

Effect of Cold Storage, Reheating, and Particle Sizes on *In Vitro* Glucose Release and Starch Digestibility among Five Rice Products in Auckland, New Zealand

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Abstract

Rice provides more than 27% of daily energy intake to more than half of the world's population. Refined and cooked rice, as a staple has been generally perceived to induce high glycaemic responses; however, it is known that cold storage of cooked rice, reheating, and mincing or chewing affects the rate and extent of starch digestion and glucose release. This *in vitro* experimental study aimed to investigate the effect of various combinations of these factors on the rate and extent of starch digestion of five rice products, medium grain white, medium grain brown, long grain white, basmati, and parboiled rice. Cold storage (at 4°C) for over ten hours significantly reduced digestibility of cooked rice starch (P=0.05). The reductions in starch digestion were dependent on the type of rice product varieties (medium grain white, medium grain brown, and long grain brown about 20% (P=0.05); basmati about 30% (P=0.05); and parboiled about 40% (P<0.001). Reheating (65°C for 15 minutes) and mincing increased starch digested by 20% (P<0.001) and 18% (P<0.001) respectively compared with the cold-stored un-minced cooked rice. The glucose released from minced freshly cooked medium grain white rice reached over 90% after 40 minutes of digestion while 24-hour cold-stored minced parboiled rice had the lowest, reaching around 40% after 180 minutes. Further study with human participants is suggested to analyse the significance of the differences between medium grain white rice and cold stored parboiled rice, for the purpose of investigating if the optimal treatment of parboiled rice (cold storage at 4°C for 24 hours), with appropriate food safety precautions (reheating to 65°C for at least 15 minutes), could be a public health recommendation that would improve the postprandial blood glucose response compared with the response to the more popular medium grain white rice.

Keywords: Rice starch digestion; Rapidly digested starch; Slowly digested starch; Resistant starch; Cold-store; Particle size; *In vitro* glucose release; Starch digestibility profile; Cooking method; Cold storage; Mincing; Reheating

Abbreviations

GI: Glycaemic Index; GL: Glycaemic Load; iAUC: Incremental Area under the Curve; RDS: Rapidly Digested Starch; RS: Resistant Starch; SDS: Slowly Digested Starch; TAS: Total Available Starch; TS: Total Starch

Introduction

As the primary carbohydrate source that supports more than half of the world population daily energy intake, rice plays an important role in meeting the energy requirement and nutrition intake. Like other dietary carbohydrates, rice products are digested and absorbed at different rates and to different extents in the human small intestine, depending on their botanical source and the physical form of the food [1]. Diets containing large amounts of rapidly digested starch (RDS i.e. starch that can be digested within 20 minutes after ingestion) may release glucose and elevate blood glucose rapidly and be detrimental to health [2], while the inclusion of foods in daily diet that have a slow release of glucose is considered beneficial. Previous studies have provided the evidence that slow starch digestion and slow glucose

release are favourable for dietary management of individuals suffering from impaired blood glucose homeostasis [3-5].

Various intrinsic and extrinsic factors have been reported in a number of studies to impact on the trajectory of glucose release from rice [6-8], which is directly associated with the starch digestibility. Two important intrinsic factors are the quantity of dietary fibre present and the amylose to amylopectin ratio which depends on the botanical origin of the rice. The dietary fibre content of brown rice may significantly reduce the susceptibility to enzymatic degeneration (i.e. amyolytic attack) both in the mouth and the small intestine slowing the rate of digestion and reducing the postprandial glycaemic response [9-11]. Rice that exhibits high amylose to amylopectin ratio (e.g. long grain rice and basmati rice) tends to resist enzymatic attack longer and produce a lower postprandial glycaemic response than rice with a lower amylose to amylopectin ratio (i.e. most medium and short grain rice products). Altering rice starch structure by thermal processing, such as cooling cooked rice or storing cooked rice at low temperature, may transform gelatinized rice starch from an amorphous state to a more ordered state (i.e. crystalline state) which persists on reheating [12]. The crystallized starch form can resist enzymatic degradation in small intestine to up to 3 hours [13]. This retrogradation process may spontaneously lower the concentration of digestible starch in cooked rice and subsequently reduce the potential of postprandial glycaemic response and glycaemic index (GI) value [14]. Different degrees of particle size reduction can significantly affect rice starch digestibility

[4]. It was also hypothesized that whole grain rice with outer bran intact may resist digestion longer than chopped whole grain rice and well-polished rice grain [15].

Previous studies of the variations in starch digestibility and absorption of glucose have largely based on the measurements of GI and estimates of the glycaemic load (GL). Englyst and Englyst [16] has introduced an *in vitro* definition of starch digestibility that mimics the way starch is digested in the human gastrointestinal tract. This *in vitro* digestion method determines nutritional starch fractions, rapidly digestible starch (RDS, can be digested within 20 minutes after ingestion), slowly digestible starch (SDS, can be digested between 20 and 180 minutes after ingestion), and resistant starch (RS, can resist digestion to up to 180 minutes), by measuring the amount of glucose released from one test food during incubation with amylase enzymes under standardized *in vitro* conditions over 180 minutes. The total amount of starch digested to glucose in 180 minutes is termed total available starch (TAS=RDS + SDS +RS).

The aim of this study was to obtain proof of principle that storing cooked rice at 4°C for 24 hours and reheating can reduce the rate and extent of starch digestion and glucose release and to discover the optimal combination of factors (rice type, particle size, cooking method, and storing condition) to improve the health profile of cooked rice to reduce the glycaemic load of rice consumers.

Method

Rice products tested

Five rice products (1 kg each) were purchased from a New Zealand local high-turnover supermarket (Pak'n'save): medium grain white rice (Sun Rice®), pure white basmati rice (King's Choice®), medium grain brown rice (Sun Rice®), long grain brown rice (Sun Rice®), and parboiled rice (Real Rice®). All rice products were produced within one year and in good condition. The selection of rice products was based on empirical information gathered from an Auckland Indian community nutritionist and shelf space for these products in supermarkets frequented by Indian and Chinese customers.

Comparison of treatments

Two series of twelve experiments were applied to each of the five rice products to investigate 1. the effect of time of cold storage at 4°C for 4, 8, 10, 12, and 24 hours and 2. the combination of the effects of mincing the freshly cooked or 24 h cold stored rice to 2.5 mm in diameter on the glucose release trajectory and starch digestibility profile (i.e. the proportions of RDS, SDS, and RS) of each cooked rice product (Table 1).

Group 1: Investigate the cold storage time effect on rice starch digestibility	
1	Freshly cooked rice
2	Cooked rice stored at 4°C for 4 hours
3	Cooked rice stored at 4°C for 8 hours
4	Cooked rice stored at 4°C for 10 hours
5	Cooked rice stored at 4°C for 12 hours
6	Cooked rice stored at 4°C for 24 hours
Group 2: Investigate the effect of mincing and cold storage on rice starch digestibility profile	
7	Freshly cooked rice at 37°C, minced grain structure
8	Freshly cooked rice at 37°C, intact grain structure
9	Cooked rice stored at 4°C for 24 hours, reheated to 65°C for 15 minutes, minced grain
10	Cooked rice stored at 4°C for 24 hours, reheated to 65°C for 15 minutes, intact grain
11	Cooked rice stored at 4°C for 24 hours, minced grain structure
12	Cooked rice stored at 4°C for 24 hours, intact grain structure
Samples 1–6: grains reheated to 65°C and minced before digesting	

Table 1: Summary of treatments and mincing used in testing the effects of rice processing on starch fractions in five types of rice (medium grain white rice, basmati rice, medium grain brown rice, long grain brown rice, and parboiled long grain white rice).

Each experiment was duplicated so all results and statistical analyses were from the average of duplicated samples.

Rice cooking

Each raw rice product (100.0 g) and 110 mL of distilled water was weighed into a 60 mL glass beaker (KIMAX, USA). All beakers were tightly sealed and immersed in boiling water in a large cooking pan

and covered during cooking. Basmati, parboiled and medium-grain white rice samples were boiled for 25 minutes; and, medium-grain and long-grain brown rice samples were boiled for 45 minutes to achieve complete gelatinisation. Freshly cooked rice (at 100°C) was removed from large cooking pan and rapidly cooled by running cold tap water around the glass beaker until the centre of the rice was cooled to 37°C.

Mincing

Mincing was achieved by rubbing the rice grains gently through a 2 mm sieve on cooking paper. The minced particles (2.5 mm in diameter) of each rice product (5.0 g) were quickly collected into 10 separate plastic pots (70 mL; Lab Serve LBS 30002) and tightly capped to prevent moisture loss.

Cold storage

Tightly capped pots containing intact or minced cooked rice grains were immediately placed in the refrigerator at 4°C. The temperature of the refrigerator was monitored every two hours during the storage time.

Reheating

At the end of each cold storage time period (4-hour, 8-hour, 10-hour, 12-hour and 24-hour), two plastic pots for each of the five rice products (5.0 g) were taken out of the refrigerator and kept tightly capped to prevent moisture loss. The pots were put into a warm water bath at 65°C for at least 15 minutes until the rice was completely heated to 65°C.

Starch digestion

Total starch and moisture content: The total starch (TS) content was determined by the Megazyme TS enzymatic procedure (AA/AMG) AACC Method 76-12 [17,18]. The moisture content (%) was determined as the moisture removed from intact uncooked rice grains by drying for 24-hr in a vacuum oven at 60°C attached to a freeze-drier.

***In vitro* digestion and glucose analysis:** The *in vitro* digestion adopted a timed digesta sampling procedure (20, 40, 60, 120, 180 min) to show the relatively susceptibility of starch digestibility [2,19]. This starch digestion method and the glucose analysis method (dinitrosalicylic acid colorimetric method) used were based on the methods published by Mishra et al. [20].

Starch calculation: The proportion of RDS was expressed as the reducing sugar measured in the 20 minutes aliquots as a proportion of TS, and SDS as reducing sugar released between 20 and 180 minutes as a proportion of TS. The proportion of RS was estimated by $(TS - RDS - SDS) / TS * 100\%$.

Statistics

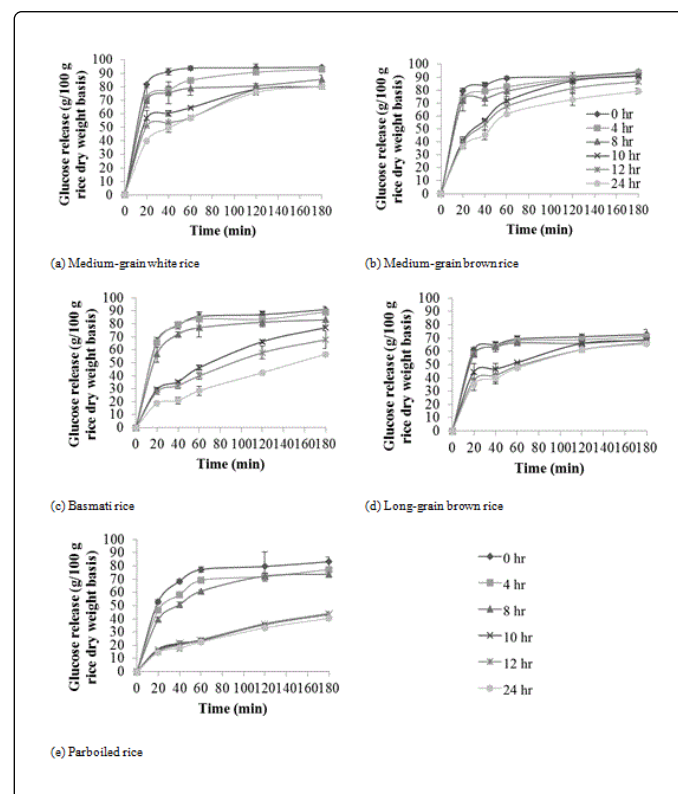
Summary of glucose release over time (g/100 g rice dry weight basis) and starch digestibility profiles (proportions of RDS, SDS, and RS) were subjected to repeated measures one-way analysis of variance (ANOVA) followed by a T-test for independent samples. The significance concentration was $P < 0.05$. All analyses were performed using Excel for Windows XP, version 2010 (Microsoft, USA) and Statistical Package for the Social Science (SPSS) for Windows XP, version 2.0 (IBM, USA).

Results

Effects of cold storage time on glucose release

The extent and rate of glucose release decreased with cold storage (Figure 1). For minced medium-grain white, medium-grain brown and

basmati rice the glucose release rate was higher than that of long-grain brown and parboiled rice for the same cold storage time. More than 10 hours of cold storage reduced glucose release around 30% ($P = 0.05$). For parboiled rice, the glucose release was less than for other rice product varieties at every time point (Figure 1) and the proportional reduction with cold treatment was greater (40%, $P < 0.001$). No further reduction was observed after 12 hours and 24 hours of cold storage (Figure 1e).



Effects of cold storage time on starch digestibility profiles

Increasing cold storage time from 0 hour to 10 hours gradually reduced the TAS and RDS whereas increased SDS and RS. The most significant change in digestibility was found in minced parboiled rice, of which both TAS and RDS dropped by around 50% ($P < 0.001$) (Figures 2a and 2b) and RS almost doubled ($P < 0.001$) after 10 hours of cold storage. Minced medium grain white and basmati rice had a more significant reduction in TAS (by average 20%, $P < 0.001$) and RDS (by average 25%, $P < 0.001$) compared with whole grain rice (medium-grain and long-grain brown rice, average 5% reduction in both TAS and RDS, $P = 0.1$) following more than 10 hours of cold storage. Both medium-grain white and basmati rice had an average 20% increase to around 40% of SDS after 10 hours of cold storage ($P < 0.001$), and both whole grain rice (medium-grain and long-grain brown rice) had around 10% increase ($P < 0.001$) (Figure 2c). An insignificant but steady increase of RS among medium-grain white rice, medium-grain brown rice, basmati rice and long-grain brown rice was observed (from 5% to 10%, $P = 0.1$) (Figure 2d).

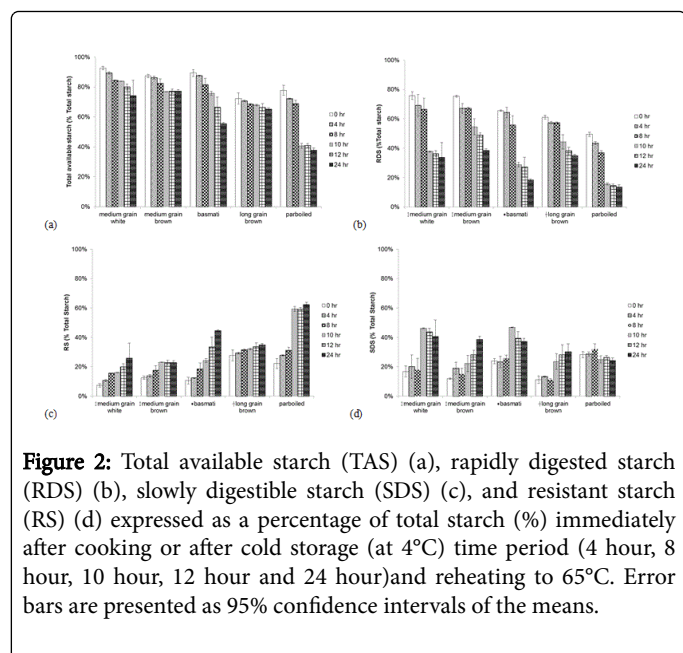


Figure 2: Total available starch (TAS) (a), rapidly digested starch (RDS) (b), slowly digestible starch (SDS) (c), and resistant starch (RS) (d) expressed as a percentage of total starch (%) immediately after cooking or after cold storage (at 4°C) time period (4 hour, 8 hour, 10 hour, 12 hour and 24 hour) and reheating to 65°C. Error bars are presented as 95% confidence intervals of the means.

Effect of various combined treatments (rice product varieties, 24-hr cold storage, reheating and rice grain particle size interruption) on glucose release

Cold storage over 24 hours without reheating treatment significantly reduced glucose release from all rice products by up to 40% ($p < 0.001$) (Figure 3). Reheating to 65°C after 24 hours of cold storage treatment reversed around 20% the effect of cold storage i.e., increased RDS and SDS in all five rice products ($p < 0.001$). Mincing also increased the rate and extent (RDS + SDS) of glucose release. The increase was larger in whole grain rice products (medium-grain brown by around 10%, $P < 0.001$; long-grain brown by around 18%, $P < 0.001$) compared with refined grain (medium-grain white and basmati by approximately 4%, $P < 0.001$; Figure 3).

When the same combination of treatments applied, both parboiled rice and long-grain brown rice had significantly lower (around 10%, $P < 0.001$) overall rates and extents of glucose release than the other three rice products (Figure 3). Between parboiled and long-grain brown rice, when reheating was applied, parboiled rice had a similar glucose release trajectory to that of long-grain brown; however, when no reheating was applied, parboiled rice had a slightly lower (around 5%, $p < 0.001$) rate and extent of glucose release (Figures 3d and 3e).

Effects of rice product variety, cold storage, reheating and mincing on starch digestibility

Between un-reheated and reheated rice: Twenty-four hours cold storage promoted the formation of starch retrogradation (i.e., increase in RS by up to 40%, $P < 0.001$) and the reduction of TAS (by around 25%, $P < 0.001$) and RDS (by up to 40%, $P < 0.001$), while reheating reversed the starch retrogradation and increased the proportion of TAS (by up to 20%, $P = 0.01$) and RDS (by up to 10%, $P < 0.001$), depending on rice types and the structure of cooked rice grains (Figures 4a-4c).

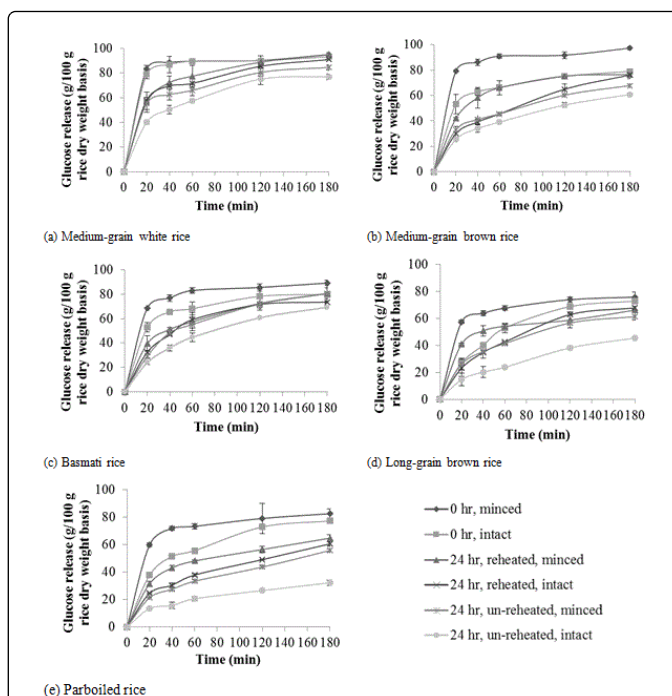


Figure 3: Rapidly digested starch (RDS) (%) expressed as a percentage of total starch immediately after cooking or after cold storage (at 4°C) time period (4 hour, 8 hour, 10 hour, 12 hour and 24 hour) and reheating to 65°C. Error bars are presented as 95% confidence intervals of the means.

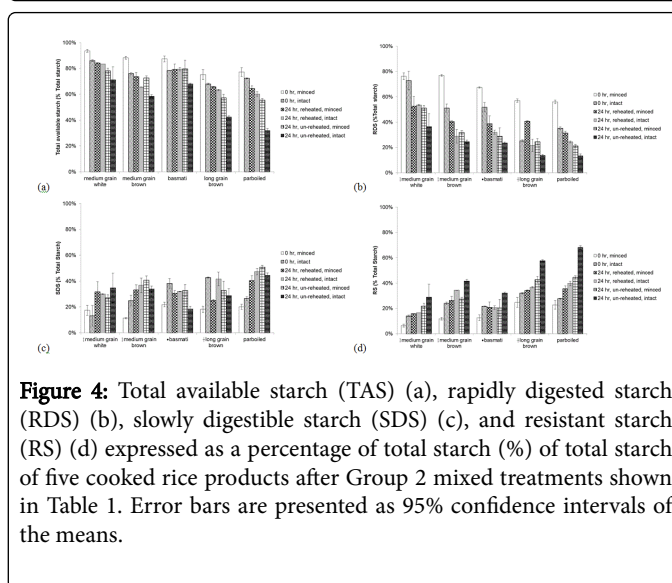


Figure 4: Total available starch (TAS) (a), rapidly digested starch (RDS) (b), slowly digestible starch (SDS) (c), and resistant starch (RS) (d) expressed as a percentage of total starch (%) of total starch of five cooked rice products after Group 2 mixed treatments shown in Table 1. Error bars are presented as 95% confidence intervals of the means.

The most significant change in starch digestibility profile following 24-hour cold storage was observed in long-grain brown rice and parboiled rice. TAS reduced by 25% ($P < 0.001$) and RDS reduced by up to 40% ($P < 0.001$), and RS increased by around 40% ($P < 0.001$; Figures 4a-4c). The cold storage also promoted the formation of SDS; however, the increase was not consistent. Among these five rice products, parboiled rice had the highest proportion of SDS (around 40%) (Figure 4c). The largest effect of reheating was observed for intact parboiled

rice (around 30% increase in TAS and RDS, $P < 0.001$), and the smallest change was observed in minced basmati rice (around 2% increase in TAS and RDS, $P = 0.5$; Figures 4a and 4b). However, the reversion did not bring the TAS and RDS to the freshly cooked values. Still around 10% of starch retained the retrogradation status in all rice products (Figure 4a).

Between intact and minced rice: Minced rice, compared with their intact counterparts, had overall a higher proportion of TAS (around 15% higher, $P < 0.001$) and RDS (around 40% higher, $P < 0.001$) and lower proportion of RS (around 20% lower, $P < 0.001$; Figures 4a, 4b and 4d). The impact of mincing on the increase of TAS content was more significant in un-reheated whole grain rice (medium-grain and long-grain brown rice) and parboiled rice (around 20% increase after mincing, $P < 0.001$; Figure 4a). Similarly, mincing had a more significant impact on the reduction of RS content among un-reheated whole grain rice and parboiled rice (around 20% decrease after mincing, $P < 0.001$; Figure 4d). A large increase in RDS and SDS following mincing treatment was again observed among whole grain rice and parboiled rice (more than 20% RDS increase after mincing, $P < 0.001$; around 10% SDS decrease after mincing, $P < 0.001$; Figures 4b and 4c). However, the change of SDS between minced and intact medium-grain white rice was statistically insignificant ($P = 0.37$; Figure 4c).

Effects of combined factors: Cold storage strongly promoted starch retrogradation in long-grain brown rice and parboiled rice, both of which had a larger decrease in TAS and RDS and a larger increase in RS compared with medium-grain white rice, basmati rice and medium-grain brown rice. Mincing and reheating, however, reduced the difference. As a result, the reheated minced long-grain brown rice and parboiled rice had a similar proportion of TAS (around 60%; Figure 4a).

The increase of TAS in minced rice was related to an increase of RDS. The mincing of brown rice almost doubled the RDS concentration and significantly reduced the SDS concentration in long- and medium-grain brown rice. Compared with the starch fractions in long-grain brown rice, the RDS, SDS and RS concentrations in parboiled rice were only slightly affected by the mincing process. (Figures 4a and 4b).

The effect of mincing also varied among different rice types. After the cold storage and reheating cycle, minced long-grain brown rice had much less RS than the intact rice. Compared with long-grain brown rice, the effect was much less significant in well-polished rice and medium-grain brown rice. The particle size did not affect the RS proportion in parboiled rice.

Discussion

This study has demonstrated that rice product variety, larger particle size (extent of mincing or chewing rice grains), 24-hour cold storage at 4°C and reheating to 65°C might also substantially lower the rate and extent of starch digestion observed by others for rice [4,21,22]. The two rice products that were most different were the widely consumed medium-grain white rice (SunRice®) and Real Rice® parboiled rice. Medium grain white rice had the highest TAS and RDS compared and 24-hour cold storage the least impact on TAS and *in vitro* glucose release reduction compared with parboiled. Generally speaking, whole grain rice products (medium-grain brown rice and long-grain brown rice) were more responsive to cold storage and mincing treatments than refined grain rice (medium-grain white rice and basmati). The

main source of these differences was in the proportions of RS and SDS, which were both directly affected by parboiling treatment (pre-cooked) and milling and polishing processes (refined vs. encapsulated whole grains).

Twenty four hour cold storage at 4°C substantially reduced *in vitro* glucose release in all rice products which is consistent with previous research findings [23,24]. The slowdown of rice starch digestion (i.e., hydrolysis) and thus the *in vitro* starch digestibility due to cold storage has been shown by others to be caused by retrogradation or recrystallisation of previously gelatinised starch [8,12,13,25].

Parboiling could also induce irreversible retrogradation of amylose, which could lead to the formation of type 3 RS (RS3) and SDS [10]. The slower *in vitro* glucose release and higher RS and lower TAS and RDS of parboiled rice confirms the previous observation that parboiling (i.e., pre-cooking, drying, cooling, and then polishing) can increase starch retrogradation and alter the chemical structures of starch in ways that limit the rate of enzyme action. Furthermore, the gelatinisation of long-grain rice starch (i.e., high amylose starch) followed by hydrothermal processing (i.e., parboiling) can result in recrystallisation of starch and significantly rearranges the retrograded starch chain, thus increasing the proportion of RS [12,25,26].

Differences in starch digestibility can also be explained by the proportion of amylose-amylopectin present which depends largely on the botanical source, the rice grain particle size and the storage conditions [13,27,28]. Long-grain rice has a relatively higher proportion of amylose, of which the irreversible retrogradation reaches peak limit after 48 hours [23] but cold storage (over 48 hours) may further induce the formation of RS and impacts on rice sensory properties making the rice unacceptable for consumption.

Particle size is inversely correlated with the accessibility of the digestive enzymes such as amylase. Previous studies have cited particle size reduction as a factor reducing retrogradation and increasing digestibility of rice starch [23]. We have shown that the effect of particle size reduction was greater for whole grain rice than well-polished rice. The bran or the whole grain outer layer acts to encapsulate the starch and protect it from enzymatic attack in the small intestine. When the encapsulation is broken down by chewing or mincing, the amylase penetrates and initiates the digestion more quickly.

Feri et al. [23] and Eerlingen et al. [27] both found a high concentration of RS in retrograded waxy maize starch after 120 minutes of *in vitro* enzymatic incubation but did not extend their studies beyond this time. This study has also shown that the digestion of rice starch was not complete at 120 minutes and for some rice varieties was still not complete at 180 minutes. Previous studies have recommended 4°C as the optimal and safe storage temperature that is compatible with our daily life. However, reheating to 65°C is required to prevent the growth of *Bacillus cereus*, a bacterium that has often been associated with food poisoning in cooked rice products.

The strength of this study is that it followed precise and valid starch digestion techniques [20]. This group of experiments extended the research by for the first time, simulating a series of rice consumption scenarios: cold storage times, reheating before consumption and chewing. The potential alternative cooking, cold-storage and reheating procedure in this study was demonstrated to be effective in reducing the rate and extent of the glucose release during the *in vitro* digestion of cooked rice products, and thus may be effective in reducing the rate and extent of postprandial glycaemic response in humans. It is

particularly relevant for individuals suffering from problematic carbohydrate metabolism. These findings should be further consolidated through GI measurement and chewing tests with human participants. Moreover, as slow digestion of starch may be associated with a reduced sensation of hunger [12] this cooking procedure may help reduce total daily food intake, increase the chewing length of each mouthful and prolong the intervals between food intake [15,29,30]. Further studies on the satiety of reheated rice and viscosity within the gastrointestinal tract are recommended.

The limitation is that this study is restricted to the *in vitro* laboratory experimental conditions. The inter-and intra- individual variations, including glucose disposal rate, chewing rate, chewed particle size distributions, etc., could not be considered as covariant variables in starch digestibility profile analysis. This study used mincing to simulate the average disruption of food achieved by chewing. Mincing broke down the outer layer of brown rice and disrupted the physical form, which directly affected the extent and rate of starch digestion *in vitro*. However, the disruption of cooked rice grains by the mincer pan gave a rice particle size less than or equal to 2.5 mm in diameter, which could be significantly different in human trials. Future study could use the samples masticated by participants till the point at which they felt the need to swallow [15]. Furthermore, further study is required to investigate the rate and extent to which the starch of optimal rice choice is digested and absorbed in the human gastrointestinal tract and released into the bloodstream as free glucose. The inter-and intra-individual variations need to be investigated in order to provide a more constructive recommendation on the optimal combination of rice choice and cooking and preparation method.

Conclusion

This study has demonstrated that the rate and extent of the starch digestibility of common rice products in New Zealand can be suppressed by reducing the physical form of the cooked rice grain and by prolonged cold storage (at 4°C for 24 hours). Reheating (to 65°C for 15 minutes) after cold storage can slightly increase the starch digestibility of cold-stored rice. The formation of RS and SDS in cooked rice products after cold storage or reheating does appear to be affected by the physical form of the rice grain. Mincing brown rice with interrupted bran structure becomes more susceptible to digestion than intact brown rice grain. The physical form interruption appears to have less impact on white rice. Among these four New Zealand popular rice products (medium-grain white rice, basmati rice, medium-grain brown rice and long-grain brown rice), parboiled long-grain white rice had the lowest overall concentration of available starch over the time course after *in vitro* digestion starts. The findings suggest that replacing freshly cooked medium-grain white rice with cold-stored and reheated cooked parboiled rice should be encouraged as it has the potential to offer significant nutritional benefits to people in New Zealand who have rice in their main diet.

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Contributions of the Authors

Louise W Lu, who designed and conducted the research and wrote the article; Elaine Rush, who helped with study development, edited

the article and added knowledge; John A Monro, who helped design the experiment and kindly contributed time and resource to all the experiments; Jun Lu, who helped with editing.

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