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In Vitro Starch Digestion of Cooked Rice Grain Following the Addition of Various Vegetable Oils

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Abstract

The present study aimed to investigate the *in vitro* starch digestion of cooked rice grain with added vegetable oils. Polished rice grain (400 g) was cooked with 500 mL of ultrapure water only or with one of six types of vegetable oils (cocoa butter [CB], coconut oil, corn oil [CO], olive oil [OO], palm oil, and rapeseed oil [RO]), and then was frozen and stored. The frozen grain was reheated using a microwave and used for texture analysis, *in vitro* gastro-small intestinal digestion, and microstructure observation. The cooked grain with added CB had significantly higher resistant starch content (0.70% d.b.) than the control (0.19% d.b.). Although the surface firmness and overall adhesiveness of the cooked grain were not significantly different between the samples, the overall firmness of the grain cooked in RO was significantly lower than that of all others. Cooked grain with added RO (70.35%) indicated a significant reduction in starch hydrolysis compared with the cooked grain with added OO (95.04%) and CO (83.94%) at 210 min of *in vitro* digestion. However, no difference was observed in tissue structure of the digested cooked grain between the samples.

Discipline: Food
Additional key words: amylose–lipid complex, frozen food, *in vitro* gastro-small intestinal digestion, texture, tissue structure

Introduction

Changes in lifestyle, such as the increase of nontraditional households and advancement of women in society, reduced and simplified the burden of household chores. Trends in dietary consumption are increasingly dependent on ordering in, take-away, and eating out rather than home-cooking. Rice consumption, the main staple of Japanese diet, has decreased overall, but the amount of rice used in take-away and eating out has increased. The amount of frozen fried rice produced in 2019 was 83 kt, which has become popular among frozen foods (Japan Frozen Food Association 2020).

Because starch, the main component of rice, is considered to increase the risk of lifestyle-related diseases, such as type II diabetes, cardiovascular diseases, and obesity, consumers have restricted their starch intake instead of their lipid intake in recent years. The research assessed the risk of lifestyle-related diseases associated with rice consumption using the starch hydrolysis percentage and estimated glycemic index (eGI) using an *in vitro* digestion model (Chung et al. 2006, Kumar et al. 2018, Tamura et al. 2016b, 2019a), as well as the glycemic index (GI), determined from the blood glucose level *in vivo*. Because most rice varieties are categorized as high-GI compared with legumes, pasta, fruits, and dairy products (Atkinson et al. 2008), these indexes are critical indicators that influence the purchase and consumption of rice and rice products.

Fried rice grain is prepared by gelatinizing rice starch that has absorbed sufficient water and adding lipids during heating. Cooked grain supplemented with cooking oil during storage has shown less variation in texture and moisture loss compared with nonsupplemented cooked grain (Suzuki 2005), whereas cooked grain with lysolecithins added has shown increased firmness and decreased adhesion (Ito et al. 1995). The amylose–lipid

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complex produced when nonwaxy rice starch gelatinizes in the presence of lipid (palm oil) reduces starch hydrolysis, enhancing the resistant starch (RS) content and decreasing the amounts of rapid (RDS) and slow digestive starch (SDS) (Farooq et al. 2018). The addition of coconut oil (CCO) and rice bran oil to rice grain produces a higher SDS content and lower eGI than that of the control (Luangsakul & Ritudomphol 2018). Soong et al. (2013) have also observed a reduction in starch digestibility when rice starch is complexed with palmitic acid (C16:0). However, several of these studies used rice samples prepared as purified starch for *in vitro* digestion, which is different from the rice grain in general, wherein the tissue structure is maintained at ingestion. Previous studies have revealed that the tissue structure of the cooked grain affects the digestibility of rice starch (Tamura et al. 2016a, 2016b). Thus, the present study aimed to investigate the *in vitro* starch digestion of grain with the tissue structure maintained and cooked experimentally with six types of vegetable oil.

Materials and methods

1. Sample preparation

The fried rice was prepared experimentally. In brief, 400 g of polished rice grain (*Oryza sativa* L., cv. Kirara 394, harvested in 2015 from Hokkaido, Japan) was soaked in 500 g of ultrapure water at room temperature (approximately 20°C) for 90 min. The soaked grain was cooked using a rice cooker (Zojirushi, NP-CB18, Osaka, Japan) in regular cooking mode for 40 min with 40 g of one of the following vegetable oils: cocoa butter (CB; containing 36.8% stearic acid [C18:0], 32.8% oleic acid [C18:1], and 25.4% palmitic acid [16:0]), CCO (containing 45.8% lauric acid [C12:0] and 18.0% myristic acid [C14:0]), corn oil (CO; containing 55.1% linoleic acid [C18:2] and 28.7% oleic acid [C18:1]), olive oil (OO; containing 77.6% oleic acid [C18:1] and 11.1% palmitic acid [C16:0]), rapeseed oil (RO; containing 61.7% oleic acid [C18:1] and 19.7% linoleic acid [C18:2]), or palm oil (PO; containing 43.8% palmitic acid [C16:0] and 39.7% oleic acid [C18:1]); it was then frozen in a freezer at −35°C. Cooked grain without vegetable oil was also prepared as a control. The frozen grain was stored in a freezer at −20°C for 1-6 months and used in 2016. The frozen grain (60 g) was then placed in a Ziploc bag and reheated at 500 W for 70 s using a microwave oven (NE-M264, Panasonic, Osaka, Japan). The microwave-heated sample grain was incubated in a hot air dryer (LC-122, Tabai Espec, Osaka, Japan) at 30°C for 30 min to measure moisture content and starch digestibility and for 2 h for texture analysis. For component analysis, the reheated sample grain was frozen overnight at −35°C, freeze–dried (RLE-52, Kyowa Vacuum Engineering, Tokyo, Japan), ground, and passed through a 0.5 mm sieve to obtain powdered samples.

2. Moisture content

Approximately 1 g of the powdered sample and 10 g of the cooked grain were dried at 135°C for 24 h in an air oven (LC-122, Tabai Espec). The moisture content (% w.b.) was calculated as the percentage weight loss after drying divided by the initial sample weight.

3. Total starch and resistant starch contents

The total starch (TS) and RS contents of the powdered samples were measured using a Resistant starch assay kit (K-RSTAR 08/11, Megazyme International Ireland).

4. *In vitro* gastro-small intestinal digestion

An *in vitro* gastro-small intestinal digestion model, simulated gastric fluid (SGF), and simulated intestinal fluid were used to simulate starch hydrolysis of the cooked grain according to Dartois et al. (2010), with minor modifications (Tamura et al. 2016a, 2016b). The supernatants (0.5 mL) of digested fluid were collected to analyze glucose content after 5 and 30 min of gastric digestion and after 5, 10, 15, 30, 60, 120, 180, and 240 min of small intestinal digestion. The percentage of starch hydrolysis was calculated using the following equation:

$$SH = S_h / S_i = 0.9\ G_p / S_i \tag{1}$$

where SH is the percentage of starch hydrolysis, S_h is the mass of hydrolyzed starch, S_i is the initial mass of TS, and G_p is the mass of glucose produced, measured using a D-glucose assay kit (GOPOD Format K-GLUK, Megazyme International Ireland) after pretreatment (Tamura et al. 2016a, 2016b). A conversion factor of 0.9, calculated from the molecular weight of the starch monomer/molecular weight of glucose (162/180 = 0.9) (Goñi et al. 1997), was used.

5. Texture analysis

A low and high two-bite test on an individual grain was conducted using a Tensipresser (TTP-50BX, Taketomo Electric, Tokyo), referring to Okadome et al. (1996). A single cooled cooked grain was placed on a base plate and compressed with a planar plunger (Φ30 mm) at a 1 mm/s compression speed, using a 10 kg load cell. It was first compressed to 25% of the initial grain height, pulled and recompressed to 90%, and then pulled to obtain a force–time curve containing the positive and negative peaks. The following parameters were calculated from the force–time curve: surface firmness (SF: the peak force at the first compression), overall firmness (OF: the peak force at the second compression), surface adhesiveness (SA: the negative peak force during pulling after the first compression), and overall adhesiveness (OA: the negative peak force during pulling after the second compression).

6. Microscopy

Before and after *in vitro* digestion, the cooked grain was fixed in 10% formaldehyde and dehydrated in a graded ethanol series (Tamura & Ogawa 2012). The fixed and dehydrated grain was mounted on a stub with double-sided sticky tape and examined under a scanning electron microscope (TM3030, Hitachi High-Tech, Tokyo, Japan), and then, the surface of the grain was observed at the same magnification. The captured images were processed and analyzed using a graphic software (Photoshop CC2014, Adobe, San Jose, CA, USA).

7. Statistical analysis

Results were calculated as mean ± standard deviation. After outliers were removed using the Smirnov–Grubbs test, Tukey's test was used in conjunction with analysis of variance to identify differences among means, at an *a priori* significance level of $P < 0.05$, using R software (R Core Team 2014). Pearson's product–moment correlation coefficient for the relationship between the different rice grain properties was calculated using R software (R Core Team 2014).

Results and discussion

1. Components

Table 1 shows the moisture, RS, and TS contents of cooked rice grain with different types of vegetable oil added. The cooked grain with six types of vegetable oil (53.74%-55.90% w.b.) had lower moisture content than the control (56.78% w.b.). The reduction of the moisture content was likely due to the hindrance of water absorption by the hydrophobic property of the oils. The cooked grain with CB added had significantly higher RS content (0.70% d.b.) than the control (0.19% d.b.). Farooq et al. (2018) also showed that the RS content tends to increase when PO is added to rice starch and cooked. The increase in RS content is likely due to heat–moisture treatment with a low moisture content during cooking (Wang et al. 2018) and/or the formation of an amylose–lipid complex (Farooq et al. 2018, Luangsakul & Ritudomphol 2018). Cooked grain induced with the oils tended to have a lower TS content (74.36%-77.65% d.b.) than the control (83.06% d.b.), which may have resulted from a relative decrease in TS content because of the addition of the oils to the cooked grain.

2. Texture

Table 2 summarizes the texture of cooked rice grain with different types of vegetable oil added. The SF and OA of the cooked grain were 6.81-7.36 and 60.15-66.63 kPa, respectively, with no significant differences

Table 1. Moisture, RS, and TS contents of cooked rice grain with different types of vegetable oil added

	Moisture content (% w.b.)	RS content (% d.b.)	TS content (% d.b.)
Control	56.78 ± 0.62 a	0.19 ± 0.08 b	83.06 ± 5.44 a
CB	54.10 ± 0.85 cd	0.70 ± 0.20 a	75.43 ± 2.79 a
CCO	55.90 ± 0.75 ab	0.16 ± 0.04 b	76.16 ± 1.28 a
CO	55.46 ± 0.57 abc	0.58 ± 0.29 ab	76.99 ± 3.43 a
OO	54.65 ± 1.10 bcd	0.57 ± 0.20 ab	76.64 ± 3.29 a
PO	53.74 ± 0.93 d	0.28 ± 0.09 ab	74.36 ± 4.54 a
RO	55.10 ± 0.57 bcd	0.24 ± 0.11 ab	77.65 ± 2.87 a

Mean ± standard deviation. The sample number (n) was as follows: moisture content (n = 5-10), RS content (n = 3-4), TS content (n = 3-4). Different letters (a-d) within the same column indicate significant differences ($P < 0.05$). RS: resistant starch; TS: total starch; CB: cocoa butter; CCO: coconut oil; CO: corn oil; OO: olive oil; PO: palm oil; RO: rapeseed oil

Table 2. Texture of cooked rice grain with different types of vegetable oil added

	SF (kPa)	OF (kPa)	SA (kPa)	OA (kPa)
Control	6.90 ± 1.75 a	306.87 ± 76.75 ab	0.99 ± 0.54 ab	60.15 ± 11.71 a
CB	6.81 ± 2.32 a	281.50 ± 63.54 b	1.23 ± 0.46 a	62.11 ± 8.46 a
CCO	7.09 ± 2.43 a	322.20 ± 76.77 ab	1.13 ± 0.56 ab	64.30 ± 8.54 a
CO	7.36 ± 1.17 a	308.09 ± 80.33 ab	0.82 ± 0.36 b	61.44 ± 13.20 a
OO	7.09 ± 1.80 a	296.22 ± 74.69 ab	1.34 ± 0.41 a	66.63 ± 7.10 a
PO	6.98 ± 2.51 a	344.28 ± 70.37 a	0.84 ± 0.34 b	63.34 ± 10.82 a
RO	7.30 ± 2.23 a	225.21 ± 53.60 c	1.05 ± 0.39 ab	61.45 ± 9.64 a

Mean ± standard deviation (n = 28-30). Different letters (a-c) within the same column indicate significant differences ($P < 0.05$). SF: surface firmness; OF: overall firmness; SA: surface adhesiveness; OA: overall adhesiveness; CB: cocoa butter; CCO: coconut oil; CO: corn oil; OO: olive oil; PO: palm oil; RO: rapeseed oil

among the samples. The OF of the cooked grain with RO added was 225.21 kPa, which was significantly lower than that of the other samples. The cooked grain with OO and CB added was higher in SA than the cooked grain with added CO or PO. Because the surface of the cooked grain was considered to be coated with the oil, it was inferred that the cooked grain texture reflected the properties of the oil. According to previous research (Ito et al. 1995, Yamada et al. 1994), monoglycerides scarcely penetrate the cooked grain, whereas lysolecithin penetrates the cooked grain extensively. This means that penetration and dispersion into the cooked grain could differ depending on the oil; thus, the surface and overall texture may vary between samples.

Furthermore, because the cooked grain was allowed to cool at 30°C for 2 h after microwave heating in this study, starch retrogradation would probably occur at 60°C or lower (Hizukuri 1970). Generally, the firmness of cooked grain increases, whereas the adhesiveness decreases with the storage time after cooking due to retrogradation. Hibi (1993) found that cooked grain with OO added suppresses the increase in firmness and decrease in cohesiveness due to retrogradation, whereas the decrease in adhesion of the cooked grain is enhanced. Cooked grain with OO added is considered to have suppressed the changes in texture by retrogradation compared with the other grains. Changes in texture due to retrogradation can also affect the starch digestibility of the cooked grain (Tamura et al. 2019a).

3. Starch hydrolysis during *in vitro* gastro-small intestinal digestion

Figure 1 presents changes in starch hydrolysis of the cooked rice grain with different types of vegetable oil added during *in vitro* gastro-small intestinal digestion. During simulated gastric digestion, for the first 30 min, no increase in starch hydrolysis was observed because of a lack of starch-degrading enzymes in SGF. However, it is considered that the physical properties and moisture content of the grain can change (Tamura et al. 2019b). Starch hydrolysis increased with time during the simulated small intestinal digestion process. Starch hydrolysis exceeding 100% at 270 min was an error and was used as a reference value. During the process, cooked grain supplemented with OO and CO tended to have a relatively high starch hydrolysis, whereas cooked grain supplemented with RO and PO tended to have a relatively low starch hydrolysis. At 210 min, cooked grain with added RO indicated a significant reduction in starch hydrolysis (70.35%) when compared with the cooked grain with added OO (95.04%) and CO (83.94%). Vegetable oils had no significant effect on the starch hydrolysis of the cooked grain compared with the control. No significant correlation coefficient was found among the moisture content, RS content, TS content, texture, and starch hydrolysis at any digestion time. Generally, amorphous starch, which has a chemically and physically unstable structure due to gelatinization, is hydrolyzed rapidly, whereas the amylose–lipid complex is resistant to hydrolysis as RS type 3 or 5 (Panyoo & Emmambux 2017). Chen et al. (2017) explained that the addition of CO to corn starch decreases RDS content and increases SDS and RS content. Crowe et al. (2000) also showed that starch digestibility decreases when potato amylose is complexed with palmitic acid (C16:0) but not with capric (C10:0), lauric (C12:0), myristic (C14:0), or stearic acids (C18:0). For rice starch, the addition of PO significantly reduces the starch digestibility of nonwaxy rice (Farooq et al. 2018). Furthermore, Soong et al.

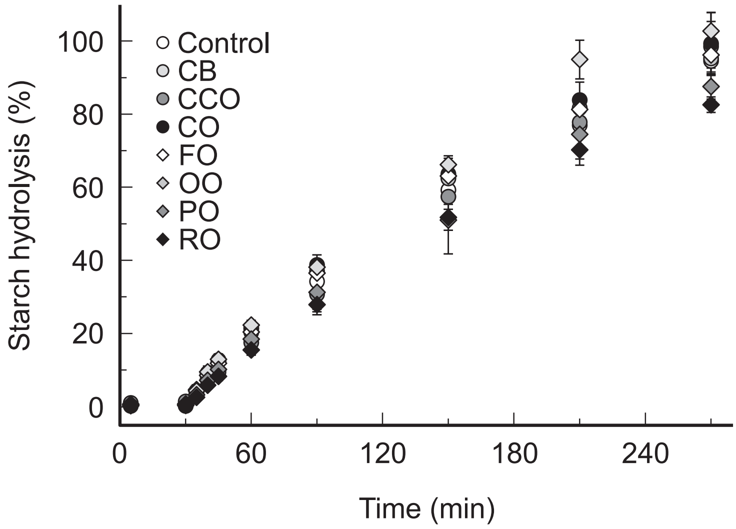

Fig. 1. Changes in starch hydrolysis of cooked rice grain with different types of vegetable oil added during *in vitro* gastro-small intestinal digestion
Error bars represent standard deviation (n = 4-6). Starch hydrolysis (%): ratio the mass of hydrolyzed starch to the initial mass of total starch; CB: cocoa butter; CCO: coconut oil; CO: corn oil; OO: olive oil; PO: palm oil; RO: rapeseed oil.

(2013) reported that a decrease in starch digestibility is observed when rice starch is complexed with palmitic acid (C16:0) instead of capric (C10:0), lauric (C12:0), myristic (C14:0), and stearic acids (C18:0). In this study, no relationship between starch hydrolysis and the composition ratio of the major fatty acids in vegetable oil was observed, as described below. The PO-added cooked grain containing the highest amount of palmitic acid (C16:0) had the second lowest starch hydrolysis level. However, cooked grain with added CB, which had the second highest amount of palmitic acid (16:0), had relatively high starch hydrolysis. The cooked grain with RO, which showed the lowest starch hydrolysis, contained the second highest amount of oleic acid (C18:1), whereas the cooked grain with OO, which contained the largest amount of oleic acid (C18:1), showed the highest starch hydrolysis. Previously, Kawai et al. (2017) revealed that the reduction in starch digestibility *in vitro* is caused by the extent of complex formation between starch and fatty acids, the numbers of carbons and double bonds, and the enzyme-annealing effect. Conversely, Tang and Copeland (2007) showed that a high amount of lipid tends to self-associate rather than form starch–lipid complexes. Furthermore, cooked grains with vegetable oils added, which maintained their tissue structure, were used in this study instead of a reaction between purified starch and lipids. Thus, it is possible that the degree of penetration of vegetable oil into the grain and interactions between components of the cooked grain, such as starch, proteins, cell walls, and lipids, may affect starch hydrolysis. Additionally, varied freezing storage periods of up to 6 months may have affected variability in starch hydrolysis, which should be investigated in further studies.

4. Microstructure

Figure 2 shows changes in the histological characteristics of cooked rice grain with different types of vegetable oil added before and after *in vitro* gastro-small intestinal digestion. The surface of the tissue structure of cooked grain with OO (Fig. 2B), PO (Fig. 2C), and RO (Fig. 2D) appeared to be smooth, similar to that of the control (Fig. 2A). It is considered that both the formation of a coating layer by gelatinization of starch (Tamura & Ogawa 2012) and coating by vegetable oil occurred on the surface of rice grain during the cooking process. However, no differences were observed between the sample grains. Innumerable honeycomb-like structures of the aleurone layer were observed on the cooked grain surface after *in vitro* digestion (Fig. 2a-d). These structures could have been created by starch hydrolysis by digestive enzymes during small intestinal digestion, which left the cell wall intact (Tamura et al. 2016a, 2016b, 2019b), and no differences between the sample grains were identified.

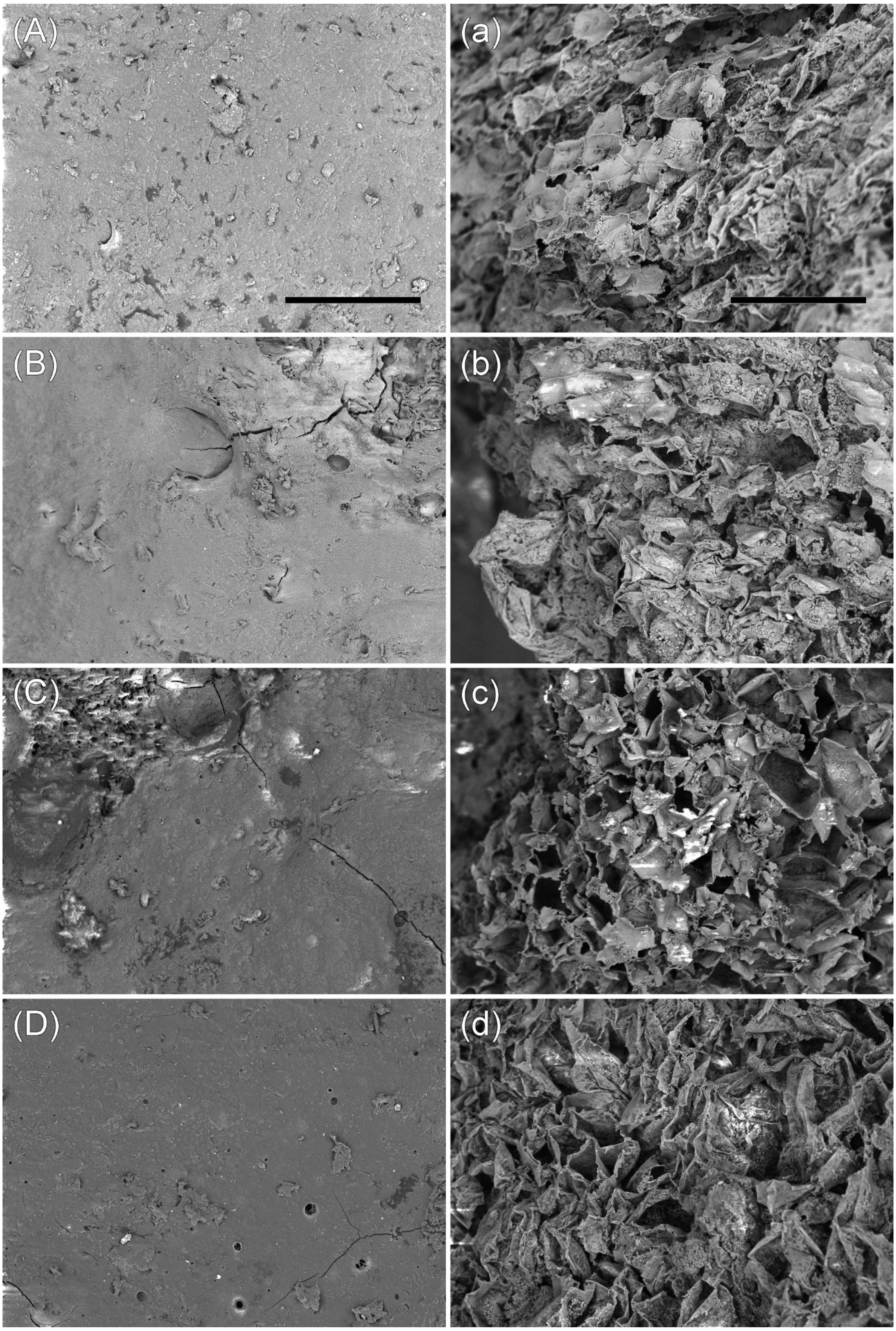

Fig. 2. Changes in histological characteristics of cooked rice grain with different types of vegetable oil added before and after *in vitro* gastro-small intestinal digestion

Panels A-D show a cooked grain sample and a-d show a digested grain sample at 270 min. A and a represent cooked grain (control); B and b represent cooked grain with added OO; C and c represent cooked grain with added PO; D and d represent cooked grain with added RO. Scale bars show 100 µm (A-D and a-d). OO: olive oil; PO: palm oil; RO: rapeseed oil

Conclusions

Cooked rice grain with added RO exhibited the lowest starch hydrolysis during *in vitro* gastro-small intestinal digestion among the samples. Supplementing the cooked grain with RO also reduced OF but did not significantly affect moisture, RS and TS contents, other textural properties, and tissue structure before and after *in vitro* digestion. Unlike the reaction between purified starch and lipids, the starch hydrolysis of the cooked grain with added vegetable oils could not explain a relation with the major fatty acid composition of the oils. The results also show that the degree of penetration of vegetable oil into the grain and interactions between components of the cooked grain, such as starch, proteins, cell walls, and lipids, may affect starch hydrolysis.

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