC[a]	–	550.0
WR[a]	–	561.0
NC[b]	21.6a	589.2a
NC1U[b]	15.8b	567.0b
NC3U[b]	15.6b	567.0b
NC6U[b]	15.6b	567.0b
RS[c]	21.0a	587.7a
RS1U[c]	13.1b	567.0b
RS3U[c]	13.9b	557.0c
RS6U[c]	14.4b	557.0c

Means followed by different letter in the same column in the same section are significantly different ($p < 0.05$).

[a] Potato amylose (AM) and amylopectin (AP), waxy corn (WC) and waxy rice (WR) starches.

[b] Native and modified corn starch gels at different enzyme concentrations (1, 3, and 6 U/g starch d.b.).

[c] Native and modified rice starch gels at different enzyme concentrations (1, 3, and 6 U/g starch d.b.).

from different botanical sources showed different spectral patterns with characteristic λ_{max} values, whereas TAαGT-modified starches developed the same pattern characterized by λ_{max} around 567 nm, which were close to those of waxy starches (Fig. 3, Table 2). The AC of TAαGT-modified starches decreased significantly compared with those of native starches but showed almost no difference among enzyme-treated samples (Table 2). Although the term "amylose content" is used in this context because we adopted the method of AC measurement, it represents a qualitative comparison of absorbance equivalent to AC. Similar to the amylopectin branch-chain distribution, iodine absorption spectra appeared to reach a plateau at a faster rate than the rate of change of the Mw distribution (Fig. 1). Although the modification reached a plateau, at which point the absorption spectra did not change significantly, the absorbance values of TAαGT-modified starches remained significantly higher than those of waxy starches. This indicated that enzymatic products of amylose still governed the iodine-binding capabilities of TAαGT-modified starches. There are several possible fates of amylose on TAαGT treatment (Kaper et al., 2004; Park et al., 2007; Takaha et al., 1996): (a) segments of amylose chains could be transferred to amylopectin branch chains by intermolecular transglycosylation, (b) cyclic glucans could form by intramolecular transglycosylation, and (c) smaller linear chains could remain through (a) and (b). A well-known feature of starch molecules responsible for iodine-binding is the formation of single helices in aqueous environments. Furthermore, it has been reported that large-ring cycloamyloses can also possess helical structures in their unique three-dimensional (3D) conformations, showing affinity to iodine (Kitamura et al., 1999). The shift of λ_{max} to that near amylopectin, with significantly higher absorbance than amylopectin, indicated that TAαGT disassembled long-chain amylose into a large amount of shorter helical glucans through disproportionation, possibly supplementing longer amylopectin branch chains (Fig. 2) and cycloamyloses, as previously reported (Kaper et al., 2004; Park et al., 2007; Takaha et al., 1996).

3.3. Starch gel textural attributes

TPA test was conducted to determine the starch gel textural properties because they have been shown to correlate with sensory evaluation (Lawless & Heymann, 2010). The measured textural properties of the native and TAαGT-modified starch gels after gel-setting (0 day) and during storage (1, 3, and 7 days) were shown in Fig. 4. Five main TPA parameters (fracture strain, fracturability, hardness, adhesiveness, and cohesiveness) which showed significant changes among the samples were listed in Table 3. It was noted that native and TAαGT-treated waxy starches did not form gels under the same experimental conditions used for the native and TAαGT-modified normal starch gel formation.

Both TAαGT treatment and storage time had substantial effects on the textural properties of starch gels. There were significant differences in textural properties between the native samples (corn vs. rice starch gel) and among the native and TAαGT-modified starch gels. The control native rice starch gel exhibited lower fracturability and hardness but considerably higher adhesiveness and cohesiveness than control corn starch gel. It has been shown that the development of a starch gel is affected by both the nature of the amylopectin as well as the amylose-to-amylopectin ratio of the starch gel (Gudmundsson, 1994; Jane & Chen, 1992; Leloup et al., 1992; Miles et al., 1985; Mua & Jackson, 1998; Oates et al., 1993; Richardson, Kidman, et al., 2004; Srichuwong & Jane, 2007). However, these large differences in textural properties between control rice and corn starch gels almost disappeared upon enzyme treatment; that is, textural property differences between enzyme-treated rice and corn starch gels were much smaller than those between two control starch gels. Although small differences between TAαGT-treated corn and rice starch gels existed, their textural properties were largely governed by the degree of enzyme treatment. TAαGT seemed to shuffle native molecular structures within starch until they had somewhat comparable textural properties. General changes upon TAαGT treatment were a decrease in fracture strain and an increase in hardness, indicating that gels became rigid and brittle on enzyme treatment. For both starch samples, as enzyme concentration increased, fracture strain and fracturability decreased, while hardness, adhesiveness, and cohesiveness increased. During 1-week storage, almost all the textural parameters increased, except for fracture strain. The changes during storage could be explained by a retrogradation process of starch gels. However, it should be noted that changes of TAαGT-treated starch gels during storage differed markedly from those of control starch gels. An increase in fracturability and hardness during storage was more dramatic for TAαGT-treated gels compared with the control gels. The adhesiveness and cohesiveness of TAαGT-treated gels showed opposite trends during storage compared with control gels. Different or even opposite trends of textural changes during storage might indicated that TAαGT-treated starch gels had considerably different gel network structures composed of modified starch molecules compared with unmodified starch gels.

Structural changes in both Mw and amylopectin branch chain length distributions (Table 1) could positively influence the increase of gel rigidity and brittleness of the TAαGT-modified starch gels. The rigidity increase of TAαGT-modified starch gels could be explained by their gel-forming matrix due to amylose chain aggregation and hydrogen bonding (Leloup et al., 1992; Miles et al., 1985; Richardson, Sun, et al., 2004), although this explanation does not exclude co-precipitation and co-crystallization of the cleaved amylose chains with the partially modified amylopectin clusters within the gel as an alternative process (Miles et al., 1985). The increasing values of textural properties, such as fracturability and hardness of TAαGT-modified starch gels during storage revealed the role of modified amylopectin in starch recrystallization.

3.4. Starch gel microstructures

The microstructures of outer and fractured inner surfaces of freeze-dried starch gels are shown in Fig. 5. The native corn and rice starch gels (1st row) exhibited continuous porous structures in which a reticulated 3D network formed isotropically. At the

outer surface of TAαGT-treated starch gels (1st and 3rd columns in 2nd through 4th row), this continuous reticulated porous network disappeared, and a partly dense, but discontinuous, structure with many cracks was observed. These surface cracks became more severe as enzyme concentration increased. Observations of fractured inner surfaces (2nd and 4th columns in 2nd through 4th row) revealed that the enzyme transformed the continuous porous network of native starch gels into oriented layers or fibrous structures, which were completely different from the native gels. These structural changes upon enzyme treatment could be related to the textural changes of the enzyme-treated gels, from elastic-soft to brittle-hard.

The fibrous structure was presumably due to changes in the molecular structures of TAαGT-treated starch gels (Fig. 1). The intermediate-Mw amylopectin clusters with re-organized chain length distributions, as well as the essential breakdown of long-chain amylose, resulted in the disappearance of the continuous network. Thus, we propose that thick crystalline fibers with small cracks detected in 4αGT-treated starch gels may be attributable to co-precipitation and/or co-crystallization of the shorter chains of amylose and intermediate-Mw amylopectin clusters with re-organized chain length distributions, which may be related to the structural and mechanical properties of 4αGT-treated starch gels.

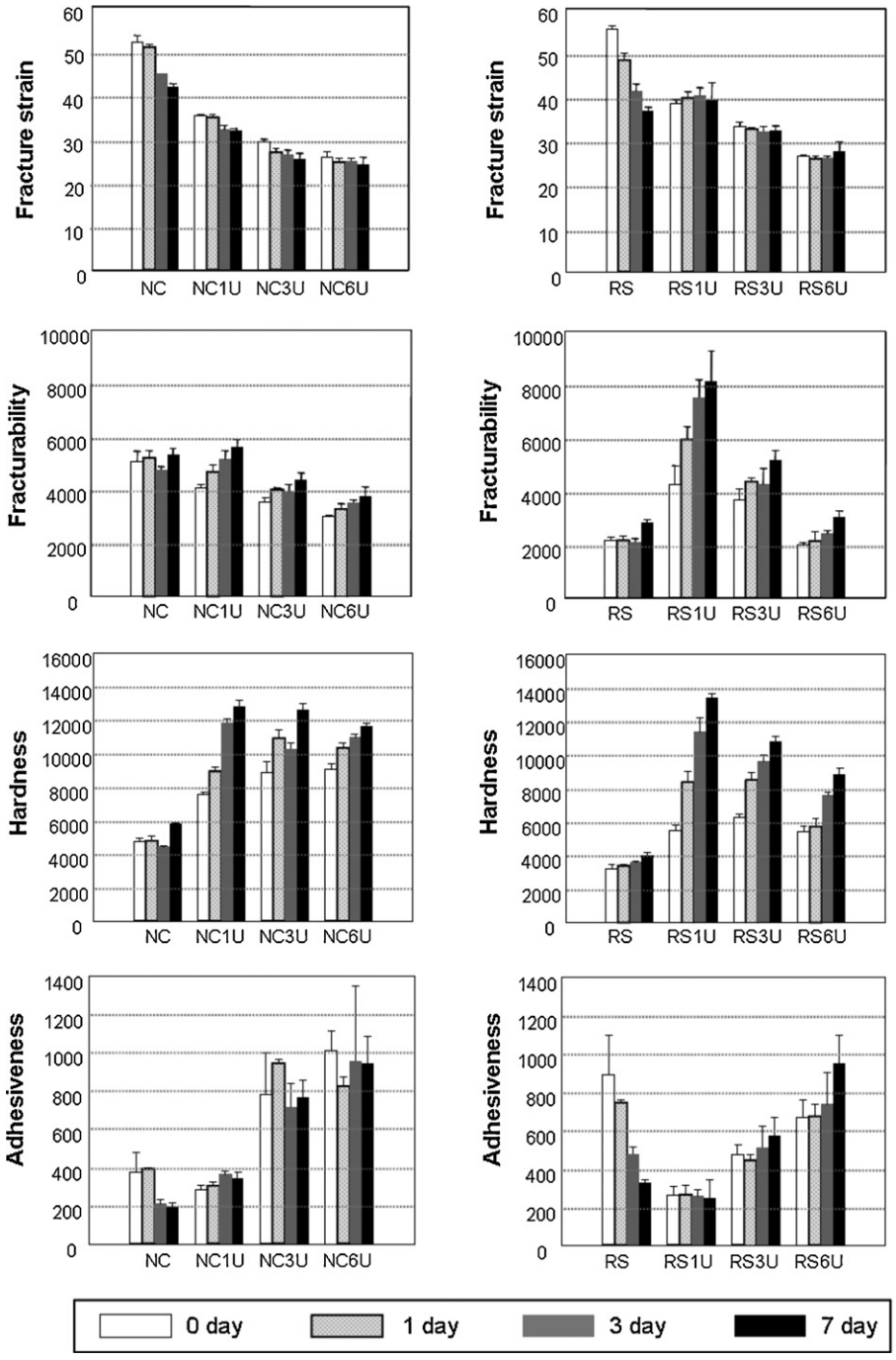

Fig. 4. TPA analyses for native and TAαGT-modified corn (left) and rice (right) starch gels after 24 h of gel setting (0 day) during storage (1, 3, and 7 days). NC: normal corn starch, RS: normal rice starch, 1, 3, or 6U: different enzyme concentration (1, 3, or 6 U/g starch, respectively) used for modification.

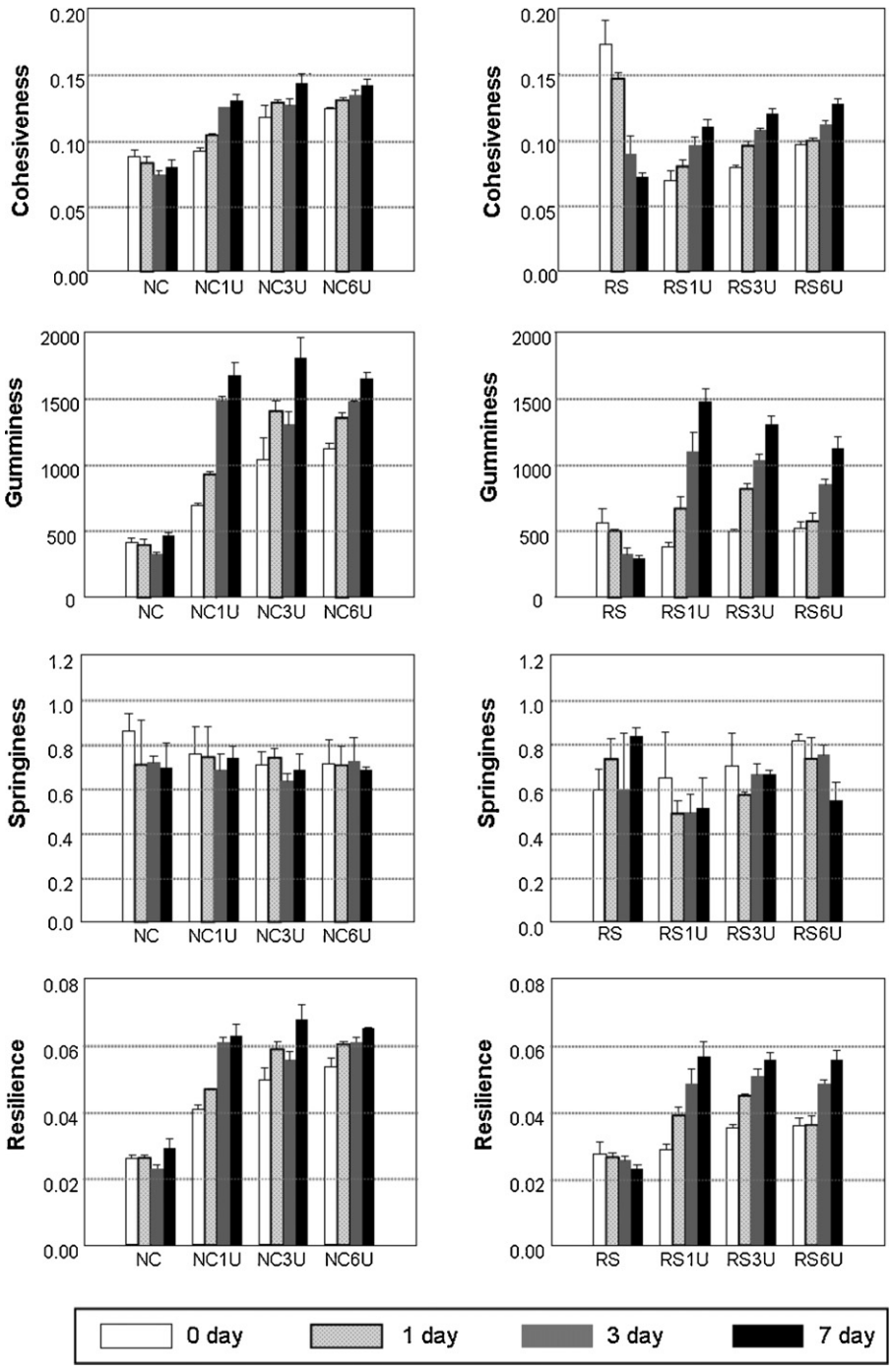

Fig. 4. (*Continued*).

Table 3
Textural properties of native and TAαGT-modified starch gels (10%, 2-h incubation) after 24 h for gel-setting at 4 °C.

Samples	Fracture strain (%)	Fracturability (g)	Hardness (g)	Adhesiveness (g s)	Cohesiveness
NC[a]	51.89a	5054.2a	4728.4c	373.4b	0.088b
NC1U[a]	35.25b	4083.1b	7548.2b	286.0b	0.091b
NC3U[a]	29.30c	3517.3c	8885.5a	780.2a	0.117a
NC6U[a]	25.86d	2986.1d	9042.0a	1009.3a	0.124a
RS[b]	55.35a	2173.3b	3226.1c	891.7a	0.173a
RS1U[b]	38.51b	4250.7a	5519.4b	260.6c	0.069c
RS3U[b]	33.01c	3684.8a	6254.8a	473.9bc	0.079bc
RS6U[b]	26.48d	1984.8b	5425.3b	670.0ab	0.096b

Means followed by different letter in the same column in the same section are significantly different ($p < 0.05$).
[a] Native and TAαGT-modified corn starch gels at different enzyme concentrations (1, 3, and 6 U/g starch d.b.).
[b] Native and TAαGT-modified rice starch gels at different enzyme concentrations (1, 3, and 6 U/g starch d.b.).

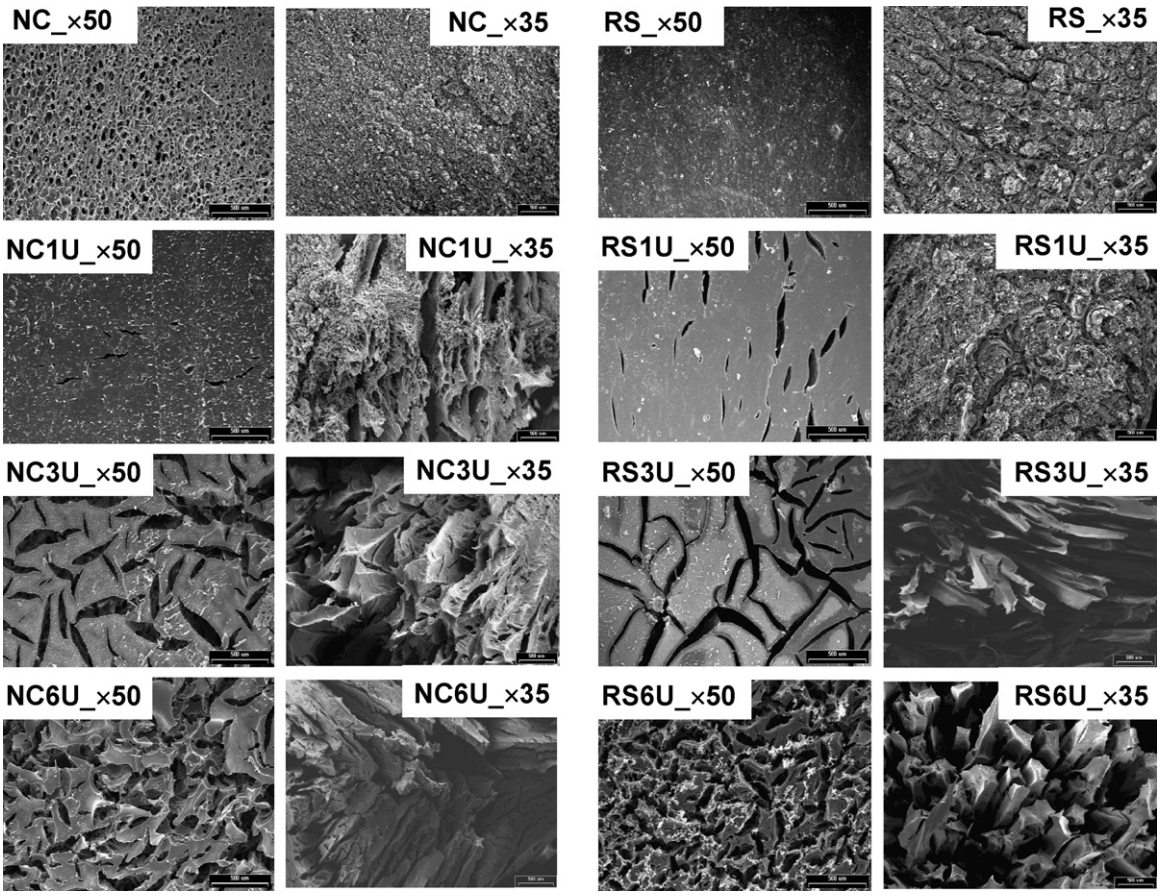

Fig. 5. SEM micrographs of the freeze-dried native and TAαGT-modified starch gels observed for outer surface (1st and 3rd columns) and fractured inner surface (2nd and 4th columns) at 50× and 35× magnifications, respectively (bar = 500 μm). NC: normal corn starch, RS: normal rice starch, 1, 3, or 6U: different enzyme concentration (1, 3, or 6 U/g starch, respectively) used for modification.

Acknowledgments

This work was supported by the Korea Science and Engineering Foundation (KOSEF) grant funded by the Korea government (MEST) (no. 20090059709). This work was also conducted with the support of the "Cooperative Research Program for Agricultural Science & Technology Development" (project no. 200803A01033092), RDA, Republic of Korea.

References

Bertoft, E. (1991). Chains of intermediate lengths in waxy-maize amylopectin. *Carbohydrate Research*, *212*, 245–251.

Bertoft, E., Laohaphatanalert, K., Piyachomkwan, K., & Sriroth, K. (2010). The fine structure of cassava starch amylopectin. Part 2. Building block structure of clusters. *International Journal of Biological Macromolecules*, *47*, 325–335.

Bradford, M. (1976). A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein–dye binding. *Analytical Biochemistry*, *72*, 248–254.

Cho, K. H., Auh, J. H., Kim, J. H., Ryu, J. H., Park, K. H., Park, C. S., et al. (2009). Effect of amylose content on corn starch modification by *Thermus aquaticus* 4-α-glucanotransferase. *Journal of Microbiology and Biotechnology*, *19*, 1201–1205.

Cho, K. H., Auh, J. H., Ryu, J. H., Kim, J. H., Park, K. H., Park, C. S., et al. (2009). Structural modification and characterization of rice starch treated by *Thermus aquaticus* 4-α-glucanotransferase. *Food Hydrocolloids*, *23*, 2403–2409.

Choi, S. G., & Kerr, W. L. (2003). Water mobility and textural properties of native and hydroxypropylated wheat starch gels. *Carbohydrate Polymers*, *51*, 1–8.

Chrastil, J. (1987). Improved colorimetric determination of amylose in starches or flours. *Carbohydrate Research*, *159*, 154–158.

Deffenbaugh, L. B. (1997). Carbohydrate/emulsifier interactions. In G. L. Hasenhuettl, & R. W. Hartel (Eds.), *Food emulsifiers and their applications* (pp. 67–94). New York: Chapman & Hall.

Durrani, C. M., & Donald, A. M. (1995). Physical characterization of amylopectin gels. *Polymer Gels and Networks*, *3*, 1–27.

Fujii, K., Minagawa, H., Terada, Y., Takaha, T., Kuriki, T., Shimada, J., et al. (2005). Protein engineering of amylomaltase from *Thermus aquaticus* with random and saturation mutagenesis. *Biologia*, *60*, 97–102.

Gudmundsson, M. (1994). Retrogradation of starch and the role of its components. *Thermochimica Acta*, *246*, 329–341.

Hansen, M. R., Blennow, A., Farhat, I., Nørgaard, L., Pedersen, S., & Engelsen, S. B. (2009). Comparative NMR relaxometry of gels of amylomaltase-modified starch and gelatin. *Food Hydrocolloids*, *23*, 2038–2048.

Hansen, M. R., Blennow, A., Pedersen, S., Nørgaard, L., & Engelsen, S. B. (2008). Gel texture and chain structure of amylomaltase-modified starches compared to gelatin. *Food Hydrocolloids*, *22*, 1551–1566.

Hizukuri, S. (1986). Polymodal distribution of the chain lengths of amylopectins, and its significance. *Carbohydrate Research*, *147*, 342–347.

Hoover, R., & Vasanthan, T. (1994). Effect of heat-moisture treatment on the structure and physicochemical properties of cereal, legume, and tuber starches. *Carbohydrate Research*, *252*, 33–53.

Jane, J. L., & Chen, J. F. (1992). Effect of amylose molecular size and amylopectin branch chain length on paste properties of starch. *Cereal Chemistry*, *69*, 60–65.

Kaper, T., Talik, B., Ettema, T. J., Bos, H., van der Maarel, M. J. E. C., & Dijkhuizen, L. (2005). Amylomaltase of *Pyrobaculum aerophilum* IM2 produces thermoreversible starch gels. *Applied and Environmental Microbiology*, *71*, 5098–5106.

Kaper, T., van der Maarel, M. J. E. C., Euverink, G. J., & Dijkhuizen, L. (2004). Exploring and exploiting starch-modifying amylomaltases from thermophiles. *Biochemical Society Transactions*, *32*, 279–282.

Kitamura, S., Nakatani, K., Takaha, T., & Okada, S. (1999). Complex formation of large-ring cyclodextrins with iodine in aqueous solution as revealed by isothermal titration calorimetry. *Macromolecular Rapid Communications*, *20*, 612–615.

Kuriki, T., Takata, H., Yanase, M., Ohdan, K., Fujii, K., Terada, Y., et al. (2006). The concept of the α-amylase family: A rational tool for interconverting glucanohydrolases/glucanotransferases, and their specificities. *Journal of Applied Glycoscience*, *53*, 155–161.

Lawless, H. T., & Heymann, H. (2010). *Sensory evaluation of food: Principles and practices* (2nd ed.). New York: Chapman & Hall. (Chapter 11).

Lee, K. Y., Kim, Y. R., Park, K. H., & Lee, G. L. (2006). Effects of α-glucanotransferase treatment on the thermoreversibility and freeze–thaw stability of a rice starch gel. *Carbohydrate Polymers*, *63*, 347–354.

Lee, B. H., Oh, D. K., & Yoo, S. H. (2009). Characterization of 4-α-glucanotransferase from *Synechocystis* sp. PCC 6803 and its application to various corn starches. *New Biotechnology*, *26*, 29–36.

Leloup, V. M., Colonna, P., Ring, S. G., Roberts, K., & Wells, B. (1992). Microstructure of amylose gels. *Carbohydrate Polymers*, *18*, 189–197.
Liebl, W., Feil, R., Gabelsberger, J., Kellermann, J., & Schleifer, K. H. (1992). Purification and characterization of a novel thermostable 4-α-glucanotransferase of *Thermotoga maritima* cloned in *Escherichia coli*. *European Journal of Biochemistry*, *207*, 81–88.
Ma, Y., Cai, C., Wang, J., & Sun, D. W. (2006). Enzymatic hydrolysis of corn starch for producing fat mimetics. *Journal of Food Engineering*, *73*, 297–303.
Miles, M. J., Morris, V. J., Oxford, P. D., & Ring, S. G. (1985). The roles of amylose and amylopectin in the gelation and retrogradation of starch. *Carbohydrate Research*, *135*, 271–281.
Morikawa, K., & Nishinari, K. (2000). Effects of concentration dependence of retrogradation behavior of dispersions for native and chemically modified potato starch. *Food Hydrocolloids*, *14*, 395–401.
Mua, J. P., & Jackson, D. S. (1998). Retrogradation and gel textural attributes of corn starch amylose and amylopectin fractions. *Journal of Cereal Science*, *27*, 157–166.
Mun, S., Kim, Y. L., Kang, C. G., Park, K. H., Shim, J. Y., & Kim, Y. R. (2009). Development of reduced-fat mayonnaise using 4αGTase-modified rice starch and xanthan gum. *International Journal of Biological Macromolecules*, *44*, 400–407.
Oates, C. G., Lucas, P. W., & Lee, W. P. (1993). How brittle are gels. *Carbohydrate Polymers*, *20*, 189–194.
Oh, E. J., Choi, S. J., Lee, S. J., Kim, C. H., & Moon, T. W. (2008). Modification of granular corn starch with 4-α-glucanotransferase from *Thermotoga maritime*: Effects on structural and physical properties. *Journal of Food Science*, *73*, 158–166.
Ortega-Ojeda, F. E., Larsson, H., & Eliasson, A. C. (2004). Gel formation in mixtures of high amylopectin potato starch and potato starch. *Carbohydrate Polymers*, *56*, 505–514.
Park, J. H., Kim, H. J., Kim, Y. H., Cha, H., Kim, Y. W., Kim, T. J., et al. (2007). The action mode of *Thermus aquaticus* YT-1 4-α-glucanotransferase and its chimeric enzymes introduced with starch-binding domain on amylose and amylopectin. *Carbohydrate Polymers*, *67*, 164–173.
Richardson, G., Kidman, S., Langton, M., & Hermansson, A. M. (2004). Differences in amylose aggregation and starch gel formation with emulsifiers. *Carbohydrate Polymers*, *58*, 7–13.
Richardson, G., Laugton, M., Bark, A., & Hermansson, A. M. (2003). Wheat starch gelatinization: The effects of sucrose, emulsifier and the physical state of the emulsifier. *Starch/Stärke*, *55*, 150–161.
Richardson, G., Sun, Y., Langton, M., & Hermansson, A. M. (2004). Effects of Ca- and Na-lignosulfonate on starch gelatinization and network formation. *Carbohydrate Polymers*, *57*, 369–377.
Rosalina, I., & Bhattacharya, M. (2002). Dynamic rheological measurements and analysis of starch gels. *Carbohydrate Polymers*, *48*, 191–202.
Srichuwong, S., & Jane, J. L. (2007). Physicochemical properties of starch affected by molecular composition and structures: A review. *Food Science and Biotechnology*, *16*, 663–674.
Takaha, T., Yanase, M., Takaha, H., Okada, S., & Smith, S. M. (1993). Disproportionating enzyme (4-α-glucanotransferase; EC 2.4.1.25) of potato: Purification, molecular cloning, and potential role in starch metabolism. *Journal of Biological Chemistry*, *268*, 1391–1396.
Takaha, T., Yanase, M., Takaha, H., Okada, S., & Smith, S. M. (1996). Potato D-enzyme catalyzes the cyclization of amylose to produce cycloamylose, a novel cyclic glucan. *Journal of Biological Chemistry*, *271*, 2902–2908.
Takaha, T., Yanase, M., Takaha, H., Okada, S., & Smith, S. M. (1998). Cyclic glucans produced by the intramolecular transglycosylation activity of potato D-enzyme on amylopectin. *Biochemical and Biophysical Research Communications*, *247*, 493–497.
Terada, Y., Fujii, K., Takaha, T., & Okada, S. (1999). *Thermus aquaticus* ATCC 33923 Amylomaltase gene cloning and expression and enzyme characterization: Production of cycloamylose. *Applied and Environmental Microbiology*, *65*, 910–915.
van der Maarel, M. J. E. C., Capron, I., Euverink, G. J., Bos, H. T., Kaper, T., Binnema, D. J., et al. (2005). A novel thermoreversible gelling product made by enzymatic modification of starch. *Starch/Stärke*, *57*, 465–472.