Biota: Jurnal Ilmiah Ilmu-Ilmu Hayati, Vol. 9(2): 210-218, Juni 2024
p-ISSN 2527-3221, e-ISSN 2527-323X, https://ojs.uajy.ac.id/index.php/biota/issue/view/453
DOI: 10.24002/biota.v9i2.9095

Modification of Starch with Amylosucrase: Methods, Physicochemical Properties and Health Implications

Angela Myrra Puspita Dewi[1*], Eduard Fransisco Tethool[1]

[1]Faculty of Agricultural Technology, Papua University
Jl. Gunung Salju, Amban, manokwari, Papua Barat, Indonesia
Email: a.puspita@unipa.ac.id **Penulis Korespondensi*

Abstract

Amylosucrase is a transglucosylase enzyme that utilizes sucrose as a substrate to produce α-1,4 glucan (amylose-like polymer). Modification of starch with Amylosucrase can increase the degree of polymerization, degree of crystallinity, heat stability, and increase the proportion of slow digestible starch (SDS) and resistant starch (RS). Thus, Amylosucrase (ASase) modified starch has enormous potential to be developed in the food industry because the consumption of ASase modified starch can improve insulin sensitivity and reduce blood glucose response, making it suitable for consumption by people with Diabetes Mellitus. In addition, consumption of ASase-modified starch also has the potential to prevent obesity and improve blood lipid profile.

Keywords: amylosucrase, resistant starch, SDS, starch.

Submitted : 3 May 2024 ; Revised : 13 May 2024 ; Accepted : 31 May 2024

Introduction

Starch is a natural polysaccharide composed of amylose and amylopectin. Amylose is a linear-chain polymer composed of α-1,4-D-glucose, while amylopectin is a forked chain polymer composed of α-1,4-D-glucose and α-1,6-D-glucose. Glucose produced from starch digestion plays a role in metabolism. Generally, foods with the same amount of starch can cause differences in post-prandial blood glucose contents and insulin response after consumption (Lim *et al.*, 2019). Starch is classified into three principal fractions based on its digestibility: rapid digesting starch (RDS), slow digesting starch (SDS), and resistant starch (RS). RDS can increase post-prandial blood glucose and insulin contents. SDS triggers a slow increase in post-prandial blood glucose levels, while RS is not absorbed in the small intestine but can be fermented in the large intestine. RDS is associated with a high glycemic index (GI), while SDS and RS enhance glucose tolerance, reduce insulin resistance, lower blood lipid levels, and have prebiotic effects (Kim *et al.*, 2016). Of these three fractions, resistant starch (RS) has received the most attention regarding its role in improving or inhibiting the digestibility of native starch by digestive enzymes (Clerici, Maria T., P & Schmiele, 2019). Starch digestibility can be controlled by changing the physicochemical properties of native starch, such as chemical composition, amylopectin structure, crystallinity, and surface area (Zhang, Wang, *et al.*, 2017). Structural modification of starch by enzymes is one of the alternative approaches to modify the physicochemical properties of native starch so as to obtain the desired starch digestibility. One of the enzymes that can be used in enzymatic modification of starch is amylosucrase (EC, AS 2.4.1.4). Amylosucrase (ASase) is a glucosyltransferase enzyme that produces α-1,4 glucan (amylose-like polymer) from sucrose as the sole substrate (Zhang *et al.*, 2016).

ASase synthesizes amylose and lengthens the amylopectin chain by transferring glucose from its substrate to the end of amylopectin. The elongation of amylopectin chains will result in changes in the crystallinity and digestibility properties of its native starch (Lee *et al.*, 2018).

How to Cite : Dewi, A. M. P. & Tethool, E. F.(2024). Modification of Starch with Amylosucrase: Methods, Physicochemical Properties and Health Implications. Jurnal Ilmiah Ilmu-Ilmu Hayati 9(2): 210-218.

This unique property of ASase can be applied in the food industry because of its ability to extend starch polymers to produce starch that is difficult to digest so that it can be classified in the resistant starch group (Park *et al.*, 2019). Due to its enormous potential, especially for the food industry, this review presents information on the method of making amylosucrase-modified starch, the physicochemical properties of the resulting modified starch, and the health implications of amylosucrase-modified starch.

Method of Modifying Starch with Amylosucrase

ASase modified starch involves a substrate in the form of sucrose, which is a disaccharide consisting of glucose and fructose as glucosyl donors (Ryu *et al.*, 2010). ASase can produce α-glucan with α-1,4 bonds and does not require primers or nucleotide sugars such as ADP and UDP-glucose (Zhang, Wang, *et al.*, 2017). ASase has transglucosylation activity by taking a glucose molecule from sucrose as its substrate and joining it to the acceptor glycosyl on starch and other polysaccharide molecules (Figure 1). This transglucosylation activity is the key to the generation of novel polysaccharides and bioactive carbohydrates (Seo *et al.*, 2017).

Starch modified with Amylosucrase (ASase) can increase the degree of polymerization and increase the proportion of SDS and RS. The success of starch modification with Amylosucrase is influenced by the type of ASase-producing microorganisms, starch source, and process conditions (time, pH, and temperature). The majority of ASases are not resistant to high temperatures, but it is reported that DG-ASase (*Deinococcus geothermalis* Amylosucrase) is the most resistant to high temperatures than other ASases (Lee *et al.*, 2018).

The procedure for making ASase-modified starch is that the starch sample is suspended in a buffer containing sucrose as a donor. The starch dispersion was autoclaved at 121 C for 20 min and then cooled at reaction temperature. This substrate dispersion was then added the enzyme ASase and the reaction was carried out at 30-50 C for 7-48 hours with constant stirring. Inactivation of enzyme and precipitation of glucan polymer was done by adding ethanol. The modified starch was collected through centrifugation and followed by drying with a freeze dryer. Some process conditions of producing modified starch by Amylosucrase and the characteristics of the resulting modified starch are presented in Table 1.

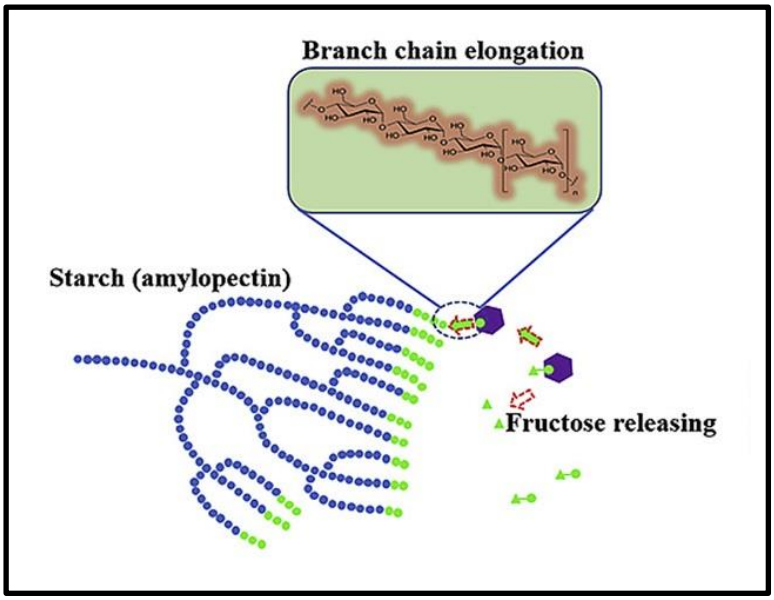


Figure 1. Mechanism of modified starch production by Amylosucrase (Zhang *et al.*, 2019).

Table 1. Starch modification methods by Amylosucrase from various starch sources

Microorganism produced ASase	Source of starch	Reaction condition	Polymerization degree distribution (%)	RS (%)	SDS (%)	Reference
DG-ASase (*Deinococcus geothermalis* Amylosucrase)	Pati chesnut	48 jam, pH 8, 30-50^{o}C	NR	24	24	(Lee *et al.*, 2018)
NpAS (*Neisseria polysaccharea* Amylosucrase)		1-45 jam, pH 7, 35^{o}C	6-12 : 8.4-20.9 13-24 : 53.2-55.8 25-36 : 19-28.8 >37 : 6.5-7	9-11	9-40	(B. K. Kim *et al.*, 2014)
	Pati jagung pulut	24 jam, pH 7, 35^{o}C	NR	14.9-51.8	NR	(Ryu *et al.*, 2010)
		24 jam, pH 7, 30^{o}C	6-12 : 10.1 13-24 : 57.1 25-36 : 26.0 >37 : 6.2	26.1	43.7	(Yoo *et al.*, 2018)
		24 jam, pH 7, 35^{o}C	NR	15.3-43.1	NR	(Ryu *et al.*, 2010)
	Pati jagung	4 jam, pH 7, 35^{o}C	6-12 : 1.1-9.0 13-24 : 35.8-48.2 25-36 : 21.5-38 >37 : 21.3-25	28.4-54.2	17.8-31.4	(Zhang *et al.*, 2019)
	Pati beras ketan	40 jam, pH 6, 30^{o}C	6-12 : 1.2 13-24 : 32.2 25-36 : 43.3 >37 : 23.3	49.8	29.1	(Shin *et al.*, 2010)
	Pati beras	40 jam, pH 6, 30^{o}C	6-12 : 3.6 13-24 : 38.6 25-36 : 37.9 >37 : 19.9	46.2	12.4	
	Pati kentang	40 jam, pH 6, 30^{o}C	6-12 : 1.9 13-24 : 32.7 25-36 : 45.3 >37 : 20	52.6	10.2	
	Waxy potato starch	40 jam, pH 6, 30^{o}C	6-12 : 1.9 13-24 : 28.4 25-36 : 47.5 >37 : 22.1	48.7	24.2	
	Pati beras modifikasi HTT	13 jam 40 menit, pH 7, suhu 30^{o}C	6-12 : 2.4 13-24 : 49.5 25-36 : 38.9 >37 : 6.4	NR	NR	(J. H. Kim *et al.*, 2016)
	Pati beras ketan modifikasi hydrothermal treatment	13 jam 40 menit, pH 7, suhu 30^{o}C	6-12 : 2.9 13-24 : 47.1 25-36 : 41.4 >37 : 6.8	NR	NR	

Lanjutan Tabel 1.

Microorganism produced ASase	Source of starch	Reaction condition	Polymerization degree distribution (%)	RS (%)	SDS (%)	Reference
NsAS (*Neisseria subflava Amylosucrase)*	Pati jagung tinggi amilosa (Amylomaize VII)	24 jam, pH 7-9, 35-45^{o}C	NR	55	NR	(Park *et al.*, 2019)
	Pati jagung	24 jam, pH 7-9, 35-45^{o}C	NR	60.9	NR	
	Pati jagung pulut	24 jam, pH 7-9, 35-45^{o}C	NR	68.7	NR	
	Pati beras	24 jam, pH 7-9, 35-45^{o}C	NR	63.5-69.2	NR	

*NR : not reported

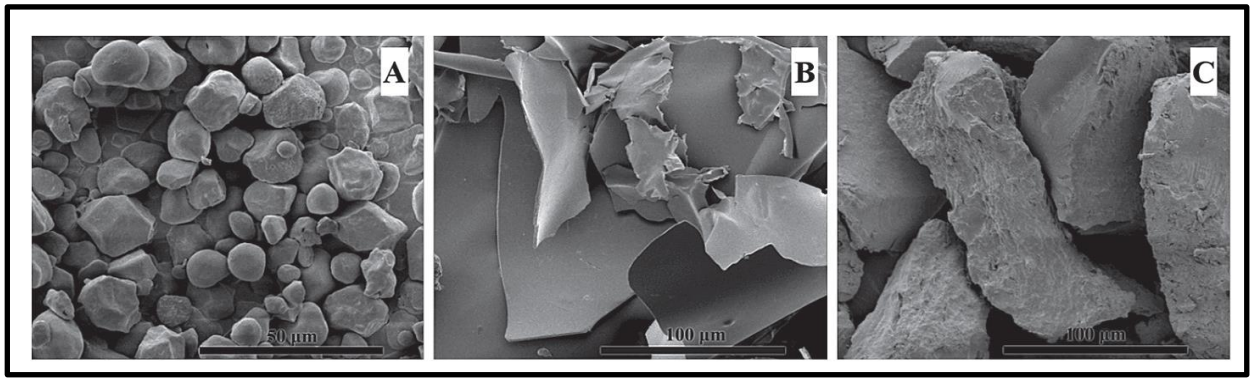


Figure 2. Granule morphology of native starch (A), pre-gelatinized starch (B), and ASase-modified starch (C) (Zhang, Wang, *et al.*, 2017)

Physicochemical properties of Amylosucrase Modified starch

ASase modified starch can reduce the fraction of short-chain starch (DP 6-12) and increase the fraction of medium-chain (DP 25-26) and long-chain (DP>37) starch (Kim *et al.*, 2014; Kim *et al.*, 2015; Shin *et al.*, 2010). Starch with high amylose content results in lower DP than starch with high amylopectin content. This is because the speed of Amylosucrase in transferring glucose at the amylopectin end is faster than the speed of transferring glucose at the amylose end (Park *et al.*, 2019; Ryu *et al.*, 2010). Because of this phenomenon, the ability of ASase-modified starch increases the iodine binding index (IBI) (B. Kim *et al.*, 2013; Lee *et al.*, 2018). In addition, ASase-modified starch also changes the morphology of starch granules. Zhang *et al.* (2017) reported that ASase-modified corn starch produced a granule appearance resembling retrograded starch with a larger particle size than native starch. This is due to the amylose-like properties of ASase-modified starch. During retrogradation, the lengthened side chains of amylopectin can be entrapped to form a network structure. This contributes to the modified starch's particle size after drying and milling (Figure 2).

ASase modification changes the crystal type of native corn starch from A-type crystals toward forming B-type crystals (Figure 4), which contributes to the ease of retrogradation (Zhang *et al.*, 2019). Zhang *et al.* (2017) reported that changing crystal type to B-type crystallite due to AS modification can facilitate and strengthen inner chain association in starch molecules, speculated to increase SDS and RS fractions. The crystallization properties of the ASase-modified starch improved its thermal properties with increasing gelatinization temperature due to chain elongation, which increases crystallinity and double helix formation in the ASase-modified starch. Modification of starch with Amylosucrase is also reported to affect the digestibility of starch.

Increasing the branch chain length of starch polymers can reduce the fraction of RDS but increase the fraction of SDS and RS (Lim *et al.*, 2019).

The longer the reaction time, the higher the SDS and RS content (Zhang *et al.*, 2018). The increased resistant starch content of the ASase-modified starch is due to the random elongation of the starch polymer chain through α-1,4 glycosidic bonds and the formation of type B crystals (Zhang, Zhou, *et al.*, 2017).

An increase in the proportion of degree of polymerization (DP) 13-30 contributes to the ease of retrogradation and the formation of crystalline structures, thereby diminishing digestive enzyme accessibility (Kim *et al.*, 2016). Starch with longer branching chains has better high temperature stability because it produces longer double helices more resistant to amylolytic hydrolysis.

Health Implications of Amylosucrase-Modified Starch

Lowering the glycemic index

The glycemic index indicates the change in blood glucose levels from high-carbohydrate foods. High carbohydrate foods can be rapidly digested and absorbed produce a high glycemic index (equivalent to blood glucose levels >70). Meanwhile, if the food is slowly digested and absorbed, it will produce a low glycemic index (equivalent to a blood glucose level of 55). Modification of starch with Amylosucrase can produce a lower glycemic index than native starch due to the increased resistant starch (RS) content (H. Li *et al.*, 2019).

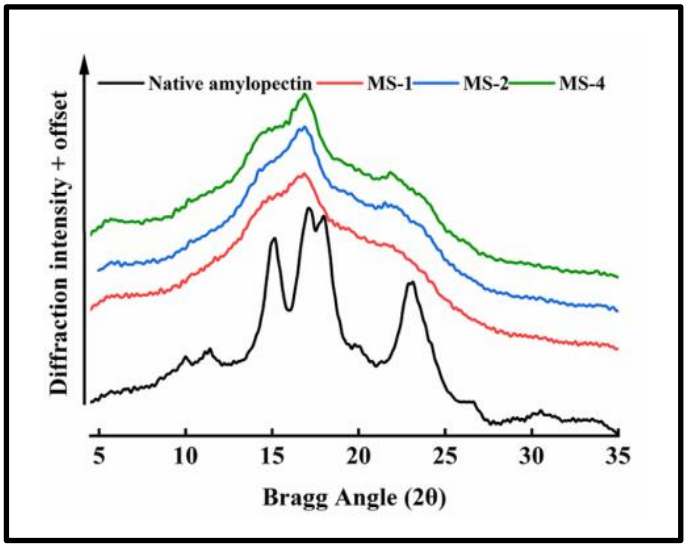


Figure 3. X-ray diffraction patterns of amylosucrase treatment at various time reactions (Zhang et al, 2019)

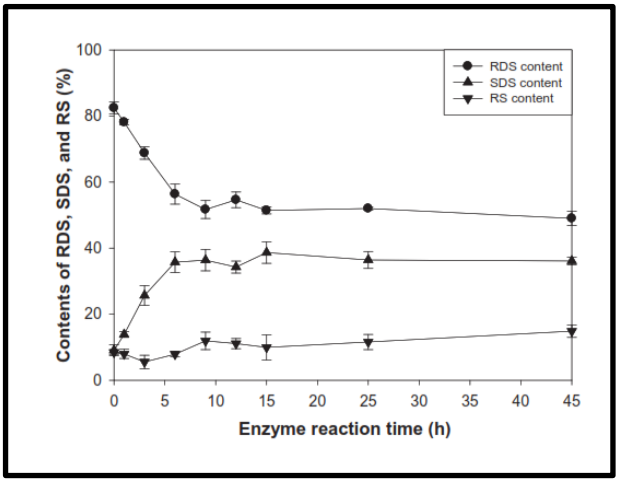


Figure 4. Effect of ASase modification reaction time on RDS, SDS and RS content (Kim et al, 2014)

Kim *et al.* (2016) reported a negative correlation between resistant starch levels and blood glucose contents. Blood glucose contents in native starch and gelatinized starch showed an increase in post-prandial blood glucose levels during the first 30 minutes after consumption, which decreased drastically by about 50 mg/dL after the next 30 minutes. In contrast, ASase-modified starch caused gradual changes with stable blood glucose levels over 30-90 minutes. It is similar to the Novelose 240 pattern, which contains 54% RS, indicating the predominant RS characteristics of the ASase-modified starch (Figure 5). ASase-modified starch may play a role in further developing starch-based products with a relatively low GI, which may be suitable for people with diabetes, obesity and colon cancer.

Enhanced Insulin Sensitivity

Lee *et al.* (2018) reported that high-fat consumption accompanied by consumption of ASase-modified chestnut starch was able to suppress blood glucose levels, resulting in decreased secreted insulin levels and lower HOMA-IR (insulin resistance index). Resistant starch cannot be digested in the small intestine; it is fermented in the colon by microbiota and produces short-chain fatty acids (SCFA). These short-chain fatty acids play a role in increasing insulin sensitivity through the mechanism of 1). Increasing the secretion of GLP-1 and PYY incretins, which are incretin hormones that send signals to the hypothalamus so as to delay hunger and impact on lowering blood glucose levels, and 2). SCFA, mainly acetate, plays a role as a signal transduction receptor GPR43 to suppress the work of insulin receptors to increase insulin sensitivity (Zhou *et al.*, 2015).

Improvement of Lipid Profile

Consumption of ASase-modified starch was able to reduce LDL levels in mice fed a high-fat diet, while triglyceride and total cholesterol levels were not significantly different compared to the control (without a high-fat diet and consumption of modified starch) (Lee *et al.*, 2018). This is due to the production of short-chain fatty acids from resistant starch fermentation in the colon, especially acetate, which inhibits adipose lipolysis (Roberfroid, 2008). In addition, low blood glucose levels due to consumption of resistant starch cause a decrease in blood glucose levels, thus preventing hepatic de novo lipogenesis, which is the process of fatty acid synthesis from non-lipid compounds by activating AMP kinase and suppressing SREBP-1c expression (Li *et al.*, 2011). Consumption of resistant starch will inhibit gluconeogenesis, so glucose is used for glycogen synthesis to decrease blood glucose levels. Blood glucose reduction inhibits fatty acid synthesis and lipid biosynthesis and increases beta oxidation. Thus, homeostatic glucose and cholesterol will be produced (Zhou *et al.*, 2015).

Body weight loss

Consumption of ASase-modified starch was reported to reduce body weight in mice on a high-fat diet. This weight loss is due to the production of short-chain fatty acids, which are transduction signals in activating GPR43, which plays a role in controlling the secretion of the incretin hormones GLP-1 and PYY (Zhou *et al.*, 2015). Lee *et al.* (2018) reported that consumption of ASase-modified starch increased GLP-1 expression but showed no apparent effect on PYY expression. Increased secretion of GLP-1 and PYY leads to inhibition of gastric emptying, provides a satiety effect and decreases appetite, thus may contribute to weight loss (Li *et al.*, 2017).

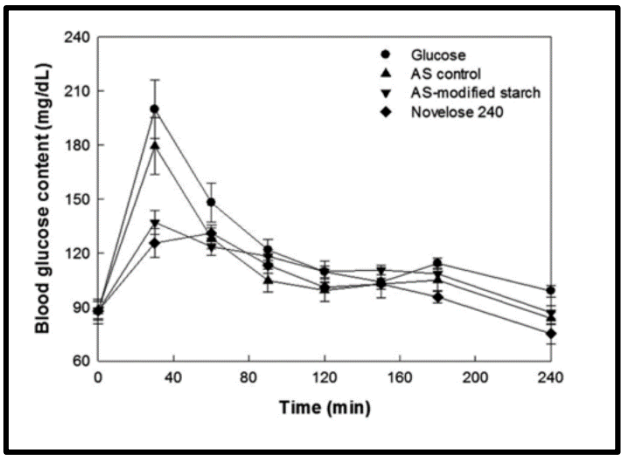
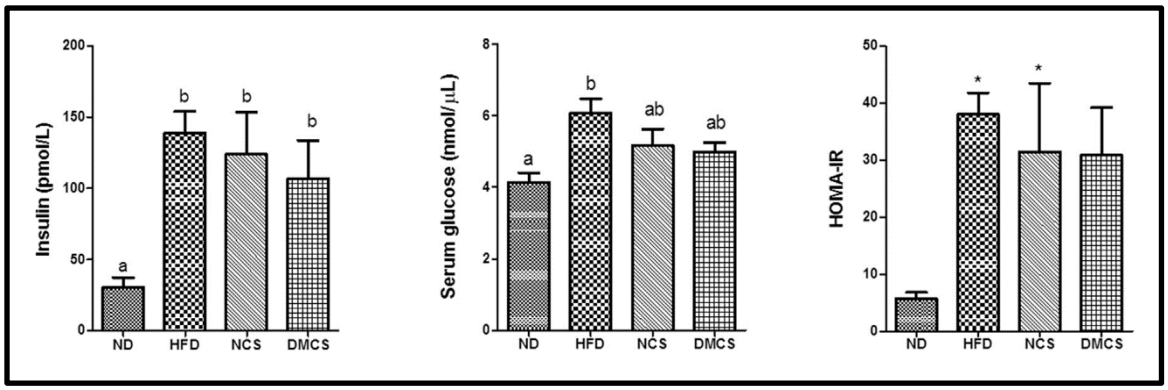


Figure 5. changes in blood glucose levels in adlay starch (AS control), ASase modified starch (AS-modified starch) and Novelose 240 (Kim *et al.*, 2016)



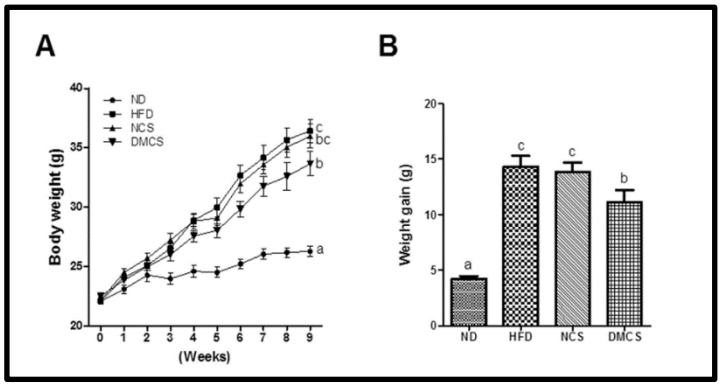
*ND: normal diet; HFD: high-fat diet; NCS : high fat diet + native chesnut starch; DMCS : high fat diet + ASase chesnut starch

Figure 6. Effect of consumption of high-fat diet with ASase-modified starch on serum insulin (A), serum glucose (B), and HOMA-IR (C) (Lee *et al.*, 2018)

Table 2. Composition of blood lipids in diabetic rats after RS consumption (Zhou *et al.*, 2015)

Group	Triglyceride (mmol/L)	Total cholesterol (mmol/L)	High Density Lipoprotein-cholesterol (mmol/L)
Normal diet	0.29 ± 0.00**	2.96 ± 0.07**	2.90 ± 0.2.35**
High fat diet	2.92 ± 1.27	5.57 ± 0.47	1.32 ± 0.45
RS consumption	1.01 ± 0.39**	4.48 ± 0.41 **	2.35 ± 0.43**

** Values in each column followed by different superscripts are significantly different at P<0.01



*ND: normal diet; HFD: high-fat diet; NCS : high fat diet + native chesnut starch; DMCS : high fat diet + ASase chesnut starch

Figure 7. Effect of chestnut starch on body weight change (A) and weight gain (B) of High-fat diet mice (Lee *et al.*, 2018)

Conclusion

Amylosucrase starch modification can change the structure of native starch by decreasing the fraction of short-chain starch (DP 6-12) and increasing the fraction of medium-chain (DP 25-26) and long-chain starch (DP>37), crystallinity, and granule size. Increasing the fraction of long-chain starch inhibits digestive enzymes from hydrolyzing starch molecules, thus decreasing the proportion of fast-digestible starch (RDS) and increasing the proportion of slow-digestible starch (SDS) and resistant starch (RS). The increase in SDS and RS contributes to the good health effects of decreased glycemic index, improved insulin sensitivity, lipid profile, and weight loss.

References

Clerici, Maria T., P, S., & Schmiele, M. (2019). *Starches for Food Application : Chemical, Technological and Health Properties.* CRC Press. New York.

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, B., Kim, H., Hong, J., Huber, K. C., Shim, J., & Yoo, S. (2013). Effects of amylosucrase treatment on molecular structure and digestion resistance of pre-gelatinized rice and barley starches. *Food Chemis* 138: 966–975.

Kim, B. S., Kim, H. S., & Yoo, S. H. (2015). Characterization of enzymatically modified rice and barley starches with amylosucrase at scale-up production. *Carbohydrate Polymers* 125: 61–68.

Kim, E. J., Kim, H. R., Choi, S. J., Park, C. S., & Moon, T. W. (2016). Low digestion property of amylosucrase-modified waxy adlay starch. *Food Science and Biotechnology* 25(2): 457–460.

Kim, J. H., Kim, H. R., Choi, S. J., Park, C., & Moon, T. W. (2016). Production of an in Vitro Low-Digestible Starch via Hydrothermal Treatment of Amylosucrase-Modi fi ed Normal and Waxy Rice Starches and Its Structural Properties. *Journal of Agricultral and Food* Science 64: 5045–5052.

Lee, E., Lee, B., Uk, D., Lim, M., Chung, W., Park, C., Baik, M., Nam, Y., & Seo, D. (2018). Amelioration of obesity in high-fat diet-fed mice by chestnut starch modi fi ed by amylosucrase from Deinococcus geothermalis. *Food Hydrocolloids* 75: 22–32.

Li, H., Li, J., Xiao, Y., Cui, B., Fang, Y., & Guo, L. (2019). In vitro digestibility of rice starch granules modified by β-amylase, transglucosidase and pullulanase. *International Journal of Biological Macromolecules* 136: 1228–1236.

Li, L., Ma, L., & Fu, P. (2017). Gut microbiota-derived short-chain fatty acids and kidney diseases. *Drug Design, Development and Therapy* 11: 3531–3542.

Li, Y., Xu, S., Mihaylova, M. M., Zheng, B., Hou, X., Jiang, B., Park, O., Luo, Z., Lefai, E., Shyy, J. Y. J., Gao, B., Wierzbicki, M., Verbeuren, T. J., Shaw, R. J., Cohen, R. A., & Zang, M. (2011). AMPK phosphorylates and inhibits SREBP activity to attenuate hepatic steatosis and atherosclerosis in diet-

induced insulin-resistant mice. *Cell Metabolism* 13(4): 376–388.

Lim, J. H., Kim, H. R., Choi, S. J., Park, C.-S., & Moon, T. W. (2019). Complexation of Amylosucrase-Modified Waxy Corn Starch with Fatty Acids: Determination of Their Physicochemical Properties and Digestibilities. *Food Chemistry* 0(0): 1–9.

Park, M., Chandrasekaran, M., & Yoo, S. (2019). Production and characterization of low-calorie turanose and digestion- resistant starch by an amylosucrase from Neisseria subflava. *Food Chemistry* 300: 125–225.

Roberfroid, M. B. (2008). *Prebiotics: Concept, definition, criteria, methodologies, and products. In Handbook of Prebiotics.* CRC Press. New York.

Ryu, J., Lee, B., Seo, D., Baik, M., Park, C., Wang, R., & Yoo, S. (2010). Production and characterization of digestion-resistant starch by the reaction of Neisseria polysaccharea amylosucrase. *Starch/Starke* 62: 221–228.

Seo, D., Jung, J., & Park, C. (2017). Fluorescence detection of the transglycosylation activity of amylosucrase. *Analytical Biochemistry* 532: 19–25.

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.

Yoo, H. J., Kim, H. R., Choi, S. J., Park, C. S., & Moon, T. W. (2018). Characterization of low-digestible starch fractions isolated from amylosucrase-modified waxy corn starch. *International Journal of Food Science and Technology* 53(3): 557–563.

Zhang, H., Chen, Z., Zhou, X., He, J., & Wang, T. (2018). Anti-digestion properties of amylosucrase modified waxy corn starch. *International Journal of Biological Macromolecules* 109: 383–388.

Zhang, H., Wang, R., Chen, Z., & Zhong, Q. (2017). Enzymatically modified starch with low digestibility produced from amylopectin by sequential amylosucrase and pullulanase treatments. *Food Hydrocolloid* 95: 195–202.

Zhang, H., Wang, R., Chen, Z., & Zhong, Q. (2019). Enzymatically modified starch with low digestibility produced from amylopectin by sequential amylosucrase and pullulanase treatments. *Food Chemistry* 95: 195–202.

Zhang, H., Zhou, X., He, J., Wang, T., Luo, X., Wang, L., Wang, R., & Chen, Z. (2017). Impact of amylosucrase modification on the structural and physicochemical properties of native and acid-thinned waxy corn starch. *Food Chemistry* 220: 413–419.

Zhang, H., Zhou, X., Wang, T., Luo, X., Wang, L., & Li, Y. (2016). New insights into the action mode of amylosucrase on amylopectin. *International Journal of Biological Macromolecules* 88: 380–384.

Zhou, J., Martin, R. J., Raggio, A. M., Shen, L., McCutcheon, K., & Keenan, M. J. (2015). The importance of GLP-1 and PYY in resistant starch's effect on body fat in mice. *Molecular Nutrition and Food Research* 59(5): 1000–1003.

Zhou, Z. K., Wang, F., Ren, X. C., Wang, Y., & Blanchard, C. (2015). Resistant starch manipulated hyperglycemia/hyperlipidemia and related genes expression in diabetic rats. *International Journal of Biological Macromolecules* 75: 316–321.