

High resistant starch content in crossbred red rice and its downstream products

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Abstract

Resistant starch (RS) has drawn considerable attention over the past decade due to its positive physiological properties as well as accessibility in many starchy foods. RS is the starch portion resistant to enzymatic hydrolysis in the human gut. Increased levels of RS in rice grains lower the glycemic index (GI) value ensuring a negative correlation with amylose content (AC). UKMRC-9 is a cross-bred red rice with GI=46 and an intermediate AC has been clinically proven to decrease postprandial blood glucose after ingestion. The objective of this study was to investigate the RS contents for cooked UKMRC-9 and its downstream products in the form of RTE (ready-to-eat). With the intention to optimise the RS contents through retrogradation of starch, these cooked rice and rice-based products were subjected to cooked-freeze and thawed cycles to simulate the modus operandi of food manufacturing and food service industry. Applying a method of enzymatically degrading starch samples according to the Association of Official Analytical Collaboration (AOAC) International, a total of 8 samples of UKMRC-9 and its downstream products were analysed for their RS contents. Cooked and frozen UKMRC-9 with coconut oil, UKMRC-9 raw and red rice noodles were computed with the highest RS at 16.52 g/100 g, 14.24 g/100 g and 11.42 g/100 g, respectively. The red rice bread, wholegrain bread and Kueh Angku have a RS value of 4.99 g/100 g, 4.97 g/100 g and 3.12 g/100 g. Based on RS classification, the RS contents of the former and latter can be categorised as high and intermediate. This study showed that UKMRC-9 was a good source of RS on its own, additional cooking and freezing processes and using it as an ingredient will further enhance the RS content of the final food products.

1. Introduction

Gluten-free food production has got a lot of attention in the recent decade, due to more accurate celiac disease diagnosis and widespread discussion about the health benefits of gluten-free diets. Celiac disease and food allergies are becoming more common, and patients, healthcare providers and the general public are becoming more aware of these conditions (Rosell and Matos, 2015). According to scientific publications, the ideal alternative for gluten-free products is a combination of modified or functional starches or flours, hydrocolloids, fibres, proteins, and co-texturizers supplemented with fibres and different types of proteins (Dar, 2013).

Rice has been widely used to replace wheat for the development of gluten-free products due to its low cost,

good digestibility, white colour, neutral flavours and hypoallergenic properties (Crockett *et al.*, 2011). Recent research has also shown that gluten-free bread made from low starch-damaged rice will have good, rheological, structural, and textural characteristics (Yano *et al.*, 2017). Thus, the focus of this experiment has been to improve the physiological and nutritional aspects of red rice products by increasing the resistant starch (RS) contents through cycles of food preparation processes.

RS is the type of starch that resists digestion by human enzymes in the small intestine and will partially or wholly ferment in the large intestine. It is the starch portion resistant to enzymatic hydrolysis that escapes the small intestine and reaches the large intestine where it gets fermented by gut-colonising bacteria. RS is also

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expected to prevent and improve type 2 diabetes and poor glucose tolerance, as well as help with obesity and weight gain control (Higgins *et al.*, 2004). It has a good impact on the digestive tract's function, gut microbial flora, blood cholesterol levels, glycemic index (GI) and diabetes management (Fuentes-Zaragoza *et al.*, 2010). It is worth noting that microbial fermentation of RS in the colon produces short-chain fatty acids that are beneficial to colonic health (Zhang *et al.*, 2012).

Resistant starch may also help lower the glycemic index (GI) of some foods. GI is a measurement of how quickly digestion causes a jump in blood glucose levels. Rice-eating populations consume starchy milled rice, which is generally high GI in nature due to low-amylose rice varieties with lower content of RS (Guzman *et al.*, 2017). According to Denardin *et al.* (2012), rice varieties with high amylose had lower GI than those with low amylose varieties. It has been shown that RS and amylose affect starch digestion. The high amylose and high RS food can alter starch digestion rates and consequently lower estimated glycemic indices (GIs).

RS can be classified into five forms based on the physical and chemical characteristics. RS1-physically inaccessible starch; RS2-starch of raw (non-gelatinised) granules of some plant species; RS3-retrograded starch; RS4-chemically or physically modified starch; RS5-amylose-lipid complex that resists amylolytic hydrolysis. The sizes and structural properties of different types of RS can be obtained by subjecting samples to a scanning electron microscope (SEM). In this study, the RS contents of different forms of UKMRC-9 products were investigated to understand how cooking and further processes could improve the nutritional properties of wholegrain red rice and its derivative food products. Besides red rice gluten-free bread and noodles, the RS content of traditional kueh made with red rice flour was also determined. Png kueh is made with rice flour and shaped into a peach shape while angku is made with glutinous rice but moulded into tortoise shape. Due to their symbolism of longevity among Chinese communities in Malaysia and Singapore, they are often classified as traditional snacks served during festivals and celebrations.

2. Materials and methods

2.1 Preparation of samples

Wholegrain bread was purchased locally from commercial products in Malaysia. The preparation of milled UKMRC-9 raw rice: one kilogram of UKMRC-9 was placed into a miller and blended at high speed for 5 mins. Only rice flour that passed through 120-160 mesh sieves (<125 µm) was used as samples for analysis. The

eight samples of UKMRC-9 and its downstream products were analysed for their RS contents.

2.1.1 Cooked rice

A total of 50 g of UKMRC-9 was cooked in a covered saucepan with 300 g filtered water. The cooked rice was kept at room temperature without going through the freezing process.

2.1.2 Cooked and frozen red rice with coconut oil (UKMRC-9 C0)

A total of 50 g of UKMRC-9 was cooked in a covered saucepan with 300 g of filtered water. A total of 10 g of coconut oil (Coconut Empire Sdn Bhd, Malaysia) was added to the cooked rice (2.1.3), both cooked rice were then placed in a blast freezer AK05-D (Eong Huat Corporation, Penang) and frozen at -25°C, and then kept in freezer at -16°C.

2.1.3 Png kueh and Kueh angku

The preparation methods for Png kueh involved cooking 90 g starch, 90 g UKMRC-9, 100 g modified starch and 450 g water, whereas for Kueh Angku, the preparation involved cooking 100 g glutinous rice powder, 50 g UKMRC-9, 100 g native starch and 300 g water. Then, coconut oil (Coconut Empire Sdn Bhd, Malaysia) was added at 10% of the total weight until the starch paste had swelled into a dough and the texture was pliable for moulding. The cooled dough was then rolled thinly and used as wraps for different fillings (UKMRC-9 was also used as 40% fillings for Png kueh). After wrapping, the kueh was steamed for 5 to 8 mins, placed in a blast freezer for freezing to -25°C and then kept in the freezer at -16°C.

2.1.4 Red rice gluten-free bread

This formulation involved 70% of rice as total dry weight with pulsed UKMRC-9 accounting for 25% and the rest were starches which accounted for total dry weight. A total of 130 g of UKMRC-9 flour, 50 g of white rice flour and 100 g of starches were dry mixed. Then the mixture was fermented with instant dry yeast and water (600 g) for 4 hrs in a temperature control mixer (Kenwood KCC9040S) at 40°C. Hydrocolloid mixture (xanthan gum and guar) was then added while mixing until the dough was stable enough to be shaped and placed into a loaf tin for further proofing until the height doubled its original. The tin was then baked in a preheated oven (Kenwood MOM880bs) at temperatures of 160°C (top) and 180°C (bottom) for 45 mins until an inserted testing stick came out clean. Since the base product was predominantly rice, the cooling process took longer to achieve as compared to conventional wheat

bread. The bread was blast chilled to 3°C, sliced and blast frozen to -25°C, and then kept in the freezer at -16°C. The sample for analysis was taken from the thawed bread that had been in the chiller overnight.

2.1.5 Red rice gluten-free noodle

This formulation involved 10% of UKMRC-9 flour as total dry weight. Red rice was ground into flour with a grinder machine (FFC-45A, Qingdao Dahua Double Cycle Machinery Co. Ltd). The red rice flour was added to wheat flour, xanthan gum and water. The mix was run through a noodle machine and sheeting process. The dough was then cut into various noodle sizes and dehydrated in the oven to form dry noodles. To prepare a sample for analysis, the noodle was cooked in boiling water for 5 mins until softened, water was drained and the noodle was kept in a container, with the lid covered, overnight in a chiller for 12 h before freeze-drying.

2.2 Measurement of resistant starch contents

The RS Assay Kit (Megazyme, Wicklow, Ireland) following the AOAC method 2002.02 (Nakayoshi *et al.*, 2015) was used to degrade the starch. Absorbance at 505 nm was measured using a UV-1800 spectrophotometer (Shimadzu Scientific Instruments Inc.). All samples except for UKMRC-9 raw rice were subjected to three days of freeze-drying (Labfreez Instruments Co. Ltd.). The freeze-dried samples were then milled in an industry-scale blender until fine, only particles that passed through 120-160 mesh sieves (<125 µm) were used as samples for analysis. The eight samples and one control with almost the same weight as the AOAC method in dry weight were used for the measurement of RS. The enzyme reaction time with pancreatin and amyloglucosidase was specified as 16 hrs in the AOAC method. Approximately 4 mL of α -amylase (10 mg/mL) containing amyloglucosidase (3 U/mL) was added to 100 mg of each RS sample, the mixture was vortexed followed by incubation at 37°C in a shaking water bath (200 strokes/min) for 16 hrs to hydrolyze digestible starch. Then, ethanol (4 mL) was added to deactivate the enzymes followed by centrifugation at 1,500×g for 10 mins. The pellet obtained was washed twice with 50% ethanol to remove the digested starch. The sediment was dissolved in KOH (2 mL, 2 M) by vigorous stirring for 20 mins. The solution was neutralized with sodium acetate buffer (8 mL, 1.2 M). Then 0.1 mL of amyloglucosidase (3300 U/mL) was added followed by incubation for 30 mins at 50°C. The samples were centrifuged at 3000×g for 10 mins. GOPOD (3 mL) was added to aliquots (0.1 mL) of the supernatant and incubated at 50°C for 20 mins. Absorbance was measured at 510 nm using a spectrophotometer. The RS contents were measured following the AOAC method.

2.3 Statistical analysis

Statistical analysis of the results was performed using AOAC-supplied software and statistical analysis software, SPSS (ver.15). Significant differences were analyzed by multiple comparisons using a one-way analysis of variance (ANOVA) with a significance level of 5%. Data reported are the averages of triplicate observations.

3. Results and discussion

3.1 Formation of different resistant starch types of UKMRC-9 and its correlation with glycemic index (GI)

Figure 1 shows the comparison of RS contents of raw red rice, cooked red rice, cooked-frozen-chilled red rice and cooked-frozen-chilled red rice added with coconut oil and control is a high amylose maize-starch provided in the RS Assay Kit (Megazyme, Wicklow, Ireland). Both raw rice and cooked rice with coconut oil added showed the highest resistant starch contents, ranging from 14.24±1.55 g/100 g to 16.52±0.91 g/100 g in dry samples, respectively. Statistical analyses of the UKMRC-9 and its downstream products are listed in Table 1. It shows the RS contents of cooked red rice and frozen red rice ranging from 8.52±0.62 g/100 g and 9.25±0.52 g/100 g without significant difference between them (one-way ANOVA). All these samples can be categorised as high based on the classification of material according to the range of RS content (Goni *et al.*, 1996). The RS content of raw red rice was high most probably due to the existing resistant starch 1 (RS1) that is derived from starch granules trapped within the protein matrix and cell wall material. Oftentimes, these physical structures hinder the digestibility of starch and reduce glycemic response (Lineback, 1999). According to Khossousi *et al.* (2008), the quality of carbohydrates in the diet is a key element in influencing the rate of glucose absorption and changing the postprandial glycemic response. RS type 2 (RS2) refers to starch molecules having type B or C polymorphs that can be found in a variety of sources, including undercooked potato, green banana and high-amylose corn. These substances have some proposed mechanisms to reduce glycemic response which are the capacity to form viscous solutions, delay gastric emptying and/or inhibit lipase activity (Lairon *et al.*, 2007). Other possible mechanisms such as modulating intestinal microbiota, appetite hormones, viscosity or gastric emptying could play a role in glycemic response (Sajilata *et al.*, 2006).

One of the focuses of this experiment was to determine if the formation of RS in rice could be affected by altering the cooking conditions. The results showed the increment of RS could be optimized by subjecting

cooked rice to rapid freezing and thawing before consumption, and RS can be further increased by adding 10% of coconut oil to the grains during cooking and subjecting the cooked rice to the freeze-thaw-chilled process. The results corroborate two previous studies on the effect of cooling cooked white rice on RS content and glycemic response (Sonia *et al.*, 2015) and the effect of oil addition on in vitro starch digestibility and physicochemical properties of instant rice (Luangsakul and Ritudomphol, 2018). In these studies, however, the rice used for experiments was white rice. Data for low GI wholegrain rice have not been extensively reported.

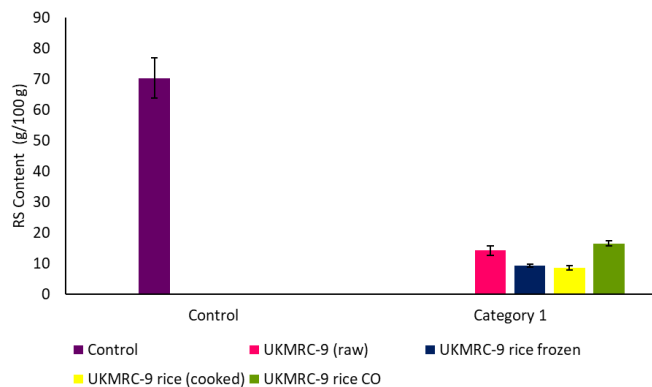


Figure 1. RS content of raw and cooked UKMRC-9 (g/100 g on a dry weight basis). Flour from dried, milled cooked rice and rice grains were prepared as the sample forms for analysis, and the RS contents were measured. Values are presented as mean±SD (n = 4).

Table 1. Resistant starch content.

Types of samples	RS Content (g/100 g on a dry
Control	70.42±6.50 ^a
UKMRC-9 (raw)	14.24±1.55 ^{bc}
UKMRC-9 rice (cooked)	8.52±0.62 ^{bde}
UKMRC-9 rice frozen	9.25±0.56 ^{bde}
UKMRC-9 rice CO	16.52±0.91 ^c
Png kueh	7.86±0.53 ^{bde}
Kueh Angku	3.12±0.29 ^d
Red rice bread	4.99±0.71 ^{de}
Wholegrain bread	4.97±2.53 ^{de}
Red rice noodles	11.42±0.87 ^{bcc}

Values are presented as mean±SD of three replicates. Values with different superscripts are statistically significantly different at $p < 0.05$ level.

In a previous study on evaluating crossbred red rice variants for postprandial glucometabolic responses (Se *et al.*, 2016), UKMRC-9 showed the most desirable glucometabolic response in the acute postprandial insulin sensitivity among other commercially available red rice which is supported by high RS content of UKMRC-9 in this study. The RS contents may exist in multiple forms such as RS2 which is composed of retrograded starch and RS5, the amylose-lipid complex that was formed

with the addition of coconut oil. The amylose-lipid combination found in starch granules promotes enzyme resistance by limiting granule swelling during cooking (Hasjim *et al.*, 2013).

According to Parween *et al.* (2020), RS is a valuable trait that was found to have a significant positive correlation with amylose content. There is also evidence that the GI value of rice is negatively correlated with its RS. Figure 2 shows the strong correlation between RS and GI of five different rice varieties. The graph was plotted using data from Kumar *et al.* (2020) providing strong support for the role of RS in glycemic response. Hence, the consumption of rice high in RS can help persons suffering from diabetes, colon cancer or obesity in improving their health.

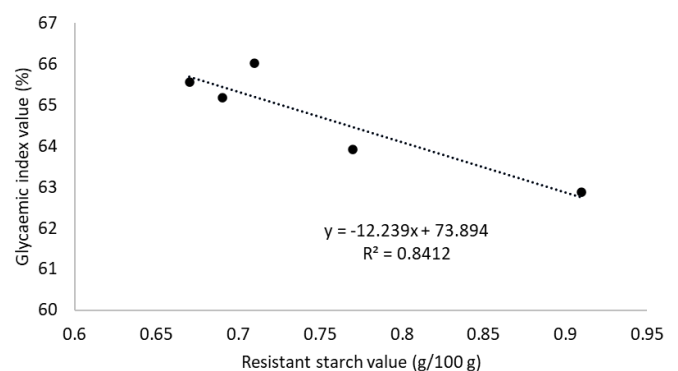


Figure 2. Glycemic index vs resistant starch in rice.

3.2 Formation of resistant starch in UKMRC-9 downstream products

Figure 3 shows RS formed in two types of frozen Chinese kueh and gluten-free loaf bread whose primary ingredients were rice and tapioca starches. A commercially available wholemeal sandwich loaf was also analysed as a comparison. All samples were lyophilized and milled to fine particles for analysis. The results show that Png kueh has the highest RS contents, 7.86±0.53 g/100 g as compared to Kueh Angku, 3.12±0.29 g/100 g. This was due to the base formulation of Png kueh which uses 35% of UKMRC-9 as compared to Kueh Angku which uses only 15%. The red rice gluten-free bread that utilized 30% of UKMRC-9 showed an intermediate RS content, 4.99±0.71 g/100 g which was comparable to the wholegrain bread, 4.97±2.53 g/100 g weight to weight without significant difference between them (Table 1). The effects on the sensory properties and qualities of the final products have been evaluated and were acceptable by testers in another study (unpublished). Based on RS classification by Goni *et al.* (1996), both RS values of the bread can be categorised as intermediate. Figure 3 also showed the comparison of RS contents on gluten-free noodles made with UKMRC-9, the contents for UKMRC-9 were 10%

on a dry weight basis. The RS values for red rice noodles were 11.42 ± 0.87 g/100 g, respectively. After cooking, the noodle was chilled overnight and during this time, the RS was formed due to the recrystallization process of amylose in a partially crystalline system (Eerlingen *et al.*, 1993). UKMRC-9 can serve as a fibre-enriching ingredient in gluten-free noodles.

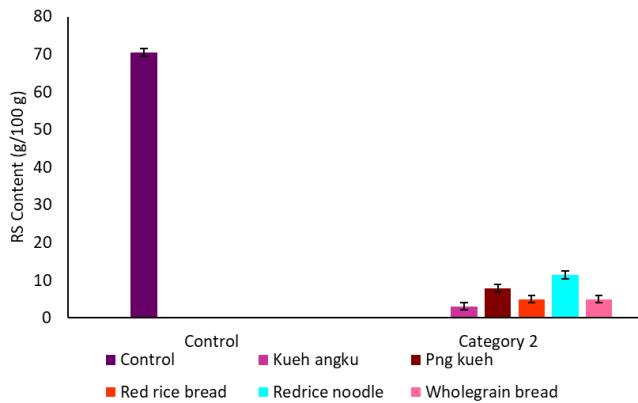


Figure 3. RS contents of UKMRC-9 downstream products (g/100 g on a dry weight basis).

4. Conclusion

The findings in the study show that UKMRC-9 on its own was a good source of carbohydrate that consists of highly resistant starches, by subjecting the cooked red rice through cycles of cooking processes and food handling will further increase its RS formation. The addition of UKMRC-9 will improve the RS contents of food products by replacing formulations that traditionally use white rice flour. The use of UKMRC-9 constitutes a promising approach to producing fiber-rich formulations of high-quality gluten-free products. This study is suitable to serve as a guideline to foodservice operators with the intention to include whole grain products in their menu.

Conflict of interest

The authors have declare no conflicts of interest.

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