# DEVELOPMENT OF NUTRITIONALLY COMPLETE ORAL NUTRITIONAL SUPPLEMENT USING TAPIOCA MALTODEXTRIN AS A CARBOHYDRATE SOURCE

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A Dissertation Submitted in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy in Food and Nutrition
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# การพัฒนาอาหารเสริมทางปากที่มีสารอาหารครบถ้วนโดยใช้มอลโทเดกซ์ทรินจากแป้งมันสำปะหลังเป็นแหล่งคาร์โบไฮเดรต

น.ส.จูนัยดา แอสทินา



วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต
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จูนัยดา แอสทินา : การพัฒนาอาหารเสริมทางปากที่มีสารอาหารครบถ้วนโดยใช้มอลโทเดกซ์ทรินจากแป้งมันสำปะหลังเป็นแหล่งคาร์โบไฮเดรต. ( DEVELOPMENT OF NUTRITIONALLY COMPLETE ORAL NUTRITIONAL SUPPLEMENT USING TAPIOCA MALTODEXTRIN AS A CARBOHYDRATE SOURCE) อ.ที่ปรึกษาหลัก : รศ. ดร.สุวิมล ทรัพย์วโรบล, อ.ที่ปรึกษาร่วม : พล.อ.ต. นพ.วิบูลย์ ตระกูลฮุน

คาร์โบไฮเดรตเป็นหนึ่งในสารอาหารหลักที่สำคัญในอาหารทางการแพทย์ ดังนั้นการใช้รีซิสแทนท์ มอลโทเด็กซ์ทรินที่ได้จากการแปรรูปแป้งมันสำปะหลัง ซึ่งมีคุณสมบัติเป็นใยอาหารชนิดละลายน้ำ อาจมีส่วนช่วยในการลดระดับการตอบสนองต่อกลูโคสได้ งานวิจัยนี้จึงมีวัตถุประสงค์ในการศึกษาผลของรีซิสแทนท์ มอลโทเด็กซ์ทริน และอาหารทางการแพทย์ที่มีส่วนประกอบของรีซิสแทนท์ มอลโทเด็กซ์ทรินต่อระดับการตอบสนองต่อระดับน้ำตาล ความเต็มอิ่ม และความทนทานของระบบทางเดินอาหาร (gastrointestinal tolerability) ในอาสาสมัครที่มีสุขภาพดี นอกจากนี้ยังศึกษาคุณสมบัติทางกายภาพและเคมี และการยอมรับทางประสาทสัมผัสของผู้บริโภคที่มีต่ออาหารทางการแพทย์อีกด้วยผลการศึกษาระยะที่ 1 พบว่าเมื่ออาสาสมัครที่มีสุขภาพดีจำนวน 16 คน รับประทานรีซิสแทนท์ มอลโทเด็กซ์ทริน 50 กรัม ส่งผลให้ระดับน้ำตาลและอินซูลินหลังมื้ออาหารลดลงอย่างมีนัยสำคัญทางสถิติ เมื่อเปรียบเทียบกับกลุ่มที่รับประทานกลูโคส 50 กรัมและกลุ่มที่รับประทานมอลโทเด็กซ์ทริน 50 กรัม โดยระดับน้ำตาลในเลือดสูงสุด (peak of plasma glucose) ของอาสาสมัครในกลุ่มที่รับประทานรีซิสแทนท์ มอลโทเด็กซ์ทริน กลุ่มที่รับประทานกลูโคส และกลุ่มที่รับประทานมอลโทเด็กซ์ทรินมีค่า 104.60±2.63 135.87±4.88 และ 127.93±4.05 mg/dl ตามลำดับ และมีระดับอินซูลินสูงสุด (peak of serum insulin) เท่ากับ 13.001±2.12 47.90±11.93 และ 52.96±17.68 µIU/ml ตามลำดับ อย่างไรก็ตามการรับประทานรีซิสแทนท์ มอลโทเด็กซ์ทรินไม่มีผลต่อระดับความหิว ความอิ่ม และความอยากอาหารในช่วง 180 นาทีขณะที่ทำการศึกษา นอกจากนี้รีซิสแทนท์ มอลโทเด็กซ์ทริน 50 กรัมไม่ทำให้เกิดอาการท้องเสีย แต่ก่อให้เกิดอาการท้องอืด ท้องเฟ้อในอาสาสมัครบางราย. การศึกษาระยะที่ 2 อาหารทางการแพทย์ได้รับการพัฒนาขึ้นจำนวน 3 สูตร โดยมีปริมาณรีซิสแทนท์ มอลโทเด็กซ์ทรินแตกต่างกัน ได้แก่ อาหารทางการแพทย์ที่ไม่มีส่วนประกอบของรีซิสแทนท์ มอลโทเด็กซ์ทริน (original) อาหารทางการแพทย์ที่มีส่วนประกอบของรีซิสแทนท์ มอลโทเด็กซ์ทริน 15 กรัมและ 30 กรัม (RMD15 และ RMD30 ตามลำดับ) จากการทดสอบความหนืด (viscosity) พบว่า RMD30 มีความหนืดเท่ากับ 34.07±0.09 cP ซึ่งต่ำที่สุดเมื่อเทียบกับ original และ RMD15 ที่มีค่าความหนืด 36.37±0.25 และ 34.60±0.06 cP ตามลำดับ และ RMD30 มีค่า water activity สูงที่สุด (0.37±0.01) เมื่อเทียบกับ original และ RMD15 (0.35±0.01 และ 0.33±0.01 ตามลำดับ) ผลการทดสอบการยอมรับทางประสาทสัมผัสของผู้บริโภคที่มีต่ออาหารทางการแพทย์ ระบุว่าอาหารทางการแพทย์ทุกสูตรได้รับการยอมรับจากอาสาสมัคร โดยมีค่าเฉลี่ยคะแนนการยอมรับโดยรวมมากกว่า 7

การศึกษาระยะที่ 3 ศึกษาผลของการรับประทานอาหารทางการแพทย์ทั้ง 3 สูตรต่อระดับน้ำตาลและอินซูลินหลังมื้ออาหารในอาสาสมัครที่มีสุขภาพดีจำนวน 17 คน พบว่า กลุ่มที่ได้รับ RMD30 มีระดับน้ำตาลในเลือดหลังมื้ออาหารต่ำที่สุด (113.33±4.44 mg/dl) เมื่อเปรียบเทียบกับกลุ่มที่ได้รับ original และ RMD15 (119.25±4.67 และ 114.42±6.43 mg/dl) ในขณะที่พื้นที่ใต้กราฟ (area under the curve) ของระดับอินซูลินในกลุ่มที่ได้รับ RMD30 ลดลง 33.1% เมื่อเปรียบเทียบกับกลุ่มที่ได้รับ original (2,320±570.76 และ 3,470.12±531.86 µIU/ml) อย่างไรก็ตามการรับประทานอาหารทางการแพทย์ทั้ง 3 สูตรไม่มีผลต่อระดับความหิว ความอิ่ม และความอยากอาหารของอาสาสมัคร อีกทั้งไม่ก่อให้เกิดอาการไม่พึงประสงค์ของระบบทางเดินอาหารในอาสาสมัครอีกด้วย. การศึกษาระยะที่ 4 ศึกษาผลของการรับประทานอาหารทางการแพทย์ที่มีส่วนประกอบของรีซิสแทนท์ มอลโทเด็กซ์ทริน 30 กรัม ต่อระดับน้ำตาล อินซูลิน น้ำหนักตัวและการรับประทานอาหารในอาสาสมัครที่มีสุขภาพดีและผู้ที่ภาวะก่อนเบาหวาน ผลการศึกษาไม่พบการเปลี่ยนแปลงของน้ำหนักตัวเมื่อรับประทาน RMD30 (พลังงาน 252 กิโลแคลอรี่ต่อหน่วยบริโภค) 1 ซองต่อวันเป็นระยะเวลา 3 สัปดาห์ (66.56±3.66 และ 66.99±3.69 กก. p=0.069) อีกทั้งยังพบว่าการรับประทานอาหารของอาสาสมัครเพิ่มขึ้นอย่างมีนัยสำคัญทางสถิติในสัปดาห์ที่ 3 (1,144.87±87.10 และ 1,477.76±94.16 กิโลแคลอรี่ p=0.001) อาสาสมัครส่วนใหญ่ขับถ่ายเป็นปกติ 1-2 ครั้งต่อวัน (รูปแบบ 3 และ 4 ของ Bristol Stool Scale) และการรับประทาน RMD30 ไม่ก่อให้เกิดอาการไม่พึงประสงค์ของระบบทางเดินอาหารในอาสาสมัคร. จากผลการศึกษาจึงสรุปได้ว่ารีซิสแทนท์ มอลโทเด็กซ์ทรินมีส่วนช่วยในการลดการตอบสนองของระดับน้ำตาลและอินซูลินในอาสาสมัครที่มีสุขภาพดี การทดแทนมอลโทเด็กซ์ทรินด้วยรีซิสแทนท์ มอลโทเด็กซ์ทรินในอาหารทางการแพทย์ยังส่งผลให้ความหนืดของอาหารทางการแพทย์ลดลง และ water activity เพิ่มขึ้น และยังเพิ่มคะแนนการยอมรับทางประสาทสัมผัสของผู้บริโภคอีกด้วย อาหารทางการแพทย์ที่มีส่วนประกอบของรีซิสแทนท์ มอลโทเด็กซ์ทริน 30 กรัม ลดการตอบสนองของอินซูลิน แต่ไม่มีผลต่อระดับน้ำตาลในเลือดและความหิว ความอิ่ม และความอยากอาหารของอาสาสมัครอย่างมีนัยสำคัญ การรับประทานอาหารทางการแพทย์ที่มีส่วนประกอบของรีซิสแทนท์ มอลโทเด็กซ์ทริน 30 กรัม เป็นระยะเวลา 3 สัปดาห์ส่งผลให้การรับประทานอาหารของเพิ่มขึ้นและไม่ก่อให้เกิดอาการไม่พึงประสงค์ของระบบทางเดินอาหารของอาสาสมัครที่มีสุขภาพดีและผู้ที่ภาวะก่อนเบาหวาน



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| สาขาวิชา | อาหารและโภชนาการ | ลายมือชื่อนิสิต ..... |
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KEYWORD: oral nutritional supplement, plasma glucose, serum insulin, tapioca resistant maltodextrin, tolerability, tapioca maltodextrin

Junaida Astina : DEVELOPMENT OF NUTRITIONALLY COMPLETE ORAL NUTRITIONAL SUPPLEMENT USING TAPIOCA MALTODEXTRIN AS A CARBOHYDRATE SOURCE. Advisor: Assoc. Prof. SUWIMOL SAPWAROBOL, DrPH., M.Sc., RDN Co-advisor: AVM Vibul Trakulhoon, M.D., Ph.D

Carbohydrate is a major composition in oral nutrition supplement. Modification of carbohydrate composition using tapioca resistant maltodextrin (TRM) may improve the glycemic response. This study aimed to investigate the effect of TRM and TRM containing-ONS on glycemic response, satiety, and gastrointestinal (GI) tolerability in healthy individuals. Physicochemical properties and sensory evaluation of developed ONS were also investigated. In Phase I, the peak of plasma glucose and insulin response after TRM (50 g) ingestion was lowest compared to 50 g glucose (GL) and tapioca maltodextrin (TM) in 16 healthy participants. Peak of plasma glucose of TRM, GL, and TM were 104.60±2.63, 135.87±4.88, and 127.93±4.05 mg/dl, respectively, while peak of serum insulin was 13.001±2.12, 47.90±11.93, and 52.96±17.68 μIU/ml. No significant effects were observed on subjective appetite during 180 min of study. Fifty grams of TRM increased flatulence in healthy participants. In phase II, three formulas were developed: original (0 g TRM), RMD15 (2.7 g TRM), and RMD30 (5.4 g TRM). The RMD30 significantly had lowest viscosity (viscosity of original, RMD15, RMD30 were 36.37±0.25, 34.60±0.06, 34.07±0.09 cP, respectively) and highest water activity (0.35±0.01, 0.33±0.01, 0.37±0.01 respectively) compared to original formula. The overall acceptability scores of developed formula were more than 7. In phase III, seventeen healthy participants were included to examine the acute effect of 1 serving of three developed formulas on glycemic response and tolerability. The RMD30 formula showed lowest postprandial blood glucose compared to original and RMD 15 (113.33±4.44 vs 119.25±4.67 and 114.42±6.43 mg/dl, respectively). Meanwhile, the Area Under Curve of serum insulin in RMD30 group was lower by 33.1% compared to original group (2,320±570.76 vs 3,470.12±531.86 μIU/ml). Subjective appetite responses were not significantly different among three formulas. All formulas were well tolerated by healthy participants. In phase IV, RMD30 formula was chosen to be supplemented in normoglycemic and prediabetic participants. Bodyweight did not significantly change after ingestion of 1 serving/d of RMD30 for 3 weeks (66.56±3.66 vs 66.99±3.69 kg, p=0.069). Food intake significantly increased at week 3 following supplementation compared to baseline (1,144.87±87.10 vs 1,477.76±94.16 kcal, p=0.001), without affecting habitual food intake. There were no significant changes in gastrointestinal symptoms. In conclusion, TRM lowers the postprandial plasma glucose and insulin response in healthy participants. Incorporation of TRM in nutritionally complete ONS decreased viscosity and increased water activity, while increased the sensory acceptability score. The replacement of TM using TRM by 30% in ONS decreased the insulin response, without significantly affected the glucose and satiety. Supplementation of RMD30 daily for 3 weeks increased total food intake in healthy and prediabetic participants. Developed formula was well-tolerated in acute and three-weeks study.

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| Field of Study: | Food and Nutrition | Student's Signature ..... |
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| | | Co-advisor's Signature ..... |

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# CHAPTER I

# INTRODUCTION

## 1.1 Background

European Society for Parenteral and Enteral Nutrition (ESPEN) defined oral nutritional supplement (ONS) as specially formulated liquid, crème, or powder supplement which is given under medical supervision (Cederholm et al., 2017). Oral nutritional supplement is beneficial to improve body weight, fat mass, fat-free mass, quality of life, nutrition status, lower hospital readmission, hospital length of stay, and cost in malnourished patients (Elia, Normand, Laviano, & Norman, 2016; Lauque, Arnaud-Battandier, et al., 2004; Rozentryt et al., 2010; Rebecca J. Stratton, Hebuterne, & Elia, 2013). According to ESPEN, ONS can be categorized as nutritionally incomplete formula which provides specific nutrients in high amount while lacking in some other nutrients; and nutritionally complete formula which provides balanced nutrition composition both macro- and micronutrients. Meanwhile, disease-specific formula is formulated for specific disease, which provides complete or incomplete nutrients (Cederholm et al., 2017). Standard nutritionally complete formula is the most commonly-used ONS in patients (Brown, Roehl, & Betz, 2015). Furthermore, ONS is available in many styles, such as juice type, milkshake type, high-energy powders, high-protein, soup type, and high density type (BAPEN, 2016).

Carbohydrate is a major component of standard ONS which is contributed up to 50-55% total energy (Forbes & Valentini, 2016). Rapidly digestible starch, including corn syrup, corn syrup solids, sucrose, and maltodextrin are widely used as carbohydrate source in ONS (Savino, 2018). However, long-term use of those rapidly digestible starch in standard ONS leads to increase risk of hyperglycemia, both in diabetic and normoglycemic patients (Voss et al., 2008). In addition, it was reported that the rise of blood glucose in patients with or without diabetes mellitus receiving enteral nutrition is caused by the increasing of hepatic glucose production and the decreasing of peripheral glucose utilization (Gosmanov & Umpierrez, 2013).

Modification of macronutrient composition of ONS may help to improve blood glucose (Gosmanov & Umpierrez, 2013). Lansink et al., showed that fasting plasma glucose was increased in type 2 diabetes subjects receiving standard ONS formula, but not in diabetes-specific formula ONS (Lansink, van Laere, Vendrig, & Rutten, 2011). Similarly, Hofman et al., (2004) reported that diabetes-specific formula had lower postprandial glucose response in healthy and diabetic subjects (Z. Hofman, Van Drunen, De Later, & Kuipers, 2004). However, it cannot be concluded whether the beneficial effect was mediated by low carbohydrate content or increased fiber and monounsaturated fatty acid (MUFA) content in diabetes-specific formula (Z. Hofman et al., 2004). Addition of dietary fiber, particularly soluble fiber,

would attenuate the glycemic response by delaying carbohydrate digestion and absorption (Ahmed, Sairam, & Urooj, 2011; Cassidy, McSorley, & Allsopp, 2018).

Resistant maltodextrin (RMD) is a novel non-viscous soluble dietary fiber. Commercially available RMD are derived from cornstarch, potato starch, and wheat starch. Incorporating 3-10 g/meal of RMD into carbohydrate containing beverages or meals decreased glycemic response (Livesey & Tagami, 2008). Resistant maltodextrin contains α-1,2, α-1,3 glucosidic linkage which is resistant to human carbohydrate digestive enzymes in small intestine (Ohkuma & Wakabayashi, 2001). The undigested part of RMD is then fermented by gut microbiota in the colon resulting short-chain fatty acids (SCFAs), mainly acetate, propionate, and butyrate (Carlson, Hospattankar, Deng, Swanson, & Slavin, 2015; Goda, Kajiya, Suruga, Tagami, & Livesey, 2006). Colonic fermentation of RMD increases Glucagon-Like-Peptide (GLP)-1 secretion thus improve the glucose tolerance in rats (Hira, Ikee, Kishimoto, Kanahori, & Hara, 2015). In a randomized clinical trial, daily supplementation of 27 g RMD for 12 weeks significantly decreased fasting plasma glucose and HOMA-IR in adults with metabolic syndrome (Hashizume et al., 2012).



Resistant maltodextrin also showed potential benefit on lipid metabolism in some animal and human studies (Kishimoto, Oga, Tagami, Okuma, & Gordon, 2007; Kishimoto et al., 2009). Previous study showed that 5 g of RMD significantly lowered postprandial triacylglycerol following high fat meals in healthy subjects (Kishimoto et

al., 2007). Later, it was known that RMD lower the triacylglycerol by inhibiting lipid absorption and increasing lipid excretion into feces (Kishimoto et al., 2009). In addition, fermentation of RMD may decrease hunger and increase satiety, yet the results were inconsistent (Guérin-Deremaux et al., 2013; Ye, Arumugam, Haugabrooks, Williamson, & Hendrich, 2015). However, fermentation of RMD, in high dose (1.1 g/kg body weight), promoted bloating, abdominal pain, or even diarrhea (Kishimoto, Kanahori, Sakano, & Ebihara, 2013).

In this study, we used tapioca maltodextrin since Thailand is one of the largest tapioca-exporting country with exports value of USD 38.3 M in 2019 (The Observatory of Economic Complexity, 2019). Tapioca maltodextrin and RMD have been discovered recently. Previous study showed that tapioca RMD (TRM) contained 86.62% of dietary fiber (Toraya-Avilés, Segura-Campos, Chel-Guerrero, & Betancur-Ancona, 2017). Hofman et al., reported that different sources of maltodextrin and manufacturing process may have different effect on its physicochemical properties (D. Hofman, Van Buul, & Brouns, 2015). Moreover, it was reported that the variability effect of RMD on glycemic response and tolerability may be affected by sources of RMD due to differences in amylose content and also, molecular weight (Kishimoto et al., 2013; Wolf, Wolever, Bolognesi, Zinker, & Garleb, 2001). However, the report of health benefit of TRM is lacking. Therefore, we aimed to investigate the effect of TRM on glucose and insulin response. In addition, we also aimed to examine the

physicochemical properties of ONS using TRM in different proportion. The acute- and long-term effect of ONS were also evaluated on glucose and insulin, as well as its tolerability and acceptability in adults.

**1.2 Objectives**

- To investigate the effect of TRM on postprandial plasma glucose and serum insulin in healthy participants.
- To develop nutritionally complete ONS containing TRM and to evaluate the physicochemical characteristic of the developed ONS.
- To determine the potential benefits of the developed ONS containing TRM on plasma glucose and serum insulin in healthy participants.
- To evaluate the effect of ONS supplementation on body weight, food intake, and tolerability of the developed ONS in normoglycemic and prediabetic adults.



**1.3 Hypothesis**

- TRM could decrease postprandial plasma glucose and serum insulin in healthy participants.
- The developed ONS containing TRM would decrease the viscosity, increase the water activity content, and acceptability by the participants.
- The developed ONS containing TRM would decrease postprandial plasma glucose and insulin in the healthy participants.

- The developed ONS increased the body weight without affecting daily food intake and well-tolerated in long-term use in normoglycemic and prediabetic participants.

# CHAPTER II

# LITERATURE REVIEW

## 2.1 Oral nutritional supplement

Oral Nutritional Supplement (ONS) is categorized as enteral nutrition according to ESPEN or known as Foods for Special Medical Purposes (FSMP) in Codex Standard. Enteral nutrition is specialized food intended for the medical purposes which can be delivered via tube (tube feeding) or oral intake (oral nutritional supplement). Oral nutritional supplement is defined as dietary food designed for medical purpose which is consumed as oral supplement, in addition to regular diet (Lochs et al., 2006). Therefore, ONS consumption should be under medical supervision.

Oral nutritional supplement is divided into three categories based on the nutrient content of the ONS, including nutritionally incomplete, nutritionally complete with standard nutrient formula, and nutritionally complete with a nutrient-adapted formula for specific disease (EFSA Panel on Dietetic Products & Allergies, 2015). Nutritionally incomplete ONS provides only one or two ingredients hence nutritionally incomplete ONS cannot be used as a sole source of nutrient. It is used only as complementary to normal diet. In contrast, nutritionally complete ONS provides all macro- and micronutrients in balanced nutrition composition of carbohydrate, protein, fat, vitamin, and minerals which is provided in 1,500-2,000 kcal/day (Iacone et al., 2015; Kapala, Choruz, & Klek, 2014). Nutritionally complete

ONS for specific disease provides modified nutrients designed for specific diseases. Currently, there are available ONS specific for diabetes, renal failure, cancer, liver failure, pancreas and biliary tract, respiratory disease, and pre-surgery (Kapala et al., 2014). According to its form, ONS are categorized in several types of form, such as powder, liquid, semi-liquid, and solid form as snack which are available in the market as shown in the table below.

Table 1. Type of oral nutritional supplements (BAPEN, 2016)

| Type | Notes |
|---|---|
| High-energy powders | Energy density is 1.5-2.5 kcal/ml with volume ranging from 125-350 ml/serving. Can be made up with addition of full cream milk to add more energy. |
| Milkshake type | Energy density is 1-2.4 kcal/ml with volume ranging from 125-220 ml/serving. Fiber-enriched ONS is available. |
| High-protein | Contains 11-20 g protein in volume ranging from 30-220 ml. Available in jellies, shots, and milkshake type ONS. |
| Soup type | Energy density is 1-1.5 kcal/ml in volume ranging from 200-330 ml. Available in ready mixed and powder form which can be reconstituted with water or milk. |
| Juice type | Energy density is 1.25-1.5 kcal/ml in volume ranging from 200-220 ml. This type does not contain fat (fat-free). |

| Type | Notes |
|---|---|
| Semi-solid | Energy density is 1.4-2.5 kcal/ml in thickened liquid (stage 1 and 2) or smooth pudding style (stage 3). |
| Low volume high concentration | Made up from fat and protein which is consumed in small quantities (30-40 ml/serving) with dose 3-4 times per day. |

General characteristics of standard ONS are 50-55% energy from carbohydrate, up to 30% energy from fat, 15-20% energy from whole protein, with or without fiber (Forbes & Valentini, 2016).

Oral nutritional supplement has been shown to improve the clinical outcome as well as increase the body function in some disease groups, such as muscle function and cognitive function (Baldwin, Spiro, Ahern, & Emery, 2012; Beck, Wijnhoven, & Lassen, 2011). In the hospital setting, ONS decreased postoperative complications, shorten hospital length of stay, and decreased mortality (Baldwin et al., 2012; Mullin, Fan, Sulo, & Partridge, 2019). On the other hand, the benefits of ONS on functionality, such as improved muscle strength, walking ability, decreased number of falls, were more obvious in the malnourished patients with BMI less than 20 kg/m$^2$ who gain weight at least 2 kg during supplementation (Rebecca J Stratton & Elia, 2000).

*Manufacturing process of ONS*

There are several methods to produce ONS, including wet-mix process, dry-mix process, and combined process (Cordier, 2008). Wet-mix process combines all raw ingredients in liquid form followed by heating, drying, and filling process. Dry-mix process allows separately processed ingredients which followed by dry blending and filling process. Meanwhile, combined process is the combination between wet- and dry mixing where unprocessed raw ingredients are processed by wet mixing to get the base powder followed by addition of separately processed ingredients to get the final product (Cordier, 2008).

In current study, we follow dry-mixing method to obtain the ONS. Dry-mixing method is more efficient in energy consumption and more flexible in formulation (FAO/WHO Expert Meeting, 2004). However, it is important to note that microorganism control is important point in dry-mixing method since there is no further heat treatment after blending ingredients. All materials used in dry mixing should follow thermal inactivation process to eliminate the contamination of microorganisms. Suppliers of ingredients should comply with current Good Manufacturing Practice (cGMP) and Hazard Analysis Critical Control Point (HACCP) standard (FAO/WHO Expert Meeting, 2004).

To ensure the safety of ONS from pathogenic bacteria, U.S Food and Drug Administration (FDA) set the criteria for unacceptable limit of bacteria, including more than $10^4$ CFU/ml of any aerobic bacteria plate count, three or more samples

containing >$10^3$ CFU/ml, and any culture containing pathogenic bacteria, such as L. monocytogenes, S. aureus, or coliforms (Bankhead et al., 2009; U.S. Food and Drug Administration, 2008).

Table 2. Microbial standard in ONS (U.S. Food and Drug Administration, 2008)

| Indicators | Standard of US FDA |
|---|---|
| Aerobic plate count | |
| Any subsample | <$10^4$ CFU/g |
| Three or more subsamples | <$10^3$ CFU/g |
| Coliforms | <3 CFU/g |
| Salmonella | Not present |
| Listeria monocytogenes | Not present |
| Escherichia coli | Not present |
| Staphylococcal enterotoxin | Not present |
| Bacillus cereus | Not present |



*Physical characteristic of ONS*

Oral nutritional supplements are available in many forms, such as liquid, semi-solid, and solid. Each form has different physical characteristic. In powder form, ONS is more stable during storage compared to liquid form due to the moisture content and water activity. Baez, *et al*., found that the initial moisture content of powder

formula in their study was 4.02% (Baéz, Rojas, Sandoval-Guillén, & Valdivia-López, 2012). In addition, Bal, *et al* evaluated the effect of moisture on stability during storage in immune enhancing formula from natural sources. The result showed that it was recommended to keep the moisture at 4-5% (Bal, Nath, Radhakrishna, Indiramma, & Vijayalakshmi, 2009).

Viscosity of oral nutritional supplement varies depends on the form of ONS. According to National Dysphagia Task Force, thin liquid ONS has viscosity range between 1-50 cP; nectar-like ONS has viscosity range between 51-350 cp; honey-like ONS has viscosity between 351-1750 cP, and; spoon-like ONS has viscosity more than 1750 cP (National Dysphagia Diet Task Force, 2002). Viscosity of the ONS affect to palatability and sensory of the product. High viscosity of oral nutritional supplement tends to decrease food intake since it gives more satiety to the subjects (Nieuwenhuizen, Weenen, Rigby, & Hetherington, 2010; Zijlstra, Mars, De Wijk, Westerterp-Plantenga, & De Graaf, 2008). Meanwhile, ONS which provide high energy tends to have higher viscosity and less palatable compared to low viscosity ONS (Chapman, Dewille, Lowe, & Mazer, 2015).

Osmolality of the oral nutritional supplement is one of the factors that affect to gastrointestinal tolerability of the patients or subjects since higher osmolality may lead to osmotic diarrhea. Osmolality of standard polymeric formula should be close to physiological (200-350 mOsm/kg $H_2O$). However, the osmolality of the ONS may

be higher in higher energy density formula and in oligomeric formula (300-600 mOsm/kg $H_2O$) (Kapala et al., 2014).

Table 3. Comparison of standard ONS available in the market

| Brand | Osmolality | Caloric distribution | | | Fiber | Nutrient sources | | | |
|---|---|---|---|---|---|---|---|---|---|
| | (mOsm/kg) | Pro | Fat | CHO | (g/ 1000 kcal) | Protein | Fat | CHO | Fiber |
| Blendera MF | 390 | 16 | 29 | 55 | 4,95 | SPI 92%<br>sodium caseinate 8% | Rice bran oil 92%<br>MCT 8% | Corn maltodextrin 64%<br>sucrose 32% | FOS |
| Ensure | 390 | 15 | 29 | 56 | 10 | milk protein isolated 71%<br>SPI 29% | HOSO<br>Soy oil<br>Canola oil | Corn maltodextrin<br>Corn syrup solid<br>Sucrose | FOS<br>Inulin |
| Ensure (FOS) | 395 | 15 | 29 | 56 | 8,4 | milk protein isolated 71%<br>SPI 29% | HOSO 62%<br>Soy oil 38% | Corn maltodextrin 35%<br>Corn syrup solid 35%<br>Sucrose 24% | FOS |
| Isocal | 300 | 13 | 37 | 50 | | Na & Ca caseinates 61%<br>Milk protein 25% | Sunflower oil 77%<br>Coconut oil 18% | Corn maltodextrin 80%<br>Glucose syrup 11% | |

| Brand | Osmolality | Caloric distribution | | | Fiber | Nutrient sources | | | |
|---|---|---|---|---|---|---|---|---|---|
| | (mOsm/kg) | Pro | Fat | CHO | (g/ 1000 kcal) | Protein | Fat | CHO | Fiber |
| | | | | | | Soy protein concentrate 14% | MCT 5% | Sucrose 9% | |
| Boost optimum | 310 | 16 | 35 | 49 | 12,3 | Whey protein 50% | HOSO | Corn maltodextrin 72% | FOS |
| | | | | | | Casein 50% | Rapeseed oil | Sucrose 18% | Inulin |
| Pan-enteral | 270 | 12 | 45 | 43 | | Na caseinate 100% | Soybean oil 50% | Maltodextrin 51% | |
| | | | | | | | MCT 50% | Sucrose 49% | |

*Fiber enrichment in ONS*

Dietary fiber is important part in daily diet since it plays important role in controlling blood glucose, lipid, satiety, and gut health. World Health Organization recommended to consume at least 25 g/d of dietary fiber, meanwhile American Dietetic Association (ADA) recommended to consume at least 14 g of fiber per 1,000 kcal (Jones, 2014; J. L. Slavin, 2008). There are several sources of fiber used in ONS, such as soy polysaccharide, mixed fiber, psyllium, FOS, inulin, partially hydrolyzed guar gum, oat and soy fiber, and galactomannan with dose of 5.2-39.0 g/d (Kamarul Zaman, Chin, Rai, & Majid, 2015).

Diarrhea is one of the most complications in patients receiving enteral support. A meta-analysis study showed that addition of fiber in ONS provides clinical benefits and physiological effect, especially in hospital settings. It reduced the incidence of diarrhea and bowel frequency in patients. The beneficial effect of fiber in reducing diarrhea is mediated by SCFAs which promotes the absorption of water and electrolyte in the colon (Elia, Engfer, Green, & Silk, 2008; Kevin Whelan & Schneider, 2011). Absorption of SCFA in apical membrane is mediated by ionic and non-ionic diffusion. Ionic diffusion of SCFA is facilitated by the exchange of SCFA-$HCO_3$ (Binder, 2010). Meanwhile, non-ionic diffusion in gut apical membrane takes place after protonation of $Na^+$, $K^+$, $H^+$ thus SCFA may enhance water and electrolyte absorption in diarrhea (Bowling, Raimundo, Grimble, & Silk, 1993; Kevin Whelan &

Schneider, 2011). In addition, fiber-enriched ONS promoted the balance of gut microbiota and prevented pathogenic bacteria overgrowth thus reduces risk of diarrhea (Elia et al., 2008).

In addition, adding of dietary fiber in ONS also showed beneficial effect on controlling blood glucose. Previous study showed that including slowly digestible carbohydrate (isomaltulose or sucromalt) in ONS (47% carbohydrate, 34% fat, and 4 g fiber/200 ml) had a comparable effect on plasma glucose with diabetes-specific formula (33% carbohydrate, 50% fat, 2.9 g fiber/200 ml). The peak of plasma glucose for ONS containing isomaltulose, sucromalt were 189 ± 3.6 mg/d and 196.2±3.6 mg/dl, while peak plasma glucose of diabetes-specific formula was 187.2 ± 3.6 mg/dl (Vanschoonbeek et al., 2009). Isomaltulose and sucromalt are hydrolyzed at slower rate by carbohydrate digestive enzymes therefore, it reduces the peak of plasma glucose in similar way of diabetes specific formula. However, different type and amount of dietary fiber would have different effect on plasma glucose and insulin response (Vanschoonbeek et al., 2009).



**2.2 Resistant maltodextrin**

Resistant maltodextrin is carbohydrate polymer which is indigestible by carbohydrate digestive enzymes due to the presence of random 1,2- or 1,3- or 1,4-alpha and beta glucosidic linkage (Toraya-Avilés et al., 2017). The digestive enzyme hydrolyze carbohydrate by cleaving at alpha-1,4 or 1,6 glucosidic linkage, but not at alpha-1,2,

or 1,3 or beta linkage. It is classified as resistant starch type 4 which is obtained by modifying the temperature and pressure. Unlike other kinds of soluble fibers, resistant maltodextrin has low viscosity and high solubility thus it is superior in palatability compared to other fibers (Toraya-Avilés et al., 2017).

There are several sources of starch which have been used for resistant starch production, including wheat, potato, corn, and tapioca starch. Resistant maltodextrin used in this research is derived from tapioca pyrodextrin. The process of making resistant maltodextrin is known as pyrodextrinization which involves different chemical reactions, such as hydrolysis, transglucosidation, and repolymerization, followed by enzymatic hydrolysis (Kapusniak & Jane, 2007; Wurzburg, 1986). Hydrolysis can be done by heating in the presence of acid. Following hydrolysis, the moisture and reducing sugar content will be vary depend on the time. Kapusniak and Jane found that heating corn starch at 130°C for 10 min decreased the moisture content and then stabilized which reflects the hydrolysis process (Kapusniak & Jane, 2007). After 30 min of heating, the moisture content was increased again. At high temperature in the presence of acid and anhydrous conditions, the small moieties re-polymerize to form larger, highly branched molecules. Meanwhile, the reducing sugar content was increased at the first 80 min and then the reducing sugar content was stable or relatively decreased indicating involvement of new formed reducing

ends in the formation of random α, and β 1⟶2, 1⟶3, 1⟶6 glycosidic linkages (Kapusniak & Jane, 2007).

The TRM used in this study has white color, non-viscous, with neutral odor and taste which make it suitable for incorporation to the formula without affecting the organoleptic parameters. Dextrose equivalent of the present resistant maltodextrin is 8-12 and the carbohydrate composition are mainly higher polysaccharides (95%), disaccharides (4%), and glucose (1%). Molecular weight of TRM is 2,895 Da. Each 100 gr of TRM contains 180 kcal of energy. In addition, TRM used in this study contains 84.8-89.7% of dietary fiber. Resistant maltodextrin is safe to consumed (GRAS US code of Federal Regulations 21 CFR 184.1444). Toraya- Avilés reported that the resistant starch content and dietary fiber in TRM were 56.06% and 86.62% (Toraya-Avilés et al., 2017). Similarly, retrograded TRM was reported to have 59.4% of resistant starch which is fermentable by gut microbiota (Fred Brouns et al., 2007).



There are some health benefits of RMD, such as lowering blood glucose and blood lipid profile, as well as maintaining gut health (Kishimoto et al., 2007; Livesey & Tagami, 2008; Watanabe, Suzuki, Yamaguchi, & Egashira, 2018). However, most of studies used resistant maltodextrin from either corn, potato, or wheat. Resistant maltodextrin is hardly digested by intestinal digestive enzymes thus it attenuates the postprandial hyperglycemia. A meta-analysis conducted by Livesey (2008) showed

that incorporating 3-10 g of RMD into meals or beverages attenuates the postprandial glycemia. The effect tends to be better when RMD is combined with beverages than with meals. Addition of resistant maltodextrin to meal or beverage gives better palatability since it is less viscous compared to other types of fiber, such as guar gum and pectin. Resistant maltodextrin does not stimulate insulin secretion thus the hypoglycemic effect of RMD is not mediated by insulin secretion stimulation (Livesey & Tagami, 2008). The glucose lowering mechanism of RMD has not been clearly explained. Most of dietary fiber are viscous thus promote bulk formation in the intestine and interfere the absorption of glucose. However, glucose lowering effect of RMD is not likely from the similar mechanism since unlike other dietary fiber, RMD is not viscous. Resistant maltodextrin responds on glucose absorption in the small intestine by reversibly inhibiting the cooperative transport system of the glucose and by the presence of indigestible starch in it (Toraya-Avilés et al., 2017; Toraya-Avilés, Segura-Campos, Chel-Guerrero, & Betancur-Ancona, 2016).



Tapioca RMD is fermentable in the colon and, thus, produces short chain fatty acids (SCFAs), such as lactic acid, acetic acid, propionic acid, and butyric acid. Major SCFAs from TRM fermentation was acetic acid, followed by lactic acid, butyric acid, and propionic acid (Sorndech, Rodtong, Blennow, & Tongta, 2019). Furthermore, acetic acid content in TRM was the highest compared to debranched-retrograded starch (RS 3) and starch citrate (RS 4) due to different molecular structure of starch

(including configuration, chemical bonding, molecular weight, and α-glucans aggregation) and hydrolytic utilization by gut microbiota (Sorndech et al., 2019).

Brouns et al., investigated the effect of retrograded TRM on SCFA production and the result showed that retrograded TRM had lower total SCFA content compared to cornstarch RMD following 24 h incubation, yet the butyrate production of retrograded TRM was significantly higher compared to cornstarch RMD (309 vs 213 μmol/100 mg substrate, respectively) (Fred Brouns et al., 2007). In addition, SCFA produced by fermentation of RMD stimulates the growth of bifidobacterial and lactobacilli which are known to benefit to host health. Therefore, TRM is potential to have bifidogenic effect (Sorndech et al., 2019).

Short-chain fatty acid confers several health benefits, such as increasing insulin sensitivity in the liver and skeletal muscle, increasing satiety, and decreasing inflammation through the activation of its receptor, G-protein coupled receptor (GPR) 41/43 (Canfora, Jocken, & Blaak, 2015). Consequently, it is potential to prevent some noncommunicable diseases, such as diabetes, obesity, and cardiovascular disease (Braquehais & Cava, 2011).

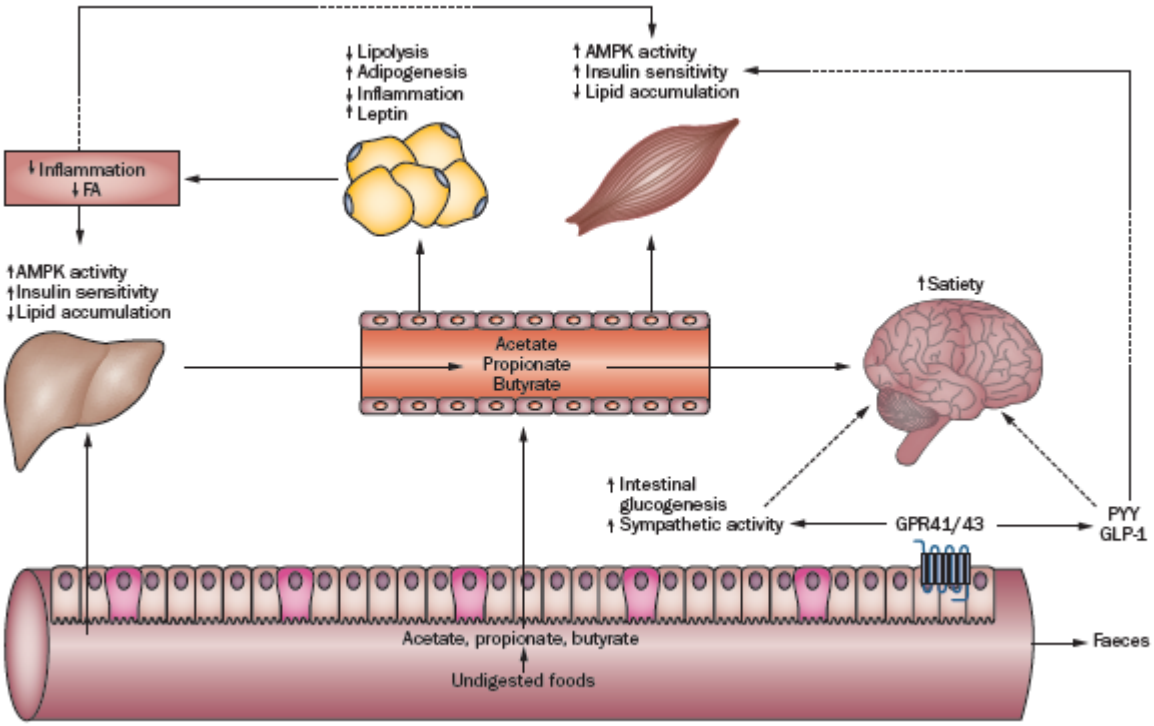



Figure 1. Crosstalk between SCFA between organs (Canfora et al., 2015)

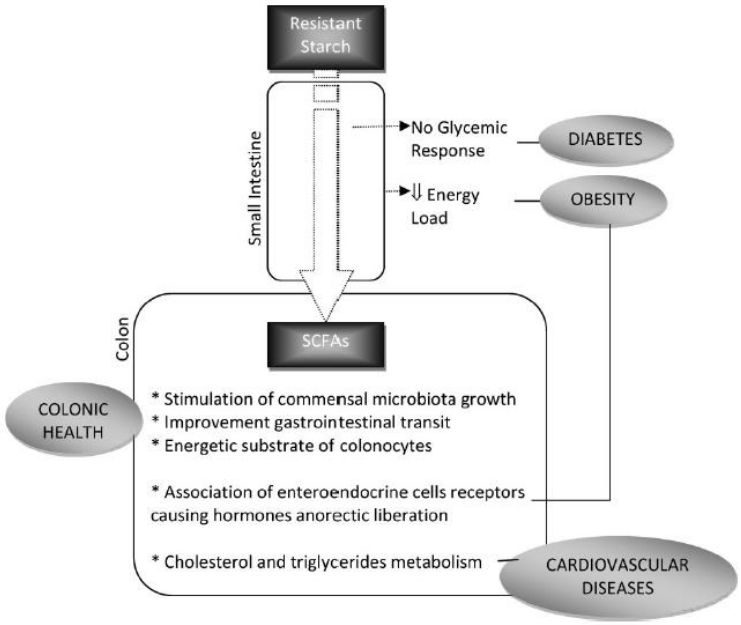



Figure 2. Benefit of resistant starch on host health (Braquehais & Cava, 2011)

Previous study, cornstarch RMD was found to stimulate the secretion of GLP-1 from enteroendocrine L-cells (Hira et al., 2015). Hira, *et al.,* showed that RMD 50 g/kg (5%) diet supplementation for 6 weeks in rats improved the glucose tolerance and increase insulin sensitivity. Treatment of 50 g/kg diet resistant maltodextrin in intestinal L-cells directly increased GLP-1 secretion and this effect was possibly mediated by the branching structure of resistant maltodextrin which similar to glucose since glucose, but not sucrose, stimulates GLP-1 secretion. The branching structure in resistant maltodextrin is identified by Toll-like receptor or orphan G protein-coupled receptor thus stimulate the secretion of GLP-1. In addition, this resistant maltodextrin also lowered the luminal pH of the rats indicating that resistant maltodextrin is fermented in the lumen thus produced SCFA. Short-chain fatty acids has been known to stimulate GLP-1 secretion and improved glucose tolerance (Hira et al., 2015). In human study, daily consumption 27 g of corn RMD for 12 weeks was also found to lower fasting plasma glucose and HOMA-IR in subjects with metabolic syndrome. In addition, visceral fat and serum triacylglycerol were also decreased (Hashizume et al., 2012).



Similarly, the elevation of postprandial triacylglycerol following high-fat meal consumption also diminished in healthy subjects consumed corn RMD. The Area Under Curve (AUC) of triacylglycerol over 6 h following placebo, 5 g, and 10 g of cornstarch RMD were 6.85±0.95 mmol x h/L, 4.99±1.18 mmol x h/L, and 5.48±0.90

mmol x h/L, respectively (Kishimoto et al., 2007). This effect might be due to the inhibition of chylomicron micelle formation by resistant maltodextrin (Kishimoto et al., 2007). Further study confirmed that resistant maltodextrin decreased the solubility of dietary lipid components, such as 1-mono-oleoylglycerol, oleic acid, and phosphatidylcholine in bile salt micelles. In addition, resistant maltodextrin also decreased the diffusion rate of bile salt micelles. Therefore, the lipid absorption was inhibited by RMD (Ikeda et al., 2016).

**2.3 Blood glucose homeostasis**

Glucose is major source of energy in most of cells in human's body. Normally, the level of glucose is maintained at the equilibrium by several hormones, such as insulin and glucagon which affect to intestinal glucose absorption, peripheral glucose uptake, and hepatic glucose production (Szablewski, 2011). Glucose homeostasis is defined as the process of maintaining blood glucose at steady-state level. Hyperglycemia is a condition which blood glucose level rise over the normal range due to lack of insulin or insulin resistance. Chronic hyperglycemia increases risk of diabetes mellitus which has several symptoms, such as polyuria, polyphagia, and unintentional weight loss. On the other hand, hypoglycemia is a condition which the blood glucose level is low with the symptoms of shakiness, sweating, anxiety, rapid heartbeat, hunger, and nausea (Szablewski, 2011).

It is recommended to control blood glucose level, including glycated hemoglobin ($HbA_{1C}$), fasting plasma glucose (FPG), and postprandial glucose (PPG) to the normal range (Ceriello & Colagiuri, 2008). Glycated hemoglobin describes the level of blood glucose over the previous 8-12 weeks. $HbA_{1C}$ can be used to diagnose diabetes with cut-off value 6.5%, while normal level of HbA1C is <5.7% (American Diabetes Association, 2015). This method does not need fasting prior to measurement hence it is more convenience for the participants. However, the result from $HbA_{1C}$ can be interfered by genetic variety, hematologic and illness-related factors, such as haemoglobinopathies, certain anemia, and malaria (World Health Organization, 2011).

Postprandial glucose is determined 2 h following the meal consumption. Normal postprandial glucose is necessary to prevent diabetic complications, such as retinopathy. Meanwhile, postprandial hyperglycemia (>200 mg/dL) increases the risk of diabetic complications hence both pharmacotherapy and non-pharmacotherapy are important to be maintained in diabetic people (Ceriello & Colagiuri, 2008). One option to lower postprandial glycemia is to consume low glycemic load (GL) food, which is an estimate of the glycemic effects of foods, accounting for both the type of carbohydrate consumed, such as the glycemic index (GI), and the amount of food consumed (Ceriello & Colagiuri, 2008).

Both fasting and postprandial plasma glucose are associated with HbA1C since HbA1C reflects the mean glucose in fasting and postprandial state (Monnier, Lapinski, & Colette, 2003). Fasting plasma glucose is defined as plasma glucose after no caloric intake for at least 8 h. Normal fasting plasma glucose is defined as plasma glucose less than 100 mg/dL after 8 h of fasting. Fasting plasma glucose higher than 126 mg/dL is considered as hyperglycemia and can be used as the cut-off value to diagnose diabetes and confirmed by another glucose measurement on a subsequent day. The criteria for fasting plasma glucose and post-meal glucose are described on the table below (American Diabetes Association, 2018).

Table 4. Fasting plasma glucose and postprandial glucose criteria (American Diabetes Association, 2018)

| Category | Fasting | 2h post-prandial | $HbA_{1C}$ |
|---|---|---|---|
| Normal | <100 mg/dL | <140 mg/dL | <5.7% |
| Impaired glucose tolerance | 100-125 mg/dL | 140-199 mg/dL | 5.7-6.4% |
| Diabetic | ≥126 mg/dL | ≥200 mg/dL | ≥6.5% |



There are many organs in human body involved in maintaining glucose homeostasis by secreting hormones that affect to food intake, digestion, absorption, and utilization (Zhao et al., 2019). Following the consumption of food-containing glucose and amino acids, blood glucose will be increased and stimulate the insulin

secretion from β-cell of pancreas. Insulin lowers the postprandial blood glucose by stimulating glucose uptake into the peripheral cells, inhibiting the glucose production from the liver (gluconeogenesis), and promoting the glycogen synthesis in the liver and skeletal muscle (glycogenesis) (Szablewski, 2011). Insulin will not be released if blood glucose concentration is less than 3 mmol/L, but insulin release will be increased when blood glucose concentration is over than 5 mmol/L. In contrast, glucagon is released from α-cell of pancreas when blood glucose is low (hypoglycemia). Glucagon will stimulate the gluconeogenesis and glycogenolysis, while inhibit the glycogenesis thus resulting blood glucose increases (Szablewski, 2011).

Gut also plays important role in glucose homeostasis since they excrete incretin hormones in response of food intake. When incretin hormones are released, it will stimulate the insulin secretion and thus helps to control postprandial plasma glucose level. There are GLP-1 (glucagon-like-peptide 1) and GIP (glucose-dependent-insulinotropic polypeptide). The incretin hormones are secreted at low level in the fasting state, while increased rapidly following the food intake and rapidly degraded by dipeptyl peptidase (DPP) IV and kidney clearance (Szablewski, 2011).

The amount and composition of nutrients in food play important role in regulating blood glucose level. Blood glucose level is quite affected by carbohydrate content in the food, including the amount, type of sugar (glucose, fructose, and

lactose), source of the starch, cooking process, and food form (liquid or solid) (American Diabetes Association, 2004). Simple carbohydrate is digested and absorbed as glucose in the brush border membrane hence it may rise the blood glucose level rapidly. However, complex carbohydrate such as soluble and insoluble fiber is hardly digested and slowly increase blood glucose level. Therefore, it is necessary to include dietary fiber into daily diet to maintain normal blood glucose level. In addition, dietary fiber consumption has been proposed to have favorable effect to prevent hypoglycemia and reduce hyperglycemia (American Diabetes Association, 2004).

Since simple carbohydrate is known to rapidly increase blood glucose level, many studies tried to modify the composition of the meals to maintain blood glucose level by substituting carbohydrate to monounsaturated fatty acids (MUFAs). The result showed substituting MUFA for carbohydrate is beneficial to lower the post-prandial glycemia and triglyceridemia. However, high intake of fat may affect to weight gain hence individualized nutrition is important to reach the target of treatment (American Diabetes Association, 2004).

Besides carbohydrate and fat, dietary protein may also affect to glycemic response, mainly whey, which acts as insulin secretagogue thus promotes insulin secretion. A study showed that supplementation of whey with simple carbohydrate stimulated insulin secretion and reduced glucose response in type 2 diabetic subjects (Frid,

Nilsson, Holst, & Björck, 2005). The underlying mechanism of insulinotropic effect of whey has not been clear. It is proposed that branched-chain amino acids and bioactive peptides in whey are responsible to stimulate the insulin secretion following whey ingestion, without affecting the incretin hormones (GLP-1 and GIP) secretion (Luhovyy, Akhavan, & Anderson, 2007).

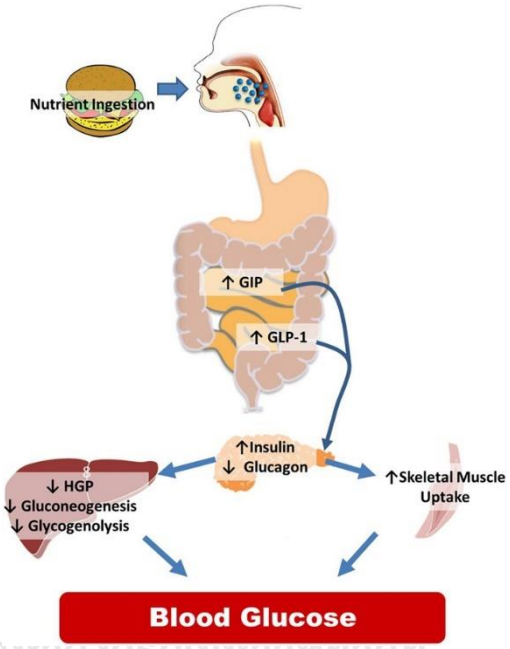





Figure 3. Glucose homeostasis regulation (Arble & Sandoval, 2013)

**2.4 Blood lipid homeostasis**

Dietary fat provides essential fatty acid and support the absorption of fat-soluble vitamins which are essential to maintain normal physiological function. However, imbalance between monounsaturated fatty acid, polyunsaturated fatty acid, and saturated fatty acid may lead to dyslipidemia (Abumrad & Davidson, 2012). Lipid homeostasis is controlled by many factors, from absorption, metabolism, and its

excretion. Dietary fat is emulsified by bile acid to form mixed micelle which subsequently hydrolyzed by pancreatic lipase in small intestine to form two-free fatty acids and one sn-2-monoacylglycerol (2-MG). Fatty acids and monoacylglycerol are absorbed through apical membrane of enterocyte and go to endoplasmic reticulum for resynthesize into triglyceride which subsequently packaged together with cholesterol and apolipoprotein for secretion into chylomicron. Triglyceride of chylomicron is hydrolyzed by lipoprotein lipase (LPL) at the surface of capillaries, while free fatty acid is absorbed by the peripheral cells. Some part of chylomicrons are repackaged into other lipoproteins, such as apolipoprotein is transferred to HDL and the rest of chylomicron remnants are transported back to the liver and taken by the receptors (Abumrad & Davidson, 2012).

Both dietary triglycerides and endogenous triglycerides are transported as chylomicron in the circulation. Meanwhile, low density lipoprotein (LDL) is responsible to transport cholesterol to all of the circulation. Most of LDL is taken up by the receptor in the liver and the free cholesterol is released. The activity of LDL receptor is regulated by β-Hydroxy β-methylglutaryl (HMG) CoA reductase, LDL receptor synthesis, and acyl-coenzyme A cholesterol acyltransferase (ACAT) which converts free cholesterol into cholesterol ester. Meanwhile, high density lipoprotein (HDL) is responsible to transport cholesterol back to the liver (Kingsbury, 2007). It is necessary to maintain cholesterol level at the normal level since high level of non-

HDL cholesterol is associated with increased risk of cardiovascular disease. Lipid profile measurement can be done following 9-12 hours fasting. The result is classified as depicted on the table below (National Health Lung and Blood Institiute, 2001).

Table 5. Adult Treatment Panel (ATP) III criteria of lipid profile (National Health Lung and Blood Institiute, 2001)

| Parameters | Level | Category |
|---|---|---|
| Total Cholesterol | < 200 mg/dl | Desirable |
| | 200-239 mg/dl | Borderline high |
| | ≥ 240 mg/dl | High |
| LDL Cholesterol | < 100 mg/dl | Optimal |
| | 100-129 mg/dl | Near optimal/above optimal |
| | 130-159 mg/dl | Borderline high |
| | 160-189 mg/dl | High |
| | ≥ 190 mg/dl | Very high |
| HDL Cholesterol | < 40 mg/dl | Low |
| | ≥ 40 mg/dl | Normal |
| Triglycerides | <150 mg/dl | Normal |
| | 150-199 mg/dl | Borderline high |

| Parameters | Level | Category |
|---|---|---|
| | ≥ 200 mg/dl | High |

Lipid profile is mainly affected by *de novo* lipogenesis in the liver. Carbohydrate is the main substrate for *de novo* lipogenesis in the liver and adipose tissue. Carbohydrate activates carbohydrate responsive element binding protein (ChREBP) and translocate to the nucleus to bind with carbohydrate responsive elements which present in promoter of lipogenic enzymes. As a result, *de novo* lipogenesis increased. In addition, carbohydrate stimulates insulin which upregulates SREBP-1 and leads to lipogenesis. On the other hand, polyunsaturated fatty acid may downregulate SREBP-1 thus inhibits lipogenesis (Ameer, Scandiuzzi, Hasnain, Kalbacher, & Zaidi, 2014).

**2.5 Satiety**



Food intake is affected by satiety, which involved in the initiation and termination process of eating. There are two processes of satiety cascade: satiation and satiety. Satiation (intra-meal satiation) is the process leading to meal termination. Meanwhile, satiety (between-meal satiety) is defined as the state of meal termination when a person does not have desire to eat anymore due to feeling full (Amin & Mercer, 2016). There are two highlights of satiety cascade: early and late satiety which happen between period of meal. Meanwhile, hunger is defined as a signal which

initiate the eating process. There are some methods to evaluate the satiety, such as measures of energy intake, subjective appetite rating, and biomarkers, such as appetite-related hormones since satiety and hunger are controlled by gut-brain axis (Gibbons, Finlayson, Dalton, Caudwell, & Blundell, 2014). The first signal that respond to hunger is vagus nerve which then stimulates other signals, such as the release of ghrelin hormone and low blood glucose level (hypoglycemia). Further, when meals enter the stomach and expand as the food fills it, it promotes the satiety signal to reduce the food intake. Hormones (GLP-1, CCK, and PYY) play important role in medium-term satiety. As food is ingested through gastrointestinal tract, those hormones will be release and inhibit further food intake. Meanwhile, long-term satiety is promoted by the insulin, glucose, and concentration of amino acid in blood. The brain will gather all the signals received from sensory, cognitive, and metabolic satiety during food processing. Further food reformulation is needed to give healthier food choices to prevent obesity by controlling the appetite (Amin & Mercer, 2016). The hormones and nutrients regulation on satiety and food intake is shown in figure below.

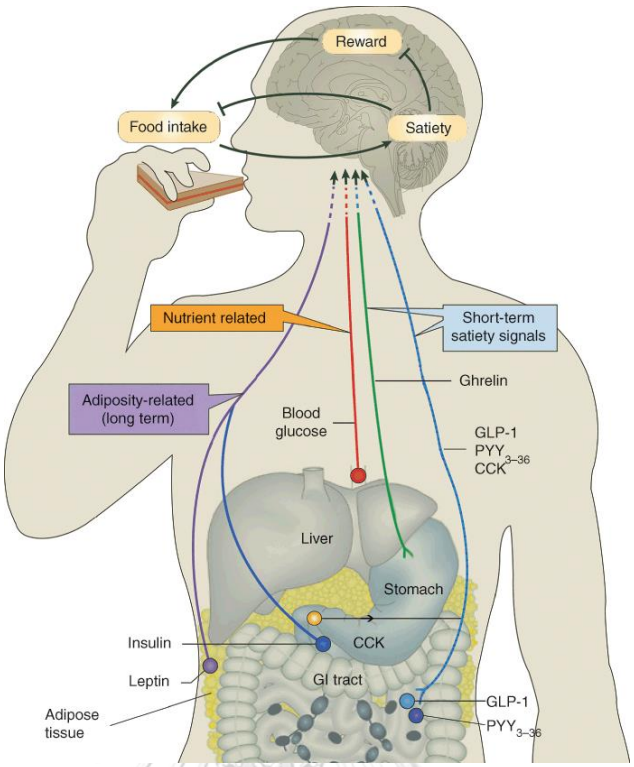



Figure 4. Influence of hormones and nutrients on satiety control (Wilkinson & Imran, 2019)

Currently, protein has been used to promote greater satiety compared to glucose and fat. High protein meal has been recommended to promote satiety, suppress appetite, and induce weight loss. In addition, the source and type of protein also affect to the outcome of satiety (Burton-Freeman, 2008). Preloads of whey protein was significantly suppressed food intake greater than soy protein and egg albumin. However, the outcome was not only related to source of protein, but also time of consumption, quantity, and composition of meals (Anderson, Tecimer, Shah, & Zafar, 2004). Whey contains high amount of branched-chain amino acids, especially leucine, which stimulates the muscle protein synthesis, maintain glucose

level, and suppress food intake. Leucine rapidly increases amino acid concentration in hypothalamus via mTor-dependent mechanism (Luhovyy et al., 2007). Glycomacropeptide in whey was found to stimulate CCK secretion and reduce subsequent food intake in women, but not in men (Burton-Freeman, 2008).

In addition, some dietary fibers promote the satiety by adding bulk into the diet as well as increasing the luminal viscosity. Adding bulky fibers into the diet increase chewing time and distend the stomach which are important for satiation process. Meanwhile, increasing luminal viscosity slows the rate of gastric emptying and nutrient absorption thus prolong the satiety (J. Slavin & Green, 2007). In a randomized cross-over study, healthy participants had a higher mean fullness following 14 days consumption of sole source of enteral formula containing 10 g/l of pea fiber and 5 g/l of FOS compared to enteral formula without fiber (K. Whelan, Efthymiou, Judd, Preedy, & Taylor, 2006). However, the effect of dietary fiber on satiety depends on its physicochemical properties of fiber (Kristensen & Jensen, 2011).



Viscosity properties of dietary fiber is important to promote the satiety (Kristensen & Jensen, 2011). However, inconsistent finding of non-viscous fiber on satiety were reported in several studies (Alexander, 2012; Guérin-Deremaux, Li, et al., 2011; Klosterbuer, Thomas, & Slavin, 2012; Pasman, Wils, Saniez, & Kardinaal, 2006; Raben, 1994; Ye et al., 2015). Alexander evaluated the effect of porridge containing

corn resistant starch (3.1%, 8.4%, or 28.9%) on postprandial glucose, satiety, and food intake in healthy adults with normal BMI and overweight. The results showed that porridge containing 28.9% resistant starch lowered peak of postprandial blood glucose but did not significantly affect the satiety and food intake in participants (Alexander, 2012). Similarly, Klosterbuer did not find any effect of breakfast containing 25 g of resistant starch or soluble corn fiber with or without pullulan on postprandial satiety in healthy participants (Klosterbuer et al., 2012). In contrast, consumption of a meal with tea containing 10 g of digestion-resistant maltodextrin significantly delayed hunger and increased satiety at 1.5-2 h postprandially. In addition, plasma peptide YY (PYY) and GLP-1 were significantly increased at 1-4 h following 10 g of digestion-resistant maltodextrin consumption (Ye et al., 2015). Supplementation of 14-24 g/day non-viscous soluble fiber from wheat dextrin for 21 day dose-dependently decreased hunger and increased satiety in healthy adults (Guérin-Deremaux, Pochat, et al., 2011).



**2.6 Gastrointestinal (GI) tolerability**

Undigested food leads to unpleasant reaction to our body, particularly on gastrointestinal tract, known as gastrointestinal tolerance. The symptoms are including bloating, abdominal pain, flatulence, and diarrhea following certain food consumption (National Health Service, 2019). The composition of ONS, including dietary fiber, influences the GI tolerability in patients (Savino, 2018). There are many

factors affecting gastrointestinal tolerability of foods containing dietary fiber, including the characteristic of the fiber, conditions of consumption, and consumer characteristic. Characteristic of fiber which may affect to gastrointestinal tolerability includes the molecular weight, degree of polymerization (DP), and debranching (Grabitske & Slavin, 2009).

Addition of fermentable oligo-, di-, monosaccharides and polyols (FODMAPs) into ONS improves the sensory attribute and clinical benefits, but it also may increase risk of GI intolerance (Savino, 2018). The type and amount of dietary fiber added to ONS is important since different type of fiber has different effect in GI function (Elia et al., 2008). Some type of soluble fiber cannot be digested and absorbed in the small intestine, but it can be fermented by gut microbiota. Fermentation of soluble fiber by gut microbiota promotes gut health by altering the gut microbiota composition, lowering the pH, and producing SCFAs (Ruiz et al., 2016). However, it also produces some gases ($CH_4$, $H_2$, and $CO_2$) that stimulates the GI discomfort, such as bloating, abdominal pain, diarrhea, and flatulence as shown in figure below (Eswaran, Muir, & Chey, 2013). However, these effects are not life-threatening thus prescriptive warning is not necessary (Livesey, 2001). Previous study showed that cornstarch resistant maltodextrin had higher tolerability compared to sugar alcohols and oligosaccharides due to the higher molecular weight of cornstarch resistant maltodextrin (Kishimoto et al., 2013). In addition, dietary fiber has better

tolerability when it is incorporated into solid meal compared to liquid form (Grabitske & Slavin, 2009).

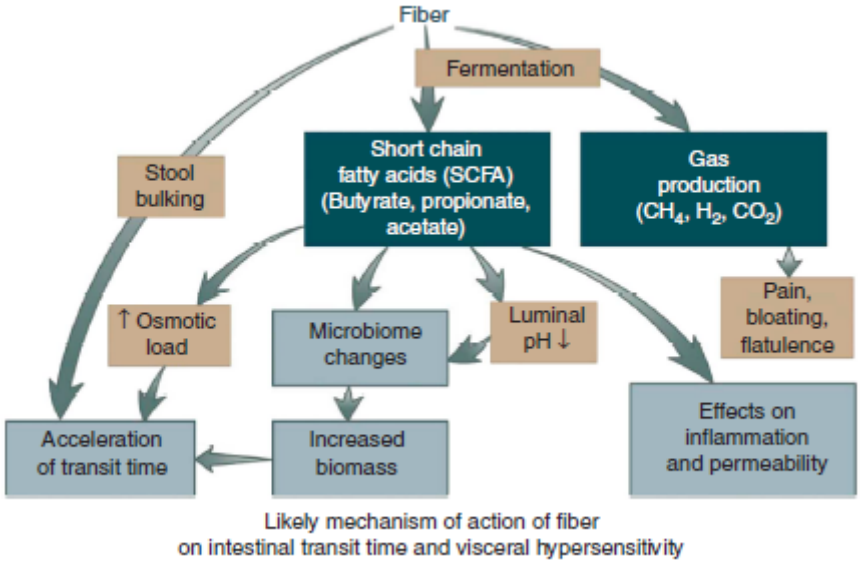



Figure 5. Mechanism of action of fiber on intestinal transit time and visceral hypersensitivity (Eswaran et al., 2013)



**2.7 Liver function**

Liver is the second largest organ in human body. It plays important role in elimination of toxins, protein and lipid metabolism, bile production, xenobiotic metabolism, regulation of red blood cells, and glucose homeostasis (Mulaikal & Emond, 2012). Liver is one of the main organs for storing glucose as glycogen via glycogenesis pathway. In fasting state, glycogen storage will be converted to glucose by glycogenolysis pathway in the presence of glucagon to maintain normal blood

glucose level. Liver is a major organ for glucose production from lactate, pyruvate, amino acids, and glycerol, which known as gluconeogenesis (Mulaikal & Emond, 2012). It also plays an important role in lipid metabolism since it controls the de novo lipogenesis, lipid absorption, blood lipid homeostasis, and lipid excretion through bile acid (Mulaikal & Emond, 2012).

There are several enzymes that associated with liver function, such as alanine aminotransaminase (ALT), aspartate aminotransaminase (AST), alkaline phosphatase (ALP), and gamma-glutamyl transaminase (GGT). In addition, there are some additional test to indicate the liver function, such as serum bilirubin, prothrombin time (PT), and international normalized ratio (INR). The increased of AST and ALT indicate hepatocellular disease, meanwhile, the increased of ALP and bilirubin indicated a cholestatic pattern (Lala, Goyal, Bansal, & Minter, 2020). Aminotransferases (AST and ALT) play role in gluconeogenesis by catalyzing the conversion of alanine or aspartic acid to ketoglutaric acid through amino group transfer. Aspartate aminotransaminase is not only found in liver, but also in skeletal muscle, cardiac muscle, brain, kidney, lungs, pancreas, red cells, and leukocyte. Therefore, AST is not a specific marker for liver injury. Meanwhile, ALT is highly found in liver that is released, together with AST, when liver is injured (Lala et al., 2020). Liver injury is one of the parameters that is evaluated in pre-marketed drug. Liver injury is defined as increases in serum ALT or AST more than 3x of upper level of

normal ULN, together with the increases of serum bilirubin to more than double of ULN (Avigan et al., 2014).

Table 6. Normal reference value of liver function test (Lala et al., 2020)

| Liver function test | Reference ranges |
|---|---|
| Alanine transaminase | 0-45 IU/L |
| Aspartate transaminase | 0-35 IU/L |
| Alkaline phosphatase | 30-120 IU/L |
| Gamma-glutamyltransferase | 0-30 IU/L |
| Bilirubin | 2-17 µmol/L |
| Prothrombin time | 10.9-12.5 sec |
| Albumin | 40-60 g/L |

**2.8 Kidney function**

Blood urea nitrogen (BUN) and serum creatinine are markers to indicate kidney function, particularly to estimate glomerulus filtration rate (GFR). Urea and creatinine are metabolites of protein metabolism. Urea is by-product of dietary protein and muscle protein turnover, while creatinine is by-product of muscle creatinine degradation (Adrian O Hosten, 1990). Dietary protein is absorbed as peptides and amino acids in the small intestine which then transported to the liver for further metabolism. Amino acids are deaminated and transaminated resulting excess nitrogen which then converted to urea, by urea cycle. Urea is distributed through

urea pool (total body water). Excess urea is excreted mostly by kidney through glomerular filtration process. The GFR determines the amount of urea reabsorption. The urea reabsorption is increased as the GFR slower, which indicated abnormality urinary tract obstruction or increased in antidiuretic hormone (ADH) (Adrian O Hosten, 1990).

The synthesis of creatinine is initiated from the transfer of amidine group from arginine to glycine resulting guanidoacetic acid (GAA) where this process occurs mostly in kidney. Further, GAA is methylated in the liver by S-adenosyl methionine resulting creatine. Creatine is transported to blood circulation and stored in the muscle as creatine phosphate. This creatine phosphate storage can be catabolized as creatinine and excreted by kidney. Unlike urea, creatinine is not normally reabsorbed by kidney. The serum creatinine level is influenced by production and excretion rates (Adrian O Hosten, 1990).



Table 7. Normal reference value for BUN and serum creatinine (Adrian O Hosten, 1990)

| Parameters | Reference ranges |
|---|---|
| BUN | 5-20 mg/dl |

| | |
|---|---|
| Serum creatinine | |
| Male | 0.6-1.2 mg/dl |
| Female | 0.5-1.1 mg/dl |

**2.9 Body composition**

Body composition is associated with nutrition intake and energy expenses, and therefore, also related to nutrition status (Dogu, Sirzai, Usen, Yilmaz, & Kuran, 2015; Thibault, Genton, & Pichard, 2012). Human body is composed of fat mass (20%) and fat-free mass (80%), including total body protein (13%), intracellular water (36%), extracellular water (24%), and bone tissue (7%) (Thibault et al., 2012). Reduction of fat-free mass (FFM) is observed in undernourished patients and positively correlated with the duration of undernutrition (Wells, 2019). It increased the risk of mortality, hospital length of stay, complications, and health cost (Pichard et al., 2004).



The evaluation of body composition is recommended in clinical practice since anthropometric measurement to evaluate the nutrition status is inadequate. In some cases, patients may reduce FFM together with increase FM, which is known as sarcopenic obesity. This kind of patients may have normal or high BMI (Thibault et al., 2012). In addition, body composition also reflects the total body protein changes in acute and chronic diseases which indicates the muscle catabolism (Thibault et al., 2012).

Body composition is useful in evaluating the efficiency of nutritional support in patients (Thibault et al., 2012). It helps to characterize the changes of either FM or FFM in accordance with the change of BMI (Thibault et al., 2012). Kim et al., (2019) reported that 8 weeks supplementation of ONS which provided energy 400 kcal, 18 g protein, 12 g fat, and 58 g carbohydrate, increased FM without changing the body weight in pancreatic cancer patients (Kim et al., 2019). In another study, it was reported that soy or casein supplementation for 3 months did not change the body weight in postmenopausal women, however, casein supplementation significantly increased total and subcutaneous abdominal fat (Sites et al., 2007).

There are several methods to evaluate the body composition, such as bioelectrical impedance analysis (BIA), Quantitative Magnetic Resonance (QMR), Air Displacement Plethysmography (ADP), Dual-energy X-ray Absorptiometry (DXA), Magnetic Resonance Imaging (MRI), Nuclear Magnetic Resonance (NMR) spectroscopy, and Positron Emission Tomography-Computed Tomography (PET-CT) (Lemos & Gallagher, 2017). Bioelectrical impedance analysis is widely used in clinical and research studies due to its fast and simple measurement (Lemos & Gallagher, 2017). In addition, BIA was superior than anthropometry and biomarkers (albumin and transthyretin) in detecting undernutrition in patients (Thibault, Le Gallic, Picard-Kossovsky, Darmaun, & Chambellan, 2010).

## 2.10 Conceptual framework

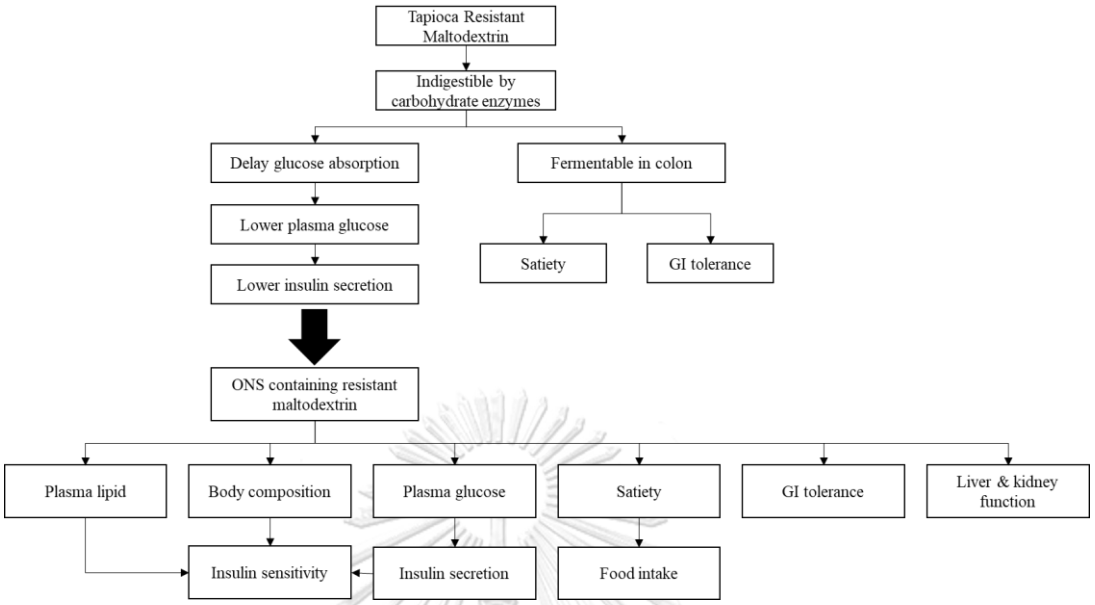



Figure 6. Conceptual framework of the study

## 2.11 Scope of work

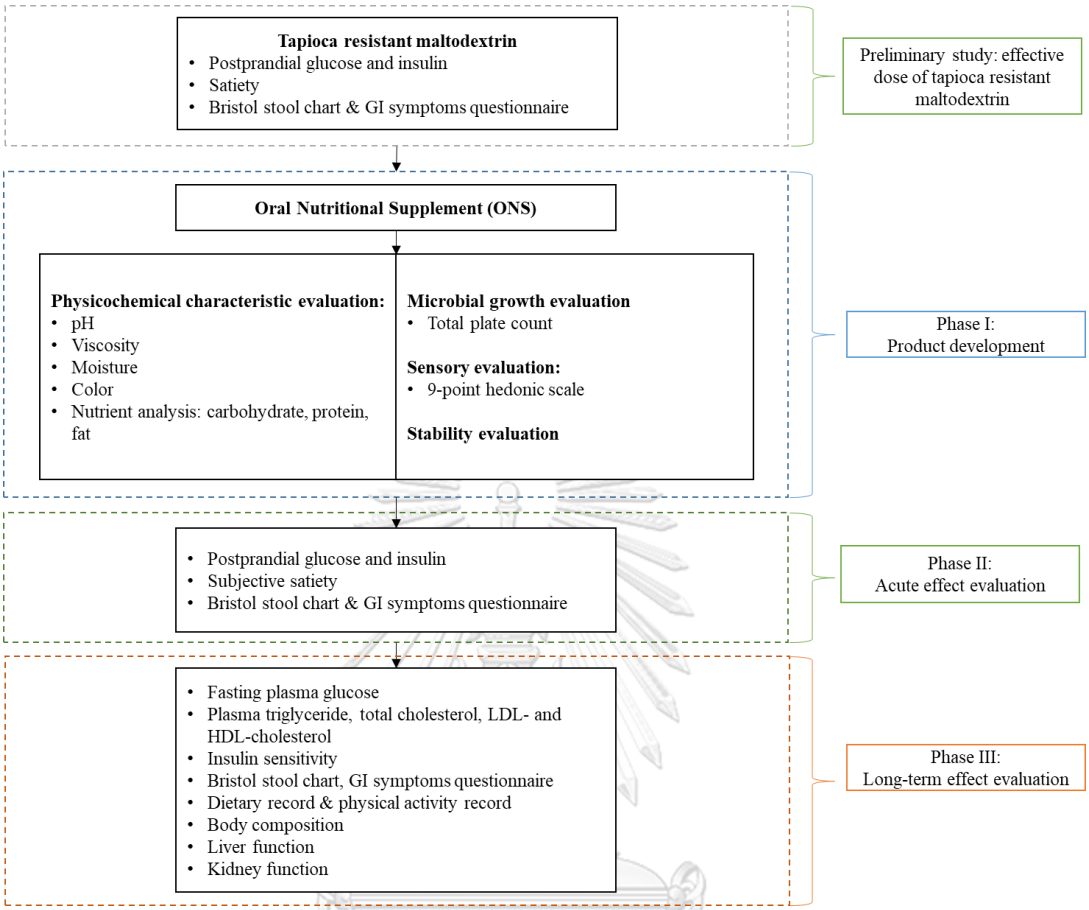



Figure 7. Scope of work of the study

# CHAPTER III

# METHODOLOGY

## 3.1 Phase I: Effect of TRM on postprandial glucose and insulin response in healthy participants

Objective: To investigate the effect of TRM on postprandial plasma glucose and serum insulin in healthy participants.

*Participants*

Participants who met the inclusion criteria, including healthy adults, aged 18-55 years old, had normal fasting plasma glucose (less than 100 mg/dl), did not use any medications/ herbal supplements or insulin injection to lower plasma glucose, did not have any allergies to study product (cassava), and did not smoke or drink alcohol were included in this study. Eligible participants were informed about the details of the study, including the study procedure, risks, and benefits of this study. Participants voluntarily signed the informed consent before participating in this study. All personal data of participants were kept confidentially, and this study had been approved by Research Ethics Review Committee for Research Involving Human Subjects Chulalongkorn University (protocol no. 196.2/60).



*Sample size calculation*

Sample size was calculated using following equation (Berggren, 2012):

$$n = \frac{(Z_{1-\alpha/2} + Z_{1-\beta})^2 \sigma^2}{\delta^2}$$

n= number of sample size

$Z_{1-\alpha/2}$= constant of two-sided significance level

$Z_{1-\beta}$= constant of power

$\sigma^2$= pooled variance

$\delta$= mean difference

$$n = \frac{(1.96 + 0.84)^2 x 42.5}{(25\% \; x \; 23.1)^2}$$

$n = 10 \rightarrow$ 10 participants

Based on previous study, a minimum sample size of 10 participants was required to detect 25% difference of peak plasma glucose response with 80% power at significance level of 0.05 (Wakabayashi, Kishimoto, Nanbu, & Matsuoka, 1999). However, the sample size was increased by 30% for estimating dropout.



*Study design*

Randomized, cross-over controlled trial was used in this study design. Participants were cross-over with three days washout period between treatments. Order of treatment was created with a Latin-square designed. Eligible participants were randomized into 5 groups which contained 50 g of either: glucose (GL) (Glucolin, The British Dispensary (L.P), Co. Ltd., Thailand), tapioca maltodextrin (TM) (Caleen-D19, Banpong Novitat, Co. Ltd., Thailand), tapioca resistant maltodextrin (TRM) (Cal-DM,

Banpong Novitat, Co. Ltd., Thailand), MIX15% (42.5 g tapioca maltodextrin + 7.5 g tapioca resistant maltodextrin), or MIX50% (25 g tapioca maltodextrin + 25 g tapioca resistant maltodextrin) which dissolved in drinking water (100 ml).

Participants were reminded to fast overnight prior to clinic day. Upon their arrival, participants collected the baseline blood samples (7 ml) and filled the appetite questionnaire. Then, participants drank the test drink within 2 min. Subsequently, blood samples and appetite data were collected at 30, 60, 120, and 180 min following the first sip of the test drink. In addition, participants rated the gastrointestinal symptoms 24-h following test drink consumption. Participants were allowed to drink 1000 ml of water within 180 of test session.

Table 8. Description of test drinks

| Group | Glucose (g) | Tapioca maltodextrin (g) | TRM (g) |
|---|---|---|---|
| GL | 50 | - | - |
| TM | - | 50 | - |
| TRM | - | - | 50 |
| MIX15% | - | 42.5 | 7.5 |
| MIX50% | - | 25 | 25 |

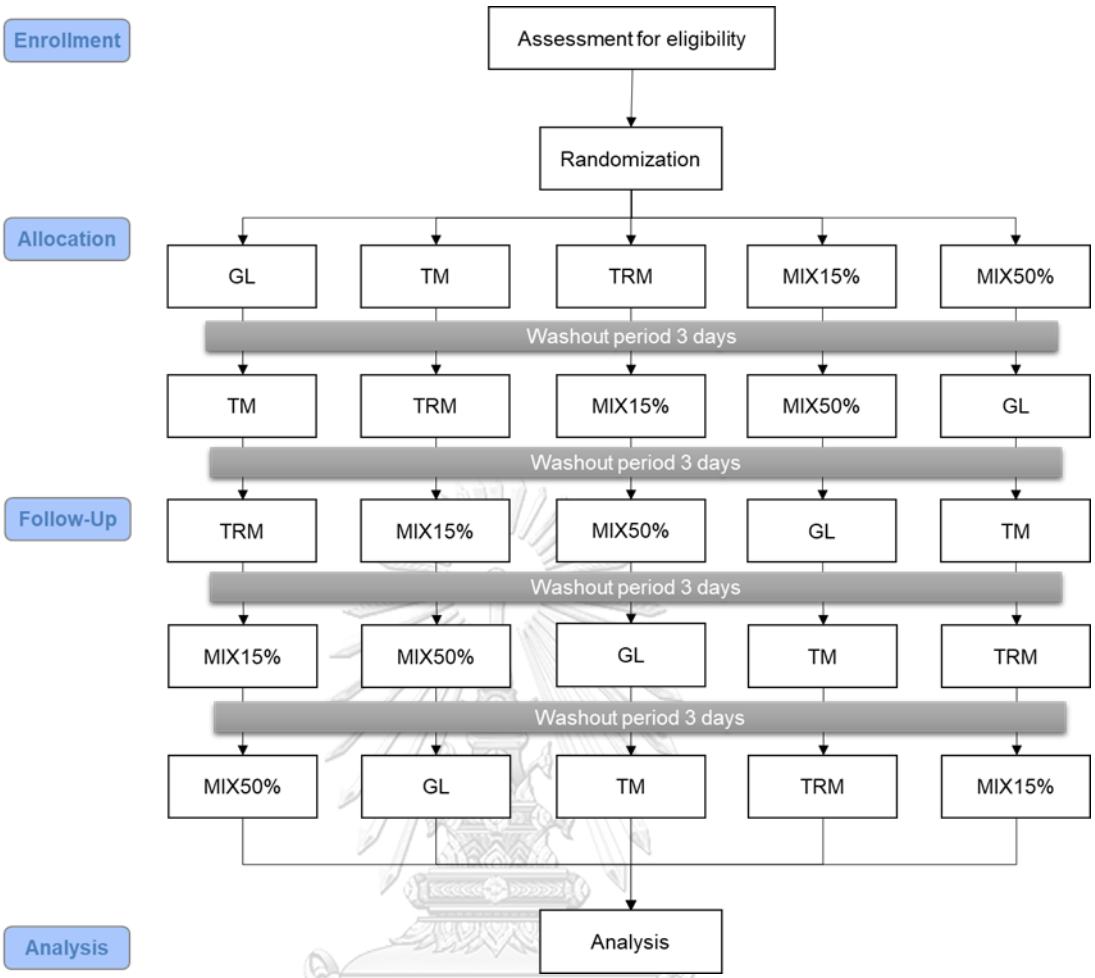



Figure 8. CONSORT flow diagram of preliminary study. GL (50 g glucose), TM (50 g tapioca maltodextrin), TRM (50 g tapioca resistant maltodextrin), MIX15% (42.5 g TM (85%) + 7.5 g TRM (15%)), or MIX50% (25 g TM (50%) + 25 g TRM (50%))



*Plasma glucose and serum insulin analysis*

Blood samples were centrifuged at 3,000 rpm for 10 min and then, followed by separation of plasma and serum. Sample was kept at -20°C for further analysis. Plasma glucose was analyzed by hexokinase method using a clinical chemistry analyzer (Beckman Coulter AU480, USA). Meanwhile, serum insulin was analyzed by

chemiluminescence immunoassay (CLIA) method (Padwal, Murshid, Nirmale, & Melinkeri, 2015).

*Subjective appetite evaluation*

Subjective appetite was measured by using a paper-based visual analogue scale (VAS) (Blundell et al., 2010). Participants answered four questions associated with hunger, satiety, desire to eat, and prospective food consumption, such as “how hungry do you feel right now?”; “how satiated are you now?”; “how strong is your desire to eat?”; and “how much food you can eat right now?”. Participants put a vertical line ( | ) on a 100 mm line scale anchored with opposite words at each end of line (for example: not hungry at all – extremely hungry) to rate their subjective appetite.

*Gastrointestinal symptoms*



Gastrointestinal symptoms inclusive of nausea, abdominal pain, vomiting, flatulence, and bloating were observed using a questionnaire 24h before and after the clinic day (Jacqz-Aigrain et al., 2015). Participants were asked to rank the intensity of symptoms from 0 (none) to 3 (severe) (Jacqz-Aigrain et al., 2015). In addition, Bristol Stool Scale with description for each type of stool form and pictures was used to evaluate the stool form 24 h before and after the test session (Lewis & Heaton, 1997).

*Statistical analysis*

Plasma glucose and serum insulin value at time point was subtracted with the value at fasting condition to obtain the incremental value of plasma glucose and serum insulin. In addition, incremental Area Under the Curve (iAUC) of plasma glucose and serum insulin over 180 min were calculated following trapezoidal rule (Braunstein et al., 2018; F Brouns et al., 2005). Data were calculated and analyzed using Microsoft Excel and Statistical Package for Social Sciences (SPSS) version 22.0 (IBM Corp, NY, USA). Normality test was performed using Shapiro-Wilk test. Non-normally distributed data were analyzed by using Friedman's two-way ANOVA. P-value of 0.05 was set to detect statistical significance. Data were expressed as mean±SEM.

**3.2 Phase II: Utilization of TRM in the development of ONS**

**3.2.1 Effect of different dextrose equivalent (DE) of tapioca maltodextrin in ONS development**

Objective: to determine the effect of tapioca maltodextrin with different DE on physical properties and acceptability of ONS

*Materials*

Tapioca maltodextrin DE7 and DE19, rice bran oil, whey protein isolate, vitamins and minerals premix, soy lecithin, flavor were obtained from Banpong Novitat, Co Ltd.

*ONS emulsion preparation*

There were two formulations used in this study which differed in DE of tapioca maltodextrin (DE 7 and DE19). The ONS emulsion was prepared by mixing water-soluble ingredients (water, tapioca maltodextrin, whey protein, and vitamins minerals) and oil-soluble ingredients (rice bran oil, soy lecithin, and flavor) separately for 20 min. The oil mixture was incorporated into water-soluble mixture for 10 min prior to homogenization using Ultra-Turrax (Ultra-Turrax, Germany) at 16,000 rpm for 3 min (Drapala, Mulvihill, & O'Mahony, 2018). The emulsion was pasteurized at 72$^{o}$C for 15 seconds.

Table 9. Formulation of ONS emulsion in one serving

| **Ingredients** | **Amount (g)** |
|---|---|
| Whey Protein Isolate | 10.5 |
| Tapioca maltodextrin | 37 |
| Rice Bran Oil | 8.5 |
| Vitamin & Mineral Premix | 0.2 |
| Lecithin | 0.625 |
| Flavor | 0.125 |



*Physical properties evaluation*

- Color

The color of ONS was determined using chromameter (Konica Minolta CR-400 Japan). Chromameter was calibrated before being used using white calibration plate (Y = 93.5, x = 0.3114 and y = 0.3190). The ONS was poured into plastic cells. The color was expressed by Lab hunter color coordinates which L* indicates lightness from black (0) to white (100), a* indicates redness from greenness (-a*) to redness (+a*), and b* indicates yellowness from blueness (-b*) to yellowness (+b*). The measurements were done in triplicate.

- pH

  A pH meter (Mettler Toledo, Switzerland) was used to determine the pH of ONS. The pH meter was calibrated using buffer solution of pH 4 and 7 before used. Thereafter, the probe of pH meter was immersed in the ONS and pH was read. Measurements were done in triplicate.

- Viscosity

  Viscometer (Fungilab Premium Series, Spain) was used to analyze the viscosity of ONS emulsion. Sample was placed in a beaker with a volume 125 ml. The spindle R2 was used with speed rotation was set at 200 rpm to determine the viscosity of ONS. Measurement was done in triplicate at room temperature.

*Sensory evaluation*

Sensory evaluation was done in healthy volunteers at Nutrition and Dietetics Department, Faculty of Allied Health Sciences, Chulalongkorn University. Participants

were instructed about the details of the ONS and they voluntarily signed the inform consent prior to do the sensory evaluation. This study had been approved by Research Ethics Review Committee for Research Involving Human Subjects, Chulalongkorn University.

Two formulas of ONS were served at the same time in front of participant. Participants were instructed to take a sip of water before tasting each ONS. After tasting the ONS, participants were asked to rate their acceptance on a 9-point hedonic scale (1= extremely dislike, 2= very much dislike, 3= moderately dislike, 4= slightly dislike, 5= neither like nor dislike, 6= slightly like, 7= moderately like, 8= very much like, 9= extremely like). In addition, participants were asked to rank their preference between formulas.

**3.2.2 Development of ONS using TRM**

Objective: To develop nutritionally complete ONS containing TRM and to evaluate the physicochemical characteristic of the developed ONS.

*Materials*

Tapioca maltodextrin (Caleen-D19), soy protein isolate, TRM (Cal-DM), and whey protein isolate, were obtained from Banpong Novitat, Co., Ltd (Thailand). Sucrose was obtained from local manufacture. Rice bran oil creamer was produced from Thai Edible Oil Co, Ltd., (Thailand). Omega-3 oil blend powder was produced by Ming Chyi Biotechnology Ltd (Taiwan). Trace amount of deoiled soy lecithin powder (Solae

LLC.,) was used to improve the stability of fat in the product. In addition, trace amount of vanilla and milk flavor were used to improve the sensory of ONS. Vitamin and mineral premix (DSM Nutritional Products Malaysia Sdn Bhd, Malaysia) were added according to recommended dietary intake (RDI).

*ONS preparation*

Three formulas of ONS were manufactured by dry blending method which differed in the proportion of TRM and digestible tapioca maltodextrin. Original formula was the basic formula which used tapioca maltodextrin as the main carbohydrate source; RMD15 replaced 15% of tapioca maltodextrin using TRM; and RMD30 replaced 30% of tapioca maltodextrin using TRM. Based in our preliminary result, replacement of tapioca maltodextrin using tapioca resistant maltodextrin slightly reduced the postprandial glucose and insulin response in healthy participants. All ingredients were weighed on digital scale and mixed thoroughly using ribbon blender (Littleford Day Inc, Florence, KY) for 30 min. Samples were kept in single aluminum foil bag and stored at room temperature. The composition of ONS used in this study is shown in table below.



Table 10. Formulation of nutritionally complete ONS

| Ingredients | Original | RMD15 | RMD30 |
|---|---|---|---|
| Carbohydrate (g) | 32.73 | 32.73 | 32.73 |

| Ingredients | Original | RMD15 | RMD30 |
|---|---|---|---|
| TRM (% of carbohydrate) | 0.00 | 11.74 | 23.48 |
| Tapioca Maltodextrin (% of carbohydrate) | 78.26 | 66.52 | 54.78 |
| Sucrose (% of carbohydrate) | 21.74 | 21.74 | 21.74 |
| Protein (g) | 9.85 | 9.85 | 9.85 |
| Whey protein isolate (% of protein) | 49.11 | 49.11 | 49.11 |
| Soy protein isolate (% of protein) | 50.89 | 50.89 | 50.89 |
| Fat (g) | 9.05 | 9.05 | 9.05 |
| Blended omega-3 oil powder (% of fat) | 49.78 | 49.78 | 49.78 |
| Rice bran creamer (% of fat) | 49.78 | 49.78 | 49.78 |
| Soy lecithin (% of fat) | 0.45 | 0.45 | 0.45 |

One serving size of ONS (60 g) was poured into 1 glass (190 ml) of drinking water and stirred well until all powder dissolved.



*Physical properties analysis*

- Proximate analysis

  Protein was analyzed by Kjeldahl method based on AOAC 991.20 (AOAC, 2005). The principle of Kjeldahl method is liberating nitrogen from the substance since nitrogen is presented in protein, but not in other

macronutrients. There are 3 steps of Kjeldahl method: digestion, neutralization, and titration (Nielsen, 2014).

Firstly, the sample was heated with sulfuric acid in a digestion flask, which breakdown the organic substance to liberate the reduced nitrogen into ammonium sulfate. To accelerate the reaction, anhydrous sodium sulfate and a catalyst (such as selenium, copper, mercury, or titanium) will be added. Adding anhydrous sodium sulfate increased the boiling point, hence accelerate the reaction. This digestion process converts any nitrogen in the sample (either nitrite or nitrate) into ammonium sulfate, and other organic substance into $CO_2$ and $H_2O$. The color will become clear or colorless when the chemical decomposition is complete (Nielsen, 2014).

Secondly, the solution was distilled with a sodium hydroxide to convert ammonium sulfate into ammonia gas. The ammonia presents the amount of nitrogen in the sample. The end of the condenser will be dipped into boric acid solution to capture the ammonia. The ammonia gas will react with the acid and turn ammonia gas into ammonium ion, and continuously converts boric acid into borate ion (Nielsen, 2014).



Lastly, to estimate the nitrogen content, the remainder ammonium borate was titrated with standard sulfuric or hydrochloric acid, using suitable indicator, such as phenolphthalein (Nielsen, 2014). The amount of hydrogen ion (mol) needed to reach end-point is equivalent to the concentration of the

amount of nitrogen content in sample. The equation to determine nitrogen concentration:

$$\%N = \frac{x\ moles}{1000\ cm^3}\ x\ \frac{(vs - vb)cm^3}{mg}\ x\ \frac{14\ g}{moles}\ x\ 100$$

Where vb and vs are volume of the blank and sample, and 14 represents the molecular weight of nitrogen. The amount of nitrogen (%N) then will be multiplied by conversion factor, with the calculation: % protein = %N x conversion factor (Nielsen, 2014). Conversion factor used in this study was 6.25 (Mæhre, Dalheim, Edvinsen, Elvevoll, & Jensen, 2018).

- Total fat

  Total fat and saturated fat were analyzed by in-house method based on AOAC (2019) 996.06.

- Total carbohydrate

  Total carbohydrate was calculated by difference (total carbohydrate = 100 – % crude protein – % total fat – % moisture – % ash) (BeMiller, 2010).

- Moisture

  Moisture of ONS powders were measured by using HB43-S halogen moisture analyzer which operates with a halogen heating module thus ensure fast heating of the samples (Mettler Toledo, Switzerland). Approximately 3±0.1 g of powder sample was evenly placed onto the pan. Once the lid is closed, the water is evaporated by the heat generated by the machine at 105°C until

the stable weight was reached. The measurements were done in triplicate for each sample.

- Water activity (Aw)

  Water activity of ONS powder was measured by using Aw meter (Mettler Toledo, Switzerland).

- Color

  Color of reconstituted ONS was determined using chromameter (Konica Minolta CM600D Japan). Chromameter will be calibrated before being used using white calibration plate. ONS was prepared by dissolving in drinking water and poured into plastic cells. The color was expressed by Lab hunter color coordinates which L* indicates lightness from black (0) to white (100), a* indicates redness from greenness (-a*) to redness (+a*), and b* indicates yellowness from blueness (-b*) to yellowness (+b*). The measurements were done in triplicate.



- pH

  A pH meter (Mettler Toledo, Switzerland) was used to determine the pH of reconstituted ONS. The pH meter was calibrated using buffer solution of pH 4 and 7 before used. Thereafter, the probe of pH meter was immersed in the reconstituted ONS and pH was read. Measurements were done in triplicate.

- Viscosity

Viscometer (Fungilab Premium Series, Spain) was used to analyze the viscosity of reconstituted ONS. Sample was placed in a beaker with a volume 125 ml. The spindle R2 was used with speed rotation was set at 200 rpm to determine the viscosity of ONS. Measurement was done in triplicate at room temperature.

*Microbial analysis*

Total plate count was analyzed following Bacterial Analytical Manual (Food and Drug Administration, 2001). Sample was diluted using 0.1% peptone water to $10^{-1}$ until $10^{-7}$ by serial dilution. Diluted sample (1 ml) was poured into petri dish and subsequently, 12-15 ml plate count agar (45±1°C) was added within 15 min and mixed thoroughly by alternate rotation. Agar was let to solidify in room temperature and petri dish was inverted and incubated for 48±2 h at 35°C. Data was expressed as colony forming units (CFU) per gram.



*Stability evaluation*

ONS powder (RMD30) was kept in aluminum foil bag at three different storage temperature for accelerated shelf-life studies: 30, 40, and 50°C with relative humidity of 75% (World Health Organization, 1996). Periodically, the sample was analyzed for its water activity, color, and pH. The measurements were done in duplicate. Data was expressed as mean ± SEM.

The data were plotted into scatter plot and linear equations were obtained $y = bx + a$, where;

y= value of the sample

x= storage time (days)

a= initial value of sample

b= changes rate of sample

The linear equation and R2 value were used to calculate the shelf-life following ordo null (ordo 0) on Arrhenius model based on water activity (Aw) (Annisa et al., 2020).

$$\ln k = -\frac{Ea}{RT} + \ln k_0$$

k= chemical reaction rate constant

$k_0$= rate constant (independent of temperature)



Ea= activation energy

R= Gas constant

T= temperature ($^{o}$K)

The rate constant (k) was then used to calculate the shelf-life of the ONS as below

$$t = \frac{(A_0 - A_t)}{k}$$

$A_0$= initial value

$A_t$= value at final period (Aw= 0.6)

t= shelf life (days)

k= chemical reaction rate constant

*Statistical analysis*

All data was presented as mean ± standard deviation (SEM). The number of micronutrients per serving were compared to RDI for Thai population (year 2541) and multiplied by 100. Independent t-test was used to analyze the differences between formulas in part 3.2.1. Analysis of variance (ANOVA) was performed to analyze the normally distributed data in part 3.2.2. Furthermore, the stability test was analyzed by using RM ANOVA. Level of significance (*p*-value) was set at 0.05. Statistical analysis was performed using SPSS 22.0 (IBM Corp, NY, USA).

**3.3 Phase III: Acute effect evaluation of developed ONS on postprandial glycemic response, acceptability, and GI tolerability**

Objective: To determine the potential benefits of the developed ONS containing TRM on plasma glucose and serum insulin in healthy participants.

*Participants*

The inclusion criteria of the study participants were:

1. Healthy adults aged 18-55 years old
2. BMI of 18.5-22.9 kg/$m^2$

3. Fasting plasma glucose less than 100 mg/dl

Exclusion criteria of the study participants were:

1. Used any medications/ dietary supplements/ insulin injection to lower plasma glucose
2. Had any allergies to products containing whey, soy, or cassava
3. Smoking or drinking alcohol

The protocol of this study was approved by the Research Ethics Review Committee for Research Involving Human Subjects Chulalongkorn University (protocol no. 196.2/60) and was registered at Thai Clinical Trials (registry number TCTR20210330005). This study was conducted at the Nutrition and Dietetics Department, Faculty of Allied Health Sciences, Chulalongkorn University, Bangkok, Thailand. All participants were informed about the details of the study, including procedures, risk, benefits, and they were allowed to withdraw at any time from the study. Participants signed the informed consent prior to starting the study. Personal data of participants were kept confidentially.



*Sample size calculation*

Based on previous study, nutritional supplement significantly reduced peak plasma glucose compared to control (Devitt, Williams, Choe, Hustead, & Mustad, 2013). Minimum sample size required was 13 subjects to detect 30% difference with 80%

power and significance level $\alpha$= 0.05. However, sample size was increased by 20% for estimating dropout. Therefore, 16 participants would be recruited in this study.

Sample size was calculated using following equation (Berggren, 2012):

$$n = \frac{(Z_{1-\alpha/2} + Z_{1-\beta})^2 \sigma^2}{\delta^2}$$

n= number of sample size

$Z_{1-\alpha/2}$= constant of two-sided significance level

$Z_{1-\beta}$= constant of power

$\sigma^2$= pooled variance

$\delta$= mean difference

$$n = \frac{(1.96 + 0.84)^2 x 418.48}{(30\% \; x \; 53.3)^2}$$

$n = 12.8 \rightarrow$ 13 participants



*Study protocol*

The information of participants recruitment was done using flyers nearby Chulalongkorn University. Interested participants came to Nutrition and Dietetics Department, Faculty of Allied Health Sciences, Chulalongkorn University for initial screening for eligibility and having a detail explanation about the study. Eligible participants were randomized into 3 groups and followed all treatments separated with at least three days washout period.

Participants were reminded to have overnight fasting (10-12 h) prior to test day. Participants came to university at 7.30-8.00 a.m. to check anthropometry and baseline blood sample collection. The IV catheter was inserted in antecubital vein by registered nurse. Following blood sample collection, the catheter was flushed using 10 ml normal saline solution 0.9% to prevent the blood clot in the catheter line. Thereafter, participants were briefed to fill the subjective appetite questionnaire and GI symptoms questionnaire. Following baseline blood sample collection and subjective appetite evaluation, participants consumed the ONS (252 kcal in 250 ml) within 2 min. Blood sample (7 ml) was collected at baseline (0), 30, 60, 120, and 180 min following the first sip. Drinking water was allowed for 1,000 ml for 180 min during the test session. Blood sample was collected in 2 separate tubes: tubes containing sodium fluoride to preserve the glucose in plasma and tubes containing clot activator gel for serum insulin analysis. At the same time point, the subjective satiety, hunger, and appetite were recorded using visual analogue scale. Gastrointestinal symptoms and stool form were evaluated 24 h before and after ONS consumption using gastrointestinal symptoms questionnaire and Bristol Stool Scale.

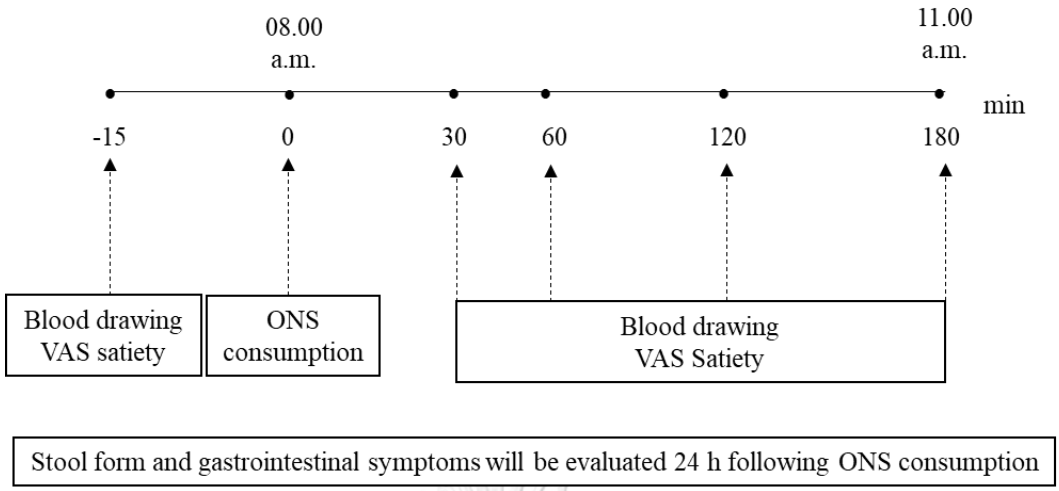



Figure 9. Blood sample collection time points

*Plasma glucose and serum insulin analysis*

Blood samples were immediately centrifuged (3,000 rpm) for 10 min at 4°C and plasma was separated and kept at -20°C for further analysis. Plasma glucose was determined according to the hexokinase method using a clinical chemistry analyzer (Beckman Coulter AU480, USA). Serum insulin was analyzed by chemiluminescence immunoassay (CLIA) (Padwal et al., 2015).



*Subjective appetite evaluation*

Subjective appetite was measured by using a paper-based visual analogue scale (VAS) (Blundell et al., 2010). Participants answered four questions associated with hunger, satiety, desire to eat, and prospective food consumption, such as “how hungry do you feel right now?”; “how satiated are you now?”; “how strong is your

desire to eat?"; and "how much food you can eat right now?". Participants put a vertical line ( | ) on a 100 mm line scale anchored with opposite words at each end of line (for example: not hungry at all – extremely hungry) to rate their subjective appetite.

*Sensory evaluation*

The acceptability of ONS was evaluated by using 9-point hedonic scale which 9 showed like extremely, 8 showed like very much, 7 showed like moderately, 6 showed like slightly, 5 showed neither like nor dislike, 4 showed dislike slightly, 3 showed dislike moderately, 2 showed dislike very much, and 1 showed dislike extremely (Lawless & Heymann, 2013; Singh-Ackbarali & Maharaj, 2014). Participants were allowed to write their comments down on the form.

*Gastrointestinal symptoms*



Gastrointestinal symptoms inclusive of nausea, abdominal pain, vomiting, flatulence, and bloating were observed using a questionnaire 24h before and after the clinic day (Jacqz-Aigrain et al., 2015). Participants were asked to rank the intensity of symptoms from 0 (none) to 3 (severe) (Jacqz-Aigrain et al., 2015). In addition, Bristol Stool Scale with description for each type of stool form and pictures was used to evaluate the stool form 24 h before and after the test session (Lewis & Heaton, 1997).

*Statistical analysis*

Incremental plasma glucose and serum insulin were calculated as the value at time point minus baseline value. Incremental area under the curve (iAUC) was calculated based on trapezoidal rule from 0 to 180 min for glucose and insulin (F Brouns et al., 2005). Data were expressed as mean values with standard errors of the means (SEM) represented as vertical bars. Data were analyzed by using Statistical Package for Social Sciences version 22.0 (IBM Corp, NY, USA). The normality of data was analyzed by using a Shapiro-Wilk test. A repeated measures ANOVA was performed to analyze the normally distributed data followed by bonferroni as post hoc analysis. Meanwhile, Friedman's two-way ANOVA was performed to test for differences between treatments in non-normally distributed data. Statistical significance was set at $p$-value <0.05.

### 3.4 Phase IV: Long-term evaluation of developed ONS on body composition, food intake, and GI tolerability in healthy and prediabetic participants.



Objective: To evaluate body weight, body composition, food intake, and tolerability of the developed ONS in healthy and prediabetic adults.

*Participants*

The inclusion criteria were:

1. Adult aged 18-55 years old
2. Never been diagnosed with serious illnesses, e.g. cancers, heart disease, liver disease, kidney disease, or severe GI disease

3. Not taking any medication/ dietary supplements/ insulin injections to lower plasma glucose
4. Did not have allergies to products containing whey, soy, or cassava

The exclusion criteria were pregnancy or breastfeeding

The study was conducted at the Nutrition and Dietetics Department, Faculty of Allied Health Sciences, Chulalongkorn University, Bangkok, Thailand. Participant recruitment was advertised by online social media. Interested participants contacted researcher and screened for the eligibility. Prior to screening, participants were informed about the details of the study, including the study procedure, risks, and benefits from this study. All eligible participants signed informed consent before participating in this study. Eligible participants received a booklet to record food intake, physical activity, gastrointestinal symptoms, and stool consistency during the study.



*Sample size calculation*

A minimum of 18 participants was required to evaluate the significance on fasting plasma glucose with effect size of 0.7083, level of significance at 0.05, and power was set at 80% (Pohl et al., 2005). Sample size was estimated using G* power version 3.1 (Faul, Erdfelder, Lang, & Buchner, 2007). Dropout rate was estimated about 30% thus 24 participants were recruited in this study.

*Intervention*

Eligible participants were invited to come to Nutrition and Dietetics Department, Chulalongkorn University to do baseline measurements, including blood sample collection, anthropometric measurement, body composition measurement, physical activity measurement, and received the booklet for dietary and GI symptoms record throughout the study. Before collecting the blood sample, participants were reminded to fasting overnight (12 h). Similar measurements were done at midpoint (week 6) and endpoint (week 12). In addition, once in every 3 weeks, participants received the ONS and did the body composition measurement. Participants should drink the developed ONS (1 serving/day) for 12 weeks. They were advised to maintain their habitual dietary intake and physical activity throughout the study. Food intake, GI symptoms, and physical activity were recorded every 3-week throughout the study. Food intake was evaluated by using 3-days food record (2 weekdays+1 weekend) (Mahan, Escott-Stump, & Krause, 2007), gastrointestinal symptoms by using questionnaire. Physical activity was evaluated by using Global Physical Activity Questionnaire (GPAQ). Stool consistency will be determined by using Bristol Stool Chart (Lewis & Heaton, 1997). Furthermore, participants were asked to record the intake of ONS every day throughout 12 weeks and kept the empty sachet to ensure the adherence.



*Blood chemistry analysis*

Ten milliliters of blood sample were drawn at each measurement day. Blood sample for glucose was collected in NaF vacutainer tube; blood sample for insulin, lipid biomarkers, BUN, creatinine, ALT, AST was collected in clot activator vacutainer tube. Blood sample for HbA1C was collected in EDTA-containing tube. Blood sample was centrifuged at 3,000 rpm for 10 min at 4°C to separate the plasma and serum. Subsequently, blood samples were kept at -20°C for further analysis (Wiedmeyer, 2003). Plasma glucose was determined according to the hexokinase method using a clinical chemistry analyzer (Beckman Coulter AU480, USA). HbA1C was determined by using enzymatic (Matsumoto, Uchino, & Kato, 2013). Serum insulin was analyzed by chemiluminescence microparticle immunoassay (CMIA) (Moriyama et al., 2006). Serum creatinine and BUN were evaluated enzymatically (Adrian O. Hosten, 1990). Lipid profile, including triglyceride, total cholesterol, HDL-cholesterol were evaluated enzymatically, while LDL-cholesterol was calculated using Friedewald equation as LDL= total cholesterol - HDL cholesterol - (triglyceride/5) (Friedewald, Levy, & Fredrickson, 1972). Furthermore, AST and ALT were evaluated enzymatically (Lala et al., 2020).

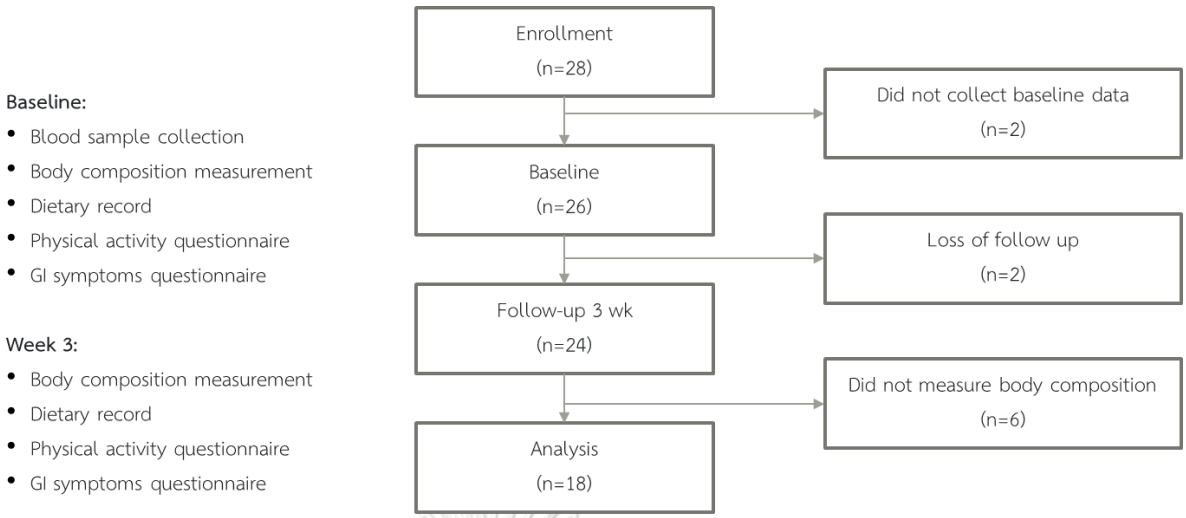



Figure 10. Study flow of participants enrollment and follow up for 12 weeks

*Anthropometric measurement*

Body weight and body composition, including fat mass, fat-free mass, muscle mass and total body water were assessed by body impedance analyzer Tanita MC-980 MA (Tanita corp, Japan) with precision of 0.1 kg. Body mass index was calculated as body weight (kg)/ body height ($m^2$). Principle measurement of body impedance analyzer is to passage the electric current to the body at a differential rate which depends on the body composition. Human body is composed of water and ion which able to pass the electric current. In addition, fat in human body may resist the electric current, thus the extracellular part of the adipose tissue plays role as resistor. Meanwhile, bone and muscle are more conductive than adipose tissue. Therefore, the body composition could be estimated through the conductivity and resistance in human body (Dehghan & Merchant, 2008).

*Blood pressure*

Blood pressure was measured by using digital sphygmomanometer (Omron model HBP-9020, Japan). Participants rested for at least 10 min before the measurement. Participant was seated on a chair, arm was inserted to arm cuff, and elbow was placed on the elbow rest. The cuff was automatically wrapped around arm. Systolic and diastolic blood pressure were recorded for further analysis.

*Gastrointestinal symptoms*

Gastrointestinal symptoms including abdominal pain, nausea, vomiting, bloating, and flatulence were evaluated using a questionnaire (Jacqz-Aigrain et al., 2015). Participants rated the intensity of symptoms from 0 (none), 1 (mild), 2 (moderate), to 3 (severe). Total score was calculated for the intensity of all symptoms. A score of 0 defined no symptoms at all, while a score of 15 reflected all symptoms rated as severe (Jacqz-Aigrain et al., 2015). Stool form was evaluated using the Bristol Stool Scale with a picture and description for each type of stool form (Lewis & Heaton, 1997).



*Food intake*

Participants recorded their food intake for 3 days, which include 2 days of weekday and 1 day of weekend. Participants wrote the name of food, amount in household measurement, and the way of cooking to assess the food intake more accurately.

Data from diary food intake were analyzed using INMUCAL, a software developed by Institute of Nutrition Mahidol University to analyze the dietary composition among Thai population (Institute of Nutrition Mahidol University).

*Physical activity*

Physical activity was assessed by using Global Physical Activity Questionnaire (GPAQ) developed by WHO and shows a good validity and reliability (Chu, Ng, Koh, & Müller-Riemenschneider, 2015). GPAQ covers 3 domains: activity at work, travel, and recreational activities. The time spent and intensity for each activity was recorded as minute and hour which then calculated and expressed as metabolic equivalents (MET). Energy at rest is defined as 1 MET which equivalent to 1 kcal/kg/hour. Meanwhile, moderate and vigorous activity are equal to 4 MET and 8 MET, respectively. Physical activity was expressed as MET-minutes per week (Armstrong & Bull, 2006).



*Statistical analysis*

Data was expressed as mean ± SEM. Normality of data was determined by using Shapiro-Wilk. Data with normal distribution was analyzed by using paired t-test, while not normally distributed data was analyzed by using Wilcoxon-signed rank test. Statistical significance was set at p-value $<0.05$. All statistical analysis was performed by Statistical Package for Social Sciences version 22.0 (IBM Corp, NY, USA).

# CHAPTER IV

# RESULTS

## 4.1 Phase I: Effect of TRM on postprandial glucose and insulin response in healthy participants

### 4.1.1 Participants

In total, eighteen participants were randomized into three groups. However, two of participants were excluded from the study due to loss of follow up. The majority of participants were female (56%) with mean age of 25.81 ± 1.19 years old and mean BMI of 22.88 ± 0.87 kg/$m^2$. At baseline, all participants were categorized as normoglycemia as mean fasting plasma glucose and mean fasting serum insulin were 84.60 ± 1.39 mg/dL and 4.94 ± 0.72 µIU/mL, respectively (Table 11).

Table 11. Baseline characteristic of participants (n=16)

| Baseline characteristic | Mean | SEM |
|---|---|---|
| Sex (male/ female) | 7 / 9 | |
| Age (year) | 25.81 | 1.19 |
| Body weight (kg) | 60.84 | 3.13 |
| BMI (kg/$m^2$) | 22.88 | 0.87 |
| Fasting plasma glucose (mg/dL) | 84.60 | 1.39 |
| Fasting serum insulin (µIU/mL) | 4.94 | 0.72 |

### 4.1.2 Postprandial plasma glucose response

The plasma glucose and serum insulin were similar in the baseline (baseline glucose of GL: 84.60±1.39, TM: 84.67±1.30, TRM: 84.87±1.51, MIX15: 83.73±1.78, and MIX50: 84.33±1.61 mg/dl; baseline insulin of GL: 4.94±0.72, TM: 4.98±0.59, TRM: 4.62±0.49, MIX15: 3.36±0.54, and MIX50: 4.19±0.62 μIU/L), then significantly increased following test drink consumption and reached the peak at 30 min. The highest peak of plasma glucose was reached following GL consumption (135.87 ± 4.88 mg/dl), followed by TM (127.93 ± 4.05 mg/dl), and MIX15% (124.67 ± 5.73 mg/dL), while peak plasma glucose of TRM (104.60 ± 2.63 mg/dL) was significantly the lowest compared to other treatments ($p<0.05$).

The trends of incremental plasma glucose were similar to plasma glucose. The incremental plasma glucose of TRM (19.73 ± 1.98 mg/dL) significantly lowest compared to GL (51.27 ± 4.56 mg/dL, *p= <0.001*), TM (43.27 ± 3.58 mg/dL, *p= 0.002*), MIX15% (40.93 ± 5.34 mg/dL, *p= 0.032*), and MIX50% (45.00 ± 5.06 mg/dL, *p= 0.003*). The incremental plasma glucose of TRM (6.67 ± 2.52 mg/dL) at 60 min was also lower than GL (36.33 ± 5.97 mg/dL, *p= 0.002*), TM (31.47 ± 7.43 mg/dL, *p= 0.027*), and MIX15% (30.73 ± 7.70 mg/dL, *p*= 0.027).

The iAUC $Glucose_{0\text{-}180\ min}$ following TRM (1,126.00 ± 191.51 mg x min/dL was the lowest compared to GL (3,481.00 ± 391.48 mg x min/dL, *p= 0.001*) and TM (3,273.00 ± 406.42 mg x min/dL, *p= 0.004).* Meanwhile, MIX15% (3,045.00 ± 497.47

mg/dL x min, *p= 0.943*) and MIX50% (2,541.43 ± 362.67 mg/dL x min, *p= 0.198*) had lower iAUC Glucose$_{0\text{-}180min}$ when compared to GL but was not significantly different.

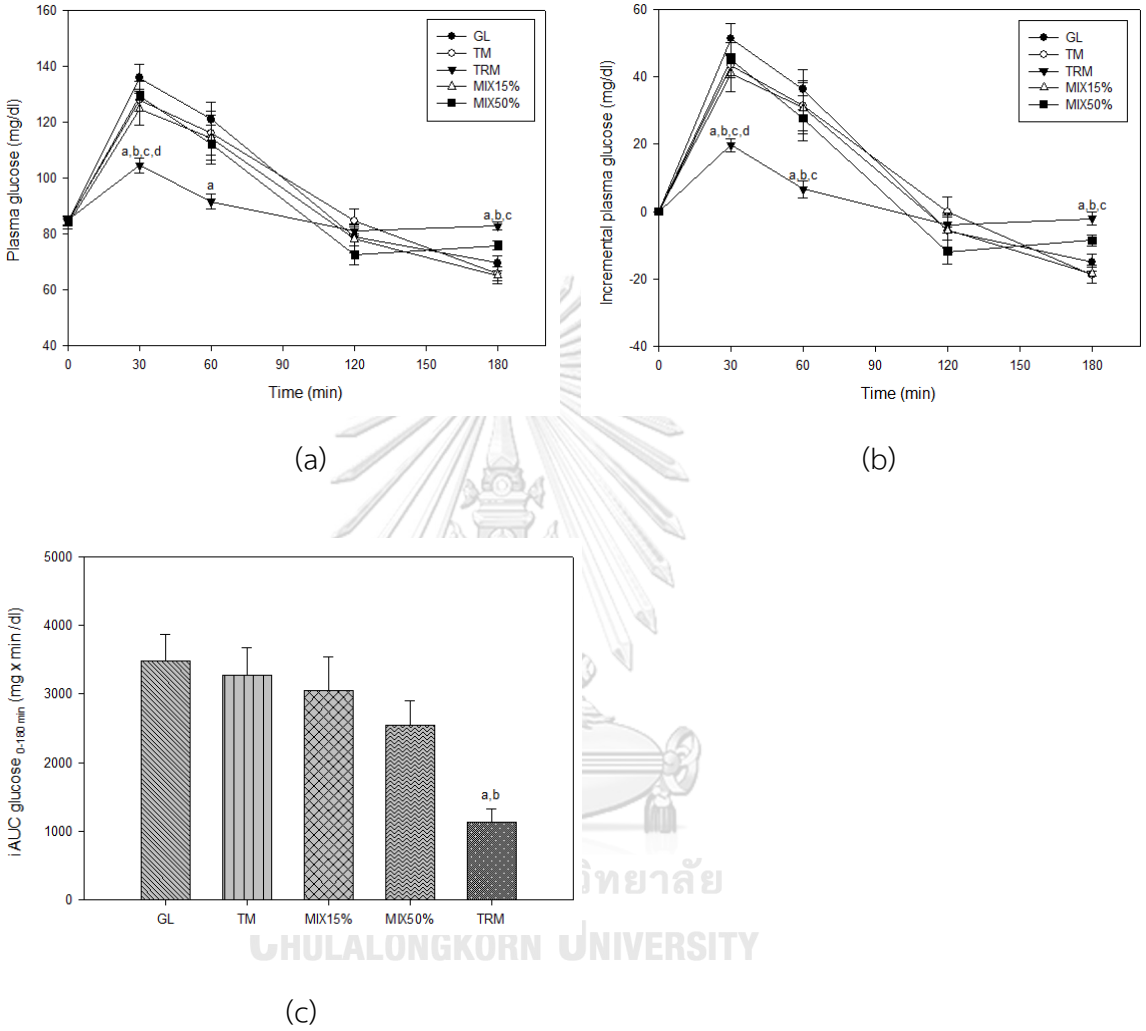



Figure 11. Postprandial glucose response following test drinks in healthy participants: (a) Plasma glucose response over 180 min, (b) incremental plasma glucose over 180 min, and (c) incremental area under the curve of glucose$_{0\text{-}180\ min}$. Data are presented as mean±sem. [a] TRM was significantly different from GL ($p<0.05$), [b] TRM was

significantly different from TM ($p<0.05$), [c] TRM was significantly different from MIX15% ($p<0.05$), [d] TRM was significantly different from MIX50% ($p<0.05$).

**4.1.3 Postprandial serum insulin response**

The peak of serum insulin was significantly lowest following TRM (13.01 ± 2.12 μIU/ml) than GL (47.90 ± 11.93 μIU/ml, *p= 0.013*), TM (52.96 ± 17.68 μIU/ml, *p= 0.002*), and MIX50% (33.16 ± 4.99 μIU/ml, *p= 0.008*). Furthermore, TRM (8.29 ± 1.16 μIU/ml) also decreased the serum insulin response at 60 compared to GL (36.91 ± 7.12 μIU/ml, *p= 0.001*), TM (40.91 ± 8.62 μIU/ml, *p= 0.008*), and MIX15% (30.65 ± 6.79 μIU/ml, *p= 0.028*).

Tapioca RMD (8.40 ± 1.77 μIU/mL) significantly decreased the peak of incremental serum insulin when compared to GL (42.96 ± 11.74 μIU/mL, *p*= 0.003), TM (47.99 ± 17.48 μIU/mL, *p*= 0.013), and MIX50% (28.98 ± 4.58 μIU/mL, *p*= 0.013). It also lowered the incremental serum insulin at 60 and 120 min compared to GL and TM. Meanwhile, MIX50% (0.09 ± 0.88 μIU/mL) significantly decreased the incremental serum insulin at 120 min when compared to GL (10.20 ± 2.54 μIU/mL, *p*= 0.023) and TM (13.14 ± 3.58 μIU/mL, *p*= 0.010).

Similarly, TRM (505.29 ± 75.14 μIU/ml x min) also reduced the iAUC $insulin_{0\text{-}180\ min}$ compared to GL (3,372.03 ± 452.46 μIU x min/ml, *p*= <0.001), TM (3,854.54± 798.26 μIU x min/ml, *p*=0.001), and MIX15% (2,543.94 ± 438.17 μIU x min/ml, *p*= 0.008), yet

MIX50% (1,913.26 ± 292.13 μIU x min/ml) was not significantly different to TRM (*p*=0.314).

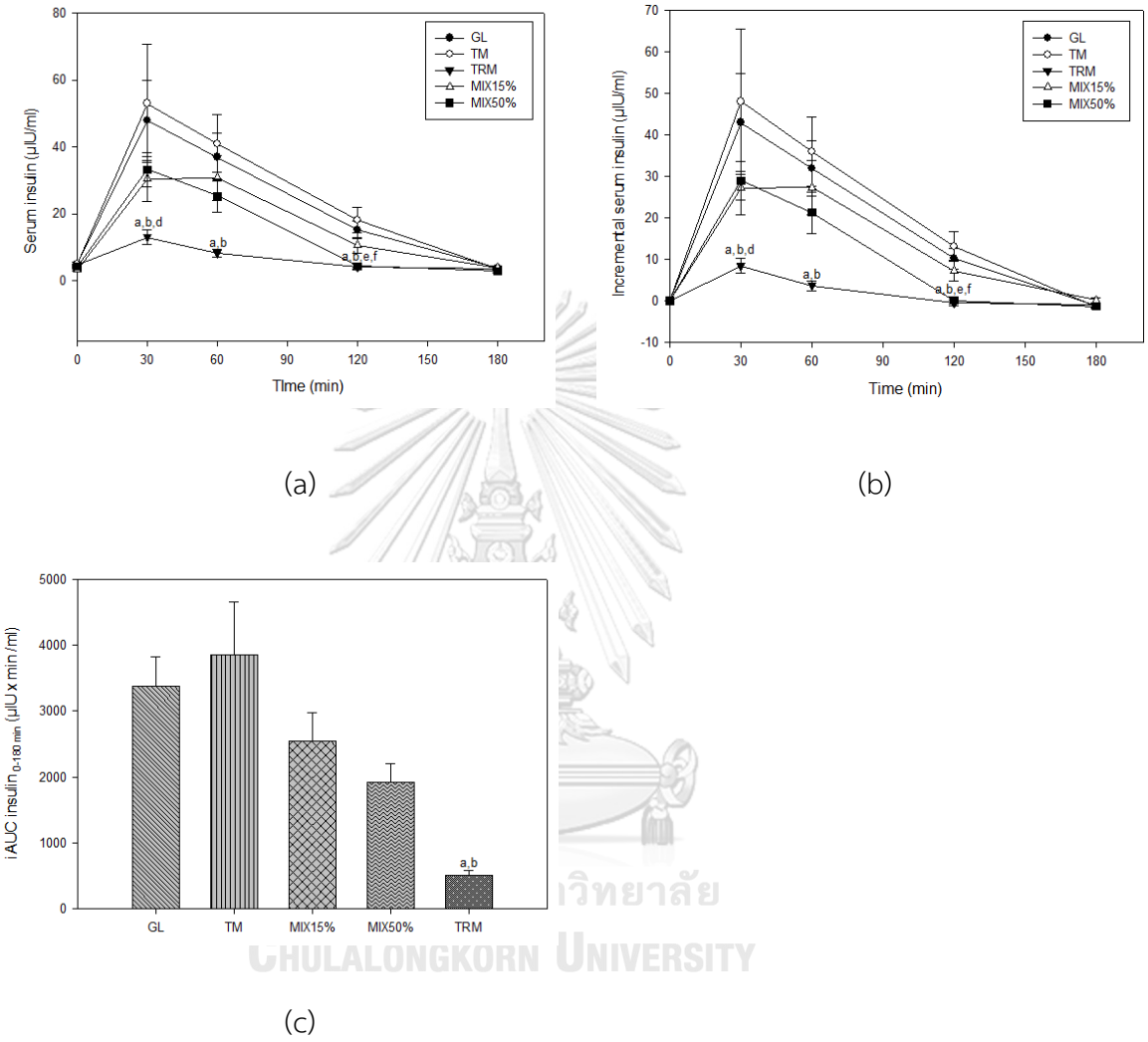
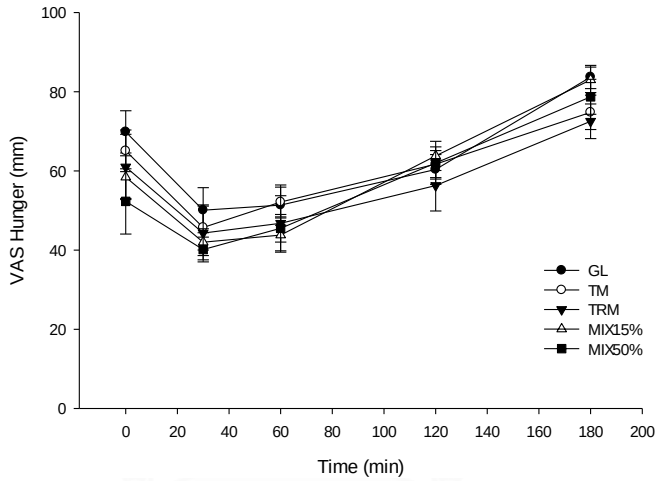



Figure 12. Postprandial insulin response following test drinks in healthy participants: (a) serum insulin response over 180 min, (b) incremental serum insulin over 180 min, and (c) incremental area under the curve of insulin$_{0\text{-}180\ min}$. Data are presented as mean±sem. [a] TRM was significantly different from GL ($p<0.05$), [b] TRM was significantly different from TM ($p<0.05$), [c] TRM was significantly different from MIX15% ($p<0.05$), [d]

TRM was significantly different from MIX50% ($p<0.05$), [e] MIX50% was significantly different from GL ($p<0.05$), [f] MIX50% was significantly different from TM ($p<0.05$).

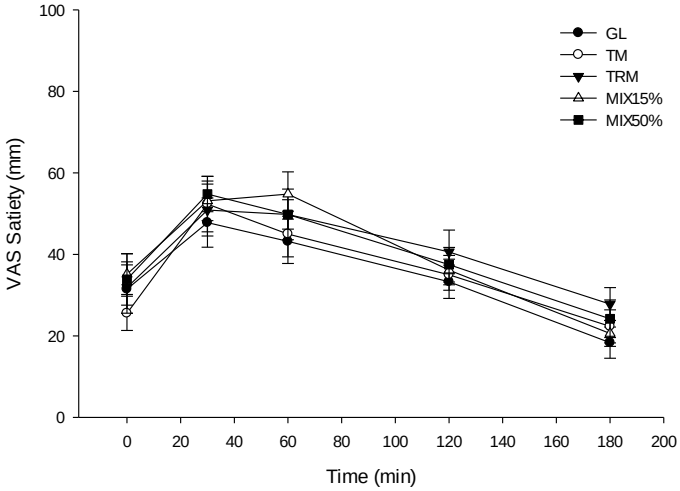
### 4.1.4 Subjective appetite

The effect of TRM on subjective appetite, including hunger, satiety, desire to eat, and prospective consumption, was not significantly different compared to other test drinks when evaluated over 180 in this study ($P>0.05$).

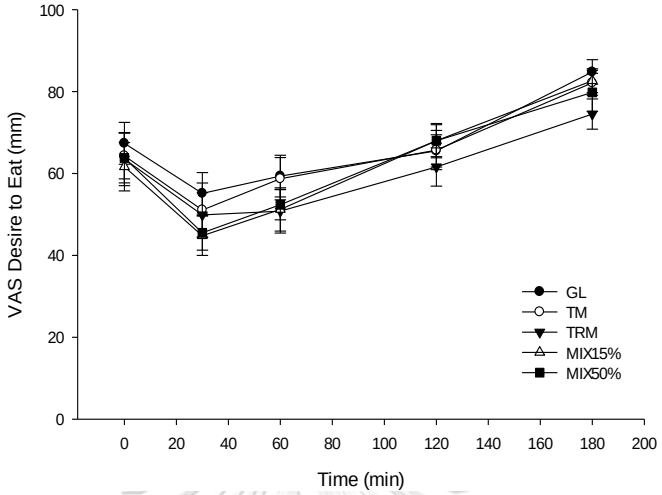




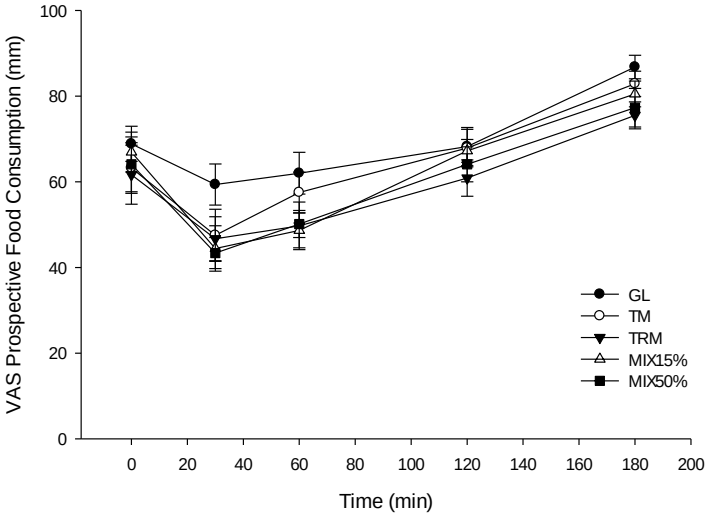

(a)



(b)




(c)




(d)

Figure 13. Subjective appetite as evaluated by visual analogue scale (mm) over 180 min, including: (a) hunger, (b) satiety, (c) desire to eat, and (d) prospective food consumption, in healthy adults. Data are presented as mean±sem.

### 4.1.5 Gastrointestinal symptoms

Some gastrointestinal symptoms were experienced in few participants, including flatulence, abdominal pain, and bloating. The flatulence intensity following TRM consumption was significantly increased when compared to GL, TM, and MIX15 (TRM 0.19±0.14, GL 0.25±0.12, TM 0.25±0.12, and MIX15 0.25±0.12, respectively, *p= 0.001*). The intensity of bloating and abdominal pain was also increased, but not significant, after TRM consumption. Total GI symptom score was significantly higher following TRM consumption when compared to GL, TM, and MIX15 (TRM 1.69±0.52, GL 0.32±0.16, TM 0.5±0.21, MIX15 0.25±0.12, *p*=0.003). The stool form was not significantly changed following test drink consumption. Most participants had normal stool form as indicated by scale 3 and 4 in Bristol Stool Scale.

Table 12. Gastrointestinal symptoms following test drink consumption

| | Abdominal pain | Nausea | Vomiting | Bloating | Flatulence | Total score | BSS |
|---|---|---|---|---|---|---|---|
| GL | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.07±0.07 | 0.25±0.12 | 0.32±0.16 | 4.2±0.18 |
| TM | 0,07±0,07 | 0.00±0.00 | 0.00±0.00 | 0.19±0.11 | 0.25±0.12 | 0.50±0.21 | 4.32±0.24 |
| TRM | 0,19±0,14 | 0.00±0.00 | 0.00±0.00 | 0.63±0.24 | 0.88±0.23[a,b,c] | 1.69±0.52[a,b,c] | 4.25±0.29 |
| MIX15 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.25±0.12 | 0.25±0.12 | 4.19±0.23 |
| MIX50 | 0,07±0,07 | 0.00±0.00 | 0.00±0.00 | 0.19±0.11 | 0.44±0.19 | 0.69±0.32 | 3.94±0.25 |

Data are presented as mean±sem. [a] TRM was significantly different from GL ($p<0.05$), [b] TRM was significantly different from TM ($p<0.05$), [c] TRM was significantly different from MIX15% ($p<0.05$).

## 4.2 Phase II: Utilization of TRM in the development of ONS

### 4.2.1 Effect of different dextrose equivalent (DE) of tapioca maltodextrin in ONS development

Difference in DE used in ONS resulted in different physicochemical characteristics, including colour and pH. The ONS formula containing TM-DE19 (formula 2) was significantly lighter compared to the formula with TM-DE7 (formula 1) (L* values, 53.14 ± 1.80 vs. 46.11 ± 2.31 respectively, p=0.004). In addition, TM-DE19 (formula 2) was significantly more yellowish compared to the formula with TM-DE7 (formula 1) (b*, 2.17 ± 0.22 vs. 1.58 ± 0.23 respectively, p=0.004). Data was shown in table 13.

*Viscosity*

The developed ONS with TM-DE7 (formula 1) was significantly higher in viscosity compared to the formula with TM-DE19 (formula 2) 34.48 ± 0.85 cP vs. 31.98 ± 1.11 cP, respectively, p-value <0.05. Data was shown in table 13.



*pH*

There was no significant difference in pH of developed ONS formula whether using TM-DE7 or TM-DE19 (6.31 ± 0.05 vs 6.29 ± 0.07, p-value = 0.052). Developed ONS in our study were classified as low acidity (AOAC, 2012). Data was shown in table 13.

Table 13. Physical characteristic of nutritionally complete ONS with different DE maltodextrin

| **Physical characteristic** | **Formula 1 (TM-DE7)** | **Formula 2 (TM-DE19)** | ***p*-value** |
|---|---|---|---|
| Colour | | | |
| L* | 46.11 ± 2.31 | 53.14 ± 1.80[a] | 0.004 |
| a* | -0.83 ± 0.23 | -0.74 ± 0.02 | 1.000 |
| b* | 1.58 ± 0.23 | 2.17 ± 0.22[a] | 0.004 |
| Viscosity (cP) | 34.48 ± 0.85 | 31.98 ± 1.11[a] | 0.001 |
| pH | 6.31 ± 0.05 | 6.29 ± 0.07 | 0.052 |

Data are presented as mean±sem. [a] indicates significant differences compared to ONS TM DE7 (p-value <0.05)

Participants were healthy adults aged 22 to 71 years old (mean age of 31.5 ± 10.8 years old). Most of the participants were female (59%). There was no significant difference in acceptability including appearance, taste, smell, and aftertaste between formula 1 and 2 as shown in table 14. In addition, 51% of participants in our study preferred ONS with TM-DE19.



Table 14. Hedonic ratings for developed ONS using TM-DE7 and TM-DE19

| **Hedonic parameters** | **Formula 1 (TM-DE7)** | **Formula 2 (TM-DE19)** | ***p*-value** |
|---|---|---|---|
| Appearance | 6.23 ± 0.22 | 6.38 ± 0.19 | 0.549 |
| Taste | 5.74 ± 0.26 | 5.85 ± 0.27 | 0.555 |

| Hedonic parameters | Formula 1 (TM-DE7) | Formula 2 (TM-DE19) | *p*-value |
|---|---|---|---|
| Smell | 6.51 ± 0.28 | 6.67 ± 0.28 | 0.648 |
| Viscosity | 6.15 ± 0.20 | 6.36 ± 0.21 | 0.313 |
| Aftertaste | 6.10 ± 0.25 | 5.95 ± 0.26 | 0.811 |
| Overall acceptability | 6.13 ± 0.24 | 6.08 ± 0.25 | 0.984 |

Data are presented as mean±sem.

**4.2.2 Development of ONS using TRM**

*Physicochemical properties of ONS*

Replacement of tapioca maltodextrin using TRM slightly decreased the viscosity of developed ONS. The viscosity of original formula was 36.37±0.25 cP, while viscosity of RMD15 and RMD30 were 34.60±0.06 cP and 34.07±0.04 cP, respectively ($p<0.001$). Furthermore, replacement of tapioca maltodextrin using TRM increased the water activity of ONS powder, as the water activity of original formula, RMD 15, and RMD30 were 0.33±0.01, 0.35±0.01 ($p= 0.012$), and 0.37±0.01 ($p<0.001$), respectively. In contrast, there were no significant differences on moisture and pH between formulas.



Table 15. Physical properties of developed ONS

| Formula | Viscosity (cP) | Moisture (%) | Aw | pH |
|---|---|---|---|---|
| Original | 36,37±0,25 | 4,51±0,04 | 0,33±0,01 | 6,36±0,02 |

| Formula | Viscosity (cP) | Moisture (%) | Aw | pH |
|---|---|---|---|---|
| RMD15 | 34,60±0,06 | 4,49±0,08 | 0,35±0,01[a] | 6,50±0,01 |
| RMD30 | 34,07±0,09[a] | 4,22±0,04 | 0,37±0,01[a,b] | 6,29±0,01 |

Data were presented as mean±SEM. [a] indicates significantly different compared to original ($p<0.05$), [b] indicates significantly different compared to RMD15 ($p<0.05$)

The developed ONS contains 251.80 kcal per serving, 32.73 g carbohydrate, 9.85 g protein, 9.05 g fat, vitamins and minerals to meet 25-29% of Thai RDI.

Table 16. Nutritional composition of ONS

| Nutrients | Amount per serving (60 g) | Thai RDI (%) |
|---|---|---|
| Energy (kcal) | 251.80 | 12.59 |
| Carbohydrate (g) | 32.73 | 10,91 |
| Protein (g) | 9.85 | 19,70 |
| Fat (g) | 9.05 | 13,92 |
| Na (mg) | 279.74 | 11.66 |
| K (mg) | 235.95 | 6.74 |
| Mg (mg) | 91.34 | 26.10 |
| P (mg) | 249.04 | 31.13 |
| Ca (mg) | 206.83 | 25.85 |
| Cl (mg) | 213.58 | 6.28 |
| Se (mcg) | 17.95 | 25.65 |

| Nutrients | Amount per serving (60 g) | Thai RDI (%) |
|---|---|---|
| Zn (mg) | 3.85 | 25.64 |
| Fe (mg) | 4.47 | 29.81 |
| Cu (mg) | 0.51 | 25.64 |
| Cr (mcg) | 33.34 | 25.65 |
| I (mcg) | 38.47 | 25.64 |
| Mn (mg) | 0.90 | 25.65 |
| Mo (mcg) | 41.03 | 25.65 |
| Choline (mg) | 176.31 | 32.06 |
| Vit B1 (mg) | 0.44 | 29.10 |
| Vit B2 (mg) | 0.50 | 29.15 |
| Vit B3 (mg) | 5.82 | 29.10 |
| Vit B5 (mg) | 1.75 | 29.10 |
| Vit B6 (mg) | 0.58 | 29.10 |
| Vit B12 (mcg) | 0.61 | 30.36 |
| Vit C (mg) | 17.57 | 29.28 |
| Vit E (mg) | 2.91 | 29.14 |
| Vit D (mcg) | 1.47 | 29.32 |
| Vit K1 (mcg) | 23.28 | 29.10 |

| Nutrients | Amount per serving (60 g) | Thai RDI (%) |
|---|---|---|
| Vit A (mcg RE) | 24.93 | 3.12 |
| β-carotene (mg) | 1.40 | - |
| Biotin (mcg) | 45.54 | 30.36 |
| Folic acid (mcg) | 60.72 | 30.36 |

Macronutrients were analyzed by AOAC method, micronutrients were results of calculation.

**4.2.3 Microbial evaluation**

Microbial evaluation was done by total plate count following Food and Drug Administration (Food and Drug Administration, 2001). Total plate count result shows that the estimated aerobic bacteria in the ONS powder was 10 colony forming unit (CFU) per gram.



**4.2.4 Stability evaluation**

As the temperature increased, the constant reaction was also increased (Table 17). A zero ordo reaction was choose to calculate the shelf-life based on water activity (Aw). The maximum water activity (At) was determined as 0.6 since no microorganism could grow at Aw below than 6.

Table 17. Attributes in Arrhenius equation

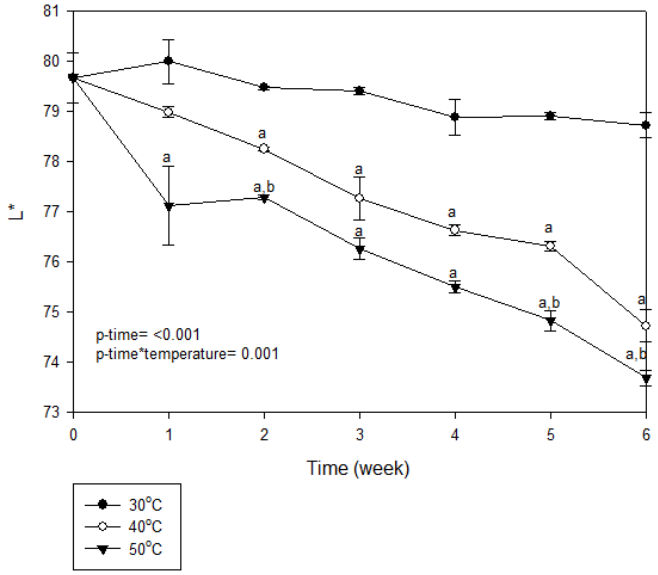
| Temperature | | 1/T | k | ln k |
|---|---|---|---|---|
| (°C) | (°K) | | | |
| 30 | 303 | 0,00330 | 0,00060 | -7,419 |
| 40 | 313 | 0,00319 | 0,00090 | -7,013 |
| 50 | 323 | 0,00310 | 0,00100 | -6,908 |

The value of ln k and 1/T of each temperature were then plotted on a linear regression which yield linear equation of $y = -2514,5x + 0,9258$ and $R^2 = 0,9078$. This equation was used to determine the shelf-life. The results showed that the ONS was shelf-stable at room temperature (25-30°C) for more than 1 year. The shelf-life decreased as the storage temperature increased.

Table 18. Linear regression of ln k against temperature

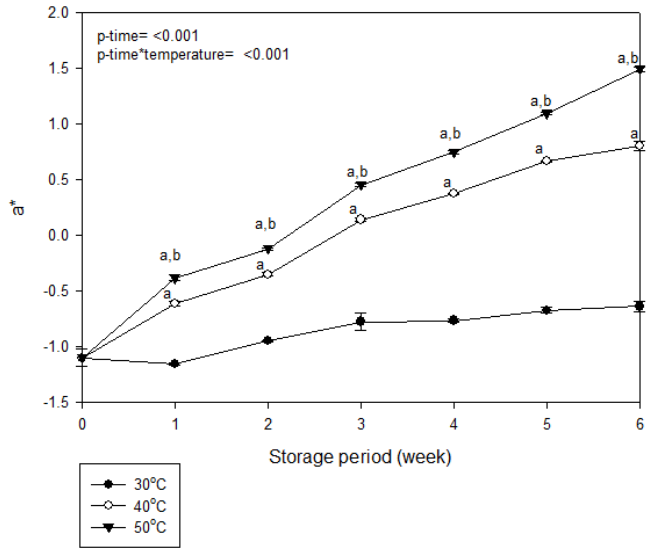
| Temperature | | ln k | k | Shelf life | |
|---|---|---|---|---|---|
| (°C) | (°K) | | | days | years |
| 4 | 277 | -8,15182 | 0,000288 | 969,78 | 2,66 |
| 10 | 283 | -7,95936 | 0,000349 | 800,00 | 2,19 |
| 25 | 298 | -7,51212 | 0,000546 | 511,51 | 1,40 |
| 30 | 303 | -7,37288 | 0,000628 | 444,23 | 1,22 |
| 40 | 313 | -7,10775 | 0,000819 | 340,77 | 0,93 |
| 50 | 323 | -6,85903 | 0,00105 | 265,73 | 0,73 |

During the storage period, the changes of color were observed. It was noticed that storage temperature and period significantly affected the yellowness ($p<0.001$) and redness ($p<0.001$) of the ONS. The lightness decreased significantly as the time and temperature increased. Meanwhile, the yellowness and redness increased with the increased of temperature and time (Fig. 12). On the other hand, no significant changes on pH at different temperature and storage period (data not shown).

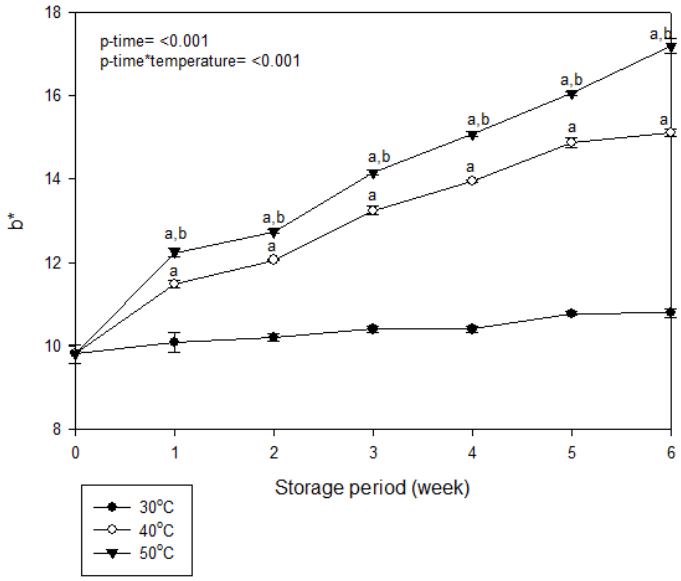





(a)


p-time= <0.001
p-time*temperature= <0.001
a*
Storage period (week)
30°C
40°C
50°C

(b)


p-time= <0.001
p-time*temperature= <0.001
b*
Storage period (week)
30°C
40°C
50°C

(c)

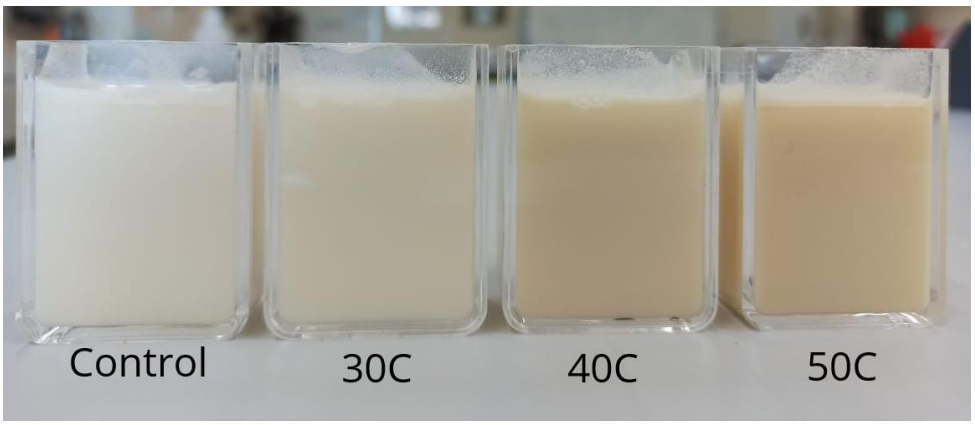



Figure 14. Color changes during storage for 6 weeks at different temperature, including lightness (a), yellowness (b), and redness (c). The color of ONS at 6 week of storage (d). Data are presented as mean±sem. [a] represents significantly different from 30°C ($p<0.05$), [b] represents significantly different from 40°C ($p<0.05$)

(d)

## 4.3 Phase III: Acute effect evaluation of developed ONS on postprandial glycemic response, acceptability, and GI tolerability

### 4.3.1 Participants

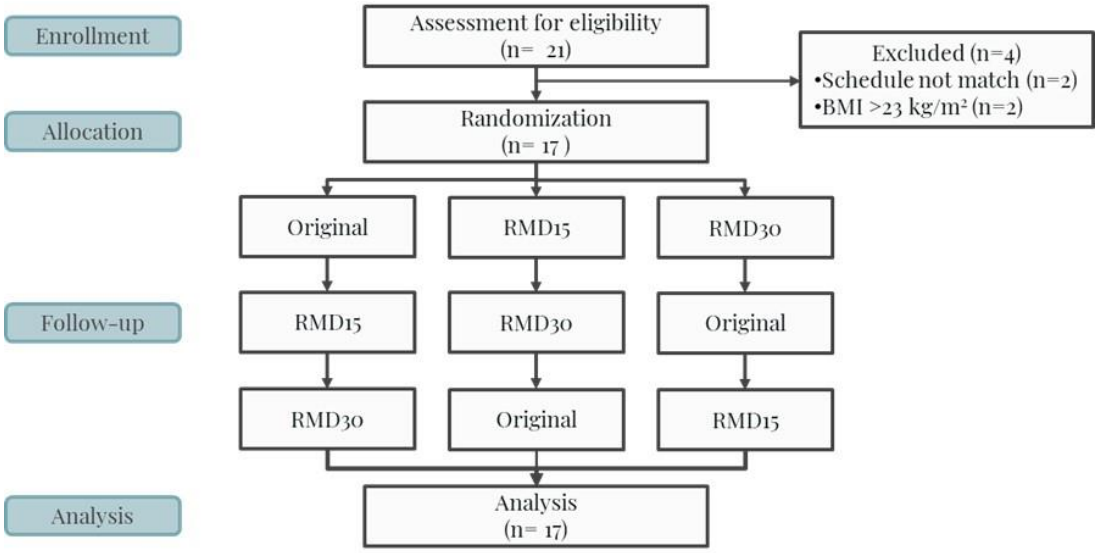



Figure 15. CONSORT diagram of acute effect evaluation of developed ONS

In the randomized cross-over study, 21 participants were interested to join the study. However, four of them were excluded due to did not match with the eligible criteria (n= 2 participants) and did not match with the schedule of clinic day (n= 2 participants). Therefore, seventeen participants were eligible and randomized into three groups: original, RMD15, and RMD30 which switched to other treatments on the following visits. Most of participants in this study were female (58.8%) aged 26.24 ± 0.62 years old. The mean BMI, fasting plasma glucose, and fasting insulin of participants were 21.29±0.50 kg/m$^2$, 91.62 ± 1.46 mg/dl, and 4.87 ± 0.68, respectively (Table 19).

Table 19. Baseline characteristic of participants

| Baseline characteristic | All participants (n= 17) |
|---|---|
| Sex (male/ female) | 7/10 |
| Age (year) | 26.24 ± 0.62 |
| Body weight (kg) | 56.05 ± 2.02 |
| BMI (kg/m$^2$) | 21.29 ± 0.50 |
| Fasting plasma glucose (mg/dl) | 91.62 ± 1.46 |
| Fasting insulin (μIU/ml) | 4.87 ± 0.68 |

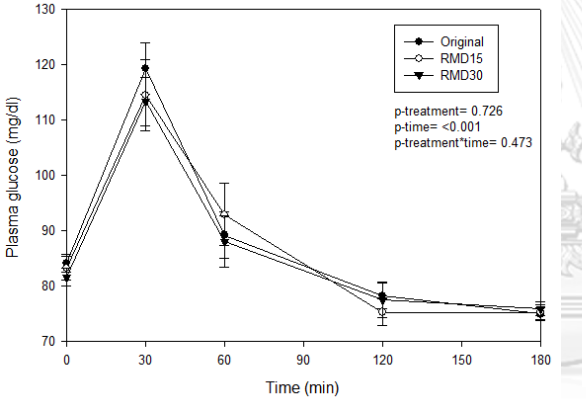
Data are presented as mean±sem.

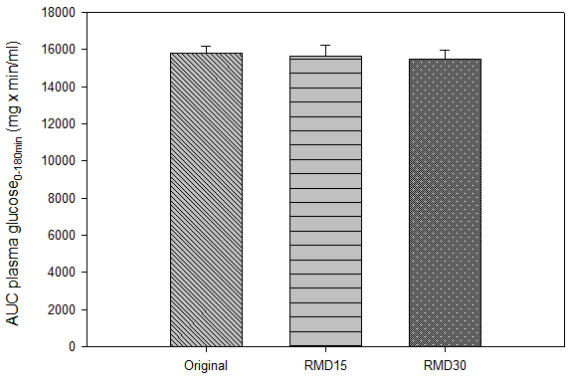
**4.3.2 Plasma glucose response**

The peaks of plasma glucose were reached at 30 min following ONS consumption for all formulas. The highest peak of plasma glucose was obtained following original formula consumption (119.25±4.67 mg/dl). However, the replacement of tapioca maltodextrin using TRM 15 and 30% reduced the peak of plasma glucose from 119.25±4.67 mg/dl to 114.42±6.43 mg/dl (p=0.640) and 113.33±4.44 mg/dl (p=0.557) for RMD15 and RMD30, respectively. Similarly, the incremental plasma glucose following original formula reached the highest peak at 30 min (35.08±4.06 mg/dl). Meanwhile, RMD15 and RMD30 insignificantly decreased the peak of incremental

plasma glucose by 10.9% and 9.5% to 31.25±5.37 mg/dl and 31.75±3.54 mg/dl ($p>0.05$).

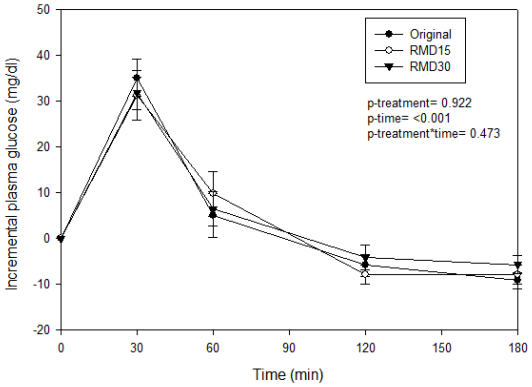
The AUC glucose over 180 min following original formula was 15,795±395.94 mg x min/ml. Meanwhile, the AUC glucose$_{0\text{-}180\ min}$ of RMD15 and RMD30 were not significantly different to original formula as 15,633.75±595.11 mg x min/ml and 15,503.75±449.84 mg x min/ml for RMD15 and RMD30, respectively ($p>0.05$).

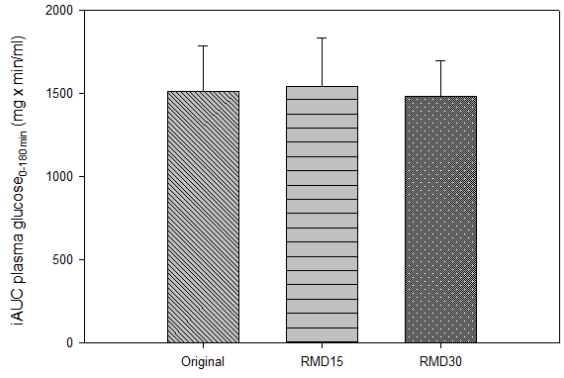





(a) (b)



(c) (d)

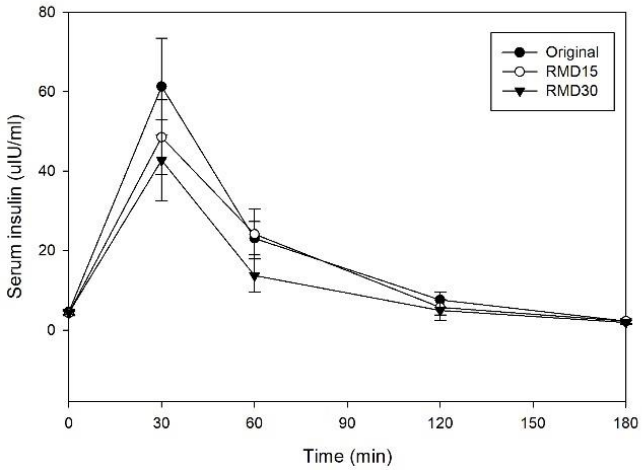
Figure 16. Postprandial plasma glucose response (a), area under the curve (AUC) of plasma glucose over 180 min (b), incremental plasma glucose (c), and incremental AUC of plasma glucose over 180 min of developed ONS in healthy subjects. Data are presented as mean±sem.

**4.3.3 Insulin response**

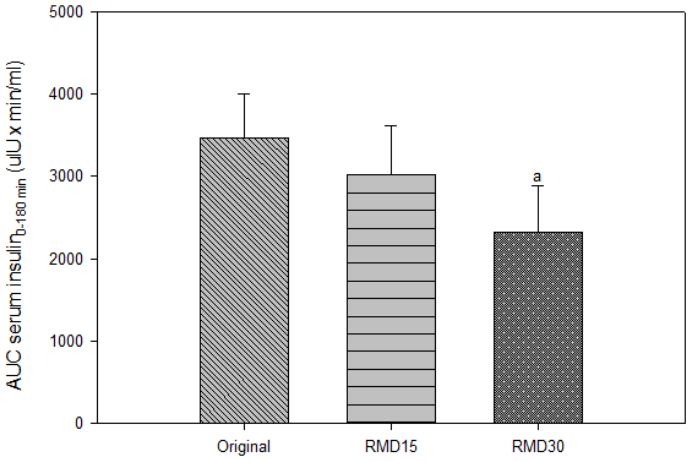
The peak of serum insulin was reached at 30 min following ONS consumption for all formula. The highest peak was reached following original formula with serum insulin of 61.30±12.14 µIU/ml. Meanwhile, 20.8% and 30.3% reduction of peak serum insulin were observed following RMD15 and RMD30 formula as peak of serum insulin were 48.53±9.41 µIU/ml and 42.74±10.24 µIU/ml, respectively. The peak of incremental serum insulin of original was 56.73±11.92 µIU/ml, while replacement of 15% and 30% of tapioca maltodextrin by TRM decreased the peak of incremental serum insulin by 22% and 32.4% to 44.22±9.14 µIU/ml and 38.35±10.23 µIU/ml, respectively.

The AUC $insulin_{0\text{-}180min}$ of original formula was 3,470.12±531.86 µIU x min/ml. Meanwhile, RMD15 and RMD30 significantly decreased the AUC $insulin_{0\text{-}180min}$ by 13.0% and 33.1% to 3,020.10±600.17 µIU x min/ml and 2,320.71±570.76 µIU x

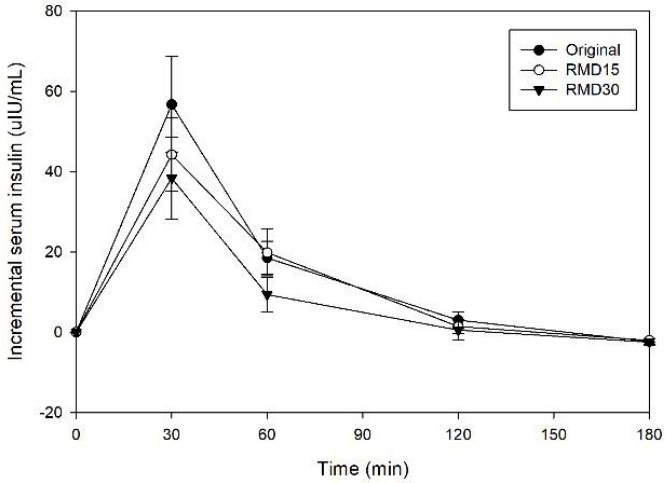
min/ml (p=0.039). The iAUC insulin$_{0\text{-}180min}$ of original formula was 2,825.64±468.24 µIU/ml. Meanwhile, RMD15 and RMD30 significantly decreased the AUC insulin$_{0\text{-}180min}$ by 15.6% and 35.3% to 2,386.05±520.68 µIU/ml and 1,827.31±528.49 µIU/ml.



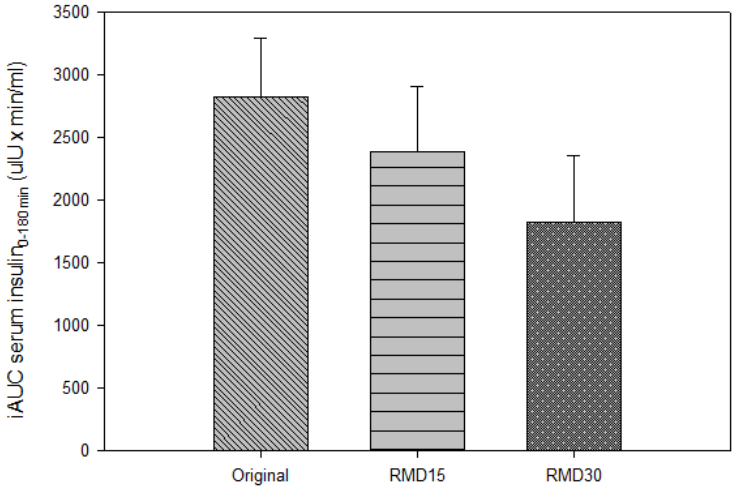

(a)




(b)




(c)

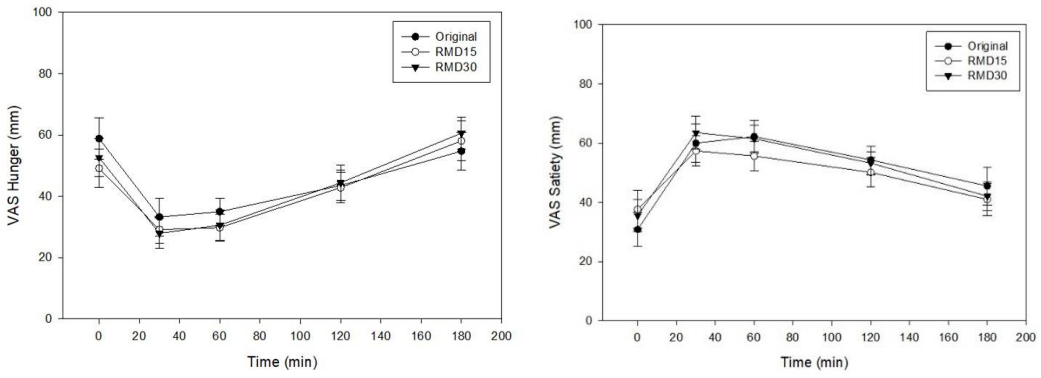



(d)

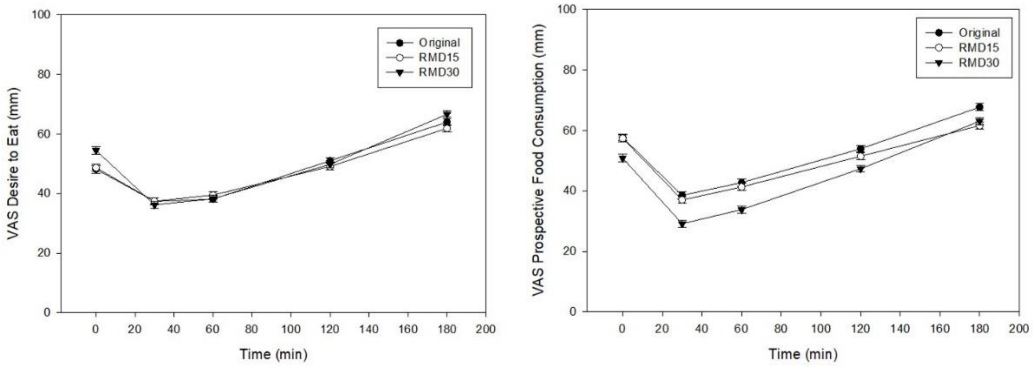
Figure 17. Postprandial serum insulin response (a), area under the curve (AUC) of serum insulin over 180 min (b), incremental serum insulin (c), and incremental AUC of serum insulin over 180 min of developed ONS in healthy subjects. Data are presented as mean±sem. [a] indicates significant different from original ($p<0.05$).

### 4.3.4 Subjective appetite

The hunger ratings for the first 60 min following original formula were slightly higher compared to RMD15 and RMD30, however, not significant differences were observed throughout 180 min of study ($p>0.05$). The satiety rating increased at 30 min following ONS consumption, despite of the formula given. There were no significant differences of satiety rating between ONS formula during 180 min ($p>0.05$). Similarly, the replacement of tapioca maltodextrin using TRM by 15% and 30% did not significantly affect to desire to eat of the participants throughout the study for 180 min ($p>0.05$). Furthermore, the prospective food consumption of RMD30 was slightly lower at the first 60 min compared to original and RMD15 formula, however, there was no significant differences were observed during the study period ($p>0.05$).




(a) (b)




(c) (d)

Figure 18. Subjective appetite was measured on visual analogue scale, including hunger (a), satiety (b), desire to eat (c), and prospective food consumption over 180 min of developed ONS in healthy participants. Data are presented as mean±sem.

### 4.3.5 Sensory evaluation

Seventeen participants were recruited in acute ONS study evaluation. They were asked to rate the acceptability of the ONS using 9-point hedonic scale. The result shows that the acceptability score between formula were not much different, except for viscosity. The viscosity rating of RMD30 formula significantly rated higher when compared to original formula (8.00±0.20 vs 6.54±0.41 for RMD30 and original, respectively, $p$=0.006).

Table 20. Sensory evaluation of developed ONS

| Formula | Appearance | Taste | Smell | Viscosity | Aftertaste | Overall |
|---|---|---|---|---|---|---|
| Original | 7.40±0.20 | 7.8±0.32 | 7.54±0.31 | 6.54±0.41 | 6.94±0.36 | 7.6±0.23 |
| RMD15 | 7.32±0.29 | 7.94±0.20 | 7.82±0.17 | 7.32±0.29 | 7.63±0.16 | 7.69±0.15 |
| RMD30 | 7.60±0.28 | 8.34±0.19 | 8.00±0.20 | 8.00±0.20[a] | 7.67±0.28 | 7.87±0.15 |

Data are presented as mean±sem. [a] indicates significant different from original (p= 0.006)

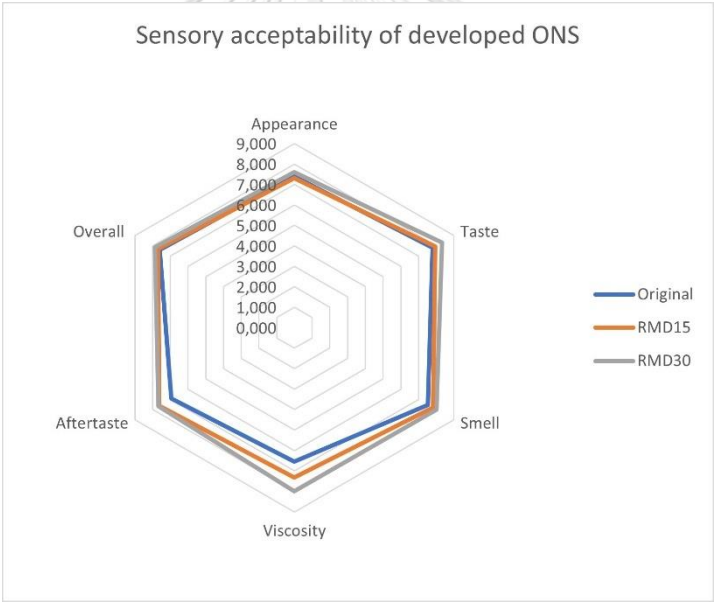



Figure 19. Sensory acceptability of developed ONS

### 4.3.6 Gastrointestinal tolerability

There were no significant differences of gastrointestinal symptoms, including abdominal pain, nausea, vomiting, bloating, and flatulence when compared before

and after ONS consumption (p>0.05). In addition, the GI symptoms were not significantly different between ONS formula (p>0.05). Most of the participants have normal stool form as indicated by Bristol Stool Scale (original 4.47±0.14, RMD15 3.82±0.15, and RMD30 3.94±0.23, respectively).

Table 21. Gastrointestinal tolerability of developed ONS in healthy participants

| Formula | Abdominal pain | Nausea | Vomiting | Bloating | Flatulence | Total score |
|---|---|---|---|---|---|---|
| Original | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0,06±0,06 | 0,18±0,1 | 0,24±0,14 |
| RMD15 | 0.00±0.00 | 0.00±0.00 | 0,06±0,06 | 0.00±0.00 | 0,06±0,06 | 0,12±0,09 |
| RMD30 | 0,06±0,06 | 0.00±0.00 | 0,06±0,06 | 0,06±0,06 | 0,18±0,1 | 0,30±0,19 |

Data are presented as mean±sem.

### 4.4 Phase IV: Long-term evaluation of developed ONS on body composition, food intake, and GI tolerability in healthy and prediabetic participants.



#### 4.4.1 Baseline characteristics

In total, there were 26 participants joined in this study. Most of participants in long-term study were female (57.7%) aged 31.53±1.74 years old. Of 26 participants, 61.5% (n= 16) were normoglycemic with fasting plasma glucose of 87.20±1.58 mg/dl, while 38.4% (n=10) were prediabetic with fasting plasma glucose of 99.60±2.27 mg/dl (Table 22). The fasting plasma glucose and HbA1C of prediabetic participants were significantly higher compared to normoglycemic participants (p<0.001), while the

insulin level was not different between normoglycemic and prediabetic participants (8.09±1.08 μIU/ml and 12.22±2.53 μIU/ml, respectively p>0.05). Interestingly, 84% of participants had LDL-cholesterol above the optimal level with mean value of 121.6±4.56 mg/dl. Furthermore, the LDL-cholesterol tended to be higher in prediabetic compared to normoglycemic participants (130.90±6.27 mg/dl and 115.40±5.98 mg/dl, respectively p<0.10).

Table 22. Baseline characteristic of participants in study phase IV

| Parameter | Prediabetic (n = 10) | Normoglycemic (n = 16) | All (n = 26) | p-value |
|---|---|---|---|---|
| Age (years) | 35.90±3.90 | 28.33±1.12 | 31.54±1.74 | 0.038 |
| Fasting glucose (mg/dl) | 99.6±2.27 | 87.2±1.58 | 92.16±1.78 | <0.001 |
| HbA1C (%) | 5.81±0.06 | 5.31±0.05 | 5,51±0,06 | <0.001 |
| Insulin (μIU/ml) | 12.22±2.53 | 8.09±1.08 | 9,74±1,24 | 0.104 |
| Total cholesterol (mg/dl) | 197.2±6.91 | 187.47±6.58 | 191.36±4.82 | 0.333 |
| HDL (mg/dl) | 45.7±3.18 | 50.27±3.5 | 48.44±2.45 | 0.373 |
| LDL (mg/dl) | 130.9±6.27 | 115.4±5.98 | 121.6±4.56 | 0.097 |
| TG (mg/dl) | 103.2±13.76 | 108.47±15.45 | 106.36±10.59 | 0.813 |
| AST (U/L) | 22.9±2.86 | 19.27±2.04 | 20.72±1.68 | 0.299 |

| | | | | |
|---|---|---|---|---|
| ALT (U/L) | 31.5±5.03 | 21.47±4.08 | 25.48±3.26 | 0.135 |
| BUN (mg/dl) | 10.7±0.45 | 10.27±0.71 | 10.44±0.46 | 0.653 |
| Creatinine (mg/dl) | 0.79±0.04 | 0.8±0.05 | 0,8±0,03 | 0.839 |
| GFR (ml/min) | 109.7±4.42 | 111±3.21 | 110.48±2.56 | 0.809 |

Data are presented as mean±sem.

### 4.4.2 Effect of developed ONS supplementation on body weight, BMI and body composition

The mean body weight and BMI of participants at baseline were 66.56±3.66 kg and 24.84±1.01 kg/m$^2$ (Table 23). There were no significant differences on body weight and body composition between normoglycemic and prediabetic participants ($p>0.05$). Following 3 weeks of ONS supplementation, mean body weight insignificantly increased by 0.6% (66.56±3.66 kg to 66.99±3.69 kg, $p=0.069$) in all participants. Fat mass and muscle mass also insignificantly increased from 19.44±2.02 and 44.55±2.53 kg to 19.71±2.03 and 44.69±2.51 kg (p-value were 0.220 and 0.233, respectively).



Table 23. Body weight, BMI and body composition of participants at baseline (week 0) and week 3 (n=18)

| **Parameter** | **Week 0** | **Week 3** | **p-value** |
|---|---|---|---|
| Weight (kg) | 66.56±3.66 | 66.99±3.69 | 0.069 |
| BMI (kg/m$^2$) | 24.84±1.01 | 25.02±1.01 | 0.055 |

| Parameter | Week 0 | Week 3 | p-value |
|---|---|---|---|
| Fat (%) | 28.6±2.12 | 28.83±2.1 | 0.328 |
| Fat mass (kg) | 19.44±2.02 | 19.71±2.03 | 0.220 |
| FFM (kg) | 47.12±2.65 | 47.28±2.63 | 0.214 |
| Muscle mass (kg) | 44.55±2.53 | 44.69±2.51 | 0.233 |
| Bone mass (kg) | 2.57±0.12 | 2.58±0.13 | 0.180 |
| BMR (kcal) | 1392.56±69.14 | 1397.5±68.89 | 0.144 |

Data are presented as mean±sem.

### 4.4.3 Effect of developed ONS supplementation on daily food intake and physical activity

Table 24 shows that the mean of daily energy intake insignificantly increased by 7% from 1,144.87±87.1 to 1,225.77±94.16 kcal (p=0.305). Accordingly, the fat and protein intake were insignificantly higher at week 3 from 39.47±3.72 and 46.94±4.09 g to 45.98±4.74 and 53.58±6.08. It should be noted that the amount of food intake at week 3 did not include the energy and nutrient contribution from the ONS. However, the energy intake significantly increased when ONS included in the total energy intake compared to baseline (1,477.76±94.16 vs 1,144.87±87.1 kcal, p= 0.001).

Table 24. Energy and nutrient intake from food intake at baseline (week 0) and week 3 (n =18)

| Nutrients | Week 0 | Week 3 | p-value |
|---|---|---|---|

| Nutrients | Week 0 | Week 3 | p-value |
|---|---|---|---|
| Energy (kcal) | 1144.87±87.1 | 1225.77±94.16 | 0.305 |
| Carbohydrate (g) | 150.15±13.69 | 152.74±13.64 | 0.836 |
| Fat (g) | 39.47±3.72 | 45.98±4.74 | 0.120 |
| Protein (g) | 46.94±4.09 | 53.58±6.08 | 0.224 |

Data are presented as mean±sem.

Physical activity of participants is shown on table 25. Total physical activity of participants at baseline tends to be higher than at week 3 (1,582.35±368.63 vs 1,211.76±271.74 MET-min/week, p=0.081). The decreased of physical activity was contributed by all domains, particularly recreation domain. The recreation domain decreased by 31.9% from 1,025.88±402.05 to 698.82±279.98 MET-min/week.

Table 25. Physical activity of participants at baseline and 3 weeks (n = 17)

| Physical activity domain (MET-min/week) | Week 0 | Week 3 | p-value |
|---|---|---|---|
| Work | 131.76±67.98 | 145.88±76.23 | 0.461 |
| Travel | 424.71±108.54 | 367.06±125.01 | 0.484 |
| Recreation | 1,025.88±402.05 | 698.82±279.98 | 0.161 |
| Total physical activity | 1,582.35±368.63 | 1,211.76±271.74 | 0.081 |

Data are presented as mean±sem.

### 4.4.4 Effect of developed ONS supplementation on GI tolerability

There were no significant differences on GI tolerability between baseline and week 3. The score of nausea decreased at week 3 as number of participants experiencing nausea decreased from 2 (at baseline) to 1 (at week 3). The score of bloating increased at week 3 as number of participants experiencing bloating increased from 3 (at baseline) to 5 (at week 3). Meanwhile, the number of participants experiencing flatulence decreased at week 3 as number of participants experienced nausea decreased from 5 participants with mild-moderate intensity (at baseline) to 4 participants with mild intensity (at week 3). Most of participants have normal stool form as indicated by type 3-4 on Bristol Stool Scale.

Table 26. Gastrointestinal symptoms at baseline and week 3 (n = 18)

| Parameter | Week 0 | Week 3 | p-value |
|---|---|---|---|
| Abdominal pain | 0.12±0.08 | 0.12±0.08 | 1.000 |
| Nausea | 0.12±0.08 | 0.06±0.06 | 0.317 |
| Vomiting | 0.00±0.00 | 0.00±0.00 | 1.000 |
| Bloating | 0.18±0.1 | 0.29±0.11 | 0.157 |
| Flatulence | 0.41±0.17 | 0.24±0.11 | 0.257 |
| Number of defecation/day | 1.32±0.13 | 1.15±0.1 | 0.083 |
| Bristol Stool Scale | 3.88±0.28 | 3.94±0.28 | 0.854 |

Data are presented as mean±sem.

# CHAPTER V

# DISCUSSION

## 5.1 Phase I: Effect of TRM on postprandial glucose and insulin response in healthy participants

### 5.1.1 The effect of TRM on postprandial plasma glucose and serum insulin in healthy participants

In the preliminary study, we found that 50 g TRM significantly decreased the peak of postprandial glucose and insulin response in healthy participants when compared to TM and GL. The TRM lowered peak of plasma glucose by 23 and 18% compared to GL and TM respectively, while peak of serum insulin was decreased by 73% compared to GL and 75% compared to TM. In addition, the replacement of MIX15 and MIX50 slightly, but not significant, decreased the postprandial insulin response when compared to TM and GL. The TRM contains 1,2 and 1,3 alpha- and beta-glycosidic linkage that makes TRM partly indigestible by human carbohydrate digestive enzymes (Toraya-Avilés et al., 2016).



Pyroconversion process allows the molecular rearrangement of the starch leading to a decline in the proportion of α-1,4 glycosidic linkage and increase the proportion of α-1,2 and 1,3 glycosidic linkage thus decrease the susceptibility to hydrolyzed by carbohydrate digestive enzymes (Toraya-Avilés et al., 2016). In addition, molecular rearrangement during dextrinization process allow RMD acts as

α-glucosidase inhibitor (Uenaka, Yagi, Takabe, & Yonei, 2020; Wolf et al., 2001). The TRM used in this study contains 84.8-89.7% of dietary fiber. This is consistent with previous study that reported that total dietary fiber content of TRM was 86.62% which represents the sum of insoluble dietary fiber, high molecular weight dietary fiber, and low molecular weight dietary fiber (Toraya-Avilés et al., 2017). The high content of dietary fiber makes TRM classified as medium glycemic index food with GI value of 59 (Toraya-Avilés et al., 2017).

Several studies reported the effect of cornstarch and potato starch RMD on postprandial plasma glucose (Livesey & Tagami, 2008; Wakabayashi et al., 1999; Wolf et al., 2001). Wakabayashi et al., reported the reduction of peak postprandial plasma glucose following addition of 5-10 g of potato starch RMD in test meal challenge (Wakabayashi et al., 1999). Similarly, Livesey and Tagami found that 6 g of RMD decreased the glucose response by 10% and 20% when added to solid food and drink, respectively (Livesey & Tagami, 2008). In contrast, Wolf et al., did not observe a significant reduction on postprandial glucose response following 16 g RMD from cornstarch consumption when compared to control (Wolf et al., 2001). It was hypothesized that different source of starch had different chemical differences following dextrinization, therefore, resulting in different glucose response (Wolf et al., 2001). Different starch may have different morphology, pores and channels on the surface. The proportion of amylose and amylopectin, and the supramolecular

structure, such as crystallinity and packing in cell may also affect the starch digestibility (Toutounji et al., 2019).

The iAUC $_{glucose0\text{-}180\ min}$ is correlated with HbA1C and represents glucose excursions (Schernthaner et al., 2010). The iAUC glucose over 180 min following TRM consumption was significantly lower compared to GL and TM showing that TRM may help to control the blood glucose response better than GL or TM during 3 h of study period. The iAUC of TRM was 67.6% and 65.6% lower than GL and TM, respectively. In consistent with this study, Nazare et al., reported 13.5% reduction of AUC glucose$_{0\text{-}150\ min}$ following consumption of breakfast containing cornstarch RMD (50 g) than breakfast containing maltodextrin (50 g) (Nazare et al., 2011). Similarly, 25 g of cornstarch RMD decreased the AUC glucose response over 3 h by 12% in healthy participants following low fiber meal consumption (Klosterbuer et al., 2012). Previous study showed that long term consumption of low glycemic index food has beneficial impact on HbA1C management (Wang, Xia, Zhao, & Zhang, 2015).



In response to alteration of plasma glucose, the peak of postprandial serum insulin was also lower following TRM consumption compared to GL, TM, and MIX50%. The TRM contains less amount of readily digestible starch compared to TM and GL, therefore, it requires less insulin to facilitate the glucose uptake into cells. However, further mechanism of TRM in reducing plasma glucose and insulin response were not investigated in this study. Previous study revealed that 48 g of resistant

starch decreased the postprandial insulin response without influencing the C-peptide level (Bodinham, Frost, & Robertson, 2010). It indicated that resistant starch did not decrease the insulin secretion from β-cell pancreas, but it increased the hepatic insulin clearance thus lower the postprandial insulin response (Bodinham et al., 2010). The effect of TRM on lowering the postprandial insulin response would be beneficial to improve the insulin resistant which predominant in type 2 diabetic participants. However, the use of TRM in long term study is remain unclear. Previous study on cornstarch RMD showed that supplementation of 27 g RMD daily significantly improved the risk factor of insulin resistant (Hashizume et al., 2012).

**5.1.2 The effect of TRM on subjective appetite**

Tapioca RMD in our preliminary study did not pose any significant effect on subjective appetite, including hunger, satiety, desire to eat, and prospective food consumption, over 180 min of the study. Similarly, Emilien et al., did not find any significant difference of cornstarch RMD (0, 20, 40 g) on subjective appetite over 150 min in healthy adults (Emilien, Zhu, Hsu, Williamson, & Hollis, 2018). In agreement with our present finding and Emilien's study, Klosterbuer et al., also failed to show significant effect of muffin containing 25 g of fiber from cornstarch RMD on satiety over 180 min compared to control group (Klosterbuer et al., 2012). In addition, Monsivais et al., did not found any significant effect of 12 g fiber from cornstarch RMD on appetite during 220 min of the study, but it significantly decreased the food

intake compared to isoenergetic control (Monsivais, Carter, Christiansen, Perrigue, & Drewnowski, 2011). In contrast, 10 g of cornstarch RMD decreased subjective hunger and increased satiety at 90-120 min following consumption. This effect was not observed in low dose RMD (0 or 5 g) (Ye et al., 2015).

However, several studies in a long-term period of RMD supplementation showed significant effect on appetite and incretin hormone release (Guérin-Deremaux, Li, et al., 2011; Guérin-Deremaux, Pochat, et al., 2011; Guérin-Deremaux et al., 2013). Previously, supplementation of wheat RMD (8-24 g) increase the satiety in time- and dose-dependent manner in healthy adult (Guérin-Deremaux, Pochat, et al., 2011). Furthermore, Guerin-Deremaux et al., also reported that consumption of 17 g wheat RMD twice daily for 12 weeks significantly decreased hunger, food intake, and body weight in overweight men (Guérin-Deremaux, Li, et al., 2011). Several types of dietary fiber were known to improve the satiety through increasing luminal viscosity, increasing the mastication, and modulating the release of SCFAs (Joanne L Slavin, 2005). However, RMD is not viscous and well soluble in water, thus it is less likely to affect the luminal viscosity and mastication. The effect of RMD on increasing satiety was mediated by the increasing of satiety hormones, including PYY and GLP-1 (Bosch-Sierra et al., 2019; Ye et al., 2015). However, the effect of TRM on SCFAs production and satiety hormones release were not clarified in present study. Further research to clarify this effect is necessary.

Resistant maltodextrin is fermentable by several types of gut microbiota thus increasing SCFA production, including acetate, butyrate, and propionate which further stimulate the release of PYY and GLP-1 from intestinal L-cells (Hira et al., 2015; Ye et al., 2015). In addition cornstarch RMD was found to directly increase GLP-1 release from intestinal L-cells due to branching-structure of RMD that is recognized by the cells (Hira et al., 2015). The type of dietary fiber, dose, and duration of supplementation may affect to the satiety response in human (Bosch-Sierra et al., 2019). Every type of dietary fiber may have different optimal dose to induce the satiety in human. The variability in fiber composition may influence the effective dose of fiber to promote satiety (Clark & Slavin, 2013).

**5.1.3 The effect of TRM on gastrointestinal tolerability**

Consumption of a high dose TRM (50 g) significantly increased the flatulence in healthy participants. However, the symptom was mild to moderate in TRM group. In this study, none of participants experienced diarrhea following all test drink. Previous study on cornstarch RMD (Fibersol-2) found the maximum dose that was tolerated and did not cause diarrhea was 1.0 g/kg body weight in men, however it caused stomach rumbling and flatulence (Kishimoto et al., 2013). Another cornstarch RMD (Promitor) was well tolerated up to 40 g for acute single dose consumption and up to 65 g per day which divided by several doses (Housez et al., 2012). Similarly, it was reported that supplementation of 30-45 g/d of wheat RMD (Nutriose) was well

tolerated and did not cause diarrhea. However, several participants reported stomach rumbling following 45 g of wheat RMD supplementation as the GI rumbling score increased from 0.6±0.7 times a day before the treatment to 1.1±1.3 times a day at day 21 following the supplementation (Pasman et al., 2006).

Consumption of fiber, particularly fermentable fiber, may cause some gastrointestinal discomfort, including abdominal pain, bloating/ rumbling, flatulence, or even diarrhea due to the fermentation of RMD in colon that produces gases and increased of osmotic load (Eswaran et al., 2013). Different characteristic of resistant starch may have different tolerability dose. The tolerability of resistant starch is affected by its molecular weight. The lower molecular weight increases the risk of osmotic diarrhea (Kishimoto et al., 2013). Tapioca RMD used in current study has molecular weight of 2,895 Da. Meanwhile, cornstarch RMD has molecular weight of 1,600-2,000 Da (Housez et al., 2012; Kendall et al., 2008; Kishimoto et al., 2013). Resistant maltodextrin from wheat has molecular weight of 2,480 Da (Pasman et al., 2006). Therefore, the tapioca RMD is well tolerable and may induce less osmotic diarrhea. The tolerability of RMD is higher than other fermentable fiber, such as inulin. Inulin was reported to be tolerated up to 10 g/d in native inulin form or 5 g/d in oligosaccharide form (Bonnema, Kolberg, Thomas, & Slavin, 2010).

There are several other factors that contribute to the tolerability of RMD in healthy participants, including the dose, form of meal (liquid/solid), duration,

fermentability, and chain length of the dietary fiber (Bonnema et al., 2010). Higher dose of RMD may increase the risk of gastrointestinal discomfort (Kishimoto et al., 2013). Consumption of inulin incorporated in solid meal was found to be more tolerable than in liquid or beverage (Bonnema et al., 2010). Furthermore, habituation of fiber consumption by gradually increase the RMD was thought to improve the tolerability (Bonnema et al., 2010; Pasman et al., 2006). In addition, the tolerability is also improved when the high dose is divided into smaller dose throughout the day rather than giving high dose in single consumption (Housez et al., 2012).

**5.2 Phase II: Utilization of TRM in the development of ONS**

**5.2.1 Effect of different dextrose equivalent (DE) of tapioca maltodextrin in ONS development**

The results demonstrated that incorporation of TM-DE7 increased viscosity of ONS. This may be due to the higher percentage of polysaccharides with lower degree of polymerization (DP) in TM-DE19. In addition, TM-DE19 has a shorter chain of saccharides and narrower saccharides fraction distribution. The intrinsic viscosity of TM-DE19 was therefore lower (Dokic, Jakovljevic, & Dokic, 2004). These ranges of viscosity in both formulas were aligned with most commercial liquid ONS available in the market. They can be also classified as thin liquid (less than 50 cP) according to National Dysphagia Diet Task Force (National Dysphagia Diet Task Force, 2002). The current study demonstrated that both TM-DE7 and TM-DE19 could be used as a

carbohydrate source of ONS. However, the pH of both the developed formulas was also similar.

In addition, ONS formula with TM DE19 was lighter in color and more yellowish compared to the formula with TM DE7. A previous study demonstrated that reducing sugar in carbohydrate may interact with amino groups in protein. This interaction may change the color of the formula. Tapioca maltodextrin DE-19 contains more reducing sugar, thus producing more interaction with amino group in whey protein and forming a yellowish color. Moreover, this color formation can be accelerated by the heating process during emulsion preparation (Kearsley & Dziedzic, 1995). The color of maltodextrin can also be influenced by the hydrolysis conditions, such as acid, heat, and time. The darker color of maltodextrin was resulted from higher acidity, longer processing time, and higher temperature used in the production. The darker color formation during hydrolysis of maltodextrin is associated with caramelization reaction. However, in commercial production of maltodextrin, decolorization will be done in the final step to create white color (Trithavisup, Krusong, & Tananuwong, 2019).



The pH of developed nutritionally complete ONS utilizing TM-DE7 and TM-DE19 as a carbohydrate source were similar. The level of pH in ONS determines the solubility of protein, as well as the interaction with other substances, such as medication. Most of ONS was developed at pH 6.8, which is close to neutral pH,

while lower pH may decrease protein solubility (Henriques, Miranda, Generoso, Guedes, & Jansen, 2017; Klang, McLymont, & Ng, 2013).

Oral nutritional supplements (ONS) are frequently prescribed for patients at risk of malnutrition. Palatability is thus an important factor to maintain long-term compliance. The semi-trained panellist in the current study reported similar sensory evaluation including appearance, taste, smell, and aftertaste of formulas with TM-DE7 and TM-DE19. Overall, the acceptability of those two formulas was also similar.

**5.2.2 Physical properties of developed ONS**

There were three formulas developed in this study which varied in the proportion of TRM and tapioca maltodextrin, including original, RMD15, and RMD30. The amount of tapioca maltodextrin in original, RMD15, and RMD30 were 18 g, 15.3 g, and 12.6 g, while the amount of TRM in original, RMD15, and RMD30 were 0 g, 2.7 g, and 5.4 g, respectively. The ONS were developed using dry blending method due to less energy required and its flexibility to adjust the formulation (FAO/WHO Expert Meeting, 2004). The developed formulas were similar in terms of energy and nutrient content. They contain 251.8 kcal, 9.85 g protein, 9.05 g fat, 32.7 g carbohydrate, vitamins, and minerals 25-30% of Thai RDI.

The replacement of tapioca maltodextrin by TRM in the ONS affected the viscosity and water activity of the ONS. The viscosity of the ONS slightly decreased

following the replacement of tapioca maltodextrin using TRM. This possibly due to the difference of viscosity between TRM and tapioca maltodextrin. In current study, the viscosity of 10% TRM (w/w) was 1.43 cP. This is similar to previous study which reported that cornstarch RMD had very low viscosity (less than 15 cP in concentration 10% (w/w)) (Nishitani, Sato, Fukuyama, & Sasaki, 2018). The viscosity of ONS depends on the molecular weight of maltodextrin which increased as the molecular weight increased. The longer chain of maltodextrin increases resistance to flow (Avaltroni, Bouquerand, & Normand, 2004; Castro, Durrieu, Raynaud, & Rouilly, 2016). The molecular weight of TRM and tapioca maltodextrin used in this study were 2,895 Da and 3,254 Da, respectively. Since TRM has lower molecular weight compared to TM, thus the viscosity of TRM is lower than TM. As a result, the higher proportion of TRM in RMD15 and RMD30 formula resulting the less viscous ONS compared to original formula. However, all formulas developed in this ONS are categorized as thin liquid since the viscosity were less than 50 cP (National Dysphagia Diet Task Force, 2002).



In addition, the higher proportion of TRM in ONS increased the water activity of the powder. Resistant maltodextrin is highly hygroscopic material due to its hydrophilic groups and shorter chain length (Pai, Vangala, Ng, Ng, & Tan, 2015). Similarly, Shaaruddin et al., reported that the hygroscopicity of RMD is higher than those in maltodextrin (Shaaruddin, Ghazali, Hamed Mirhosseini, & Muhammad, 2017).

The water activity has been known to influence the stability of vitamins in ONS, such as thiamin, vitamin E, and vitamin A (Frias, Peñas, & Vidal-Valverde, 2009). Thiamin degradability increased with the increased water activity from 0.44 to 0.65 (Barbosa-Cï, Fontana Jr, Schmidt, & Labuza, 2007). Therefore, it is important to consider the water activity as one of the factor to determine the shelf-life of the ONS (Frias et al., 2009).

The moisture content of the ONS were not significantly different among these three formulas. The moisture content in this study was approximately 4% in all formulas. This finding is similar to Baez et al., that reported the initial moisture of casein-based ONS was 4.02% (Baéz et al., 2012). It is important to keep the moisture to be as low as possible to maintain the physical and chemical stability of the ONS. It was recommended to keep the moisture content of ONS between 4-5% to prevent from the lipid oxidation (Bal et al., 2009).



**5.2.3 Microbial evaluation of developed ONS**

Microbiological evaluation is an important factor in medical food production. It ensures the stability and safety of the product. The developed ONS in current study meets the criteria for total plate count (TPC) bacteria evaluation. The maximum value for TPC in medical food is 1,000 cfu/g (U.S. Food and Drug Administration, 2008). Controlling the hygiene and sanitation of ingredients, manufacturing, storing,

and preparing the ONS are critical to ensure the safety from pathogenic bacteria (Baniardalan, Sabzghabaee, Jalali, & Badri, 2014).

**5.2.4 Stability evaluation**

Shelf life study is necessary to ensure the safety of the consumer, to maintain the physicochemical characteristic of the food, and to follow the regulation of food labeling (Phimolsiripol & Suppakul, 2016). Accelerated shelf-life testing was conducted in this study due to its fast and inexpensive. The stability evaluation was done in RMD30 formula since it was the formula chosen for the long-term study. The stability study reveals that the RMD30 was shelf-stable for approximately 1 year when kept in room temperature (25-30°C). The shelf-life of ONS depends on the water activity, pH, ingredients, packaging, storage temperature and relative humidity (RH), and also the initial microbial counts (Phimolsiripol & Suppakul, 2016).

The water activity of ONS increased with the increased of storage period and temperature. Similarly, Syamaladewi also reported that water activity of all-purpose flour and peanut butter elevated with the elevated temperature (Syamaladevi et al., 2016). The increased of temperature leads to damage the bond between hydrophilic molecules and water thus increase the free water availability and water activity (Syamaladevi et al., 2016). As the water activity increased, the probability of microorganism growth is going to increase as well, such as bacteria at Aw 0.91 and

fungi at Aw 0.6 (Allen Jr, 2018). Therefore, the cut off value of water activity to determine the shelf-life of ONS in this study was 0.6.

In addition, the increased storage temperature and period may affect to physical properties of ONS, such as color. The color of ONS changed to be more brownish when stored at higher temperature at the longer storage period. Similar finding was reported by Jia et al., that showed infant formula stored at 42°C and 50°C significantly increased in a* (redness) and b* (yellowness) value, while decreased in L* (lightness) value which might occur due to the oxidation during the storage in high temperature (Jia, Chen, Qi, & Su, 2019). Rufian-Henares et al., also reported that the yellowness (b*) and yellowing index (YI) of enteral formula increased with the increasing heating temperature and time which could be mediated by the Maillard's reaction that occur in high temperature (Rufian-Henares, Garcia-Villanova, & Guerra-Hernandez, 2001). Maillard's reaction occurs between carbonyl group of reducing sugar and amino group from protein during heating resulting Maillard's Reaction Products (MRPs) that produces brownish color, called melanoidin. In addition, some MRPs acts as natural antioxidant, such as melanin-like reductant and volatile-nitrogen containing heterocyclic compound. However, Maillard's reaction also produces harmful substances including carboxymethyl lysine (CML) and acrylamide which is associated with increased risk of cardiovascular

disease, diabetes mellitus, and cancers (Liu et al., 2020). However, the MRPs in developed ONS were not evaluated in our study.

### 5.3 Phase III: Acute effect evaluation of developed ONS on postprandial glycemic response, acceptability, and GI tolerability

#### 5.3.1 The effect of developed ONS on postprandial glucose and insulin response

In this study, a replacement of tapioca maltodextrin by TRM by 15% (2.7 g TRM) and 30% (5.4 g TRM) in ONS did not significantly reduce the postprandial blood glucose response. Similarly, a previous study reported that blood glucose response was not significantly different following consumption of bread containing 6 g of resistant starch (test bread) and placebo bread in healthy subjects (Y. Yamada et al., 2005). However, when it was stratified based on the baseline blood glucose, participants with impaired fasting glucose (fasting plasma glucose 100-125 mg/dl) experienced a significantly decreased peak of postprandial plasma glucose following test bread when compared to placebo bread. Meanwhile, no significant difference of blood glucose between bread consumptions were observed for normal fasting blood glucose participants (Y. Yamada et al., 2005). This is consistent with the finding of Bhoite, which showed that nutritional drink containing 5.2 g of wheat fiber decreased the peak of postprandial plasma glucose in overweight adults with impaired fasting plasma glucose (Bhoite, 2020). In our study, we only recruited participants with

normal fasting blood glucose (<100 mg/dl). Healthy participants with normal blood glucose have tight control of postprandial blood glucose, while participants with impaired fasting blood glucose impaired control on blood glucose (Bock et al., 2006; Juntunen et al., 2003). It is believed that a tight glucose homeostasis in healthy participants, might be the reason of these insignificant changes.

In addition, Visek et al., reported that the glycemic index (GI) of ONS containing fiber (2.3 g/100 ml) was not different from ONS without fiber (Visek, Zourek, Lacigova, & Rusavy, 2007). The probability of high intraindividual variability within participants could affect the blood glucose response. It would be beneficial to have three rounds of repeated measures of the test sample and calculate the average to obtain the GI value. However, it takes a lot of time and expense to have repeated measurements of glycemic index in food (Visek et al., 2007).

In contrast, previous study reported that incorporating 5-8 g of RMD into foods and beverages decreased the glucose response by 10-20%, respectively (Livesey & Tagami, 2008). Previous study showed that resistant maltodextrin may decrease plasma glucose response due to the structure of RMD that contains α-1,2 and α-1,3 glycosidic linkage which cannot be digested by human digestive enzymes (Ohkuma & Wakabayashi, 2001). In addition, RMD is also fermentable in the colon by gut microbiota and promotes short-chain fatty acids (SCFAs) (Baer et al., 2014). In

addition, the increase in SCFA increases GLP-1 release and improves insulin release thus lowers the plasma glucose response (Canfora et al., 2015).

The effect of modified carbohydrate contained in ONS decreased the insulin response over 180 min in the current study. Similarly, Kishimoto et al., reported that cornstarch RMD (5 and 10 g) significantly decreased the postprandial insulin response without altering glucose response when compared to placebo following high fat-meal consumption (Kishimoto et al., 2007). This finding indicates that less insulin is needed to control the postprandial blood glucose. It remains unclear how RMD30 formula decreased the insulin response without altering the postprandial plasma glucose response in our study. Previous study showed that the difference of the starch structure might influence the insulin response for healthy participants (Juntunen et al., 2003). Resistant maltodextrin had lowered insulin response when compared to digestible starch since it is less susceptible for hydrolysis by amylolytic enzyme (Juntunen et al., 2003). Therefore, it is slowly digested in the small intestine and, thus, decreases the glucose-dependent insulinotropic polypeptide (GIP) level as well as the insulin response. The decrease of GIP was correlated with a slower glucose clearance rate (GCR) following resistant starch consumption, therefore the glucose response might not be significantly reduced, although insulin was decreased following resistant starch consumption (Eelderink et al., 2012). Foods with lower insulin response was beneficial to control long-day insulin response compared to foods with higher insulin response in similar glycemic response, therefore it is

potential to improve the beta cell function and insulin sensitivity (Bell, Bao, Petocz, Colagiuri, & Brand-Miller, 2015).

**5.3.2 The effect of developed ONS on subjective appetite**

Dietary fiber, including RMD, has been reported to prolong satiety in human (Guérin-Deremaux, Li, et al., 2011; Guérin-Deremaux, Pochat, et al., 2011; Guérin-Deremaux et al., 2013; Ye et al., 2015). The SCFAs resulted from fermentation of RMD stimulates the release of gut hormones that promotes satiety, such as glucagon-like peptide 1 (GLP-1) and peptide YY (PYY) (Ye et al., 2015). In contrast, this effect was not observed in this study as no significant difference of hunger, satiety, desire to eat, and prospective food consumption was found between ONS formulas. It was hypothesized that either the dose or duration in this study was inadequate to pronounce the significant effect. It takes about 6-8 hours for RMD to reach the colon and be fermented by gut microbiota following RMD consumption (Goda et al., 2006). In the current study, we only observed the subjective appetite for 180 min, therefore, the fermentation effect was not observed. In addition, satiety hormones were not evaluated in this study. Therefore, we could not confirm the clear mechanism of developed ONS on satiety in this study. Similarly, Emilien et al., never found any effect of 10-20 g RMD on subjective appetite over 150 min and there was no effect on food intake for the rest of the day following RMD consumption (Emilien et al., 2018). Meanwhile, Ye et al., reported the increase of PYY and GLP-1 in

accordance with the higher satiety response following 10 g, but not 5 g, consumption of RMD with a meal (Ye et al., 2015). A previous study showed that ONS containing pea fiber (10 g/L) and fructo-oligosaccharide (5 g/L) significantly increased satiety in patients (K. Whelan et al., 2006). However, ONS aims to increase the energy and nutrients consumption food intake in addition to normal diet, thus, the satiety effect of ONS should be minimal to maintain the food intake on the next meal (K. Whelan et al., 2006).

**5.3.3 Sensory evaluation**

Sensory evaluation was conducted in seventeen participants during the acute effect study. The result shows that the sensory acceptability of the developed ONS was not significantly different among formulas. The RMD30 formula was rated highest in sensory evaluation compared to original and RMD15 formula. This is possibly due to the lower viscosity of RMD30. Previous study reported that the viscosity of the beverage affected to the mouth coating and mouthfeel. The higher viscosity increases the mouthcoating, thickness, and stickiness thus may reduce the palatability (He, Hort, & Wolf, 2016). In addition, it was found that thin ONS was preferred over thick ONS (den Boer, Boesveldt, & Lawlor, 2019). All formulas in this study were moderately liked by the participants as the mean overall acceptability score were higher than 7.

### 5.3.4 The effect of developed ONS on gastrointestinal tolerability

Mild gastrointestinal symptoms, including abdominal pain, bloating, vomiting, and flatulence were experienced in 5.88%-17.65% of the participants. However, there was no significant difference of gastrointestinal symptoms among three ONS formulas. It indicated that composition of modified carbohydrate in ONS using TRM did not significantly affect the tolerability of ONS. Similarly, Mayr et al., showed that 14 days supplementation of 2.5 g/100 ml inulin and oat fiber containing ONS was well-tolerated in healthy participants. In addition, the stool form following all formula was categorized as the ideal stool form (Continence Foundation of Australia, 2020). The amounts of TRM in this formula (2.7-5.2 g) were much lower than the maximum tolerable dose of RMD that does not cause diarrhea (1.0 g/kg body weight for males and 1.1 g/kg body weight for females) (Kishimoto et al., 2013).



## 5.4 Phase IV: Long-term evaluation of developed ONS on body composition, food intake, and GI tolerability in healthy and prediabetic participants.

### 5.4.1 Effect of developed ONS supplementation on body composition

Oral nutritional supplement has been known to increase the body weight in acutely ill or older patients (Rebecca J. Stratton & Elia, 2007). However, three weeks supplementation of developed ONS did not significantly increase the body weight of participants. Previous study have shown that the increment of weight gain following

ONS supplementation depends on the amount of ONS given and duration of supplementation (Rebecca J. Stratton, 2000). In current study, we supplemented 252 kcal/day, which may be insufficient to increase the body weight significantly in participants. Moreover, the compliance in our study reached 94.44% (76.19-100%). Recently, Ellis et al., reported that supplementation with 180 kcal/d of ONS for 24 weeks insignificantly increased body weight in healthy elderly (p=0.063) (Ellis, Hunter, Goss, & Gower, 2019). In addition, the effect of ONS on body weight gain would be greater when supplemented more than 400 kcal/day (Rebecca J. Stratton & Elia, 2007). The research study of Lauque et al., also found that 300-500 kcal/day significantly increased body weight by 1.90 and 1.57 kg following 3 and 6 months supplementation in patients with Alzheimer's Disease (Lauque, Arnaud-Battandier, et al., 2004).

In addition, the study duration in current study was relatively short to see the changes in body weight. The short duration of supplementation (3 weeks) due to the COVID-19 pandemic situation limit the mobility of the people. Previous study showed that the increment of body weight increased as the increasing duration of daily-432 kcal ONS supplementation in malnourished patients (Huynh et al., 2015).

**5.4.2 Effect of developed ONS supplementation on food intake and physical activity**

Supplementation of developed ONS (252 kcal/day) did not significantly suppress the food intake as indicated by small increase of daily energy, fat, and protein intake by the participants at week 3 compared to baseline. Participants were instructed to consume the ONS between meals, therefore, it did not significantly affect the food intake. Similarly, Pouyssegur et al., reported that supplementation of ONS (244 kcal/day) did not affect to habitual food intake in elderly at nursing homes (Pouyssegur et al., 2015). It is important to consider the timing and composition of ONS thus it will not affect the habitual intake of participants.

Resistant maltodextrin from other sources, including cornstarch and wheat dextrin, suppressed the appetite in healthy and overweight adults (Guérin-Deremaux, Pochat, et al., 2011; Guérin-Deremaux et al., 2013). Fermentation of RMD by gut microbiota in the colon stimulates the production of SCFAs which then increases the release of GLP-1 and PYY by activation of GPR41/43 (Ye et al., 2015). However, the dose of TRM used in previous study was 7 times higher compared to dose used in our study (5.4 g/day) (Guérin-Deremaux, Li, et al., 2011).

Physical activity was evaluated to ensure that the participants maintain their habitual physical activity throughout the study, thus minimalize the effect of confounding factor. The total physical activity at week 3 decreased by 23.4%

compared to baseline, mainly on recreation domain. Recreation domain covers the activities in leisure time, such as sports and fitness. The recreation domain decreased by 31.9% within 3 weeks in this study due to Covid-19 pandemic situation. Strict confinement that limits people to do outdoor activities is necessary to control the spread of Covid-19. Several reports showed that physical activity decreased by 26.5-47.04% during covid-19 pandemic (Castañeda-Babarro, Arbillaga-Etxarri, Gutiérrez-Santamaría, & Coca, 2020; Martinez-Ferran, de la Guía-Galipienso, Sanchis-Gomar, & Pareja-Galeano, 2020; Srivastav, Sharma, & Samuel, 2021; M. Yamada et al., 2020). The reduction of physical activity may influence the physical and mental health (Duncan, Avery, Seto, & Tsang, 2020; Martinez-Ferran et al., 2020). Physical inactivity increases risk of insulin resistant, visceral obesity, inflammatory cytokines, while decreases muscle mass (Martinez-Ferran et al., 2020). Previous study reported that reduction of physical activity in just two weeks in healthy young adults decreased the peripheral insulin sensitivity due to impairment of insulin-stimulated muscle *Akt* phosphorylation (Krogh-Madsen et al., 2009).



**5.4.3 Effect of developed ONS supplementation on GI tolerability**

The developed ONS was well-tolerated by all (n=18) participants in this study. The amount of the TRM in the study did not significantly change the GI symptoms in participants. Similarly, Cuesta-Triana reported that eight-week supplementation of fiber-containing formula (1.5 g fiber/100 g ONS) was well-tolerated by the subjects

(Cuesta-Triana et al., 2017). In addition, the developed ONS supplementation also did not change the defecation frequency and stool form as evaluated by Bristol Stool Scale. Most of participants defecated 1-2 times per day and had type 3 and 4 of Bristol Stool Scale, which is classified as normal shape. Mayr et al., showed two weeks supplementation of fiber-containing ONS (2.5 g/100 ml) was well-tolerated as it softened the stool thus less constipation occurred following the supplementation (Mayr, Kalde, Vogt, & Kuhn, 2000).

## CONCLUSION

Tapioca RMD used in this study has been shown to decrease the postprandial glucose and insulin response in healthy participants. Replacement of tapioca maltodextrin by TRM slightly, but not significant, decreased the postprandial glucose and significantly decreased insulin response without affecting the subjective appetite in healthy participants over 180 min. However, high dose (50 g) of TRM increased the occurrence of flatulence thus the amount of TRM included in food or beverages should be taken with care.

Furthermore, three formulas of ONS (original, RMD15, and RMD30) were developed by differing the proportion of tapioca maltodextrin and TRM. It was found that the amount of TRM in ONS affected to the viscosity and water activity. The replacement of tapioca maltodextrin by TRM improved the sensory acceptance as RMD30 formula rated the highest for sensory acceptability score of the viscosity. The developed formula also complied with the regulation as evaluated by total plate count method which is important to ensure it safety and stability of the ONS. Based on its water activity evaluation, RMD30 formula is shelf stable for 1 year when stored in sealed aluminum foil bag at room temperature (25-30$^{o}$C).



When examined clinically in healthy participants, three developed ONS showed similar glucose response, however, the insulin response of RMD30 was significantly lowest compared to other formulas. The AUC insulin0-180 min was decreased by 33% following RMD30 compared to original formula. It indicated that

less insulin needed to control the blood glucose. The different proportion of TRM in the ONS did not pose any significant effect to subjective appetite, including hunger, satiety, desire to eat, and prospective food consumption when observed over 180 min. All developed ONS were well-tolerated in healthy participants as there were no diarrhea reported and there was no significant difference of gastrointestinal symptoms between formulas.

Three weeks supplementation of developed ONS (1 serving/day) did not significantly increase the body weight in healthy participants within three weeks. Supplementation of developed ONS also did not affect to normal food intake therefore it may help to improve the food intake without affecting the satiety. Lastly, TRM-containing ONS was well-tolerated in participants throughout the study.

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