PRODUCTION AND PROPERTIES OF RESISTANT MALTODEXTRIN FROM CASSAVA STARCH

Miss Kamonrat Trithavisup

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การผลิตและสมบัติของมอลโทเด็กซ์ทรินชนิดทนย่อยจากสตาร์ชมันสำปะหลัง

น.ส.กมลรัตน์ ไตรทวิทรัพย์

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ลิขสิทธิ์ของจุฬาลงกรณ์มหาวิทยาลัย

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Thesis Advisor	Associate Professor Kanitha Tananuwong, Ph.D.

Accepted by the FACULTY OF SCIENCE, Chulalongkorn University in Partial Fulfillment of the Requirement for the Doctor of Philosophy

.... Dean of the FACULTY OF SCIENCE
(Professor POLKIT SANGVANICH, Ph.D.)

DISSERTATION COMMITTEE

.... Chairman
(Associate Professor Jirarat Anuntagool, Ph.D.)

.... Thesis Advisor
(Associate Professor Kanitha Tananuwong, Ph.D.)

.... Examiner
(Assistant Professor Thanachan Mahawanich, Ph.D.)

จุฬาลงกรณ์มหาวิทยาลัย

.... Examiner
(Assistant Professor DARIS KUAKPETOON, Ph.D.)

.... External Examiner
(Natthiya Deeying, Ph.D.)

.... External Examiner
(Professor Yong-Cheng Shi, Ph.D.)

กมลรัตน์ ไตรทวิทรัพย์ : การผลิตและสมบัติของมอลโทเด็กซ์ทรินชนิดทนย่อยจากสตาร์ชมันสำปะหลัง. (PRODUCTION AND PROPERTIES OF RESISTANT MALTODEXTRIN FROM CASSAVA STARCH) อ.ที่ปรึกษาหลัก : รศ. ดร.ขนิษฐา ธนานุวงศ์

งานวิจัยนี้มีวัตถุประสงค์เพื่อศึกษาผลของภาวะการผลิตต่อโครงสร้างโมเลกุลและสมบัติบางประการของเด็กซ์ทรินชนิดทนย่อยและมอลโทเด็กซ์ทรินชนิดทนย่อยจากสตาร์ชมันสำปะหลัง เตรียมเด็กซ์ทรินชนิดทนย่อย โดยผสมสตาร์ชมันสำปะหลังกับสารละลายกรดไฮโดรคลอริกให้ได้ความเข้มข้นสุดท้ายของกรดเท่ากับ 0.04-0.10% โดยน้ำหนักของสตาร์ชแห้ง จากนั้นนำตัวอย่างไปทำแห้งที่อุณหภูมิ 50°C จนมีความชื้นไม่เกิน 5% แล้วจึงนำไปให้ความร้อนที่อุณหภูมิ 100-120°C เป็นเวลา 60-180 นาที จากผลการทดลองพบว่า เมื่อความเข้มข้นของกรด อุณหภูมิและเวลาในการให้ความร้อนเพิ่มขึ้น ความสามารถในการละลายน้ำ ปริมาณน้ำตาลรีดิวซ์ ปริมาณใยอาหารทั้งหมดและสัดส่วนของใยอาหารที่มีน้ำหนักโมเลกุลสูงของเด็กซ์ทรินชนิดทนย่อยมีค่าเพิ่มขึ้น ในขณะที่ดัชนีความขาวของตัวอย่างมีค่าลดลง สำหรับสารละลายของเด็กซ์ทรินชนิดทนย่อยที่เตรียมจากภาวะที่ไม่รุนแรง (ความเข้มข้นกรด 0.04-0.08%, 100°C, 60 นาที) ตรวจพบพีคการดูดความร้อนที่ 45-70°C และมีค่า enthalpy 1.66-2.14 จูลต่อกรัมโดยน้ำหนักตัวอย่างแห้ง แต่ไม่พบพีคการดูดความร้อนของสารละลายเด็กซ์ทรินชนิดทนย่อยที่เตรียมจากภาวะที่รุนแรงขึ้น การดัดแปรภายใต้ภาวะที่รุนแรงส่งกว่าผลให้เด็กซ์ทรินชนิดทนย่อยมีน้ำหนักโมเลกุลเพิ่มขึ้นเล็กน้อย แต่มีสายโซ่กิ่งที่สั้นลง กระบวนการเด็กซ์ทริไนเซชันส่งผลให้เกิดพันธะไกลโคซิดิกที่ตำแหน่ง α-1,2, α-1,6, β-1,2, β-1,4 และ β-1,6 ในเด็กซ์ทรินชนิดทนย่อย สัดส่วนของพันธะ a-1,4 ไกลโคซิดิกและ a-/ β-reducing ends มีค่าลดลง ในขณะที่สัดส่วนของพันธะ β-1,2, β-1,4, α-/ β-1,6 ไกลโคซิดิกและระดับความเป็น กิ่งแขนงมีค่าเพิ่มขึ้นในเด็กซ์ทรินชนิดทนย่อยที่เตรียมจากภาวะที่รุนแรงขึ้นในขั้นตอนเด็กซ์ทริไนเซชัน แม้ว่าส่วนที่ทนย่อยมีสัดส่วนเพิ่มขึ้นจากขั้นตอนเด็กซ์ทริไนเซชัน แต่เด็กซ์ทรินชนิดทนย่อยที่เตรียมได้ยังคงมีใยอาหารทั้งหมดเพียง 7-47% เพื่อเพิ่มปริมาณใยอาหารทั้งหมด จึงนำตัวอย่างเด็กซ์ทรินชนิดทนย่อยที่เตรียมจากภาวะต่างๆ 5 ภาวะ (ความเข้มข้นกรด 0.04% และ 0.06, 120°C, 60-180 นาที) และมีใยอาหารทั้งหมดมากกว่า 30% มาผลิตเป็นมอลโทเด็กซ์ทรินชนิดทนย่อย โดยย่อยสารละลายของตัวอย่างที่ความเข้มข้น 30% ด้วยเอนไซม์อัลฟาแอมิเลสทางการค้า ที่อุณหภูมิ 70°C pH 6 เป็นเวลา 90 และ 120 นาที เพื่อให้ได้ตัวอย่างที่มีค่าสมมูลเด็กซ์โทรส 8 และ 12 ตามลำดับ จากนั้นจึงทำให้บริสุทธิ์และทำแห้งด้วยวิธีทำแห้งแบบพ่นฝอย การย่อยด้วยอัลฟาแอมิเลสทำให้ได้มอลโทเด็กซ์ทรินชนิดทนย่อยที่มีสายโซ่กิ่งสั้นกว่า มีน้ำหนักโมเลกุลเฉลี่ยและค่าอุณหภูมิการเปลี่ยนสถานะคล้ายแก้วของเฟสที่ไม่แข็งตัวที่ผ่านการทำให้เข้มข้นโดยการแช่เยือกแข็งในระดับสูงที่สุด (T_g') ที่ต่ำกว่า แต่มีใยอาหารทั้งหมด ใยอาหารส่วนน้ำหนักโมเลกุลต่ำและสัดส่วนของน้ำที่ไม่แข็งตัวสูงกว่าเด็กซ์ทรินชนิดทนย่อย และยังพบว่ามอลโทเด็กซ์ทรินชนิดทนย่อยมีสัดส่วนของพันธะ β-ไกลโคซิดิกทั้งหมด ระดับความเป็นกิ่งแขนง และสัดส่วนของ α-/ β-reducing ends สูงกว่าเด็กซ์ทรินชนิดทนย่อย นอกจากนี้ยังพบว่ามอลโทเด็กซ์ทรินชนิดทนย่อยที่มีสายโซ่กิ่งสั้น (DP < 5) และใยอาหารส่วนน้ำหนักโมเลกุลต่ำในสัดส่วนที่สูงกว่า แต่มีระดับความเป็นกิ่งแขนงต่ำกว่า มีค่าแอคทิวิตีของพรีไบโอติกสูงกว่าด้วย

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สาขาวิชา	เทคโนโลยีทางอาหาร	ลายมือชื่อนิสิต
		....
ปีการศึกษา	2563	ลายมือชื่อ อ.ที่ปรึกษาหลัก
		....

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Kamonrat Trithavisup : PRODUCTION AND PROPERTIES OF RESISTANT MALTODEXTRIN FROM CASSAVA STARCH. Advisor: Assoc. Prof. Kanitha Tananuwong, Ph.D.

This research aimed to determine the effects of processing conditions on molecular structure and selected properties of cassava-derived resistant dextrins (RDs) and resistant maltodextrins (RMDs). Cassava starch was mixed with hydrochloric acid solution to obtain the final acid concentration of 0.04-0.10% dry starch basis, dried at 50°C until having <5% moisture, and dextrinized at 100-120°C for 60-180 min. As acid concentration, heating temperature and time increased, water solubility, reducing sugar content, total dietary fiber (TDF) content and proportion of high molecular weight fiber fraction of RDs increased while their whiteness decreased. For the solution containing RDs produced under mild conditions (0.04-0.08% HCl, 100°C, 60 min), an endothermic peak at 45-70°C, having enthalpy of 1.66-2.14 J/g was detected. However, no endotherm was detected from the solution of RDs processed under extreme conditions. Harsher dextrinization conditions resulted in the RDs with slightly higher molecular weight but containing shorter branched chains. Dextrinization resulted in the formation of α-1,2, α-1,6, β-1,2, β-1,4 and β-1,6 in RDs. Lower proportion of α-1,4 linkage and α-/β-reducing ends with higher proportion of β-1,2, β-1,4, α-/β-1,6 linkages and degree of branching (DB) were found in RDs prepared under stronger dextrinization conditions. Although indigestible portion was enhanced by dextrinization, TDF of the RDs were only 7-47%. To increase the TDF content, RDs prepared from 5 different conditions (0.04% and 0.06% HCl, 120°C, 60-180 min), having >30% TDF, were selected for RMD production. The 30% RD solutions were treated with commercial α-amylase enzyme at 70°C, pH 6, for 90 and 120 min, to obtain the final dextrose equivalent of 8 and 12, respectively. The samples were then purified and spray dried. Alpha-amylase treatment produced RMD with shorter branched chains, lower molecular weight and glass transition temperature of maximally freeze-concentrated unfrozen phase (T_g'), but having higher TDF content, low molecular weight dietary fiber (LMWDF) content and unfrozen water content, comparing to its RD counterpart. RMDs had higher proportion of all β-glycosidic linkages, DB and α-/β-reducing ends than RDs. It was also found that RMDs with higher proportion of shorter branched chains (DP < 5) and LMWDF fraction, but having lower DB, had higher prebiotic activity score.

Field of Study:	Food Technology	Student's Signature ....
Academic Year:	2020	Advisor's Signature ....

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CHAPTER 1
Introduction

1.1 Overview of the Ph.D. dissertation

Research study in this Ph.D. dissertation focused on the production and properties of soluble dietary fiber namely resistant maltodextrin from cassava starch. The production of resistant maltodextrin comprised of 2 main parts, which were dextrinization and enzymatic treatment. Therefore, this research work was divided into 2 main parts in accordance with major processing steps. The first part was the preparation of resistant dextrins from cassava starch using various acid concentrations, temperatures and time in the dextrinization step. Effects of dextrinization conditions on molecular structure as well as physical, chemical and thermal properties were determined. Research article from the first part, as shown in Chapter 2, was published in Food Chemistry in 2019. The second part was the preparation of resistant maltodextrin via α-amylase, using resistant dextrin containing >30% dietary fiber as substrates. In this part, effects of dextrinization and enzymatic modification on molecular structure and some properties, including dietary fiber content, prebiotic activity and thermal properties of the frozen systems, of cassava-derived resistant dextrins and resistant maltodextrins were evaluated. Research article from this part was present in Chapter 3. The article has been summited for publication in Carbohydrate Polymers. Both research articles were used to partially fulfill the requirements for the degree of Doctor of Philosophy in Food Technology, Department of Food Technology, Faculty of Science, Chulalongkorn University.

1.2 Rationales of the research study

Resistant maltodextrin, also known as a soluble dietary fiber, has been widely used in food and pharmaceutical industries. This is due to its desirable properties including low viscosity, good water solubility and heat, acid and/or enzymes stable characteristic. Resistant maltodextrin can be produced by the combination of dry heat (≤5% moisture, ≥100°C) and acid (≥0.1% dry starch basis (db)) (Bai & Shi, 2016; Chen et al., 2020; Jochym, Kapusniak, Barczynska, & Śliżewska, 2012; Kapuśniak & Jane, 2007; Laurentin, Cárdenas, Ruales, Pérez, & Tovar, 2003; Wurzburg, 1995), called dextrinization or pyroconversion, following by enzymatic treatment. During dextrinization, hydrolysis, transglucosidation and repolymerization will occur. Acid-catalyzed hydrolysis caused the breaking of α-1,4 or 1,6-glucosidic linkages. Then, the aldehydic groups of the ruptured fragment react with free hydroxyls on other fragments to produce branched polymers. This step is known as tranglucosidation. The last step called repolymerization induces the formation of highly-branched polymers with higher molecular weight. (Wurzburg, 1995). After dextrinization, the resulting dextrin solution is hydrolyzed by alpha-amylase. This step involves scission of α-1,4-glycosidic bonds. Therefore, resistant maltodextrin is a highly branched polysaccharides, containing short branched chains linked by α-1,2, α-1,3, α-1,4- α-1,6 and β-glycosidic bonds, and has dextrose equivalent <20 (Hashizume & Okuma, 2009). However, the resulting dextrin from dextrinization without enzymatic treatment can also be categorized as dietary fiber, namely “resistant dextrin” or “pyrodextrin” (Bai & Shi, 2016; Kapuśniak & Jane, 2007; Laurentin et al., 2003; Tomasik, Wiejak, & Pałasiński, 1989; Wurzburg, 1995).

Resistant dextrin and resistant maltodextrin have physiological properties associated with soluble dietary fiber. They can improve the intestinal regularity and moderation of postprandial blood glucose levels (Hashizume & Okuma, 2009; Lefranc-Millot, Wils, Roturier, Le Bihan, & Saniez-Degrave, 2009; Pasman, Wils, Saniez, & Kardinaal, 2006). Moreover, Kishimoto, Oga, Tagami, Okuma, and Gordon (2007) found that resistant maltodextrin can suppresses postprandial elevation of blood triacylglycerol and cholesterol levels. Miyazato, Nakagawa, Kishimoto, Tagami, and Hara (2010) reported that resistant maltodextrin led to promotion of some mineral absorption in rat. In addition, resistant maltodextrin possesses prebiotic activity, as they can promote the growth of probiotic bacteria including *Bifidobacterium* spp. (Hashizume & Okuma, 2009). The example of commercially available resistant dextrin is Nutriose®. It is a soluble dextrin obtained from wheat or corn, Nutriose® contains about 42% less energy than starch (Fouache, Duflot, & Looten, 2003; Vermorel et al., 2004) due to its high dietary fiber content (≤85%) (Roquette, 2017). Whereas Fibersol® is one of the commercially available corn-based resistant maltodextrins containing approximately 90% dietary fiber. (ADM, 2020; Roquette, 2017).

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Previous studies reported that both starch sources and processing conditions affected the molecular structure and properties of resistant dextrins. Laurentin et al. (2003) found that dietary fiber content and molecular weight profiles of resistant dextrins prepared under similar conditions varied with starch source. Several research studies (Bai, Cai, Doutch, Gilbert, & Shi, 2014; Han, Kang, Bai, Xue, & Shi, 2018; Kapuśniak & Jane, 2007; Lin, Lin, Zeng, Wu, & Chang, 2018; Toraya-Avilés, Segura-Campos, Chel-Guerrero, & Betancur-Ancona, 2017b) reported that increasing

heating temperature and time during dextrinization process led to an increase in water solubility, reducing sugar content and indigestible fraction of the derived products while the product had lower molecular weight. Lin et al. (2018) reported that dextrinization with higher acid concentration yielded the resistant dextrins with increasing water solubility and enzyme-resistant fraction but decreasing molecular weight. Moreover, Laurentin et al. (2003); Lin et al. (2018) and Toraya-Avilés et al. (2017b) found that resistant dextrins had darker color when prepared under stronger conditions. New glycosidic linkages that could not be hydrolyzed by enzymes in human gastrointestinal tract were found in resistant dextrins (Bai & Shi, 2016; Han et al., 2018). This could be used to explain the presence of indigestible fraction after dextrinization. However, reports on the effects of various processing parameters on the structure and properties of resistant maltodextrins were still scarce.

Cassava is one of the important crops in Thailand. Its starch has been widely utilized in food and non-food industries. This type of starch can be a potential raw material for production of resistant dextrins and resistant maltodextrins. There have been several reports on the production of resistant dextrins from cassava starch (Laurentin et al., 2003; Toraya-Avilés, Segura-Campos, Chel-Guerrero, & Betancur-Ancona, 2017a; Toraya-Avilés et al., 2017b). However, research studies regarding the production of resistant maltodextrin from cassava starch are still limited. In the current study, this topic was thus focused on.

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1.3 Objectives of the research study

This research aimed to determine the effects of dextrinization and α-amylase hydrolysis on molecular structure and selected properties, including physical,

chemical and thermal properties of resistant dextrins and resistant maltodextrins from cassava starch.

1.4 Scope of the research study

This research was divided into 2 main parts. The first part was to determine physical, chemical and thermal properties, as well as molecular structure of the cassava-derived resistant dextrins prepared under different conditions. Acid concentration, temperature and time during dextrinization step were varied to 0.04-0.10% HCl, 100-120°C, 60-180 min, respectively. The resistant dextrins containing more than 30% of total dietary fiber were selected as raw materials for resistant maltodextrin productions in the second part. The α-amylase treatment was the main step of the production, which helped eliminate the digestible portion containing α-1,4-glycosidic bonds and thus increasing dietary fiber content of the resistant maltodextrins. The suitable hydrolysis time was selected as the time that provided the resisitant maltodextrin with highest dextrose equivalent. Molecular structure, dietary fiber content, prebiotic activity score and thermal properties of the frozen systems of resistant maltodextrins were determined.

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1.5 Beneficial outcome(s) of the research study

Effects of processing parameters, during dextrinization and/or enzymatic hydrolysis steps, on some important properties of resistant dextrins and resistant maltodextrins from cassava starch were obtained. New insights into their structure-function relationships were also be achieved. Overall information from this study could be applied for preparing the starch-derived soluble dietary fibers with specific molecular structures and desirable properties, especially those related to health benefits.

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CHAPTER 2

In-depth study of the changes in properties and molecular structure of cassava starch during resistant dextrin preparation

Kamonrat Trithavisup[a], Kuakarun Krusong[b] and Kanitha Tananuwong[a,*]

[a] *Department of Food Technology, Faculty of Science, Chulalongkorn University, Bangkok, 10330, Thailand*

[b] *Structural and Computation Biology Research Unit, Department of Biochemistry, Faculty of Science, Chulalongkorn University, Bangkok, 10330, Thailand*

*Corresponding author. Tel.: +66-2-218-5515-6. Fax: +66-2-254-4314

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Abstract

Physical, chemical and thermal properties, as well as molecular structure of cassava-based resistant dextrins prepared under different dextrinization conditions (0.04-0.10% HCl, 100-120ºC, 60-180 min) were determined. Increasing acid concentration, temperature and heating time resulted in the products with darker color, higher solubility, reducing sugar content, total dietary fiber and proportion of high molecular weight fiber fraction. An endothermic peak at 45-70°C, having enthalpy of 1.66-2.14 J/g, was found from the samples processed under mild conditions (0.04-0.08% HCl, 100°C, 60 min). However, harsher dextrinization conditions eliminated this endotherm. Dextrinization led to 1000-fold decrease in weight-average molecular weight (M_w) of the products, comparing to the native starch. Stronger processing conditions yielded the resistant dextrins with slightly higher M_w but composing of shorter branched chains. During dextrinization, hydrolysis was a predominant step, while transglucosidation and repolymerization played key roles in modifying molecular structure and properties, especially dietary fiber content, of resistant dextrins.

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Keywords: Resistant dextrin, Pyrodextrin; *Manihot esculenta*; Dextrinization; Properties; Dietary fiber; Molecular structure

1. Introduction

Resistant dextrin, generally known as pyrodextrin, is a starch-derived product that partially resists the enzymatic hydrolysis in human gastrointestinal tract. This highly-branched carbohydrate polymer contains relatively short chains of glucose oligomers/polymers linked together with many types of non-digestible glycosidic bonds, in addition to the α-1,4- and α-1,6-glycosidic bonds found in starch molecules (Han et al., 2018; Nunes et al., 2016). Resistant dextrin is produced via pyroconversion or dextrinization, using the combination of dry heat (≤5% moisture, ≥100°C) and acid (≥0.1% dry starch basis (db)) (Jochym et al., 2012; Kapuśniak & Jane, 2007; Laurentin et al., 2003; Wurzburg, 1995). Resistant dextrin has been recognized as a soluble dietary fiber with distinct properties. It has been widely used in functional food and beverage products since it provides low viscosity, excellent water solubility and exceptional heat stable characteristic. A number of physiological benefits from this starch-derived dietary fiber have also been proven. These include the ability to reduce glycemic effect of food products, decrease the level of plasma triglycerides, increase the absorption and retention of mineral and its prebiotic activity (Lefranc-Millot et al., 2009; Pasman et al., 2006).

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Characteristics of resistant dextrins depend on both starch sources and processing conditions. Laurentin et al. (2003) studied the production of resistant dextrins via dextrinization of cassava, cocoyam, lentil, maize, sagu and sorghum starch. Under similar processing conditions (140ºC, 180 min, 0.182% HCl, db), non-digestible fraction of the derived products ranged from 42.6 to 67.4%, depending on starch type used. Major carbohydrate fractions (52-63%) of the resistant dextrins obtained from cassava, cocoyam, lentil, maize and sagu starch had the molecular

weight distributed from 8 to 105 kDa, while molecular weight of the major fraction found in the sorghum-derived resistant dextrin was greater than 105 kDa. Numerous studies showed that processing conditions greatly affected chemical structure, physicochemical properties and digestibility of resistant dextrins. Higher temperature and longer time used in the pyroconversion process resulted in the resistant dextrin with greater solubility, reducing sugar content and non-digestible fraction, but the product had lower molecular weight (Bai, Cai, et al., 2014; Han et al., 2018; Kapuśniak & Jane, 2007; Lin et al., 2018; Toraya-Avilés et al., 2017b). As acid concentration increased, water solubility and enzyme-resistant fraction of the derived products increased while their molecular weight decreased (Lin et al., 2018). Resistant dextrin produced under harsh conditions (greater acid concentration, higher temperature and/or longer processing time) usually had darker color (Laurentin et al., 2003; Lin et al., 2018; Toraya-Avilés et al., 2017b). However, under the similar processing condition, resistant dextrin prepared with acetic acid was shown to have lighter color comparing to that modified with HCl (Lin et al., 2018).

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Cassava is one of the major food crops utilized in starch production. Previous studies determined the application of cassava starch in resistant dextrin production (Laurentin et al., 2003; Toraya-Avilés et al., 2017a; Toraya-Avilés et al., 2017b). However, detailed studies of the molecular structure as well as the structure-function relationship of the resistant dextrins prepared from cassava starch using different processing parameters are still limited. Therefore, this research aimed to investigate the physical, chemical and thermal properties, as well as molecular structure of the cassava-derived resistant dextrins prepared under different conditions. In comparison with the previous studies, milder conditions (lower acid concentration and

temperature range) were applied for the preparation of resistant dextrins in current study. Data from this research could provide better understanding in structural modification during dextrinization process. A guideline for producing the resistant dextrin with desirable properties and functions could also be obtained.

2. Materials and methods

2.1 Materials

Cassava starch was obtained from General Starch, Ltd. (Nakhon Ratchasima, Thailand). The starch was prepared from mixed varieties of cassava roots grown in Nakhon Ratchasima province, Thailand, using industrial scale production line. Moisture content and amylose content of the cassava starch samples were 12-13% and 34-37%, respectively.

2.2 Preparation of resistant dextrins

Overall process of resistant dextrin production was explained as followed. For each batch, three kilograms of cassava starch were mixed with 60 mL of HCl solution. After an overnight equilibration at room temperature, the sample was dried at 50ºC in a rotary roaster for 6 h until its moisture content was less than 5%. Dextrinization process was then performed in the same equipment. Acid concentration used in this study varied from 0.04 to 0.1% db. Dextrinization temperatures and durations were 100-120ºC and 60-180 min, respectively. Counting of the heating time started when sample temperature reached the target temperature. Moisture content was determined with hot air oven, according to AOAC 925.10 method (AOAC, 2012). The resulting resistant dextrins had 0.90-4.15% moisture.

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2.3 Physical and chemical properties of native starch and resistant dextrins

The following physical and chemical properties of the samples before and after pyroconversion were determined. Water solubility was determined by centrifugation method (Kapuśniak & Jane, 2007). One gram of the sample was mixed with 10 mL of distilled water. The mixture was stirred at room temperature for 30 min, and centrifuged with benchtop centrifuge (C2006, Centurion Scientific, UK) at 3000×g for 20 min. The separated supernatant was then dried in a hot air oven using 2-step drying, 60°C overnight and 110°C for 1 h. Water solubility was calculated as percentage of solid content in supernatant based on dried sample weight. Reducing sugar was determined by the method of Nelson (1944), with slight modification. Briefly, one milliliter of the sample solution (30% solid content) was mixed with 1 mL of Nelson's copper reagent. The mixture was then heated in a boiling water bath for 20 min. After cooling down, one milliliter of arsenomolybdate color reagent was added. The final volume of the mixture was adjusted to 25 mL. Absorbance of the sample was measured at 745 nm. Standard curve using glucose solution (10 - 100 µg/mL) was constructed for the calculation of reducing sugar content.

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Color of powder samples and 10% (w/v) resistant dextrin solution were measured in CIELAB (L*, a*, b*) system using Minolta Chroma Meter CR-300 (Konica Minolta Sensing, Osaka, Japan). Color difference (ΔE) of resistant dextrin samples was calculated with the following equation (Marcos, Kerry, & Mullen, 2010).

$$\text{Color difference } (\Delta E) = \sqrt{(L^* - L_c^*)^2 + (a^* - a_c^*)^2 + (b^* - b_c^*)^2} \quad (1)$$

Where L*, a* and b* represented color parameters of the resistant dextrin samples and L_c^*, a_c^* and b_c^* represented color parameters of the control, which was resistant dextrin prepared by using 0.04% HCl, and heated at 100ºC for 60 min.

The calculation of whiteness index of samples was performed as followed (Judd & Wyszecki, 1963).

$$\text{Whiteness index} = 100 - \sqrt{(100 - L^{*2}) + a*^{2} + b*^{2}} \quad (2)$$

Where $L*$, $a*$ and $b*$ represented color parameters of the resistant dextrin samples.

2.4 Total dietary fiber content of resistant dextrins

Total dietary fiber content was determined according to AOAC official 2009.01 methods (AOAC, 2012) using enzymatic assay kit (K-INTDF, Megazyme International Ireland Ltd., County Wicklow, Ireland). Briefly, the sample was hydrolyzed by pancreatic α-amylase, amyloglucosidase and protease to remove the digestible part. The sample was then precipitated in 78% aqueous ethanol. Dietary fiber fractions in the supernatant and the remaining residue were separately determined.

In this study, dietary fiber of the resistant dextrins was categorized into 2 main fractions, high molecular weight dietary fiber (HMWDF) and low molecular weight dietary fiber (LMWDF). HMWDF fraction was defined as sum of the dried residues of the insoluble dietary fiber and the dietary fiber that was soluble in water but precipitated in 78% ethanol (aq.). This fraction was gravimetrically determined. LMWDF, consisting of non-digestible oligosaccharides, was the dietary fiber that was still soluble in 78% ethanol (aq.). This fraction was quantitatively determined by high performance liquid chromatography according to AOAC official 2009.01 methods (AOAC, 2012). D-sorbitol was used as an internal standard for the analysis.

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2.5 Thermal properties of resistant dextrins

Thermal properties of the samples were determined according to the method of Tananuwong and Malila (2011) with slight modification. Differential scanning

calorimeter (DSC) (Diamond DSC, Perkin-Elmer Co., Norwalk, CT, USA) equipped with Intracooler 2P (Perkin-Elmer) was used in the analysis. Nitrogen was used as purged gas. Starch or resistant dextrin sample (3 mg) was mixed with deionized water (9 mg) in an aluminum DSC pan (part no. 02190062) and equilibrated overnight at room temperature. The sample was heated at 10ºC/min from 30ºC to 90ºC. An empty aluminum DSC pan was used as reference. Onset temperature (T_o), peak temperature (T_p), conclusion temperature (T_c) and enthalpy of gelatinization (ΔH) were analyzed by Pyris™ operation software version 12 (Perkin-Elmer Co., Norwalk, CT, USA). The range of gelatinization temperature (ΔT) was calculated by the difference between T_o and T_c.

2.6 Characterization of resistant dextrin structures

2.6.1 Molecular weight distribution

The molecular weight distributions of resistant dextrins were obtained by a high-performance size-exclusion chromatography (HPSEC) system (Agilent 1100 Series, Agilent Technologies, Inc., Waldbronn, Germany) according to the method of Saeaurng and Kuakpetoon (2018) with slight modification. Ten milligrams of the resistant dextrin samples were dissolved in 1 mL of deionized water. The solution was filtered through 0.45 µm membrane filter. The sample was injected into HPSEC system, comprising of gel filtration column (TSK G4000 PWXL, Tosoh Corp., Tokyo, Japan) (21.5 mm ID x 30 cm, 17 µm) and refractive index detector (Agilent 1100 Series, Agilent Technologies, Inc., Waldbronn, Germany). The temperature of the detector was 40ºC. Sodium azide solution (0.02% w/v) was used as eluent at a flow rate of 3 mL/min. Pullulan (Shodex standard P-82 kit, Showa Denko K.K., Kanagawa, Japan) with weight-average molecular weights from 5,900 to

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788,000 g/mol were used as standards. The weight-average molecular weight (M_w), number-average molecular weight (M_n) and polydispersity index (I) of samples was calculated with the following equation.

$$M_w = \frac{\sum n_i M_i^2}{\sum n_i M_i} \quad (3)$$

$$M_n = \frac{\sum n_i M_i}{\sum n_i} \quad (4)$$

$$I = \frac{M_w}{M_n} \quad (5)$$

When M_i represented the molecular weight of samples which was calculated from standard curve and n_i represented the signal of refractive index detector.

2.6.2 Chain length distribution

The chain-length distribution of resistant dextrins was characterized by high-performance anion-exchange chromatography equipped with a pulsed amperometric detector (HPAEC-PAD) according to the method of Kuakpetoon and Wang (2007) and Jochym et al. (2012) with some modifications. Fifty milligrams of resistant dextrin samples were mixed with 4.79 mL of 20 mM acetate buffer (pH 3.5) and 10 µL of isoamylase (activity of 600 enzyme units, Hayashibara Biochemical Laboratories Inc. Okayama, Japan). The mixture was incubated at 40ºC for 24 h. The enzyme was inactivated by adding 0.2 mL of 0.1 M sodium hydroxide. Five milliliters of ultrapure water were added. The sample was then filtered through a 0.45 µm membrane filter. Chromatographic separation was performed using an ICS-5000 ion chromatograph (Dionex, Sunnyvale, CA, USA) with a Carbopac PA-100 column (4 mm × 250 mm) (Dionex). Mixtures of solvent A (100 mmol/L sodium hydroxide) and

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solvent B (100 mmol/L sodium hydroxide and 1 mol/L sodium acetate), at a flow rate of 0.5 mL/min, were used as mobile phase. The following gradient elution was used: 100% A at 0 min; 100-65% A + 0-35% B at 0.1-45 min; 65% A + 35% B at 45-68 min; 65-100% A + 35-0% B at 68-70 min; 100% A at 70-73 min.

2.7 Statistical analysis

All experiments were performed in triplicates. Completely randomized design was applied for the experiments, except chain length distribution analysis. Analysis of variance was performed. Mean comparison was done using Duncan’s new multiple range test. All statistical analyses were performed by IBM SPSS statistics software, version 22 (Windows, SPSS Inc., Chicago, IL, USA).

3. Results and discussion

3.1 Physical and chemical properties of resistant dextrins

Due to the technical issue regarding processing equipment used in this study, resistant dextrin samples could not be obtained from some of the strong conditions. At 120°C, stronger acid concentration (0.08-0.10%) and longer heating time (180 min) resulted in burnt resistant dextrin powder (having black or dark brown color). Those samples were thus excluded from the study.

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Physical and chemical properties of resistant dextrins were shown in Table 1. The dextrin samples prepared under milder conditions (100ºC, 60 min, 0.04-0.08% HCl) had significantly lower water solubility (88-94%) ($p \leq 0.05$). In contrast, the samples prepared under stronger conditions had nearly 100% solubility, comparable to that of the commercially available resistant dextrin sample (data not shown). Commercial food-grade resistant dextrin used in this study was claimed to produce from wheat or corn starch via dextrinization process, followed by a chromatographic

fractionation step (Fouache et al., 2003). Higher acid concentration, higher temperature and longer heating time also resulted in the samples with significantly greater reducing sugar content ($p \leq 0.05$). Lin et al. (2018) prepared resistant dextrin by heating corn starch at 140-180ºC for 180 min in presence of acids (0.5-2.5% acetic acid or 0.05-0.2% HCl). They found that solubility of the resistant dextrins increased with increasing pyrolytic temperature and acid concentration. In addition, Kapuśniak and Jane (2007) studied the dextrinization of corn starch using 0.1% HCl (db) and heating temperature of 130ºC. They found that increasing process time from 0 to 180 min resulted in increasing solubility and reducing sugar of the dextrins.

Three major stages of structural changes during dextrinization are hydrolysis, transglucosidation and repolymerization. Hydrolysis involves acid-catalyzed breaking of α-1,4- or α-1,6-glycosidic bonds (Tomasik et al., 1989; Wurzburg, 1995). Initial moisture content of starch and water molecules released via dehydration during initial period of dextrinization are likely to induce the hydrolysis (Bai & Shi, 2016). The aldehydic groups on the molecular fragments can further combined with free hydroxyls on other fragments, yielding structures that may contain random α- and β-linkages at the position of 1,2-, 1,3-, 1,4- and 1,6-glycosidic bonds. This step is called transglucosidation (Tomasik et al., 1989; Wurzburg, 1995). Mixed types of glycosidic linkages within the resistant dextrin structure was reported in the previous study. Bai and Shi (2016) reported the preparation of resistant dextrin sample from waxy maize starch by adjusting the pH of the starch suspension to 3.0 with 0.5 M HCl. The suspension was further filtered and dried at 40°C, 24 h, to reach final moisture content of 10 - 15%. The sample was then dextrinized at 170°C for 4 h. Based on NMR spectroscopic technique, the authors showed that new glycosidic linkages, including

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α-1,6, β-1,6, α-1,2 and β-1,2, were identified in the resistant dextrin. The last step named as repolymerization leads to the formation of the highly-branched dextrins with greater molecular weight. This step will occur at greater extent under stronger conditions, including higher temperature and longer time (Tomasik et al., 1989; Wurzburg, 1995). Repolymerization via the formation of α-1,6-glycosidic linkages has been proposed as one possible reaction leading to the highly-branch structure (Bai & Shi, 2016). Nevertheless, significant increase in solubility and reducing sugar after extensive dextrinization could imply that hydrolysis was the predominant step. This presumption could further be supported by the results of molecular structure in the following section.

Effects of processing parameters on color of the resistant dextrins were determined from color difference (ΔE), which was calculated based on the sample prepared with mildest condition (0.04% HCl, 100ºC, 60 min). Selection of this resistant dextrin sample, instead of raw cassava starch, as a reference material for ΔE calculation was mainly based on its color and solubility. Color of this reference in the powder form was nearly similar to that of the cassava starch, as shown by insignificant difference between whiteness index of this sample and the original starch ($p > 0.05$) (Fig. 1). Moreover, unlike highly turbid starch suspension, this reference had ~94% solubility (Table 1), providing the 10% solution with negligible turbidity. Therefore, this reference was appropriate for the ΔE calculation of both powder and 10% solution samples. Data from Table 1 indicated that ΔE of the samples in both forms tended to increase with increasing acid concentration, temperature and heating time. These results corresponded with the appearance and whiteness index of the resistant dextrins (Figure 1a, b). Harsher conditions resulted in darker color of both

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powdered samples and the 10% solution (Figure 1a). At the acid concentration beyond 0.04%, higher temperature and longer duration of dextrinization led to significantly lower whiteness index of the powdered resistant dextrins ($p \leq 0.05$) (Figure 1b). Our results corresponded to the results from previous studies. Lin et al. (2018) reported that resistant dextrins prepared with higher acid concentration and temperature were more intense brown and had higher ΔE value. Toraya-Avilés et al. (2017b) found that cassava resistant dextrin prepared from extreme reaction conditions (80:1 acid concentration, 110ºC temperature and 3 hours reaction time) produced the highest ΔE value. Darker color developed during dextrinization could be related to caramelization reaction. Rate of this reaction depended on temperature and pH (Laurentin et al., 2003). It was also important to note that most of the powdered samples and their 10% solution had ΔE > 5, indicating that the color difference could be visibly detected (Luchese, Abdalla, Spada, & Tessaro, 2018). Decolorization step was thus an important step for commercial production of resistant dextrins with high dietary fiber content (see the dietary fiber data in the following section).

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Table 1. Solubility, reducing sugar and color difference (ΔE) of cassava-derived resistant dextrins prepared under different conditions.

Acid conc. (%, dsb)	Temp. (°C)	Time (min)	Solubility (%, db)	Reducing sugar (mg/g dry dextrin)	ΔE (Powder)	ΔE (solution, 10% (w/v))
0.04	100	60	$93.98^{d} \pm 0.72$	$12.75^{i} \pm 0.14$	-	-
		120	$97.69^{c} \pm 1.90$	$14.29^{h} \pm 0.40$	1.45	3.00
		180	$99.26^{abc} \pm 0.09$	$18.16^{ef} \pm 0.39$	2.64	3.56
0.06	100	60	$87.96^{e} \pm 1.65$	$14.19^{h} \pm 0.44$	1.16	4.03
		120	$97.82^{c} \pm 0.62$	$15.07^{h} \pm 0.48$	3.61	4.38
		180	$99.47^{ab} \pm 0.58$	$18.43^{def} \pm 1.28$	7.44	4.60
0.08	100	60	$94.32^{d} \pm 1.10$	$14.28^{h} \pm 0.57$	3.49	4.54
		120	$99.10^{abc} \pm 1.17$	$16.28^{g} \pm 1.09$	6.76	5.02
		180	$99.66^{ab} \pm 0.20$	$20.72^{bc} \pm 1.30$	8.10	5.32
0.10	100	60	$98.19^{bc} \pm 1.23$	$14.57^{h} \pm 0.41$	7.27	5.45
		120	$98.45^{abc} \pm 0.74$	$17.25^{fg} \pm 0.49$	13.02	6.00
		180	$99.83^{a} \pm 0.26$	$21.74^{ab} \pm 0.54$	15.01	7.57
0.04	120	60	$99.27^{abc} \pm 0.66$	$18.94^{de} \pm 0.62$	10.39	5.16
		120	$99.42^{ab} \pm 0.78$	$19.62^{cd} \pm 0.67$	12.88	6.91
		180	$99.85^{a} \pm 0.12$	$21.89^{ab} \pm 0.70$	14.94	7.60
0.06	120	60	$98.22^{abc} \pm 1.22$	$17.34^{fg} \pm 0.84$	13.35	6.61
		120	$99.06^{abc} \pm 0.68$	$22.06^{a} \pm 0.32$	19.07	9.52

Means with different letters within the same column are significantly different ($p \leq 0.05$)

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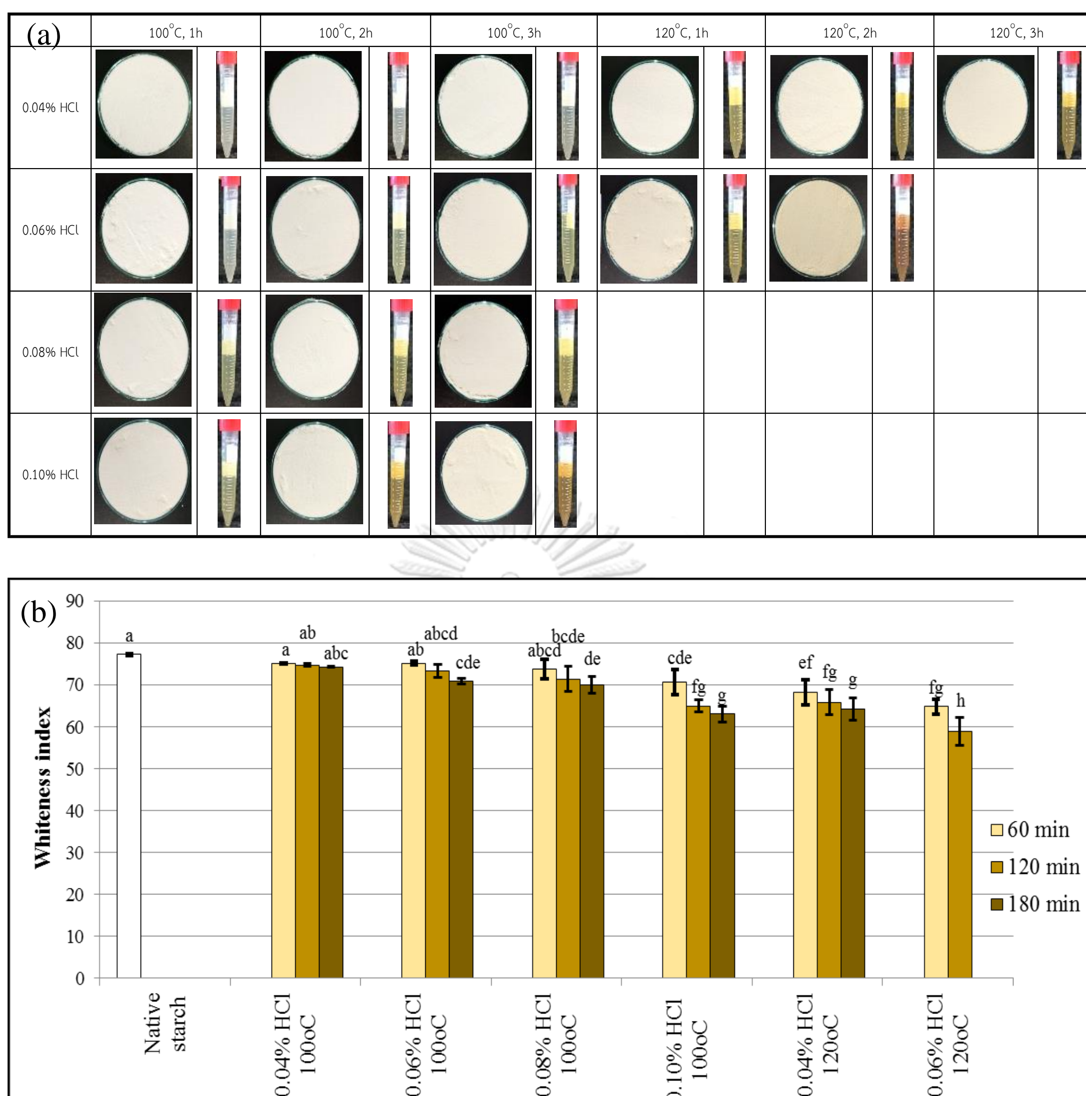

Fig. 1 Appearance of powdered and solution (10% w/v) of resistant dextrins prepared under different conditions (a) and whiteness index of powdered cassava starch and resistant dextrins (b). Means with different letters are significantly different ($p \leq 0.05$).

3.2 Thermal properties of resistant dextrins

According to DSC investigation (Table 2, Fig S1), single endothermic peak was found from each resistant dextrin sample prepared under mild condition (0.04-0.08% HCl, 100ºC, 60 min). Endotherm from those resistant dextrin samples had

lower onset, peak, conclusion temperature and melting enthalpy than the parameters found in the native cassava starch. For the samples prepared under stronger conditions, no endotherm was found. Disappearance of the endothermic peak after dextrinization at strong condition was reported. Laurentin et al. (2003) acidified cassava starch with 0.182% HCl (db) and heated the sample at 140ºC for 180 min. No endotherm was detected in this resistant dextrin sample. Result from the DSC experiment implied that resistant dextrins prepared from milder conditions might still have some ordered structures. Those mild conditions might not be sufficient to induce extensive hydrolysis, leading to complete destruction of the crystalline lamella and other ordered structures (i.e. double helical structure of amylopectin). However, the ordered structures were much weaker and remained in much lower amount in comparison with those in the native starch. Stronger conditions: higher acid concentration, temperature and longer time, could cause the greater degree of hydrolysis and transglucosidation, resulting in the complete loss of those ordered structure. These results corresponded well with the solubility of resistant dextrins (Table 1). Resistant dextrins that provided DSC endothermic peak had less than 95% solubility. However, resistant dextrins produced under extreme conditions had nearly 100% solubility, indicating the complete loss of ordered structure after dextrinization.

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Table 2. Thermal properties of cassava-derived resistant dextrins prepared under different conditions.

Acid conc. (%, dsb)	**Temp. (ºC)**	**Time (min)**	**T_o (ºC)**	**T_p (ºC)**	**T_c (ºC)**	**ΔT (ºC)^{ns}**	**ΔH (J/g dry dextrin)**
Native cassava starch	-	-	$56.50^{a} \pm 0.78$	$68.12^{a} \pm 0.19$	$80.66^{a} \pm 0.83$	24.17 ± 0.64	$15.60^{a} \pm 0.45$
0.04	100	60	$45.08^{b} \pm 0.67$	$55.97^{b} \pm 0.11$	$68.07^{c} \pm 0.14$	22.99 ± 0.78	$1.66^{b} \pm 0.42$
0.06	100	60	$45.68^{b} \pm 0.88$	$56.87^{b} \pm 0.90$	$69.03^{bc} \pm 0.77$	23.36 ± 0.85	$2.14^{b} \pm 0.39$
0.08	100	60	$45.13^{b} \pm 0.48$	$56.25^{b} \pm 0.19$	$69.94^{b} \pm 0.60$	24.81 ± 0.55	$1.8\ 1^{b} \pm 0.28$

Means with different letters within the same column are significantly different ($p \leq 0.05$).

T_o = onset gelatinization temperature, T_p = peak gelatinization temperature, T_c = conclusion gelatinization temperature, ΔT = gelatinization temperature range (T_c-T_o), ΔH=enthalpy of gelatinization

^{ns}Means within the same column are not significant difference (p >0.05).

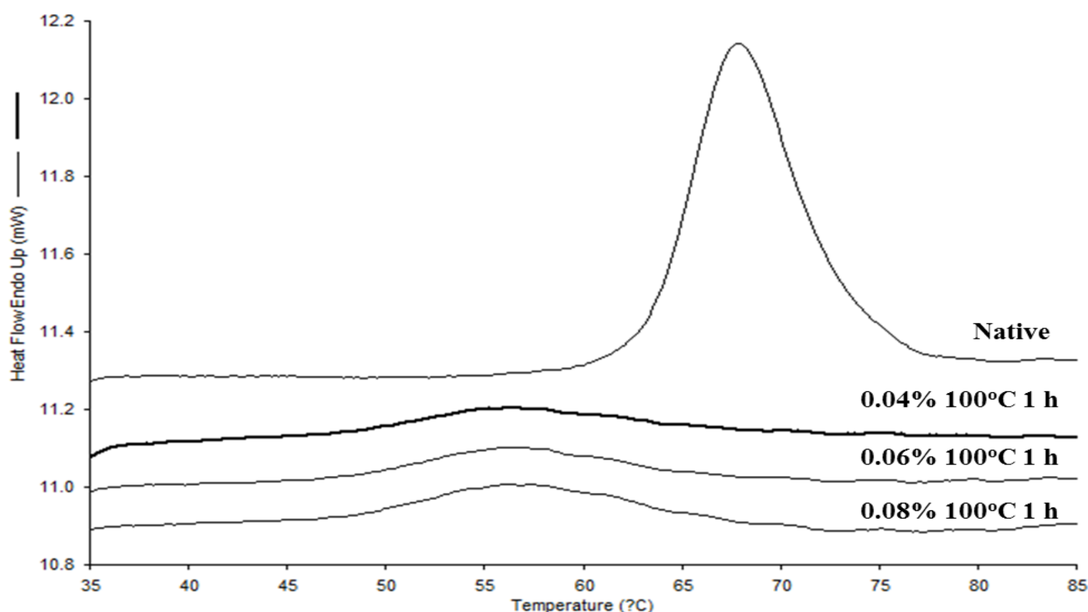

Fig. S1 DSC diagrams of cassava-derived resistant dextrins prepared under different dextrinization conditions.

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3.3 Indigestible fraction of resistant dextrins

Total dietary fiber content of resistant dextrins apparently increased with increasing acid concentration, heating temperature and time (Figure 2, Table S1). This trend corresponded to the previous report. Cao et al. (2018) prepared the resistant dextrin samples using the same procedure as reported by Han et al. (2018), except varying heating time within the range of 0.5 – 4 h. They found that total dietary fiber content of pyrodextrin samples increased from 28.4% to 46.3% as heating time increased. However, proportion of the high molecular weight dietary fiber (HMWDF) and low molecular weight dietary fiber (LMWDF) in the resistant dextrins depended on the modification conditions. Dextrinization at any milder conditions (100ºC, 60 and 120 min, 0.04-0.06% acid) yielded a sample with nearly similar proportion of the HMWDF and LMWDF within the same sample. However, further increase in

temperature, heating time and acid concentration resulted in the resistant dextrins that contained HMWDF as a major fraction (60-80% of DF). As for the mechanistic-based aspect, transglucosidation and repolymerization could be crucial steps that increased the overall dietary fiber content and governed the molecular weight of those indigestible portions.

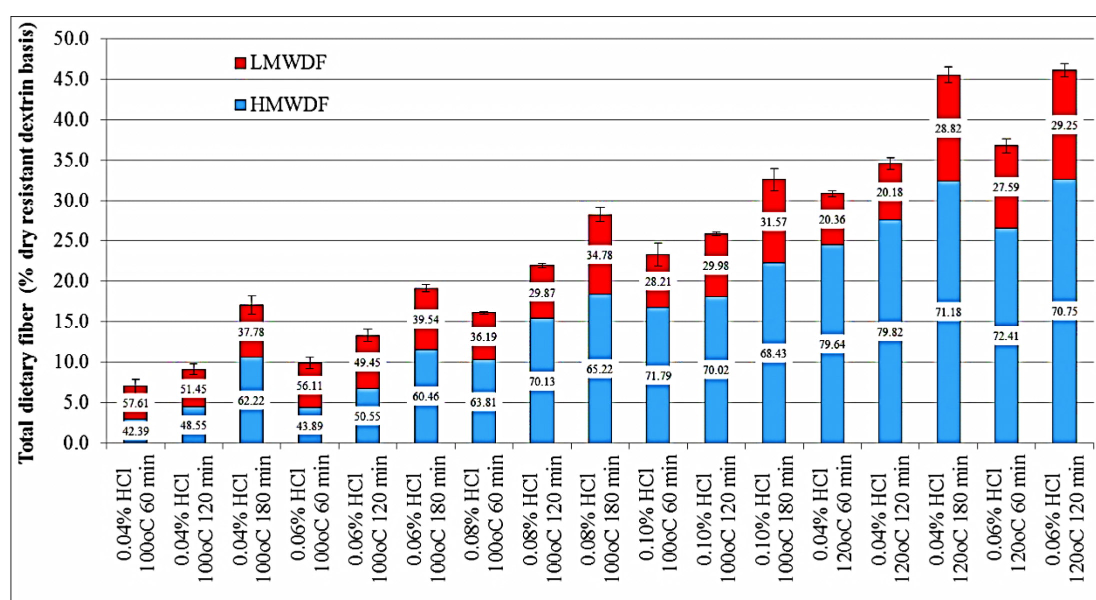

Fig. 2 Total dietary fiber of cassava-derived resistant dextrins prepared under different conditions.

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Although HMWDF was the major indigestible portion found in resistant dextrins prepared under extreme conditions (0.08-0.1% HCl, 100-120ºC), content of this fraction tended to fluctuate as heating time increased. At similar acid concentration and temperature applied, increasing heating time from 60 to 120 min led to an increase in the percentage of HMWDF fraction. However, further extension from 120 min to 180 min caused a slight decrease in this value (Figure 2, Table S1).

In these cases, prolonged heating could induce greater degree of transglucosidation, which might overcome the effects of repolymerization, thus resulted in small reduction of HMWDF fraction in the samples. As water molecules become scarce, longer heating time could induce greater extent of chain scission with the arising of oxocarbenium cations or free radicals. These newly formed structures were not only able to attack the hydroxyl groups of the surrounding molecules, causing an increase in branching structure, but could also induce the formation of intramolecular glycosidic linkages. An oxocarbenium cation or a free radical could react with an intramolecular C6 hydroxyl group, causing the formation of 1,6-anhydro-β-d-glucopyranosyl unit (levoglucosan) (Tomasik et al., 1989). This reaction could cause a decrease in reducing ends, which might retarded the repolymerization step and eventually resulted in slightly lower HMWDF fraction. The existence of levoglucosan end group in the resistant dextrin structure was proven by the NMR spectroscopic technique (Bai & Shi, 2016).

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Table S1. Dietary fiber content of cassava-derived resistant dextrins prepared under different dextrinization conditions.

Acid conc. (%, dsb)	Temp. (°C)	Time (min)	% HMWDF	% LMWDF	% TDF
0.04	100	60	2.97^{m} ± 0.08	4.43^{h} ± 0.83	7.40^{l} ± 0.88
		120	4.42^{l} ± 0.16	5.30^{gh} ± 0.49	9.72^{k} ± 0.64
		180	10.6^{j} ± 0.39	7.32^{def} ± 1.48	17.94^{i} ± 1.79
0.06		60	4.35^{l} ± 0.30	6.43^{fg} ± 0.70	10.79^{k} ± 0.77
		120	6.72^{k} ± 0.52	7.47^{def} ± 0.45	14.20^{j} ± 0.84
		180	11.58^{i} ± 0.09	8.49^{de} ± 0.47	20.08^{h} ± 0.38
0.08		60	10.27^{j} ± 0.27	6.57^{efg} ± 0.41	16.84^{i} ± 0.15
		120	15.38^{h} ± 0.15	7.41^{def} ± 0.30	22.79^{g} ± 0.36
		180	18.41^{f} ± 0.15	10.49^{bc} ± 1.00	28.91^{e} ± 1.12
0.10		60	16.73^{g} ± 1.07	7.54^{def} ± 0.38	24.27^{g} ± 1.45
		120	18.11^{f} ± 0.39	8.73^{cd} ± 0.50	26.84^{f} ± 0.34
		180	22.28^{e} ± 0.44	11.07^{b} ± 2.28	33.36^{d} ± 2.15
0.04	120	60	24.57^{d} ± 0.16	6.93^{defg} ± 0.39	31.51^{d} ± 0.30
		120	27.60^{b} ± 0.26	7.74^{def} ± 0.49	35.35^{c} ± 0.75
		180	32.43^{a} ± 0.03	13.37^{a} ± 2.31	45.80^{a} ± 2.33
0.06		60	26.62^{c} ± 0.01	10.97^{b} ± 0.97	37.59^{b} ± 0.97
		120	32.62^{a} ± 0.12	14.18^{a} ± 1.30	46.80^{a} ± 1.25

Means with different letters within a column are significantly different ($p \leq 0.05$).

3.4 Molecular weight and chain length distribution of resistant dextrins

Resistant dextrins prepared from specific conditions, using 0.04-0.06% HCl, were selected for studying effect of processing conditions on their molecular structures. Most of the selected samples contained relatively high total dietary fiber (>30%). Molecular weight distribution of the resistant dextrins determined by HPSEC

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was shown in Table 3 and Fig S2. M_w and M_n of the resistant dextrins prepared from this study were within the range of 11-15 kDa and 6.3-8.1 kDa, respectively. These M_w values were much smaller than M_w of native cassava starch (21,870 kDa) (Xia, Li, & Gao, 2017). Polydispersity index of the resistant dextrins (I), representing the width of the molecular mass distribution, ranged from 1.7 to 1.9. These I values were relatively similar to those of the resistant dextrins from potato starch (I values of 1.2-1.6) (Kapusniak & Nebesny, 2017). In addition, M_w and M_n slightly increased as heating time increased. Kapuśniak and Jane (2007) also reported that extended dextrinization of corn starch (0.1% HCl, 130ºC, 60-180 min) resulted in slight increase of M_w of the resistant dextrins.

Table 3. Molecular weight of cassava-derived resistant dextrins prepared under different conditions.

Acid conc. (%, dsb)	Temp. (ºC)	Time (min)	Weight average molecular weight (M_w) (kDa)	Number average molecular weight (M_n) (kDa)	I^{ns} (M_w/ M_n)
0.06	100	120	$11.5^{c} \pm 2.1$	$6.3^{e} \pm 0.2$	1.8 ± 0.3
0.04	120	60	$12.7^{bc} \pm 0.1$	$6.9^{d} \pm 0.0$	1.8 ± 0.0
0.04	120	120	$14.4^{ab} \pm 0.1$	$7.6^{b} \pm 0.0$	1.9 ± 0.0
0.04	120	180	$15.4^{a} \pm 0.6$	$8.1^{a} \pm 0.1$	1.9 ± 0.1
0.06	120	60	$11.1^{c} \pm 0.1$	$6.4^{e} \pm 0.0$	1.7 ± 0.0
0.06	120	120	$14.1^{ab} \pm 0.1$	$7.4^{c} \pm 0.0$	1.9 ± 0.0

Means with different letters within the same column are significantly different ($p \leq 0.05$).

^{ns}Means within the same column are not significant difference ($p > 0.05$).

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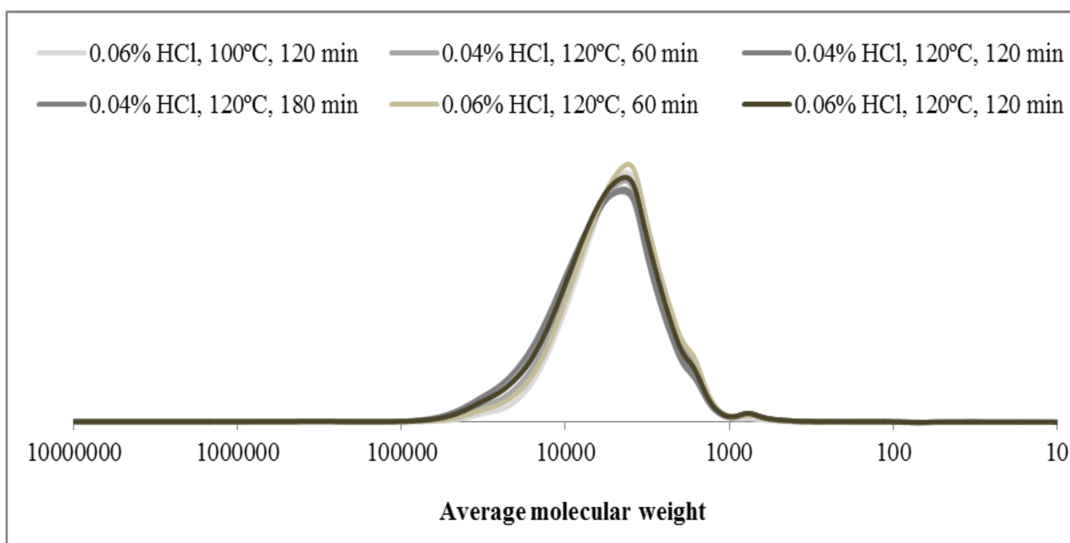

Fig. S2 HPSEC chromatograms of some cassava-derived resistant dextrins prepared under different conditions.

Chain length distribution of the resistant dextrins, as determined by HPAEC, was shown in Table 4 and Fig S3. According to the amylopectin cluster model (Hanashiro, Abe, & Hizukuri, 1996), linear portion of the debranched amylopectin chains can be classified by their length into four groups, A type (DP 6-12), B1 type (DP 13-24), B2 type (DP 25-36), and B3+ type (DP ≥ 37). The major component of the crystalline lamellae are A and B1 chains, while the composition of amorphous lamellae are mainly B2 and B3+ chains (Hizukuri, 1986). In comparison with native cassava starch, resistant dextrins prepared from two milder dextrinization conditions, 0.06% HCl/100°C/120 min and 0.04% HCl/120°C/60 min, had greater proportion of the branched chains with DP≤12, and smaller proportion of the longer branched chains (DP ≥ 13). However, the other samples obtained from the stronger conditions contained higher proportion of the short branched chains (DP 1-5) with lower proportion of the longer branched chains (DP ≥ 6). For the dextrinization at 120ºC,

increasing heating time and acid concentration resulted in decreasing amount of the longer chains (DP ≥ 6) with increasing fractions of smaller chains/molecules (DP 1-2).

The overall results from HPSEC and HPAEC techniques provided better insights into the changes in molecular structure of resistant dextrins during dextrinization. We speculated that hydrolysis was a predominant step, resulting in the products with much lower M_w and shorter chain length in comparison with those of the native starch. At milder condition (i.e. lower temperature, 100ºC), hydrolysis could occur at lower degree, thus yielding the product that contained higher proportion of long branched chains. At stronger conditions, extensive degree of hydrolysis occurred, leading to the products that contained lower M_w, with decreasing fractions of longer branched chains. Greater degree of transglucosidation and repolymerization could also occur under those conditions, thus resulted in slight increase in M_w and M_n found in HPSEC. Moreover, we could hypothesize that harsher conditions induced the formation of larger and highly-branched resistant dextrins that recombined from shorter branched chains.

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Moreover, changes in molecular structure were well-related with the physicochemical properties modification via dextrinization. In comparison with native starch, low molecular weight resistant dextrin had much higher solubility at room temperature, higher reducing sugar content and disappearance of endothermic peak in DSC (Table 1 and 2). This research demonstrated that acid concentration, temperature and heating time affected the molecular structure and physicochemical properties of resistant dextrins.

Table 4. Chain length distribution of cassava-derived resistant dextrins prepared under different conditions

DP	Chain length distribution (%)						
	Native cassava starch	**0.06% HCl, 100ºC, 120 min**	**0.04% HCl, 120ºC, 60 min**	**0.04% HCl, 120ºC, 120 min**	**0.04% HCl, 120ºC, 180 min**	**0.06% HCl, 120ºC, 60 min**	**0.06% HCl, 120ºC, 120 min**
1	2.61 ± 0.51	2.58 ± 0.34	8.68 ± 0.69	11.44 ± 0.24	15.84 ± 0.88	11.56 ± 0.35	14.23 ± 0.38
2	7.36 ± 0.21	15.51 ± 0.19	35.06 ± 1.66	49.41 ± 0.87	53.21 ± 0.58	53.13 ± 0.55	53.84 ± 0.40
3	1.19 ± 0.12	3.07 ± 0.63	7.17 ± 0.38	7.36 ± 0.19	6.08 ± 0.47	6.75 ± 0.31	6.74 ± 0.42
4	0.67 ± 0.10	2.86 ± 0.07	3.16 ± 0.81	2.40 ± 0.89	2.92 ± 0.15	2.35 ± 0.18	2.48 ± 0.05
5	0.85 ± 0.16	1.67 ± 0.26	1.13 ± 0.33	2.41 ± 0.66	2.83 ± 0.22	1.85 ± 0.04	2.14 ± 0.11
6-12	21.14 ± 0.37	29.13 ± 1.02	25.15 ± 0.60	12.18 ± 0.86	7.56 ± 0.60	11.29 ± 0.66	7.83 ± 0.33
13-24	44.33 ± 0.89	27.83 ± 1.04	15.88 ± 0.47	13.31 ± 1.53	11.21 ± 0.95	12.43 ± 0.39	12.21 ± 0.66
25-36	14.61 ± 0.89	12.14 ± 0.27	3.71 ± 0.16	1.44 ± 0.22	0.27 ± 0.07	0.59 ± 0.03	0.50 ± 0.09
≥ 37	7.24 ± 0.68	5.21 ± 0.19	0.05 ± 0.01	0.06 ± 0.01	0.06 ± 0.01	0.06 ± 0.01	0.03 ± 0.01

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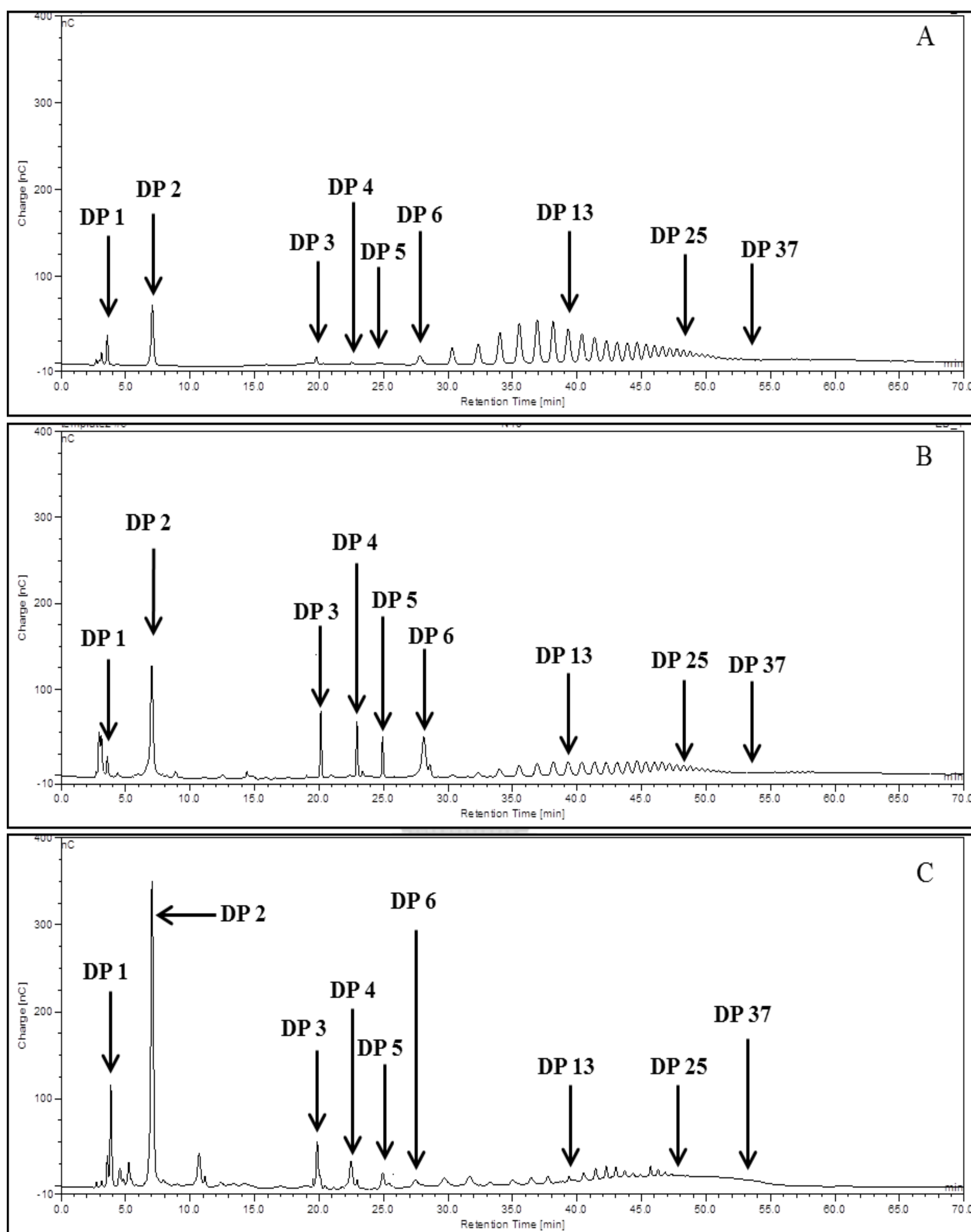

Fig. S3 HPAEC chromatograms of native starch (A), cassava-derived resistant dextrins prepared by using 0.06% HCl, 100°C, 120 min (B) and 0.06% HCl, 120°C, 120 min (C).

4. Conclusions

Pyroconversion of the cassava starch under stronger conditions (high acid concentration, high temperature and long reaction time) resulted in the resistant dextrins with darker color, higher water solubility, reducing sugar content and total dietary fiber. At milder conditions (≤0.06% HCl, 100°C, ≤120 min), the proportion of HMWDF was nearly equal to that of LMWDF. Stronger dextrinization condition resulted in the products with greater proportion of HMWDF. DSC results confirmed that harsher condition effectively destroy the ordered structure within the starch granules. Great reduction in M_w and M_n of the resistant dextrins, in comparison with the native starch, indicated that hydrolysis was the predominant reaction during dextrinization. For harsher condition, greater extent of transglucosidation and repolymerization could occur, resulting in the resistant dextrins with slightly increased M_w and M_n but containing shorter branched chains. Overall results from this research provided some new insights into the mechanism of dextrinization and the modification of structure and properties of cassava starch through this process. Nevertheless, dietary fiber content of the resistant dextrins obtained from this study was still lower than 50%. It is important to note that dietary fiber content in commercial resistant dextrin sample is currently ≥85% (Roquette, 2018). Further study might be required to enhance the total dietary fiber content of the products, and to determine the detailed structure of those high-fiber resistant dextrins.

Conflict of interest

The authors have no conflicts of interest.

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CHAPTER 3

Impact of processing conditions on molecular structure and properties of resistant dextrins and resistant maltodextrins from cassava starch

Kamonrat Trithavisup[a], Yong-Cheng Shi[b], Kuakarun Krusong[c] and Kanitha Tananuwong[a,*]

[a] *Department of Food Technology, Faculty of Science, Chulalongkorn University, Bangkok, 10330, Thailand*

[b] *Department of Grain Science and Industry, Kansas State University, Manhattan, Kansas, 66506, USA*

[c] *Department of Biochemistry, Faculty of Science, Chulalongkorn University, Bangkok, 10330, Thailand*

*Corresponding author. Tel.: +66-2-218-5515-6. Fax: +66-2-254-4314

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Abstract

Molecular structure and selected properties of cassava-derived resistant dextrins (RDs) and resistant maltodextrins (RMDs) were determined. RDs were prepared from various dextrinization conditions (0.04% and 0.06% HCl, 120°C, 60-180 min). RMDs, produced via α-amylase hydrolysis of RDs, had shorter branched chains, lower molecular weight and glass transition temperature of maximally freeze-concentrated unfrozen phase (T_g'), higher total dietary fiber content, low molecular weight dietary fiber (LMWDF) content and unfrozen water content than RDs. New glycosidic linkages, α-1,2, α-1,6, β-1,2, β-1,4 and β-1,6, were found in RDs and RMDs. Stronger dextrinization conditions decreased proportion of α-1,4 linkage and α-/β-reducing ends, but increased fraction of α-/β-1,6, β-1,4, β-1,2 linkages and degree of branching (DB) in RDs. Alpha-amylase treatment increased the proportion of all β-glycosidic linkages, DB and α-/β-reducing ends in RMDs. Higher prebiotic activity score was found in RMDs rich in shorter branched chains (DP ≤ 5) and LMWDF fraction, but possessed lower DB.

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Keywords: Resistant dextrins; Resistant maltodextrin; *Manihot esculenta*; Dextrinization; Alpha-amylase; Molecular structure; Prebiotic activity; Dietary fiber; Glass transition

1. Introduction

Starch derivatization yielding resistant dextrin/maltodextrin, dietary fiber with prebiotic activities, has gained more interest in food industry. Major derivatization step is pyroconversion or dextrinization. In this step, dry heat (≤5% moisture, ≥100°C) and acid (≥0.1% dry starch basis (db)) induces hydrolysis of α-1,4- or α-1,6-glycosidic bonds, rearrangement of the cleaved glycosidic bonds, resulting in the formation of some linkages that are indigestible in human gastrointestinal tracts including β-linkages and α-1,2-, α-1,3-glycosidic bonds, and eventually repolymerization, yielding the highly-branched dietary fiber generally called resistant dextrin (Bai & Shi, 2016; Kapuśniak & Jane, 2007; Laurentin et al., 2003; Tomasik et al., 1989; Wurzburg, 1995). The resistant dextrin can be further hydrolyzed with α-amylase until alpha-limit dextrin is obtained; the dextrose equivalent (DE) of the hydrolyzed product is generally less than 20. The resulting product is normally named as resistant maltodextrin (Hashizume & Okuma, 2009). Both of these functional ingredients are claimed to possess excellent water solubility, providing low viscosity and can tolerate several conditions, including heat and low pH (Bai, Cai, et al., 2014; Chen et al., 2020; Hashizume & Okuma, 2009; Kapuśniak & Jane, 2007; Kapusniak & Nebesny, 2017; Laurentin et al., 2003; Lefranc-Millot et al., 2009). Therefore, resistant dextrin/maltodextrin have widely been used to enhance dietary fiber content, and/or sources of prebiotics, in many food and beverage products.

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Our previous study reported the preparation of resistant dextrins from cassava starch using relatively lower acid concentration (0.04-0.10%) and temperature range (100-120°C) in the dextrinization step, which could avoid excessive burning of the products during preparation (Trithavisup, Krusong, & Tananuwong, 2019). Structure-

function relationship of the resistant dextrins was also discussed. It was found that higher acid concentration, temperature and longer heating time resulted in the resistant dextrins with higher total dietary fiber, greater proportion of high molecular weight fiber fraction and slightly higher weight-average molecular weight (M_w), but the products composed of shorter branched chains. Due to the 1000-fold decrease in M_w of the products in comparison with its native starch counterpart, we hypothesized that hydrolysis was predominate during dextrinization. However, the dextrinization conditions used in the previous study might not induce extensive transglucosidation and repolymerization, resulting in the resistant dextrins with <50% dietary fiber content. Treatment with α-amylase was shown to eliminate some of the digestible portions (via hydrolysis of α-1,4-glycosidic bonds) in resistant dextrins (Huang, Kou, Li, & Wang, 2007; Park, Park, & Park, 2017). This approach was thus applied in the current study to help enhance dietary fiber proportion of this cassava starch-derived product. The resulting resistant maltodextrin could have greater dietary fiber content comparing to its resistant dextrin counterpart.

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Previous studies regarding resistant maltodextrin mainly focus on evaluating its health benefits, including improvement of intestinal regularity, moderation of postprandial blood glucose levels, reduction of serum cholesterol and triacylglycerol levels and enhancement of the absorption and retention of mineral and its prebiotic activity (Hashizume & Okuma, 2009; Lefranc-Millot et al., 2009; Pasman et al., 2006). Reports on the effects of processing conditions on the molecular structure and properties of the resistant maltodextrins, as well as the relationship between their structures and properties, are still scarce. The objectives of this study were to evaluate the effects of dual modification, dextrinization and enzymatic modification, on

molecular structure, dietary fiber content, prebiotic properties and thermal properties of cassava-derived resistant maltodextrins. Detailed discussion regarding the relationship between molecular structure and the selected properties was also provided. Information obtained from this study could be applied for tailoring the resistant maltodextrin with desirable properties.

2. Materials and methods

2.1 Materials

Cassava starch was obtained from General Starch, Ltd. (Nakhon Ratchasima, Thailand). The mixed varieties of cassava roots grown in Nakhon Ratchasima province were used to prepare this cassava starch on an industrial scale.

2.2 Preparation of resistant dextrins

Resistant dextrins were prepared by the same process as explained in Trithavisup et al. (2019), using the dextrinization conditions shown in Table 1. Briefly, cassava starch (3 kg) was mixed with 2% and 3% hydrochloric acid solutions (60 mL) to obtain the final acid concentration of 0.04 and 0.06% dry starch basis, respectively. The samples were preheated at 50°C in a rotary roaster to achieve the final moisture content of < 5%, and heated at 120°C for 60-180 minutes.

2.3 Preparation of resistant maltodextrins

Resistant dextrins prepared with the conditions listed in Table 1 contained more than 30% dietary fiber (Trithavisup et al., 2019). Therefore, they were selected to prepare resistant maltodextrin. The 30% solution of the selected resistant maltodextrin was prepared. The sample solution was hydrolyzed with commercial α-amylase (enzyme activity 480 KUN/g, BAN® 480L, Novozyme, Bagsvaerd, Denmark) at 70°C, pH 6 until reaching the final DE of 8 or 12 (Table 1). The enzyme

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was heat-inactivated at 95°C. Final pH of the solution was adjusted to 6-7. The hydrolyzed samples were treated with 3% activated carbon (Rasayan Laboratories, Anand, Gujrat, India) to remove color and odor, using the modified method of Huang et al. (2007). Small saccharide molecules were then removed by centrifugation and filtration, using Macrosep® advance centrifugal device composing of 1kDa modified polyethersulfone membrane (1K Omega™) in 50 mL centrifuge tube (Poll Laboratory, Port Washington, NY, USA). Twenty mL of sample solution were added into the device and centrifuged at 3,500xg for 60 min. Resistant maltodextrin solutions were then spray-dried in mini spray dryer (Buchi B-290, Switzerland). Moisture content was determined by hot air oven method (AOAC, 2012). All resistant maltodextrin samples had 1.5-1.7% moisture content.

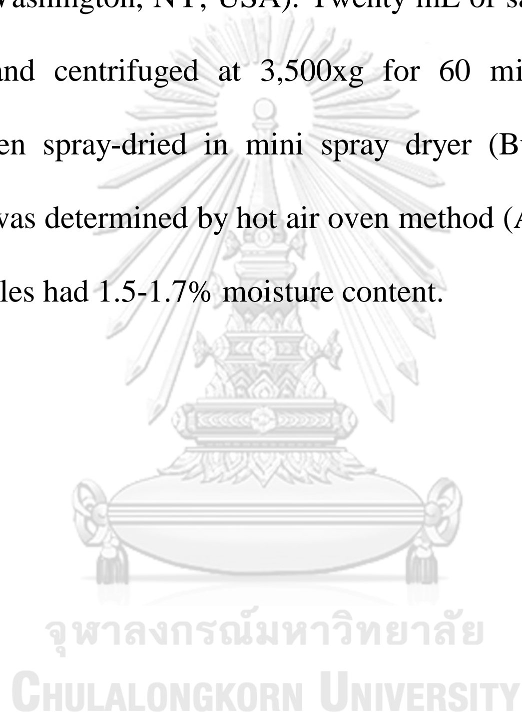

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Table 1. Processing conditions and dextrose equivalent (DE) of the resistant dextrin and resistant maltodextrins prepared from cassava starch.

Processing condition	Dextrinization				α-amylase treatment	
	Acid concentration (%, dsb)	Temperature (°C)	Heating time (min)	DE (after dextrinization)	Hydrolysis time (min)	DE (after α-amylase treatment)
1	0.04	120	60	$1.89^{b} \pm 0.06$	120	$12.16^{a} \pm 0.50$
2	0.04	120	120	$1.96^{b} \pm 0.06$	120	$11.96^{a} \pm 0.77$
3	0.04	120	180	$2.19^{a} \pm 0.07$	90	$7.95^{b} \pm 0.28$
4	0.06	120	60	$1.89^{b} \pm 0.05$	90	$8.51^{b} \pm 0.50$
5	0.06	120	120	$2.21^{a} \pm 0.03$	90	$8.31^{b} \pm 0.34$

Means with different letters within a column are significantly different ($p \leq 0.05$).

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2.4 Structural determination

2.4.1 Relative molecular weight distribution

Relative molecular weight distribution of resistant maltodextrin samples were determined by gel permeation chromatography (GPC) using the method of Bai, Kaufman, Wilson, and Shi (2014) with slight modification. The sample (4 mg) was dissolved in DMSO (4 mL) and stirred at room temperature for 24 h. The solutions were filtered through a 2 μm filter and injected by an autosampler into a PL-GPC 220 system with three Phenogel columns (00H-0642-K0, 00H-0644-K0 and 00H-0646-K0; 7.8 mm x 300 mm; Phenomenex, Inc., Torrance, CA, USA) and a differential refractive index detector. DMSO (200 mL) containing lithium bromide (5.5 g) was used as eluent at a flow rate of 0.8 mL/min. The column oven temperature was 80°C. Pullulan (Shodex standard P-82 kit, Showa Denko K.K., Kanagawa, Japan) and maltose standard with different molecular weights with molecular weight from 342 to 1,720,000 g/mol were used for molecular weight calibration. GPC results were analyzed using Cirrus GPC software version 3.0 (Agilent Technologies, Santa Clara, CA, USA). The relative M_w, relative number-average molecular weight (M_n) and polydispersity index (I) of samples were calculated with the following equation.

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$$M_w = \frac{\sum n_i M_i^2}{\sum n_i M_i} \quad (1)$$

$$M_n = \frac{\sum n_i M_i}{\sum n_i} \quad (2)$$

$$I = \frac{M_w}{M_n} \quad (3)$$

When M_i represented the molecular weight of samples which was calculated from standard curve and n_i represented the signal of refractive index detector.

2.4.2 Chain length distribution

High-performance anion-exchange chromatography equipped with a pulsed amperometric detector (HPAEC-PAD) was used to determine the chain-length distribution of resistant maltodextrins, using the modified method of Kuakpetoon and Wang (2007)). The sample (50 mg) was mixed with 0.02 M acetate buffer (4.79 mL; pH 3.5) and isoamylase (10 µL; activity of 600 enzyme units, Hayashibara Biochemical Laboratories Inc. Okayama, Japan). The mixture was incubated at 40ºC for 24 h. The 0.1 M sodium hydroxide solution (0.2 mL) was added to the mixture in order to inactivate the enzyme. Ultrapure water (5 mL) was then added. The mixture was filtered through 0.45 µm membrane filter, and injected to an ICS-5000 ion chromatograph (Dionex, Sunnyvale, CA, USA) with a Carbopac PA-1 column (4 mm × 250 mm) (Dionex). The following gradients of solvents A (150 mmol/L sodium hydroxide) and B (150 mmol/L sodium hydroxide and 500 mmol/L sodium acetate) at a flow rate of 1 mL/min were used for elution: 100% A at 0 min; 0–100% B at 30 min. Glucose, maltose, maltotriose, maltotetraose, maltopentaose, maltohexose and maltoheptaose (Hayashibara Biochemical Laboratories Inc. Okayama, Japan) were used as standards representing the branched chains with degree of polymerization (DP) of 1 – 7.

2.4.3 Nuclear magnetic resonance (NMR) spectroscopy

The type and proportion of glycosidic linkages in resistant dextrins and resistant maltodextrins were determined by NMR spectroscopy using the method of Bai and Shi (2016) with slight modification. The samples (1 mg) were exchanged with D_2O (10 mL) twice, followed each time by freeze-drying, to attenuate the signal from water proton. The deuterium-exchanged samples were dissolved in D_2O (10 %

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w/w). The analysis was performed via Varian 500 NMR System (Palo Alto, CA) at 25°C, using the frequency of 499.839 MHz for ^{1}H. The NMR spectrometer was equipped with a cryogenic 5-mm carbon-enhanced triple resonance inverse detection pulse field gradient probe. The ^{1}H spectra were collected in 32 individual scans with a sweep width of 16 ppm and a delay time of 1 s. Tetramethylsilane (TMS) was used as an internal reference at 0 ppm, and chemical shift were reported in parts per million (ppm).

Proportion of the chemical structures corresponding to the specific chemical shift position was calculated from the area of each peak divided by sum of the areas of total anomeric proton. The degree of branching (DB, %) was calculated from sum of the areas of all internal non α-1,4 linkages divided by that of the total anomeric proton. Average chain length (CL) was calculated from total areas of anomeric protons divided by the sum of the areas of reducing end and branching points (Han et al., 2018). The anomeric signal from 1,6-anhydro-β-D-glucopyranosyl groups that formed during dextrinization was considered reducing end for the calculation purpose (Bai, Cai, et al., 2014; Han et al., 2018) and the other internal anomeric signals excluding α-1,4 linkages was counted as parts of branching points.

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2.5 Dietary fiber content

Total dietary fiber content, high molecular weight dietary fiber (HMWDF) and low molecular weight dietary fiber (LMWDF) fractions of resistant maltodextrins were determined according to AOAC official 2009.01 methods (AOAC, 2012) using enzymatic assay kit (K-INTDF, Megazyme International Ireland Ltd., County Wicklow, Ireland) as previously described (Trithavisup et al., 2019).

2.6 Prebiotic activity

Prebiotic activity of resistant maltodextrins was determined by the modified method of Huebner, Wehling, and Hutkins (2007). *Lactobacillus casei*-01 (Chr Hansen®, Denmark) and *Lactobacillus reuteri* TBRC 291 (Thailand Bioresource Research Center, Thailand) were used as probiotic representatives in this study. Cocktail of *Escherichia coli* ATCC 25922, *Escherichia coli* TISTR 073 and *Escherichia coli* TISTR 074 (Thailand Institute of Scientific and Technological Research, Thailand) were used as reference enteric mixture for calculating prebiotic activity score. Each lactobacillus strain was cultivated in de *Man*, *Rogosa* and *Sharpe* (MRS) broth at 37°C for 48 h. The enteric mixture, containing 1:1:1 ratio of *E. coli* ATCC 25922, *E. coli* 073 and *E. coli* 074, was cultivated in tryptic soy broth at 37°C for 24 h. The initial viable cells for probiotic bacteria and the enteric mixture were then adjusted to 1×10^8 CFU/mL, to be used as the test culture. For each probiotic strain, the 0.1 mL of the test culture was added to 10 mL of MRS broth containing 1% w/v glucose or 1% w/v resistant maltodextrin. The 0.1 mL of the enteric mixture was diluted into 10 mL of M9 broth fortified with 1% w/v glucose or 1% w/v resistant maltodextrin. For both cases, the initial viable cell count was approximately 1×10^6 CFU/mL. All samples were incubated overnight at 37°C. The viable cells were then determined by pour-plate technique, using MRS agar and nutrient agar for probiotic bacteria and enteric bacteria, respectively. The prebiotic activity score was determined using the following equation:

$$Prebiotic\ activity\ score = \frac{(\log P_x 24 - \log P_x 0)}{(\log P_g 24 - \log P_g 0)} - \frac{(\log E_x 24 - \log E_x 0)}{(\log E_g 24 - \log E_g 0)} \quad (4)$$

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When P_x and P_g represented the viable prebiotic bacteria (CFU/mL) cultivated in 1% w/v resistant maltodextrin of and 1% w/v glucose, respectively; E_x and E_g represented the viable enteric bacteria (CFU/mL) cultivated in 1% w/v resistant maltodextrin and 1% w/v glucose, respectively; 0 and 24 represented incubation time at 0 h and 24 h.

2.7 Thermal properties at subzero temperatures

Glass transition temperature of maximally freeze-concentrated unfrozen phase (T_g') and unfrozen water of resistant dextrin and resistant maltodextrin solution at 30% concentration were determined using differential scanning calorimeter (DSC) (Perkin-Elmer, Diamond DSC, USA) with modified method of Roos (1993) and Wang and Wang (2000). Ten microliters of the sample solution were pipetted into DSC aluminum pan (Perkin-Elmer kit no. 02190062) and hermetically sealed. The sample, as well as an empty reference pan, was subjected to the following temperature program. The sample was cooled to -50°C at 30°C/min, then heated from -50°C to 20°C at 5°C/min (first scan), to determine onset temperature of ice melting peak (T_o). The sample was then cooled down to -50°C at 30°C/min, rescanned from -50°C to the annealing temperature (1°C - 2°C below T_o) at 10°C/min, and held for 15 min to enhance ice crystal formation. Lastly, the sample was cooled to -50°C at 30°C/min and rescanned from -50°C to 20°C at 5°C/min (second scan). DSC thermogram was analyzed using Pyris^{TM} software version 12 (Perkin-Elmer Co.). T_g', midpoint, was determined from the peak temperature of the first-derivative heat flow curve obtained from the second scan (Figure S1). Frozen water was calculated as shown in the

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equation 5. Unfrozen water was calculated as the difference between total water and frozen water in the sample.

$$Frozen\ water\ content\ \left(\frac{g_{water}}{g_{dried\ RMD}}\right) = \frac{\Delta H_I}{\Delta H_L \times W} \quad (5)$$

When ΔH_I represented the enthalpy of ice melting (mJ) obtained from the second scan; ΔH_L represented the latent heat of ice melting (333.55 mJ/mg water); W represented the weight of dried RMD (mg).

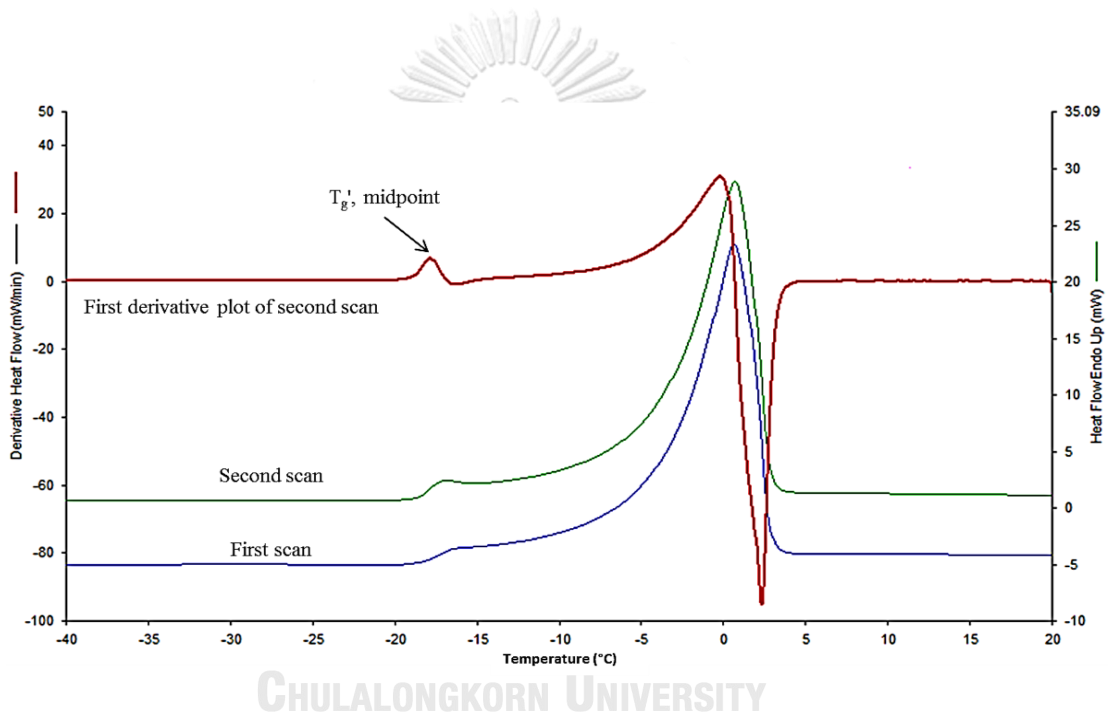

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Fig. S1 Example of the DSC thermograms obtained from 30% solution of resistant maltodextrin. The sample was prepared from the first condition shown in Table 1. Glass transition temperature of maximally freeze-concentrated unfrozen phase (T_g', midpoint) was located at the peak found in the first derivative plot of the second scan.

2.8 Statistical analysis

All experiments were performed in triplicates, except the NMR analysis which was done in duplicates. Completely randomized design was applied for the experiments, except chain length distribution, molecular weight determination and NMR analysis. Analysis of variance was performed. Mean comparison was done using Duncan's new multiple range test. All statistical analyses were performed by IBM SPSS statistics software, version 22 (Windows, SPSS Inc., Chicago, IL, USA).

3. Results and discussion

3.1 Processing condition and final DE of resistant dextrins and resistant maltodextrins

Conditions of dextrinization and enzymatic treatment significantly affected DE of resistant dextrins and resistant maltodextrins, respectively ($p \leq 0.05$). Dextrinization resulted in the resistant dextrins with DE ranged from 1.86-2.21 (Table 1). Longer heating time led to significant increase in DE values of resistant dextrin ($p \leq 0.05$). In case of α-amylase treatment, the hydrolysis time of the resistant dextrins was selected to achieve the highest DE (Table 1). For the resistant dextrins obtained from milder dextrinization condition (0.04% HCl, 120°C, 60-120 min), the suitable hydrolysis time was 120 min, yielding the resistant maltodextrins with the final DE of 12. However, the appropriate hydrolysis time reduced to 90 min for the resistant dextrins prepared from stronger dextrinization condition (0.04% HCl, 120°C, 180 min and 0.06% HCl, 120°C, 60-120 min). The latter group of the resistant maltodextrins thus had lower DE. Extending hydrolysis beyond those selected durations did not increase the DE of the products. Difference in the hydrolysis times could be due to the structural difference among the resistant dextrin substrates. During dextrinization, the

formation of new α- and β-1,2-, 1,3-, and 1,6-glycosidic linkages (Tomasik et al., 1989; Wurzburg, 1995) may block the action of α-amylase due to steric hindrance (Chen et al., 2020). Stronger dextrinization condition could induce the formation of those novel glycosidic linkages in greater extent (see further discussion in section 3.3). Moreover, the samples obtained from the stronger conditions contained higher proportion of the short branched chains (DP 1-5) (Trithavisup et al., 2019), which could be unfavorable for α-amylase hydrolysis. Okada, Kitahata, Higashihara, and Fukumoto (1969) reported that the liquefying α-amylase that produce from *Bacillus amyloliquefaciens* hydrolyses α-1,4-glucosidic linkage of starch containing more than 7 glucose molecules and does not attack α-1,4-glucosidic linkage adjacent to α-1,6-glucosidic linkage in starch (Umeki and Yamamoto, 1975). Due to those structural changes, limited extent of α-amylase hydrolysis was obtained. Suitable hydrolysis time for the resistant dextrins prepared from harsher dextrinization conditions was thus shorter.

3.2 Relative molecular weight and chain length distribution

The relative molecular weight distribution of the samples determined by GPC was shown in Table 2. The relative M_w and M_n of resistant maltodextrins were within the range of 3.48-4.69 kDa and 1.89-2.46 kDa, respectively. These M_w and M_n values of all samples were higher than those of the commercial resistant maltodextrin. Polydispersity index (*I*) of the samples, indicating the molecular weight distribution pattern, ranged from 1.81 to 1.95 (Table 2). *I* value of samples was similar to *I* value of commercial resistant maltodextrin sample. In comparison with the relative M_w and M_n of resistant dextrins prepared with dextrinization condition 1-5 (Table 1), as reported in our previous study (Trithavisup et al., 2019), relative M_w and M_n of each

resistant maltodextrin sample were lower than those of its resistant dextrin counterpart. This could be due to the loss of digestible portion, containing α-1,4-glycosidic bonds, via α-amylase hydrolysis. Moreover, effects of dextrinization time on the relative M_w and M_n of the resistant maltodextrins tended to follow the same trend as found in resistant dextrins (Trithavisup et al., 2019). At similar acid concentration, increasing dextrinization time from 60 to 120 min led to an increase in the relative M_w and M_n of the resistant maltodextrins. Impact of transglucosidation and repolymerization steps, which were amplified under stronger dextrinization conditions, on the molecular weight could still be observed after α-amylase treatment.

Chain length distribution of resistant maltodextrins determined by HPAEC was shown in Table 2 and Figure S2. The chain length distribution profile of resistant maltodextrin samples prepared in this study was relatively similar to that of the commercial resistant maltodextrin. In comparison with its resistant dextrin counterpart as previously reported (Trithavisup et al., 2019), α-amylase treatment resulted in apparently increasing proportion of the DP 4-24 branched chains together with disappearance of the longer chain fractions (DP ≥ 25). These results suggested that the enzymatic hydrolysis yielded the resistant maltodextrin with shorter side chain. Effect of α-amylase treatment on chain length distribution were in an agreement with M_w and M_n data. Enzymatic hydrolysis of the resistant dextrins resulted in the reduction of the portion with intramolecular α-1,4 linkage, yielding the resistant maltodextrin with shorter branched chains and lower molecular weight. It is also interesting to note that stronger dextrinization conditions resulted in increasing fraction of smaller chains/molecules (DP ≤ 5) with decreasing proportion of the longer branched chain (DP ≥ 6) in both resistant dextrins (Trithavisup et al., 2019) and resistant

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maltodextrins (Table 2). Although the purification step was performed, relatively high proportion of glucose (DP1) and maltose (DP2) were found in the debranched resistant maltodextrin samples. Generally, fungal isoamylase could not remove a branched glucosyl unit and slowly hydrolyzed a maltosyl unit attached by α-1,6 linkage (Robyt, 2009). Therefore, those small sugar molecules might not be completely removed via the purification step.

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Table 2. Molecular weight and chain length distribution of resistant maltodextrins prepared under different conditions.

Measured parameter	Commercial RMD	Processing condition				
		1	2	3	4	5
Molecular weight						
Weight average molecular weight (M_w) (kDa)	3.23 ± 0.01	3.70 ± 0.14	4.69 ± 0.03	4.67 ± 0.10	3.48 ± 0.05	4.26 ± 0.07
Number average molecular weight (M_n) (kDa)	1.72 ± 0.03	1.97 ± 0.05	2.46 ± 0.03	2.39 ± 0.07	1.89 ± 0.07	2.36 ± 0.03
I **(M_w/M_n)**	1.88 ± 0.03	1.88 ± 0.04	1.91 ±0.01	1.95 ± 0.03	1.84 ± 0.05	1.81 ± 0.05
Chain length distribution (%)						
DP 1	13.47 ± 0.80	15.04 ± 0.54	15.25 ± 0.40	15.44 ± 0.23	15.67 ± 0.10	16.92 ± 0.54
DP 2	6.60 ± 0.15	6.47 ± 0.68	8.02 ± 0.83	8.30 ± 0.44	7.73 ± 0.12	7.93 ± 0.74
DP 3	5.08 ± 0.48	5.93 ± 0.63	6.20 ± 0.19	7.59 ± 0.11	6.34 ± 0.07	7.56 ± 0.25
DP 4	11.42 ± 0.39	6.22 ± 1.00	6.41 ± 0.21	6.66 ± 0.28	6.66 ± 0.39	6.74 ± 0.48
DP 5	8.20 ± 0.67	5.57 ± 0.54	6.24 ± 0.45	6.72 ± 0.22	6.37 ± 0.32	7.26 ± 0.45
DP 6-12	41.09 ± 0.56	41.93 ± 0.28	41.35 ± 0.92	39.42 ± 0.39	40.20 ± 0.44	39.02 ± 0.26
DP 13-24	14.14 ± 0.60	18.83 ± 0.89	16.53 ± 0.32	15.87 ± 0.40	17.03 ± 0.39	14.57 ± 0.51

The processing conditions listed in this Table (1-5) correspond to the conditions shown in Table 1.

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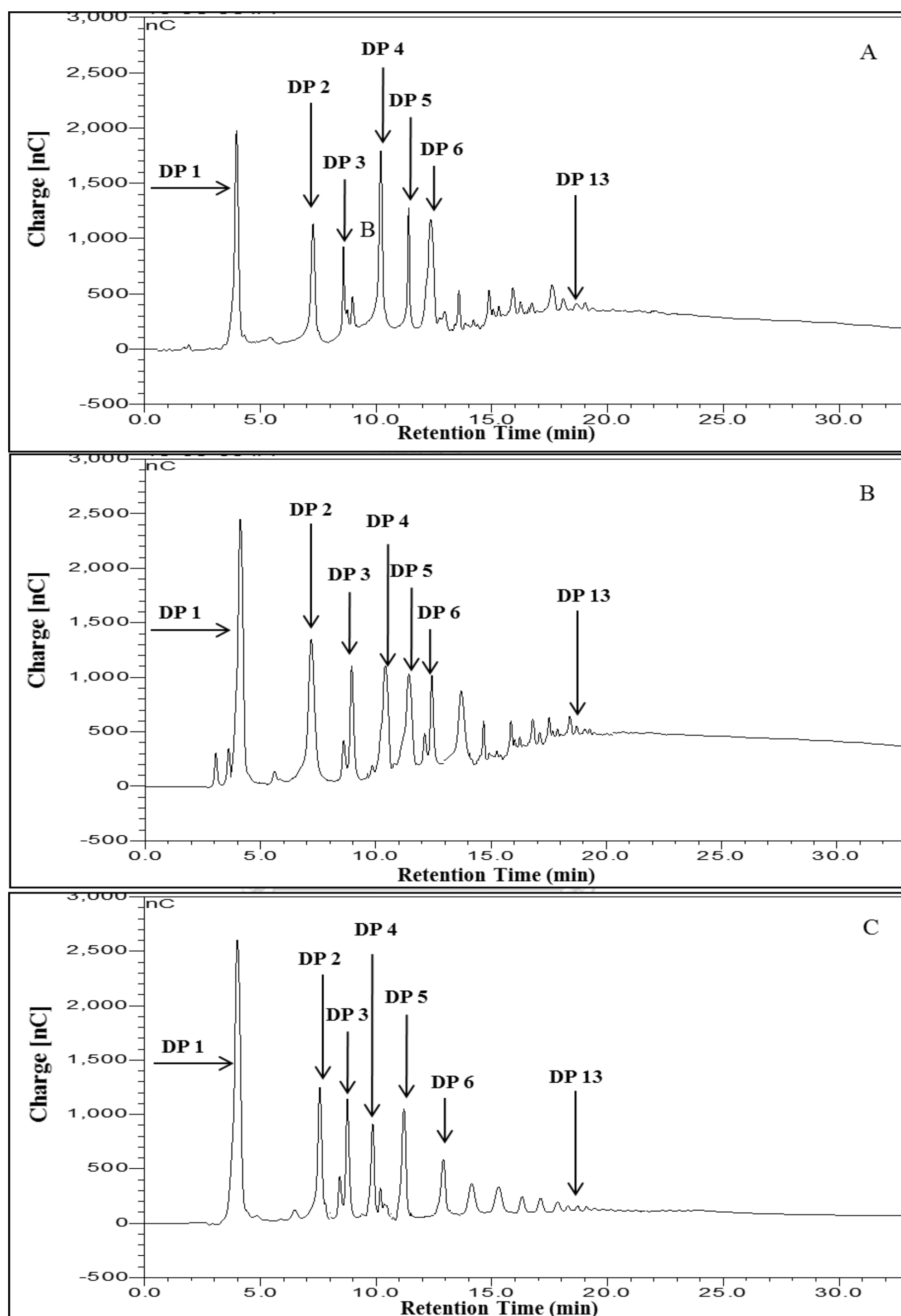

Fig. S2 HPAEC chromatograms of commercial resistant maltodextrin (A), cassava-derived resistant maltodextrins dextrinized from the 2nd condition (B) and 5th condition (C) shown in Table 1.

3.3 NMR spectroscopic analysis

According to the NMR spectroscopic technique, the ^1H NMR spectra of the samples were record at 25°C. All the protons of samples were presented in the area of 3.3-5.5 ppm (Figure S3). The spectra in the low-field region between 4.4 and 5.5 ppm, which was arisen from anomeric protons, were well separated while the other protons were overlapped in the area of 3.3-4.2 ppm. Peaks at 5.45, 5.11, 5.07 and 4.67-4.35 ppm were found in ^1H NMR spectrum of resistant dextrins and resistant maltodextrins. These results indicated that new bonds or linkages were formed during dextrinization. New linkages were identified and assigned with the assistance of 2D NMR system including HSQC, HMBC, COSY and TOCSY techniques (data not shown) as previously described (Bai & Shi, 2016; Han et al., 2018). In this research, both peaks at 5.11 and 5.07 ppm were assigned to the anomeric protons of α-1,2 linkages. Bai and Shi (2016) also reported that a peak at 5.09 ppm in the ^1H NMR spectrum of pyrodextrin corresponded to an anomeric proton of α-1,2 linkage. According to the separation of the signals into 2 peaks at 5.11 or 5.07 ppm, we postulated that one peak could arise from the anomeric proton involved in α-1,2 linkage in adjacent to the reducing end. The other peak could occur from the anomeric proton involved in α-1,2 linkage at the middle position of a starch chain.

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The position and integration of the anomeric protons of resistant dextrins and resistant maltodextrins prepared in this study were summarized in Table 3. During dextrinization, new linkages including α-1,6, β-1,6, α-1,2, β-1,2 and β-1,4 were found as previously reported (Bai & Shi, 2016; Chen et al., 2020). For resistant dextrins, changes in acid concentration and dextrinization time affected proportion of the reducing ends and the intramolecular glycosyl-linked group. At shorter heating time

(60 min, RD1 and RD 4), as acid concentration increased, the content of α- and β-anomeric reducing ends and α-1,6 linkage increased while the content of 1,6-anhydro-β-D-glucopyranose group and α-1,4 linkage decreased. Greater extent of the reduction was found for the proportion of α-1,4 linkage. These results suggested that higher acid concentration could induce greater degree of hydrolysis. This presumption was supported by decreasing average CL of the RD4. Increasing acid concentration was previously shown to decrease proportion of the longer chains (DP $\geq$ 6) in resistant dextrins (Trithavisup et al., 2019). However, the increasing of content of α-1,6 linkage in RD4 might be due to the greater extent of tranglucosidation. When considering the treatment with the same acid concentration, longer heating time caused decrease in proportion of internal α-1,4-glycosidic linkage as well as α- and β-reducing ends, but led to increase in the α-1,6, β-1,6, β-1,4 and β-1,2 linkages. These might be due to larger degree of hydrolysis, transglucosidation and repolymerization at prolonged heating time. These structural changes were in an agreement with our previous report regarding chain length distribution and M_w of the resistant dextrins (Trithavisup et al., 2019). At similar acid concentration, increasing dextrinization time yielded the resistant dextrins with higher M_w and smaller fraction of longer branched chains. It is interesting to note that the content of α- and β-anomeric reducing ends, and the 1,6-anhydro-β-D-glucopyranisyl units found in the commercial resistant dextrin sample was 3.6% and 2.9%, respectively, which were nearly similar to those of RD1 sample. Proportion of the glycosidic linkages found in the commercial sample was within the range of the values found in RD1 – RD5. Nevertheless, in comparison with the commercial sample, RD5 sample prepared from the harsh condition had

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approximately 10% lower proportion of α-1,4 linkage, with approximately 10% higher proportion of other types of glycosidic linkages.

After hydrolyzing with α-amylase, the content of α-1,4 linkage in resistant maltodextrin decreased while the content of α- and β-reducing ends increased as compared with its resistant dextrin counterpart. The results agreed with those reported by Chen et al. (2020), as the level of α-1,4 linkage of waxy maize derived resistant dextrins (HCl 8%, 170°C, 5 h) decreased while the level of α- and β-reducing ends increased after α-amylase and amyloglucosidase hydrolysis. However, the resistant maltodextrin samples still had 32.8-44.0% of α-1,4 linkage. These might be due to highly branched structure of the resistant maltodextrin. The newly intra- and intermolecular glycosidic linkages might block the action of α-amylase due to steric hindrance (Chen et al., 2020). Moreover, the samples obtained from the stronger conditions contained higher proportion of the short branched chains, which could be unfavorable for α-amylase hydrolysis (Okada et al., 1969; Umeki & Yamamoto, 1975). Nevertheless, proportion of the α-1,4 linkage found in RMD4 was relatively similar to that of the commercial resistant maltodextrin sample. After α-amylase hydrolysis, while the proportion of the α-1,4 linkage decreases, the proportion of the of the β-1,2, β-1,4 and β-1,6 linkages in resistant maltodextrin increased when comparing to the values found in its resistant dextrin counterpart (Table 3).

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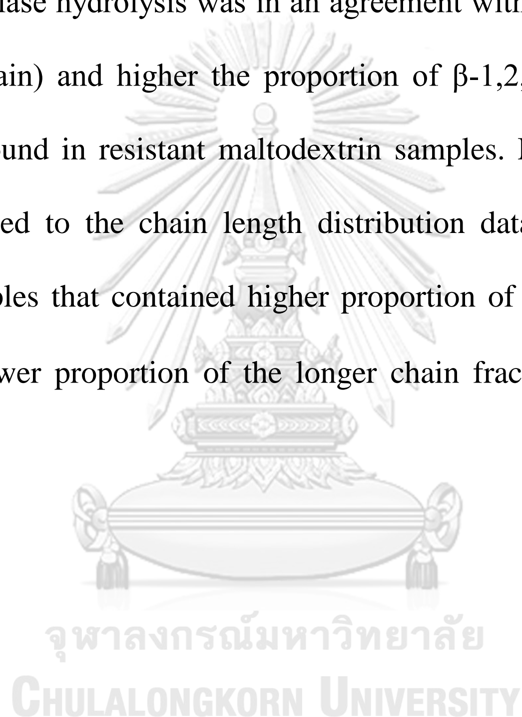
The DB of the resistant dextrins and resistant maltodextrins reflected the degree of transglucosidation and repolymerization. On the contrary, average CL of samples reflected the degree of hydrolysis, which affected their properties such as solubility and prebiotic effect. As the heating time and acid concentration increased during dextrinization, the DB of resistant dextrins increased but the average CL

reduced (Table 3). Han et al. (2018) also reported that, as dextrinization duration increased (30 - 240 min), DB of waxy maize-derived resistant dextrins (pH 3.0, 170°C) increased while the CL decreased. Comparing to its resistant dextrin counterpart, resistant maltodextrin had higher DB with lower average CL (Table 3). These results were in an agreement with the previous study on the waxy maize-derived resistant dextrins and resistant maltodextrin (Chen et al., 2020). An increase in DB after α-amylase hydrolysis was in an agreement with lower proportion of α-1,4 linkage (linear chain) and higher the proportion of β-1,2, β-1,4 and β-1,6 linkages (branch chains) found in resistant maltodextrin samples. Moreover, the average CL results corresponded to the chain length distribution data (Table 3). The resistant maltodextrin samples that contained higher proportion of the chains/molecules with DP 1 - 5 with lower proportion of the longer chain fractions (DP ≥ 6) had lower average CL.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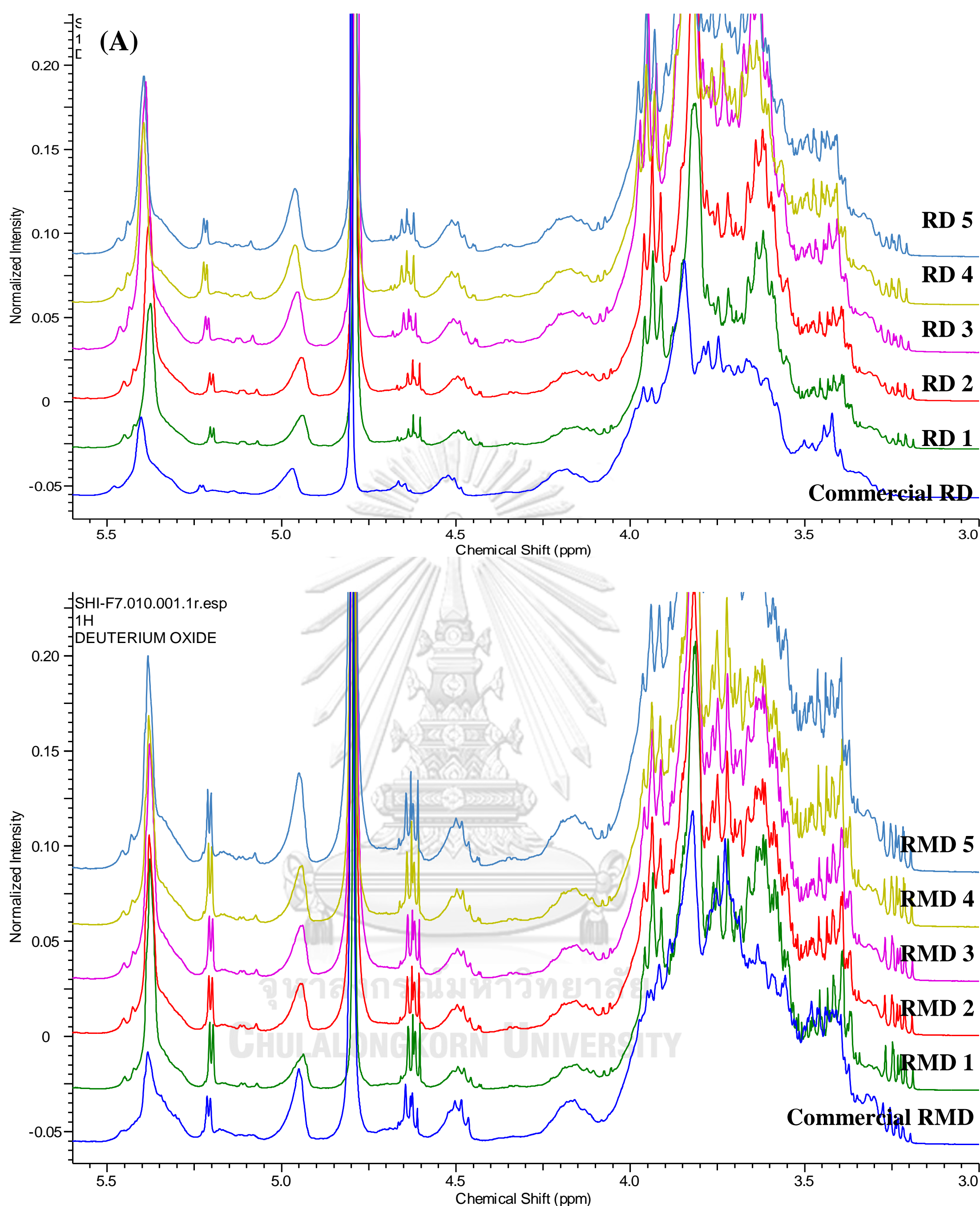

Fig. S3 ^{1}H NMR spectra of resistant dextrins (RD) (A) and resistant maltodextrin (RMD) (B). All spectra were recorded at 25°C. For the samples derived from cassava starch (RD 1 – 5; RMD 1 – 5), number 1 – 5 represented the processing conditions shown in Table 1. RD samples were processed via dextrinization step only.

Table 3 Chemical shifts of anomeric protons, proportion of the corresponding molecular structures, degree of branching and average chain length of resistant dextrins and resistant maltodextrins prepared under different conditions

Samples	**Chemical shift of anomeric protons (δ, ppm) and the corresponding molecular structure**						**DB (%)**	**Average CL**
	5.45	**5.37**	**5.20 & 4.65**	**5.11 & 5.07**	**4.94**	**4.67-4.35**		
	(1,6 anhydro-β-D-glucopyranosyl unit)	**(α-1,4 internal)**	**(α- & β-reducing end)**	**(α-1,2)**	**(α-1,6)**	**(β-1,6; β-1,4; β-1,2)**		
Commercial RD	2.9	47.0	3.6	2.0	18.4	26.1	51.6	1.7
RD1	2.8	47.8	3.5	2.9	16.5	26.5	46.0	1.9
RD2	2.4	47.2	3.2	3.5	16.9	26.8	47.3	1.9
RD3	2.6	45.7	3.2	4.0	17.3	27.2	48.5	1.8
RD4	2.2	39.8	4.5	4.1	19.3	30.1	53.5	1.7
RD5	2.3	37.0	3.8	3.8	20.9	32.2	56.9	1.6
Commercial RMD	2.3	36.1	5.9	3.6	23.3	28.9	57.2	1.5
RMD1	1.9	44.0	6.8	2.1	16.9	28.2	47.3	1.8
RMD2	2.1	42.4	6.1	2.9	17.5	29.0	49.4	1.7
RMD3	2.3	41.4	5.7	3.2	17.6	29.8	50.6	1.7
RMD4	1.8	36.0	6.9	2.8	19.6	32.9	55.2	1.6
RMD5	2.1	32.8	6.2	3.8	20.4	34.6	58.8	1.5

RD1 – RD5 represent the resistant dextrin samples obtained from the condition 1-5 as shown in Table 1, using only dextrinization step.

RMD1 – RMD5 represent the resistant maltodextrin samples obtained from the condition 1-5 as shown in Table 1, using both dextrinization and α-amylase hydrolysis.

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3.4 Dietary fiber fractions

Dietary fiber content of resistant maltodextrins is shown in Table 4. TDF, LMWDF and HMWDF of the resistant maltodextrin samples ranged from 48-62%, 26-35% and 22-30%, respectively. In comparison with its resistant dextrin counterpart, TDF of the resistant maltodextrins was 14-26% higher. After α-amylase treatment, HMWDF of the samples decreased (1-4%) while LMWDF increased (17-28%). It is interesting to note that the sample dextrinized with 0.04% acid/ 120°C/ 120 min and hydrolyzed by α-amylase for 120 min had the largest increase in the proportion of LMWDF (+28%) and TDF (+26%).

During dextrinization, it involves hydrolysis, transglucosidation and repolymerization (Wurzburg, 1995), causing new linkages formation in resistant dextrin that cannot be digested with enzymes in the human, as discussed in our previous studied (Trithavisup et al., 2019). These results were in an agreement with the data from NMR analysis, New glycosidic linkages including α-1,2, α-1,6, β-1,2, β-1,4 and β-1,6 could correspond to the indigestible fraction of resistant dextrins. An α-amylase treatment of those resistant dextrins resulted in decreasing proportion of α-1,4-glycosidic linkages with increasing proportion of β-1,2, β-1,4, β-1,6 and α-1,6-glycosidic linkages (Table 3). Moreover, the hydrolyzed samples had much lower M_w and shorter branched chains in comparison with those before α-amylase hydrolysis, as stated in section 3.2. These data correspond well with decreasing HMWDF and increasing LMWDF of the resistant maltodextrins (Table 4).

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Table 4. Dietary fiber content of resistant maltodextrins prepared under different conditions and the relative changes in comparison with the resistant dextrin counterparts.

Processing condition	% LMWDF	Change in LMWDF	% HMWDF	Change in HMWDF	% TDF	Change in TDF
1	$26.28^{d} \pm 0.30$	+20.00	$22.23^{e} \pm 0.17$	-2.34	$48.51^{e} \pm 0.27$	+17.65
2	$35.28^{a} \pm 0.24$	+28.30	$25.59^{c} \pm 0.19$	-2.02	$60.86^{b} \pm 0.22$	+26.27
3	$30.29^{c} \pm 0.47$	+17.16	$29.93^{a} \pm 0.19$	-2.50	$60.22^{c} \pm 0.49$	+14.65
4	$26.68^{d} \pm 0.07$	+16.54	$25.39^{cd} \pm 0.15$	-1.24	$52.07^{d} \pm 0.21$	+15.30
5	$32.98^{b} \pm 0.19$	+19.49	$28.78^{b} \pm 0.12$	-3.85	$61.76^{a} \pm 0.11$	+15.65

Means with different letters within a column are significantly different ($p \leq 0.05$).

The processing conditions listed in this Table (1-5) correspond to the condition shown in Table 1.

Change in LMWDF, HMWDF and TDF after α-amylase hydrolysis was calculated based on the corresponding data of resistant dextrin samples reported in the previous study (Trithavisup et al., 2019).

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3.5 Prebiotic activity

The prebiotic activity scores of all resistant maltodextrin samples were positive (Figure 1), indicating that all samples had prebiotic activity. Prebiotic activity scores of the samples obtained from culturing *Lactobacillus casei*-01 and *Lactobacillus reuteri* TBRC 291 were 0.16-0.33 and 0.19-0.37, respectively. Figueroa-Gonzalez, Rodríguez-Serrano, Gomez-Ruiz, Garcia-Garibay, and Cruz-Guerrero (2019) reported that prebiotic activity scores of commercial saccharides as prebiotic, including Frutafit HD (mainly insulin), Oligomate 55 (galactooligosaccharide 55%, lactose 40% and monosaccharide 5%) and Regulact (lactulose) under the same incubation conditions in this study (anaerobiosis, addition of 1% prebiotic, incubation temperature of 37°C) were approximately 1-3 for 2 strains of *L. casei*. Moreover, prebiotic activity score of Regulact and Oligomate 55 obtained from culturing *L. casei* Shirota were approximately 0.3-0.5.

Fermentation rate for carbohydrate depended on many factors, including the degree of polymerization, sugar and glycosidic linkage and degree of branching of the carbohydrate molecules (Al-Sheraji et al., 2013). According to the current study, the resistant maltodextrin samples that contained smaller proportion of the chains/molecules with DP 1 – 5, greater DB and/or lower LMWDF content tended to have lower prebiotic activity score. Our results correspond well with those reported in the previous study. Van Laere, Hartemink, Bosveld, Schols, and Voragen (2000) monitored the fermentation of nondigestible oligosaccharides from several plant cell wall and polysaccharides, with widely different sugar composition and molecular size, by several strains of *Bifidobacterium spp.*, *Lactobacillus spp.*, *Bacteroides spp.* and *Clostridium spp.* They found that linear oligosaccharides were catabolized to a larger

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extent than those with branched structure. For instance, *Lactobacillus spp*. fermented (arabino)-galactooligosaccharide to a greater extent than the arabinogalactan-enriched polysaccharide fraction. B*ifidobacteria also better* utilized arabinooligosaccharides, in comparison with the arabinan-enriched polysaccharide fraction (Van Laere et al., 2000).

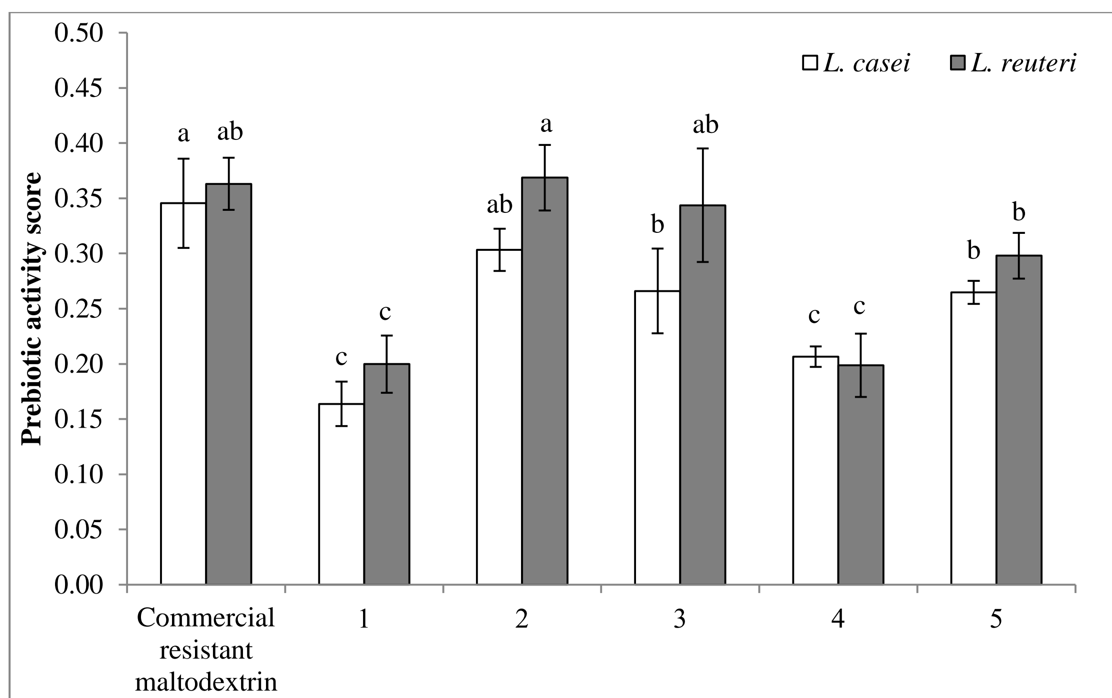

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Fig. 1 Prebiotic activity score of resistant maltodextrins prepared under different conditions. Means with different letters are significantly different ($p \leq 0.05$). Number 1-5 represented the processing conditions shown in Table 1.

Interestingly, resistant maltodextrin sample having its prebiotic activity score similar to that of the commercial sample ($p > 0.05$), regardless of the probiotics used, was the sample prepared from the second condition in Table 1. Note that this resistant dextrin sample had significantly highest LMWDF content ($p \leq 0.05$) (Table 4),

relatively high proportion of shorter branched chains (Table 2) and considerably low DB (Table 3).

3.6 Thermal properties at subzero temperatures

Glass transition temperature of maximally freeze-concentrated unfrozen phase (T_g'), and unfrozen water contents of 30% resistant maltodextrin solutions were shown in Table 5. T_g' is regarded as reference parameters determining the stability of frozen foods. As frozen system was kept below T_g', the unfrozen phase transformed to a glassy state. In the glassy unfrozen phase, molecular motion was extremely low, further ice crystallization and chemical reactions associated with the molecular diffusion of water and other reactants were retarded; therefore, long-term stability may be expected. (Charoenrein & Harnkarnsujarit, 2017; Li, Dickinson, & Chinachoti, 1998; Roos, 2010). As shown in Table 5, T_g' of the resistant maltodextrin was significantly lower than that of its resistant dextrin counterpart ($p \leq 0.05$). This could be due to the molecular weight reduction after α-amylase treatment. The relative M_w values of RD1 – RD5 ranged from 11.1 to 15.4 kDa (Trithavisup et al., 2019), whereas those of RMD1 – RMD5 were less than 5 kDa (Table 2). For the carbohydrate polymers, especially for the molecules with limited degree of chain entanglement in the solution and cannot form gels, T_g' generally decreases with decreasing molecular weight, (Levine & Slade, 1986; Roos, 1993). However, T_g' of the resistant maltodextrins prepared under different conditions (RMD1 – RMD5) did not follow this trend. Small difference of the relative M_w (<2kDa) among the resistant maltodextrins might have little or no effect on their T_g' values.

In the amorphous unfrozen phase, the remaining unfrozen water was considered as immobilized water which was not frozen at -40°C or lower

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(Charoenrein & Harnkarnsujarit, 2017; Li et al., 1998; Roos, 2010). According to Table 5, the solution of resistant dextrins and/or resistant maltodextrins with lower T_g' tended to have higher amount of unfrozen water. The similar trend was previously reported for the T_g' and the unfrozen water content of the corn syrup samples with DE ranged from 26 to 95 (Levine & Slade, 1986).

Table 5. Thermal properties of frozen solutions containing 30% (w/v) resistant dextrins or resistant maltodextrins obtained from different processing conditions

Sample	T_g' (°C)	Unfrozen water (g water/ g dried sample)
RD1	$-14.94^{c} \pm 0.03$	$0.27^{bc} \pm 0.03$
RD2	$-14.83^{c} \pm 0.04$	$0.25^{c} \pm 0.03$
RD3	$-13.02^{a} \pm 0.05$	$0.23^{c} \pm 0.02$
RD4	$-13.35^{b} \pm 0.09$	$0.24^{c} \pm 0.01$
RD5	$-13.12^{ab} \pm 0.12$	$0.23^{c} \pm 0.08$
RMD1	$-17.84^{g} \pm 0.04$	$0.41^{a} \pm 0.03$
RMD2	$-17.58^{f} \pm 0.13$	$0.39^{ab} \pm 0.02$
RMD3	$-15.51^{d} \pm 0.10$	$0.32^{abc} \pm 0.05$
RMD4	$-16.06^{e} \pm 0.07$	$0.29^{abc} \pm 0.08$
RMD5	$-16.03^{e} \pm 0.12$	$0.26^{bc} \pm 0.03$

Means with different letters within a column are significantly different ($p \leq 0.05$).

RD1 – RD5 represent the resistant dextrin samples obtained from the condition 1-5 as shown in Table 1, using only dextrinization step.

RMD1 – RMD5 represent the resistant maltodextrin samples obtained from the condition 1-5 as shown in Table 1, using both dextrinization and α-amylase hydrolysis.

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The overall results from this part could provide an insight into additional role of resistant dextrins and resistant maltodextrins in frozen food products. Those highly-branched carbohydrate polymers may not only be added as a source of dietary fiber and prebiotics but may also help increase T_g' of the sugar-rich frozen products, such as frozen dairy desserts. Frozen system with higher T_g' usually has slower rate of ice recrystallization during storage (Levine & Slade, 1986), and the quality deterioration due to this phenomena can be retarded.

4. Conclusions

Alpha-amylase treatment of the resistant dextrins enhanced indigestible carbohydrates, especially the LMWDF fraction. Selected chromatographic techniques and NMR spectroscopy provided insight into molecular structure modification after the enzymatic hydrolysis. In comparison with its resistant dextrin counterpart, resistant maltodextrin had lower molecular weight, shorter branched chains, smaller proportion of digestible α-1,4-glycosidic linkages, with greater proportion of indigestible β-1,2, β-1,4, β-1,6 and α-1,6-glycosidic linkages as well as higher DB. Moreover, modification of the molecular structure of resistant maltodextrins could also be obtained from dextrinization step. For instance, resistant maltodextrins derived from stronger dextrinization conditions tended to have higher molecular weight, DB and proportion of indigestible fraction but contained shorter branched chains.

Relationship between molecular structure and selected properties of resistant maltodextrins was also revealed. Resistant maltodextrin with higher prebiotic activity score tended to contain greater proportion of short branched chains (DP 1 - 5) and LMWDF fraction, but having less DB. According to thermal properties in frozen systems, despite of their structural difference, little variation in T_g' and unfrozen water

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content of the resistant maltodextrins was found. Nevertheless, the impact of decreasing molecular weight on T_g' reduction was clearly seen for any resistant maltodextrin sample comparing to its resistant dextrin counterpart.

Conflict of interest

The authors have no conflicts of interest.

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CHAPTER 4
Conclusion

4.1 Overall conclusions of the research study

Overall results from this study clearly showed the effects of processing conditions on molecular structure and properties of resistant dextrins and resistant maltodextrins. According to the first part, resistant dextrins obtained from stronger dextinization conditions (high acid concentration, high temperature and long reaction time) had darker color, higher water solubility, reducing sugar content and total dietary fiber. No endothermic peak was found in the solution of resistant dextrin prepared under stronger conditions, indicating the loss of crystalline structure in starch granules. The resistant dextrins had much lower molecular weight and shorter branch chains in comparison with the native starch. However, stronger dextrinization conditions induced slight increase in molecular weight but led to a decrease in the fractions of longer branched chains. These results suggested that the predominant reaction during dextrinization was hydrolysis. Nevertheless, transglucosidation and repolymerization could be enhanced under stronger conditions.

The resistant dextrins prepared from this study contained <50% dietary fiber. This could indicate that great extent of tranglucosidation and repolymerization did not occur. Results from the second part showed the importance of α-amylase treatment on improving properties of the soluble dietary fiber, especially enhancing indigestible portion of the products. Resistant maltodextrins contained 48 - 62% of total dietary fiber, which accounted for 15 – 26% increase in the dietary fiber content, comparing to resistant dextrin. Alpha-amylase treatment greatly enhanced the low molecular

weight dietary fiber fraction. Moreover, the enzymatic hydrolysis caused the reduction in molecular weight and branched chain length of resistant maltodextrin.

The H^1 NMR spectra clearly indicated the formation of new glycosidic linkages, α-1,2, α-1,6, β-1,2, β-1,4 and β-1,6, during dextrinization step. For both resistant dextrins and resistant maltodextrins, degree of branching tended to increase but the average chain length reduced at stronger dextrinization conditions. According to thermal properties of the frozen system, resistant maltodextrin solution tended to have lower T_g' and higher unfrozen water content in comparison with the solution from its resistant dextrin counterpart. In case of additional health benefits from resistant maltodextrins, it was found that prebiotic activity score was higher in the resistant maltodextrin with greater proportion of short branched chains and LMWDF fraction, but had lower DB.

According to the overall results from this study, the resistant maltodextrin dextrinized with 0.06% HCl, 120°C, 120 min and hydrolyzed for 90 min contained highest total dietary fiber content. Nevertheless, the product prepared from the dextrinization condition at 0.04% HCl, 120°C, 120 min and hydrolyzed for 120 min had highest prebiotic activity score. These data could serve as guidelines for selecting an appropriate processing condition, depending on the targeted properties of the resistant maltodextrins.

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4.2 Limitation of the research study

Commercial resistant dextrins and resistant maltodextrins usually contain >80% dietary fiber. However, the highest TDF content of resistant maltodextrins obtained from this study was 62%. This might result from relatively

mild dextrinization conditions used in this study, in order to avoid excessive pyrolysis.

4.3 Recommendations of the research study

Modification of the rotary cooker used for preparing resistant dextrins are required to prevent burning of the sample at stronger dextrinization conditions. Therefore, higher acid concentration, temperature, and/or longer reaction time during dextrinization can be applied. Those stronger conditions might help enhance the total dietary fiber content of the resistant dextrin and resistant maltodextrin products.

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CHULALONGKORN UNIVERSITY

VITA

NAME	Miss Kamonrat Trithavisup
DATE OF BIRTH	26 July 1988
PLACE OF BIRTH	Bangkok
INSTITUTIONS ATTENDED	Department of Food Science and Technology, Faculty of Agro-Industry, Kasetsart University (2007-2011; B.S. holder) Department of Food Science and Technology, Faculty of Agro-Industry, Kasetsart University (2012-2015; M.S. holder) Department of Food Technology, Faculty of Science, Chulalongkorn University (2016-2020; Ph.D. holder)
HOME ADDRESS	27/709 Kanjanapisek Road, Lak Song, Bangkae, Bangkok 10160
PUBLICATION	Trithavisup, K., K. Krusong and K. Tananuwong. 2019. In-depth study of the changes in properties and molecular structure of cassava starch during resistant dextrin preparation. Food Chemistry. 297: DOI: org/10.1016/j.foodchem.2019.124996. Trithavisup, K. and S. Charoenrein. 2019. The effect of acid treatment and freeze-thaw cycle on textural properties and microstructure of cooked aged rice. Starch – Stärke. 71: DOI: 10.1002/star.201800098. Trithavisup, K. and S. Charoenrein. 2016. Influence of acid treatment on physicochemical properties of aged rice flour. International Journal of Food Properties. 19: 2074–2086.
AWARD RECEIVED	Thailand Research Fund (TRF) and General Starch Ltd. through Research and Researchers for Industries Ph.D. Program (Grant no. PHD60I0004), 2017 - 2020 Graduate School, Kasetsart University (Research Grant for International Publication), 2012 – 2015 Special Prize of Poster Presentation in KU-UT Student Symposium 2015 “The Future of Sustainable Food and Farming”, awarded by Faculty of Agriculture, Kasetsart University, 2015

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Third Prize for Graduate Poster Presentation in KU-UT Student Symposium 2015 “The Future of Sustainable Food and Farming”, awarded by Faculty of Agriculture, Kasetsart University, 2015

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Chulalongkorn University