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Meta-analysis of retrogradation effect on starches of white rice, and comparative study of different cooking oils and cooking methods on *in vitro* glucose release from white rice

[1]Lian, Y. P., [2,3]Loo, K. W., [4]Tan, T. C. and [1]*Lye, H. S.

[1]*Department of Agricultural and Food Science, Faculty of Science, Universiti Tunku Abdul Rahman, Kampar Campus, 31900 Kampar, Perak, Malaysia*
[2]*Department of Biological Science, Faculty of Science, Universiti Tunku Abdul Rahman, Kampar Campus, 31900 Kampar, Perak, Malaysia*
[3]*Centre for Biomedical and Nutrition Research (CBNR), Faculty of Science, Universiti Tunku Abdul Rahman, Kampar Campus, 31900 Kampar, Perak, Malaysia*
[4]*Food Technology Division, School of Industrial Technology, Building G07, Persiaran Sains, Universiti Sains Malaysia, 11800 Pulau Penang, Malaysia*

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Abstract

Cooling is a technique employed to reduce the high glucose release from white rice by altering the proportion of rapidly digestible (RDS), slowly digestible (SDS), and resistant (RS) starches. Retrogradation of the gelatinised starches to a crystalline structure increases the RS content that does not spike blood glucose on consumption. The meta-analysis showed that following different retrogradation durations, there were significant mean differences (MD), *viz*. an increase in RS (MD: 4.17 g/100 g, $p < 0.00001$) and decrease in RDS (MD: -7.09 g/100 g, $p < 0.0001$). The addition of cooking oil to rice retards the release of glucose due to the formation of an amylose-lipid complex (ALC), and together with retrogradation, further lowers glucose release. In the present work, palm oil (PO) and coconut oil (CO) were added to steamed rice by three methods: (A) stir-frying raw rice with oil before steaming, (B) adding oil in cooking water during steaming, and **(C)**: stir-frying the steamed rice with oil, following by refrigeration at 4°C for 12 h. For nutritional composition, the moisture, fat, and carbohydrate contents of white rice were affected upon treatments. Besides, oil-treated rice released glucose slower than control in the *in vitro* digestibility test, showing increased RS and decreased RDS. Moreover, rice with CO added by Method A might serve as a potential prebiotic as it increased the growth of *Lacticaseibacillus casei* and *Lacticaseibacillus rhamnosus* in tandem with the decrease in oligosaccharides over 24 h. Coconut oil was the better cooking oil for reducing glucose release from white rice due to its greater ability to form ALC. The addition of CO *via* Method C is recommended as the lower moisture content in the rice aided formation of perfectly crystalline starch during retrogradation.

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Introduction

White rice (*Oryza sativa* L.) is a high glycaemic index (GI) staple food in Asian countries. Frequent consumption of high GI rice may increase the risk of non-communicable diseases (NCDs) owing to the rapid increase in blood glucose upon consumption. Therefore, low GI rice with a slower and sustained glucose release is more suitable, especially in the diets of health-conscious consumers and patients with NCDs.

Rice grain contains three starch fractions categorised based on their *in vitro* digestibility: rapidly digestible (RDS), slowly digestible (SDS), and resistant (RS) starches. A high proportion of RDS in white rice contributes to a spike in glucose release after consumption (Lovegrove *et al*., 2019). In contrast, white rice with a high content of SDS and

*Corresponding author.
Email: lyehs@utar.edu.my

RS results in a slower and sustained glucose release during digestion. Thence, different processing technologies and cooking methods have been developed to reduce the high glycaemic response of white rice *via* alteration of the starch proportions. For instance, cooling is a remarkable processing technology that allows the retrogradation of gelatinised starch into a high degree of crystalline structure, thereby increasing the RS content in white rice (Sonia *et al*., 2015). The significance of the effect of retrogradation on the starches of treated white rice was examined in this meta-analysis. Relevant studies were retrieved from research databases to collate treatment effects with increased statistical power from the pooled results. This did not merely increase the generalisability of the pooled results, but also served as statistical evidence for future research.

The recent advocacy for adding cooking oil to white rice is increasing because this method not only improves the flavour of white rice but also retards the release of glucose due to the formation of an amylose-lipid complex (ALC), one of the resistant starches (type v) that can be formed through the addition of cooking oil to starch during heating. Fatty acids in the cooking oil fit into the helical cavities of gelatinised amylose chains, forming ALCs that resist enzymatic digestion (Krishnan *et al*., 2020). Additionally, 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 cooking oils can be tested to determine the most effective option for lowering the high GI of white rice.

The combination of retrogradation and oil treatment may further lower the GI of white rice. Reed *et al*. (2013) combined the two by stir-frying 24-h retrograded rice with 10% corn oil. As a result, the RS content of the treated rice was higher than that of freshly steamed rice, 15.8 ± 0.4% and 0.7 ± 0.5%, respectively. However, the issue of overgrown bacteria may arise with prolonged retrogradation duration (Chiu and Stewart, 2013). Later, Anugrahati *et al*. (2015) combined coconut (CO) treatment with a 12-h retrogradation. Notably, the RS content of the treated rice was significantly higher than that of untreated control, 1.90 ± 0.09% and 1.25 ± 0.28%, respectively. To date, relevant research is limited. As such, there is still a need to further study the combined effects of retrogradation and different oil treatments on the starches of white rice.

Since increased RS in the treated rice remains in its original form throughout the human digestive system, it may be a potential prebiotic that helps to support the growth of beneficial bacteria in the human body. The growth of good bacteria serving as probiotics in the human gut may decrease the risk of NCD due to their anti-diabetic effect. Additionally, several studies also indicate that certain probiotics effectively prevent colorectal cancer.

The experimental aspect of the present work was aimed at determining *in vitro* starch digestibility of white rice upon the combination treatments of different cooking oils and retrogradation. Proximate analysis was conducted to examine the changes in the nutritional composition of the treated rice. The ability of the treated rice to act as a potential prebiotic was also examined using two commercial probiotic strains, *Lacticaseibacillus casei* and *Lacticaseibacillus rhamnosus*.

Materials and methods

Meta-analysis of retrogradation treatment effects on different types of rice starches

Meta-analysis was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. The inclusion and exclusion criteria for eligible article were established, and six combinations of keywords were used to retrieve articles from three databases. A total of 132 articles were retrieved from Scopus (n = 80), PubMed (n = 38), and Google Scholar (n = 14). After deduplication, the remaining 70 articles were screened for their titles and abstracts following the prerequisite criteria. Two books, four reviews, and 23 articles were removed because they featured incongruent research objects that included rice flour (n = 9), rice products (n = 9), and other types of rice (n = 5). Subsequently, the remaining 41 articles were screened for their full texts, and 39 articles were excluded owing to two reasons. Firstly, 30 of the 39 full articles did not meet the prerequisite criteria of involving starch analysis in retrograded white rice. Another nine articles were excluded due to insufficient information such as standard deviation (n = 5) and untreated controls (n = 4).

Nonetheless, an additional eight topic-related articles were found from the reference searching. Four articles were excluded due to the lack of standard deviation (n = 3) and sample size (n = 1).

Besides, another two articles were removed because they combined retrogradation with oil treatment. Hence, a total of four eligible articles were included in the meta-analysis model. The unit of measurement for the starches was g/100 g of rice.

Upon data extraction, random effects models were applied for three types of starches, *i.e.*, RS, SDS, and RDS. Different durations of retrogradation and different types of white rice, categorised as "L" for long-grain, "M" for medium-grain, and "S" for short-grain, were included in the analysis model. Funnel plots were constructed to determine the heterogeneity of these studies. All statistical analyses were conducted using Review Manager Software 5.4 (Cochrane Collaboration, England).

Rice varieties and cooking oils

Cap Rambutan local long-grain white rice (OEL Distribution (Kedah) Ltd., Malaysia), Medella brand coconut cooking oil (Bountiful Ventures Ltd., Malaysia), and Alif brand palm oil (Sime Darby Oil, Malaysia) were purchased from a local supermarket.

Preparation and treatment of rice with cooking oils

The rice samples were prepared using two types of cooking oils that were added by three cooking methods. The cooking oil volume used in the present work was 3% (Tanjor and Hongsprabhas, 2021). The three cooking methods were (i) Method A - raw rice was stir-fried with cooking oil for 1 min, followed by steaming with filtered water for 20 min; (ii) Method B - raw rice was steamed with filtered water and cooking oil for 20 min; and (iii) Method C - raw rice was steamed with filtered water for 20 min and then stir-fried with cooking oil for 1 min (Kaur *et al.*, 2015). The non-oil-treated rice sample served as the control in each cooking method. All samples were then refrigerated at 4°C for 12 h before conducting subsequent analyses.

Nutritional composition of treated rice

The nutritional composition of the treated rice was determined according to Association of Official Analytical Chemist methods (AOAC, 2002). The protein factor for rice was 5.26 (Fujihara *et al.*, 2008). The total carbohydrate content was calculated by the difference method. The total calorie test was performed using the IKA C200 bomb calorimeter (IKA Works (Asia) Ltd., Malaysia).

In vitro starch digestibility of treated rice

The glucose release of the treated rice was determined *in vitro* as described by Kaur *et al*. (2020). The surface area of the samples was increased by passing through a sieve to simulate the mastication of rice. The samples (2.5 g) were added into volumetric flasks containing 30 mL of Milli-Q water, and immersed in a circulating water bath maintained at 37°C for 30 min. The oral phase digestion was initiated by adding 100 µL of 10% (w/v) alpha-amylase (≥ 10 units/mg solid) in Milli-Q water (Sigma-Aldrich, USA), and left for 1 min. Subsequently, 0.8 mL of 1 M hydrochloric acid (HCl) (R&M Chemicals Ltd, UK) was added to achieve pH 2.5 (± 0.2). Then, 1 mL of 10% (w/v) pepsin (≥ 250 units/mg solid) in 0.05 M HCl (Sigma-Aldrich, USA) was added to the samples, and stirred for another 30 min. The low pH in the gastric phase was neutralised by adding 2 mL of 1 M sodium bicarbonate ($NaHCO_3$) and 5 mL of 0.2 M acetic buffer (pH 6). The environment of pancreatic digestion was imitated by adding 5 mL of 10% (w/v) oxgall in Milli-Q water (Sisco Research Laboratories Ltd., India), and volume up to 55 mL mark with Milli-Q water. After 15 min, 0.1 mL of amyloglucosidase (≥ 260 U/mL) (Sigma-Aldrich, USA) and 1 mL of 5% (w/v) pancreatin in acetate buffer (Sigma-Aldrich, USA) were added to initiate the pancreatic digestion. After 16 h, the samples were centrifuged at 3,000 *g* for 15 min, and the supernatants were discharged to collect pellets. The pellets were resuspended with 3 mL of Milli-Q water. Then, 6 mL of 2 M potassium hydroxide (KOH) (Sigma-Aldrich, USA) and 0.1 mL of amyloglucosidase were added and incubated for 45 min. Aliquots of 0.25 mL, from baseline, the end of the oral and the gastric phases, and the 20, 60, 90, 120, 180th min of the pancreatic phase were collected in 1 mL ethanol-containing tubes.

Aliquot samples were centrifuged at 1,000 *g* for 10 min, and 50 µL of 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. Then, 0.75 mL of dinitrosalicylic acid (DNS) mixture was added into the samples, and heated at 100°C for 15 min. Milli-Q water (4 mL) was then added to the samples. The absorbances of the samples and the standards were determined at 540 nm against blank using a UV/Vis spectrophotometer (BMG LABTECH, Germany). The stoichiometric constant used for calculating starch from glucose content was 0.9. Rapidly digestible starch was calculated using the glucose released at the first 20 min of the pancreatic phase; SDS was calculated by the difference in glucose between 20 and 120 min of the pancreatic phase; and RS was determined from the glucose released after 16 h of the pancreatic phase. Total available starches were the sum of RS, SDS, and RDS.

Growth of probiotics and quantification of oligosaccharides in treated samples

The ability of the treated samples to act as potential prebiotics was determined by quantifying its oligosaccharide using DNS colorimetric method as described by Ng *et al*. (2008). The samples (2 g) were homogenised and added to a centrifuge tube containing 8 mL of 0.1% (w/v) phosphate-buffered saline solution (pH 7.4). The rice starch solutions (1 mL) were transferred into the universal bottles containing *Lacticaseibacillus casei* and *Lacticaseibacillus rhamnosus*. The growth of the probiotics and the oligosaccharide concentration were observed every 4 h over 24 h. The growth of probiotics was measured by absorbance at 600 nm against blank. To quantify the oligosaccharide concentration, 3 mL of aliquot samples from each 4 h interval were collected and centrifuged at 4,000 *g* at 4°C for 5 min. Next, 1 mL of supernatant was mixed with 3 mL of DNS mixture, and heated at 100°C for 15 min. Another 1 mL OF supernatant was hydrolysed with alpha-amylase and amyloglucosidase for 60 min before adding the DNS mixture. Then, 4 mL OF Milli-Q water was added to each sample after heating. The absorbances of the samples and the standards were determined at 540 nm against blank. The oligosaccharide concentration of the sample was the difference between the reducing sugar concentrations of the non-hydrolysed and the hydrolysed samples.

Scanning electron microscopy analysis of starch granules

Scanning electron microscopy (SEM) analysis was conducted as described by Golding *et al*. (2016). The samples were dried at 40°C overnight, and cooled in a desiccator. The dried rice grains were fixed on carbon adhesive tape, coated with platinum by the Jeol Auto Fine Coater (Jeol USA Inc., USA), and subjected to Field Emission Scanning Electron Microscope JSM-6701F (Jeol USA Inc., USA). The acceleration voltage was set to 4 kV. The microstructure of the outer layer of the treated rice was observed at 350 - 500× magnification.

Statistical analysis

All analyses were performed in duplicate with three separate runs ($n = 6$). The data were expressed as mean ± standard deviation, and analysed by one-way analysis of variance (ANOVA), using the IBM SPSS Statistics software version 26 (International Business Machines Co, USA). Significant difference between means was determined by Tukey's test with $p < 0.05$.

Results and discussion

Meta-analysis of retrogradation treatment effect on different types of rice starches

Resistant starch

There was a significant mean difference (MD) following different retrogradation durations for RS, *i.e.*, MD: 4.17 (95%; CI: 2.77 to 5.56) at $p < 0.00001$. Heterogeneity was detected across the pooled results. Specifically, the MD for the 24-h retrogradation was 4.29 (95%; CI: 2.05 to 6.54), while that for the 72-h retrogradation was 3.97 (95%; CI: 2.42 to 5.53). Heterogeneity was detected in different hours of retrogradation. Comparing both retrogradations, the 24-h retrogradation showed a higher MD. This could have been due to the irreversible crystallisation of amylose in short-term retrogradation (24-h retrogradation), and the reversible crystallisation of amylopectin in long-term retrogradation (72-h retrogradation). The retrograded amylopectin was not as resistant as the retrograded amylose due to the short-branch structure, hence lower RS was observed in the 72-h retrogradation (Dobosz *et al*., 2018).

Among the eligible studies, the results of Hsu *et al*. (2015)-L(4)-72 h and Chiu and Stewart (2013)-L(1)-72 h showed significant heterogeneity. The types of rice reported by Chiu and Stewart (2013) showed the opposite effect to those of Hsu *et al*. (2015) in similar 72-h retrogradation. This was due to the high proportion of F1 subfraction in the high-amylose Indica rice [L(4)] that could transform an imperfect crystalline structure into perfect crystallite during retrogradation, resulting in higher RS in retrograded rice (Hsu *et al*., 2015). In contrast, Chiu and Stewart (2013) opined that the digestibility of rice was influenced by the amylose content in rice grains. Therefore, jasmine rice [L(1)] with low amylose content resulted in a low rate of retrogradation.

Slowly digestible starch

There was no significant MDs in SDS upon retrogradation treatment, *viz*. -0.82 (95%; CI: -5.81 to 4.16; $p = 0.75$) for different retrogradation durations, -0.35 (95%; CI: -8.56 to 7.86; $p = 0.93$) for 24-h retrogradation, and -1.22 (95%; CI: -6.19 to 3.75; $p = 0.63$) for 72-h retrogradation. Heterogeneities were detected across the pooled results for the different durations of retrogradation.

The results of Hsu *et al*. (2015)-L(4)-72 h and Hsu *et al*. (2015)-S(1)-24 h showed significant heterogeneity. According to the authors, the F1 subfraction contents in the high-amylose Indica rice [L(4)] and the low-amylose Japonica rice [S(1)] were 26.7 and 12.3%, respectively. The high proportion of F1 subfraction in [L(4)] transformed the imperfect crystalline structure into perfect crystallite during retrogradation, decreasing SDS content in L4. On the contrary, the high F2 subfraction in [S(1)] tended to form more SDS. Therefore, an opposite effect was observed in [L(4)] and [S(1)] rice.

Rapidly digestible starch

The different retrogradation durations showed significant differences in RDS upon treatment, where negative effect was observed at -7.09 (95%; CI: -10.24 to -3.94; $p < 0.0001$). However, it is important to take note that this MD was solely calculated from six individual results extracted from Hsu *et al*. (2015). In addition, heterogeneity was reported with I^2 of 97%. The MD for 24-h retrogradation was -6.29 (95%; CI: -11.21 to -1.37), while the MD for 72-h retrogradation was -8.16 (95%; CI: -10.93 to -5.40). Heterogeneity was detected in different hours of retrogradation. Comparing both retrogradations, the higher MD was shown in the 72-h retrogradation. This was due to the additional crystallisation of amylopectin in the long-term retrogradation as the retrograded amylopectin also resisted amylolysis (Dobosz *et al*., 2018). Therefore, the RDS was further decreased in the 72-h retrogradation.

The result of Hsu *et al*. (2015)-L(2)-24 h showed significant heterogeneity. According to the authors, the minor change in the RDS of high-amylose Indica rice [L(2)] was due to the higher proportion of long-chain amylopectin that helped to maintain the structure of starch granules during short-term retrogradation.

Nutritional composition of the treated rice

Protein

The crude protein content of cooking oil-treated rice and controls ranged from 2.62 to 2.71 g/100 g. However, no significant ($p > 0.05$) difference was observed following the treatments (Table 1). This result corresponded with the study of Sun *et al*. (2014) where the authors reported that the crude protein content of the white rice cooked with groundnut oil was similar to that of untreated rice. Besides, Kim *et al*. (2019) also observed that there was no significant ($p > 0.05$) difference between untreated control, and sesame and olive oil-treated rice. Similarly, Sonia *et al*. (2015) and Strozyk *et al*. (2022) demonstrated that retrogradation did not affect the protein content of rice. This was attributed to the strong peptide bonds found in the chains of amino acids. The partial double bond character of peptide bonds makes amino acid chains resistant to high heat and sodium concentration (Damodaran, 2017), and therefore, similar nitrogen content was detected across the treatments. Other domestic cooking methods like ordinary, high-pressure, and microwave cooking also rarely affect the protein content of rice (Liu *et al*., 2019).

Ash

There was no significant ($p > 0.05$) difference in the ash contents observed between rice cooked with oil and untreated controls (Table 1). This result was consistent with the findings of Tanjor and Hongsprabhas (2021) who reported that the ash contents of rice cooked with coconut oil and rice bran oil were similar to those of untreated controls. Besides, Suman and Boora (2015) also reported that the differences between the ash contents of rice cooked with different methods of ordinary, pressure, microwave, and solar approaches were not significant ($p > 0.05$). This might relate to the high stability of minerals in high heat and pressure, owing to their strong covalent bonding. Therefore, cooking methods rarely influence the ash content of rice.

Moisture

Both the coconut oil and palm oil-treated rice exhibited lower moisture contents in all three cooking methods (Table 1). This was attributed to the

Table 1. Nutritional composition of controls and cooking oil-treated rice cooked by three methods.

	Method	Water	Palm oil	Coconut oil
Protein (g/100 g)	A	2.62 ± 0.27^{Aa}	2.65 ± 0.35^{Aa}	2.69 ± 0.41^{Aa}
	B	2.66 ± 0.38^{Aa}	2.68 ± 0.33^{Aa}	2.71 ± 0.36^{Aa}
	C	2.63 ± 0.22^{Aa}	2.67 ± 0.44^{Aa}	2.70 ± 0.46^{Aa}
Ash (g/100 g)	A	0.12 ± 0.01^{Aa}	0.11 ± 0.01^{Aa}	0.12 ± 0.01^{Aa}
	B	0.12 ± 0.01^{Aa}	0.12 ± 0.01^{Aa}	0.12 ± 0.01^{Aa}
	C	0.12 ± 0.01^{Aa}	0.12 ± 0.01^{Aa}	0.12 ± 0.01^{Aa}
Moisture (g/100 g)	A	68.88 ± 0.09^{Ab}	66.62 ± 0.23^{Bb}	66.49 ± 0.17^{Bb}
	B	70.78 ± 0.37^{Aa}	68.48 ± 0.33^{Ba}	68.38 ± 0.29^{Ba}
	C	66.47 ± 0.29^{Ac}	65.92 ± 0.06^{Bc}	64.84 ± 0.13^{Cc}
Fat (g/100 g)	A	0.10 ± 0.01^{Ba}	1.07 ± 0.03^{Ab}	1.09 ± 0.04^{Ab}
	B	0.10 ± 0.01^{Ba}	1.71 ± 0.07^{Aa}	1.73 ± 0.13^{Aa}
	C	0.10 ± 0.01^{Ba}	1.05 ± 0.15^{Ab}	1.07 ± 0.08^{Ab}
Crude fibre (g/100 g)	A	0.21 ± 0.01^{Aa}	0.21 ± 0.01^{Aa}	0.21 ± 0.01^{Aa}
	B	0.20 ± 0.01^{Aa}	0.21 ± 0.01^{Aa}	0.21 ± 0.01^{Aa}
	C	0.21 ± 0.01^{Aa}	0.21 ± 0.01^{Aa}	0.21 ± 0.01^{Aa}
Carbohydrate (g/100 g)	A	28.07 ± 0.25^{Bb}	29.34 ± 0.37^{Ab}	29.40 ± 0.30^{Ab}
	B	26.14 ± 0.53^{Bc}	26.79 ± 0.29^{Ac}	26.85 ± 0.20^{Ac}
	C	30.47 ± 0.21^{Ba}	30.02 ± 0.34^{Ba}	31.08 ± 0.48^{Aa}
Total calorie (kcal/g)	A	4.00 ± 0.05^{Ba}	4.06 ± 0.03^{Aa}	4.06 ± 0.04^{Aa}
	B	3.97 ± 0.05^{Aa}	4.00 ± 0.05^{Ab}	4.00 ± 0.05^{Aa}
	C	3.88 ± 0.05^{Cb}	3.95 ± 0.01^{Bb}	4.02 ± 0.04^{Aa}

Data are mean ± standard deviation (n = 6). Means in the same column followed by different lowercase superscripts are significantly different (p < 0.05) within the same cooking oil treatment. Means in the same row, followed by different uppercase superscripts are significantly different (p < 0.05) within the same cooking method.

formation of the ALC, impacting the moisture content of the treated rice. During cooking, the hydrocarbon chain of fatty acid bonds with the hydrophobic moiety of amylose chain, thereby limiting the binding of water molecules (Ronie and Hasmadi, 2022). In addition, ALC also limits the swelling of rice starch by the entanglement of amylopectin molecules during starch gelatinisation, and thus fewer water molecules are engaged (Huang *et al*., 2020).

The dry heat in stir-frying using Method C caused the partial evaporation of moisture from the surface of rice grains, resulting in the rice retaining the lowest moisture content among the three cooking methods. Furthermore, the addition of CO in Method C resulted in even lower moisture content. This was related to the ability of fatty acid to form ALC. Shorter chains of saturated fatty acids have higher ability to form ALC due to their higher solubility in water (Chumsri *et al*., 2022). Coconut oil contains a high quantity of medium-chain fatty acids, consisting of 49% lauric (C-12:0), 8% myristic (C-14:0), and 8% palmitic (C-16:0) acids, whereas palm oil contains only 44% palmitic (C-16:0) and 5% stearic (C-18:0) acids (Boeteng *et al*., 2016). Therefore, CO-treated rice had lower moisture content compared with PO-treated rice due to the greater ability of CO to form ALC which decreased the binding of water molecules, and the swelling of starch granules.

Fat

Rice treated with both types of cooking oil had significantly (p < 0.05) higher crude fat contents than that of control (Table 1). The same crude fat contents were determined in both oil-treated rice of each cooking method, coinciding with the results of Tanjor and Hongsprabhas (2021). Method B retained the highest crude fat contents among the three cooking methods. The lower crude fat contents in Methods A and C could have been due to the losses during stir-frying. Method A involved stir-frying of raw rice before steaming. The oil particles could only adhere to the surface of rice grains as it was hard to penetrate the ordered crystalline structure of ungelatinised starches (Li *et al*., 2022). As such, some cooking oil was left on the utensil (frying pan) during the transfer. Besides, the cooking temperature of stir-frying was as high as 160 to 250°C (Nugrahedi *et al*., 2017). This temperature could vaporise a small amount of cooking oil during stir-frying (Adeniran *et al*., 2020). In contrast, the steamed rice in Method C was fully gelatinised. The strong hydrogen bonding in the ordered crystalline region of raw rice had been disrupted, leading to amylose leaching and starch granule swelling (Li *et al*., 2022). The leached amylose and the swollen starch promoted sticking on the stainless-steel pan in high heat due to the bond forming between the carbohydrates and the pan surface. This resulted in some oil being left on the pan.

Fibre

Crude fibre is a complex combination of insoluble cellulose polymers and lignin. These polymers are heat- and chemical-resistant due to their strong hydrogen bonding. They are only susceptible to strong acids, alkalis, and enzymes (Boarino and Klok, 2023). However, the studied cooking methods and oil treatments did not create an extreme pH environment that could alter the crude fibre content of white rice. Therefore, the crude fibre of the treated rice did not vary across different cooking methods and oil treatments (Table 1). This result also corresponded to those of Suman and Boora (2015) and Tanjor and Hongsprabhas (2021).

Carbohydrates

Method C-treated rice showed the highest carbohydrate content, followed by Methods A and B (Table 1). This was mainly the result of the lower moisture content in Method C cooking. The carbohydrate contents of the cooking oil-treated rice in Methods A and B increased due to the formation of ALC which limited the leaching of amylose chains out of the rice grain during starch gelatinisation (Shi *et al*., 2020). In addition, the large quantity of medium-chain fatty acids in CO formed a greater amount of ALC in the gelatinised rice, contributing to the highest carbohydrate content in the CO-treated rice cooked with Method C.

Total calories

The 3% cooking oil did not cause a spike in the calories of treated rice. Nevertheless, the oil-treated rice cooked with Methods A and C showed higher calorie than that of the controls (Table 1). In contrast, no significant ($p > 0.05$) difference was observed between the cooking oil-treated rice and the untreated control in Method B. This might be related to the indirect contact between the rice grain and the cooking oil in Method B. The cooking oil floated on the water due to its non-polar properties, thus reducing accessibility and consequently forming less bound fat in white rice. On the other hand, the calories of rice cooked by Method C were the lowest among the three cooking methods. This could have been due to the dextrinisation of starch since shorter carbohydrate chains have slightly lower calorie. For instance, hexose has lower calorie of 3.9 kcal/g (Roberfroid, 1999). Troy (2020) also demonstrated that long-chain starch contains higher calories of 4.20 kcal/g, whereas the monosaccharide glucose contains 3.74 kcal/g.

In vitro digestibility of treated rice

Glucose released from treated white rice during in vitro digestion

The glucose released from rice treated with cooking oil was relatively lower in each digestion phase compared to that of the controls across all cooking methods (Figure 1). In a previous study of Krishnan *et al*. (2020), it was also found that the glucose release of white rice was reduced when cooked with ghee, CO, virgin CO, and rice bran oil at three different times of addition: before, during, and after steaming. Kaur *et al*. (2015) similarly observed that the steamed rice added with cooking oil released glucose at a slower rate compared to the untreated control. This was mainly due to the strong electrostatic bonds formed between the fatty acid chains and glucose units in ALC which competed against amylolysis by digestive enzymes (Panyoo and Emmambux, 2016). Moreover, ALC exhibited a higher gelatinisation temperature than native starch, significantly retarding the accessibility of digestive enzymes (Kumar *et al*., 2020).

The medium-chain fatty acids in CO have higher ability to form ALC owing to its higher hydrophilicity; therefore, CO-treated rice demonstrated slower rate of glucose release than PO-treated rice (Figure 1). In addition, the higher melting point of long-chain fatty acids in PO restricted the dispersion of free fatty acids in cooking water, leading to reduced availability to form ALC during steaming (Hasjim *et al*., 2013). Moreover, the

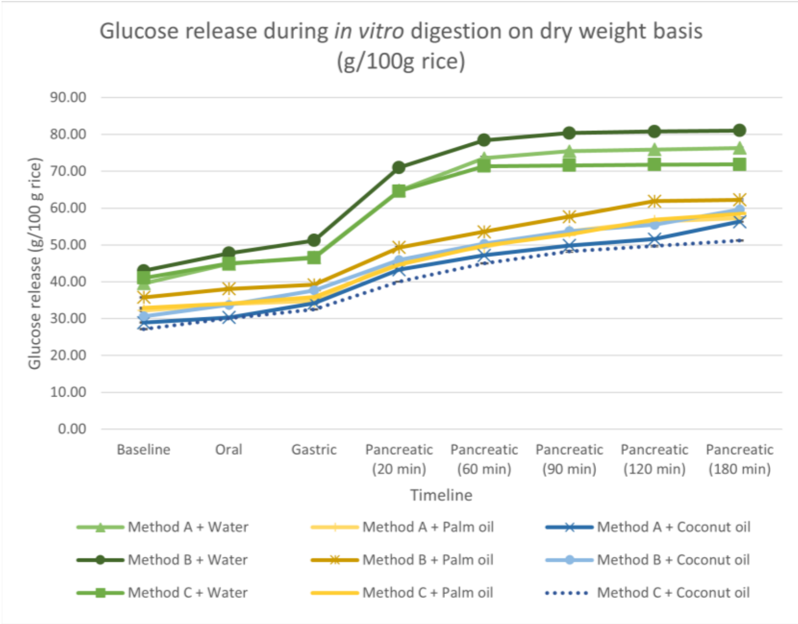

Figure 1. Glucose release from rice treated with different cooking oils and methods at different *in vitro* digestion phases on dry weight basis (n = 6).

formation of ALC from long-chain fatty acids might inhibit the retrogradation process, potentially resulting in a less crystalline structure formation in PO-treated rice during the cooling process (Wang *et al*., 2015).

Compared to Methods A and B, Method C exhibited the lowest rate of glucose release (Figure 1). This result was consistent with the findings of Kaur *et al*. (2015) who reported that the cooking oil-treated rice cooked with the "after" cooking condition (similar to Method C) showed the lowest glucose release. This was attributed to the direct contact between gelatinised rice and cooking oil in Method C which increased the chances of forming more ALC in rice. In contrast, although there was direct contact between cooking oil and rice grains in Method A, the ordered structure of raw rice only allowed the cooking oil to adhere to the surface of the rice grains. Therefore, the formation of ALC was less effective in rice cooked by Method A. Furthermore, Yan *et al*. (2021) found that the degree of retrogradation increased as the moisture content of rice decreased. Consequently, rice cooked using Method C, which had the lowest moisture content, might form more perfect crystalline structure during retrogradation compared to Methods A and B.

Total available, resistant, slowly digestible, and rapidly digestible starches of treated rice

Glucose release of white rice was lowered upon cooking oil treatment and retrogradation due to alternation of the starch fraction (Figure 2). The total available starch decreased by 15 - 17% in CO-treated rice and 8 - 13% in PO-treated rice for three different cooking methods compared with the controls (Figure 2A).

In detail, the RDS of cooking oil-treated samples significantly (p < 0.05) decreased in all cooking methods (Figure 2B), with CO treatment exhibiting greater decrease of 33 - 35%, while PO treatment exhibited 29 - 31% decrease. This resulted from the collapsed helical conformation in the ALC, which restricted the binding of the amylolysis enzyme (Hasjim *et al*., 2013). Furthermore, the entanglement of amylopectin by ALC also affected the swelling of starch, which further lowered the degree of gelatinisation in white rice (Huang *et al*., 2020). A crystalline structure in amylose and amylopectin molecules that resisted enzymatic hydrolysis was reformed during retrogradation, converting RDS to SDS or RS depending on the crystallinity (Jayawardena *et al*., 2017).

The formation of the ALC resulted in a significant (p < 0.05) gradual increase in the RS of rice treated with cooking oils, as it resisted the amylolysis of digestive enzyme (Figure 2C). Specifically, the medium-chain fatty acids in CO had the advantage of forming a greater amount of ALC in white rice due to their greater hydrophilicity and lower melting point (Chumsri *et al*., 2022). Therefore,

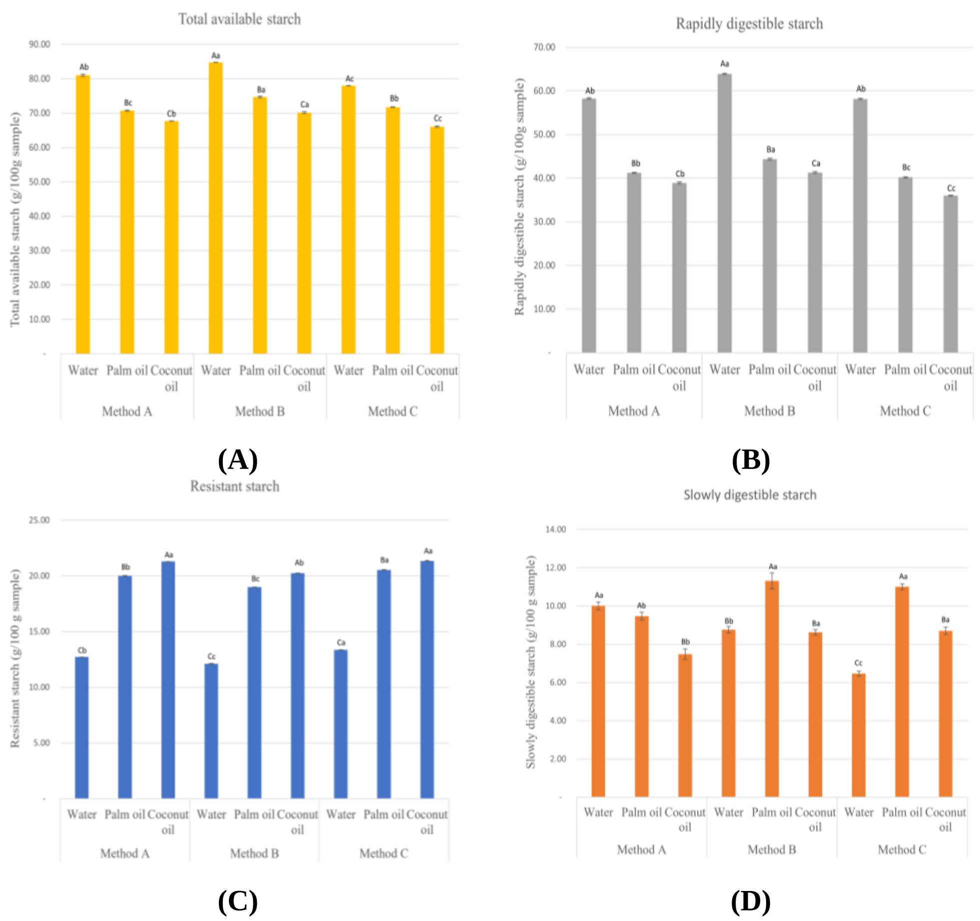

Figure 2. Starch fraction of **(A)** total available starch, **(B)** rapidly digestible starch, **(C)** resistant starch, and **(D)** slowly digestible starch of white rice after treated with different cooking methods and oils ($n = 6$). Bars with different uppercase letters are significantly different ($p < 0.05$) in the same cooking method. Bars with different lowercase letters are significantly different ($p < 0.05$) in the same oil treatment.

the RS of white rice cooked with CO was increased by 60 - 70%, while that of rice treated with PO was increased by 54 - 57% compared to the controls. Additionally, retrogradation promoted the reassociation of crystalline structures in amylose molecules, resulting in the formation of type III RS in the treated rice (Hsu *et al*., 2015). Since ALC formed by long-chain fatty acids restricted the retrogradation of starch (Wang *et al*., 2015), PO-treated rice had a lower RS content compared with CO-treated rice.

The increase in SDS in the PO-treated rice cooked with Methods B and C was noteworthy, providing a sustainable glucose release upon consumption (Figure 2D). While this might be attributed to the ability of unsaturated fatty acids to form an ALC, the kink structure in the *cis* double bond of unsaturated fatty acids might also affect the stability of ALC, contributing to a slight retardation of enzyme hydrolysis during digestion (Hasjim *et al*., 2013). The formation of ALC by long-chain fatty acids led to imperfect crystallinity in amylose and amylopectin molecules during retrogradation (Wang *et al*., 2015), leading to a significant ($p < 0.05$) increase in SDS in PO-treated rice.

Prebiotics analysis

Oligosaccharides of the treated rice

The highest oligosaccharide concentration was observed in the CO-treated rice cooked with Method A (Figure 3A). This might indicate a higher chance of medium-chain fatty acids in CO forming the ALC, preventing amylose from leaching out from the rice grain during gelatinisation (Chumsri *et al*., 2022). In addition, Method A involved direct contact between cooking oil and rice grains before steaming. This gave fatty acids in CO an advantage to react with amylose before it leached out of the grains during gelatinisation, contributing to the highest oligosaccharide concentration in this treated rice.

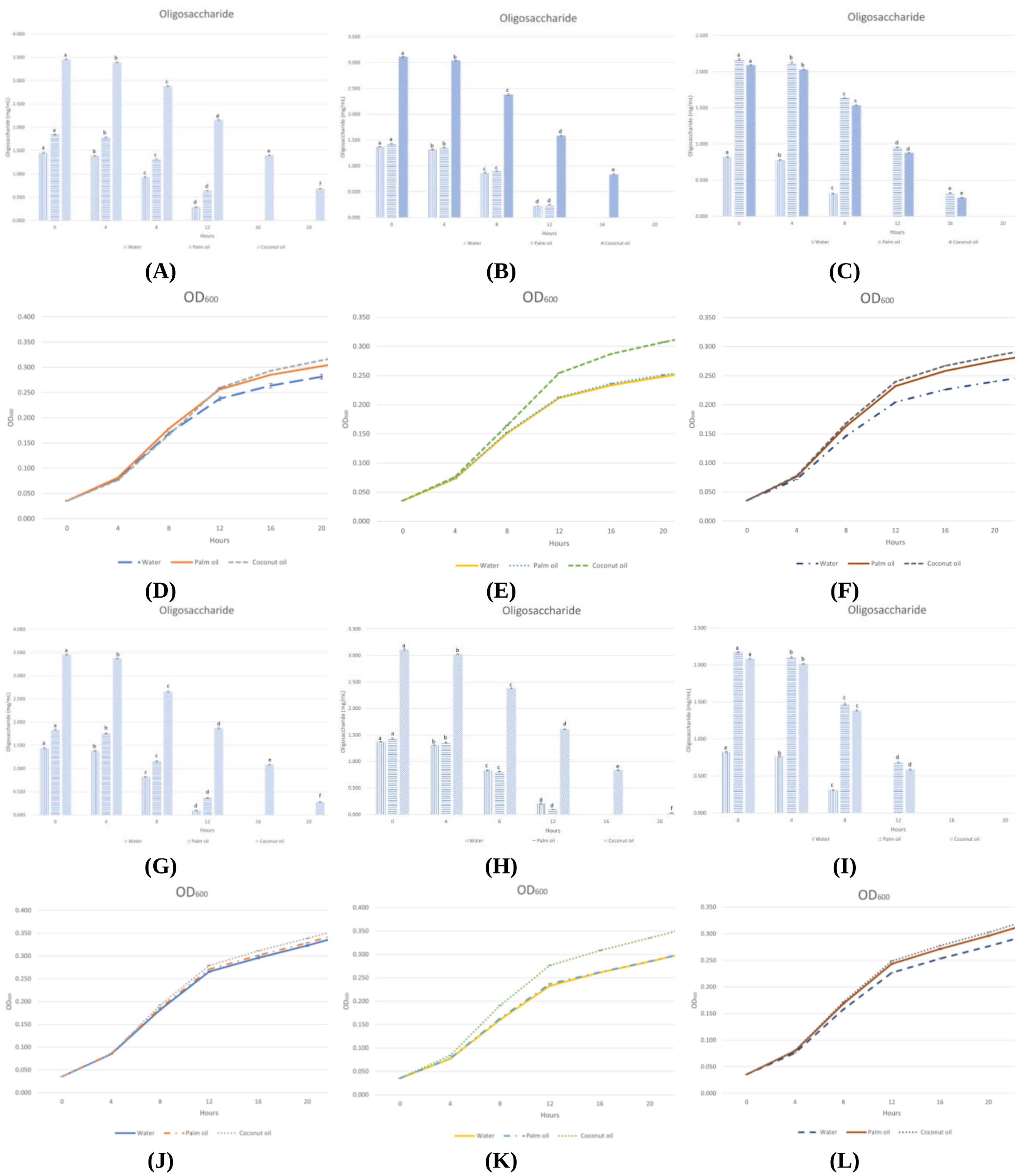

Figure 3. Oligosaccharide concentrations and growth curves (OD_{600}) of *Lacticaseibacillus casei* **(A - F)** and *Lacticaseibacillus rhamnosus* **(G - L)** after 24 h incubation. **(A)**, **(D)**, **(G)**, and **(J)** are samples cooked by Method A; **(B)**, **(E)**, **(H)**, and **(K)** are samples cooked by Method B; **(C)**, **(F)**, **(I)**, and **(L)** are samples cooked by Method C (n = 6). Bars with different lowercase letters are significantly different ($p < 0.05$) between hours in the treated rice samples.

Growth of probiotics and oligosaccharide concentration in 24 h

Lacticaseibacillus casei and *L. rhamnosus* were able to hydrolyse the oligosaccharides of all treated rice at each time point (Figure 3). The growth of both probiotic strains increased over time. Most of the oligosaccharides were fully hydrolysed after 16 h, except the oligosaccharides from CO-treated rice cooked by Method A due to them being the highest amount of oligosaccharides within the sample.

Microstructure

An uneven and rougher surface was observed in both stir-fried controls (Figures 4A and 4C), possibly due to the moisture evaporation by dry heat during stir-frying. No hollows or voids were observed in the cooking oil-treated samples, facilitating the leaching of amylose and amylopectin (Figure 4D-I) (Yang *et al*., 2016). Additionally, a greater integrity of swollen starch granules was observed in PO and CO-treated rice (Figure 4D-I). In comparison, the starch granules of CO-treated rice were more compact and intact than PO-treated rice. This indicated a limited amylose chain leaching out of the CO-treated rice (Hsu *et al*., 2015).

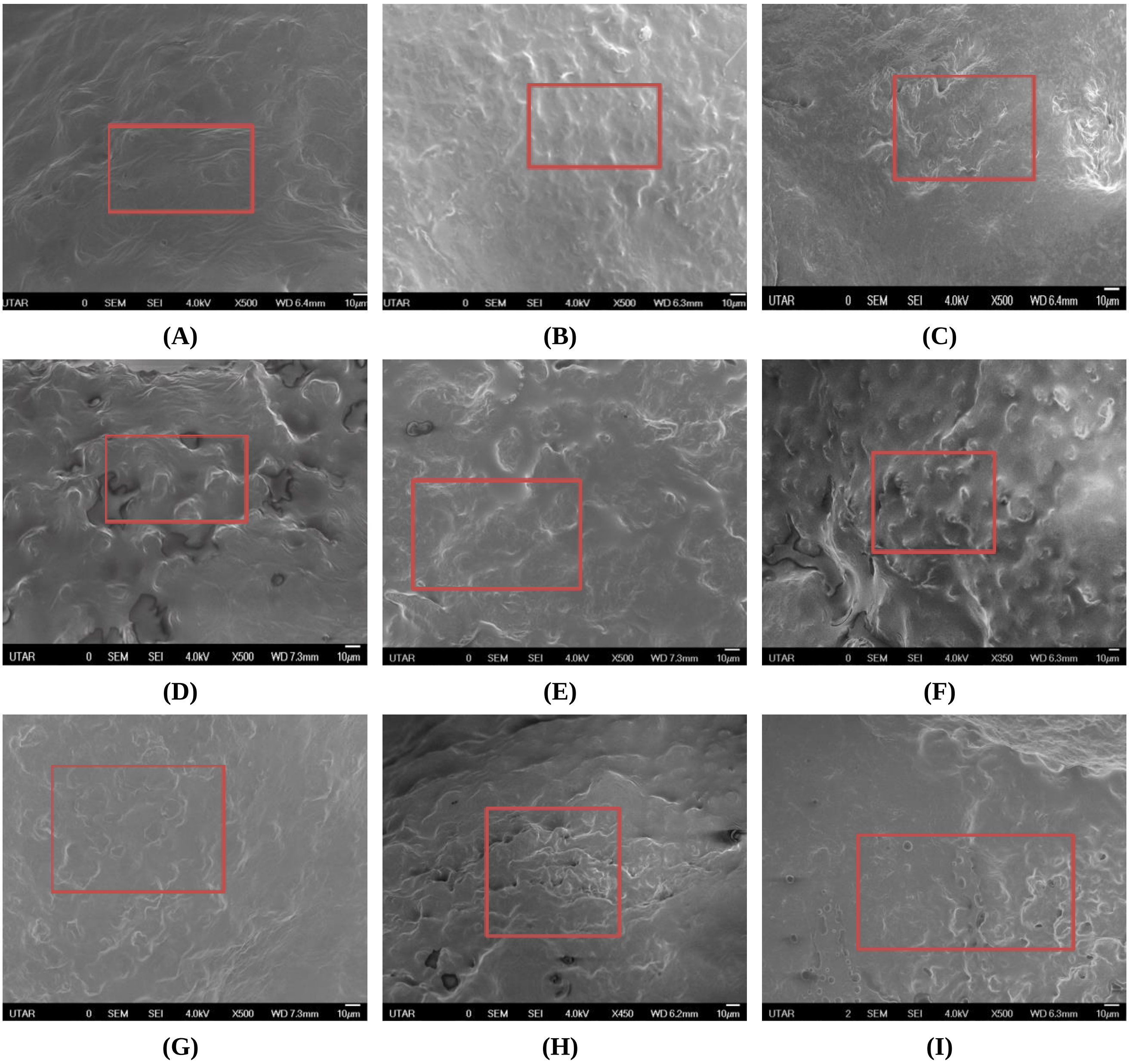

Figure 4. Scanning electron micrographs of rice after being treated with different cooking oils and methods followed by retrogradation. Images in the first row are controls with ascending Method A to C **(A, B, C)**; second row are PO-treated rice samples **(D, E, F)**; and third row are CO-treated rice samples **(G, H, I)**.

Conclusion

Meta-analysis models showed a significant MD following different retrogradation durations for RS and RDS, *i.e.*, MD: 4.17 (95%; CI: 2.77 to 5.56; $p < 0.00001$) and -7.09 (95%; CI: -10.24 to -3.94; $p < 0.0001$), respectively. Heterogeneity was determined across the pooled results. Concerning nutritional composition, the moisture, crude fat, and carbohydrate contents of treated rice were affected by different cooking methods and oil treatments. Besides, an increase in the calorie of cooking oil-treated rice was observed, but the 3% oil treatment did not cause a spike in the calorie content. Moreover, both cooking oil-treated rice was found to release glucose slower than that of controls in all cooking methods, especially added by Method C due to the direct contact between cooking oil and gelatinised rice, which aided in the formation of ALC. In addition, the low moisture content created by Method C facilitated the degree of retrogradation, forming a greater perfect crystalline structure in starch during retrogradation. On the other hand, CO showed a greater ability to form ALC than PO due to the shorter chain of fatty acids and high hydrophilicity property. Therefore, the addition of CO to white rice by Method C is recommended to lower the glucose release of white rice. Moreover, the CO-treated rice cooked with Method A might be a potential prebiotic as it promoted the growth of *L. casei* and *L. rhamnosus* over 24 h.

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References

Adeniran, J. A., Yusuf, R. O., Abdulkadir, M. O., Yusuf, M. N. O., Abdulraheem, K. A., Adeoye, B. K., ... and Du, M. X. 2020. Evaporation rates and pollutants emission from heated cooking oils and influencing factors. Environmental Pollution 266(2): 115169.

Anugrahati, N. A., Pranoto, Y., Marsono, Y. and Marseno, D. W. 2015. *In vitro* digestibility of Indonesian cooked rice treated with cooling-reheating process and coconut milk addition. International Research Journal of Biological Sciences 4(12): 34-39.

Association of Official Analytical Chemists (AOAC). 2002. Official methods of analysis of AOAC International. 17th ed. United States: AOAC.

Boarino, A. and Klok, H. A. 2023. Opportunities and challenges for lignin valorization in food packaging, antimicrobial, and agricultural applications. Biomacromolecules 24(3): 1065-1077.

Boeteng, L., Ansong, R., Owusu, W. B. and Steiner-Asiedu, M. 2016. Coconut oil and palm oil's role in nutrition, health and national development: A review. Ghana Medical Journal 50(3): 189-196.

Cervantes-Ramírez, J. E., Cabrera-Ramirez, A. H., Morales-Sánchez, E., Rodriguez-García, M. E., Reyes-Vega, M. L., Ramírez-Jiménez, A. K., ... and Gaytán-Martínez, M. 2020. Amylose-lipid complex formation from extruded maize starch mixed with fatty acids. Carbohydrate Polymers 246(2020): 116555.

Chiu, Y. T. and Stewart, M. L. 2013. Effect of variety and cooking method on resistant starch content of white rice and subsequent postprandial glucose response and appetite in humans. Asia Pacific Journal of Clinical Nutrition 22(3): 372-379.

Chumsri, P., Panpipat, W., Cheong, L. Z. and Chaijan, M. 2022. Formation of intermediate amylose rice starch-lipid complex assisted by ultrasonication. Foods 11(16): 2430.

Damodaran, S. 2017. Amino acids, peptides, and proteins. In Damodaran, S. and Parkin, K. L. (eds). Fennema's Food Chemistry, p. 217-329. United States: CRC Press.

Dobosz, A., Sikora, M., Krystyjan, M., Tomasik, P., Lach, R., Borczak, B., ... and Lukasiewicz, M. 2018. Short- and long-term retrogradation of potato starches with varying amylose content. Journal of the Science of Food and Agriculture 99(5): 2393-2403.

Fujihara, S., Sasaki, H. Aoyagi, Y. and Sugahara, T. 2008. Nitrogen-to-protein conversion factors for some cereal products in Japan. Journal of Food Science 73(3): C204-209.

Golding, C. G., Lamboo, L. L., Beniac, D. R. and Booth, T. F. 2016. The scanning electron microscope in microbiology and diagnosis of infectious disease. Scientific Reports 6: 26516.

Hasjim, J., Ai, Y. F. and Jane, J. L. 2013. Novel applications of amylose-lipid complex as resistant starch type 5. In Shi, Y. C. and Maningat, C. C. (eds). Resistant Starch: Sources, Applications and Health Benefits, p. 70-94. United States: John Wiley and Sons, Ltd.

Hsu, R. J. C., Chen, H. J., Lu, S. and Chiang, W. C. 2015. Effects of cooking, retrogradation and drying on starch digestibility in instant rice making. Journal of Cereal Science 65: 154-161.

Huang, Q., Chen, X., Wang, S. and Zhu, J. 2020. Amylose-lipid complex. In Wang, S. (ed). Starch Structure, Functionality and Application in Foods, p. 57-76. United States: Springer.

Jayawardena, N., Herath, P. N., Watawana, M. I. and Waisundara, V. Y. 2017. Effects of processing and storage conditions on the *in vitro* digestibility and other functional properties of six South Asian starches. Journal of Food Processing and Preservation 41(4): 1-11.

Kaur, B., Lim, J., Chusak, C. and Henry, C. J. 2020. Microwave cooking enhances glycaemic potential of rice: An *in vitro* study. Malaysian Journal of Nutrition 26(1): 117-128.

Kaur, B., Ranawana, V., Teh, A. L. and Henry, C. J. K. 2015. The glycemic potential of white and red rice affected by oil type and time of addition. Journal of Food Science 80(10): 2316-2321.

Kim, J. S., Nam, K. and Chung, S. J. 2019. Effect of nutrient composition in a mixed meal on the postprandial glycemic response in healthy people: A preliminary study. Nutrition Research and Practice 13(2): 126-133.

Krishnan, V., Mondal, D., Bollinedi, H., Srivastava, S., Ramesh, S. V., Madhavan, L., ... and Praveen, S. 2020. Cooking fat types alter the inherent glycaemic response of niche rice varieties through resistant starch (RS) formation. International Journal of Biological Macromolecules 162(2020): 1668-1681.

Kumar, A., Panda, P. A., Lal, M. K., Ngangkham, U., Sahu, C., Soren, K. R., ... and Sharma, S. 2020. Addition of pulses, cooking oils, and vegetables enhances resistant starch and lowers the glycemic index of rice (*Oryza sativa* L.). Starch 72(9-10): 1900081.

Li, C., Yu, W. W. and Gilbert, R. G. 2022. The effects of starch molecular fine structure on thermal and digestion properties of rice starch. Foods 11(24): 4012.

Liu, K. L., Zheng, J. B. and Chen, F. S. 2019. Effect of domestic cooking on rice protein digestibility. Food Science and Nutrition 7(2): 608-616.

Lovegrove, A., Kosik, O., Bandonill, E., Abilgos-Ramos, R., Romero, M., Sreenivasulu, N. and Shewry, P. 2019. Improving rice dietary fibre content and composition for human health. Journal of Nutritional Science and Vitaminology 65: S48-S50.

Ng, K. H., Lye, H. S., Easa, A. M. and Liong, M. T. 2008. Growth characteristics and bioactivity of probiotics in tofu-based medium during storage. Annals of Microbiology 58: 477-487.

Nugrahedi, P. Y., Oliviero, T., Heising, J. K., Dekker, M. and Verkerk, R. 2017. Stir-frying of Chinese cabbage and pakchoi retains health-promoting glucosinolates. Plant Foods for Human Nutrition 72(4): 430-444.

Panyoo, A. E. and Emmambux, M. N. 2016. Amylose-lipid complex production and potential health benefits: A mini-review. Starch 69(7-8): 1-24.

Reed, M. O., Ai. Y. F., Leutcher, J. L. and Jane, J. L. 2013. Effects of cooking methods and starch structures on starch hydrolysis rates of rice. Journal of Food Science 78(7): H1076-H1081.

Roberfroid, M. B. 1999. Caloric value of inulin and oligofructose. The Journal of Nutrition 129(7): 1436-1437.

Ronie, M. E. and Hasmadi, M. 2022. Factors affecting the properties of rice flour: a review. Food Research 6(6): 1-12.

Shi, S. X., Dong, Y. Y., Li, Q., Liu, T. T. and Yu, X. Z. 2020. Morphology, structural, thermal and rheological properties of wheat starch-palmitic acid complexes prepared during steam cooking. RSC Advances 10: 30087-30093.

Sonia, S., Witjaksono, F. and Ridwan, R. 2015. Effect of cooling of cooked white rice on resistant starch content and glycemic response. Asia Pacific Journal of Clinical Nutrition 24(4): 620-625.

Strozyk, S., Rogowicz-Frontczak, A., Pilacinski, S., LeThanh-Blicharz, J., Koperska, A. and Zozulinska-Ziolkiewicz, D. 2022. Influence of resistant starch resulting from the cooling of rice on postprandial glycemia in type 1 diabetes. Nutrition and Diabetes 12(1): 21.

Suman and Boora, P. 2015. Effect of cooking methods on the nutritional quality of rice (*Oryza sativa*) varieties. Asian Journal of Dairy and Food Research 34(2): 136-140.

Sun, L., Ranawana, D. V., Leow, M. K. S. and Henry, C. J. 2014. Effect of chicken, fat and vegetable on glycaemia and insulinaemia to a white rice-based meal in healthy adults. European Journal of Nutrition 53(8): 1719-1726.

Tanjor, S. and Hongsprabhas, P. 2021. Effect of rice bran oil or coconut oil on *in vitro* carbohydrate and protein digestion of cooked fragrant rice. Agriculture and Natural Resources 55: 496-507.

Troy, E. 2020. Measuring calories from food: Protein, fats, and carbohydrates. Retrieved on July 20, 2023 from Culinary Lore website: https://culinarylore.com/food-science:measuring-calories-from-food-protein-fats-and-carbohydrates/

Wang, S. J., Wang, J. R., Yu, J. L. and Wang, S. 2015. Effect of fatty acids on functional properties of normal wheat and waxy wheat starches: A structural basis. Food Chemistry 190: 285-292.

Yan, W. J., Yin, L. J., Zhang, M. H., Zhang, M. and Jia, X. 2021. Gelatinization, retrogradation and gel properties of wheat starch–wheat bran arabinoxylan complexes. Gels 7(4): 200.

Yang, L., Sun, Y. H., Liu, Y., Mao, Q., You, L. X., Hou, J. M. and Ashraf, M. A. 2016. Effects of leached amylose and amylopectin in rice cooking liquid on texture and structure of cooked rice. Brazilian Archives of Biology and Technology 59: e16160504.