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Research article

Effect of rice bran oil or coconut oil on *in vitro* carbohydrate and protein digestion of cooked fragrant rice

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Abstract

The influences were investigated of rice bran oil (RBO) or coconut oil (CO) on the digestion of cooked rice having different final lipid contents (0.07–7.32% dried weight basis). Cooked rice was ground for 10 s or 300 s to represent different degrees of mastication. The rice ground for 10 s had a lower surface area than the rice ground for 300 s and retained the amyloid-like network of protein and non-starch carbohydrate in the endosperm cell wall. Grinding rice for 10 s released glucose up to 80% of the carbohydrate while rice ground for 300 s released glucose of only 60% during *in vitro* digestion for 152 min. Although the oil type and concentration did not significantly ($p \geq 0.05$) affect the release of glucose from rice ground for 300 s, principal component analysis suggested that RBO lowered the release of glucose from rice ground for 10 s due to the high contents of phytosterols and long-chain fatty acids. Rice cooked in the presence of RBO or CO and ground for 300 s released the NH_2 group during the late intestinal phase to a greater extent than rice cooked in water alone. The interplay among the integrity of the amyloid-like network in the endosperm cell wall resulting from different degrees of mastication or grinding time, together with the oil type and contents during rice cooking, the digestion of rice proteins and the surface-active nutrients released during digestion in the presence of bile extracts, were significant ($p < 0.05$) in the release of glucose from cooked rice.

Introduction

Rice (*Oryzae sativa* L.) is one of the most important crops for human consumption as a carbohydrate source (Ziegler and Barclay, 2008). However, rice is classified as a high glycemic index (GI) food (Foster-Powell et al., 2002). Nonetheless, high-amylose rice varieties can be used in low GI diets (Miller et al., 1992). Attempts have been made to modify the GI of rice by altering the starch structure, that is by enhancing the retrogradation of amylose and the formation of the amylose-lipid complex and resistant starch (Boers et al., 2015; Toutounji

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et al., 2019) and ageing the rice grain to alter the protein network (Azizi et al., 2019). Extensive rice breeding programs have been enabled in many countries to modify the amylose and amylopectin fine structure that could lower the GI of rice (Kong et al., 2015).

Rice grains can be cooked differently at a household level, depending on the rice and water ratios according to consumer preference and ethnicity. In addition to water, rice can be cooked with ghee, coconut milk and vegetable oils to achieve desired flavor, aroma and texture. The addition of oil during rice cooking can affect starch digestibility. For example, red rice boiled with ghee for 20 min or fried with ghee for 1 min before boiling reduced starch digestion (Kaur et al., 2015, Kumar et al., 2018). In addition, white rice mixed with rice bran oil (Krishnan et al., 2020) or palm oil and heated at 95°C for 30 min reduced starch digestibility (Farooq et al., 2018). Amylose-lipid complex formation has been explained, in part, as being responsible for the lower degree of starch digestion of cooked rice grains (Kaur et al., 2015; Farooq et al., 2018; Kumar et al., 2018; Krishnan et al., 2020).

The influences of physical parameters have been investigated such as the cooked rice microstructure, particle size and non-starch constituents such as the endosperm cell wall structure and protein network, as a physical barrier for gut enzyme accessibility, (Tamura et al., 2016; Ogawa et al., 2018; Azizi et al., 2019; Miraji et al., 2020). In raw rice, starch granules are encased within the endosperm cell wall structure (Ogawa et al., 2018). Upon cooking, starch granules, cell wall constituents and protein bodies take up the water, solubilize, diffuse and disperse within the cooked rice matrix composed of swollen rice starch granules and alter the whole grain microstructure in both the starch and non-starch fractions (Tamura et al., 2016; Zhou et al., 2016; Likitwattanasade and Hongsprabhas, 2010; Miraji et al., 2020). Thus, the alterations in the non-starch fractions could also affect the fate of starch digestibility (Tamura et al., 2016; Zhou et al., 2016; Ogawa et al., 2018; Azizi et al., 2019; Miraji et al., 2020).

Variability among individuals during mastication before swallowing could influence the starch digestibility and glycemic responses (GR) of cooked rice (Ranawana et al., 2010). A low degree of rice mastication, for which around 37% of rice particles are larger than 2,000 μm, resulted in lower contents of rapidly digestible starch (RDS), which was detected as less than half during the early stage of *in vitro* digestion for 30 min. In contrast, masticated rice having a particle size of less than 500 μm after a high degree of mastication could substantially increase the RDS during *in vitro* digestion in the early stage. The *in vivo* GR after ingestion for 30 mins indicated that habitual mastication also influenced the magnitude and the pattern of GR in addition to the chemical characteristics of rice grains (Ranawana et al., 2010).

Although a high postprandial glucose level is expected when white rice containing low to intermediate amylose is cooked for an extended time to soften the grains (Boers et al., 2015), the consumption of newly cooked warm rice is preferential in a population eating rice as a staple carbohydrate source. Therefore, it is crucial to better understand the fates of starch digestion of rice grains cooked and consumed at a household level.

The current study further addressed the influences of the integrity in the endosperm cell wall and the differences in surface-active nutrients, particularly the oil-based compounds, generated during *in vitro* digestion in the presence of bile extracts on the release of glucose from rice cooked with rice bran oil (RBO) or coconut oil (CO) that are commonly used in rice cooking in Asia. The rice endosperm cell walls are composed of cellulose, hemicellulose and pectic substances, as well as hydroxyproline-containing glycoproteins that bind to cellulose microfibrils (Shibuya and Iwasaki, 1978). The mesh-like network of the amyloid structure of proteins and cellulose microfibril can be visualized by staining with alkaline Congo red and observation under a light microscope (Clark and Dodds, 1982, Klunk et al., 1989). Thai fragrant rice has an intermediate amylose content of 17–18% (Likitwattanasade and Hongsprabhas, 2010). Both RBO and CO have different ratios of saturated to polyunsaturated fatty acids and phytosterol contents (Dayrit, 2014; Liang et al., 2014; Eyres et al., 2016; Sohail et al., 2017; Wallace, 2019) that may alter the amphiphilic characteristics of the digesta upon digestion. Understanding the complex dynamic phenomena during the digestion of cooked rice in the presence of cooking oil, different matrix continuity and consequential interfacial characteristics of the digesta may shed more light on the release characteristics of glucose during the digestion of a carbohydrate-rich meal.

Materials and Methods

Materials

Polished fragrant rice (Maboonkrong™; Patum Rice Mill and Granary Public Co., Ltd.; Pathum Thani, Thailand), two brands of rice bran oil (RBO; Thai Edible Oil Co., Ltd.; Bangkok, Thailand) designated as RBOA and RBOB and coconut oil (CO; ThaiPure™; Thai Pure Coconut Co., Ltd.;

Samut Sakorn, Thailand) were purchased from a local store in Bangkok, Thailand. Porcine pancreas α-amylase type VI–B, (≥ 5 units/mg solid), pepsin (from porcine gastric mucosa in lyophilized powder, 3,200–4,500 units/mg protein), pancreatin (from porcine pancreas, activity 4×USP/g), porcine bile extract, invertase (from baker's yeast (*S. cerevisiae*), grade VII, ≥ 300 units/mg solid), phenylmethanesulfonyl fluoride (PMSF) solution (~0.1 M in EtOH) and orlistat (≥ 98% solid) were purchased from Sigma-Aldrich Ltd. (St. Louis, MO, USA). Amyloglucosidase (3,260 U/mL) and D-glucose assay kit (glucose oxidase/peroxide, GOPOD format) were purchased from Megazyme International Ireland Co. Ltd. (Wicklow, Bray, Ireland). All chemicals were of reagent grade.

Preparation of cooked rice

Samples (100 g each) of rice grain were washed, added with 140 g of drinking water and then cooked using an automatic rice cooker (Sharp KSH-Q03 Candy™; Thaicity Co., Ltd.; Bangkok, Thailand) for approximately 15 min. The cooked rice was kept warm using the warm mode in the rice cooker for approximately 10 min, similar to the at-home cooking procedure commonly used in Thailand. For the samples containing oil, 7.5 g and 15 g of water were substituted with the equivalent oil weight to yield around 3% and 6% oil, respectively, in the cooked rice.

After warming for 10 min in the rice cooker, the rice was cooled to room temperature (27°C), ground for 10 s or 300 s using a household blender (Philips HR2118; Koninklijke Philips N.V., Co., Ltd.; Bangkok, Thailand) to simulate chewing to different degrees of mastication before swallowing and classified according to particle size as described by Israkarn et al. (2015).

Proximate analysis

Moisture, protein (N-factor of 5.95), fat, crude fiber and ash contents were determined (Association of Official Analytical Chemists, 2000). Carbohydrate was calculated as the difference (Food and Agriculture Organization of the United Nations, 2003).

Fatty acid analysis

The fatty acid composition was determined as ester derivatives of fatty acids using gas chromatography with a flame ionization detector (GC-FID; Agilent 7890 B; Agilent Technologies, Inc.; Santa Clara, CA, USA) based on the Compendium of Methods for Food Analysis in Thailand (Department of Medical Sciences and National Bureau of Agriculture Commodity and Food Standards, 2003). Mixed chloroform and methanol (ratio of 2:1) was used to extract the non-polar lipid from the RBO and CO, which were later esterified in 14% boron trifluoride.

Phytosterol analysis

Phytosterols, namely *β*-sitosterol, stigmasterol, campesterol, stigmastanol and brassicasterol in the RBO and CO were determined using GC-FID (Agilent 6850; Agilent Technologies, Inc.; Santa Clara, CA, USA) using methods described by Laakso (2005).

In vitro starch digestion

The standardized static *in vitro* method developed by Minekus et al. (2014) was used to monitor the digestion. Samples (5 g each) of cooked rice were weighed and dispersed in 5 mL of simulated salivary fluid (SSF) electrolyte stock solution (pH 7) containing salivary α-amylase. The reaction mixture was incubated in a shaking water bath (Memmert GmbH + Co. KG; Schwabach, Germany) at 37°C for 2 min to simulate the oral phase.

After 2 min incubation in the oral phase, 10 mL of simulated gastric fluid (SGF) electrolyte stock solution (pH 3) containing pepsin was added to the digesta and incubated for 30 min at 37°C. After 30 min incubation in the gastric phase, 20 mL were added of simulated intestinal fluid (SIF) electrolyte stock solution (pH 7) containing pancreatin and porcine bile extract. The digestion in the intestinal phase was allowed to proceed for another 120 min. The total time of digestion from the oral phase until the end of the intestinal phase was 152 min. The SSF, SGF and SIF were prepared to contain electrolytes and enzymes as detailed by Minekus et al. (2014). A sample (0.5 mL) of the digesta was withdrawn at 2 min, 17 min, 32 min, 37 min, 42 min, 47 min, 62 min, 92 min, 122 min and 152 min during the digestion for analyses as described below.

Carbohydrate digestion

The digesta (0.5 mL) was mixed with 3 mL EtOH to inactivate the enzymes and precipitate dextrin for 30 min. The supernatant was hydrolyzed by amyloglucosidase and invertase to yield glucose (Dartois et al., 2010) that was determined by glucose determination reagent (McCleary and Codd, 1991). The

absorbance at 510 nm was monitored using a microplate reader (TECAN™; Tecan Trading AG; Männedorf, Switzerland). The cumulated released glucose content at each time increment was reported as the weight in grams of released glucose per 100 g of carbohydrate on a dried weight basis. The area under the digestion curve (AUC) was determined graphically.

Protein digestion

The digesta (0.5 mL) sampled at 0 min, 32 min, 47 min, 62 min and 122 min were analyzed for the NH_2 group content. A solution (10 µL) of 1 M $NaHCO_3$ was added to neutralize the digesta at 0 min and 32 min to inhibit enzyme activity. A solution (10 µL) of inhibitor mix (0.1 M PMSF:10 mM orlistat in EtOH at the volume ratio of 1:1) was added to the digesta at 47 min, 62 min and 122 min to inactivate the enzymes. After that, the samples were precipitated by 0.83 mL of 5% trichloroacetic acid (Mulet-Cabero et al., 2017) and centrifuged at 10,000×*g* for 30 min at room temperature (Hercuvan™, TT-14500 Pro Microcentrifuge; Selangor Darul Ehsan, Malaysia). The supernatants were filtered through a syringe filter (PVDF membrane; Whatman™; GE Healthcare UK Ltd.; Sheffield, UK) and determined for NH_2 group content using the *o*-phthaldialdehyde spectrophotometric assay (Nielsen et al., 2001) with D-leucine as a standard. The absorbance at 340 nm was recorded using a microplate spectrophotometer (TECAN™; Tecan Trading AG; Männedorf, Switzerland). The NH_2 group content at each time interval was reported as cumulated released NH_2 group content in millimoles equivalent to leucine.

Appearance of digesta

Paprika oleoresin (20,000 CU as oil-soluble; TCS Pacific Co., Ltd.; Bangkok, Thailand) was used as an indicator for the oil phase. Photographs of the digesta after 152 min digestion were taken and observed for lipid separation.

Light microscope and staining of amyloid-like structure

The cooked rice ground for 10 s and 300 s was stained with alkaline Congo red prepared as described by Clark and Dodds (1982). A powder (50 mg) of the Congo red was solubilized in 50 mL of 80% EtOH containing 3% NaCl, stirred for 15 min and added with 0.5 mL of 1% NaOH. Alkaline Congo red was used to locate the amyloid-like structure (Klunk et al., 1989) in the cooked rice based on observation under a light microscope (Zeiss™ Axio A1; Carl Zeiss AG; Oberkochen, Germany).

Statistical analysis

The experiments were carried out in two separate trials using a completely randomized design. Each trial was run in duplicate. The data were analyzed using analysis of variance with significance tested at $p < 0.05$. Subsequently, wherever the F-values were significant, means were compared using Duncan's Multiple Range test. Later, the data were subjected to a data reduction procedure using principal component analysis (PCA) using 28 variables: AUC, carbohydrate, protein, fat, ash, crude fiber, caprylic, capric, lauric, myristic, palmitic, palmitoleic, heptadecanoic, stearic, oleic, linoleic, linolenic-n6, linolenic-n3, arachidic, eicosenoic, eicosadienoic, behenic, lignoceric, brassicasterol, campesterol, stigmasterol, *β*-sitosterol and stigmastanol. The statistical analyses were performed using the IBM SPSS Statistics software (version 25; SPSS Inc.; Chicago, IL, USA).

Results and Discussion

Effect of grinding time on in vitro digestion curves of cooked fragrant rice containing different oil types and concentrations

The cooked rice samples contained 6.71–7.20 % protein, 0.07–7.32 % lipid and 85–92 % carbohydrate on a dried weight basis but the rice samples had similar ash and crude fiber contents (Table 1). The addition of oil also decreased the carbohydrate from 92.05% in the control sample (rice cooked with water) to 85.09–87.84% in oil-added samples ($p < 0.05$). Then, the cumulated released glucose at each time increment was calculated based on the carbohydrate content in each sample for the entire study.

The appearance of ground cooked rice obtained from the different grinding times is shown in Fig. 1. Cooked rice ground for 10 s (Fig. 1A) had large millimeter-sized particles visibly separated into small pieces, while cooked rice ground for 300 s was homogeneously liquified (Fig. 1D) after grinding. Small pieces of ground rice obtained after grinding for 10 s showed a red mesh-like network of the endosperm cell wall (Figs. 1B, 1C). Alkaline Congo red is commonly used to specifically stain amyloid proteins or peptide fibril having a beta-pleated sheet conformation in pathological tissue specimens (Klunk et al., 1989). The rice glutelin and glycoproteins bound to cellulose microfibril in the endosperm cell wall reported by Shibuya and Iwasaki (1978) may have been responsible for the stacking interactions of Congo red onto the fibrillar structure of the protein and cellulose fibrils.

Table 1 Proximate analysis of cooked rice containing different oil types and concentrations

Oil type	Added oil (%)	Content (g/100 g cooked rice on dried weight basis)				
		Protein	Fat	Crude fiber	Ash	Carbohydrate (by difference)
Control	0	7.20±0.09^{a}	0.07±0.01^{c}	0.34±0.07^{a}	0.35±0.06^{a}	92.05±0.09^{a}
CO	3	6.71±0.14^{b}	4.99±0.47^{b}	0.30±0.17^{a}	0.32±0.05^{a}	87.69±0.39^{b}
RBOA	3	6.95±0.16^{ab}	4.57±0.45^{b}	0.33±0.06^{a}	0.32±0.06^{a}	87.84±0.60^{b}
RBOB	3	6.72±0.22^{b}	4.99±0.85^{b}	0.30±0.10^{a}	0.34±0.02^{a}	87.64±1.20^{b}
CO	6	6.98±0.28^{ab}	7.32±1.25^{a}	0.36±0.04^{a}	0.27±0.06^{a}	85.09±1.07^{c}
RBOA	6	6.74±0.08^{b}	5.89±0.51^{ab}	0.24±0.03^{a}	0.28±0.01^{a}	86.86±0.47^{bc}
RBOB	6	6.78±0.04^{b}	7.13±1.02^{a}	0.29±0.11^{a}	0.31±0.03^{a}	85.48±1.07^{c}

CO = coconut oil; RBOA = rice bran oil brand A; RBOB = rice bran oil brand B;
Means ± SD (n = 2) in the same column superscripted with different lowercase letters are significant ($p < 0.05$) different.

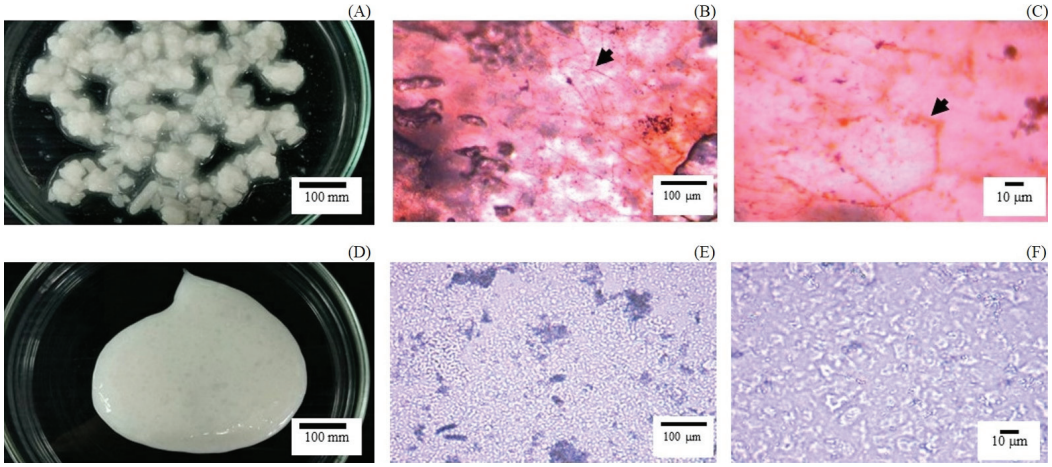

Fig. 1 Appearance and light micrographs of cooked rice samples ground for 10 s (A–C) and 300 s (D–F) at different magnifications; (A, D) cooked rice after grinding; (B, E) light micrographs of cooked rice stained with alkaline Congo red; (C, F) light micrographs of cooked rice stained with alkaline Congo red, where a black arrow indicates red endosperm cell wall network

Grinding the cooked rice for 300 s, which simulated a high degree of mastication, disintegrated the cooked rice grain internal structure, resulting in samples having a higher surface-to-volume ratio than those ground for 10 s. The cooked rice ground for 300 s lost the red color of an amyloid-like network of the endosperm cell wall (Figs. 1E and 1F). Cell wall remnants and rice granule ghosts were observed at high magnification (Fig. 1F).

The cooked rice samples after grinding for 10 s released glucose more slowly than the cooked rice ground for 300 s that had lost the amyloid-like network and had smaller particles, regardless of the oil type and concentration (Fig. 2A). An increase in the grinding time from 10 s to 300 s resulted in a sharp increase in the cumulated released glucose that reached a plateau at around 60% of total carbohydrate within 50 min of digestion (the early stage of the intestinal phase).

The increased lipid content in the cooked rice when 3% RBO or CO were added did not significantly affect the glucose released from rice sample ground for 10 s (Fig. 2B). However, increasing the oil content to 6% altered the digestion curve of cooked rice ground for 10 s (Fig. 2C). The rice cooked with RBO showed slower carbohydrate digestion and lower glucose released than rice cooked with CO. Glucose released from the rice cooked with RBO reached a plateau of 60% carbohydrate at the end of *in vitro* digestion at 152 min. However, the rice cooked with CO released glucose up to 80% of carbohydrate at the end of digestion.

Fig. 3 summarizes the area under the carbohydrate digestion curve (AUC) of rice cooked with different oil types and concentrations and ground for 10 s. Increasing the added CO content to 6% increased the AUC of cumulated released glucose. However, the addition of 6% RBO resulted in a lower AUC than for CO ($p < 0.05$). It was most likely that the different components in CO and RBO were responsible for the different patterns of glucose released during digestion.

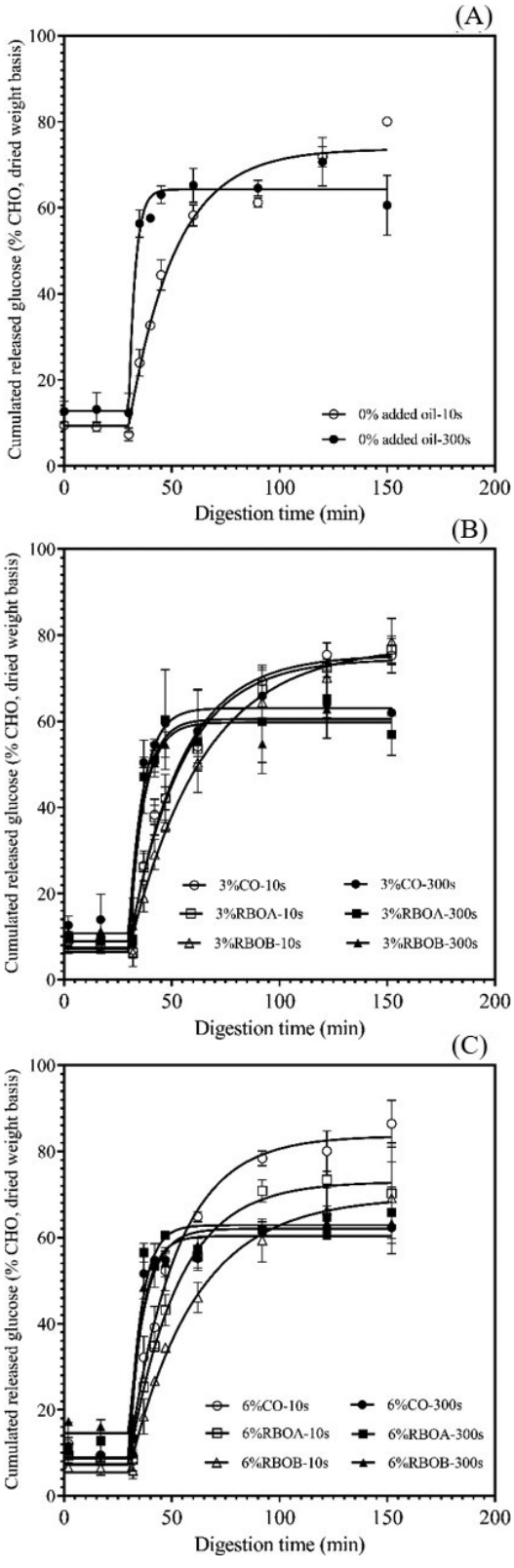

Fig. 2 Effect of lipid type and concentration on carbohydrate digestion determined as cumulated released glucose from cooked rice ground for 10 s versus 300 s: (A) no oil added; (B) 3% oil added; (C) 6% oil added, where CO = coconut oil, RBOA = rice bran oil brand A, RBOB = rice bran oil bran B and error bars = ± SD

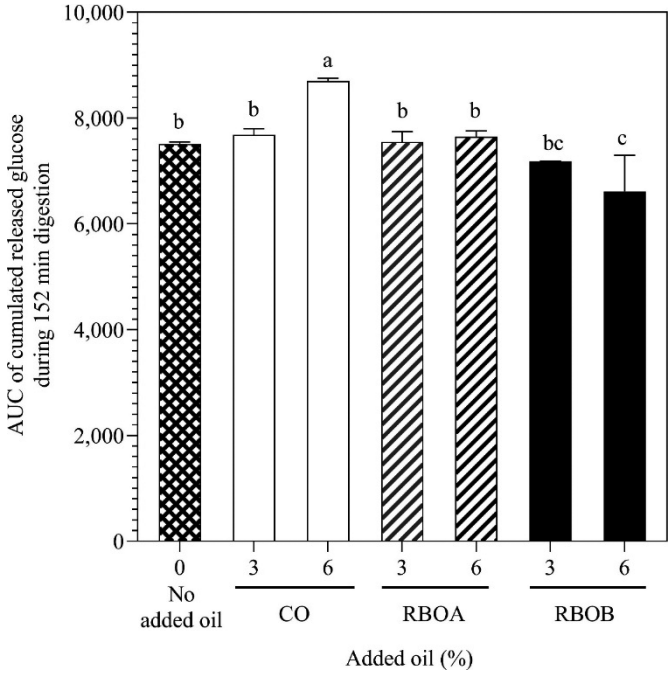

Fig. 3 Effect of oil type and concentration on area under the curve of cumulated released glucose of cooked rice ground for 10 s, where different lowercase letters above columns indicate significant ($p < 0.05$) differences, CO = coconut oil, RBOA = rice bran oil brand A, RBOB = rice bran oil brand B and error bars represent ± SD

At the end of the digestion period of 152 min, the cooked rice ground for 10 s had an orange oily layer of fat-soluble paprika oleoresin separated from the aqueous phase (Figs. 4A, 4C). Notably, all samples contained a similar bile salt concentration of 10 mM in the final digestion mixture. The disintegration of the cooked rice by grinding for 300 s increased the surface area-to-volume ratio and the viscosity of the sample before digestion. The digesta showed better emulsification and oil-holding capacity than cooked rice ground for 10 s, suggesting that the digesta had different surface properties.

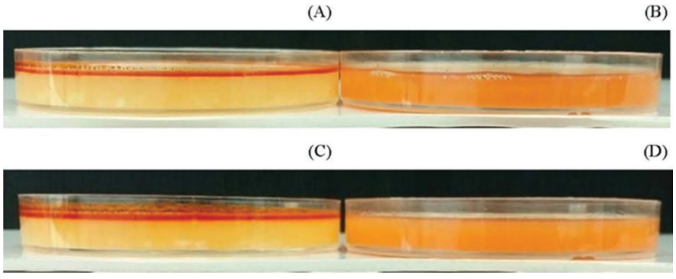

Fig. 4 Effect of grinding time on oil phase separation in digesta containing 6% CO (A, B) or RBOA (C, D) after digestion for 152 min: (A, C) cooked rice ground for 10 s; (B, D) cooked rice ground for 300 s, where paprika oleoresin was added to indicate the oil phase

The influence of the lipid type on different digestion curves of gelatinized rice and colored rice grains have been investigated by many investigators (Kaur et al., 2015; Farooq et al., 2018; Kumar et al., 2018; Krishnan et al., 2020), who suggested that the added lipid induced the formation of an amylose-lipid complex responsible for the low starch digestibility, depending on the type of lipid. The dominant fatty acids in CO consisted of lauric acid and myristic acid, while oleic acid and linoleic acid were the major fatty acids in RBO (Table 2). RBO contained high contents of phytosterols (*β*-sitosterol, stigmasterol, campesterol, stigmastanol, brassicasterol) compared to those in CO. The surface-active nutrients such as oligopeptides, fatty acids, phytosterol, mono- and diglycerides generated during digestion may have played important roles in determining the fate of the glucose released from the cooked rice subjected to a different grinding regime. Thus, the lipid composition roles on carbohydrate digestion in rice ground for 10 s and 300 s were considered separately.

Effect of fatty acid and phytosterol compositions on in vitro digestion of cooked rice ground for 10 s (representing a low degree of mastication) and digesta having low emulsifying properties

PCA was used to visualize the interplay among the components of the cooked rice (Table 1), lipid composition (Table 2) and total AUC of the cooked rice ground for 10 s (Fig. 3) by categorizing the data into two or three uncorrelated dimensions or principal components (PCs). In the current study, two PCs could explain a total variation of 85.87%, with PC1 explaining 55.02% of the variation and PC2 30.85% of the variation. Fig. 5 illustrates that the AUC, capric acid, lauric acid, myristic acid and fiber contents were grouped in the quadrants of positive PC1 and negative PC2. In contrast, the long-chain fatty acids (arachidic, oleic, linoleic, linolenic acids) and phytosterols (stigmasterol, *β*-sitosterol, campesterol, brassicasterol) were grouped in the positive PC1 and positive PC2 quadrants. Apparently, multivariate analysis using PCA suggested that RBO and CO may have affected the carbohydrate digestion of cooked rice via different processes.

Table 2 Fatty acid and phytosterol composition of commercial coconut oil and rice bran oil

Constituent	CO	RBOA	RBOB
Fatty acid (g/100g)			
Caprylic acid (8:0)	0.58±0.12	n.d.	n.d.
Capric acid (10:0)	3.05±0.09	n.d.	n.d.
Lauric acid (12:0)	46.59±0.21	n.d.	n.d.
Myristic acid (14:0)	21.56±0.24^{a}	0.31±0.01^{b}	0.31±0.00^{b}
Palmitic acid (16:0)	10.92±0.01^{c}	18.29±0.01^{b}	18.54±0.02^{a}
Heptadecanoic acid (17:0)	n.d.	0.04±0.0^{a}	0.04±0.00^{a}
Stearic acid (18:0)	3.82±0.03^{a}	2.11±0.03^{b}	2.16±0.00^{b}
Arachidic acid (20:0)	0.13±0.01^{c}	0.81±0.01^{b}	0.92±0.01^{a}
Behenic acid (22:0)	n.d.	0.27±0.01^{a}	0.29±0.01^{a}
Lignoceric acid (24:0)	n.d.	0.43±0.01^{a}	0.43±0.00^{a}
Total saturated fatty acid	86.63±0.27^{a}	22.24±0.07^{b}	22.68±0.02^{b}
Palmitoleic acid (16:1)	n.d.	0.23±0.01^{a}	0.23±0.01^{a}
Oleic acid (18:1)	7.53±0.15^{b}	42.55±0.03^{a}	42.48±0.01^{a}
Linoleic acid (18:2)	1.44±0.12^{c}	28.97±0.04^{a}	28.67±0.01^{b}
Linolenic acid (18:3)	n.d.	0.94±0.01^{a}	0.88±0.00^{b}
Eicosenoic acid (20:1)	n.d.	0.62±0.00^{a}	0.34±4.00^{a}
Eicosadienoic acid (20:2)	n.d.	0.05±0.00^{a}	0.06±0.01^{a}
Total unsaturated fatty acid	8.96±0.27^{b}	73.35±0.06^{a}	72.65±0.38^{a}
Phytosterols (mg/100g)			
β-sitosterol	39.56±3.12^{b}	298.67±16.43^{a}	296.27±47.77^{a}
Stigmasterol	10.98±1.39^{b}	82.93±2.77^{a}	95.24±8.00^{a}
Campesterol	5.55±0.50^{b}	63.87±1.63^{a}	64.11±1.36^{a}
Stigmastanol	30.79±4.00^{a}	37.01±8.26^{a}	38.11±8.53^{a}
Brassicasterol	n.d.	13.20±0.90^{ab}	24.82±13.03^{a}
Total selected phytosterol	86.87±9.00^{b}	495.68±28.19^{a}	518.54±19.54^{a}

CO = coconut oil; RBOA = rice bran oil brand A; RBOB = rice bran oil brand B; n.d. = not detected

Mean ± SD (n = 2) in the same row superscripted by different lowercase letters are significant ($p < 0.05$) different.

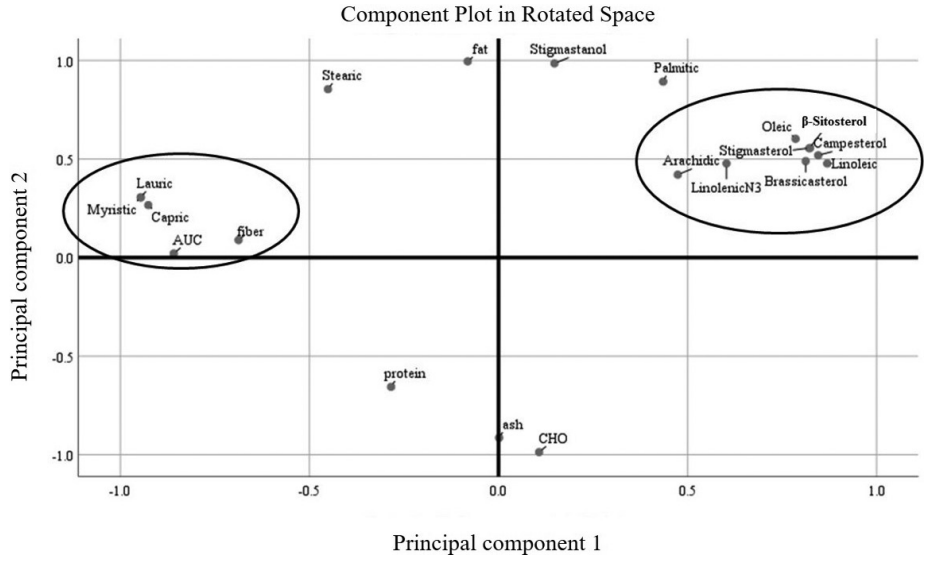

Fig. 5 Eigenvector plot of principal components that cumulatively explain more than 85% of total variance, where the area under the curve (AUC) data were calculated from cooked rice ground for 10 s

Upon lipid digestion in the intestinal phase, the longer carbon chain length of the fatty acids and phytosterols in the RBO could affect the micellar formation of surface-active oil-based hydrolyzed nutrients and their stability in the aqueous phase differently from the CO-based micelles due to the differences in the contents of phytosterols and fatty acids. Tamesue and Juniper (1967) and Yuan et al. (2018) reported that the long-chain fatty acid-based micelles were 400 nm in size. These micelles were most likely deposited on the surface of cooked rice the millimeter-sized particles and hindered the accessibility of the gut enzymes responsible for cell wall breakdown and starch digestion. Consequently, RBO indirectly lowered the glucose release from the cooked rice ground for 10 s compared to that of the control sample and rice containing CO.

The fatty acids in CO, which were statistically grouped with the AUC, suggested their direct involvement in carbohydrate digestion and glucose release. In contrast to the influence of RBO, CO increased the AUC and the content of released glucose from the cooked rice ground for 10 s. Notably, the low contents of long-chain fatty acids and phytosterols in CO may have limited the formation of phytosterol and long-chain fatty acid-based micelles. The presence of 6% CO enhanced the release of glucose up to 80% of carbohydrate towards the end of digestion, compared to the control sample, which released glucose of approximately 70% of carbohydrate and had a lower AUC ($p < 0.05$), suggesting that the amylose-lipid complex formation induced by the C10–C14 saturated fatty acids may not have occurred in the newly cooked fragrant rice subjected to immediate *in vitro* digestion. For the first time, the current study has shown that the low carbohydrate digestion of fragrant rice cooked with 6% RBO may have been influenced by the low surface area, the integrity of the endosperm cell wall and the shielding effect of the long-chain fatty acid-based micelles on the surface of the rice particles that hindered the digestion compared to the cooked rice containing CO.

Effect of fatty acid and phytosterol composition on in vitro digestion of cooked rice ground for 300 s (representing a high degree of mastication) and digesta having high emulsifying properties

The current study showed that grinding cooked rice for 300 s increased the surface area-to-volume ratio and enhanced the carbohydrate digestion of the cooked rice, resulting in the rapid release of glucose of approximately 60% of carbohydrate within 50 min of digestion, regardless of the oil type and concentration. These results were in good agreement with Tamura et al. (2016) and Thuengtung et al. (2018), who reported that the rice endosperm cell wall played a significant role as a physical barrier for enzyme accessibility. The destruction of the endosperm cell wall structure as a result of homogenizing the cooked rice resulted in higher starch digestibility than for the intact cooked rice (Tamura et al., 2016, Thuengtung et al., 2018).

Nonetheless, the present study further showed that the endosperm cell wall contained an amyloid-like structure of proteins in addition to non-starch carbohydrate, with the

cumulated released glucose reaching a plateau of only 60% of carbohydrate when the cooked rice was ground for 300 s. The better oil-holding ability and emulsification of the digesta obtained from grinding the cooked rice for 300 s suggested dynamic changes in the surface properties of the hydrolyzed nutrients during intestinal digestion. From a colloid science perspective, the surface-active hydrolyzed nutrients resulting from lipid digestion (mono- and di-glycerides, fatty acids, phytosterols) and protein digestion (peptides, amino acids) were most likely re-assembled in the presence of bile salts in the intestinal phase (Wongekalak et al., 2019). The rapid release of glucose within the first 10 min in the intestinal phase, which increased the osmotic pressure of the digesta during the early stage of the intestinal phase, further enhanced the flocculation of the re-assembled hydrolyzed molecules. Consequently, the carbohydrate digestion was limited to 60% of carbohydrate, in contrast with the slower release of glucose in the cooked rice ground for 10 s that could release glucose up to 80% of carbohydrate.

Fig. 6 shows that the free NH_2 groups released from the cooked rice ground for 300 s gradually increased during the gastric and early intestinal phases due to protease digestion of the rice cooked with water and the rice cooked with 3% RBO or CO. The oil addition did not affect protein digestion during the gastric and early intestinal phases ($p \geq 0.05$). However, prolonging the *in vitro* digestion to 122 min or during the late intestinal phase increased the protein digestion of rice cooked with 3% RBO. The current study showed that RBO, which had higher amounts of polyunsaturated fatty acids and phytosterols than CO, may also determine the fate of protein digestion. Based on current knowledge, the association between the peptides and phytosterol and the long-chain fatty acid-based micelles on the digestion of carbohydrates when RBO was added is largely not known and merits in-depth investigation.

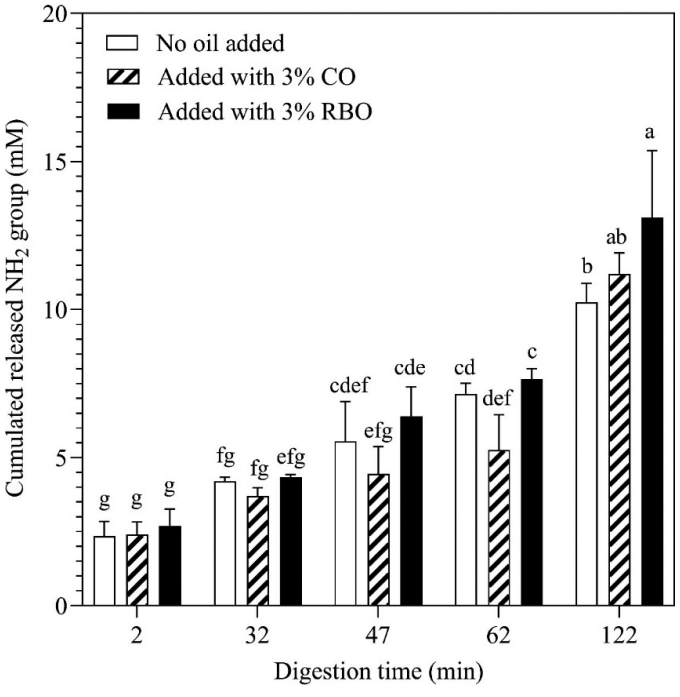

Fig. 6 Effect of 3% oil addition before rice cooking on protein digestibility determined as cumulated released free NH_2 group during *in vitro* digestion, where different lowercase letters above columns indicate significant ($p < 0.05$) differences, CO = coconut oil, RBO = rice bran oil, cooked rice was ground for 300 s and error bars represent ± SD

In summary, cooked rice samples subjected to different grinding times had differences in their *in vitro* digestion due to the presence of physical barriers (amyloid-like network of protein and cellulose fibril in the endosperm cell wall, matrix continuity, hindrance from phytosterol and long-chain fatty acid-based micelles) against the accessibility of gut enzymes. The addition of RBO could hinder the glucose released from cooked fragrant rice, while CO enhanced starch digestion when the rice was masticated to small millimeter-sized particles. The current study suggested the significance of surface-active hydrolyzed nutrients from lipid and protein digestion regarding the fate of carbohydrate digestion. These insights helped to suggest the tools to modulate the glucose released from rice cooked at a household level, which can be achieved by the presence of long-chain fatty acids and phytosterols and even by the degree of rice mastication. The roles of endosperm proteins and amino acids on the carbohydrate digestion of rice from different cultivars are currently being investigated.

Conflict of Interest

The authors declare that there are no conflict of interest.

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