Preparation of slowly digestible sweet potato starch by dual enzyme modification using branching enzyme and amylosucrase

Tae Wha Moon
Seoul National University

Department of Agricultural Biotechnology

Starch

Starch in a wide range of products

as a raw material or food additive;

thickener, gelling agent, absorber of water,

energy source of fermentation,

bulking agent, sticky agent

Functional properties of starch for food industry

To stand high shear rates/shear forces/low pH

To tolerate high temperature (sterilization or microwave oven)

To tolerate low temperature (storage in the refrigerator or freezer)

Physiological functionality

Starch

- The major polysaccharide carbohydrate in the diet
- Nutritional classification of starch

- Rapidly digestible starch (RDS)

rapidly digested and absorbed in the small intestine

- Slowly digestible starch (SDS)

digested slowly through the entire small intestine

- Provides sustained glucose release
- Controls postprandial glucose level
- Prevents hyperglycemia - Type II diabetes
- Prolongs satiety

- Resistant starch (RS)

not digested in the small intestine, but fermented in the large intestine into short-chain fatty acids

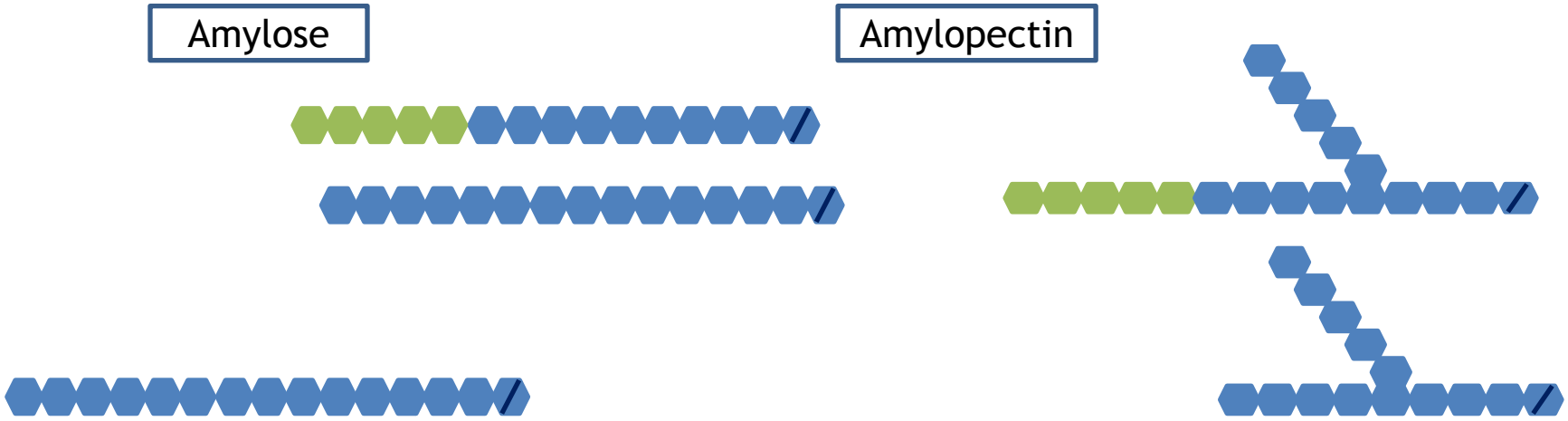
- Similar to dietary fiber
- Produces short-chain fatty acids
- Prevents colon cancer

Starch modification

Method	Advantage	Example
Physical	- Easy treatment - Economical - Harmless - Limited damage	- Hydrothermal treatment - Pregelatinization
Chemical	- Fast reaction - Well-established	- Acid treatment - Cross linking
Enzymatic	- Environment-friendly - Safe - High-yield - Little by-product	- Elongation - Branching - Debranching - Cyclization

Branching enzyme (EC 2.4.1.18)

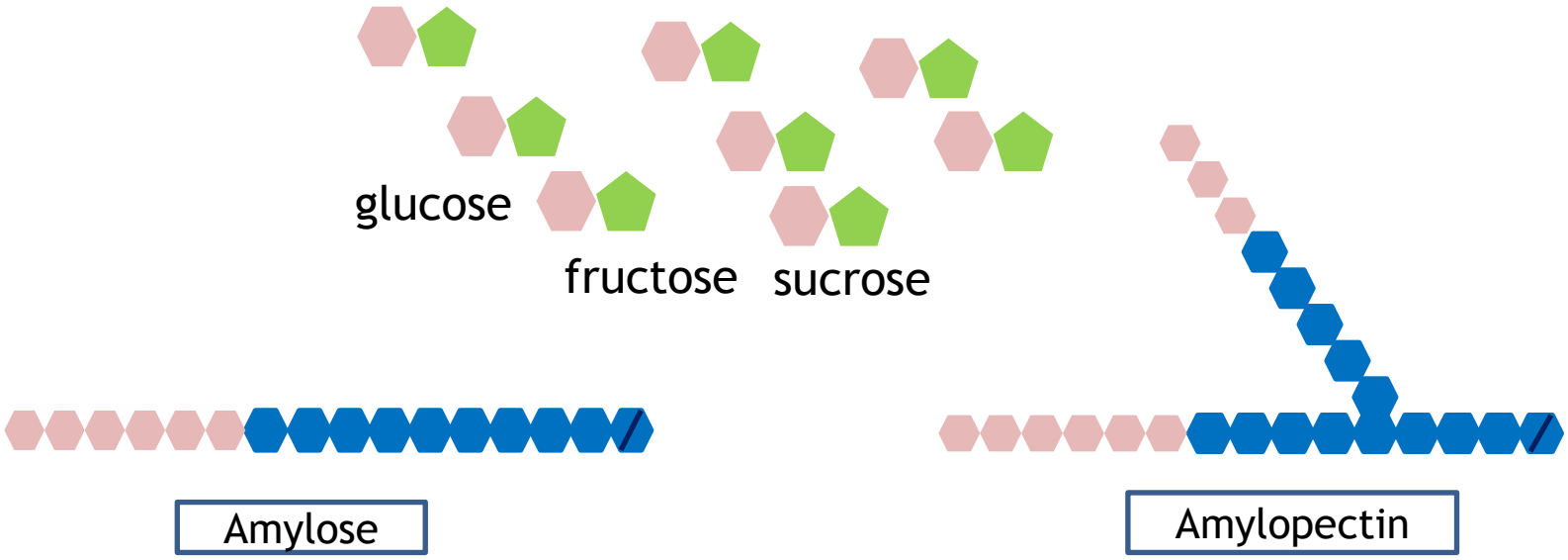
- Catalyzes the cleavage of α-1,4 linkages
- Transfers some of the cleaved oligosaccharides to form a new α-1,6 branch linkage



Amylosucrase (EC 2.4.1.4)

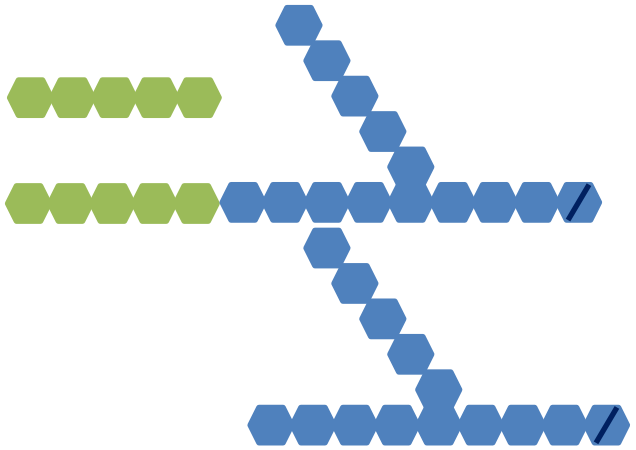
- Catalyzes the transglycosylation reaction to produce an insoluble α-1,4-glucan using sucrose, releasing fructose

- Accelerates the elongation of some external chains at their non-reducing ends

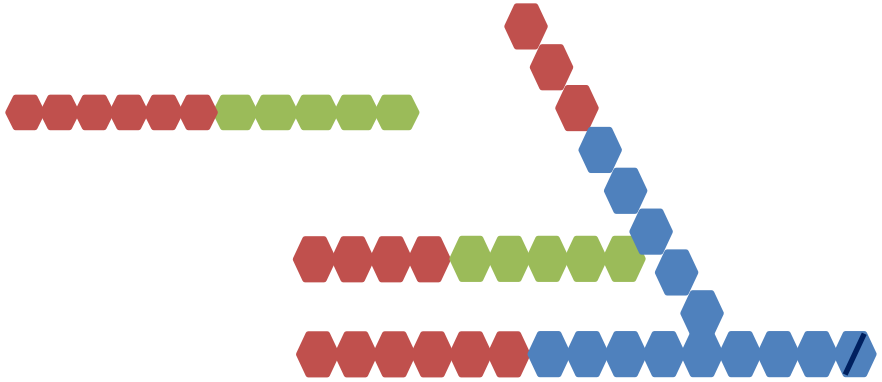


Objectives

- To enhance SDS content of sweet potato starch by dual enzymatic modification
- To elucidate the relationship between its physicochemical properties and digestibility



BE-treated starch



BEAS-treated starch

Materials & Methods

- Sweet potato: Daeyumi (a Korean cultivar)
- Enzyme-treated starch
  - Glycogen branching enzyme from *Streptococcus mutans*
  - Amylosucrase from *Neisseria polysaccharea*
  - Sucrose

BE-treated starch

- Boiling a starch suspension (2%)
- Adding BE (4u, 16u, 32u, 48U[1)]/mL)
- Incubating (30℃, 1 hr)
- Stopping the reaction by boiling

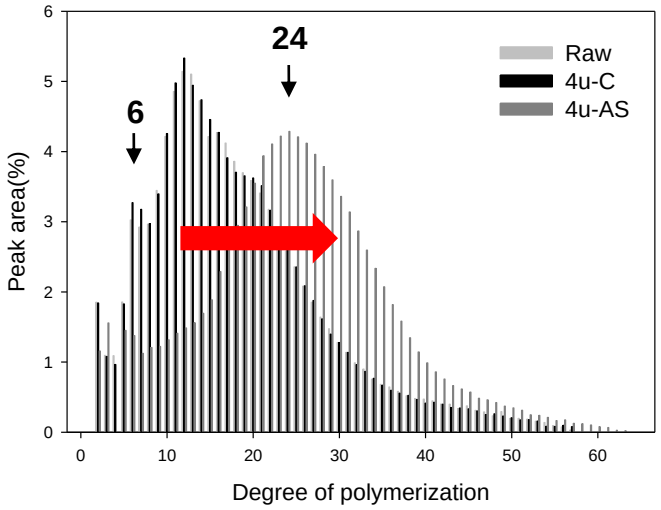
BEAS-treated starch

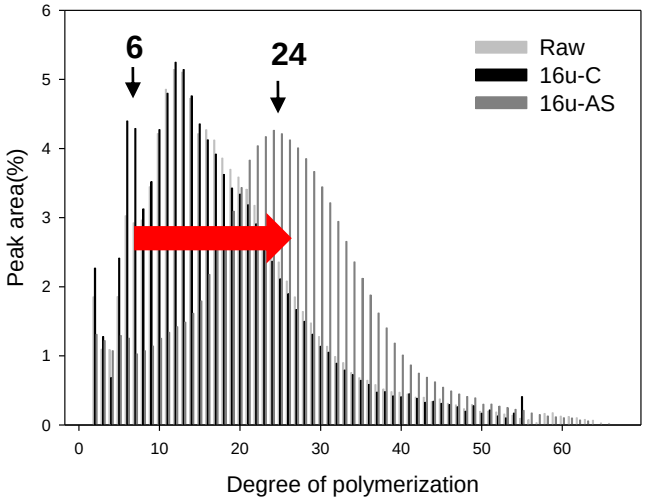
- Adjusting pH to 7
- Adding sucrose (200mM) and AS (1333U[2)]/mL)
- Incubating (30℃, 24 hr)
- Stopping the reaction by EtOH addition, and removing the soluble fraction by centrifugation
- Freeze drying

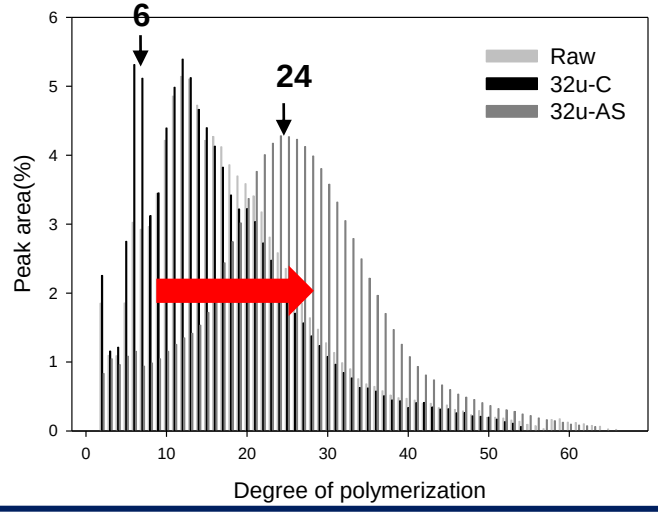
[1)] 1(U) of BE: amount of enzyme degrading 1μg/mL of amylose per min.

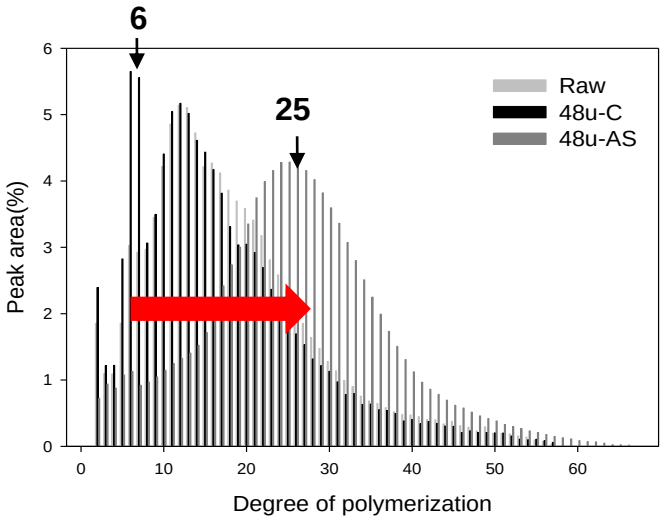
[2)] 1(U) of AS: amount of enzyme that catalyzes the consumption of 1μmole of sucrose per min.

Branch chain distributions









Percent distributions

Sample	Percent distributions[1] (%)				
	≤DP5	DP6-12	DP13-24	DP25-36	DP37≤
C-C	5.9	26.6	45.6	15.8	6.2
C-AS	5.2	8.9	36.5	36.5	12.9
4U-C	5.7	27.4	45.5	15.6	5.8
4U-AS	4.2	9.1	36.3	37.9	12.5
16U-C	6.6	29.6	43.8	14.3	5.6
16U-AS	4.9	8.5	35.3	38.5	12.9
32U-C	7.4	31.8	42.4	13.3	5.1
32U-AS	3.9	7.9	34.6	39.8	13.8
48U-C	7.7	32.4	41.5	13.1	5.3
48U-AS	3.6	7.8	34.3	40.1	14.2

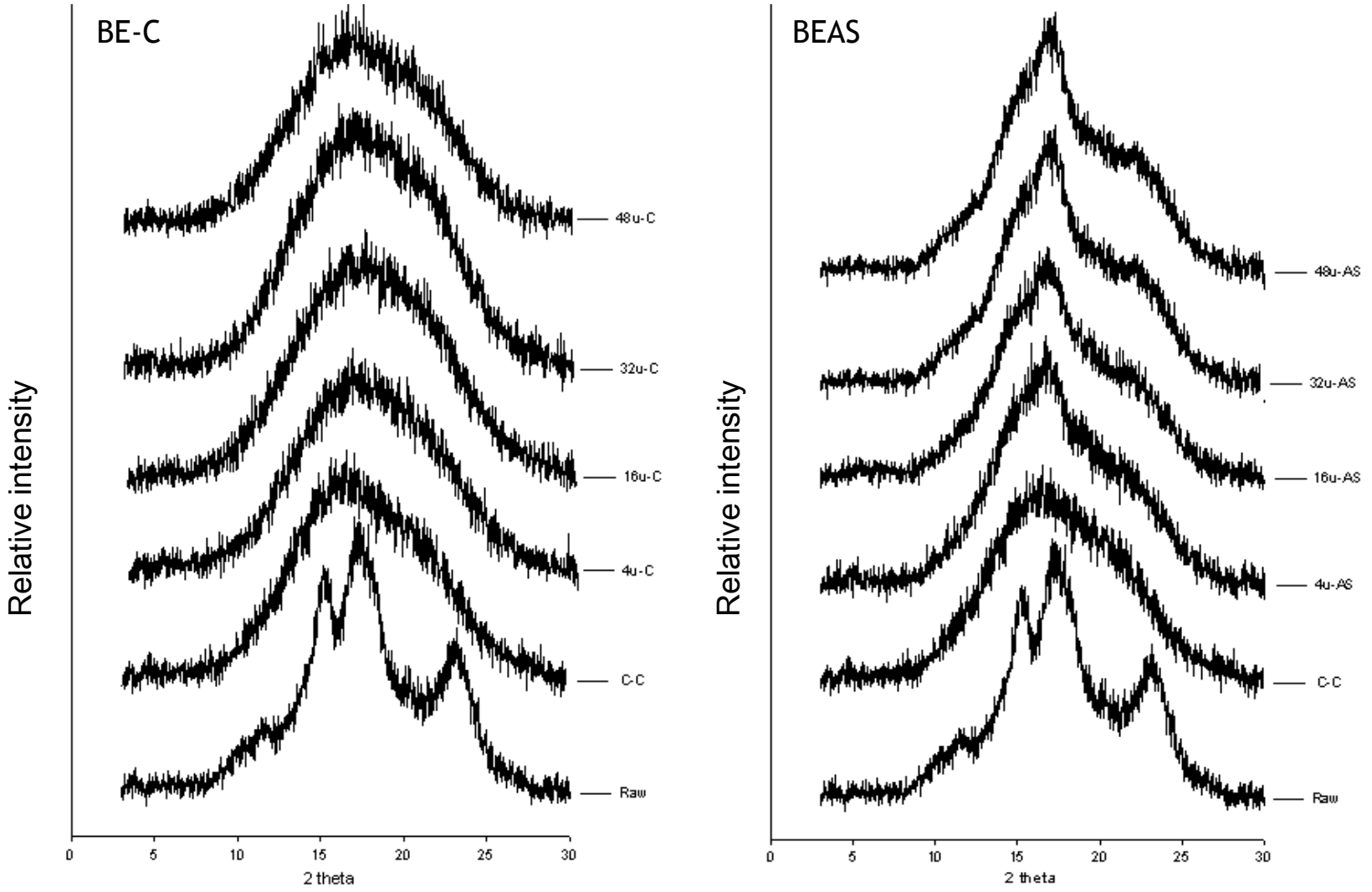
[1] DP, degree of polymerization

Apparent amylose content

Sample	Apparent amylose content (%)
Raw	18.8±0.8
4U[1)]-C[2)]	17.2±1.3
4U-AS[3)]	37.1±1.4
16U-C	18.1±1.5
16U-AS	37.7±0.3
32U-C	19.3±1.2
32U-AS	34.5±0.5
48U-C	22.5±1.1
48U-AS	31.7±1.2

[1)] Branching enzyme unit/mL
[2)] Starch with no amylosucrase treatment.
[3)] Branching enzyme treatment for followed by amylosucrase treatment.

X-ray diffractograms



Relative crystallinity

Sample	Relative crystallinity (%)	
	BE[1)]	BEAS[2)]
Raw	25.4	
C-C[3)]	15.9	
4U[4)]	16.4	18.6
16U	16.3	19.9
32U	15.3	22.3
48U	12.5	25.3

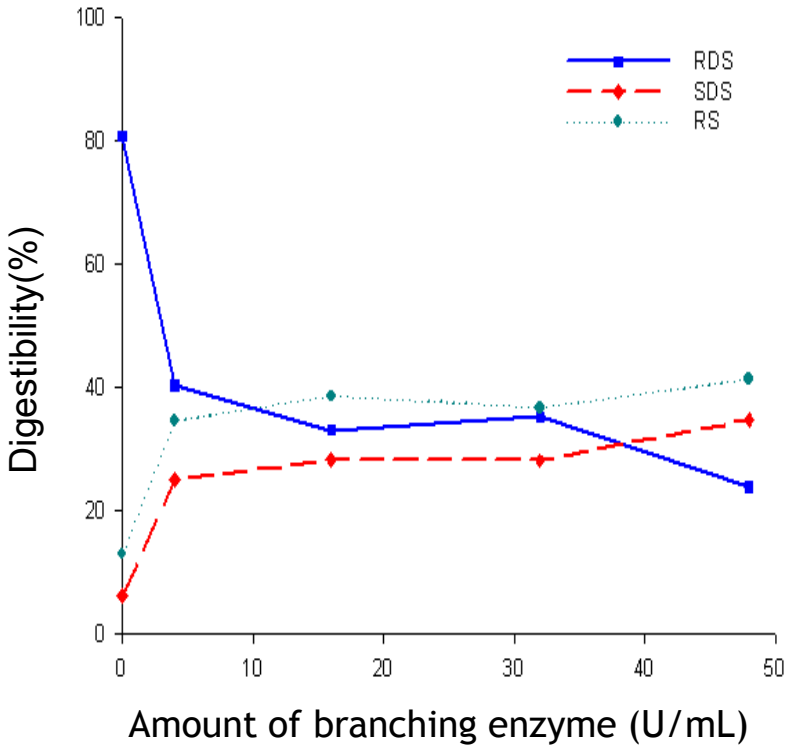
[1)] Branching enzyme treatment.
[2)] Branching enzyme treatment for 1 hr followed by amylosucrase treatment.
[3)] Control, Daeyumi starch with no enzymatic treatment.
[4)] Branching enzyme unit/mL.

Thermal properties

Sample	T_o (℃) [1]	T_p (℃)	T_c (℃)	T_c-T_o (℃)[2]	ΔH (J/g)
Raw	61.7 ± 0.6	70.0 ± 0.2	82.3 ± 0.4	20.7 ± 0.9	16.6 ± 1.6
C-C	46.7 ± 0.3	54.1 ± 0.3	65.3 ± 0.1	18.6 ± 0.2	2.9 ± 0.2
C-AS	72.1 ± 1.0	91.1 ± 0.1	106.1 ± 0.3	34.0 ± 1.2	8.5 ± 0.4
4U-C			N.D.[3]		
4U-AS	70.5 ± 0.8	89.8 ± 0.5	108.3 ± 2.3	37.8 ± 1.5	8.3 ± 0.5
16U-C			N.D.		
16U-AS	72.6 ± 0.4	90.6 ± 0.4	109.1 ± 1.1	36.4 ± 1.1	8.5 ± 0.6
32U-C			N.D.		
32U-AS	71.7 ± 0.6	89.2 ± 0.6	108.3 ± 2.8	37.6 ± 2.2	9.2 ± 0.7
48U-C			N.D.		
48U-AS	70.7 ± 1.3	91.5 ± 1.8	110.4 ± 1.0	39.6 ± 1.3	12.2 ± 1.9

[1] T_o, T_p and T T_c indicate the onset, peak and conclusion temperature of gelatinization, respectively.
[2] T_c-T_o indicates the temperature range of gelatinization. ΔH, gelatinization enthalpy.
[3] Not detected within the temperature range.

In vitro digestion profiles

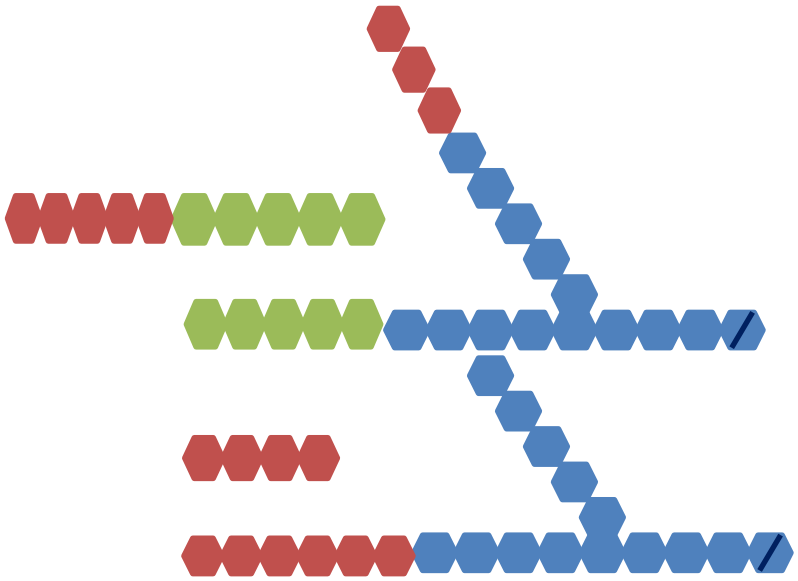


Sample	RDS (%)	SDS (%)	RS (%)
C-C[1)]	80.8±0.8[a]	6.3±0.2[ed]	12.9±0.7[d]
C[2)]-AS	40.9±0.8[c]	**18.4**±1.0[c]	40.6±0.4[a]
4U[3)]-C[4)]	76.1±1.7[b]	8.5±2.0[d]	15.4±3.5[dc]
4U-AS[5)]	40.4±0.7[d]	**25.0**±1.8[b]	34.6±1.0[b]
16U-C	79.7±1.4[a]	4.8±2.5[e]	15.5±2.6[dc]
16U-AS	33.1±0.6[e]	**28.3**±0.5[b]	38.6±1.1[ba]
32U-C	77.6±1.0[b]	5.7±1.3[ed]	16.7±1.4[c]
32U-AS	35.2±0.9[d]	**28.2**±1.3[b]	36.7±0.4[b]
48U-C	75.7±0.6[b]	6.4±1.8[ed]	17.8±1.2[c]
48U-AS	23.9±1.3[f]	**34.8**±3.1[a]	41.3±3.1[a]

[1)] Control, Daeyumi starch with no enzymatic treatment.
[2)] Branching enzyme unit/mL
[3)] Starch with no amylosucrase treatment.
[4)] Branching enzyme treatment for 1 hr followed by amylosucrase treatment.

Conclusion

Branching enzyme
&
Amylosucrase
treatment



- Branch-chain length ⬆
- Crystalline region and heterogeneity ⬆
  ⇒ Slowly digestible starch 6.3 → 34.8% ⬆
  ⇒ Industrial applications: beverages and low GI foods

Acknowledgment

Bioenergy Crop Research Center, National Institute of Crop Science, Rural Development Administration

Dr. Joon Seol Lee, Dr. Mi Nam Chung, Ms. Seon Kyeong Han

Food Chemistry Lab., Seoul National University

Ms. Ara Cho, Dr. Seung Jun Choi

Thank you