A Thesis for the Degree of Master of Science

Physicochemical and digestion properties of amylosucrase-treated starch mixed with galactomannan

아밀로수크레이스 처리 전분의 갈락토만난 첨가 후 특성 분석

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지도교수 문 태 화
이 논문을 석사학위 논문으로 제출함

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Physicochemical and digestion properties of amylosucrase-treated starch mixed with galactomannan

by

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ABSTRACT

Starch can have lowered digestibility when galactomannan (guar gum or locust bean gum) is added. The objective of this study is to elucidate the digestive patterns and physicochemical characteristics of amylosucrase-treated starch mixing with galactomannan. In this study, the digestibility of starch decreased to a greater extent with added galactomannan after amylosucrase treatment. It could be attributed to the increased contact between starch and galactomannan due to elongated branch chains of amylopectin after amylosucrase treatment. Guar gum was more effective on lowering digestibility of starches, because guar gum can interact more easily with starch compared to locust bean gum, which could be connected to the results of glucose release and rheology test. In the steady shear rheological analysis, guar gum exhibited more shear thinning behavior. Presumably, guar gum was in more extended form because of more galactose branches which can prevent the formation of intramolecular hydrogen bonding, compared with locust bean gum. Fewer galactose branches of locust bean gum led to the formation of a coil and the consequent decrease in interaction with starch. Dynamic shear rheological analysis revealed that AS-treated starches could form more stable gels than AS control starches. The stability of AS-treated starch was higher with guar gum than with locust bean gum. According to the glucose release measurement, guar gum had a greater ability to suppress glucose release and to adsorb more glucose than locust bean gum. Besides, the results of

differential scanning calorimetry showed that the onset temperature of starch gelatinization was higher, and its endothermic enthalpy was reduced after mixing starch with galactomannan. It indicates that galactomannan reduced free water required for starch gelatinization.

In short, this study presented an appropriate methodology for lowering starch digestibility by mixing starch and galactomannan.

Keywords: slowly digestible starch, amylosucrase, galactomannan, *in vitro* digestibility, glucose diffusion, rheology

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CONTENTS

LIST OF FIGURES

LIST OF TABLES

INTRODUCTION

Starch is the main natural storage source of carbohydrates in plants (Webb, 1981). Starch is composed of two polymers, amylose and amylopectin. Amylose is a linear glucose polymer linked with α-1,4 linkage and amylopectin is a branched glucose polymer linked with α-1,6 linkage.

In general, starch is nutritionally classified according to the rate of digestion into rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistance starch (RS) (Shin et al., 2010). RDS is digested within 10 min, SDS is digested between 10 min and 240 min, and RS cannot be digested even after 240 min. RS can act as a dietary fiber. In addition, SDS and RS can help remain low glycemic index (Shin et al., 2007).

Amylosucrase (AS) catalyzes elongation of the external chain of starch by attaching glucose to the non-reducing end of the chain (Kim et al., 2014). As a result, AS-treated starches have reduced RDS and increased SDS and RS. Also, AS-treated starch is more compatible with other polysaccharides because of its elongated external chains. Amylose and amylopectin with long exterior chain (amylose-like) can interact with the galactomannan (Funami et al., 2005). Waxy corn starch was used in the present study, because it not only

is most commonly used in the food industry, but also has the most reduced range of digestibility after AS-treatment (Shin et al., 2010).

Hydrocolloids, a class of non-starch polysaccharides, have the long hydrophilic chain with high molecular weight. Hydrocolloid is often used in starch-based food processing. Starch gelation could be strongly influenced by adding hydrocolloid (BeMiller and James, 2011). Therefore, hydrocolloid can compensate the defect of starch by increasing freeze-thaw stability, water mobility, and emulsion stability (Lee et al., 2002). Also, sensory properties of starch can be improved by adding hydrocolloid (Meyer et al., 2011; Lee and Chang, 2016). Use of hydrocolloids in food system has been enhanced because of the increased concern about wellbeing. Hydrocolloids have many health functional effects such as lowering cholesterol level and regulating blood glucose (Jenkins et al., 2000). The properties of starch mixed with hydrocolloid is related to the molecular weight of each component as well as the ratio of starch to hydrocolloid (Annable et al., 1994).

Galactomannans, one of the most common hydrocolloids, are composed of mannopyranose units joined by β-D-(1→4) linkages, with α-D-galactopyranose units attached to the chain by (1→6) linkages. In this study, guar gum (GG) and locust bean gum (LBG) were used. Guar gum is obtained from the guar seed *Cyamopsis tetragonolobus* and locust bean gum is obtained

from the seeds of the tree *Ceratonia silliqua*. Guar gum and locust bean gum have the same composition, but they have different ratio of mannose to galactose, 1:2 and 1:4, respectively (Kulicke et al., 1996). Guar gum and locust bean gum reduce syneresis and improve freeze-thaw stability of starches (Sudhakar et al, 1996). According to a previous study (Weber et al, 2009), the covalent bond between galactomannan and starch is not observed by infra-red absorption spectra. The only interaction between galactomannan and starch is supposed to the hydrogen bonding.

Understanding rheological properties and digestibility of starch mixed with galactomannan is important for starch-based products (Brennan et al, 1996). There have been plenty of studies to elucidate the effect of galactomannan on starches, but studies about the effect of two kinds of galactomannan on AS-treated starch are scarce. Especially, the relationship between digestibility and rheology characteristics has not been clarified for the AS-treated starch mixed with galactomannan at a low concentration (< 1%). This study was designed because elongated external chains of AS-treated starch can have great effects on the digestive patterns when galactomannan are added (Funami et al., 2005). Therefore, the objective of the present study is to reveal the effects of mixing locust bean gum or guar gum on the digestibility and physicochemical properties of amylosucrase (AS)-treated starch.

MATERIALS AND METHODS

1. Materials

Waxy corn starch was obtained from Ingredion (Westchester, IL, USA). Guar gum and locust bean gum were obtained from MSC Corp. (Yangsan, Korea). Sucrose was purchased from Junsei Chemical (Tokyo, Japan). Pancreatin (P7545, activity 8 x USP/g) and dialysis tubing (D9777, flat width 25 mm) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Isoamylase (activity 1,000U), amyloglucosidase (AMG 300 L, activity 300 AGU/mL) and GOD-POD assay kit were supplied by Megazyme (Bray, Ireland), Novozymes (Bagsvaerd, Denmark), and Embiel Co. (Gunpo, Korea), respectively.

2. Methods

2.1. Amylosucrase activity assay

The gene of AS from *Neisseria polysaccharea* was cloned and expressed in *Escherichia coli* BL21 (DE3). The enzyme was purified using affinity chromatography with nickel-nitrilotriacetic acid (Ni-NTA) resin (Qiagen, Hombrechtikon, Switzerland) as described in a previous article (Jung et al., 2009). Activity of AS was measured according to the method of van der Veen et al. (2004) with slight modification. The mixture of diluted 0.05mL of AS, 0.1 mL of 4% sucrose, 0.1 mL of 1% glycogen, and 0.25 mL of 100 mM sodium citrate buffer (pH 7.0) was incubated in a water bath at 30°C and 80 rpm for 10 min. The amount of released fructose was quantified by dinitrosalicylic acid method (Miller, 1959). One unit (U) of AS was the amount of enzyme that catalyzes the production of 1 μM of fructose per min from sucrose.

2.2. Preparation of samples

2.2.1. Preparation of AS-treated starches

Starch suspension (2%, w/v) with 100 mM sucrose in 100 mM sodium citrate buffer (pH 7.0) was boiled with intermittent vortexing for 30 min and cooled at 30°C. AS was added to the starch suspension (5,000 U/30 mL) and

incubated in a water bath at 30°C and 80 rpm. After 24 h, enzyme reaction was stopped by adding 3 volumes of ethanol. AS-treated starch was obtained by centrifuging at 10,000 x g for 10 min and washing with distilled water 3 times. The pellet was freeze-dried, ground, and passed through a 100-mesh sieve.

AS unit to use was set at 5,000U based on the largest unit remaining soluble in boiling water.

AS control starch was prepared following the same process of AS-treated starches except adding amylosucrase.

2.2.2. Preparation of starches mixed with galactomannans

Guar gum or locust bean gum was dissolved in distilled water or in 0.1M sodium acetate buffer (in case of digestion) to make 0.5 and 1% solution. Galactomannan was used in concentrations of 0.5% and 1% according to the previous study (Gularte et al., 2011).

The galactomannan solution was boiled for 30 min with intermittent vortexing and then cooled at room temperature for 30 min. The starch sample (80 mg) was weighed in a 50 mL centrifuge tube containing 2 mL of galactomannan solution and then mixed. The sample dispersion was boiled for 30 min with vortexing, cooled at room temperature for 30 min, and then stored at 4°C for 1 h.

AS control starch without galactomannan, AS control starch with 0.5% guar gum, AS control starch with 1% guar gum, AS control starch with 0.5% locust bean gum and AS control starch with 1% locust bean gum were abbreviated as C-0, C-G0.5, C-G1, C-LB0.5, and C-LB1, respectively. AS starch without galactomannan, AS starch with 0.5% guar gum, AS starch with 1% guar gum, AS starch with 0.5% locust bean gum, and AS starch with 1% locust bean gum were abbreviated as AS-0, AS-G0.5, AS-G1, AS-LB0.5, and AS-LB1, respectively.

2.3. Analysis of branch chain length distribution

Starch sample (15 mg) was dispersed in 90% dimethyl sulfoxide (3 mL) and boiled for 30 min. Ethanol (15mL) was added to the suspension. After centrifuging (10,000 x g, 10 min), the pellet was boiled for 15 min with distilled water (1.5 mL) and then with 50 mM sodium acetate buffer (1.5 mL, pH 4.3) for 15 min. The suspension was equilibrated at 45°C for 30 min. Isoamylase (30 μL) was added to the suspension and incubated for 2 h in a shaking water bath (45°C, 30 rpm). The reaction was stopped by boiling for 10 min.

The debranched starch suspension was passed through a 45 μm syringe filter (DISMIC-13CP, Advantec, Tokyo, Japan) and analyzed using a high-

performance anion-exchange chromatography (HPAEC) system. The HPAEC system was equipped with a pulsed amperometric detector (PAD; ED40 electrochemical detector, Dionex, Sunnyvale, CA, USA) and a Carbo-pak PA1 anion-exchange column (4x250 mm, Dionex). The debranched sample was eluted at a rate of 1 mL/min using 600 mM sodium acetate in 150 mM NaOH with gradients as follows: 0-20 % for 0-5 min, 20-45 % for 6-30 min, 45-55 % for 31-60 min, 56-60 for 61-80 min, 61-65 % for 81-90 min, 66-80 % for 91-95 min, and 81-100 % for 96-100 min.

2.4. Determination of x-ray diffraction patterns and relative crystallinity

X-ray diffraction pattern was analyzed using a powder X-ray diffractometer (Model New D8 Advance, Bruker, Karlsruhe, Germany) at 40 kV and 40 mA. The sample was measured through 2θ range from 3° to 33° with a 0.02° step size and a count time of 2 sec. The relative crystallinity was calculated according to the equation (Eq 1) of Nara and Komiya (1983). The area for crystallinity was determined with the software developed by the instrument manufacturer (EVA, 2.0).

$$\text{Relative crystallinity (\%)} = \frac{A_c}{A_a + A_c} \times 100 \qquad \text{(Eq 1)}$$

A_a: area of amorphous region, A_c: area of crystalline region

2.5. Measurement of thermal transition properties

Thermal transition properties were measured using a differential scanning calorimeter (Diamond DSC, Perkin-Elmer, Waltham, MA, USA). A starch sample (10 mg) was put into a stainless steel pan (03190029, Perkin-Elmer, Waltham, MA, USA) with 40 μL of distilled water or glucose solution (0.5 or 1%). The pan was sealed and kept at room temperature overnight for moisture equilibrium. An empty stainless steel pan was used as a reference. The sample was heated from 10°C to 180°C at a rate of 10°C/min. The onset temperature (T_o), the peak temperature (T_p), the conclusion temperature (T_c), and the melting enthalpy (ΔH) were obtained.

2.6. Assessment of diffused glucose through dialysis tube

In this study, diffused glucose was defined as the amount of glucose released from dialysis tube.

Glucose was dissolved in distilled water or galactomannan solution (0.5 and 1%) to make 2% glucose solution (w/v). Glucose solution (8 mL) was poured into a dialysis tube. Both ends of the dialysis tube were closed and then immersed into a 100 mL glass vial containing 100 mL of distilled water. The vial was incubated in a shaking incubator at 240 rpm, 37°C. An aliquot of released glucose through the dialysis tube was obtained after 0, 10, 20, 30, 60,

120, 180, 240, 300, and 360 min. The released glucose concentration (mg/mL) was determined using a GOD-POD kit.

With the experimental data, K_m (mass transfer coefficient) was determined using Eq 2 (Dhital et al., 2014). A and B are constants determined by radius (R) and length of dialysis tube (L) and volumes of inside (V_i) and outside (V_o) of dialysis tube. t indicates time (min).

$$\% \text{ Glucose diffusion} = A\left[1 - \frac{1}{e^{BK_m t}}\right] \quad \text{(Eq 2)}$$

$$A=(100(V_iV_o))/(V_i(V_i+V_o))$$

$$B=((V_i+V_o)(120{,}000\pi RL)/V_iV_o)$$

2.7. Measurement of rheological properties

Dynamic rheological properties were measured using an oscillatory rheometer (Rheostress 1, Thermo HAAKE, Karlsruhe, Germany), equipped with a cone-plate geometry (gap: 0.052 mm; cone angle: 1°; diameter: 35 mm). Each sample was loaded onto the rheometer plate at 25°C, followed by wiping off the excess material before measurement. Frequency sweep and shear rate sweep were performed.

2.7.1. Steady shear rheological analysis

Dynamic shear rate tests were performed over a shear rate range of 1.0 - 300 s^{-1}. The data was fitted with the power law model to analyze the flow property using the following Eq 3.

$$\sigma = K \times \dot{\gamma}^{n} \quad \text{(Eq 3)}$$

where σ is the shear stress, $\dot{\gamma}$ is the shear rate, K is the consistency index, and n is the flow behavior index.

2.7.2. Dynamic shear rheological analysis

Dynamic frequency sweep tests were carried out from 0.1 to 10 Hz at a constant stress of 1 Pa, which was within the linear viscoelastic region of all samples. Storage modulus (G′), loss modulus (G″) and loss tangent were obtained from frequency sweep.

Frequency dependence was calculated using the following Eq 4 (Tunick, 2010).

$$\log G' = D \log\omega + C \quad \text{(Eq 4)}$$

where ω is frequency and D is the degree of frequency dependence

Loss tangent was calculated by Eq 5.

$$\text{Loss tangent } (\tan\delta) = G''/G' \quad \text{(Eq 5)}$$

2.8. Analysis of *in vitro* digestibility of starch

The degree of hydrolysis was measured at the certain time points (10 and 240 min) following the method of Shin et al. (2007) with modification. Pancreatin (1.5 g) was dispersed in distilled water (18 mL) and stirred for 10 min. The suspension was centrifuged at 1,500 xg for 10 min, and the supernatant (15 mL) was mixed with amyloglucosidase (0.3 mL) and distilled water (2.7 mL). The mixed enzyme solution was incubated in a water bath at 37°C for 10 min.

The starch sample (80 mg) was dispersed in 2 mL of 0.1M sodium acetate buffer (pH 5.2) containing guar gum or locust bean gum and mixed with 3 glass beads. Guar gum or locust bean gum was dissolved in 0.1M sodium acetate buffer (pH 5.2) to make 0.5 and 1% solution. The starch sample (80 mg) was dispersed. The sample dispersion was equilibrated in a shaking incubator (240 rpm) at 37°C for 10 min, and the enzyme solution (2 mL) was added for hydrolysis reaction. The sample was incubated in a shaking incubator (240 rpm) at 37°C for certain times (10 and 240 min). The hydrolysis reaction was stopped by boiling for 10 min. The sample was centrifuged at 10,000xg for 10 min, and the supernatant was used for determining the glucose released from hydrolysis of starch using a GOD-POD kit.

Starch fractions were determined following the method of Shin et al. (2007)

and classified into RDS, SDS, and RS according to the degree of hydrolysis. RDS was the amount of glucose after reaction for 10 min. SDS was the fraction digested between 10 and 240 min. RS was the undigested fraction after 240 min.

2.9. Statistical analysis

All experiments were conducted in triplicate. The data were expressed as mean ± standard deviation. The data were analyzed using analysis of variance (ANOVA). Significance was analyzed by the Duncan's multiple range test of IBM SPSS statistics version 21.0 (IBM, New York, NY, USA) at a significance level of 0.05.

RESULTS AND DISCUSSION

1. Branch chain length distribution

The branch chain length distributions determined by HPAEC-PAD are displayed in Figure 1. Amylopectin chain length was classified into 4 groups depending on degree of polymerization (DP) and is shown as AS control in Table 1; A chain (DP 6-12), B_1 chain (DP 13-24), B_2 chain (DP 25-36) and B_3 chain (DP $\geq$37) (Hanashiro et al., 1996). Branch chain length distribution was largely changed after the AS treatment. AS-treated starch had a less proportion of short chain (A chain), and a larger proportion of long chains (B_1, B_2, B_3 chain) compared with the AS control starch. The portion of A chain decreased by approximately 3 quaters. Both B_2 and B_3 chains increased more than twofold after the AS treatment. The ratio of short chains to long chains was reduced to a quarter of its original value after the AS treatment, while degree of polymerization increased from 17.1 to 24.1.

These results corresponded with the previous report that amylosucrase catalyzes the elongation of non-reducing end of amylopectin chain by attachment of 12 to 18 glucosyl units (Potocki et al., 1999). Also, short chains like A chain and B_1 chain of amylopectin can be easily accessed by amylosucrase, because they are external chains of the cluster structure. (Kim

et al., 2014). Also, these elongated external chains of amylopectin (amylose-like components) could more easily contact with galactomannan upon heating (Rolland et al., 2004; Funami et al., 2005).

Table 1. Branch chain length distributions of AS-treated starch samples

Sample[1)]	Percent distribution (%)				S/L[3)]	DP_n[4)]
	DP[2)] 6-12	DP 13-24	DP 25-36	DP ≥ 37		
Control	36.7±0.3	47.0±0.1	11.4±0.3	4.9±0.2	0.580±0.008	17.1±0.1
AS 5000U	9.9±0.1	48.6±0.4	29.9±0.4	11.6±0.1	0.110±0.001	24.1±0.1

[1)] Control = AS control starch without amylosucrase addition; AS 5000U = Amylosucrase 5,000 U/30 mL-starch suspension;

[2)] DP = degree of polymerization.

[3)] S/L = ratio of short chains (DP 6-12) to long chains (DP ≥ 13).

[4)] Number-based average degree of polymerization

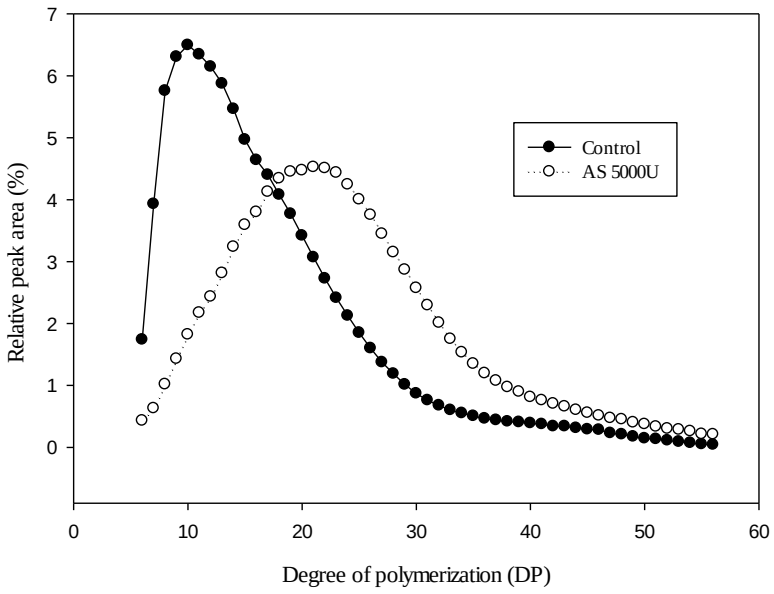

Figure 1. Branch chain length distributions of starch samples. Control = AS control starch without amylosucrase addition; AS 5000U = Amylosucrase 5000 U/30 mL-starch suspension

2. X-ray diffraction patterns and relative crystallinity

The x-ray diffraction patterns of the AS control and AS-treated starch samples are presented in Figure 2. The native waxy corn starch displayed a typical A-type x-ray diffraction patterns with peaks at 15°, 17°, 18°, and 23°. The AS control starch did not show any peaks. These results corresponded with the previous study indicating that AS control starch was mainly composed of amorphous regions. (Shin et al., 2010). After the AS treatment, partially resolved two peak appeared at 22° and 23.9°, while the peaks at 15° and 23° were of relatively low intensity indicating that means x-ray diffraction pattern of starches was altered to the B-type after AS treatment (Kim et al., 2014). According to a previous study, A-type starches have a larger amount of the short chains than those of the B-type starches (Hizukuri, 1985).

The relative crystallinity of samples calculated from the x-ray diffraction patterns is shown in Table 2. The relative crystallinity of native waxy corn starch was greatly reduced in the AS control starch. After the AS treatment, relative crystallinity increased more than threefold compared with the AS control starch. Relative crystallinity of starch can be related to the digestibility (Englyst et al., 1992).

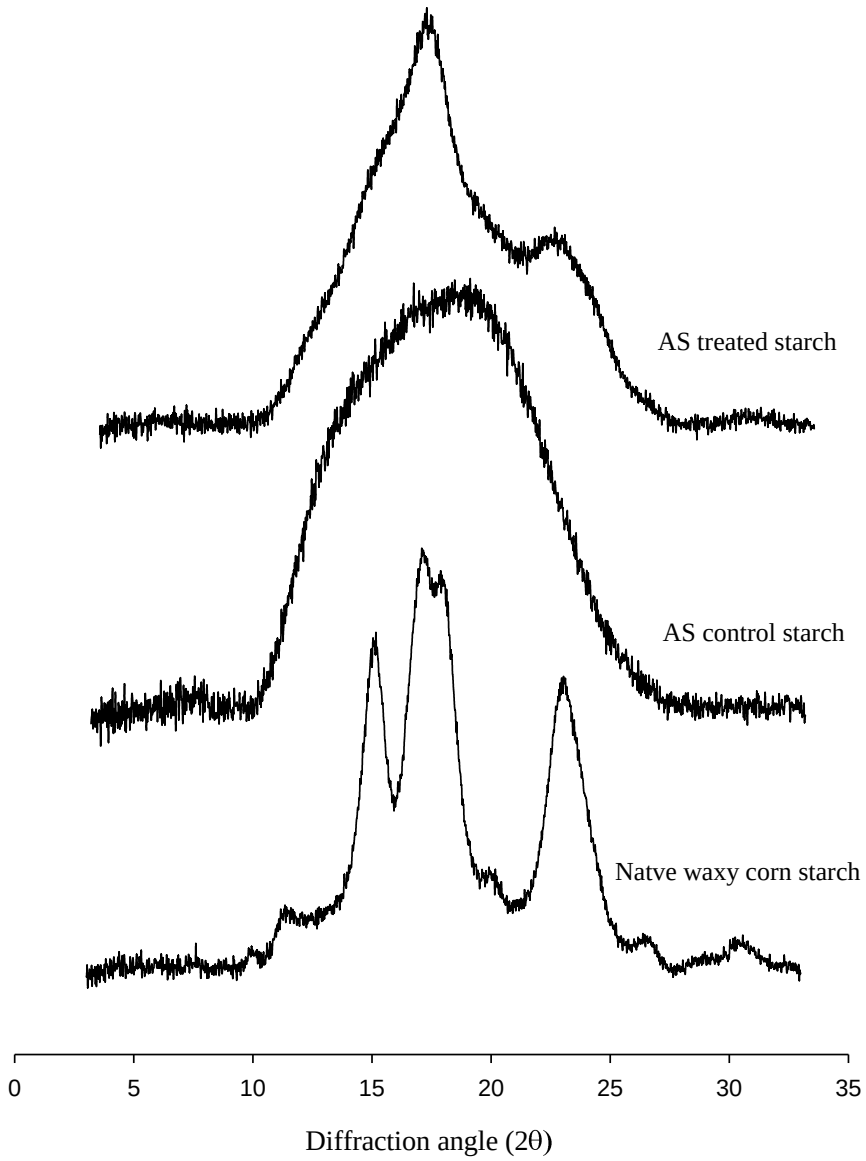

Figure 2. X-ray diffractograms of starches. AS = amylosucrase

Table 2. Relative crystallinity of starch samples

	Crystallinity (%)
Waxy corn[1)]	$42.0^{a2)}$±0.21
AS control	7.7^{c}±0.28
AS 5000U	24.6^{b}±0.14

1)Waxy corn = native waxy corn starch, control = AS control starch without amylosucrase addition; AS 5000U = Amylosucrase 5,000 U/30 mL-starch suspension
2) The values with different superscripts are significantly different ($p<0.05$).

3. Thermal transition properties

The onset (T_o), peak (T_p), and conclusion (T_c) temperatures, and the gelatinization enthalpy (ΔH) determined by the DSC for the samples are depicted in Table 3. Elongated branch chains of amylopectin can show different patterns in pasting properties (Jane et al., 1999). The AS control starches did not show any peaks. After AS treatment, the peaks were observed. It can be attributed to the fact that double helices of elongated chains require a higher temperature to dissociate completely (Yamin et al., 1999). The T_o values were increased in the presence of a galactomannan, regardless of type and concentration. This can be due to a less amount of free water because of the water molecules immobilized by added galactomannan and the interaction between galactomannan and starch (Rojas et al., 1999; Yoshimura et al., 1996). Also, galactomannan has ability to absorb water, leading to a lower heat transfer rate and mass transfer of water (Krüger et al., 2003). These factors were supposed to hinder starch gelatinization (Chaisawang et al., 2005). However, the values had differences among the samples. The T_o values were significantly increased only with guar gum ($p < 0.05$). The highest value was observed for the AS-G1. This could be attributed to the difference in the ability of water absorption depending on the type of gums. According to the previous studies, guar gum has greater water binding capacity than locust bean gum

(Wallingford and Labuza, 1983; Sánchez et al., 1995). Wallingford and Labuza (1983) reported that water binding capacity of guar gum (40.0 mL/g) is more than threefold of that of locust bean gum (11.6 mL/g), which seems to be not enough to significantly ($p < 0.05$) increase T_o values. The galactomannan had little effect on the T_p of AS-treated starch. The similar result has been reported in the previous study (Yoshimura et al., 1999).

A significant change ($p < 0.05$) in ΔH was not observed in AS-LB0.5 and AS-LB1. A significant decrease ($p < 0.05$) in ΔH was only shown in AS-G0.5 and AS-G1 because of greater water absorption ability of guar gum than locust bean gum. In the previous study, ΔH of corn starch decreased to a greater extent in the presence of guar gum compared with locust bean gum (Šubarić et al., 2011). Water absorption reduces free water for starch causing partial gelatinization of crystalline regions in the starch (Biliaderis et al., 1980; Huang, 2009).

Table 3. Thermal transition properties of samples

Sample[1)]	T_o[2)] (°C)	T_p (°C)	T_c (°C)	ΔH (J/g)
C-0				
C-G0.5				
C-G1		Not detected		
C-LB0.5				
C-LB1				
AS-0	60.62^{b}[3)] ±2.17	77.51^{a}±0.41	89.34^{a}±6.76	13.67^{a}±1.23
AS-G0.5	65.55^{a}±2.18	77.26^{a}±0.14	92.72^{a}±3.13	10.53^{b}±1.03
AS-G1	64.45^{a}±1.60	75.84^{b}±0.49	89.21^{a}±5.19	2.79^{c}±0.73
AS-LB0.5	63.40^{ab}±0.60	76.16^{b}±0.35	91.66^{a}±0.81	13.74^{a}±2.92
AS-LB1	63.36^{ab}±0.13	75.77^{b}±0.32	91.18^{a}±0.71	12.93^{ab}±0.42

[1)] AS = amylosucrase, C-0 = AS control starch without galactomannan, C-G0.5 = AS control starch with 0.5% guar gum, C-G1 = AS control starch with 1% guar gum, C-LB0.5 = AS control starch with 0.5% locust bean gum, C-LB1 = AS control starch with 1% locust bean gum, AS-0 = AS starch without galactomannan, AS-G0.5 = AS starch with 0.5% guar gum, AS-G1 = AS starch with 1% guar gum, AS-LB0.5 = AS starch with 0.5% locust bean gum, AS-LB1 = AS starch with 1% locust bean gum

[2)] The onset T_o = onset temperature, T_p = peak temperature, T_c = conclusion temperatures, ΔH = gelatinization enthalpy

[3)] The values with different superscripts in the same column are significantly different (p<0.05).

4. Glucose diffusion

Glucose liberated from dialysis tube is presented in Figure 3. The amount of diffused glucose concentration increased with time and approximately approached a plateau after 240 min. The amount of diffused glucose at 360 min indicates galactomannan's ability of glucose adsorption (Ou et al., 2001). The amount of adsorbed glucose increased with galactomannan concentration, and in addition, it showed a considerable increase when guar gum was added (Fabek et al., 2014). It can be attributed to a relatively large molecular weight of guar gum containing relatively high proportion of galactose side chain compared to locust bean gum. The molecular weight of hydrocolloid is related to glucose adsorption capacity. The hyrocolloid with high molecular weight could decrease available glucose concentration to a greater extent than hydrocolloid with low molecular weight (Zhang et al., 2016). The galactose branch seemed to have ability to catch glucose. K_m increased with increasing galactomannan concentration and decreased to a greater extent in the presence of guar gum compared with locust bean gum.

Glucose diffusion was relatively lower when guar gum was added at the same concentration. This can be explained that guar gum had a greater ability to resist to the glucose release through dialysis tube (Fabek et al., 2014). The difference in glucose release between guar and locust bean gum at the same

concentration became smaller over time. In other words, the resistance of guar gum against glucose release was higher at the initial stage.

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Table 4. Diffused glucose concentration with galactomannan

mg/mL	0[1)]	G0.5	G1	LB0.5	LB1
10	0.54^{a}[2)]±0.02	0.28^{bc}±0.01	0.23^{c}±0.01	0.32^{b}±0.05	0.24^{c}±0.03
20	0.89^{a}±0.03	0.46^{c}±0.01	0.35^{e}±0.02	0.58^{b}±0.03	0.40^{d}±0.02
30	1.11^{a}±0.03	0.65^{c}±0.04	0.45^{e}±0.03	0.76^{b}±0.03	0.52^{d}±0.02
60	1.44^{a}±0.03	0.95^{c}±0.03	0.67^{e}±0.03	1.12^{b}±0.02	0.76^{d}±0.05
120	1.57^{a}±0.03	1.18^{c}±0.02	0.87^{e}±0.01	1.31^{b}±0.03	1.00^{d}±0.03
180	1.57^{a}±0.00	1.26^{c}±0.02	0.93^{e}±0.02	1.36^{b}±0.06	1.05^{d}±0.06
240	1.57^{a}±0.01	1.26^{c}±0.04	0.97^{e}±0.02	1.36^{b}±0.04	1.05^{d}±0.05
300	1.56^{a}±0.01	1.25^{c}±0.04	0.99^{e}±0.02	1.34^{b}±0.04	1.05^{d}±0.03
360	1.58^{a}±0.02	1.28^{c}±0.00	1.01^{d}±0.01	1.34^{b}±0.06	1.05^{d}±0.02
Km	**2.32×10^{-7}**	**3.54×10^{-8}**	**1.82×10^{-8}**	**3.97×10^{-8}**	**2.38×10^{-8}**

[1)] 0 = glucose without galactomannan solution, G0.5 = glucose with 0.5% guar gum solution, G1 = glucose with 1% guar gum solution, LB0.5 = glucose with 0.5% locust bean gum solution, G1 = glucose with 1% locust bean gum solution

[2)] The values with different superscripts in the same column are significantly different (p<0.05).

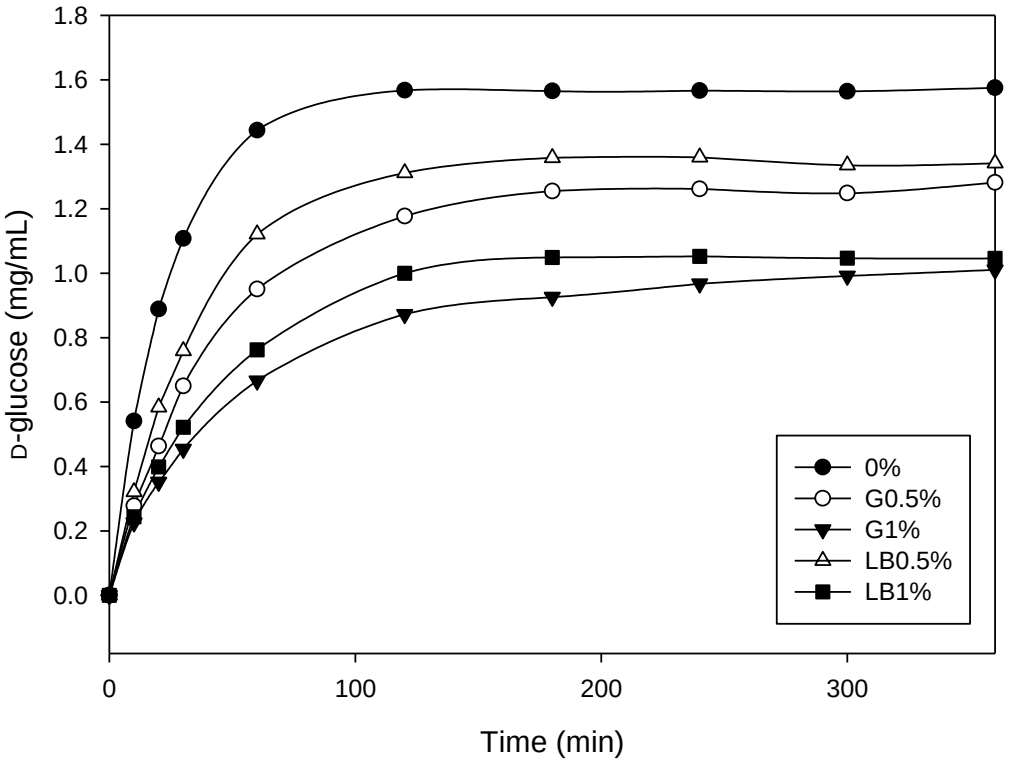

Figure 3. Glucose diffusion with/without galactomannan

0 = glucose without galactomannan solution, G0.5 = glucose with 0.5% guar gum solution, G1 = glucose with 1% guar gum solution, LB0.5 = glucose with 0.5% locust bean gum solution, G1 = glucose with 1% locust bean gum solution

5. Rheological properties

5.1. Steady shear rheological analysis

Apparent viscosity measured using shear rate sweep is represented in Figure 4. Apparent viscosity was a little higher when adding locust bean gum than guar gum. These data were fitted to the power model (Eq 5), and N (flow behavior index) and k (consistency index) determined by the power law equation are shown in Table 5 and Table 6. All samples had shear-thinning behavior with values of flow behavior indexes below 1. The flow behavior indexes of AS control starches were in the range of 0.56-0.63, while those of AS-treated starches were 0.22-0.30. AS-treated starches exhibited more pseudoplastic behavior compared with AS control starches. Starches mixed with guar gum had lower N values than those mixed with locust bean gum. Namely, starches mixed with guar gum were more pseudoplastic than those mixed with locust bean gum (Sajjan and Rao, 1987). It can be attributed to more extended conformation of guar gum than locust bean gum. More galactose branches of guar gum prevent forming intramolecular hydrogen bonding, and, thereby, guar gum can be of extended form (Yoo et al., 2005). The extended guar gum can easily interact with amylose-like structure of starch by hydrogen bonding (Dakia and Paquot, 2010; Kim and Yoo, 2011). Locust bean gum has a relatively low proportion of galactose branch, and thus

it can form many hydrogen bonding within the molecule than guar gum can. Locust bean gum has many coiled structure and has less hydroxyl groups which can interact with starch. Therefore, locust bean gum undergoes relative less interaction with starch compared with guar gum. These results are in agreement with other studies about mixing acorn starch with galactomannan (Kim and Yoo, 2011) and mixing water chestnut starch with galactomannan (Lee and Yoon, 2015). A concentration below 0.5% of guar gum seems to form composite network with less number of junction zones between galactomannan and starch. Also, the concentration of galactomannan higher than 1% induces phase separation when mixed with starch. (Kulicke et al., 1996, Alloncle and Doublier, 1991).

Shear rates below 10 s^{-1} can be representative of the properties during swallowing by human, so viscoelasticity of the samples at the low shear rate can be applicable to the assessment of mouthfeel (Yaseen et al., 2005).

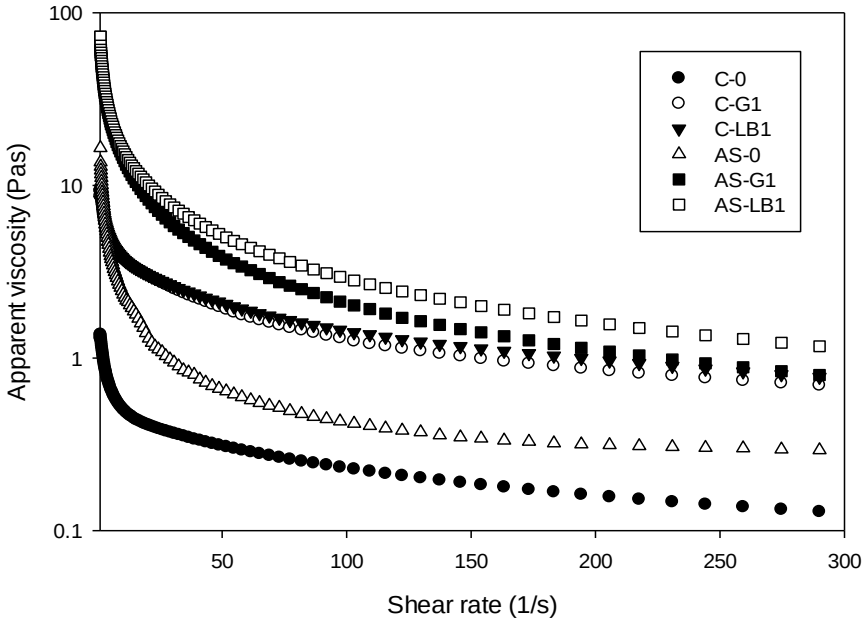

Figure 4. Apparent viscosity measured using shear rate sweep

AS = amylosucrase, C-0 = AS control starch without galactomannan, C-G1 = AS control starch with 1% guar gum, C-LB1 = AS control starch with 1% locust bean gum, AS-0 = AS starch without gum, AS-G1 = AS starch with 1% guar gum, AS-LB1 = AS starch with 1% locust bean gum

Table 5. Constant value determined from power law model of AS control starches mixed with galactomannan

	C-0[1)]	C-G0.5	C-G1	C-LB0.5	C-LB1
N	0.6274	0.5919	0.5654	0.6055	0.5988
k	1.25	4.67	9.86	5.29	9.27

[1)] C-0 = AS control starch without galactomannan, C-G0.5 = AS control starch with 0.5% guar gum, C-G1 = AS control starch with 1% guar gum, C-LB0.5 = AS control starch with 0.5% locust bean gum, C-LB1 = AS control starch with 1% locust bean gum

Table 6. Constant value determined from power law model of AS-treated starches mixed with galactomannan

	AS-0[1)]	AS-G0.5	AS-G1	AS-LB0.5	AS-LB1
N	0.2692	0.2428	0.2205	0.3013	0.3071
k	13.04	40.45	78.47	44.46	72.61

[1)] AS = amylosucrase, AS-0 = AS starch without gum, AS-G0.5 = AS starch with 0.5% guar gum, AS-G1 = AS starch with 1% guar gum, AS-LB0.5 = AS starch with 0.5% locust bean gum, AS-LB1 = AS starch with 1% locust bean gum

5.2. Dynamic shear rheological analysis

Storage modulus (G′) and loss tangent were measured using frequency sweep. The G′ values of AS control and AS-treated starches are shown in Figure 5 and Figure 6, respectively. G′ increased with the addition of galactomannan. Also, G′ increased with increasing galactomannan concentration. All AS-treated starches had higher G′ than AS control starches, indicating increased gel strength after AS treatment (Shin et al., 2010). This results can be attributed that elongated branch chains of amylopectin behave like amylose (Rolland et al., 2004). The results of storage modulus can be related to the results of *in vitro* digestibility which indicated AS-treated starches had lower digestibility than AS control starches in section 6. When the same amount of galactomannan was added, the differences in G′ between guar gum and locust bean gum were bigger in AS-treated starches compared with those in the AS control starches. These results indicate that the pattern of interaction between galactomannan and the AS control and that between galactomannan and AS-treated starches was different. It can be attributed to the difference in chain length distribution between AS control and AS-treated starches. The AS control starch had more short chains which were difficult to interact with galactomannan. The proportion of DP 6-12 in the AS control was threefold over the AS-treated starch. After AS treatment, elongated branch

chains of the AS-treated starch could interact with galactomannan. AS-G0.5 and AS-G1 showed higher storage modulus values than AS-LB0.5 and AS-LB1, because guar gum can have more interaction with starch because of its extended form as mentioned in section 5.1.

Frequency dependence was calculated and presents in Table 7 and Table 8.

The degree of frequency dependence (D) was higher in the AS control starches than in the AS-treated starches. It indicated that the AS-treated starches formed more stable gels with galactomannan than the AS control starches (Rosalina and Bhattacharya, 2002). Also, the value of D in the AS-treated starches was smaller with guar gum than with locust bean gum, suggesting the formation of more stable gels with guar gum. These results were also consistent with digestibility patterns which revealed that the mixture with guar gum led to a greater decrease compared with the one with locust bean gum. Also, D values decreased with galactomannans added to the AS control starches. It means that adding galactomannan facilitated the making of a stable gel with AS control starches. On the other hand, when galactomannans were added to the AS-treated starches, D values increased, implying that the gels of AS-treated starches became weaker with the addition of galactomannans. However, the AS-treated starches showed D values lower than AS control starches even after galactomannans were added.

Loss tangent was calculated and shown in Figure 7. Loss tangent is a good indicator of the solid or liquid like behaviors of samples (Achayuthakan and Suphantharika, 2008). Loss tangent of AS control starch without added galactomannan ranged from 1.0 to 1.7. The loss tangent of AS control starch decreased with galactomannan concentration. It indicates that AS control starch displayed more elastic behavior after galactomannans were added. The loss tangent values of the AS-treated starch without galactomannan were below 0.2. In contrast to the AS control starch, the loss tangent of AS-treated starch increased with galactomannan concentration, implying that the addition of galactomannans induced more viscous behavior of the AS-treated starch. The loss tangent values of the AS-treated starch were higher in AS-LB0.5 and AS-LB1 than in AS-G0.5 and AS-G1, respectively. It can be explained by the incompatible network structure established between the AS-treated starch and locust bean gum. Meanwhile, as stated in an earlier section, guar gum had more interaction with AS-treated starch, and thus more compatible network was formed between the AS-treated starch and guar gum.

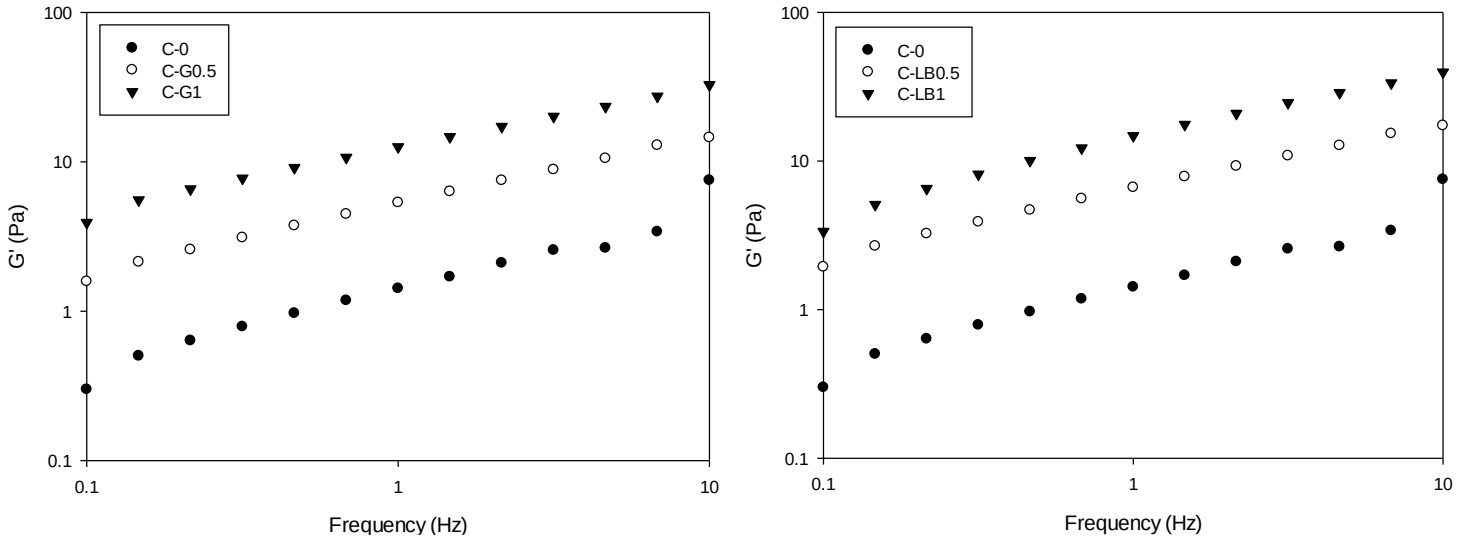

Figure 5. Storage modulus (G′) determined by frequency sweep of the AS control starch mixed with galactomannan

C-0 = AS control starch without galactomannan, C-G0.5 = AS control starch with 0.5% guar gum, C-G1 = AS control starch with 1% guar gum, C-LB0.5 = AS control starch with 0.5% locust bean gum, C-LB1 = AS control starch with 1% locust bean gum

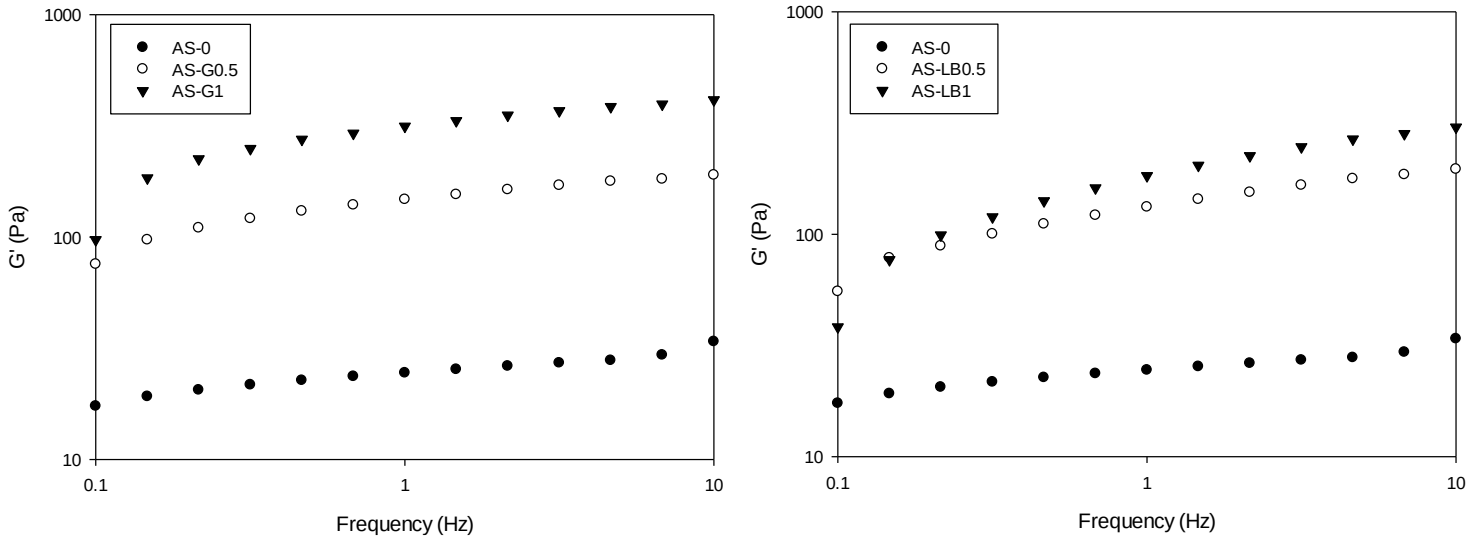

Figure 6. Storage modulus (G′) measured by frequency sweep of AS-treated starch mixed with galactomannan

AS-0 = AS starch without galactomannan, AS-G0.5 = AS starch with 0.5% guar gum, AS-G1 = AS starch with 1% guar gum, AS-LB0.5 = AS starch with 0.5% locust bean gum, AS-LB1 = AS starch with 1% locust bean gum

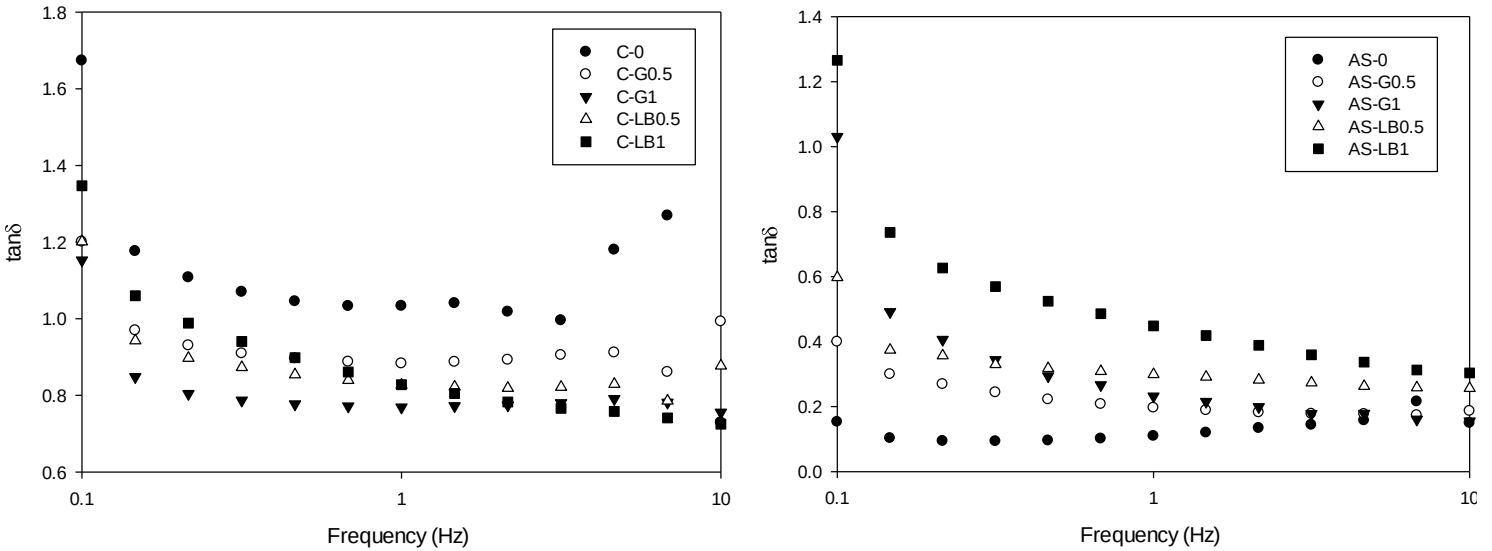

Figure 7. Tanδ (G″/G′) determined by frequency sweep of starch mixed with galactomannan

C-0 = AS control starch without galactomannan, C-G0.5 = AS control starch with 0.5% guar gum, C-G1 = AS control starch with 1% guar gum, C-LB0.5 = AS control starch with 0.5% locust bean gum, C-LB1 = AS control starch with 1% locust bean gum, AS-0 = AS starch without gum, AS-G0.5 = AS starch with 0.5% guar gum, AS-G1 = AS starch with 1% guar gum, AS-LB0.5 = AS starch with 0.5% locust bean gum, AS-LB1 = AS starch with 1% locust bean gum

Table 7. Frequency dependence (D) of AS control starches mixed with galactomannan

	C-0[1)]	C-G0.5	C-G1	C-LB0.5	C-LB1
D	0.5741	0.4701	0.4332	0.4595	0.5058

[1)] C-0 = AS control starch without galactomannan, C-G0.5 = AS control starch with 0.5% guar gum, C-G1 = AS control starch with 1% guar gum, C-LB0.5 = AS control starch with 0.5% locust bean gum, C-LB1 = AS control starch with 1% locust bean gum.

Table 8. Frequency dependence (D) of AS-treated starches mixed with galactomannan

	AS-0[1)]	AS-G0.5	AS-G1	AS-LB0.5	AS-LB1
D	0.1207	0.175	0.2358	0.2439	0.3108

[1)] AS-0 = AS starch without gum, AS-G0.5 = AS starch with 0.5% guar gum, AS-G1 = AS starch with 1% guar gum, AS-LB0.5 = AS starch with 0.5% locust bean gum, AS-LB1 = AS starch with 1% locust bean gum

6. *In vitro* digestibility

Starch fractions of AS control and AS-treated starch are shown in Table 9 and Table 10, respectively. After AS treatment, low digestible starch increased remarkably (Kim et al., 2014). It is related to the results of relative crystallinity in section 2. Relative crystallinity of starches increased more than triplet after AS treatment which can lower digestibility. RDS of AS-treated starch reduced by 13 to 26% compared with RDS of AS control starch. Elongated branch chains of amylopectin by AS can make helices inhibiting digestive enzyme access (Kim et al., 2014). RDS was reduced to a greater extent in AS-treated starch compared with AS control starch when galactomannan was added. RDS in C-G0.5 and C-LB0.5 decreased by 10% compared to C-0. In the case of RDS in AS-G0.5 and AS-G1, it was reduced by 17% compared as AS-0. These results indicate that galactomannans have more contact with the elongated chains of amylopectin of AS-treated starch. When the amount of added guar gum or locust bean gum was doubled, RDS was reduced by 10 and 5%, respectively compared with that of the AS control starches. Also, an increased addition of galactomannan caused RDS to decrease drastically after AS treatment. When the amount of added guar gum or locust bean gum was doubled to the AS-treated starches, RDS was reduced by 33% and 29%, respectively. It can be attributed to the more contact space of elongated

amylopectin chains with galactomannan after AS treatment. Also, RDS of AS-treated starches was always relatively lower when guar gum was added than locust bean gum. It can be explained by the results of rheological analyses. In the steady shear rheological test, starches mixed with guar gum showed greater shear thinning, compared to the starches mixed with locust bean gum suggesting that guar gum can have more interaction with starches. It can be attributed to more extended form of guar gum, because of its more galactose branches. Galactose in the guar gum hindered intramolecular interaction through hydrogen bonding. In other words, locust bean gum has fewer galactose branches allowing intramolecular hydrogen bonding and the consequent forming of a coil. Hydroxyl groups to interact with starch can be removed because of coiled form in locust bean gum, and thus locust bean gum seems to interact with starch relative weakly, compared to guar gum (Lee and Yoon, 2015).

The extent of increase in RS was higher when adding guar to the control than to the AS-treated starches. The content of RS increased 10 to 13% in the AS control starches when galactomannan was added. In the AS-treated starches, RS increased only up to 9% with the addition of galactomannan. It can be explained that glucose adsorption of galactomannan was more efficient in the AS control starches than in AS-treated starches. The reason can be that

galactomannan could contact to a lesser extent with AS control starch because of its short branch chains compared to the AS-treated starch. In the result, galactomannan can adsorb more glucose in AS control starch. After 240 min of enzymatic reaction, some of starch was hydrolyzed into glucose. Some of galactomannans could adsorb glucose, and adsorbed glucose by galactomannan was not detected by GOD-POD assay. This *in vitro* circumstance was similar to that in the intestine. Thus, digestibility would be relatively lower when the starches mixed with galactomannan are consumed.

Table 9. Contents of RDS, SDS, and RS of AS control starch

Sample[1)]	RDS[2)] (%)	SDS (%)	RS (%)
C-0	$89.69^{a3)}$±1.71	3.75^{c}±1.42	6.56^{c}±1.17
C-G0.5	79.10^{b}±2.51	4.55^{bc}±3.31	16.34^{a}±0.88
C-G1	69.10^{d}±3.85	11.25^{a}±6.16	19.64^{a}±3.68
C-LB0.5	79.02^{b}±1.72	10.79^{ab}±2.62	10.19^{b}±0.95
C-LB1	74.53^{c}±0.84	8.56^{abc}±0.78	16.92^{a}±0.35

[1)] C-0 = AS control starch without galactomannan, C-G0.5 = AS control starch with 0.5% guar gum, C-G1 = AS control starch with 1% guar gum, C-LB0.5 = AS control starch with 0.5% locust bean gum, C-LB1 = AS control starch with 1% locust bean gum

[2)] RDS = rapidly digestible starch, SDS = slowly digestible starch, RS = resistance starch

[3)] The values with different superscripts in the same column are significantly different ($p<0.05$).

Table 10. Contents of RDS, SDS, and RS of AS-treated starch

Sample[1)]	RDS[2)] (%)	SDS (%)	RS (%)
AS-0	76.38[a3)]±1.47	3.81[d]±1.01	19.81[c]±0.91
AS-G0.5	59.46[b]±2.65	21.34[b]±1.98	19.20[c]±0.69
AS-G1	43.33[d]±1.99	28.41[a]±1.18	28.26[a]±0.90
AS-LB0.5	59.40[b]±0.79	16.65[c]±1.06	23.95[b]±1.76
AS-LB1	47.41[c]±1.20	27.72[a]±1.18	24.87[b]±0.10

[1)] AS-0 = AS starch without galactomannan, AS-G0.5 = AS starch with 0.5% guar gum, AS-G1 = AS starch with 1% guar gum, AS-LB0.5 = AS starch with 0.5% locust bean gum, AS-LB1 = AS starch with 1% locust bean gum

[2)] RDS = rapidly digestible starch, SDS = slowly digestible starch, RS = resistance starch

[3)] The values with different superscripts in the same column are significantly different ($p<0.05$).

CONCLUSION

The present study clearly demonstrated that galactomannans can lower starch digestibility, especially guar gum mixed with AS-treated starches. Guar gum was better for resisting glucose release than locust bean gum and adsorbed more glucose than locust bean gum. Rheology tests showed that AS-treated starch, possessing elongated branched chains of amylopectin through AS modification, formed more stable gel when mixed with guar gum than with locust bean gum. More galactose branches, causing guar gum to be rather extended form, easily led to the interaction with elongated amylopectin chains. In the results, when adding galactomannan to the AS–treated starches, reduction range of digestibility was relative high because of elongated branch chains of AS-treated starches.

In this study, the effect of galactomannan on *in vitro* starch digestibility was investigated. Therefore, further study such as *in vivo* digestibility is required to completely figure out the changes in blood glucose concentration after consuming starch mixed with galactomannan.

References

Achayuthakan, P., & Suphantharika, M. (2008). Pasting and rheological properties of waxy corn starch as affected by guar gum and xanthan gum. *Carbohydrate Polymers, 71*, 9-17.

Ahmad, F. B., & Williams, P. A. (2001). Effect of galactomannans on the thermal and rheological properties of sago starch. *Journal of Agricultural and Food chemistry, 49*, 1578-1586.

Alloncle, M., & Doublier, J. L. (1991). Viscoelastic properties of maize starch/hydrocolloid pastes and gels. *Food Hydrocolloids, 5*, 455-467.

Annable, P., Fitton, M. G., Harris, B., Phillips, G. O., & Williams, P. A. (1994). Phase behaviour and rheology of mixed polymer systems containing starch. *Food Hydrocolloids, 8*, 351-359.

BeMiller, J. N. (2011). Pasting, paste, and gel properties of starch–hydrocolloid combinations. *Carbohydrate Polymers, 86*, 386-423.

Biliaderis, C. G., Maurice, T. J., & Vose, J. R. (1980). Starch gelatinization phenomena studied by differential scanning calorimetry. *Journal of Food Science, 45*, 1669-1674.

Brennan, C. S., Blake, D. E., Ellis, P. R., & Schofield, J. D. (1996). Effects of guar galactomannan on wheat bread microstructure and on the in

vitro and in vivo digestibility of starch in bread. *Journal of Cereal Science, 24*, 151-160.

Bordoloi, A., Singh, J., & Kaur, L. (2012). In vitro digestibility of starch in cooked potatoes as affected by guar gum: Microstructural and rheological characteristics. *Food Chemistry, 133*, 1206-1213.

Chaisawang, M., & Suphantharika, M. (2005). Effects of guar gum and xanthan gum additions on physical and rheological properties of cationic tapioca starch. *Carbohydrate Polymers, 61*, 288-295.

Dakia, P. A., Wathelet, B., & Paquot, M. (2010). Influence de la teneur en galactose sur les interactions moléculaires et sur les propriétés physico-chimiques des galactomannanes en solution. *Biotechnologie, Agronomie, Société et Environnement, 14*, 213-223.

De Montalk, G. P., Remaud-Simeon, M., Willemot, R. M., Planchot, V., & Monsan, P. (1999). Sequence analysis of the gene encoding amylosucrase from Neisseria polysaccharea and characterization of the recombinant enzyme. *Journal of Bacteriology, 181*, 375-381.

Dhital, S., Dolan, G., Stokes, J. R., & Gidley, M. J. (2014). Enzymatic hydrolysis of starch in the presence of cereal soluble fibre polysaccharides. *Food & Function, 5*, 579-586.

Englyst, H. N., Kingman, S., & Cummings, J. (1992). Classification and

measurement of nutritionally important starch fractions. *European Journal of Clinical Nutrition, 46*, S33-50.

Fabek, H., Messerschmidt, S., Brulport, V., & Goff, H. D. (2014). The effect of in vitro digestive processes on the viscosity of dietary fibres and their influence on glucose diffusion. *Food Hydrocolloids, 35*, 718-726.

Funami, T., Kataoka, Y., Omoto, T., Goto, Y., Asai, I., & Nishinari, K. (2005). Effects of non-ionic polysaccharides on the gelatinization and retrogradation behavior of wheat starch. *Food Hydrocolloids, 19*, 1-13.

Guarda, A., Rosell, C. M., Benedito, C., & Galotto, M. J. (2004). Different hydrocolloids as bread improvers and antistaling agents. *Food Hydrocolloids, 18*, 241-247.

Gularte, M. A., & Rosell, C. M. (2011). Physicochemical properties and enzymatic hydrolysis of different starches in the presence of hydrocolloids. *Carbohydrate Polymers, 85*, 237-244.

Hanashiro, I., Abe, J.-i., & Hizukuri, S. (1996). A periodic distribution of the chain length of amylopectin as revealed by high-performance anion-exchange chromatography. *Carbohydrate Research, 283*, 151-159.

Hardacre, A. K., Yap, S. Y., Lentle, R. G., & Monro, J. A. (2015). The effect

of fibre and gelatinised starch type on amylolysis and apparent viscosity during in vitro digestion at a physiological shear rate. *Carbohydrate Polymers, 123*, 80-88.

He, H., Zhang, Y., Hong, Y., & Gu, Z. (2015). Effects of hydrocolloids on corn starch retrogradation. *Starch-Stärke, 67*, 348-354.

Hizukuri, S. (1985). Relationship between the distribution of the chain length of amylopectin and the crystalline structure of starch granules. *Carbohydrate research, 141*, 295-306.

Huang, C. C. (2009). Physicochemical, pasting and thermal properties of tuber starches as modified by guar gum and locust bean gum. *International Journal of Food Science & Technology, 44*, 50-57.

Jane, J., Chen, Y. Y., Lee, L. F., McPherson, A. E., Wong, K. S., Radosavljevic, M., & Kasemsuwan, T. (1999). Effects of amylopectin branch chain length and amylose content on the gelatinization and pasting properties of starch 1. *Cereal Chemistry, 76*, 629-637

Jenkins, D. J., Kendall, C. W., Axelsen, M., Augustin, L. S., & Vuksan, V. (2000). Viscous and nonviscous fibres, nonabsorbable and low glycaemic index carbohydrates, blood lipids and coronary heart disease. *Current Opinion in Lipidology, 11*, 49-56.

Jenkins, D. J., Wolever, T. M., Leeds, A. R., Gassull, M. A., Haisman, P.,

Dilawari, J., ... & Alberti, K. G. (1978). Dietary fibres, fibre analogues, and glucose tolerance: importance of viscosity. *British Medical Journal*, *1*, 1392-1394.

Jung, J. H., Seo, D. H., Ha, S. J., Song, M. C., Cha, J., Yoo, S. H., ... & Park, C. S. (2009). Enzymatic synthesis of salicin glycosides through transglycosylation catalyzed by amylosucrases from Deinococcus geothermalis and Neisseria polysaccharea. *Carbohydrate Research*, *344*, 1612-1619.

Kim, B. K., Kim, H. I., Moon, T. W., & Choi, S. J. (2014). Branch chain elongation by amylosucrase: Production of waxy corn starch with a slow digestion property. *Food Chemistry*, *152*, 113-120.

Kim, W. W., & Yoo, B. (2011). Rheological and thermal effects of galactomannan addition to acorn starch paste. *LWT-Food Science and Technology*, *44*, 759-764.

Krüger, A., Ferrero, C., & Zaritzky, N. E. (2003). Modelling corn starch swelling in batch systems: effect of sucrose and hydrocolloids. *Journal of Food Engineering*, *58*, 125-133.

Kulicke, W. M., Eidam, D., Kath, F., Kix, M., & Kull, A. H. (1996). Hydrocolloids and rheology: regulation of visco-elastic characteristics of waxy rice Starch in mixtures with galactomannans.

Starch-Stärke, 48, 105-114.

Leeman, A. M., Karlsson, M. E., Eliasson, A. C., & Björck, I. M. (2006). Resistant starch formation in temperature treated potato starches varying in amylose/amylopectin ratio. *Carbohydrate Polymers, 65*, 306-313.

Lee, M. H., Baek, M. H., Cha, D. S., Park, H. J., & Lim, S. T. (2002). Freeze–thaw stabilization of sweet potato starch gel by polysaccharide gums. *Food Hydrocolloids, 16*, 345-352.

Lee, Y., & Chang, Y. H. (2015). Effects of galactomannan addition on rheological, pasting and physical properties of water chestnut starch. *Journal of Texture Studies, 46*, 58-66.

Lee, Y., & Chang, Y. H. (2016). Influence of guar gum addition on physicochemical, microbial, rheological and sensory properties of stirred yoghurt. *International Journal of Dairy Technology*.

Liu, H., Eskin, N. M., & Cui, S. W. (2003). Interaction of wheat and rice starches with yellow mustard mucilage. *Food Hydrocolloids, 17*, 863-869.

Meyer, D., Bayarri, S., Tárrega, A., & Costell, E. (2011). Inulin as texture modifier in dairy products. *Food Hydrocolloids, 25*, 1881-1890.

Miller, G. L. (1959). Use of dinitrosalicylic acid Reagent for determination

of reducing sugar. *Analytical Chemistry, 31*, 426-428.

Nara, S., & Komiya, T. (1983). Studies on the relationship between water-satured state and crystallinity by the diffraction method for moistened potato starch. *Starch-Stärke, 35*, 407-410.

Ou, S., Kwok, K. C., Li, Y., & Fu, L. (2001). In vitro study of possible role of dietary fiber in lowering postprandial serum glucose. *Journal of Agricultural and Food Chemistry, 49*, 1026-1029.

Potocki de Montalk, G., Remaud-Simeon, M., Willemot, R. M., Sarçabal, P., Planchot, V., & Monsan, P. (2000). Amylosucrase from Neisseria polysaccharea: novel catalytic properties. *FEBS letters, 471*, 219-223.

Rojas, J. A., Rosell, C. M., & De Barber, C. B. (1999). Pasting properties of different wheat flour-hydrocolloid systems. *Food Hydrocolloids, 13*, 27-33.

Rolland-Sabaté, A., Colonna, P., Potocki-Veronese, G., Monsan, P., & Planchot, V. (2004). Elongation and insolubilisation of α-glucans by the action of Neisseria polysaccharea amylosucrase. *Journal of Cereal Science, 40*, 17-30.

Rosalina, I., & Bhattacharya, M. (2002). Dynamic rheological measurements and analysis of starch gels. *Carbohydrate Polymers, 48*, 191-202.

Sajjan, S. U., & Rao, M. R. (1987). Effect of hydrocolloids on the

rheological properties of wheat starch. *Carbohydrate Polymers, 7*, 395-402.

Sasaki, T., & Kohyama, K. (2012). Influence of non-starch polysaccharides on the in vitro digestibility and viscosity of starch suspensions. *Food Chemistry, 133*, 1420-1426.

Sánchez, V. E., Bartholomai, G. B., & Pilosof, A. M. R. (1995). Rheological properties of food gums as related to their water binding capacity and to soy protein interaction. *LWT-Food Science and Technology, 28*, 380-385.

Shin, H. J., Choi, S. J., Park, C. S., & Moon, T. W. (2010). Preparation of starches with low glycaemic response using amylosucrase and their physicochemical properties. *Carbohydrate Polymers*, *82*, 489-497.

Shin, S. I., Lee, C. J., Kim, D. I., Lee, H. A., Cheong, J. J., Chung, K. M., ... & Moon, T. W. (2007). Formation, characterization, and glucose response in mice to rice starch with low digestibility produced by citric acid treatment. *Journal of Cereal Science, 45*, 24-33.

Slaughter, S. L., Ellis, P. R., Jackson, E. C., & Butterworth, P. J. (2002). The effect of guar galactomannan and water availability during hydrothermal processing on the hydrolysis of starch catalysed by pancreatic α-amylase. *Biochimica et Biophysica Acta (BBA)-General*

Subjects, 1571, 55-63.

Šubarić, D., Babić, J., Ačkar, Đ., Piližota, V., Kopjar, M., Ljubas, I., & Ivanovska, S. (2011). Effect of galactomannan hydrocolloids on gelatinization and retrogradation of tapioca and corn starch. *Croatian Journal of Food Science and Technology, 3*, 26-31.

Sudhakar, V., Singhal, R. S., & Kulkarni, P. R. (1996). Starch-galactomannan interactions: functionality and rheological aspects. *Food Chemistry, 55*, 259-264.

Tunick, M. H. (2010). Small-strain dynamic rheology of food protein networks. *Journal of Agricultural and Food Chemistry, 59*, 1481-1486.

Van der Veen, B. A., Potocki-Véronèse, G., Albenne, C., Joucla, G., Monsan, P., & Remaud-Simeon, M. (2004). Combinatorial engineering to enhance amylosucrase performance: construction, selection, and screening of variant libraries for increased activity. *FEBS Letters, 560*, 91-97.

Wallingford, L., & Labuza, T. P. (1983). Evaluation of the water binding properties of food hydrocolloids by physical/chemical methods and in a low fat meat emulsion. *Journal of Food Science, 48*, 1-5.

Webb, W. L. (1981). Relation of starch content to conifer mortality and

growth loss after defoliation by the Douglas-fir tussock moth. *Forest Science, 27*, 224-232.

Weber, F. H., Clerici, M. T. P., Collares-Queiroz, F. P., & Chang, Y. K. (2009). Interaction of guar and xanthan gums with starch in the gels obtained from normal, waxy and high-amylose corn starches. *Starch-Stärke, 61*, 28-34.

Yamin, F. F., Lee, M., Pollak, L. M., & White, P. J. (1999). Thermal properties of starch in corn variants isolated after chemical mutagenesis of inbred line B73 1. *Cereal Chemistry, 76*, 175-181.

Yaseen, E. I., Herald, T. J., Aramouni, F. M., & Alavi, S. (2005). Rheological properties of selected gum solutions. *Food Research International, 38*, 111-119.

Yoo, D., Kim, C., & Yoo, B. (2005). Steady and Dynamic Shear Rheology of Rice Starch-Galactomannan Mixtures. *Starch-Stärke, 57*, 310-318.

Yoshimura, M., Takaya, T., & Nishinari, K. (1996). Effects of konjac-glucomannan on the gelatinization and retrogradation of corn starch as determined by rheology and differential scanning calorimetry. *Journal of Agricultural and Food Chemistry, 44*, 2970-2976.

Yoshimura, M., Takaya, T., & Nishinari, K. (1999). Effects of xyloglucan on the gelatinization and retrogradation of corn starch as studied by

rheology and differential scanning calorimetry. *Food Hydrocolloids, 13*, 101-111.

Zhang, Y., Zhang, H., Wang, L., Qian, H., Qi, X., Ding, X., ... & Li, J. (2016). The effect of oat β-glucan on in vitro glucose diffusion and glucose transport in rat small intestine. *Journal of the Science of Food and Agriculture, 96*, 484-491.

국문초록

본 연구에서는 아밀로수크레이스 처리 전분에 갈락토만난 (구아검, 로커스트콩검)을 첨가 후 나타나는 소화 특성을 포도당 확산도, 열적 특성, 물성 등을 통해 해석하고자 하였다.

아밀로수크레이즈 처리 전분을 사용하였을 때 연장된 전분의 가지 사슬에 의해 더 낮은 소화율을 보였다. 이는 아밀로수크레이스 처리 전분은 연장된 가지 사슬로 인해 친수 콜로이드와 더 많은 결합을 할 수 있기 때문으로 보인다. 또한 갈락토만난 가지가 로커스트콩검보다 두 배 더 많은 구아검을 전분에 첨가하였을 경우가 로커스트콩검보다 더 낮은 소화율을 보였다. 이는 포도당 확산도 결과와 연관된다. 포도당 확산 저해 능력은 구아검이 로커스트콩검보다 컸고, 포도당 흡착 또한 구아검이 로커스트콩검보다 뛰어났다. 또한 물성 측정 결과, 로커스트콩검보다 구아검이 아밀로수크레이즈 처리 전분과 더 안정한 젤을 형성하였고, 전단 감소 효과가 컸다. 이를 통해 구아검이 갈락토스 가지 사슬로 인해 분자 내 수소 결합이 적어 뻗은 구조를 형성하기 때문에 전분과 보다 많은 수소 결합을 형성할 수 있다는 것을 확인하였다. 이외에도 갈락토만난을

섞은 전분은 시차주사열량계 분석 결과 흡열 피크가 더 높은 온도 쪽으로 이동하고, 흡열 엔탈피가 감소하였다. 이는 검의 물 결합력으로 인해 자유수가 부족하여 전분의 호화가 어려워지기 때문으로 판단되었다.

또한 위의 결과를 종합하면, 구아검을 아밀로수크레이스 처리 전분에 첨가하였을 때 소화율이 가장 낮아지는 것을 확인하였다.

본 연구는 향후 전분과 갈락토만난을 함께 사용하여 전분질 식품의 물성과 소화율을 조절하는데 활용될 수 있을 것이다.

주요어: 지소화성 전분, 아밀로수크레이스, 갈락토만난, 소화율, 포도당 확산도, 물성

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