

Which rice and why? A healthier choice

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A thesis submitted to Auckland University of Technology in fulfilment of
the requirements for the degree of

Doctor of Philosophy (PhD)

2016

School of Health and Environmental Sciences

Abstract

Rice has been a staple grain of the human diet for 9,000 years. Currently, more than half of the world's population derives one-third of their total daily dietary energy intake from rice. The most popular rice product, white rice, has the oil-rich bran and germ layer removed to prolong shelf life and, compared with brown rice, requires less cooking and preparation. However, many product varieties of freshly cooked white rice are composed of rapidly digestible starch and when consumed may trigger a rapid and prolonged rise of blood glucose among people with impaired glucose tolerance or diabetes. Regular and long-term consumption of freshly cooked white rice increases the glycaemic load (GL) of the diet and may be associated with an increased risk of hyperglycaemia and the development of type 2 diabetes, obesity and other metabolic diseases. In multicultural societies such as Auckland, New Zealand, the consumption of rice per capita is increasing. This series of experiments aimed to investigate *in vitro* and *in vivo* the effect of popular rice products and method of preparation on the digestibility of starch and glycaemic responses. The rice product that had the best starch profile and lowest GL was then investigated for sensory acceptability by participants who commonly consume cooked rice.

The first series of *in vitro* experiments investigated five popular rice products: medium-grain white rice, medium-grain brown rice, long-grain brown rice, basmati rice and parboiled rice. Samples of each cooked product had the starch digestibility profile and glucose release measured when rice was freshly prepared (Chapter 4) and then when cooked rice was stored for two to 24 hours at 4 °C, reheated, and minced (Chapter 5). The velocity and the extent of glucose release during *in vitro* enzymatic starch digestion over 180 minutes were compared. No significant difference in total starch content was observed among the five uncooked rice products. After full gelatinisation (i.e., cooking), the *in vitro* glucose release (gram glucose / gram dry weight base) within the first 20 minutes of digestion showed that medium-grain white rice reached 78.4% (SD \pm 3.9%), basmati rice 41.5% (\pm 6.8%), medium-grain brown rice 36.5% (\pm 0.2%), long-grain brown rice 26.6% (\pm 2.3%) and parboiled rice 27.5 (\pm 5.8%) of the starch as glucose. After 180 minutes, the *in vitro* glucose release from whole grains of medium-grain white rice reached 98.3% (\pm 1.1%), basmati 87.7% (\pm 3.1%), medium-grain brown 76.5% (\pm 1.8%), long-grain brown 71.5% (\pm 2.5%) and parboiled 81.2% (\pm 1.0%).

Up to eight hours of cold storage at 4 °C and mincing the cold rice to 2,400 μ m did not significantly change the glucose release trajectory in any of the five rice products

(within 10% reduction, $P = 0.1$). When stored for more than 10 hours, the trajectory was reduced significantly. For medium-grain white, medium-grain brown and long-grain brown, the reduction was around 20% ($P = 0.05$), for basmati around 30% ($P = 0.05$) and for parboiled around 40% ($P = 0.01$). Conversely, for all five rice products, reheating the rice to 65 °C for at least 15 minutes increased the rate and extent of glucose release by around 20% ($P = 0.01$) and mincing significantly increased glucose release in various degrees. Among the rice products, minced freshly cooked medium-grain white rice had the highest overall glucose release trajectory, reaching 90% of the total available glucose after 40 minutes, whereas minced parboiled rice that had been stored at 4 °C for 24 hours and then reheated had the lowest at 60% after 180 minutes. Clinical recommendations to help improve post prandial blood glucose concentrations could include replacement of freshly cooked medium-grain white rice with parboiled rice with the optimal treatment (cold storage at 4 °C for 24 hours) and appropriate food safety precautions (reheating to 65 °C for at least 15 minutes). The next step was to measure the glucose response to rice prepared this way in healthy participants.

Twenty-eight apparently healthy participants had two-hour (0, 30, 60, 90 and 120 minutes) postprandial glycaemic responses measured on three separate occasions when they consumed 140 g ($140 \text{ g} \pm 0.3 \text{ g}$) of either freshly cooked medium-grain white rice, freshly cooked parboiled rice or reheated parboiled rice that had been stored for 24 hours at 4 °C. All rice was served warm at 65 °C. Chewing time, chewed particle size distribution, and perceptions of satiety and palatability over two hours were also assessed. The 24-hours cold-stored and reheated parboiled rice resulted in a significantly lower blood glucose concentration trajectory (42%, $P < 0.001$) than freshly cooked medium-grain white rice and 12% lower ($P = 0.001$) than freshly cooked parboiled rice. Longer chewing time (6.34 seconds/10 g of rice compared with freshly cooked medium-grain white ($P = 0.026$) and higher palatability score (“visual appeal” was 2.0 higher ($P = 0.001$), “smell” was 1.0 higher ($P = 0.034$), “taste” was 1.5 higher ($P = 0.023$), and “overall palatability” was 1.9 higher ($P = 0.003$)) might have impacted on the slower rise of glucose response of the reheated parboiled rice. Further study is required to investigate the sensory acceptability of the rice products to investigate whether rice prepared by the optimal treatment could be accepted as part of the diet.

Finally (Chapter 7), 64 participants rated their acceptance and liking of the freshly cooked and also reheated medium-grain white rice, medium-grain brown rice and parboiled rice, that is, six treatments all served warm at 65 °C. All six rice samples

(three freshly cooked and three reheated) were perceived to be similarly acceptable by participants (average 5.3 out of 10 score \pm 0.2). No significant difference in overall liking was found in any of the six rice samples. Similarly, no significant differences in the acceptability of colour and sweetness were observed. Among all rice samples, the texture and the flavour of freshly cooked warm medium-grain white were less preferred (scored 4.6 ± 0.7 out of 10; 4.3 ± 0.6 , respectively) compared with other samples ($P < 0.05$). Over 50% of all participants preferred both reheated parboiled rice (5.6 ± 0.6 out of 10) and freshly cooked parboiled rice (5.2 ± 0.6 out of 10) as a daily regular staple grain to freshly cooked medium-grain white rice (4.5 ± 0.6 out of 10, $P < 0.05$).

This series of experiments suggests that reheated parboiled rice, which has the lowest starch digestibility and glucose impact (both *in vitro* glucose release and *in vivo* glucose response), can be accepted as a healthier alternative to freshly prepared medium-grain white rice.

The strength of this study is the *in vitro* and then *in vivo* exploration of the glycaemic impact of rice products freshly prepared or stored for 24 hours and reheated. While limited by small sample sizes, this is a proof of concept and principle that needs now to be tested in real world settings with those most at risk.

Reheated parboiled rice could be a staple rice that offers solutions for long-term glycaemic management and contributes to solutions for the grand challenges (e.g., type 2 diabetes and obesity) to global health because of its relatively better nutrient profile, low *in vitro* starch digestibility, low postprandial glycaemic response, promotion of satiety and palatability, and generally positive overall likeability.

Keywords: Rice, parboiled, reheated, minced, *in vitro* glucose release, starch digestibility, rapidly digestible starch, slowly digestible starch, resistant starch, *in vivo* glucose response, chewing, satiety, liking preference.

List of Abbreviations

ANOVA	Analysis of variance
ASTM	American Society for Testing and Materials
AUC	Area under the curve
AUT	Auckland University of Technology
BCMRC	Body composition and metabolic responses composition
BMI	Body mass index
CCK	Cholecystokinin
CDKAL1	CDK5 regulatory subunit associated protein 1-like 1
CI	Confidence interval
DCCT	Diabetes Control and Complication
DP	Degree of polymerisation
eGDR	Estimated glucose disposal rate
EGS	Estimated glycaemic score
EPIC	European Prospective Investigation Into Cancer
ETE	Emetic toxin
FAO	Food and Agriculture Organisation of the United Nation
FAPRI	Food and Agricultural Policy Research Institute
FV	Final volume
GD	Glucose disposal
GDM	Gestational diabetes mellitus
GI	Glycaemic index
GIP	Glucose-dependent insulintropic peptide
GL	Glycaemic load
GLP-1	Glucagon-like-peptide-1
GT	Gelatinisation temperature
GWAS	Genome-wide association studies
HHEX	Homeobox hematopoietically expressed
HSD	Tukey's honest significant difference
iAUC	Incremental area under the curve
LSD	Least significant difference
NCD	Non-communicable disease
NEFA	Non-esterified fatty acids
NGSP	National Glycohemoglobin Standardisation Program
NHS I	Nurses' Health Study I
NHS II	Nurses' Health Study II
NZ	New Zealand
MC	Moisture content
OGTT	Oral glucose tolerance test
PYY	Peptide YY
RCT	Randomised controlled trial
RDS	Rapidly digested starch
RR	Relative risk
RS	Resistant starch
SE	Standard error
SD	Standard deviation
SDS	Slowly digested starch
SLC30A8	Solute carrier family 30, member 8
SPSS	Statistical Package for the Social Sciences
TAS	Total available starch
TS	Total starch
T2DM	Type 2 diabetes mellitus
VAS	Visualised analogue scale
WHO	World Health Organization of the United Nation

Table of Contents

Abstract.....	i
List of Abbreviations.....	iv
List of Figures.....	x
List of Tables.....	xiii
Attestation of Authorship	xv
Acknowledgements.....	xvi
Ethics Approval	xvii
Chapter 1 Introduction.....	1
Chapter 2 : Literature review: How to lower the gap between current and future knowledge for rice choice? A modelling prediction	5
2.1 High glycaemic load diet: An ongoing health challenge in a multi-ethnic population in Auckland, New Zealand	7
2.1.1 Asian populations in New Zealand	7
2.1.2 Increasing prevalence of type 2 diabetes and metabolic syndrome among Asians in New Zealand.....	8
2.1.3 The pathogenesis of type 2 diabetes mellitus.....	10
2.2 Rice is related to high glycaemic index diet among people living in Auckland.....	24
2.2.1 Increasing rice consumption in New Zealand	24
2.2.2 Application of dietary management of high blood glucose concentration....	25
2.2.3 Modelling the long-term glycaemic impact of rice	27
2.3 Digestibility of starch in rice and blood glycaemic responses	30
2.3.1 Background of rice as a food.....	30
2.3.2 Rice starch digestibility	32
2.3.3 Starch digestibility profile: Rapidly digestible starch, slowly digestible starch and resistant starch.....	33
2.4 Satiety and dietary management relating to rice products prepared in different ways	38
2.4.1 Satiety and palatability in short-term food intake regulation.....	38
2.4.2 Satiety and its association with carbohydrates in food	40
2.5 Sensory evaluation of the liking of rice cooked in different ways	42
2.5.1 Sensory attributes evaluation of cooked rice products.....	42
2.5.2 Diverse rice flavour preferences	43
2.5.3 Consumer liking and acceptability of cooked rice products	44
2.5.4 Sensory evaluation of rice in New Zealand	44
2.6 Aim and study design	45
Chapter 3 : Freshly cooked rice: Starch and moisture profile.....	48

Abstract	48
3.1 Introduction	49
3.2 Method.....	52
3.2.1 Selection of rice products.....	52
3.2.2 Determination of total starch in rice as purchased	54
3.2.3 Sample preparation.....	55
3.2.4 Phase one.....	55
3.2.5 Phase two.....	56
3.2.6 Determination of moisture content (%) of uncooked rice.....	56
3.3 Determination of starch profile of freshly cooked rice.....	58
3.3.1 Sample preparation.....	58
3.3.2 Determination of moisture content (%) of cooked rice products	58
3.3.3 <i>In vitro</i> digestion process to measure glucose release over 180 minutes	58
3.3.4 Measuring glucose released during digestion	61
3.3.5 Determination of starch digestibility profile derived from glucose release over time.....	63
3.4 Statistical analysis.....	65
3.4.1 Comparison of glucose release curve.....	65
3.4.2 Comparison of starch digestibility profile.....	65
3.4.3 Determination of the differences	65
3.5 Results	65
3.5.1 Determination of total starch content (g/100 g rice)	65
3.5.2 Determination of moisture content by freeze-drying (%)	67
3.5.3 Determination of total starch content (g/100 g rice) after freeze-drying	67
3.5.4 Determination of relative cost (NZ dollar).....	67
3.5.5 Comparison of glucose release (g/100 g rice dry weight basis), net glucose release (g/100 g rice dry weight basis), time returning to glucose disposal baseline (min), and area under the curve (AUC) during <i>in vitro</i> digestion process.....	67
3.5.6 Comparison of starch digestibility profiles	70
3.6 Discussion.....	73
3.7 Conclusion	77
Chapter 4 : Effect of rice product varieties, cold storage, reheating and grain particle sizes on starch digestibility profile and <i>in vitro</i> glucose release	78
Abstract	78
4.1 Introduction	79
4.2 Method and materials	81
4.2.1 Selection of rice products.....	83

4.2.2	Determination of total starch and moisture content (%) in rice as purchased.....	83
4.2.3	Determination of starch profile of cooked rice treated in different ways	84
4.3	Results	89
4.3.1	Comparison of glucose release (g/100 g rice dry weight basis) during <i>in vitro</i> digestion process after various cold storage periods	89
4.3.2	Comparison of starch digestibility profiles among five rice products after various cold storage periods.....	92
4.3.3	Comparison of glucose release (g/100 g rice dry weight basis) during <i>in vitro</i> digestion process after various combined treatments (rice product varieties, cold storage, reheating and rice grain particle size interruption).....	98
4.3.4	Comparison of rice starch digestibility profile after various combined treatments (rice product varieties, cold storage, reheating and rice grain particle size interruption)	100
4.4	Discussion.....	106
4.5	Conclusion	109
Chapter 5 : Effect of rice cooking method on postprandial glycaemic response, satiety and palatability, and chewed particle distribution		110
	Abstract	110
5.1	Introduction	111
5.2	Method and material	113
5.2.1	Study design	113
5.2.2	Ethics.....	113
5.2.3	Participants	116
5.2.4	Rice cooking and cold storage	117
5.2.5	Data collection.....	119
5.2.6	Statistics analysis.....	121
5.3	Results	122
5.4	Discussion.....	142
5.5	Conclusion	146
Chapter 6 : Which rice and why? Consumer preference and acceptability towards freshly cooked medium-grain white rice, freshly cooked parboiled rice and reheated parboiled rice.....		148
	Abstract	148
6.1	Introduction	148
6.2	Method.....	150
6.2.1	Experiment methodology	150
6.2.2	Study design	151
6.2.3	Ethics.....	151
6.2.4	Participants characteristics	151

Inclusion criteria	152
6.2.5 Rice sample preparation	154
6.2.6 Sensory evaluation: Affective testing.....	155
6.2.7 Consumer data analysis	156
6.3 Results	156
6.3.1 Participants characteristics and rice consumption frequency.....	156
6.3.2 Participants' liking of six cooked rice samples	159
6.3.3 Participants' future consumption intent.....	163
6.4 Discussion.....	163
6.5 Conclusion.....	166
Chapter 7 : Overall discussion and conclusion.....	167
7.1 Reheated parboiled rice has a higher proportion of slowly digestible starch and resistant starch and slower <i>in vitro</i> and <i>in vivo</i> glucose release.....	173
7.2 Reheated parboiled rice has a requirement for a longer chewing time resulting in a more uniform breakdown of rice into intermediate-sized particles.....	174
7.3 Reheated parboiled rice has longer and more prominent satiety responses, including feelings of fullness.....	175
7.4 Reheated parboiled rice has increased liking and acceptance from rice consumers	175
7.5 Cold-storage and food safety of cooked rice	179
7.6 Overall conclusion.....	180
Glossary.....	181
References	182
Appendices	211
Appendix 1: Reagent list of Total Starch Assay Kit (AA/AMG).....	211
Appendix 2: Ethical Approval Forms for Study “Effect of Rice Cooling Method on Glycaemic Response, Satiety and Chewing Test”	212
Appendix 3: Advertisement and Information Sheet for Study “Effect of Rice Cooking Method on Glycaemic Response, Satiety, and Chewing Tests”	216
Appendix 4: Study Participants Eligibility Questions for Study “Effect of Rice Cooking Method on Glycaemic Response, Satiety, and Chewing Tests”	219
Appendix 5: Satiety and Palatability Questionnaires for Study “Effect of Rice Cooking Method on Glycaemic Response, Satiety, and Chewing Tests”	222
Appendix 6 Ethical Approval Forms for Study “Which rice and why? Consumer preference and acceptability towards freshly cooked medium grain white rice, freshly cooked parboiled rice, and reheated parboiled rice”	224
Appendix 7: Advertisement and Information Sheet for Study “Which rice and why?”	227
Appendix 8: Study Participants Screening Questions for Study “Which Rice and Why?”	229

List of Figures

Figure 2-1 The possible pathogenic process of type 2 diabetes mellitus (T2DM) development Adapted from the context of “Pathophysiology and treatment of type 2 diabetes perspectives on the past, present, and future” (Kahn, Cooper, & Del Prato, 2014).	11
Figure 2-2 The rate of digestion of starch to glucose. Figure was adapted from Jenkins et al. (1987).	19
Figure 2-3 Model of the relationship between staple rice replacement and fasting blood glucose concentration after around 26 weeks.	27
Figure 2-4 Structure of rice grain. Adapted from “The rice grain and its gross composition.” by Juliano & Bechtel (1985b).	30
Figure 2-5 The association between rice starch digestibility profile and the glucose response. Modelled based on the concept from “Classification and measurement of nutritionally important starch fractions” by (Englyst, Kingman, & Cummings, 1992).	34
Figure 2-6 Relationship between appetite hormone regulation and energy balance	39
Figure 2-7 Study design and structure of the thesis	46
Figure 3-1 Flow chart of the experiment design and analytical steps to determine the starch digestibility profile of five rice products.	53
Figure 3-2 Protocol for <i>in vitro</i> release of glucose over 180 minutes.	60
Figure 3-3 Estimated glucose release curve of one rice sample.	63
Figure 3-4 Estimation of the starch digestibility profile (rapidly digestible starch, slowly digestible starch and resistant starch) was derived from the glucose release (g per 100 g of cooked rice dry weight basis) over time.	64
Figure 3-5 Average glucose release as a percentage of available starch within 180 minutes (at 0, 20, 40, 60, 120 and 180 minutes) of <i>in vitro</i> digestion process (oral, gastric and small intestinal digestion process) of the five freshly cooked rice products. Interpolation between measured points was plotted (N=14 for each rice sample) using the Excel function. Error bars show the SD of the mean measured value.	69
Figure 3-6 Comparison of starch digestibility profiles among five freshly cooked rice products ((a) TAS total available starch, (b) RDS rapidly digestible starch, (c) SDS slowly digestible starch and (d) RS resistant starch).	72
Figure 4-1 Flow chart of the experiment design.	82
Figure 4-2 Average glucose release (g) as a percentage of rice (g dry weight basis, n = 14) within 180 minutes (at 0, 20, 40, 60, 120 and 180 minutes) of <i>in vitro</i> digestion process of the five cooked rice products after cold storage at 4 °C (at 0, 4, 8, 10, 12, 24 hours). (Error bars are the 95% confidence intervals of the means.)	91
Figure 4-3 Total available starch (TAS) (%) of five cooked rice (n = 14) 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.	93

Figure 4-4 Rapidly digestible starch (RDS) (%) of five cooked rice (n = 14) 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.....	94
Figure 4-5 Slowly digestible starch (SDS) (%)of five cooked rice (n = 14) 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.....	96
Figure 4-6 Resistant starch (RS) (%)of five cooked rice (n = 14) 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.	97
Figure 4-7 Average glucose release as a percentage of total starch within 180 minutes (at 0, 20, 40, 60, 120 and 180 minutes) of <i>in vitro</i> digestion process of the five cooked rice products (g dry weight basis, n = 14) after Group 2 mixed treatment (Table 4-2).	99
Figure 4-8 Average total available starch (TAS) (%) expressed as a percentage of total starch of five cooked rice products (n=14) after Group 2 mixed treatments shown in Table 4-2. Error bars are presented as 95% confidence intervals of the means.....	102
Figure 4-9 Average rapidly digestible starch (RDS) (%) expressed as a percentage of total starch of five cooked rice products (n=14) after Group 2 mixed treatments shown in Table 4-2. Error bars are presented as 95% confidence intervals of the means.....	103
Figure 4-10 Average slowly digestible starch (SDS) (%)expressed as a percentage of total starch of five cooked rice products (n=14) after Group 2 mixed treatments shown in Table 4-2. Error bars are presented as 95% confidence intervals of the means.....	104
Figure 4-11 Average resistant starch (RS) (%) expressed as a percentage of total starch of five cooked rice products (n=14) after Group 2 mixed treatments shown in Table 4-2. Error bars are presented as 95% confidence intervals of the means.....	105
Figure 5-1 Flow chart of the experiment design for human experiments (glycaemic responses, chewed particle distribution, and satiety and palatability study).....	115
Figure 5-2 Calculation of incremental area under the blood glucose response curve (iAUC) (mmol/L) over the baseline.	122
Figure 5-3 Finger prick blood glucose response (means ± standard error) in 28 healthy participants to three different rice meals (140 g): freshly cooked medium-grain white rice (control), freshly cooked parboiled rice, and reheated parboiled rice. (Test of homogeneity of variances had P-value = 0.001 at 15 minutes and 0.014 at 90 minutes.).....	127
Figure 5-4 Mean satiety scores (VAS, visualised analogue scale scores) change over 120 minutes for freshly cooked medium-grain white rice, freshly cooked parboiled rice and reheated parboiled rice, and each satiety VAS question. Error bars show the standard error of the mean VAS score.	137

Figure 5-5 Mean palatability scores (VAS, visualised analogue scales scores) immediately after finishing eating prepared control and test rice samples (freshly cooked medium-grain white rice, freshly cooked parboiled rice and reheated parboiled rice) for each palatability VAS question.	141
Figure 6-1 Flow chart of the experiment design for sensory evaluation (liking preference).....	153
Figure 6-2 Diagrammatic presentation of liking for sensory attributes (colour, texture, flavour, sweetness and overall) of six rice samples by 64 participants. Sensory attribute with $P < 0.05$ indicates significant difference among the six rice samples. Visualised analogue scales (VAS) scores were used (VAS score = measurement from extremely unlike on the left end to participant's marking (mm)/100 (mm); score 0 = least acceptable/dislike extremely; score 10 = highly acceptable/like extremely.).....	162
Figure 7-1 Venn diagram of the demonstration that reheated parboiled rice may be considered a plausible and healthier alternative for the public.	168
Figure 7-2 Influencing food choice: A multi-level framework of factors that influence eating behaviours. (Gidding et al., 2009; Glanz & Bishop, 2010; Kumanyika, 2008; Story et al., 2008; Swinburn et al., 2005).....	178

List of Tables

Table 2-1 Criteria for increased risk for diabetes (i.e., prediabetes) and for diagnosis of diabetes mellitus (T2DM).....	22
Table 2-2 Comparative nutrient composition of rice grains of different varieties	31
Table 3-1 Sample used in <i>in vitro</i> starch digestibility study all purchased from PAK'nSAVE (Albany, Auckland, New Zealand) on 20 May 2012.....	54
Table 3-2 Calculation of total starch concentration (g/100 g rice dry weight basis) in rice as purchased.	57
Table 3-3 Calculation of glucose release (g/100 g rice dry weight basis).	62
Table 3-4 Total starch content, moisture content and cost for the five rice products (mean and 95% Confidence Interval). A total of 14 experiments repeated on the same rice product (N=14).	66
Table 4-1 Sample used in <i>in vitro</i> starch digestibility study.....	83
Table 4-2 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).	84
Table 5-1 Sample details and preparation methods based on the results from glycaemic responses study.	114
Table 5-2 Characteristics of participants in glycaemic response human experimental trail (N = 28).....	124
Table 5-3 Means of blood glucose responses (mmol/L) at baseline and incremental blood glucose response (mmol/L) at each time point after consuming three rice samples and the incremental area under the glucose responses curve (iAUC) (mmol/L * min). The means in the same column with the same letter are significantly different (P < 0.05).	126
Table 5-4 Before-swallowing rice particle size distribution (%), chewing time (sec), and area under the two-hour glucose response curve. (N = 28) Values are mean and standard deviation, unless otherwise stated.	129
Table 5-5 Correlations between particle size distributions (%) (as dependent variable) and chewing time (seconds) (as explanatory variable) (N = 28) by multiple linear regression analysis.	131
Table 5-6 Area under the curve (AUC) of satiety (mean, SD) over 120 minutes for freshly cooked medium-grain white rice, freshly cooked parboiled rice and reheated parboiled rice, and each visualised analogue scale question.	138
Table 5-7 Association between satiety and palatability responses (visualised analogue scales (VAS) scores) (as dependent variable) and blood glucose concentration (mmol/L) (as explanatory variable) (N = 28) by linear regression analysis.	139
Table 6-1 Preparation methods for three rice samples.	154
Table 6-2 Characteristics of participants (N = 64) participated in this study.	158
Table 6-3 Participants liking score (N = 64) of colour, texture, flavour and sweetness and overall liking score of each cooked plain rice sample.....	161

Table 6-4 Frequency of participants' intention to eat the rice sample as regular staple meal.....	163
Table 7-1 Summary of physical and physiological factors of reheated parboiled rice that may contribute to lower glycaemic responses.	168
Table 7-2 Selected studies relevant to investigation of starch digestibility profiles and <i>in vitro</i> glucose release, postprandial glycaemic responses, satiety and palatability, chewing (chewed particle size distribution and chewing length), and sensory evaluation (i.e., consumer liking preference) of rice products.	169

Attestation of Authorship

I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which to a substantial extent has been accepted for the qualification of any other degree or diploma of a University or other institution of higher learning, except where due acknowledgement is made in the acknowledgements.

Acknowledgements

First and foremost I want to thank my primary supervisor Professor Elaine Rush, who has been a tremendous mentor to me. I would like to thank her for encouraging my proactive thinking and for allowing me to develop to an independent research scientist. Her advice on both research strategy as well as future prospect of nutrition and health promotion have been priceless. Most importantly, I appreciate all her contributions to time, ideas, and funding to make my Ph.D. journey productive and exciting. Her enthusiasm for her research was so contagious and inspiring for me, even during the most difficult times in my pursuit of Ph.D. She has given an excellent example as a successful woman professor and scholar.

I would also like to thank my supervisor/advisor Dr. John Monro and Dr. Suman Mishra, who have contributed immensely to my in vitro experiments at Food & Plant Research in Palmerston North. I would like to thank Dr. Bernard Venn and his GI lab team, who have helped me through each step in my in vivo experiments. I would like to show my gratitude to Assoc Prof. Jun Lu, who supported my thesis journey, and Michael Rush for contributing so much time on critiquing my method section to ensure that it was flawless. I am especially grateful to the fun group of BCMRC team for the professional and moral support, particularly during the writing retreats, of which each was a valuable opportunity that pushed me to achieve a higher level of writing and presentation skills.

Last but not least, I would like to show my gratitude to my family and friends. Words cannot express how grateful I am to my mother for all of the sacrifices that you have made on my behalf. Your love for me has what sustained me thus far. I would also like to thank all my friends who supported me in writing, and incentivised me to strive towards my goal. In the end, I would like to express appreciation to my beloved husband who I spent countless sleepless nights with and was always my support in the moments when there was no one to answer my queries.

Ethics Approval

This body of work included two research studies involving human participants and collecting human blood. The ethics approvals for both studies were obtained.

1. “Effect of rice cooling method on glycaemic response, satiety and chewing testes” was approved by the AUT Human Ethics Committee on 8 February 2012 (Reference number: 13/05, Appendix 2)
2. “Effect of rice cooling method on glycaemic response, satiety and chewing testes” was approved by the University of Otago Human Ethics Committee (Reference number: 4695068, Appendix 2)
3. “Which rice and why?” was approved by the AUT Human Ethics Committee on 8 August 2013 (Reference number: 13/183, Appendix 6)

Chapter 1 Introduction

Rice has played a predominant role in the human diet for more than 9,000 years. It is the main staple food for over half of the world's population, particularly for people living in the 15 countries in the Asia and Pacific area (Food and Agricultural Policy Research Institute (FAPRI), 2004). A cooked rice meal provides 27% of the average daily energy intake of the world's population and, by volume, contributes 60% of the daily food quantity in Southeast Asia and 35% in East Asia and South Asia (Wailes & Chavez, 2012). In East and South Asian populations, cooked rice is also a major source of nutrients, including 20% of dietary protein, 3% of dietary fat and a significant amount of micronutrients, including thiamine, riboflavin, niacin and zinc (Fresco, 2005; Kennedy, Burlingame, & Nguyen, 2004). Over 65 kg per capita per year are consumed in many countries, including China, India, Korea, Japan, Malaysia and Indonesia.

From 1996 to 2003, the global production of rice increased by 1.0% each year while the global per capita consumption stayed unchanged (Wailes & Chavez, 2012), suggesting that the increase has been driven solely by world population growth. From 1996 to 2003, in European Union countries, total rice consumption increased slowly but steadily, by 14%, from 4.2 to 4.8 million tonnes (Fresco, 2005), while the annual per capita rice consumption for Europeans increased from 5.0 to 6.0 kg per capita (Wailes & Chavez, 2012). In Australia, the per capita rice consumption increased from 17 kg to 19 kg per year (Wailes & Chavez, 2012). In New Zealand, from 1997 to 2014, the total domestic rice consumption rose by 6.7% (from 27 to 45 tonnes), while the annual per capita rice consumption increased from 7.1 kg to 10.0 kg per year (United States Department of Agriculture, 2014). It has also been suggested that growth is supported by increased consumption of rice in countries where rice is not a major food crop. Demographic factors, including socio-economic transition, immigration, lifestyle changes, changing ethnic composition to include immigrants with high per capita rice consumption, and cultural influences, often result in changes in the dietary profile of a community or population

In New Zealand, the numbers of East and South Asian populations are projected to increase from 0.54 million in 2013 (Statistics New Zealand, 2014a) to between 0.81 to 0.92 million in 2025, and to between 1.06 and 1.26 million in 2038 (Statistics New Zealand, 2015), which may place increased demands for importation of rice into New Zealand.

In New Zealand and globally refined or white rice continues to constitute nearly 80% of rice grains consumed globally (Wailes & Chavez, 2012). Rice is refined by passing whole grain rice through a series of mechanised processes, including hulling and milling and the bran layer removed. Without the bran layer refined or white rice has better storage properties than unrefined/whole grain rice but is less nutritious as it is primarily starch (80%) and the majority of the oil and the micronutrients of the whole grain are in the bran: 80% of vitamin B1, 67% of vitamin B3, 90% of vitamin B6, 50% of phosphorus, 50% of iron, and all the dietary fibres and essential fatty acids (Babu, Subhasree, Bhakayaraj, & Vidhyalakshmi, 2009; Heinemann et al., 2005). As rice is primarily starch it is digested to glucose and therefore has a post-prandial effect on blood glucose.

The effect of a food on blood glucose can be estimated by a measure called glycaemic index (GI). Introduced in 1981 GI, measured in at least 10 healthy people, is a comparison of the rate of digestion and absorption of a standardised dose of carbohydrate in a specific food (e.g., rice) compared with the rate of digestion and absorption of the same amount of glucose on another occasion (Jenkins et al., 1981). Glycaemic load (GL) was introduced to quantify the cumulative “glucose-response-increasing potential of carbohydrate-containing foods” (Englyst, Liu, & Englyst, 2007) consumed over time. It is indirectly calculated as the grams of available carbohydrates in each food consumed multiplied by the glycaemic index (GI) of that carbohydrate food and summed for the period of time considered. Both GI and GL are current guiding tools to provide instruction on a healthy diet in order to improve blood glucose control and insulin sensitivity. A diet of low GI and GL is recommended from a nutritional point of view (Aston et al., 2010; Esfahani et al., 2009; Grant, Wolever, O'Connor, Nisenbaum, & Josse, 2011; Laville & Nazare, 2009).

The GI values of cooked refined rice or white rice are higher on average (64, SD = 7) than those of whole grain rice such as brown rice (55, SD = 5) (Atkinson, Foster-Powell, & Brand-Miller, 2008). This poses the possibility that cooked refined rice or white rice is a growing and major contributor to dietary GL for populations that consume rice as a staple food. It is known that the consumption of freshly cooked white rice induces a sharp and prolonged rise in blood glucose concentration in apparently healthy people with normal glucose homeostasis (Barclay et al., 2008; Chen, Sun, Wong, & Huang, 2010).

It is also known that the digestion and absorption potency (i.e., GI) of rice product varieties depends on the composition of the properties of the starch, that is, the profile or proportions of rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS) in its kernel (Chung, Shin, & Lim, 2008; Lehmann & Robin, 2007; Zhang & Hamaker, 2009). The starch profile of rice can be changed by the amylose-to-amylopectin ratio of the rice product, the polishing and milling process, parboiling and the cooking method and storage time which impact on the amount of retrogradation. In addition, compared with refined white rice, whole grain rice, which has a higher content of dietary fibre, has been reported to delay the digestion and absorption process, thus reducing glycaemic potency (Babu et al., 2009). It has also been reported that parboiled long grain rice also has a lower GI and is useful in glycaemic management (Heinemann et al., 2005). Parboiling treatment is applied to the whole grain before milling in the same way as white rice to remove the outer bran. Parboiling treatment preserves around 80% of the micronutrients found in brown rice.

Genetically, cultivars of long-grain rice and basmati rice have higher amylose to amylopectin ratio (around 22% amylose vs. around 78% amylopectin), while medium and short grain rice have lower amylose to amylopectin ratios (around 15% amylose vs. around 85% amylopectin) (Vandeputte, Vermeulen, Geeroms, & Delcour, 2003a, 2003b). Long-grain white rice also has a lower GI and has been shown a protective effect on glycaemic management (Heinemann et al., 2005; Sajilata, Singhal, & Kulkarni, 2006b). Studies have also suggested that cooling cooked starch induces starch retrogradation, which in turn reduces the proportion of RDS and increases the proportions of SDS and RS (Chung, Lim, & Lim, 2006; Hu, Zhao, Duan, Linlin, & Wu, 2004; Landon, 2007; Perdon, Siebenmorgen, Buescher, & Gbur, 1999). The chemistry provides a rationale but the question is: would rice prepared in a different way to lower the GI be acceptable to the population and be consumed in sufficient quantities to reduce the GL of the diet?

The next chapter reviews the literature concerning what is known about the following:

- The ongoing challenge of a high GI diet in a multi-ethnic population in Auckland, New Zealand
- The association between a long-term high GI diet and the risk for type 2 diabetes mellitus in New Zealand
- The relation of rice to a high GI diet among people living in Auckland

- How changes in the rice starch digestibility (by different rice preparation methods) may affect the change of *in vitro* starch digestibility and postprandial glycaemic responses, satiety and palatability
- The sensory evaluation of cooked rice with different rice preparation methods

Chapter 2: Literature review: How to lower the gap between current and future knowledge for rice choice? A modelling prediction

From 1980, the rapidly increasing prevalence of non-communicable chronic diseases, including type 2 diabetes mellitus (T2DM), obesity and cardiovascular disease, has reduced the quality and possible length of life for a large proportion of the world's population (Dixon, 2015). Adverse combinations of foods, including staple foods that induce glycaemia and have low nutrient density, are major contributors along with a high energy intake and low physical activity to these health challenges (Ley et al., 2014). The nutrition and lifestyle approaches in recent diabetes prevention strategies in Finland and the United States have been proven to be as effective as drug therapies in delaying the onset of the disease and related complications (Barclay et al., 2008; Burton, Monro, Alvarez, & Gallagher, 2011; Moran, 2004). Adoption of low glycaemic index (GI) meals has underpinned nutritional recommendations for two decades (Augustin et al., 2015). A meta-analysis of prospective cohort studies suggested that a low GI diet is positively associated with lower risk of type 2 diabetes, independent of the amount of cereal fibre in the diet; (Chen, Magliano, & Zimmet, 2012). In public health the GI can help inform dietary recommendations for the management and prevention of obesity and type 2 diabetes. For clinicians it may be used to advise modifications in an individual's dietary pattern.

Rice has been generally perceived as a high GI food (Brand-Miller, Holt, Pawlak, & McMillan, 2002; Brand-Miller, McMillan-Price, Steinbeck, & Caterson, 2009), and several studies have linked the regular high intake of high GI rice to increased risk of developing hyperglycaemia and T2DM (Chen et al., 2010; Fitzgerald et al., 2011; E. Hu, Pan, Malik, & Sun, 2012; Nanri et al., 2010; Tamura et al., 2005; Villegas et al., 2007), particularly among sedentary people. The current globalisation of New Zealand, particularly of Auckland, has seen the consumption of rice increase and become a regular staple grain. This includes an increased variety of cooked rice products in the supermarket and increased popularity of many different Asian takeaways that include cooked rice. Rising immigration, particularly of East and South Asians, has brought a new dietary culture (Parackal, Smith, & Parnell, 2015).

Previous studies have suggested that product varieties of rice and rice products that have a GI at least 10 units below that of medium-grain white rice (Ranawana, Henry,

Lightowler, & Wang, 2009; Tamura et al., 2005) could be part of the nutritional management of T2DM and help prevent the onset of the condition. Furthermore, the cold storage of cooked rice products and other starch meals has been proven to increase starch retrogradation and reduce overall GI, and cold storage and reheating may be a healthier alternative to freshly cooked rice meals.

This literature review will present the evidence base for the need to investigate the associations and the possibilities for practicable and feasible intervention. The following aspects are covered:

- The current glycaemic load (GL) of the diet and nutrition-associated health challenges, including non-communicable diseases, among diverse populations in Auckland, New Zealand
- Genetic and environmental factors associated with the development of obesity, type 2 diabetes and gestational diabetes with a focus on carbohydrate quality.
- The possible solutions (optimal rice choice and cooking and preparation methods) to improve the glycaemic profile of rice meals (i.e., improving postprandial glycaemic management), satiety and sensory responses in order to translate the bench research to the real world
- The starch digestibility profile and glycaemic potency of widely available and consumed rice products, and their effect on long-term glycaemic management
- The sensory evaluation of the liking and acceptance of rice cooked in different ways

Relevant literature for review was obtained initially via computer online search on Medline, Embase and the Auckland University of Technology (AUT) library online resource. Database systems were assessed using the following key words, their truncated forms and American spelling: glycaemic responses, blood glucose, glycaemic index (GI), glycaemic load (GL), type 2 diabetes, non-insulin dependent diabetes mellitus, rice starch digestibility, resistant starch, rapidly digested starch, slowly digested starch, total available starch, whole grain rice, parboiled rice, rice nutrient, satiety, palatability, liking preference, sensory evaluation and consumer acceptability. The literature search was designed to maximise retrieval of all relevant studies by using several combinations of key words. Further relevant studies were obtained from reference lists of the publications retrieved.

2.1 High glycaemic load diet: An ongoing health challenge in a multi-ethnic population in Auckland, New Zealand

Diet quality has long been acknowledged to be one of the key contributors to the risk of a wide range of non-communicable diseases (World Health Organization, 2003). One measure of diet quality that researchers have reached consensus (Augustin et al., 2015) based on evidence accumulated over the last 20 years is that the glycaemic load of the diet is positively associated with the development of chronic diseases, such as type 2 diabetes. Some of the more recent randomised controlled trials that have shown that low GI and low GL diets improved glycaemic control among people with diabetes and reduced risk of cardiovascular diseases include (Ajala, English, & Pinkney, 2013; Jenkins, Kendall, & Augustin, 2012; Reynolds, Tekinkaya, & Venn, 2014; Zhang, Kane, Liu, & Venn, 2015).

Meta-analysis and systematic reviews of observational studies have also found the positive relationship between high GI and high GL diets and increased risk of obesity, diabetes, and cardiovascular diseases (Barclay et al., 2008; Livesey et al., 2013; Schwingshackl & Hoffmann, 2013).

Refined carbohydrate food, such as white rice, has replaced whole-food as a staple in Asian diets (Alhazmi, Stojanovski, McEvoy, & Garg, 2014). At the same time, there has been an increase in the prevalence of non-communicable diseases especially among Asian populations at younger ages. This has occurred in New Zealand (Parackal et al., 2015; Scragg & Northern District Health Board, 2010; Wong, 2015). There is a need to understand better the association between starch digestibility and long-term blood glucose control to better inform dietary recommendations.

2.1.1 Asian populations in New Zealand

The definition of the term “Asians” in New Zealand contains a wide range of ethnic groups. This may encompass anyone originating from the Asian continent, which includes people with origins from the Middle East to Japan in the east and Indonesia and India in the south (Wong, 2015). For the past two decades, the diversity of New Zealand’s population has increased and the Asian population in New Zealand has progressed from being a small group to emerging as an important demographic group of New Zealand society. Eleven percent of the New Zealand population is Asian and 23% were born in New Zealand, which leaves around 66% of migrants who were born in Asia and 11% in other areas (Statistics New Zealand, 2014b). Currently the Asian population in New Zealand is approximately one-third Chinese, one-third South Asian

and one-third “other Asian”. Although Asian ethnic groups, such as the two major Asian communities, Chinese and Indians, have been settling in New Zealand since the late 19th century, health and nutrition information and statistics on the status of these ethnic groups have not received the same attention as Māori and Pacific (Rasanathan, Ameratunga, & Tse, 2006; Scragg & Maitra, 2005; Scragg & Northern District Health Board, 2010).

2.1.2 Increasing prevalence of type 2 diabetes and metabolic syndrome among Asians in New Zealand

T2DM has been described as “a global epidemic”, and in 2010, an estimated 6.4% of adults aged between 20 and 79 years were known to have the disease (Shaw, Sicree, & Zimmet, 2010). In developed countries, it is estimated that the prevalence will increase by 20% by 2030, which is nearly twice the annual rate of growth of the total adult population (Shaw et al., 2010). In New Zealand, the number of people with diabetes almost doubled in the 10 years from 2001 to 2011, from about 110,000 to 243,125 (Diabetes New Zealand, 2010, 2013b). The average increase in prevalence is approximately 8–9% per annum over the past decade, with the highest growth in Auckland (about 14%) (Diabetes New Zealand, 2013a). The Diabetes Policy Model, which states that approximately 500,000 New Zealanders will be affected by diabetes by 2036, has confirmed that diabetes is a top health priority (Diabetes New Zealand, 2013b). In conjunction with the epidemic of type 2 diabetes, there is an increase in women with gestational diabetes mellitus (GDM), particularly among Chinese and Indian populations. At National Women’s Hospital, Auckland, in 2009 the prevalence of GDM by ethnicity was Chinese 10%, Indian 17% and European 3% respectively (Auckland District Health Board, 2010). These mothers and their offspring are at high risk of T2DM in their later life. One of the main forms of treatment for GDM is dietary management, which includes reduced intake of “white foods”: foods that contain a high concentration of easily digestible carbohydrates (D. Simmons, Rowan, Reid, & Campbell, 2008).

While the prevalence of diabetes and obesity and metabolic syndrome (a major risk factor for cardiovascular disease and diabetes) is rising rapidly in East (including China and Korea) and South Asian countries, the risk of having the metabolic syndrome and diabetes appears to further increase after migration from Asia. Insulin resistance level is positively associated with the length of stay by Taiwanese women in Australia (Lee, Lingard, & Bermingham, 2007). Similarly, the mortality related to metabolic syndrome is 40–50% higher among South Asians in the United Kingdom compared with the rest

of the population (Dodani, 2009; Lip et al., 2007). Both morbidity and mortality rates relating to metabolic syndrome are increasing in South Asians living in North America compared with other ethnic groups, whose rates are declining (Bainey & Jugdutt, 2009; Ramaraj & Chellappa, 2008).

A similar pattern exists in New Zealand. Based on the most recent report (Ministry of Health, 2014), the best estimation of the prevalence of obesity and diabetes in New Zealand, in 2011–2012 (approximately 1 million adults):

- 28% of adults were obese, compared with 26% in 2006–2007.
- 4% of Chinese (a twofold increase since 2006–2007), 12% of South Asians (a fourfold increase since 2006–2007) and 5% of other Asians (a twofold increase since 2006–2007) were on medication for diabetes, compared with 4% of Europeans.
- 5% of adults (almost 200,000 adults) had been diagnosed with diabetes, compared with 2% in 2006–2007.

There is a pattern of increased prevalence of self-reported diagnosed diabetes in Asian people compared with Europeans in Auckland, particularly in South Asian and Other Asians (Scragg & Northern District Health Board, 2015). Three factors among many that contribute to the development of insulin resistance maybe high energy high carbohydrate and high GI diets, reduced time spent in physical activity, and a genetic susceptibility (Metcalf et al., 2008; Wahlqvist, 2002; Yang, Chung, Kim, Bianchi, & Song, 2007). The report also indicated that the Chinese ethnic group is likely to experience elevated prevalence of diabetes in the future if the dietary and lifestyle patterns continue with increasing acculturation. It is also suggested that because Asian people are not accessing the health services to the same degree as non-Asians in New Zealand, it is likely that the prevalence of diabetes and metabolic syndrome is underreported.

With the increasing Asian population in Auckland, New Zealand, their unique health issues and needs have increased (Scragg & Northern District Health Board, 2015). The health issues identified include nutrition-related risk factors of a high proportion of calories from carbohydrates and a diet of high GI foods that may be associated with obesity, diabetes, heart diseases and other major chronic health problems (Parackal et al., 2015; Scragg & Northern District Health Board, 2010, 2015) which place a social

and economic burden on individuals and societies (Bainey & Jugdutt, 2009; Dodani, 2009; Lip et al., 2007; Ramaraj & Chellappa, 2008).

2.1.3 The pathogenesis of type 2 diabetes mellitus

The complexity of the specific aetiologies of T2DM are not yet well understood however, the consensus from the evidence is that the development of type 2 diabetes is associated with both genetic (Tuomi et al., 2014) and environmental factors (Zimmet, Magliano, Herman, & Shaw, 2014). The hyperglycaemia in T2DM is associated with impaired beta-cell function and diminished tissue sensitivity to insulin (Ali, Ferris, Naran, & Crowther, 2011; American Diabetes Association, 2014; Williams & Pickup, 1997; Yoon, Pickup, & Williams, 1997). An imbalance in nutrition and physical activity drives the deposition of excess body fat which precedes the development of type 2 diabetes (Alhazmi et al., 2014). (Figure 2-1, adapted from Kahn, Cooper, and Del Prato (2014))

The development of type 2 diabetes mellitus may be slow (Blundell et al., 2015; Kahn et al., 2014; Ley et al., 2014). Because of this, many of the classic symptoms are not detected in individuals in the early stages (American Diabetes Association, 2014; Williams & Pickup, 1997; Yoon et al., 1997). The factors are outlined below (Figure 2-1).

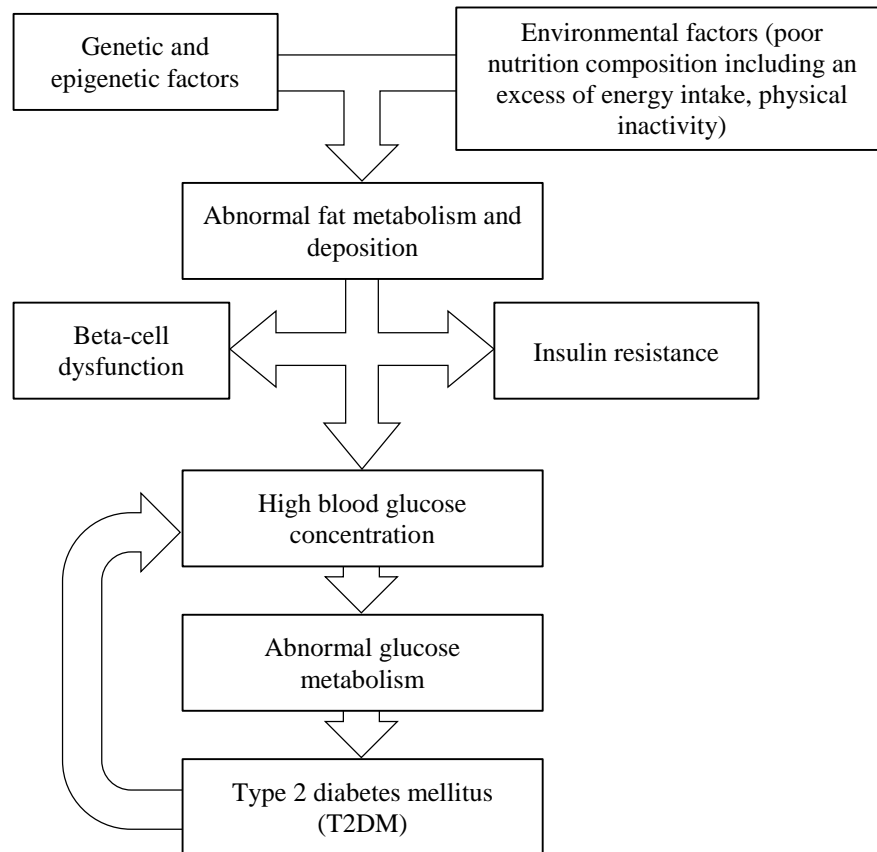


Figure 2-1 The possible pathogenic process of type 2 diabetes mellitus (T2DM) development Adapted from the context of “Pathophysiology and treatment of type 2 diabetes perspectives on the past, present, and future” (Kahn et al., 2014).

Genetic factors

Genome-wide association studies (GWAS) and meta-analyses (Basile, Johnson, Xia, & Grant, 2014; Schierding & O’Sullivan, 2015) have identified common variants that are associated with higher risk for type 2 diabetes mellitus (T2DM). In 2014 Basile, Johnson, Xia and Grant (2014) reviewed gene analysis and family-based linkage studies that identified loci-harboring genes associated with T2DM. The genes identified included CDK5 regulatory subunit associated protein 1-like 1 (CDKAL1), solute carrier family 30 (zinc transporter), solute carrier family 30, member 8 (SLC30A8), homeobox hematopoietically expressed (HHEX), insulin- like growth factor 2 mRNA-binding protein 2 (IGF2BP2), and cyclin-dependent kinase inhibitor 2A/2B (CDKN2A/2B). They also suggested that because some ethnic populations have a higher prevalence of T2DM they may be genetically predisposed to T2DM. The strongest association with T2DM and obesity in ethnic groups such as South and East Asian occurs in the wnt-signaling pathway member: transcription factor 7-like 2 (TCF7L2 as confirmed in two large-scale GWAS in Asian populations (27,715 individuals) (Basile, Johnson, Xia, & Grant, 2014). The expression of these genes is dependent on interactions with the

environment and it could be that there is potential to reduce risk, particularly for Asian, by modifying the dietary and physical activity environment and reducing other risk factors such as poor sleep, stress and smoking. Aging and a genetic predisposition are not modifiable.

Environmental factor: Obesity

Many longitudinal and cross-sectional studies have shown that obesity, especially truncal obesity, has a strong association with the development of type 2 diabetes mellitus (Ali et al., 2011; Boden & Shulman, 2002; Brand-Miller et al., 2002; Dowse et al., 1991). The body mass index (BMI), which is calculated by $\text{weight}/(\text{height})^2$, is the most common and standardised measurement used to measure level of obesity. The recommended healthy range is defined as a BMI of 20–25 kg/m², overweight as a BMI of 25–29.9 kg/m², obesity as a BMI of 30–40 kg/m², and morbid obesity as a BMI > 40 kg/m². Other parameters, including waist-to-height ratio and waist-to-hip ratio have been recently introduced to the screening measurements for risk.

A simple explanation of obesity in humans is that it is caused by greater energy intake than energy expenditure, especially in diets with a high glycaemic load and high fat content (Schwingshackl & Hoffmann, 2013, Brand-Miller, Pawlak, & McMillan, 2002), and it is also partly inherited and polygenetic (Basile, Johnson, Xia, & Grant, 2014). Moreover, emerging data also suggested beyond extragenetic (i.e. in the form of societal, cultural or familial habits) both genetic and epigenetics inheritance may be important contributors to the emergence of obesity and the associated complications (Bays & Scinta, 2015). The genomewide association studies (GWASs) have identified that the strongest genetic signal for weight change is located in the FTO locus, of which polymorphic differences in non-coding nucleotide sequences may alter the base function of adipocytes from energy storage to energy utilisation via enhanced thermogenesis (Rosen & Ingelfinger, 2015; Claussnitzer, 2015). Several studies suggested the monogenic diseases might also cause severe cases of obesity including leptin and leptin receptor deficiency SH2B1 mutations, and carboxypeptidase E mutations (Herrera, Keildson & Lindgren, 2011; Farooqi & O’Rahilly, 2014; Alsters et al., 2015). Some specific promoters could potentially contribute to epigenetic alteration to an obesogenic environment. The promoters may include exposure to obesogens, including toxins (e.g. brominated diphenyl ether 47, polycyclic aromatic hydrocarbons, tributyltin) and endocrine disrupting chemicals (such as cortisol and excessive oestrogens in the womb) (Bays & Scinta, 2015). Other promoters may include

nutritional abnormalities, lack of physical activity, medications, and infection at conception, during pregnancy, or even after pregnancy (e.g. post-partum breastfeeding and future pregnancy), and sperm epimutations (Bays & Scinta, 2015). The review by Van Dijk et al. (2015) provided epidemiological evidence supporting that maternal undernutrition during foetal development increased the risk of both obesity and T2DM of the offspring later in life.

Obesity, or excess body fat, has a multitude of effects including increasing the likelihood of insulin resistance. One possibility is that with obesity and physical inactivity, insulin resistance in skeletal muscle increases. Insulin resistance may be induced by non-esterified fatty acids (NEFA) liberated by lipolysis, acting through the Randle cycle (also called the “glucose-fatty acid” cycle) to reduce glucose utilisation (Weir & Bonner-Weir, 2004). Truncal obesity, in particular, is related to the efflux of a large amount of NEFA, which reach the liver directly through the portal system and impair glucose utilisation; nevertheless, hepatic gluconeogenesis and glucose production also increase with truncal obesity (Ali et al., 2011; Boden & Shulman, 2002; Brand-Miller et al., 2002; Dowse et al., 1991).

Insulin resistance stimulates insulin secretion, and high NEFA levels may also inhibit the extraction of insulin by the liver, thus aggravating high peripheral insulin concentrations (hyperinsulinaemia). Hyperinsulinaemia may be able to maintain normal blood glucose levels, but individuals who have a defective beta-cell function will develop an elevated blood glucose concentration, which leads to impaired glucose tolerance and, eventually, T2DM. Insulin resistance coupled with obesity increases the functional demand for insulin from beta-cells of the pancreas and the increased demand may accelerate beta-cell dysfunction (Cerf, 2013). In addition, high plasma insulin concentrations per se, generated in an attempt to overcome insulin resistance, may reduce insulin sensitivity by down-regulating insulin receptors, and perhaps induce secondary post-binding defects (Cerf, 2013).

Obesity is one of the major risk factors for type 2 diabetes mellitus. However, lean individuals can also develop T2DM. It has been suggested that ectopic fat accumulation in organs (such as liver, skeletal muscles, heart, kidneys, and pancreas) and visceral fat accumulation around the organs are associated with impaired β -cell function, and thus, insulin resistance, at different BMIs and different levels of total adiposity (Genser et al., 2016; Sattar & Gill, 2014). Recent genetic data also contributed evidence that supported the concept of lower BMI individuals with diminished subcutaneous capacity being

linked to a greater metabolic risk (Yaghootkar et al., 2014). The rapid increase in central obesity coupled with low muscle mass for a given modest BMI among Chinese (Ley et al., 2014; Ma, Lin, & Jia, 2014) compared to European may explain the high prevalence of diabetes in Chinese population despite the lower prevalence of obesity compared with European population. With the current rapid increasing Chinese migrants into New Zealand, this adverse effect from acculturation is likely to continue into the future, and impact on future generations and the prevalence of T2DM observed in Asians increase.

Environmental factor: Physical inactivity

Evidence that total low physical activity (low sum of activity time) is a risk factor for T2DM has been included in 55 cohort studies (Aune, Norat, Leitzmann, Tonstad, & Vatten, 2015) and 26 observational studies (Mansoubi, Pearson, Biddle, & Clemes, 2014), and 53 randomised controlled trials (diet and physical activity promotion programmes) have shown that combined diet and physical activity promotion programmes are effective at decreasing diabetes incidence (Balk et al., 2015). The mechanism by which increased physical activity reduces the risk of T2DM may be related to increased insulin sensitivity and glucose tolerance (Ali et al., 2011; Aune et al., 2015; Boden & Shulman, 2002), and prevention of accumulation of excess body fat (Balk et al., 2015; Boden, 2011; Mansoubi et al., 2014).

The studies have also shown that the responsiveness to regular physical activities varies from person to person, and physical activities with higher levels of intensity promote greater improvements in insulin sensitivity and glucose tolerance than physical activities with lower levels of intensity. The most popular recommendation among population health advice, is for physical activities of moderate intensity with regular activities on most days in a week (Balk et al., 2015; Mansoubi et al., 2014).

Environmental factors: Nutritional factors

Carbohydrate quality has been shown to be associated with the development of T2DM. Some evidence suggests that a low dietary fibre intake may relate to T2DM, but this remains controversial. Three factors related to fibre and glucose homeostasis are firstly that the presence of fibre in food may reduce the rate of digestion and therefore the GI and GL of the food. Secondly that fibre itself takes a long time to digest and will add to the glucose supply (8 kJ/g) at a later stage of digestion. Finally, fibre is complex and varies by source, solubility, fermentability and other factors such as fibre size or function so cannot be considered as a single entity. For instance, a “smoothie” would have a higher glycaemic impact than eating the whole fruit.

A high glycaemic diet and a diet with high fat (especially high saturated fat) have been reported to be the two most consistent risk factors associated with the development of T2DM (Alhazmi et al., 2014; Ley et al., 2014; Schwab et al., 2014). The association between diet quality and T2DM may be mediated by obesity (Ley et al., 2014; Ma et al., 2014).

A number of prospective cohort studies have investigated the association of GI and GL with risk for T2DM after adjusting for confounders (e.g. difference baseline characteristics of participants, different duration of the studies, dietary behaviours, and physical activities). Meta-analysis by Barclay et al. (2008) examined 37 prospective cohort studies and concluded that low-GI and low-GL diets are independently associated with a reduced risk of diabetes and heart disease and the protection is comparable with diets high in whole grains or dietary fibre. A later meta-analysis by Livesey, Taylor, Livesey, and Liu (2013) of 24 prospective cohort studies prior to August 2012 reported that GL was positively associated with relative risk of T2DM of 1.45 (95% CI: 1.31, 1.61) for a 100 g of glucose equivalent increase in GL ($P < 0.001$). Bhupathiraju et al. (2014) reported three prospective trials comprising 74,248 women from Nurses' Health Study (1984-2008), 90,411 women from the Nurses' Health Study II (1991-2009), and 40,498 men from the Health Professionals Follow-Up Study (1986-2008) (at baseline with no diabetes, cardiovascular disease, or cancer) and assessed their diets using a validated questionnaire and updated every four years. This meta-analysis of three cohorts found that highest quintile of energy-adjusted GI is associated with 33% higher risk (95%CI: 26%, 41%) of T2DM than those in low-GI quintile, the highest quintile of energy-adjusted GL had a 10% higher risk (95% CI: 2%, 18%) of T2DM, and diets high in GI or GL and low in cereal fibre was associated with around 50% higher risk of T2DM (Bhupathiraju et al., 2014). This epidemiological evidence all supported that higher dietary GI and GL are associated with increased risk of T2DM.

Furthermore, a number of randomized controlled trials have supported the benefit of low GI or low GL diets. The randomised trial by Chiasson et al. (2002) reduced the GI in diets by using acarbose to inhibit starch digestion to glucose and reduced the incidence of T2DM by 36% in high risk individuals. The later randomised trials have also shown significant reduction in HbA1c levels with lower GI or GL diets (Thomas & Elliott, 2010). Other recent clinical studies have also shown that low GI and low GL diets improved glycaemic control among people with diabetes and reduced risk of cardiovascular diseases (Grundy, 2012; Jenkins et al., 2008; Olokoba, Obateru, &

Olokoba, 2012; White et al., 2015). Trials by McMillan-Price et al. (2006) and systematic review on trials by Mirrahimi et al. (2013) reported high GL diets were associated with higher incidences of cardiovascular diseases.

Overall the association between GI, GL of diets and risk for non-communicable disease is strong but other considerations such as food security, social norms and the overall quality of the diet need to be considered in the prevention of NCD.

One measure of the nutritional quality of a diet is the intake of plant foods and dietary fibre such as wholegrain staple foods, legumes, nuts, fruits and vegetables and a variety of these foods is important in reducing the risk of non-communicable diseases. Dietary fibre is not a single entity but a mixture of different chemical structures forming a complex physical structure. Both insoluble dietary fibre such as rice bran (i.e. hemicellulose) and plant cell walls (i.e. cellulose) and soluble dietary fibre such as β glucan in oat bran and other fibres (including gums, pectin, mucilage and algal polysaccharides, and lignin) have shown similar benefit in improving long-term glycaemic management and lipid profiles and reducing blood pressure and risk of central obesity (Maćkowiak¹, Torlińska-Walkowiak, & Torlińska, 2016). Many whole grain foods are composed of significant amount of insoluble fibre (e.g. hemicellulose and lignin), which are the major components of the starchy endosperm, germ and bran of brown rice (Okarter & Liu, 2010). The benefit of dietary fibre in whole grains and foods is to reduce the starch digestibility and therefore slow and lower the glycaemic response compared to refined grain.

One large-scale epidemiological study, the European Prospective Investigation Into Cancer and Nutrition (EPIC)-Postdam Study by (Schulze et al., 2007) has reported that the consumption of dietary fibre rich cereal foods was associated with a 27% reduced risk of T2DM (RR = 0.73 (CI95%: 0.57, 0.94) after adjusting for other dietary and lifestyle factors. In the NHS I and NHS II report, Sun et al. (2010) reported that consuming ≥ 2 servings of brown rice per week was associated with 11% reduced risk of T2DM (RR of 0.89 (95%CI: 0.81-0.97)) compared to lowest category of brown rice intake after adjustment for confounding factors. The large scale eight-year follow-up study, The Women's Health Initiative Observational Study, reported that women who consumed more than two servings of whole grain daily reduced the risk for 37% compared to those who consumed little or none after adjustment for age, ethnicity and lifestyle and dietary factors (Parker et al., 2013).

Long term randomised controlled trials of the effects of whole grains on glucose homeostasis in humans are not feasible or ethical as they could cause harm to the control group. A number of small randomised controlled trials have observed the association between whole grain consumption and improved glycaemic control. Ye et al (2012) report for 11 RCT ranging from 4 to 16 weeks that there was a reduction in fasting glucose of 0.93 (95% CI -1.65, -0.21) mmol/L.

Long-term effects of high carbohydrate consumption on blood glucose concentration

Dietary carbohydrates, found in all plant foods, vary in chemical and physiological properties. In foods they are also commonly referred to as simple carbohydrates such as sugar, complex carbohydrates such as the starch in rice and fibre such as the husk and bran. They are an important macronutrient (alongside protein, fat (and alcohol)) and normally provide the largest single source of energy in the diet (at least 55% of the energy of the average New Zealander or Australian) (Australian National Health and Medical Research Council & New Zealand Ministry of Health, 2005; Ministry of Health, 2003, 2011). Carbohydrates can be classified into four principal groups, monosaccharides (1 sugar unit), disaccharides (2 sugar units), oligosaccharides (3–5 sugar units) and polysaccharide digestible carbohydrate (disaccharides and starch polysaccharides). Digestible carbohydrates can be easily degraded to monosaccharides by enzymes (disaccharidases, dextrinase and amylase) in the gastrointestinal tract (mouth and small intestine) and then absorbed into the small intestine.

Monosaccharides are absorbed in the small intestine, and fructose and galactose are converted to glucose in the liver. Following a meal containing digestible carbohydrates, the systemic blood glucose concentration rises and reaches a peak after 15 to 45 minutes, depending on the rate of digestion and absorption (Englyst, Kingman, & Cummings, 1992; Englyst, Quigley, Hudson, & Cummings, 1992; Englyst, Englyst, Hudson, Cole, & Cummings, 1999). It is important to maintain blood glucose levels within a narrow range (between 4.4 and 7.0 mmol/L) because blood glucose levels that are too high will damage tissues and result in acute complications and chronic complications in the long term, and those that are too low will cause symptoms of hypoglycaemia, such as shakiness, nervousness or anxiety (Brand-Miller, McMillan-Price, et al., 2009; Williams & Pickup, 1997; Yoon et al., 1997).

Among many anabolic roles, insulin is the main hormone involved in regulating postprandial blood glucose level, amino acids, and fats. Insulin is produced in beta cells of the pancreas, and its most important function is lowering blood glucose levels by

increasing glucose uptake by muscle and adipose cells, and suppressing hepatic glucose output. Insulin release is mainly stimulated by raised blood glucose concentration, and is also influenced by other hormones. The quantity and the rate of digestion of carbohydrates consumed are the main determinant of the blood glucose concentration after eating (Figure 2-2, the figure was adapted from “Digestibility of carbohydrate foods in an ileostomate: relationship to dietary fibre, in vitro digestibility, and glycaemic response” by Jenkins et al. (1987)) (Goncalves Reis & Dullius, 2011; Laville & Nazare, 2009; Livesey, Taylor, Hulshof, & Howlett, 2008). Furthermore, the concentrations of postprandial blood glucose and insulin are not only affected by the quantity of carbohydrates in the meal, but also by the quality of carbohydrates. There are many classifications, chemical, structural and functional of carbohydrate that is resistant to digestion. The resistant starch in the rice kernel does not meet the FAO definition of dietary fibre and does contribute to glycaemia when digested in the large intestine. It is now recognised that consumption of intrinsic plant cell wall polysaccharides should be the basis for dietary guidelines for public health and consumption of dietary fibre (Englyst et al., 2007) and they have an important role to play in the health of the microbiota (Young et al., 2016).

Currently, the most popular measurement and ranking systems for the glycaemic potency of food carbohydrate is the GI and the GL (DJ Jenkins et al., 1981; Wolever & Jenkins, 1986).

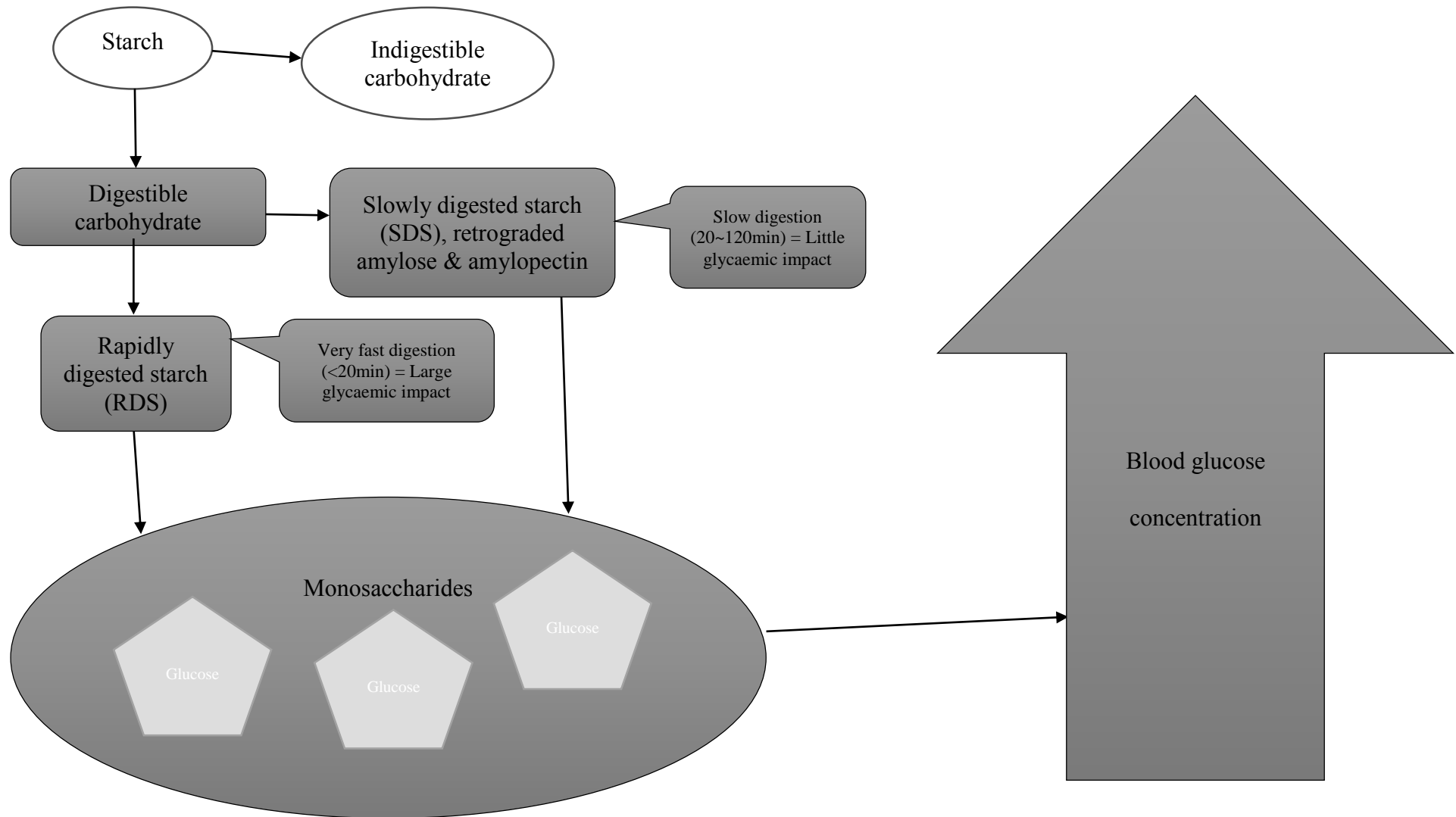


Figure 2-2 The rate of digestion of starch to glucose. Figure was adapted from Jenkins et al. (1987).

Definition of glycaemic index

The GI of a food is determined, for at least 10 healthy volunteers, as the average two-hour glycaemic impact of 50 g of available carbohydrate in a food compared with the same amount of available carbohydrate in a reference, which is usually white bread or a glucose drink (Brand-Miller et al., 2002; Brouns et al., 2005; Wolever, Jenkins, Jenkins, & Josse, 1991), and is expressed as a percentage. Jenkins et al. (1981) tested 56 foods with 50 g carbohydrate portions in non-diabetic volunteers, and the GI for the foods showed that great variation between foods existed within most of the food groups with the exception of dairy products.

Dietary carbohydrate foods have been classified into three categories according to their GI values: high GI food ($GI > 70$), medium GI food ($55 \leq GI \leq 70$) and low GI foods ($GI < 55$) (Brand-Miller, Colagiuri, & Foster-Powell, 1998). Foods with a high GI elicit higher blood glucose concentrations rapidly, and foods with low GI produce lower but more sustained postprandial blood glucose concentrations (Brand-Miller et al., 1998). Refined sucrose has a GI of 60, which is less than the index of some starch staples such as rice (between 50 and 80) and hot potatoes (around 60) (Truswell, 1992).

Definition of glycaemic load

GL was introduced to quantify the cumulative “glucose-response-increasing potential of carbohydrate-containing food” (Englyst et al., 2007). It is indirectly calculated by multiplying the amount of available carbohydrates in food by the average GI of carbohydrate food consumed over a period of time. For example, the GL of 100 g of cooked medium-grain rice with a GI of 80 has around 29 g of total carbohydrate, making the calculation “ $29 \text{ g} * 80/100 \text{ g}$ ” equals the GL of 23.2 units.

Both GI and GL are current guiding tools to provide instruction on a healthy diet in order to improve blood glucose control and insulin sensitivity. A diet of low GI and GL should be recommended from a nutritional point of view (Esfahani et al., 2009; Grant et al., 2011; Laville & Nazare, 2009).

Strengths and limitations of glycaemic index

Among the many dietary management strategies (including the carbohydrate exchange system established by the American Diabetes Association and the United States Public Health Service in 1950), the physiological classification of carbohydrates in terms of glycaemic responses or blood glucose concentration following ingestion (postprandial glycaemia) (Jenkins et al., 1981) has long been recognised as useful physiologically as opposed to merely considering the chemical structure, and has implications in health

and disease prevention. A study by Henry et al. (2008) offered strong evidence to show the relative constancy of the ranking of the acute glycaemic response of foods across different cultures, emphasising the importance of the global use of the GI system and the application of universal values to different ethnic groups. Despite the controversies over the initial implementation of the intake of low GI foods for the treatment of diabetes (Burton et al., 2011), the general concept of GI has grown increasingly in both public health and clinical interest because of its convenient and effective application to human health and performance.

Although from a nutrition point of view it is not recommended that sugar replace starch foods, the GI allows foods of similar composition to be ranked according to their impact on blood glucose responses. Nevertheless, GI ranks foods by the impact on blood glucose responses under equi-carbohydrate conditions, and does not change with food weight. The GI can be misleading when it is used in isolation because, in practical use, it is necessary to consider both the amount of available carbohydrate in the food, the quantity of the food being consumed and how the food is consumed (ie. In a meal with other foods or in isolation). Furthermore, because GI for individual food was calculated among a small number of healthy individuals, the inter-individual variation may impact on the fasting blood glucose and postprandial glycaemic responses which limits the use of GI and GL.

Pathogenesis of long-term high blood glucose concentration in type 2 diabetes mellitus and gestational diabetes mellitus and the diagnosis criteria

Studies have identified that the underlying biological factors are impaired function of the pancreatic cells that produce insulin (beta cells) and the reduced ability of tissue cells to take up glucose (i.e., insulin resistance and impaired glucose tolerance) (Porte & Kahn, 2001). The diagnosis of T2DM and GDM relies on measurements of blood glucose.

Diagnostic criteria were first published in 1985, by a WHO study group on diabetes mellitus (World Health Organization, 1985), and the most recent diagnostic criteria for diabetes mellitus emerged in 2013 (American Diabetes Association, 2013), and for gestational diabetes in the same year (World Health Organization, 2013). The aim of the new criteria is to simplify diagnosis procedures in order to reduce the morbidity and mortality associated with diabetes, as well as to provide clinicians, patients, researchers, payers and other interested individuals with components of diabetes care, general treatment goals and tools to evaluate the quality of care. The diagnosis criteria are still

based on the measurement of elevated blood glucose concentration, as shown in Table 2-1 (American Diabetes Association, 2016).

Table 2-1 Criteria for increased risk for diabetes (i.e., prediabetes) and for diagnosis of diabetes mellitus (T2DM).

Categories of increased risk for diabetes (prediabetes)	
1	Fasting plasma glucose between 5.6 mmol/L and 6.9 mmol/L (as impaired fasting glucose). Fasting is defined as no caloric intake for at least 8 hours. OR
2	2-hour plasma glucose in the 75 g oral glucose tolerance test (OGTT) between 7.8 mmol/L and 11.0 mmol/L (as impaired fasting glucose). The test should be performed as described by the World Health Organization (WHO), using a glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water. OR
3	Plasma HbA1C between 5.7% and 6.4%.
Criteria for the diagnosis of diabetes mellitus	
1	Plasma HbA1C \geq 6.5%. The test should be performed in a laboratory using a method that is National Glycohemoglobin Standardisation Program (NGSP) certified and standardised to the Diabetes Control and Complication (DCCT) assay. OR
2	Fasting plasma glucose \geq 7.0 mmol/L. OR
3	2-hour plasma glucose \geq 11.1 mmol/L during an OGTT. OR
4	In a patient with classic symptoms of hyperglycaemia or hyperglycaemic crisis, a random plasma glucose \geq 11.1 mmol/L.
The criteria was derived from the “Classification and Diagnosis of Diabetes” by American Diabetes Association (2016).	

In normal glucose metabolism, fasting blood glucose concentration is in a range of 4 to 6 mmol/L and rises up to 7 mmol/L after a meal. The development of hyperglycaemia is complex and is an interplay of disordered fat metabolism and rising free fatty acid concentrations and inhibition of insulin signalling and glucose transport (Boden & Shulman, 2002). At the same time, disordered fat metabolism is associated with the deposition of fat in ectopic areas such as in the liver and around the abdominal organs. On the other hand, the accumulation of subcutaneous fat is thought to be beneficial (Boden & Shulman, 2002; Hajer, van Haeften, & Visseren, 2008) and associated with lower plasma-free fatty acid and glucose concentrations.

Normal pregnancy is associated with increasing insulin resistance; therefore, in women who are unable to increase their insulin secretion further, to overcome the insulin resistance, GDM develops (Buchanan, 2001; Kautzky-Willer et al., 1997; Ryan, 2011; Ryan et al., 1995). GDM typically develops in the second half of pregnancy (Buchanan, 2001; Homko, Sivan, Chen, Reece, & Boden, 2001). Changes in adiposity, hormones

associated with the placenta and the inflammatory response to pregnancy are factors in the increasing insulin resistance (Buchanan, 2001; Fernández-Morera, Rodríguez-Rodero, Menéndez-Torre, & Fraga, 2010; Jovanovic & Pettitt, 2001; McIntyre et al., 2000).

Intergenerational effect of long-term high blood glucose concentration

The recent rapid growth in the prevalence of T2DM and GDM at a younger age in New Zealand and in other developing countries has suggested a strong association between changing environmental factors and poor blood glucose regulation. A number of researchers have investigated the foetal origin or epigenetics of chronic diseases in adulthood, suggesting intergenerational influences of intrauterine growth on T2DM and GDM later in life (Ben-Shlomo, 2007; Ben-Shlomo & Kuh, 2002; Kuh, Ben-Shlomo, Lynch, Hallqvist, & Power, 2003). Exposure to an unbalanced excess nutrient supply (i.e., excess carbohydrates), one of the major intrauterine environmental factors, could be associated with a greater risk of T2DM and GDM in offspring (Deierlein, Siega-Riz, Chantala, & Herring, 2011; Hunger-Dathe, Mosebach, Samann, Wolf, & Muller, 2006; Silverman et al., 1991). From conception, the amount of blood glucose available for metabolism may affect the risk across the life course of developing T2DM and GDM.

In the case of GDM, glucose travels freely from the mother to the foetus, but maternal insulin does not. As a result, maternal GDM exposes the foetus to higher concentrations of glucose than normal. This condition may lead to foetal stress, which subsequently gives rise to rapid foetal growth and high fat synthesis and deposition, a baby of larger than gestation age or macrosomia, and very possibly, obesity in childhood (Ben-Ziv & Hod, 2008; Deierlein et al., 2011). High foetal blood glucose concentrations may also force the foetus to increase its own insulin production and cause an excessively high foetal insulin concentration (i.e., foetal hyperinsulinemia) (Weintrob, Karp, & Hod, 1996). Excess production of insulin may cause some damage to foetal pancreatic islet cells, and may also have an impact on insulin secretion capacity later in life. Studies indicate that children of women with GDM have a significantly higher risk (more than fourfold) of high blood glucose concentrations, obesity and diabetes mellitus than those of healthy mothers (Ben-Ziv & Hod, 2008; Deierlein et al., 2011).

Furthermore, one theory has proposed that foetal exposure to high blood glucose may lead to alteration of normal gene expression for transcription factors that regulate pancreas development and pancreatic beta-cell differentiation, glucose metabolism proteins and molecules of the insulin signalling pathway (Colomiere, Permezel, &

Lappas, 2010; Park, Stoffers, Nicholls, & Simmons, 2008; Shaat et al., 2006; Simmons, 2007). Expression of these genes in the foetus may be suppressed and cause progressive pancreatic cell damage through their life. The damage may eventually lead to persistence of glucose intolerance and an insulin-resistant phenotype in the foetus. The phenotype could be a result of nutritionally induced epigenetic changes that may be related to long-term high blood glucose concentration that influences the expression of key genes in their offspring (Fernández-Morera et al., 2010; Fraga, 2009).

The rapidly increasing annual incidence of T2DM and GDM among New Zealand Chinese and Indian women implies that they are exposed to higher risk of developing high blood glucose (i.e., hyperglycaemia). Consequently, their offspring have a higher chance to be exposed to high blood glucose concentration in utero and have a glucose-intolerant and insulin-resistant phenotype, which may be passed on to their next generations. In order to minimise the adverse effect of high blood glucose concentration, urgent action is required to understand the aetiological factors.

2.2 Rice is related to high glycaemic index diet among people living in Auckland

2.2.1 Increasing rice consumption in New Zealand

Despite the long history of Asian migration in New Zealand, only a few studies have reported the dietary patterns and nutrition intakes of this rapid growing population. Of the 1977 National Diet survey, the 1989 Life in NZ survey, the 1997 National Nutrition Survey and the 2008/09 Adult National Nutrition Survey, only the 2008/09 Adult National Nutrition Survey attempted to provide representative samples to report the dietary intake of Asians in New Zealand (Parackal et al., 2015). The report provided a detailed comparison of common food items intake (including fruit, vegetables, milk, bread, chicken, red meat, seafood, etc.) and daily nutrient intake (e.g., carbohydrates) among South Asians, East Asians, and Europeans but did not include the intake of rice, which is the main element in the traditional diet of East and South East Asian and part of traditional diet of South Asian nor the quality of the carbohydrates (i.e., average GI of the daily diet) (Parackal et al., 2015). The study by Foroughain (2010), investigating adolescents' daily diet reported that rice was the main after-school food and that the East Asian focus group stated that they were "having more rice and noodles and less bread in their traditional diet" and considered that "to eat rice and noodles is good to heart". Moreover, Chinese takeaways, including fried rice and freshly cooked warm rice, were their favourite food.

More studies have indicated that the amount of total carbohydrate consumed is increasing, particularly in the New Zealand Asian populations (Parackal et al., 2015; Scragg & Northern District Health Board, 2010). A great proportion of carbohydrates in their diet comes from rice, which is a staple carbohydrate source for more than 50% of the world's population and provides 27% of the world's calories. In 2004, it was reported that Asian countries consumed around 90% of the total rice produced (Food and Agricultural Organization of the United Nations (FAO), 2004) and the global paddy production has increased each year since then. It was reported by Kennedy et al. (2004) that the average Chinese and Indian adult each day consumes 250 g (uncooked weight) of rice (about 4 cups of cooked rice) and 200 g (about 3 cups of cooked rice) respectively. Rice intake in New Zealand has also increased in recent years alongside the rapid increase in the Chinese and Indian population and increase in the prevalence of diabetes.

To counteract the increasing prevalence of type 2 diabetes and related complications, there is an urgent need for implementation of effective dietary adjustment (lowering the GI of rice) in diabetes prevention and management among people who consume rice regularly (Diabetes Prevention Program Research Group, 2002). Some studies have also shown that replacing high GI foods with lower glycaemic index foods may be as effective as the use of antihyperglycaemic medication for improving overall glycaemic control among patients with T2DM (Brand-Miller, Hayne, Petocz, & Colagiuri, 2003).

2.2.2 Application of dietary management of high blood glucose concentration

Dietary management is fundamental for the treatment of hyperglycaemia and plays a critical role in maintaining optimal blood glucose concentrations. The glycaemic targets are fasting blood glucose ≤ 5.3 mmol/L, one hour postprandial glucose ≤ 7.8 mmol/L, two-hour postprandial glucose ≤ 6.7 mmol/L (D. Simmons & Campbell, 2007; D. Simmons et al., 2008). One dietary strategy aimed at improving diabetes prevention and control is the use of a low GI diet (Brand-Miller, Hayne, Petocz, & Colagiuri, 2003; Liu et al., 2000). According to the definition of GI, foods with low GI that produce slower and flatter blood glucose responses may facilitate better glycaemic control in people with high fasting blood glucose concentration.

There is strong evidence that a high intake of foods with high GI, especially refined white rice, the main carbohydrate contributor in Chinese and Indian populations, may increase the risk of chronic insulin resistance and glucose intolerance that may lead to T2DM among Chinese and Indian women (Grant et al., 2011; Isharwal, Misra, Wasir, &

Nigam, 2009; Khosravi-Boroujeni et al., 2013; Lin, Wu, Lu, & Lin, 2010; Mani et al., 1990; Salmeron, Ascherio, et al., 1997; Salmeron, Manson, et al., 1997; Schulze et al., 2004; Soriguer et al., 2013; Villegas et al., 2007). A number of large-scale observational studies among various populations (Nanri et al., 2010; Villegas et al., 2007) have observed a significant positive association between white rice consumption and risk of diabetes among Chinese and Japanese women. Two studies in European populations with lower consumption of white rice than Asian populations also suggested the possibility of mixed association (Hodge, English, O'Dea, & Giles, 2004; Q. Sun et al., 2010). Hu et al. (2012) summarised the evidence on the association between white rice consumption and risk of T2DM, which included a total of 13,284 research cases of T2DM among 352,384 participants with follow-up period between 4 and 22 years. It was reported that Asian populations (Chinese and Japanese) who consumed three to four servings of white rice per day had 1.55-fold relative risk of developing T2DM, whereas European populations who commonly consume one to two servings per week had 1.12 relative risk (E. Hu et al., 2012). Hu et al. (2012) also reported a dose-response association that for consumption of each serving of white rice per day there would be 1.11-fold increased relative risk of developing T2DM.

It is possible that Chinese and Indian women, whose rice-based diets have a high glycaemic load, are exposed to a higher risk of developing GDM and T2DM. A number of randomised controlled trials that intervened in participants' diets by replacing high GL food with low GL food (a GI difference of 20-plus units, i.e., most low GI diets were 20 or more GI units lower than the high GI diets) have shown that a low glycaemic diet is effective in controlling postprandial blood glucose responses and improves the overall long-term glycaemic status among people with T2DM (Du et al., 2008; Ebbeling, Leidig, Feldman, Lovesky, & Ludwig, 2007; Goncalves Reis & Dullius, 2011; Grant et al., 2011; Huang, Hsu, Wang, & Shin, 2010; Jenkins et al., 2008; Livesey et al., 2008; Thomas & Elliott, 2009). Analysis of outcomes in these studies were adjusted for potential confounders, including age, sex, smoking, physical activity, cohort, total energy, dietary fibre, alcohol, and cholesterol intake, fatty acids, plant-and animal-based protein, polysaccharides, and mono- and disaccharides, therefore, the observed associations were not likely to be affected by different dietary composition or lifestyle factors.

However, caution must be used when comparing the values of the GI for a food among the studies because the value of the GI varies from centre to centre, from individual to

individual and according to the reference foods used (Foster-Powell & Brand-Miller, 1995). Other limitations of the studies were that the longest study period was 12 weeks and the number of subjects in each study was small. In addition, even though researchers tried to keep the macronutrients similar between two diets, this was not always the case. For example, the lower GI diet often had a higher fibre content than the higher GI diet (Jenkins et al., 1988).

2.2.3 Modelling the long-term glycaemic impact of rice

The question “What would be the reduction of fasting blood glucose if medium grain white rice was replaced by another rice variety or prepared in a different way? The researcher (L.W.L.) modelled the answer to this question based on Livesey’s model (reference) and the GI and GL database from Sydney University (2012).

Following the Livesey argument (next section), the effects of parboiled medium-grain white rice, medium-grain brown rice, and cooked and cooled medium-grain white rice on blood glucose were modelled (Figure 2-3). This model predicts that in subjects habitually consuming three cups of hot white rice a day, fasting blood glucose concentration can be reduced progressively and substantially from 0.2 to 1.2 mmol/L with the replacement of one to three cups of rice prepared to reduce the availability of available starch (see Figure 2-3).

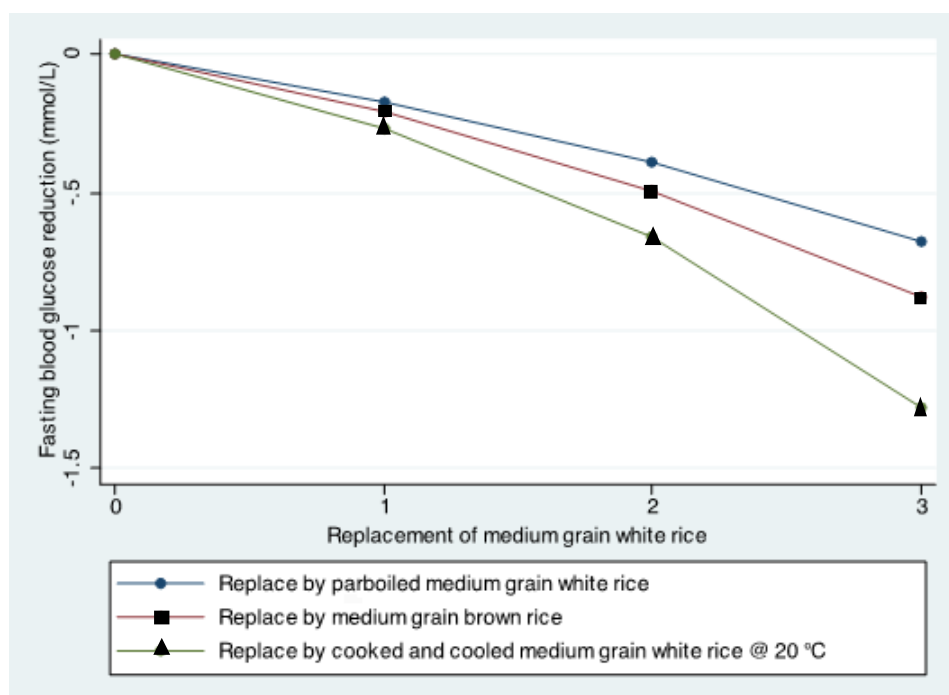


Figure 2-3 Model of the relationship between staple rice replacement and fasting blood glucose concentration after around 26 weeks.

The Livesey model

In a systematic review by Livesey et al. (2008), the association between glycaemic response and health outcomes was systematically examined and a mathematical model to describe the effects of altered GI of the diet derived. A total of 45 controlled dietary intervention trials which took place before January 2005 and a total of 972 participants in each arm met the review criteria. Seventeen studies involved participants with type 2 diabetes, 7 studies with type 1 diabetes, 13 studies with healthy participants, and 8 studies with participants had impaired glucose tolerance. There was a total of 972 participants aged between 10 and 63 years in each treatment arm. An overall effect of a reduction in fasting blood glucose concentration (in either capillary blood or venous plasma blood) was achieved when daily GI was reduced by up to 32 units (GL was reduced by up to 134 g glucose equivalents) after up to 26 weeks. The authors then developed a mathematical model to show the difference in fasting blood glucose concentration at the end of treatment with either lower GI or GL after stratified by age (years), body weight (kg), BMI (kg/m^2). All participants were free living and not hospitalized. The equation is shown in the box below.

For the change of one unit of GI (Δ GI), the change of fasting capillary blood glucose (mmol/L) after up to 26 weeks of treatment would be:

$$\Delta \text{ Fasting capillary blood glucose } \left(\frac{\text{mmol}}{\text{L}} \right) = K + \text{Mean slope}^1 * \text{Fasting blood glucose (mmol/L)} + \text{slope}^2 * \text{covariate} \pm \text{Tau} \pm \text{SE residual}$$

$K = \text{constant} = 18$

$\text{Mean slope}^1 = -0.30$

$\text{Fasting blood glucose (mmol/L)} = \text{baseline venous plasma fasting blood glucose (mmol/L)} = 1.06 * \text{fasting capillary blood glucose (mmol/L)} + 0.65$

$\text{Slope}^2 = 0$ (The univariate model before adjustments was the same as the bivariate model, therefore, constraining slope2 to 0.)

$\text{Covariate} = K - 2 = 16 - 2 = 14$

$\text{Tau} = \text{the between-study SE} = 0.63 \text{ mmol/L}$ (The univariate model before adjustment was heterogeneous, with a between-studies error of 0.63 mmol/L.)

$\text{SE residual} = \text{the SE of the fitted values or within-study SE} = 0.12 \Delta \text{ mmol/L per mmol/L}$

For the change of one unit of GL (Δ GL (g equivalent carbohydrate/day)), the change of fasting capillary blood glucose (mmol/L) after up to 26 weeks of treatment would be:

$$\Delta \text{ Fasting capillary blood glucose } \left(\frac{\text{mmol}}{\text{L}} \right) = K + \text{Mean slope}^1 * \text{Fasting blood glucose (mmol/L)} + \text{slope}^2 * \text{covariate} \pm \text{Tau} \pm \text{SE residual}$$

$K = \text{constant} = 16$

$\text{Mean slope}^1 = -0.34$

$\text{Fasting blood glucose (mmol/L)} = \text{baseline venous plasma fasting blood glucose (mmol/L)} = 1.06 * \text{fasting capillary blood glucose (mmol/L)} + 0.65$

$\text{Slope}^2 = 0$ (The univariate model before adjustments was the same as the bivariate model, therefore, constraining slope² to 0.)

$\text{Covariate} = K - 2 = 18 - 2 = 16$

$\text{Tau} = \text{the between-study SE} = 0.63 \text{ mmol/L}$ (The univariate model before adjustment was heterogeneous, with a between-studies error of 0.63 mmol/L.)

$\text{SE residual} = \text{the SE of the fitted values or within-study SE} = 0.12 \Delta \text{ mmol/L per mmol/L}$

2.3 Digestibility of starch in rice and blood glycaemic responses

2.3.1 Background of rice as a food

All rice sold in New Zealand is imported. The popular rice product varieties are generally classified as long-, medium- or short-grain and white or brown in accordance with grain dimension and milling. Long-grain rice is typically dry and fluffy when cooked. Medium-grain rice is more moist and tender than long-grain rice, and is typically used in Asian households for daily staple meals. Short-grain rice is soft, plump and almost round, and is traditionally used for dessert dishes.

Rice is harvested as paddy rice, which consists of individual rice kernels with an adherent hull that is not edible. The hull includes the lemmae, the palea, and the larger lemma, and the caryopsis. The caryopsis, which is still present on brown rice, comprises the pericarp, seed coat, nucellus, aleurone layer, endosperm and embryo. Although the composition of rice varies greatly depending on the variety and growing environment, milled (white) rice consists at 14% moisture of approximately 77.6% starch, 6.3–7.1% protein, 0.3–0.5% crude fat, 0.3–0.8% ash and 0.2–0.5% crude fibre (Figure 2-4) (Juliano & Bechtel, 1985b). Unmilled or brown rice has double the fibre and more than double the calcium, iron, magnesium and B vitamins than that of white rice (Table 2-2). In addition parboiling, which is applied prior to milling or polishing, involves soaking the rice in warm water, steaming and drying which conserves the B vitamins and some of the minerals in particular calcium (Table 2-2). The micronutrients are transferred from the aleurone and germ into the starchy endosperm (Juliano, 1993; Juliano & Bechtel, 1985a).

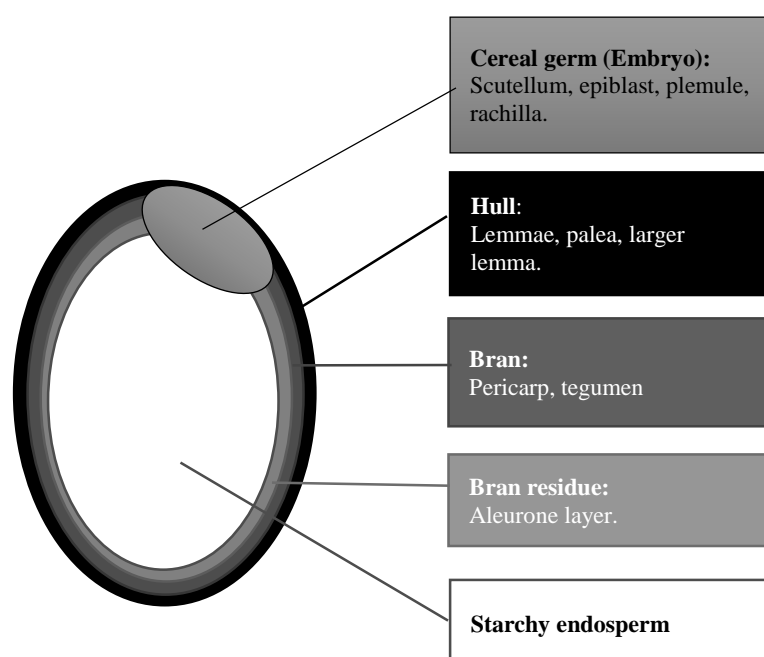


Figure 2-4 Structure of rice grain. Adapted from “The rice grain and its gross composition.” by Juliano & Bechtel (1985b).

Table 2-2 Comparative nutrient composition of rice grains of different varieties

Parameter	Medium grain white rice	Medium grain brown rice	Basmati	Long grain brown rice	Parboiled rice	Recommended daily intake
Per 100g uncooked rice						Per day
Carbohydrate (g)	79.34	76.17	79.95	76.25	80.89	
Protein (g)	6.61	7.50	7.13	7.54	7.51	
Total lipid (fat) (g)	0.58	2.68	0.66	3.20	1.03	
Calcium (Ca) (mg)	9.00	33	28	9	71	1000
Iron (Fe) (mg)	0.80	1.80	0.80	1.29	0.74	15
Magnesium (Mg) (mg)	35.00	143	25	116	27	350
Phosphorus (P) (mg)	108	264	115	311	153	1000
Potassium (K) (mg)	86	268	115	250	174	3500
Sodium (Na) (mg)	1	4	5	5	2	2400
Zinc (Zn) (mg)	1.16	2.02	1.09	2.13	1.02	15
Vitamin C (mg)	0.0	0.0	0.0	0.0	0.0	75
Thiamine (Vitamin B1)(mg)	0.070	0.413	0.070	0.541	0.224	1.4
Riboflavin (Vitamin B2)(mg)	0.048	0.043	0.049	0.095	0.050	1.6
Niacin (Vitamin B3) (mg)	1.600	4.308	1.600	6.494	5.048	18
Vitamin B6 (mg)	0.145	0.509	0.164	0.477	0.452	2
Folate (µg)	9	20	8	23	8	400
Vitamin B-12(µg)	0.0	0.0	0.0	0.0	0.00	6
Vitamin A(µg)	0.0	0.0	0.0	0.0	0.0	600
Vitamin E(α-tocopherol)(mg)	0.0	0.0	0.11	0.60	0.03	10
Vitamin D (D2, D3) (µg)	0.0	0.0	0.0	0.0	0.0	5
Vitamin K(phyloquinone)(µg)	0.6	0.0	0.1	0.6	0.1	80
Dietary fibre (g)	1.40	3.40	1.30	3.60	1.80	25

The data was extracted from “Appetite and food intake: a human experimental perspective. Appetite and food intake: behavioural and physiological considerations.” By *Yeomans and Bertenshaw (2008)*.

2.3.2 Rice starch digestibility

Starch

Starch, the major constituent of rice, is made up of two polymers: essentially, linear amylose (20–30%) and highly branched amylopectin (70–80%). These two polymers are packed into discrete particles (granules) naturally (sizes range from 2 to 100 μm). The rice amylose has an average degree of polymerisation (DP) of 115 glucose units with 8–11 branches (Magnus & Ann-Charlotte, 2006; Sajilata et al., 2006b). The amylose content of milled rice varies and is affected by the genetic background and growing environment (Fitzgerald et al., 2011). Generally, the amylose content of long-grain, medium-grain and short-grain rice are 20–25%, 15–23% and 14–18% respectively (Boers, ten Hoorn, & Mela, 2015). Amylose is present in an amorphous form intertwined and interspersed with the amylopectin (Tamura, Singh, Kaur, & Ogawa, 2015) in crystalline and amorphous zones within the starch granule. Rice amylopectin molecules are broadly categorised into small (DP 700–2100), medium (DP 4400–8400) and large (DP 13,400–26,500) ranges but typically consist of all three. Regardless of rice type, rice contains a greater proportion of amylopectin with a wide range of DP. Long-grain rice generally has a greater proportion of small range than the medium range of amylopectin (Fitzgerald et al., 2011; Takeda, Shibahara, & Hanashiro, 2003). Amylopectin is found in the highest proportion in medium-grain and short-grain rice, and causes these types of rice to be softer and have a greater tendency to cling (Fitzgerald et al., 2011; Takeda, Hizukuri, Takeda, & Suzuki, 1987; Takeda et al., 2003).

Transformation of starch during cooking

Starch undergoes five stages of transformation during cooking: glass transition, gelatinisation, swelling, leaching and retrogradation (Tamura et al., 2015; Fitzgerald et al., 2011). Glass transition occurs when the amorphous regions in the starch transition from a rigid to a viscous, rubbery state in the presence of water. Water acts as a plasticiser that lowers the starch glass transition temperature through thermal transition depression (Tamura et al., 2016; Delcour et al., 2010; Vandeputte et al., 2003a; Biliaderis, Maurice, & Vose, 1980). Gelatinisation is an irreversible process in which starch crystals collapse from hydration and excess heat. Once gelatinised, starch undergoes swelling by absorbing more water, and at the same time, amylose and amylopectin molecules leach out of the granules into the continuous phase, resulting in an increase in viscosity. Long-grain rice (with higher “amylose:amylopectin” ratio) typically has an intermediate to high gelatinisation temperature (GT) range of 70 °C to 79 °C (Zhu et al., 2011; Juliano, 1992; Juliano & Bechtel, 1985b). Medium-grain and

short-grain rice product varieties with low “amylose:amylopectin” ratio rice have low GTs at 55 °C to 69.5 °C (Zhu et al., 2011; Juliano, 1992; Juliano & Bechtel, 1985b).

When cooked rice starch cools, the amylose and amylopectin chains which disaggregated during gelatinization process reassociate to form more ordered crystalized structure (Wang, Li, Copeland, Niu, & Wang, 2015). The reformation of these crystals consequently affects the pasting properties and cooked rice texture through hardening and gel formation (Delcour et al., 2010; Panchan & Naivikul, 2009; Vandeputte et al., 2003b). The gelatinisation temperature of starch is between 52 °C and 85 °C, depending on the source. The starch granules are dense and insoluble in cold water. In order to dissolve the starch granules, heat has to be applied. At 80 °C, unmodified starch granules form a paste with very high viscosity (Tamura et al., 2016; Biliaderis et al., 1980). The rate of retrogradation is dependent on the molecular ratio of amylose to amylopectin, structures of the amylose and amylopectin molecules (source of starch), temperature, starch concentration, and presence and concentration of other ingredients such as surfactants and salts. Generally, the higher the linear amylose content is, the greater the extent of retrogradation. The rate of retrogradation in rice starch paste increases as the temperature is reduced (Zhu et al., 2011; Delcour et al., 2010; Jacobson, Obanni, & Bemiller, 1997). Therefore, long-grain rice cooled to a low temperature would be expected to undergo more retrogradation than short- and medium-grain rice.

2.3.3 Starch digestibility profile: Rapidly digestible starch, slowly digestible starch and resistant starch

Rice is typically considered a highly digestible source of carbohydrate, but the rate of digestion and the resulting glycaemic response varies among rice cultivars and preparation techniques (Brand-Miller, Stockmann, Atkinson, Petocz, & Denyer, 2009; Panlasigui et al., 1991). Starch may be divided into three categories based on digestibility and absorbability of the breakdown products: rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS) (Figure 2-2). RDS is starch that is rapidly and completely digested in the small intestine. SDS is starch that is slowly but completely digestible in the small intestines. RS is the sum of starch and starch degradation products (such as dietary fibre) that, on average, reach the human large intestine relatively undigested.

The profile of digestibility of starch, the proportions of RDS, SDS and RS, in rice directly determines the glycaemic impact of rice (Englyst, Kingman, et al., 1992; Englyst et al., 1999) (Figure 2-5). The type of starch that is digested within 20 minutes by α -amylase *in vitro* is defined as RDS (Englyst et al., 1999). The starch that can be

digested within between 20 and 180 minutes are defined as SDS (Englyst, Kingman, et al., 1992; Englyst et al., 1999). Starch that can resist digestion for up to two hours and escapes digestion in the small intestine is defined as RS (Englyst, Kingman, et al., 1992; Englyst et al., 1999). RS is calculated as the difference between the total starch (TS) obtained from a homogenised and chemically treated sample and the sum of RDS and SDS (Englyst et al., 1999). The sum of RDS and SDS is called total available starch (TAS), by measuring the amount of glucose released from one test food during incubation with amylase enzymes under standardised *in vitro* conditions over 180 minutes.

$$“TS = RS + TAS = RS + RDS + SDS”$$

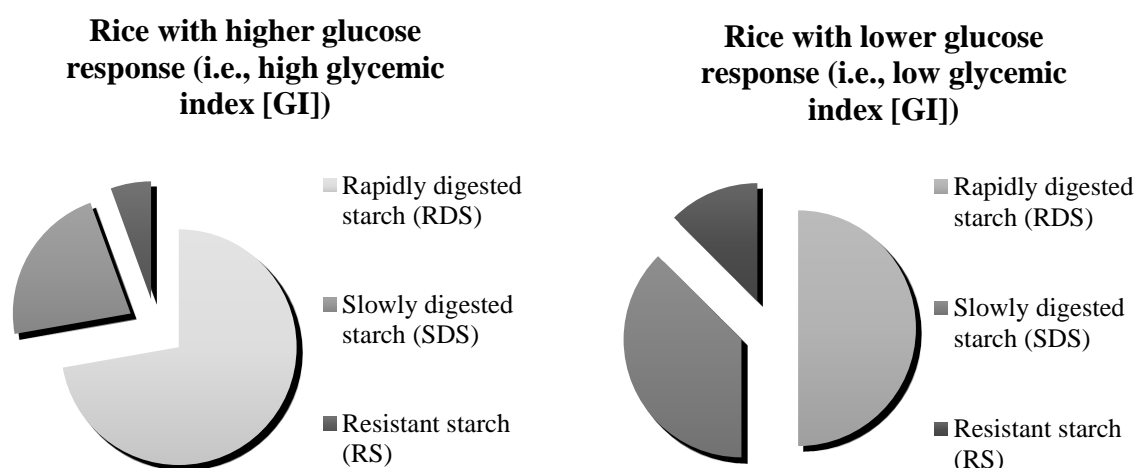


Figure 2-5 The association between rice starch digestibility profile and the glucose response. Modelled based on the concept from “Classification and measurement of nutritionally important starch fractions” by (Englyst, Kingman, & Cummings, 1992).

Resistant starch (RS) is the fraction of starch that survives the enzyme attack in the digestive tract. It directly determines the available carbohydrate (i.e., TAS) in starchy food. RS has been classified into four types according to the nature of the enzyme resistance and the structure of starch (Eggum, Juliano, Perez, & Acedo, 1993; Englyst, Trowell, Southgate, & Cummings, 1987). RS type 1 (RS1) is physically inaccessible starch, which is protected by a protein matrix or cell wall material, particularly that in whole grain rice (Englyst et al., 1987). Resistant starch type 2 (RS2) is a native, uncooked semi-crystalline granular starch that displays the B-type (i.e. with long side chains and distant branching points of amylopectin) and some C-type polymorphs (i.e. combination of amylopectin with short lateral chains and closed branching and amylopectin with long side chains and distant branching points). These B and C type polymorphs are found in uncooked rice starch and high amylose maize starch. RS type 2

(RS3) is the retrograded amylose formed in cooked starchy food. RS type 4 (RS4) is chemically modified or cross-linked starch, which is less accessible for enzyme hydrolysis.

In summary, the bioavailability of glucose from rice starch can be affected by a number of physical factors: the physical form of the rice, the amylose-to-amylopectin ratio, particle size, parboiling, and retrogradation during cold storage and cold storage time length.

Physical form of rice: whole grain rice versus refined grain rice

Factors that determine whether rice starch is resistant to digestion include the physical form of grains in which starch is located, particularly whether these are whole or partially disrupted (Slavin, 2004). Whole grain rice products are rich sources of unavailable carbohydrates, including dietary fibre and RS (RS1), which can only be digested when they reach the colon and are fermented by intestinal microflora to short-chain fatty acids and gases. Because of the protective effect of the rice bran, which works as a barrier between endogenous digestive enzymatic attack and rice starch in the gastrointestinal tract, the starch in whole grains is apparently more resistant to digestion than refined grains (Englyst & Englyst, 2005; Slavin, 2004).

Medium-grain white rice (with all of the outer kernel layer removed), freshly cooked by moist heat and which contains a high proportion of RDS (in a range from 75% to 87 %) consisting mainly of amorphous and dispersed starch, and low proportions of SDS and RS, induces a relatively high glucose response (Englyst, Kingman, et al., 1992; Sajilata et al., 2006b). Brown rice, which is partially milled so that only the outermost layer is removed, contains a higher level of undigested RS than refined rice, reducing glucose release during *in vivo* and *in vitro* digestion (de Munter et al., 2007; Murtaugh et al., 2003; Rave et al., 2007).

Studies have also shown that postprandial blood glucose and insulin responses are greatly affected by food structure (Slavin, Martini, Jacobs, & Marquart, 1999). Any process that disrupts the physical or botanical structure of rice grains (i.e., polishing, milling and chewing) increases the plasma glucose and insulin responses. Food structure was discovered to be more important than gelatinisation or presence of viscous dietary fibre in determining glycaemic response in a study by Granfeldt et al. (Granfeldt, Hagander, & Björck, 1995). A study by Järvi et al. (1995) demonstrated that preserving the original structure of foods was an important determinant of glycaemic

response in patients with T2DM. In contrast, consumption of refined grains tends to increase glycaemic response (Slavin et al., 1999).

Amylose-to-amylopectin ratio and digestion

Rice starch exists in the form of starch granules, which are capable of surviving milling and staying relatively intact because of their comparatively small size. As a result, the form of the crystalline structure of rice starch granules is still an important determinant of the rice starch digestibility, despite the disruption of the bran during the polishing and milling process (Englyst & Englyst, 2005; Slavin, 2004). The form of the rice starch crystalline structure is determined by the ratio of amylose to amylopectin, as amylose tends to form secondary structures that are difficult to disperse (Panlasigui et al., 1991). When gelatinised (i.e., heated in the presence of water), starch granules are disrupted and transformed into a form easily available to pancreatic amylase (Zenel & M. Stewart, 2015). Although cooling does not reverse this process, some starch, especially those with a high amylose-to-amylopectin ratio, may retrograde into forms less susceptible to digestion (i.e., the formation of RS type 3 (RS3)). A study by Zhu and others (L. Zhu, Liu, Wilson, Gu, & Shi, 2011) found a positive correlation between amylose content and RS (RS3).

Particle size

Mastication plays an important role in the digestion process. Recent studies have shown that chewed food particle sizes, number of chews per mouthful and the chewing length per mouthful all have a clear impact on postprandial blood glucose responses (Ranawana, Monro, Mishra, & Henry, 2010; Suzuki et al., 2005; Tan, Ooi, et al., 2015). Furthermore, although particle size reduction and mastication time length during normal mastication differ from individual to individual, it has been suggested that such differences may contribute to the observed individual variability seen in the postprandial glycaemic responses to a single food (Ranawana et al., 2010; Read et al., 1986; Suzuki et al., 2005). The degree of oral breakdown may directly affect the protective character of RS type 1 (RS1) and the *in vivo* starch digestion rate.

There is a need to explore in commonly consumed rice product varieties combinations of safe and practicable cooking and storage methods (New Zealand Food Safety Authority, 2009) that may induce the most retrogradation of amylose and amylopectin, and to compare rice with different degrees of milling (Lake, Hudson, & Cressey, 2004; New Zealand Food Safety Authority, 2009). There are a number of *in vitro* ways of determining the digestibility and glucose availability of rice and Dr John Monro's

laboratory at Massey University in Palmerston North has validated methods of measuring this in various foods and has shown that the *in vitro* starch digestion and glucose release method is appropriate to study rice digestion (Ranawana et al., 2010).

Parboiling

Parboiling is a hydrothermal treatment that traditionally involves soaking below 60 °C, heating through steam and drying of rough rice (i.e. intact rice within the husk). The main purposes of parboiling are to increase total and head rice yield, to reduce the moisture content of the rough rice (to around 30–35% for effective parboiling), to better retain nutrients after milling, to salvage wet or damaged rough rice, and to prepare rice according to the consumer's preference (Kong et al., 2015). During steam heating, water absorption and swelling causes the husk to split and water-soluble nutrients such as the B vitamins migrate into the endosperm, and lipids migrate outward (Kong et al., 2015). Sufficiently soaked starch undergoes gelatinisation when exposed to high pressure from steam (Mahanta, Ali, Bhattacharya, & Mukherjee, 1989; Mahanta & Bhattacharya, 1989). Increased steam pressure and duration of treatment actively increases the starch retrogradation (i.e., the formation of RS3) and amylose complexes, thus increasing the hardness of parboiled rice grain (Mahanta et al., 1989; Mahanta & Bhattacharya, 1989). After drying and cooling, the parboiled starch transits from the rubbery state back to a glassy state. The parboiling process significantly improves milling yield because gelatinised starch and denatured protein bodies from the steaming process seal any fissures and reduce loss of starch.

Retrogradation of cooked starch during cold storage

Resistant starch can be precipitated from cooled pastes and gels that contain mainly amylose through retrogradation. The hydrogen bonds within hydrated starch interact and result in physical-chemical changes; however, no permanent chemical bond is created (Zhang & Hamaker, 2009). Amylopectin retrogrades very slowly. The higher the amylose content is, the greater the retrogradation. It is also found that high amylose starch is more resistant to digestion than amylopectin because of its compact linear structure (Rashmi & Urooj, 2003).

The rate of retrogradation of cooked rice grains can be modified by changing the storage temperature for nucleation, propagation and maturation of starch crystallites (Slade & Levine, 1987). The nuclei of the starch crystallites form more rapidly at a lower temperature (close to the glass transition temperature), whereas the propagation of the starch crystallites is greater at a higher temperature (close to the melting temperature).

Water molecules are required as plasticisers to lower the glass transition temperature of the starch molecules and to increase the mobility of starch molecules (Lehmann & Robin, 2007; Tamura et al., 2015; Zhang & Hamaker, 2009). The maximum degree of starch retrogradation is achieved at an intermediate moisture content (50–60%) at room temperature.

Although the degree of digestibility can be manipulated by selection of different rice products and methods of preparation and storage, the production of a low glycaemic rice is only useful if that rice is acceptable to the consumer in terms of time, cost and palatability. As dietary management is the fundamental treatment for the prevention and control of type 2 diabetes and metabolic syndrome (including hyperglycaemia and obesity), interventions must stem from the management of food intake, with foods that are palatable and increase postprandial satiety (Blundell & V. Burley, 1987; Jones, 2008).

2.4 Satiety and dietary management relating to rice products prepared in different ways

2.4.1 Satiety and palatability in short-term food intake regulation

Short-term food intake is regulated by psycho-physiological mechanisms controlling satiety and appetite (Clark & Slavin, 2013; Hetherington, 2007; Polivy & Herman, 1987). Unlike hunger, which is a subjective feeling that occurs when the sensation signals food deprivation to a degree that food consumption should be initiated, appetite is a psychological desire to eat and is related to the oro-sensory acceptability of a specific appetising food (Blundell & Bellisle, 2013). Satiety is the inhibition of the feelings of hunger and appetite (i.e., the feeling of fullness that persists after eating) in postprandial phase (Blundell & Bellisle, 2013; Holt & Brand-Miller, 1995; Holt, Brand, Soeveny, & Hansky, 1992) and the primary mechanisms that lead to termination of food intake and directly determine daily meal frequency and the quantity eaten on each occasion.

In the 1950s and 1960s, it was hypothesised that the control of satiety was driven by the hypothalamic ‘dual centre’, through the initiation and inhibition of food intake (Blundell & Burley, 1987). After further identification of the neurotransmitter pathways in the brain, the two-centre hypothesis was replaced by Blundell’s model based on catecholaminergic and serotonergic aminergic systems (Blundell & Bellisle, 2013; Blundell & Burley, 1987). The later discovery of further neuropeptides and receptors supported the current understanding that satiety control is based on a complex

interaction among many factors including adipose tissue, adipokines and peripheral episodic signals from intestinal peptides (Blundell & Bellisle, 2013; Näslund & Hellström, 2013).

Satiety, or reduced hunger, is regulated by a complex mechanism beginning with the consumption of food and continues after the digestion and absorption phases (Näslund & Hellström, 2013). Blundell and Bellisle (2013) suggested that postprandial satiety consists of two phases: the early phase, which is a result of sensory and cognitive factors associated with food, and the late phase, which is related to biochemical signals initiated during gastrointestinal digestion and absorption. Sensory and cognitive factors are related to the expectations for the reward and pleasure of the meal quality, including taste, texture, aroma and appearance of the food and the association with previous experience (Levitsky, 2002). Then, as digestion continues in the stomach and intestine, the volume of the meal stretches the stomach and increases the osmotic load within the digestive tract. In response, a series of satiety hormones including cholecystokinin (CCK), glucagon-like-peptide-1 (GLP-1), glucose-dependent insulintropic peptide (GIP), peptide YY (PYY), and ghrelin is secreted (Blundell, 2010; Näslund & Hellström, 2013; Woods, Seeley, Porte, & Schwartz, 1998) (Figure 2-6, Figure was adapted from (Blundell et al., 2015) “Appetite control and energy balance: Impact of exercise”). In the post-absorptive stage, nutrients (e.g., glucose, amino acids) are detected by specialist receptors (e.g., insulin receptors) in various sites of the body and further impact on the sensation of postprandial satiety (Levitsky, 2002). After the fermentation and absorption stage, satiety may also be affected by insulin and leptin, which convey information about the fat storage in the body (Blundell & Bellisle, 2013; Levitsky, 2002).

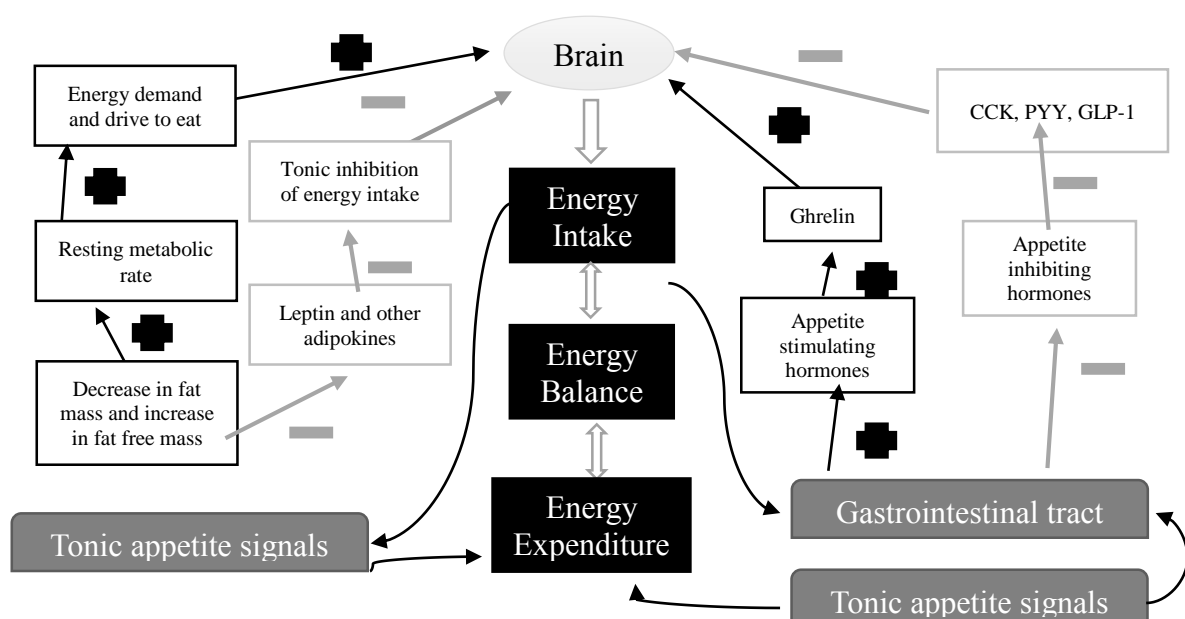


Figure 2-6 Relationship between appetite hormone regulation and energy balance
The figure was adapted from “Appetite control and energy balance impact of exercise” by Blundell et al. (2015).

In addition to these satiety responses, internal appetite signalling may be enhanced by food quality, quantity and palatability of the food and be negatively associated with the postprandial satiety response. The more palatable food is, the less satiety, and vice versa (Blundell & Bellisle, 2013). Palatability refers to one's evaluation of the pleasurable experience of consuming food under normal circumstances (Blundell & Bellisle, 2013; Levitsky, 2002; Polivy & Herman, 1987). Palatability of food stimulates a positive emotional response and increases the possibility for consumers to consume more after eating a meal (Levitsky, 2002). Palatability could be enhanced by a number of factors, including meal size, meal duration, number of bites and time spent chewing, increased sweetness, and increased fat content (Simone, Hennink, & Jeroen, 2013). Drewnowski (1998) found a positive association between energy density and the palatability suggesting humans' innate preference for high energy-density food, furthermore, as the negative association between satiety and palatability could potentially increase the consumption of energy-rich food. However, the review by Benelam (Levitsky, 2002), suggested that the positive association between food energy density and palatability could be manipulated by altering the taste and appearance of the food to improve the palatability, without significantly increasing the energy density of the food. Poortvliet et al. (2008) also reported the possibility of increasing both postprandial palatability and satiety responses after a meal while maintaining total energy. In the study, a healthy meal with high dietary fibre content and high protein content and low energy density was designed to be compared with a control food low in dietary fibre and protein, but significantly higher in energy density (approximately 2.16-fold higher). In response to the healthy meal, participants reported reduced palatability responses (i.e., reduced hunger, desire to eat, and prospective consumption (how much they thought they could eat?)) as well as significantly reduced subsequent energy intake after the meal (i.e., increased satiety) (Poortvliet, Bérubé-Parent, & Drapeau, 2007).

2.4.2 Satiety and its association with carbohydrates in food

The rate and extent of carbohydrate digestibility is directly and positively associated with satiety and, thus, appetite regulatory systems. As it is a direct energy source for the brain and body, blood glucose concentration is tightly controlled through integration of information from the glucose receptors in various sites in the body to the brain through neuronal pathways, including the pathways affecting energy intake (Marty, Dallaporta, & Thorens, 2007). This mechanism means that food with a slow glycaemic response is generally absorbed more slowly than food with a high glycaemic response resulting in

less variation in postprandial blood glucose concentration which in turn could increase satiety.

The review by Bornet, Jardy-Gennetier & Jacquet (2007) reported that of 26 short-term studies (within one day) that investigated the association between glycaemic response and its impact on satiety, 16 studies reported a positive association between a low glycaemic response and increased satiety after a meal despite some possible confounders, such as fibre content and palatability of food. In contrast, three randomised controlled trials (Alfenas & Mattes, 2005; Aston, Stokes, & Jebb, 2008; Das, Gilhooly, & Golden, 2007) compared the long-term satiety responses between diets consumed for eight days to six months with low or high glycaemic responses, and found no associations between meals with low glycaemic responses and long-term satiety responses. However, in all three randomised controlled trials, intervention and control diets were matched for macronutrient and dietary fibre proportions, and it is possible that dietary fibre and resistant starch could be the confounding factors that influence the long-term satiety (Alfenas & Mattes, 2005). In epidemiological studies, increased dietary fibre has been found to be associated with weight reduction and long-term glycaemic control over a period of months (Ludwig, 2003; Ludwig, Pereira, & Kroenke, 1999; Ludwig, 2002), while in short-term randomised controlled trials, dietary fibre has also been found to promote satiety responses and reduce food intake (Freeland, Anderson, & Wolever, 2009; Hamedani, Akhavan, & Samra, 2009; Samra & Anderson, 2007). Dietary fibre, a component of whole grain food, such as brown rice, delays gastric emptying and creates gastric distension (Darwiche, Björgell, & Almér, 2003) and the satiety-related hormone are released to signal fullness. Resistant starch, which is defined as a type of starch that resists starch digestion to up to 180 minutes after ingestion may have a similar satiating effect. Therefore, both of these two components may act as satiating ingredients in foods that are mainly carbohydrate.

Epidemiologic studies support an association between fibre intake and lower body weight. A systematic review of the effects of diets containing higher dietary fibre and whole grains with body weight reduction and the prevention of weight gain (Clark & Slavin, 2013). This review identified 44 randomised controlled trials and analysed 107 treatments and 38 sources of fibre. They reported that most acute fibre treatments (within 24 hours) did not enhance satiety (61%) or reduce food intake (78%) and concluded that neither fibre type or fibre dose were related to satiety response or food intake (Clark & Slavin, 2013). The authors suggested that favourable effects may be a

result of enhanced satiety and decreased food consumption in relation to specific fibre types and effects on fermentation and changes in gut hormones as possible mechanisms driving appetite control (Clark & Slavin, 2013; Näslund & Hellström, 2013). The two studies that examined resistant starch derived from corn however did not report enhanced satiety but did report reduced food intake.

Rice is generally considered as a highly digestible carbohydrate source, which induces rapid and high glycaemic response. However, the digestibility of rice could be reduced by a number of factors (see Sector 2.3 “Digestibility of starch in rice and blood glucose responses”), including retaining the whole grain physical form of rice (higher dietary fibre content in whole grain rice compared to refined rice), increased amylose to amylopectin ratio, increased particle size, parboiling treatment, and increased starch retrogradation (increase resistant starch and slowly digested starch) during prolonged cold storage. The study by (Chiu & Stewart, 2013) demonstrated in healthy adults no significant difference in appetite or blood glucose over two hours between short grain rice with 0.4g RS and rice containing 4.4 g RS. However, a more recent study by (Zenel & Stewart, 2015) reported compared to short grain rice (1 g RS), high amylose rice (2g RS) consumption was associated with significantly less feelings of hunger at 30 minutes and lower blood glucose at 60 minutes.

It is hypothesised that the reduced rice starch digestibility could lead to slow glycaemic responses and, therefore, a series of satiating factors could be triggered to promote satiety and reduce appetite. This is of particular interest in reducing the total energy intake and managing dietary intake by promoting and extending the postprandial satiety with long term implications for weight and glycaemic control and the prevention of chronic disease such as T2DM. Further research is necessary to understand the association between rice starch digestibility and the satiety and palatability responses.

2.5 Sensory evaluation of the liking of rice cooked in different ways

2.5.1 Sensory attributes evaluation of cooked rice products

Sensory analysis utilises different tools or tests that involve humans in assessing the quality characteristics of foods or food ingredients (Thompson, Drake, Lopetcharat, & Yates, 2004). Mainstream tests can be divided into two categories: affective and analytical. Affective tests utilise consumers to assess their perceptions of acceptability and are useful in exploring the role of flavour, texture and appearance in choice, acceptability and response (Heymann & Lawless, 2013). Analytical sensory tests are more objective than affective tests as they are required to have descriptive and

discriminatory responses such as intensity or duration of sensory stimulation. (Heymann & Lawless, 2013).

The three basic categories of sensory quality are texture, colour and flavour. Flavour is further divided into aroma and taste. A study performed to check the sensory and instrumental relationships of cooked rice texture revealed that rice texture was influenced by a variety of factors such as cultivar characteristics, postharvest handling practices, milling degree, drying conditions, final moisture and cooking method. Combined sensory and instrumental data revealed that sensory attributes accounted for most variation, and sensory descriptive analysis was more sensitive to subtle changes in initial texture perception parameters relating to stickiness and adhesiveness (Lyon, Champagne, Vinyard, & Windham, 2000).

Subsequent sensory evaluation of cooked rice indicated that the intensity of sensory hardness was the most important characteristic of cooked rice (Srisawas & Jindal, 2007). The overall acceptability based on appearance, texture and flavour attributes reached peak levels corresponding to optimum cooked moisture content for different rice cultivars, and was highly correlated with sensory hardness and stickiness. The study indicated that the acceptability ratings of cooked rice could be reliably predicted from the physicochemical properties such as the apparent amylose content, protein content, gel consistency, alkali-spreading value and grain elongation ratio of milled rice.

2.5.2 Diverse rice flavour preferences

Flavour perception is a response to compounds present in a particular food. It involves a complex series of reactions with the food and our nose, tongue and other parts of our mouth. The main ingredients that determine rice texture are rice starch, proteins and lipids that affect its cooking and eating quality and the proportion of amylose (Zhou, Robards, Helliwell, & Blanchard, 2002). Previous studies have reported that aroma and appearance were the most important acceptance factors for cooked rice, for Asian consumers living in the United States (Champagne, Wood, Juliano, & Bechtel, 2004; Zhou et al., 2002).

The headspace of fragrant rice products, such as basmati or jasmine rice, has shown that 2-acetyl-1-pyrroline is the main cause of the distinctive fragrance (Vandeputte et al., 2003a). Basmati grains contain 0.09 parts per million of the chemical compound 2-acetyl-1-pyrroline, which is about 12 times more than concentrations found in unscented rice products and gives basmati its distinctive spicy fragrance (Widjaja, Craske, & Wootton, 1996). The aroma combined with fine, slender grains and a soft,

fluffy texture after cooking has made basmati the world's most sought-after rice, commanding prices up to 10 times more than common rice on international markets (Yadav, Yadav, & Chaudhary, 2011). The desirability of fragrance has resulted in strong human preference and selection of aromatic rice.

2.5.3 Consumer liking and acceptability of cooked rice products

Consumer overall acceptability and cost are critical for survival of any product.

Consumer testing indicates liking or preference for a particular product and if there will be repeat buys. Liking or preference can be measured directly by comparing two or more products or indirectly by comparing product scores (Sidel & Stone, 1993).

Consumer perception and acceptance of food quality depends on a number of factors such as risk associated with different foods and ethical concerns (Frewer, Scholderer, & Bredahl, 2003). Consumers may either turn to food they commonly consume or that provides reassurance in terms of risk perception or they might switch to food with stronger images of health benefits (Aaker, 2009; Michell, King, & Reast, 2001).

2.5.4 Sensory evaluation of rice in New Zealand

Rice consumption is increasing in New Zealand, due in large part to the growing ethnic populations, particularly of South and East Asian peoples who eat rice as a staple in their diets. Asian cultivated rice has evolved into three eco-geographic races (indica, japonica and javanica). These cultivars have different quantities of amylose and amylopectin, which affect cooking quality and possible uses (Yadav et al., 2011).

Aromatic rice can be identified by its distinctive, nutty flavour, and a "popcorn-like" aroma. Basmati is an aromatic rice type that has had increased consumer acceptance in recent years. However, there is little information about the factors that contribute to the acceptability of rice flavour and how consumer preferences differ among rice products commonly available in local supermarkets.

Furthermore, the consumption of rice product varieties and rice cooking culture is not homogeneously distributed among various populations and strong regional differences have been observed. For example, after cooking, the parboiled rice grains stay firm and do not stick together like medium-grain white rice grains do, and they are more nutritious because the proteins and vitamins are diffused through the centre of the grain after parboiling pre-treatment. However, the hydrothermal process leaves the parboiled rice a pale yellowish colour, with a harder texture even after cooking and a typical flavour, which have been suggested as disadvantages for East Asian populations (Ong & Blanshard, 1995; Pillaiyar, 1988). In contrast, a past study has suggested that parboiled

rice with firmer texture and more separated grains and typical flavour is favoured by South Asian populations (Juliano, 1985). On the other hand, milled rice has the bran layer removed, resulting in partial loss of its micronutrients, including some minerals and vitamins, but the polished white kernel provides a creamy flavour and sticky texture, which may be generally liked by rice consumers in East and Southeast Asia (Barber & De Barber, 1991; Marshall & Wadsworth, 1993). For example, japonica product varieties, which are characterised by an adhesive and softer texture, are greatly favoured in Japan and other countries with a similar temperate climate (Barber & De Barber, 1991). Therefore, a study concerning the liking and the possibility of accepting “healthier” rice as a feasible alternative to the regular daily rice meal is important to obtain a better understanding of local population preferences and acceptance in Auckland, New Zealand.

2.6 Aim and study design

The overarching goal of the proposed series of experiments and investigations (Figure 2-7) was to determine whether rice selected and prepared in ways that have the potential to reduce the effect on blood glucose will be accepted by frequent rice consumers living in Auckland as an alternative to the more commonly consumed freshly cooked medium-grain white rice.

Based on the preliminary model of the relationship between fasting blood glucose concentration and daily GL (see Figure 2-4), the study investigated the digestible starch profile (i.e., the proportion of RDS, SDS and RS of four commonly consumed product varieties of rice), as well as *in vitro* the rate of glucose release and the rate of glucose assimilation in human participants and the chewing patterns and satiety and palatability and liking that may affect the general population’s choice.

In this study, the following research questions were addressed (Figure 2-7):

- Do different rice types and different cooking-storing methods affect the availability of glucose *in vitro* (digestion) and rice starch digestion profile?
- What is the optimal combination of rice type, cooking method and storing condition to effectively increase SDS and RS in cooked rice and reduce the speed and extent of human glucose responses (absorption and assimilation)?
- Is replacing rice of high glycaemic availability with rice of low glycaemic availability going to be accepted and adopted easily into their lives by the general New Zealand population living in Auckland?

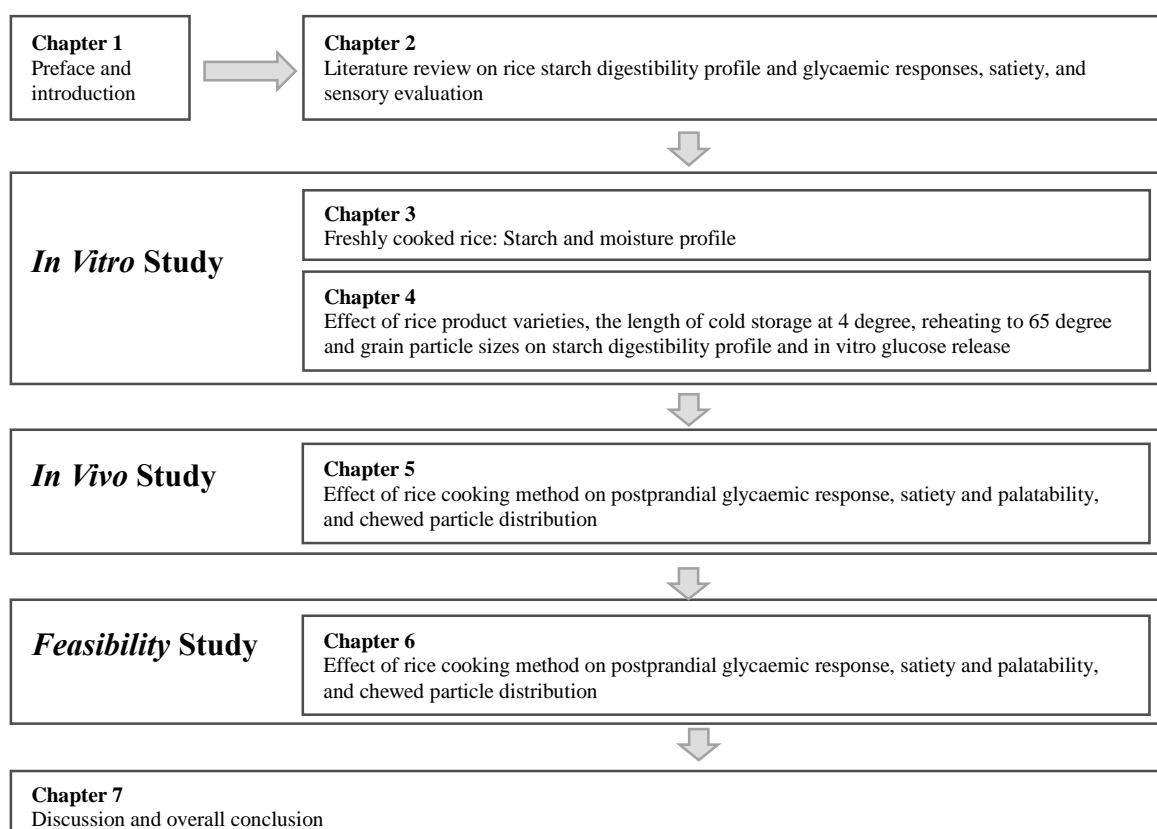


Figure 2-7 Study design and structure of the thesis

This group of studies hypothesised that:

1. The length of time of storage of cooked rice at 4 °C (0–24 hours) changes the level of SDS and RS and lowers the level of RDS and *in vitro* glucose availability (glycaemic glucose equivalent) in commonly consumed rice products (medium-grain white rice, medium-grain brown rice, parboiled long-grain white rice and basmati rice) to various degrees.
2. Compared with the most commonly consumed rice product, freshly prepared medium-grain white rice, the “cold-stored reheated rice” will have a slower rate of glucose absorption.
3. The reheated parboiled, medium grain brown, and medium grain white rice will have similar sensory attributes as the freshly cooked parboiled, medium grain brown, and medium grain white rice.

The primary objective of the body of work was to find a practical way to lower the proportion of rapidly digestible carbohydrates in rice and reduce the rate of glucose release.

This body of work (experimental chapters 3 to chapter 6) investigated:

1. The *in vitro* glycaemic potency of popular rice product varieties in New Zealand (medium-grain white rice, medium-grain brown rice, parboiled long-grain white rice, long-grain brown rice and basmati rice) and the impact of rice product varieties and storing-reheating on *in vitro* glucose release
2. The human glycaemic responses to popular rice product varieties and the impact of rice product varieties and cooling-reheating on human glycaemic response, chewing, satiety and palatability
3. The sensory liking and acceptance of popular rice product varieties and the influences of rice product varieties and the cooling-reheating process on consumer's liking and acceptability

This thesis aimed to add knowledge to current understanding of rice starch digestibility and *in vitro* and human glucose responses to various popular rice types and different cooking-storing methods. It also aimed to provide an evidence base for dietary management of blood glucose for the New Zealand population.

Chapter 3: Freshly cooked rice: Starch and moisture profile

Abstract

Globally rice provides approximately 27 % of the total dietary energy and more than 30 % for Indian and Chinese populations. Starch in refined white rice is rapidly digested. The consumption of white rice may be associated with a higher initial rate (within 20 minutes) and the extent (peak) of glucose release and starch digestion. This *in vitro* experimental study aimed to compare five commonly consumed rice products and describe the velocity and the extent of glucose release during *in vitro* enzymatic starch digestion over 180 minutes. No significant difference in total starch content was observed among the five uncooked rice products. After full gelatinisation (i.e., cooking), the *in vitro* glucose release (gram glucose / gram dry weight base) within the first 20 minutes of digestion showed that medium-grain white rice reached 78.4% (SD \pm 3.9%), basmati rice 41.5% (\pm 6.8%), medium-grain brown rice 36.5% (\pm 0.2%), long-grain brown rice 26.6% (\pm 2.3%) and parboiled rice 27.5 (\pm 5.8%) of the starch as glucose. After 180 minutes, the *in vitro* glucose release from whole grains of medium-grain white rice reached 98.3% (\pm 1.1%), basmati 87.7% (\pm 3.1%), medium-grain brown 76.5% (\pm 1.8%), long-grain brown 71.5% (\pm 2.5%) and parboiled 81.2% (\pm 1.0%) of total starch content. Among these five rice products, freshly cooked medium-grain white rice had the highest initial rate and final extent of *in vitro* glucose release over 180 minutes. Therefore, changing the daily staple grain to whole grain brown rice or parboiled rice may be a healthier eating choice. Other factors, such as cold storage time, reheating and grain particle disruption, need to be investigated further to determine the optimal cooking and preparation method.

Keywords: Rice, rapidly digestible starch, slowly digestible starch, resistant starch, glucose release.

3.1 Introduction

Rice is the primary grain and staple food for more than half of the world's population and provides approximately 27.0% of total dietary energy (Wailes & Chavez, 2012). It has been reported that, every day, the average Indian and Chinese adult consumes 208 g (31% of dietary energy) and 251 g (30% of dietary energy) of cooked rice respectively (Kennedy et al., 2004). Rice consumption is expected to increase globally in the next few years because it is cheaper than other staple grains (e.g., wheat and rye) and can be transported, stored and cooked more efficiently (Matsumura et al., 2009). In New Zealand, rice consumption is also expected to rise alongside the rapidly growing proportion of ethnic groups in the population that commonly consume rice (e.g., Indian, Chinese and Pacific populations), and vulnerable (low socio-economic status) populations, because of its low cost (Villegas et al., 2007).

The most widely consumed rice products (i.e., refined rice or white rice) (Kearney, 2010; Nanri et al., 2010; United States Department of Agriculture, 2014; Villegas et al., 2007) have the outer bran and germ portions of rice grains removed, which leaves the primarily starchy endosperm (Slavin, 2004). The endosperm contains more than 80% starch and less than 5% fibre and minimal micronutrients (Juliano, 1985; Slavin, 2004). White rice starch can be digested and absorbed rapidly, and this may be associated with a rise in postprandial blood glucose in healthy people with normal glucose regulation (Brand-Miller, McMillan-Price, et al., 2009; Nanri et al., 2010; Slavin, 2004).

The rate of rise of postprandial blood glucose is important because of its direct association with overall blood glucose homeostasis and the prevention and management of type 2 diabetes and the associated cardiovascular diseases and obesity (Bjorck, Granfeldt, Liljeberg, Tovar, & Asp, 1994; Brand-Miller, McMillan-Price, et al., 2009). The average glycaemic response of a group of people to a food is commonly estimated using the measures of glycaemic index (GI) and glycaemic load (GL). The GI relates the response to a test food containing a standardised quantity of available carbohydrate to that of pure glucose, or a reference food (e.g., fresh white bread) (Wolever et al., 1991). It was introduced by Jenkins (1981) to rank carbohydrate-based food products according to the postprandial glucose responses over three hours following consumption of a standardised quantity of carbohydrate in a food. Foods with a GI of > 70 were classified as high GI, GI 55–GI 70 were classified as medium GI, and GI < 55 was classified as low GI. The GL was introduced later as a measurement derived from GI to quantify the cumulative glucose-response-increasing potential of carbohydrate-

containing foods for the overall daily diet (Englyst et al., 2007). It is calculated by multiplying the amount of available carbohydrates in a specific food by the average GI for that food. This may be summed for all carbohydrate foods consumed over a day giving an estimate of the GL.

Rice is known as a starchy food with a relatively high GI (ranging from 60 to 100). Jenkins and colleagues reported a GI of 83 for freshly cooked medium-grain brown rice and 83 for white rice (when high GI food with values ranging from 60 to 100) (DJ Jenkins et al., 1981). Miller et al. have also indicated the high GI (ranging from 64 to 93) property of freshly cooked medium-grain white rice in their study (Miller, Pang, & Bramall, 1992). Slavin and colleagues (1999) reported that the mean GL of a cup (150 g) of cooked medium-grain white rice and basmati rice was equivalent to consuming 28 and 23 g of glucose respectively. Freshly cooked medium-grain white rice alone is estimated to contribute more than half the daily energy intake for Chinese and almost half for Indian ethnic groups (Abdullah, Ito, & Adhana, 2004; Kearney, 2010).

From a glycaemic status point of view, inclusion of foods in the daily diet that have a low glycaemic response (i.e., low GI and low GL) is considered beneficial, especially for individuals suffering from impaired blood glucose homeostasis. Consumption of white rice at least once a day by Indian and Chinese ethnic groups and vulnerable populations in New Zealand may pose an obstacle to the dietary management of people with abnormal glucose regulation (impaired fasting glucose or impaired glucose tolerance) and type 2 diabetes mellitus.

In order to lower the GI and GL of the daily diet without reducing daily carbohydrate energy intake, a number of studies have recommended replacing high GI carbohydrate rich foods with low GI carbohydrate foods (Grant et al., 2011; Stamatakis, Ludwig, Meier, & Wolf, 2002; Weiss, Diedrich, & Ludwig, 2002; Wolever et al., 1992). Replacement with other foods may be more expensive or not meet the other requirements for human consumption (Matsumura et al., 2009) and taste (Ludwig, 2002). An alternative would be to reduce the postprandial glycaemic response (GI and GL) to cooked white rice.

The GI or “glucose-response-increasing potential” of cooked rice can be lowered by changing the digestibility of the rice starch (Chung et al., 2006; Hu et al., 2004; Perdon et al., 1999). A number of studies have reported that both intrinsic and extrinsic factors may mediate the digestibility (Asp, 1996; Englyst & Hudson, 1996; Sajilata et al.,

2006b). Two important intrinsic factors are the quantity of dietary fibre present and the nature of the rice starch itself.

Firstly, brown rice, or whole grain rice, has a higher concentration of dietary fibre than white rice (i.e., refined rice) (Englyst & Hudson, 1996). The dietary fibre content of brown rice may significantly reduce the susceptibility to enzymatic degradation (i.e., amylolytic attack) both in the mouth and in the small intestine, slowing the rate of digestion and reducing the postprandial glycaemic response (Eggum et al., 1993; Englyst et al., 1987).

Secondly, rice starch in the endosperm is a mixture of the alpha-glucan polysaccharides, amylose and amylopectin. The amylose-to-amylopectin ratio depends on the botanical origin of the rice. 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). When starch is ingested and orally broken down during mastication, the amylose and amylopectin in starch granules are firstly partially hydrolysed by breaking down the α 1-4 glycosidic bonds by salivary α -amylase in saliva. The partially digested oligosaccharides and shorter polysaccharides reach the lumen of small intestine and are further hydrolysed by pancreatic α -amylase by breaking down the α 1-4 glycosidic bonds, by maltase by breaking down the maltose, by sucrase or isomaltase by breaking down the α 1-6 glycosidic bonds, leaving the glucose molecules to be absorbed in small intestine and the resistant starch to be passed into colon for fermentation in bacterial biota.

Previous studies of the variations in starch digestibility and absorption of glucose have been largely based on the measurements of GI and estimates of the GL. Englyst and Englyst (2005) 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), slowly digestible starch (SDS) and resistant starch (RS), that together constitute the total available starch (TAS), by measuring the amount of glucose released from one test food during incubation with amylase enzymes under standardised *in vitro* conditions over 180 minutes. RDS is defined as starch that can be digested within 20 minutes after ingestion. SDS is defined as starch that can be digested between 20 and 180 minutes after ingestion. RS is defined as starch that can resist digestion for up to 180 minutes. TAS is the sum of RDS + SDS.

The primary aim of this study was to compare the glycaemic impact (i.e., *in vitro* glucose release over 180 minutes) of five popular rice products – medium-grain white rice, medium-grain brown rice, basmati rice, long-grain brown rice and parboiled rice – and their starch digestibility profiles, by measuring the proportions and the amounts of RDS, SDS and RS obtained by timed *in vitro* digestion of the rice products.

It was hypothesised that:

- The magnitude of overall glycaemic impact (i.e., *in vitro* glucose release over 180 minutes) of five freshly cooked types of rice would be significantly different.
- Among the five freshly cooked rice products, medium-grain white rice would have the highest proportion of RDS among basmati rice, medium-grain brown rice, parboiled rice and long-grain brown rice.

3.2 Method

3.2.1 Selection of rice products

Five rice products from one of New Zealand's local high-turnover supermarkets (PAK'nSAVE, Palmerston North), medium-grain white rice (SunRice®), pure white basmati rice (King's Choice®), medium-grain brown rice (SunRice®), long-grain brown rice (SunRice®), and parboiled rice (Real Rice®) were purchased for all the experiments as single batches (see Table 3-1). The selection of rice products was based on empirical information gathered in 2013 from an Auckland Indian community nutritionist (Mrs Purvi Chchichia) and observation of the proportion of shelf space used for each product in supermarkets frequented by Indian and Chinese customers.

While it is recognised that the protein and lipid biosynthesis of the same rice cultivar/product were more sensitive to the different agro-climatic zones (e.g. environmental temperature), the starch fine structures (i.e. degree of branching) and the amylose content are not greatly affected (Seila et al., 2014). Therefore, the sample of rice product that were purchased at the time of experiment was able to represent the rice product cultivar.

Sample size (n=14) for each rice product was based on previous work by Mishra, Monro & Hedderley (2008) and standard operating procedure in laboratory practice, which determines the precision of a method, which is not reliant on biological variability.

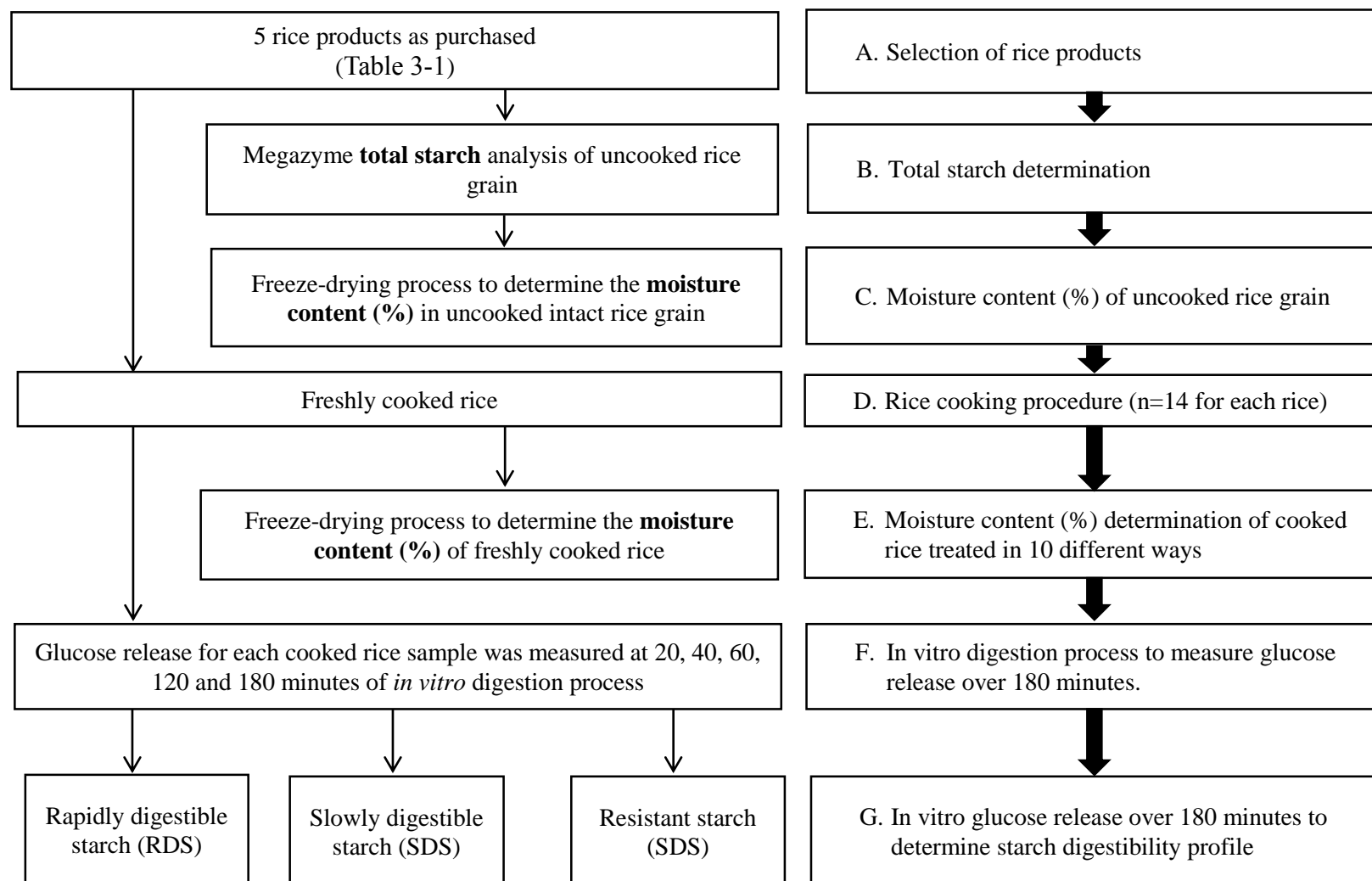


Figure 3-1 Flow chart of the experiment design and analytical steps to determine the starch digestibility profile of five rice products.

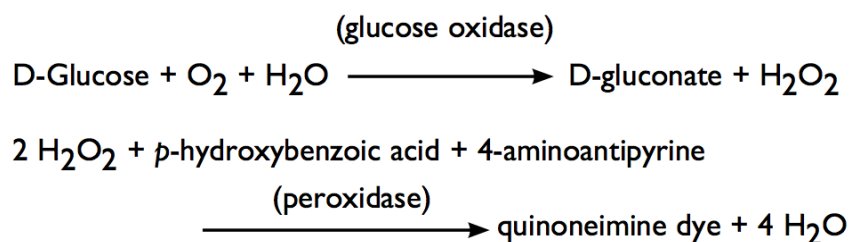
Table 3-1 Sample used in *in vitro* starch digestibility study all purchased from PAK'nSAVE (Auckland, New Zealand) on 20 May 2012.

Rice product	Country of origin	Brand	Batch no.	Expiry date
Medium-grain white	Australia	SunRice®	9310140283746	12/02/2014
Medium-grain brown	Australia	SunRice®	9310140283869	20/02/2014
Basmati	India	King's Choice®	9310130014923	18/03/2014
Long-grain brown	Australia	SunRice®	9310140283906	01/03/2014
Parboiled long-grain white	Thailand	Real Rice®	9421014797440	19/08/2014

3.2.2 Determination of total starch in rice as purchased

A highly reproducible and reliable enzymatic and acid hydrolysis procedure, the Megazyme total starch (TS) analysis procedure (AA/AMG) AACC Method 76-12 (McCleary, Gibson, Solah, & Mugford, 1994; McCleary, Solah, & Gibson, 1994) to determine the TS in 100 g of milled rice powder.

The method of total starch digestion followed the Megazyme method for total starch (McCleary, Gibson, et al., 1994). All assays were repeated at least twice and a third time if not within a pre-set tolerance to ensure precision. Digestion of rice to glucose under aqueous conditions was achieved in two phases: (1) partial hydrolysis (α -amylase) to maltodextrins; (2) complete enzymatic (amylglucosidase) hydrolysis of the dextrins to glucose. In the first phase, the RS was first converted to maltodextrins by stirring the powder sample with dimethyl sulphoxide at 100 °C. Starch was partially hydrolysed into soluble branched and unbranched maltodextrins by the addition of thermostable α -amylase (at pH 7.0, 100 °C). In the second phase, the assay of D-glucose was oxidised to D-gluconate with the release of one mole of hydrogen peroxide (H₂O₂), which was measured quantitatively in a colourimetric reaction that used the peroxidase process to produce a quinoneimine dye (two moles of H₂O₂ is equivalent to one mole of quinoneimine dye). The Megazyme Total Starch Assay Kit (AA/AMG) was used for TS determination (see Reagent list, Appendix 1).



3.2.3 Sample preparation

The five rice products were each milled to a very fine crystallised powder (of size estimated 100 nm in diameter) using a grain mill (Model K5-A, Whirlpool®, UK). To ensure no carry-over, the mill was dismantled and carefully cleaned by brushing between products. As is standard analytical laboratory practice all assays were duplicated and if varied by more than a predefined tolerance would be repeated. This method was standardised in this laboratory and used by others in other experiments so it was known what precision should be expected.

Duplicates of each milled product, 200 mg (± 0.9 mg), were weighed into glass tubes (16 \times 120 mm). Pure starch samples (202.1 ± 2.1 mg; provided in the Megazyme Total Starch Assay Kit) were also weighed into two glass tubes as the starch standard, and one reagent blank glass tube (containing 3 mL of distilled water only) was prepared.

All glass tubes were tapped to ensure that all the samples and standards dropped to the bottom of 13 tubes.

3.2.4 Phase one

Pre-treatment of resistant starch

Aqueous ethanol (0.2 mL 80% v/v, Megazyme Total Starch Assay Kit; see Reagent list, Appendix 1) was added to each glass tube containing milled rice powder or starch standard to aid dispersion. The glass tubes were mixed (vortex mixer, MT19, Chiltern, UK) for around 10 seconds. Immediately after mixing, 2.0 mL of dimethyl sulphoxide was added to each glass tube and mixed for around 10 seconds and placed in a vigorously boiling water bath (between 95 °C and 100 °C) for 5 minutes.

Partial hydrolysis

Thermostable α -amylase in 6 ml of MOPS buffer (Megazyme Total Starch Assay Kit; see Reagent list, Appendix 1) was added and the tubes were incubated in the boiling water bath for 6 minutes. After 6 minutes, the tubes were taken out of the boiling water bath (at 8, 10, 12 and 14 minutes) and immediately mixed vigorously by vortex.

3.2.5 Phase two

The glass tubes were then placed in a water bath at a maximum temperature of 50 °C, and 0.1 mL of the amyloglucosidase (AMGDF, Megazyme Total Starch Assay Kit; see Reagent list, Appendix 1) solution was added to each tube and mixed for 10 seconds, and incubated at 50 °C for 30 minutes. After the incubation, 10 mL of distilled water was added to dilute the solution to give a total of 20 mL in each glass tube. The tubes were then centrifuged at 3,000 rpm (Megafuge 2.0 R, HERAEUS, UK) for 10 minutes.

Two aliquots (0.1 mL) of the supernatant from each of the 13 tubes were transferred to clean glass tubes (26 clean glass tubes in total), and 3.0 mL of GOPOD Reagent (Megazyme Total Starch Assay Kit; see Reagent list, Appendix 1) was added to each of the glass tubes and incubated at a maximum of 50 °C for 20 minutes.

One D-glucose standard and one colour blank were prepared. The D-glucose standard was prepared by mixing 0.1 mL of D-glucose standard solution (1 mg/mL in 0.2% (w/v) benzoic acid, Megazyme kit) and 3.0 mL of GOPOD Reagent. The colour blank was prepared by mixing 0.1 mL of water and 3.0 mL of GOPOD Reagent.

The absorbance for each sample (starch standards, rice samples and blank, 28 tubes in total) and D-glucose standards, colour blank and the reagent blank were read in 1.5 mL microcuvettes at 510 nm (Unico 4802, Japan). The starch concentration was calculated accordingly (see Table 3-2). All results were averaged to obtain means. Standard errors of $\pm 2\%$ are achieved routinely with this method (McCleary, Gibson, et al., 1994).

3.2.6 Determination of moisture content (%) of uncooked rice

The moisture content (%) determined was the moisture removed by 24 hours of freeze-drying of intact uncooked rice grains. The moisture content (%) of around 40 g of uncooked rice was determined in duplicate. For each analysis, uncooked intact rice grains were weighed into a pre-weighed aluminium pot. Ten pots were sealed with aluminium foil. Ten to eight tiny holes (around 1mm in diameter) were punched on the aluminium foil sealed on each pot to allow water evaporation and prevent rice grains from escaping from the sealed pots. Samples were vacuum dried at 60 °C for 24 hours in a vacuum oven (Lab-Line Instruments Inc, USA) attached to a high-vacuum freeze drier (Flexi-Dry™ Microprocessor controlled, USA). The pots were left in the vacuum oven under vacuum until room temperature (21 °C to 23 °C) was reached. One at a time, foils were removed, and the pot and vacuum-dried rice samples were immediately weighed (to 0.001 g).

The amount of water in uncooked rice samples (g) was calculated by deducting the weight of “freeze-dried rice samples + pot” from the weight of “uncooked rice sample + pot”. The experiments were repeated if the duplicate differences in moisture content (%) were more than 1% precision (Table 3-2).

Table 3-2 Calculation of total starch concentration (g/100 g rice dry weight basis) in rice as purchased.

A.	Calculation of absorbance (A)
A _{Sample}	Samples (five rice products in duplicate)
A _{Starch standard}	Starch standard (pure starch powder in duplicate)
A _{Reagent blank}	Reagent blank absorbance (distilled water with reagent)
A _{D-glucose standard}	D-glucose standard (1 mg/mL) solution (1 mg/mL in 0.2% (w/v) benzoic acid; 2 × 1.5 mL microcuvettes; each was read five times)
A _{Colour blank}	Colour blank absorbance (2 × 1.5 mL microcuvettes; each was read five times)
ΔA_{Sample}	$= A_{\text{Sample}} - A_{\text{Colour blank}}$
$\Delta A_{\text{Starch standard}}$	$= A_{\text{Starch standard}} - A_{\text{Colour blank}}$
$\Delta A_{\text{Reagent blank}}$	$= A_{\text{Reagent blank}} - A_{\text{Colour blank}}$
B.	Calculation of moisture content (MC%)
$\Delta W_{\text{water removed (g)}}$	$= (W_{\text{unfreeze-dried rice + pot (g)}} - W_{\text{pot (g)}}) - (W_{\text{freeze-dried rice + pot (g)}} - W_{\text{pot (g)}})$
Moisture content (MC%)	$= \Delta W_{\text{water removed (g)}} / (W_{\text{freeze-dried rice + pot (g)}} - W_{\text{pot (g)}}) * 100\%$
C.	Calculation of total rice starch (g/100 g rice dry weight basis)
F (conversion factor for absorbance to μg)	$= 1 (\mu\text{g}/\mu\text{L of D-glucose}) / (A_{\text{D-glucose standard}} - A_{\text{Colour blank}})$
FV	$= \text{Final volume (mL)} = 20 \text{ mL}$
W	$= \text{The weight in (g) of the powder analysed}$
0.9 (Adjustment from free D-glucose to anhydro D-glucose (as occurs in starch))	$= 162/180$
Moisture content (MC%)	$= \text{Moisture content of uncooked rice sample in pot}$
Starch (g/100 g)	$= \Delta A * F * FV * 0.9 * (1/W)$
Starch (g/100 g rice wet weight basis)	$= \text{Starch}_{\text{sample (g/100 g)}} - \text{Starch}_{\text{Reagent blank (g/100 g)}}$
Starch (g/100 g rice dry weight basis)	$= \text{Starch (g/100 g rice wet weight basis)} * 100\% / (100\% - \text{MC}\%)$

3.3 Determination of starch profile of freshly cooked rice

3.3.1 Sample preparation

Each rice product (100.0 g) was weighed into a 600 mL glass beaker (KIMAX, USA) and 110 mL of distilled water was added into each beaker. The beakers were tightly sealed with aluminium foil to minimise moisture loss during cooking. All beakers were immersed in boiling water in a large cooking pan with a lid cover. Basmati, parboiled and medium-grain white rice samples were taken out of the cooking pan after 25 minutes. Medium-grain and long-grain brown rice samples were boiled for a further 20 minutes to achieve complete gelatinisation. Freshly cooked rice (at 100 °C) was rapidly cooled by running cold tap water around the glass beaker placed in the sink until the centre of the rice was cooled to 37 °C.

3.3.2 Determination of moisture content (%) of cooked rice products

Around 40 g (average 41.7 ± 4.7 g) of rice from the middle of each beaker were weighed into an aluminium container (100 mL) and immediately sealed with aluminium foil for the determination of moisture content (MC%). The same procedure as described in Section 3.2.6, “Determination of moisture content (%) of uncooked rice”, was followed (see Table 3-2).

3.3.3 *In vitro* digestion process to measure glucose release over 180 minutes

Two rice samples (5.0 ± 0.0 g each) from the middle of each beaker were weighed into two separate plastic pots (70 mL; Lab Serve LBS 30002) and tightly capped. The plastic pots were immediately transferred to the wells in a 15-well aluminium-heating block (in a water bath at 37 °C). A total of 10 pots were prepared. All plastic pots were kept tightly capped to prevent moisture loss until all plastic pots were ready for digestion.

All caps were removed from the pots. A single insulating sheet was placed on top of the pots. One magnetic stirrer was placed in each specimen pot. The mixture in each plastic pot was stirred slowly (130 rpm) at 37 °C while digestion proceeded. The digestion sequence involved a two-step digestion: gastric digestion followed by small intestine digestion (Figure 3-2).

Thirty millilitres of water and 0.8 mL of 1 M HCl were mixed with the rice sample to adjust to pH 2.5 (± 0.2). One millilitre of 10% pepsin solution in 0.05 M HCl was added to the sample while the mixture was stirred constantly (130 rpm) for 30 minutes at 37 °C to complete gastric digestion. Two millilitres of 1M NaHCO₃ and 5 mL 0.1 M Na maleate buffer were added to each specimen pot to neutralise the mixture to pH 6 to start the small intestinal digestion phase. To start amylolysis, 0.1 mL amyloglucosidase

(E-AMGDF, Megazyme Total Starch Assay Kit; see Reagent list, Appendix 1) and 5 mL of 2.5% pancreatin (P-7545, Sigma, St. Louis, MO, USA) in 0.1 M maleate buffer pH 6 were added, and distilled water was added immediately to the 55 mL mark on the specimen pot and the digestion continued at 37 °C. The insulation sheet was removed when the reagents were being added to each plastic pot and replaced when incubation started (Figure 3-2).

At 20, 40, 60, 120 and 180 minutes from the start of the pancreatic digestion, digesta aliquots (1.0 mL) were extracted to measure the time course of release of glucose, and added to 4 mL absolute ethanol in a clean test tube to stop the digestion immediately. After at least 30 minutes, the tubes were centrifuged for 10 minutes at $1000 \times g$ at 20 °C (Centrifuge Omnifuge 2.0 RS, Heraeus Sepatech, Hanau, Germany) to clarify the supernatant before the sugar analysis. All aliquots were duplicated for higher precision and minimising between sample variations. The whole experiment was then repeated again.

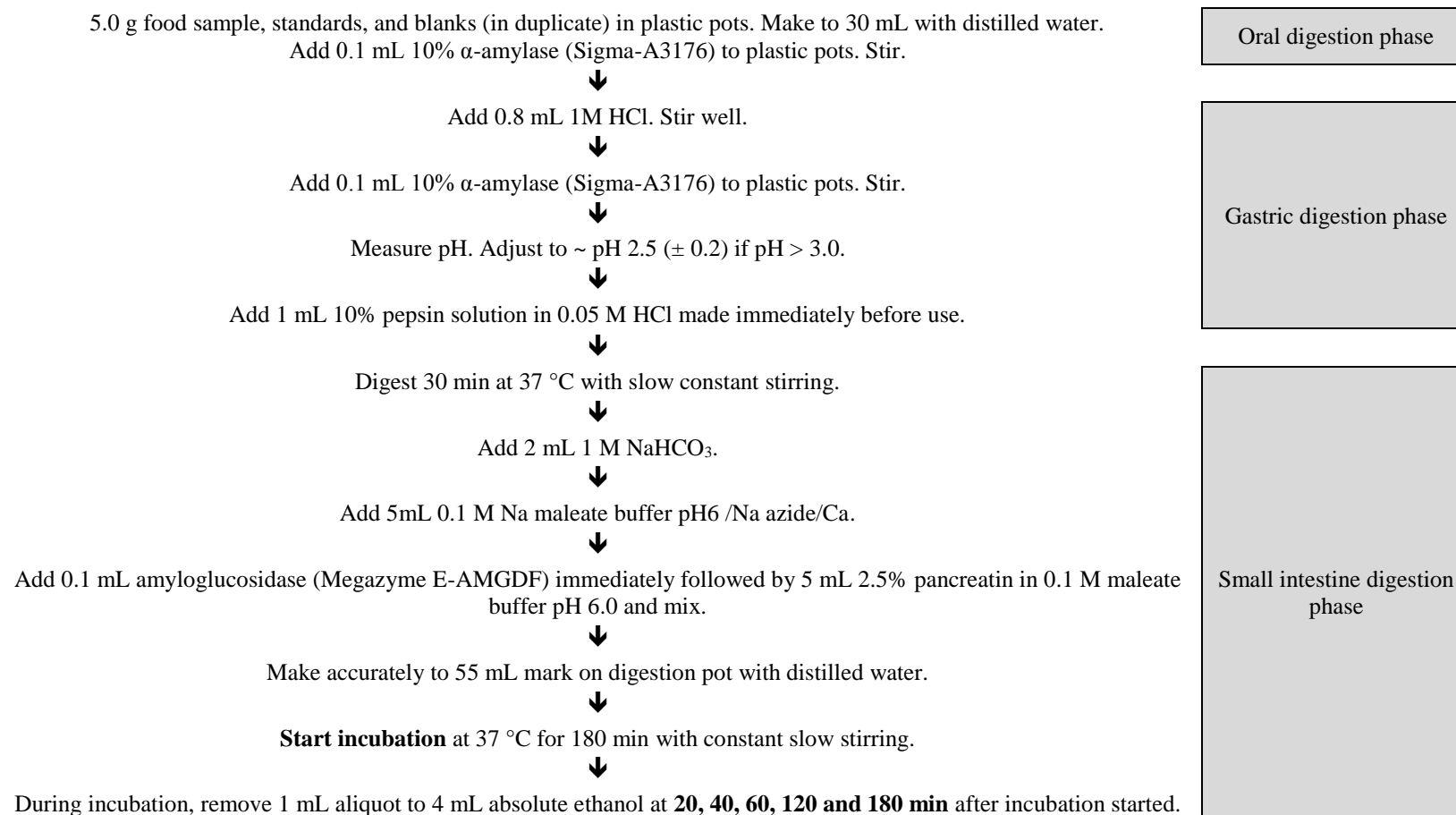


Figure 3-2 Protocol for *in vitro* release of glucose over 180 minutes.

3.3.4 Measuring glucose released during digestion

The absorbance was measured for each of the aliquots. The amount of glucose released during digestion was measured as monosaccharaides by a small-scale modification of the dinitrosalicylic colourimetric method (Englyst, Kingman, Hudson, & Cummings, 1996). A 0.05 mL aliquot of the ethanolic sample was added to 0.25 mL enzyme solution A (1% invertase + 1% amyloglucosidase in acetate buffer pH 5.2) in a test tube, mixed and rested for 10 minutes at room temperature for secondary digestion (depolymerisation of maltose and limit dextrins to monosaccharaides) before adding 0.75 mL dinitrosalicylic reagent (0.5 mg/mL glucose:4M NaOH:3,5-dinitrosalicylic acid reagent mixture in ratio 1:1:5) to the mixture. The tubes were mixed, covered and heated for 15 minutes at 95 °C to 100 °C. The tubes were cooled and 4.0 mL water was added to each tube. The absorbance of the mixture was read at 530 nm (Jenway 6100 UV-Spectrophotometer, Felsted, UK). The experimental procedures were repeated with D-glucose standard (1 µg/µL glucose) and colour blank (mixture of distilled water and reagent).

The absorbance for each sample was derived from the average absorbance of duplicate aliquots. The sample absorbance (ΔA) and the standard absorbance (ΔA standard) were calculated using these formulas. The concentration of glucose released from cooked rice samples at 20, 40, 60 120 and 180 minutes was calculated by the formula shown in Table 3-3.

Table 3-3 Calculation of glucose release (g/100 g rice dry weight basis).

A.		Absorbance (A)
A_{Sample}	= Samples (5 rice products * 5 aliquots at 20, 40, 60, 120 and 180 min * duplicate)	
$A_{\text{D-glucose standard}}$	= D-glucose standard (1 $\mu\text{g}/\mu\text{L}$) solution (1 mg/mL in 0.2% (w/v) benzoic acid; 2*1.5 mL microcuvettes; each was read five times)	
$A_{\text{Colour blank}}$	= Colour blank absorbance (2*1.5 mL microcuvettes; each was read five times)	
ΔA_{Sample}	= $A_{\text{Sample}} - A_{\text{Colour blank}}$	
B.		Calculation of moisture content (MC%)
$\Delta W_{\text{water removed (g)}}$	= $(W_{\text{unfreeze-dried rice + pot (g)}} - W_{\text{pot (g)}}) - (W_{\text{freeze-dried rice + pot (g)}} - W_{\text{pot (g)}})$	
Moisture content (MC%)	= $\Delta W_{\text{water removed (g)}} (W_{\text{freeze-dried rice + pot (g)}} - W_{\text{pot (g)}}) * 100\%$	
C.		Calculation of <i>in vitro</i> glucose release (g/100 g rice dry weight basis) over 180 minutes
F (conversion factor for absorbance to μg)	= $1 (\mu\text{g}/\mu\text{L of D-glucose}) / (A_{\text{D-glucose standard}} - A_{\text{Colour blank}})$	
FV @ 20 min = Final volume (mL)	= 55 mL	
FV @ 40 min	= 54 mL	
FV @ 60 min	= 53 mL	
FV @ 120 min	= 52 mL	
FV @ 180 min	= 51 mL	
W (g)	= The weight of cooked rice sample in plastic pot	
Moisture content (MC%)	= Moisture content of cooked rice sample	
Glucose (g/100 g rice wet weight basis)	= $\Delta A_{\text{Sample}} * F * FV * (1/W) * 5\text{-fold dilution}$	
Glucose (g/100 g rice dry weight basis)	= $\text{Glucose (g/100 g rice wet weight basis)} * 100\% / (100\% - \text{MC}\%)$	

The glucose release (g/100 g rice dry weight basis) was then plotted against the duration of digestion (0, 20, 40, 60, 120 and 180 minutes; see Figure 3-3). The glucose release (g/100 g rice dry weight basis) at time 0 of incubation was estimated to be 0 g starch/100 g rice dry weight basis for the purpose of determining the starch profile. The starch digested from the rice sample in the plastic pot in the oral phase of digestion was included in the first 20 minutes of glucose release in order to accurately determine the RDS fraction in the total digestible starch.

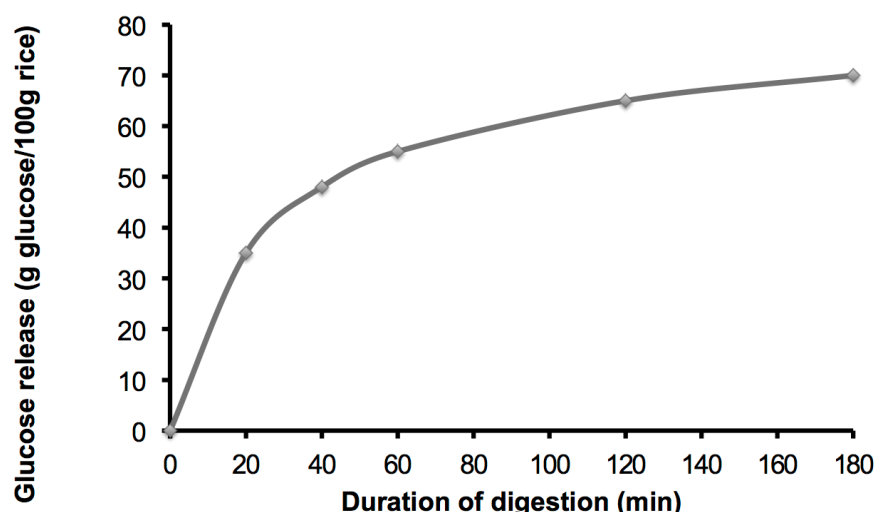


Figure 3-3 Estimated glucose release curve of one rice sample.

3.3.5 Determination of starch digestibility profile derived from glucose release over time

The amount of starch in 100 g of cooked rice on a dry weight basis that was released during the *in vitro* digestion process at each time point was then calculated. The concentration of starch (g/100 g rice wet weight basis) and starch digested (g/100 g rice dry weight basis) in cooked rice was calculated by the following formula:

Moisture content (MC%)	= Moisture content of cooked rice sample in pot
0.9 = Adjustment from free D-glucose to α -D-glucose (as occurs in starch)	162/180
Starch (g/100 g rice wet weight basis)	= Glucose (g/100 g rice wet weight basis) * 0.9
Starch digested (g/100 g rice dry weight basis)	= Glucose (g/100 g rice wet weight basis) * 0.9 * $\frac{1}{(100\% - MC\%)} * 100\%$

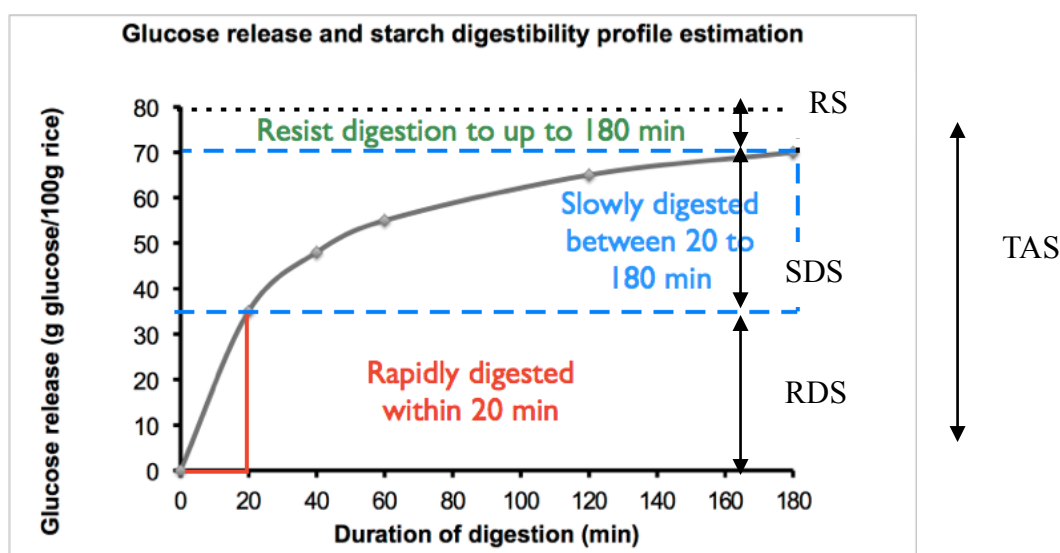


Figure 3-4 Estimation of the starch digestibility profile (rapidly digestible starch, slowly digestible starch and resistant starch) was derived from the glucose release (g per 100 g of cooked rice dry weight basis) over time.

Four rice fractions were calculated accordingly. These were TAS (can be digested within 180 minutes), RDS (can be digested within 20 minutes), SDS (can be digested within 20 to 180 minutes) and RS (undigested at 180 minutes; see Figure 3-4). The starch fractions (TAS, RS, RDS and SDS) and the proportions of each starch fraction were calculated by the following formulas:

Total available starch (TAS) (g/100 g rice dry weight basis)	= Starch digested within 180 min (g/100 g rice dry weight basis)
Resistant starch (RS) (g/100 g rice dry weight basis)	= Total starch (g/100 g rice dry weight basis) – TAS (g/100 g rice dry weight basis)
Rapidly digestible starch (RDS) (g/100 g rice dry weight basis)	= Starch digested within 20 min (g/100 g rice dry weight basis)
Slowly digestible starch (SDS) (g/100 g rice dry weight basis)	= TAS (g/100 g rice dry weight basis) – RDS (g/100 g rice dry weight basis)
Total available starch (TAS) (%)	= Total available starch (TAS) (g/100 g rice dry weight basis) / Total starch (TS) (g/100 g rice dry weight basis) * 100%
Rapidly digestible starch (RDS) (%)	= Rapidly digestible starch (RDS) (g/100 g rice dry weight basis) / Total starch (TS) (g/100 g rice dry weight basis) * 100%
Slowly digestible starch (SDS) (%)	= Slowly digestible starch (SDS) (g/100 g rice dry weight basis) / Total starch (TS) (g/100 g rice dry weight basis) * 100%
Resistant starch (RS) (%)	= Resistant starch (RS) (g/100 g rice dry weight basis) / Total starch (TS) (g/100 g rice dry weight basis) * 100%

3.4 Statistical analysis

3.4.1 Comparison of glucose release curve

In vitro glucose release of each test rice product was plotted against time. Comparisons were made among values from five freshly cooked rice products to test the hypothesis that the medium-grain white rice has the highest glucose release over time. Differences in glucose release among the five different rice products ($n = 14$) at each time point (0, 20, 40, 60, 120, and 180 min) were determined by repeated measures ANOVA.

3.4.2 Comparison of starch digestibility profile

Comparisons were made among proportions of TS, TAS, RDS, SDS and RS (g/100 g rice dry weight basis) and MC (%) from five freshly cooked rice products ($n = 14$) to test the hypothesis that the medium-grain white rice has the least healthy profile among the five rice products. The differences were assessed by one-way analysis of variance (ANOVA) followed by a t-test for independent samples.

3.4.3 Determination of the differences

TS, TAS, RDS, SDS and RS (g/100 g rice dry weight basis) and MC (%) were subjected to repeated measured one-way analysis of variance (ANOVA) followed by a t-test for independent samples ($n = 14$). The significance level was $P < 0.05$. The retrogradation effect analyses were carried out by Duncan's multiple range test with the significant level of $P < 0.05$. All analysis was performed using Excel for Windows XP, version 2010 (Microsoft, USA) and SPSS for Windows XP, version 2.0 (IBM, USA)

3.5 Results

3.5.1 Determination of total starch content (g/100 g rice)

All five uncooked rice products contained over 80% starch (Table 3-4); the overall average was 85.4 ± 2.3 g/100 g. Medium-grain white and basmati rice products were similar, but parboiled had the highest (87.4 ± 2.5 g/100 g) and medium-grain brown rice had the lowest TS content (82.9 ± 0.8 g/100 g, $P < 0.001$). Refined white rice products (medium-grain white rice, basmati rice and parboiled long-grain white rice) had an average starch content of 87.1 ± 0.4 g/100 g, and wholegrain rice products (medium-grain brown rice and long-grain brown rice) had a higher average starch content of 83.0 ± 0.1 g/100 g ($P = 0.01$).

After cooking to full gelatinisation, the average starch content as a percentage of total mass dropped by more than 50% to 40.1 ± 1.5 g/100 g (Table 3-4) with no significant differences among the five cooked rice products.

Table 3-4 Total starch content, moisture content and cost for the five rice products (mean and 95% Confidence Interval). A total of 14 experiments repeated on the same rice product (N=14).

Rice products	Total starch (g)	Cost (NZ\$) ³	Moisture content (%) ¹	Total starch (g)	Cost (NZ\$) ³
	mean	mean	mean	mean	mean
	(95% Confidence Interval)		(95% Confidence Interval)	(95% Confidence Interval)	
	/100 g rice wet weight basis ¹	/100 g rice wet weight basis ¹	Of rice wet weight basis	/100 g rice dry weight basis ²	/100 g rice dry weight basis ²
Uncooked					
Medium-grain white	87.1 (86.1, 88.1) a	\$0.27	12.5 (12.5, 12.6) a	97.6 (96.6, 98.7) a	\$0.31
Medium-grain brown	82.9 (82.5, 83.3) abc	\$0.36	11.4 (11.4, 11.4) b	91.7 (91.2, 92.2) ab	\$0.42
Basmati	86.6 (86.0, 87.2) b	\$0.37	8.9 (8.8, 9.1) abc	93.3 (92.9, 93.7) ab	\$0.39
Long-grain brown	83.0 (81.4, 84.6) abc	\$0.30	9.4 (8.8, 10.0) abc	89.8 (88.6, 91.0) ab	\$0.33
Parboiled	87.4 (86.1, 88.7) c	\$0.19	11.4 (11.1, 11.7) c	96.8 (95.0, 98.6) b	\$0.21
Cooked					
Medium-grain white	39.8 (38.7, 40.9)	\$0.13	57.8 (58.7 to 58.8)		
Medium-grain brown	38.8 (38.8, 38.8)	\$0.18	57.5 (57.4, 57.6)		
Basmati	41.9 (39.4, 44.4)	\$0.18	55.4 (52.9, 57.9)		
Long-grain brown	38.6 (37.0, 40.1)	\$0.14	53.4 (52.5, 54.2)		
Parboiled	41.3 (39.5, 43.1)	\$0.09	55.9 (55.1, 56.7)		

¹ Wet weight basis = rice grain without freeze-drying process

² Dry weight basis = rice grain after being freeze-dried

³ The price is based on purchase price at Countdown (Woolworths) supermarkets in August 2012. For this chain, prices are the same throughout New Zealand.

Within a column of uncooked or cooked rice products, means (95% confidence interval) followed by the same lowercase letter are significantly different compared with other values with the same letter (P < 0.05, independent t test).

3.5.2 Determination of moisture content by freeze-drying (%)

The average moisture content (%) determined by freeze-drying of the five uncooked rice products was 10.7% ($\pm 1.5\%$). Moisture content of medium-grain rice products (medium grain white and brown rice products) and parboiled rice was $\sim 2\%$ higher than the long-grain rice products (basmati and long grain brown).

After cooking, the average moisture content increased approximately fivefold to 57.3% ($\pm 2.8\%$, $P < 0.001$). Long-grain brown rice had the lowest moisture content ($53.4 \pm 1.7\%$, $P = 0.01$).

3.5.3 Determination of total starch content (g/100 g rice) after freeze-drying

After freeze-drying, the TS content of medium grain white and parboiled rice were higher than those in medium grain brown, basmati, and long grain brown rice products ($P < 0.05$), though the differences in TS content by weight was around 5.0% (Table 3-4).

3.5.4 Determination of relative cost (NZ dollar)

The cost of 100 g of rice (as purchased – non-freeze-dried uncooked) ranged from 19 to 37 cents (see Table 3-4), with parboiled rice being half the price of basmati rice. For 100 g of cooked rice, parboiled rice cost the least (9 cents/100 g), and basmati and wholegrain rice cost the most (17 cents/100 g).

3.5.5 Comparison of glucose release (g/100 g rice dry weight basis), net glucose release (g/100 g rice dry weight basis), time returning to glucose disposal baseline (min), and area under the curve (AUC) during *in vitro* digestion process

Medium-grain white rice had significantly higher overall *in vitro* glucose release compared with the other four rice products ($P < 0.001$). Basmati rice had the second highest overall *in vitro* glucose release compared with the other four rice products ($P < 0.001$). Long-grain brown rice had the lowest overall glycaemic release ($P = 0.01$, Figure 3-5).

By 20 minutes, medium-grain white rice had a significantly sharper rise in glucose release (from 0.0 to 78.4 g of glucose release per 100 g freeze-dried rice) (Figure 3-5). At 20 minutes, medium-grain brown rice glucose release was also significantly higher (around 10%) than long-grain brown and parboiled rice ($P=0.010$) (Figure 3-5). After around 50 minutes, the glucose release of parboiled rice started to climb significantly faster than long-grain brown rice (10% higher at each time point between 50 ($P=0.05$) and 180 minutes ($P=0.010$; Figure 3-5).

At 40 minutes, glucose release of medium-grain white rice reached 94.5% (± 4.8) of the available starch with only a very slow and insignificant increase occurring in the

following two hours from 94.5% (± 4.8) to 98.3% (± 0.8) of the available starch (Figure 3-5). Compared with medium-grain white rice, the other four rice products had significantly slower increases in glucose release from 40 to 180 minutes ($P < 0.05$). Basmati rice reached 85% of the available starch at 120 minutes, increasing to approximately 90% at 180 minutes (Figure 3-5).

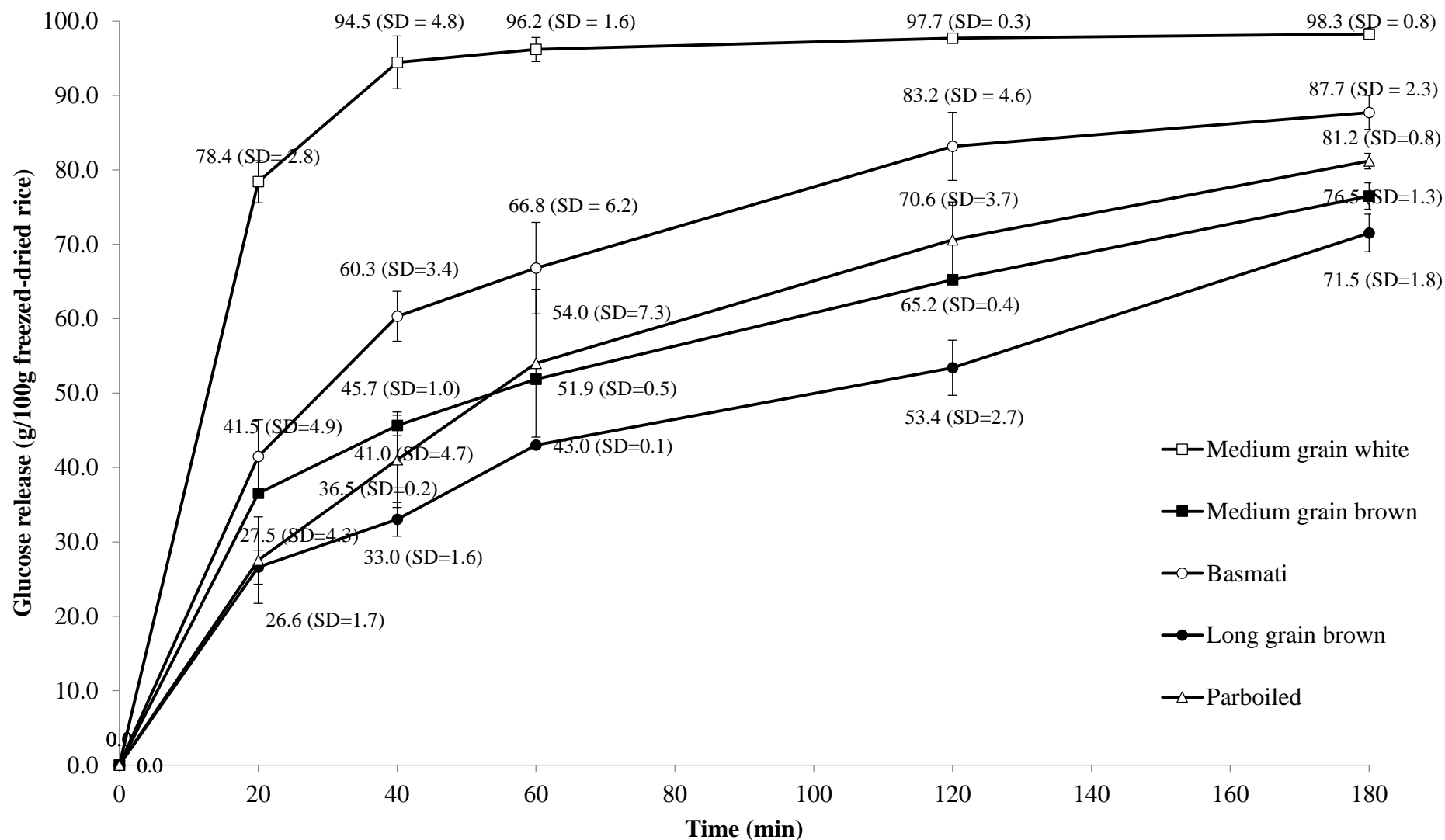


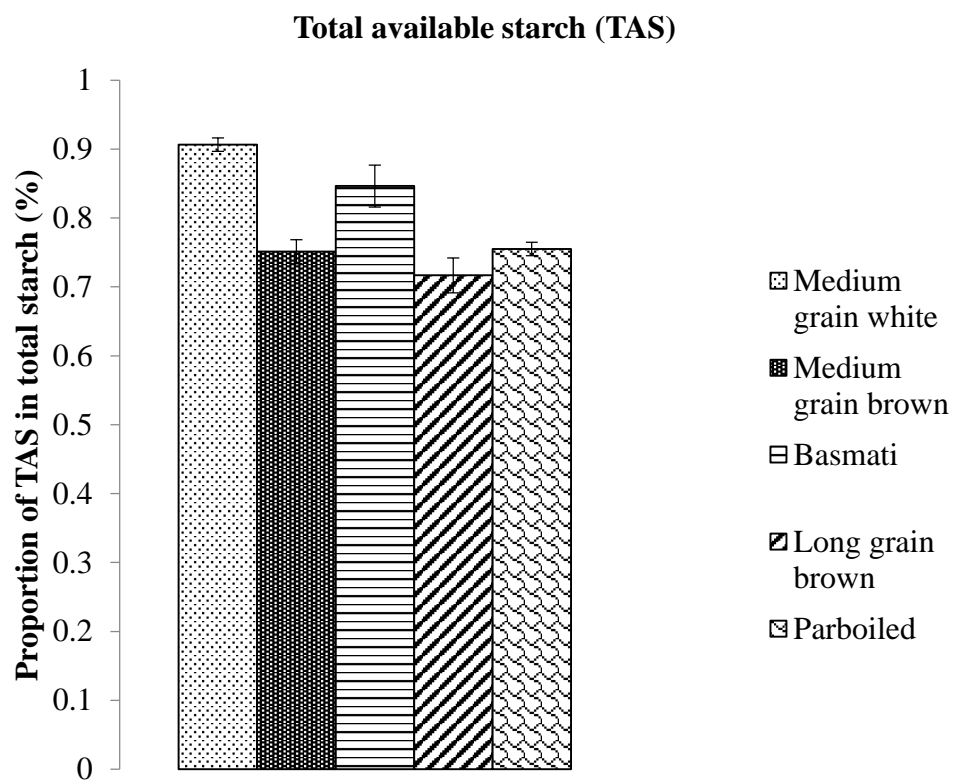
Figure 3-5 Average glucose release as a percentage of available starch within 180 minutes (at 0, 20, 40, 60, 120 and 180 minutes) of *in vitro* digestion process (oral, gastric and small intestinal digestion process) of the five freshly cooked rice products. Interpolation between measured points was plotted (N=14 for each rice sample) using the Excel function. Error bars show the SD of the mean measured value.

3.5.6 Comparison of starch digestibility profiles

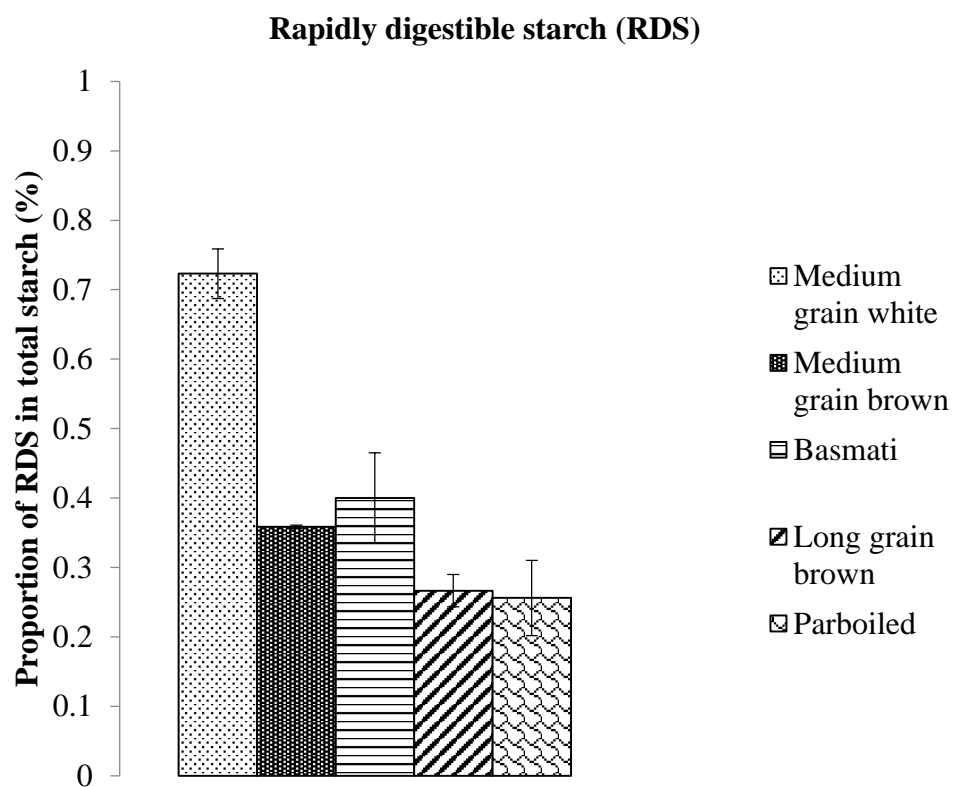
For all rice products, the TAS (i.e., starch that can be released as glucose in simulated gastrointestinal digestion over 180 minutes) was over 70% of the TS (Figure 3-6a).

Medium-grain white rice had a slightly higher (approximately 5%, $P = 0.040$) TAS than basmati rice, which in turn was nearly 15% higher than the other rice products. No significant differences were found among parboiled, long-grain brown and medium-grain brown rice.

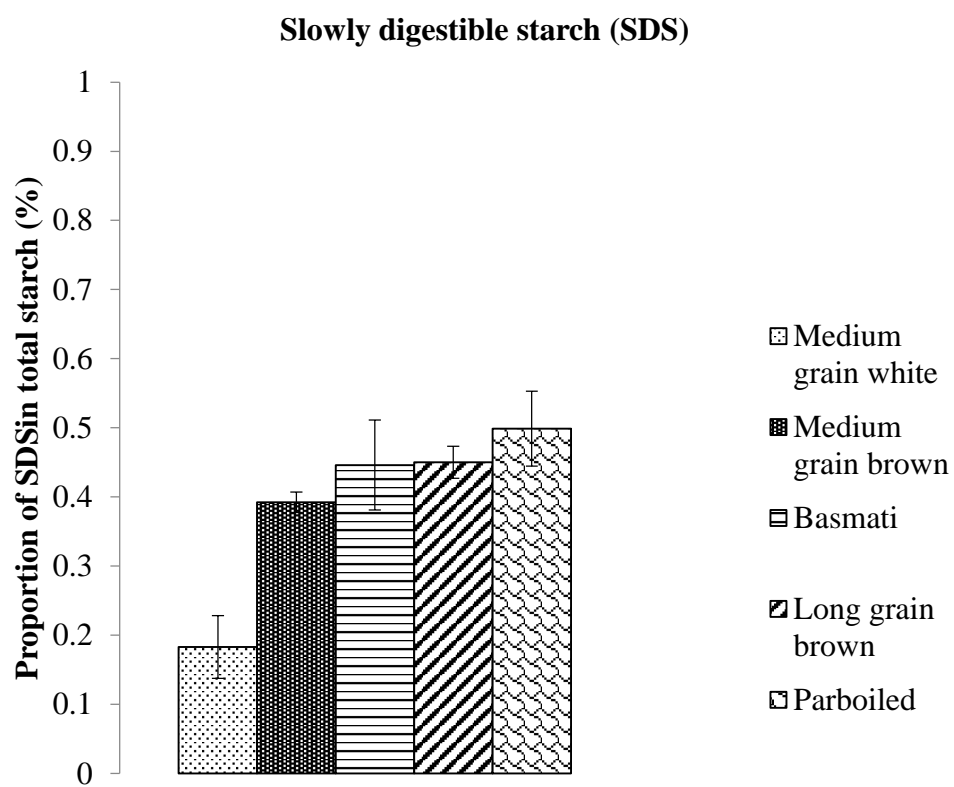
When compared with the other four rice products, medium-grain white rice had a significantly greater proportion (35%, $p < 0.001$) of RDS (starch released as glucose in the first 20 minutes) (Figure 3-6b). Basmati and medium-grain brown rice had similar RDS content ($P > 0.05$). Medium grain brown rice had slightly higher RDS content than long grain brown and parboiled rice ($P < 0.05$). However, RDS in basmati was not significantly higher than long grain brown and parboiled rice ($P = 0.05$). Basmati, medium-grain brown, long-grain brown and parboiled rice had similar SDS (20 to 180 minutes) content, which were significantly higher (approximately 25% greater) than for medium-grain white rice ($P < 0.001$) (Figure 3-6 c). Medium-grain brown, long-grain brown and parboiled rice had approximately 15% higher RS (undigested at 180 minutes) content than medium-grain white and basmati rice ($P < 0.001$) (Figure 3-6d). No significant differences in RDS, SDS or RS were found among medium-grain brown, long-grain brown and parboiled rice (Figure 3-6 b, c & d). Overall, parboiled rice had the highest proportion of SDS (approximately 50%) and a lower proportion of RS (approximately 5% lower, $P < 0.001$) (Figure 3-6d).



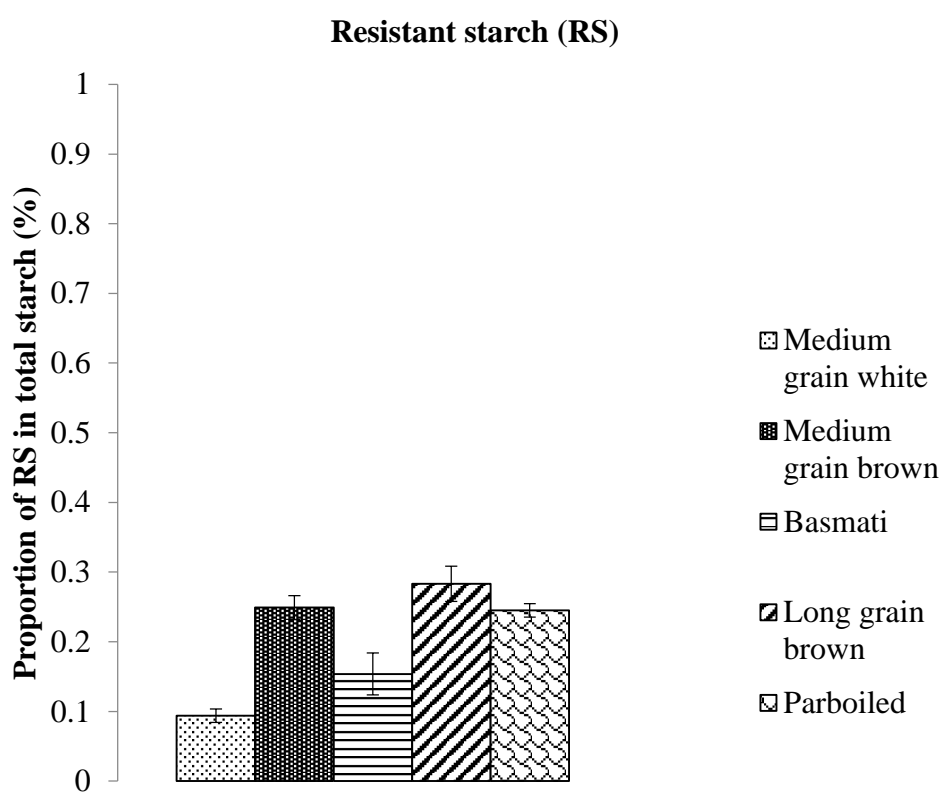
(a)



(b)



(c)



(d)

Figure 3-6 Comparison of starch digestibility profiles among five freshly cooked rice products (n = 14) ((a) TAS total available starch, (b) RDS rapidly digestible starch, (c) SDS slowly digestible starch and (d) RS resistant starch).

3.6 Discussion

The glucose release trajectory over 180 minutes for cooked medium-grain white rice was clearly faster than it was for the other four rice product varieties (medium-grain brown, basmati, long-grain brown and parboiled rice) despite less than 5g/100g differences in TS content (all >89% of dry weight) of the uncooked rice products. The rate and the extent or quantity of the glucose release determines the starch digestibility profile. Compared with medium grain brown, long grain brown, and parboiled rice, the TAS of medium-grain white rice was one-fifth greater (Figure 3-6a), that is, the amount of glucose released over 180 minutes. Within the first 20 minutes (Figure 3-6b), 70% was released; this RDS was double that of the other product varieties. Only half as much glucose was released from medium grain rice compared with others between 20 and 180 minutes (Figure 3-6 c) and half as much starch remained undigested after 180 minutes (Figure 3-6 d, 10%). The three main explanations for these different trajectories is that the starch digestion and the following glucose release can be directly affected by (1) the milling and polishing process (refined vs. whole), (2) the rice grain structure (grain size medium vs. long) and (3) parboiling treatment (pre-cooked). Each of these factors in relation to medium-grain white rice will be considered in turn.

First, physical structures, such as the bran, may decrease enzyme accessibility (Syahariza et al., 2013; Goddard, Young, & Marcus, 1984; Miller et al., 1992) and therefore reduce enzyme hydrolysis over 180 minutes. There are four types of RS (RS1 to RS4), which determine the starch availability in rice. White grain (refined) rice is milled and polished, which destroys or removes the physically inaccessible or digestible RS (RS1) and in turn accelerates the rate of starch breakdown and glucose release (Sajilata et al., 2006b). Consistent with previous studies (Eggum et al., 1993; Lehmann & Robin, 2007; Walter, da Silva, & Denardin, 2005; Zhang & Hamaker, 2009), in this study, within 20 minutes after *in vitro* digestion began, the glucose release from refined rice products (medium-grain white rice and basmati but not parboiled, which was pre-treated) was more than twice that of the whole grain rice (medium-grain and long-grain brown rice); after 40 minutes, the glucose release of medium-grain white rice almost reached 94.5% ($\pm 4.8\%$) and basmati rice reached only 60.3% ($\pm 4.6\%$), whereas the whole grain rice product varieties reached around 30%; after 180 minutes, medium-grain white rice and basmati released most of the glucose (98% and 80% respectively). In comparison, the whole grain rice released only 60%. Both white rice products (medium-grain white and basmati rice) had more than 20% higher proportion of TAS

than both brown rice products (medium-grain and long-grain brown rice), which in terms of energy/bioavailability but not glycaemia is an advantage.

Secondly, grain shape, size (long- versus medium-grain rice) and internal structure can also impact on the different glucose release trajectory and thus starch digestibility. A number of previous studies have reported that starch in long-grain rice has higher amylose-to-amylopectin ratios than medium-grain rice (Kim, Mullan, Hampson, & Pluske, 2006; Sajilata et al., 2006b). The grain structural difference can be attributed to the different amount of RS (RS2) in cooked rice starch. Polymerisation is higher in amylose ($950\text{--}1000 \times 10^3$ glucose) than in amylopectin ($19\text{--}22 \times 10^3$ glucose) (Juliano, 1992); the tightly twisted helix of amylose chains is less accessible to pancreatic α -amylase (Black, 2001; Goddard et al., 1984; Miller et al., 1992; Stephen & Phillips, 2006), and as a result, cooked long-grain rice starch which has a high amylose-to-amylopectin ratio can resist enzymatic attack and starch digestion longer than medium-grain (Chung et al., 2006; Hu et al., 2004).

This study has shown that basmati rice and long-grain brown rice had overall less TS digestion and at a lower digestion rate than counterparts (medium-grain white rice and medium-grain brown rice) respectively (around 25% and 15%) which could be explained by the higher amylose content of long grain rice. A higher proportion of RS (RS2) (around 10%) was also found in long-grain rice than in the medium-grain rice products (medium-grain white rice and medium-grain brown rice), which was in agreement with previous studies (Kim, Je, Jernigan, Buckley, & Whitten, 2006; Walter et al., 2005) and within the range of a previous systematic review (Landon, 2007).

One interesting observation was that basmati rice, compared with medium-grain white rice, and had 25% less TAS. Compared with medium-grain brown rice, long-grain brown rice and parboiled rice, basmati had around 7% more TAS. As a result, basmati rice had half the RDS but double the RS of medium-grain white rice and a slightly higher proportion of RDS but significantly lower RS than the other three. It has been suggested that high amylose starch in long-grain rice can interfere with the starch digestion of cooked rice because of starch being encapsulated in the tight helical twists of amylose chains (Lehmann & Robin, 2007; Zhang & Hamaker, 2009). In this case, it is likely that the higher proportion of RS2 in the high amylose starch of long-grain rice (i.e., basmati) made a similarly significant contribution to the protection of starch hydrolysis within 20 minutes compared with RS1 in whole grain rice products (bran

encapsulation) (Butterworth, Warren, & Ellis, 2011; Hu et al., 2004; Kim et al., 2006; Gudmundsson Magnus & Eliasson Ann-Charlotte, 2006).

Thirdly, the special case of parboiled rice, which is a refined but pre-treated rice product, had a lower glucose release than other refined rice (50% lower than medium-grain white and 20% lower than basmati rice), and similar TAS and RS compared to medium grain and long grain brown rice, and this could be attributed to the pre-treatment process (i.e., parboiling). The parboiling process induces starch retrogradation, resulting in the formation of more RS (RS3) and SDS (Lehmann & Robin, 2007; Riva, Fessas, & Schiraldi, 2000; Sajilata et al., 2006b). It has been suggested that gelatinisation of high amylose starch (i.e., pre-cooking) followed by hydrothermal processing at a low temperature (i.e., cooling) can result in recrystallisation of starch and significant rearrangements in the retrograded starch chain, thus limiting the rate of enzyme attack and increasing the proportion of SDS and RS3 (Frei, Siddhuraju, & Becker, 2003; Kim et al., 2006; Lehmann & Robin, 2007; Walter et al., 2005; Zhang & Hamaker, 2009). The results of the parboiled rice in this study, having a similarly slow glucose release to medium grain and long grain brown rice (with around 28% at 20 minutes, 50% at 60 minutes and 78% at 180 minutes), is in accordance with this prediction. In parboiled rice, a maximum of 44% of SDS (digested between 20 and 120 minutes after starch hydrolysis begins) was achieved in previous studies (Lehmann & Robin, 2007), which was similar to the results from this study (45%).

In contrast to other studies that used minced parboiled rice (Kim et al., 2006) the RS content in the NZ parboiled rice was around 5% lower than in whole grain rice (i.e., long-grain brown rice). This difference may be due to grain size, whereas in this study none of the grains were disrupted (i.e., minced or chopped) so starch in the whole grain rice was still being protected by grain encapsulation (i.e., high RS1 content). Therefore, particle size (i.e., mincing to emulate mastication) is suggested as a factor for further investigation in the next study (Chapter 4, “Effect of rice product varieties, cold storage, reheating and grain particle sizes on starch digestibility profile and *in vitro* glucose release”).

The strength of this research study is that it followed the *in vitro* starch digestion techniques whose precision and validity have been previously studied (Monro, 2005; Wallace, Monro, Brown, & Frampton, 2008). The *in vitro* glucose release and determination of starch digestibility fractions is an objective, non-invasive way to

define the bioavailability of starch in rice in a way that models digestion in the human gastrointestinal tract. There are, however, other factors that affect starch availability and digestibility, such as particle size and preparation (parboiling, prolonged cold storage and reheating) (Englyst & Englyst, 2005; Landon, 2007; Walter et al., 2005). A study by Seila et al (2014) reported the protein and lipid biosynthesis of the same rice cultivar/product were more sensitive to the different agro-climatic zones (e.g. environmental temperature), the starch fine structures (i.e. degree of branching) and the amylose content are not greatly affected. Therefore, the sample of rice products that were purchased at the time of experiment was able to represent the rice product cultivar. However, other factors, such as storage time and season of growth were likely to affect the starch composition and moisture content. An assessment of variations in amylose to amylopectin ratio of rice varieties available in New Zealand would also add value.

Another strength of this study is that, unlike past research, this *in vitro* experimental research used whole cooked rice instead of milled rice powder to best represent the starch digestibility of the rice cooked by a common method. One limitation of this research study is that the effect of simulated chewing was not examined, although it may have altered the rice glucose release trajectory and digestibility profile. In addition, it is not known whether rice preparation practice involves delayed consumption and storage and reheating prior to consumption. It was suggested that mincing to reduce the particle size of cooked rice grains, prolonged storage time and reheating are the three factors that need to be considered as variables (investigated in Chapter 4). Further study is required to investigate particle size (mincing) and preparation and storage conditions on the overall *in vitro* glucose release trajectory and starch digestibility profiles (see Chapter 4).

Furthermore, in humans, the rate and extent to which starch is digested and absorbed in the small intestine and released into the bloodstream as free glucose has considerable inter-individual variation (Tan, Wu, Henry, & Lee, 2015). Further study is required to investigate the human glucose responses among healthy people with normal glucose regulation (see Chapter 5, “Effect of rice cooking method on postprandial glycaemic response, satiety and palatability, and chewed particle distribution”) and explore possible genetic or environmental influences such as overall diet.

This *in vitro* study has provided the results that the rate and extent of glucose release from different rice product varieties have meaningful differences. Freshly prepared medium-grain white rice is likely to induce a more rapid release of glucose into the

bloodstream in humans than other product varieties, which may be associated with elevated blood glucose, particularly in those who are insulin resistant. Further work is required to determine the effects of particle size (chewing) and preparation technique on the rate of release of glucose from rice.

3.7 Conclusion

This study has shown that in terms of overall glucose availability during 180 minutes *in vitro* digestion, the five freshly cooked rice products examined may be ranked in the following order: medium-grain white rice > basmati rice > parboiled rice > medium-grain brown rice > long-grain brown rice. Medium-grain white rice had the highest proportion of RDS, followed by basmati rice, medium-grain brown rice, parboiled rice and long-grain brown rice. We hypothesise from these results that the rice products may be ranked in a similar order in terms of their glycaemic effect *in vivo*.

The rice starch digestibility profile reported in the present study with standard cooking methods can be used as guide for further research in the method of post-cooking interventions, including cooking and reheating, cold storage time and particle size of grains, to achieve the desired level of starch digestibility profile and glucose response trajectory.

Existing evidence has suggested that the direct association between SDS and RS value and metabolic response is most conducive to optimal health (Englyst & Englyst, 2005). SDS digestion leads to a low rate of blood glucose loading, which does not exceed the GD capacity of the body, while RS does not contribute to the glycaemic carbohydrate load and may have benefits for colonic health. The increasing prevalence of energy-dense carbohydrate foods that dominate many diets and their contribution to the rise in hyperglycaemia and related complications have sparked concern in many health professionals. Investigating starch content in staple foods and the factors that modulate starch digestion to change the proportions of RDS, SDS and RS that they contain is needed if these health concerns are to be addressed (McKeown, 2004).

Chapter 4: Effect of rice product varieties, cold storage, reheating and grain particle sizes on starch digestibility profile and *in vitro* glucose release

Abstract

During 180 minutes *in vitro* digestion, medium-grain white rice released glucose faster (higher rapidly digested starch) and to a greater extent (higher slowly digested starch) than medium-grain brown rice, basmati rice, long-grain brown rice and parboiled rice when freshly cooked. This *in vitro* experimental study aimed to investigate the effect of cold storage time, reheating and grain particle size disruption on the rate and extent of *in vitro* starch digestion, that is, the trajectory of glucose release over 180 minutes. The rate and extent of glucose release was not significantly changed within eight hours of cold storage (at 4 °C) among all five rice products (within 10% reduction, $P = 0.1$). When cold-stored for more than 10 hours, the starch digestibility and rate of digestion were reduced significantly. Dependent on the rice products, the reduction of starch digested from medium-grain white, medium-grain brown and long-grain brown was around 20% ($P = 0.05$), from basmati around 30% ($P = 0.05$) and from parboiled around 40% ($P < 0.001$) compared with the freshly cooked warm rice product varieties. Conversely, reheating to 65 °C for at least 15 minutes and mincing both increased total starch digested by around 20% ($P < 0.001$) and up to 18% ($P < 0.001$) respectively among all five rice products compared with the chilled cooked rice. Compared with the other rice products, minced freshly cooked medium-grain white rice released the greatest amount of glucose after rice digestion (reached over 90% after 40 minutes), and minced parboiled rice that had been stored at 4 °C for 24 hours had the lowest (reached 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 whether 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: Rapidly digestible starch, slowly digestible starch, resistant starch, *in vitro*, reheating, mincing, cold storage, glucose release, starch digestibility profile.

4.1 Introduction

As the primary carbohydrate source that supports more than 27% of the daily energy intake of the world's population, rice plays an important role in meeting energy requirements 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 (Elia & Cummings, 2007). From a glycaemic status point of view, diets containing large amounts of rapidly digestible 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 (Englyst et al., 1999). On the other hand, the inclusion of foods in the daily diet that have a slow release of glucose is considered beneficial. Previous studies have provided evidence that slow starch digestion and slow glucose release are favourable for dietary management of glycaemia in individuals suffering from impaired blood glucose homeostasis (Asp, 1996; Bjorck et al., 1994; Wolever & Jenkins, 1986).

Various intrinsic and extrinsic factors have been reported in a number of studies to impact on the glucose release trajectory in rice (Guraya, Kadan, & Champagne, 1997) (Hu et al., 2004; Marsono & Topping, 1993), which is directly associated with the rate and extent of starch digestion. Two important intrinsic factors are the quantity of dietary fibre present and the nature of the rice starch itself.

First, brown rice, or whole grain rice, has a higher concentration of dietary fibre than white rice (i.e., refined rice) (Englyst & Hudson, 1996). The dietary fibre content of brown rice may significantly reduce the susceptibility to enzymatic degradation (i.e., amylolytic attack) both in the mouth and in the small intestine, slowing the rate of digestion and reducing the postprandial glycaemic response (Eggum et al., 1993; Englyst et al., 1987).

Secondly, rice starch in the endosperm is a mixture of the alpha-glucan polysaccharides, amylose and amylopectin. The amylose-to-amylopectin ratio depends on the botanical origin of the rice. Rice that exhibits a 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).

Thirdly, altering rice starch structure by thermal processing may also change postprandial glycaemic responses (Lehmann & Robin, 2007). When raw rice products

are heated in water, starch granules are gelatinised. Starch gelatinisation disrupts the starch structure and increases the susceptibility to starch degradation (Lehmann & Robin, 2007). Cooling cooked rice or storing cooked rice at low temperatures, for example, in the refrigerator, can transform gelatinised rice starch from an amorphous state to a more ordered state (i.e., crystalline state) that persists on reheating. The crystallised starch form can resist enzymatic degradation in the small intestine for up to three hours (Sajilata et al., 2006b). This retrogradation process may spontaneously lower the concentration of digestible starch in cooked rice and subsequently reduce the postprandial glycaemic response and glycaemic index (GI) value (Riva et al., 2000).

Fourthly, different degrees of particle size reduction can significantly affect rice starch digestibility (Bjorck et al., 1994). Rice grains of a smaller size tend to be digested faster than grains of a bigger size (Ranawana et al., 2010). It is also hypothesised that whole grain rice with the outer bran intact may resist digestion longer than chopped whole grain rice and well-polished rice grain.

Previous studies of the variations in starch digestibility and absorption of glucose have been largely based on the measurements of GI and estimates of the glycaemic load (GL). Englyst and Englyst (2005) 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, RDS, slowly digestible starch (SDS) and resistant starch (RS), by measuring the amount of glucose released from one test food during incubation with amyloid enzymes under standardised *in vitro* conditions over 180 minutes. Rapidly digested starch (RDS) is defined as starch that can be digested within 20 minutes after ingestion. Slowly digested starch (SDS) is defined as starch that can be digested between 20 and 180 minutes after ingestion. Resistant starch (RS) is defined as starch that can resist digestion to up to 180 minutes.

The primary aim of this study was to prove-in-principle that storing cooked rice at 4 °C for 24 hours and reheating it can reduce the rate and extent of *in vitro* starch digestion and reduce the rate of *in vitro* glucose release. The secondary aim was to discover the optimal combination of factors (rice type, particle size and wholeness of cooked rice grain, cooking method and storing condition) to optimise the starch digestion profile of rice.

It was hypothesised that for five different rice products:

- Larger particle size, parboiling and cold storage would decrease the available starch (i.e., RDS and SDS) and increase the RS.
- The effect of particle size, preparation and storing method will vary by rice product.

4.2 Method and materials

The aim of this set of experiments was to investigate the effect of rice product variety, cooking and cooling treatment, reheating and rice grain particle size in various combinations, on the glycaemic potency of five rice products: medium-grain white rice, medium-grain brown rice, basmati rice, long-grain brown rice and parboiled rice. A series of *in vitro* experiments (5 rice products \times 12 experiments; see Figure 4-1) measured the rate of *in vitro* glucose release over 180 minutes and compared the starch profile (i.e., the proportions of RDS, SDS and RS) of each cooked and treated rice product. The same rice samples and products as in Chapter 3 were utilised. The rice starch digestibility profiles were compared in order to determine the optimal combination of the factors that may induce the lowest postprandial glucose release trajectory over 180 minutes.

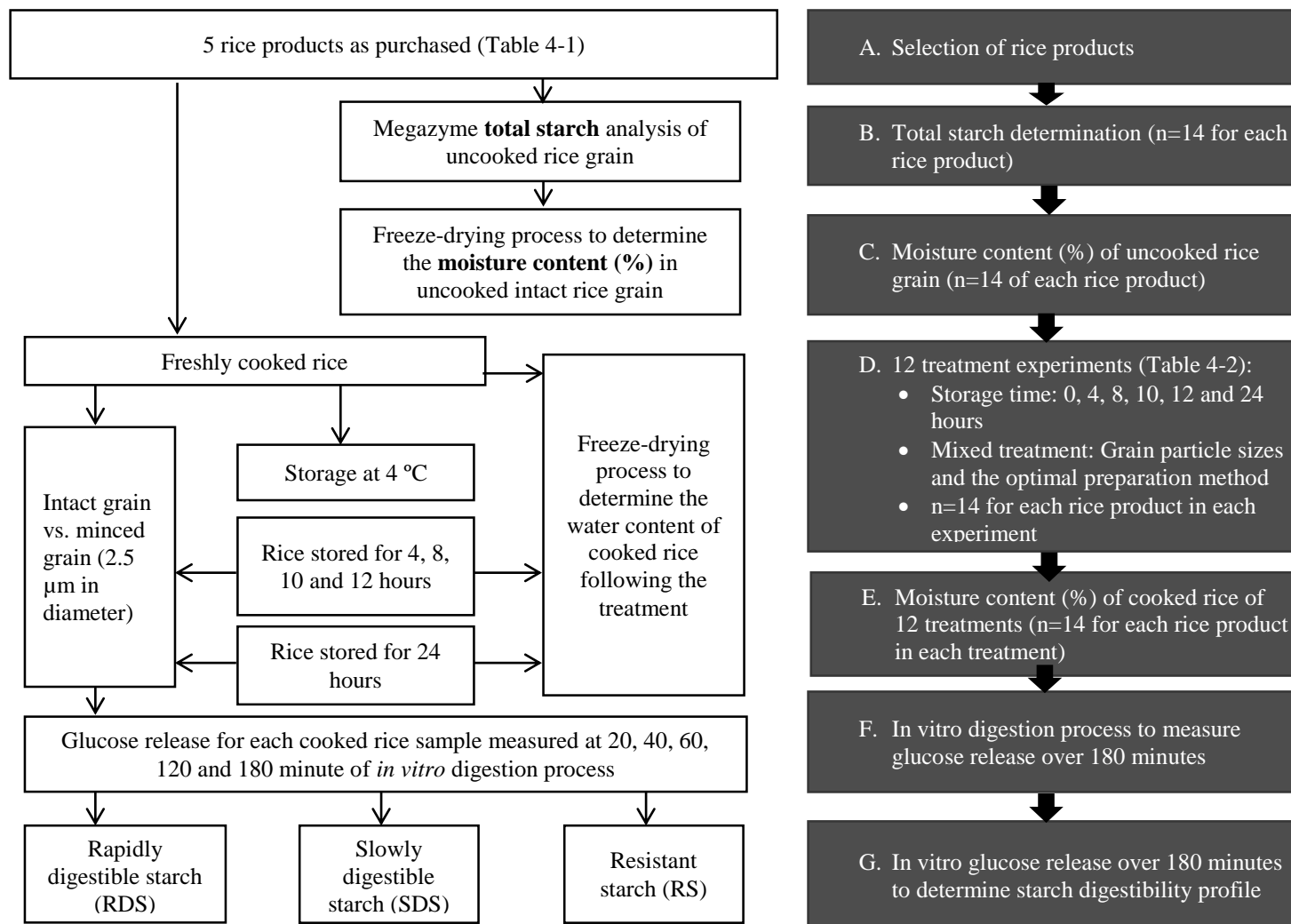


Figure 4-1 Flow chart of the experiment design.

4.2.1 Selection of rice products

Five rice products were purchased from a New Zealand local high-turnover supermarket (PAK'nSAVE) for all the experiments: medium-grain white rice (SunRice®), pure white basmati rice (King's Choice®), medium-grain brown rice (SunRice®), long-grain brown rice (SunRice®) and parboiled rice (Real Rice®) (see Table 4-1). The selected rice products were the same as in the rice starch digestibility profile experiment (Chapter 3).

4.2.2 Determination of total starch and moisture content (%) in rice as purchased

TS (gram per 100 g uncooked rice) was determined as described in Chapter 3 (starch and moisture profile). This experiment used a highly reproducible and reliable enzymatic and acid hydrolysis procedure, the Megazyme total starch analysis procedure (AA/AMG) AACC Method 76-12 (McCleary, Gibson, et al., 1994; McCleary, Solah, et al., 1994), which requires the use of milled rice powder.

The moisture content (%) determined was the moisture removed by 24-hr of freeze-drying of intact uncooked rice grains. The experiment and calculation procedure were followed as described in Chapter 3, "Freshly cooked rice: Starch and moisture profile".

Table 4-1 Sample used in *in vitro* starch digestibility study.

Rice types	Country of origin	Brand	Batch no.	Supermarket
Medium-grain white	Australia	SunRice®	9310140283746	PAK'nSAVE, Auckland
Medium-grain brown	Australia	SunRice®	9310140283869	PAK'nSAVE, Auckland
Basmati	India	King's Choice®	9310130014923	PAK'nSAVE, Auckland
Long-grain brown	Australia	SunRice®	9310140283906	PAK'nSAVE, Auckland
Parboiled long-grain white	Thailand	Real Rice®	9421014797440	PAK'nSAVE, Auckland

Table 4-2 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).

Treatments for each rice product	
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 mixed treatment 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	

4.2.3 Determination of starch profile of cooked rice treated in different ways

Three factors, grain particle disruption, cold storage time and reheating, were applied to five rice products (medium-grain white rice, medium-grain brown rice, basmati rice, long-grain brown rice and parboiled long-grain white rice). Two groups of a total of 12 experiments were applied to each of the five rice products (see Table 4-2):

Group 1: Investigating the combination effect of cold (at 4 °C) storage time (freshly cooked, and stored for 4, 8, 10, 12 and 24 hours) on minced rice (2.5 µm in diameter) glucose release trajectory and starch digestibility profile

Group 2: Analysing the combined effect of the prolonged (24 hours) cold storage (at 4 °C) and reheating (to 65 °C) and the degree of rice grain particle disruption (intact cooked rice grain and minced rice grain to 2.5 µm in diameter) on rice starch digestion and glucose release

The optimal cold storage time Group 1 was determined from the *in vitro* digestion of minced rice grain. The aim of Group 2 was to investigate the effect of reheating on intact and minced rice grain cold stored for an optimal time period, which was determined in Group 1 experiments.

Group 1: Storage time effect on rice starch digestibility profile

A total of six experiments (no cold storage, stored for 4 hours, 8 hours, 10 hours, 12 hours and 24 hours) were carried out for each of the five rice products (see Table 4-2). For each rice product each experiment below was undertaken in duplicate – this means that the rice product was cooked on separate days for the experiment to be repeated. A total of 14 repeated cooking and measurements.

Rice cooking

Each rice product (100.0 g) was weighed into a 600 mL glass beaker (KIMAX, USA), and 110 mL of distilled water was added into each beaker. The cooking procedures for each of the rice products as described in Chapter 3, “Freshly cooked rice: Starch and moisture profile” were followed. For each experiment, a new beaker of rice was cooked for each rice product. As is standard analytical laboratory practice all assays were duplicated and if varied by more than a predefined tolerance would be repeated. This method was standardised in this laboratory and used by others in other experiments so it was known what precision should be expected. Each cooked rice sample was stored for 0, 4, 8, 10, 12, and 24 hour at 4°C. To analyse the rice starch digestibility after each storage period, the 0 to 24-hour storage experiments for each rice product were repeated 14 times. The total n was 14.

Determination of starch profile of freshly cooked minced rice

Degree of grain particle disruption (mincing) treatment

Around 150.0 g of freshly cooked rice products from the middle of each beaker were weighted onto a piece of cooking paper. Mincing was achieved by rubbing the rice grains gently through the 2 mm sieve on the cooking paper. The rice grains were not pushed through the sieve too hard so that the structure of minced particles was retained to prevent the rice from turning to paste. The minced particles (2.0 mm in diameter) of each rice product (5.0 g) were quickly collected into two separate plastic pots (70 mL; Lab Serve LBS 30002) and tightly capped. All plastic pots were kept tightly capped to prevent moisture loss until all plastics pots were ready for *in vitro* digestion.

Determination of moisture content (%) of minced freshly cooked rice products

Around 40.0 g (average 40.9 g, SD = 3.7) from each of the remaining minced rice products were weighed into an aluminium container (100 mL) and immediately sealed with aluminium foil for the determination of moisture content (MC%).

The same procedure as described in Section 3.2.6, “Determination of moisture content (%) of uncooked rice”, was followed to determine the water content (%) in each cooked rice sample. The water content (%) of each rice variety was calculated by the “amount of water in uncooked rice sample (g) / weight of uncooked rice sample \times 100%”. The experiments were duplicated, from taking a sample of the rice product to cook, in order to achieve more reliable results.

In vitro digestion process of minced freshly cooked rice products and calculation

Immediately after the mincing treatment, 2 samples (i.e. duplicates) from each of the 5 cooked rice products were taken and the *in vitro* digestion process administered and glucose release measured over 180 minutes as a standard laboratory practice to ensure precision. The *in vitro* digestion process, glucose release measurement and determination of starch digestibility profile derived from the glucose release over 180 minutes followed the same procedure as described in Section 3.3, “Determination of starch profile of freshly cooked rice”.

Determination of starch profile of minced rice after cold storage and reheating treatment

Degree of grain particle disruption (mincing) treatment and cold storage treatment

Around 150.0 g of freshly cooked rice products from the middle of each beaker were weighed onto a piece of cooking paper. Mincing was achieved by rubbing the rice grains gently through the 2 mm sieve on the cooking paper. The minced particles (2.0 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. A total of 50 plastic pots (2 \times 5 rice products \times 5 storage time periods: 4 hours, 8 hours, 10 hours, 12 hours and 24 hours) of minced freshly cooked rice (5.0 g) were immediately transferred and placed in the refrigerator at -4°C . The temperature of the refrigerator was monitored every two hours during the storage time.

Determination of moisture content (%) of minced reheated rice products

Around 40.0 g (average 40.9 g, SD = 3.7) from each of the remaining minced rice products were weighed into an aluminium container (100 mL) and immediately covered with aluminium foil for the determination of moisture content (MC%). The same procedure as described in Section 3.2.6, “Determination of moisture content (%) of uncooked rice”, was followed to determine the water content (%) in each cooked rice

sample. The water content (%) of each rice variety was calculated by “amount of water in uncooked rice sample (g) / weight of uncooked rice sample \times 100%”. The experiments were duplicated in order to achieve more reliable results.

Reheating treatment

At the end of each cold storage time period (4 hours, 8 hours, 10 hours, 12 hours and 24 hours), two plastic pots for each of the five rice products (5.0 g) were taken out of the refrigerator while tightly capped to prevent moisture loss. The specimen 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. A thermometer was used to measure the temperature of the minced rice.

In vitro digestion process of minced reheated rice products and calculation

The plastic pots were taken for the *in vitro* digestion process immediately after the reheating treatment to measure the glucose release over 180 minutes. The *in vitro* digestion process, glucose release measurement and determination of starch digestibility profile derived from the glucose release over 180 minutes followed the same procedure as that described in Section 3.3, “Determination of starch profile of freshly cooked rice”. The rate and the extent of glucose release were compared among the rice samples to determine the optimal cold storage time.

Group 2: Effect of mixed treatments on rice starch digestibility profile

Group 2 experiments aimed to compare the effect of mixed treatments on the rice starch digestibility profile. A total of six experiments (freshly cooked minced, freshly cooked intact, reheated minced, reheated intact, un-reheated minced and un-reheated intact) were carried out for each of the five rice products (see Table 4-2).

According to the results from the Group 1 experiments, the most effective safe storage time for the lowest rate and extent of rice glucose release and slowest rice starch digestibility was estimated to be 24 hours. Hence, in Group 2, only two storage times, 0 hours and 24 hours, were compared. In addition, according to the New Zealand safety guideline for rice meal cooking and preparation (Lake et al., 2004), cooked rice products should be reheated at 65 °C for at least 15 minutes before ingestion. The un-reheated rice samples were compared with the reheated rice samples to determine the effect of reheating on increasing the glucose release trajectory during 180 minutes *in vitro* digestion. All samples were processed quickly at room temperature with care to prevent moisture loss.

Rice cooking

Each rice product (100.0 g) was weighed into a 600 mL glass beaker (KIMAX, USA), and 110 mL of distilled water was added into each beaker. The cooking procedures for each rice product were followed as described in Chapter 3, “Freshly cooked rice: Starch and moisture profile”. For each experiment, a new beaker of rice was cooked for each rice product. A total of 30 beakers of cooked rice (6 experiments \times 5 rice products) were prepared for the Group 2 experiments.

Determination of starch digestibility profile of freshly cooked intact grains

Determination of moisture content (%)

Around 40.0 g (average 40.9 g, SD = 3.7) from each of the remaining minced rice products were weighed into an aluminium container (100 mL) and immediately sealed with aluminium foil for the determination of moisture content (MC%).

The same procedure as described in Section 3.2.6, “Determination of moisture content (%) of uncooked rice”, was followed to determine the water content (%) in each cooked rice sample. The water content (%) of each rice variety was calculated by the “amount of water in uncooked rice sample (g) / weight of uncooked rice sample \times 100%”.

In vitro digestion process of minced freshly cooked rice products and calculation

A total of 10 plastic pots (2 \times 5 rice products) were taken for the *in vitro* digestion process to measure the glucose release over 180 minutes. The *in vitro* digestion process, glucose release measurement and determination of the starch digestibility profile derived from the glucose release over 180 minutes followed the same procedure as described in Section 3.3, “Determination of starch profile of freshly cooked rice”.

Determination of starch digestibility profile of freshly cooked minced grains

The same procedure as described above in “Determination of starch profile of freshly cooked minced rice” was followed.

Determination of starch digestibility profile of intact grains after 24-hr cold storage

The same 24-hour cold storage, determination of moisture content (%) and *in vitro* digestion procedure as described above in “Degree of grain particle disruption (mincing) treatment and cold storage treatment” was followed.

Determination of starch digestibility profile of minced grains after 24-hr cold storage

The same mincing, 24-hour cold storage, determination of moisture content (%) and *in vitro* digestion procedure as described above in “Degree of grain particle disruption (mincing) treatment and cold storage treatment” was followed.

Determination of starch digestibility profile of reheated intact grains

The same 24-hour cold storage, reheating, determination of moisture content (%) and *in vitro* digestion procedure as described above in “Degree of grain particle disruption (mincing) treatment and cold storage treatment” was followed.

Determination of starch digestibility profile of reheated minced grains

The same mincing, 24-hour cold storage, reheating, determination of moisture content (%) and *in vitro* digestion procedure as described above in “Degree of grain particle disruption (mincing) treatment and cold storage treatment” was followed.

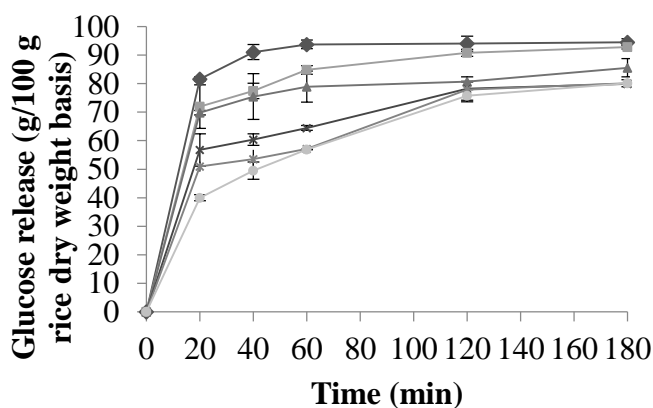
4.3 Results

4.3.1 Comparison of glucose release (g/100 g rice dry weight basis) during *in vitro* digestion process after various cold storage periods

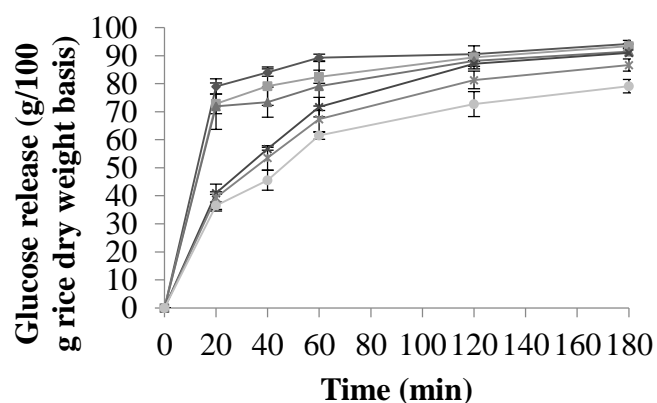
The overall glucose release rate of minced medium-grain white, medium-grain brown and basmati rice were considerably higher than that of long-grain brown and parboiled rice when the same cold storage time was applied (see Figure 4-2). The glucose release graphs demonstrated that the increasing cold storage time gradually reduced overall glucose release in various degrees depending on the rice product variety. After the first four hours and eight hours of cold storage (at 4 °C), there was only a minor reduction (within 3%, $P = 0.01$) in the amount of glucose released from minced long-grain brown rice during digestion, and the other rice products had similarly insignificant reduction (within 10%, $P < 0.001$; see Figure 4-2a and b). The reduction was likely to be between 20 and 120 minutes after *in vitro* digestion began; the total glucose release reached a similar level to the un-stored rice after 180 minutes of *in vitro* digestion.

The rate and the extent of glucose release reduction was more significantly reduced after more than 10 hours of cold storage. The most significant changes were found in parboiled rice, for which the overall glucose release during *in vitro* digestion was reduced by an average of 40% ($P < 0.001$) on storage, at each time point (20, 40, 60, 120 and 180 minutes). No further reduction was observed after 12 hours and 24 hours of cold storage (Figure 4-2e). The 10 hours of storage contributed to around 30% reduction of the glucose release after 20, 40 and 60 minutes of *in vitro* digestion ($P = 0.05$). Unlike

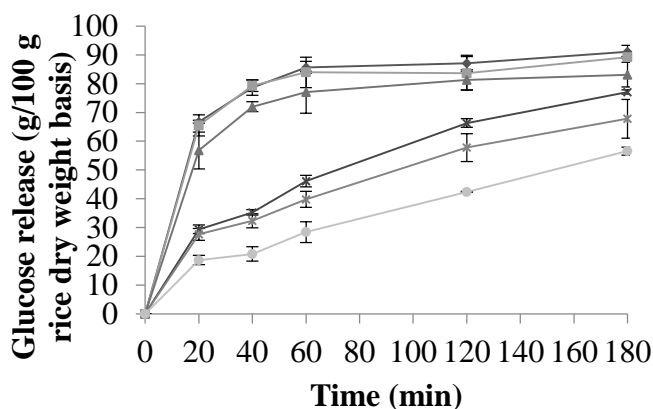
parboiled rice, the glucose release of these four rice products suddenly increased at 120 minutes and at 180 minutes reached a similar level to the ones without cold storage treatment.



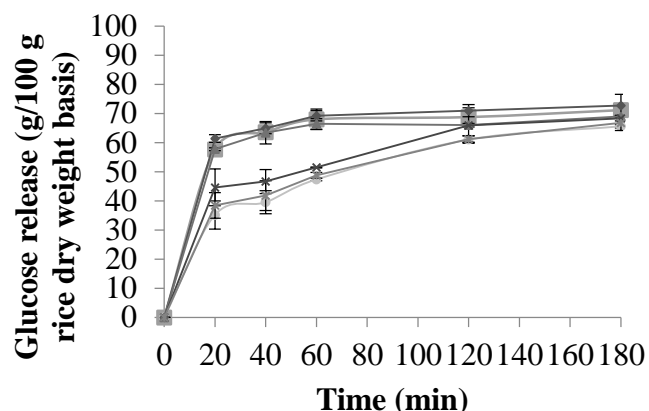
(a) Glucose release of medium-grain white rice



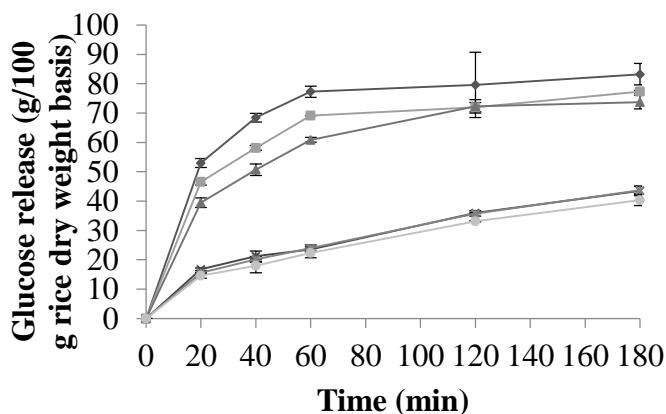
(b) Glucose release of medium-grain brown rice



(c) Glucose release of basmati rice



(d) Glucose release of long-grain brown rice



(e) Glucose release of parboiled rice

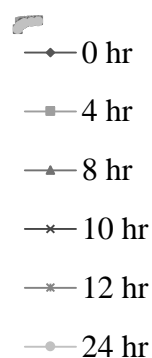


Figure 4-2 Average glucose release (g) as a percentage of rice (g dry weight basis, $n = 14$) within 180 minutes (at 0, 20, 40, 60, 120 and 180 minutes) of *in vitro* digestion process of the five cooked rice products after cold storage at 4 °C (at 0, 4, 8, 10, 12, 24 hours). (Error bars are the 95% confidence intervals of the means.)

4.3.2 Comparison of starch digestibility profiles among five rice products after various cold storage periods

With increasing cold storage time from 0 hour to 24 hours, the total available starch (TAS) (i.e., starch that can be digested within 180 minutes of *in vitro* digestion process) gradually reduced. The reduction after 24 hours storage was small in minced whole grain rice products (around 5% reduction in minced medium-grain brown rice, $P = 0.02$; around 10% reduction in minced long-grain brown rice, $P=0.02$) compared with refined grain (medium-grain white rice, basmati rice and parboiled rice) (reduction ranged from around 20% to 40%, $P < 0.001$; Figure 4-3). After the first eight hours of cold storage, the TAS content of minced whole grain rice products remained at a similar level (around 76% for medium-grain brown rice and 60% for long-grain brown rice) and minced refined grain rice continued to fall (by average 20%, $P < 0.001$) until 24 hours. The most significant reduction was found in parboiled rice (from around 80% to 40%) after eight hours of storage at 4 °C. The reduction slowed down after 10 hours of storage and remained at around 40% (Figure 4-3).

With increasing cold storage time from 0 hour to 24 hours, the proportion of RDS significantly decreased (Figure 4-4) whereas SDS and RS gradually increased (Figures 4-5 and 4-6). Within eight hours of cold storage, the extent of RDS (%) reduction was trivial (an average of 5% reduction, $P = 0.1$) compared with the cold storage for more than 10 hours (an average of 15% reduction, $P < 0.001$). Refined rice products (medium-grain white, basmati and parboiled rice) had RDS reduced following more than 10 hours of cold storage (average 25%, $P < 0.001$) compared with a trivial change in whole grain rice (medium-grain and long-grain brown rice, average 5% reduction, $P = 0.1$). The largest reduction in RDS was found in parboiled rice, which dropped to around 18% of total starch after 10 hours of cold storage (Figure 4-4).

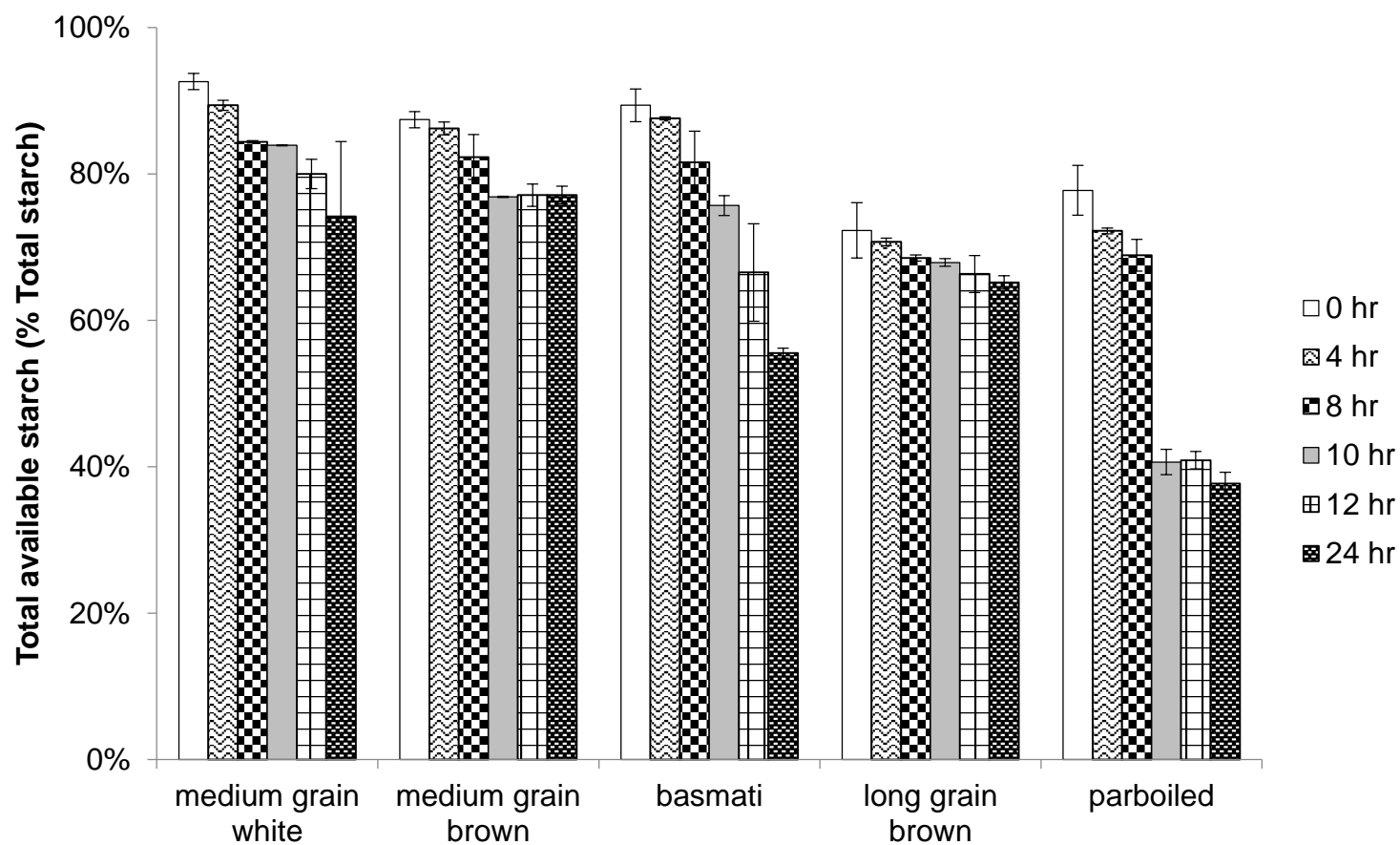


Figure 4-3 Total available starch (TAS) (%) of five cooked rice (n = 14) expressed as a percentage of total starch immediately after cooking or after cold storage (at 4 °C) time period (4 hour, 8 hour, 10 hour, 12 hour and 24 hour) and reheating to 65 °C. Error bars are presented as 95% confidence intervals of the means.

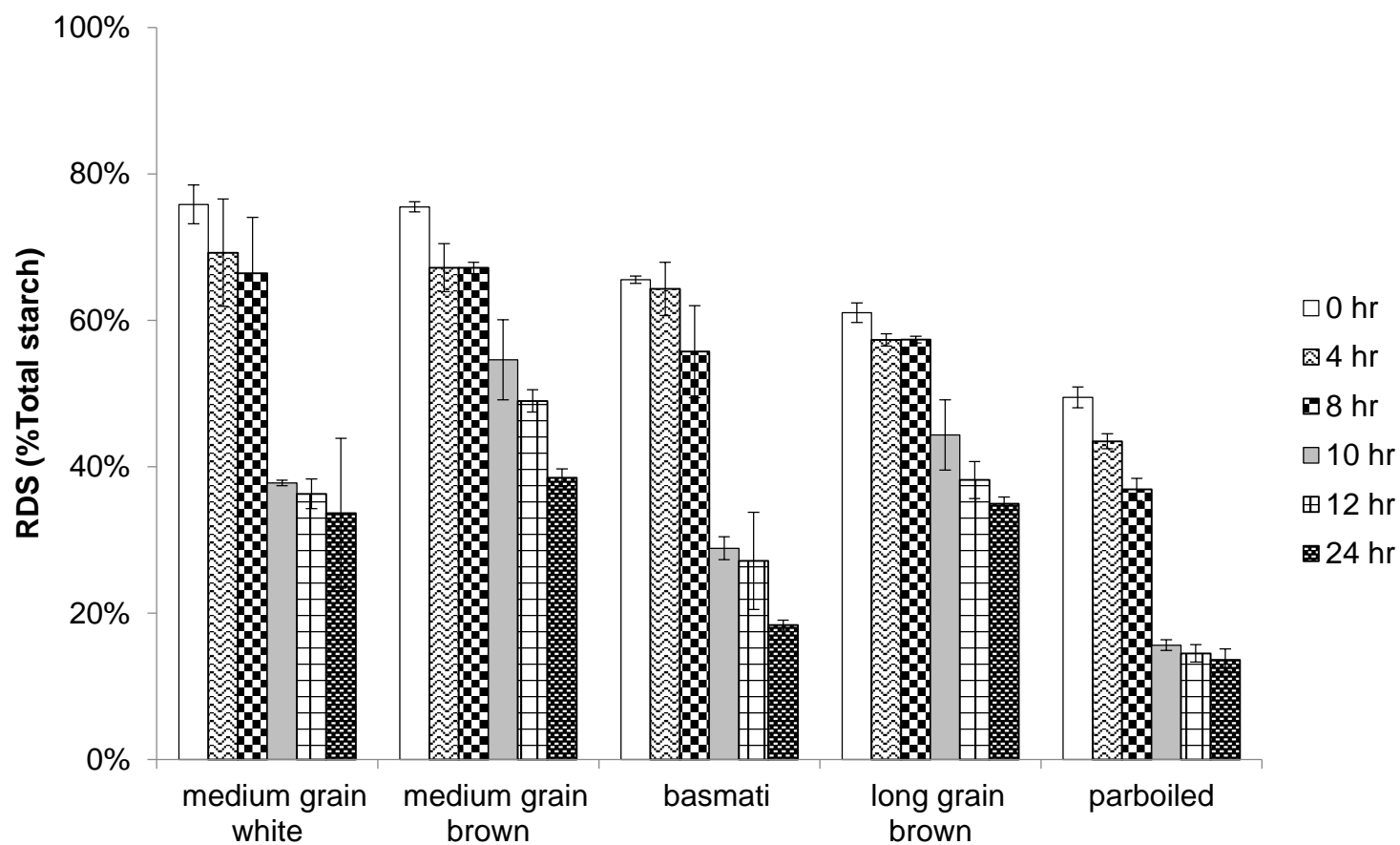


Figure 4-4 Rapidly digestible starch (RDS) (%) of five cooked rice (n = 14) expressed as a percentage of total starch immediately after cooking or after cold storage (at 4 °C) time period (4 hour, 8 hour, 10 hour, 12 hour and 24 hour) and reheating to 65 °C. Error bars are presented as 95% confidence intervals of the means.

The SDS of parboiled rice did not change significantly (remained at around 22%) with increasing cold storage time. In all other four rice products, SDS increased after cold storage for more than 10 hours ($P < 0.001$). Both refined grain rice (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 4-5).

The RS in parboiled rice increased significantly from around 30% to 60% when cold storage time extended to 10 hours and remained at 60% when cold storage time extended further. The RS in both medium-grain rice (white and brown rice) reached 20% after 24 hours of storage, and both long-grain rice (basmati and brown rice) reached over 30% (Figure 4-6).

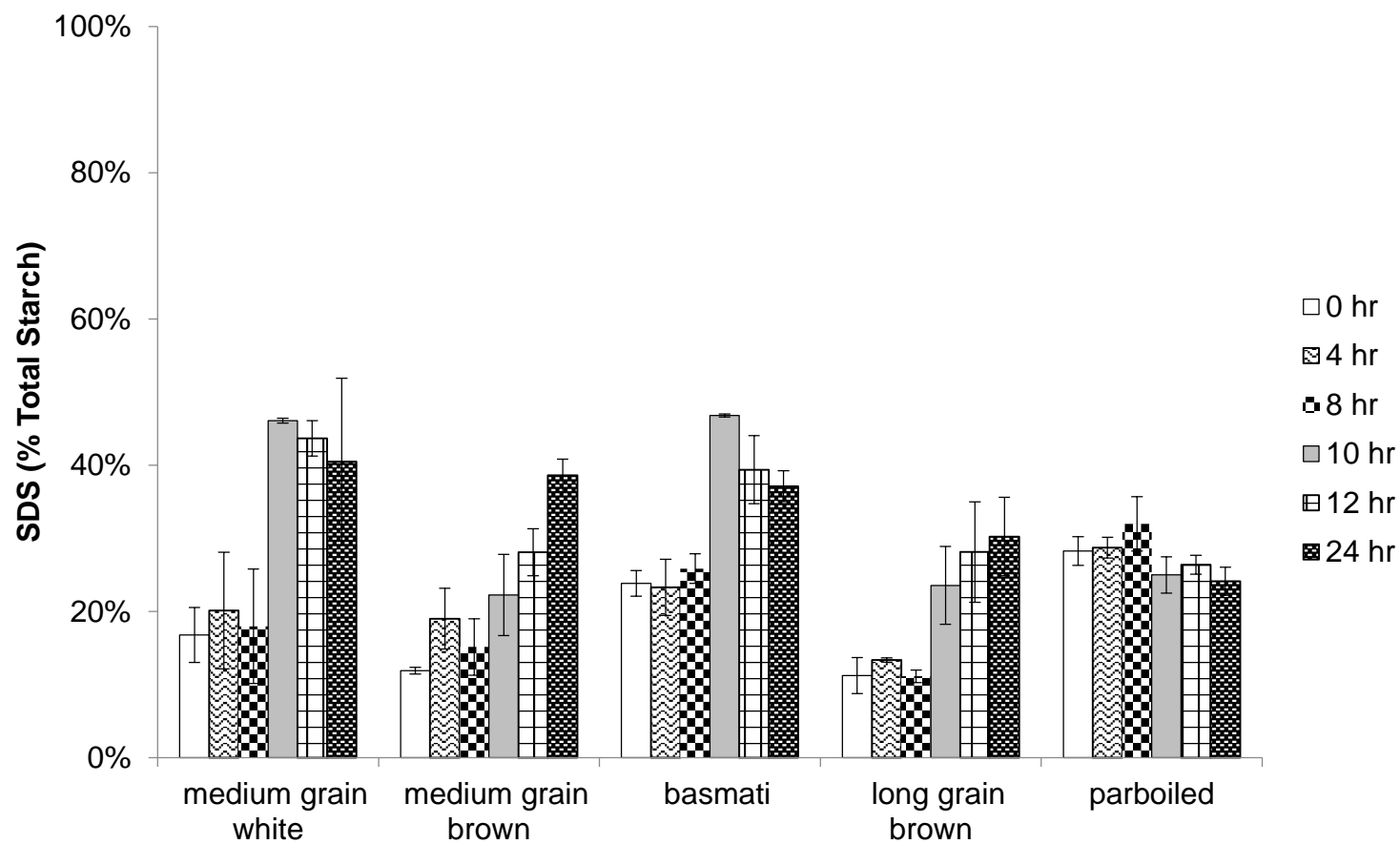


Figure 4-5 Slowly digestible starch (SDS) (%) of five cooked rice (n = 14) expressed as a percentage of total starch immediately after cooking or after cold storage (at 4 °C) time period (4 hour, 8 hour, 10 hour, 12 hour and 24 hour) and reheating to 65 °C. Error bars are presented as 95% confidence intervals of the means.

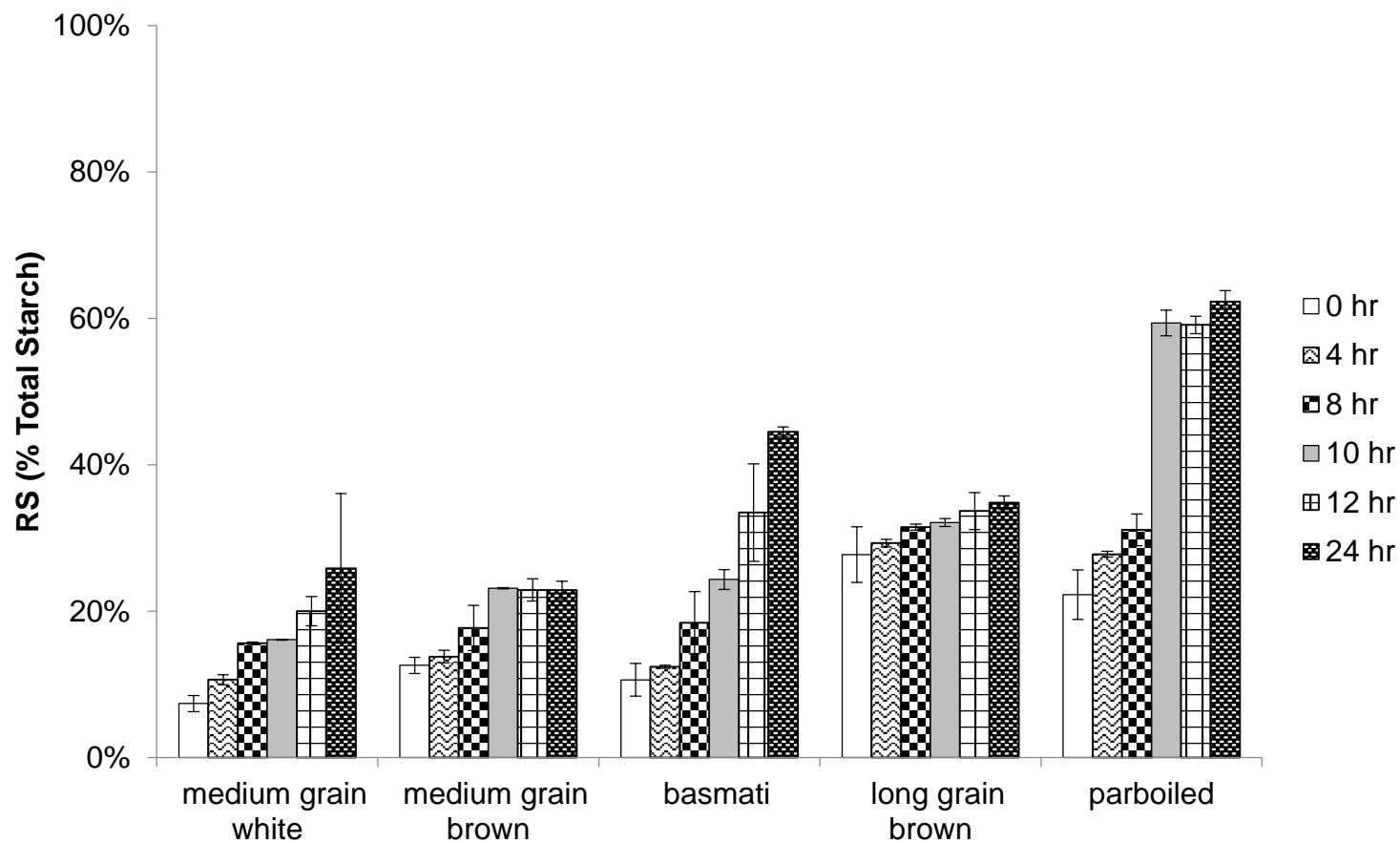


Figure 4-6 Resistant starch (RS) (%) of five cooked rice (n = 14) expressed as a percentage of total starch immediately after cooking or after cold storage (at 4 °C) time period (4 hour, 8 hour, 10 hour, 12 hour and 24 hour) and reheating to 65 °C. Error bars are presented as 95% confidence intervals of the means.

4.3.3 Comparison of glucose release (g/100 g rice dry weight basis) during *in vitro* digestion process after various combined treatments (rice product varieties, cold storage, reheating and rice grain particle size interruption)

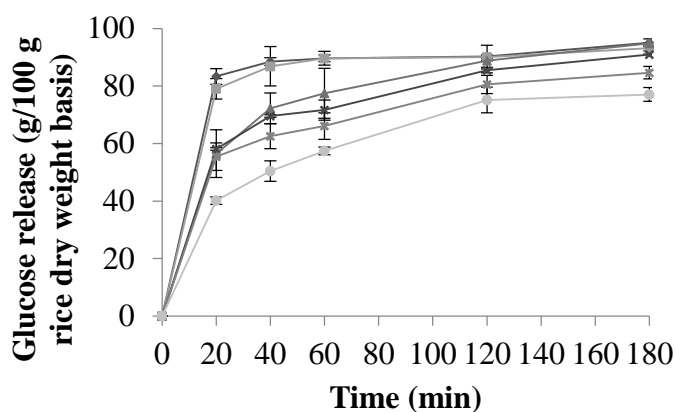
From the results obtained from the investigation of the cold storage time effect on starch digestibility profiles, it was estimated that 24-hour storage was able to reduce both the rate and the extent of glucose digestion during *in vitro* digestion, probably through the process of starch retrogradation, and produce the healthiest starch digestibility profile – the lowest fractions of RDS and TAS and the highest fractions of SDS and RS.

Therefore, the experiment of investigating the combination of factors (rice product varieties, cold storage, reheating and rice grain particle size interruption (i.e., mincing)) adopted 24-hour cold storage.

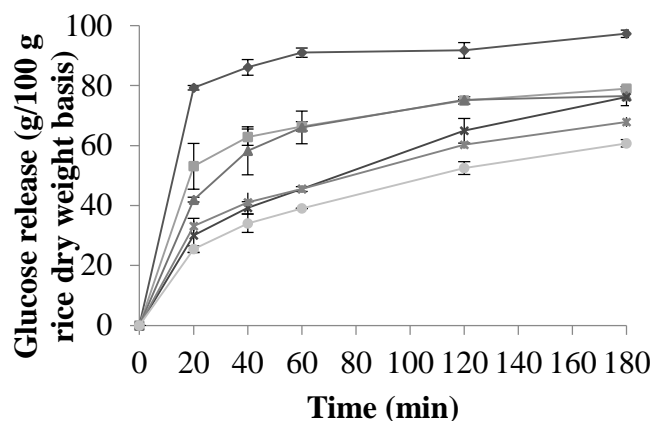
The trajectory of the graphs reflected the changes that cold storage caused: a reduction in RDS, an increase in SDS, a reduction in RDS + SDS, and an increase in RS leading to flatter and prolonged digestion curves (see Figure 4-7). Cold storage over 24 hours without reheating treatment significantly reduced glucose release from all rice products by up to 40% ($p < 0.001$). Reheating to 65 °C after 24 hours of cold storage treatment reversed the effect of cold storage and increased the average glucose release (RDS + SDS) by around 20% in all five rice products ($p < 0.001$).

Mincing (i.e., rice grain particle size disruption) also increased the rate (RDS) and extent (RDS + SDS) of glucose release in various degrees. The increase was more significant 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%, $P < 0.001$; see Figure 4-7).

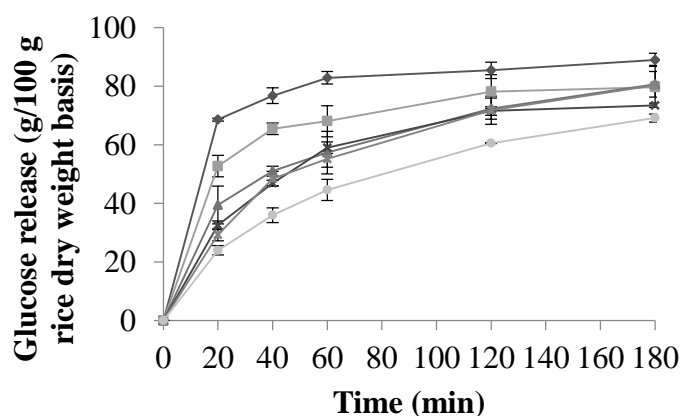
When the same combination of treatments applied, both parboiled rice and long-grain brown rice had significantly lower (around 10%, $P < 0.001$) overall rates and extents of glucose release than the other three rice products (medium-grain white and brown rice and basmati rice; see Figure 4-7). Between 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 (see Figure 4-7d & e).



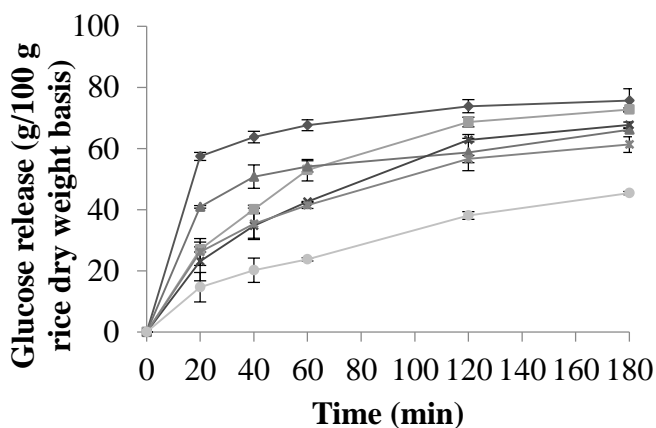
(a) Glucose release of medium-grain white rice



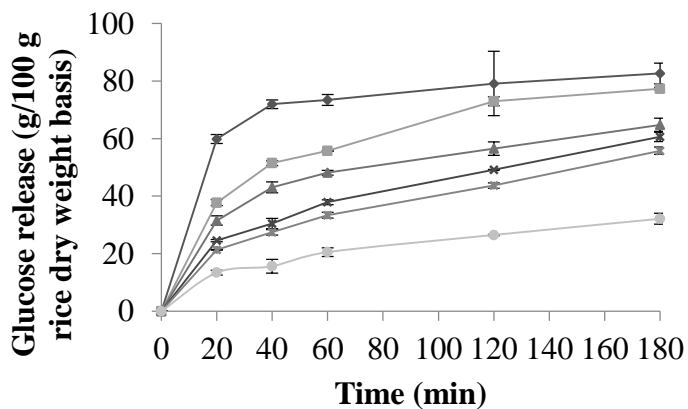
(b) Glucose release of medium-grain brown rice



(c) Glucose release of basmati rice



(d) Glucose release of long-grain brown rice



(e) Glucose release of parboiled rice

- 0 hr, minced
- 0 hr, intact
- ▲— 24 hr, reheated, minced
- ×— 24 hr, reheated, intact
- *— 24 hr, un-reheated, minced
- 24 hr, un-reheated, intact

Figure 4-7 Average glucose release as a percentage of total starch within 180 minutes (at 0, 20, 40, 60, 120 and 180 minutes) of *in vitro* digestion process of the five cooked rice products (g dry weight basis, n = 14) after Group 2 mixed treatment (Table 4-2).

4.3.4 Comparison of rice starch digestibility profile after various combined treatments (rice product varieties, cold storage, reheating and rice grain particle size interruption)

Comparison rice starch digestibility profiles between un-reheated and reheated rice

After 24-hour cold storage treatment, the most significant change in starch digestibility profile 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% ($P = 0.001$; Figure 4-8, 4-9 and 4-11). 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 4-10).

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 (see Figure 4-8 and 4-9). The most significant reverse was observed in intact parboiled rice (around 30% increase in TAS and RDS, $P < 0.001$), and the least significant change was observed in minced basmati rice (around 2% increase in TAS and RDS, $P = 0.5$; see Figure 4-8 and Figure 4-9). However, the reverse did not bring the TAS and RDS to the original level (i.e., without cold storage). Still around 10% of starch retained the retrogradation status in all rice products (see Figure 4-8).

Comparison of rice starch digestibility profiles 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, $P < 0.001$; see Figure 4-8, 4-9 and 4-11). 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, $P < 0.001$; see Figure 4-8). 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, $P < 0.001$; see Figure 4-11). A significant change of 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, $P < 0.001$; see Figure 4-9 and 4-10). However, the change of SDS between minced and intact medium-grain white rice was statistically insignificant ($P = 0.37$; see Figure 4-10).

Comparison of effects of mixed factors on rice starch digestibility

Cold storage strongly promoted starch retrogradation in long-grain brown rice and parboiled rice, both of which decreased in TAS and RDS and increased RS more than medium-grain white rice, basmati rice and medium-grain brown rice. Mincing and reheating, however, reduced the changes. As a result, the reheated minced long-grain brown rice and parboiled rice had a similar proportion of TAS (around 60%; Figure 4-8).

The smaller decline of TAS in minced rice was related to an increase in RDS. The disruption of encapsulation of brown rice almost doubled the RDS level and reduced the SDS level in long- and medium-grain brown rice. Compared with the starch fractions in long-grain brown rice, the RDS, SDS and RS levels in parboiled rice were only slightly affected by the mincing process.

The effect of particle size also varied among different rice types. Long-grain brown rice was more likely to be affected. After cold storage and reheating cycle, minced long-grain brown rice had much less RS than 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.

Therefore, the encapsulation of the rice starch within intact tissue portions played an important role in SDS and RS formation. The disruption of the rice grain structure enhanced the formation of SDS and RS. The enhancement was less significant in parboiled rice. As a result, cooked medium-grain white rice had the highest proportion of TAS and RDS, which may contribute to significant glucose release within 180 minutes of *in vitro* digestion, and the lowest proportion of RS and SDS among five rice product varieties. Cooked parboiled rice had the lowest proportion of TAS and RDS and the highest proportion of RS and SDS. Mincing did not affect significantly either of the rice product varieties.

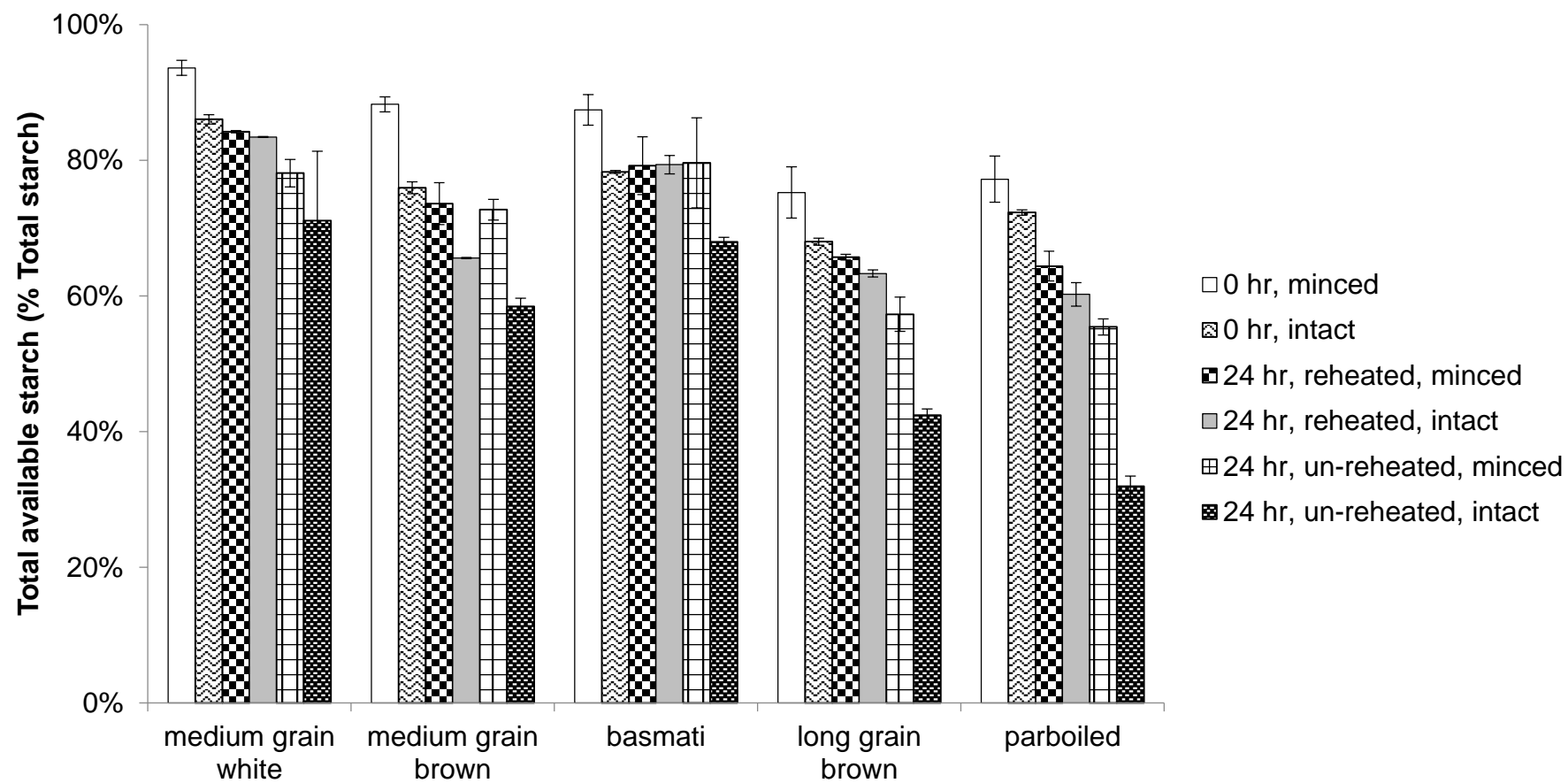


Figure 4-8 Average total available starch (TAS) (%) expressed as a percentage of total starch of five cooked rice products (n=14) after Group 2 mixed treatments shown in Table 4-2. Error bars are presented as 95% confidence intervals of the means.

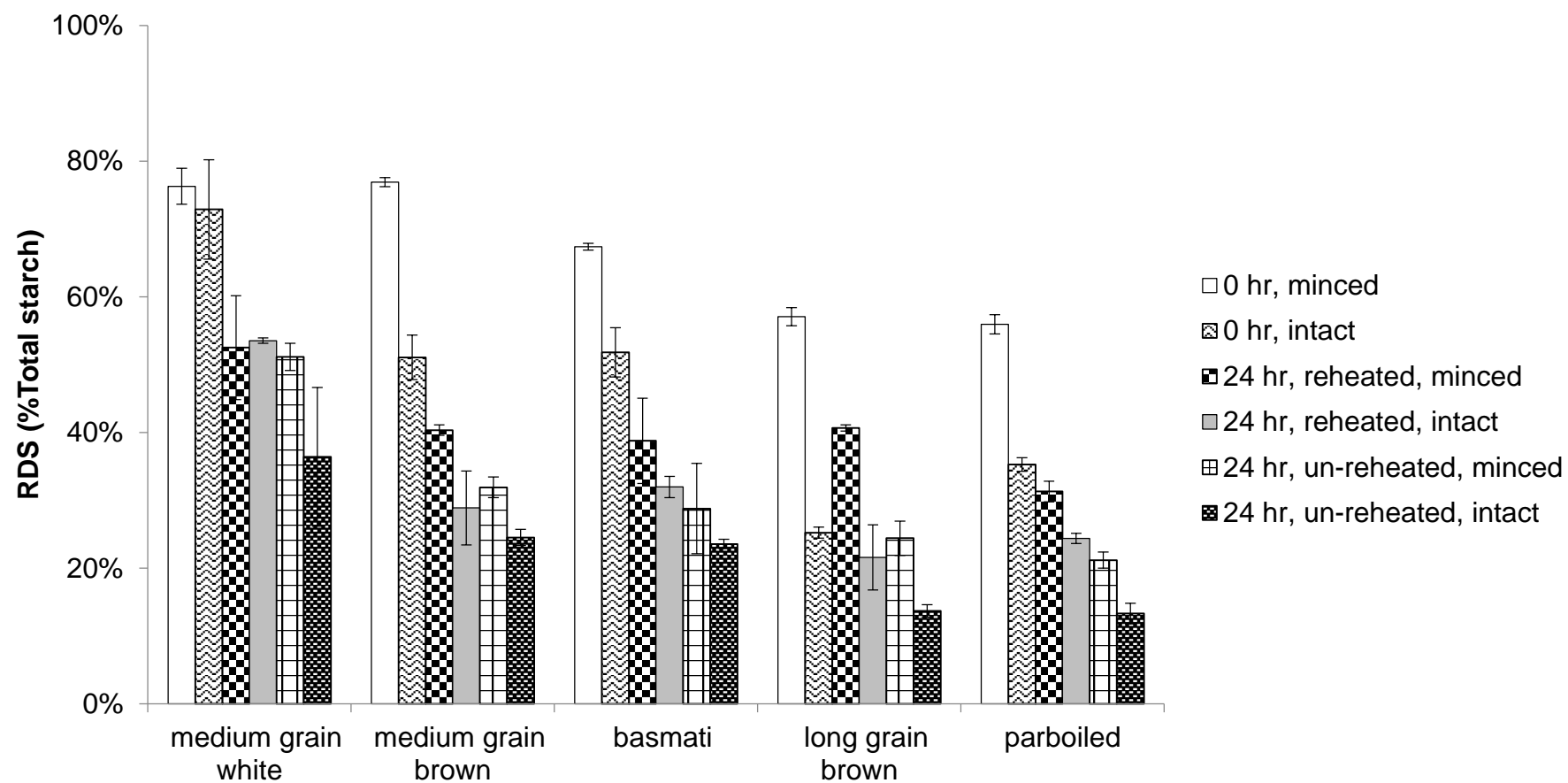


Figure 4-9 Average rapidly digestible starch (RDS) (%) expressed as a percentage of total starch of five cooked rice products (n=14) after Group 2 mixed treatments shown in Table 4-2. Error bars are presented as 95% confidence intervals of the means.

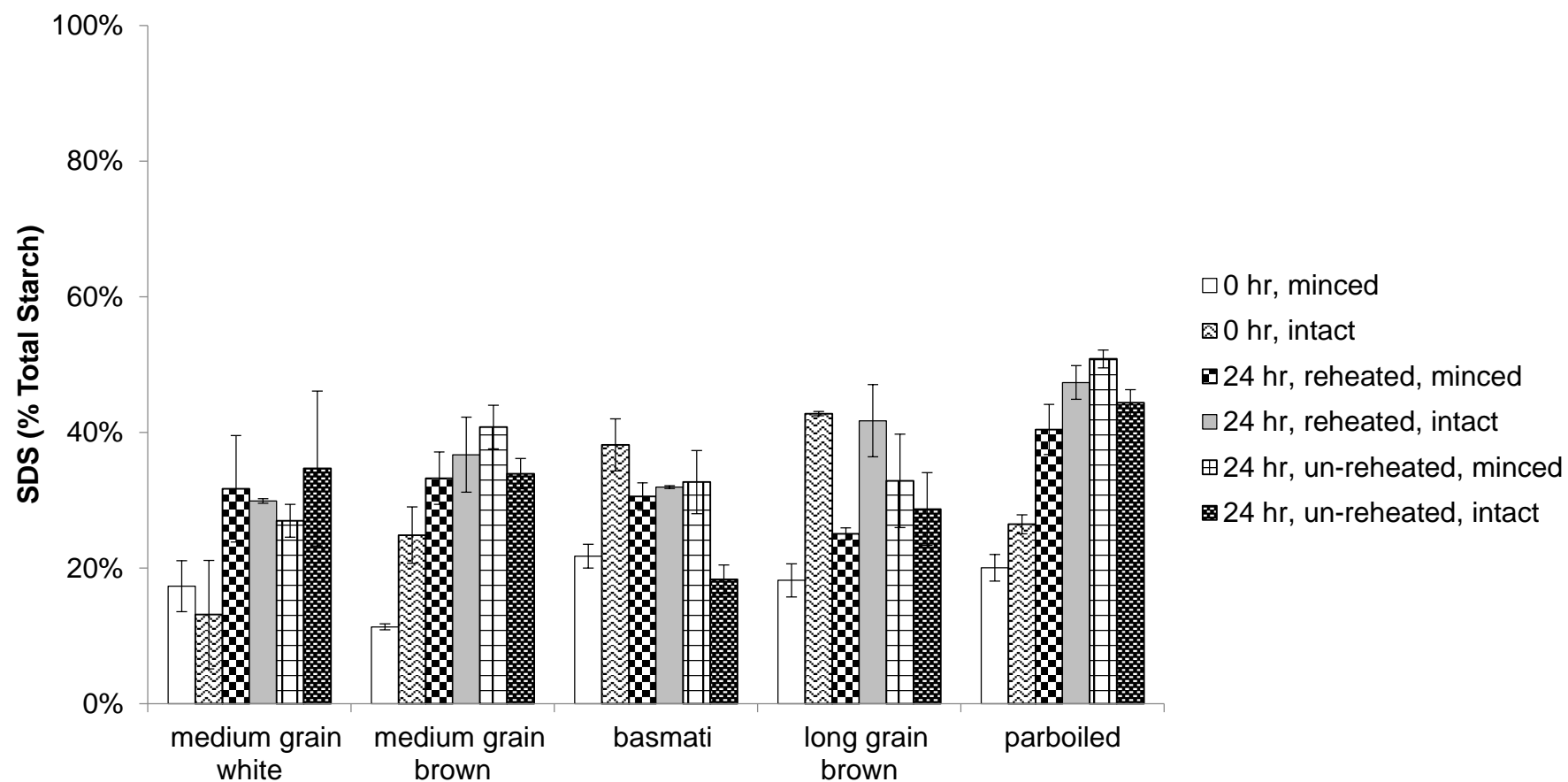


Figure 4-10 Average slowly digestible starch (SDS) (%) expressed as a percentage of total starch of five cooked rice products (n=14) after Group 2 mixed treatments shown in Table 4-2. Error bars are presented as 95% confidence intervals of the means.

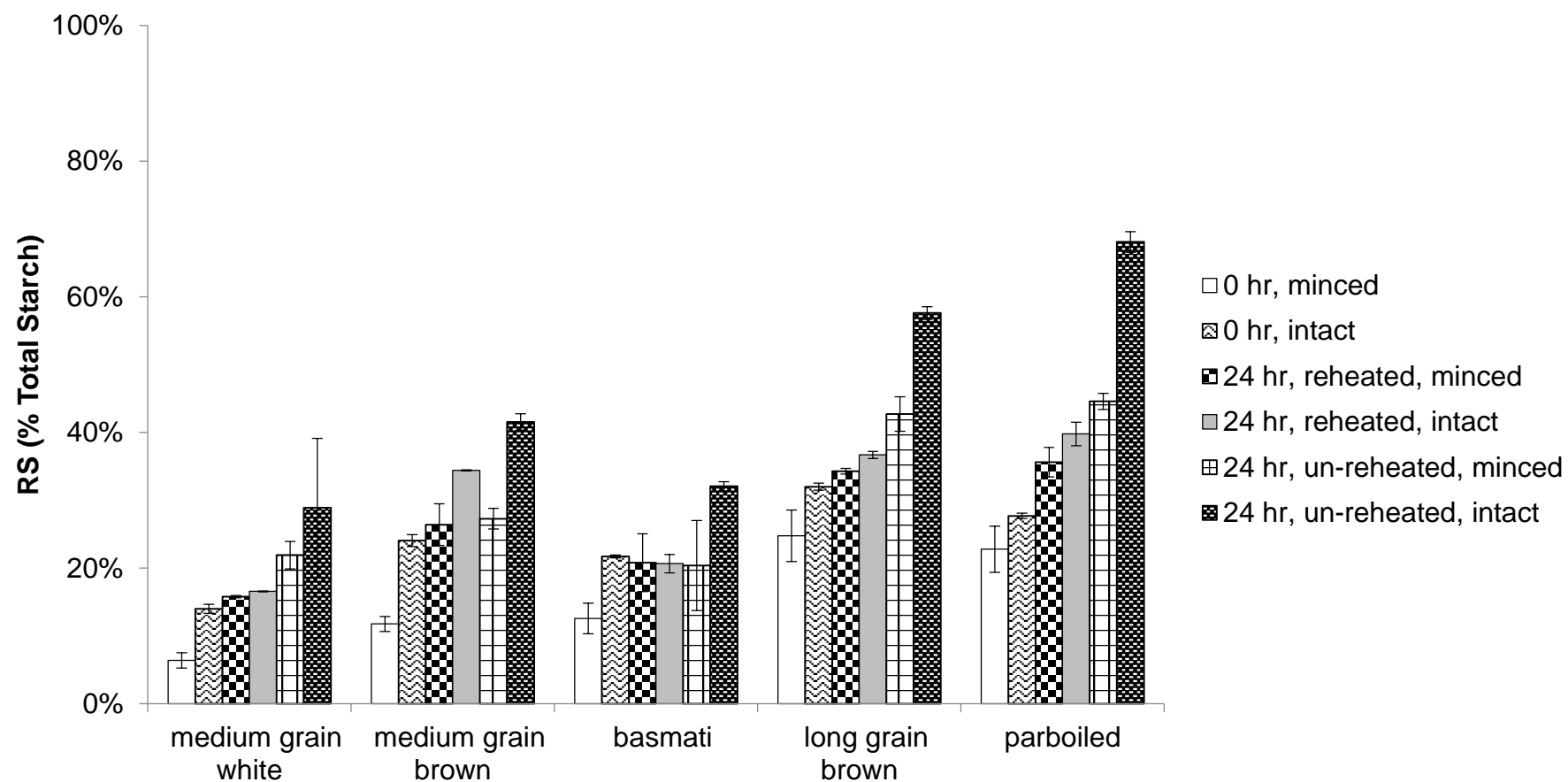


Figure 4-11 Average resistant starch (RS) (%) expressed as a percentage of total starch of five cooked rice products (n=14) after Group 2 mixed treatments shown in Table 4-2. Error bars are presented as 95% confidence intervals of the means.

4.4 Discussion

Previous studies on starch digestibility in cooked rice products have led to the conclusion that rice is generally classified as a staple food producing high postprandial glycaemic responses (Bjorck et al., 1994; Jenkins et al., 1981; Miller et al., 1992). In contrast, results from the present study showed that rice product variety, larger particle size (i.e., mincing or chewing rice grains), 24-hour cold storage at 4 °C and reheating to 65 °C might also lower the rate and extent of starch digestion. This investigation confirmed the hypothesis that the Group 1 treatment (i.e., prolonged cold storage, which promotes *in vitro* glucose release reduction) and Group 2 treatments (i.e., reheating and mincing, which both act as factors that counteract the effects of cold storage) have an impact on the rice starch digestibility profile, as reflected in the physiologically distinctive starch fractions, RDS, SDS and RS.

The investigated samples included a currently widely consumed rice product as control (SunRice® medium-grain white rice) and four rice products (SunRice® long-grain and medium-grain brown rice, basmati rice and Real Rice® parboiled rice) as alternatives.

Substantial differences in starch digestibility based on rice product varieties were noticeable. It was demonstrated that the widely consumed medium-grain white rice had relatively higher TAS and higher RDS compared with the other alternatives.

Furthermore, prolonged cold storage (0, 2, 4, 8, 10, 12 and 24 hours) had less impact on TAS and *in vitro* glucose release reduction of medium-grain white rice compared with other rice products. Generally speaking, whole grain rice products (medium-grain brown rice and long-grain brown rice) were more vulnerable to cold storage and mincing treatments than refined grain rice (medium-grain white rice and basmati.) Among all rice products, parboiled rice was found to have undergone the most marked reduction of starch digestibility. The main explanation for these differences is the proportion of RS and SDS, which are affected by parboiling treatment (pre-cooking), milling and polishing processes (refined vs. whole grain), mincing and cold storage.

First, cold storage at 4 °C is a significant factor that reduced *in vitro* rice starch digestibility among all five rice products. The study results are consistent with previous research findings that cold storage at 4 °C over 24 hours can significantly reduce the glucose release during the *in vitro* digestion process in rice (Frei et al., 2003; Fresco, 2005). 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 (Hu et al., 2004; Lehmann & Robin, 2007; Sajilata et al., 2006b; Zhang & Hamaker, 2009).

The effect of the parboiling treatment should be considered a key factor. Irreversible retrogradation of amylose, which can be induced by the pre-treatment process (i.e., parboiling), in previously gelatinised rice (parboiled rice) can lead to the formation of type 3 RS (RS3) and SDS (Englyst et al., 1987). The results for the parboiled rice, having a slower *in vitro* glucose release and higher RS and SDS content than other rice after the same treatment, also confirmed the previous observation that pre-cooking, drying and cooling 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 (Chung et al., 2008; Lehmann & Robin, 2007; Zhang & Hamaker, 2009).

This study has also demonstrated the significant difference in starch digestibility between the rice product varieties cooked and processed using the same procedures. This may be due to the different amylose-amylopectin proportion in medium-grain and long-grain rice. Medium-grain white rice has a higher proportion of amylopectin than long-grain white rice. Retrogradation of amylopectin is an unstable phenomenon and depends largely on the botanical source, the rice grain particle size and the storage conditions (Fried, Ludwig, Psenner, & Schleifer, 2002; Landon, 2007; Sajilata et al., 2006b). Retrogradation of amylopectin is reversible and occurs slowly, whereas the irreversible retrogradation of amylose reaches peak limit after 48 hours. It is suggested that longer cold storage (over 48 hours) may further induce the formation of RS; however, it may also have a negative impact on rice sensory properties, making the rice unacceptable for consuming (Frei et al., 2003).

This study also reported an inverse correlation between particle size and the *in vitro* starch digestion rate of cooked rice. It is suggested that the larger particle size of the cooked rice grain may limit the accessibility of the amylase in the small intestine. The results from this study are consistent with previous studies that cited particle size reduction as a factor reducing retrogradation and increasing digestibility of rice starch (Frei et al., 2003). This study also found that the effect of particle size reduction is more significant on whole grain rice than well-polished rice. It is suggested that the bran, the whole grain outer layer, is acting 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.

The study suggests that the digestion of rice starch was not terminated after 120 minutes. This supports the findings of Feri et al. (2003) and Eerlingen et al. (2007), who both found a high level of RS in retrograded waxy maize starch after 120 minutes of *in vitro* enzymatic incubation. 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. This study suggests that reheating reversed the retrogradation and crystallisation of rice starch to some extent; however, the extent of the reverse action was not significant in minced rice.

Twenty four-hour cold storage of cooked rice meal before consumption 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. It is particularly relevant for individuals suffering from impaired glucose metabolism. These findings should be further consolidated through GI measurement and chewing tests with human participants. Moreover, as was suggested in a previous study, that slow starch digestibility may be associated with a reduced sensation of hunger following ingestion (Lehmann & Robin, 2007), this cooking procedure may help reduce total daily food intake, increase the chewing length of each mouthful and prolong the intervals between food intakes (Collier & O'Dea, 1982; Ranawana et al., 2010; Read et al., 1986). Further studies on the satiety of reheated rice and viscosity within the gastrointestinal tract are recommended.

The strength of this study is that it followed *in vitro* starch digestion techniques whose precision and validity were tested and proven by previous studies, as indicated in the previous chapter. This group of experiments extended the research by simulating a series of rice consumption circumstances in real life, by investigating the impact of various cold storage times, reheating before consumption and chewing. The purpose of mincing was to simulate the average disruption of food achieved by simulating chewing. 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. 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, further study is required to investigate the rate and

extent to which starch of the 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.

4.5 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. Minced 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 could be explored further as it has the potential to reduce the glycaemic impact of rice for those who consume rice frequently.

Chapter 5: Effect of rice cooking method on postprandial glycaemic response, satiety and palatability, and chewed particle distribution

Abstract

When cooked rice is consumed, the rate of *in vitro* starch digestion and the rate of glucose released over 180 minutes is affected by the time the cooked rice is stored in the refrigerator before being reheated and the particle size of the rice. A group of simulated digestion tests showed that compared with freshly cooked medium-grain white rice, parboiled rice cold stored for 24 hours had a significantly lower rate of glucose release. This randomised cross-over experiment trial testing three different rice samples on three different occasions with 28 healthy human volunteers aimed to investigate at 0, 30, 60, 90 and 120 minutes the changes in postprandial blood glucose concentration when eating approximately 140 g ($140 \text{ g} \pm 0.3 \text{ g}$) of rice. The three rice samples were freshly cooked medium-grain white rice, freshly cooked parboiled rice, and parboiled rice stored overnight at 4 °C. All rice was served warm at 65 °C. Chewing time was recorded. The 24-hours cold-stored and reheated parboiled rice resulted in a significantly lower blood glucose concentration trajectory (42%, $P < 0.001$) than freshly cooked medium-grain white rice and 12% lower ($P = 0.001$) than freshly cooked parboiled rice. Longer chewing time (6.34 seconds/10 g of rice compared with freshly cooked medium-grain white ($P = 0.026$) and higher palatability score (“visual appeal” was 2.0 higher ($P = 0.001$), “smell” was 1.0 higher ($P = 0.034$), “taste” was 1.5 higher ($P = 0.023$), and “overall palatability” was 1.9 higher ($P = 0.003$)) might have impacted on the slower rise of glucose response of the reheated parboiled rice. Further study is required to investigate the sensory acceptability of the rice products to investigate whether rice prepared by the optimal treatment could be accepted as part of the diet.

Keywords: Parboiled rice, medium-grain white rice, cold stored, reheating, blood glucose concentration chewing time, satiety, palatability.

5.1 Introduction

Globally, the consumption of rice, which currently provides the majority of daily energy intake and carbohydrate for at least half of the world's population (O'Neil, Nicklas, & Fulgoni III, 2013; Parackal et al., 2015), has been increasing. Since the Joint FAO/WHO Expert Consultation on Carbohydrates in Human Nutrition in April 1997 (Englyst et al., 2007), there has been increased understanding of the diverse physiological roles that carbohydrates have on the rate and extent of digestion in the gut and the relationship between dietary carbohydrates and various non-communicable diseases, including hyperglycaemia, insulin resistance, obesity, metabolic syndrome and type 2 diabetes (Barclay et al., 2008; Bell & Sears, 2003; Burton et al., 2011; Moran, 2004).

The metabolic quality of carbohydrate sources such as rice may be directly determined by the effect on biomarkers of carbohydrate metabolism, such as glycaemic response (i.e., postprandial blood glucose concentration). The key determinants of the postprandial blood glucose response are the amount, rate and extent of carbohydrate digestion (Wolever & Jenkins, 2010), insulin secretory response (Grundy, 2012), and gastric emptying (Phillips et al., 2015; Zhu, Hsu, & Hollis, 2013). The extent of digestion and thus the glycaemic response are determined by the particle size of the food, the cooking method, the size of the mouthful, the extent of chewing and digestion that takes place in the mouth, and the physical and chemical properties of the starch (Bornhorst & Singh, 2012; Tamura et al., 2016; Tan et al., 2015).

One measure of a healthier cooked rice meal would be reduction of the glycaemic load (GL, i.e., slower and lower sustained release of glucose) related to a lower proportion of rapidly digestible starch (RDS, starch that can be digested within 20 minutes following ingestion) and higher proportions of slowly digestible starch (SDS, starch that can be digested between 20 and 180 minutes following ingestion) and resistant starch (RS, starch that can resist digestion for up to 180 minutes following ingestion) as well as to promote satiety and reduce palatability to reduce hunger and delay voluntary feeding behaviour.

As reported in Chapter 3, “Freshly cooked rice: Starch and moisture profile of rice”, and Chapter 4, “Effect of rice product varieties, cold storage, reheating, and grain particle sizes on starch digestibility profile and *in vitro* glucose release”, the rate and extent of *in vitro* rice starch digestion and release of glucose varied by rice product variety, processing techniques (i.e., the extent of milling and polishing), and pre- and post-treatments (e.g., parboiling and reheating after cold storage). This group of *in vitro*

digestion tests has shown that freshly cooked warm rice was digested more rapidly than cold-stored and reheated rice and minced reheated parboiled rice was more resistant to digestion. Three previous studies, by Wolever (1986), Larsen (2000), and Chitra, Singh, & Ali (2010), have investigated the commercially obtained parboiled rice products (processed by modernised parboiling process) and found lower glycaemic response in the freshly prepared parboiled rice samples compared with non-parboiled. However, the glycaemic response of cooked parboiled rice after cold storage and reheating process and the effect of chewed particle size on glucose release have not been investigated.

The rate and extent of blood glucose response, the energy density of carbohydrates (i.e., the proportion of available starch in a cooked rice meal), and the proportion of RS may all influence satiety and palatability and ultimately feeding behaviour (Elia & Cummings, 2007). Studies have found that low energy density carbohydrate food (e.g., low GI and high RS) is associated with promoting satiety and reduced palatability (Anderson et al., 2010; Elia & Cummings, 2007; Higgins, 2004; Raben et al., 1994; Willis, Eldridge, Beiseigel, Thomas, & Slavin, 2009). Reduced satiety and increased palatability have both been linked to the improved quality of food that is consumed in the general diet and thus influence the long-term daily total energy intake (Anderson et al., 2010; Elia & Cummings, 2007; Willis et al., 2009). Previous studies have suggested that a healthy diet that promotes weight management should avoid high glycaemic and high energy density carbohydrate foods with a lower proportion of non-digestible starch (i.e., RS and dietary fibre), on the basis that they may cause postprandial hyperglycaemia that, in turn, stimulates appetite and palatability, reduces satiety and makes weight loss more difficult (Anderson & Woodend, 2003; Ball et al., 2003; Blundell & V. Burley, 1987; Drewnowski, 1998; Duncan, Bacon, & Weinsier, 1983; Raben, Holst, Christensen, & Astrup, 1996). However, few studies have compared the satiety and palatability responses of the same food that was prepared in different ways (i.e., cold storage and reheating) in the same subject population while the glycaemic responses were examined.

The findings of the previous in vitro experiments: that freshly cooked parboiled rice and cold stored and reheated parboiled rice compared with freshly cooked medium grain white rice had a reduced glycaemic impact led to the need to the aim to test the glycaemic response in a trial with healthy participants. While reheated medium grain rice could also be tested it was considered that an extra visit would add to participant

burden and reduce retention of participants in the trial – hence only three conditions. The aim of the study was to compare the test rice (freshly cooked parboiled rice, and reheated parboiled rice) to the control rice, freshly cooked medium grain white rice in order to investigate the possible rice alternative and cooking alternative.

It was hypothesised that after cooking rice, the cold storage (at 4 °C for 24 hours) and reheating process (to 65°C for 15 minutes) would significantly reduce the glycaemic response to a rice meal and that parboiled rice compared with non-parboiled rice would also lower the response. Moreover, reheated parboiled rice would enhance satiety and reduce palatability after a meal. Thus, consuming reheated rice, especially parboiled rice, would reduce postprandial blood glucose concentration.

5.2 Method and material

5.2.1 Study design

This experimental trial tested three rice samples, freshly cooked medium grain white rice, freshly cooked parboiled rice, and reheated parboiled rice, was divided into two parts: the investigation of the primary outcome, postprandial blood glucose concentration; and the relationship with the secondary outcomes, satiety response, palatability response and chewed particle size distribution (Figure 5-1). The design of the primary experiment was based on the GI methodology (Brouns et al., 2005; Venn et al., 2006; Wolever et al., 1991). The secondary outcome measures were based on several previous satiety investigations using visualised analogue scales (VAS) (Flint, Raben, Blundell, & Astrup, 2000; B. Parker et al., 2004; Wewers & Lowe, 1990). The study was randomised by group allocation. Each study day was seven days apart from the previous study day to minimize any possible ordering effect. Full randomisation was not practical given the restricted availability of the laboratory, staff and resources.

5.2.2 Ethics

This study was conducted according to the guidelines of the human ethics committees of the University of Otago and AUT. Both university ethics committees approved all procedures involving human participants. Ethical approval for the study was obtained from the University of Otago Ethics Committee and the Human Ethics Committee of AUT (see Appendix 2).

Table 5-1 Sample details and preparation methods based on the results from glycaemic responses study.

Sample	Rice types	Product origin	Brand	Supermarket	Rice:Water ¹	Average cooking time ² (min) (95% CI)	Preparation method
Control	Medium-grain white	Australia	SunRice®	New World, Dunedin	1:1.5	33.5 (31.1, 35.9)	Freshly cooked warm at 65 °C
Test 1	Parboiled long-grain white	Thailand	Real Rice®	Countdown, Auckland	1:2.3	46.8 (44.4, 49.1)	Freshly cooked warm at 65 °C
Test 2	Parboiled long-grain white	Thailand	Real Rice®	Countdown, Auckland	1:2.3	46.3 (43.6, 48.9)	24-hr storage, reheated to 65 °C

Notes.

¹ Rice-to-water ratio for cooking was recommended by SunRice® and Real Rice® provided on rice product package.

² Cooking time (min) of each rice product was measured by timing the cooking duration in each rice cooker (rice cooker A and B) in two separate experiment sessions. For each rice type, four cooking times (min) were collected to calculate the average.

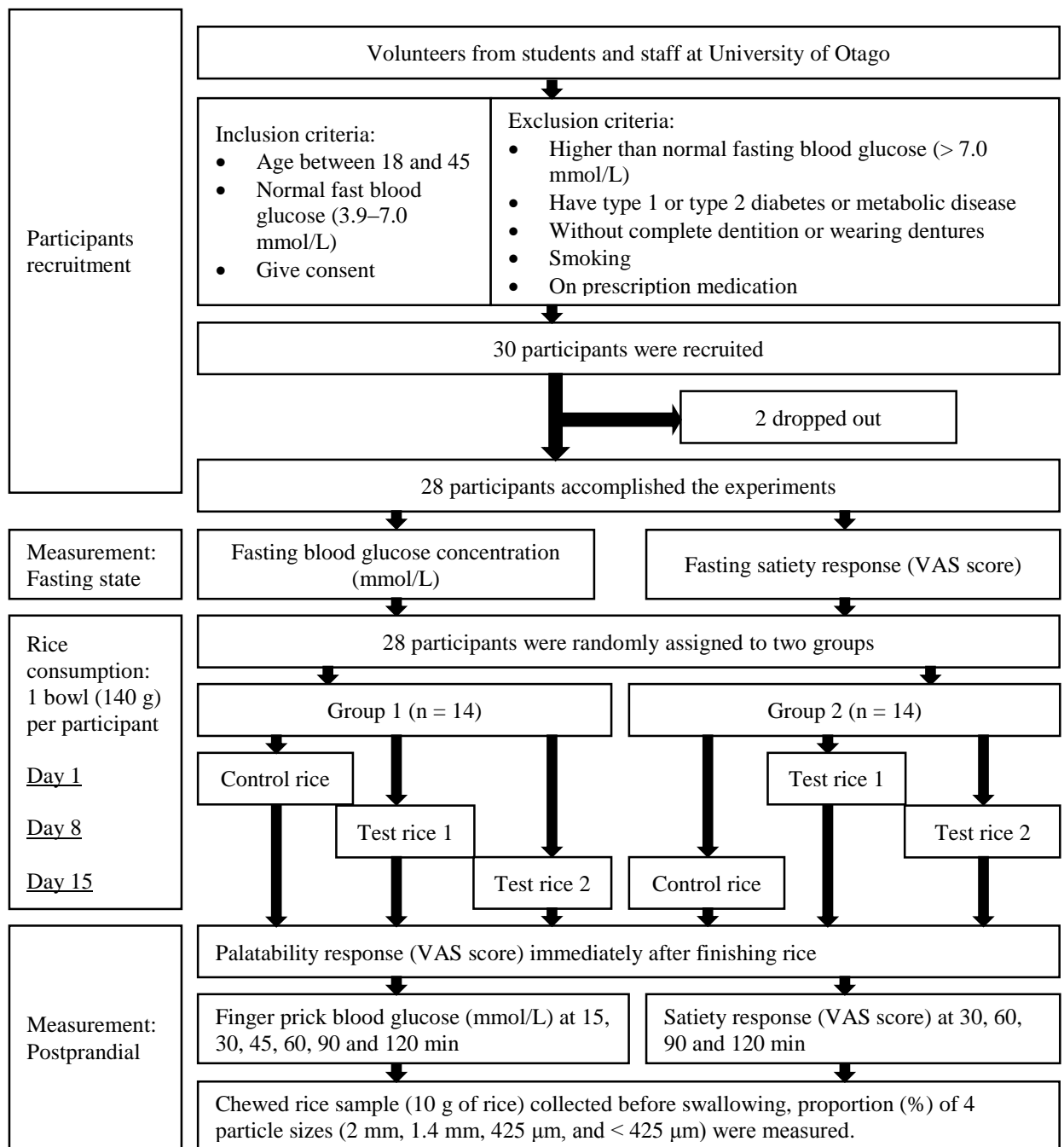


Figure 5-1 Flow chart of the experiment design for human experiments (glycaemic responses, chewed particle distribution, and satiety and palatability study).

5.2.3 Participants

Recruitment method

The study population target was young multi-ethnic healthy adults living in Dunedin, New Zealand. To control for body mass index (BMI) effects on glycaemic responses and to broaden generalisability, around half of the participants were screened for normal weight and half for being overweight or obese (WHO criteria). Thirty healthy participants were recruited between March and April 2013 by means of advertisements, flyers, information sheets, notices and Internet postings. Flyer advertisements, which clearly explained the purpose and inclusion and exclusion criteria of this experimental study, were posted on University of Otago department bulletin boards on campus and in the library (see Appendix 3). Emails containing the same message were sent to university departments and to the Otago student associations.

Screening

Volunteers who expressed interest were contacted via telephone and email, and a brief description of this study, “Information Sheet”, was provided in hard copy (see Appendix 4). Those who remained interested in the study were asked to complete a recruitment questionnaire on Survey Monkey (see Appendix 5) and to come for screening for fasting finger prick blood glucose (using a HemoCue reader, HemoCue® Hb 201 System, Sweden), weight and height. People who met the eligibility criteria were included.

Eligibility

Inclusion criteria

The eligible population consisted of staff and students, aged between 18 and 45 years, from the University of Otago, Dunedin, New Zealand. Before inclusion in the study, potential participants were briefed with all aspects of the experiments (finger prick blood glucose measurement, satiety and palatability scales, and chewing test) and were given an opportunity to ask questions. The second procedure was a health assessment, which included anthropometric measurement and a health questionnaire (including whether they had diabetes). Those who met all the inclusion criteria (50% of participants were normal weight ($BMI < 25$) or 50% overweight ($BMI \geq 25$), fasting finger prick glucose between 3.9 and 7.0 mmol/L; age, 18–45 years old; complete dentition; not wearing dentures; non-smoking; not on prescription medication; no genetic or metabolic diseases) were included in the study. The blood pressure and waist were not recorded. However, none of the participant had high fasting blood glucose or abnormal glycaemic response after eating the rice meal. No metabolic syndrome or insulin resistance was observed.

The selection of half overweight or obese was for a concurrent study (Amy White) where chewing dynamics were compared and not part of the question for this study.

Exclusion criteria

People were excluded from this study if they were younger than 18 or older than 46 years old, pregnant, smokers or taking prescription medications.

All participants gave written informed consent before starting. After completion of all experiments, all participants were provided with a light breakfast and compensated for their time.

The light breakfast was a mixture of liquid breakfast, one low-sugar bar, and one piece of fruits of choice. The light breakfast could not have altered the glucose response to the rice because there was seven days apart between two study visits.

5.2.4 Rice cooking and cold storage

Materials

Three 1 kg bags of medium-grain white and two 2 kg of parboiled long-grain rice were purchased from New Zealand Dunedin supermarkets (see Table 5-1). Both rice products were from the same batch to avoid inter-batch variation.

Rice cooker

Two standard automatic rice cookers (Tefal® R07), cooker A and cooker B, were used for rice cooking. Turning on the power switch started the cooking process by boiling the water and rice mixture in the removable inner cooking pan using the bottom build-in heating plate. The heat of the content of the inner cooking pan was monitored by a spring-loaded thermometer sensor. During the cooking process, the majority of the water in the inner cooking pan was absorbed by the rice (i.e., the rice gelatinisation process) and a small amount of the water evaporated through the holes in the lid. Once all the water was absorbed by the rice, the temperature inside the inner cooking pan started to rise. The sensor detected a temperature rise and automatically switched the rice cooker to the warming cycle. Initiation of the warming cycle was indicated by an indicator light turning from green to amber.

Control rice: Freshly cooked preparation for medium-grain white rice

Three standard measuring cups (Farberware® Classic Measuring Cup, 3 cups = 600 g) of medium-grain white rice (SunRice®, Ricegrowers Ltd.) were taken out of the package and poured into the clean removable inner cooking pan in automatic rice cooker A (Tefal® R07). Four and half standard measuring cups (Farberware® Classic Measuring Cup, 4.5 cups = 1,700 ml) of room temperature tap water were added to the

same pan. The same amount of water and rice was added to the clean removable inner cooking pan in rice cooker B. After the lids of both rice cookers were fully closed, the rice cookers were turned on at the same time and the cooking duration was timed for each (Table 5-1).

When the rice was cooked, the cooker continued to keep the rice warm. One hundred and forty grams of warm rice (65 °C) was weighed on a scale (Sartorius®, CP4202S, USA) and placed into a warm bowl (pre-heated to 50 °C in a convection oven, Sanyo®, MOV-212F, Japan) and checked by a clean thermometer before being served to each participant. The thermometer was cleaned by alcohol wipes and then rinsed under tap water to remove the rice residues. Between servings, the lids of the rice cookers were kept fully closed to prevent heat loss and the top evaporation hole was wrapped in plastic food wrap to reduce moisture loss. Two rice cookers were used to cook and prepare the sufficient amount of rice for each study day. The cooking times (min), rice-to-water ratio, and temperatures were recorded to ensure cooking procedures were not different.

Test rice 1: Freshly cooked parboiled rice

Three standard measuring cups (Farberware® Classic Measuring Cup, 3 cups = 600 g) of parboiled long-grain white rice (Real Rice®, Rice Importer Ltd.) and seven standard measuring cups (Farberware® Classic Measuring Cup, 7 cups = 2625 ml) of cold tap water were added to the clean removable inner cooking pan in automatic rice cooker A (Tefal® R07). The same amount of water and rice was added to the clean removable inner cooking pan in rice cooker B. The same cooking and serving procedures as used in the control rice experiment were followed.

Test rice 2: Cold storage and reheating preparation for parboiled rice

Following the cooking procedure, approximately 140 g of freshly cooked warm parboiled long-grain white rice was weighed on a balancer (Sartorius®, CP4202S) and placed into a shallow bowl (4 cm deep, pre-cooled to 4 °C in the refrigerator) to ensure instant cooling. Plastic food wrap was wrapped around the bowl to seal the moisture. The sealed rice bowls were placed in the refrigerator for rapid cooling to 4 °C and for 24-hour storage. The temperature of the cooled rice was checked three times: at three hours, six hours and 24 hours after refrigeration, using a clean wiped thermometer. Before consumption, the bowls were removed from the refrigerator and reheated in a convection microwave (Sharp®, R99) at 1,000 W power until the temperature after

stirring reached 65 °C. The thermometer cleaning procedures used in the control rice experiment were followed in this experiment.

5.2.5 Data collection

Participants were randomly assigned to two groups in equal sample size (14 participants in each group, n=28) using block randomisation to ensure a balance in sample size across groups over time. The randomisation was undertaken using a random number generator program in Excel. Each group was pre-assigned one of the three rice treatments in random order.

Each group attended the Glycaemic Index Laboratory, Science Building One, University of Otago, Dunedin, New Zealand, for three separate sessions, from 23 April to 3 May 2013. No specific diet control was required of participants during the study period. Participants were asked not to eat heavy meals on the previous day, fast for at least 10 hours before study, and no vigorous physical activity during the study period. In each session, participants completed a series of three separate tests on one rice product: blood glucose responses test, satiety and palatability VAS, and chewing test (Figure 5-1). A total of three samples were tested (freshly cooked medium-grain white rice, freshly cooked parboiled long-grain rice and reheated parboiled long-grain rice). Each session was seven days apart to increase the accuracy of the blood glucose response (Brouns et al., 2005). Three experiments were performed during one session (Figure 5-1).

Recording blood glucose responses

Three HemoCue analysers (HemoCue® Hb 201 System; HemoCue No.1 SN: 1103122022, HemoCue No.2 SN: 1336122063, and HemoCue No.3 SN: 1336122062) were calibrated using an instrument-matched calibration cuvette, and three concentrations of control solutions (2.0 mmol/L, 4.5 mmol/L and 8.0 mmol/L) that were provided by the HemoCue manufacturer were used in glucose precision test to verify the accuracy of the meter reading on a daily basis before all tests began. The glucose precision test reported the mean, standard deviation (SD), and coefficient variation (CV) range of three concentrations of control solutions on these three HemoCue analysers. The lower concentration (2.4 mmol/L, range 1.6-3.2) gave mean 2.47 mmol/L (SD = 0.007, CV = 2.73%), 2.58 mmol/L (SD = 0.06, CV = 2.45%), and 2.57 mmol/L (SD = 0.05, CV = 1.88%) respectively, the medium concentration (5.9 mmol/L, range 5.0-6.8) gave mean 5.71 mmol/L (SD = 0.09, CV = 1.65%), 5.79 mmol/L (SD = 0.16, CV = 2.73%), and 5.83 mmol/L (SD = 0.11, CV = 1.89%) respectively, the high concentration (9.9 mmol/L, range 8.7-11.1) gave mean 9.31 mmol/L (SD = 0.14, CV = 1.56%), 9.37

mmol/L (SD = 0.16, CV = 1.75%), and 9.34 mmol/L (SD = 0.15, CV = 1.61%) respectively.

Participants arrived at the laboratory after eight-hour overnight fasting (between 10 pm and 6 am). Each participant was provided with a copy of the Lab Visit Checklist (see Appendix 6) with their participant ID, rice sample and lab technician name written on top of the sheet. After letting participants rest in a warm room for 15 to 20 minutes, their fasting capillary blood sample was sampled twice five minutes apart from fingers using sterilised disposable lancets and a 5 µl drop was collected using a microcuvette (HemoCue® Hb 201 Microcuvette). The two measurements were averaged to provide the baseline fasting blood glucose concentration (at T0).

Immediately after the second fasting blood glucose concentration was measured, the participants were given a bowl of warm cooked plain rice sample (140 ± 0.1 g at 37 °C), which they consumed at an even pace over 15 minutes. Blood glucose concentrations were measured after eating over a period of 120 minutes: at 15, 30, 45, 60, 90 and 120 minutes. For time efficiency participants were asked to record all blood glucose concentration readings (baseline and the other six readings) on the Lab Visit Checklist immediately after the reading and this was checked by the investigator.

Satiety and palatability response

Participants were provided with six VAS questionnaires (five satiety questionnaires and one palatability questionnaire; see Appendix 5). Each participant was asked to complete the first satiety questionnaire before the first baseline blood glucose concentration was measured and then the palatability questionnaire immediately after finishing the rice sample. The other four satiety questionnaires were completed at 30, 60, 90 and 120 minutes immediately after each blood measurement. For each question, participants were asked to mark their relative response on the visual analogue score line. After completion questionnaire was turned face down on the desk to prevent participants from reviewing the previous results.

The VAS score was analysed by measuring the length (mm) from the left end of the scale to the mark. The length (mm) was divided by the total length of the scale (100 mm) to give a score of the VAS out of ten.

Chewed particle size distribution test

At the end of the testing (120 min), participants were given a tablespoonful of rice sample (10.6 ± 0.1 g) and asked to chew it as they normally would, but instead of swallowing, to expectorate the rice into a labelled container. Time (seconds) spent on

chewing was recorded by the researcher. A sip of lukewarm water was then given to the participants to rinse the mouth and to then expectorate the remaining chewed particles in the mouth into the same container. All participants were asked to perform the chewing test only once. They were asked to repeat the chewing test if they accidentally swallowed part of the rice sample.

Expectorated samples were washed over three stainless steel laboratory sieves with a mesh aperture of 2,000 µm, 1,400 µm and 425 µm. The rice particles retained on the sieve were carefully washed (to eliminate the salivary α-amylase residues) and collected and placed into a metal dish for drying in the convection oven. The samples were oven-dried at 70 °C and measured twice at 24 and 48 hours.

A non-expectorated duplicate sample was taken from the cooked rice samples and used for a moisture content determination. For the weight of the dried sample and the moisture content, the proportions of the rice sample that passed through the sieves (“particle diameter > 2,000 µm”, “particle diameter ≤ 2,000 µm and > 1,400 µm”, “particle diameter ≤ 1,400 µm and > 425 µm”, and “particle diameter ≤ 425 µm”) were calculated. For each diameter, the same sieve was used each time to minimise cumulative errors and reduce variability.

5.2.6 Statistics analysis

Calculation of incremental area under the glucose response curve

The method used to calculate the incremental area under the curve (iAUC) is illustrated in Figure 5-2, and this involved accumulating the areas of triangles and rectangles compassed by the curve, following the trapezoidal rule described by Wolever et al. (2010; 1986). The equation can be written as:

$$Area = a * \frac{t1}{2} + a * t2 + (b - a) * \frac{t2}{2} + b * t3 + (c - b) * \frac{t3}{2} \text{ etc}$$

where *a*, *b*, *c*, *d* and *e* are the blood glucose increment values above fasting at each time period, and *t* is the time interval between blood samples (15 minutes in the first four sets and 30 minutes in the last two sets). When the blood glucose value is negative (i.e., less than fasting), the last area must be calculated as the proportion of the triangle that is above 0 (fasting state).

The formula was modified for each individual blood glucose curve, such as when the blood glucose fell below the fasting level earlier, later or not at all. As recommended by Wolever and Jenkins (1986) and Brouns et al. (2005), only the area under the curve that

was above the fasting level was calculated, and any areas below the fasting level were ignored.

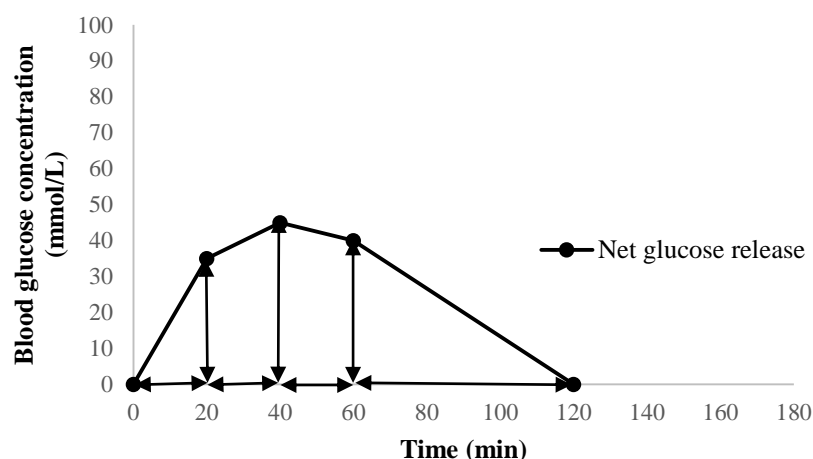


Figure 5-2 Calculation of incremental area under the blood glucose response curve (iAUC) (mmol/L) over the baseline.

All statistical analyses were performed using SPSS version 12.0 (SPSS Inc, Chicago, Ill). The finger prick blood glucose concentrations and area under the glucose response curve (AUC) were compared among three rice samples by repeated measurement analysis of variance (ANOVA). The chewing particle distributions, chewing time, satiety and palatability scores were also analysed by repeated measurement ANOVA. Univariate regression models with a random effect for participants were used to examine the association between the iAUC for rice and variables including age, sex, BMI, chewing particle distributions, chewing time, and satiety and palatability scores.

5.3 Results

All of the rice samples were well received and completely consumed by the participants. The volume of each rice meal was not large (140 ± 0.3 g) and all participants took less than five minutes (3.5 ± 0.7 minutes) to complete eating each serve.

Participant characteristics

Twenty-eight young volunteers: 18 females and 10 males, aged $22.1 (\pm 4.3)$ and $25.3 (\pm 6.5)$ years respectively, participated. All participants had normal fasting blood glucose (male, 5.0 ± 0.3 mmol/L; female, 4.9 ± 0.3 mmol/L) according to the World Health Organisation classification (normal fasting blood glucose between 3.9 and 5.5 mmol/L), and there were no differences in the fasting blood glucose concentrations by gender ($F < 0.001$, $P = 1.000$; Table 5-2). None of the participants had fasting blood glucose over 6.1 mmol/L during the study period. All participants had fasting blood glucose between 3.9 and 5.5 mmol/L.

By design for another study, half the participants were overweight or obese ($\text{BMI} \geq 25 \text{ kg/m}^2$). No significant difference was found between the two BMI groups in fasting blood glucose ($P = 0.589$) (There was also no difference in incremental the area under the curve by overweight status).

The self-identified ethnic groups of the participants were either European (71.4%) or Asian (Chinese and South Asian, 28.6%). European participants were of European descent and were born in Europe, Australia or New Zealand. The South Asian participants also originated from several countries, including India, Nepal and Sri Lanka. One Chinese participant came from Hong Kong and the other from Mainland China.

Table 5-2 Characteristics of participants in glycaemic response human experimental trail (N = 28).

	Male	Female	P-value	Total
n	10	18		28
Age (y)	25.3 (6.5)	22.1 (4.3)	0.197	23.2 (5.4)
Height (cm)	175.1 (4.0)	164.0 (8.3)	< 0.001	167.9 (8.8)
Weight (kg)	76.7 (12.3)	69.4 (14.8)	0.178	72.0 (14.1)
BMI ¹ (kg/m ²)	24.9 (3.6)	25.8 (4.7)	0.589	25.5 (4.3)
Fasting finger prick glucose ² (mmol/L)	5.0 (0.3)	4.9 (0.3)	0.501	4.9 (0.3)
BMI categories				
Normal weight ³ (n)	5	8		13
Overweight and obese ⁴ (n)	5	10		15
Ethnicities				
European (n)	6	14		20
South Asian (n)	3	3		6
Chinese (n)	1	1		2

Note: Measures are presented as mean (SD) or are indicated otherwise.

¹ Body mass index was calculated as weight in kilogram/height in square metres.

² Fasting finger prick glucose was measured at the recruitment interview.

³ Normal weight was defined by BMI (kg/m²) < = 25.

⁴ Overweight and obese were defined by BMI (kg/m²) > 25.

Comparison of glycaemic response trajectory and the incremental area under the blood glucose response curve

For all experimental treatments, the fasting blood glucose concentrations were similar prior to consumption of each of the three rice meals (freshly cooked medium-grain white, 4.9 ± 0.4 mmol/L; freshly cooked parboiled rice, 5.0 ± 0.4 mmol/L; reheated parboiled rice, 4.8 ± 0.3 mmol/L; see Table 5-3) and were not significantly different ($F = 1.020$, $P = 0.356$).

The glycaemic response trajectory for each rice treatment was plotted as mean postprandial finger prick blood glucose against time (Figure 5-3). Reheated parboiled rice was consistently lower and freshly cooked medium grain white rice higher throughout the 120 minutes. The repeated measures ANOVA test showed significant differences in the mean blood glucose concentrations (mmol/L) of the three rice meals at 15 minutes ($F = 3.070$, $P = 0.052$), at 45 minutes ($F = 3.255$, $P = 0.044$), at 60 minutes ($F = 6.540$, $P = 0.002$), at 90 minutes ($F = 10.831$, $P < 0.001$), and at 120 minutes ($F = 6.343$, $P = 0.003$). At 30 minutes after consumption, no significant difference among the three rice treatments was found ($F = 0.795$, $P = 0.455$). With increasing time (minutes after consumption), there was an increasing amount of variance that may be explained by the rice meals (from around 15% of variance ($\eta^2 = 0.157$ at baseline) to around 40% ($\eta^2 = 0.459$ at 90 minutes). No overall significant difference was found between freshly cooked medium-grain white rice and freshly cooked parboiled rice (both least

significant difference (LSD) and Bonferroni tests $P > 0.05$). Significant differences were observed in the iAUC for the three rice treatments ($F = 9.555$, $P < 0.001$).

The incremental change in glycaemic response (see Table 5-3) shows that reheated parboiled rice had the lowest overall glucose response trajectory compared with freshly cooked parboiled rice, that is, the control ($P < 0.05$). Conversely, the trajectory and iAUC of the control rice, freshly cooked medium-grain white rice, was the highest: 1.7-fold higher (1.7, 2.0) than reheated parboiled rice ($P < 0.001$) and 1.1-fold higher (1.0, 1.3) than freshly cooked parboiled rice ($P = 0.001$; Table 5-3). In other words, the 24-hours cold-stored and reheated parboiled rice resulted in a significantly lower blood glucose concentration trajectory (42% lower, $P < 0.001$) than freshly cooked medium-grain white rice and 12% lower ($P = 0.001$) than freshly cooked parboiled rice.

Fifteen minutes after consumption finished, the glycaemic response to freshly cooked medium-grain rice (control) was 0.2 mmol/L higher than it was to the freshly cooked parboiled group ($P = 0.219$) and 0.4 mmol/L higher than it was to reheated parboiled ($P = 0.015$; Figure 5-3). This initial faster and prolonged higher glycaemic response (over 5.0 mmol/L) for the control rice was sustained over 120 minutes. Contrarily, the mean glycaemic response to reheated parboiled rice dropped below 5.0 mmol/L at 90 minutes after consumption finished.

Table 5-3 Means of blood glucose responses (mmol/L) at baseline and incremental blood glucose response (mmol/L) at each time point after consuming three rice samples and the incremental area under the glucose responses curve (iAUC) (mmol/L * min). The means in the same column with the same letter are significantly different (P < 0.05).

		Incremental area under the curve (iAUC) (mmol/L*min)	Incremental blood glucose responses (mmol/L)						
Cooking method	Rice product varieties		Baseline	15 min	30 min	45 min	60 min	90 min	120 min
Control rice									
Freshly cooked	Medium-grain white	144.7 a b (119.8, 169.6)	4.9 (4.8, 5.1)	1.0 (0.7, 1.3)	2.1 (1.7, 2.5)	2.1 a b (1.7, 2.5)	1.5 a b (1.1, 1.9)	0.8 a b (0.5, 1.1)	0.4 a b (0.1, 0.6)
Test rice									
Freshly cooked	Parboiled	94.9 a (75.5, 114.4)	5.0 (4.8, 5.1)	0.7 (0.5, 0.9)	1.7 (1.4, 2.0)	1.5 a (1.2, 1.9)	0.8 a (0.5, 1.2)	0.3 a (0.0, 0.5)	0.0 a (-0.1, 0.2)
Reheated	Parboiled	83.5 b (63.4, 103.6)	4.8 (4.7, 5.0)	0.6 (0.4, 0.8)	1.9 (1.5, 2.3)	1.6 b (1.2, 2.0)	0.7 b (0.3, 1.1)	0.0 b (-0.1, 0.2)	0.0 b (-0.2, 0.2)

95% confidence intervals for each mean of blood glucose responses (mmol/L) are in the brackets.

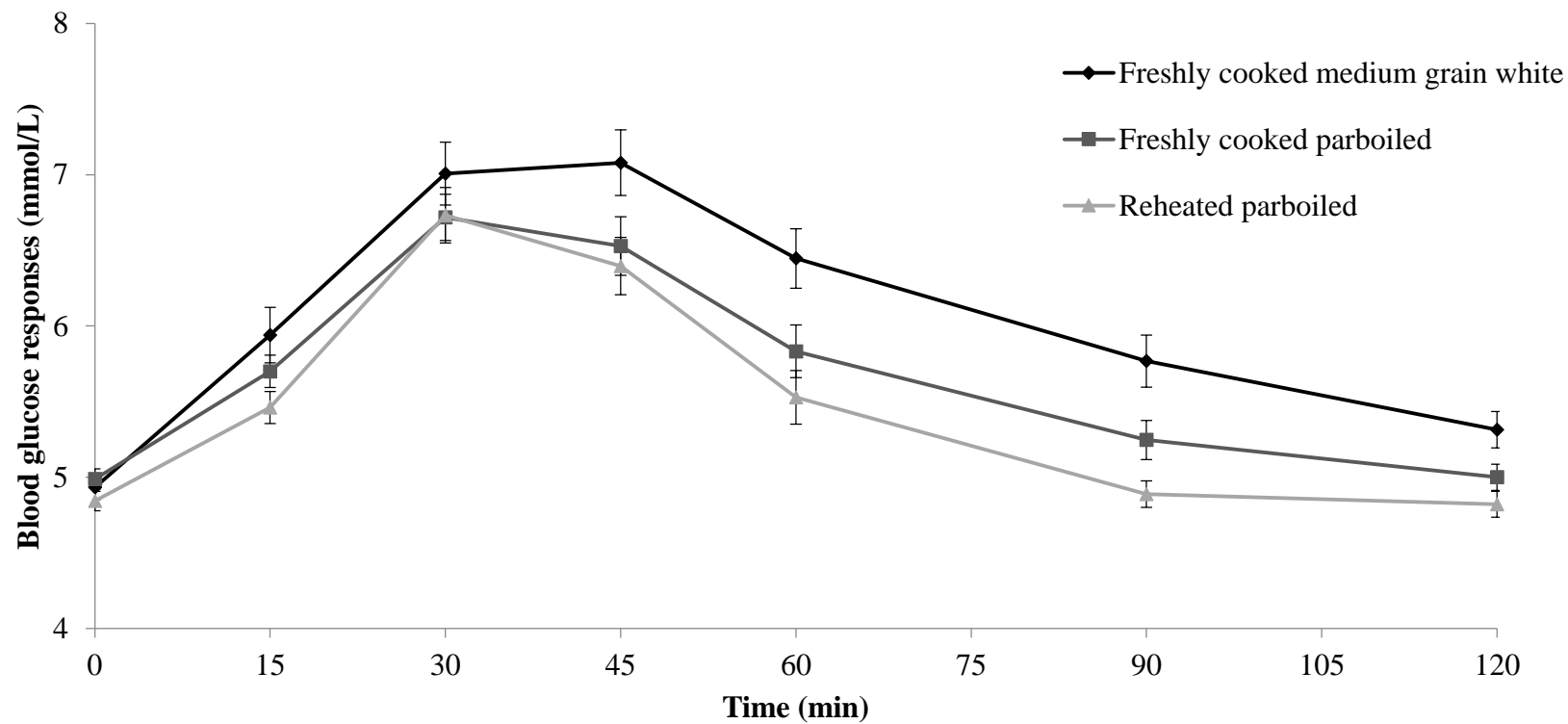


Figure 5-3 Finger prick blood glucose response (means \pm standard error) in 28 healthy participants to three different rice meals (140 g): freshly cooked medium-grain white rice (control), freshly cooked parboiled rice, and reheated parboiled rice. (Test of homogeneity of variances had P-value = 0.001 at 15 minutes and 0.014 at 90 minutes.)

The highest blood glucose concentrations (around 2.0 mmol/L increase above fasting for all three rice products, $P = 0.389$) were measured within the 30 minutes following consumption (Table 5-3 and Figure 5-3). From 45 minutes after consumption (Figure 5-3) the average blood glucose response to reheated parboiled rice was lower (~ 0.3 mmol/L, $P = 0.004$) than freshly cooked parboiled rice.

Chewing time and proportions of particle size distributions

Chewing time varied widely within each rice treatment group and an insignificant between-treatment and individual interaction was observed ($F = 2.966$; $P = 0.057$). The chewing time (overall average was around 30 seconds) required for swallowing reheated parboiled white rice was around 6.4 seconds longer than it was for the other two freshly cooked rice products. However, the only statistically difference ($P = 0.026$) was between reheated parboiled and freshly cooked medium-grain white rice.

The particle size of the rice masticated by the 28 individuals was weighed into three categories (over 2,000 μm , between 2,000 μm and 1,400 μm , between 1,400 μm and 425 μm , and less than 425 μm) (Table 5-4). These particle sizes were chosen based on a previous study carried out in the same laboratory as this investigation. (Ranawana et al., 2010).

Approximately half (around 48%) of the mouthful rice was disintegrated into less than 425 μm and this was not different by rice treatment ($F = 0.030$, $P = 0.970$). Similarly, the proportion (around 45%) of essentially intact particles ($> 2,000$ μm) for all three rice samples was not different by rice ($F = 0.392$, $P = 0.677$) and represented almost half of the masticated sample. Only small amounts (less than 10% by weight) of intermediate particle sizes (particle sizes between 2,000 μm and 1,400 μm ; between 1,400 μm and 425 μm) were found.

Table 5-4 Before-swallowing rice particle size distribution (%), chewing time (sec), and area under the two-hour glucose response curve. (N = 28) Values are mean and standard deviation, unless otherwise stated.

	Freshly cooked medium-grain white rice	Freshly cooked parboiled white rice	Reheated parboiled white rice	F-value	P-value ¹
Particle size distributions (% by weight)	Mean (SD) [Range of particle sizes (%)]				
> 2,000 µm	46.1 (3.2) [0.5–71.2]	44.0 (2.2) [21.4–74.1]	42.6 (3.1) [17.2–83.6]	0.392	0.677
< 2,000 µm ~ > 1,400 µm	2.9 (0.3) [0.3–5.8] a b	4.4 (0.3) [1.5–7.7] a	4.9 (0.4) [0.9–8.5] b	6.278	0.003 *
< 1,400 µm ~ > 425 µm	3.1 (0.3) [1.2–6.1] a b	4.6 (0.4) [1.4–9.4] a	5.3 (0.4) [0.3–12.6] b	9.120	< 0.001 *
< 425 µm	47.9 (2.9) [25.0–96.7]	47.0 (2.3) [17.0–73.0]	47.8 (3.0) [10.9–70.8]	0.030	0.970
Moisture content (%)	Mean (SD)				
	62.1 (4.7) a	66.6 (3.3) b	67.7 (1.5) a b	21.146	< 0.001 *
Chewing time (sec)	Mean (SD)				
	26.8 (1.4) a	27.8 (1.6)	33.1 (2.7) a	2.966	0.057
iAUC (mmol/L*min)	Mean (SD)				
	144.7 (12.1)	94.9 (9.5)	83.5 (9.8)	9.555	< 0.001

¹ ANOVA test. P-value with * (< 0.05) indicates that the mean in the same row is significantly different from others. The means with the same letter in the same row indicates significant difference between these two means (P < 0.05).

Generally, the proportions of particle size between 1,400 μm and 425 μm of all rice samples were around 0.2% higher than the proportion of particle size between 2,000 μm and 1,400 μm . Around 90% of the particles were either larger than 2,000 μm or smaller than 425 μm reflecting the effectiveness of the chewing. The proportion of rice of each particle size was consistent between rice samples for each individual i.e. the either chewed the rice well (small particle size) or not very well (large particle size).

The regression analysis was undertaken within rice sample with the particle size distribution (% the proportion of chewed rice particles) as the dependent variable and the chewing time (seconds) as an explanatory variable. The comparison for each rice samples was across four rice particle size categories, which are '>2,000 μm ', '<2,000 μm and >1,400 μm ', '<1,400 μm and >425 μm ', and '<425 μm ' (Table 5-5) for each rice sample separately. For each rice sample the proportion (%) of the particles over 2,000 μm was inversely correlated with chewing time (seconds). However, the correlation was strongest in reheated parboiled rice ($P = 0.005$). Other particle sizes were positively correlated with chewing time. All r squared values did not exceed 0.5, which indicated that less than 50% of the variations in particle size distributions were explained by chewing time. Therefore, the relationship was insignificant because of the wide inter-individual variation.

Table 5-5 Correlations between particle size distributions (%) (as dependent variable) and chewing time (seconds) (as explanatory variable) (N = 28) by multiple linear regression analysis.

	Coefficient	Constant	r	F-value	P-value ¹
Control rice	Freshly cooked medium-grain white				
> 2,000 µm	-13.985	33.197	0.307	2.701	0.112
< 2,000 µm ~ > 1,400 µm	168.442	21.859	0.367	4.057	0.054
< 1,400 µm ~ > 425 µm	337.431	16.178	0.649	18.871	< 0.001*
< 425 µm	10.365	21.789	0.220	1.326	0.260
Test rice 1	Freshly cooked parboiled				
> 2,000 µm	-32.266	41.963	0.435	6.052	0.021
< 2,000 µm ~ > 1,400 µm	309.665	14.204	0.624	16.555	<0.001*
< 1,400 µm ~ > 425 µm	248.154	16.396	0.583	13.373	0.001*
< 425 µm	16.946	19.785	0.234	1.504	0.231
Test rice 2	Reheated cooked parboiled				
> 2,000 µm	-43.571	51.654	0.516	9.449	0.005*
< 2,000 µm ~ > 1,400 µm	112.205	28.286	0.149	0.592	0.449
< 1,400 µm ~ > 425 µm	298.233	17.178	0.490	8.197	0.008*
< 425 µm	40.906	13.557	0.457	6.880	0.014

¹ ANOVA test for multiple linear regression and residue. P-value with * (< 0.05) indicates there is a significant linear relationship between particle size distribution and chewing time (seconds).

Comparison of satiety and palatability responses (VAS scores) over 120 minutes

For all three rice treatments, all participants had similar baseline satiety responses (scored out of 10) to the feelings of hunger, fullness and satisfaction (average score 5.5 (SD 0.3), $F = 0.659$, $P = 0.520$; 3.1 (0.2), $F = 0.305$, $P = 0.738$; and 2.8 (0.2), $F = 0.515$, $P = 0.600$ respectively). Thirty minutes after consumption of the rice meals, the general satiety response (feelings of hunger (Figure 5-4a) and the amount of food participants would like to eat Figure 5-4d)) decreased whereas the general satisfaction increased (Figure 5-4b). After 60 minutes, the feeling of hunger started to rise, and the fullness and satisfaction steadily declined approximately to the baseline level.

The feeling of hunger (How hungry do you feel?) differed among the three meals (Figure 5-4a). On average, participants who had reheated parboiled rice felt hungry approximately 30 minutes later than the other two freshly cooked rice products (medium-grain white and parboiled; $F = 3.281$, $P = 0.043$).

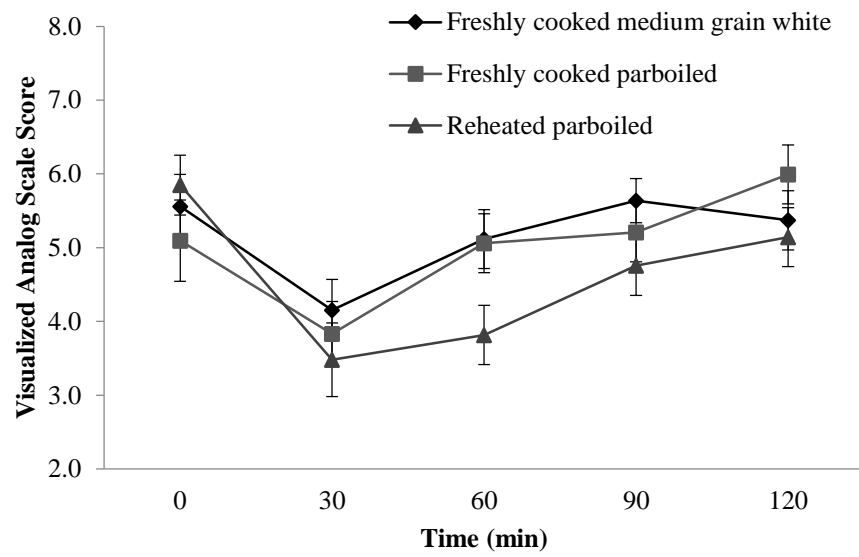
At baseline, the participants' average satiety responses to sweet ($F = 1.415$, $P = 0.249$) and savoury ($F = 1.648$, $P = 0.199$) taste showed between-group variations; however, these were not significant, and the average responses to salty and fatty tastes were not different ($F = 0.085$, $P = 0.918$; $F = 0.858$, $P = 0.428$ respectively). However, the average satiety responses to these four tastes over 120 minutes followed a similar trend. The willingness to eat sweet food slightly declined, whereas salty, savoury and fatty food stayed at a similar level to baseline. No clear pattern of responses to questions about sweet, salty, savoury and fatty foods was observed over the two hours (Table 5-7).

Overall, no significant difference in area under the VAS curve (AUC) was found among the control rice and two test rice products ($P > 0.05$) for satiety or palatability. The two largest between-group variances were found in the VAS responses to "How hungry do you feel?" ($F = 1.401$) and "How much do you think you can eat?" ($F = 1.054$; see Table 5-6). The feeling of hunger and the amount of food participants could eat tended to be higher after eating freshly cooked medium-grain white. However, neither were statistically significant.

Simple linear regression analysis showed no correlations between any of the mean satiety scores and the blood glucose concentration when iAUCs were used. Satiation and blood glucose (mmol/L) at each time point up to 120 minutes negatively correlated with "How hungry do you feel?" and "How much can you eat?" and positively correlated with how satisfied do you feel and how full do you feel (Table 5-7).

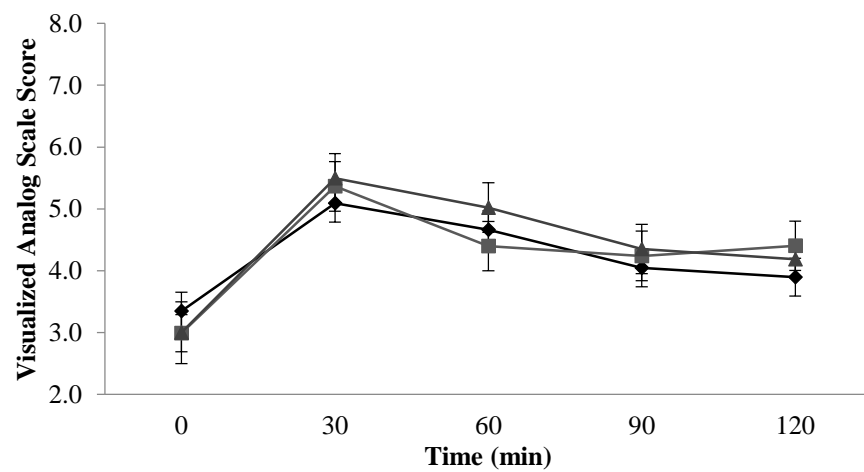
Palatability responses were examined once immediately after participants finished the assigned rice meals. Reheated parboiled rice showed generally higher VAS scores than the other two freshly cooked meals (Figure 5-5). The visual appearance of reheated parboiled rice score was around 2.0 times higher than freshly cooked medium-grain white rice ($P = 0.001$) and 1.7 times higher than freshly cooked parboiled rice ($P = 0.003$). The smell of reheated parboiled rice was preferred to the other two freshly cooked rice meals (around 1.0 times higher than the control rice ($P = 0.034$) and around 1.0 times higher than the freshly cooked parboiled rice ($P = 0.029$)). Its taste scored around 1.5 times higher than freshly cooked medium-grain white rice ($P = 0.023$). However, no significant difference was found in the palatability of the aftertaste. Overall, reheated parboiled rice was preferred compared with medium-grain white rice ($P = 0.003$) and freshly cooked parboiled rice ($P = 0.012$).

How hungry do you feel?



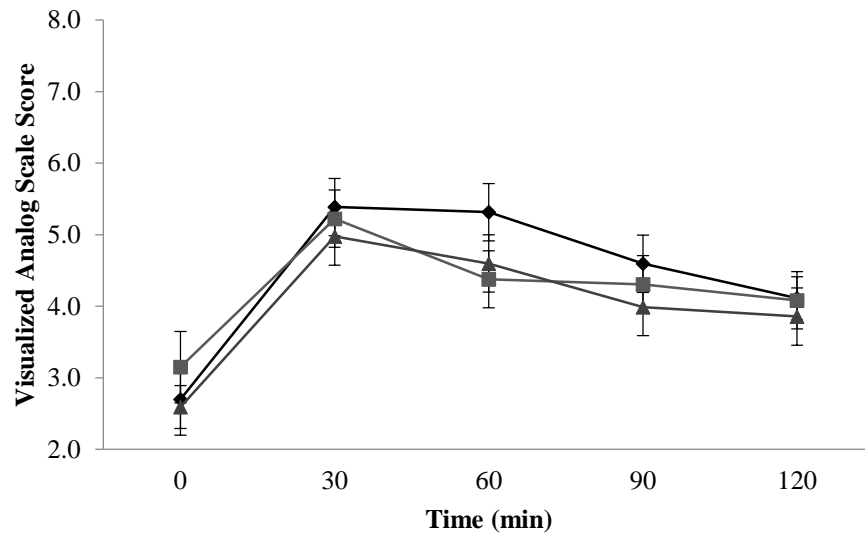
(a)

How satisfied do you feel?



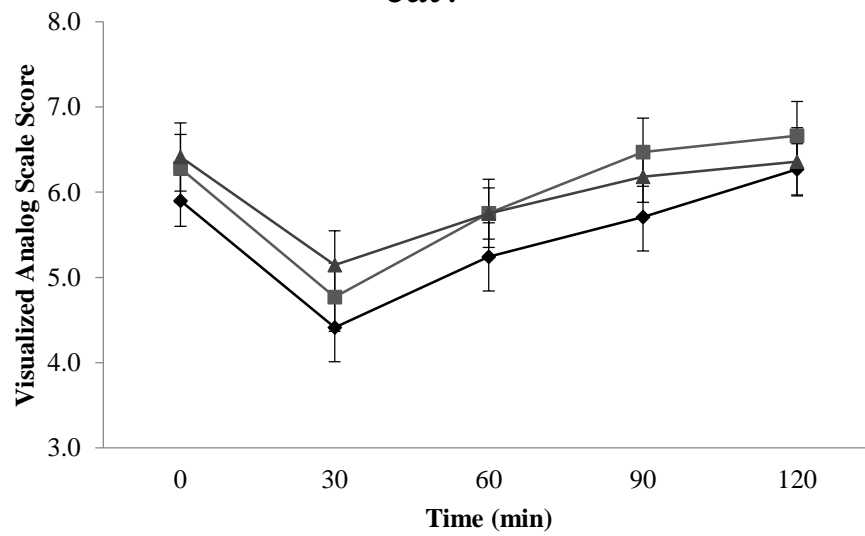
(b)

How full do you feel?



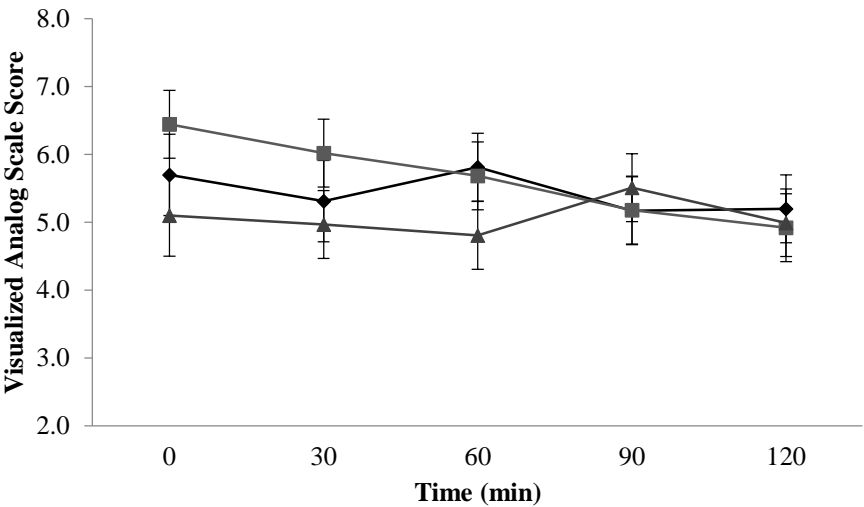
(c)

How much do you think you can eat?



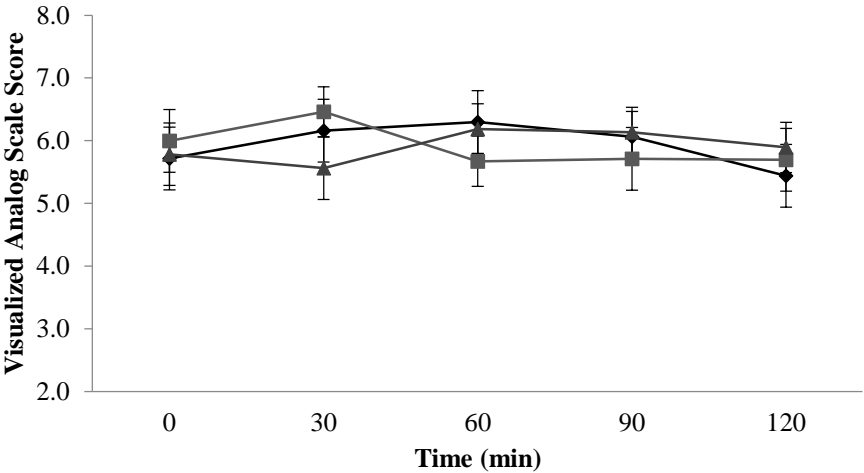
(d)

Want to eat something sweet?



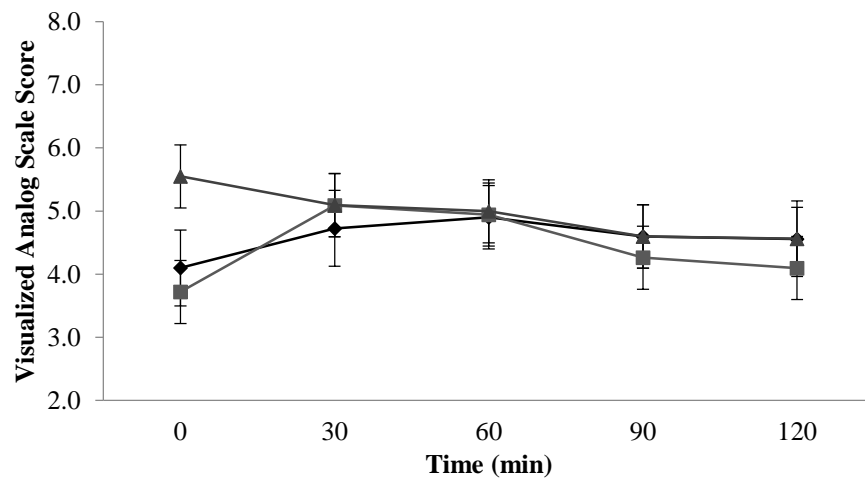
(e)

Want to eat something salty?



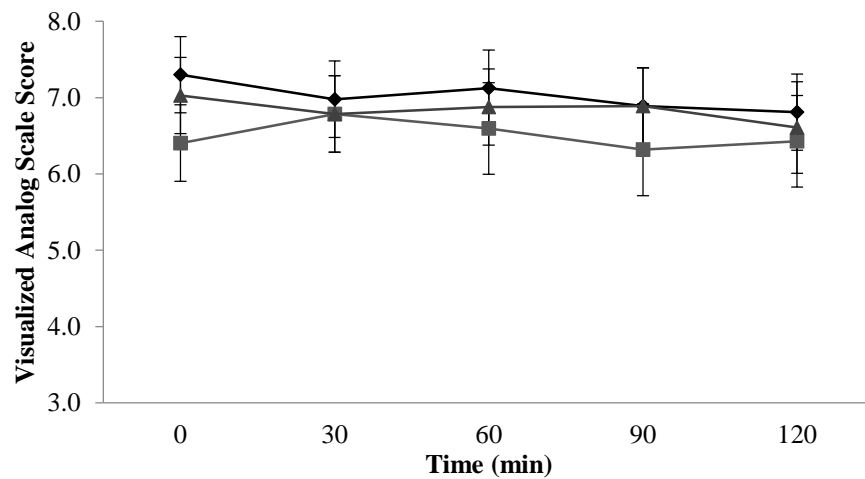
(f)

Want to eat something savoury?



(g)

Want to eat something fatty?



(h)

Figure 5-4 Mean satiety scores (VAS, visualised analogue scale scores) change over 120 minutes for freshly cooked medium-grain white rice, freshly cooked parboiled rice and reheated parboiled rice, and each satiety VAS question. Error bars show the standard error of the mean VAS score.

Table 5-6 Area under the curve (AUC) of satiety (mean, SD) over 120 minutes for freshly cooked medium-grain white rice, freshly cooked parboiled rice and reheated parboiled rice, and each visualised analogue scale question.

	Freshly cooked medium-grain white rice	Freshly cooked parboiled white rice	Reheated parboiled white rice	F-value	P-value
	(score*min)	(score*min)	(score*min)		
Satiety	Mean (SD)				
How hungry do you feel?	611.1 (33.2)	589.3 (40.2)	526.9 (37.2)	1.401	0.252
How satisfied do you feel?	522.7 (33.1)	531.0 (37.6)	553.5 (35.0)	0.203	0.817
How full do you feel?	561.0 (36.3)	525.7 (41.8)	502.7 (33.5)	0.616	0.543
How much do you think you can eat?	664.1 (37.5)	703.9 (40.1)	769.3 (90.2)	1.054	0.353
Would you like to eat something sweet?	652.0 (59.0)	676.9 (53.0)	610.0 (57.2)	0.358	0.700
Would you like to eat something salty?	723.0 (51.5)	710.7 (49.6)	711.7 (47.2)	0.019	0.981
Would you like to eat something savoury?	556.8 (57.4)	546.1 (54.5)	592.3 (58.5)	0.181	0.835
Would you like to eat something fatty?	841.6 (57.1)	783.5 (61.8)	819.6 (58.1)	0.248	0.781

The AUC was calculated by the trapezoidal rule and was compared, in SPSS, using a mixed effects linear model with a random subject effect. Within each question, the difference was insignificantly at $P > 0.05$ among three treatments.

Table 5-7 Association between satiety and palatability responses (visualised analogue scales (VAS) scores) (as dependent variable) and blood glucose concentration (mmol/L) (as explanatory variable) (N = 28) by linear regression analysis.

	Coefficient	Constant	r	F-value	P-value¹
Control rice	Freshly cooked medium-grain white				
How hungry do you feel?	-1.282	7.602	0.888	11.187	0.044*
How satisfied do you feel?	1.180	-3.996	0.988	124.321	0.002*
How full do you feel?	0.703	-2.147	0.915	15.357	0.030*
How much do you think you can eat?	-1.065	6.820	0.923	17.235	0.025*
Would you like to eat something sweet?	-0.127	1.648	0.043	0.006	0.945
Would you like to eat something salty?	1.745	-9.404	0.781	4.703	0.119
Would you like to eat something savoury?	2.172	-8.990	0.759	4.073	0.137
Would you like to eat something fatty?	-0.716	5.988	0.163	0.082	0.793
Test rice 1	Freshly cooked parboiled				
How hungry do you feel?	-0.807	4.629	0.890	11.399	0.043*
How satisfied do you feel?	0.675	-2.328	0.806	5.568	0.099
How full do you feel?	0.809	-2.852	0.850	7.820	0.068
How much do you think you can eat?	-0.912	6.053	0.968	44.237	0.007*
Would you like to eat something sweet?	0.364	-1.491	0.306	0.310	0.617

	Coefficient	Constant	r	F-value	P-value¹
Would you like to eat something salty?	1.525	−8.466	0.743	3.708	0.150
Would you like to eat something savoury?	1.133	−4.446	0.911	14.655	0.031*
Would you like to eat something fatty?	3.313	−20.917	0.925	17.771	0.024*
Test rice 2	Reheated cooked parboiled				
How hungry do you feel?	−0.740	3.925	0.845	7.483	0.002*
How satisfied do you feel?	0.689	−2.525	0.785	4.817	0.116
How full do you feel?	0.681	−2.220	0.750	3.856	0.144
How much do you think you can eat?	−1.450	9.165	0.979	69.360	0.004*
Would you like to eat something sweet?	−1.299	7.116	0.406	0.590	0.498
Would you like to eat something salty?	−1.807	11.218	0.521	1.115	0.369
Would you like to eat something savoury?	0.514	−2.029	0.234	0.175	0.704
Would you like to eat something fatty?	−0.370	3.048	0.068	0.014	0.914

¹ ANOVA test for multiple linear regression and residue. P-value with * (< 0.05) indicates there is a significant linear relationship between particle size distribution and chewing time (seconds).

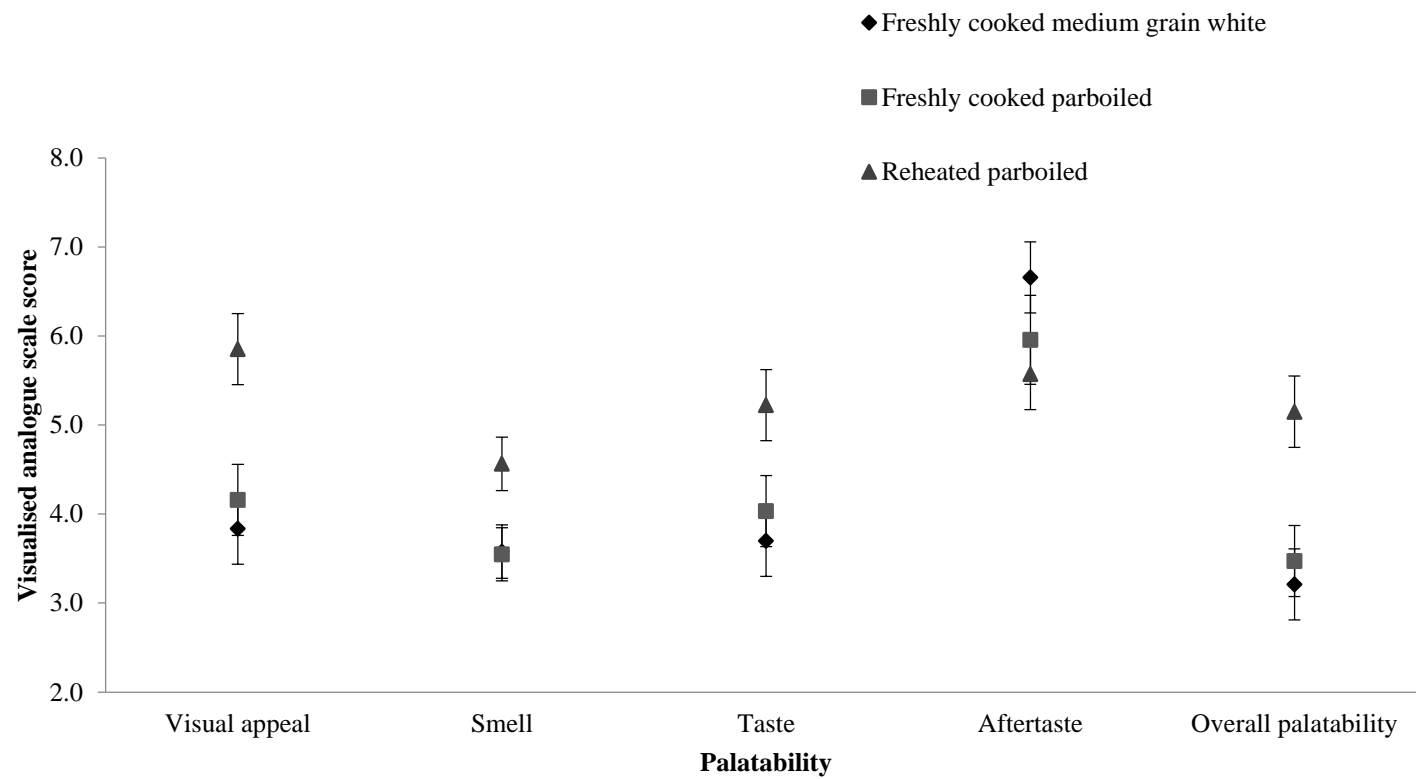


Figure 5-5 Mean palatability scores (VAS, visualised analogue scales scores) immediately after finishing eating prepared control and test rice samples (freshly cooked medium-grain white rice, freshly cooked parboiled rice and reheated parboiled rice) for each palatability VAS question.

5.4 Discussion

The present study found that the postprandial glycaemic response trajectory of the reheated parboiled rice sample was significantly lower (42% lower) than that of the non-parboiled medium-grain white rice. Moreover, the differences in glycaemic response trajectories between reheated and freshly cooked parboiled rice samples started to develop and increase with increasing postprandial time after the assumed peak time (30 minutes) was reached (Bornet et al., 2007). Reheated parboiled rice also required significantly longer time (around 6 seconds per mouthful) to chew and resulted in a significantly lower proportion of chewed large particle size ($> 2,000 \mu\text{m}$) compared with the other two freshly cooked rice samples. The feeling of the general response of hunger (“How hungry do you feel?”) was negatively correlated with the glycaemic response trajectory over 120 minutes, whereas the feeling of “How much do you think you can eat?” was negatively correlated.

This study found that cold storage and reheating (stored at 4°C for 24 hours and evenly reheated to 65°C for 15 minutes) of parboiled rice might lead to a meaningful and large effect on postprandial blood glucose response. The explanation could be that cold storage promoted the retrogradation of rice starch and increased the hardness of the rice grain, which in turn would prolong the chewing time for each mouthful, slow down starch breakdown, promote the stomach emptying, and reduce the glycaemic responses.

The overall trajectory of glycaemic response to reheated parboiled rice was consistently low, being reduced over two hours by about 34% ($P < 0.001$) compared with non-parboiled medium-grain white rice, similar to the 30%, $P < 0.001$ reported in a study (Larsen et al., 2000) comparing the effect of severe pressure parboiling with traditional parboiling. However, the Larsen study (2000) found no difference between the GI of the non-parboiled rice and that of the traditionally parboiled rice, and all rice was freshly cooked before consumption.

The role of the parboiling and cold storage in relation to reduced glycaemic response trajectories may be explained by the considerably higher proportion (approximately 30% higher) of RS (starch that can resist digestion for up to 180 minutes) in reheated parboiled rice than in freshly cooked medium-grain white rice reported in the *in vitro* experiment in Chapter 5, “Effect of rice product varieties, cold storage, reheating and grain particle sizes on starch digestibility profile and *in vitro* glucose release”). It was possible that the parboiling pre-treatment and reheating preparation method led to specific changes to specific organoleptic properties of the rice (i.e., increased the

amylopectin and amylose crystallisation) and thus reduced the starch digestibility (Boers et al., 2015; Tamura et al., 2016; Derycke et al., 2005) and glycaemic responses (Boers et al., 2015; Sonia, Witjaksono, & Ridwan., 2015; Heinemann et al., 2005). It is suggested that cold storage significantly increased the amylose and amylopectin crystallisation (Wang et al., 2015; Sajilata et al., 2006b) and enhanced its resistance to digestion (Wang et al., 2015; Chung et al., 2006; Riva et al., 2000). The reheating (to 65 °C) in the study might have broken some of the amylopectin crystallites and reversed the impact; however, the magnitude of the effect was not explored because the un-reheated parboiled rice was not tested on participants because of food safety concerns. It is possible that some of the amylopectin crystallites retained the associating forces during reheating and were partly responsible for the low glycaemic response observed for reheated parboiled rice (Wang et al., 2015; Zhang & Hamaker, 2009).

Although the degree of habitual mastication and the time of chewing required for preparing a food suitable for swallowing varied considerably among participants, they were relatively constant within individuals as reported by others (Bornhorst & Singh, 2012; Ranawana et al., 2010; Woda et al., 2006). The primary purpose of mastication is the reduction of food to particles small enough to form a cohesive, liquid-coated bolus that can be swallowed (Van der Bilt et al., 2006; Peyron, Mishellany, & Woda, 2004). Thus, the degree of habitual mastication (i.e., particle sizes) and the chewing time required for mastication would depend on the food type, the saliva flow and the ability to form a bolus (Bornhorst & Singh, 2012). Masticated rice with a higher proportion of RDS has been reported to form a bolus more easily than those with a lower proportion of RDS (Ranawana et al., 2010). Harder reheated parboiled rice grains in the study with a lower proportion of RDS would require more chewing time (i.e., less chewed particle > 2,000 µm), and softer freshly cooked medium grain white rice with higher moisture content (%) and higher RDS would require much less mastication time (i.e., more chewed particle > 2,000 µm).

Surprisingly, an inverse correlation between chewing time and postprandial glucose response was found. As hypothesised based on the *in vitro* results, thorough mastication (i.e. increased chewing time) breaks rice grains into small particle size, stimulates salivation, and increases mixing rice particles with salivary enzymes, improving hydrolysis of starch in mouth and stomach and, thus, is expected to increase the acute glycaemic responses. However, in this study, lower glycaemic responses was associated with longer chewing time and smaller chewed particle size in our healthy participants.

Furthermore, the magnitude of the correlation was stronger for reheated parboiled rice than for freshly cooked parboiled rice and freshly cooked medium-grain white rice.

Others have also found that a chewing effect on *in vivo* digestion rate and the subsequent postprandial glycaemic responses (i.e., the longer to chew, the slower the glycaemic response to rise) (Madhu et al., 2016; Kataoka et al., 2013, Suzuki et al., 2005). One previous study, by Suzuki et al. (2005), compared the effect of mastication (proportions of chewed particle size) on the glucose response to a rice meal and observed an inverse correlation between postprandial glucose and chewing (increasing thorough mastication and decreasing glycaemic responses) in the group of participants with normal glucose tolerance and participants with T2DM. The explanation by Suzuki et al. (2005) for these results was that the insulin-impaired participants showed a greater glycaemic response after shorter chewing time compared with participants with normal insulin tolerance and extended chewing time, which confirmed that the degree of chewing may have a direct and positive effect on the rate of *in vivo* digestion and the glycaemic response.

One theory is that increase in chewing time can increase both the time of the food in oral cavity and oral amylase action and may further reduce the postprandial glycaemia (Madhu et al., 2016; Alberti et al., 2015; Kataoka et al., 2013). Oral amylase activity has been reported to have minor effect on early increasing serum insulin level immediately after starch consumption (Alberti et al., 2015; Mandel et al., 2012). Increased amylase activity and early insulin release have been reported to be linked to a lower area under 120-minute glycaemic curve (AUC) (Alberti et al., 2015). It was proposed in some studies that the glucose release from starch digestion in oral cavity might send signal to the body to be ready for incoming starch through vagal activation and result in early insulin secretion (i.e., preabsorptive insulin or cephalic phase of insulin secretion) and thus glycaemia (Madhu et al., 2016; Mandel et al., 2012; Just et al., 2008; Åhrén & Holst, 2001; Teff & Engelman, 1996).

It is also suggested that less thorough mastication (i.e. shorter chewing time) and swallowing a food bolus with a high proportion of large particles ($> 2,000\ \mu\text{m}$) may delay gastric emptying (Madhu et al., 2016; Phillips et al., 2015; Marathe et al., 2013). This could be because the viscosity of the gastric contents is increased and pyloric outflow is decreased. Increased viscosity reduces sedimentation of solids in liquids and impairs the ability of the antrum to preferentially empty liquids faster than solids (Bornhorst & Singh, 2012). In addition delayed gastric emptying increases the time for

the antrum of the stomach to grind solid food to smaller particles (less than 1,000 μm) and delays the interaction between macronutrients, such as starch molecules, amylopectin and amylose and small intestine, thus delays the stimulation of vagus nerve and the secretion of gut hormones (GLP-1, CCK, and PYY) from entero-endocrine cells located most densely in the proximal small intestine and distal small intestine/colon and prolongs the subsequent gastric emptying (Marathe et al., 2013; Ma et al., 2012). In turn, both retardation of gastric emptying and the digestion of starch and absorption of blood glucose in the small intestine reduce the secretion of gut hormones and delays insulin secretion and glucagon suppression and, thus, delays postprandial blood glucose expenditure (Ma et al., 2012; Gonlachanvit et al. 2003).

The participants' glycaemic responses and the *in vivo* digestibility of cooked rice significantly influenced their subjective feelings of the general satiety response ("How hungry do you feel?" and "How much do you think you can eat?") over 120 minutes. It has previously been stated that changes in satiety after a carbohydrate load may be mediated through an effect of plasma glucose on specific gluco-sensitive cells in the brain (Blundell, 2010). Previous studies have also demonstrated a satiating effect of carbohydrate (Blundell & Bellisle, 2013; Anderson & Woodend, 2003). Feinle, O'Donovan, & Horowitz (2002) reviewed the effect of carbohydrate (both glucose infusion and oral ingestion of carbohydrates) on satiety and suggested that satiety was associated with ingestion of carbohydrate appears to result primarily from gastrointestinal signals. A recent systematic review by Miquel-Kergoat et al. (2015) further suggested that longer chewing may decrease self-reported hunger and possibly alter the gut hormone response related to satiety. However, the present study did not find a significantly increased satiety effect of the reheated parboiled rice compared to other two rice samples despite the significant different AUC. It was suggested that the way in which chewing was analysed, including the intensity of chewing and the chewing speed, may also influence the acute satiety. A better control of this parameter (or covariant) could have aided the interpretation. In addition, different ratings of taste, visual appeal and texture have been reported in previous studies to influence satiety and the sensation which motivates consumption and can be present even without physiological requirement (Blundell et al., 2010). The present study also found that increased palatability ratings (visual appeal, smell, taste and overall palatability rating) were associated with an increased amount of food the participants perceived that they could eat ("How much do you think you can eat?") and decreased feelings of fullness ("How full do you feel?").

The strength of this study is that it followed a glycaemic response method whose precision and validity were tested and validated by previous studies. The group of participants tested each rice sample on separate experiment days (7 days apart) to minimise possible intra-individual variation and improve precision. On each experiment day, the volumes of the test meals were similar, the time spent on consuming the meals was restricted to less than five minutes, all rice meals were fully gelatinised, and the rice grains all retained their original shape when they were served to participants to minimise the influences on glycaemic response and satiating power of each rice meal.

Limitations of the study include lack of full randomisation, and that each condition including the chewed sample was measured only once. Participant compliance was high and no one did not complete. However, this relatively small study is only applicable to these specific brands of rice cooked in this specific way. It should be acknowledged that the volunteers may not necessarily be representative of the population at large that some unrecognized attributes of this convenience sample could explain the marked differences in glycaemic responses among three rice samples. The variation among the brands or rice (the rice origin, storage time/condition, and humidity etc.) and the methods of cooking also needs to be explored further in bigger sample size to find significant associations. The two factors that might have affected the gastric emptying rate, glycaemic response, and the feelings of satiety and palatability are the chewed particle size and the net energy of the rice meals served to each participant. Although chewed particle size is impossible to control, the net energy may be monitored by dietary measurements in order to explain changes in the subjective satiety score in the future.

5.5 Conclusion

The effect of reheating on reduced glycaemic response, extended chewing time and improved palatability shown in the present study may be considered a positive effect with regard to better blood glucose and appetite regulation and perhaps also long-term weight management. Reheated parboiled rice replacing freshly cooked medium-grain white or parboiled rice in the habitual diet may reduce the risk of fast blood glucose peaks and the glycaemic overload in the daily diet. Because a high GI diet has been shown to increase energy intake and body weight and the risk of obesity and hyperglycaemia, a lower GL replacement may be desirable in long-term glycaemia regulation (e.g., for people with type 2 diabetes and pregnant women with gestational diabetes). Further study is required to investigate the sensory acceptability of the rice

products to investigate whether rice prepared by the optimal treatment could be accepted as part of the diet.

Chapter 6: Which rice and why? Consumer preference and acceptability towards freshly cooked medium-grain white rice, freshly cooked parboiled rice and reheated parboiled rice

Abstract

Previous *in vitro* and *in vivo* studies have confirmed that the optimal rice preparation method, reheating to 65 °C after 24 hours of cold storage at 4 °C, may significantly reduce starch digestibility and overall glucose release over 180 minutes and blood glucose response over 120 minutes. Sixty-four volunteers participated in a sensory acceptability test to investigate the acceptability for sensory characteristics of three cold-stored and reheated rice products (medium-grain white, medium-grain brown and parboiled rice) and their likeability was compared with their controls (freshly cooked and served warm at 65 °C). All six rice samples (three freshly cooked and three reheated) were accepted by participants (average 54.0%, \pm 2.5%). No significant difference in overall liking was found among any of the six rice samples. Similarly, no significant difference in the acceptability of colour and texture was observed. Among all the rice samples, the sweetness and the flavour of freshly cooked warm medium-grain white were less preferred (scored 42.1% \pm 6.0% and 45.0% \pm 6.0% respectively) compared with other samples ($P = 0.05$). Over 50% of all participants preferred both reheated parboiled rice (51.6%) and reheated medium-grain brown rice (53.1%) as a daily regular staple grain over medium-grain white rice (48.4%). It is suggested that reheated parboiled rice, with the lowest starch digestibility and glucose impact (both *in vitro* glucose release and *in vivo* glucose response) could be accepted as a healthier alternative for the daily staple meal.

Keywords: Plain cooked rice, commonly consumed rice types, cooking-storing method, affective analysis, consumer preference.

6.1 Introduction

Globally, rice is one of the most important staple foods. The crop is tolerant to a wide range of production and storage conditions. The previous experiments on *in vitro* rice starch digestion (Chapter 4, “Effect of rice product varieties, cold storage, reheating and grain particle sizes on starch digestibility profile and *in vitro* glucose release”) and human participants’ glycaemic responses to cooked rice samples (Chapter 5, “Effect of rice cooking method on postprandial glycaemic response, satiety and palatability, and

chewed particle distribution”) demonstrated that rice parboiled and cold stored for 24 hours reduced and delayed the digestion of rice starch and decreased the postprandial glycaemic response and increased satiety (i.e., fullness and palatability) responses. This evidence would support advice that replacing commonly consumed medium-grain white rice with parboiled rice and adopting the cold storage preparation method may improve postprandial glycaemic response and benefit long-term glycaemic management.

However, there is still little information about the sensory acceptability to consumers of parboiled rice and whether and how rice consumers’ preferences differ among rice products (refined grain white rice, whole grain brown rice and parboiled rice) and between cooking-storing methods (freshly served and 24-hour cold storage at 4 °C). A sensory acceptability test would provide knowledge on the liking for sensory characteristics of selected popular rice products that are cooked and stored in different ways. This testing will assist with the recommendations of a healthier option of cooked rice as a staple food.

Reported physical differences between parboiled rice and milled white rice are that parboiled rice has a pale yellow colour, a harder and firmer texture, and a stronger, unique flavour compared with white rice, which has a softer, adhesive texture and creamy starchy flavour (Ong & Blanshard, 1995; Pillaiyar, 1988; Tomlins, Manful, Larwer, & Hammond, 2005). It has been suggested that these unique characteristics of parboiled rice are disliked by rice consumers, especially those from East and Southeast Asian backgrounds (Barber & De Barber, 1991; Marshall & Wadsworth, 1993), but favoured by consumers in India, Pakistan, Brazil and Ghana (Barber & De Barber, 1991; Heinemann, Behrens, & Lanfer-Marquez, 2006; Tomlins et al., 2005). Recent studies have suggested that changing demographic factors, including ethnicity structure and cultural infiltration, age distribution, lifestyle and the shift towards more convenience in food preparation and disposable income, might affect consumer liking and demand for food (Ares et al., 2016; Heymann & Lawless, 2013). In an ethnically diverse population such as Auckland, New Zealand, these factors present difficulties when introducing a healthier dietary recommendation as the sensory acceptability to consumers may differ. Therefore, a study concerning Auckland rice consumers was proposed in order to obtain a better understanding of local consumer preferences of rice prepared in different ways.

The purpose of this study was to evaluate whether the slow-glycaemic-release reheated parboiled rice could be accepted and liked as a healthier alternative by Auckland

consumers who commonly consume plain cooked medium-grain white or brown rice as their staple grain.

Six rice samples, three rice products (medium-grain white rice, medium-grain brown rice and parboiled rice) times two preparation methods (freshly cooked and reheated) were tested in this experiment. The study aimed to investigate the following questions: (1) Would reheated parboiled rice have significant different VAS of four attributes (colour, texture, flavour and sweetness) compared with the other five rice samples (freshly cooked or reheated medium-grain white rice and medium-grain brown rice, and freshly cooked parboiled rice)? (2) Would reheated parboiled rice be acceptable to participants who commonly consume plain cooked medium-grain white or brown rice?

6.2 Method

This section describes the specific sensory data collection for six plain cooked rice samples (3 rice products \times 2 preparation methods) and the data analysis methods used for this sensory evaluation. The section explains the study design of this sensory evaluation experiment and then outlines the steps of constructing the research experiment and the specific methods used to collect and analyse data.

6.2.1 Experiment methodology

In this sensory experiment testing affect, untrained participants were chosen to be representative of the population who commonly consume plain cooked rice as a staple grain to evaluate the rice samples under naturalistic condition (O'Mahony, 1988). The affective testing method was designed to quantify the degree of liking and disliking of cooked rice products and to identify which products were preferred using participants' senses (sight, smell, taste and touch) for the purposes of subjectively evaluating the cooked rice products (liking preference of rice grain colour, texture, flavour and sweetness, and overall liking preference).

The consumer's liking represents the sum of experiences and information about the rice as well as the preparation method and the response evoked as a positive or a negative feeling towards the rice product and preparation method. Consumer attitude regarding the rice products and preparation method can be defined as the intensity of feelings in favour or against. In order to evaluate consumer behaviours and attitudes, this study used quantitative approaches. A scaling measurement method (i.e., a quantitative method), the visualised analogue scale (VAS), which describes the application of numbers and judgements that are converted to numerical values, was used to describe the degree of liking or disliking for some experience or product. Four attributes (colour,

texture, flavour and sweetness) and the overall liking were defined as primary sensory attributes and were evaluated by consumer testing to assess how well the cooked rice products were likely to be accepted. All participants had to be actual rice product consumers and likers, and be able to express their acceptance levels for products differently in each category.

6.2.2 Study design

This research experiment used a two-way treatment (or factorial treatment) structure and a completely randomised design (Heymann & Lawless, 2013). Each rice product was prepared in two different ways with a total of six treatments (three rice products by two cooking-storing methods) prior to sensory evaluation (Figure 6-1). All samples were blind labelled with random non-repeating three-digit codes using an excel random number generator (e.g., 809, 390, 672, etc.) to avoid bias. All six samples were simultaneously assigned to each participant in random order to minimise artefacts due to order of sample presentation. Each serving sequence was assigned to participants an equal number of times to ensure the sample order was counterbalanced. Sample size was calculated based on F-test (ANOVA repeated measures) with difference of 14.8% according to Gacula and Rtenbeck (2006) between rice treatments, an alpha value of 0.05, and beta value of 0.10. The sample size was 64 participants.

6.2.3 Ethics

This study was conducted according to the guidelines of the human ethics committee of AUT. The AUT Ethics Committee approved all procedures involving human participants. Ethical approval for the study was obtained from the committee (see Appendix 6).

6.2.4 Participants characteristics

The participant recruitment was based on volunteering. The recruitment location was at the AUT North Campus and City Campus. Information sheets and advertisements were distributed at the campus cafeteria, library, gymnasiums, AUT Student Movement offices and student information centres. The inclusion criteria were listed on top of the information sheet and advertisement (see Appendix 7) so participants were able to self-identify whether they were valid candidates before volunteering. Potential participants who showed interest were asked to complete an online enrolment questionnaire (see Appendix 8). In the questionnaire, they were required to provide personal information to show they met the inclusion criteria and express interest in volunteering. Anyone who did not complete the online enrolment questionnaire was excluded from enrolment.

Inclusion criteria

All participants had to be over 18 years old. They had to have been consuming plain cooked rice as a staple food at least once per week for the previous year and to consider continuing to consume rice as staple food in the future. All participants had to be willing to complete the entire tasting session (six plain cooked rice samples) and share their questionnaire results and required personal information with the researcher. Signed consent had to be obtained from all participants before the sensory experiments.

Exclusion criteria

Volunteers who were under 18 years old and did not eat plain cooked rice regularly (less than once per week) in the previous year were excluded from the study. All volunteers are self-reported healthy (with no diabetes, cardiovascular diseases, cancer, no underwent major surgeries). All volunteers were asked to carefully read and thoroughly understand the information sheet before the experiment. Volunteers who did not sign the consent were excluded from the study. Volunteers who did not complete the six tasting sessions were excluded from the final data collection.

All volunteers needed to fast for at least two hours before attending the tasting sessions. Volunteers who ate within two hours of the tasting sessions were given the option to come back on a separate day.

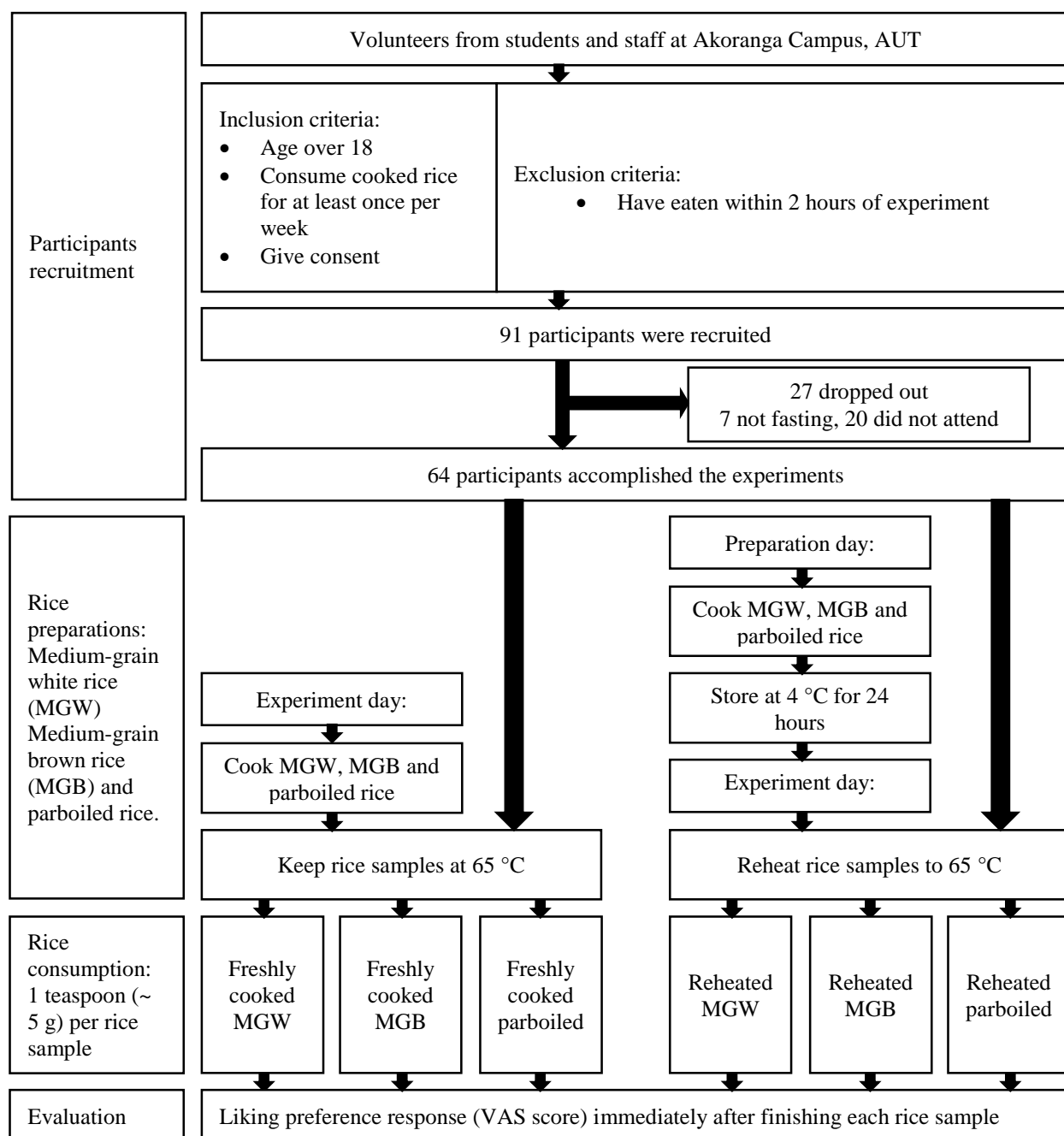


Figure 6-1 Flow chart of the experiment design for sensory evaluation (liking preference).

Table 6-1 Preparation methods for three rice samples.

Test sample	Rice products	Product origin	Brand	Preparation method
1	Medium-grain white	Australia	SunRice	Freshly cooked, kept at 65 °C
2	Medium-grain brown	Australia	SunRice	Freshly cooked, kept at 65 °C
3	Parboiled long-grain white	Thailand	Real Rice	Freshly cooked, kept at 65 °C
4	Medium-grain white	Australia	SunRice	Cooked, cooled rapidly to 4 °C, stored for 24 hours @ 4 °C, reheat to 65 °C
5	Medium-grain brown	Australia	SunRice	Cooked, cooled rapidly to 4 °C, stored for 24 hours @ 4 °C, reheat to 65 °C
6	Parboiled long-grain white	Thailand	Real Rice	Cooked, cooled rapidly to 4 °C, stored for 24 hours @ 4 °C, reheat to 65 °C

6.2.5 Rice sample preparation

Samples

Three rice products, medium-grain white rice (SunRice®, Australia), medium-grain brown rice (SunRice®), parboiled long-grain rice (RealRice®), were purchased from a large stock supermarket (Countdown Supermarket, Takapuna, Auckland). Medium-grain white rice was selected as the most commonly consumed staple rice and the control sample, medium-grain brown as the most commonly perceived healthy alternative to medium-grain white rice and with a nutty texture similar to parboiled rice, and parboiled long-grain rice as the healthiest alternative based on results from previous *in vitro* studies and human experimental trials.

The medium-grain white and medium-grain brown rice were characterised as medium-grain commercial rice (*Oryza sativa* L.) (Department of Health and Ageing, 2005), and cultivated and processed in Riverina, Australia, in 2013. The parboiled long-grain rice was cultivated and processed (parboiling) in Thailand and harvested and processed in late 2012 and 2013.

Rice cooking-storing methods

Two different convenient cooking-storing methods (suitable for all rice cookers and microwaves) were standardised: the amount of rice and water, the temperature (cooking, reheating and serving), the room temperature and humidity of the cooking environment, and the time of cooking and reheating (see Table 6-1). Considering the limitations of rice cookers, pre-preparation (e.g., soaking rice in lukewarm water prior to cooking) was not selected. Furthermore, because the aim of this study was to evaluate the sensory

values of plain cooked rice, cooking recipes involving any other ingredients, including fat (saturated and non-saturated fat), sugar, salt and any other flavours, were excluded.

Three samples were cooked in three separate domestic automated rice cookers following the instructions on the labels of the packages (Abode® Rice Cooker, BIGW_7963940). For medium-grain white rice, one measuring cup of rice ($141.9 \text{ g} \pm 5.0 \text{ g}$) was added to one and a half cups of water (375 mL) at room temperature (23°C) in a clean automatic rice cooker and cooked till the rice cooker automatically turned off. For medium-grain brown rice, one measuring cup of rice ($130.8 \pm 5.0 \text{ g}$) was cooked in two cups of water (500 mL) at room temperature (23°C). For parboiled long-grain rice, one measuring cup of rice ($135.3 \text{ g} \pm 5.0 \text{ g}$) was added to two and one-third cups of water (583.3 mL). After cooking, the cooked rice was maintained in the warm container at 65°C until the experiments were finished. The temperature was checked regularly during the cooking process and sensory test to standardise the samples on separate testing days.

Reheating methods

The cooked rice was placed in a shallow plastic pan and sealed with food wrap. All samples were transferred immediately to the refrigerator at 4°C for rapid and even cooling. The temperature of the rice samples was checked after 30 minutes and one hour. The rice samples were kept in the refrigerator (at a stable temperature) for 24 hours. After 24 hours, the temperature of the rice was checked again and then the rice samples were reheated in the microwave several times until they were over 65°C .

6.2.6 Sensory evaluation: Affective testing

The plain cooked rice samples were subjected to affective testing. Affective testing was done by an untrained participant group consisting of 64 regular weekly rice consumers of both genders among students, employees and retirees of AUT who volunteered using VAS for liking scores (see Appendix 1). Each participant was required to complete the tasting of all samples on the same day. Volunteers were asked to provide their available time and dates. They were contacted again on the day prior to their testing sessions to confirm their availability and to remind them of the pre-sensory test requirements.

On arrival, each participant was asked to collect a package of forms for completion (consent form, sensory test questionnaires for the six rice samples and personal information form) and an information sheet and to sit in individual sensory tasting booths. The booths isolated participants from each other so they were not able to talk to each other or look at other participants' evaluation results. The sensory booth setting

followed a statistical design proposed by the Standard Guide for Serving Protocol for Sensory Evaluation of Foods and Beverages (ASTM International, 2013). Participants were asked to carefully read the information sheet again and sign the consent if they agreed to proceed with the tasting session. They were also asked to provide personal information (age, ethnicity, gender, known food allergy, frequency of rice consumption and dietary pattern, such as “vegetarian or not” and “food not consumed”).

Each participant was given a sufficient amount of samples at room temperature in white plastic containers of the same size and shape. Cold filtered water was provided to participants for rinsing the mouth before and after each test. Four sensory attributes, colour, texture, flavour and sweetness, of each rice sample and overall liking of each sample were evaluated. A VAS of 100 mm was used as an instrument to measure acceptability of each attribute of each sample (see Appendix 9). Each participant was asked to mark on the scale line where his or her liking and preference were best represented. The VAS score was analysed by measuring the length (mm) from the left end of the scale to the mark. The length (mm) was divided by the total length of the scale (100 mm) to give a score of the VAS out of ten. After the tasting session, participants were asked to state whether they would eat the rice sample regularly. All the questionnaires were collected after all tasting sessions, and a light snack was provided to the participants as they left.

6.2.7 Consumer data analysis

Consumer datasets were evaluated using standard descriptive statistics (average, range, etc.) and a two-way analysis of variance (ANOVA) with product and consumer as main effects. When ANOVA indicated a significant difference between products (product $p < 0.05$), Tukey’s honest significant difference (HSD) was used to determine which products differed significantly in overall liking. Analyses were conducted using the SPSS software (SPSS Statistics 20.0, IBM, US).

6.3 Results

6.3.1 Participants characteristics and rice consumption frequency

Sixty-four participants from the AUT North Campus volunteered and completed the sensory test and questionnaire at the sensory laboratory. Participant population covered different ages, genders and ethnicities. On average, participants were normally distributed in the range of age between 18 and 78 years old (skewness = 0.5, kurtosis = -0.3). The average age of participants was 42.3 years old (SD = 14.7). As the age increased, the number of participants decreased. More than 40% of the participants who

showed interest in the rice sensory study were aged between 30 and 49 years (Figure 6-2). More female participants (60.9%) showed an interest in participating in the rice sensory evaluation than males (39.1%), and one-quarter of participants were female aged between 30 and 49 years (Table 6-2). Around 60% of participants were European, followed by 31.3% East Asian (Chinese, Korean and Japanese ethnic groups).

Participants reported consuming plain cooked rice for an average of 4.2 (\pm 4.0) times per week. The frequency of plain rice consumption was slightly skewed to the lower quartile (i.e., low frequency of rice consumption; skewness = 1.3, kurtosis = 0.9). Half of the participants consumed rice less than three times per week. Age groups between 30 and 49 years and between 50 and 69 years were the major rice consumers, reporting that they ate rice more than 4.5 times per week (Table 6-2). Male participants reported eating plain cooked rice slightly more frequently than female participants (4.9 ± 4.2 times per week vs. 3.8 ± 3.9 times per week). East Asian participants consumed rice the most frequently (7.9 ± 4.9 times per week) compared with South Asian participants (4.7 ± 2.1 times per week) and Europeans (2.4 ± 1.9 times per week).

Table 6-2 Characteristics of participants (N = 64) participated in this study.

		Gender				Total	Rice meal (n/wk(SD)) ¹
		Males (N (%))	Rice meal (n/wk(SD)) ¹	Females (N (%))	Rice meal (n/wk(SD)) ¹	(N (%))	
Age (years)	18–29	6 (9.4%)	5.0 (4.9)	11 (17.2%)	2.9 (2.8)	17 (26.6%)	3.6 (3.5)
	30–49	11 (17.2%)	4.4 (3.7)	16 (25.0%)	4.6 (4.5)	27 (42.2%)	4.5 (4.1)
	50–69	7 (10.9%)	5.3 (5.2)	9 (14.1%)	4.2 (4.4)	16 (25.0%)	4.7 (4.6)
	Over 70	1 (1.6%)	7.0 (–)	3 (4.7%)	1.0 (0.0)	4 (6.2%)	2.5 (2.4)
Ethnicity ²	European	14 (21.9%)	2.6 (2.0)	23 (35.9%)	2.2 (1.9)	37 (57.8%)	2.4 (1.9)
	East Asian	7 (10.9%)	9.3 (5.2)	13 (20.3%)	7.2 (4.7)	20 (31.3%)	7.9 (4.9)
	South Asian	3 (4.7%)	4.7 (2.1)	0 (0.0%)	0.0 (0.0)	3 (4.7%)	4.7 (2.1)
	Other	1 (1.6%)	7.0 (–)	3 (4.7%)	1.0 (0.0)	4 (6.3%)	1.0 (0.0)
Total		25 (39.0%)	4.9 (4.2)	39 (60.9%)	3.8 (3.9)	64 (100%)	4.2 (4.0)

Notes.

¹ The frequency of plain cooked rice meal consumption per week.

² All ethnicities were self-identified. European ethnicity includes New Zealand Pakeha; East Asian includes Chinese, Korean and Japanese; South Asian includes Indian, Pakistan and Sri Lanka; and Other includes Maori, Cook Island people and African American.

6.3.2 Participants' liking of six cooked rice samples

The mean “overall liking” scores were compared among the six cooked rice samples (see Table 6-3). The average overall liking scores were all above average (5.3 with 95% CI between 5.0 and 5.5), ranging from 5.1 (4.5, 5.6; reheated medium-grain white rice) to 5.8 (5.2, 6.3; freshly cooked medium-grain brown rice) out of 10 on the VAS. The repeated measures ANOVA test showed significant differences in the mean overall liking scores among the six rice samples ($F = 2.967$, $P = 0.012$). The homogeneity of variance test indicated that the variance within each rice sample was not significantly different from the others ($P = 0.817$).

Among all, freshly cooked medium-grain brown rice was the favourite of participants 5.8 (5.2, 6.3; see Figure 6-2). Freshly cooked medium-grain white rice was the least preferred 4.5 (3.8, 5.0; see Figure 6-2). It was significantly less liked compared with freshly cooked parboiled rice (-0.9 , $P = 0.036$), freshly cooked medium-grain brown rice (1.4 lower, $P = 0.001$), reheated medium-grain brown rice (-1.2 , $P = 0.005$) and reheated parboiled (1.2 lower, $P = 0.004$).

The average liking of colour was not significantly different among the six rice samples ($F = 0.201$, $P = 0.962$), and only 0.3% of variance ($\eta^2 = 0.003$) could be explained by the rice samples (see Figure 6-2). No significant difference in liking of the sweetness was observed among the six rice samples ($F = 0.781$, $P = 0.564$), and only 1% of the variance ($\eta^2 = 0.010$) could be explained by the rice samples (see Figure 6-2).

Overnight cold storage and reheating improved the liking of sweetness of all samples by up to 0.9 VAS score.

Significant differences were observed in the liking of texture among six rice samples ($F = 2.247$, $P = 0.049$; see Figure 6-2). The liking score of the texture of freshly cooked parboiled rice was slightly higher than that of freshly cooked medium-grain white rice (0.9, $P = 0.032$), and the liking scores for freshly cooked medium-grain brown rice was higher than both freshly cooked (1.2, $P = 0.005$) and reheated (1.0, $P = 0.014$) medium-grain white rice (see Figure 6-2 and Table 6-3). No difference was found between reheated parboiled rice and reheated medium-grain brown rice (0.0, $P = 0.929$). Among all, both freshly cooked and reheated medium-grain white rice samples were less favoured ($P > 0.05$).

The liking of flavour ($F = 5.565$, $P < 0.001$) was significantly different among the six rice samples (see Figure 6-2). The flavour of the freshly cooked medium-grain brown

rice was the favourite (5.9 (5.4, 6.4)) among the six rice samples. The freshly cooked and reheated medium-grain white rice samples were both significantly less liked by participants (4.3 (3.9, 5.1) and 4.5 (3.9, 5.1) respectively; see Table 6-3). The liking of flavour of the freshly cooked parboiled rice was significantly lower than that of the freshly cooked medium-grain brown rice (-0.9 , $P = 0.032$), after the cold storage and reheating treatment, the liking of flavour of the parboiled rice improved to 5.7 (5.1, 6.3), which was similar to that of the reheated medium-grain brown rice (5.7 (5.1, 6.2), difference = 0.0, $P = 0.920$; see Table 6.3).

Table 6-3 Participants liking score (N = 64) of colour, texture, flavour and sweetness and overall liking score of each cooked plain rice sample.

Rice sample characteristics		Liking of the attributes ¹ (VAS score out of 10) (Mean (95% CI))				Overall liking
Preparation	Rice product varieties	Colour	Texture	Flavour	Sweetness	
Freshly cooked	Parboiled	5.9 (5.4, 6.3)	5.5 (5.0, 6.1) ^a	5.1 (4.5, 5.6) ^a	4.8 (4.3, 5.5)	5.2 (4.7, 5.9) ^a
	Medium-grain brown	6.0 (5.5, 6.5)	5.8 (5.3, 6.3) ^{bc}	5.9 (5.4, 6.4) ^{abc}	5.1 (4.6, 5.6)	5.8 (5.2, 6.3) ^b
	Medium-grain white	5.9 (5.4, 6.4)	4.6 (4.0, 5.3) ^{ab}	4.3 (3.7, 4.9) ^{bde}	4.2 (3.7, 4.9)	4.5 (3.8, 5.0) ^{abcd}
Reheated	Parboiled	6.1 (5.6, 6.7)	5.2 (4.6, 5.9)	5.7 (5.1, 6.3) ^{dg}	5.4 (4.8, 6.0)	5.6 (5.0, 6.2) ^c
	Medium-grain brown	6.1 (5.6, 6.6)	5.2 (4.6, 5.8)	5.7 (5.1, 6.2) ^{ef}	5.4 (4.8, 6.0)	5.6 (4.5, 5.6) ^d
	Medium-grain white	5.9 (5.4, 6.3)	4.8 (4.2, 5.3) ^c	4.5 (3.9, 5.1) ^{cfg}	5.1 (3.2, 7.0)	5.1 (4.5, 5.6)
Total		6.0 (5.8, 6.2)	5.2 (5.0, 5.4)	5.2 (5.0, 5.4)	5.0 (4.7, 5.4)	5.3 (5.0, 5.5)

Note:

¹ Liking score is presented as mean (mm/100 mm) (lower 95% CI, upper 95% CI of the mean).

² The value with the same letter indicates that their mean values differ significantly ($P < 0.05$) in the same column by repeated measures ANOVA.

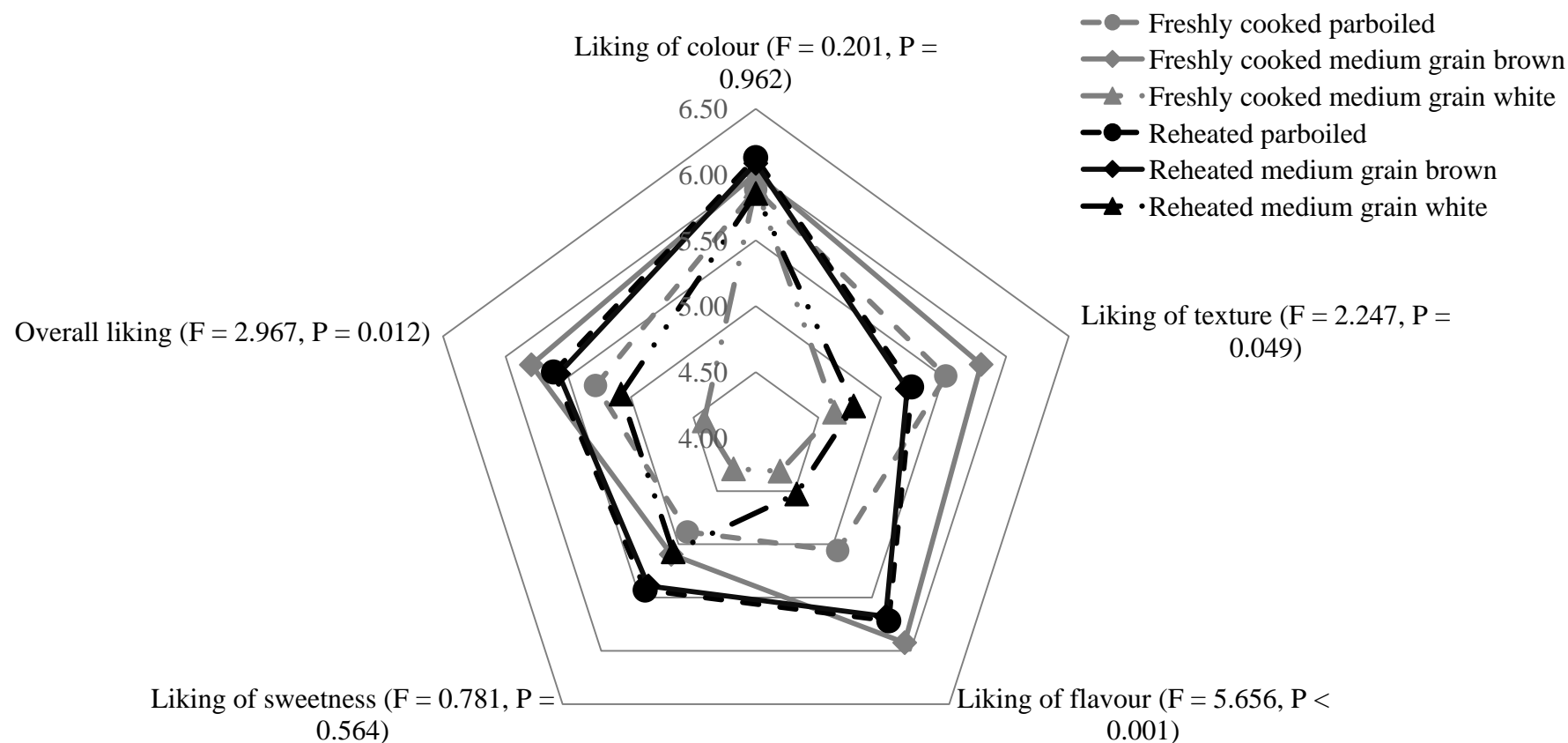


Figure 6-2 Diagrammatic presentation of liking for sensory attributes (colour, texture, flavour, sweetness and overall) of six rice samples by 64 participants. Sensory attribute with $P < 0.05$ indicates significant difference among the six rice samples. Visualised analogue scales (VAS) scores were used (VAS score = measurement from extremely unlike on the left end to participant's marking (mm)/100 (mm); score 0 = least acceptable/dislike extremely; score 10 = highly acceptable/like extremely.)

Generally, freshly cooked medium-grain white sample was less preferred by participants compared to other rice sample (Figure 6-2). Overnight cold storage and reheating improved the overall acceptance and the acceptance of colour and sweetness of the medium-grain white rice. No differences were found between the reheated medium-grain brown rice and the reheated parboiled rice. For medium-grain brown rice, participants generally preferred freshly cooked to reheated; however, participants generally preferred reheated parboiled rice samples to freshly cooked.

6.3.3 Participants' future consumption intent

When asked to comment about their acceptance of the three types of rice prepared in different ways, more than half of the participants answered that they would consider eating freshly cooked and reheated parboiled rice (56.2% and 57.8% respectively) and freshly cooked and reheated medium-grain brown rice (62.5% and 56.2% respectively) as a regular meal. The intention to eat the parboiled rice increased slightly after the cold storage treatment whereas that of the medium-grain brown rice decreased by around 6% (see Table 6-4). The intention to eat the freshly cooked medium-grain white rice was more than 10% lower compared with the other five rice samples. Overnight cold storage and reheating increased the intention to eat by 7.8% to 53.1%.

Table 6-4 Frequency of participants' intention to eat the rice sample as regular staple meal.

Rice sample		No to eat as regular meal N (%)	Yes to eat as regular meal N (%)
Freshly cooked	Parboiled rice	28 (43.8%)	36 (56.2%)
	Medium-grain brown rice	24 (37.5%)	40 (62.5%)
	Medium-grain white rice	35 (54.7%)	29 (45.3%)
Reheated	Parboiled rice	27 (42.2%)	37 (57.8%)
	Medium-grain brown rice	28 (43.8%)	36 (56.2%)
	Medium-grain white rice	30 (46.9%)	34 (53.1%)

6.4 Discussion

Reheated parboiled rice has been shown to be an equally liked alternative to freshly cooked or reheated medium grain white rice and medium grain brown rice.

Furthermore, almost 60% of the participants said that they would eat reheated parboiled rice as a regular staple meal. Combined with the favourable glycaemic properties of

reheated parboiled rice, this treatment of rice preparation could be a recommendation for a healthier diet. Previous studies have observed overall liking of rice over 5.0 on average for freshly cooked using a 10-point categorical Likert scale (1 = extremely dislike and 10 = extremely like) (Heinemann et al., 2006; Park, Kim, & Kim, 2001), which are consistent with the result found in this study.

Cold storage and reheating preparation significantly improved participants' overall liking towards both medium grain white and parboiled rice. This trend was associated with the significant increase in liking of the sweetness and the flavour of reheated parboiled rice. These findings are consistent with previous studies (Ali, Hasan, & Islam, 2008; Deveriya, 2007; Heinemann et al., 2006) where it was observed that a distinct fragrance and less sweetness and less creamy flavour added to the acceptance of reheated cold stored rice. This might be due to the increased proportion of resistant starch and slowly digested starch in cold-stored and reheated rice that reduced the sweetness in the rice, which posed a healthier image of rice meals (Behrens, Heinemann, & Lanfer - Marquez, 2007; Qingyun, Yeming, Mikami, Kawano, & Zaigui, 2007; Srisawas & Jindal, 2007; Wilkie, Wootton, & Paton, 2004). Previous studies in West Africa, Brazil (including participants of European origin) and India (including participants of South Asian origin) also found that less sweet grains and less creamy flavour were generally preferred by participants (Deveriya, 2007; Gayin, Manful, & Johnson, 2009; Heinemann et al., 2006; Tomlins, Manful, Gayin, Kudjawu, & Tamakloe, 2007). However, studies in East and South East Asia (Hori et al., 1994; Prescott, 1998; Qingyun et al., 2007) found that participants preferred increased sweetness, creamier flavour in refined grain. As the present study recruited around 25% of East Asian origin and 75% of European and South Asian origins, it is suggested that the increase in overall liking, flavour and sweetness could be contributed by the high variance in the liking preference among regions and participant population (Hori et al., 1994; Prescott, 1998).

While cold storage and reheating improved the liking of the texture of medium grain white rice, the same rice preparation procedure reduced the liking of the texture of parboiled and medium grain brown rice. It is suggested that cold storage and reheating reduced the moisture content in medium grain white rice (as also observed in the previous studies, Chapter 4, the *in vitro* starch digestibility study, and Chapter 5, human trial on postprandial glycaemic response) and may have hardened the grains and resulted in an increase in the liking of texture (Ali et al., 2008; Srisawas & Jindal, 2007)

as the firmer texture was generally favoured by participants of European and South Asian origin (Deveriya, 2007; Gayin et al., 2009; Heinemann et al., 2006; Tomlins et al., 2007). However, because both whole grain brown rice and parboiled long grain rice have lower moisture content than medium grain white rice, the cold storage and reheating may have reduced the moisture further resulting in significantly firmer texture and reduced liking of the texture.

No significant variations in the liking of colour indicated that average participants did not reject or prefer the rice samples based on the visual characteristics of the rice samples. It is suggested that the other attributes, flavour and texture, played significant roles in driving participants' liking and preference, and the sweetness had a considerable impact on the overall liking. However, a study conducted in Brazil by Heinemann et al. (2006) and two studies conducted in West Africa by Tomlins et al. (Tomlins et al., 2007; Tomlins et al., 2005) all indicated that the appearance of the rice grains (including colour and the uniform appearance of the grains) played more a significant role in liking preference compared with texture and flavour. This suggests that the location of the participant population may have played a significant role in the liking of colour.

The design and execution of the study followed the requirements for a reliable and credible laboratory-based sensory liking test (Heymann & Lawless, 2013) which was powered to detect minimal difference in the VAS ratings given for the rice sample (Flint et al., 2000; Parker et al., 2004; Heymann & Lawless, 2013). The other advantage of this study is that the selection of participants was not designed to have an even number of participants in each age, gender and ethnic group but the participant population may represent the diverse Auckland community who eat rice. All participants were asked to fast for at least two hours before testing and rinse their mouth thoroughly between testing of each sample in order to avoid possible misjudging or bias (Heymann & Lawless, 2013). The other strength of this study is the novelty of the study design. No recent study has examined the effect of a home-prepared cold storage and reheating treatment on the sensory attributes of rice (overall liking, colour, texture, flavour and sweetness).

The main limitation of this study is that it compared medium-grain white, medium-grain brown and parboiled rice only once with each participant. Previous studies have introduced a multi-sample repeated measure on one participant on separate days in order to minimise the Type II error (Heinemann et al., 2006; Heymann & Lawless, 2013). It is suggested that a repeated measure be introduced to test within-individual variance. In

addition, only five attributes, colour, flavour, texture, sweetness, and overall liking were compared, and other factors, such as mood, that may have influenced participants' liking were not measured. Because this study was not designed to compare the age, gender and ethnic effect on liking preference, these factors were not compared. There may be a natural variation in preference in different population groups.

6.5 Conclusion

There are very few reports in the literature on the liking and acceptability of parboiled rice, and no recent study has investigated the overnight cold storage and reheating preparation method in relation to the liking of attributes. For its unique nutritional characteristics (including slower and lower postprandial glycaemic responses), both freshly cooked and reheated parboiled rice may be considered a valuable alternative to whole grain rice (i.e., medium-grain brown rice). This may be of particular advantage to those at risk of type 2 diabetes and for pregnant women at risk of gestational diabetes. Based on this sensory evaluation, it was concluded that the majority of the participant population did not reject reheated parboiled rice based on its attributes (colour, texture, flavour and sweetness). Consumers did not report that they regularly consumed parboiled rice as a staple grain. This may be because of a lack of familiarity with and lack of nutritional knowledge of the parboiled rice and not appreciating that it is cheaper and has a longer shelf life.

These findings corroborate the need for marketing efforts that can effectively inform about the health advantages of overnight cold storage and reheating and the nutritional values and convenience of parboiled rice. This information may contribute to increasing public awareness and, eventually, bringing the nutritional benefit to the population.

Chapter 7: Overall discussion and conclusion

This body of work compared five commonly available rice products (medium-grain white, medium-grain brown, basmati, long-grain brown and parboiled rice) able to be purchased from New Zealand supermarkets and observed how the rice products were prepared, stored, digested and absorbed. It has presented the evidence that, when compared with other rice products, reheated parboiled rice has (see Figure 7-1):

1. a higher proportion of slowly digestible starch (SDS) and resistant starch (RS) and slower *in vitro* glucose release and postprandial glycaemic response;
2. a requirement for a longer chewing time, resulting in a more uniform breakdown of rice into intermediate-sized particles;
3. longer and more prominent satiety responses, including feelings of fullness; and
4. increased liking and acceptance from rice consumers.

These findings will be considered, in the context of other work, in turn and in combination in the following sections 7.1, 7.2, 7.3, and 7.4. To summarise, the physical and chemical properties of reheated parboiled rice and the physiology of digestion and satiety associated with rice carbohydrate are presented in Table 7-1 and referred to in the following discussion. In addition, the key papers that place the context of this work in relation to the existing body of knowledge are summarised in Table 7-2. To the best of knowledge, this body of work is the most comprehensive series of studies of starch digestibility profiles and *in vitro* glucose release, postprandial glycaemic responses, satiety and palatability, chewing (chewed particle size distribution and chewing length), and sensory evaluation (i.e., consumer liking preference) of rice products to date.

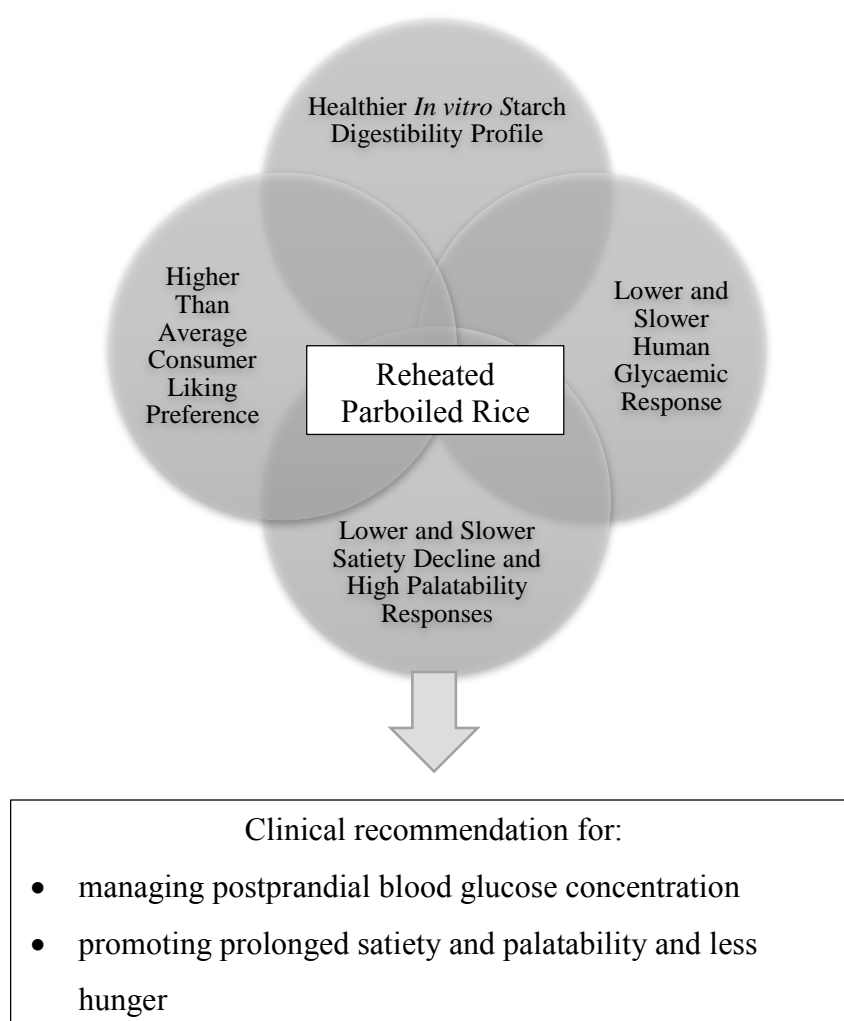


Figure 7-1 Venn diagram of the demonstration that reheated parboiled rice may be considered a plausible and healthier alternative for the public.

Table 7-1 Summary of physical and physiological factors of reheated parboiled rice that may contribute to lower glycaemic responses.

Rice physical features after cold storage @ 4 °C for 24 hr	Physical factors	Physiological factors
Firmer and harder grain texture	Longer chewing time*	Slower release and lower rise in blood glucose*
Reduced sweetness*	Higher proportion of larger particle size *	Faster and longer satiety*
Reduced moisture content*	Higher proportion of resistant starch (RS) and slowly digestible starch (SDS) *	

* Measured for this thesis.

Table 7-2 Selected studies relevant to investigation of starch digestibility profiles and *in vitro* glucose release, postprandial glycaemic responses, satiety and palatability, chewing (chewed particle size distribution and chewing length), and sensory evaluation (i.e., consumer liking preference) of rice products.

Study	Products	Cooking and preparation methods	Starch digestibility profile and <i>in vitro</i> glucose release	Postprandial glycaemic responses and/or glycaemic index (GI)	Participants characteristics	Satiety and/or palatability	Chewed particle size distribution	Consumer liking preference
Present body of work	Medium-grain white rice, medium-grain brown rice, basmati rice, long-grain brown rice, parboiled rice	Freshly boiled, cold storage at 4 °C for 24 hours at 2-hr intervals, reheating after cold storage	<i>In vitro</i> glucose release and starch digestibility profiles	Postprandial blood glucose responses among freshly cooked medium-grain white rice, freshly cooked parboiled rice and reheated parboiled rice	Healthy participants with no currently diagnosed diabetes.	Postprandial satiety and palatability responses	Chewed particle size distribution and chewing time length	Liking preference and consumer acceptance
(Tan, Wu, et al., 2015)	Jasmine and basmati rice	Freshly boiled		Blood glucose responses and GI	Healthy males with no metabolic syndrome		Length of chewing time	
(Zenel & M. Stewart, 2015)	High amylose white rice	Freshly boiled		Postprandial blood glucose concentration	Healthy participants with no currently diagnosed diabetes.	Postprandial satiety responses		
(Sudha et al., 2013)	Indian rice product varieties	Freshly boiled			Healthy participants with no currently diagnosed diabetes.			Liking preference and consumer acceptance

Study	Products	Cooking and preparation methods	Starch digestibility profile and <i>in vitro</i> glucose release	Postprandial glycaemic responses and/or glycaemic index (GI)	Participants characteristics	Satiety and/or palatability	Chewed particle size distribution	Consumer liking preference
(Anderson et al., 2010)	High amylose (long-grain) and low amylose (medium-grain) rice	Freshly boiled		Postprandial blood glucose concentration	Healthy participants with no currently diagnosed diabetes.	Postprandial satiety responses		
(Ranawana et al., 2010)	Pure basmati rice	Freshly boiled	<i>In vitro</i> glucose release	<i>In vivo</i> blood glucose responses	Healthy participants with no currently diagnosed diabetes.		Degree of mastication	
(Schroeder, Gallaher, Arndt, & Marquart, 2009)	Refined rice (white rice)	Freshly boiled			Healthy women with no currently diagnosed diabetes.	Postprandial satiety responses		
(Gayin et al., 2009)	Parboiled rice from USA	Freshly boiled			Trained panellist, healthy status was not specified.			Liking preference and consumer acceptance
(Tomlins et al., 2007)	Parboiled and raw milled rice from West Africa and USA	Freshly boiled			Trained panellist, healthy status was not specified.			Liking preference and consumer acceptance

Study	Products	Cooking and preparation methods	Starch digestibility profile and <i>in vitro</i> glucose release	Postprandial glycaemic responses and/or glycaemic index (GI)	Participants characteristics	Satiety and/or palatability	Chewed particle size distribution	Consumer liking preference
(Behrens, Heinemann, & Lanfer-Marquez, 2007)	Parboiled rice from Brazil				Not specified.			Liking preference and consumer acceptance
(Englyst et al., 2007)	Long-grain brown rice	Freshly boiled	<i>In vitro</i> starch digestibility profile					
(Heinemann et al., 2006)	Parboiled rice from Brazil and other countries in South America	Freshly cooked and cooled for 2 hours			Not specified.			Liking preference and consumer acceptance
(Chung et al., 2006)	Waxy rice, milled to fine starch powder	Freshly boiled vs. cold storage at 4 °C for 2, 4 or 7 days	<i>In vitro</i> starch digestibility profile	Estimated GI				
(Hu et al., 2004)	Indica, japonica and hybrid rice (basmati vs. long-grain white rice)	Freshly boiled vs. 24-hr cold storage at 4 °C	<i>In vitro</i> starch digestibility profile	Estimated glycaemic score (EGS)				
(Wilkie et al., 2004)	Australian fragrant, non-fragrant and imported fragrant rice	Freshly boiled			Not specified			Liking preference and consumer acceptance

Study	Products	Cooking and preparation methods	Starch digestibility profile and <i>in vitro</i> glucose release	Postprandial glycaemic responses and/or glycaemic index (GI)	Participants characteristics	Satiety and/or palatability	Chewed particle size distribution	Consumer liking preference
(Frei et al., 2003)	Philippine rice cultivars (white rice, non-parboiled)	Freshly boiled vs. cold storage at 4 °C for 24 hours	<i>In vitro</i> glucose release and total starch, resistant starch and amylose content	Estimated GI (<i>in vitro</i> kinetics of starch digestion)				
(Goñi, Garcia-Diz, Mañas, & Saura-Calixto, 1996)	Unspecified white rice	Freshly boiled vs. cooled (temperature and storage time were not specified)	<i>In vitro</i> starch digestibility profile					
(Holt & Brand-Miller, 1995)	White rice	Freshly boiled		Postprandial blood glucose concentration		Postprandial satiety responses		
(Hori et al., 1994)	Non-aromatic and aromatic rice	Freshly boiled			Healthy participants with no currently diagnosed diabetes.			Liking preference and consumer acceptance
(Miller et al., 1992)	White rice, brown rice, and parboiled rice	Freshly boiled		GI	Healthy, with normal glucose tolerance			

7.1 Reheated parboiled rice has a higher proportion of slowly digestible starch and resistant starch and slower *in vitro* and *in vivo* glucose release

Storing cooked rice for 24 hours at 4 °C decreased the rate of starch digestibility measured *in vitro* over two hours by up to 30%. This finding is in agreement with earlier *in vitro* studies on the digestibility of rice starch and glucose release, which led to the conclusion that rice should be generally classified as a high glycaemic index (GI) grain or food with high glycaemic potency (Bjorck et al., 1994; Jenkins et al., 1987; Miller et al., 1992). In a later study, by Goñi et al. (1996), researchers identified that rapid cooling of freshly cooked hot white rice can increase the proportion of RS from less than 1% to up to 2.5%. Although the intention of the study by Goñi et al. (1996) was to identify the possible errors in the method of quantifying RS (%) rather than the full profile of starch digestibility (the proportions of rapidly digestible starch (RDS), SDS and RS), the concept of rapid cooling was quickly absorbed into the consideration of possible ways to increase the RS (%) content and therefore decrease the rate of *in vitro* and *in vivo* digestion of starch products. Variations in digestibility were related to longer cold storage time, waxiness of the cultivar and higher amylose content (Frei et al., 2003).

Frei et al. (2003) and Chung et al. (2006) both identified that cold storage at 4 °C for more than 24 hours induced significant rice starch retrogradation, and thus the *in vitro* glucose release trajectory (i.e., *in vitro* starch hydrolysis trajectory) was reduced by up to 26%. The study by Frei et al. did not report the full starch digestibility profile of the cultivars; however, they did report a reduction in the estimated GI by up to 30% (Frei et al., 2003). This is in line with the present study, in which cooling reduced the rate of glucose release from reheated parboiled rice by 30% *in vitro* and 15% *in vivo*. In contrast, another study, by Chung et al. (2006), reported that in Canadian rice cultivars, there were no significant differences in the rice digestibility profile (RS%, SDS%, RDS%) after cold storage. This may be due to how the authors prepared their rice sample (the waxy rice was milled to starch powder before gelatinisation and cold storage treatment) (Chung et al., 2006), which would destroy the rice grain structure (i.e., losing RS1 and RS3) and lead to lack of protection from rapid starch hydrolysis during the *in vitro* digestion process. Similarly, Frei et al. (2003) only rubber-rolled rice into coarse, rough particles rather than mincing the rice grain to simulate chewing actions of cutting and grinding. In the present series of *in vitro* investigations, the rice

was minced, and this may have changed the *in vitro* starch digestibility characteristics compared with those found in Frei et al.'s study.

7.2 Reheated parboiled rice has a requirement for a longer chewing time resulting in a more uniform breakdown of rice into intermediate-sized particles

This work has shown, *in vitro*, an increase in the rate of glucose release and starch digestibility for all rice products (i.e., medium-grain white rice, medium-grain brown rice, basmati rice, long-grain brown rice and parboiled rice) when particle size was reduced to around 2,000 μm (i.e., simulated chewing), which may be attributed to the increased contact area between rice and the solutions containing enzymes (i.e., amylase). These observations on the effect of particle size are consistent with a recent study by Ranawana et al. (2010), which reported an inverse correlation between chewed and expectorated particle size and *in vitro* starch digestibility. Moreover, in this study, particle size of chewed rice was measured after the glycaemic response and an increase in chewing time was associated with an increased glycaemic response and reduced particle size. As expected, this study also showed that whole grains of brown rice elicited a lower rate of hydrolysis than minced grains in *in vitro* digestion. *In vitro* and *in vivo* breaking down the grains by mincing or longer chewing respectively reduced the resistance to starch hydrolysis and increased the digestion rate significantly. This apparent increase in apparent bioavailability of glucose did not, however, reach equivalence to that of the white rice product varieties. Bioavailability of the rice starch and other nutrients overall would need to be investigated further with either biomarkers or faecal samples.

In addition, the glycaemic responses trial with healthy volunteers presented the evidence that, despite high inter-individual variance, there was a tendency for the postprandial glycaemic responses to be positively associated with chewing time and the proportion of intermediate size particles (between 2,000 μm and 425 μm). This finding is supported by recent studies by Tan et al. (2015) and Ranawana et al. (2010), who both analysed the association between mastication parameters and the GI of basmati rice. They explained their findings by the fact that less efficient mastication (i.e. longer chewing time) may create larger food boluses that would slow gastric digestion and the food would not be passed into the duodenum until the particle size was reduced by the action of the stomach movement and acid. This delay in gastric emptying and duodenal digestion would therefore delay and possibly reduce postprandial glucose expenditure. The third study (Chapter 5) of this thesis further demonstrated that, when compared to

freshly cooked parboiled rice and freshly cooked medium-grain white rice, the cold-stored and reheated parboiled rice (with a higher proportion of RS and SDS) increased the chewing time and led to a significantly larger proportion of intermediate size particles (between 2,000 μm and 425 μm). Both these factors may have contributed to the significantly slower postprandial glycaemic response that was observed.

7.3 Reheated parboiled rice has longer and more prominent satiety responses, including feelings of fullness

A slower glucose response was consistently associated with greater perceived satiety and palatability by participants. Previous studies have reported that differences in satiety after low and high glycaemic loads (GLs) may be physiologically mediated through a dynamic effect of the rate of change of the plasma glucose concentration on specific gluco-sensitive cells in the brain and increased mastication and changes in gut hormones (i.e., ghrelin or glucagon-like peptide-1) (Holt & Brand-Miller, 1995), and thus the satiating effect of low GL carbohydrates (Anderson & Woodend, 2003). Another factor that may explain increased satiety is that the higher proportion of RS and SDS present in cold-stored and reheated parboiled rice and larger particle sizes may have created gastric distension and delayed gastric emptying (Schroeder et al., 2009; Zenel & M. Stewart, 2015), and satiety-related hormones in participants were then released, signalling fullness (Schroeder et al., 2009). Reheated parboiled rice, compared with medium-grain white rice, which induced higher glycaemic responses but lower satiety, required longer chewing time, which may have further delayed the gastric distension and extended the feeling of fullness (see Table 7-1).

7.4 Reheated parboiled rice has increased liking and acceptance from rice consumers

In addition to the increased satiety and acceptable palatability perceptions, if recommendations are to be made about rice preparation, it also needs to be known whether rice consumers, in general, would be willing to eat rice reheated after cold storage. The majority of participants in the final study of this thesis did not reject the parboiled rice based on its sensory attributes (colour, texture, flavour and sweetness). The cold storage and reheating preparation was associated with participants' increased overall liking towards parboiled rice; however, the liking of texture decreased compared with that of freshly cooked rice, possibly because of the hardened texture caused by some moisture loss during overnight cold storage (Hori et al., 1994). Overall, the evidence presented in this thesis is in contrast with that of other researchers (Heinemann

et al., 2006; Tomlins et al., 2007) who have suggested that participants would not be familiar with parboiling pre-treatment and its characteristics and nutritional benefits and that medium-grain brown rice would be a preferable, healthy alternative to parboiled medium-grain white rice. Similarly, others (Behrens et al., 2007; Gayin et al., 2009; Wilkie et al., 2004) have suggested that rice with a firmer texture would not be acceptable for its sensory attributes, yet the increased firmness contributes to the low glycaemic responses and improved satiety. Cold storage and reheating was associated with less perceived sweetness, and this was generally liked by participants. However, no other recent study has examined the sensory attributes of cold-stored rice and, in particular, parboiled rice.

Long-term reduction of the GL of the diet through consumption of rice with a slower and lower glycaemic response may reduce the risk for non-communicable diseases (Gogebakan et al., 2011). Parboiled rice and reheated parboiled rice are hypothesised to contribute positively to human health because of their rich RS content and SDS content and vitamins and minerals (e.g., vitamin B, D and E) (Dipti et al., 2012). In addition, the RS and SDS in parboiled rice and reheated parboiled rice may act as satiating ingredients and create gastric distention and delay gastric emptying. Epidemiological studies have suggested that the consumption of foods that are rich in RS and SDS are inversely associated with the risk of type 2 diabetes and other non-communicable diseases (Raben et al., 1994; Schroeder et al., 2009). Replacing commonly consumed freshly cooked medium-grain white rice with parboiled rice or reheated parboiled rice would provide a protective effect against type 2 diabetes or onset of the condition.

The main strength of the present body of work is the novelty and the extent of the investigation of the effect of cold storage and reheating on rice starch digestibility, glycaemic response, satiety and palatability, chewing capacity, and consumers' liking and preference. A number of studies that have investigated similar outcomes have focused on comparison among rice product varieties and neglected the cold storage effect and the storage time effect (see Table 7-1), or only studied the association of the effect among two or three of the outcomes. The present body of work ranged from measures of *in vitro* digestion to *in vivo* human glycaemic responses, and satiety to actual liking and intent to eat. In addition, the present study investigated the *in vitro* starch digestibility of five regularly available rice products in Auckland supermarkets, medium-grain white, medium-grain brown, basmati, long-grain brown and parboiled, whereas previous studies compared only limited rice product varieties.

Furthermore, this body of work investigated the possibility and plausibility of translating the findings from the *in vitro* starch digestibility and human glycaemic response study to a practicable nutritional message in order to bridge laboratory bench science with population-based community intervention. The body of work adds value to the current existing research on rice and may inspire further study on a larger scale. Another strength of this body of work is the representation within the study population. Representatives of the majority ethnic groups who commonly consume rice as a staple grain were recruited. In addition, this study introduced a safe rice cold storage method (Lake et al., 2004) that minimises the risk of *Bacillus cereus* infection, which has not been a main concern in previous studies.

The major limitation of this study is the limited scale of the human experimental trial due to restricted research funding and time. In addition, because of the limited funding, the study population in the sensory study was not large enough to provide a comparison between genders and among age and ethnic groups. The other limitation is that the responses to the questions by socio-economic status were not analysed, although this is a major contributor to consumer acceptability, cooking skills and consumer affordability (Heymann & Lawless, 2013). It is suggested that in future studies, the associations among demographic, attitude profiles of consumers, consumer acceptability and consumer affordability be analysed to obtain a better understanding of the market value of parboiled rice. Because this body of work aimed to compare frequently consumed rice products from Auckland supermarkets, it included only one parboiled rice product. It is suggested that in future studies other parboiled rice products be included.

Future work should include the investigation of the possibilities of implementing an interventional dietary change, replacing freshly cooked medium-grain white rice with reheated parboiled rice within the general population who consume rice regularly. As discovered in many previous translational studies, health and dietary messages are not well followed, in part because of lack of motivation or ability to do so or lack of access (Gidding et al., 2009; Glanz & Bishop, 2010; Kumanyika, 2008; Story, Kaphingst, Robinson-O'Brien, & Glanz, 2008; Swinburn, Gill, & Kumanyika, 2005). In 2009, the American Heart Association Nutrition Committee identified four key challenges that may interfere with food choices (see Figure 7-2): macro-environment, microenvironment, family environment and individual. Reheated parboiled rice is able to conquer each of these challenges, which proves its ability to be the next popular nutritious staple grain.

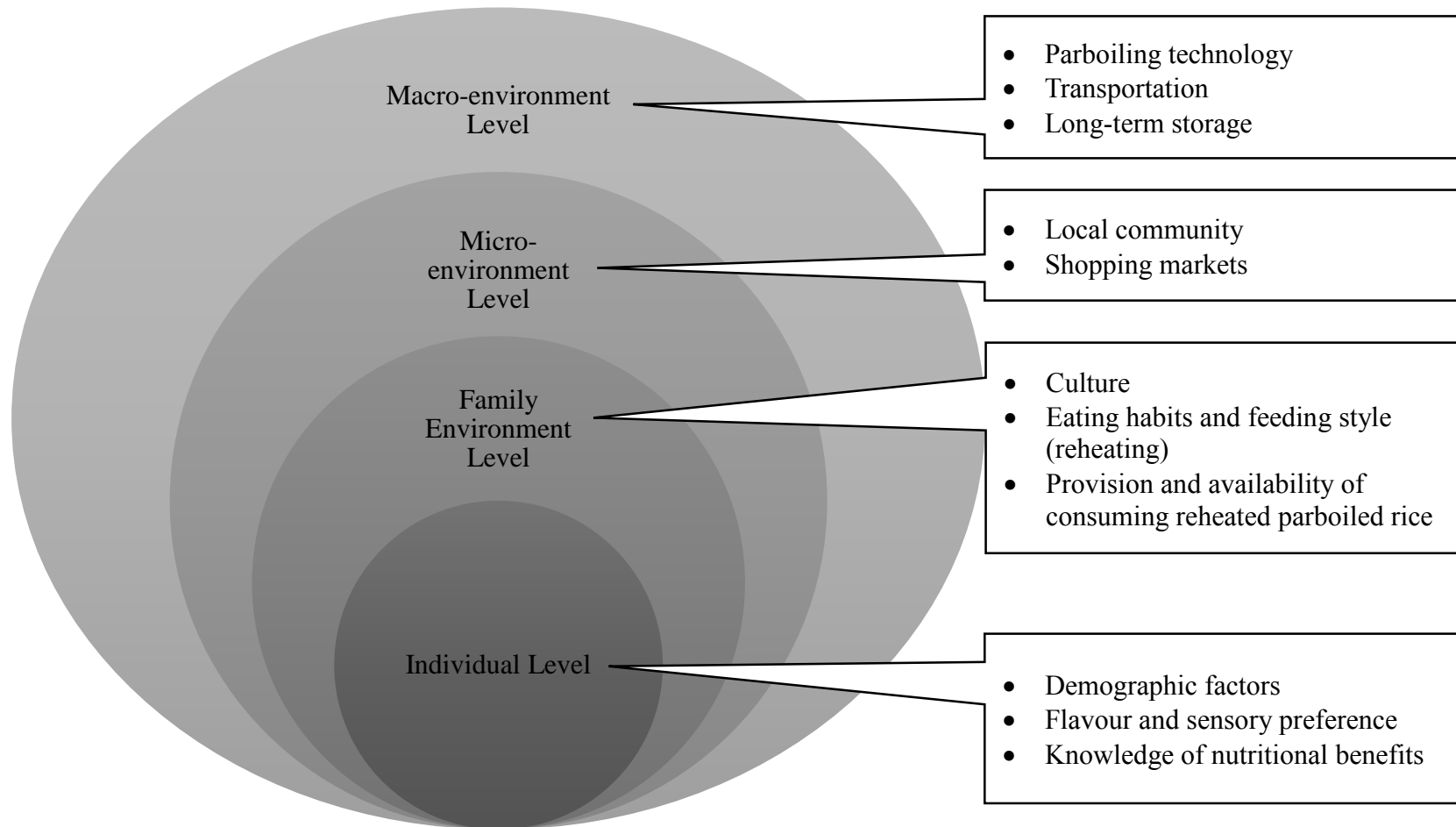


Figure 7-2 Influencing food choice: A multi-level framework of factors that influence eating behaviours. (Gidding et al., 2009; Glanz & Bishop, 2010; Kumanyika, 2008; Story et al., 2008; Swinburn et al., 2005)

There is a need to educate those who frequently consume rice about the advantages of parboiled rice for reduction of glycaemic load, especially when it is reheated. Additional advantages include the minerals and water-soluble vitamins that are retained from the parboiling process, the lower cost, and the longer storage time than both whole grain and refined rice (Tomlins et al., 2007). Recent advances in technology have improved the physical quality of the parboiled rice grain. This has also improved the availability (i.e., extending the storage time and reducing the susceptibility to insect attack, etc.) and thus reduced the price of making parboiled rice more accessible to average consumers.

Currently, the rice parboiling technique has been introduced to Thailand and other big rice-consuming countries in Southeast Asia. Parboiled rice can be conveniently introduced to and is likely to be accepted and liked by New Zealand families and individuals, especially in a rapidly growing multicultural society such as Auckland. Furthermore, the key element, a home-based cooking and preparation method (24-hour cold storage and reheating before serving), provides a simple alternative to existing rice products without major lifestyle (i.e., dietary pattern) changes or excess expenses for new rice products or product varieties. This practice can be adopted in family as well as work environments on a daily basis.

Existing investment in identifying the association between rice consumption and decreased risk factors for chronic diseases has led to a trend of nutrition promotion and finding a cost-effective alternative rice meal in every rice-consuming country. The outputs from this research may be integrated with effect to deliver an immediately available and cheap rice product and a preparation method that are acceptable to consumers. Reheated parboiled rice has been reported to be more cost effective than other whole grain or refined rice without the parboiling treatment and has been proven to meet the needs of low glycaemic response needs and the linkage to sensory needs of consumers in terms of satiety and sensory properties. These findings deliver solid nutritional information that may be used by the community, and consumers may be persuaded to adopt the home-based cold storage method and increase their consumption of reheated parboiled rice.

7.5 Cold-storage and food safety of cooked rice

Bacillus cereus, which may grow on cooked rice, produces emetic toxin (ETE) and three different enterotoxins: HBL, Nhe, and EntK that have been recognised as food toxins since the 1950s. The bacterium causes two types of food-borne illness. One is characterised by nausea and vomiting 30 minutes to 6 hours after consumption, the

second is manifested by abdominal cramps and watery diarrhoea 6 to 15 hours after consumption.

The contamination by *B. Cereus* of rice occurs when the cooked rice is not cooled quickly and thoroughly to less than 4 °C or not properly reheated at the correct temperature after cold storage. Ideal conditions for *B. Cereus* growth include warmth and moisture: 30 °C pH range (pH2-11).

New Zealand Food Safety Guideline has indicated that safe practice of preparing boiled rice is essential to stop *Bacillus cereus* growth during storage and stop toxin production. The cooked rice may be kept for a short time at more than 60 °C or cooled quickly and stored for up to one day (Lake et al., 2004). The (Ministry for Primary Industries, 2016) advised that cooked rice can be removed from the hot container and spread into clean shallow container (<10cm deep) and then placed in a refrigerator at 4 °C or lower to be cooled quickly and evenly to prevent bacteria growth and toxin production. It is required to evenly reheat the cold-stored rice to over 65 °C to destroy the bacteria and production of toxins.

7.6 Overall conclusion

Reheated parboiled rice could be a staple rice that offers solutions for long-term glycaemic management and contributes to solutions for the grand challenges (e.g., type 2 diabetes and obesity) to global health because of its relatively better nutrient profile, low *in vitro* starch digestibility, low postprandial glycaemic response, promotion of satiety and palatability, and generally positive overall likeability.

Glossary

Glycaemic index	The rate of digestion and absorption of a standardised dose of carbohydrate in food (e.g., rice) compared with the rate of digestion and absorption of the same amount of glucose calculated as a percentage – results in a measure of ranking
Glycaemic load	The amount of carbohydrate in a food, meal or diet multiplied by the glycaemic index of each food component – units are gram equivalents of glucose
Glycaemic release	The rate and extent of <i>in vitro</i> release of glucose from starch
Rapidly digestible starch	The starch fraction (%) that is digested within 20 minutes of simulated digestion process
Resistant starch	The starch fraction (%) that is able to resist a standardised simulated digestion process for up to 180 minutes
Rice product	A distinct rice product line available in local supermarkets
Slowly digestible starch	The starch fraction (%) that is digested in between 20 and 180 minutes of a standardised simulated digestion process
Total available starch	The starch fraction (%) that can be digested during a standardised simulated digestion process that equals to the sum of rapidly digestible starch and slowly digestible starch

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Appendices

Appendix 1: Reagent list of Total Starch Assay Kit (AA/AMG)

The important reagents used in total starch determination are listed below.

Reagents Used in Total Starch Determination:

Thermostable α -amylase (10 mL, 3,000 Units/mL on CERALPHA reagent at pH 6.0)

Amyloglucosidase (AMG, 10 mL, 200 Units/mL on p-nitrophenyl β -maltoside at pH 4.5) at 40 °C

Glucose Determination Reagent (GOPOD Reagent; glucose oxidase > 12,000 U/litre, peroxidase > 650 U/litre, 4-aminoantipyrine of 0.4 mM)

MOPS Buffer (50 mM, pH 7.0; prepared by dissolving sodium salt, 11.55 g (Sigma Chemical Co.; cat. No. M9381) to 900 mL of distilled water, then adjusted to pH 7.0 by the addition of 1 M (10%) HCl)

Dimethyl sulphoxide (DMSO) (Cat. No. D5879, Sigma Chemail Co.)

Appendix 2: Ethical Approval Forms for Study “Effect of Rice Cooling Method on Glycaemic Response, Satiety and Chewing Test”



11 February 2013

AUTEC SECRETARIAT

Elaine Rush Faculty of Health and Environmental Sciences

Dear Elaine Re Ethics Application: **13/05 Effect of rice cooling method on glycaemic response, satiety and chewing tests.**

Thank you for providing evidence as requested, which satisfies the points raised by the AUT University Ethics Committee (AUTEC).

Your ethics application has been approved for three years until 11 March 2016. As part of the ethics approval process, you are required to submit the following to AUTEC:

- A brief annual progress report using form EA2, which is available online through <http://www.aut.ac.nz/researchethics>. When necessary this form may also be used to request an extension of the approval at least one month prior to its expiry on 11 March 2016;
- A brief report on the status of the project using form EA3, which is available online through <http://www.aut.ac.nz/researchethics>. This report is to be submitted either when the approval expires on 11 March 2016 or on completion of the project. It is a condition of approval that AUTEC is notified of any adverse events or if the research does not commence. AUTEC approval needs to be sought for any alteration to the research, including any alteration of or addition to any documents that are provided to participants. You are responsible for ensuring that research undertaken under this approval occurs within the parameters outlined in the approved application. AUTEC grants ethical approval only. If you require management approval from an institution or organisation for your research, then you will need to obtain this. If your research is undertaken within a jurisdiction outside New Zealand, you will need to make the arrangements necessary to meet the legal and ethical requirements that apply there. To enable us to provide you with efficient service, please use the application number and study title in all correspondence with us. If you have any enquiries about this application, or anything else, please do contact us at ethics@aut.ac.nz.

All the very best with your research,

A handwritten signature in dark ink, appearing to read 'Rosemary Godbold'.

Dr Rosemary Godbold Executive Secretary

Auckland University of Technology Ethics Committee

Cc: Weiwei Louise Lu_lolu@aut.ac.nz



12/333

Academic Services
Manager, Academic Committees, Mr Gary Witte

18 December 2012

Dr B Venn
Department of Human Nutrition
Division of Sciences

Dear Dr Venn,

I am again writing to you concerning your proposal entitled "**Effect of rice cooking method on glycaemic response, satiety and chewing tests**", Ethics Committee reference number **12/333**.

Thank you for providing a copy of your documentation showing the amended wording around the payment of \$60 to participants. We confirm the amended wording in the application, the advertisement and consent form are approved.

Your proposal continues to be fully approved by the Human Ethics Committee. If the nature, consent, location, procedures or personnel of your approved application change, please advise me in writing. I hope all goes well for you with your upcoming research.

Yours sincerely,

Mr Gary Witte
Manager, Academic Committees
Tel: 479 8256
Email: gary.witte@otago.ac.nz

c.c. Emeritus Professor L J Holloway Head Department of Human Nutrition

NGĀI TAHU RESEARCH CONSULTATION COMMITTEE
Te KOMITI RAKAHAU KI KAI TAHU

Tuesday, 18 December 2012.

Dr Bernard Venn,
Department of Human Nutrition,
DUNEDIN.

Tēnā Koe Dr Bernard Venn,

Effect of rice cooking method on glycaemic response, satiety and chewing tests

The Ngāi Tahu Research Consultation Committee (The Committee) met on Tuesday, 18 December 2012 to discuss your research proposition.

By way of introduction, this response from The Committee is provided as part of the Memorandum of Understanding between Te Rūnanga o Ngāi Tahu and the University. In the statement of principles of the memorandum it states "Ngāi Tahu acknowledges that the consultation process outline in this policy provides no power of veto by Ngāi Tahu to research undertaken at the University of Otago". As such, this response is not "approval" or "mandate" for the research, rather it is a mandated response from a Ngāi Tahu appointed committee. This process is part of a number of requirements for researchers to undertake and does not cover other issues relating to ethics, including methodology they are separate requirements with other committees, for example the Human Ethics Committee, etc.

Within the context of the Policy for Research Consultation with Māori, the Committee base consultation on that defined by Justice McGechan:

"Consultation does not mean negotiation or agreement. It means: setting out a proposal not fully decided upon; adequately informing a party about relevant information upon which the proposal is based; listening to what the others have to say with an open mind (in that there is room to be persuaded against the proposal); undertaking that task in a genuine and not cosmetic manner. Reaching a decision that may or may not alter the original proposal."

The Committee considers the research to be of interest and importance.

As this study involves human participants, the Committee strongly encourage that ethnicity data be collected as part of the research project. That is the questions on self-identified ethnicity and descent, these questions are contained in the 2006 census.

The Ministry of Health website <http://www.health.govt.nz/publications> contains a list of Māori health publications. The Committee recommends you review the Māori health publications on this website, eg. Unequal Impact II: Māori and Non-Māori Cancer Statistics by Deprivation and Rural-Urban Status 2002-2006 and Tatau Kahukura: Māori Health Chart Book 2010. Another Publication, Hauora: Māori Standards of Health IV (2000-2005), has its own website, <http://www.hauora.maori.nz/>. These publications provide information on a range of Māori health issues and will assist in ensuring your research has an appropriate Māori health focus.

The Committee suggests that rice as a food may become more popular with lower socio-economic groups as it is cheaper and Māori may form a significant part of that group.

The Ngāi Tahu Research Consultation Committee has membership from:

Te Rūnanga o Ōtākou Incorporated
Kāti Huirapa Rūnaka ki Puketeraki
Te Rūnanga o Moeraki

NGĀI TAHU RESEARCH CONSULTATION COMMITTEE
Te KOMITI RAKAHAU KI KAI TAHU

The Committee suggests dissemination of the research findings to Māori health organisations regarding this study.

We wish you every success in your research and The Committee also requests a copy of the research findings.

This letter of suggestion, recommendation and advice is current for an 18 month period from Tuesday, 18 December 2012 to 18 June 2014.

Nāhaku noa, nā



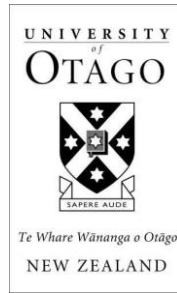
PR NTRC

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*Te Rūnanga o Ōtākou Incorporated
Kāti Huirapa Rūnaka ki Puketeraki
Te Rūnanga o Moeraki*

Appendix 3: Advertisement and Information Sheet for Study “Effect of Rice Cooking Method on Glycaemic Response, Satiety, and Chewing Tests”



HOW DOES BLOOD SUGAR CHANGE WHEN RICE PREPARED IN DIFFERENT WAYS IS EATEN?

Freshly cooked rice may be digested differently to cooked rice that has been stored in the fridge overnight. On three separate occasions we want to measure the rate of change of blood sugar (glucose) after eating one cup of rice:

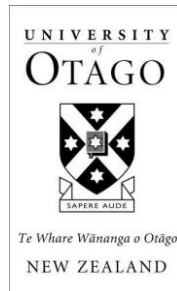
If you are aged between 18 and 45 years, not pregnant, and do not have diabetes, then you are invited to take part in this study. A total of 26 people are needed for this study.

You may want to bring a friend with you.

This project includes three separate visits to Chemistry Lab 1 of about 3 hours each on three days from 6am till 9am.

[Contact Details: Louise Lu, 0212546486 and lolu@aut.ac.nz]

[This project has been reviewed and approved by the University of Otago Human Ethics Committee. Reference: 12/333]



Effect of rice cooking method on glycaemic response, satiety and chewing tests INFORMATION SHEET FOR PARTICIPANTS

Thank you for showing an interest in this project. Please read this information sheet carefully before deciding whether or not to participate. If you decide to participate we thank you. If you decide not to take part there will be no disadvantage to you and we thank you for considering our request.

What is the Aim of the Project?

Freshly cooked rice may be digested differently to cooked rice that has been stored in the fridge overnight. On three separate occasions we want to measure the rate of change of blood sugar (glucose) after eating one cup of either:

- freshly cooked medium grain white rice, or
- freshly cooked parboiled long grain white rice, or
- cooked then stored (fridge for 24 hours) and reheated parboiled long grain white rice.

This project is being undertaken by Weiwei Lu as part of a PhD study looking at ways to prevent blood sugar concentrations becoming too high.

What Type of Participants are being sought?

If you are aged between 18 and 45 years, not pregnant, do not have diabetes, Crohn's or coeliac disease or other digestive problems you are invited to take part in this study. A total of 26 people are needed and you may bring a friend with you.

What will Participants be Asked to Do?

You will visit the Department of Human Nutrition undergraduate laboratories on three separate mornings for 3 hours with a start time of between 6am to 7:30 am and a finish time of 9 am to 10:30 am. On the first visit you will be asked to provide some personal information (contact details, date of birth, ethnicity, sex) and your height and weight will be measured.

The procedure for each visit follows:

- Arrive in the morning (between 6 and 7:30 am) after an overnight fast. This means you stop eating any food or drink (other than water) from 10pm the night before.
- On your arrival your finger will be pricked twice to obtain two separate measurements of blood glucose.
- You will be given one cup (250mL) of cooked rice to eat within 10 minutes. 100mL of water is offered with each cup of cooked rice.
- Remain seated for 2 hours after ingestion.

- We will collect a drop of blood from you by fingerpricking at 15, 30, 45, 60, 90 and 120 minutes. If your blood glucose reading has not come down to baseline by two hours, we would ask you to remain seated and undergo two further blood glucose tests at 150 and 180 minutes.
- Every half hour you will be asked questions as to how hungry you feel
- You can watch TV or read during each three hour visit.
- After the last blood test you will be given a spoonful of rice, asked to chew as usual but instead of swallowing, to spit it out into a container.

It is unlikely that you will experience any discomfort except for the finger prick. At the end of each visit, you will be offered some food and drink.

At all times you may ask questions. Please be aware that you may decide at any time not to take part in the project without any disadvantage to yourself of any kind.

What Data or Information will be Collected and What Use will be Made of it?

Personal information (date of birth, ethnicity, sex) is being collected so that the characteristics of the group can be described. All information will be kept confidential with only the named researchers having access to the data which will be stored under your code number for at least 5 years in locked filing cabinets and password protected computer files. All use of the data in reports, journal articles, conference presentations and thesis will be summary data for the entire group so that you will not be identifiable in any way.

On request and provision of your email address, you will receive a copy of your results as well of that of the overall group.

What if Participants have any Questions?

If you have any questions about our project, either now or in the future, please feel free to contact either:

Dr Bernard Venn
Human Nutrition
University of Otago
Phone 03 479 5068

Professor Elaine Rush
AUT University
Phone: 09 921 9758
Email: elaine.rush@aut.a.nz

Researcher
Weiwei Louise Lu
AUT University, Auckland
Phone 0212546486
Email: lolu@aut.ac.nz

This study has been approved by the University of Otago Human Ethics Committee. If you have any concerns about the ethical conduct of the research you may contact the Committee through the Human Ethics Committee Administrator (ph 03 479 8256). Any issues you raise will be treated in confidence and investigated and you will be informed of the outcome.

Appendix 4: Study Participants Eligibility Questions for Study “Effect of Rice Cooking Method on Glycaemic Response, Satiety, and Chewing Tests”

Thank you for your interest in participating our study, "Effect of rice cooking method on glycaemic response, satiety and chewing tests".

Study aim: Freshly cooked rice may be digested differently to cooked rice that has been stored in the fridge overnight. On three separate occasions we want to measure the rate of change of blood sugar (glucose) after eating one cup of rice.

Please note:

1. Your personal details will be kept securely and will be used for this study only. During the experiment, your identity will be protected and a code number will be used for identification.
2. This study includes three separate visits to the Department of Human Nutrition undergraduate laboratories for 3 hours (between 6am to 7.30am and 9am to 10.30am). At completion of three tests, you will be given \$60 to compensate your time.

Please take a few minutes to answer the following questions.

If you have any further questions or concerns, please contact Cecilia (samcecilia2010@gmail.com) or Louise (lolu@aut.ac.nz).

1. Please tell us about yourself.

Name:

Email Address:

Phone Number:

2. How old are you?

Younger than 18

18 to 25

26 to 30

31 to 35

36 to 40

41 to 45

Older than 45

3. Are you male or female?

Male

Female

4. What is your ethnicity? Mark one or more.

Maori

European

Chinese

Indian

Pacific Islander

Other (please specify)

5. What is your height (cm) and weight (kg)? (Remove shoes before measuring.)

Height (cm)

Weight (kg)

6. Are you pregnant?

Yes

No

I'm a male.

7. Have you ever had gestational diabetes?

Yes

No

I'm a male.

8. Do you have...?

Type 1 diabetes

Type 2 diabetes

Gestational diabetes

Crohn's disease

Coeliac disease

9. Are you taking drugs or dietary supplement that influence carbohydrate and fat metabolism?

Yes

No

10. Are you willing to fast overnight for 10 hours (between 8pm and 6am) on three separate days?

Yes

No

Stay or delete

Appendix 5: Satiety and Palatability Questionnaires for Study “Effect of Rice Cooking Method on Glycaemic Response, Satiety, and Chewing Tests”

Satiety questionnaire

Date: _____ Time: _____

I am not hungry at all	How hungry do you feel?	I have never been more hungry
<hr/>		
I am completely empty	How satisfied do you feel?	I cannot eat another bite
<hr/>		
Not at all full	How full do you feel?	Totally full
<hr/>		
Nothing at all	How much do you think you can eat?	A lot
<hr/>		
Yes, very much	Would you like to eat something sweet?	No, not at all
<hr/>		
Yes, very much	Would you like to eat something salty?	No, not at all
<hr/>		
Yes, very much	Would you like to eat something savoury?	No, not at all
<hr/>		
Yes, very much	Would you like to eat something fatty?	No, not at all
<hr/>		

Palatability questionnaire

Date: _____

Time: _____

Good

Visual appeal

Bad

Good

Smell

Bad

Good

Taste

Bad

Much

Aftertaste

None

Good

Overall palatability

Bad

**Appendix 6 Ethical Approval Forms for Study “Which rice and why?
Consumer preference and acceptability towards freshly cooked medium
grain white rice, freshly cooked parboiled rice, and reheated parboiled rice”**



26 July 2013

Elaine Rush

Faculty of Health and Environmental Sciences

Dear Elaine

Ethics Application: 13/183 Which rice and why?

Thank you for submitting your application for ethical review. I am pleased to advise that the Auckland University of Technology Ethics Committee (AUTE C) approved your ethics application at their meeting on 22 July 2013, subject to the following conditions:

1. Clarification as to whether this research constitutes part of PhD qualification;
2. Revision of the response to section B.10 and B.12 of the application giving more details on the background and to clearly explain the methodological approach being used. AUTE C also noted there was no hypothesis;
3. Reflection and revision of the response to section B.11 of the application to reflect the response given on the Information Sheet. AUTE C suggests the potential benefits to the general public are overstated on the application;

4. Clarification of the reference to survey monkey in section C.3.4 of the application;
5. Provision of revised response to section E.2, E.2.1 and E.2.2 of the application and inclusion of evidence about any consultation that has occurred;
6. Reconsideration of the response to section J.1 of the application regarding feedback. AUTECH suggests posting the report online and providing a url link rather than participants having to ask for the findings;
7. Amendment of the advertisement to advise potential participants ‘not to participate’ if they have an allergy to starch;
8. Amendment of the Information Sheet as follows:
 - a. Checking of the grammar
 - b. Advice that the data will be used for further studies as stated in section H.2 of the application;
 - c. Inclusion of advice of point 6 above;
 - d. Provision of the current Executive Secretary’s contact details as given on the exemplar on the website;
9. Amendment of the 8th bullet point on the Consent Form to reflect point 6 above.

AUTECH noted that the applicant had advised AUTECH that the responses to all the sections were very similar to another application. However, the questions in the application should be responded to specifically in relation to the research which is the focus of the individual application.

AUTECH also considered that the literature referenced needed expansion.

Please provide me with a response to the points raised in these conditions, indicating either how you have satisfied these points or proposing an alternative approach.

AUTEC also requires copies of any altered documents, such as Information Sheets, surveys etc. Once your response is received and confirmed as satisfying the Committee's points, you will be notified of the full approval of your ethics application. Full approval is not effective until all the conditions have been met. Data collection may not commence until full approval has been confirmed. If these conditions are not met within six months, your application may be closed and a new application will be required if you wish to continue with this research.

To enable us to provide you with efficient service, we ask that you use the application number and study title in all correspondence with us. If you have any enquiries about this application, or anything else, please do contact us at ethics@aut.ac.nz.

I look forward to hearing from you,

Yours sincerely

A handwritten signature in black ink, appearing to read 'K O'Connor', written in a cursive style.

Kate O'Connor

Executive Secretary

Auckland University of Technology Ethics Committee

Cc: Louise Weiwei Lu lolu@aut.ac.nz, Nazimah Hamid

Appendix 7: Advertisement and Information Sheet for Study “Which rice and why?”

How do I agree to participate in this research?

You will need to contact the researcher if you wish to participate. This information sheet will be explained further, you can ask any questions, and, if you agree to participate, a mutually convenient appointment will be arranged.

Researcher Contact Details:

Louise Lu
Phone: 0212546486
Email: lolu@aut.ac.nz

*This study has received ethical approval
from the Auckland University of
Technology Ethics Committee,
Reference number-----*

September 2013

Who to contact if you have concerns about this research?

If you have any questions about this project, either now or in the future, please feel free to contact the following:

Any concerns regarding the nature of this project should be notified in the first instance to the Project Supervisor:

Professor Elaine Rush
Phone: 921 9758
Email: elaine.rush@aut.ac.nz

Concerns regarding the conduct of the research should be notified to the Executive Secretary, AUTEC:

Kate O'Connor

Room WA505E, Level 5, WA Building
55 Wellesley Street East
Private Bag 92006
Auckland 1010
Phone +64 9 921 9999 extn: 6038

Researcher Contact Details:

Louise Lu
Phone: 0212546486
Email: lolu@aut.ac.nz

Information Sheet

Which rice?



Why?



If you want further information about this research, please contact Professor Elaine Rush and the Louise Lu

An Invitation

You are invited to participate in a sensory trial to compare the characteristics of cooked rice products and to say which ones you would eat and why. I, Louise Lu, want to know this as part of research for my PhD.

If you are 18 years or older, eat rice at least once a week and have no known allergy to rice starch, you are the right participant to take part in this trial.

Your participation is highly valued and is entirely voluntary. You may withdraw at any time without any adverse consequences.

Why are we doing this?

The food market has been changing and the demand for healthier, high quality foods associated with disease prevention and health promotion has increased. This study is designed to understand better why customers prefer some cooked plain rice products compared with others.

What will happen in this research?

- You will be asked to fill out a Survey Monkey screening questionnaire asking about your contact details, availability, age, gender, ethnicity, and rice consumption.
- You will visit the sensory lab at AUT city campus or at Akoranga once at around 11 am in the morning, at least two hours after breakfast. You will be required to have no food or drinks except water for one hour before the test.
- Food patterns (if there is anything you do not eat) will be filled out by you on the first visit.
- You will be presented with samples of six cooked rice samples and asked to describe your liking of the appearance (colour), flavour, texture, sweetness and overall liking. You will rinse your mouth with water between tasting rice samples.
- You will be asked not to discuss your ratings with others.

What are the discomforts and risks?

There are no known discomforts or risks. This will take around one hour of your time.

What are the benefits?

The information that you provide will help understanding of the liking preferences of rice prepared in different ways.

What will happen to the results?

Your results will be kept confidential to the researchers who will be the only ones to have access to your data. All use of data in reports, journal articles, and conference papers and to inform future research will be summary data so that you will not be identified in any way.

You will be emailed a copy of the grouped results with an explanation at the completion of the research.

If you want further information about this research, please contact Professor Elaine Rush and the Louise Lu

Appendix 8: Study Participants Screening Questions for Study “Which Rice and Why?”

Survey Monkey Screening Questions for “Which Rice and Why?”

Thank you for considering participating in “Which Rice and Why?” and reading this information sheet (download). If you would like to accept the invitation to participate we would appreciate you taking the time to complete the following screening questionnaire. It should take about five minutes. Your responses are voluntary and will be confidential. Responses will not be identified by individual. Please provide the following details and you will be contacted by Louise Lu to discuss further and to make an appointment.

1. :
 - a. First name
 - b. Last name
 - c. Email address.
 - d. Contact phone number (cell phone preferred)
 - e. Preferred contact : email or text or phone
 - f. Available dates (dd/mm/2013)
 - g. Available times in morning at least two hours after eating (hh:mm to hh:mm)
2. What is your age?
3. What is your gender?
4. Which ethnic group(s) do you identify with?
5. How often do you eat cooked rice products (e.g. steamed rice, boiled rice, fried rice)?
6. How is the rice product prepared normally (e.g. steamed, boiled, fried, microwaved, instant)?

<https://www.surveymonkey.com/s/G6RKLSM>

Appendix 9: Consumer Testing Questionnaire for Study “Which Rice and Why?”

ID# _____

Date ____ / ____ / 2013

Consumer Testing Which rice and why?

Please fill the shadow area ☺

Please state your age _____ yrs

Ethnicity _____

Dietary pattern (if there is anything you do not eat)?

How often do you consume cooked rice?

- ☐ Occasionally
- ☐ Once per month
- ☐ Once per week
- ☐ Several times per week
- ☐ Once per day
- ☐ Several times per day

Are you (please tick)

- ☐ Male
- ☐ Female

Do you have any allergies associated with starch ☐ Yes ☐ No

Anything else please name _____

* If you have any allergies associated with cooked rice please do not proceed with this test

Instructions: There are 6 questionnaires provided – one for each cooked rice which is identified by a number on the plate and on the questionnaire. .

Consider each cooked rice sample one at a time.

Before you taste each cooked rice sample please rinse your mouth out with water before tasting.

ID# _____

Date / / 2013

Record your rice sample number here _____

Rinse your mouth out with water first.

For your liking of this cooked rice sample please put a | mark on the line scale below to show how much you like each characteristics

Please taste the rice sample and score how much do you like or dislike the product overall.

Extremely Dislike

Extremely Like

Colour



Texture

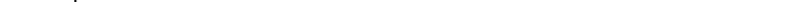
Extremely Dislike

Extremely Like

Extremely Dislike

Extremely Like

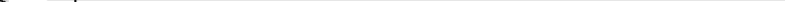
Flavour



Extremely Dislike

Extremely Like

Sweetness

[illegible]

Will you eat this rice regularly?

☐ Yes☐ No

Do you have any additional comments? (Optional)

Please rinse your mouth and go on taste the next spoon.