

**META-ANALYSIS AND COMPARATIVE STUDY OF DIFFERENT  
OILS AND COOKING METHODS ON GLYCEMIC RESPONSE OF  
BROWN AND WHITE RICE**

By

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## **ABSTRACT**

### **META-ANALYSIS AND COMPARATIVE STUDY OF DIFFERENT OILS AND COOKING METHODS ON GLYCEMIC RESPONSE OF BROWN AND WHITE RICE**

**Lian Yun Ping**

Rice, a staple food, has a high glycemic index (GI), contributing to adverse metabolic health outcomes. To mitigate this, different processing technologies and cooking methods have been developed to alter starch proportions, including rapidly digestible (RDS), slowly digestible (SDS), and resistant starches (RS). Retrogradation, achieved by cooling cooked rice, recrystallises gelatinised starch into a more resistant form. Additionally, incorporating oils to rice during cooking forms amylose-lipid complexes (ALC), slowing glucose release. However, the combined effects of retrogradation and oil treatments on nutritional composition and glycemic response remain underexplored. This study examined the impact of palm oil (PO) and coconut oil (CO) on the starch profile of brown and white rice using three cooking methods (A: stir-frying raw rice with oil before steaming, B: adding oil to cooking water during steaming, C: stir-frying steamed rice with oil), followed by refrigeration at 4°C for 12 hours. A meta-analysis showed that retrogradation significantly ( $p < 0.05$ )

increased RS and decreased RDS in white rice. Proximate analysis revealed changes in moisture, crude fat, and carbohydrate content across treatments. Brown rice with both oil treatments showed the lowest release of glucose across three cooking methods. Both oil treatments demonstrated a reduction in the RDS and an increase in the RS in both rice across three cooking methods. A greater integrity of swollen starch granules was observed in oil-treated rice across all cooking methods via scanning electron microscopy. Additionally, the indigestible RS demonstrated prebiotic potential by promoting the growth of *Lactobacillus casei* and *Lactobacillus rhamnosus* while reducing oligosaccharides over 24 hours. CO-treated white rice prepared using Method A showed the greatest probiotic growth. These findings highlight the potential for oil treatments and retrogradation to reduce the glycemic response of rice while improving gut health, addressing a critical research gap and offering practical dietary strategies for metabolic health management.

Keywords: Brown rice; white rice; retrogradation; oil treatment; starch digestibility

Subject Area: QD415-436

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## LIST OF ABBREVIATION

ALC	amylose lipid complex
ANOVA	one-way analysis of variance
AOAC	Association of Official Analytical Chemists
CI	confidence interval
CO	coconut oil
DNA	deoxyribonucleic acid
DP	degree of polymerisation
GLUT	glucose transporter
GI	glycemic index
HDL	high-density lipoprotein
HDAC	histone deacetylase
LDL	low-density lipoprotein
MD	mean difference
M <sub>w</sub>	weight-average molecular mass
OD	optical density
PO	palm oil
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
RBO	rice bran oil
RDS	rapidly digestible starch
RNA	ribonucleic acid
RS	resistant starch
SCFA	short-chain fatty acids
SDS	slowly digestible starch
SGLT	sodium-glucose linked transporter

## CHAPTER 1

### INTRODUCTION

#### 1.1 Background

Rice is a widely consumed and affordable staple food in many Asian countries, primarily cultivated from the seeds of *Oryza sativa* plant. It is rich in carbohydrate content, making it a main source of energy for human daily activities (Jukanti, et al., 2020). However, rice has a high GI, which causes a rapid increase in blood glucose levels shortly after consumption, followed by a subsequent drop (Chang, et al., 2014). This fluctuation can contribute to metabolic risk factors that lead to insulin resistance, significantly increasing the risk of non-communicable diseases (Kaur, et al., 2015). Thus, regular rice may not be suitable for health-conscious individuals or those suffering from such diseases.

Brown rice is a whole grain rice produced through minimal milling, which removes only the inedible hull, leaving the bran and germ layer intact. In contrast, white rice undergoes extensive milling, which also removes the bran layer (Rathna, et al., 2019). The retention of the bran in brown rice not only gives brown colour to the rice grain, but also provides greater nutritional values especially dietary fibre.

Food release glucose into bloodstream at varying rates, thereby being classified into high, medium, and low GI categories. Brown rice is considered as a medium GI food, while white rice is classified as high GI (Zahra and Jabeen, 2020). Consuming medium or low GI food is generally recommended for maintaining good health. Despite this, white rice is more popular due to its affordability, better taste and texture.

Rice grains contain three starch fractions categorised according to *in vitro* digestibility: RDS, SDS, and RS (Magallanes-Cruz, Flores-Silva and Bello-Perez, 2017). Total digestible starch refers to the starches that can be digested by the human digestive system, involving RDS and SDS. These starches break down into simple sugars within 20-120 minutes in the presence of enzyme amylase. Contrastingly, RS is not digested in the small intestine and instead fermented in the colon by human intestinal microbiota. A high proportion of RDS in rice contributes to a spike in glucose release upon consumption (Parween, et al., 2020). In contrast, rice with a high content of SDS and RS results in a slower and sustained glucose release during digestion.

Various strategies have been proposed to lower the GI of rice, including breeding low-GI rice cultivars, genetic modification, and using natural additives like plant fibres or polyphenols. For instance, breeding efforts have focused on altering amylose-to-amylopectin ratios to enhance RS content, while natural additives such as polyphenol-rich tea extracts have shown potential in reducing glucose release during digestion (Li, et al., 2023c; Du, et al., 2019). However, these approaches often require advanced



infrastructure, extended timeframes, or high costs, limiting their immediate applications.

In contrast, simple cooking techniques such as retrogradation through cooling and oil incorporation offer practical, low-cost solutions. Cooling promotes the retrogradation of gelatinised starch, resulting in a more crystalline structure and an increased in RS content (Sonia, Witjaksono and Ridwan, 2015). Rice starch comprises of amylose and amylopectin. During the retrogradation process, the linear structure of amylose has a high tendency to form double helices with adequate moisture content (Birt, et al., 2013). These double helices alter the original structure of starch molecules, making them inapt to fit the enzymatic binding site of amylase. Therefore, it cannot be hydrolysed by amylase and formed Type III RS (Ordonio and Matsuoka, 2016). Since human body is incapable of producing enzymes that can breakdown RS, it can serve as a potential prebiotic that promotes the growth of beneficial probiotics in the gut (Jung and Park, 2023). Studies have shown that an increase in probiotics may help reduce the risk of non-communicable diseases (Taherian, et al., 2019).

The advocacy for adding oil to rice is increasing due to its ability to slow down glucose release, primarily through the formation of an ALC. Fatty acids in the oil fit into the helical cavities of gelatinised amylose chains, forming an inclusion complex that prevents the enzymatic hydrolysis by amylase (Birt, et al., 2013). Additionally, ALC restricts the granule swelling by entangling the amylopectin, which also retard the amylase enzyme to

hydrolyse the starch granules (Ordonio and Matsuoka, 2016). The structure of six to eight glucose units per turn in amylose chains allows for interactions with saturated fatty acids of varying lengths (Cervantes-Ramírez, et al., 2020). Therefore, different oils can be tested to determine the most effective option for lowering the high GI of rice.

Palm oil and CO were selected for this study due to their local popularity in Asian diets and distinct fatty acid compositions. PO contains a balance of saturated and unsaturated fatty acids, while CO is predominantly saturated fat (Boateng, et al., 2016). These characteristics influence ALC formation, which reduces enzymatic hydrolysis of starch (Birt, et al., 2013). By evaluating these oils, this study aims to provide practical strategies for incorporating common dietary oils to reduce rice's glycemic response, with potential benefits for gut health and the prevention of non-communicable diseases.

## **1.2 Problem Statement**

Various strategies have been developed to lower the GI of white rice, including breeding and genetic modification, cooking techniques, and the use of natural additives. In low- and middle-income countries, low-cost and simple methods such as cooking techniques and the use of natural additives are more practical and accessible. The effectiveness of lowering the glycemic response of white rice by adding CO has been revealed by previous studies. However,

there is a lack of research concerning PO and even brown rice. Palm oil might be an alternative for lowering the glycemic response of rice, not only due to its domestic popularity in Malaysia but also because of its equal proportion of saturated and unsaturated fats. Additionally, there was a lack of meta-analysis reports on the effects of retrogradation and cooking oil on the glycemic response of both brown and white rice. Furthermore, research examining the prebiotic potential of treated rice was limited.

### **1.3 Hypotheses**

It was hypothesised that the type of fatty acids present in rice will influence the formation of ALC in both brown and white rice. Furthermore, it was proposed that different oil treatments and cooking methods will affect the glycemic response and nutritional composition of these rice varieties. Finally, it was hypothesised that undigested starches in rice will undergo fermentation through the action of beneficial probiotics, leading to an increase in their growth and potentially enhancing gut health.

## **1.4 Objectives**

This study embarked on the following objectives:

1. to investigate the effects of retrogradation and oil treatment on the starches of brown and white rice using meta-analysis.
2. to determine proximate analysis and starch profile of rice upon treatment of different oil treatments and cooking methods.
3. to examine the potential prebiotic property of treated rice.
4. to determine microstructure of starch granules via scanning electron microscopy analysis.

## CHAPTER 2

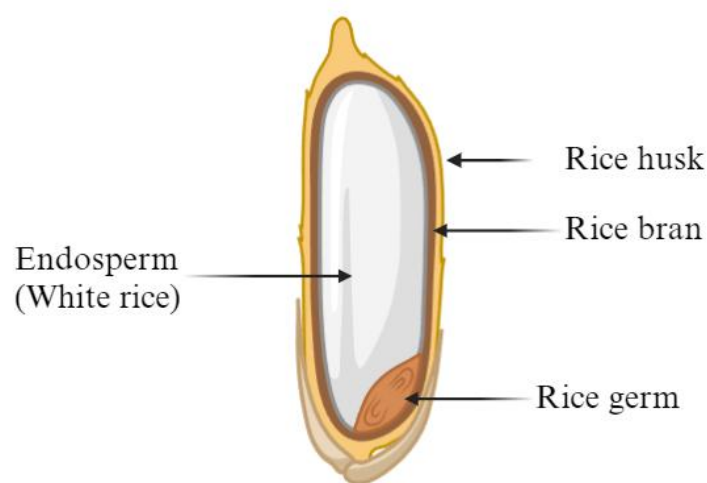
### LITERATURE REVIEW

#### 2.1 Rice

Rice is one of the cereal grains widely consumed as the main carbohydrate source to supply intensive energy for human daily activities. It is commonly harvested from the seed of two domesticated grass species *Oryza sativa* (Asian rice) and *Oryza glaberrima* (African rice). In comparison, African rice is less common in the market, mainly due to the low yield and the higher levels of grain breakage during milling (Nayar, 2014). *Oryza sativa* is widely cultivated in tropical and subtropical areas, under full sun, partially immersed in water, within fertile and loamy soils with ideal pH range of 6.5 to 7.0 (Joshi, 2018). Most cultivars take 110 to 120 days to reach the physiological maturity stage, at which the rice grain becomes firm but not brittle when squeezed between teeth (Bautista and Javier, 2008).

Rice grain consists of three main layers: the outermost rice husk, the middle germ and rice bran layer, and the innermost endosperm (Figure 2.1). After removing from the straw, the inedible rice husk is firstly removed, leaving behind the edible rice bran layer that gives brown colour to the rice grain, producing brown rice with high dietary fibre (Rathna, et al., 2019). Further friction milling and polishing processes will produce a whiter rice,

where the rice bran and germ are also removed, remaining the endosperm which is easier to be digested after cooking (de Oliveira, et al., 2020). However, this also results in a spike release of blood glucose upon consumption, increasing the risk of non-communicable diseases such as diabetes mellitus, cardiovascular diseases, and cancers (Kaur, et al., 2015).



**Figure 2.1: The structure of a rice grain**

Rice is often categorised by biometric parameters: the length, the width, and the length-to-width ratio of the rice grain (Sampaio, Almeida and Brites, 2021). The length-to-width ratio of the rice grain is internationally used to classify rice into three categories: long-grain rice, medium-grain rice, and short-grain rice. Each category exhibits distinct characteristics, such as stickiness and texture when cooked, due to varying ratios of amylose and amylopectin in the rice (Syafutri, et al., 2016). These differences influence the use of rice in various cuisines, subject to the characteristics of the cooked rice

produced (Table 2.1). Normally, rice with a high ratio of amylose content (25 to 30%) demonstrates a firm and dry texture, whereas rice with a lower proportion of amylose content (less than 20%) tends to give a softer and stickier texture upon cooking (Vici, et al., 2021).

**Table 2.1: The characteristics of different types of rice**

<b>Characteristics</b>	<b>Long-grain</b>	<b>Medium-grain</b>	<b>Short-grain</b>
Appearance	long and slender	short and plump	short, almost round shape
Length	more than 6.0 mm	5.2 mm to 6.0 mm	less than 5.2 mm
Ratio of length to width (Koutroubas, et al., 2004)	more than 3	less than 3	less than 2
Texture when cooked (Hensperger and Kaufmann, 2003)	firm and dry textures, fluffy and separate when cooked	chewy and tender textures, stick together when cooked	soft, and tender textures, stick together and clump when cooked
Common variety	basmati, jasmine	arborio, valencia	bomba, pearl
Common uses	pilaf, biryani, table rice	risotto, arancini, rice salad	sushi, mochi, rice ball

Long-grain rice is lengthy and slender, measuring over 6.0 mm in length and having a length-to-width ratio greater than three. The grains remain dry, fluffy, and separate after cooking, primarily due to the low starch content in long-grain rice (Iqbal, et al., 2022). This characteristic makes long-grain rice be a popular choice for dishes such as pilaf, biryani, and various side

dishes. Fragrant rice such as jasmine rice and basmati rice fall into this category (Gaur, et al., 2016).

Medium-grain rice looks shorter and plumper as compared to long-grain rice, with a length ranging from 5.2 mm to 6.0 mm and a length-to-width ratio less than three. The moderate level of starch in medium-grain rice contributes to creamier, chewier, and more tender textures in dishes, making it suitable for risotto, paella, arancini, and rice salad (Katzin, 2010). Arborio and valencia varieties are the most common types of medium-grain rice found in the marketplace.

Short-grain rice has a short, plump, and almost round kernel, with a length less than 5.2 mm and a length-to-width ratio less than two. The grains stick together and clump when cooked, making them easier to pick up with chopsticks, giving a sticky, soft, and tender textures when served (Hensperger and Kaufmann, 2003). Therefore, it is widely used in Japanese cuisines such as making sushi, mochi, and rice ball. The most common short-grain rice varieties in the marketplace are bomba rice and pearl rice.

### **2.1.1 Nutritional Composition of Rice**

Rice exhibits a complex nutritional profile, with significant differences between white rice and brown rice. The primary component of rice is carbohydrates, with starch being the most prevalent (Amagliani, et al., 2016).



Starch in rice consists of two main fractions: amylose and amylopectin, influencing the digestibility of the rice. The GI of rice is largely determined by its amylose content, with higher amylose varieties, such as basmati rice, exhibiting lower GI values (Kale, et al., 2015).

The protein content of rice is approximately 7-8%, but it is considered incomplete due to its deficiency in the essential amino acid lysine (Birla, et al., 2017). The lipid content in rice is low, typically less than 1%. However, brown rice contains slightly higher fat and fibre levels due to the presence of the germ and bran (Rathna, et al., 2019). Brown rice contains approximately 2-4 g of dietary fibre per 100 g of cooked rice, primarily in the form of insoluble fibre, which supports digestive health and lowers cholesterol levels (Hashimoto, et al., 2022). In contrast, white rice, after undergoing milling and polishing, loses nearly all its fibre, making it less beneficial for gastrointestinal function and more likely to contribute to rapid spikes in blood sugar levels (Mohidem, et al., 2022).

### **2.1.2 Brown Rice**

Brown rice is a whole rice grain produced from a low degree of milling process that only removes the inedible rice husk, leaving behind the rice bran and germ which provide greater nutrients to consumers (Rathna, et al., 2019). This minimal milling process makes brown rice a rich source of dietary fibre and micronutrients, including magnesium, phosphorus, and potassium. These

minerals are essential for human health, with magnesium notably reducing the risk of stroke and cardiovascular diseases (Saleh, et al., 2019). Phosphorus in brown rice supports the formation of bones and teeth, and promotes the growth, maintenance, and repair of damaged cells and tissues. It also plays essential roles in producing genetic building blocks (deoxyribonucleic acid DNA and ribonucleic acid RNA) and balancing the use of other vitamins and minerals, which helps in maintain good health (Mir, et al., 2016).

Furthermore, high potassium content in brown rice regulates kidney function, muscle contraction, and heart function. However, this potassium richness may pose a concern for individuals with kidney problems, as it can lead to heart diseases or other health issues (Malabadi, Kolkar and Chalannavar, 2022). Selenium, a trace mineral found abundantly in brown rice, domains in DNA synthesis and thyroid health. Selenium not only protects human body from free radical damage but also promotes apoptosis, the process of removing damaged or harmful cells to prevent tumour growth, thereby reducing the risk of cancer (Ravichanthiran, et al., 2018).

Moreover, brown rice rich in various vitamins and bioactive compounds found in its rice bran and germ. Especially, the high amount of vitamin B found in the germ supports human metabolism and immune system, while also improving brain function and inhibiting tumour cell proliferation (Zahra and Jabeen, 2020). Vitamin E, another key component of brown rice, mainly in two types of structure, which are tocopherols and tocotrienols,

reducing cellular ageing by scavenging lipid peroxy radicals (Lee, et al., 2019).

Additionally, the rice bran and germ in brown rice are good sources of monounsaturated and polyunsaturated fatty acids, such as oleic acid and linoleic acid (Ghazani and Marangoni, 2016). These fatty acids enhance the uptake of good cholesterol HDL (high-density lipoprotein) and suppress bad cholesterol LDL (low-density lipoprotein), which carries cholesterol to the arteries and forms plaque against vessel walls (Malabadi, Kolkar and Chalannavar, 2022). Nevertheless, the storage time of brown rice is shorter due to its susceptibility to rancidity, which is attributed to its high fat content in rice bran and germ (Saleh, et al., 2019).

Last but not least, brown rice is categorised as a medium GI food, with a GI of 55, resulting in a lower postprandial glucose response upon consumption (Zahra and Jabeen, 2020). It tends to release glucose slowly and steadily during digestion because of its high content of dietary fibre and polysaccharides such as arabinoxylan and  $\beta$ -glucan (Ravichanthiran, et al., 2018). As a result, the advocacy for brown rice consumption has gained popularity to prevent non-communicable diseases. However, the high cost of brown rice often leads people to choose white rice, despite the loss of nutrients during the extensive milling process. Typically, brown rice costs twice as much as white rice due to its shorter shelf life, which is only around six months (Tuncel, 2023). Besides, brown rice requires better storage conditions, further increasing its cost. The longer cooking time, along with the less

likeable taste and texture of brown rice, also presents additional challenges in promoting its consumption popularly (Mir, et al., 2020).

### **2.1.3 White rice**

White rice, also known as refined rice, is produced through extensive milling of brown rice, involving whitening and polishing processes. During milling, the nutrient-rich rice bran and germ are removed, remaining the starchy endosperm. Since the rice bran is firmly attached to the rice kernel, friction and abrasion are the primary methods used to separate the rice bran and germ from the grain (Afzalnia, Shaker and Zare, 2004). In traditional friction-type milling, a mixture of brown rice and paddy grains is inserted into a frictional whitener, utilising the frictional force between the grains to remove the rice bran layer (Firouzi, Allahyari and Marzban, 2021). Contrastingly, abrasion-type milling removes the rice bran by rubbing the brown rice kernel against an abrading stone. After whitening, polishing process eliminates the remaining bran residues using an abrasive polisher, further whitening the rice grain, resulting in white rice composed mostly of starch endosperm (Mohidem, et al., 2022). The whitening process causes 11-18% rice breakage, while polishing process leads to 2-4% breakage at a paddy moisture content of 8-14% (Afzalnia, Shaker and Zare, 2004). Therefore, different milling systems are optimised to minimise rice breakages during process (Firouzi, Allahyari and Marzban, 2021).

As the degree of milling increases, there is a significant loss of health-beneficial phytochemical compounds, leading to a decrease in cellular antioxidant activity. These milling processes result in the reduction of macronutrients, including 75% of dietary fibre, 85% of fat content, and 15% of protein content, as well as micronutrients such as 75% of phosphorus and 70% of B vitamins, as detailed in Table 2.3 (Ravichanthiran, et al., 2018). Additionally, other bioactive compounds, such as total phenolic compounds, are also lost during the milling process (Ukpong, et al., 2023). Nevertheless, this reduction can benefit individuals with kidney problems who need to lower their potassium intake, as well as those with digestive issues.

The exposed endosperm results in a rapid increase in blood glucose levels upon consumption, thus white rice is being classified as a high GI food with a GI of 64 (Zahra and Jabeen, 2020). Regular intake of high GI white rice is associated with an increased risk of developing non-communicable diseases, particularly type II diabetes. This is because the spike rise of glucose level promotes insulin resistance, which in turn inhibits the glucagon release and further suppresses the hepatic gluconeogenesis, increasing the risk of hyperglycaemia in individuals or patients with diabetes mellitus (Hatting, et al., 2018). Additionally, the consumption of highly processed starchy food contributes to increased level of LDL, resulting in hypercholesterolemia and a heightened risk of developing atherosclerosis and stroke (Chiu and Taylor, 2011). Despite these health concerns, white rice remains as a staple cereal in many countries due to its affordability and preferred taste and texture over brown rice.

## 2.2 Starch

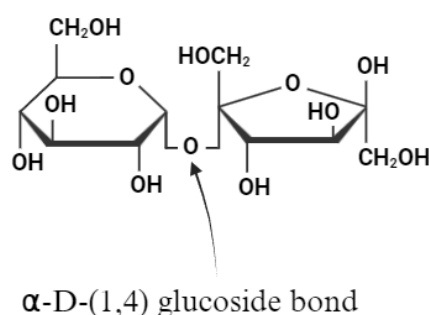
Rice endosperm serves as a chemical reservoir, primarily accumulating approximately 80-90% starch, along with 6-8% protein content and minimal dietary fibre (Alhamba, et al., 2019). Starch is the most prevalent storage carbohydrate in plants, stored as granules that vary in size and shape depending on the cereal species (Amagliani, et al., 2016). Rice starch granules are unimodally distributed, with sizes ranging from 3 to 10  $\mu\text{m}$ . They typically exhibit polyhedral shape and are stored within amyloplasts (Bhat, Chauhan and Verma, 2023). Each amyloplast accommodates 20 to 60 individual granules, forming compound granules that can reach up to 150  $\mu\text{m}$  in diameter (Bao and Bergman, 2004).

Starch is a polysaccharide polymer that mostly composed of  $\alpha$ -D-glucopyranosyl units bonded by glycosidic linkages (Hu, et al., 2022). Specifically, starch is a polymeric mixture of two  $\alpha$ -glucans, amylose and amylopectin, which together constitute 98-99% of the dry weight of starch granules (Amagliani, et al., 2016). The ratio of these two polysaccharides varies depending on the botanical source, affecting the physical and physicochemical properties of starch through interactions with other constituents in the rice endosperm (lipids, proteins, and water) during the gelatinisation and retrogradation processes (Fitzgerald, 2004). Gelatinisation is the process where starch granules absorb water and swell upon heating, disrupting their crystalline structure. Retrogradation is the subsequent reorganization of amylose and amylopectin molecules into a more ordered

structure when the starch cools, resulting in the formation of SDS and RS (Kadam, Tiwari and O'Donnel, 2015).

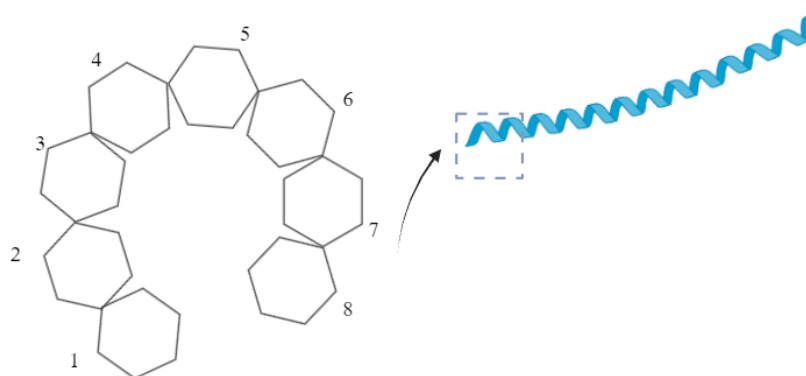
### 2.2.1 Amylose

Amylose is a linear or slightly branched polysaccharide predominantly linked by  $\alpha$ -D-(1,4) glucoside bonds, as illustrated in Figure 2.2. Rice amylose chains vary in length, generally exhibiting a degree of polymerisation (DP) that ranges from 920 to 1110 glucose residues. It has a weight-average DP of 2750 to 3320 glucoses, a number-average DP of 980 to 1110 glucoses, and an average chain length of 200 to 700 glucose units (Li, et al., 2022b; Tester, Karkalas and Qi, 2004). The weight-average molecular mass ( $M_w$ ) of rice amylose is between  $5.1$  to  $6.9 \times 10^5$  g/mol, while the number-average molecular mass ranges from  $1.4$  to  $1.8 \times 10^5$  g/mol (Amagliani, et al., 2016).



**Figure 2.2: The structure of amylose**

Amylose forms a helix in its secondary structure, with glucose units coiling into a helical shape stabilised by hydrogen bonds between hydroxyl groups, as shown in Figure 2.3 (López, de Vries and Marrink, 2012). The pitch of a helix accommodates six to eight glucose units, allowing it to trap small molecules within its core, such as fatty acid chains and iodine molecules (Bergthaller and Hollmann, 2014). Under specific conditions, such as lower temperatures or high concentrations of amylose, these single helices may pair up to form double helices. This structure is more rigid and less soluble in water, which forms when two amylose chains align parallel to each other and bonds interchain hydrogen bonds between glucose units (Fan, et al., 2021). Further aggregation of double helices leads to more ordered crystalline structures, further reducing their solubility in water (Yui, et al., 2018).



**Figure 2.3: The helical structure of amylose**



Amylose generally crystallises as double helices in different allomorphs. A-type and B-type amyloses have a left-handed double helix structure, while V-type amylose exists in a left-handed single helical conformation (Fan, et al., 2021). A-type amylose features a helical structure with a distinct, compact, and less ordered packing arrangement (Tetlow and Bertoft, 2020). This structure exhibits lower resistance to retrogradation and has specific gelatinisation properties, including a higher gelatinisation temperature and increased viscosity during cooking. B-type amylose is commonly found in tubers and roots, displaying a more ordered crystalline structure with a less compact helical arrangement (Tian, et al., 2023a). It shows a greater resistance to retrogradation and has a lower gelatinisation temperature and viscosity (Tong, et al, 2023). Moreover, V-type amylose arranges in an anti-parallel arrangement due to its complexation with small molecules (Guo, Ziegler and Kong, 2022). This configuration facilitates the formation of ALC, where amylose helices accommodate lipids within their central cavity, resulting in unique crystalline structures (López, de Vries and Marrink, 2012).

Amylose content varies among rice varieties. It is generally determined in term of apparent amylose content measured by starch-iodine colourimetry method (Villareal, De La Cruz and Juliano, 1994). Waxy rice starch contains less than 5% amylose, whereas non-waxy rice starch has an amylose content ranging from 8% to 37%. Non-waxy rice is further classified into four categories based on amylose content: very low (5-12%), low (12-20%), intermediate (20-25%), and high (>25%) (Suwannaporn, Pitiphunpong and

Champangern, 2007). Japonica rice varieties usually have low amylose content, whereas most indica varieties have intermediate or higher amylose contents compared to other rice varieties (Jukanti, et al., 2020; Zhang, et al., 2023a).

The linear structure of amylose tends to disrupt the ordered arrangement of starch molecules in rice when present in significant amounts. It interferes with the ordered alignment of amylopectin chains, thereby reducing the overall crystallinity of starch (Sasaki, Yasui and Matsuki, 2020). Despite this, the strong hydrogen bonding and the linear nature of amylose require more energy to break down during gelatinisation. As a result, rice with higher amylose content typically has a higher gelatinisation temperature, at which the starch granules begin to absorb water and swell upon heating (Chung, et al., 2011).

During gelatinisation, amylose molecules, especially those with smaller molecular sizes stored within amorphous lamellae, can easily leach out from the granule and intertwine to form a network through hydrogen bonding (Yan, et al., 2021). The formation of this network, generally known as gelling process, is crucial for determining the final texture of cooked rice and rice-based products, including the firmness and stickiness levels (Zhu, et al., 2021). Amylose retains less water molecules during swelling due to its linear and slightly branched structures, exhibiting a firmer and less sticky textures in high amylose rice (Pulgarin, Larrea-Wachtendorff and Ferrari, 2023). In contrast,

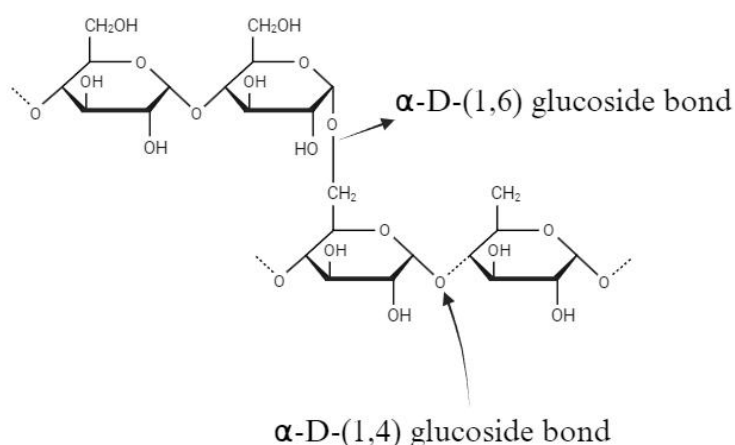
low amylose rice varieties tend to have a soft and sticky textures (Li, et al., 2023a).

Additionally, high amylose rice demonstrates lower peak viscosity and higher final viscosity. The lower peak viscosity results from the inhibition of starch swelling by amylose chains, which entangles with amylopectin and reduces the maximum viscosity during heating (Jia, et al., 2023). During cooling, a more rigid gel structure forms with the reassociation of amylose molecules, contributing to a higher final viscosity. However, Tao, et al. (2019) found that the length of amylose chain significantly affects these properties. Medium and short amylose chains demonstrate higher peak and trough viscosities, attributed to their greater mobility and enhanced ability to interact with water, which disrupt the swelling of amylopectin during gelatinisation (Biduski, et al., 2018; Lin, et al., 2023).

Furthermore, amylose content is a key factor in starch retrogradation. This process is more prominent in high amylose rice due to the linear structure of amylose, which facilitates tighter molecular packing and stronger intermolecular hydrogen bonding (Hsu, et al., 2015). The formation of RS and SDS in rice starch during retrogradation is associated with slower digestion, resulting in a slower and sustained release of glucose into the bloodstream. This characteristic makes high amylose rice potentially beneficial for managing blood sugar levels.

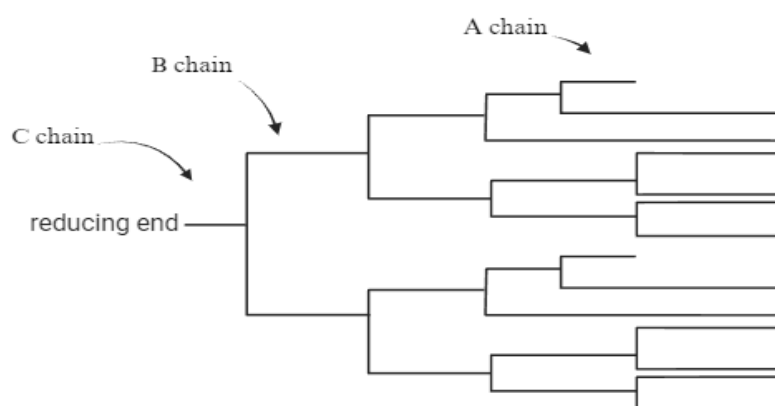
### 2.2.2 Amylopectin

Amylopectin is a branched polysaccharide bonded with both  $\alpha$ -D-(1,4) and  $\alpha$ -D-(1,6) glucoside linkages, making up 70 to 80% of rice starch granules (Lu, 2004). Its highly branched structure comprises approximately 95% linear 1,4-linked glucose units and 5-6% of  $\alpha$ -D-(1,6) glucoside linkages at branch points occurring every 24 to 30 glucose subunits, as illustrated in Figure 2.4 (Martens, et al., 2018). Amylopectin has a lower DP, generally less than 70 glucose units (Li, et al., 2016). The number-average DP of amylopectin ranges from 8200 to 12,900 glucoses, with an average chain length of 18 to 25 glucose units (Tester, Karkalas and Qi, 2004). Thus, amylopectin is relatively shorter than amylose and exhibits a broad distribution profile. Nevertheless, amylopectin molecules are considerably larger than amylose, with  $M_w$  of  $2.7 \times 10^9$  g/mol in non-waxy rice and  $5.7 \times 10^9$  g/mol in waxy rice (Amagliani, et al., 2016).



**Figure 2.4: The structure of amylopectin**

The side chains of the branched amylopectin are classified into three types: A, B, and C chains, which are connected by  $\alpha$ -D-(1,6) glucoside linkages (Amagliani, et al., 2016). C chain functions as the central backbone, carrying the only reducing group and forming the core structure of amylopectin, as illustrated in Figure 2.5 (Martens, et al., 2018). This central chain provides stability and a foundation for the attachment of B chains. B chains play a crucial role in forming the multi-tiered branching architecture of amylopectin. These chains carry one or more additional branches, which are further categorised into subtypes B1 through B4, based on their participation in side chain clusters and their respective chain lengths. In details, B1 chains are linked within a single cluster, while B2 and B3 chains span two and three clusters, respectively (Tester, Karkalas and Qi, 2004). A chains, also known as unbranched chains, are located at the outermost regions of the amylopectin molecule, primarily attached linearly to B1 chains (Ratnavathi and Komala, 2016). Comparatively, A chains tend to have shorter amylopectin branch lengths than B and C chains (Allan, Read and Johanningsmeier, 2022).



**Figure 2.5: The structure of side chains in amylopectin**

The branched structure of amylopectin results in regions with varying branch densities, where areas with high branching form crystalline zones, and areas with low branching create amorphous regions (Martens, et al., 2018). These crystalline and amorphous regions contribute to the semicrystalline nature of starch granules, which is conferred by the ordered and radial arrangements of starch molecules (Dona, et al., 2010). Crystalline regions primarily result from the formation of double helices between the outer chains of amylopectin, while amorphous regions are composed of both amylopectin and amylose chains, typically classified into three types: A-, B-, and C-type crystalline structures (Dome, et al., 2020).

A-type crystalline structure, commonly found in cereal starches, features densely packed short glucose helices that forms a compact and stable structure with parallel double helices (Nakamura, et al., 2020). These starches generally have lower gelatinisation temperature and are easier to digest. B-type crystalline starches, found in tubers, are less densely packed than A-type, with a more open structure and a hydrated helical core, allowing more interstitial water molecules between branches. Specifically, A-type crystalline starches contain four water molecules, whereas B-type can accommodate 36 water molecules per unit cell (Amagliani, et al., 2016). Therefore, B-type starches have higher gelatinisation temperature and better water retention. C-type crystalline starches, often found in legumes and roots, are a combination of A- and B-type crystalline starches (Martens, et al., 2018).

Amylopectin demonstrates a strong capacity to absorb and retain water owing to the extensive hydroxyl groups presented in its branched structure, which provide numerous sites for hydrogen bond formation with water molecules (Cornejo-Ramírez, et al., 2018). Consequently, when starch granules are heated in water, they swell and eventually rupture, releasing amylopectin molecules into the surrounding liquid and contributing to gelling and thickening effects (Jia, et al., 2023). The strong water-binding ability and high solubility of amylopectin in water also results in the sticky and moist textures in cooked rice with high amylopectin content, such as waxy rice (Zhang, et al., 2020a). Additionally, amylopectin interacts with itself and water molecules to form a network that entraps more water molecules or other constituents, which hinders the molecule movement and enhances the viscosity (Mhaske, Majzoobi and Farahnaky, 2023). Furthermore, the structure of amylopectin predominantly influences the swelling capacity of starch granules (Vamadevan and Bertoft, 2020). The short chains of amylopectin promote starch granule swelling and are more readily leaching into the starch solution. Contrastingly, longer amylopectin chains with higher molecular weight, introduce a greater steric hindrance, thereby inhibiting the swelling of starch granules during starch gelatinisation (Zhang, et al., 2023a).

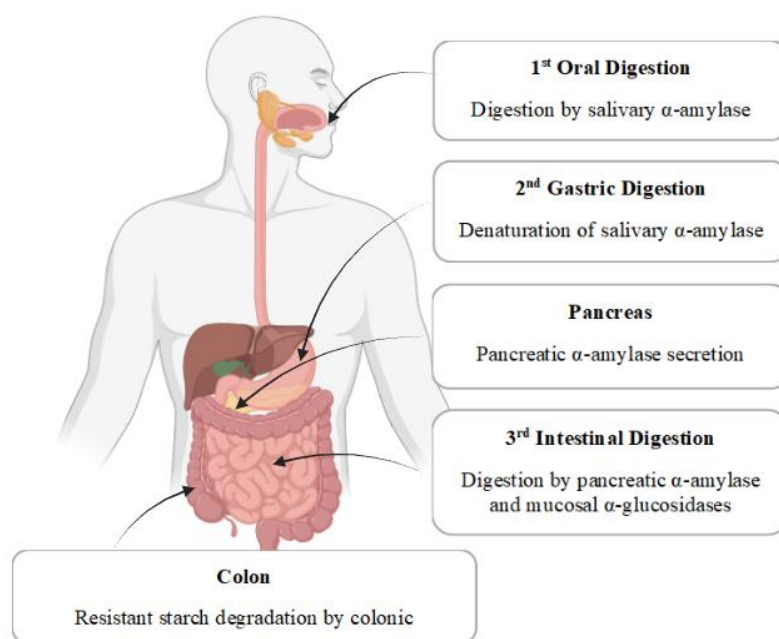
The highly branched structure of amylopectin is less prone to realignment into a crystalline form, which prevents the network from fully reorganising into crystalline regions (Tian, et al., 2023b). Besides, the high water-binding capacity of amylopectin helps to maintain the gel, preventing the formation of a rigid crystalline structure, thereby reducing the likelihood of

retrogradation (Koczoń, et al., 2022). The ability of amylopectin to resist retrogradation also contributes to its freeze-thaw stability, allowing products containing amylopectin to withstand freezing and thawing without significant changes in texture or separation (Woo, et al., 2021).

### **2.3 Starch Digestibility**

Starch digestibility involves a complex process occurring in the human gastrointestinal tract, beginning in the mouth and continuing through the stomach and small intestines. This process is divided into three distinct phases as depicted in Figure 2.6. It is predominantly governed by two groups of digestive enzymes: (I) salivary and pancreatic  $\alpha$ -amylases, and (II) intestinal brush border enzymes, including glucoamylases, maltase, isomaltase, and sucrase (Chi, et al., 2022). Initially, the starch complexes are hydrolysed into shorter intermediate chains by salivary and pancreatic  $\alpha$ -amylases, and are further digested into simple sugars by intestinal brush border enzymes (Freitas and Feunteun, 2019). These simple sugars are then absorbed into the bloodstream through the intestinal lining, providing energy to the human body (Peyrot des Gachons and Breslin, 2019).





**Figure 2.6: The starch digestion process in human gastrointestinal tract**

Upon food ingestion, the process of mastication mechanically fragments starch complexes into smaller pieces using the teeth, increasing surface area and enhancing accessibility to digestive enzymes (Li, et al., 2023b). During this process, the starches are mixed with saliva, forming semi-solid mass called boluses. Saliva contains salivary  $\alpha$ -amylase, an enzyme responsible for hydrolysing the  $\alpha$ -1,4 glycosidic bonds in starch complexes, breaking linear amylose chains into shorter maltose disaccharides, while converting the branched into dextrins (Chi, et al., 2022). The boluses are then rapidly transferred to the stomach via the oesophagus by peristaltic contractions (Sensoy, 2021). This process takes only a few seconds, so only a minimal amount of large starch molecules is cleaved into shorter chains by salivary  $\alpha$ -amylase before its activity is inhibited by the acidic environment of

the stomach (Peyrot des Gachons and Breslin, 2019). Moreover, gastric acid does not hydrolyse starch complexes; instead, they are likely ground into particles smaller than 2 mm in diameter by gastric contractions (Li, et al., 2023b). These acidic chymes are subsequently neutralised by bicarbonate secreted by the pancreas before it enters the small intestine.

The breakdown of starch into intermediate chains continues in duodenum, the first part of the small intestine, facilitated by pancreatic  $\alpha$ -amylase secreted by the pancreas (Kajla, Yadav and Gaur, 2024). This enzyme is more efficient than salivary  $\alpha$ -amylase due to the longer exposure time to starch (Yuan, et al., 2021). The final stage of starch digestion occurs at the brush border of the small intestine (jejunum), where the microvilli-covered surface of the intestinal epithelial cells that significantly increases the surface area for absorption (Patricia and Dhamoon, 2022). Enzymes like maltase, isomaltase, sucrase, and glucoamylase in jejunum further break down these intermediate products into simple glucoses, as demonstrated in Table 2.2. These monosaccharides are then absorbed into the bloodstream through specialised transporters in the intestinal lining, such as sodium-glucose linked transporter 1 (SGLT1) and glucose transporter 2 (GLUT2) (Navale and Paranjape, 2016).

**Table 2.2: The brush border enzymes and their respectively substrates.**

Enzyme	Reaction
Glucoamylase	Glucoamylase cleaves maltooligosaccharides and larger polysaccharides like dextrans.
Isomaltase	Isomaltase specifically targets the $\alpha$ -1,6-glycosidic bonds in isomaltooligosaccharides and $\alpha$ -limit dextrans.
Maltase	Maltase hydrolyses the $\alpha$ -1,4-glycosidic bonds in maltose and maltotriose.
Sucrase	Sucrase cleaves the $\alpha$ -1,4-glycosidic bonds in sucrose and maltose.

Starch digestibility is influenced by a range of intrinsic and extrinsic factors. Firstly, the intrinsic properties of the food matrix, such as the degree of starch-protein interactions and the ratio of amylose to amylopectin, play a crucial role in determining starch digestibility (Jia, et al., 2023). Secondly, natural non-starch components like tannins and saponins interact with starch to form V-type crystals, which serve as physical barriers to impede starch digestion (Yang, et al., 2023). Additionally, the structural features of starch, including helical, crystalline, and lamellar arrangements, also substantially slow down the binding of digestive enzymes (Chi, et al., 2022). Furthermore, processing techniques such as milling and polishing processes, along with cooking methods like roasting, steaming, and frying induce starch gelatinisation, which also increasing the rate of starch digestibility (Toutounji, et al., 2019). Consequently, based on these varying degrees of digestibility, starch can be further categorised into three different types: RDS, SDS, and RS.

### **2.3.1 Rapidly Digestible Starch**

Rapidly digestible starch is characterised by its swift breakdown and absorption in the small intestine, resulting in a rapid increase in blood glucose levels after consumption (Kim, Park and Kim, 2024). This type of starch is usually found in highly processed or cooked food, where the starch has been gelatinised through cooking or processing methods (Noraidah, et al., 2023). This increases the starch's accessibility to digestive enzymes, enabling it to be digested within 20 to 30 minutes. White rice is an example that contains high levels of RDS, which can cause rapid spikes in blood sugar and insulin levels upon consumption. Although RDS provides a quick source of energy, the associated rapid rise and subsequent fall in blood glucose levels may lead to fluctuations in energy and potentially increase hunger shortly after eating (Aller, et al., 2011). This might be a consideration for individuals managing diabetes or metabolic syndromes.

### **2.3.2 Slowly Digestible Starch**

Slowly digestible starch is a type of starch that breaks down into glucoses at a moderate rate during digestion, providing a gradual and sustained release of energy (Miao, et al., 2015). This characteristic makes SDS particularly beneficial for managing blood glucose levels, especially for individuals with insulin resistance or type 2 diabetes (Methew, Zubair and Tadi, 2023). Additionally, the prolonged energy supply contributes to an

extended feeling of fullness after meals, which helps regulate overall calorie intake and supports effective weight management.

The unique benefits of SDS are largely due to its structural characteristics which make them less accessible to digestive enzymes. These include a higher proportion of  $\alpha$ -1,6 linkages, short branch chains with a DP less than 13, longer chains with a DP of 25–36, and imperfect helical and crystalline structures (Chi, et al., 2022). These compact structures result in a slower digestion process compared to RDS, lowering sharp spikes in blood sugar levels. Moreover, SDS can be found in whole grains, legumes, and pasta, particularly when they are cooked and cooled. This cooling process promotes the retrogradation of starch molecules, leading to a more crystalline structure that resists rapid digestion (Hsu, et al., 2015).

### **2.3.3 Resistant Starch**

Unlike RDS and SDS, RS resists enzymatic hydrolysis throughout the digestive process. As a result, it passes through the small intestine largely intact and reaches the colon, where it undergoes fermentation by gut microbiota (Jin, Peng and Nie, 2023). In the colon, primarily in the cecum and large intestine, RS acts as a prebiotic selectively promoting the growth of beneficial bacteria like *Bifidobacterium* and *Lactocaseibacillus* (You, et al., 2022). This fermentation process leads to the production of short-chain fatty acids (SCFAs) such as acetate, propionate, and butyrate, along with gases like

carbon dioxide, methane, and hydrogen (Den Besten, et al., 2013). These SCFAs play crucial roles in regulating the structure of the intestinal microbiota and enhancing the function of the intestinal epithelial barrier, which is critical in the prevention and treatment of metabolic syndrome, bowel disorders, and inflammatory diseases (Nogal, Valdes and Menni, 2021).

Butyrate possesses anti-inflammatory property and acts as a histone deacetylase (HDAC) inhibitor, which can suppress inflammation and protect against HCT116 colon cancer by promoting cell differentiation and apoptosis (Zhang, et al., 2023b). Additionally, butyrate has been found to improve insulin sensitivity and significantly reduce lipolysis in adipose tissue, which is crucial for managing blood sugar levels and preventing type 2 diabetes (Wang, et al., 2019). This effect is also partly attributed to the production of acetate during fermentation, which plays a role in regulating glucose metabolism and maintaining energy balance. Propionate, another SCFA produced from the fermentation of RS, reduces cholesterol levels and further decreases the risk of cardiovascular diseases. Also, propionate has anti-proliferative effects on tumour cells, further supporting its role in reducing the risk of obesity-related cancers (Jin, Peng and Nie, 2023). Furthermore, RS helps to prevent rapid spikes in blood sugar levels after meals, contributing to better glycemic control and reduced risk of developing insulin resistance (Pugh, et al., 2023).

The distinctive properties of RS are primarily attributed to the presence of specific structural features. These features include double helix-promoting chains with DP ranging from 12–24 and  $DP \geq 37$ , as well as chains with DP

25–36 that form tightly packed double helices and crystalline structures (Chi, et al., 2022). These structures often consist of densely packed crystalline lamellae, V-type crystals, and more ordered reassembled structure formations such as ALC (Yang, et al., 2023). Furthermore, RS can be classified into five distinct types based on its sources and structural characteristics, as summarised in Table 2.3 (Pugh, et al., 2023; Birt, et al., 2013).

**Table 2.3: Classification and characteristics of different types of resistant starch.**

<b>Resistant starch</b>	<b>Description</b>
RS I (Physically inaccessible starch)	This starch is physically trapped within the fibrous cell walls, making it difficult to access by digestive enzymes, found in coarsely ground or whole grains, seeds, and legumes.
RS II (Naturally resistant starch)	Starch with B- or C crystalline granular structure, found in raw potatoes, high amylose maize starch, and unripe bananas.
RS III (Retrograded starch)	Retrograded starch forms when starchy food, such as potatoes and rice, are cooked and then cooled.
RS IV (Chemically modified starch)	Starch that is chemically modified such as cross-linked starch and octenyl succinate starch, usually used in processed food.
RS V (Amylose lipid complex)	Starch that has formed a complex with lipids (fats), making it resistant to digestion.

## **2.4 Processing Techniques Reducing Glycemic Index of Rice**

Efforts to lower the GI of rice have been the focus of extensive research due to its implications for metabolic health. Various methods have been explored, including food processing, genetic approaches, and the incorporation of additives during cooking. One of the earliest strategies involves altering rice starch fractions through processing methods such as retrogradation, parboiling, and enzymatic treatments. Sonia, Witjaksono, and Ridwan (2015) demonstrated that retrograded rice, formed by cooling cooked rice, exhibited a significant increase in RS, which lowered the GI of rice. Parboiling, which gelatinises the starch within the husk before milling, has also been shown to reduce RDS of rice (Dhital, et al., 2017).

The development of rice varieties with higher amylose content and altered starch structures via genetic modification approaches has been another promising avenue. Li, et al. (2024) reported that high-amylose rice cultivars release glucose more slowly during digestion, resulting in a lower GI. Additionally, the inclusion of natural additives such as dietary fibres, polyphenols, and fats during cooking has also been investigated. Du, et al. (2019) found that polyphenol-rich tea extracts reduced rice GI by inhibiting starch hydrolysis. Similarly, guar gum, a dietary fibre, was shown to increase RS levels when mixed with rice (Hasjim, et al., 2013).



The use of cooking oils to lower rice GI has gained attention for its practicality and efficacy. Panyoo and Emmambux (2016) observed that rice cooked with PO showed increased RS content due to the formation of ALC, which reduced the enzymatic hydrolysis of starch. Similarly, Nugrahedhi, et al. (2017) reported that CO added during stir-frying not only increased RS content but also enhanced the rice's structural stability against enzymatic digestion.

Among the various techniques, cooking oil treatments are particularly attractive due to their compatibility with everyday cooking practices and the accessibility of oils like PO and CO in many rice-consuming regions. While retrogradation and parboiling require additional processing steps, oil-based methods integrate seamlessly into existing cooking routines. Furthermore, the ability of oils to form stable ALCs during stir-frying makes this method a practical and culturally relevant option for lowering rice GI.

#### **2.4.1 Retrogradation**

Resistant starch III is primarily formed through retrogradation, which occurs when gelatinised starches reassociate into a more ordered structure during the cooling stage (Han, et al., 2021). This retrogradation process is driven by the formation of hydrogen bonds between the hydroxyl groups of neighbouring glucose units in the starch molecules, forming double helices and crystalline regions (Tako, et al., 2014). These regions are highly ordered

and thermodynamically more stable than the gelatinised starch (Martínez Sanz, et al., 2018). Therefore, digestive enzymes unable to hydrolyse the glucose units within these regions.

Retrogradation of starch can be categorised into short-term and long-term retrogradation based on the timeline and the molecular processes involved. Short-term retrogradation occurs within hours after the starch has been gelatinised and cooled (Chakraborty, et al., 2023). During this stage, the linear amylose molecules realign in parallel and form adjacent hydrogen bonds. Due to its smaller and more flexible structure, amylose has a strong tendency to form double helices, leading to the immediate formation of crystalline regions upon cooling (Cornejo-Ramírez, et al., 2018; Baptista, et al., 2024). This structural change in amylose is irreversible and significantly enhances the resistance of starch to digestion in the small intestine (Yamakuchi, et al., 2019).

Contrastingly, long-term retrogradation initiates after 12 h and extends over several days to weeks (Chakraborty, et al., 2023). This process primarily involves the retrogradation of amylopectin. Due to its branched structure, amylopectin reassociates at a low degree and slower than amylose (Lin, et al., 2023). Additionally, amylopectin with short external length (6-8 glucose residues) resists undergoing retrogradation (Vamadevan and Bertoft, 2018). Over time, the gradual formation of more organised and tightly packed structures in amylopectin increases the size of the crystalline regions, thereby

enhancing their resistance to enzymatic breakdown. (Cornejo-Ramírez, et al., 2018).

The conversion of RDS to SDS or RS depends on the degree of crystallisation during retrogradation. This degree of crystallisation is influenced by several factors, including amylose concentration, cooling rate, and other factors summarised in Table 2.4 (Han, et al., 2021). Starches with higher amylose content undergo retrogradation more rapidly because linear amylose molecules are more likely to form double helices and crystalline structures compared to branched amylopectin molecules (Baptista, et al., 2024). Addition of ingredients like sugars and salts also influence the retrogradation process. Sugar is a plasticiser, which hinders the crystallisation of starch molecules by disrupting the interaction in starch chains (Ploypetchara and Gohtani, 2018). Salts also influence the structural stability of starch with effects varying based on the types of ions due to differences in charge distribution and polarisation (Wang, et al., 2016). Furthermore, adequate moisture is necessary for retrogradation, allowing starch molecules to move and realign (Ojogbo, Ogunsona and Mekonnen, 2020).

**Table 2.4: Factors affect starch retrogradation**

Factor	Characteristics
Inherent starch properties	Starch crystallinity, molecular weight, and botanical origin
The presence of other constituents	Lipids and proteins
Additives	Sugars, salts, and acids
Storage conditions	Temperature, time, and water content

#### **2.4.2 Addition of Oil to Starch Through Different Cooking Methods**

Amylose lipid complex is a newly recognised type of RS, generally known as RS V, characterised by the formation of inclusion complexes through the interaction between amylose chains and fatty acid chains. During gelatinisation, the mobility of amylose chains increases, transitioning from a coil structure to helical conformation (Chumsri, et al., 2022). The hydrophobic cavity within the helix allows for hydrophobic interactions with the hydrophobic tail of the fatty acid chains (Liang, et al., 2023). This complex is further stabilised by the formation of strong electrostatic bonds upon cooling, forming ALC that can retard the amylolysis of digestive enzymes (Ronie and Hasmadi, 2022). Additionally, amylopectin is also capable of forming complexes with fatty acid chains. However, these complexes are less stable due to the side branches of amylopectin which interfere with the formation of a single glucan helix conformation similar to that of amylose (Mohamed, 2021).

The formation and stability of RS V are influenced by several key factors. Fatty acid chains with 12–18 carbons fit more effectively within the hydrophobic cavity (Putseys, et al., 2009). Within this range, shorter fatty acid chains exhibit a greater ability to form ALC due to their higher solubility in water (Chumsri, et al., 2022). Moreover, saturated fatty acids form more complexes than unsaturated fatty acids. This is attributed to the linear saturated fatty acids which fit neatly into the amylose helix, whereas the kink

structures in unsaturated fatty acids hinder their ability to form stable ALCs (Hasjim, et al., 2013).

The amounts of amylose and lipid also affect the formation of ALCs as fatty acid chains prone to self-associate at high concentration (Wang, et al., 2020). Thus, starches with higher amylose content and adequate lipid concentration form more complexes due to the increased availability of amylose and lipids. Additionally, amyloses with 20 to 40 glucosyl residues form more stable complexes because longer polymers can accommodate two fatty acid chains and form more helical structures within the same polymer (Sang, et al., 2021). However, amylose with a very high molecular weight exhibits reduced mobility, potentially limiting its ability to interact with fatty acid chains effectively (Li, et al., 2022b). Furthermore, other components in food matrix such as proteins may compete with lipids for binding sites on amylose, reducing the formation of ALCs (Lan, et al., 2024).

The mechanical forces such as mixing or extrusion enhance the contact between starch and lipid molecules (Cervantes-Ramírez, et al., 2020). Therefore, the addition of oil to starch through stir-frying might promote the complex formation. Stir-frying is a cooking technique that originated in China, involving the quick cooking of food in a small amount of hot oil over high heat with constant stirring (Zhou, et al., 2019). The cooking temperature during stir-frying ranges from 160 to 250°C, normally completing in minutes (Nugrahedi, et al., 2017). This high temperature increases the gelatinisation of starch also further promotes the formation of complexes (Panyoo and

Emmambux, 2016). As such, stir-frying is an appropriate cooking method for adding oil to rice, which enhances the formation of ALCs in rice through the application of additional mechanical forces.

## **2.5 Cooking Oil**

Cooking oil, also known as edible oil, is an essential ingredient in food preparation and widely available in the market place. It is derived from a variety of plant and animal sources and can exist in either liquid or solid form, depending on the composition of fatty acids (Lankatillake, Dias and Huynh, 2023). Due to its high specific heat capacity, cooking oil facilitates higher cooking temperatures, which in turn accelerates the cooking process and enhances both the flavour and texture of food (Nayak, et al., 2015). As a result, cooking oil is utilised in a broad range of culinary techniques, including baking, stir-frying, and sauce preparation.

Cooking oils are primarily composed of triglycerides, a type of fat that consists of a hydrophilic glycerol backbone attached to three hydrophobic fatty acid chains (Asokapandian, Sreelakshmi and Rajamanickam, 2021). They are essential macronutrients, providing energy and assisting in the absorption of fat-soluble vitamins, including vitamins A, D, E, and K. Fatty acids, the building blocks of these triglycerides, are categorised into three groups according to the presence and number of double bonds in their carbon chains: saturated, monounsaturated, and polyunsaturated fats (Lobb and Chow,

2008). The structure of these fatty acid chains significantly influences the physical and chemical properties of fats. Furthermore, the consumption of different types of fats has varying health implications, offering both benefits and potential risks to consumers (Calder, 2015).

Saturated fats have no double bonds between carbon atoms in their fatty acid chains, which results in a straight and tightly packed structure, typically making them solid at room temperature (Scrimgeour and Harwood, 2007). They are commonly found in animal sources and tropical plant fruit, such as coconut and palm kernel. Saturated fats are well-known for raising LDL cholesterol levels in blood, a factor often linked to an increased risk of cardiovascular diseases such as stroke and heart disease (Perna and Hewlings, 2022). However, when consumed in moderation, saturated fats may offer certain health benefits. Notably, several studies suggest that an appropriate intake of saturated fatty acids can help reduce blood-brain barrier damage and neuroinflammation (Blake, et al., 2022).

Monounsaturated fats contain a single double bond in their fatty acid chains, introducing a kink in their structure that prevents the molecules from packing tightly together (Watkins and German, 2002). As a result, they are typically liquid at room temperature but may solidify when refrigerated. Monounsaturated fats are considered beneficial for heart health, as they can reduce LDL cholesterol levels while potentially raise HDL cholesterol, which is beneficial for cardiovascular health (Banik and Hossain, 2014). Additionally, these fats exhibit anti-inflammatory properties and are a key component of the

Mediterranean diet, which is associated with a reduced risk of heart disease and other chronic conditions (Dinu, Pagliai and Sofi, 2017).

Polyunsaturated fats contain two or more double bonds in their fatty acid chains, resulting in multiple kink structures that make them more flexible but less stable than saturated and monounsaturated fats (Rustan and Drevon, 2005). They are liquid at both room temperature and when refrigerated, such as omega-3 and omega-6 fatty acids. Due to their multiple double bonds, they are more susceptible to oxidation, which can lead to rancidity if not stored properly (Shahidi and Wanasundara, 2002). Despite this, polyunsaturated fats, particularly omega-3 fatty acids, significantly lower LDL cholesterol and triglyceride levels (Drenjančević and Pitha, 2022). Furthermore, polyunsaturated fats also essential for brain function, nerve signalling, blood clotting, and cell growth (Levenson, 2021).

### **2.5.1 Coconut Oil**

Coconut oil is an edible oil extracted from the kernel or flesh of mature coconuts (*Cocos nucifera*) (Pham, 2016). It is widely used in cooking, skincare, hair care, medicinal, and industrial uses. Various forms of CO are available in the market, including virgin CO, refined CO, and fractionated CO. The primary constituents of CO are saturated fatty acids, consisting of 8% palmitic (C16) acid, 8% myristic (C14), 49% lauric acid (C12), 14-16% capric acid (C10), and 6-8% caprylic acid (C8) (Dayrit, 2014).



Coconut oil is distinct among oils due to its tendency to solidify or become semi-solid at cooler room temperatures, typically between 20°C and 25°C (Binks and Marinopoulos, 2017). In its solid state, it appears white or creamy white, while in warmer conditions it liquefies, becoming clear or slightly pale yellow. Coconut oil melts at approximately 24°C to 26°C (Dia, et al., 2005). This low melting point is due to its high content of saturated fatty acids, especially lauric acid. The boiling point of CO is relatively high due to increased London dispersion intermolecular forces, making it suitable for cooking and frying at moderate to high temperatures (Zhai and Albritton, 2020).

Coconut oil is often acclaimed for its various health benefits, although some claims remain controversial. Despite its high saturated fat content, some studies suggest that stearic acid in CO may help increase good HDL cholesterol, potentially improving cholesterol balance (Jones, 2008). However, other research advises caution regarding its long-term cardiovascular effects. Besides, the medium-chain triglycerides in CO may enhance metabolism and promote fat burning, as they are metabolised more rapidly than long-chain triglycerides, providing a quicker source of energy and reducing the likelihood of fat storage. (Duranova, et al., 2024). Additionally, CO is widely used as a moisturiser due to its natural fats, which aid in moisture retention and are frequently applied to alleviate dry skin conditions such as eczema (Varma, et al., 2019).

### 2.5.2 Palm Oil

Palm oil is derived from the fruit of oil palm tree (*Elaeis guineensis*), which is cultivated extensively in tropical regions, including Indonesia and Malaysia (Onoja, et al., 2019). Oil palms can produce 3 tonnes of oil per hectare, making PO has a high yield compared to other vegetable oils, such as soybean and sunflower (Beekmans, Molenaar, and Dallinger, 2014). The oil palm fruits are small, reddish-orange, oval-shaped, with PO extracted from the mesocarp (flesh) and palm kernel oil obtained from the seed (Onoja, et al., 2019).

Palm oil is naturally reddish due to its high carotene content (Imoisi, et al., 2015). It primarily consists of 50% saturated, 40% unsaturated, and 10% polyunsaturated fats. In contrast, palm kernel oil contains mostly lauric acid, making it chemically more similar to CO (Sabahannur and Alimuddin, 2022). Crude PO remains semi-solid at room temperature, with a melting point approximately 42°C. However, PO found in the market is typically refined and often in liquid form. It is usually pale yellow, odourless, and resistant to rancidity. Palm oil has a neutral flavour and high oxidative stability, which contribute to its widespread use in various food products, including margarine, shortening, and baked goods (Dian, et al., 2017).

The health implications of PO are controversial, primarily due to its high saturated fat content, which linked to increased LDL cholesterol. However, PO also contains beneficial compounds. Crude PO is rich source of

provitamin A carotenoids, especially beta-carotene, which can be converted into vitamin A in the body for vision, immune function, and skin health (Daud, Kaur and Khosla, 2012). Tocotrienols, a form of vitamin E found in PO, exhibit antioxidant properties and are believed to have neuroprotective and anti-cancer potential (Nesaretnam, Yew and Wahid, 2007). Furthermore, PO is one of the most versatile oils in cosmetics, personal care, biofuel, and various industrial applications.

## **2.6 Meta-analysis**

Meta-analysis is a statistical technique used to combine and analyse results from multiple studies on a particular topic to provide a more precise estimate (Paul and Barari, 2022). This approach is particularly useful in fields such as medicine, psychology, and social sciences for evaluating the efficacy of a specific treatment intervention (Ryan, et al., 2014). This process generates an overall treatment effect that is statistically stronger compared to individual studies, which are more reliable and precise owing to the accumulated effect from different articles (Borenstein, et al., 2010). However, the robustness of these overall treatment effects is linked to the quality of the included studies, making it crucial to assess study quality prior to introducing data into a meta-analysis.

In a meta-analysis, the magnitude and direction of treatment effects are consistently described as effect size, such as mean difference (MD) and odds ratio, facilitating the combination of results (McGough and Faraone, 2009). The MD is used to describe continuous data, whereas the odds ratio and relative risk are used for binary data (Higgins, Li and Deeks, 2019). The effect sizes across studies are inherently heterogeneous, owing to variations in sample size, variance, and the reliability of outcome measures (Olejnik and Algina, 2000). Thus, standard error weighting on sample size is applied to produce a more precise effect size estimate for each selected study.

### **2.6.1 Review Manager 5**

Review Manager 5 is a widely used software for conducting meta-analyses, offering two statistical models for calculating effect size estimates: fixed-effects and random-effects models (Konstantopoulos and Hedges, 2019). The effect size is interpreted differently according to the statistical models used. In the fixed-effects model, it is considered an estimate of a common fixed effect, based on the assumption that all selected studies estimate the same underlying effect (Rice, et al., 2018). Conversely, in the random-effects model, the effect size represents the mean of the distribution of true effects, acknowledging heterogeneity among studies and not assuming a single common effect (Barili, et al., 2018). Consequently, the effect size derived from the random-effects model can be generalised beyond the included studies,

whereas the result from the fixed-effects model is applicable only to the studies analysed (Firebaugh, Warner and Massoglia, 2013).

However, the decision for selecting statistical model is relatively subjective. The primary criterion is the goal of statistical inference. For instance, to generalize the results beyond the included studies, the use of a random-effects model is recommended (Nikolakopoulou, Mavridis and Salanti, 2014). The second criteria is the level of statistical heterogeneity. When considerable heterogeneity is present, random-effects model should be used to obtain a more accurate effect size estimate (Tufanaru, et al., 2015).

### **2.6.2 Analytical Technique**

Forest and funnel plots are generated by Review Manager 5 to illustrate the summary findings of the pooled studies. Forest plot provides a graphically representation of the effect size estimates for each selected study, along with an overall effect size (Israel and Richter, 2011). The x-axis of the forest plot represents the effect size estimates, while the y-axis corresponds to the line of null effect, which indicate the point at which there is no statistical significant difference between the treatment group and control groups (Cuzick, 2005). This line serves as a divider, classifying between the two interventions, either favouring the treatment or the control. For dichotomous data, the line of null effect is positioned at a value of 1 on the x-axis, whereas for continuous data in a meta-analysis, the line of null effect is located at 0 (Woodall, 2014).

The effect size of each study is represented by a horizontal line with a box. The horizontal line denotes the 95% confidence interval (CI), while the midpoint of the box signifies the effect size estimate (Cuzick, 2005). The size of the box reflects the weight of the individual studies, with larger boxes representing studies that contribute more precise and meaningful data. This weight is often related to its sample size, as it is calculated based on the inverse variance of the treatment effect (Hedges, Tipton and Johnson, 2010). Consequently, studies with larger sample sizes typically carry greater weight. A wider CI typically reflects a smaller sample size, thus lower precision effect size estimate is calculated. Conversely, a narrower CI is associated with larger sample sizes, indicating higher precision in the effect size estimate (Berben, Sereika and Engberg, 2012). However, a smaller sample size can also result in a smaller CI if the variance of the study is small.

The overall effect size is depicted by a diamond shape in the forest plot. The centre of the diamond represents the overall effect size, while its width corresponds to the 95% CI (Li, et al., 2020). The statistical significance of the overall effect size is determined by the Z-test. If the p value of the Z-test is below 0.05, or even 0.001, the overall effect size is considered statistically significant (Woodall, 2014).

The statistical heterogeneity of the pooled studies is assessed via heterogeneity test to evaluate the consistency of the estimated effect size (Schriger, et al., 2010). Heterogeneity refers to any type of variability present in the selected studies and can be classified into three categories: clinical,

methodological, and statistical heterogeneity (Higgins and Thompson, 2002). Clinical heterogeneity arises from differences in participants, interventions, and outcome definitions. Methodological heterogeneity relates to variations in study designs, while statistical heterogeneity refers to inconsistencies in the estimated intervention effects. Statistical heterogeneity is often influenced by both clinical and methodological heterogeneity (Cuzick, 2005).

In a forest plot, statistical heterogeneity can be assessed in two ways: graphically, by observing the overlap of CI, and statistically, through the chi-square and I-square tests (Favorito, 2023). A poor overlap of CI suggests the presence of statistical heterogeneity. The chi-square test evaluates whether the variability in effect size estimates is due to sampling error (chance) alone; a p value less than 0.05 indicates sufficient evidence that the variability is not driven by chance (Malone, Hines and Graff, 2014). In contrast, the I-square test quantifies the proportion of variability due to heterogeneity and is expressed as a percentage. Interpretation thresholds for I-square values are outlined in Table 2.5, as suggested by Ahn and Kang (2018).

**Table 2.5: The thresholds for I-square interpretation.**

<b>I-square (%)</b>	<b>Interpretation</b>
0-30%	Homogeneity
30-60%	Moderate heterogeneity
50-75%	Substantial heterogeneity
75-100%	Considerable heterogeneity

Publication bias, which arises from the tendency to preferentially publish statistically significant results, can significantly impact systematic reviews and meta-analyses (Gopalakrishnan and Ganeshkumar, 2013). This bias can hinder the collection of eligible articles, as non-significant studies may be suppressed, resulting in an insufficient number of articles for conducting meta-analysis. The presence of publication bias is often assessed using a funnel plot, which is a scatter plot of effect size estimates (on the x-axis) against the standard error of effect size estimates (on the y-axis) (Hoffman, 2019). Ideally, the pooled studies form a symmetrical funnel shape, as studies with varying standard errors are represented as dots on the plot. Studies with larger sample sizes and higher precision appear at the top of the funnel, while studies with smaller sample sizes and lower precision are dispersed towards the base (Deeks, Macaskill and Irwig, 2005). Asymmetry in the funnel plot indicates the presence of publication bias among the pooled studies.



## CHAPTER 3

### METHODOLOGY

#### 3.1 Meta-analysis

##### 3.1.1 Research Question

The effects of retrogradation and oil treatment on the starches of cooked brown and white rice were analysed using meta-analysis in accordance with Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guideline. The starches included RS, SDS, and RDS. The research questions were listed as:

1. Does the retrogradation significantly induce changes in the starches of cooked brown and white rice? (Meta-analysis A)
2. Does the oil treatment significantly affect the proportion of starches in cooked brown and white rice? (Meta-analysis B)

##### 3.1.2 Inclusion Criteria

The criteria for selecting eligible studies were defined in Table 3.1. Fundamentally, the eligible study should be a journal article or a conference paper reported in English language. Besides, *in vitro* starch analysis on cooked

brown and white rice grains upon retrogradation or oil treatment should be included in the study. Additionally, the starches should be reported in mean  $\pm$  standard deviation, in unit of g/100 g. Furthermore, the sample size should be clearly stated in the study.

**Table 3.1: The inclusion criteria for selecting eligible studies**

Inclusion criteria	<ol style="list-style-type: none"> <li>1. The study should be a journal article or a conference paper only.</li> <li>2. The study should be reported in English language.</li> <li>3. The research object should be cooked brown and white rice grain.</li> <li>4. <i>In vitro</i> starch analysis should be included.</li> <li>5. The mathematical data should be expressed in mean <math>\pm</math> standard deviation, in unit of g/100 g.</li> <li>6. The sample size should be included.</li> </ol>
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### 3.1.3 Search Strategy

Ten search keywords were set for both research questions (Appendix A), resulting in eight combinations of keywords for Meta-analysis A and 14 combinations for Meta-analysis B (Appendix B). Meanwhile, two search methods were employed for retrieving topic-related studies, including database search and additional search on the citation list of the studies from the eligibility phase in PRISMA flow diagram.

Three search engines were utilised for article seeking, including Google Scholar, Scopus, and PubMed, as these databases presented a vast coverage of high-quality scientific articles (Falagas, et al., 2008). In Meta-analysis A, a total of 465 outcomes were found from the databases. Most of

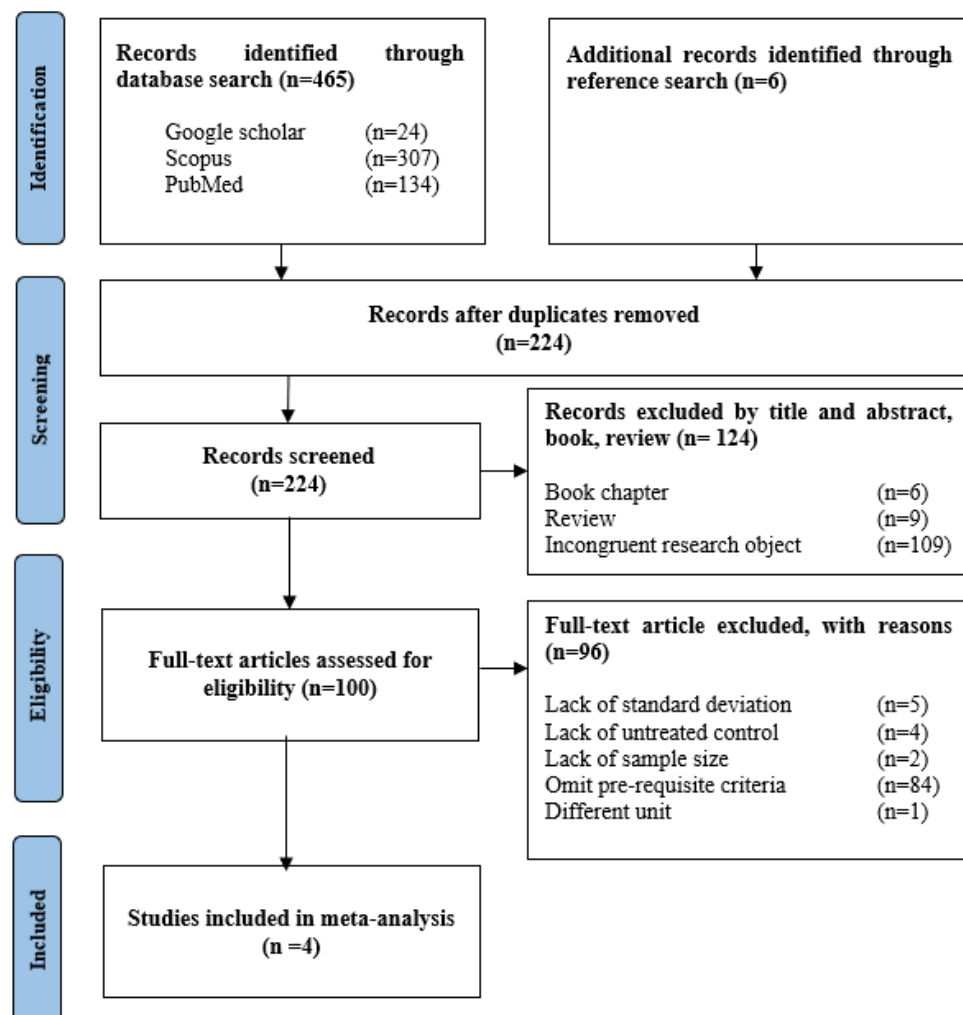
the studies were retrieved from Scopus (n=307), followed by PubMed (n=134) and Google Scholar (n=24). Simultaneously, 722 outcomes were sought in Meta-analysis B, including 220 studies from Google Scholar, 348 studies from Scopus, and 154 studies from PubMed. The last retrieve date was 2<sup>nd</sup> December 2023.

### **3.1.4 Selection Strategy**

The pooled studies were screened following PRISMA four-phase flow diagram. In Meta-analysis A, the pooled studies were firstly deduplicated and then filtered using Inclusion Criteria One to Three during the screening phase (Figure 3.1). Six book chapters, 9 reviews, and 109 studies with incongruent research objects were removed. Most of the incongruent research objects were rice products, such as flour (n=63) and starch (n=13). The other incongruent research objects had been listed in Appendix C.

In the eligibility phase, the full-texts of the filtered studies were assessed against remaining prerequisite criteria. A number of 84 studies were sorted out due to the uninvolved *in vitro* starch analysis on cooked brown and white rice grain upon retrogradation. Besides, the studies without standard deviation (n=3), untreated control (n=3), and sample size (n=1) were also omitted. In a nutshell, three eligible studies were found from the database search.

Additionally, six topic-related studies were retrieved from the addition search. However, five studies were excluded due to the different unit expression in starch (n=1) as well as the lack of standard deviation (n=2), sample size (n=1), and untreated control (n=1). Therefore, four eligible studies were found in Meta-analysis A. Overall, the keyword combination of “White rice AND Resistant starch” showed the highest effectiveness on retrieving eligible studies.



**Figure 3.1: PRISMA flow diagram of Meta-analysis A.**

In Meta-analysis B, a total of 385 studies were screened by their titles and abstracts against inclusion criteria after the duplicates were removed (Figure 3.2). Ten book chapters, 17 reviews, two errata, 10 studies reported in other languages, and 96 studies with incongruent research objects were sorted out accordingly. Major incongruent research objects were flour (n=56) and noodle (n=8), and the others were listed in Appendix C.

In the eligibility phase, the full-texts of remaining studies (n=250) were screened for their eligibility. A total of 248 studies were omitted as they did not involve *in vitro* starch analysis as stated in the prerequisite criteria. In addition, two studies were excluded due to the lack of untreated control (n=1) and standard deviation (n=1). Additionally, three topic-related studies were sought from the additional search. Nevertheless, these studies were excluded due to different unit expression. In a nutshell, there was no eligible studies found in Meta-analysis B.

The mathematical data of eligible studies in Meta-analysis A was extracted as Appendix D. It was crucial to mention that the four eligible studies only recruited white rice as research object, analysing the effect of retrogradation on the starches of white rice. The varieties of white rice were standardised based on the length of rice grain: L for long-grain, M for medium-grain, and S for short-grain. Review Manager 5.4 software (Cochrane Collaboration, England) was employed to conduct meta-analysis. Random effects models were applied to determine the overall effect of retrogradation

on the starches of cooked white rice grain upon different retrogradation duration, 24 h, and 72 h retrogradation.

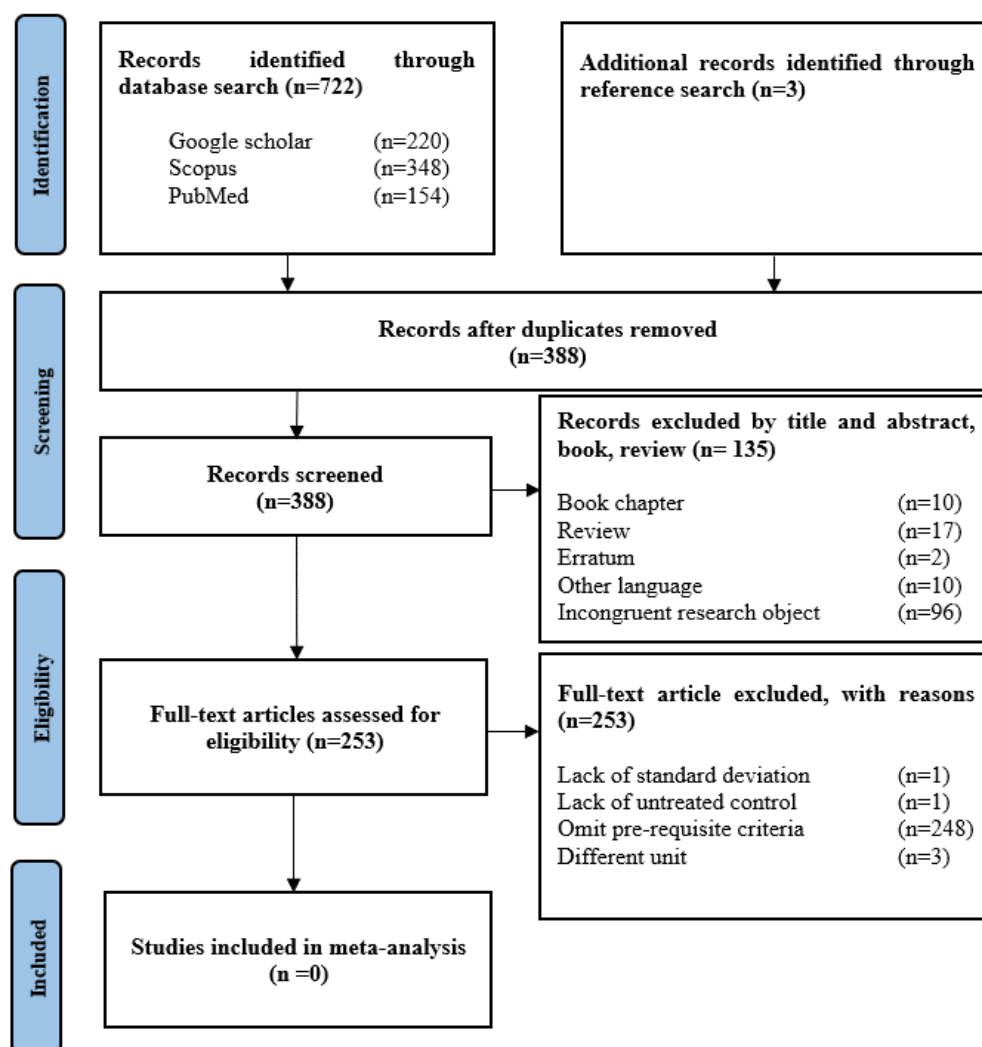


Figure 3.2: PRISMA flow diagram of Meta-analysis B.

### **3.1.5 Analytical Procedure**

#### **3.1.5.1 Forest Plot**

The statistical significance of the overall effect estimate was analysed via Z-test. When the p value of Z-test lower than 0.05, the overall effect estimate was statistically significant. Besides, the consistency of the results collected from eligible studies was determined by chi-square and I-square tests. When the p value of chi-square less than 0.05, simultaneously the percentage of I-square greater than 30%, the pooled results considered heterogenous.

#### **3.1.5.2 Funnel Plot**

The publication bias was observed through the distribution of dots in the funnel plot. When the dots were distributed in pyramid shape, representing the absence of the publication bias. Contrastingly, the presence of asymmetry distribution represented that there was publication bias existing in those pooled studies.

## 3.2 Materials

### 3.2.1 Rice and Oils

Cap Rambutan Super Special Local long-grain white rice (Oel Distribution (Kedah) Sdn. Bhd., Malaysia), ecoBrown's Original long-grain brown rice (Serba Wangi Sdn. Bhd., Malaysia), Medella coconut cooking oil (Bountiful Ventures Limited Company, Malaysia), and Alif pure palm oil (Sime Darby Oil, Malaysia) were purchased from the local supermarkets.

### 3.2.2 Enzyme

**Table 3.2: List of enzymes and their brands**

Enzyme	Specific activity	Brand
Alpha-amylase from porcine pancreas Type VI-B (A3176)	$\geq 10$ units/mg solid	Sigma-Aldrich
Amyloglucosidase <i>Aspergillus niger</i> (A7095)	$\geq 260$ U/mL	Sigma-Aldrich
Oxgall		Sisco Research Laboratories
Pancreatin from porcine pancreas (A7545)	8 x USP specification	Sigma-Aldrich
Pepsin from porcine gastric mucosa (P7125)	$\geq 250$ units/mg solid	Sigma-Aldrich



### 3.2.3 Chemicals and Consumables

**Table 3.3: List of chemicals and their brands**

Chemical	Brand
3,5-dinitrosalicylic acid ( $C_7H_4N_2O_7$ )	ChemSoln
Acetic acid glacial ( $CH_3COOH$ )	Bendosen Laboratory Chemicals
Boric acid ( $H_3BO_3$ )	ChemSoln
Copper (II) sulphate ( $CuSO_4$ )	Quality Reagent Chemicals
De Man, Rogosa and Sharpe (MRS) agar	HiMedia
De Man, Rogosa and Sharpe (MRS) broth	HiMedia
Ethanol ( $C_2H_6O$ ; 99%)	Chemiz
Glucose ( $C_6H_{12}O_6$ )	Quality Reagent Chemicals
Hydrochloric acid (HCl)	Merck
Methyl red ( $C_{15}H_{15}N_3O_2$ )	Bendosen Laboratory Chemicals
Peptone	Bendosen Laboratory Chemicals
Petroleum ether ( $C_6H_{14}$ ; 40-60°C)	R & M Chemicals
Potassium hydroxide (KOH)	Merck
Potassium sodium tartrate ( $KNaC_4H_4O_6 \cdot 4H_2O$ )	Merck
Potassium sulphate ( $K_2SO_4$ )	ChemSoln
Sodium acetate ( $C_2H_3NaO_2$ )	Quality Reagent Chemicals
Sodium bicarbonate ( $NaHCO_3$ )	Quality Reagent Chemicals
Sodium hydroxide (NaOH)	HiMedia
Sulphuric acid ( $H_2SO_4$ )	Merck

**Table 3.4: List of consumables and their brands**

<b>Consumable</b>	<b>Brand</b>
Ashless filter paper	GVS
Boiling stone	Bendosen Laboratory Chemicals
Cotton wool	Premier
Fibre bag	Gerhardt
Filter paper	GVS
Thimble	Smith

### 3.2.4 Equipment

**Table 3.5: List of equipment and their brands**

<b>Equipment</b>	<b>Brand</b>
Distillation Unit K-355	BUCHI
Field Emission Scanning Electron Microscope JSM-6701F	JEOL
FLUOstar Omega microplate reader	BMG Labtech
Gerhardt Manual Fibre Bag system FBS6	Gerhardt
Gerhardt Soxtherm	Gerhardt
IKA C200 bomb calorimeter	IKA
Jeol Auto Fine Coater	Jeol
Nabertherm Muffle furnace	Nabertherm
Speed Digestor K-436	BUCHI
Water bath WNB 22 with Shaking Device	Memmert

### 3.3 Sample Preparation

The 3% oil was added to rice by three methods as described in Table 3.6. White rice was steamed for 20 min, while brown rice for 40 min. The oil-free sample was served as the untreated control in each cooking method. All samples were refrigerated at 4°C for 12 h prior to subsequent analyses.

**Table 3.6: Cooking methods of brown and white rice**

Method	A	Raw rice was stir-fried with oil for 1 min, followed by steaming with filtered water.
	B	Raw rice was steamed with filtered water and oil.
	C	Raw rice was first steamed with filtered water, subsequently stir-fried with oil for 1 min.

### 3.4 Nutritional Composition of Treated Rice

#### 3.4.1 Protein

Kjeldahl method was conducted to determine the crude protein of treated rice according to Association of Official Analytical Chemists (AOAC) 991.20. The samples (5 g) were firstly digested with 20 mL of 98% (v/v) concentrated H<sub>2</sub>SO<sub>4</sub> and catalysts (0.8 g CuSO<sub>4</sub> and 7 g K<sub>2</sub>SO<sub>4</sub>) using Speed Digester K-436 at 470°C for 90 min until the solutions turned into clear blue-green. After cooling, the digested solutions were distilled via Distillation Unit K-355 for 5 min after adding 32% (w/v) NaOH and distilled water in the ratio of 3:2 to the volume of acid. The distillates were collected into the conical flasks containing 50 mL boric acid with 2 to 3 drops of Methyl red indicator,

turning the pink solutions to clear blue. The clear blue solutions were titrated with 0.25 M H<sub>2</sub>SO<sub>4</sub> and the volume of titrant used to turn blue solutions to pink was recorded. The crude protein content (%) was calculated using Equation 3.1. The protein factor of rice was 5.26 (Fujihara, et al., 2008).

Equation 3.1:

$$\text{Protein content (\%)} = \left[ \frac{V \times z \times c \times f \times \text{Mn}}{\text{Weight of sample}} \times 1000 \right] \times \text{PF} \times 100\%$$

V= volume of titrant used  
z = molar valance factor  
c = concentration of titrant  
f = titrant factor  
Mn = molecular weight of nitrogen  
PF = protein factor

### 3.4.2 Ash

The ash of treated rice was determined as described in AOAC 923.03. The samples (10 g) were wrapped with ashless filter papers placed in pre-dried crucibles with lids and incinerated in the Nabertherm Muffle furnace at 550°C overnight. The incinerated samples were cooled in a desiccator for 1 h before weighing. The ash content (%) was calculated by Equation 3.2.

Equation 3.2:

$$\text{Ash content (\%)} = \frac{\text{Weight of Ash}}{\text{Weight of original sample}} \times 100\%$$

### 3.4.3 Moisture

The moisture of treated rice was determined using oven-drying method (Ahn, et al., 2014). The samples (5 g) were weighed in the pre-dried crucibles with lids and dried at 105°C for 6 h. After cooling in a desiccator, the dried samples were weighed and subsequently reheated and cooled until a constant weight was gained. The moisture content of treated rice was calculated using Equation 3.3.

Equation 3.3:

$$\text{Moisture content (\%)} = \frac{\text{Initial weight of sample} - \text{Final weight of sample}}{\text{Initial weight of sample}} \times 100\%$$

### 3.4.4 Fat

The crude fat of treated rice was analysed as stated in AOAC 945.16 (Nielsen, 2010). The samples (5 g) were wrapped with filter papers and inserted into the thimbles covered with cotton wools. Subsequently, the thimbles were placed in the pre-dried extraction beakers via thimble holders. The fat content of treated rice was extracted using 90 mL of petroleum ether with 2 to 3 pieces of boiling stone via Gerhardt Soxtherm for 2 h. After extraction, the petroleum ether residue in the extraction beakers was evaporated at 105°C for 1 h and weighed after cooling in a desiccator. The extracted fat was reheated for another 15 min for constant weight. The crude fat content of treated rice was calculated by Equation 3.4.

Equation 3.4:

$$\text{Fat content (\%)} = \frac{\text{Weight of extracted fat}}{\text{Weight of sample}} \times 100\%$$

### 3.4.5 Fibre

The crude fibre of treated rice was determined using Gerhardt Manual Fibre Bag system FBS6 based on AOAC 962.09. The samples (2 g) were added into pre-dried fibre bags and firstly digested by 360 mL of 0.13 M H<sub>2</sub>SO<sub>4</sub> for 30 min. After rinsing with hot water, it was repeated with 360 mL of 0.23 M NaOH for another 30 min before drying at 105°C for 4 h. The dried digesta-containing fibre bags were placed into pre-ashed crucibles and incinerated at 600°C for 4 h. The crucibles were then cooled in a desiccator for 1 h before weighing. The crude fibre content of treated rice was calculated by Equation 3.5.

Equation 3.5:

$$\text{Fibre content (\%)} = \frac{[\text{Weight of sample ash} - \text{Weight of empty fibre bag ash}]}{\text{Weight of sample}} \times 100\%$$

### 3.4.6 Carbohydrate

The carbohydrate content of treated rice was calculated by difference method as shown in Equation 3.6 (Tanjor and Hongsprabhas, 2021).

Equation 3.6:

$$\text{Carbohydrate content (\%)} = 100\% - (\% \text{ protein} + \% \text{ ash} + \% \text{ moisture} + \% \text{ fat} + \% \text{ fibre})$$

### 3.4.7 Total Calorie

The total calorie test was performed using IKA C200 bomb calorimeter. The powdered sample (0.5 g) was combusted in the presence of oxygen. The total calorie of samples was calculated via the rising temperature after a complete combustion.

## 3.5 *In Vitro* Digestibility of Treated Rice

The glucose release of treated rice during *in vitro* digestion was conducted as described by Kaur, et al. (2020). The rice samples (2.5 g) were pressed through a sieve and added into volumetric flasks with 30 mL Milli-Q water. The volumetric flasks were immersed in a water bath maintained at 37°C and stirred for 30 min to obtain glucose baseline. In oral phase, 100 µL of 10% (w/v) alpha-amylase in Milli-Q water was added and left for 1 min. Subsequently, 0.8 mL of 1 M HCl was added to achieve pH 2.5 (±0.2). The gastric phase was initiated by adding 1 mL of 10% (w/v) pepsin in 0.05 M

HCl and stirred for another 30 min. Afterward, 2 mL of 1 M NaHCO<sub>3</sub> and 5 mL of 0.2 M acetic buffer (pH 6) were added to neutralise the low pH in gastric phase. The pancreatic enzyme secretion was imitated by adding 5 mL of 10% (w/v) oxgall in Milli-Q water, subsequently volume up to the 55 mL mark with Milli-Q water. After 15 min, the pancreatic digestion was initiated by adding 1 mL of 5% (w/v) pancreatin in acetate buffer and 0.1 mL of amyloglucosidase for 180 min.

After pancreatic digestion, the remaining sample residues were incubated for another 13 h. The pellets were collected and resuspended with 3 mL Milli-Q water after a centrifugation at 3,000 x g for 15 min. Subsequently, the remaining starch was digested using 6 mL of 2 M KOH and 0.1 mL of amyloglucosidase for 45 min. The 0.25 mL aliquots from the baseline, the end of the oral and the gastric phases, and the 20, 60, 90, 120, 180 min and 16 h of pancreatic digestion were collected in 1 mL absolute ethanol-containing tubes.

The supernatants of ethanolic aliquots were collected after a centrifugation at 1,000 x g for 10 min. Fifty microlitre supernatants were hydrolysed with 0.25 mL of 1% (v/v) amyloglucosidase in 0.1 M acetate buffer (pH 5.2) at 37°C for 10 min. Subsequently, the hydrolysed samples were added with 0.75 mL 3,5-dinitrosalicylic acid (DNS) mixture (0.5 mg/mL glucose, 4 M NaOH, and DNS reagent at ratio of 1:1:5) and heated at 100°C for 15 min. The DNS reagent was prepared by dissolving 1 g 3,5-dinitrosalicylic acid and 30 g potassium sodium tartrate in 20 mL of 2 N NaOH and topped up to 100 mL with Milli-Q water. The absorbances of



samples and glucose standards were determined at 540 nm against blank using FLUOstar Omega microplate reader.

The glucose content was converted to starch by stoichiometric constant of 0.9. Rapidly digestible starch was calculated from the glucose released at the first 20 min of pancreatic digestion; SDS was the glucose difference between the 20 and 120 min of pancreatic digestion; RS was the glucose released after 16 h of pancreatic phase.

### **3.6 Prebiotic Potential of Treated Rice**

#### **3.6.1 *Lactocaseibacillus* Strains and Culture Conditions**

The probiotic strains of *Lactocaseibacillus casei* and *Lactocaseibacillus rhamnosus* were isolated from the commercial single strain probiotic powder supplements. The probiotic powders were firstly activated in 10 mL of 0.1% (w/v) sterile peptone water for 30 min and plated in MRS agar at 37°C for 3 days. The single colony of probiotics was isolated and incubated in 10 mL of MRS broth at 37°C for 24 h and stored as bacterial stocks. All probiotics strains were sub-cultured three times prior to experimental use.

### **3.6.2 Quantification of Oligosaccharide and Growth Curve of Probiotics**

The prebiotic potential of treated sample was assessed as described by Ng, et al. (2008). Firstly, 2 mL of 20% (w/v) smashed rice starch solutions were added into 18 mL MRS broths containing *L. casei* and *L. rhamnosus* with optical density (OD) of 0.03 at 600 nm. Afterward, the growth of probiotics and the oligosaccharide concentration were observed every 4 h over 24 h.

The oligosaccharide concentration of sample was calculated from the difference between the reducing sugar concentration of non-hydrolyse and hydrolysed samples. The 3 mL aliquots from 4 h intervals were firstly centrifuged at 4,000 x *g* at 4°C for 5 min. Subsequently, 1 mL of supernatant was added with 3 mL DNS mixture and heated at 100°C for 15 min. Another 1 mL supernatant was hydrolysed by 100 µL 10% (w/v) alpha-amylase and amyloglucosidase for 60 min before mixing with DNS mixture. The absorbances of the samples and standards were determined at 540 nm against blank, while the growth of probiotics was measured at 600 nm against blank.

### **3.7 Scanning Electron Microscopy of Starch Granule**

Scanning electron microscopy analysis was carried out as described by Golding, et al. (2016). The samples were dried at 40°C overnight before observation. The rice grain was fixed on carbon adhesive tape before platinum

coating by Jeol Auto Fine Coater. The platinum-coated samples were subjected to Field Emission Scanning Electron Microscope JSM-6701F. The acceleration voltage was set to 4 kV. The microstructure of the outer layer of treated rice was observed at 350-500x magnification.

### **3.8 Statistical Analysis**

All analyses were conducted in duplicates with three separate determinations (n=6). All data were analysed using one-way analysis of variance (ANOVA) with post hoc Tukey test at a 95% CI, conducted by IBM SPSS Statistics software version 28 (International Business Machines Co, USA).

## CHAPTER 4

### RESULT

#### 4.1 Meta-analysis

##### 4.1.1 Resistant Starch

A significant ( $p < 0.00001$ ) positive MD of 4.17 (95% CI: 2.77 to 5.56) was observed in the RS of white rice upon different retrogradation durations at (Figure 4.1). Nevertheless, heterogeneous results were reported in the pooled studies. Specifically, the individual results of “Chiu & Stewart (2013)-L(1)-72h” and “Hsu et al. (2015)-L(4)-72h” showed heterogeneities as demonstrated in the funnel plot (Figure 4.2). The asymmetrical distribution of dots in the funnel plot also suggests that the presence of publication bias in the pooled studies.

In the subgroup analysis, 24 h retrogradation showed a higher MD compared to that of 72 h retrogradation, 4.29 (95% CI: 2.05 to 6.54,  $p = 0.0002$ ) and 3.97 (95% CI: 2.42 to 5.53,  $p < 0.00001$ ), respectively (Figure 4.3 and 4.5). Heterogeneity was also detected in both subgroup analyses. Obviously, “Hsu et al. (2015)-L(2)-24h” and “Jayawardena et al. (2017)-M-24h” were deviated from the overall effect estimate line (Figure 4.4). Also, the deviations of “Chiu

& Stewart (2013)-L(1)-72h” and “Hsu et al. (2015)-L(4)-72h” were demonstrated in the funnel plot (Figure 4.6).

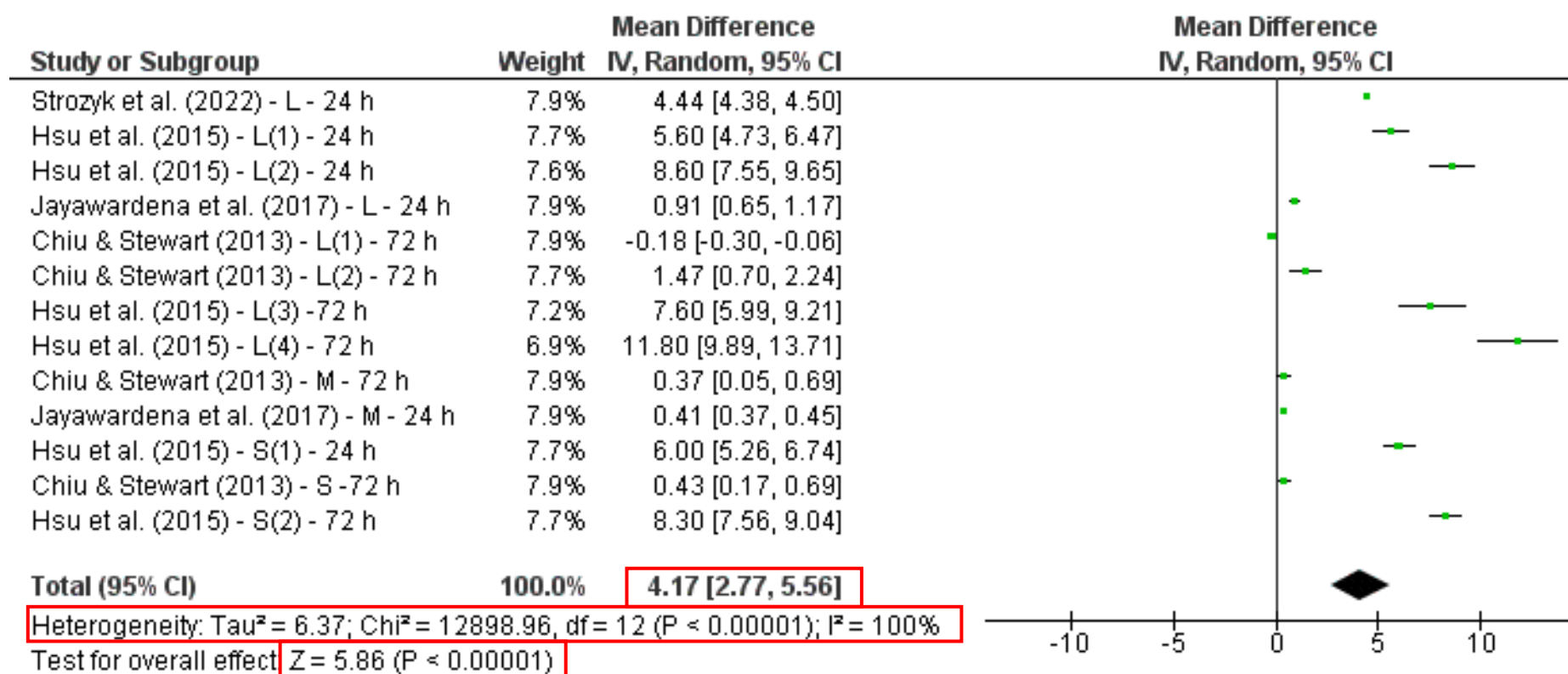


Figure 4.1: Forest plot of the effect of different retrogradation durations on the resistant starch of white rice

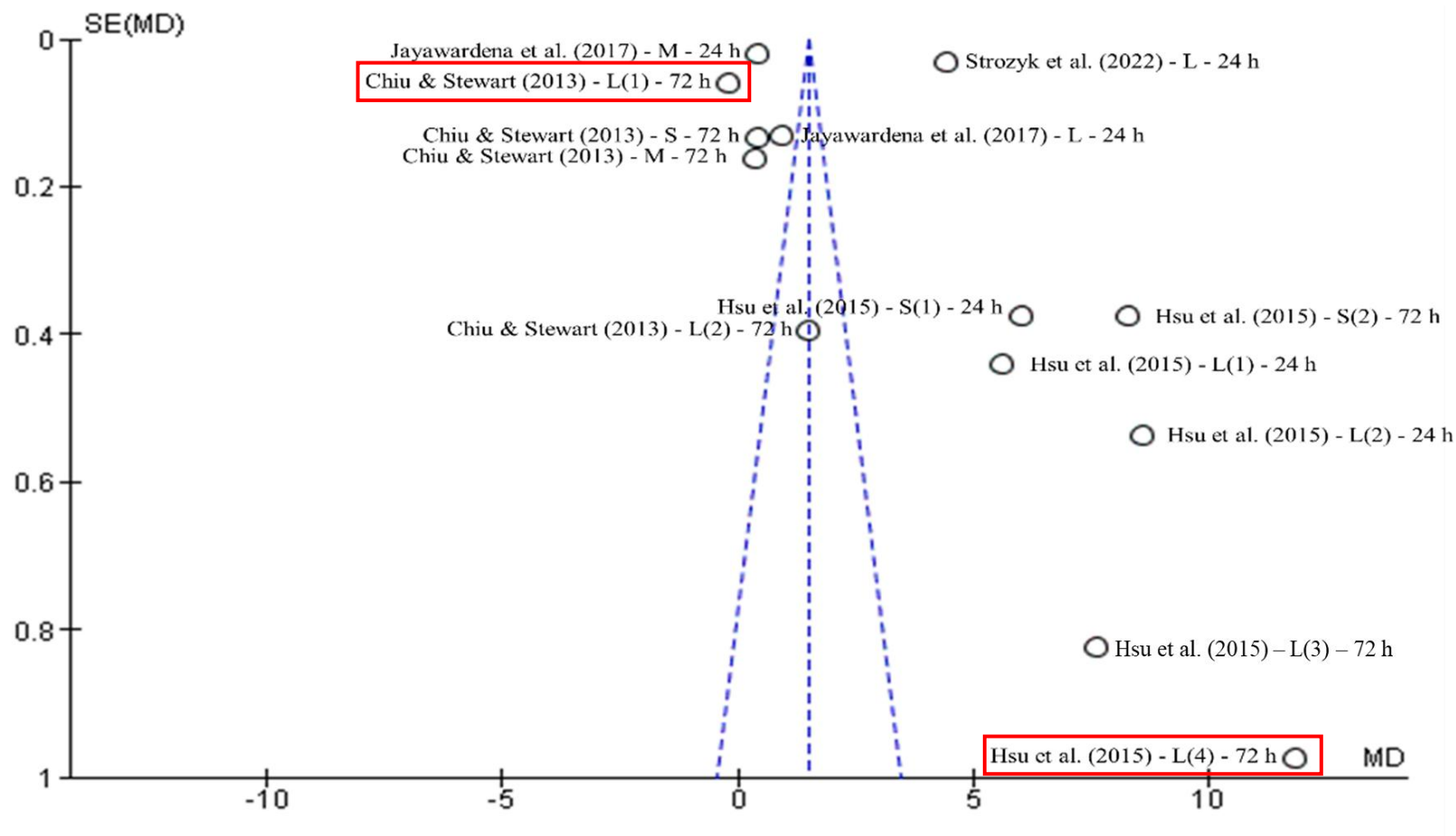


Figure 4.2: Funnel plot of the effect of different retrogradation durations on the resistant starch of white rice

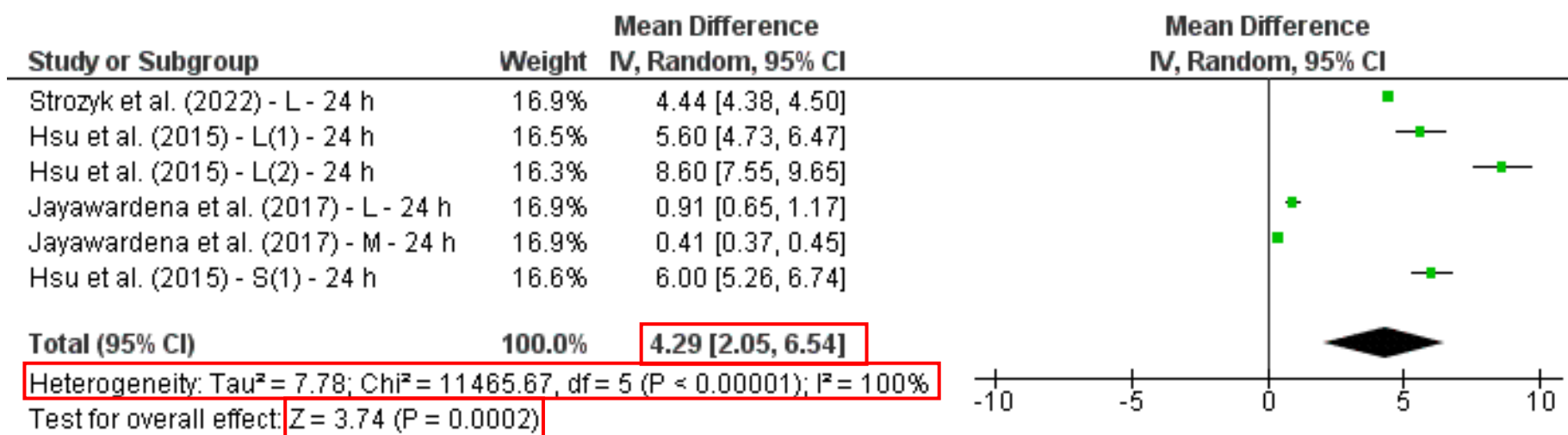


Figure 4.3: Forest plot of the effect of 24 hours retrogradation on the resistant starch of white rice



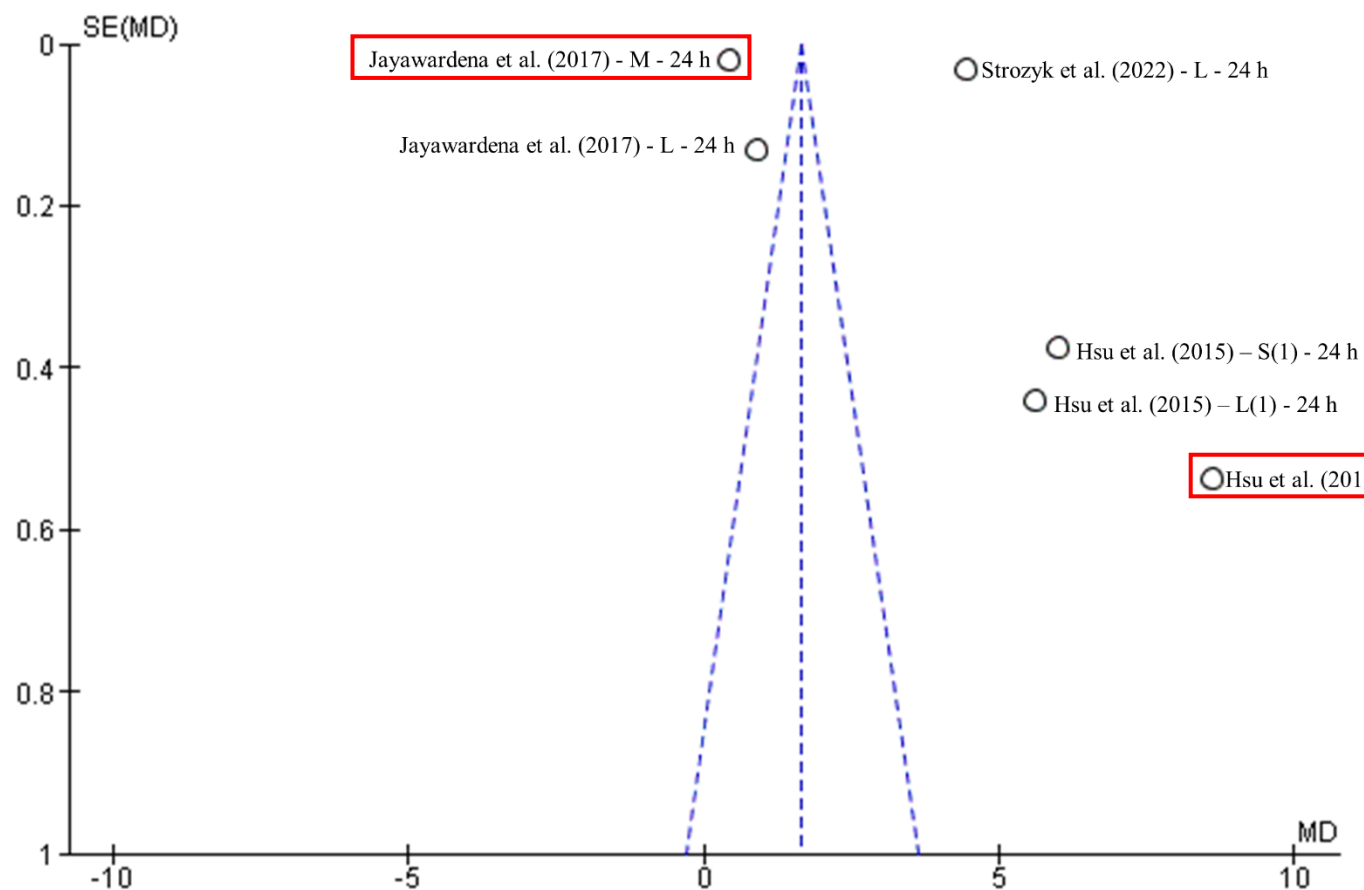


Figure 4.4: Funnel plot of the effect of 24 hours retrogradation on the resistant starch of white rice

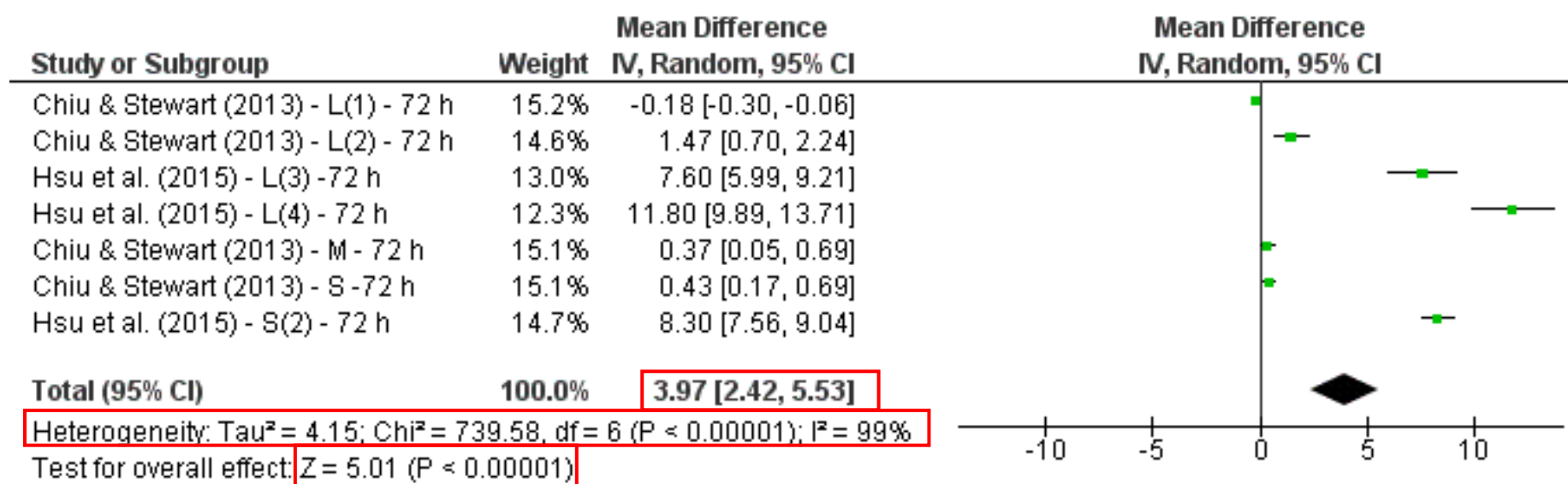
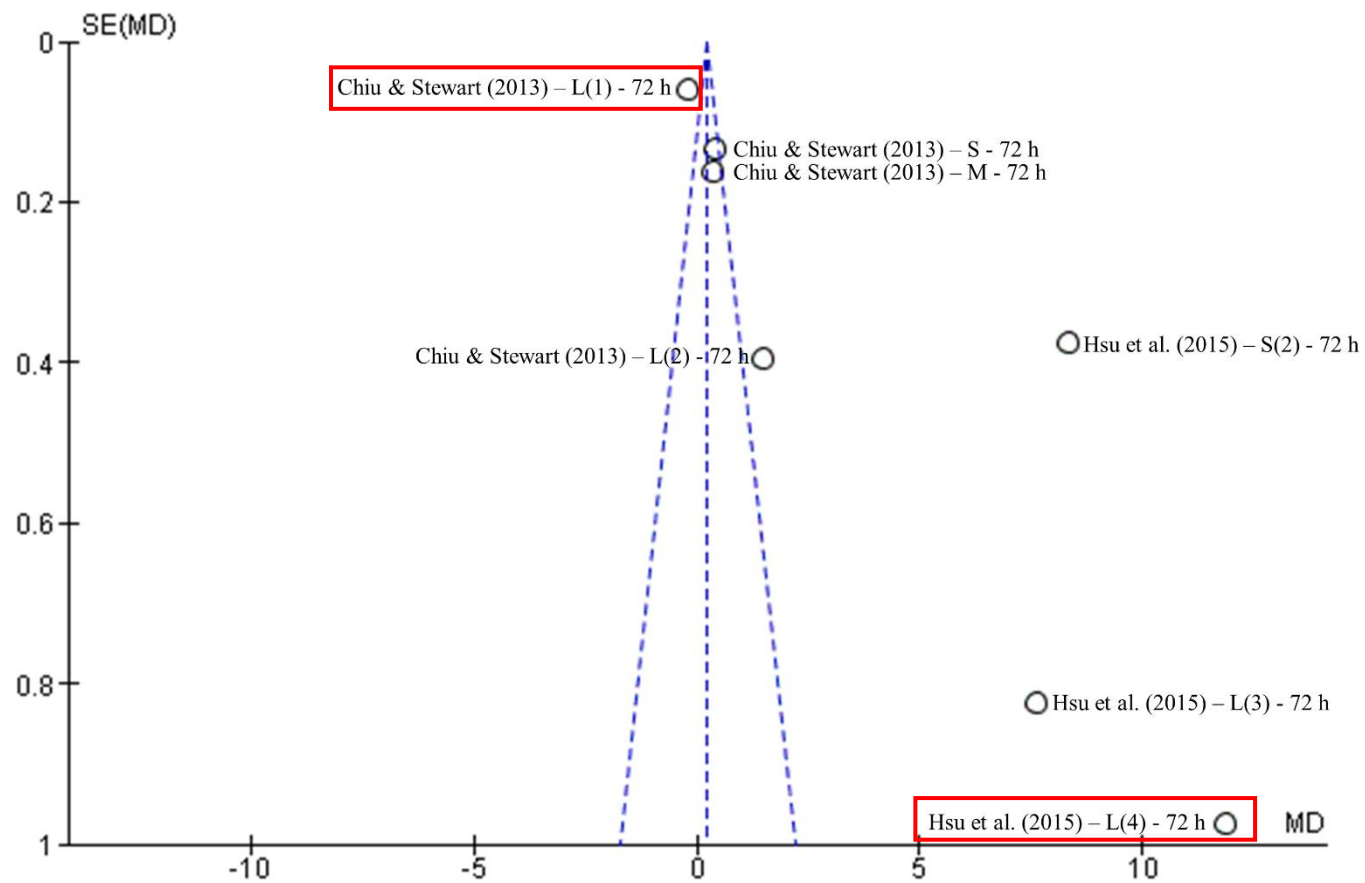


Figure 4.5: Forest plot of the effect of 72 hours retrogradation on the resistant starch of white rice



**Figure 4.6: Funnel plot of the effect of 72 hours retrogradation on the resistant starch of white rice**

### 4.1.2 Slowly Digestible Starch

There was no statistically significant MD in the SDS of white rice upon retrogradation treatment, i.e. -0.82 (95% CI: -5.81 to 4.16,  $p=0.75$ ) for different retrogradation durations (Figure 4.7); -0.35 (95% CI: -8.56 to 7.86,  $p=0.93$ ) for 24 h retrogradation (Figure 4.9); and -1.22 (95% CI: -6.19 to 3.75,  $p=0.63$ ) for 72 h retrogradation (Figure 4.11). Heterogeneities were detected across the pooled results, coincided with the deviations of individual studies in the funnel plots (Figures 4.8, 4.10, and 4.12). Especially, the individual results of “Hsu et al. (2015)-L(4)-72h” and “Hsu et al. (2015)-S(1)-24h” were deviated from the overall effect estimate line in the funnel plot (Figure 4.8). Additionally, publication bias was detected in the pooled studies.

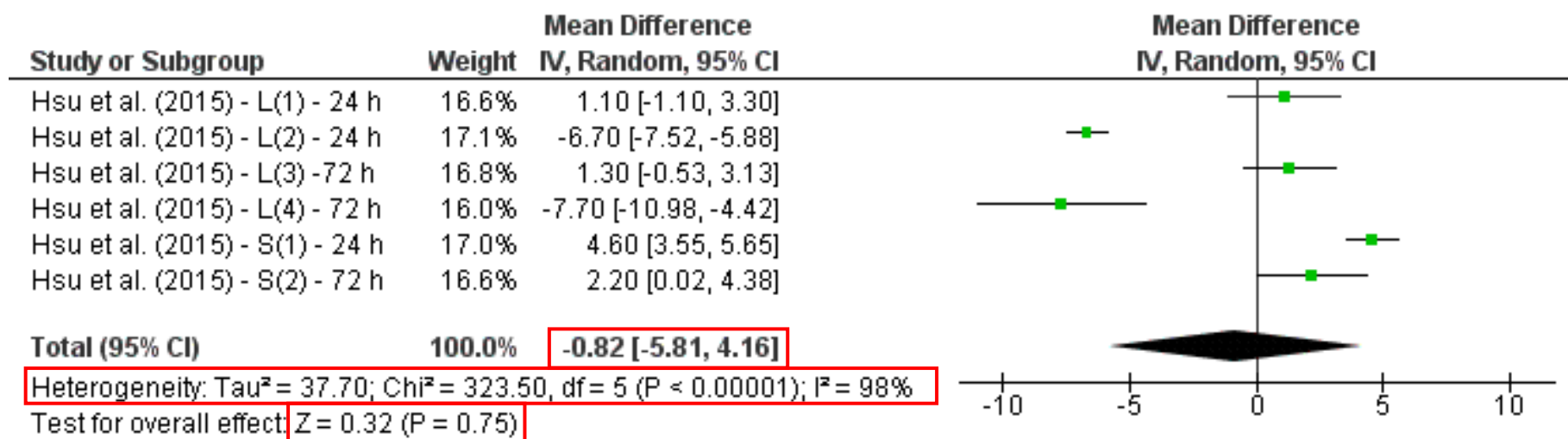
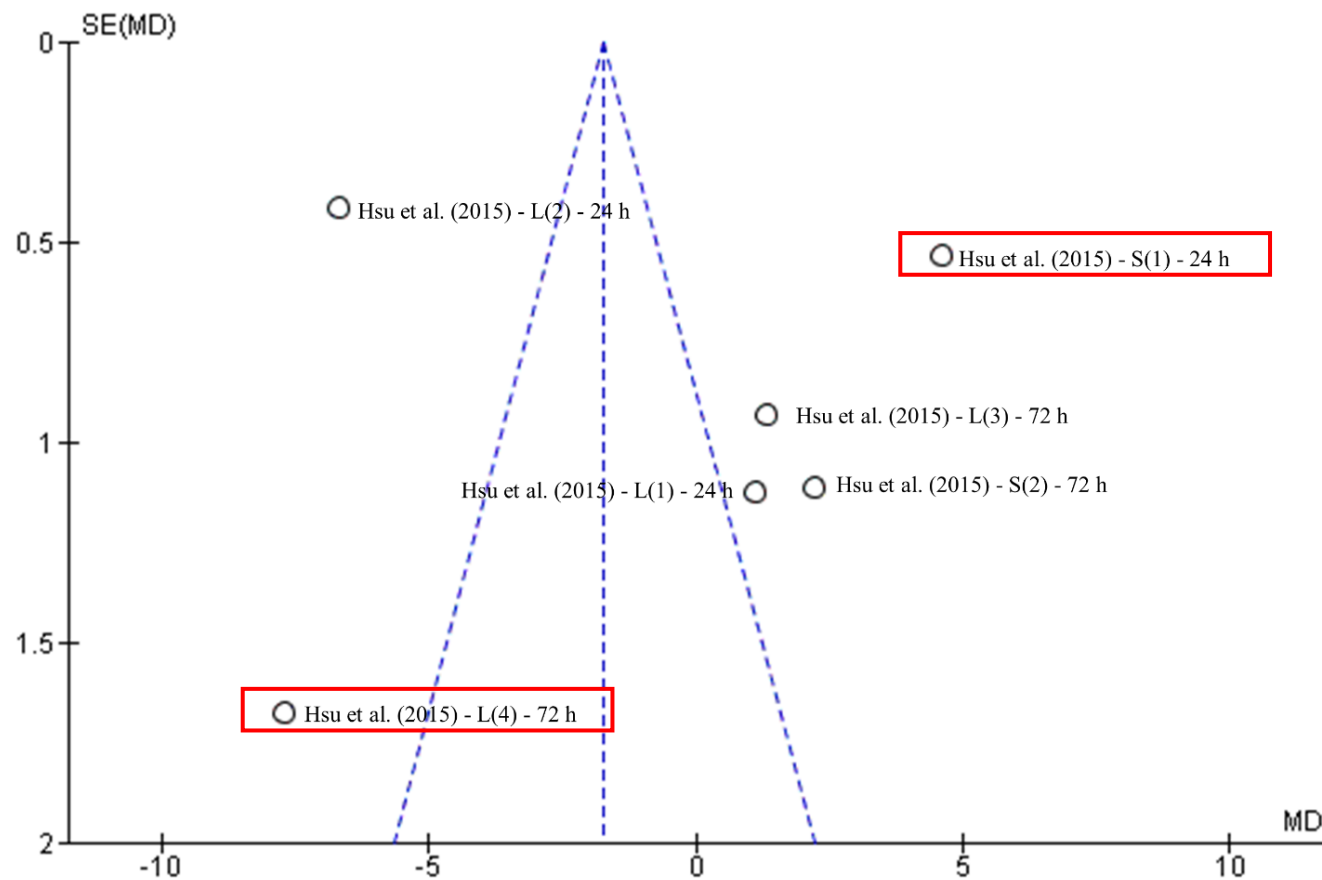


Figure 4.7: Forest plot of the effect of different retrogradation durations on the slowly digestible starch of white rice



**Figure 4.8: Funnel plot of the effect of different retrogradation durations on the slowly digestible starch of white rice**

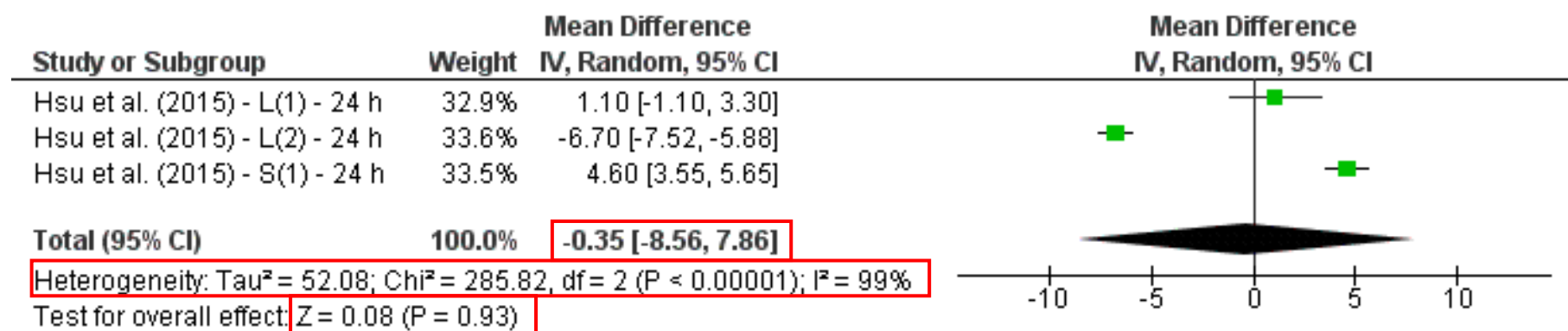
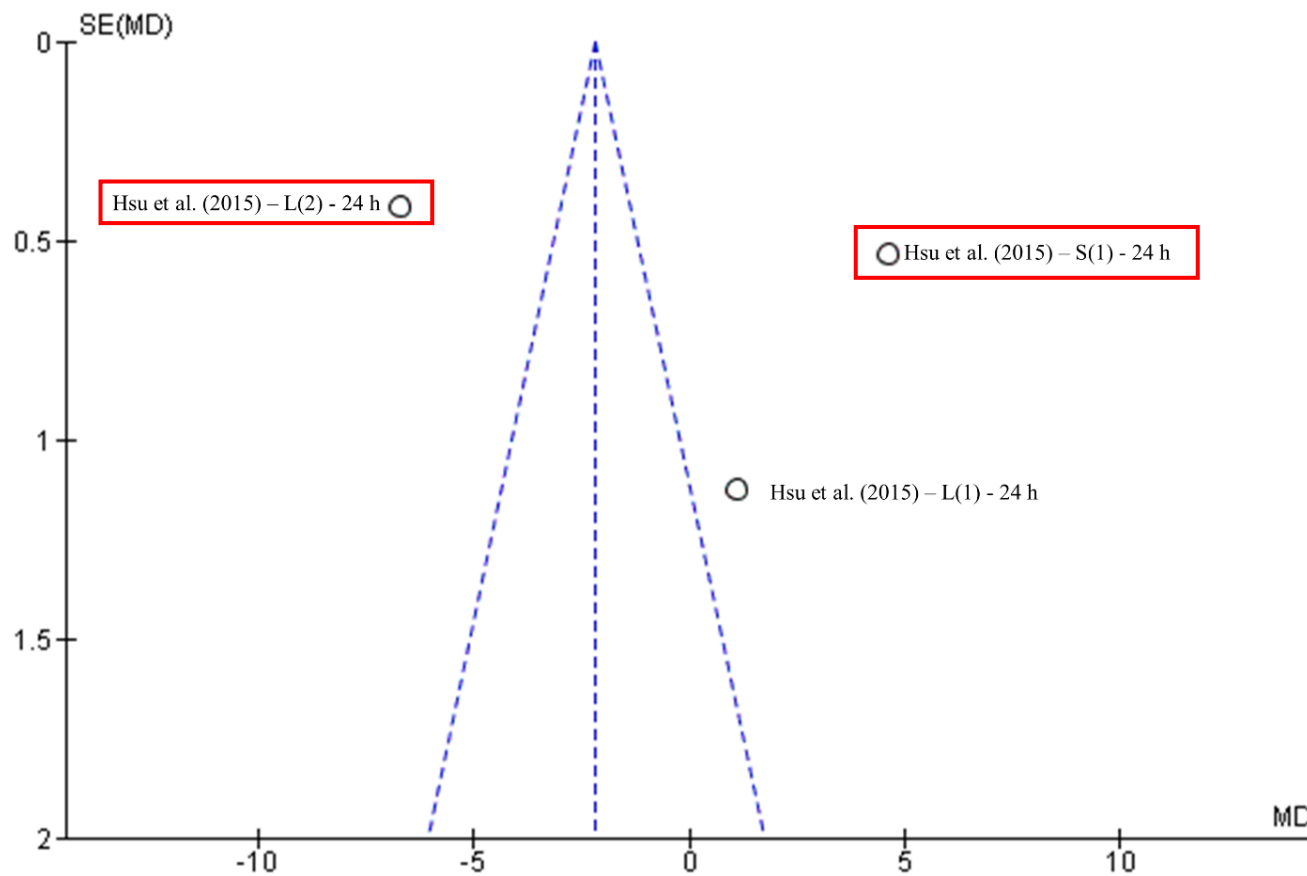


Figure 4.9: Forest plot of the effect of 24 hours retrogradation on the slowly digestible starch of white rice



**Figure 4.10: Funnel plot of the effect of 24 hours retrogradation on the slowly digestible starch of white rice**



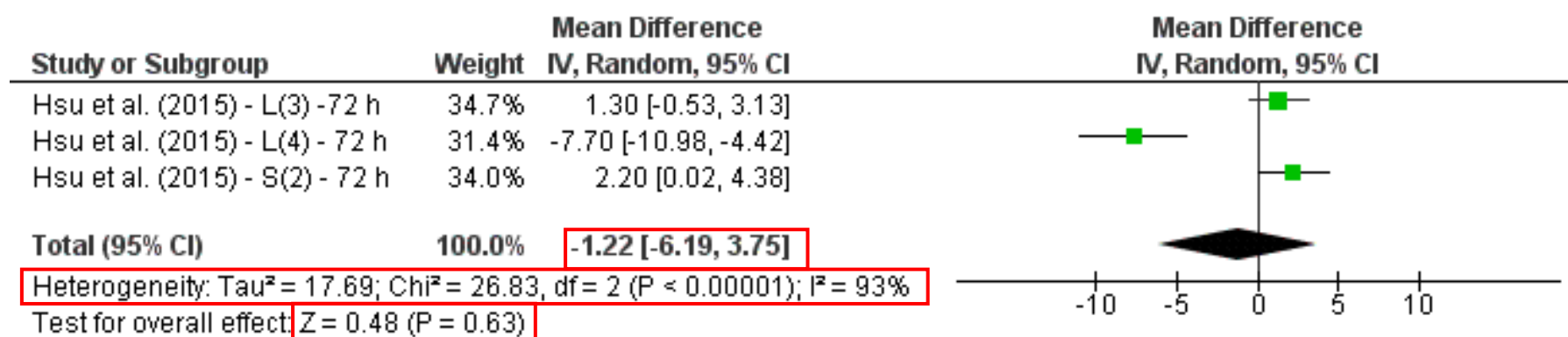
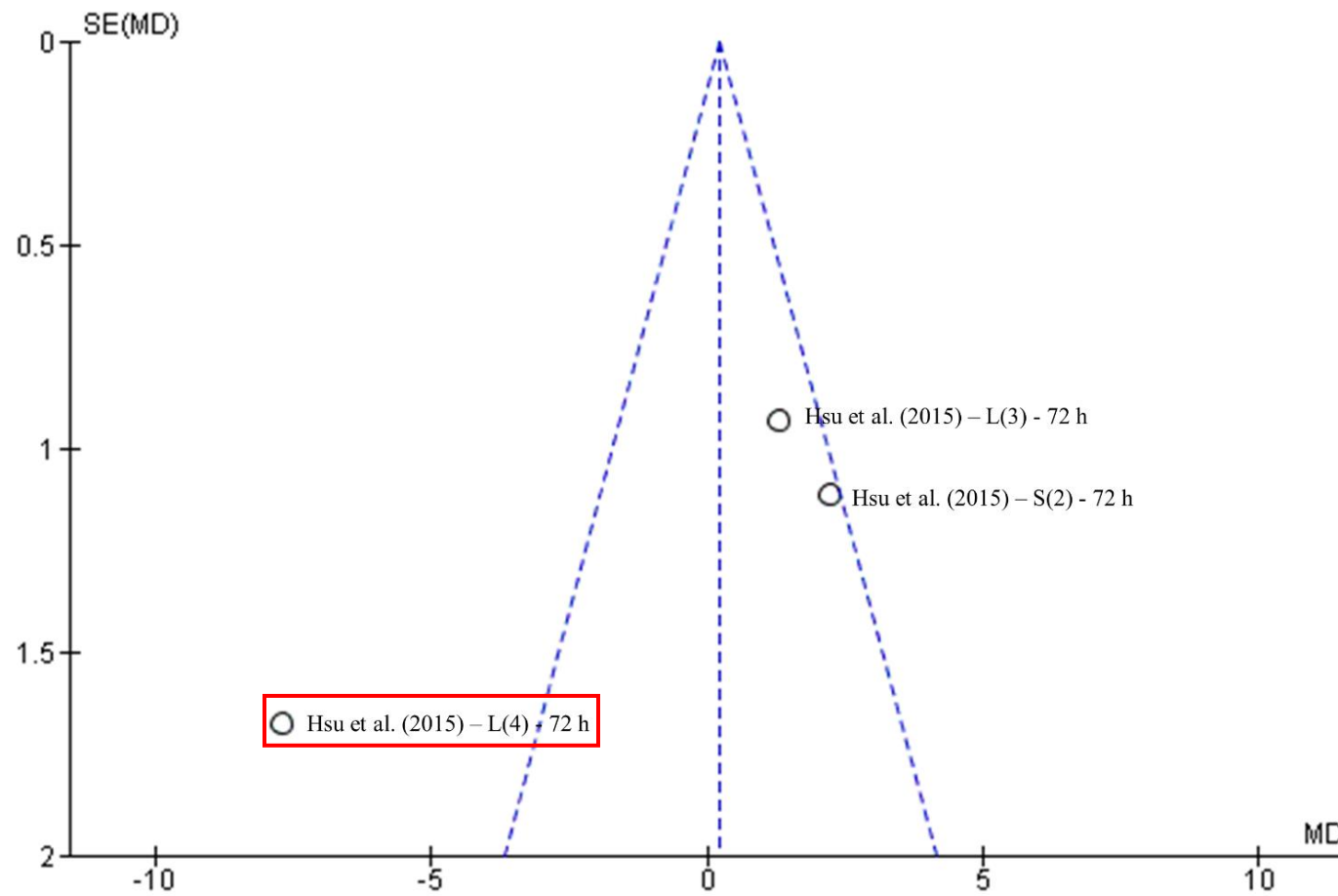


Figure 4.11: Forest plot of the effect of 72 hours retrogradation on the slowly digestible starch of white rice



**Figure 4.12: Funnel plot of the effect of 72 hours retrogradation on the slowly digestible starch of white rice**

### 4.1.3 Rapidly Digestible Starch

A negative MD was exhibited in the RDS of white rice upon different durations retrogradation, i.e. -7.09 (95% CI: -10.24 to -3.94) at  $p < 0.0001$  (Figure 4.13). Similarly, heterogeneity was reported across the pooled results, with a deviation of the individual result “Hsu et al. (2015)-L(4)-72h” observed in the funnel plot (Figure 4.14).

Comparing subgroup meta-analyses, 72 h retrogradation showed a higher negative MD, i.e. -6.29 (95% CI: -11.21 to -1.37,  $p = 0.01$ ) for 24 h retrogradation (Figure 4.15) and -8.16 (95% CI: -10.93 to -5.40,  $p < 0.00001$ ) for 72 h retrogradation (Figure 4.17). Heterogeneity was detected in the subgroup analyses. This was in tandem with the deviation of individual studies in the funnel plots (Figures 4.16 and 4.18). Specifically, the results of “Hsu et al. (2015)-L(2)-24h” and “Hsu et al. (2015)-S1-24h” were deviated from the overall effect estimate line in 24 h retrogradation funnel plot, while “Hsu et al. (2015)-L(4)-72h” in 72 h retrogradation funnel plot.

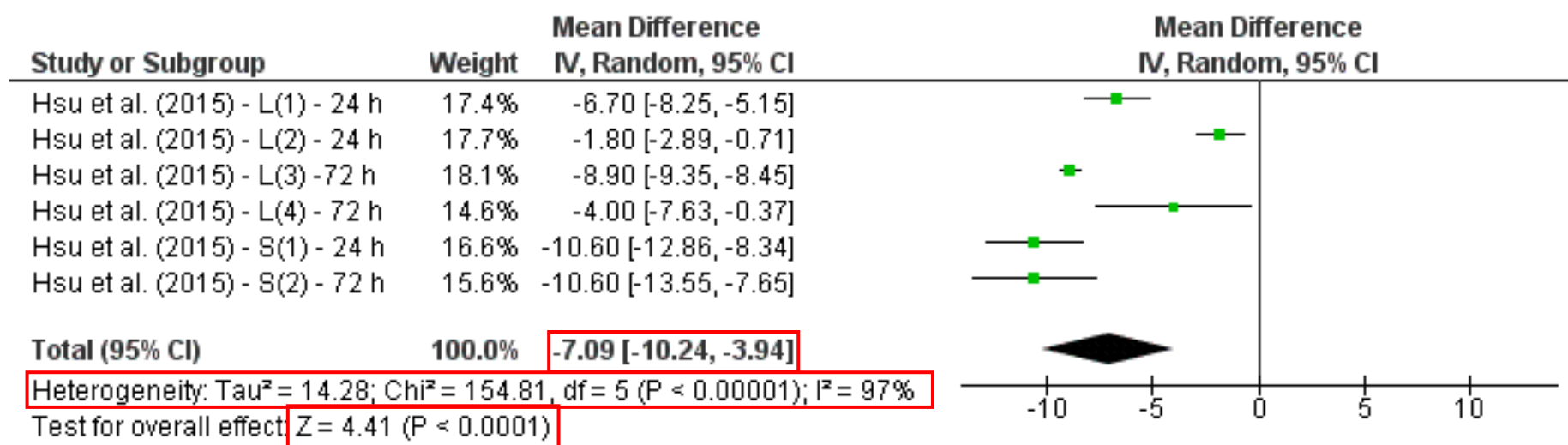
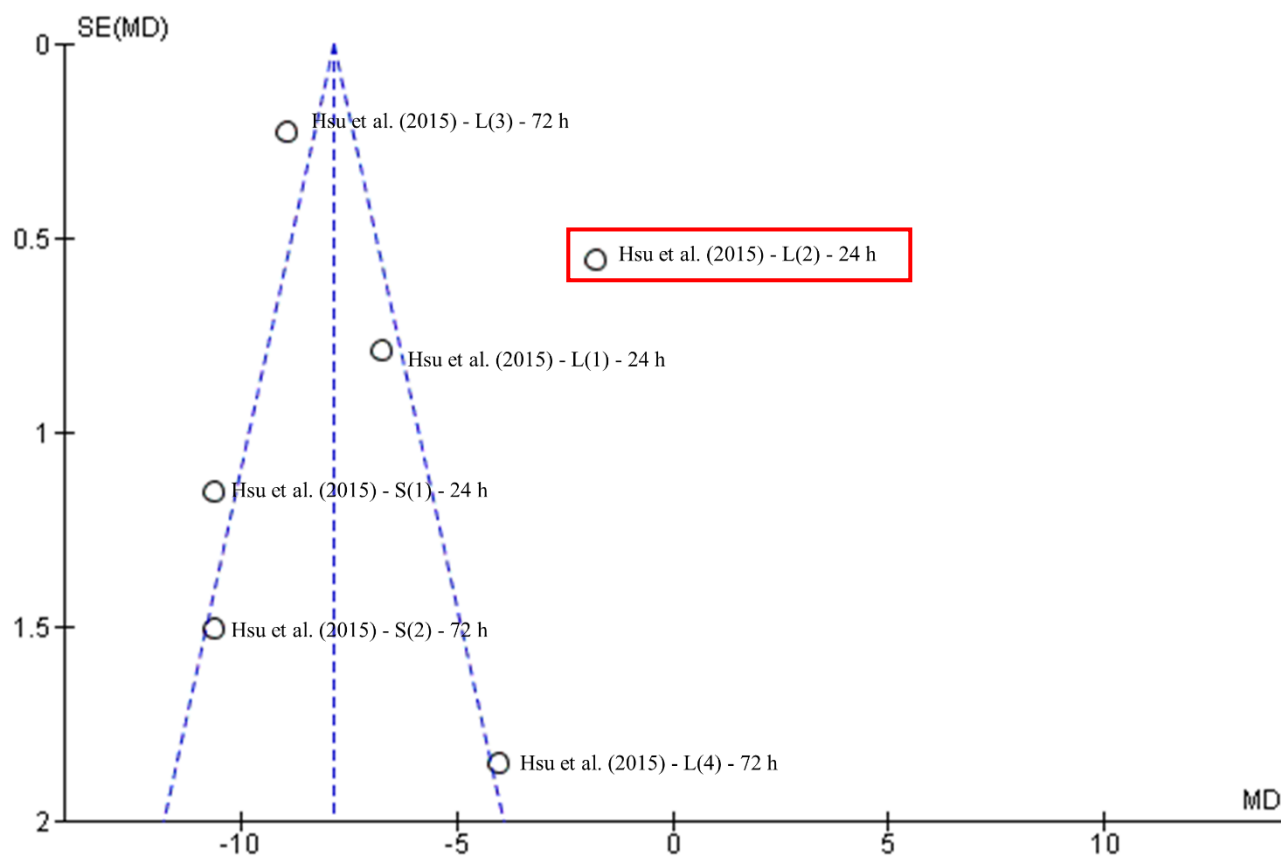


Figure 4.13: Forest plot of the effect of different retrogradation durations on the rapidly digestible starch of white rice



**Figure 4.14: Funnel plot of the effect of different retrogradation durations on the rapidly digestible starch of white rice**

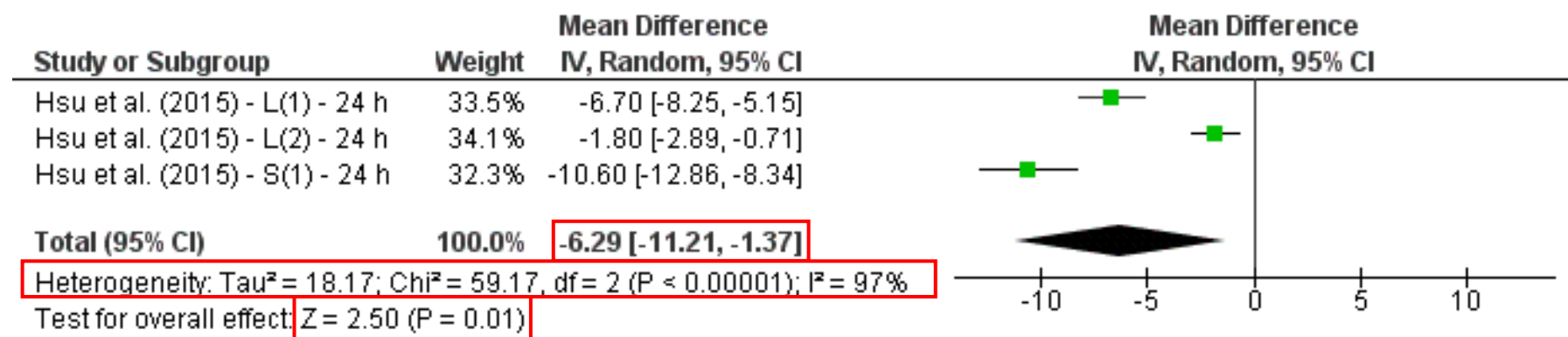


Figure 4.15: Forest plot of the effect of 24 hours retrogradation on the rapidly digestible starch of white rice

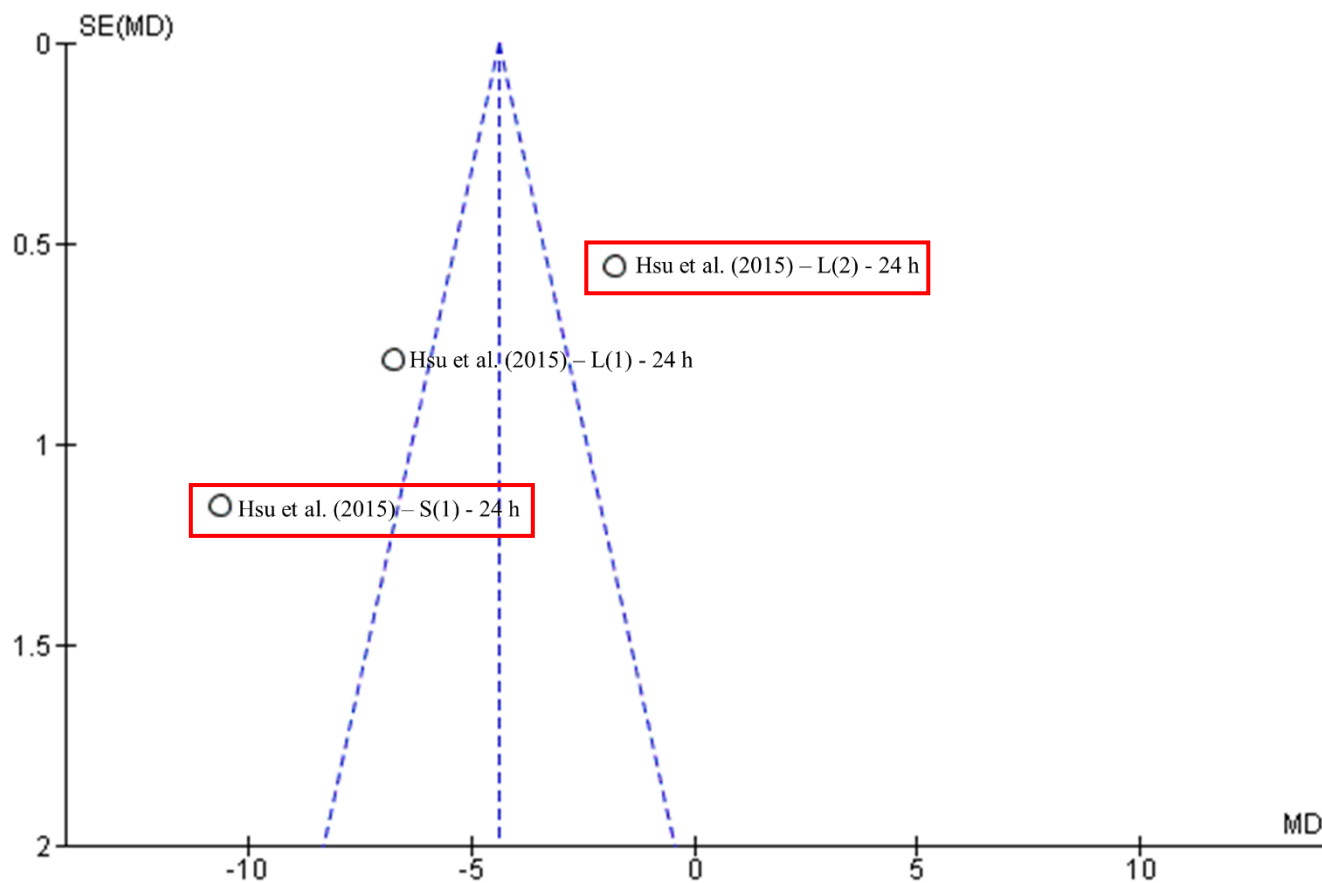


Figure 4.16: Funnel plot of the effect of 24 hours retrogradation on the rapidly digestible starch of white rice

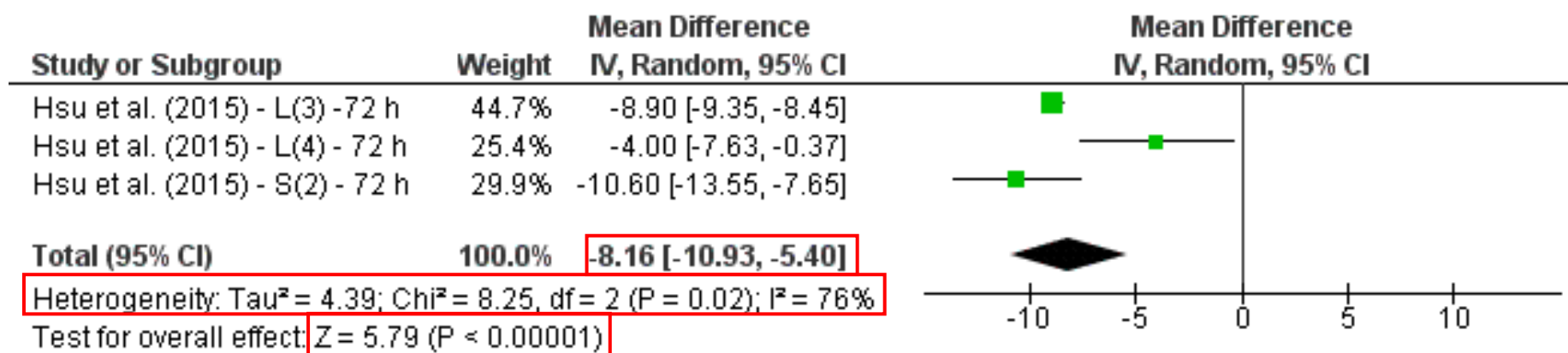
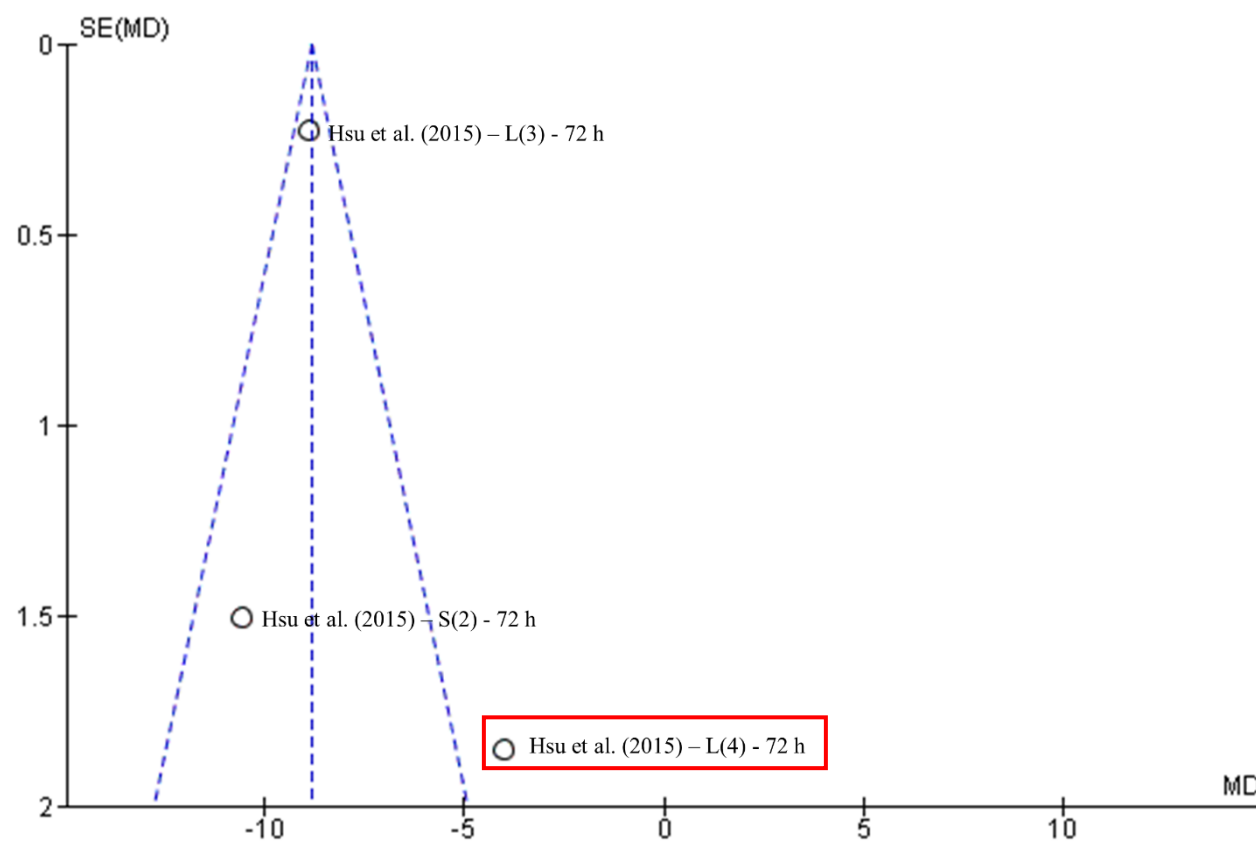


Figure 4.17: Forest plot of the effect of 72 hours retrogradation on the rapidly digestible starch of white rice





**Figure 4.18: Funnel plot of the effect of 72 hours retrogradation on the rapidly digestible starch of white rice**

## **4.2 Nutritional Composition of Treated Rice**

### **4.2.1 Protein**

There was no statistically significant ( $p>0.05$ ) difference in the crude protein content of brown and white rice upon different oil treatments in three cooking methods (Table 4.1). Similar observation was also noted across different cooking methods when comparing the oil treatments and the control groups individually. These statistics revealed that both oil treatments and cooking methods have a minimal impact on the protein content of brown and white rice.

### **4.2.2 Ash**

The ash content of brown rice, including both control and treatment groups, was higher than white rice across all cooking methods (Table 4.1). However, considering both brown and white rice, there was no significant ( $p>0.05$ ) difference observed between the control and the two oil-treated rice. Moreover, the difference between cooking methods in the control and treatment groups was also not significant ( $p>0.05$ ). This implies that the ash content of brown and white rice was less affected by oil treatments and three cooking methods.

**Table 4.1: The effect of different cooking methods and oil treatments on the nutritional composition of the control groups and the treated brown and white rice**

	Method	White rice			Brown rice		
		Control	Palm oil	Coconut oil	Control	Palm oil	Coconut oil
Protein (%)	A	2.62±0.27 <sup>Aa</sup>	2.65±0.35 <sup>Aa</sup>	2.69±0.41 <sup>Aa</sup>	2.67±0.35 <sup>Aa</sup>	2.77±0.24 <sup>Aa</sup>	2.80±0.38 <sup>Aa</sup>
	B	2.66±0.38 <sup>Aa</sup>	2.68±0.33 <sup>Aa</sup>	2.71±0.36 <sup>Aa</sup>	2.73±0.28 <sup>Aa</sup>	2.79±0.37 <sup>Aa</sup>	2.81±0.21 <sup>Aa</sup>
	C	2.63±0.22 <sup>Aa</sup>	2.67±0.44 <sup>Aa</sup>	2.70±0.46 <sup>Aa</sup>	2.69±0.32 <sup>Aa</sup>	2.77±0.38 <sup>Aa</sup>	2.80±0.39 <sup>Aa</sup>
Ash (%)	A	0.12±0.01 <sup>Ba</sup>	0.11±0.01 <sup>Ba</sup>	0.12±0.01 <sup>Ba</sup>	0.40±0.01 <sup>Aa</sup>	0.41±0.02 <sup>Aa</sup>	0.41±0.01 <sup>Aa</sup>
	B	0.12±0.01 <sup>Ba</sup>	0.12±0.01 <sup>Ba</sup>	0.12±0.01 <sup>Ba</sup>	0.39±0.01 <sup>Aa</sup>	0.39±0.03 <sup>Aa</sup>	0.39±0.03 <sup>Aa</sup>
	C	0.12±0.01 <sup>Ba</sup>	0.12±0.01 <sup>Ba</sup>	0.12±0.01 <sup>Ba</sup>	0.40±0.02 <sup>Aa</sup>	0.42±0.02 <sup>Aa</sup>	0.41±0.03 <sup>Aa</sup>
Moisture (%)	A	68.88±0.09 <sup>Ab</sup>	66.62±0.23 <sup>Cb</sup>	66.49±0.17 <sup>Cb</sup>	67.31±0.52 <sup>Ba</sup>	66.61±0.36 <sup>Cb</sup>	66.71±0.19 <sup>Cb</sup>
	B	70.78±0.37 <sup>Aa</sup>	68.48±0.33 <sup>Ba</sup>	68.38±0.29 <sup>Ba</sup>	67.67±0.16 <sup>Ca</sup>	67.05±0.03 <sup>Da</sup>	67.14±0.08 <sup>Da</sup>
	C	66.47±0.29 <sup>Ac</sup>	65.93±0.06 <sup>Bc</sup>	64.84±0.13 <sup>Dc</sup>	66.70±0.18 <sup>Ab</sup>	65.32±0.10 <sup>Cc</sup>	65.35±0.17 <sup>Cc</sup>
Fat (%)	A	0.10±0.01 <sup>Da</sup>	1.07±0.03 <sup>Cb</sup>	1.09±0.04 <sup>Cb</sup>	1.85±0.10 <sup>Ba</sup>	3.30±0.12 <sup>Ab</sup>	3.17±0.21 <sup>Aa</sup>
	B	0.10±0.01 <sup>Ca</sup>	1.71±0.07 <sup>Ba</sup>	1.73±0.13 <sup>Ba</sup>	1.82±0.07 <sup>Ba</sup>	3.69±0.20 <sup>Aa</sup>	3.49±0.31 <sup>Aa</sup>
	C	0.10±0.01 <sup>Da</sup>	1.05±0.15 <sup>Cb</sup>	1.07±0.08 <sup>Cb</sup>	1.86±0.09 <sup>Ba</sup>	3.66±0.21 <sup>Aa</sup>	3.65±0.49 <sup>Aa</sup>
Fibre (%)	A	0.21±0.01 <sup>Ba</sup>	0.21±0.01 <sup>Ba</sup>	0.21±0.01 <sup>Ba</sup>	1.10±0.04 <sup>Aa</sup>	1.12±0.03 <sup>Aa</sup>	1.14±0.04 <sup>Aa</sup>
	B	0.20±0.01 <sup>Ba</sup>	0.21±0.01 <sup>Ba</sup>	0.21±0.01 <sup>Ba</sup>	1.09±0.06 <sup>Aa</sup>	1.08±0.05 <sup>Aa</sup>	1.09±0.07 <sup>Aa</sup>
	C	0.21±0.01 <sup>Ba</sup>	0.21±0.01 <sup>Ba</sup>	0.21±0.01 <sup>Ba</sup>	1.09±0.04 <sup>Aa</sup>	1.13±0.05 <sup>Aa</sup>	1.14±0.02 <sup>Aa</sup>
Carbohydrate (%)	A	28.07±0.25 <sup>Bb</sup>	29.34±0.37 <sup>Ab</sup>	29.40±0.30 <sup>Ab</sup>	26.67±0.63 <sup>Cab</sup>	25.80±0.59 <sup>Db</sup>	25.81±0.43 <sup>Db</sup>
	B	26.14±0.53 <sup>Ac</sup>	26.79±0.29 <sup>Ac</sup>	26.85±0.20 <sup>Ac</sup>	26.30±0.44 <sup>Ab</sup>	25.01±0.58 <sup>Bb</sup>	25.04±0.44 <sup>Bb</sup>
	C	30.47±0.21 <sup>ABa</sup>	30.02±0.34 <sup>Ba</sup>	31.08±0.48 <sup>Aa</sup>	27.26±0.46 <sup>Ca</sup>	26.70±0.40 <sup>Ca</sup>	26.78±0.84 <sup>Ca</sup>
Calorie (kcal/g)	A	4.00±0.02 <sup>Ca</sup>	4.06±0.03 <sup>BCa</sup>	4.06±0.04 <sup>BCa</sup>	4.11±0.03 <sup>ABa</sup>	4.15±0.05 <sup>Aa</sup>	4.16±0.07 <sup>Aa</sup>
	B	3.97±0.05 <sup>Da</sup>	4.00±0.05 <sup>CDb</sup>	4.00±0.05 <sup>CDa</sup>	4.07±0.03 <sup>BCa</sup>	4.13±0.04 <sup>ABa</sup>	4.17±0.05 <sup>Aa</sup>
	C	3.88±0.05 <sup>Eb</sup>	3.95±0.01 <sup>Db</sup>	4.02±0.04 <sup>Ca</sup>	4.11±0.02 <sup>Ba</sup>	4.19±0.04 <sup>Aa</sup>	4.19±0.04 <sup>Aa</sup>

<sup>a</sup> The data were expressed as mean ± standard deviation (n=6). Means in the same column followed by different lowercase letters were significantly (p<0.05) different in the same oil treatment. Means in the same row followed by different uppercase letters were significantly (p<0.05) different in the same cooking method.

### 4.2.3 Moisture

The moisture content of brown and white rice was varied against different oil treatments also cooking methods (Table 4.1). Both oil-treated rice demonstrated a lower moisture content than that of the control groups in all cooking methods. Notably, a comparable moisture content was observed in both rice upon two oil treatments by Method A. However, white rice control exhibited a higher moisture content compared to brown rice control. As such, less moisture content was retained in white rice upon oil treatment. In Method B, brown rice was observed to have lower moisture content than white rice, including the control and the oil-treated rice. On the other hand, the lowest moisture content was determined in white rice treated with CO, whereas white rice added with PO retained the most moisture content among Method C-treated samples.

Regardless of the control groups or the oil treatment groups, Method C evaporated the most moisture content in both types of rice, followed by Methods A and B. This also reflects that both oil treatments did not induce significant ( $p>0.05$ ) changes in the moisture evaporation of brown and white rice resulted from the three cooking methods.

#### **4.2.4 Fat**

The addition of oil to brown and white rice increased the crude fat content of treated rice in contrast to the controls (Table 4.1). Nonetheless, it did not contribute a spike in the crude fat content of rice, ranging from 1.05% to 1.73% in white rice and 3.17% to 3.69% in brown rice upon two oil treatments incorporated with different cooking methods. In comparison, brown rice exhibited a higher crude fat content than white rice across all cooking methods, regardless of with or without oil treatments.

Both types of rice retained the highest oil when cooked with Method B. Additionally, similar crude fat content was observed in white rice treated by Methods A and C upon oil treatments. Contrarily, brown rice showed a lower oil uptake in Method A following PO treatment. However, in CO treatment, there was no significant ( $p>0.05$ ) difference in the crude fat content of brown rice against three cooking methods.

#### **4.2.5 Fibre**

The crude fibre content of brown rice was higher than white rice over all cooking methods (Table 4.1). The crude fibre content of brown rice was varied from 1.08% to 1.14%, while white rice exhibited a lower range of 0.20% to 0.21%. Moreover, it was notable that both oil treatment and cooking

method have a minimal impact on the crude fibre content of both rice, as no significant ( $p>0.05$ ) difference was detected between treatments and controls.

#### **4.2.6 Carbohydrate**

Oil treatment induced different impacts on the carbohydrate content of brown and white rice in three cooking methods (Table 4.1). It resulted in an increased carbohydrate content in white rice, conversely showed a contrast effect on brown rice in Method A. While in Method B, the carbohydrate content of oil-treated brown rice was decreased as compared to the control. However, an insignificant ( $p>0.05$ ) difference was shown in the carbohydrate content of white rice upon oil treatments. Furthermore, PO treatment reduced the carbohydrate content of white rice, while CO treatment exhibited an opposite effect following Method C. Contrastingly, an insignificant ( $p>0.05$ ) impact was observed in the carbohydrate content of brown rice over two oil treatments.

When comparing the control groups, it was observed that the carbohydrate content of both types of rice treated by Method C was the highest, followed by Methods A and B. Additionally, the addition of different oils did not alter the effect of cooking methods on the carbohydrate content of both type of rice.

#### **4.2.7 Total Calorie**

Both oil treatments increased the calorie of brown and white rice in all cooking methods (Table 4.1). However, it did not result in a spike, ranging from 3.95 kcal/g to 4.06 kcal/g in white rice and 4.13 kcal/g to 4.19 kcal/g in brown rice. Remarkably, the calorie of brown rice was higher than white rice in all cooking methods, including the control and the oil treatment groups.

Without oil treatment, white rice treated by Method C exhibited the lowest calorie compared to the other cooking methods. Following PO treatment, white rice treated by Methods B and C showed lower calorie. In contrast, there was no statistically significant ( $p>0.05$ ) difference among cooking methods beyond CO treatment. Contrarily, there was an insignificant ( $p>0.05$ ) difference in the brown rice among three cooking methods, regardless of control, PO or CO treatments.

### **4.3 *In Vitro* Digestibility of Treated Rice**

#### **4.3.1 Glucose Release During *In Vitro* Digestion of Treated Rice**

The glucose concentration was calculated using the equation derived from the standard curve with R-squared value of 0.9952 (Appendix E). Subsequently, the glucose concentration was then converted to glucose release in g/100 g rice as shown in Table 4.2.

**Table 4.2: *In vitro* glucose release of brown and white rice upon different cooking methods and oil treatments**

Method	Oil treatment	Baseline	Oral phase	Gastric phase	Pancreatic phase				
					20 min	60 min	90 min	120 min	180 min
A	White rice (Control)	39.55±0.50 <sup>Fa</sup>	44.85±0.60 <sup>Ea</sup>	46.66±0.72 <sup>Da</sup>	64.75±0.32 <sup>Ca</sup>	73.54±1.32 <sup>Ba</sup>	75.45±0.94 <sup>Aa</sup>	75.88±0.76 <sup>Aa</sup>	76.30±0.65 <sup>Aa</sup>
	White rice + Palm oil	32.21±0.71 <sup>Gb</sup>	33.99±0.36 <sup>Fb</sup>	34.75±0.28 <sup>Fb</sup>	45.83±0.22 <sup>Eb</sup>	49.48±0.45 <sup>Db</sup>	53.68±0.45 <sup>Cb</sup>	56.35±0.44 <sup>Bb</sup>	57.33±0.53 <sup>Ab</sup>
	White rice + Coconut oil	28.90±0.44 <sup>He</sup>	30.34±0.66 <sup>Ge</sup>	34.22±0.77 <sup>Fbc</sup>	43.26±0.68 <sup>Ec</sup>	47.18±0.65 <sup>Dc</sup>	49.83±0.84 <sup>Cc</sup>	51.58±0.12 <sup>Bc</sup>	56.31±0.27 <sup>Ac</sup>
	Brown rice (Control)	31.67±0.18 <sup>Gb</sup> <sub>c</sub>	33.05±0.16 <sup>Fc</sup>	33.62±0.16 <sup>Fc</sup>	36.12±0.14 <sup>Ed</sup>	42.36±0.36 <sup>Dd</sup>	49.29±0.92 <sup>Cc</sup>	50.51±0.42 <sup>Bd</sup>	51.42±0.45 <sup>Ad</sup>
	Brown rice + Palm oil	31.21±0.15 <sup>Gc</sup>	32.49±0.15 <sup>Fcd</sup>	32.77±0.08 <sup>Fd</sup>	35.13±0.15 <sup>Ee</sup>	41.57±0.25 <sup>Dd</sup> <sub>e</sub>	46.87±0.57 <sup>Cd</sup>	48.10±0.27 <sup>Be</sup>	49.08±0.15 <sup>Ae</sup>
	Brown rice + Coconut oil	29.97±0.17 <sup>Hd</sup>	32.09±0.10 <sup>Gd</sup>	32.64±0.19 <sup>Fd</sup>	35.04±0.19 <sup>Ee</sup>	40.80±0.19 <sup>De</sup>	45.07±0.22 <sup>Ce</sup>	47.07±0.28 <sup>Bf</sup>	48.92±0.19 <sup>Ae</sup>
B	White rice (Control)	42.97±0.45 <sup>Ga</sup>	47.73±0.52 <sup>Fa</sup>	51.20±0.22 <sup>Ea</sup>	71.02±0.39 <sup>Da</sup>	78.41±0.25 <sup>Ca</sup>	80.34±0.23 <sup>Ba</sup>	80.76±0.13 <sup>AB</sup> <sub>a</sub>	81.04±0.11 <sup>Aa</sup>
	White rice + Palm oil	35.80±0.38 <sup>Gb</sup>	38.10±0.73 <sup>Fb</sup>	39.18±0.24 <sup>Eb</sup>	49.31±0.52 <sup>Db</sup>	53.60±0.60 <sup>Cb</sup>	57.66±0.68 <sup>Bb</sup>	61.89±0.67 <sup>Ab</sup>	62.24±0.64 <sup>Ab</sup>
	White rice + Coconut oil	30.64±0.16 <sup>He</sup>	33.72±0.36 <sup>Gd</sup>	37.67±0.45 <sup>Fc</sup>	45.89±0.52 <sup>Ec</sup>	50.32±0.72 <sup>Dc</sup>	53.72±0.42 <sup>Cc</sup>	55.47±0.72 <sup>Bc</sup>	59.64±0.47 <sup>Ac</sup>
	Brown rice (Control)	32.82±0.16 <sup>Hc</sup>	34.47±0.17 <sup>Gc</sup>	35.61±0.12 <sup>Fd</sup>	38.49±0.14 <sup>Ed</sup>	44.13±0.19 <sup>Dd</sup>	50.34±0.26 <sup>Cd</sup>	51.83±0.16 <sup>Bd</sup>	52.43±0.10 <sup>Ad</sup>
	Brown rice + Palm oil	31.75±0.17 <sup>Hd</sup>	33.11±0.10 <sup>Gde</sup>	33.39±0.14 <sup>Fe</sup>	36.38±0.14 <sup>Ee</sup>	42.84±0.16 <sup>De</sup>	48.90±0.12 <sup>Ce</sup>	50.39±0.12 <sup>Be</sup>	51.32±0.12 <sup>Ae</sup>
	Brown rice + Coconut oil	31.42±0.17 <sup>Hd</sup>	32.98±0.15 <sup>Ge</sup>	33.64±0.14 <sup>Fe</sup>	36.02±0.10 <sup>Ee</sup>	42.43±0.10 <sup>De</sup>	45.09±0.18 <sup>Cf</sup>	47.28±0.26 <sup>Bf</sup>	49.44±0.27 <sup>Af</sup>
C	White rice (Control)	41.02±0.41 <sup>Ea</sup>	44.96±0.53 <sup>Da</sup>	46.49±0.15 <sup>Ca</sup>	64.61±0.44 <sup>Ba</sup>	71.36±0.51 <sup>Aa</sup>	71.57±0.18 <sup>Aa</sup>	71.79±0.10 <sup>Aa</sup>	71.85±0.10 <sup>Aa</sup>
	White rice + Palm oil	32.96±0.25 <sup>Hc</sup>	34.07±0.09 <sup>Gc</sup>	35.84±0.19 <sup>Fc</sup>	44.68±0.37 <sup>Ec</sup>	49.85±0.14 <sup>Db</sup>	52.85±0.21 <sup>Cb</sup>	56.91±0.15 <sup>Bb</sup>	58.47±0.16 <sup>Ab</sup>
	White rice + Coconut oil	27.11±0.34 <sup>Hf</sup>	30.11±0.44 <sup>Gf</sup>	32.49±0.34 <sup>Fe</sup>	40.01±0.24 <sup>Ed</sup>	44.99±0.50 <sup>Dc</sup>	48.26±0.57 <sup>Cd</sup>	49.68±0.44 <sup>Bd</sup>	51.20±0.46 <sup>Ad</sup>
	Brown rice (Control)	38.47±0.15 <sup>Hb</sup>	40.84±0.14 <sup>Gb</sup>	42.53±0.15 <sup>Fb</sup>	47.42±0.18 <sup>Eb</sup>	49.94±0.10 <sup>Db</sup>	51.93±0.15 <sup>Cc</sup>	53.01±0.14 <sup>Bc</sup>	53.56±0.14 <sup>Ac</sup>
	Brown rice + Palm oil	31.00±0.18 <sup>Hd</sup>	32.54±0.14 <sup>Gd</sup>	33.80±0.10 <sup>Fd</sup>	37.82±0.13 <sup>Ee</sup>	41.93±0.24 <sup>Dd</sup>	45.44±0.51 <sup>Ce</sup>	47.21±0.28 <sup>Be</sup>	48.86±0.29 <sup>Ae</sup>
	Brown rice + Coconut oil	29.97±0.09 <sup>He</sup>	31.12±0.13 <sup>Ge</sup>	31.89±0.10 <sup>Ff</sup>	33.96±0.13 <sup>Ef</sup>	36.39±0.13 <sup>De</sup>	37.36±0.09 <sup>Cf</sup>	39.70±0.15 <sup>Bf</sup>	41.35±0.13 <sup>Af</sup>

<sup>a</sup> The data were expressed as mean ± standard deviation in g/100 g rice (n=6). Means in the same column followed by different lowercase letters were significantly (p<0.05) different in the same cooking method. Means in the same row followed by different uppercase letters were significantly (p<0.05) different in different digestion phases.

<sup>b</sup> Rapidly digestible starch was calculated from the glucose released at the first 20 min of pancreatic digestion; SDS was the glucose difference between the 20 and 120 min of pancreatic digestion; RS was the glucose released after 16 h of pancreatic phase.



When comparing the glucose baseline of the control groups across three cooking methods, results showed that all white rice controls exhibited a higher glucose baseline than brown rice controls. Upon two oil treatments, both types of rice demonstrated a lowered glucose baseline as compared to the control groups. Especially, white rice treated with CO showed the lowest glucose baseline in all cooking methods, followed by brown rice treated with CO. This implies that CO showed a greater reducing effect on both types of rice compared to that of PO. Subsequently, similar observation was demonstrated in the oral phase upon the addition of alpha-amylase enzyme.

In the pancreatic phase, brown rice with oil treatments showed the lowest release of glucose across the three cooking methods. Additionally, white rice showed a sustained release of glucose compared to the controls following oil treatments. It was conspicuous when a significant ( $p < 0.05$ ) increase in glucose release was observed in the oil-treated white rice during the first 60 min to 180 min of the pancreatic phase.

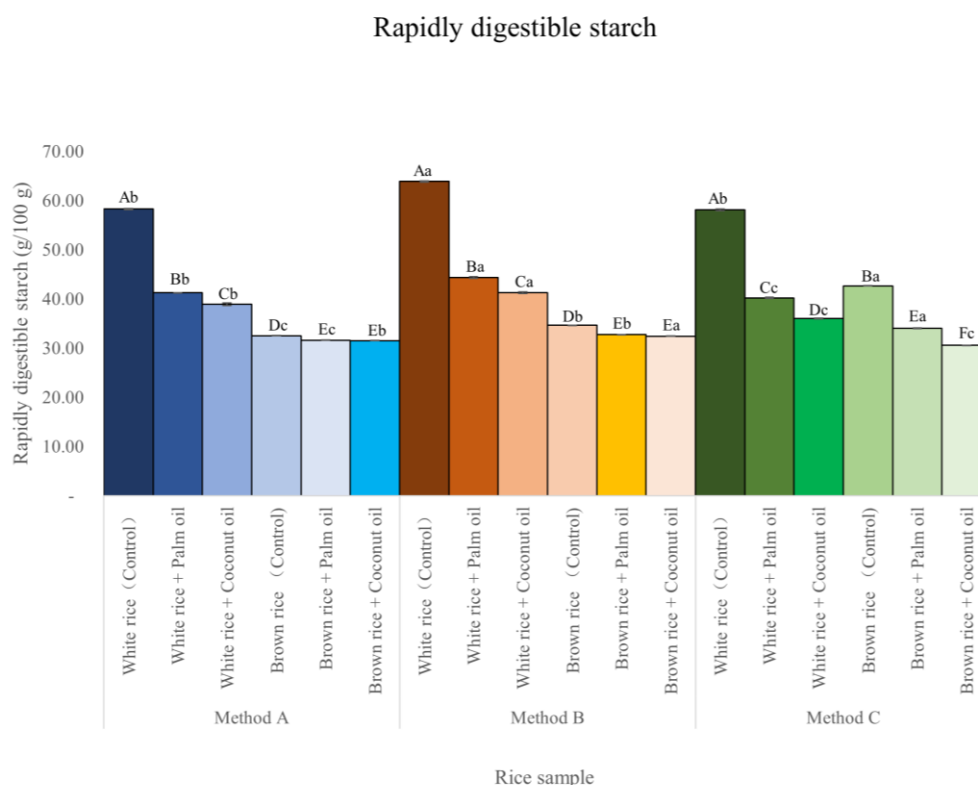
### **4.3.2 Starch Proportion in Treated Rice**

#### **4.3.2.1 Rapidly Digestible Starch**

The RDS of both types of rice was lowered upon oil treatments in the three cooking methods (Figure 4.19). Overall, CO treatment resulted in a greater reduction in the RDS of white rice across the three cooking methods,

while a notable effect was observed in brown rice only when treated with Method C. In Methods A and B, both oil-treated brown rice showed the lowest RDS, followed by brown rice control and the oil-treated white rice. Notably, brown rice control in Method C was higher than both oil-treated white rice. The lowest RDS was exhibited in brown rice treated with CO, followed by PO-treated brown rice.

Comparing both rice controls, white rice exhibited the highest RDS when cooked by Method B, whereas brown rice showed the highest RDS upon Method C. Following PO treatment, white rice cooked with Method C showed the lowest RDS, whereas brown rice treated with Method A showed similar observation. Nevertheless, both brown and white rice showed the lowest RDS in Method C upon CO treatment.



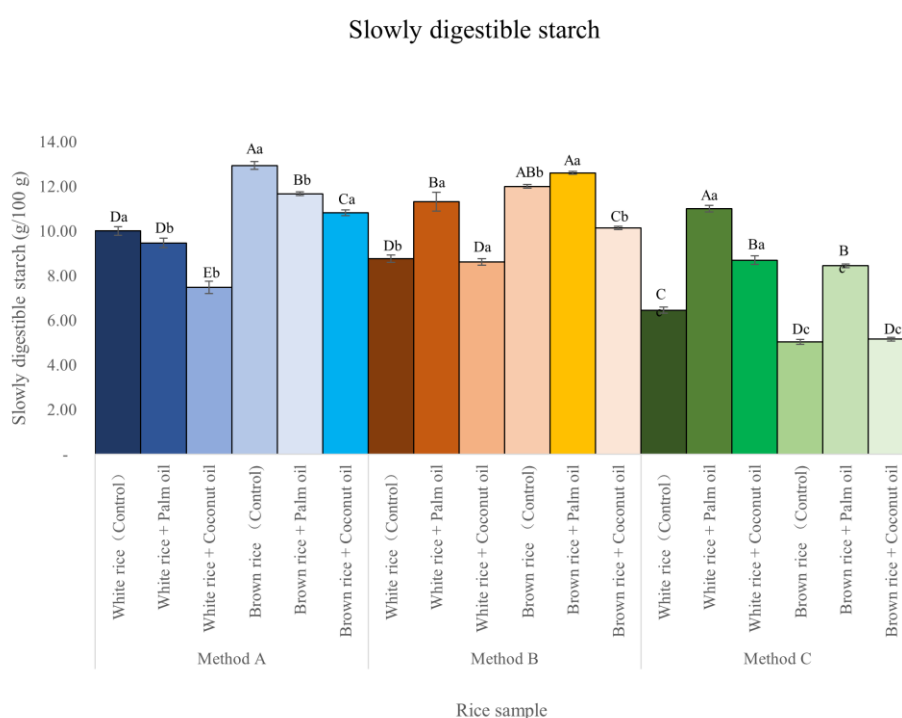
**Figure 4.19: Rapidly digestible starch of brown and white rice following different oil treatments incorporated with different cooking methods (n=6). Bars with different uppercase letters were significantly ( $p<0.05$ ) different in the same cooking method. Bars with different lowercase letters were significantly ( $p<0.05$ ) different in the same oil treatment.**

#### 4.3.2.2 Slowly Digestible Starch

Oil treatments induced different effects on the SDS of brown and white rice (Figure 4.20). In Method A, the highest SDS was observed in brown rice control, while both oil treatments showed a reducing effect on the SDS of both types of rice. Especially, white rice treated with CO showed the lowest SDS among Method A-treated samples. In Method B, a significant ( $p<0.05$ ) increase was observed in the SDS of PO-treated rice, while the highest SDS was exhibited in brown rice treated with PO. Moreover, both oil-treated white

rice showed an increased SDS upon treatments in Method C. However, in brown rice, the SDS content only increased upon the addition of PO.

Following PO treatment, Method C demonstrated the highest increase in the SDS of white rice, whereas Method B exhibited the highest incremental effect on brown rice. Conversely, Methods B and C showed the greatest increase in the SDS of white rice upon CO treatment, while Method A exhibited the highest increment in SDS of brown rice.

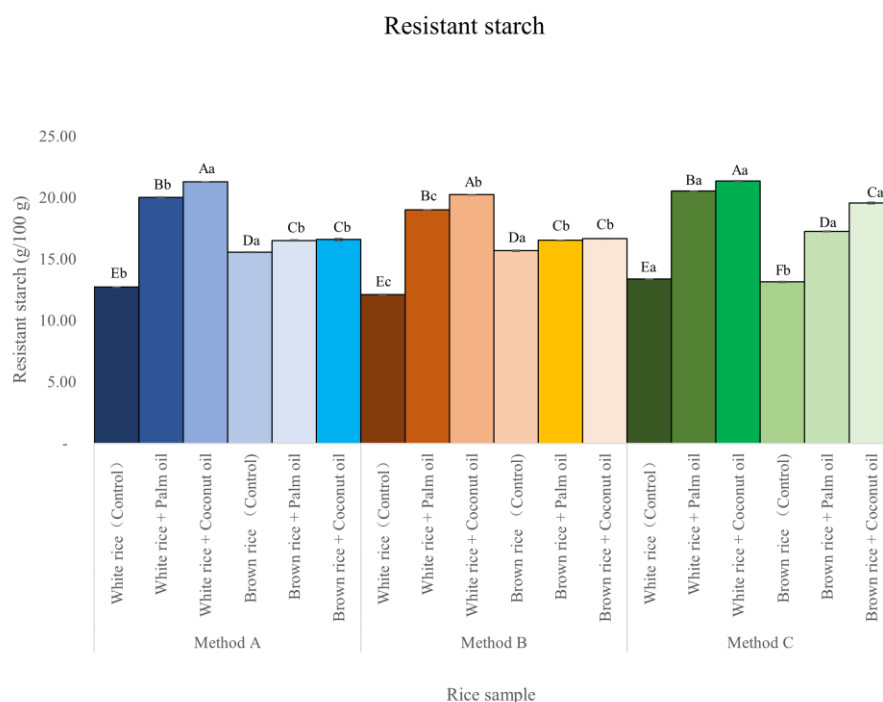


**Figure 4.20: Slowly digestible starch of brown and white rice following oil treatments incorporated with different cooking methods (n=6). Bars with different uppercase letters were significantly ( $p<0.05$ ) different in the same cooking method. Bars with different lowercase letters were significantly ( $p<0.05$ ) different in the same oil treatment.**

#### **4.3.2.3 Resistant Starch**

The RS of brown and white rice was increased upon two oil treatments, especially an obvious increment was observed in white rice in all cooking methods (Figure 4.21). The highest RS was observed in white rice with CO treatment, followed by white rice PO treatment. However, both oils showed a similar effect on brown rice treated with Methods A and B. While in Method C, CO treatment showed a greater increase in RS of brown rice.

Comparing the control groups, Method A resulted in the highest RS content for white rice, while Method C for brown rice. In PO treatment, the RS of brown and white rice was increased the most upon Method C. Contrastingly in CO treatment, both Methods A and C increased the RS the most in white rice, while Method C in brown rice.



**Figure 4.21: Resistant starch of brown and white rice following different oil treatments incorporated with different cooking methods (n=6). Bars with different uppercase letters were significantly ( $p<0.05$ ) different in the same cooking method. Bars with different lowercase letters were significantly ( $p<0.05$ ) different in the same oil treatment.**

#### 4.4 Prebiotic Potential of Treated Rice

The oligosaccharide concentration of rice samples (mg/mL) was determined using the equation constructed from the standard curve with R-squared value of 0.9941 (Appendix F).

##### 4.4.1 Initial Oligosaccharide Concentration of Treated Rice

Comparing the control treatment, all brown rice samples showed a higher initial oligosaccharide concentration than white rice (Table 4.3). Brown

rice treated with Method A showed the highest initial oligosaccharide, followed by Methods B and C. However, with PO treatment, brown rice prepared with Method C showed the highest initial oligosaccharide concentration, subsequently by white rice with Method C. Contrarily, white rice treated with Methods A and B showed the greatest initial oligosaccharide concentration in CO treatment, and simultaneously, the highest initial oligosaccharide concentration among all 18 samples.

Regardless of brown or white rice, CO treatment demonstrated the greatest initial oligosaccharide concentration in both rice prepared by Methods A and B. On the other hand, PO treatment showed a greater initial oligosaccharide concentration on both rice upon Method C.

**Table 4.3: The initial oligosaccharide concentration of brown and white rice upon different cooking methods and oil treatments**

Oil treatment	White rice			Brown rice		
	A	B	C	A	B	C
<b>Control</b>	1.46±0.01 <sup>Dc</sup>	1.37±0.01 <sup>Ec</sup>	0.82±0.01 <sup>Fc</sup>	1.86±0.01 <sup>Ab</sup>	1.65±0.01 <sup>Bc</sup>	1.62±0.01 <sup>Cb</sup>
<b>Palm oil</b>	1.84±0.01 <sup>Db</sup>	1.43±0.01 <sup>Eb</sup>	2.17±0.01 <sup>Ba</sup>	1.86±0.01 <sup>Cb</sup>	1.83±0.01 <sup>Cb</sup>	2.29±0.01 <sup>Aa</sup>
<b>Coconut oil</b>	3.46±0.01 <sup>Aa</sup>	3.11±0.01 <sup>Ba</sup>	2.09±0.01 <sup>Fb</sup>	2.34±0.01 <sup>Da</sup>	2.42±0.01 <sup>Ca</sup>	2.29±0.01 <sup>Ea</sup>

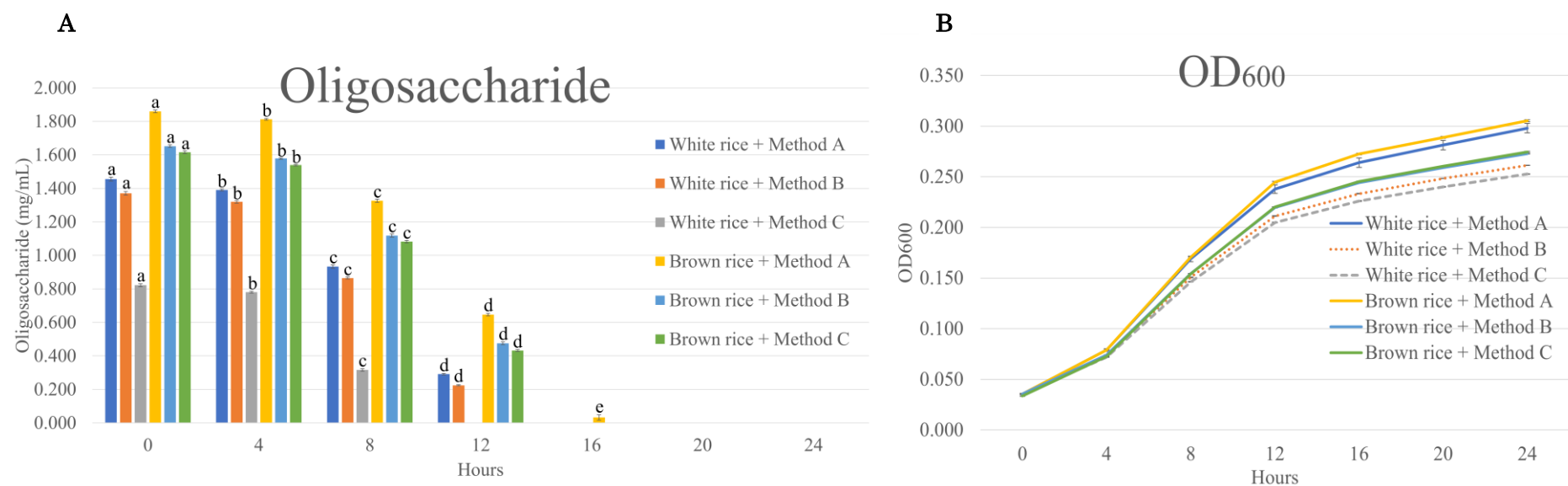
a The data were expressed as mean ± standard deviation (n=6). Means in the same column followed by different lowercase letters were significantly (p<0.05) different in the same cooking method. Means in the same row followed by different uppercase letters were significantly (p<0.05) different in the same oil treatment.

#### 4.4.2 Oligosaccharide Concentration and Growth Curve upon 24 hours

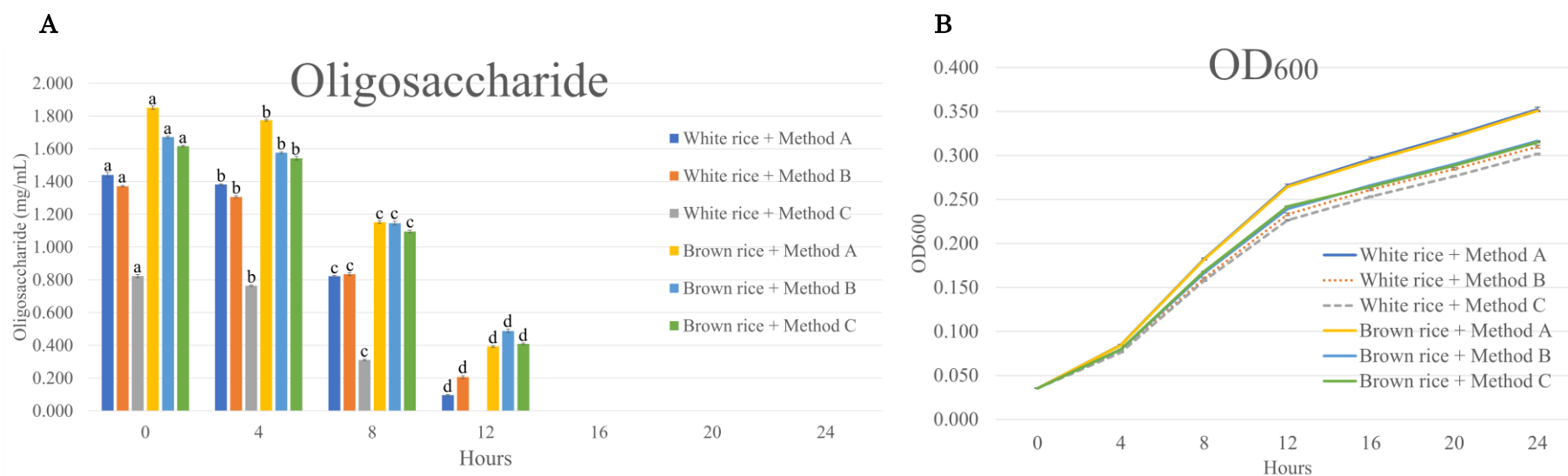
Both probiotic strains *Lacticaseibacillus casei* and *Lacticaseibacillus rhamnosus* hydrolysed the oligosaccharides from all treated brown and white

rice at four-hour intervals over 24 h, along with the increase in bacterial growth over time (Figures 4.22 to 4.27). Most oligosaccharides were being hydrolysed within 20 h except CO-treated white rice prepared by Methods A and B.

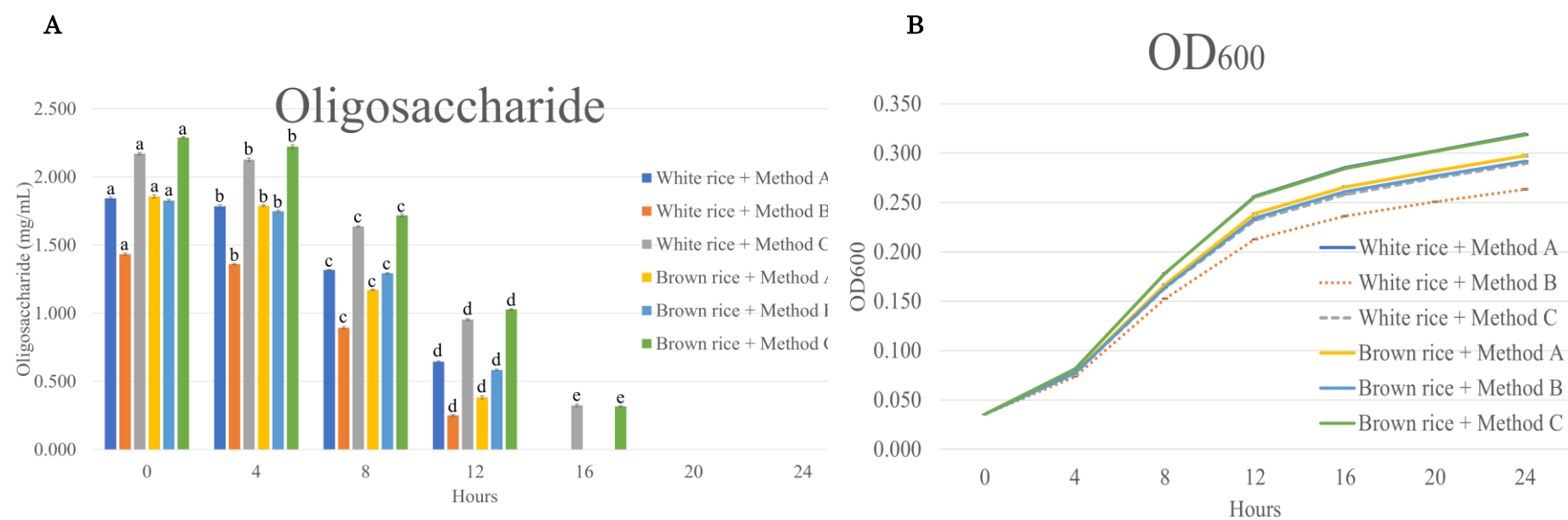




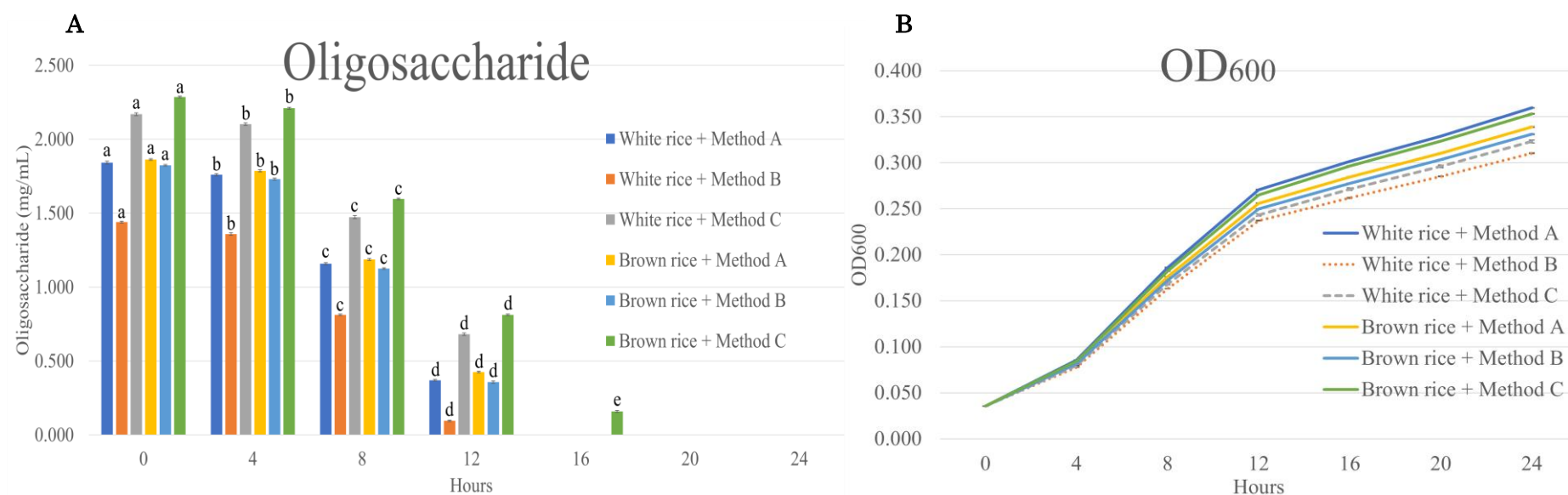
**Figure 4.22: Oligosaccharide concentration of the control samples without oil treatment following different cooking methods (A) and growth curve of *Lacticaseibacillus casei* (B) upon 24 hours (n=6). Bars with different lowercase letters were significantly ( $p<0.05$ ) different between four-hour intervals.**



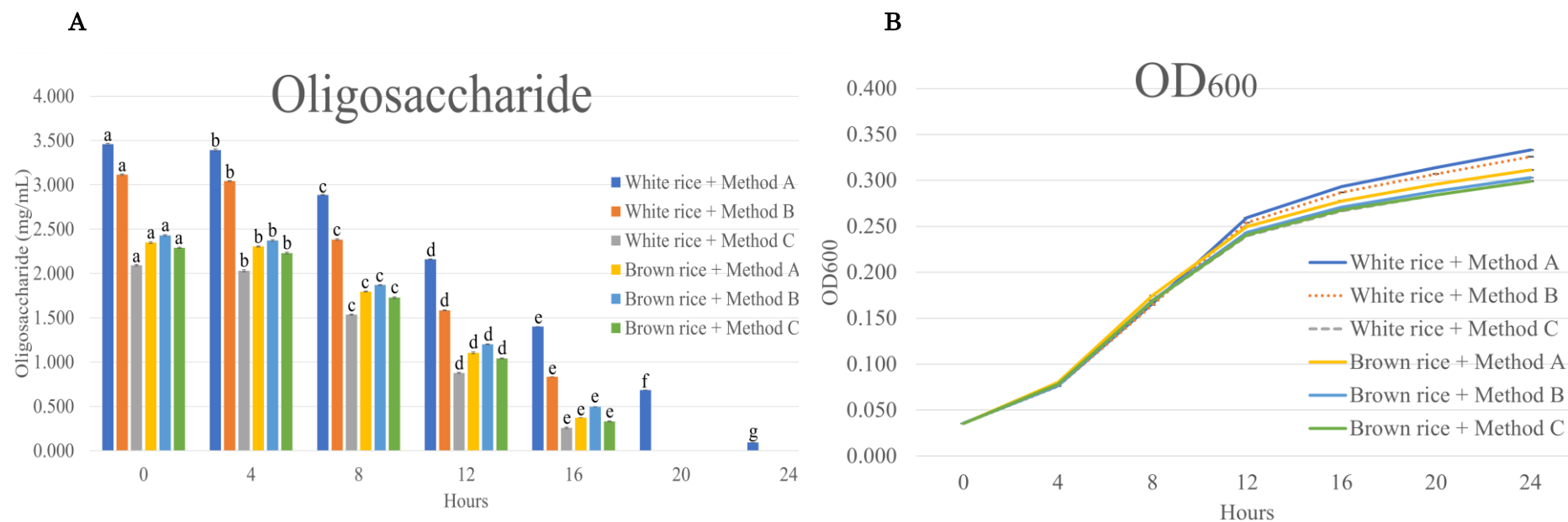
**Figure 4.23: Oligosaccharide concentration of the control samples without oil treatment following different cooking methods (A) and growth curve of *Lactocaseibacillus rhamnosus* (B) upon 24 hours (n=6). Bars with different lowercase letters were significantly ( $p<0.05$ ) different between four-hour intervals.**



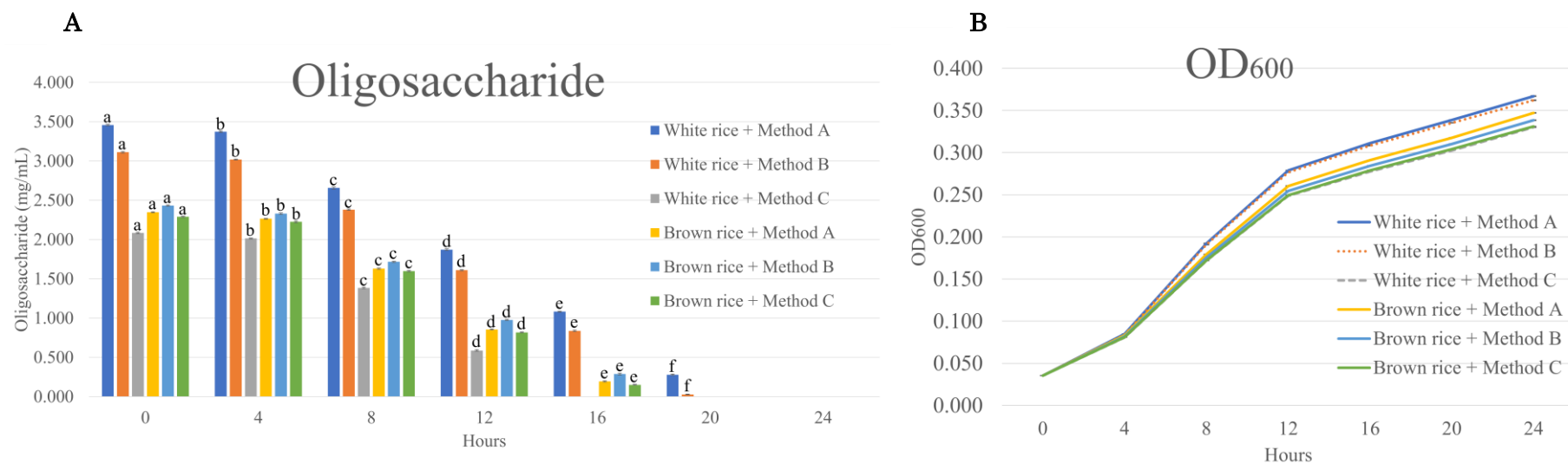
**Figure 4.24: Oligosaccharide concentration of the palm oil-treated samples following different cooking methods (A) and growth curve of *Lacticaseibacillus casei* (B) upon 24 hours (n=6). Bars with different lowercase letters were significantly ( $p < 0.05$ ) different between four-hour intervals.**



**Figure 4.25: Oligosaccharide concentration of the palm oil-treated samples following different cooking methods (A) and growth curve of *Lacticaseibacillus rhamnosus* (B) upon 24 hours (n=6). Bars with different lowercase letters were significantly ( $p < 0.05$ ) different between four-hour intervals.**



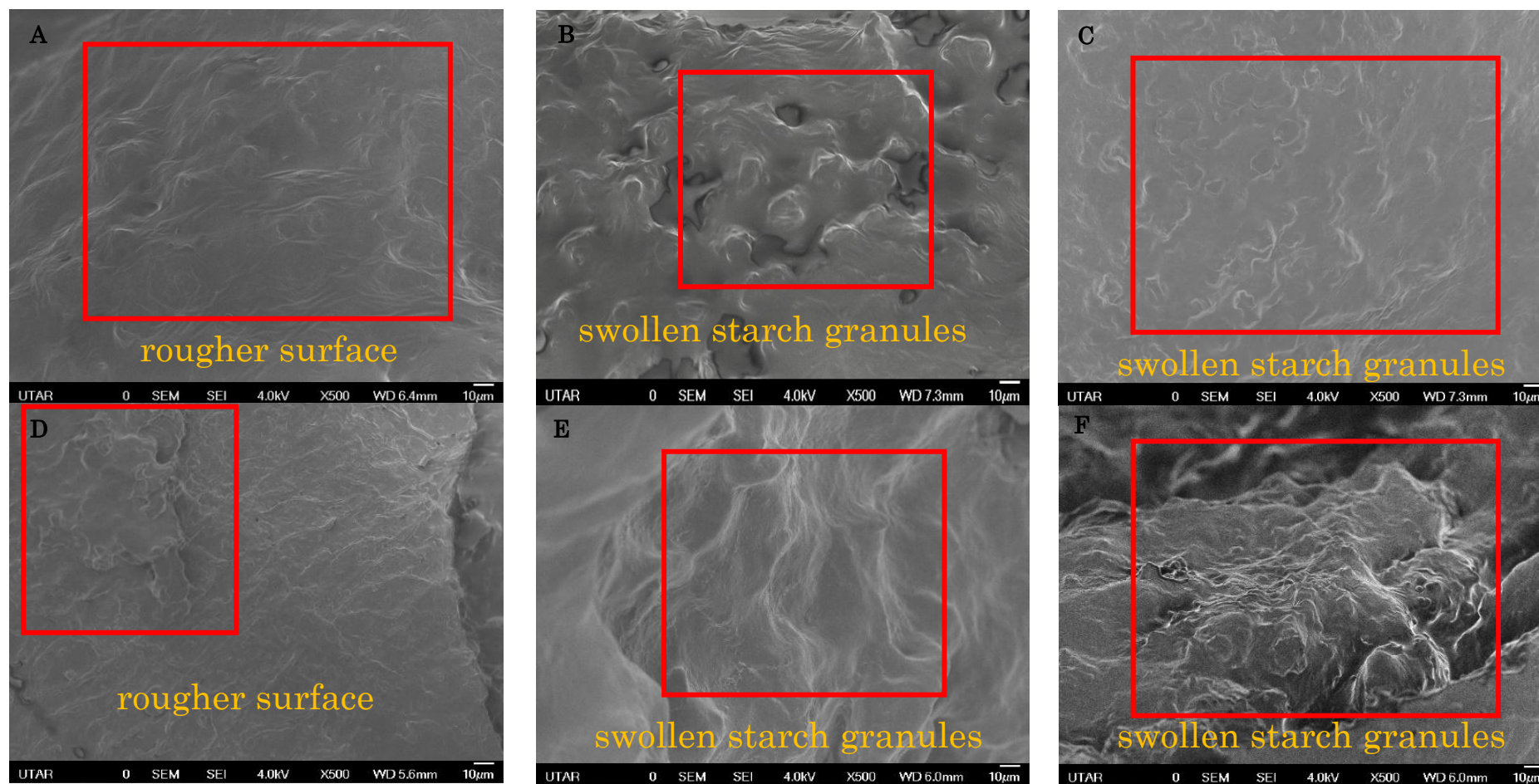
**Figure 4.26: Oligosaccharide concentration of the coconut oil-treated samples following different cooking methods (A) and growth curve of *Lacticaseibacillus casei* (B) upon 24 hours (n=6). Bars with different lowercase letters were significantly ( $p < 0.05$ ) different between four-hour intervals.**



**Figure 4.27: Oligosaccharide concentration of the coconut oil-treated samples following different cooking methods (A) and growth curve of *Lacticaseibacillus rhamnosus* (B) upon 24 hours (n=6). Bars with different lowercase letters were significantly ( $p<0.05$ ) different between four-hour intervals.**

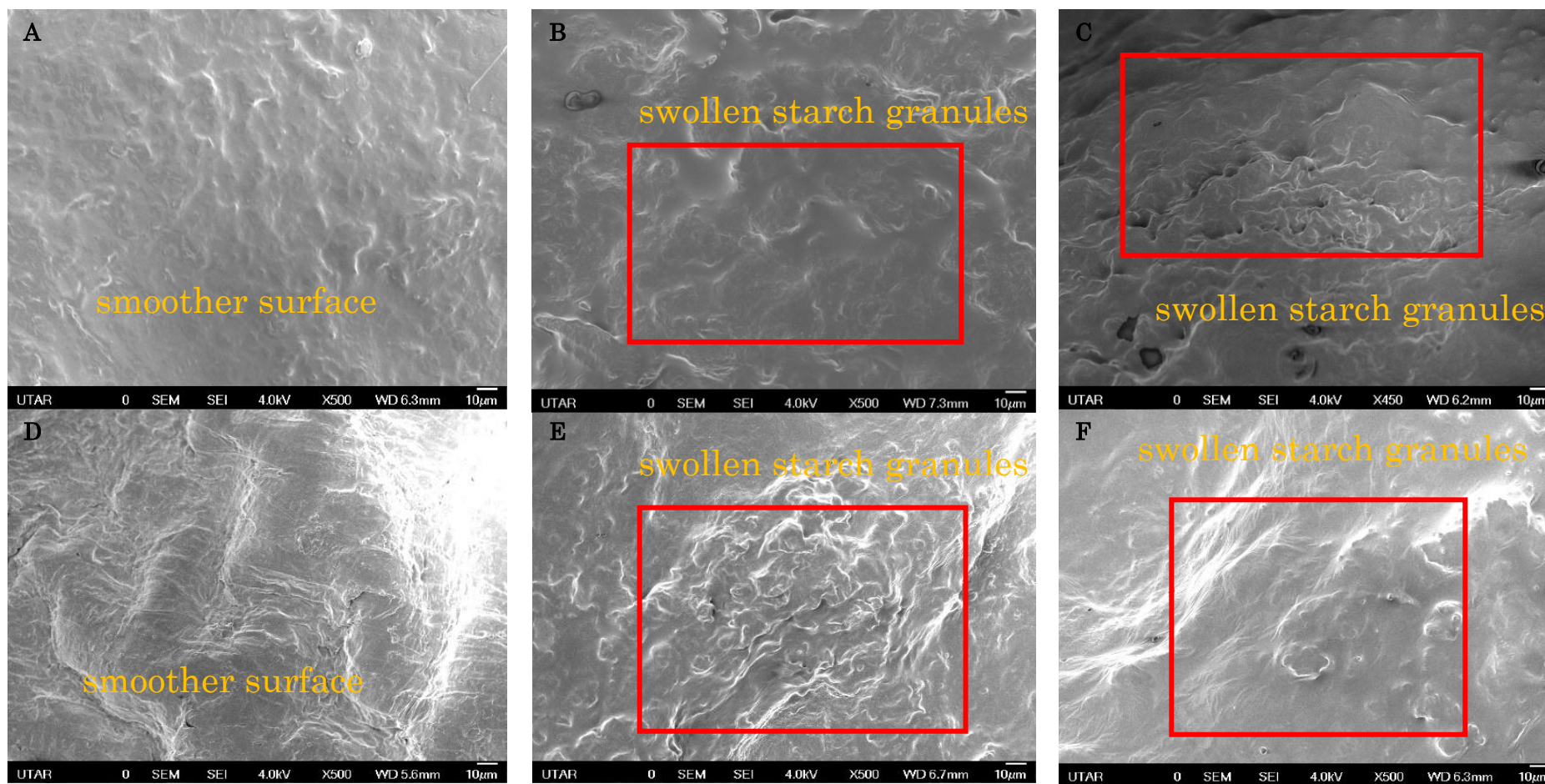
#### **4.5 Scanning Electron Microscopy of Starch Granule**

Both stir-fried controls (Figures 4.28 and 4.30) showed an uneven and rougher surface compared to steamed rice control (Figure 4.29). Besides, all oil-treated rice demonstrated swollen starch granules on the rice surface as shown in Figures 4.28, 4.29 and 4.30. As comparison, a greater integrity of swollen starch granules was observed in CO-treated rice across the three cooking methods. Additionally, there was no voids and hollows observed on the surfaces of all rice samples as showed in the findings of Yang, et al. (2016).

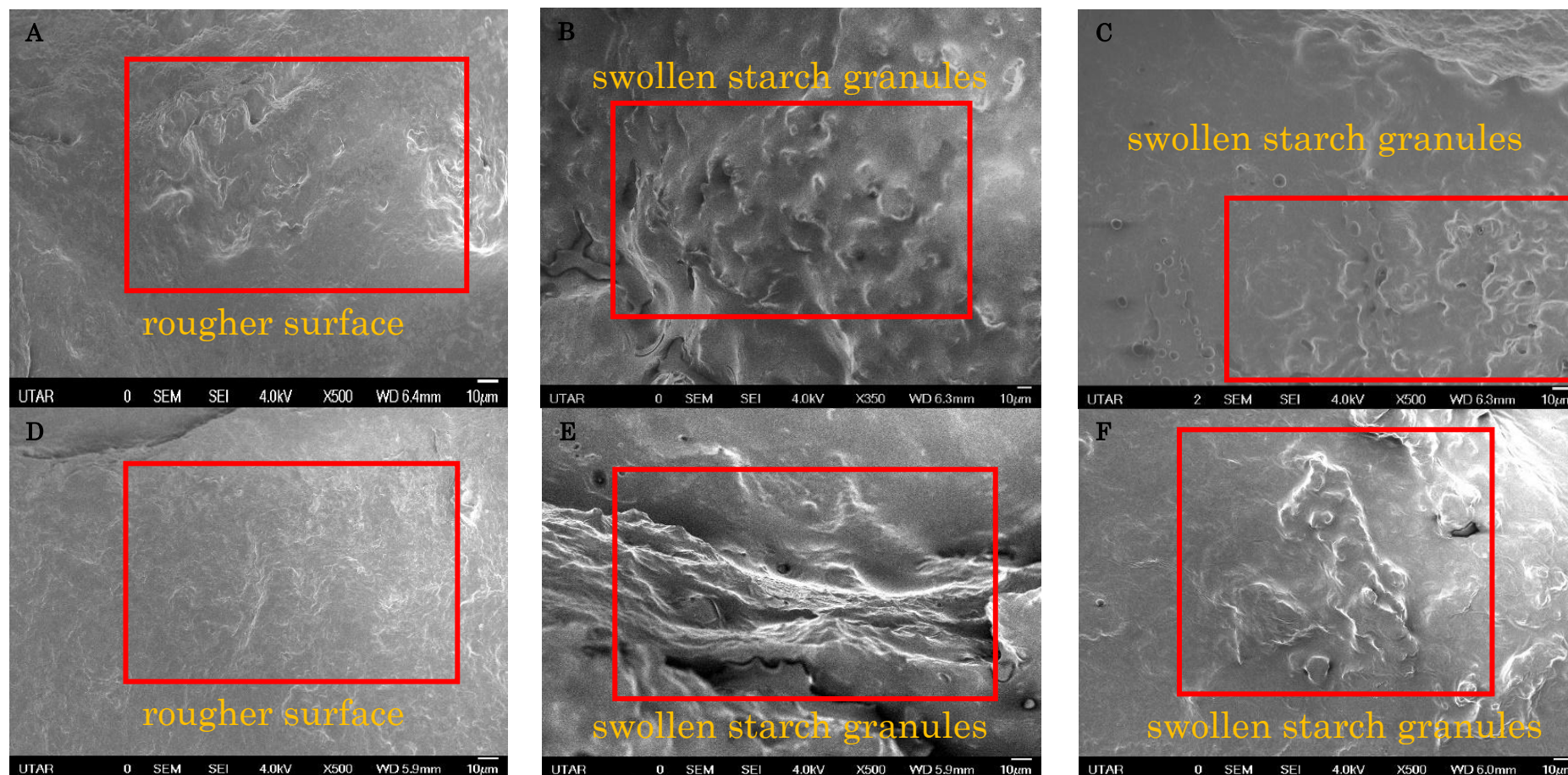


**Figure 4.28:** Micrographs of the Method A-treated rice cooked with different oils followed by retrogradation. The images of the first row were white rice ascending control, palm oil, and coconut oil treatments (A-C); the second row were brown rice samples (D-F).





**Figure 4.29:** Micrographs of the Method B-treated rice cooked with different oils followed by retrogradation. The images of the first row were white rice ascending control, palm oil, and coconut oil treatments (A-C); the second row were brown rice samples (D-F).



**Figure 4.30: Micrographs of the Method C-treated rice cooked with different oils followed by retrogradation. The images of the first row were white rice ascending control, palm oil, and coconut oil treatments (A-C); the second row were brown rice samples (D-F).**

## CHAPTER 5

### DISCUSSIONS

#### 5.1 Meta-analysis

##### 5.1.1 Resistant Starch

Retrogradation by cooling enables the reassociation of the amylose and amylopectin in gelatinised rice via two stages: short-term retrogradation and long-term retrogradation (Channab, et al., 2023). This process results in a higher degree of crystalline structure, which is more resistant to amylolysis during digestion. Short-term retrogradation predominantly involves the retrogradation of amylose and typically completes within a few hours, subsequently by long-term retrogradation, which initiates after 12 h of retrogradation (Chakraborty, et al., 2023). Thereby, pooled studies with more than 12 h of retrogradation showed an increase in RS, specifically type III RS, as demonstrated in the meta-analysis of different retrogradation durations.

Heterogeneous results reported in the pooled studies were primarily attributed to differences in the amylose content of rice, which significantly influenced the formation of the RS during retrogradation. This was clearly illustrated not merely by the deviations of “Hsu et al. (2015)-L(2)-24h” and “Jayawardena et al. (2017)-M-24h” in 24 h retrogradation funnel plot, also by

the deviations of “Hsu et al. (2015)-L4-72h” and “Chiu & Stewart (2013)-L(1)-72h” in 72 h retrogradation funnel plot, which displayed opposing effects despite undergoing same duration of retrogradation. Referring to Hsu, et al. (2015), the higher amylose content in L2 and L4 indica rice (16.4% and 29.3%), along with a high proportion of F1 subfraction (15.4% and 26.7%), transformed an imperfect crystalline structure into a more perfect crystallite, contributing to a higher formation of RS upon retrogradation. Correspondingly, the low formation of RS in rice reported by Chiu and Stewart (2013) and Jayawardena, et al. (2017) was attributed to the lower amylose content in rice, as claimed by the authors.

Publication bias detected in this meta-analysis might result from the following reasons: reporting bias, true heterogeneity, and editorial bias. The high preference for reporting significant results, suppressing negative results, and selective reporting only results with distinct treatment effect are the major reasons for the asymmetry in funnel plots. Additionally, the high amylose rice introduces true heterogeneity, which further contributes to this asymmetry. Reviewer bias toward statistically significant findings also restricts the publication of small studies and suppresses negative results, leading to asymmetry in the funnel plot.

Theoretically, 72 h retrogradation was expected to show a higher MD than 24 h retrogradation due to the accumulation of irreversible amylose crystallisation in short-term retrogradation and reversible amylopectin crystallisation in long-term retrogradation. However, the lower MD observed

in 72 h retrogradation during the subgroup meta-analysis might be due to several reasons. Firstly, the amylose content of rice significantly influences the formation of RS during retrogradation. Therefore, varying amylose content across different rice varieties in the pooled studies may have contributed to these results. This was evident from the study by Hsu, et al. (2015), where the same rice variety exhibited an increasing MD from 24 h to 72 h retrogradation. Additionally, factors such as moisture content of rice and environmental humidity also affect the starch retrogradation (Dong, et al., 2020).

#### **5.1.2 Slowly Digestible Starch**

In this meta-analysis, the pooled results were solely extracted from the study by Hsu, et al. (2015). Therefore, factors such as the moisture content of rice and environmental humidity were constant and would not affect starch retrogradation. During the retrogradation process, the recrystallisation of amylopectin tends to form SDS due to its short-branch structure, which induces a less stable structure that is more susceptible to amylolysis (Dong, et al., 2020). Simultaneously, Hsu, et al. (2015) claimed that the greater amount of the F2 subfraction in low-amylose japonica rice (S1 and S2) and high-amylose indica rice (L1 and L3) prior to forming SDS during retrogradation. As such, positive results were demonstrated upon 24 and 72 h of retrogradation. Contrastingly, the high proportion of the F1 subfraction content in high-amylose indica rice (L2 and L4) transformed SDS to RS during retrogradation, decreasing SDS content as showed in the forest plot.

Therefore, these opposite effects contributed to the insignificant ( $p>0.05$ ) difference in SDS upon retrogradation in this meta-analysis.

### **5.1.3 Rapidly Digestible Starch**

The recrystallisation of amylose and amylopectin involves the formation of double helices between 40 to 70 glucose units via hydrogen bonding, turning the gelatinised starches into a more ordered structure during the retrogradation process (Wang, et al., 2015). The formation of an ordered structure through strong hydrogen bonding restricts the binding of amylase during digestion (Patel, et al., 2017). This lowered the proportion of RDS to SDS and RS, depending on the degree of crystallisation, which corresponds to the negative MD in the meta-analysis model.

Factors such as the moisture content of rice and environmental humidity were omitted, which could contribute to the heterogeneity in the pooled results, as these results were solely extracted from Hsu, et al. (2015). This heterogeneity might be attributed to the variety of rice corresponding to the F1 and F2 subfractions, which played different roles in transforming the proportion of RDS to RS and SDS, as aforementioned. Nevertheless, the low decrement in the RDS of high-amylose indica rice (L2) during 24 h retrogradation was due to the higher proportion of long-chain amylopectin, which helped maintain the structure of starch granules during short-term retrogradation, as claimed by the authors.



The additional recrystallisation of amylopectin during long-term retrogradation increased the MD for 72 h retrogradation compared to 24 h retrogradation (Dobosz, et al., 2018). Long-term retrogradation decreases the water-holding capacity of starch, creating an ideal environment for the retrogradation process and converting more RDS to SDS and RS (Zhai, et al., 2023). Moreover, longer retrogradation durations are required for starches with higher branching degrees and shorter branch chain lengths to form an ordered structure (Li, Yu and Gilbert, 2022). Furthermore, extended retrogradation allows for greater co-crystallisation between amylose and amylopectin molecules, forming junctions between the two polysaccharides that resist amylolysis (Fu, et al., 2014).

## **5.2 Nutritional Composition of Treated Rice**

### **5.2.1 Protein**

Both oil treatments and cooking methods incorporated with 12 h of retrogradation demonstrated an insignificant ( $p>0.05$ ) effect on the protein content of brown and white rice. This was attributed to the partial double bond character of peptide bonds found in the chains of amino acids, which made amino acid chains resist high heat and sodium concentration by restricting the rotation of the molecules (Damodaran, 2017). Additionally, the nature of the double bond in peptide bonds further increases molecular stability (Forbes and Krishnamurthy, 2023). As a result, more energy is required to break the strong

peptide bond and release free amino acids. As such, similar nitrogen content was detected across the treatments. This observation was also supported by the findings of Sun, et al. (2014), where the protein content of white rice added with groundnut oil was comparable to that of untreated rice. Kim, Nam and Chung (2019) also revealed similar findings using olive oil. Furthermore, Sonia, et al. (2015) and Strozyk, et al. (2022) claimed that retrogradation did not affect the protein content of rice due to the absence of factors that could break the strong peptide bonds. Additionally, Liu, Zheng and Chen (2019) found that domestic cooking methods, such as ordinary, high-pressure, and microwave cooking, rarely affected the protein content of rice.

### **5.2.2 Ash**

Rice bran and germ are abundant in minerals such as potassium, phosphorus, and magnesium (Sapwarabol, Saphyakhajorn and Astina, 2021). As a result, brown rice demonstrated a higher ash content than refined white rice. Food minerals cannot be destroyed by heat and pressure because they are inorganic elements that maintain their chemical structures even under high temperatures and pressure. This stability is attributed to the accumulation effects of covalent bonds, ionic bonds, and crystal lattice structures (Klein, 2024). However, most minerals are lost through leaching during food preparation due to their water solubility (Fellows, 2017). Therefore, insignificant ( $p>0.05$ ) changes in the ash content of brown and white rice were exhibited during retrogradation, as well as with different cooking methods and



oil treatments. This result was similar to the study by Tanjor and Hongsprabhas (2021), where rice treated with CO and rice bran oil (RBO) showed similar ash content compared to untreated controls. Furthermore, Suman and Boora (2015) reported that different methods, such as ordinary, pressure, microwave, and solar approaches, rarely influenced the ash content of rice.

### **5.2.3 Moisture**

The addition of PO and CO to brown and white rice reduced moisture content of rice via the formation of the ALC. During the gelatinisation process, amylose forms hydrogen bonds with water molecules, contributing to the swelling of starch granules in rice (Tako, et al., 2014). However, the addition of oil limits the binding of water with amylose because the hydrocarbon chains of fatty acids bond with the hydrophobic moiety of amylose chains, forming an ALC (Ronie and Hasmadi, 2022). Moreover, ALC also limits the swelling of rice starch by entangling neighbouring amylopectin molecules, restricting their ability to bond with water molecules (Huang, et al., 2020). Furthermore, ALC promotes the crystallisation of starch during retrogradation. When starch undergoes retrogradation, the increased crystallinity reduces their ability to hold water molecules. Crystalline regions are less capable of binding and retaining water compared to amorphous regions, resulting in reduced moisture content in rice (Wang, et al. 2015).

Method A involved stir-frying raw rice with oils before the steaming process. The direct contact between hot oil and the ordered structure starch may destroy the rice surface by creating seals on the surface of the grains, inhibiting water absorption in subsequent cooking (Bodden, 2022). Additionally, the dextrinization of starch may occur due to the dry heat during stir-frying, breaking down the ordered structure into smaller starch chains, which also affects water retention (Huang and Perdon, 2020). These effects are more pronounced in refined white rice due to the loss of the physical protection provided by rice bran. Therefore, less moisture content was retained in white rice cooked using Method A with different oil treatments.

In Method B, oil was added to the filtered water during the steaming process. Rice bran and germ in brown rice contain an additional source of linoleic acid and other essential fatty acids (Rathna, et al., 2019). As a result, more fatty acids are available to form more ALCs compared to refined white rice. Therefore, brown rice was observed to have lower moisture content than white rice, including both the control and the oil-treated rice.

Method C involved stir-frying steamed rice with oil, which allowed all types of fatty acid chains to form ALCs without hydrophilicity concerns. However, the presence of rice bran may act as a barrier to direct contact between gelatinised rice starch and oil, slightly slowing down the formation of ALCs compared to refined white rice. Additionally, this barrier also reduced the moisture evaporation of brown rice during the stir-frying process, resulting in brown rice demonstrating a higher moisture content than white rice.

Coconut oil contains a high quantity of medium-chain saturated fatty acids, consisting of 49% lauric (C-12:0), 8% myristic (C-14:0), and 8% palmitic (C-16:0) acids. In contrast, PO contains equal proportion of unsaturated fatty acids and long-chain saturated fatty acids, including 44% palmitic (C-16:0), and 5% stearic (C-18:0) acids (Boeteng, et al., 2016). Shorter chains of saturated fatty acids in CO have a higher ability to form ALC due to their higher solubility in water (Chumsri, et al., 2022). As such, CO-treated white rice showed the lowest moisture content. Furthermore, the ALC formed from long-chain fatty acid inhibited retrogradation during the cooling process, leading to a higher moisture retention in PO-treated rice (Wang, et al., 2015).

Regardless of the control groups or oil treatment groups, Method C resulted in the highest moisture evaporation in both types of rice. This was attributed to the dry heat in stir-frying, which caused the partial evaporation of moisture from the surface of the rice grains, leading to the lowest moisture content among the three cooking methods. In contrast, Method A involved stir-frying raw rice, which left seals on the surface of the rice grains and affected the moisture content during the subsequent steaming process (Bodden, 2022). Method B involved only steaming, thus retaining the most moisture among the three cooking methods.

#### 5.2.4 Fat

The addition of oils to brown and white rice increased the crude fat content across the three cooking methods. In comparison, brown rice showed a higher crude fat content compared to white rice. This was due to the presence of rice bran and germ, which contributed additional crude fat content to brown rice (Rathna, et al., 2019). Additionally, rice bran contains approximately 34% cellulose, which has oil adsorption properties. The abundance of hydroxyl groups on the surface of cellulose allows fatty acids to adhere to the matrix during the cooking process (Fürtauer, et al., 2021). On the other hand, similar crude fat content was determined in both rice upon two different cooking methods. This might be attributed to the percentage of oil added to rice not exceeding the capacity of rice starch for oil absorption. This finding was supported by Tanjor and Hongsprabhas (2021), who reported similar results with 3% RBO and CO treatments.

Method B retained the highest crude fat content in both types of rice due to the absence of a stir-frying process, which might induce minimal oil evaporation aroused by dry heat, as seen in Methods A and C. The cooking temperature during stir-frying was ranged from 160 to 250°C, which vaporised a minimal amount of oil during the cooking process (Nugrahedi, et al., 2017). Additionally in Method A, the ordered crystalline structure in raw rice only allowed the oil particles to adhere to the surface of rice grains (Li, Yu and Gilbert, 2022). Thus, minimal oil was lost during transfer. Contrarily, the steamed white rice in Method C allowed the leaching of amylose and the

swollen of starch. This promoted sticking to the utensil under high heat due to the covalent bond formation between the utensil surface and the carbohydrates (RSC Education, 2024). This also resulted in some oil might be left on the utensil and lost during transfer from stir-frying to the steaming process. However, this effect was diminished in brown rice due to the presence of rice bran which served a physical barrier to prevent the direct contact between gelatinised rice starch and utensil surface. Thus, there was an insignificant ( $p>0.05$ ) difference in the crude fat content of brown rice against Methods B and C upon both PO and CO treatments.

### **5.2.5 Fibre**

The low degree of milling in brown rice leaving behind the rice bran and germ, contributing to a higher crude fibre content as compared to white rice. Crude fibre is a complex combination of insoluble cellulose polymers, pentosans, and lignin (Cvrk, et al., 2022). These polysaccharides are made up of glucose units linked by  $\beta$ -1,4-glycosidic bonds and form a rigid, linear chain that contributes to their strength and resistance to acid and alkaline hydrolysis (Brown and Saxena, 2007). Therefore, they are only susceptible to strong chemicals and the presence of enzymes (Boarino and Klok, 2023). However, these cooking methods and oil treatments did not involve an extreme pH environment or the presence of enzymes that could affect the crude fibre content. Moreover, crude fibre of rice rarely leaches via food processing, such as bleaching, thus, both oil treatments and cooking methods

have a minimal impact on the crude fibre content of brown and white rice. This result was also consistent to the findings of Suman and Boora (2015) and Tanjor and Hongsprabhas (2021), who also reported that the crude fibre content of rice treated with different oils and cooking methods was similar to that of untreated controls.

#### **5.2.6 Carbohydrate**

The variation in the changes of moisture and crude fat contents in brown and white rice resulted in different impacts on their carbohydrate content across the three cooking methods. The greater increase in the crude fat content in brown rice contributed to the reduction in its carbohydrate content, as observed in Methods A and B following oil treatments. This finding was consistent with the findings of Tanjor and Hongsprabhas (2021) regarding 3% CO and RBO treatments. Furthermore, PO treatment reduced the carbohydrate content of white rice, while CO treatment exhibited an opposite effect following Method C. This was attributed to the higher moisture content retrained in PO-treated rice compared to CO-treated rice. On the other hand, the highest carbohydrate content was observed in Method C, mainly due to the loss of moisture content during the stir-frying of gelatinised rice.

### 5.2.7 Total Calorie

The total calorie content of food was derived from its carbohydrate, fat, and protein content. Fat provides a dense energy due to the greater number of carbon-hydrogen bonds, which store greater chemical potential energy than protein and carbohydrates (Melzer, 2011). Rice bran and germ in brown rice contain a good source of linoleic acid and other essential fatty acids (Rathna, et al., 2019). Consequently, brown rice demonstrated a higher calorie content than white rice across various cooking methods and oil treatments. Additionally, the oil treatment also increased the calorie content of both brown and white rice.

The lower calorie content observed in the white rice control cooked by Method C was possibly due to the dextrinization of starch, which broken down the long polysaccharides into shorter dextrin chains (Huang and Perdon, 2020). Shorter carbohydrate chains release slightly fewer calories compared to long-chain carbohydrates due to fewer glycosidic bonds and greater combustion efficiency. For instance, long-chain starch contains slightly higher calories at 4.20 kcal/g, whereas the monosaccharide glucose contains lower 3.74 kcal/g (Troy, 2020).

Following PO treatment, the addition of oil to white rice during stir-frying prevented the dextrinization of starch by providing a protective barrier against thermal breakdown (Devi, Sindhu and Khatkar, 2020). Besides, oil barrier retains the moisture content around the starch granules, reducing the

dextrinization of starch because this process is more prominent under dry conditions. Consequently, similar calorie content was observed in rice cooked by Methods B and C. Additionally, the direct contact between oil and gelatinised rice addressed the low hydrophilicity properties of PO, increasing accessibility and resulting in the formation of more bound fat in white rice. Bound fat contributes to slightly lower calorie content compared to free fat, as free fats are more readily and completely combusted because they are more accessible and not entangled with other molecules (Ruibal-Mendieta, Delacroix and Meurens, 2002). However, the ALCs formed from long-chain fatty acids affect the crystallisation of starch during retrogradation, which might slightly increase the calorie content of rice due to higher combustion efficiency (Wang, et al., 2015). Thus, similar calorie content was observed in white rice treated with Methods B and C following PO treatment.

Contrastingly, the high hydrophilicity properties of CO provided an advantage in forming ALC across the different cooking methods. Additionally, the ALC formed by shorter fatty acid chains rarely affected the retrogradation process (Wang, et al., 2015). Therefore, there was an insignificant ( $p>0.05$ ) difference in the calorie content of white rice between the three cooking methods upon CO treatment. Moreover, stir-frying brown rice did not induce the dextrinization of starch due to the presence of the rice bran and oil layer, which acted as a barrier during stir-frying. This contributed to an insignificant ( $p>0.05$ ) difference in brown rice among the three cooking methods, regardless of control, PO or CO treatments.



### **5.3     *In Vitro* Digestibility of Treated Rice**

#### **5.3.1    Glucose Release During *In Vitro* Digestion of Treated Rice**

Friction and polishing mills remove the rice bran and germ, leaving behind the mostly starchy endosperm in refined white rice (Tuncel, 2023). These intensive milling processes, particularly friction milling, break long-chain polysaccharides at the outermost surface of the rice grain into shorter chains due to the use of physical force to abrade the outer layers of the rice kernel (Mohapatra and Bal, 2004). Consequently, following cooking processes broke these shorter chains more easily, releasing more glucose and resulting in a higher glucose baseline in white rice controls (Berg, 2023).

The addition of oil to brown and white rice lowered the glucose baseline. This might be due to the interaction of fatty acid chains with shorter carbohydrate chains. The hydrocarbon chains of fatty acids form strong electrostatic bonds with the hydrophobic moiety of amylose chains, forming ALCs resist the higher kinetic energy from the heating process (Ronie and Hasmadi, 2022). Thus, less simple sugar was released during the cooking process, as shown in oil-treated brown and white rice. In comparison, CO showed a greater lowering effect on the glucose baseline in both types of rice. This was mainly due to the greater ability of medium-chain fatty acids in CO to form ALCs because of their higher hydrophilicity properties, as aforementioned earlier (Chumsri, et al., 2022).

Brown rice with oil treatments showed the lowest release of glucose across the three cooking methods. The rice bran and germ in brown rice contributed to higher crude fibre content, which slow down the digestion of carbohydrates, leading to a slower release of glucose (Battle, 2024). Additionally, the formation of the ALCs further retard the release of glucose due to the amylolysis resistance resulting from the strong electrostatic bonds between fatty acid chains and glucose units in the amylose chain (Panyoo and Emmambux, 2016). Moreover, the degree of gelatinisation of the oil-treated rice decreases because the gelatinisation temperature of ALCs is relatively higher than that of native starch, indirectly creating another hurdle to limit amylolysis by digestive enzymes. Furthermore, retrogradation induces the crystallisation of starch in rice, forming a higher degree of crystalline structure, which also decreases amylolysis.

White rice also showed a sustained release of glucose compared to the controls following oil treatments due to the formation of the ALCs and retrogradation process which slowed the release of glucose, as aforementioned. Similar observations were reported by Kaur, et al. (2015) and Krishnan, et al. (2020), who treated white rice with different cooking oils: ghee, CO, virgin CO, and RBO.

### 5.3.2 Starch Proportion in Treated Rice

#### 5.3.2.1 Rapidly Digestible Starch

Oil treatment reduced the RDS in both types of rice across the three cooking methods, owing to the formation of the ALC and their effect on the retrogradation process (Hsu, et al., 2015). The ALCs resist amylolysis due to the strong electrostatic bond between fatty acid chains and glucose units in the amylose chain, which convert the RDS to SDS or RS, depending to the stability of the ALCs (Panyoo and Emmambux, 2016). The stability of the ALCs is closely related to the type and the saturation of the fatty acid chain. The ALCs formed by unsaturated fatty acids are less stable than those formed by saturated fatty acids due to the kink structure in the *cis* double bond. This results in SDS that only slightly retards the amylolysis during digestion (Hasjim, Ai and Jane, 2013). Regarding saturation, a decrease in the saturation of long-chain fatty acid tends to result in the formation of structures with lower stability when forming an ALC (Zabar, et al., 2009). Furthermore, ALCs promote the retrogradation process by lowering the water retention of neighbouring starch granules, which restricts the mobility of starch molecules and further improves the rate of starch crystallisation (Cui, et al., 2024).

Coconut oil exhibits a greater ability to form ALCs due to the abundance of the medium-chain fatty acids which have higher hydrophilicity properties (Chumsri, et al., 2022). The greater formation of ALCs promotes the retrogradation process by reducing the availability of free amylose chains

for recrystallisation (Cui, et al., 2024). Thus, CO treatment resulted in a greater reduction in the RDS of white rice across the three cooking methods, followed by PO treatment. This might be attributed to the ALCs formed by long-chain fatty acids, which retard retrogradation by interfering with the co-crystallisation of neighbouring amylopectin molecules, thereby directly slowing down the crystallisation of gelatinised starch in rice (Wang, et al., 2015; Arik Kibar, Gönenç and Us, 2013).

Brown rice showed a lower RDS across the cooking methods, owing to its lower degree of milling and the presence of bioactive compounds in the rice bran (Pereira, et al., 2021). However, the presence of rice bran served as a barrier, slowing down the formation of ALC in Methods A and B. Conversely, Method C facilitated direct contact between oils and rice, thereby increasing the chances of forming ALCs. Additionally, the lower moisture content associated with Method C accelerated the rate of retrogradation by limiting the mobility of starch molecules in rice grains (Dong, et al., 2020). As a result, an obvious effect was observed only in brown rice when treated with Method C.

High heat in Method C might release simple glucose from gelatinised rice without oil treatment due to the dextrinization of starch by dry heat. Consequently, brown rice showed the highest RDS upon Method C. In comparison, white rice exhibited the highest RDS when cooked by Method B. The higher moisture content in Method B-treated rice slowed down the retrogradation process due to higher mobility of starch molecules in rice grains (Dong, et al., 2020).

### 5.3.2.2 Slowly Digestible Starch

Fundamentally, brown rice contains a greater amount of SDS due to its lower degree of milling. The presence of rice bran and germ increases the crude fibre content, resulting in a slower and more sustained release of glucose during digestion (Battle, 2024). Palm oil treatment promotes the formation of SDS because the long-chain fatty acids in PO require additional amylose space, leading to a less stable structure during the formation of ALC (Chumsuri, et al., 2022). Moreover, the unsaturated fatty acids in PO tend to form SDS due to the kinked structure of the *cis* bonds (Hasjim, et al., 2013). Furthermore, ALCs formed by long-chain fatty acids retard retrogradation by interfering with the co-crystallisation of neighbouring amylopectin molecules. This interference directly slows the crystallisation of gelatinised starch in rice, resulting in unstable structures upon retrogradation. Consequently, in Methods B and C, an increase in the SDS was observed in PO-treated rice, with the highest SDS noted in brown rice treated with PO.

However, Method A induced a reduction in the formation of SDS in both types of rice. Especially, white rice treated with CO showed the lowest SDS among Method A-treated samples. In Method A, although direct contact increased the chance of forming ALCs, the ordered crystalline structure in raw rice allowed the oil particles to adhere mainly to the surface of rice grains, resulting in fewer ALCs being formed during stir-frying (Li, Yu and Gilbert, 2022). Additionally, the hydrophobic effect of long-chain fatty acid increases when the temperature is raised to approximately 140°C (Lesmes, et al., 2009).

This promotes the aggregation of hydrophobic tails into micelles, which affects the formation of ALC during steaming (Fan, et al., 2019). Consequently, this contributed to a decrease in SDS, even with PO treatment.

Contrastingly, shorter fatty acid chains exhibit increased solubility in water because high heat disrupts their aggregates. As heating raises their kinetic energy, it can break weak hydrophobic interactions, leading to reduced aggregation (Romero and Suárez, 2009). The shorter-chain fatty acids appear less hydrophobic due to the absence of stable aggregated structures, which facilitates the formation of more ALCs and the conversion of SDS to RS. Therefore, CO treatment exhibited the most reduction in the formation of SDS in both types of rice.

### **5.3.2.3 Resistant Starch**

The RS content of brown and white rice was increased with two oil treatments incorporated with 12 h of retrogradation. This was attributed to the formation of the ALCs and their effects on retrogradation, as aforementioned. The highest RS content was observed in white rice with CO treatment, followed by white rice with PO treatment across the three cooking methods. This was not merely owing to the greater ability of CO to form ALCs but also the severe milling process in white rice, which facilitated direct contact between starches and fatty acid chains, further accelerating the formation of ALCs (Chumsri, et al., 2022). Nevertheless, the equal proportion of

unsaturated and saturated fatty acids in PO provides fewer medium-chain fatty acids for forming stable ALCs, resulting in lower RS content upon PO treatment.

Both oils showed a similar effect on brown rice treated with Methods A and B due to the presence of rice bran, which served as a barrier that slowed down the formation of ALCs. Conversely, Method C facilitated direct contact between oils and rice, thereby increasing the chances of forming ALCs. Additionally, the lower moisture content associated with Method C accelerated the rate of retrogradation (Dong, et al., 2020). Thus, an obvious effect was observed only in brown rice when treated with Method C.

## **5.4 Prebiotic Potential of Treated Rice**

### **5.4.1 Initial Oligosaccharide Concentration of Treated Rice**

Brown and white rice illustrated an increase in initial oligosaccharide concentration following oil treatments, indicating low leaching of medium-chain glucose due to the formation of the ALCs and the entanglement of neighbouring starch by ALCs (Huang, et al., 2020). The highest oligosaccharide concentration was observed in the CO-treated white rice cooked with Method A, owing to the combined effects of starch dextrinization and the decreased hydrophobicity of CO resulting from the dry heat during stir-frying. Stir-frying raw rice allowed the dextrinization of starch on the

surface of the rice grains, forming a greater amount of oligosaccharides in white rice at high temperature. Additionally, the decreased hydrophobicity of CO accelerated the formation of ALCs, which prevented the leaching of medium-chain glucose from rice (Romero and Suárez, 2009).

Conversely, in the PO treatment, brown and white rice prepared with Method C showed the highest initial oligosaccharide concentration. This might be attributed to the lower hydrophilicity and higher melting point of long-chain fatty acids in PO, which restricted the distribution of fatty acids in the cooking water, reducing the ability to form ALCs in rice (Hasjim, et al., 2013). Thus, the direct contact between gelatinised starch and fatty acid chains in Method C was more likely to prevent some medium-chain glucose leaching out from the rice.

#### **5.4.2 Oligosaccharide Concentration and Growth Curve upon 24 hours**

Both probiotic strains *Lacticaseibacillus casei* and *Lacticaseibacillus rhamnosus* were able to hydrolyse the oligosaccharides of all treated rice at each time point. This was in tandem with the increase in the growth of probiotics over time. This was mainly due to the lower molecular weight of oligosaccharides, which were more easily hydrolysed by probiotics (You, et al., 2022).



## 5.5 Scanning Electron Microscopy Analysis

The rougher surfaces observed in the controls cooked by Methods A and C were possibly due to the moisture evaporation caused by dry heat and the interaction with hot cooking oil during the stir-frying process (Kaláb, 2018). Additionally, the absence of voids and hollows, along with the presence of swollen starch granules, indicated limited amylose leaching during the cooking process as suggested by Yang, et al., 2016. This might be attributed to the formation of the ALC, which prevented the leaching of amylose chain from the matrix (Hsu, et al., 2015). Lee et al. (2017) have shown that the leaching of amylose is more prominent in rice subjected to boiling, where starch granules rupture and release amylose into the surrounding water. This contrasts with the limited amylose release observed in this study, likely due to the formation of the ALC, which prevented amylose chains from leaching out of the matrix (Hsu, et al., 2015).

Additionally, the differences between CO- and PO-treated rice can be explained by the findings of a study by Zhang, et al. (2018), which demonstrated that cooking oils with higher hydrophilicity helped reduce amylose leaching by promoting the formation of a more compact starch matrix. In this study, CO-treated rice exhibited more intact and compact starch granules compared to PO-treated rice, indicating that the high hydrophilicity of CO may have facilitated the formation of the ALC and limited amylose chain leaching. This suggests that the type of oil and cooking method plays a

significant role in regulating amylose leaching during cooking, as noted in other studies (Kaur, et al., 2019).

## **5.6 Limitations and Recommendations**

The effect of oil treatment on the starches of brown and white rice could not be determined via meta-analysis due to the limited number of eligible studies. Thus, these meta-analyses may be conducted in the future when more relevant papers are published. Besides, more relevant papers can be included in the current meta-analysis in the future, particularly those examining the effect of retrogradation on the SDS and RDS of and white rice. This would increase the cumulative data, thereby strengthening the statistic evidences. Once sufficient information is accumulated, a meta-analysis of rice classified by amylose content can be carried out because amylose content plays an important role in affecting the retrogradation and the formation of ALCs.

For future research, it is recommended that a feasibility study be conducted to assess the scalability of this combination of retrogradation and oil treatment for industrial applications. This would involve evaluating whether the effects observed in the laboratory can be replicated on a larger scale, considering factors such as cost, consistency, and large-scale processing time. Additionally, sensory evaluation should be carried out to determine the acceptability of treated rice among consumers. Sensory assessments, including

taste, texture, and visual appeal, will be crucial in understanding whether treated rice can meet consumer preferences. Moreover, texture analysis and pasting properties should be evaluated to determine the impact of retrogradation and oil treatments on the rice's texture and cooking quality. Pasting properties such as gelatinisation temperature and viscosity can provide valuable insights into the quality and mouthfeel of the rice. Lastly, exploring the effects of a wider range of cooking oils, including those with varying compositions of saturated and unsaturated fatty acids, could provide further insights into which oils are most effective for reducing glycemic impact and enhancing the prebiotic properties of rice. By investigating these areas, future studies can optimize the oil treatment method for broader applications in both the food industry and consumer health.

## **CHAPTER 6**

### **CONCLUSION**

This study highlighted the significant influence of the types of fatty acids and cooking methods on the starch profile, glycemic response, and potential prebiotic properties of brown and white rice. The meta-analysis demonstrated that retrogradation resulted in an increase in RS and a decrease in RDS in white rice. However, the effect of oil treatment on the starches of brown and white rice could not be determined via meta-analysis due to the limited number of eligible studies. The proximate analysis further revealed that crude protein, ash, and crude fibre contents were remained unaffected by oil treatments, although both types of rice exhibited lower moisture content after oil treatment. Importantly, oil treatments did not result in a spike increases in crude fat or calorie content, suggesting their suitability for maintaining the nutritional balance of rice.

From a glycemic perspective, rice treated with oil, particularly CO, exhibited a reduced glucose baseline and slower glucose release compared to that of controls. The CO-treated white rice showed the lowest glucose baseline across all cooking methods, while brown rice demonstrated the slowest glucose release across all cooking methods. Coconut oil, when combined with Methods A and C, resulted in the highest levels of RS, suggesting its superior role in the formation of ALC and in reducing the glycemic response. Scanning

electron microscopy showed that oil-treated rice maintained a more compact and intact starch granule structure, particularly in CO-treated samples, where greater starch granule integrity was observed compared to PO treatment.

Moreover, CO-treated white rice prepared by Method A demonstrated potential prebiotic properties, enhancing the growth of *Lactobacillus casei* and *Lactobacillus rhamnosus*, while reducing oligosaccharides after 24 h. This suggests that CO-treated rice, especially when prepared using Method A, may have applications in promoting gut health through prebiotic activity. Overall, CO, in combination with Method C, was recommended for optimal RS formation, reduced glycaemic impact, and the development of prebiotic potential in rice.

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## APPENDIX

### Appendix A

**Table A: The search keywords used to retrieve eligible articles in the meta-analysis**

Search keywords	Brown rice
	White rice
	Retrogradation
	Stir-frying
	Cooking oil
	Coconut oil
	Palm oil
	Resistant starch
	Slowly digestible starch
	Rapidly digestible starch

## Appendix B

**Table B: The combinations of search keywords for two meta-analyses and the number of findings from three databases**

Combination		Number of findings		
		Google Scholar	Scopus	PubMed
<b>Retrogradation</b>				
1.	Brown rice AND retrogradation	9	41	13
2.	Brown rice AND resistant starch	10	89	37
3.	Brown rice AND slowly digestible starch	0	17	6
4.	Brown rice AND rapidly digestible starch	0	13	5
5.	White rice AND retrogradation	1	35	13
6.	White rice AND resistant starch	4	80	39
7.	White rice AND slowly digestible starch	0	19	11
8.	White rice AND rapidly digestible starch	0	13	10
<b>Total</b>		<b>24</b>	<b>307</b>	<b>134</b>
<b>Oil treatment</b>				
1.	Brown rice AND cooking oil	100	22	10
2.	Brown rice AND coconut oil	29	8	2
3.	Brown rice AND palm oil	25	18	6
4.	Brown rice AND stir-frying	0	0	0
5.	Brown rice AND resistant starch	10	89	37
6.	Brown rice AND slowly digestible starch	0	17	6
7.	Brown rice AND rapidly digestible starch	0	13	5
8.	White rice AND cooking oil	33	31	16
9.	White rice AND coconut oil	11	6	1
10.	White rice AND palm oil	8	27	9
11.	White rice AND stir-frying	0	5	2
12.	White rice AND resistant starch	4	80	39
13.	White rice AND slowly digestible starch	0	19	11
14.	White rice AND rapidly digestible starch	0	13	10
<b>Total</b>		<b>220</b>	<b>348</b>	<b>154</b>

## Appendix C

**Table C: The incongruent research objects from the pooled study in data search**

Meta-analysis A	Apple pomace	1
	Barley	1
	Bibimbap	1
	Bread	9
	Bulgur	1
	Cake	2
	Cereal	1
	Cracker	2
	Fettuccine	1
	Flour	63
	Meal replacement powder	1
	Noodle	5
	Pasta	4
	Porridge	1
	Rice cake	3
	Starch	13
Meta-analysis B	Barley	1
	Bibimbap	1
	Biscuit	1
	Bread	5
	Brown rice oil	1
	Bulgur	1
	Cake	1
	Cassava	1
	Cereal	1
	Cracker	2
	Fettuccine	1
	Flour	56
	Noodle	8
	Pasta	2
	Porridge	1
	Rice bran oil	5
	Rice cake	3
	Snack bar	1
	Starch	4

## Appendix D

**Table D: The mathematical data extracted from the eligible articles in Meta-analysis A**

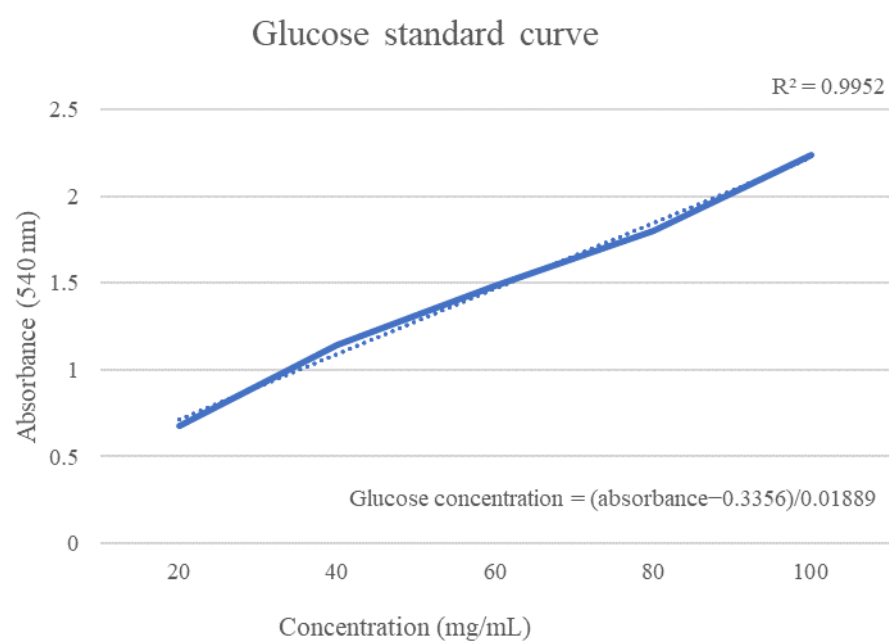
Study	Variety	Sample	Duration (Hours)	Resistant starch		Slowly digestible starch		Rapidly digestible starch	
				Control	Treatment	Control	Treatment	Control	Treatment
Strozyk, et al.– L	-	4	24	7.52±0.05	11.96±0.04	-	-	-	-
Hsu, et al. – L (1)	Low amylose indica	6	24	4.70±0.90	10.30±0.60	12.20±0.90	13.30±2.60	83.10±0.40	76.40±1.90
Hsu, et al. – L (2)	High amylose indica	6	24	3.00±1.30	11.60±0.20	20.20±0.20	13.50±1.00	76.70±1.10	74.90±0.80
Jayawardena, et al. – L	Basmati	9	24	1.24±0.08	2.15±0.39	-	-	-	-
Chiu and Stewart – L (1)	Jasmine	4	72	0.92±0.02	0.74±0.12	-	-	-	-
Chiu and Stewart – L (2)	-	4	72	1.08±0.27	2.55±0.74	-	-	-	-
Hsu, et al. – L (3)	Low amylose indica	6	72	4.70±0.90	12.30±1.80	12.20±0.90	13.50±2.10	83.10±0.40	74.20±0.40
Hsu, et al. – L (4)	High amylose indica	6	72	3.00±1.30	14.80±2.00	20.20±0.20	12.50±4.10	76.70±1.10	72.70±4.40
Chiu and Stewart – M	-	4	72	0.49±0.12	0.86±0.30	-	-	-	-
Jayawardena, et al. – M	-	9	24	0.84±0.02	1.25±0.06	-	-	-	-
Hsu, et al. – S (1)	Low amylose japonica	6	24	2.90±0.90	8.90±0.20	11.50±1.30	16.10±0.20	85.60±2.80	75.00±0.40
Chiu and Stewart – S	-	4	72	0.38±0.06	0.81±0.26	-	-	-	-
Hsu, et al. – S (2)	Low amylose japonica	6	72	2.90±0.90	11.20±0.20	11.50±1.30	13.70±2.40	85.60±2.80	75.00±2.40

<sup>a</sup> L – long-grain; M – medium-grain; S – short-grain

<sup>b</sup> Data were expressed in mean ± standard deviation in g/100 g.

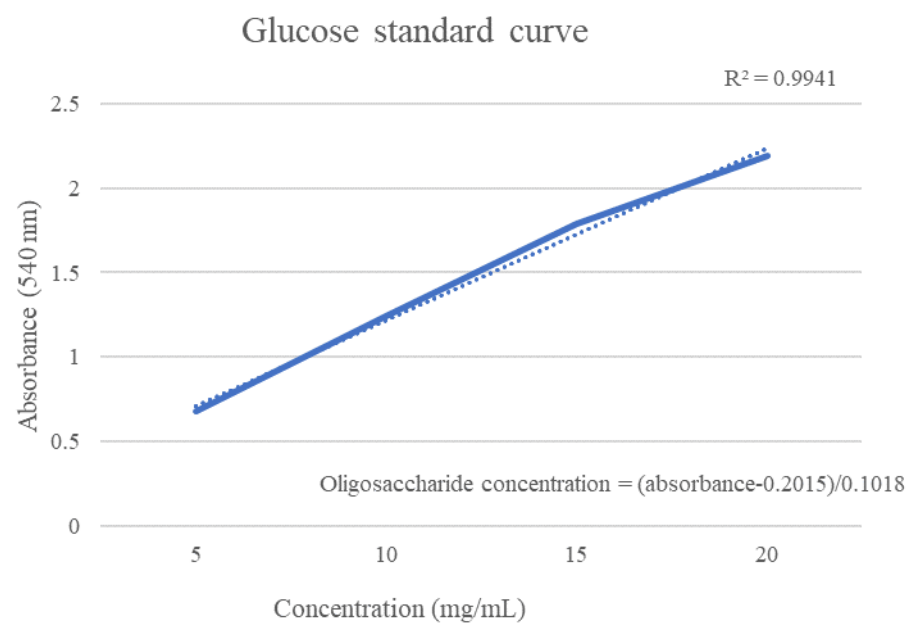


## Appendix E



**Figure E: Glucose standard curve for *in vitro* glucose release assay**

## Appendix F



**Figure F: Glucose standard curve for oligosaccharide assay**