Digestion characteristics of grains and flours for breads of low glycaemic potency

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Abstract

In a survey of supermarket breads containing grain particles in New Zealand it was evident that grain structure reduced the starch digestibility of the breads, but the effect was small unless the inclusion rate of the large grain particles (>1 mm) was high (65% or more). The factors that influenced starch digestibility and glycaemic potency of grain particles of various sizes were studied in this research. It was shown that relatively large particle size and incomplete gelatinization of starch in the grains studied make them more resistant to digestion than others. From the knowledge gained in preliminary research, a range of kibbled (large grain particles) grain breads was developed containing 75% of large (> 2.8 mm) grain particles. The digestibility of starch in the prototype kibbled grain breads was examined *in-vitro* by simulated small intestinal digestion either with the grains intact or after homogenizing the breads. The results showed that the glycaemic potency of kibbled grains bread was low in comparison to white breads provided that the grain particles remained intact. However, when the kibbled grain breads were completely homogenized to eliminate grain structure the estimated glycaemic index (GI) increased by 31% and was similar to that calculated for white bread. An *in-vivo* study was conducted to compare the *in-vitro* estimates of glycaemic potency with the *in-vivo* findings and showed that the results from the *in-vivo and in-vitro* studies were highly correlated. This showed that endosperm structure played a significant role in reducing the glycaemic potency of the breads but only when the grain particles were large (>2.8 mm) and when they were swallowed intact. This suggested that the glycaemic potency of breads cannot be significantly reduced by adding high proportions of large grain particles as chewing reduces particle size and destroys much of the endosperm structure that would restrict digestion of the unchewed grain particles. Hence, increasing the proportion of large grain particles in bread is, in itself, ineffective in reducing the rate of starch digestion and consequently, blood glucose response *in-vivo*. However, in all cases, it was found that adding large grain particles to breads made in this work or sourced commercially increased the proportion of non-starchy ingredients (mostly insoluble and soluble fibre) so reducing the proportion of available starch for digestion, in the bread.

The *in-vitro* results showed that reducing the proportion of available starch in the bread, either by increasing the proportion of whole-grain particles or by increasing the proportion of fibre or protein added to the breads is an effective strategy to reduce the glycaemic potency of breads. As expected from the earlier experiments, the glycaemic potency of the carbohydrates

(GI) present in these breads was similar to that of a standard commercial white bread. Therefore, reducing the proportion of carbohydrate in bread formulations by increasing the proportion of protein and/or fibre is an effective strategy for reducing glycaemic potency of breads even though the glycaemic index, which shows the relative glycaemic potency of the available carbohydrate portion of the bread, is unchanged.

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List of Abbreviations

AUC	Area under the curve
CHO	Available carbohydrates
C_i	Proportion of starch digested in i^{th} particle
$C\infty$	Total proportion of starch digested
D	Diameter
d.w.b	Dry weight basis
DNS	Dinitrosalicylic acid
DSC	Differential scanning calorimetry
g	Gram
GD	Glucose disposal
GE	Glucose equivalents
GGE	Glycaemic glucose equivalents
GI	Glycaemic index
GL	Glycaemic load
h	Hours
H/h*	Homogenized
HCl	Hydrochloric acid
I	Intact
iAUC	Incremental area under the curve
k_i	Fractional-digestion rate coefficient
LOS	Logarithm of Slope
min	Minutes
ml	Millilitre
mm	Millimetre
mmol/L	Millimoles per litre
NSP	Non-starch polysaccharides
NZ	New Zealand
RDS	Rapidly digested starch
RH	Relative humidity
rpm	Rotation per minute
RS	Resistant starch

RVA	Rapid Visco Analyser
SD	Standard deviation
SDS	Slowly digested starch
sec	Seconds
SEM	Standard error of mean
U	Units of enzymes
V	Volume
XRD	X-ray Diffraction

List of Publications

Srv, A., Mishra, S., Hardacre, A., Matia-Merino, L., Goh, K., Warren, F., & Monro, J. (2019). Kernel structure in breads reduces in vitro starch digestion rate and estimated glycaemic potency only at high grain inclusion rates. *Food Structure, 21*, 100109. doi: https://doi.org/10.1016/j.foostr.2019.100109.

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Oral presentation- Structural properties of grains and flours for breads of low glycaemic potency - Riddet Institute Student Colloquium, Auckland 5th November 2017.

Poster presentation- The role of grain structure in the glycaemic potency of New Zealand breads was presented at the 7th International Symposium on Delivery of Functionality (DOF), Auckland – 5-8 November 2017.

Oral presentation - Structural properties of milled grains and flours for cereal products of low glycaemic potency – Baking Industry Research Trust- The New Zealand Institute for Plant & Food Research Limited, Annual Research Meeting, Lincoln – 22nd February 2018.

Oral presentation - Can the structure be exploited to modulate digestibility of starch in whole grain breads? Riddet Institute Conference, Wellington, 10- 12 July 2018.

Oral presentation – Human intervention study of kibbled grain breads – Baking Industry Research Trust- The New Zealand Institute for Plant & Food Research Limited, Annual Research Meeting, Auckland – 23rd May 2019.

Poster presentation- The effects of chewing on kibbled-grain breads- An in vivo study was presented at the 8th International Symposium on Delivery of Functionality (DOF), Portugal – 9-11 July 2019.

Glossary

Glycaemic response - Glycaemic response refers to the effect that a food has on postprandial blood glucose concentration.

Glycaemic potency - Glycaemic potency is the tendency of a 100 g of food consumed in a single serving, to induce a postprandial glycaemic response in comparison to 100 g of glucose, expressed as glycaemic glucose equivalents (GGE/ 100 g of food) in this thesis.

The glycaemic index (GI) - GI is the postprandial effect of the available carbohydrate component of a food on blood glucose concentration expressed as a percentage of the effect of glucose equal in amount to the available carbohydrate in the food. It may be expressed as GGE/100 g of carbohydrates and is calculated as such in this thesis based on *in-vitro* determinations of GGE.

Rapidly digested starch (RDS) - RDS is characterised as starch that can be hydrolyzed within 20 min of the start of a defined *in-vitro* pancreatic digestion, and it is of major interest in this thesis. It is highly digestible and hence highly glycaemic starch and can correlate with postprandial blood glucose response.

Actually available carbohydrates - Actually available carbohydrates content of the food were measured as reducing sugar released into the digestion medium at 120 min after adding the digestive enzymes during *in-vitro* amylolysis.

Potentially available carbohydrates - Potentially available carbohydrates of the food were measured as glucose released after one hour of incubating the homogenized samples of 120 min digesta with the addition of amyloglucosidase, to access any remaining digestible carbohydrate.

Wholegrain/ Intact/ Kibbled grains - In the study, these words refer to products that are identifiable by the presence of intact or partially intact grain particles that retains complete or partially complete kernel structure. Terms such as kibbled grains and large grains are interchangeable.

Chapter 1

Chapter 1: Introduction

Chronic diseases such as cardiovascular disease, diabetes, obesity, and stroke are currently responsible for about 60% of disease-related deaths worldwide (WHO, 2003). Since diet can be causally linked with physical and mental health, there is an increasing interest in using diet to reduce the risk of chronic diseases (Cheraskin, Ringsdorf Jr, & Clark, 1995; Mera, 1994). Additionally, the market for foods claiming to prevent disease is increasing rapidly. A great deal of research has been performed on the relationship between the quantity and quality of carbohydrates in the diet which indicated that eating carbohydrates of low glycaemic potency, that is carbohydrates that have a relatively small effect on blood glucose concentrations, and are usually digested relatively slowly, increase satiety and reduce hunger by maintaining stable blood glucose levels (Brand-Miller, Dickinson, Barclay, & Celermajer, 2007; Brand-Miller, Hayne, Petocz, & Colagiuri, 2003; Reynolds, Stockmann, Atkinson, Denyer, & Brand-Miller, 2009; Wilde, 2009). The inclusion of whole-grains and whole-grain-based products into foods has been suggested as a method of achieving this (Borneo & Leon, 2012; Jacobs Jr, Pereira, Slavin, & Marquart, 2000) as the grain particles of increased size are digested more slowly hence lowering of glycaemic response to those foods (Liljeberg, Granfeldt, & Björck, 1994). In this thesis, kibbled grains refer to intact or partially intact grain particles that retain complete or partially complete kernel structure. Terms such as kibbled grains and large grains are interchangeable.

Breads contain a high proportion of finely milled flour, and when cooked fully, the contained starch is almost fully gelatinised. As a consequence, they have a GI approaching 70 GGE/ 100 g of carbohydrates (white and wholemeal breads). One strategy used to reduce the glycaemic potency of breads has been to use large grain particles by substituting a proportion of the flour with coarse or kibbled grains. Many of the “whole grain” breads sold in New Zealand and for that matter elsewhere in the world contain grain particles that create an impression of wholesomeness and are often assumed to have low glycaemic potency. Such breads are often considered, and in advertising are claimed to be ‘healthy’ and in some cases to have a low glycaemic potency (Eckelkamp, 2016; Shoemaker, 2015). These wholemeal or whole-grain breads differ widely in texture and the proportion of large (>1 mm) grain particles they contain. However, the role and contribution of kibbled grains in reducing the glycaemic potency of breads are not well documented. Also, the path to making low glycaemic breads is not clear despite marketing claims to date. Thus, to address this knowledge gap, this thesis aimed at

answering the research question "What is the contribution of kibbled grain particles in reducing the glycaemic potency of breads?" Test breads were compared to standard commercial or test white breads using a standardised simulated *in-vitro* method that simulates gastric and small intestinal digestion (Monro & Mishra, 2010). Other specific topics addressed within this framework are listed below.

Chapter 1 is an overview of the thesis, along with the research questions addressed and the hypotheses tested. **Chapter 2** focuses on the review of the literature and the purpose of the study. General experimental techniques are explained in **Chapter 3** in the thesis.

1.1 Research questions and hypotheses.

Chapter 4

Research question

1. Do New Zealand breads have enough structure to significantly reduce starch digestibility and glycaemic potency compared with the reference white bread?

Hypothesis

1. New Zealand breads do not contain enough kibbled grain structure to reduce glycaemic potency of the breads substantially.

Chapter 5

Research question

1. For a given size of cooked grain particles, how do different cereals differ in the rate and yield of glucose during *in-vitro* digestion?
2. Do factors such as plant structure and gelatinization govern the glycaemic potency in cooked grains as a function of particle size?
3. Does plant structure alone reduce the glycaemic potency of milled grains when completely gelatinized?

Hypotheses

1. Cooked grains differ in digestibility as a function of particle size.
2. Kernel structure and degree of gelatinization both have a substantial effect on influencing the glycaemic potency of milled-cooked grains.

3. Plant structure of kibbled grains can reduce starch digestibility, even when the grains are completely gelatinized.

Chapter 6

Research question

1. At a given temperature of hydration, how do different types of grain differ in the rate and yield of glucose during *in-vitro* digestion?
2. Can limiting hydration during soaking and cooking, reduce the rate and yield of glucose during *in-vitro* digestion?

Hypotheses

1. Grains differ in digestibility as a function of hydrating temperature during cooking.
2. The rate and yield of glucose can be reduced by limiting hydration during *in-vitro* digestion.

Chapter 7

Research questions

1. Do starch digestibility and glycaemic potency differ for breads made with different particles sizes of grain?
2. What is the role of the proportion of larger grain particles in the bread matrix in determining the glycaemic potency of the bread?
3. Can prototype low glycaemic breads be developed by increasing the proportion of larger grain particles?

Hypotheses

1. Breads made with larger grain particles have lower glycaemic potency than breads made with smaller particles.
2. Greater proportions of larger grain particles in a bread matrix reduce glycaemic potency.
3. Prototype low glycaemic breads can be developed by formulating with high proportions (>60%) of larger grain particles.

Chapter 8

Research questions

1. Is the potential effect of large grain particles in the prototype breads on glycaemic response maintained following normal ingestion processes including chewing and still be able to lower the glycaemic potency of the breads *in-vivo*?

Hypotheses

1. During normal ingestion, sufficient large grain particles survive chewing to reduce the glycaemic response of the bread.

Chapter 9

Research questions

1. What is the role of addition of non-flour components, such as proteins, fibres and non-starch polysaccharides (NSP) in determining the glycaemic potency of the bread matrix?

Hypotheses

1. Substituting carbohydrates with proteins, fibres and NSP can reduce the glycaemic potency of breads.

These questions were explored and answered using *in-vitro* and *in-vivo* techniques.

Chapter 2

Chapter 2: Literature review

2.1 Introduction

It is estimated that chronic diseases including cardiovascular disease, diabetes and obesity will contribute to about 73% global deaths annually by 2020 (WHO, 2014). Nutritionally unbalanced diets, physical inactivity and environmental changes have all been implicated in chronic diseases (Bauer, Briss, Goodman, & Bowman, 2014) and with balanced nutrition, the risk of these diseases may be reduced (WHO, 2003). Carbohydrate is the major source of energy, but in excess, it can lead to postprandial glycaemia and may eventually lead to chronic glycemia. Reducing the proportion and digestibility of highly digestible carbohydrates such as starch in the diet may reduce glycaemia and therefore contribute to the development of nutritionally improved, healthier foods.

This literature review considers starch related to diet, and factors associated with the digestibility of starchy foods. The literature reviewed was sourced from databases including, Web of Science, Google Scholar and Science Direct. Literature was searched across various disciplines and was published after 1985 and included academic papers and books.

2.2 Carbohydrates

Carbohydrate is a general term applied to a very large range of macromolecules with the general formula $(C_6H_{12}O_6)_n$ for hexoses, but pentoses $(C_5H_{10}O_5)_n$ are also common. Saccharides range from the simplest monosaccharides, where the number of bound sugar residues (n) is n =1 (glucose, fructose) to sucrose n = 2 (Glucose-fructose) but may range to millions (starch, gums, cellulose). Simple sugars (n = 1) can be absorbed through the gut wall, but all larger polymers must be digested to monosaccharides using a number of enzyme systems prior to absorption. As the value for n increases, the carbohydrates tend to be less soluble and are digested more slowly or not at all in the small intestine, depending on their composition and structure, although most can be fermented either completely or partially in the large intestine (Brennan, 2005).

Starch is an exception among the large complex polysaccharides because it consists only of glucose and when gelatinized is rapidly hydrolyzed to glucose in the small intestine, which is in turn rapidly absorbed so increasing blood glucose levels. Sugars and starches are found in

most plant material and may be present at high levels in storage structures such as fruits and grains. They are less abundant in animal tissues and products where glucose and glycogen, sometimes occur although milk contains appreciable quantities of lactose. Starches are mostly consumed in a form where some of the original structure of the plant tissue from which it was derived remains, e.g. flour. When the plant structures remain relatively intact the remaining cell walls may reduce the digestibility of the contained starch by restricting access by digestive enzymes (Edwards et al., 2015; Grundy, Edwards, et al., 2016; Grundy, Lapsley, & Ellis, 2016; Lemmens, Van Buggenhout, Van Loey, & Hendrickx, 2010; Tydeman et al., 2010). Accessibility by enzymes to the starch contained in plant tissues depends on several factors (Palou, Bonet, & Pico, 2009) that include processing conditions and the origin and degree of gelatinization of the starch (Lehmann & Robin, 2007). These factors which will be discussed in greater detail later in this chapter.

2.3 Carbohydrate metabolism and glucose regulation

Carbohydrate metabolism begins with digestion in the small intestine where monosaccharides are absorbed into the blood stream. The metabolic process called glycoysis begins if the glucose is needed immediately upon entering the cells to supply energy, the end products of glycolysis are pyruvic acid and ATP. Since glycolysis releases relatively little ATP, further reactions continue to convert pyruvic acid to acetyl CoA and then to citric acid in the citric acid cycle. The majority of the ATP is made from oxidations in the citric acid cycle in connection with the electron transport chain. During strenuous muscular activity, pyruvic acid is converted into lactic acid rather thatn acetyl CoA and during rest, the lactic acid is converted back to pyruvic acid. The pyruvic acid is in turn is converted back to glucose by the process called gluconeogenesis. If the glucose is not needed at that moment, glucose is converted into glycogen by glycogenesis and is stored in muscles (Maughan, 2013). Sugars absorbed in the gut may therefore be either used immediately, or stored in muscles as glycogen or elsewhere in the body.

Glucose regulation or glucose homeostasis is the process by which blood glucose levels are maintained within a narrow range. This is achieved by three hormones: insulin, glucagon, and epinephrine. If the concentration of glucose in the blood is above about 7.8mmol/L, insulin is secreted by the pancreas. Insulin stimulates the transfer of glucose into the cells, especially those of the liver and muscles, although all other organs are also able to store and metabolize

glucose. In the liver and muscles, most of the glucose is changed into glycogen by the process of glycogenesis and is stored until needed later when glucose levels are low. If blood glucose levels are below about 4 mmol/L, then eqinephrine and glucogon hormones are secreted to stimulate the conversion of glycogen to glucose. This process is called glycogenolysis (Aronoff, Berkowitz, Shreiner, & Want, 2004).

2.4 Measuring the impact of carbohydrates on blood glucose response

The definitions of Glycaemic Potency, Glucose Glycaemic Equivalents, Glycaemic Index, and Glycaemic Load used in this work are all as for Monro and Shaw (2008). And as stated by these authors they are "based on the currently accepted measurement of glycaemic response as the incremental area under the blood glucose response curve (IAUC) for 2–3 h after consuming food, (but) they each express a different aspect of glycaemic potency:

Briefly: The change in blood glucose after food intake is called the glycaemic response.

Glycaemic potency is the glycaemic response elicited by 100 g of a carbohydrate-containing food in comparison with the response elicited by an amount of glucose equal in weight to the whole food. It is usually derived from the area under the plot of blood sugar with time for 2 h after consuming a serving of the food in comparison with the response elicited by a known weight of glucose, or other carbohydrate reference material of known glycaemic potency, such as white bread which is frequently used in this thesis.

The glycaemic response resulting from the consumption of a given food can then be characterised in terms of glycaemic glucose equivalents (GGEs) in terms of an equivalent amount of glucose consumed (Monro & Shaw, 2008). Diets, physical activity and environmental changes have all been implicated in affecting the reading of blood sugar response (Bauer et al., 2014).

The glycaemic index (GI) is the blood glucose response to a portion of food containing 50 g of available carbohydrate expressed as a percentage of the response to 50 g of glucose. It is a ratio that does not take into account the quantity of carbohydrate/glucose consumed and is expressed as GGE/100 g of carbohydrates present in a food (**Figure 2.1**). Glycaemic load (GL) is calculated from GI, and it takes into account both the quantity and the digestibility of the CHO in the meal in a meal (GL) = (GI*total CHO in the food) (Barclay et al., 2008; Brand-Miller, 2003; Monro & Shaw, 2008). Though GL takes into account the carbohydrate consumed in the

meal, it is not the same as glycaemic potency although it approximates glycaemic potency (GGE/weight of food) as it is an indirect calculation based on GI, which is experimentally determined from blood glucose concentration after consuming 50g of glucose, rather than following the consumption of a more realistic food serving. Glycaemic potency is GGE per weight of the food. Thus, GGE can be used to establish glycaemic potency as a food property (GGE/100 g), as it is based on food composition and its effect on blood glucose.

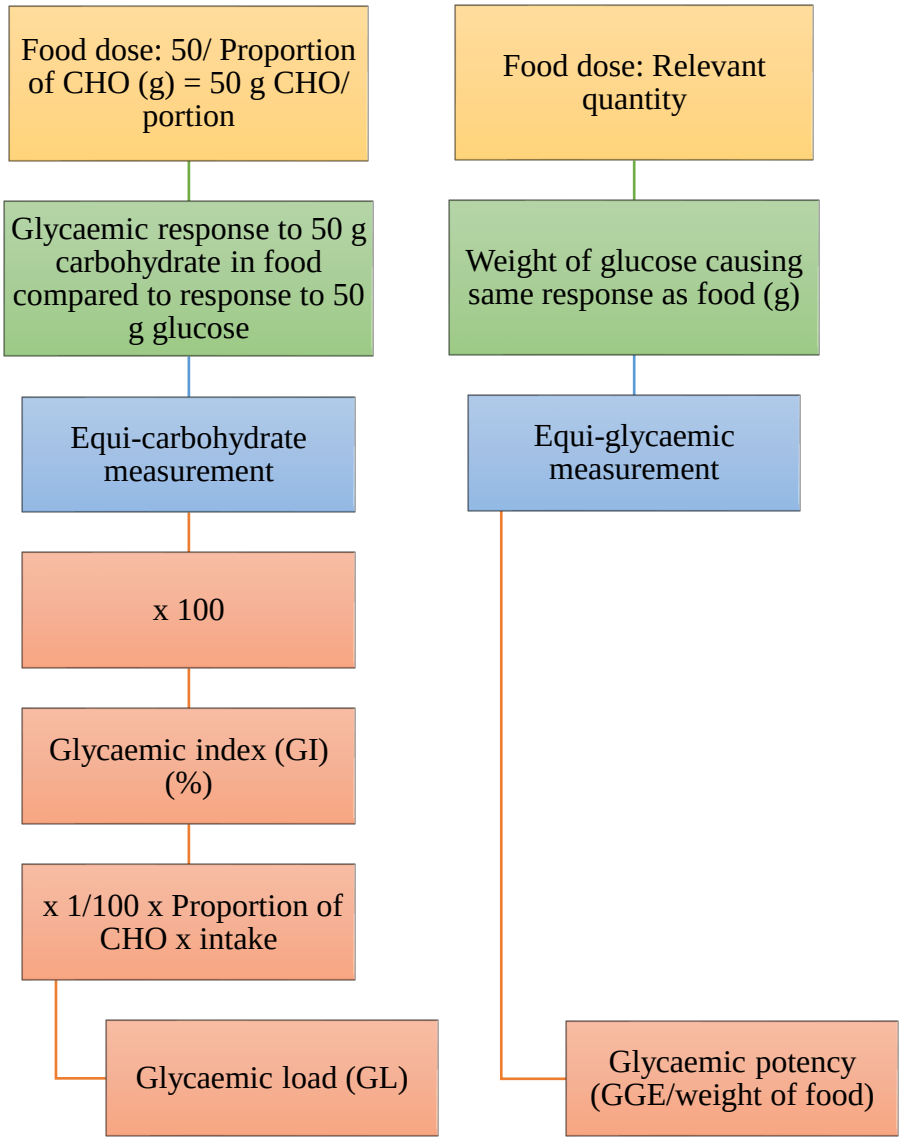


Figure 2.1 Difference between GI, GL and glycaemic potency of a food. CHO is potentially available carbohydrate. It is adapted from (Monro & Shaw, 2008).

The factors that influence the glycaemic response to a food are the type of CHO in the food, its rate of digestion, its availability for digestion (affected by food structure) or the effects of other macromolecules such as fat, NSP and protein, all of these "food factors" are highly mediated by physiological differences in the test subjects (Moghaddam, Vogt, & Wolever,

2006; Wolever & Jenkins, 1986; Wolever & Bolognesi, 1996). Large differences exist in the degree to which different starch-containing foods affect blood glucose, and these differences have been shown to relate to the digestibility of the starch (Thorne, Thompson, & Jenkins, 1983). All of which will be discussed in more detail in the following sections.

2.5 Whole-grains and their use in foods

Grain particles derived from cereals and pseudo-cereals (legumes) can be either intact whole-grains or milled to some degree to make large kibbled particles, or ground to coarse or fine flours (Fardet, 2010). The grain particles contain carbohydrates that are mainly starch (70%-80 %), minerals, lipids (2-9%), and proteins (7-15%) (Grains & Legumes Nutrition Council, 2012). However, legume seeds may contain up to 35% protein and lower proportions of starch.

Cereal grains are an important source of food in the human diet (Hawkins & Johnson, 2005; Rehman & Shah, 2005) and contain essential nutrients including, vitamins, minerals and dietary fibres (Anderson, 2003). The grains are composed of the endosperm, germ, and bran. The outer most layer of the cereals is the fibre-rich bran also containing vitamins, minerals and other phytochemicals. The endosperm comprises the bulk of the grain and serves to nourish the germinating seedling, it is non-living tissue in the cereals but living and dormant in legumes. The germ is the living tissue that will develop into the new plant when germination occurs.

The structure of cereal grains makes them resistant to digestion due to the presence of cell walls, dense structures that resist hydration and limit the diffusion of digestive enzymes to sites of activity (Edwards et al., 2015; McDougall, Morrison, Stewart, & Hillman, 1996). This is discussed in detail in the later sections of the literature review. According to Livesey et al. (1995), starch granules differ in shape and size and in the ratio of amylose to amylopectin with plant species. The endosperm of some cereals is hard and permeability low due to the dense packing of starch and protein in the endosperm. Digestive enzymes can act on the surface of endosperm fragments, but penetration into the dense particles is slow so that the particles may be digested slowly or in some cases remain largely undigested in the small intestine and passes into the colon where the present bacteria ferments it. Processing such as grinding or cooking the grains facilitates digestion (Mishra, Hardacre, & Monro, 2012).

In legumes, starch is present inside the living cotyledon cells, which are bounded by robust cell walls consisting of cellulose, hemicelluloses and pectin (Berg, Singh, Hardacre, & Boland, 2012). The non-cellulosic compounds present in the legume cell walls are responsible for the integrity and the stability of the cell walls as they act as an adhesive and hold the microfibrils of cellulose together (Carpita & Gibeaut, 1993). Starch granules situated in the cells are surrounded by protein bodies which further restricts swelling during hydration or gelatinization during cooking (Berg et al., 2012) so reducing rates of digestion (Grewal & Jood, 2009) thereby reducing the glycaemic potency of foods containing high proportions of relatively intact legume flour (Mishra et al., 2012).

Increasing the particle size of kibbled grains has been associated with lowering of glycaemic responses in some studies (Irika binti Idril, Diana, & Firmansyah Wargahadibrata, 2013; Jenkins et al., 1981; Liljeberg, Granfeldt, & Bjork, 1992), which is one of the possible benefits of consuming whole-grain products (Jacobs, Pereira, Slavin, & Marquart, 2000). Additionally, whole-grain provides a full spectrum of cereal nutrients that are partially lost by refining to white flour (Pedersen, Krudsen, & Eggum, 1989). As a result, whole-grain products have been widely and justifiably promoted for their potentially low glycaemic impact and their nutritional attributes (Richardson, 2000; Slavin, 2004).

Whole-grains are widely consumed in the form of bread, pasta, flour, breakfast cereals, porridges, soups, cakes or on its own. According to a key finding from the 2008/09 New Zealand Adult Nutrition Survey conducted by University of Otago and The New Zealand Ministry of Health (2011), whole grain-based foods such as breads are an important contributor to dietary energy intake (11%), protein intake (11%), fat intake (bread-based dishes- 6%), dietary fibre intake (17%) and contributed 17% of the carbohydrate intake. White breads contain 78-80% of starch, which is rapidly digested and may lead to a high postprandial glycaemic response. However, in comparison to white breads, starch from breads containing kibbled grain particles may be digested slowly leading to a low to moderate postprandial glycaemic responses due to slower rates of starch digestion (Fardet, Leenhardt, Lioger, Scalbert, & Remesy, 2006) but often, as will be discussed in this research, starch in breads made with kibbled grains are digested at rates similar to those of white bread.

2.6 Starch

Starch is the major carbohydrate storage material in higher plants. It is synthesised from glucose produced during photosynthesis by enzymatic condensation. It is temporarily stored in the leaf chloroplasts and but rapidly exported in the form of sugars and recondensed to starch organised in granules in **Figure 2.2 (A) and (B).** Starch in the amyloplasts of seeds, roots and tubers (Wang, Bogracheva, & Hedley, 1998). Starch is insoluble in cold water and consists of D-glucopyranose polymers linked by α-1,4 and α-1,6 glycosidic bonds. The α linkage is determined by the arrangement of the hydroxyl (-OH) group on C1 of the pyranose ring, bond angles form the starch molecules as a helical structure (Thomas & Atwell, 1999).

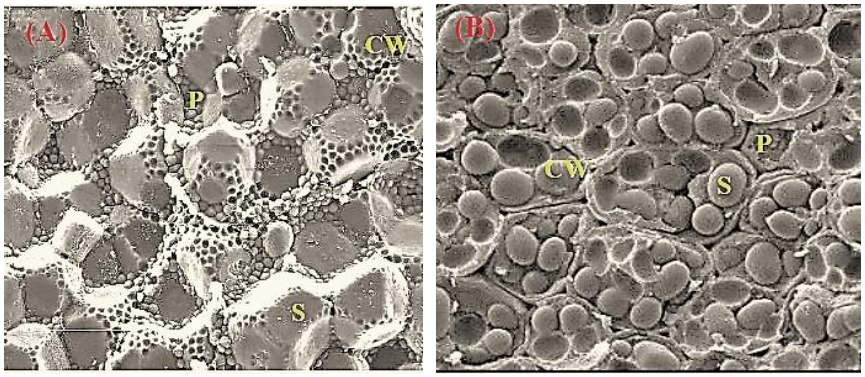


Figure 2.2 An electron micrograph of starch granules in (A) the endosperm of sorghum showing the protein matrix with embedded protein bodies surrounding each granule (Black, 2001). (B) Sections of the cotyledon cell of raw navy beans (Berg et al., 2012). CW- cell wall, P- protein and S – starch.

2.6.1 Molecular structure of starch

In starch, glucose polymerization results in two types of polymers, amylose and amylopectin. Unbranched linear chains with 500-600 glucose residues linked by α-1,4 glycosidic bonds are called amylose while branched starch polymers that contain a proportion of α-1,6 glycosidic bonds have DP's in the millions and are called amylopectin. The molecular weight of amylose is less than 10^4 Dalton while that of amylopectin is much greater at 10^7 -10^9 Dalton (Hoover, 2001). Studies of starch structure using debranching enzymes to hydrolyse the α-1,6 glycosidic bonds (e.g. pullulanase) and using isoamylase to hydrolyse α-1,4 glycosidic bonds followed by size exclusion chromatography have shown that amylopectin contains branched chains of which the frequency and length of the branches vary with the botanical source (Wang et al., 1998).

Amylose is primarily a linear chain-linked mainly by α-1,4 glycosidic bonds with occasional α-1,6 linkages that result in short branches (**Figure 2.3)**. It exists in three forms: a disordered

amorphous conformation or as two helical forms. In the disordered conformation, amylose forms a polymer with a hydrodynamic radius of 7-22 nm. However, it is generally chiral forming a single left-handed helix or parallel left-handed double helix (Imberty, Chanzy, Pérez, Buléon, & Tran, 1988). Single helical amylose contains hydrogen bonding atoms (O2 and O6) on the outer surface of the helix with the ring oxygen pointing inwards, the centre of the helix tends to be hydrophobic and can sequester fats and other hydrophobic moieties.

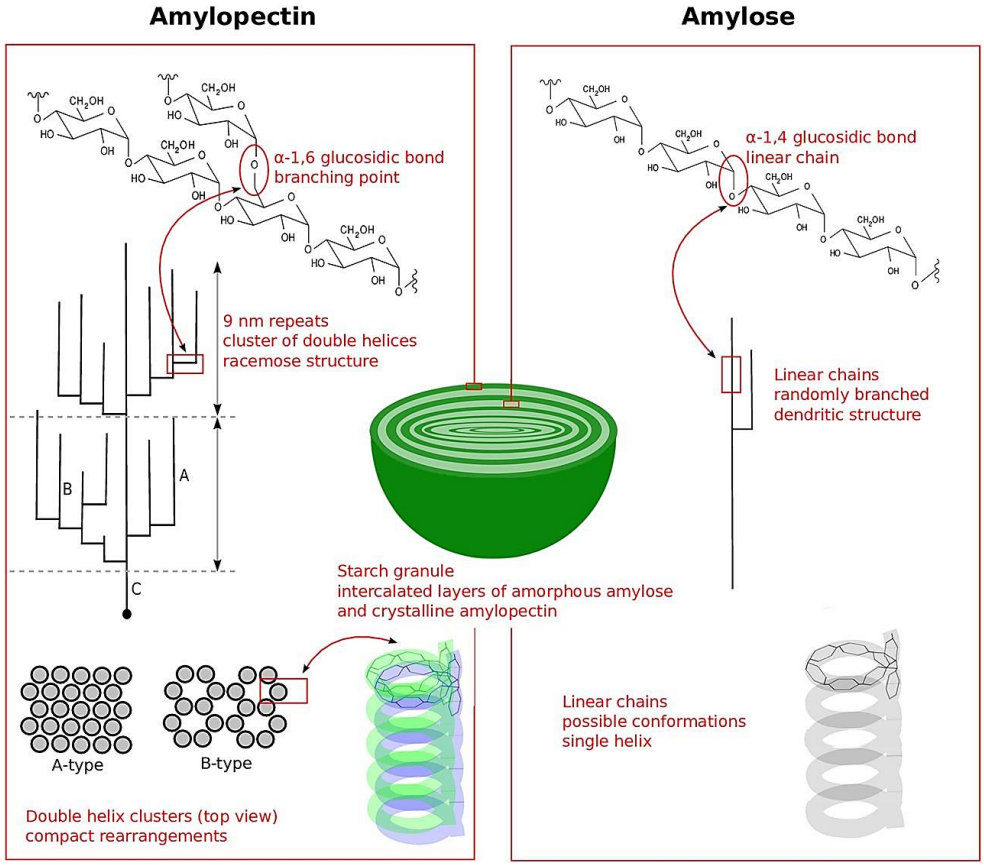


Figure 2.3 Structure of Amylose and Amylopectin (Raguin & Ebenhöh, 2017).

Amylopectin contains up to 2 million glucose residues with a hydrodynamic radius of 12-75 nm. Size exclusion chromatography revealed that branching within amylopectin is not random (Thompson, 2000). The cluster model describes the 3-dimensional structure of amylopectin and classifies amylopectin into three classes of glucose chains based on their length and branching points: Terminal branches (A chains) are short, unbranched and approximately contain 15 -25 glucose residues (Bertoft, Piyachomkwan, Chatakanonda, & Sriroth, 2008). The B chains (contains 23-35 glucose residues) connect between the A and C chains, the latter being a single chain forming the backbone of the molecule and containing the single reducing group (**Figure 2.3)**. The terminal A chains form regions of high molecular order are also known as pseudo-crystalline regions. The A chains of amylopectin are linked only through an a-1-6 bond to the C6 of a glucose unit of a B chains. The B chains are linked in the same way, but

the B chain has at least one other chain (either an A or a B chain) attached through an a-1-6 bond to a C6 of one of its glucose units. The C chain carries other chains as branches and contains the sole reducing terminal residue. The average chain lengths of amylopectin between 1-6 links or terminations typically range from 19 and 31sugar residues, depending on the botanical source (Ao, Simsek, Zhang, Venkatachalam, Reuhs, & Hamaker, 2007).

The proportion of amylose and amylopectin differs with the botanical source, but most starch comprises between 27-30% of amylose in cereals and up to 70% of amylose for high amylose types and between 0 and 2% of amylose for waxy starch types (Martens, Gerrits, Bruininx, & Schols, 2018). The proportion of amylose and amylopectin in a starch affects the architecture of starch granules, gelatinization viscosity, gelatinization temperature, texture and digestibility of the cooled pastes or gels.

2.6.2 Starch granule organization

Starch granules (S) are typically embedded in a matrix of protein bodies (P) and are surrounded by a cell wall (CW) (**Figure 2.2 A** and **B**) which may be partially degraded in cereals or present intact in the living cotyledons of legumes. Although chemically and structurally similar to cellulose present in the cell walls of the plant, only starch can be directly digested by animals, including humans (Martin & Smith, 1995). Amylose and amylopectin formed into semi-crystalline aggregates comprise the starch granule, and these are the primary for starch storage in all plants. The size, shape, and structure of starch granules vary with the type of plant. Rice has the smallest granules 2-5 µm in diameter, but these are further organised into larger granule aggregates about 40 µm in diameter, tubers such as potato have the larger starch granules ranging between 22-85 µm in diameter (Buléon, Colonna, Planchot, & Ball, 1998; Thomas & Atwell, 1999). Cereals typically have starch granules 15 -25 µm in diameter.

Under polarized light or using X-ray diffraction, native starch granules (unmodified by processing) exhibit birefringence that suggests an ordered or crystalline-like structure with a radial orientation within the granule (Kim, Kim, & Baik, 2012). This property is lost when the starch is gelatinized or heated to its melting point. The granule comprises alternating rings (growth rings) in the form of concentric shells of increasing diameter extending from the hilum at the centre of the granule towards the surface. The concentric shells have alternating hard-shells which is the pseudo-crystalline region and softshell which is the amorphous regions (**Figure 2.4**) (Ridout, Gunning, Parker, Wilson, & Morris, 2002). This formation especially

visible after enzyme or acid etching, followed by microscopic imaging. The growth rings are assumed to be formed due to the circadian rhythms associated with sugar and starch synthesis resulting from photosynthesis (Buléon et al., 1998; Ridout et al., 2002). The thickness of the rings ranges from 120 to 400 nm. The low-density amorphous rings consist of a disordered arrangement of amylose and amylopectin and contain a high frequency of α 1-6 glycosidic branches.

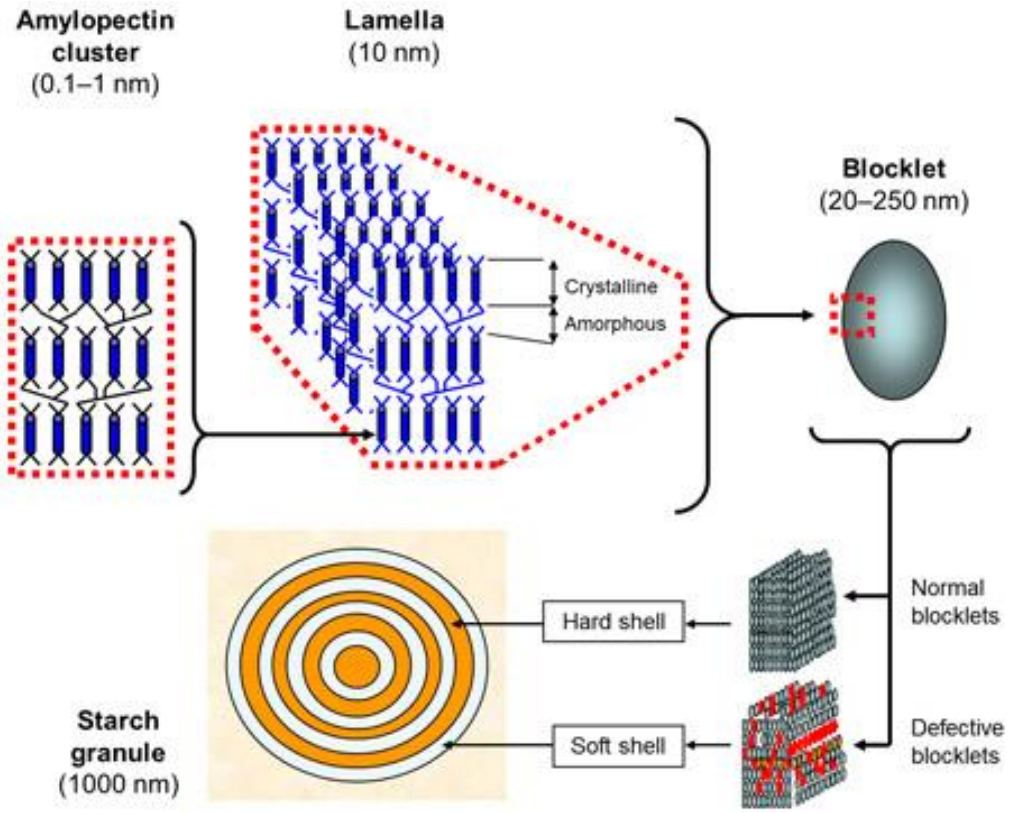


Figure 2.4 Molecular structure of a starch granule (Lincoln Taiz, Eduardo Zeiger, Ian M. Møller, & Murphy, 2015)

The starch granule consists of lamellar structures of alternating concentric layers of pseudo-crystalline and amorphous starch (Zhang, Venkatachalam, & Hamaker, 2006). The pseudo-crystalline lamellae consist of double helices of amylopectin A chains whereas the amorphous lamellae contain amylose and amylopectin branching points associated with B and C chains. Although the structure of starch is broadly understood, there are some aspects of the organizational structure that are not clear.

An important model proposed to describe the organization of starch granules is the theory of blocklets. New images show that blockets consist of more or less spherical bodies with diameters ranging from 50-250 nm depending on the plant source. Blocklets consist of crystalline lamellae (5-6 nm in depth) alternating with amorphous lamellae (2-5 nm in depth)

the lamellae repeat distance is about 9 nm. The blocklets contain amylopectin clusters near the surface of the granule and protrude slightly from the surface of the starch granule (Gallant, Bouchet, & Baldwin, 1997; Juszczak, Fortuna, & Krok, 2003).

2.6.3 Starch crystallinity

The pseudo-crystalline structure of starch is classified into A, B, and C- type starches (not to be confused with A, B and C chains) based on packing arrangement of amylopectin double helices and the X-ray diffraction patterns that these cause (**Figure 2.5 A and B**). The A-type diffraction pattern results from A chains packed into monoclinic structures (a rhombic pattern) while the B-type diffraction pattern results from A chains packed hexagonally. The C-type is the combination of A-and B-type diffraction patterns.

Figure 2.5 X-ray powder diffraction patterns of (A) A- (monoclinic) type and (B) B- (hexagonal) type crystalline starch from uncooked maize starch granules (Damager, Engelsen, Blennow, Lindberg Møller, & Motawia, 2010). Red dots denote the presence of water (Lopez-Rubio, Flanagan, Gilbert, & Gidley, 2008).

The B-type structure has 36-42 water molecules per unit structure in comparison to the A structure with 8 molecules per unit cell (Gous, Gilbert, & Fox, 2015). The amylopectin molecules of B-type diffraction pattern starches contain less short branch chains than the A-type. Another difference in the amylopectin structure between the A- and B-type starch is that the amylopectin of the A-type starch has some branch linkages located in the crystalline region,

but that of the B-type starch has almost all the branch linkage located in the amorphous region. This makes the granules containing a high proportion of starch with the A-type diffraction pattern (cereal starches) more porous on the surface and more loosely packed than those containing a high proportion of starch with the B- (tuber starches) and C-type diffraction patterns (legume starches) (Butterworth, Warren, & Ellis, 2011).

Granules containing a high proportion of A-type starch are digested more quickly than B-type starches, while the digestibility of C-type starch was intermediate (Zhang & Hamaker, 2009). This is due partly to the increased porosity of the granules containing A-type starch and partly due to easier access of digestive enzymes to starch sub-chains in the pseudo-crystalline region (Srichuwong, Sunarti, Mishima, Isono, & Hisamatsu, 2005).

2.6.4 Microstructure of starch

Starch granules contain distinctive microstructural characteristics discernible using light and electron microscopy (**Figure 2.6 A and B**). The microstructures include pores, pits and channels. Scanning electron microscopic analysis revealed that the pores present on the surface of the cereals were usually blocked by protein residues; these were removed by digesting with protease (Kim et al., 2012).

Figure 2.6 (A) Fluorescence of a protein stain intensified along pore channels in the interior channels and hilum and (B) Scanning electron microscopic image of corn starch granule showing surface pores (Kim et al., 2012).

The pores present on the surface of the starch granules (**Figure 2.6 B**) lead to the centre of the granule, i.e. the hilum. The channels leading to the interior of the granules of corn and wheat were also lined with protein. This protein was considered to be remnants of the granule-bound starch synthetase enzyme. The pores enhanced the accessibility of fluorescence dyes into the interior of the granule (**Figure 2.6 A**) (Kim & Huber, 2008) and presumably would facilitate

hydration and access by digestive enzymes, which is discussed in detail in the later sections of the literature review.

2.6.5 Interaction of starch with macromolecules

Proteins and lipids are the other major compounds present in starch granules, albeit in minimal quantities. Wheat, a typical cereal starch contains about 0.4% of protein and 0.9% of lipids while tuber starch such as tapioca contains about 0.1% of protein and 0.1% of lipid, considerably less than for cereal starches (Morrison, 1988). The protein and lipid components have a small influence on gelatinization temperatures and viscosity; however, the most important effect is on the flavour profile of the starch. Tuber starches have a very bland flavour in comparison the cereal starches, and this is due to the small proportion of lipid and protein present tubers.

Starch granule proteins, which are termed granule-associated proteins, are either present on the surface of the granules, e.g. Gliadin and gluten or integrated with amylose-amylopectin structures, probably as the starch is deposited in the amyloplast. Storage proteins can be extracted using salt solutions, while the granule-associated proteins can be removed only with detergents such as sodium dodecyl sulphate. Granule-associated proteins such as puroindolines are related to the softness of the endosperm texture (Anjum & Walker, 1991; Greenwell & Schofield, 1986). Other granule-associated proteins have been shown to leftover from amylose synthesis (Rahman et al., 1995). As noted earlier, the lumen of the starch helix is hydrophobic, and monoacyl lipids (starch-lipids) may bind in this space while other lipids are associated with the surface of the granule or endosperm in general (surface lipids and non-starch lipids). During processing and purification of starches, the levels of di- and tri-acyl lipids decrease to negligible levels. Starch surface lipids form inclusion complexes with amylose at the surface of the granule (Panyoo & Emmambux, 2017). Lipids that are associated with a single amylose helix are termed V-amylose lipids. These amylose-lipid complexes have 6-glycosyl residues per turn of the amylose helix and a guest molecule within the helix. Monoglycerides, fatty acids and fatty acid esters can complex within the helical cavity of amylose (López, de Vries, & Marrink, 2012), altering the interaction of starch with water, and are important in altering the texture and staling properties of bakery products. Hydrophilic hydroxyl groups project away from the helix of amylose (Immel & Lichtenthaler, 2000).

The amylose-lipid complexes can significantly alter the functional properties of starch in several ways that are of interest to the food industry, i.e. the amylose-lipid complexes reduce the ability of amylose to form networked double-helical structures resulting in a looser gel with higher final paste viscosity (Richardson, Kidman, Langton, & Hermansson, 2004). Amylose-lipid complexes enhance water and oil absorption capacities, however, they decrease the solubility of high amylose maize starch. Lipid-binding to amylopectin is relatively weaker than to amylose as longer acyl groups (>12C) will not fit within the short helices of the A chains. The mechanism of interaction and influence on starch functionality lacks literature data. Addition of lipids to starch reduces retrogradation and staling of starch due to inability of lipid-complexed amylose molecules to form junction zones across the starch molecules that are responsible for short-term starch retrogradation. Potential health benefits of amylose-lipid complexes in terms of lowered digestibility and improved glycaemic control are discussed in the later sections of this chapter.

2.6.6 Gelatinization of starches

Starch granules are highly organized structures; however, they lose order and structure when heated with water, although the change depends on the temperature and the type of starch. Starch contains many hydroxyl groups at the exterior of the coil with an affinity for other hydroxyl groups, which is the driving force bringing starch chains together by strong hydrogen bonding, this is responsible for the structural integrity of starch granule and its ability to form gels when gelatinized in water. This makes the native starch insoluble in water due to the unavailability of hydroxyl groups to participate in intermolecular hydrogen bonding with other (solvent/water) molecules (Bart, Gucciardi, & Cavallaro, 2012).

When the starch is subjected to heat and moisture, there is a change in its pseudo-crystalline and organized structure, i.e. an order-disorder transition occurs, the phenomenon called gelatinization (Sablani, 2009). When gelatinization occurs in excess water (> 70% w/w water) (Parker & Ring, 2001), a series of temperature-dependent processes begin (Belitz, Grosch, & Schieberle, 2004). Between 55°C- 60°C the intermolecular hydrogen bonds between hydroxyl moieties on the starch molecule are disrupted, and the free hydroxyl groups begin to interact with water molecules. As the temperature increases, more water is absorbed, causing the chains to move further apart, causing irreversible disruption of the original organised structure (Taggart & Mitchell, 2009) and the hydrolysis of the starch interact with the polar oxygen atom of the water molecule.

During the early stages of gelatinization, (~60°C) amylose diffuses from the granules leaching into the surrounding water (Varavinit, Shobsngob, Varanyanond, Chinachoti, & Naivikul, 2003). The structural changes of the starch molecules during gelatinization are caused by swelling of the amorphous region during hydration. As the amorphous region swells, it disrupts the connected pseudo-crystalline region, thereby creating disorganization in the structure of the granules (Donald, 2004). As the granules absorb water and swell, viscosity increases as is readily seen during the pasting of a starch suspension. When fully gelatinized in water, the granules soften and if their concentration is above about 3% in water will form a paste that may set as a gel on cooling.

Structural changes to the granules during gelatinization in water include swelling, loss of birefringence in polarizing light, unwinding and insertion of water into helical structures, loss of amylose from the granules into the surrounding fluid, loss of endotherms measured by differential scanning calorimetry and changes in X-ray diffraction. At higher temperatures and in conditions where water is limited (< 30% w/w water), there is insufficient water for full hydration. Gelatinization may also occur when the starch melts, particularly in high shear temperature conditions. The temperature at which this occurs is around 120°C and is typical of the processes occurring during extrusion processing of starchy foods used to make crisp snack and breakfast foods.

Based on wide-angle X-ray diffraction and small-angle X-ray scattering, Waigh, Gidley, Komanshek, and Donald (2000) proposed a framework for the mechanism of granule disassembly during starch gelatinization based on the assumption that hydrated amylopectin had a structure similar to a branched side-chain liquid crystalline polymer of smectic (alternating regions of crystalline and amorphous material) or nematic type. When excess water is present (> 70% w/w water), starch undergoes a two-stage gelatinization process that involves a slow dissociation of helices (slow smectic-nematic/isotropic transition) followed by fast helix-coil transition. At intermediate water conditions (34–66% w/w water), there is insufficient water for full hydration; gelatinization occurs when the starch may melt, the first endotherm is associated with the smectic–nematic/isotropic transition and the second endotherm corresponds to the helix–coil transition. When water is limited (< 34% w/w water), a direct transition from glassy nematic/isotropic to amorphous state occurs at elevated temperatures for A and B type starches (Wang & Copeland, 2013).

Optical microscopy was employed to study the gelatinization behaviour of starch; however, the gelatinizing temperature cannot be precisely controlled, and energy absorbed during gelatinization could not be measured. Thus, differential scanning calorimetry (DSC) is used to monitor endotherms associated with the loss of organized structure (Higley, Love, Price, Nelson, & Huber, 2003). Gelatinization temperature (GT) and enthalpy (ΔH) are conveniently measured by DSC, as it is experimentally convenient and precise. Due to starch's pseudo-crystalline nature, X-ray diffraction can be employed to study changes occurring in the periodicity of the pseudo-crystalline lamella and associated loss of crystallinity. Nuclear magnetic resonance spectroscopy can be used to measure the proportion of double-helices and loss of crystalline and molecular order during gelatinization by assessing the mobility of water within the structure (Cooke & Gidley, 1992).

2.6.7 Retrograded starch

When gelatinized starch is subsequently cooled, the disrupted amylose and amylopectin chains slowly re-associate into an ordered structure different from native starch, this process is called retrogradation. Retrogradation involves a change in the structure of the starch, i.e. re-association of the linear segments of α-1,4 linked glucose units of glucan polymer by hydrogen bonds to form an ordered structure which becomes more resistant to digestion (Chung, Lim, & Lim, 2006; Zhu & Wang, 2012). Hydrogen bonding between adjacent starch chains, particularly amylose, results in a realignment of the starch molecules as water is expelled from the structure, a process known as syneresis. The starch molecules become aligned in chains of double-stranded crystallites which are more resistant to amylase digestion than the fully gelatinized starch. The interactions between the bonds during retrogradation occurs over time and proceeds faster at temperatures down to about 4°C. There is a negative relationship between the amylose content of starch and the minimum gelatinization temperature. High amylose maize starch gelatinizes at temperatures above 100°C, greater than high amylopectin starch (~80°C) and it retrogrades more readily (Ishiguro, Noda, Kitahara, & Yamakawa, 2000).

DSC is an extremely useful tool to characterize starch retrogradation by the quantitative measurements of enthalpy and transition temperatures. The pasting properties of the retrograded starch can be measured using rapid-visco analyzer or the Brabender amylograph high-quality rheometers capable of implementing small strain oscillation measurements, The order in the crystalline region and the state of organization of double helices inside the

crystalline region can be measured using the Fourier transforms infrared spectroscopy or X-ray diffraction. Molecular changes of starch gels during retrogradation can be measured using Raman or nuclear magnetic resonance (NMR) spectroscopy. The structural morphology of retrograded starch granules can be measured using scanning electron microscopy or atomic force microscopy. Upon retrogradation and dehydration, fractal-like networks and well-defined pores can be observed in retrograded starch using environmental SEM (Wu, Lin, Chen, Wu, & Xiao, 2012).

2.7 Digestion of starches

Starch is digested in several stages in the human gut (**Figure 2.7)**. Mastication or chewing is important as it breaks down the food and mixes it with saliva to form a paste, portions of which are swallowed as food boluses. The initial digestion is in the buccal cavity by salivary amylases secreted by the salivary glands. Since gelatinised starch, the form in which it is most commonly eaten has moderate to high moisture content, its digestion is a solid-liquid phase reaction.

Amylase enzymes are classified as alpha (α)-Amylase and beta (β)-Amylase. These enzymes are glycoside hydrolases and act on α-1,4-glycosidic bonds. α-Amylase is considered as a major digestive enzyme in the mammalian digestive tract. Human salivary and pancreatic amylases are α-amylases while plants, fungi and bacteria can also produce β-amylases. Animals do not produce β-amylase. The optimum pH of α-Amylase is 6.7–7.0. α-amylase can break down long-chain carbohydrates such as starch amylose into maltotriose and maltose or amylopectin into maltose, glucose and limit dextrins formed by starch remnants near α-1,6-glycosidic bonds.

For salivary digestion, α-amylase diffuses into the hydrated starchy food mass, binding to the starch and cleaving the α-1,4-glycosidic linkages of the starch molecules in amylose and amylopectin, hydrolysing them into shorter particles or dextrins and oligosaccharides. This process contributes to reducing the viscosity of the bolus and aiding gastric mixing (Butterworth et al., 2011). α- amylase binds to 5 adjacent glucose subunits linked by α-1-4 glycosidic bonds cleaving subterminal maltose units from the starch molecules. Digestion by salivary amylase may continue until gastric acidification and pepsins render the amylases inactive. After food intake, pH in the stomach rises to pH 4-5, which is then followed by the secretion of hydrochloric acid (HCl) and acid proteases from the parietal cells in the mucus layer causing the pH to decrease to pH 1.2 - 2.0. Proteins present in the starch granules are

hydrolysed by the gastric proteases, which assist with exposing starch present in the food matrix. Low pH begins starch hydrolysis, assisting the digestion by α-amylase as the food passes into the small intestine (Wursch, Del Vedovo, & Koellreutter, 1986).

In the small intestine, bicarbonate secreted by the pancreas neutralizes acid secreted by the stomach allowing pancreatic enzymes secreted by the pancreas into the duodenum almost completely hydrolyzed starch in the digesta. Enzymes hydrolyse amylose and amylopectin, resulting in the production of mono and disaccharides (maltose, maltotriose). The latter, along with ingested disaccharides and limit dextrins, are hydrolyzed to monosaccharides by brush border glucogenic enzymes including amyloglucosidase and invertase secreted by the intestinal cells that line the villi. Only monosaccharides can be absorbed by the sodium-glucose co-transporter (SGLT-1) located on the luminal surface of enterocytes and transported across the gut wall into the bloodstream (Lehmann & Robin, 2007). Brush border enzymes cleave both α-1-4 and α-1-6 glycosidic bonds of limit dextrins, although cleaving α-1-6 glycosidic bonds is slower in comparison to cleaving α-1-4 glycosidic bonds. Starch that is resistant to small intestinal digestion arrives at the colon where it is fermented by bacteria, this resistant starch may have prebiotic properties by providing nutrition to the colon bacteria which in turn produce essential fatty acids.

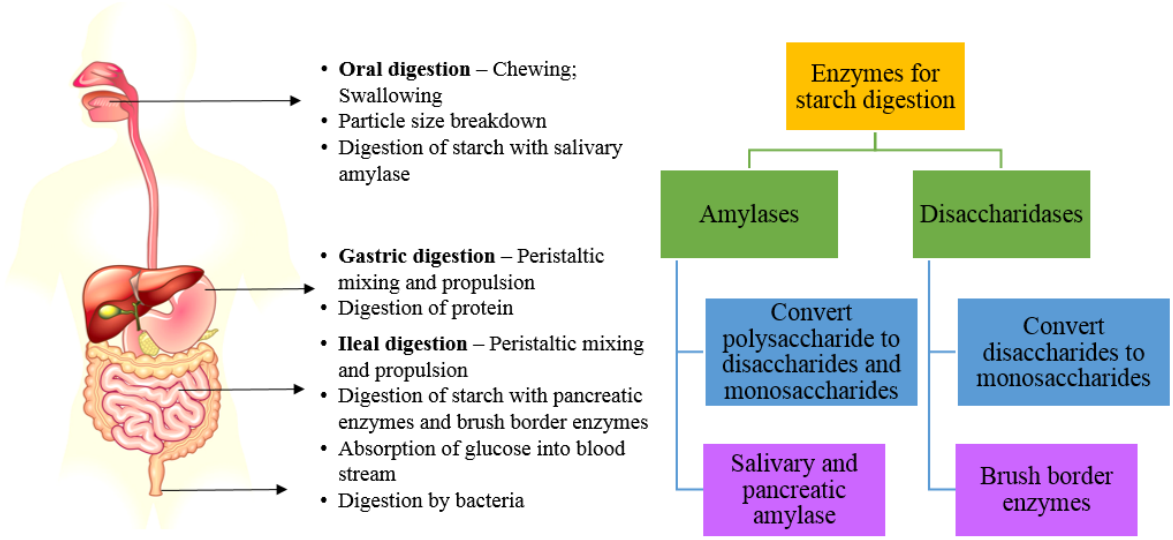


Figure 2.7 Digestion of starches and the enzymes responsible for digestion.

2.8 The rate of digestion of starches

Starches are often crudely characterized in terms of the rate of *in-vitro* digestion of gelatinized starch and are classified as rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS). The divisions between these groups are arbitrary and represent a

continuum of digestibility, which may change depending on how that starch is prepared/processed.

RDS is characterised as starch that can be hydrolyzed within 20 min of the start of a defined *in-vitro* pancreatic digestion and can be highly correlated with postprandial blood glucose response (Englyst, Kingman, & Cummings, 1992). Starch that is hydrolyzed more slowly is classed as either SDS or RS. SDS is characterised as starch that can be hydrolysed between 20 and 120 min of the start of a defined *in-vitro* pancreatic digestion method and the remaining proportion of starch that is undigested after 120 min exposure to the enzymes is the RS. The relatively slow digestion of SDS causes a moderate and steady slow release of glucose into the blood. Foods containing a high proportion of SDS show lower post-prandial blood glucose and insulin responses in comparison with the foods with a higher proportion of RDS (Englyst et al., 1992; Miao, Jiang, Cui, Zhang, & Jin, 2015; Zhang & Hamaker, 2009; Zhang, Ao, & Hamaker, 2006).

RS is further classified into RS 1, 2, 3, 4 and 5 starches. RS 1 starches are not accessible because they are trapped by the intact cell wall of the cellular matrix. RS 2 starches are native starches that are resistant to enzyme digestion due to their pseudo-crystalline organisation that resists hydration and access by enzymes. These starches can only be partially digested, and a resistant portion may remain after passing through the small intestine. RS 3 starch is made by retrogradation of cooled gelatinised starch after cooking. During cooling of gelatinized starch, the starch polymer re-aggregates into a semi-crystalline form. The steric hindrance created by re-aggregation makes the starch more resistant to digestive enzymes. RS 4 starches are chemically modified for industrial purposes (Fuentes-Zaragoza, Riquelme-Navarrete, Sánchez-Zapata, & Pérez-Álvarez, 2010; Perera, Meda, & Tyler, 2010; Raigond, Ezekiel, & Raigond, 2015; Sajilata, Singhal, & Kulkarni, 2006). Amylose-lipid complexes have been proposed as resistant starch (RS) type 5, because of their resistance to enzyme hydrolysis.

2.9 Factors affecting the rate of digestibility

Edible starches which we consume in a food matrix are often cooked (gelatinized), milled or extruded. The granular structure of the starch is destroyed by these treatments during the processing that involves hydration, heat and shear. The structural features of the starch both at the molecular and granular levels, plant structure in the form of intact/partially grain particles

and the interaction of starch with protein/lipids are all important factors that affect starch digestibility. The structure of the food matrix is one of the most important factors that influence the digestibility of starch.

2.9.1 Granule morphology (size and shape)

The size and the surface area of the native starch granule influences the mechanism of starch hydrolysis (Al-Rabadi, Gilbert, & Gidley, 2009; Dhital, Shrestha, & Gidley, 2010), i.e. the smaller the granule size, the greater the enzyme susceptibility and increased digestibility due the larger surface area to volume ratio compared to large particles for enzymatic action (Franco & Ciacco, 1992; Mahasukhonthachat, Sopade, & Gidley, 2010). In larger particles, radial penetration of digestive enzymes reaches a smaller proportion of the starch than in smaller particles, so the rate of digestion is lower.

2.9.2 The surface of the starch granule

Starch contains proteins and lipids which together are highly organized so they form a coating resistant to water and digestive enzymes, reducing the digestibility of the granule. Though the starch granules are covered with proteins and lipids (Debet & Gidley, 2006), they do contain pores on the surface that act as entrance points for digestive enzymes and once the enzyme enters the granule digestion proceeds. The presence of pores, channels and cavities increase the surface area which is potentially available for chemical and enzymatic reactions. The walls of the pores are the initial sites of enzyme attack that allow enzyme molecules direct access to the granule interior (hilum) (Planchot, Colonna, Gallant, & Bouchet, 1995).

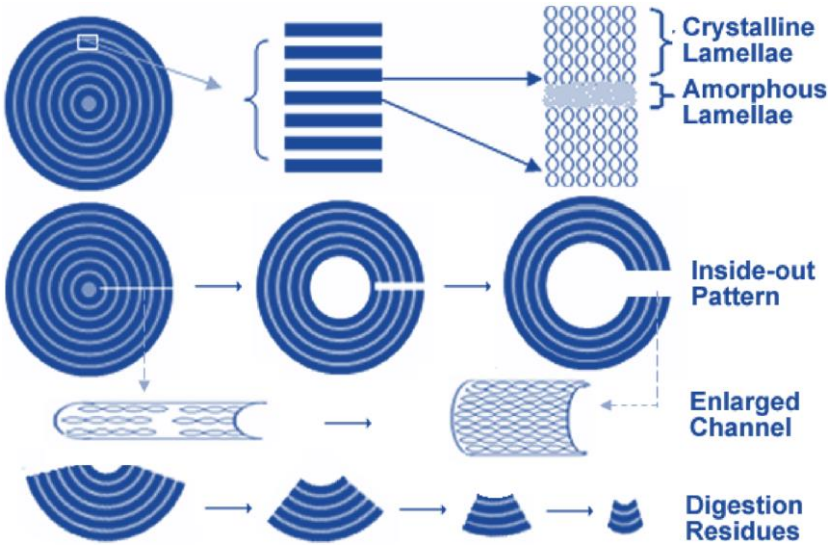


Figure 2.8 Schematic representation of inside out and layer by layer digestion in starches. Adopted from (Zhang, Ao, et al., 2006).

The pore size present on the surface of the starch granule plays an important role in digestion, by allowing the penetration of enzymes and water into the centre of the granules (Huber & BeMiller, 2000). This makes the native starch appear to be digested inside out (Benmoussa, Suhendra, Aboubacar, & Hamaker, 2006). Native cereal starches undergo inside out and layer by layer digestion where the enzymes start on the pores on the surface and inside as shown in **Figure 2.8** (Han & Hamaker, 2001; Huber & BeMiller, 2000; Zhang, Ao, et al., 2006). Then it is digested side-by-side where the channel enlarges and concurrently digests the pseudo-crystalline and amorphous regions. Having said that, the digestion of native potato starch and high amylose maize starch (~70% amylose is resistant to enzymatic digestion as the granules are large (smaller surface area to volume ratio for enzymatic action) and smooth (slower penetration of enzymes as it lacks pits and pores on the surface) when compared to the cereal starches *(Oates, 1997)*. The other morphological parameter that influences starch digestibility is the shape of the starch granule, which affects the surface area to volume ratio significantly and hence the potential for amylase digestion, i.e. spherical starch granule has smaller surface area to volume ratio while polyhedral has larger surface area to volume ratio *(Tester, Qi, & Karkalas, 2006)*.

2.9.3 Molecular structure of starch

Major factors acting at the molecular level that influence starch hydrolysis are the pseudo-crystalline or, amorphous structure and the amylose and amylopectin ratio, both of which affect the enzyme susceptibility (Colonna, Leloup, & Buleon, 1992; Park & Rollings, 1994). When starch is subjected to an enzyme or acid reaction, the amorphous regions are degraded more easily than the pseudo-crystalline regions, as the pseudo-crystalline regions are orderly and closely packed, which makes them more resistant to digestion with α- amylase (Donald, 2004).

Double helical amylopectin packing in A, B and C crystallite patterns of starches affect starch hydrolysis based on the length of double helices and interior crystallites. When compared to the A- and C- type crystallites, B- type crystallites are more resistant to enzymatic hydrolysis, as they have long chains which form longer and more stable double helices (Lehmann & Robin, 2007) which are further stabilized by hydrogen bonds distributed over the entire crystalline region, causing decreased digestibility (Miao et al., 2015; Zhang & Hamaker, 2009; Zhang, Ao, et al., 2006; Zhang, Sofyan, & Hamaker, 2008; Zhang, Venkatachalam, et al., 2006).

Semi-crystalline starches such as high amylose maize and green banana starch have a B- or C-type polymorph that is resistant to digestion by α-amylases (RS 2) (Birt et al., 2013). Though being less crystalline, high amylose starch resists starch hydrolysis more effectively than native

starch due to the distribution of crystallites in these starch granules. According to Sajilata et al. (2006), starch with a higher amylose content has a lower digestibility due to closer and more stable packing of the starch molecules in the granule.

In amylopectin branching reduces close packing in the amorphous regions, which makes it more susceptible to enzymatic hydrolysis (Ao, Simsek, Zhang, Venkatachalam, Reuhs, & Hamaker, 2007). Chain length, branch density and degree of polymerization of amylose and amylopectin also affect starch digestibility. The increase of the starch branch density and the crystalline structure in the starched treated with α-amylase or maltogenic-α-amylase, with or without a combination of transglucosidase, likely contribute to their slow digestion property (ao, Simsek, Zhang, Venkatachalam, Reuhs, & R Hamaker, 2007).

2.9.4 Interaction of starch with macromolecules

The presence of other food components ingested withy starch such as proteins, lipids, phenolic compounds and NSPs can also affect the rate of digestion (Butterworth, Warren, Grassby, Patel, & Ellis, 2012). The rate of enzymatic hydrolysis can be affected by surface proteins (e.g. in cereals and legumes) acting as a protective coating around starch granules, so blocking adsorption sites, and slowing starch digestibility (Singh, Dartois, & Kaur, 2010). Free fatty lipids and phospholipids present on the starch surface may complex with amylose, thereby making the amylose chains less accessible to the active site of α- amylase.

Hydrocolloids such as guar gum and xanthan gum slow starch digestion as they increase the viscosity of the digesta in the small intestine so reducing rates of diffusion and absorption (Dartois, Singh, Kaur, & Singh, 2010; Hardacre, Yap, Lentle, & Monro, 2015; Hong et al., 2016). However, insoluble fibres such as cellulose and lignin have a small effect in influencing the rate of starch digestion (Sajilata et al., 2006). Using guar galactomannan had a direct non-competitive inhibitory effect on alpha-amylase due to the direct binding of the enzyme to galactomannan which causes galactomannan-amylase complex being inactive.

Thus, the effects of NSP in lowering postprandial glycaemia not only involve modifications of gut physiology, but also include direct inhibition of the first stage in the biochemical degradation of starch (Slaughter, Ellis, Jackson, & Butterworth, 2002).

2.9.5 Food structure and digestibility

The food matrix, which has also been called the secondary structure, plays an important role in altering the accessibility of digestive enzymes to starch (Mishra et al., 2012) which is explained in detail in the following sections.

2.9.5.1 Dense food matrix

According to Fardet, Leenhardt, Lioger, Scalbert, and Remesy (2006), breads prepared with a dense, compact structure have a relatively low GI because dense foods of low porosity inhibit the penetration of the digestive enzymes into the food matrix. Starchy foods with a compact or dense structure show delayed digestion *in-vitro*, as the enzymes only have access to the surface layer before penetrating into the matrix. This causes dense foods such as pasta, whole grains, intact vegetables and legumes to have low GI. The dense, compressed, food matrices with few pores or channels contribute to lowering the digestibility and GI (Granfeldt & Björck, 1991; Monro, Mishra, & Hardacre, 2011).

2.9.5.2 Porous food matrix

Processed foods such as cakes, breads, puffed, and flaked breakfast cereals are highly porous. These porous structures facilitate penetration of digestive enzymes into the products which therefore digest rapidly. Moreover, the processed foods are hydrated then pre-cooked, and thus the starch in the food matrix is gelatinised, which in turn, increases the rate of digestion by 8 to 10 times (Mishra et al., 2012).

2.9.6 Effect of processing techniques and treatments on starch digestibility

Many of the factors mentioned above will be affected to a greater or lesser extent by food processing conditions such as milling and hydrothermal treatments. The latter gelatinizes the starch and makes it more susceptible to amylolysis.

2.9.6.1 Milling

Cereals are usually milled before being incorporated into foods. Milling fractures the endosperm and removes protective bran layers. Particle size is an important factor that contributes to the rate of digestion. For instance, milling of grains to smaller particle size increases the rate of digestion and subsequently postprandial glucose and insulin levels. An *in-*

vivo analysis of ten healthy subjects who consumed different size grades of cooked wheat flour showed that the glycaemic and insulin responses decreased and the satiety increased as particle size increased (Holt & Miller, 1994). The effect of particle size on digestion is due to increase in surface area per unit volume of the starch and fracturing of the starch granules, i.e. the degree of starch access is increased by a decrease in the particle size (Blasel, Hoffman, & Shaver, 2006). This can result in an increase in the rate of starch digestion and fermentation in the monogastric digestive tract (Al-Rabadi, Torley, Williams, Bryden, & Gidley, 2012). In homogenized gelatinized starchy foods, there is almost unlimited access to digestives enzymes to starch, so it is rapidly digested (Monro et al., 2011). According to Fardet, Leenhardt, Lioger, Scalbert, and Remesy (2006), either by limiting digestive enzyme mobility in a solid food matrix or by incorporating dense, non-porous materials in the matrix, like larger intact particles of whole grains in breads, the rate of digestion can be decreased.

2.9.6.2 **Hydration - Soaking**

Hydration is an essential step in cereal processing, which can have a potential effect on starch digestibility. As seeds are hydrated water enters the intact or partially intact grains, producing swelling of the tissues without cell separation, when fully hydrated the moisture content of the seed is about 50%. Prolonged hydration causes a partial loss of integrity of starch granules due to swelling. Hydrated legume starches gelatinize more easily than un-soaked starch because starch which is unavailable before soaking due to the crystalline C-type structure present in legumes becomes more available for enzyme hydrolysis after hydration. The RS content in soaked and gelatinised legumes is lower than for seed that is not hydrated as the starch is converted to a more digestible form by gelatinization (Eyaru, Shrestha, & Arcot, 2009).

2.9.6.3 **Heat – moisture treatment (Cooking and baking)**

The amorphous regions in starch have connecting bonds with the pseudo-crystalline regions which when subjected to heat-moisture treatment (cooking and baking) (> 65% w/w water; > 55°C), swell and tends to dissociate the pseudo-crystalline regions (Anklam, 2005). Due to this swelling and dissociation, the starch becomes increasingly accessible to digestive enzymes (Mishra et al., 2012). Based on the susceptibility to gelatinization, starch has been classified into those that swell rapidly, those that have restricted swelling due to macromolecules such as lipids and proteins and those that do not swell significantly at temperatures below 100 °C due to high amounts of amylose (high semi-crystalline content). When starch is treated with limited

moisture (<35% w/w water) for a certain period at a temperature above the glass transition temperature, but below the gelatinization temperature (55-70°C), the crystalline packing of starch is altered without destroying granule structure and results in the formation of SDS, a treatment called annealing.

Baking breads in a lower temperature for a longer time (20 h, 120°C) can double RS formation compared with the ordinary baking process (40 min, 200°C) (Liljeberg, Åkerberg, & Björck, 1996). This could be due amylose leaching out of the starch retrogrades in the first few hours of baking, thus causing low starch digestibility.

2.9.6.4 **Low-temperature storage**

Cooking, baking and other heat treatments can cause a reduction in RS content while the storage of starch or starchy products after cooking can increase RS 3 level due to retrogradation (Wang, Li, Copeland, Niu, & Wang, 2015). Cooling heat-treated starch below -20°C may result in the formation of high levels of RS. The linear chains recrystallize and become resistant to digestion, while branched chains recrystallize incompletely and remain susceptible to digestion (Mishra et al., 2012). During retrogradation, the starch regains an ordered structure and becomes resistant to enzymatic digestion. Although the molecular structure of retrograded starch does not return to the original native starch structure, the recrystallization step does result in fewer available α-glucan chains for α-amylase to bind to, thereby reducing starch digestibility (Lovegrove et al., 2017). As the retrograded starch decreased in digestibility, there is a decrease in RDS and SDS with very little change in RS (Chung, Shin, & Lim, 2008; Zhu & Wang, 2012) although increases in RS have been reported by (Park, Baik, & Lim, 2009). In several studies temperature cycling was used to increase the RS content (Tian et al., 2012; Zhu & Wang, 2012) and SDS content (Tian et al., 2009).

According to a study conducted by Park et al. (2009), temperature cycling between 4°C and 30°C for 16 days increased RS with decreases in s RDS and SDS. The increasing organisation within the granule from amorphous to pseudo-crystalline reduces digestibility even when the starch is fully gelatinized, probably by reducing the accessibility of the relatively large enzyme molecules to the starch molecule in the correct orientation to effect cleavage of the α-1-4 bonds (Englyst, Vinoy, Englyst, & Lang, 2003). Having said that, re-heating the starchy foods can increase starch digestibility again.

2.10 Manipulating food structure to control starch digestibility

Accessibility of starch due to plant tissue structure in cereals can be retarded by retaining the particles intact/whole in a food matrix, by limiting gelatinization, adding other macromolecules (protein, lipids and dietary fibres) or starch structural modifications. In practice, with most starchy foods, these techniques are used to make the starch inaccessible for digestion and absorption. The contributions that each may play in determining the rate of carbohydrate digestion are discussed in this section of the work.

2.10.1 By retaining intact tissue structure in foods

Retaining intact cell structure in cooked food is most widely used in the baking industries by retaining particles of intact/kibbled grain particles in breads. Kibbling of kernels that would otherwise be ground to flour has found a place in food processing for populations with high rates of obesity and glucose intolerance, where there is a need to reduce both the rate and extent of carbohydrate digestion. However, when breads made with white flour, conventional whole grain flour and ultrafine whole grain flour were compared it was found that they were all highly glycaemic with no difference between the breads in glycaemic effect (Behall, Scholfield, & Hallfrisch, 1999).

According to Bhattarai, Dhital, Mense, Gidley, and Shi (2018), an individual cell wall was sufficient to provide a barrier to the ingress of amylolytic enzymes inside cells in cereal endosperm. Theoretically, flours can be substituted with partially intact kibbled kernels in bakery products such as breads to potentially reduce the rate of digestion and glycaemic potency potentially. Although reductions in digestibility have been associated with increasing particle size. Lanzerstorfer et al. (2018) showed that breads containing 90% of fine (< 1 mm), medium (< 1.4 mm) or coarse (>1.4 mm) grain particles did not significantly reduce glycaemic response compared with white breads.

In contrast, separate studies have shown that increasing the proportion of intact barley and wheat grains to between 40 and 80% in breads reduced the glycaemic index *in-vivo* from 92 to 33 (Liljeberg, Granfeldt, & Bjorck, 1992). Pumpernickel consists of 60-70% of partially intact rye grains and has low digestibility and much lower glycaemic index than most other breads (Jenkins et al., 1987).

Thus, reductions in the glycaemic potency of breads cannot be assumed to result from increasing the size of flour particles, but there is evidence that glycaemic potency is reduced when larger kernel particles are used in which internal structures based on cell walls survive to protect the starch (Fardet, Leenhardt, Lioger, Scalbert, & Remesy, 2006). Having said that, there are no or few intact cell walls in wheat and other cereal starches.

2.10.2 By controlled gelatinization

As starch gelatinization requires heat and moisture, the degree of gelatinization may be controlled by limiting the level of hydration, controlling the cooking time and temperature. Kernels cooked with limited water levels can remain ungelatinized/partially gelatinized, thereby digesting slowly in the gut when compared to completely gelatinized starch. (Zhang, Ao, et al., 2006) as explained in previous sections.

2.10.3 By controlled retrogradation

After gelatinization, cooling of starches causes the linear sections of amylose and amylopectin chains to form hydrogen-bonded alignments which limit access by digestive enzymes. Cooked potatoes that were chilled lead to the partial retrogradation of amylopectin in potato starches, which thereby reduced the glycaemic potency of potatoes (Mishra, Monro, & Hedderley, 2008). Retrogradation is now used industrially to produce resistant starches (RS 3) in nutritionally enhanced bakery and other products (Mishra et al., 2012).

2.10.4 By adding macro-molecules

Added proteins, including albumins and globulins, can form a matrix surrounding the starch granules, thereby acting as a barrier and limiting starch digestibility (Hamaker & Bugusu, 2003). Addition of lipids, in particular, monoglycerides with a shorter fatty acid chain (~ C14) to the starch, can decrease hydrolysis due to the formation of complexes between starch and fatty acids, that increase the hydrophobicity of the starch. Digestive enzymes cannot form enzyme-substrate complexes with amylose-lipid complexes as the α-1-4 glycosidic bonds are not exposed to allow enzyme-substrate complex formation. According to Hasjim, Ai, and Jane (2013), the consumption of breads with amylose-lipid complexes showed lower postprandial blood glucose and insulin levels after digestion in human subjects in comparison with white breads. This can be attributed either to the formation of resistant starch due to amylose-lipid complexes or delayed gastric emptying.

Addition of slowly digestible starch such as native potato starch and high amylose maize starch which are resistant to enzymatic digestion can reduce starch digestibility due to its granule morphology and crystalline nature (Miao, Jiang, Cui, Zhang, & Jin, 2013; Smrčková et al., 2014).

Addition of dietary fibres such as guar gum to starch may slow the rate of glucose release through their high viscosity, which in turn slows the absorption of digested products in the small intestine. According to Brennan (2005), whole grains are highly nutritious, partly because of their dietary fibre component, and they tend to digest slowly if they are not chewed. Soluble dietary fibre has the effect of reducing the rate and extent of digestion, as shown in (Dhital, Dolan, Stokes, & Gidley, 2014; Hardacre et al., 2015). Some of this was discussed earlier and does not have to be reintroduced.

Soluble and insoluble dietary fibre can be used as an ingredient in food manufacturing to reduce the energy density of a product, as well as to introduce functional properties. Non Starch Polysaccharide (NSPs) such as xanthan and guar gums are often blended into gluten-free rice bread formulations as a substitute for gluten to improve bread (Ahlborn, Pike, Hendrix, Hess, & Huber, 2005; Hager & Arendt, 2013). NSPs have the potential to increase the viscosity of food products so they are expected to alter the accessibility of starch granules to the digestive enzyme (Brennan, 2005). The effect of NSPs (xanthan gum, guar gum, pectin, and konjac glucomannan) on *in-vitro* digestibility of starch suspensions and gelatinized starch has been previously evaluated, for example, the addition of xanthan gum at ≥1% has been found to decrease starch digestibility in a breads (mixed system of starch and NSPs), and it was a more pronounced suppressive effect, which was concentration-dependent (Sasaki, 2018).

2.10.5 By altering the secondary structure

Breads are highly susceptible to enzymatic digestion because the endosperm becomes fine during milling into flour and the starch gelatinised during cooking. In addition, the bread matrix is highly porous and disintegrates rapidly in the gut. This aids in the rapid penetration of digestive enzymes and subsequent digestion. Starch digestion is relatively slow in food matrices with dense and low porosity, as it is related to food geometry, because little enzyme penetration is possible, and any digestion is dependent on surface area (Monro et al., 2011). Dense foods such as pumpernickel have grains soft enough to be swallowed partially intact, which allows the influence of surface area on digestion rate to be retained (Burton, Monro,

Alvarez, & Gallagher, 2011; Jenkins et al., 1988). Moreover, white breads, brown breads and pastas are made from similar ingredients, but the GI of the pasta is much lower than that of the breads, due partly to the density of pasta, which is less porous than bread and requires superficial enzymatic erosion (Monro et al., 2011). These techniques are useful in delivering CHO without an acute postprandial blood glucose response (Mishra et al., 2012).

2.10.6 By starch modification

Native starches are modified physically, chemically and enzymatically to be used by the industries practically in all starch applications. Much of the literature focuses on discussing new functional properties that are suitable for various industrial applications, while the focus here is on modifications that were made to increase SDS and RS fractions, with an emphasis on the emerging physical treatments. Enzymatic modifications are performed by hydrolysing α-1-6 linkages of amylopectin with debranching enzymes such as pullulanase and isoamylase (Ick Shin et al., 2004) to form linear chains, which are then retrograded to increase SDS or RS fractions. Hydrolysis time and enzyme concentration play an important role in producing SDS and RS fractions. High enzyme concentrations (20 or 40 ASPU/g) and shorter debranching times (3 to 6 h) increased the amount of SDS, whereas longer times accelerated the production of RS (Miao, Jiang, & Zhang, 2009). Change in enzyme concentration altered the level of imperfect packing helices in crystallites, hence altering the proportion of amorphous and ordered packed regions that are associated with SDS. Maize starch was subjected to dual-enzymatic modification with β-amylase and transglucosidase (0.5% and 8 TGU/g, respectively) for 6 h. This enzymatic modification not only increased the number of short chains but also increased the proportion of α-1-6 linkages, which led to slower digestion (Miao et al., 2014).

Chemical modifications including cross-linking, hydroxypropylation, acetylation, and esterification which involves the addition of functional groups within the starch molecule, have been successfully employed to change starch structure to reduce starch digestion by increasing SDS and RS fractions (Han & BeMiller, 2007). However, such procedures give rise to issues concerning consumers and the environment. Therefore, there is intense interest in developing novel methods of starch modification to reduce starch digestibility (Kaur, Ariffin, Bhat, & Karim, 2012). Physically modifying starch is simple, cost-effective and environmentally friendly. The most common processes used for physical modification include heat-moisture treatment, high-pressure treatment, extrusion, microwave irradiation, ultraviolet light

irradiation, and radiation. Heat-moisture treatment requires low moisture levels (<35% w/w water) and high temperatures (100°C to 130°C) for a wide range of time (15 min to 16 h) (Zavareze & Dias, 2011) to obtain SDS that is intended as an additive for foodstuffs. Ultra-High Pressure induces partial gelatinization in which the crystalline regions of starch granules undergo incomplete disintegration, showing upwardly shifted gelatinization temperatures, which is a common characteristic of annealed starch granules, due to the changes in the starch polymorphism (A to B), which will produce SDS.

2.11 Starch digestibility and glycaemia

Various food macromolecules have different glycaemic potencies and induce a different glycaemic response. Highly proteinaceous foods such as meats and eggs contain very little CHO (Moghaddam et al., 2006) and are not associated with glycemia. Fats or soluble dietary fibre will reduce and delay the glycaemic response by slowing gastric emptying (Øverby, Sonestedt, Laaksonen, & Birgisdottir, 2013; Welch, Bruce, Hill, & Read, 1987) or because proteins and fats mask the starch causing slower digestion. Foods containing a high proportion of SDS and RS show lower post-meal blood glucose and insulin responses in comparison with the foods with a higher proportion of RDS content (Englyst et al., 1992; Zhang & Hamaker, 2009; Zhang, Ao, et al., 2006). RS is not affected or is very slowly affected by the enzyme action of α-amylase and is not absorbed in the intestine. However, it can be fermented by the colonic microflora (Englyst et al., 1992). The rate of starch digestion for RS is very slow, and it has a very little impact on the postprandial blood glucose. Recent research indicates that a diet characterised by SDS or RS has beneficial metabolic effects and improve glycaemic control.

2.12 Objectives of the thesis (Breads and their contribution to glycemia)

About 65% of our daily energy should come from carbohydrates, mainly from starch (Efsa Panel on Dietetic Products & Allergies, 2010). Bread is one of the major components of a balanced diet that contributes substantially to daily requirements of most nutrients and considered as an essential food (Bautista-Castaño & Serra-Majem, 2012; O'Connor, 2012) and its composition has been extensively studied. Several innovations have been performed to improve the nutritive value of bread and have transformed them into different types with varying characteristics (Wahlquist, 2010). Bread is made from cereal grains such as wheat, rye, soy, so its nutrient content is largely determined by the content of the grains and type of flour

used. According to Watson (2014), usage rates for wholemeal bread consumption increased by 7.5%; however, the consumption of white breads decreased by 11% in the US between 2004 and 2013. Adults of about 60% in the US (Watson, 2014) and 14% in New Zealand (Ministry of Health, 2015) eat breads with higher grain content. NZ has various products labelled "whole grain, multi-grain, stone-ground, 100% wheat, cracked wheat, including; breads, breakfast cereals, rice and pasta, that may not contain any intact whole grain particlesand where most of the particles are finely gound flour. Bread consumption in New Zealand is high in comparison to Australia, the UK and US with an average of about 1.4 loaves per person per week (Monro, 1994). Since the 1980s consumption of white bread has reduced: in 1981 75% ate white bread but in 1991 less than 50% did so. Since whole intact grains contain a lower proportion of starch than white flour and the starch whole grains contain is less accessible to digestive enzymes it is believed that breads containing whole grain flour also have a lower glycaemic response. Wholegrain breads are therefore often claimed to be "healthier" than white bread, due to the low proportion of CHO and intact or partially intact grain content, therefore also be assumed to have a lower glycaemic potency (Juntunen et al., 2002; Mofidi et al., 2012). Incorporating grain particles which limited the degree of starch digestibility in grain particles has been attributed to insoluble fibre (cell walls) acting as a fibrous network protecting the starch (Fardet, Leenhardt, Lioger, Scalbert, & Remesy, 2006).

Markets in New Zealand offer many breads, ranging from standard white brands containing up to 65% intact hydrated grain (**Table 2.1**). However, the role and contribution of intact/partially intact grains in reducing the glycaemic potency of the bread require further research. Also, there is little information on the detailed role of the physical factors of starch and grains that contribute to a lower glycaemic potency. Therefore, this research focussed on determining if the addition of kibbled grains can lower the digestibility of the starch and glycaemic potency of breads. Furthermore, aspects of the grain such as particle size, the degree of hydration, the degree of gelatinization were investigated. The knowledge gained in this work was used to develop breads with high proportions of large intact grains and was tested for glycaemic potency *in-vivo*. Later in this work, the effect of the addition of non-flour components such as proteins, NSP's and fibres that may alter the digestibility of the starch in breads was investigated. The flow of this thesis is given in **Figure 2.9**.

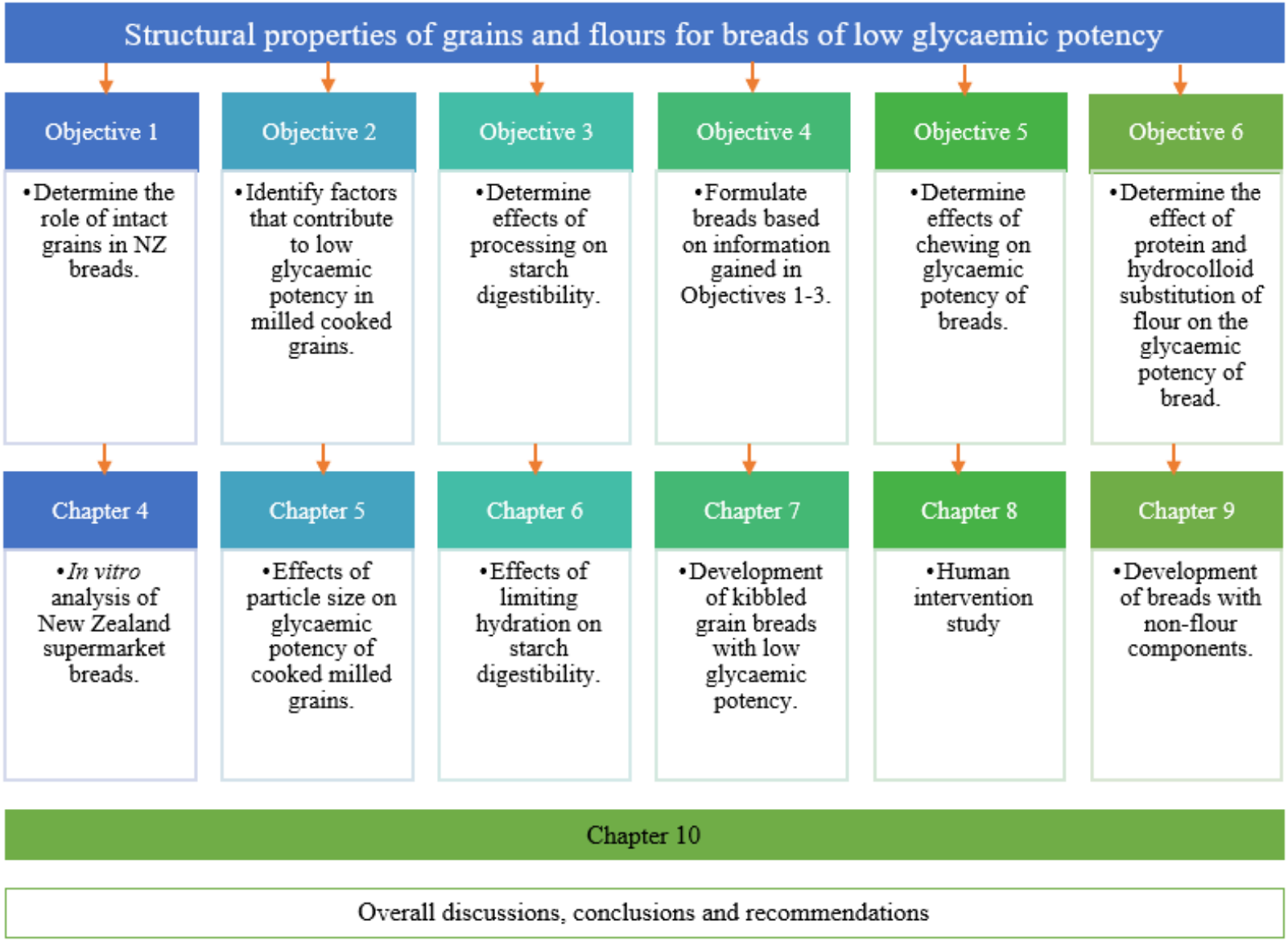


Figure 2.9 Flow diagram of the thesis

Table 2.1 List of breads available in the New Zealand market and their speciality and formulations.

Breads	Speciality	Major ingredients
Countdown	Wholemeal and grain	Wheat, soybeans, linseed, wheat bran
Countdown	Sandwich loaf	Wheat flour
Countdown	Four seed loaf	Wheat flour, Linseed, canola, sesame, poppy, soy
Home brand Woolworths	White bread Sandwich slice	Wheat flour, soy flour.
Nature's fresh	White toast	Wheat flour, soy flour, wheat fibre
Nature's fresh	Multi grain	Wheat four, mixed grains
Signature range	Wheat meal	Wheat flour and -wheat bran?
Tip top	Whole meal	Whole meal wheat flour, wheat flour, Wheat gluten, soy flour.
Tip top	Whole grains	Wheat flour, mixed whole grains, kibbled wheat, rye, purple wheat.
Tip top	9-grain seed	Linseed, wheat grain, corn grits, oat bran, sunflower seeds, whole grain wheat, barley, and rye
Tip top	Soy and linseed	Kibbles soy and linseed
Mackenzie	Purple wheat grains	Purple wheat kibbled, South Island grains, sesame seeds, kibbled soy

Signature range	Multigrain	Wheat, rye oat
Mackenzie	Seed and grain	Kibbled barley, whole grain oats, kibbled wheat, rye, sesame seeds, kibbled soy
Ploughman's bakery	Pumpkin and sunflower seeds	Wheat, pumpkin, sunflower seeds
Ploughman's bakery	Otago oats and seeds	Wheat and oats
Vogel's	Original mixed grain	Wheat flour. Mixed grain (24% wheat and rye), wheat gluten
Freya's	Tuscan Mixed Grain	Wheat flour (white and wholemeal), grains and seed (22% red wheat, purple wheat, rye, linseeds, corn), wheat gluten, soy flour
Freya's	Soy and Linseed (40% fewer carbs)	Wheat flour (white and wholemeal), kibbled soy (12%), linseed (8%), soy flour, sesame seeds
Burgen	Soy and Linseed	Wheat flour, kibbled soy (10%), linseed (10%), kibbled wheat (5%), wheat gluten
Molenberg	Grain plus	Wheat flour (white and wholemeal), kibbled grain (29% wheat, rye), seed mix (2% linseed, sunflower seed, sesame seed), soy flour
Molenberg	Light grains	Wheat flour (white and wholemeal), kibbled grains (6% wheat, rye), wheat gluten, wheat fibre, soy flour
Molenberg	Super thick	Wheat flour, kibbled grains (23% wheat, rye), oat bran (3%) wheat gluten, soy flour
Bolletje Pumpernickel	Rye bread	Broken wholemeal rye, wheat bran, wheat flour
Liberte	Wholemeal gluten free	Modified tapioca starch, rice flour, maize starch, sorghum flour, besan flour, linseed, guar gum, tara gum, flax fibre
Burgen	Mixed grains	Wheat flour, mixed whole grains (15%) (kibbled wheat, kibbled purple wheat, kibbled rye), wheat gluten, chia seeds

Chapter 3

Chapter 3: Experimental techniques

3.1 Breads

3.1.1 Moisture content

Moisture contents were determined for a sample by oven-drying at 105 °C to a constant weight (AOAC, 1995).

3.1.2 Proportion of intact/partially intact grain particles in breads

The grain particle content in breads was measured as the dry weight of particulate material retained on a 1 mm sieve after pepsin digestion. The pepsin digestion loosened the cooked gluten structure of the bread allowing the easy collection of particles by washing. Approximately 30 g of fresh bread with crusts removed were weighed and subjected to simulated gastric digestion using 50 ml of 0.2% pepsin-HCl at pH 2.5, for 30 min with constant stirring at 130 rpm. The resulting slurry was washed with water in a 1 mm wire mesh sieve, and retained particles (> 1 mm in diameter) were washed free of adhering bread matrix particles with a stream of water. The retained particles were freeze-dried before weighing and calculating the proportion of the whole freeze-dried bread sample. The results were expressed as the proportion of intact grain particles of > 1 mm in 100 g of dry bread (Srv et al., 2019).

3.1.3 *In-vitro* amylolysis of bread

The rate of *in-vitro* amylolysis of the bread samples was measured as the rate of appearance of sugars in a standardized *in-vitro* digestion system using the method of Monro and Mishra (2010). It involves two-step digestion, gastric followed by ileal.

Bread samples (5 g) were digested in 70 ml specimen pots (LabServe LBS 30002) inserted to their full depth in a 15-place aluminium heating block, covered with a single insulating sheet, and placed on a 15 place magnetic stirrer (**Figure 3.1**). Samples of breads with 30 ml of deionized water were either homogenized for 2 min with an UltraTurrax Homogenizer (IKA®-Werke, GmbH & Co.KG, Staufen, Germany) with an S18N-19G dispersing element or were left unhomogenized and essentially intact. Homogenized samples were checked to ensure that all of the samples passed through a 1 mm sieve and hence that homogenizing was effective.

Figure 3.1 *In-vitro* digestion pots

For the simulated gastric phase, 0.8 ml of 1 M HCl diluted with 30 ml of MilliQ water and 1 ml of a 10% (w/v) solution of dry pepsin powder (Pepsin EC 3.4.23. from Porcine stomach mucosa, P 7000, Sigma-Aldrich, USA; 800-2500 U/ml) dissolved in 0.05 M HCl were each added to the sample, the pH of the suspension was adjusted to 2.5, and the sample stirred at 130 rpm at 37 °C for 30 min. The small intestinal phase was initiated by neutralizing the gastric HCl with 2 ml of 1M $NaHCO_3$ and 5 ml of 0.1 M Na maleate buffer pH 6/0.2% Na azide/1 mM $CaCl_2$ (**Figure 3.2**), and the pots were immediately made to the 55 ml mark with distilled water. Starch digestion was commenced by adding, in quick succession, 0.1 ml amyloglucosidase (EC. 3.2.1.3. from A niger, Megazyme, E-AMGDF; 3260 U/mL) and 1 ml of 5% pancreatin (P7545, Sigma-Aldrich, USA; 8 x USP specifications) in 0.1 M maleate buffer pH 6, Digesta aliquots of 0.5 ml were removed before adding the amyloglucosidase-pancreatin (0 min) and at 20 and 120 min from the start of amylolysis, and were each added to 2 ml absolute ethanol in a tube to give a final concentration of 80% ethanol, and immediately mixed thoroughly. After at least 30 min the tubes were centrifuged for 10 min at 1000 *g* at 20°C (Centrifuge Omnifuge 2.0 RS Heraeus Sepatech) to clarify the supernatant before analysis of sugars released during digestion.

Available carbohydrate content of the digesta was measured as 80% ethanol-soluble sugar and dextrins released into the digestion medium at 120 min after adding the digestive enzymes, using secondary amyloglucosidase digestion to convert the 80% ethanol-soluble starch digestion products to glucose. Potentially available carbohydrates for breads were measured as glucose released after 1 h of further incubating the 120 min sample after it had been

homogenised and 0.1 ml amyloglucosidase added to depolymerise any remaining digestible carbohydrate.

Note: The *in-vitro* digestive analysis for different concentrations of pancreatin suitable for the study was performed with white and mixed grain breads (**Appendix A)**. Evaluation of the DNS assay for chromogenic interferences in the samples showed there to be none. The efficiency of the pancreatic concentrations on carbohydrate recovery at 120 min was calculated and compared to the carbohydrate's values given in the nutrient information panel (**Appendix B**).

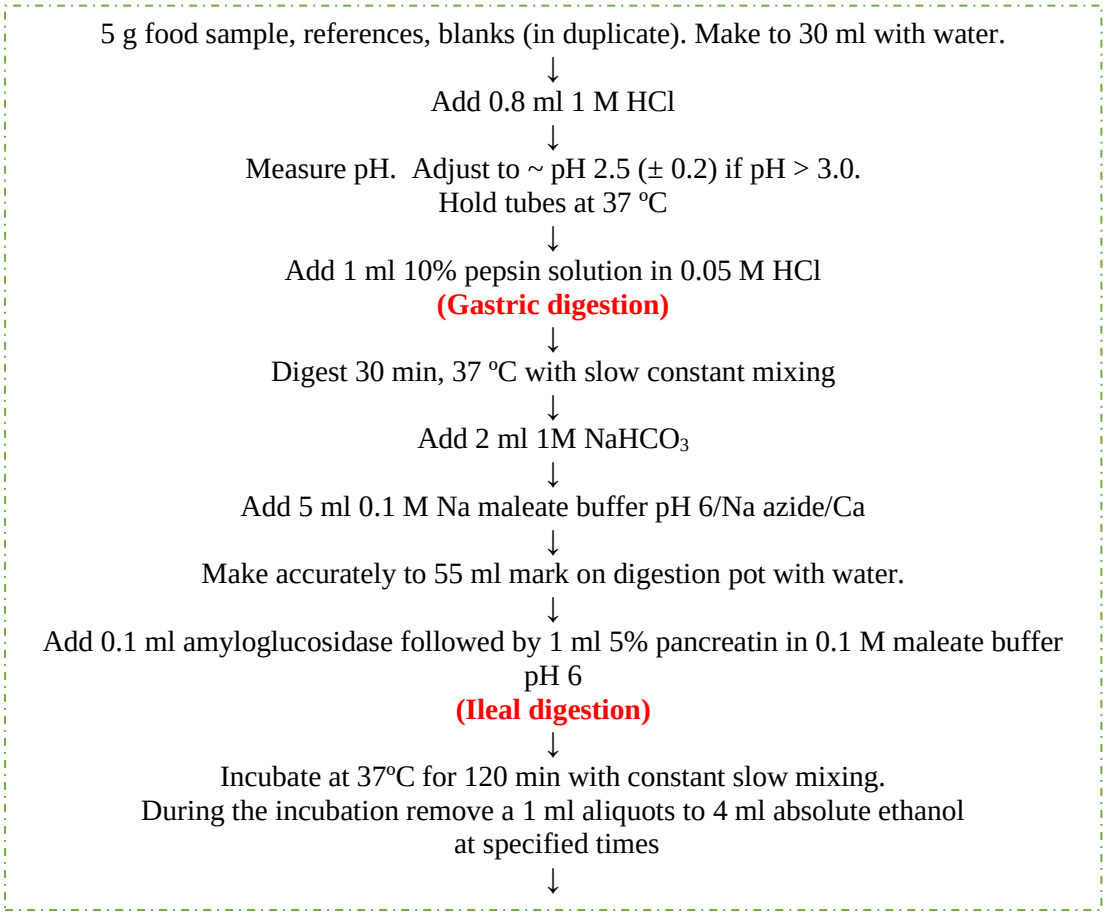


Figure 3.2 The *in-vitro* amylolysis method used throughout this thesis as a template, to which systemic modifications were made.

3.1.4 Measuring sugars released during *in-vitro* digestion

Sugars released during digestion were measured as monosaccharides by a small-scale modification of the dinitro salicylic acid (DNS) colorimetric method after an invertase + amyloglucosidase secondary digestion. A 0.05 ml aliquot of ethanolic sample from the *in-vitro* digestion, or glucose standard (5 or 10 mg/ml glucose), was added to 0.25 ml of 1% (w/v) amyloglucosidase (Megazyme E-AMGDF) + 1% (w/v) invertase concentrate (BDH 390203D) in acetate buffer pH 5.2 and incubated at 37ºC for 10 min to complete depolymerisation to

monosaccharides. Reducing sugars were then measured by adding 0.75 ml DNS mixture (containing a 1:1:5 mixture of 0.5 mg/ml glucose:4M NaOH: DNS reagent) and heated 15 min at 95-100 ºC. The DNS reagent consisted of 10 g of 3,5-dinitro salicylic acid dissolved in 1.0 L of a solution of 300 g Na-K tartrate and 16 g NaOH. The tubes were cooled, 4.0 ml water added, mixed, and absorbances read at 530 nm (Jenway 6100 Spectrophotometer) (Monro & Mishra, 2010).

3.1.5 Starch fractions

The rate and yield of starch digested were estimated using the *in-vitro* digestion method described in **Section 3.1.3.** RDS is of particular interest in our study because it is the major contributor to glycaemia and may be used to predict the glycaemic response (Englyst et al., 2003). Formulations for the breads used include sugars, and for this reason, the 0 min values from the DNS assay were subtracted from later sample values.

RDS was taken as reducing sugar measured in the 20 min aliquot of digesta after the secondary digestion, SDS as reducing sugar after 120 min (RDS + SDS) minus RDS. RS was estimated by the difference between RDS+SDS and total starch (Englyst et al., 1992). The starch digested was plotted against time (min) and rate (g/100 g starch) calculated as a linear regression of these data.

Total starch was determined by gelatinizing a subsample (100 mg) in dimethylsulphoxide using Megazyme Total starch AOAC 996.11 Method (Edwards, Warren, Milligan, Butterworth, & Ellis, 2014). The sample is incubated with dimethyl sulphoxide to gelatinize the starch, which is then completely solubilised by controlled incubation at 100°C with 0.1 ml of α-amylase. The remaining starch is then solubilised, and the starch particles are converted to sugars with 0.1 ml of pancreatin and 0.1 ml of amyloglucosidase incubation followed by DNS assay.

3.1.6 Glycaemic potency

The glycaemic potency of samples was measured from the analytical glucose equivalents (GE) measurements, and it is summarized in **Figure 3.3**:

1. The analytical GE results obtained from the DNS assay were adjusted by the GI of the contributing sugars and the mass of a customarily consumed portion of the food (**Figure 3.3 A**). This was mainly to allow for the fact that fructose and sucrose have a much lower

relative glycaemic effect than glucose (Foster-Powell, Holt, & Brand-Miller, 2002), and to convert the *in-vitro* quantity to realistic human intake. In the present study, in which nearly all of the CHO was glucose (GI = 100 %) derived from starch, the GI adjustment was not required.

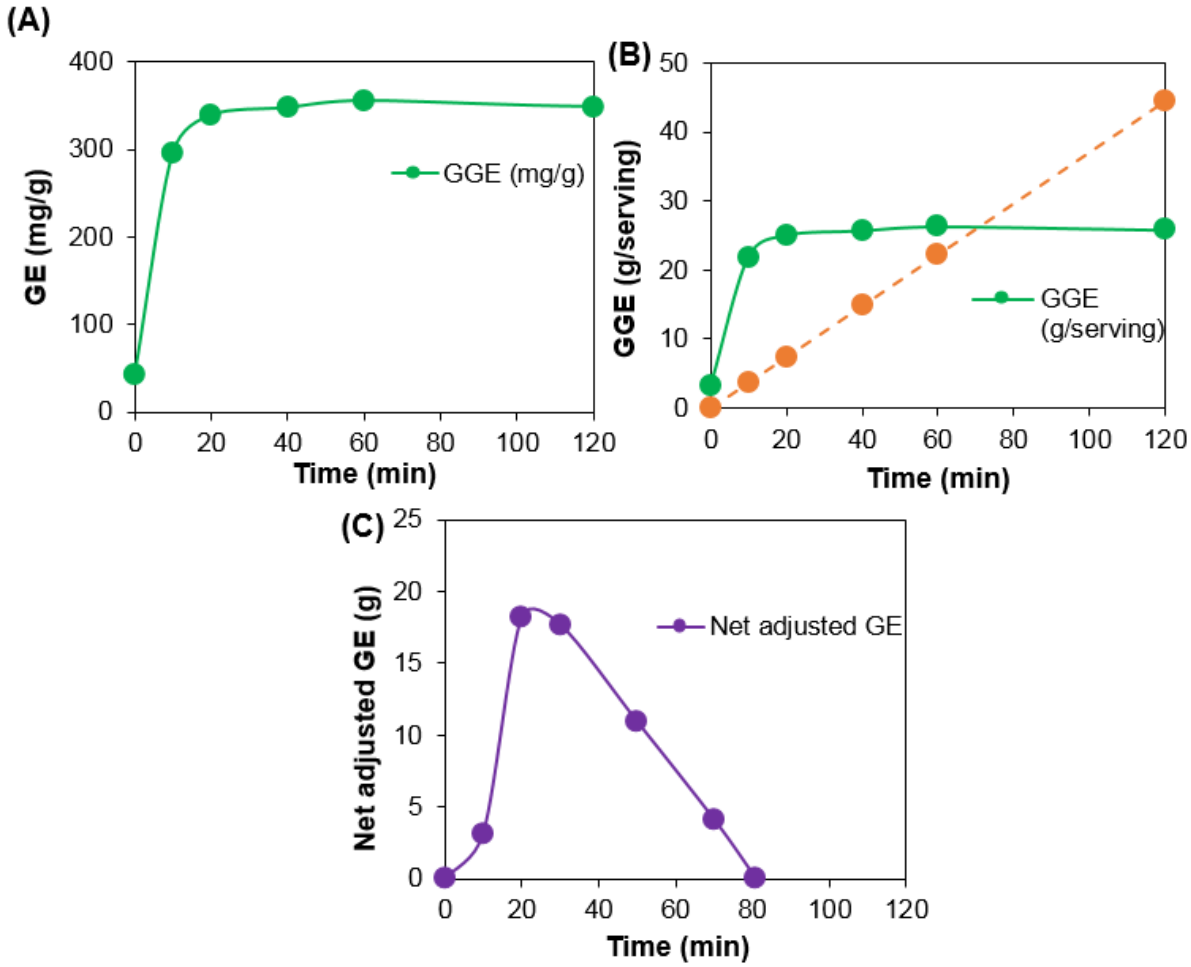


Figure 3.3 Simulated blood glucose response curves for a serving of wholemeal bread based on the *in- vitro* digestion curves. (A) Sugar release (GGE mg/g) during *in-vitro* digestion (B) Subtracting the GD from GGE (g/serving) and (C) theoretical *in vivo* blood glucose response.

2. The likely relative glycaemic response to the released glucose equivalents was estimated by taking into account concurrent apparent glucose disposal (GD) that would accompany the cumulative release of free sugar during digestion at each time point using **Equation 3.1** (Monro, Mishra, & Venn, 2010a):

$$GD = 0.0135\ x + 0.0232 \qquad \text{Equation 3.1}$$

$$\text{Glycaemic potency} = (GI * \%\ CHO) * (\text{portion weight}) \qquad \text{Equation 3.2}$$

where x was the GI/serving size-adjusted GE release at 40 min (**Figure 3.3 B**). The 40 min adjusted GE value was used in the rate equation because it represents a point at which most of the easily accessed carbohydrate has been digested, and it is the point on the *in-vitro* digestion curve that corresponds to peak *in vivo* glycaemic response (30-40 min) after which maximum net glucose disposal can be determined from the linear decline in postprandial blood glucose.

3. The GD value was subtracted from the cumulative adjusted GE value at each time point to obtain the net adjusted GE value (Monro, 2010 #107). This represents the glucose that would be able to contribute to the glycaemic response.
4. The net adjusted GE values (i.e. theoretical *in vivo* blood glucose responses) were plotted against time to obtain a simulated blood glucose response curve (**Figure 3.3 C**). The area under that curve (AUC) represents the proportion of starch in the food appearing in the blood and was calculated using the trapezoid summation technique Tai (1994). The glycaemic potency (in terms of GGE) of the various breads was then determined on an equal weight basis by comparing the AUC of the bread with the AUC of a white bread reference of known GGE content. The GGE content of the reference was estimated as GL and calculated from its GI, the content of CHO, and portion size (**Equation 3.2**) (Monro & Mishra, 2010; Monro et al., 2010a; Monro & Shaw, 2008).

3.2 Grains

3.2.1 Dehulling and milling of seeds

Grains were passed through a Kenwood Grain Mill AT941A to coarsely grind the seed and facilitate separation of the seed coat. The seed coat was winnowed from the endosperm using an Ozito 2000W Variable Temperature Heat Gun in the coolest setting. The seeds were milled using a Kenwood Grain Mill AT941A with variable feed rate which had an adjustable feeder and two fluted rollers running at different speeds. These rollers crushed the seeds into smaller size fractions. The gaps between the rollers can be adjusted by screws to get the desired size of broken seeds.

The milling was done in two stages. During the first stage, the grains were broken into larger kibbles, and unbroken seeds as the gap between the rollers were set at 3.0 mm. During the second stage of the milling, the gap between the rollers was reduced by adjusting the screws.

This produced a smaller size fractions**.** The milled samples were passed through vertically mounted multiple sieves (3.3 mm, 2.8 mm, 2 mm, 1.2 mm, 0.6 mm, 0.3 mm, 0.1 mm aperture) which were shaken by a shaker (Model RX-6-1, W.S Tyler, 8570 Tyler Blvd., Mentor, OH 44060, USA) for 5 minutes. These sieve sizes were selected to produce six sizes denoted as: >2.8 mm, >2 mm, > 1.2 mm, > 0.6 mm, > 0.3 mm and > 0.1 mm according to the upper and exact sieve apertures. These particle sizes were chosen in order to cover a broad spectrum of particle size that occurs *in-vivo* post-mastication of edible plant tissues (Ranawana, Monro, Mishra, & Henry, 2010). The sample fractions remaining on the sieves were collected and labelled for the experiments.

3.2.2 Characterization of food materials

3.2.2.1 Fat, protein, dry matter, ash and moisture content

The fat content of the seeds was estimated by the Soxtec solvent extraction method (Thiex, Anderson, & Gildemeister, 2003) and was carried out by the TELARC accredited Nutrition Laboratory at Massey University**.**

The protein content was estimated by the Dumas method (Horwitz & Latimer, 2005) using the TruSpec CN analyser (LECO Corporation, St. Joseph, MI, USA). The protein analysis was performed by the TELARC accredited Nutrition Laboratory at Massey University. The crude protein content of the sample was obtained by multiplying total nitrogen content with a suitable factor; in general, 5.83 is used**.**

The dry matter was determined by placing approximately 2 g of ground sample spread evenly on an aluminium pan at 105°C in an oven (Contherm Scientific, New Zealand) for 16 h (AOAC, 2005)**.** The ash content is determined by placing approximately 2 g of the ground sample at 500°C in an oven (Contherm Scientific, New Zealand) for 16 hrs (AOAC, 2006a)**.** Moisture contents were determined, as mentioned in **Section 3.1.1**.

3.2.2.2 Sugar, starch content and fibre content

The sugars and starch content was obtained from the *in-vitro* digestive analysis was quantified by the DNS assay **(Section 3.1.4)**. The total starch content of the cereals was determined as described in **Section 3.1.5**. The dietary fibre was calculated by the AOAC 991.43 method (AOAC, 2006b) and AOAC 994. 13 method (Theander & Åman, 1982).

Briefly, 1 g of ground samples were subjected to sequential enzymatic digestion in a volume of 40 ml by adding 0.1 ml of heat-stable α-amylase, 0.2 ml of pepsin and 0.05ml of amyloglucosidase, with appropriate pH adjustment at each stage. The digest was filtered and the supernatant treated with four volumes of 95 % ethanol to precipitate soluble fibre (SDF) and remove depolymerised protein and glucose (from starch).

The residue from filtration was washed with 78% ethanol, 95% ethanol, and acetone, dried, and weighed to provide insoluble dietary fibre (IDF). The SDS filtrate was washed with ethanol, acetone, dried and weighed The dried precipitates of IDF and SDF were hydrolysed in 12 M sulphuric acid for 1 h at 37° C which was followed by hydrolysing in 1 M sulphuric acid and heating it for 1 h at 100° C individually.

The sugars were measured colorimetrically by the DNS assay **(Section 3.1.4).** Total dietary fibre determination was the sum of the insoluble dietary fibre and soluble dietary fibre.

3.2.3 *In-vitro* starch digestion

The starch digestibility was measured using 0.2 g of samples, weighed into the 10 ml glass tubes with 4 ml of deionised water and mixed thoroughly followed by the addition of 2 ml 0.1 M Na maleate buffer pH 6/Na azide/Ca and 4 ml of 2% NaCl solution with 0.1% $CaCl_2$. The tubes were placed in an overhead shaker (Heidolph REAX 2) at 37ºC and rotated at speed setting 1. pH adjustment was performed if necessary, with 1 M NaHCO3 to maintain the pH 6.5 (± 0.2). Illeal digestion was started by adding 0.05 ml of 5% pancreatin (Sigma P-7545) in 0.1 M maleate buffer pH 6 and 0.02 ml of amyloglucosidase. Digesta aliquots of 0.05 ml were removed before adding the pancreatin (0 min) and at required intervals followed by the DNS assay **(Section 3.1.4)**. Successive 0.5 ml aliquots of digestate were taken at required time intervals following the addition of the amylolytic enzymes. Actual and potentially available carbohydrates were measured as given in **Section 3.1.5**.

3.2.4 Modelling of starch digestogram

Starch amylolysis was examined using two models for the milled cereal fractions: Logarithm of slope model and Englyst Model. Starch digestibility curves gave the proportion of glucose equivalents released during the 120 min of pancreatic digestion for milled particles.

3.2.4.1 **Logarithm of Slope (LOS) model**

According to Butterworth et al. (2012), the LOS model was based on the 1st order reaction kinetics of amylolysis, and the starch digestibility curves were fitted to a first-order equation (**Equation 3.3**). This model assumes there is continuous digestion of starch from large particles over the entire period of starch digestion, over two phases, but without the involvement of two distinct classes of starch, RDS and SDS, as proposed by Englyst et al. (1992). $C_{i(t)}$ is the starch fraction digested at digestion time (*t)* in the *i*th particle size; k_i is the fractional-digestion rate coefficient (min^{-1}). The values of C_∞ and *k* were obtained from Logarithm of Slope (LOS) plots, as described previously (Butterworth et al., 2012). Data obtained from the digestion of larger size-fractions was fitted to the modified first-order equation proposed by (Edwards et al., 2014). The modification accounts for the biphasic nature of starch digestion in heterogeneous particles, e.g., where the starch is encapsulated within intact plant cells or when some of the starch is less digestible (SDS and RS). In such materials, LOS plots reveal two or more distinct near-linear phases (rapid and slow phase), each represented by a set of enzyme-kinetic parameters which must be accounted for **Equation 3.4 and Equation 3.5** to describe the overall digestion process.

$$C_{i(t)} = 1\text{-}e^{-k_i t} \qquad \text{Equation 3.3}$$

$$C_{i(t)} = C_{1\infty}(1\text{-}e^{-k1t}), \text{ if } t \leqslant t_{int} \qquad \text{Equation 3.4}$$

$$C_{i(t)} = C_{int} + C_{2\infty}(1\text{-}e^{-k2\times(t\text{-}tint)}), \text{ if } t \geqslant t_{int} \qquad \text{Equation 3.5}$$

Starch amylolysis is represented by two consecutive linear phases, two time points for each reaction phase and C_{int} and t_{int} is the concentration and time at the intersections between the two phases. Distinct enzyme kinetic parameters are obtained for each phase (denoted $C_{1\infty}$, $C_{2\infty}$ and k_1, $k_{2)}$ using Logarithm of Slope (LOS) analysis. LOS plot analysis was performed using data expressed as a percentage of total starch. The LOS only provides a good fit over the first 20 min of digestion as is described in detail in **Chapter 5**.

3.2.4.2 **Englyst Model**

As mentioned in **Section 2.8**, Englyst et al. (1992) proposed starch digestibility with defined phases such as RDS, SDS and RS. First-order kinetics was plotted for all the particle fractions for cereals in the first 20 min of the digestion as it is the most crucial stage in which the RDS

digests rapidly (Englyst et al., 1992) and has the most acute effect on blood glucose response *in-vivo*.

3.2.5 Glycaemic potency

The glycaemic potency of grains was derived from the analytical glucose equivalents (GE) measurements (**Section 3.1.4)**.

3.2.6 Volumes of particles during digestion

The milled grains were carefully sieved to obtain particles of uniform diameter and were assumed to be spherical**.** After cooking, spherical samples for digestion were placed into a small measuring cylinder and their volumes determined by fluid displacement. Particles were counted into a measuring cylinder, their volume determined by displacement, and the mean particle diameter (D) was calculated from the mean volume (V) per particle, based on the formula for a sphere (**Equation 3.6**) (Monro et al., 2011).

$$D: (6V\pi^{-1})^{1/3} \qquad \text{Equation 3.6}$$

The samples were then digested, as mentioned in **Section 3.2.3**. The volume and diameter of the samples were measured before adding the pancreatin (0 min) and after adding the pancreatin at 20 min and 120 min.

3.2.7 Pasting Characteristics

Paste consistency profiles were determined using a Rapid Visco Analyzer (RVA) (Series 4, Newport Scientific, Warriewood, NSW, Australia). Cooked (treated) samples for each grain type and particle size before digestion were dried in an oven at 40 ° C until a constant weight was obtained and were ground and passed through a 0.5 mm sieve before analysis.

The paste consistency profile for native and treated samples was measured using the ground grain sample of 3 g (12% w/w) with distilled water added until a final weight of 25 g ± 0.01 g was reached. Each sample was held at 50 °C for one min and stirred at 960 rpm for 10 s followed by constant stirring at 160 rpm, heating to 95 °C at 4°C /min, holding at 95 °C for 3 min, cooling to 50 °C in 3 min (Al-Rabadi, Torley, Williams, Bryden, & Gidley, 2011).

3.2.8 Thermal characteristics

A Perkin-Elmer DSC 7 operated by Pyris software (Perkin-Elmer Instruments, Norwalk, CT) with an internal coolant and nitrogen purge gas was used to measure the thermal properties of the particles. Tzero hermetically sealed aluminium pans were used to study the thermal behaviour of test samples. The instrument was calibrated for temperature using indium and tin standards. Treated and native flour samples of approximately 5 g were accurately weighed in duplicate into a pan, each with 15 mg of deionised water, mixed, sealed and left at least an hour to equilibrate. The weight of milled material added was adjusted based on starch content so that all pans contained 2 mg of starch (d.w.b). Samples were heated from 30 to 120°C at a rate of 5°C/min. The slow heating rate (5°C/min) was used to minimize any temperature lag due to the large mass of the steel pans. Endotherm plots with time were generated using TA Universal Analysis 2000 software (version 4.5A, 2007© TA Instrument – Waters LLC) and the onset temperature (T_o), peak temperature (T_p), conclusion temperature (T_c) and enthalpy of dissolution (ΔH_{dis}) were calculated using them (Roman, Gomez, Li, Hamaker, & Martinez, 2017).

3.2.9 X-ray diffraction

The crystalline nature of the cooked starch in grains was measured by placing a small amount of cooked, dried, ground samples in a loop with a minimal amount of Fomblin oil and mounted on a 3-circle kappa-goniostat. Samples were oscillated in phi +/- 5° at chi=0 for 5 minutes. The generator, optics and detector used in this study were a MM007 microfocus rotating copper anode generator, Rigaku-Osmic multi-metal-layer focussing and monochromating optics (giving Cu –alpha radiation) and Spider-Rapide wrap-around image-plate detector, respectively. The samples were fixed to the detector at a distance of 124.1 mm. Software used for data collection, processing and display were Rigaku Rapid, Rigaku 2DP (background correction and reduction from 2D to 1D) and CrystalDiffract, respectively (Dankar, Haddarah, Omar, Pujolà, & Sepulcre, 2018).

3.3 Data analysis

All data manipulations were conducted in a Microsoft Excel spreadsheet (Microsoft Corp., Redmond, WA, USA). All experiments were conducted in duplicate. Analysis of variance and Pearson correlation coefficients for the relationship between different parameters was carried

out using SPSS 24.0 software (IBM Corporation, Chicago, IL, USA). Comparisons of means were determined by Tukey's test to a 5% significance level.

Chapter 4

Research article

Srv, A., Mishra, S., Hardacre, A., Matia-Merino, L., Goh, K., Warren, F., & Monro, J. (2019). Kernel structure in breads reduces *in-vitro* starch digestion rate and estimated glycaemic potency only at high grain inclusion rates. *Food Structure, 21*, 100109.

Chapter 4: Do intact grains contribute to lowering the glycaemic potency in whole grain breads?

4.1 Introduction

Incorporating grain particles into breads has been a strategy for reducing the glycaemic potency of breads as some, but not all studies have shown that increasing particle size and proportion of grain particles lowers glycaemic responses (Irika binti Idril et al., 2013; Liljeberg, Granfeldt, & Bjork, 1992). The GI of whole-grain breads has been shown to be reduced by increasing the proportion of grain particles (Jenkins et al., 1986; Liljeberg & Bjorck, 1994). In contrast, a study showed that the particle size of grain did not affect the glycaemic potency of the whole meal and white bread (Behall et al., 1999). This could be because the particles were too small, and the starch was easily accessible for digestive enzymes (Lanzerstorfer et al., 2018), causing a high glucose response.

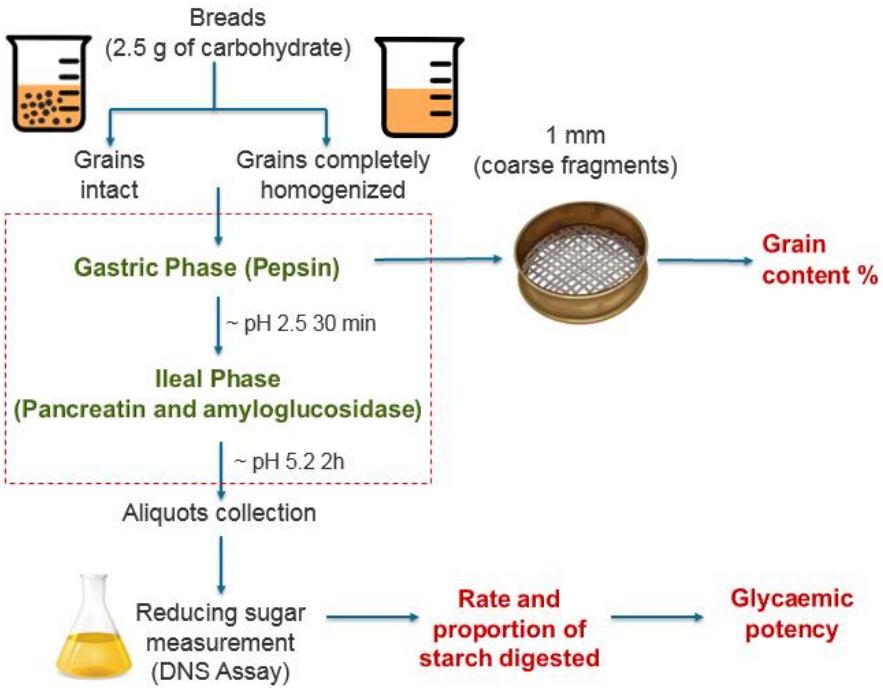


Figure 4.1 Flow chart of Chapter 4. Adapted from (Srv et al., 2019).

In the study reported here, "whole-grain breads" refers to products which are identifiable by the presence of intact or partially intact "whole" grain particles (kibbles) greater than about 1 mm in diameter, although breads labelled as "whole grain", "mixed grain" or "multi-grain" differ widely in texture and in the proportion of grain particles they contain, with values ranging from 5% and up to 65% in the New Zealand Supermarket. However, the role and contribution

of intact grain particles in reducing the glycaemic potency of currently available whole-grain breads in New Zealand are not well documented. Therefore, the overall aim of this study was to determine if the structure in intact grain particles in the New Zealand supermarket breads is sufficient to reduce their glycaemic potency, estimated as GGE release during *in-vitro* starch digestibility, and to compare the digestibility of breads off the supermarket shelf with their digestibility after homogenizing to remove kernel structure (**Figure 4.1**).

4.2 Materials and Methods

4.2.1 Samples

Various breads marketed with or without "whole-grain" claims were purchased from New Zealand supermarkets (**Table 2.1**). The standard white bread contained no grain particles of >1 mm in size while the other samples contained between 5 and 65% of hydrated coarse grain particles (>1 mm). Grain content and starch digestibility of the breads were analyzed on the day of purchase.

4.2.2 Grain particle content

The grain particle content in the breads was measured as the dry weight of particulate material retained on a 1 mm sieve after pepsin digestion **(Section 3.1.2)**.

4.2.3 *In-vitro* amylolysis

Bread samples with all crusts excluded, were cut into equal squares containing the equivalent of 2.5 g of CHO. These were weighed and placed in 70 ml digestion pots containing 30 ml of deionised water. The samples were either homogenized (H) for with an UltraTurrax Homogenizer (IKA®-Werke, GmbH & Co.KG, Staufen, Germany) with an S18N-19G dispersing element for 2 min or were left unhomogenized and essentially intact (I). Homogenized samples were sieved (1 mm sieve) to check the effectiveness of homogenizing in reducing particle size. Digestion was conducted in two phases; simulated gastric peptic digestion and an amylolytic phase simulating digestion in the small intestine **(Section 3.1.3)**. Aliquots of digestate (0.5 ml) were taken before (0 min) and after adding pancreatic enzymes (1.0 ml aliquot of 5% pancreatin dissolved in 0.1 M sodium maleate buffer and 0.1 ml of fungal amyloglucosidase) at 10, 20, 40, 60, 120 and 180 min following the addition of the amylolytic

enzymes. Potentially available carbohydrates for intact and homogenized breads were measured as mentioned in **Section 3.1.5**. The glucose released was quantified by DNS method as given in **Section 3.1.4**.

Table 4.1 Characteristics of the breads analysed

Bread names as given on the labels	Ingredients listed on the label	Carbohydrates (g/g whole fresh bread (label value))
White toast	Wheat flour, soy flour, wheat fibre	48.4
Gluten-free	Modified tapioca starch, rice flour, maize starch, sorghum flour, bean flour, linseed, guar gum, tara gum, flax fibre	46.4
Wholemeal	Whole meal wheat flour, wheat flour, Wheat gluten, soy flour.	41.7
Super thick	Wheat flour, kibbled grains (23% wheat, rye), oat bran (3%) wheat gluten, soy flour	41.5
Mixed grain	Wheat flour. Mixed grain (24% wheat and rye), wheat gluten	40.4
Grain plus	Wheat flour (white and whole meal), kibbled grain (29% wheat, rye), seed mix (2% linseed, sunflower seed, sesame seed), soy flour	39
Light grains	Wheat flour (white and whole meal), kibbled grains (6% wheat, rye), wheat gluten, wheat fibre, soy flour	38.2
Rye bread	Broken whole meal rye, Wheat bran, wheat flour	35
Soy and linseed/Low	Wheat flour (white and whole meal), kibbled soy (12%), Linseed (8%), soy flour, sesame seeds	24.1
Soy and linseed	Wheat flour, kibbled soy (10%), Linseed (10%), kibbled wheat (5%), wheat gluten	21.1

4.2.4 The rate of starch digestion

The *in-vitro* digestion method for measuring differences in rates of starch digestion was similar to the protocol described above (**Section 4.2.3)** but with more frequent sampling over the first 20 min of digestion to obtain the digestion rate during the initial linear phase of rapid starch digestion. A reduced concentration of pancreatin (0.1 ml of 5% pancreatin in 50 ml) was used

to slow the rate of digestion to increase sensitivity to differences between the breads in digestibility. All the steps were the same as used in **Section 4.2.3** except that the simulated ileal phase was initiated by adding 0.1 ml of 5% pancreatin (Sigma P-7545) in 0.1 M maleate buffer pH 6 and 0.1 ml of amyloglucosidase to slow the rates of starch digestion. Digesta aliquots of 0.5 ml were removed before adding the pancreatin (T = 0) and at 1, 2.5, 5, 7.5, 10, 15, 20, and 120 min. Sugars released during digestion were measured using the DNS method (**Section 3.1.3)**.

4.2.5 Data analysis

All data manipulations were conducted in a Microsoft Excel spreadsheet (Microsoft Corp., Redmond, WA, USA). All experiments were conducted in duplicate. Analysis of variance and Pearson correlation coefficients for the relationship between different parameters was carried out using SPSS 24.0 software (IBM Corporation, Chicago, IL, USA). Comparisons of means were determined by Tukey's test to a 5% significance level.

4.3 Results and Discussion

4.3.1 Grain content in New Zealand breads

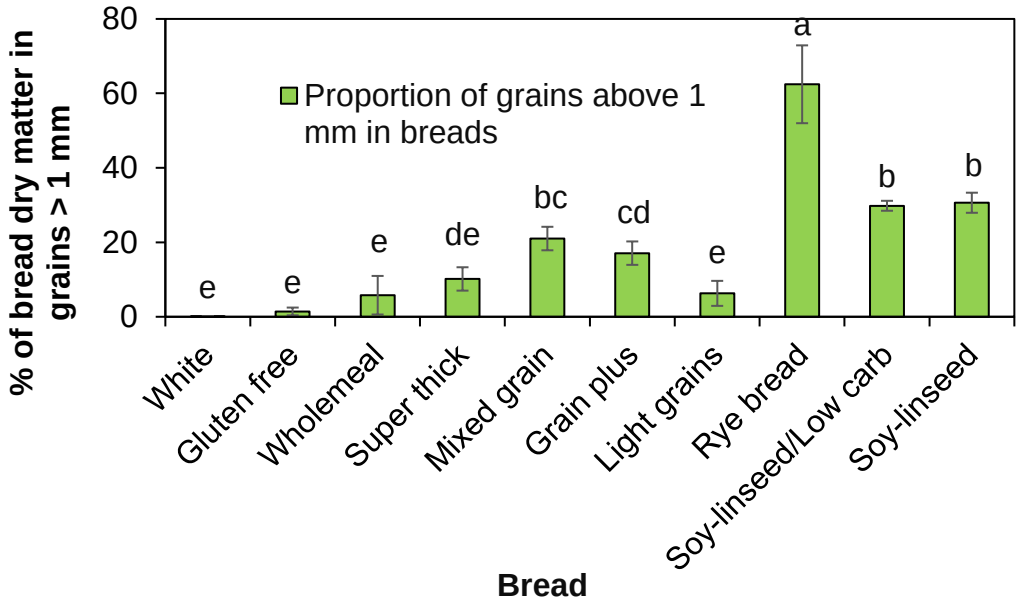


Figure 4.2 Contribution to bread dry matter (%) of particles > 1 mm. Different letters indicate significant differences between the breads ($p < 0.05$).

The proportion of intact grain particles of the sampled breads differed greatly, ranging from 0% to 65% (**Figure 4.2**). Most of the breads sampled contained less than 30% (dry weight basis) of intact grains. The rye bread (Pumpernickel) was an exception and about 65% of the dry matter content comprised of intact grain particles.

4.3.2 Effect of grain particle structure on the glycaemic potency of breads

The starch in the breads was rapidly digested by pancreatin, and the digestion profiles of the breads were similar to one another (**Figure 4.3**). The proportion of sugar released increased rapidly and linearly in the first 20 min of simulated intestinal digestion and reached a plateau between 20-120 min after adding pancreatin. In all cases, digestion of potentially available starch was about 80% complete within 20 min (**Figure 4.3**) and in most cases digestion at 10 min was greater for homogenised than non-homogenised samples. But the digestion curves for intact and homogenized breads were broadly similar except for rye bread, where the homogenized sample was more completely digested than the non-homogenized sample. The potentially available carbohydrates in the breads samples of both intact and homogenized bread after homogenizing and incubating after 120 min is denoted at "h" in **Figure 4.3**. By 180 min of digestion, nearly all of the potentially available carbohydrate had been digested in all breads (**Figure 4.3**).

The *in-vitro* study estimated glycaemic potency of 100 g of bread as the area under the net adjusted GE (adjusted GE minus glucose disposal at each time point) from time 0 to baseline **(Figure 4.4 A) (Table 4.2)**. The net adjusted GE release from intact breads tended to be less than for the homogenized samples, but differences were not significant **(Figure 4.4 A)**. As homogenization did not significantly increase GGE release from the bread, it is suggested that the proportion of grain particles >1 mm alone at inclusion rates used in the tested breads was not responsible for reducing the glycaemic potency.

Rye bread, soy-linseed/low carbohydrate and soy-linseed breads had a low adjusted GE release in comparison to other breads. The proportion of CHO in the bread was positively correlated with the calculated adjusted GE release (**Figure 4.4 B)**. Therefore, the proportion of CHO in the bread was the prime determination of glycaemic potency of the breads. The *in-vitro* GI calculated as GGE per 100 g of carbohydrates did not increase significantly as a result of homogenizing (**Figure 4.4 C**). The value for intact white bread (71.6±3.3 GGE/100g carbohydrate) was almost identical to a previously reported value of 70 for the GI of white bread (Foster-Powell et al., 2002).

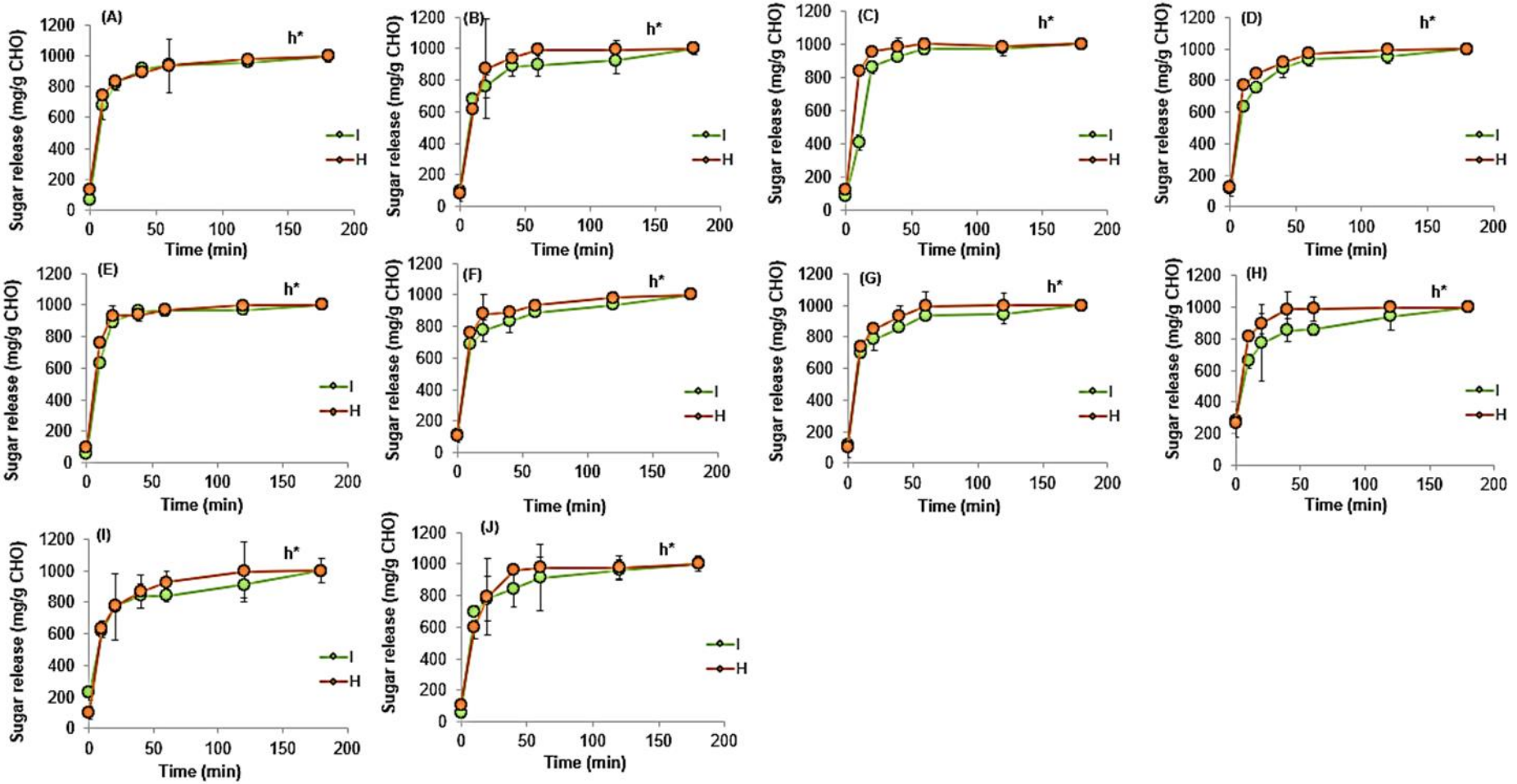


Figure 4.3 *In-vitro* digestive profile for (A) White, (B) Gluten-free, (C) Wholemeal, (D) Super thick, (E) Mixed grain, (F) Grain plus, (G) Light grain, (H) Rye bread, (I) Soy - linseed/ Low carb, (J) Soy and linseed breads digested with 1 ml of 5% pancreatin measure as intact ("I") and after homogenization ("H"). The time point (180 min) is for an aliquot taken after homogenizing after 120 min of digestion. It measures potentially available carbohydrate, indicated as (h*) on the graphs. Points are the average of 2 samples; intervals are (±) SD. CHO- Available carbohydrates.

Table 4.2 The glycaemic potency (GGE/ 100 g of bread) calculated from the area under the curve (AUC)) per 100 g bread.

Breads	AUC[1]	Glycaemic potency[2]
	Per 100 g	GGE (g/ 100 g)
Intact		
White (reference)	1156.5[a]	34
Gluten free	829.1[b]	24.3
Wholemeal	940.4[b]	27.6
Super thick	892.2[b]	26.2
Mixed grain	928.8[b]	27.3
Grain plus	845.4[b]	24.8
Light grains	815.8b	23.9
Rye bread	607.1[c]	17.8
Soy-linseed/Low carb	510.4[cd]	15
Soy-linseed	390.3[d]	11.4
Homogenized		
White	1247.1[a]	36.6
Gluten free	942.2[d]	27.7
Wholemeal	996.8[ab]	29.3
Super thick	989.2[ab]	29
Mixed grain	997.6[ab]	29.3
Grain plus	907.2[c]	26.6
Light grains	834.5[c]	24.5
Rye bread	782.3[d]	23
Soy-linseed/Low carb	486.1[d]	14.2
Soy-linseed	405.2[d]	11.9

Glycaemic potency (GGE (g)/100 g of food) = [(AUC $_{bread}$/AUC $_{white\ (100\ g)}$)* 34)]
White bread GGE = GI * % CHO. = 70 *48.4 = 33.9 (approx. 3). (Monro, 2002)
[1]Different letters indicate significant differences between the breads in each group ($p < 0.05$). [1]
[2] Significant differences between the breads in each group ($p < 0.05$) is given in Figure 4.4 A.

The breads did not differ much in glycaemic potency (GGE/100 g bread) based on the potentially available starch (GGE at 180 min digestion). The results show that if an equal quantity (100 g) of white and whole-grain breads were consumed, then the impact on blood sugar levels would be expected to differ appreciably between many of the breads independently of their differing content of grain particles, as shown in **Figure 4.4 A**. However, if an equal quantity (100 g) of CHO present in bread was digested (**Figure 4.4 C**), then the differences are largely eliminated except that the GGE release per 100 g carbohydrate for intact rye bread (pumpernickel) was lower (60.6 ± 10.9 g) in comparison to other intact breads, but increased to 78 ± 1.2 on homogenizing.

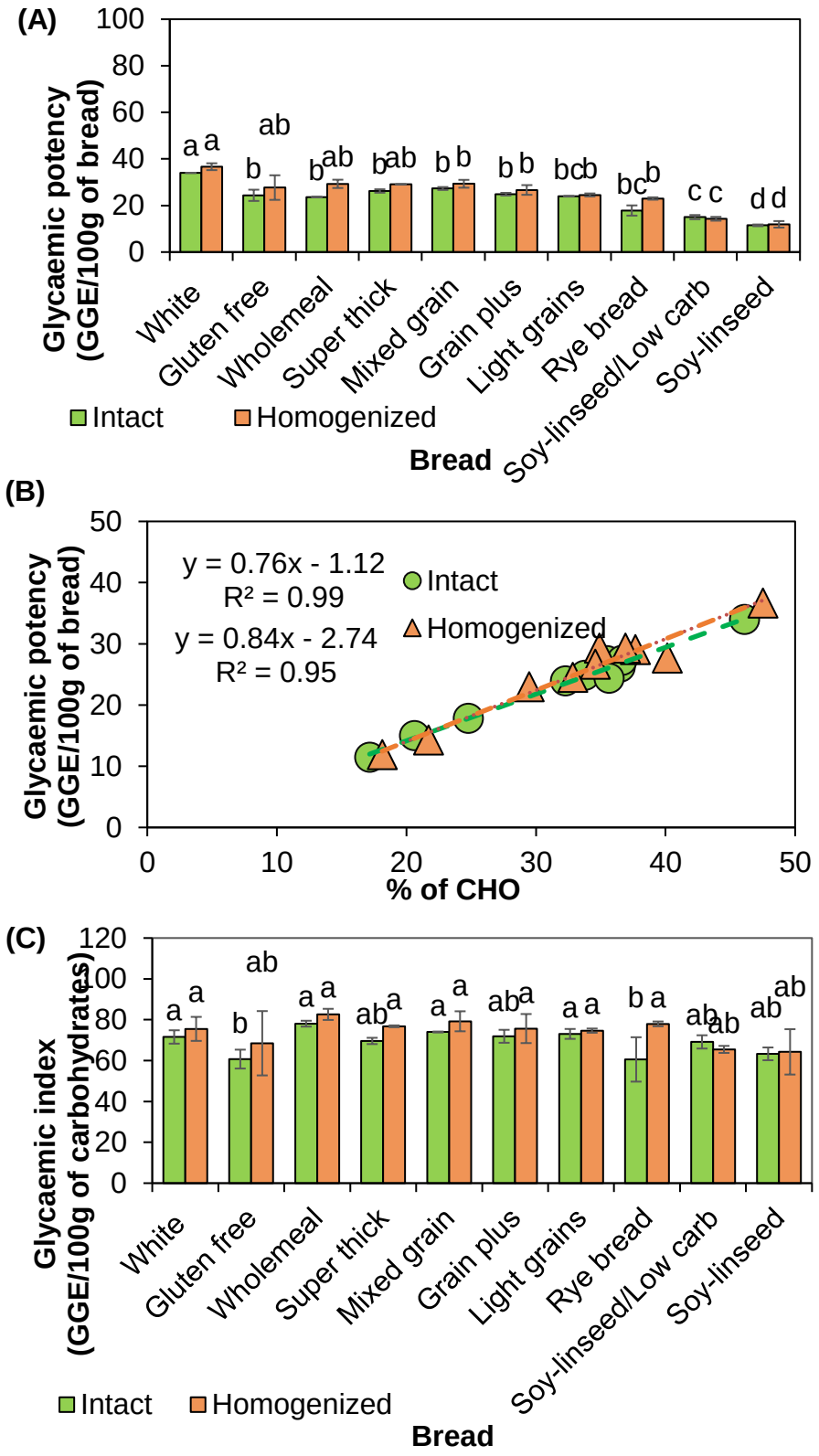


Figure 4.4 (A) Glycaemic potency of breads based on 100 g of bread. (B) Relationship of the proportion of available carbohydrates (CHO) with the glycaemic potency (GGE/ 100 g of intact and homogenized breads) (C) Estimated GI (GGE/ 100 g of CHO). Different letters indicate significant differences between the breads under the individual groups ($p < 0.05$).

These three datasets (**Figure 4.4 A, B and C**) suggest two scenarios: The first is that the most effective strategy for reducing the glycaemic potency of bread is to reduce the proportion of digestible starch. The second strategy is to include a high proportion (> 65%) of larger (> 1 mm) grain particles in the bread matrix. It is proposed that in the second case starch contained within intact grain particles is undigested or digested more slowly due to slow penetration of digestive enzymes into the intact grain endosperm (Edwards et al., 2015; Grundy, Edwards, et al., 2016; Johansson, Gutiérrez, Landberg, Alminger, & Langton, 2018). To determine the proportion and rate of starch digested *in-vitro* the intrinsic digestion rate of starch was determined, with the results presented in the following section.

4.3.3 Effect of grain particle structure on the digestibility of starch in breads

Using the *in-vitro* digestion system as described in **Section 4.2.4**, nearly 80% of the starch was converted into sugars within 20 min, and after 20 min digestion was still proceeding (**Figure 4.5**), even though the concentration of pancreatin had been reduced (from 1.0 ml to 0.1 ml of 5% pancreatin) to slow the rate of digestion. Almost 100% of CHO was digested at 120 min of the pancreatic digestion (**Appendix A3**). At reduced pancreatin levels the sugar release over the first 20 min did not differ significantly between intact and homogenized forms of white, gluten-free, wholemeal, super thick, light grain and soy-linseed/low carbohydrate breads (**Figure 4.5 A, B, C, D, I and J**). For grain plus and soy-linseed **(Figure 4.5 F and J)** rates of digestion were significantly slower to 5 min and 7.5 min respectively for the non-homogenized compared with homogenized bread, after which the starch was digested similarly to homogenized bread. However, the digestion curves of intact and homogenized rye bread (pumpernickel) were significantly different from one another (**Figure 4.5 H**). Rye bread (pumpernickel) was the only bread for which the rate and proportion of starch digested in the intact sample was substantially lower than for the homogenized sample. The results show that the proportion of intact grain particles present in some of the breads sampled was not sufficient to significantly reduce the rate of starch digestion and was not, therefore, effective in reducing the glycaemic potency. However, for the rye bread, a slow rate of starch digestion occurred when the proportion of grain particles of > 1mm exceeded 65% of the dry bread mass.

When the data shown in **Figure 4.5** was fitted to linear regressions, the slopes of the regressions estimated the rate of starch digestion over this 20 min period. The rates of digestion for the 20 min for intact and homogenized breads are shown in **Figure 4.6 A**. Amongst most of the

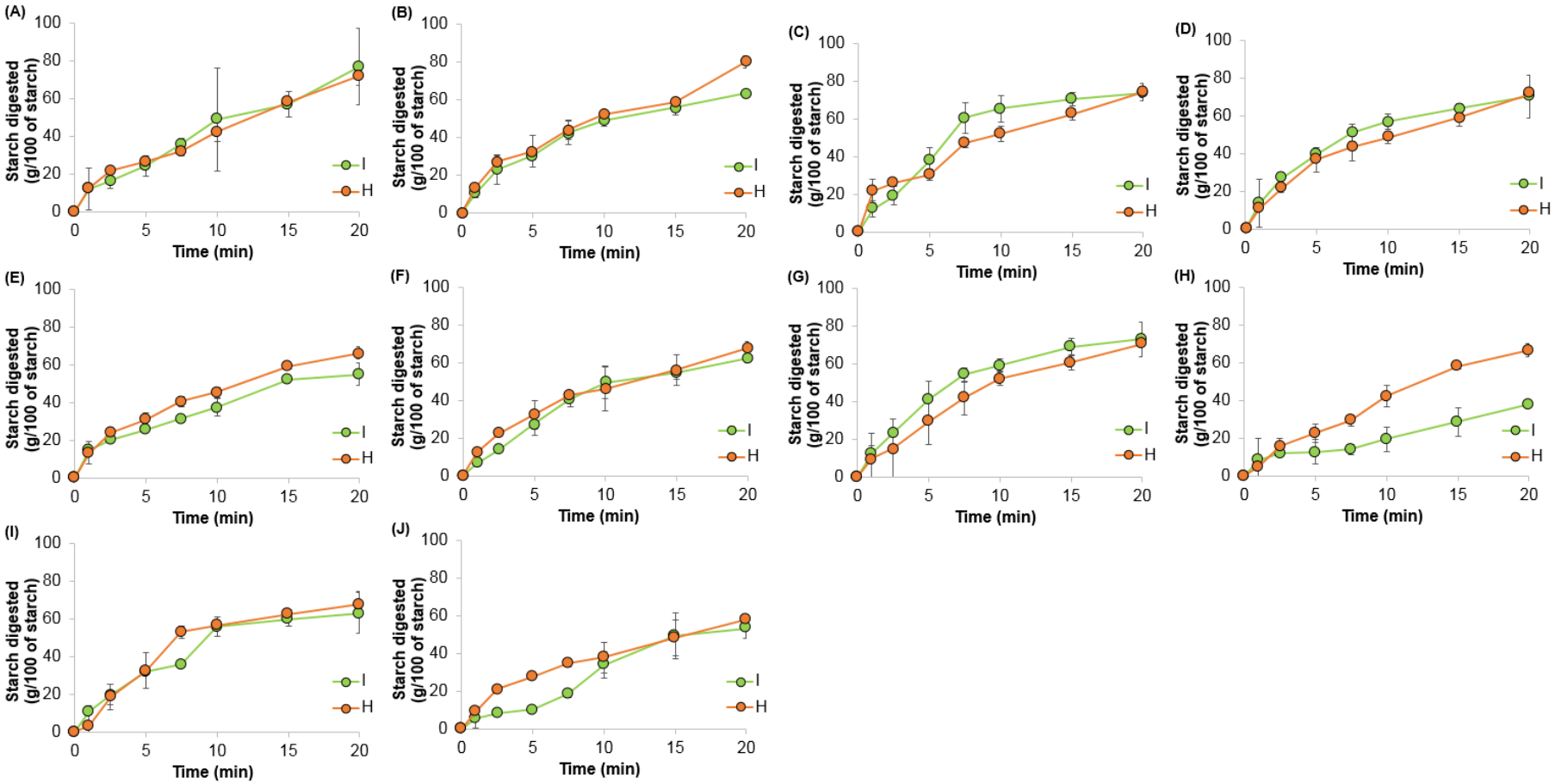


Figure 4.5 *In-vitro* digestive profile of starch (sugars eliminated at 0 min) for 0-20 min of digestion. (A) White, (B) Gluten free, (C) Wholemeal, (D) Super thick, (E) Mixed grain, (F) Grain plus , (G) Light grain, (H) Rye bread, (I) Soy-linseed/ Low carb, (J) Soy and linseed breads digested with 0.1 ml of 5% pancreatin measure as intact ("I") and after homogenized ("H"). To measure the intrinsic starch digestion, high-frequency initial sampling was performed. Points are the average of 2 samples; intervals are (±) SD.

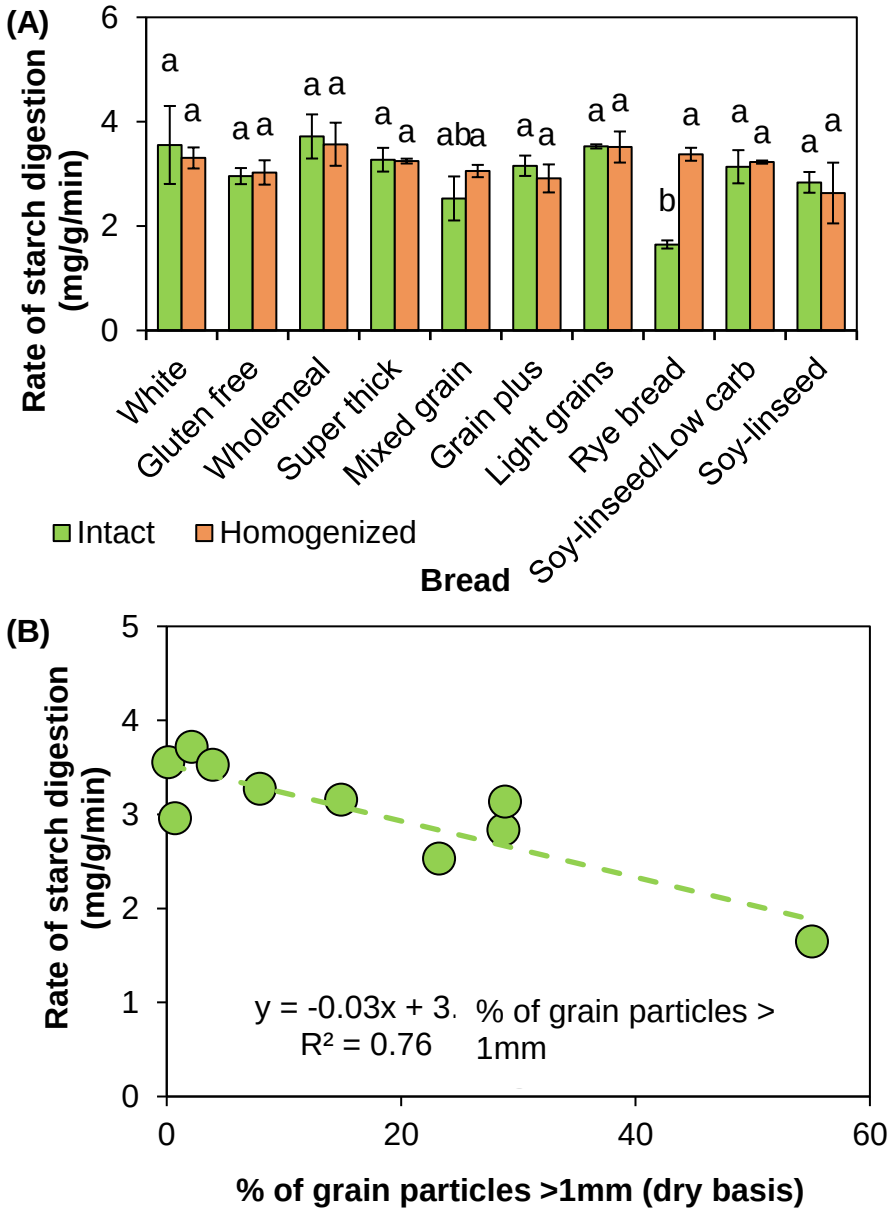


Figure 4.6 (A) Rate of starch digestion for A (White), B (Whole meal), C (Light grains), D (Super thick), E (Soy and linseed), F (Original mixed grain), G (Grain plus), H (Rye bread), I (Whole meal/gluten free), J (Soy and linseed/Low carb) breads digested with 0.1 ml of 5% pancreatin measure as intact ("I") and after homogenized ("H"). Points are the average of 2 samples; intervals are (±) SD. Different letters indicate significant differences between the breads in each group ($p < 0.05$). (B) Relationship between the proportions of grains above 1 mm in diameter with the rate of starch digestion (mg glucose/g starch/min) in intact bread samples.

breads, the rates of digestion were similar for intact and homogenized breads, and the disruption of grain particles by homogenizing did not increase in the rate of starch digestion (**Figure 4.6 A)** with the relationship strongly influenced by the pumpernickel value. Grain content was negatively correlated with the rate of starch digestion (**Figure 4.6 B)**. However, when the proportion of particles > 1 mm was less than 30%, the rate of digestion was weakly related to the proportion of particles in the bread. With > 65 % intact grain particles in rye

bread, a lower rate of starch digestion in the intact form was achieved than in other breads. It is evident that in the 0-30% range, the inclusion rate of particles was not sufficient to effectively reduce the glycaemic potency of breads, whereas, at 65% inclusion, a significant reduction was obtained.

The inclusion rate at which grain particles start to reduce the glycaemic potency of New Zealand breads substantially requires further research. Intact grain particles (> 1mm) decreased the digestibility of starches in the unhomogenized group, possibly because the starch mass in the particles had delayed enzyme penetration and required surface erosion to gain access to the starch. Cell walls may also have reduced water uptake, reducing or preventing the swelling of the starch granules, and as a result, reducing the rate of digestion (Fardet, Leenhardt, Lioger, Scalbert, & Remesy, 2006; Grundy, Edwards, et al., 2016). The free starch present in the bread matrix was as easily digested in breads with a low proportion of intact grains as in breads with a high proportion.

Rye bread contained 65% of the large whole and kibbled rye grains (>1 mm) which may have been resistant to the digestive enzymes due to the thick cell walls of its aleurone layer (Edwards et al., 2015; Johansson et al., 2018; Mishra et al., 2012). The starch is likely retained inside cells by the cell walls, which causes slower glucose release and starch digestion during the *in-vitro* digestive analysis. Starch digestion of the homogenized rye bread did not differ significantly in comparison to other breads which suggests that such structural factors alone may have retarded starch digestion in the intact rye bread. Homogenization breaks the cell walls of the grains and disperses the endosperm, thereby allowing the access of the digestive enzyme to the starch (Berg et al., 2012; Mishra et al., 2012).

In breads that contain a very high density of large intact endosperm particles, such as rye bread (pumpernickel), the grain content and particle size is, therefore, likely to be important. In our study, the maximum proportion of intact particles > 1 mm was 65% in rye breads in contrast to the other breads containing less than 30%. Rye bread showed a decrease in the rates of starch digestion by half when it was digested intact, and the estimated GGE/100 g of carbohydrates was lower by 20 points in comparison to the homogenized rye bread. The proportion of large intact particles > 1 mm was too low in the other whole-grain breads which represent what is currently available, to significantly reduce the starch digestibility and glycaemic potency. The results from the literature are consistent with our results showing that the effect of particle size was significant only above 1 mm, and this explains why the proportion of intact grains labelled

in the packaging is not associated with a reduced rate of digestion and glycaemic potency, and the bread is therefore not as beneficial from the glycaemic control perspective as consumers might think.

Having observed a negative correlation between the proportion of intact grains and rates of starch digestibility, it may be possible to reduce the starch digestibility by increasing the grain content in the bread products. Significant differences in the calculated glycaemic potency were evident, but these were largely dependent on the proportion of digestible carbohydrates present in the breads. Compared to white bread, the substitution of white flour with wholemeal flour and/or with intact grains reduced GGE release during digestion and hence the potential glycaemic potency, only by reducing the proportion of digestible starch, which shows that the proportion of CHO (digestible starch) in the breads was the major factor determining its glycaemic potency. Therefore, reducing the proportion of digestible starch in bread may be a more effective strategy than increasing particle size. The results suggest that grain dense breads with at least 65% of larger grain particles similar to the rye bread (pumpernickel) tested, will reduce the rate of starch digestion and thereby lower glycaemic potency. However, CHO was the major factor determining the glycaemic potency of the breads, and it is evident that formulating breads with a low carbohydrate content would more effectively reduce the glycaemic potency of breads.

4.4 Conclusion

The intact grain particles in most New Zealand wholemeal or whole grain supermarket breads contribute little to the reduced digestibility of starch and hence glycaemic potency of these breads. Only in rye bread, which contained more than 65% of grain particles >1 mm, the rate of starch digestion was significantly reduced. If organoleptic properties can be maintained, it may be possible to increase the proportion of grain particles to more than 65% with further reductions in glycaemic potency. High proportions of intact grain particles were only effective in reducing the glycaemic potency when the particles were intact. Chewing the bread will reduce particle size and hence increase the glycaemic potency. The most effective and reliable strategy to decrease the glycaemic potency of breads is, therefore, to reduce the proportion of digestible starch in the breads (**Figure 4.7**). At present, it is not clear how this could be achieved while maintaining organoleptic properties, especially when the effects of chewing are considered.

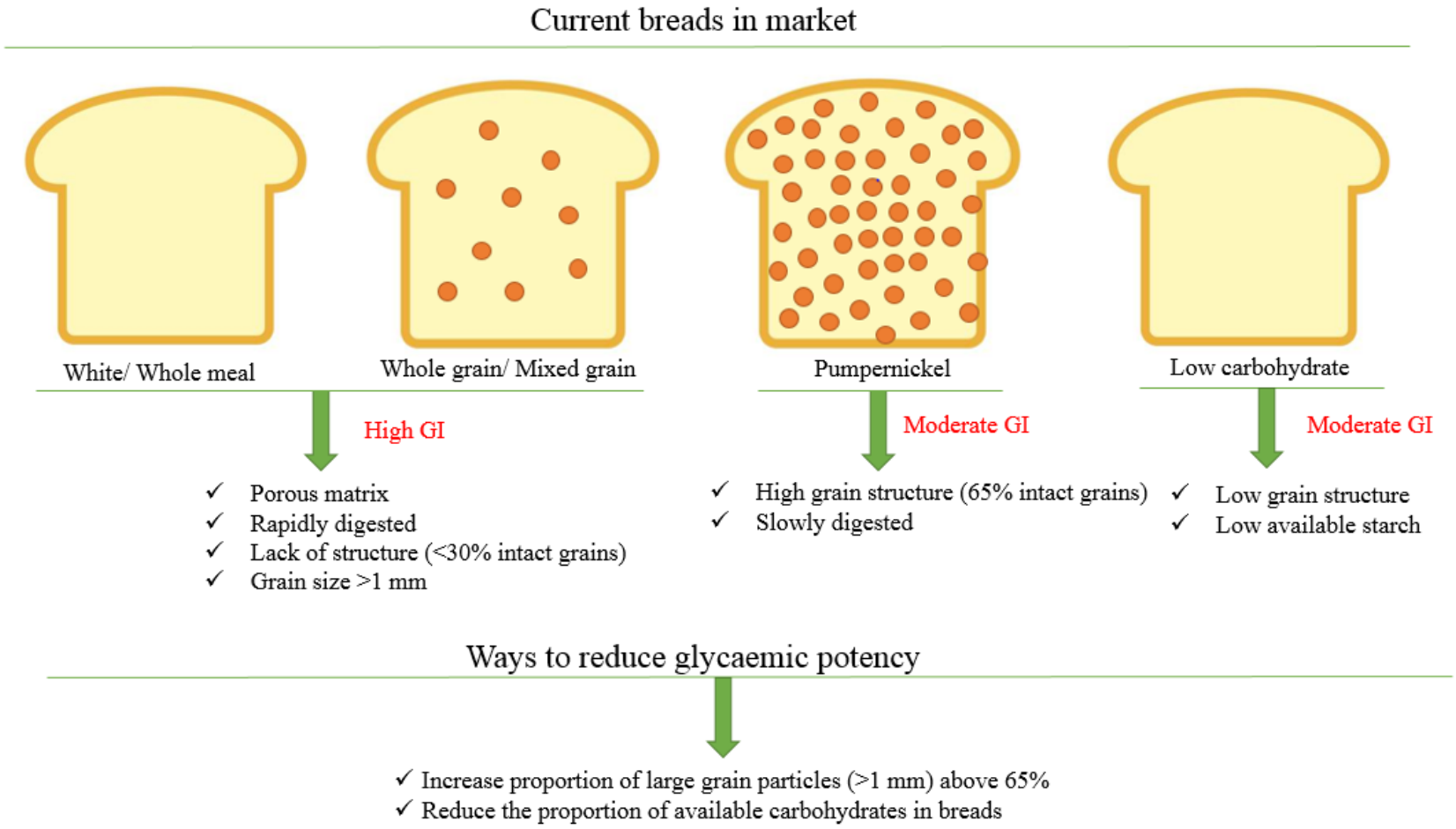


Figure 4.7 Schematic summary on the role of intact grains on the starch digestibility of New Zealand Supermarket breads

Chapter 5

Chapter 5: Can kernel structure be exploited to modulate the digestibility of starch in milled grains?

5.1 Introduction

Starch has an energy density of about 15.4 KJ/100g, a little under half that of fats (~37.7KJ/100g). In the native form, starch is digested slowly but as a result of gelatinization during processing, the rate of starch digestion in the human gut increases 8-10 times and as a result, it becomes highly glycaemic (Zhang & Hamaker, 2009). Different foods may contain similar amounts of starch but differ in the postprandial glucose and insulin levels they induce in humans (Behall & Hallfrisch, 2002). Such differences may be due to differences in the physical and/or chemical structure of the starch, the structure of the food matrix or the presence of dietary components or processing treatments including milling and gelatinization that affect the availability of the starch to the digestion process (Al-Rabadi et al., 2009; Blasel et al., 2006; Butterworth et al., 2012). The effects of processing on the digestibility of the starch can be attributed to modification or disruption of plant structure at many levels, from the molecule to the starch granule to the whole seed. One mechanism that could be exploited to decrease the glycaemic potency of grain products used in breads is the ability of intact/semi-intact kernels to protect endosperm starches from hydrolysis, but the extent to which this mechanism is valid for cereal endosperm cells is not well understood. Therefore, our research has focussed on retaining the natural structure of cereal grains to various degrees in an attempt to reduce the rate and extent of starch digestion under conditions similar to those in the small intestine (**Figure 5.1**).

The particle size of flours and kibbled products has been reported to reduce the rate of digestion and postprandial blood glucose concentrations (Edwards, Maillot, Parker, & Warren, 2018) due to plant structure and degree of gelatinization in the particles. Initially, to verify that increasing the size of grain particles may reduce glucose release during *in-vitro* digestion, twelve different cereals (Broad beans, Peas, Rice, Rye, Soy, Bagherra barley, Streaker barley, Reliance wheat, Empress wheat, Amethyst wheat, Tyrian wheat and Corn) and two extreme particle sizes (>2.8 mm and >0.1 mm) were subjected to *in-vitro* digestion as described in **Section 3.2.3.** Based on the results on the effect of extreme particle sizes on the starch digestibility **(Appendix E)**, two types of grains which showed a greater difference in digestibility as a function of particle size were chosen for detailed analysis.

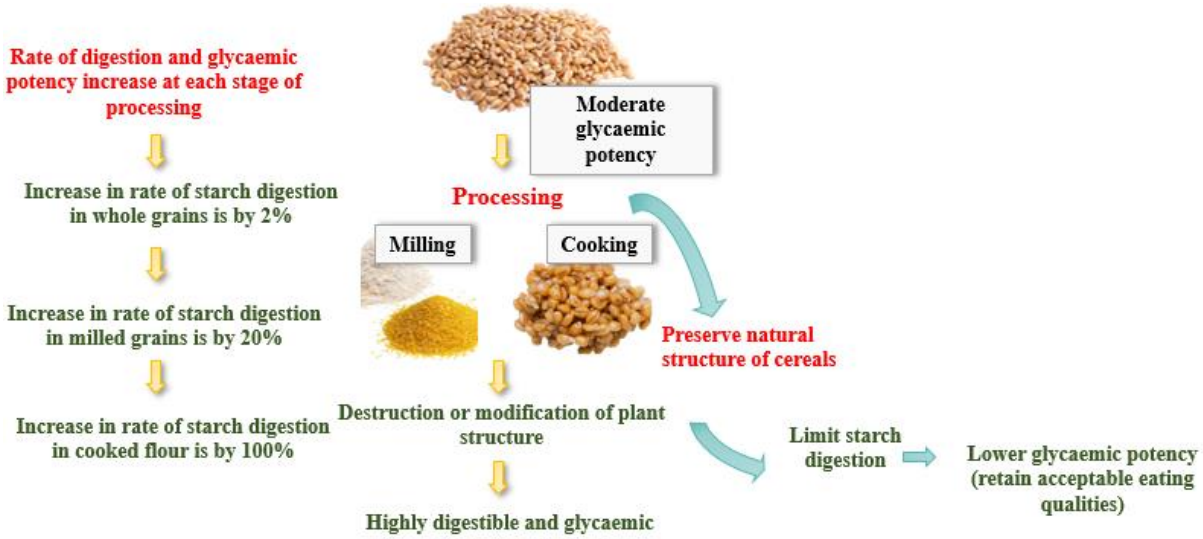


Figure 5.1 Schematic representation of processing conditions on starch digestibility

Pea and purple amethyst wheat particles were the two-grain types showing the greatest resistance to digestion in earlier trials **(Appendix E)**. The other reason to take two different cereal types is that cell walls of pea are robust and tend to remain intact whereas endosperm cell walls of wheat are degraded, so impose less of a barrier to starch digestion than in pea. Although it is known that particle size contributes to lowering glucose release during *in-vitro* digestion, the role of and relative contribution of different food processes to the way that cell wall structure (**Figure 5.1**) affects starch digestibility and glycaemic potency is unknown. This chapter will consider the effects of grain particle size and degree of gelatinization on the digestibility of the starch they contain and hence its possible effect on expected glycaemic potency.

This chapter was divided into two phases (**Figure 5.2**) in order to address two different questions:

Phase 1: Does plant structure govern starch digestibility and expected glycaemic potency of milled grain particles through its effect on starch gelatinization?

Phase 2: Will plant structure alone govern starch digestibility and glycaemic potency when the milled grain particles are completely gelatinized?

In this section of the thesis, all work is carried out *in-vitro* using digestion under simulated small intestinal conditions. In later chapters, the effect of simulated chewing, and later, consumption of the particles in breads on human glycaemic response is reported.

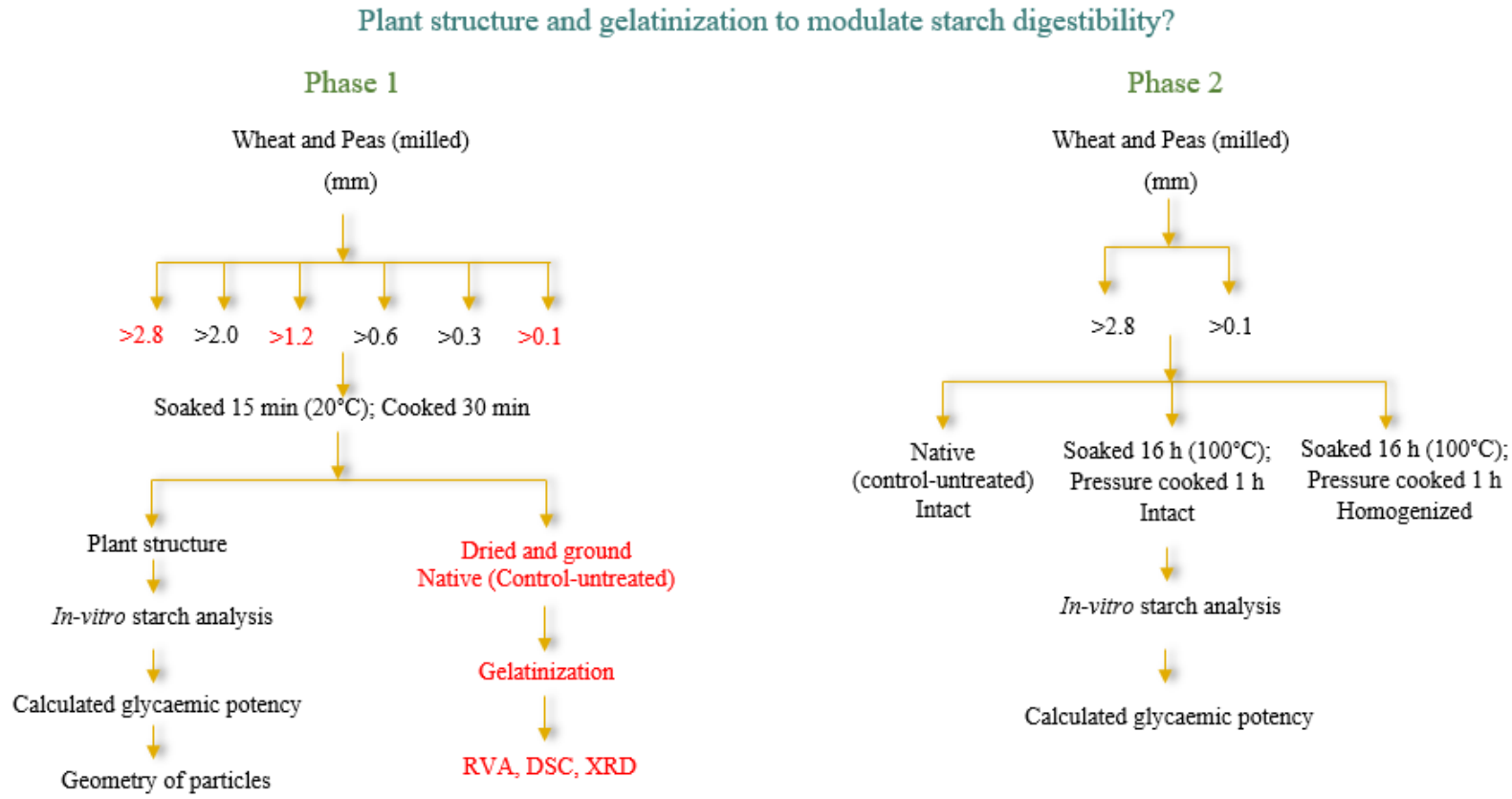


Figure 5.2 Schematic flow chart of Chapter 5. Particle sizes shown in red are used to measure gelatinization parameters in Phase 1. RVA – Rapid visco analysis, DSC – Differential scanning Calorimetric analysis and XRD – X-ray diffraction.

5.2 Materials and Methods

5.2.1 Phase 1: Do factors such as gelatinization and plant structure govern the digestibility of starch and glycaemic potency of cooked grain particles?

5.2.1.1 Materials

Whole blue peas were provided as a gift by Mr. Allan Hardacre (School of Food and Nutrition, Massey University, NZ) while amethyst purple wheat was provided as a gift by Dr. Catherine Munro (Plant & Food Research, Lincoln, NZ).

5.2.1.2 Dehulling and milling of seeds

Amethyst purple wheat was de-hulled during the time of procurement. Whole peas were milled using a Kenwood Grain Mill AT941A. The seed coats were separated from the milled product, as reported in **Section 3.2.1**. For both the pea and wheat, the milled samples were passed through standard sieves with apertures of 3.3 mm, 2.8 mm, 2 mm, 1.2 mm, 0.6 mm, 0.3 mm and 0.1 mm using a shaker (Model RX-6-1, W.S Tyler, 8570 Tyler Blvd., Mentor, OH 44060, USA) for 5 minutes. The sieve sizes were selected to produce six size classes denoted as: >2.8 mm, >2 mm, > 1.2 mm, > 0.6 mm, > 0.3 mm and > 0.1 mm according to the upper and exact sieve apertures. E.g. > 2.8 mm particles were obtained by sieving through the upper and exact sieve apertures which were 3.3 mm and 2.8 mm, respectively. The sample fractions remaining on the sieves were collected and stored at 24°C for the subsequent experiments.

5.2.1.3 Treatment

Samples of the milled grain particles (0.2 g) were placed into 10 ml falcon tubes with 4 ml of deionised water and mixed thoroughly. The samples were sealed in the tubes and left to soak for 15 min and then cooked in a hot water bath (100°C) for 30 min. The samples were then cooled to 37°C before subsequent analysis.

5.2.1.4 *In-vitro* starch digestion and glycaemic potency

Briefly, simulated illeal digestion was started for the cooked and cooled samples by adding 0.05 ml of 5% pancreatin (Sigma P-7545) in 0.1 M maleate buffer pH 6 and 0.02 ml of undiluted amyloglucosidase, and the tubes were immediately made to the 10 ml mark with distilled water **(Section 3.2.3)**. Digesta aliquots of 0.05 ml were removed before (0 min) and

after the addition of the amylolytic enzymes at 5, 10, 15, 20, 30, 40, 50, 60 and 120 min. The samples were then analysed for reducing sugars using the DNS assay (**Section 3.1.4**). Potentially digestible carbohydrates were measured as previously described **Section 3.1.5**.

5.2.1.5 Modelling starch digestion and glycaemic potency

Starch digestion has been modelled using the LOS method and Englyst methods **(Section 3.2.4)**. The glycaemic potency of the grain particles was derived from the proportion of starch digested as glucose equivalents (GE) measurements, as mentioned in **Section 3.2.5**.

5.2.1.6 Volumes of particles during digestion

Briefly, the volume of the milled and cooked samples was measured before adding the pancreatin (0 min) and after adding the pancreatin at 20 and 120 min. The volumes were measured by a fluid displacement method, as previously described in **Section 3.2.6**.

5.2.1.7 Gelatinization, thermal and crystalline properties

To measure the gelatinization (RVA), thermal (DSC) and crystalline (XRD) properties, particle samples of >2.8 mm, >1.2 mm and >0.1 mm were cooked as described in **Section 5.2.1.3** and oven-dried at 40°C until a constant weight was obtained. The samples were ground to a flour and material passing through a 0.5 mm sieve was used for analysis. Paste viscosity during gelatinization of the flours between 50°C and 95°C was measured using a Rapid Visco Analyser (RVA) (Series 4, Newport Scientific, Warriewood, NSW, Australia) as described in **Section 3.2.7**.

Thermal characteristics of flours from the 6 size fractions of milled particles were measured using a Perkin-Elmer DSC 7 using Pyris software (Perkin-Elmer Instruments, Norwalk, CT). The onset temperature (T_o), peak temperature (T_p), conclusion temperature (T_c) and enthalpy of gelatinization (ΔH_{dis}) were calculated (**Section 3.2.8)**.

Crystalline properties of the starch component in the flours were assessed using X-ray diffraction. A small amount of cooked, dried, flour in the loop with a minimal amount of Fomblin oil and mounted on 3-circle kappa-goniostat (**Section 3.2.9)**. Untreated/Uncooked wheat and pea flour were used as a control for gelatinization, thermal and crystalline analysis.

5.2.2 Phase 2: Will plant structure govern starch digestibility and glycaemic potency for completely gelatinized particles?

5.2.2.1 Treatment

Two size fractions of peas and wheat particles: >2.8 mm, and > 0.1 mm were measured (0.2 g) into 10 ml falcon tubes along with 4 ml of deionised water and mixed thoroughly. The samples in sealed tubes were heated for 16 h at 100°C in a water bath and were then cooked at 100°C in a pressure cooker for 1 h to fully gelatinize the starch in the particles. The samples were then cooled to 37°C and rates of digestion (**Section 3.2.4.2**) measured immediately. The rates of digestion obtained were compared with those of untreated samples which were assumed to be ungelatinized, these were regarded as the control samples. Rates of digestion for the control and heat-treated samples after 20 min of digestion were determined either on the particles or on samples of particles that had been homogenized for 2 min using an UltraTurrax Homogenizer (IKA®-Werke, GmbH & Co.KG, Staufen, Germany). This resulted in pea and wheat particles of two sizes either ungelatinized, gelatinized-intact, and gelatinized-homogenized, a total of 6 treatments.

5.2.2.1 Gelatinization properties

To ensure that complete gelatinization has taken place in the heat-treated samples (**Section 5.2.2.1)**, pasting analysis was carried out using homogenized and dried samples (**Section 3.2.7).** The absence of a gelatinization peak being considered as evidence of complete gelatinization of the starch in the samples.

5.2.2.2 *In-vitro* starch digestion - Rates of starch digestion and Glycaemic potency

The rate of *in-vitro* starch digestion was performed using >2.8 mm and >0.1 mm particle fractions for the pea and wheat samples in three forms: Uncooked, cooked and cooked and homogenized (**Section 3.2.3)**. Digesta aliquots of 0.05 ml were removed before (0 min) and after adding pancreatin/amyloglucosidase (5, 10, 15, 20, 30, 40, 50, 60 and 120 min), followed by the DNS assay for reducing sugars (**Section 3.1.4**). The rate of starch digestion was modelled using Englysts method (**Section 3.2.4.2)**. The glycaemic potency of grains was derived from the glucose equivalents (GE) in solution after the predetermined digestion times (**Section 3.1.6)**.

5.2.3 Data analysis

All data manipulations were conducted using Microsoft Excel spreadsheets (Microsoft Corp., Redmond, WA, USA). All experiments were conducted in duplicate. Analysis of variance and Pearson correlation coefficients for the relationship between selected parameters were carried out using SPSS 24.0 software (IBM Corporation, Chicago, IL, USA). Comparisons of means were determined using Tukey's pairwise test, differences at the 5% probability level were considered significant.

5.3 Results and discussion

5.3.1 Phase1: Factors responsible for the differences in starch digestibility and glycaemic potency of cooked-milled grains.

5.3.1.1 *In-vitro* starch digestibility and glycaemic potency

The chemical composition of the pea and wheat particles are given in **Appendix D**. In the following graphs the proportions of starch digested at the sample times 0-180 min are presented along with the calculated GE values for the treatments described above. **Figure 5.3 (A) and (B)** shows the proportion of starch digested for the six size fractions (>0.1 mm, >0.3 mm, >0.6 mm, >1.2 mm, >2.0 mm and >2.8 mm) of peas and wheat respectively. The graphs were plotted as g/100 g of potentially available starch digested after 120 min. A lower proportion of starch was digested from the larger compared with smaller intact particles before homogenizing and as expected the calculated glycaemic potency (GGE/ 100 g of the sample) was also lower **(Figure 5.3 C and D)**. The digestion mixtures, including the remnant grain particles for all treatments, were homogenized immediately after the 120 min samples were removed. The homogenized samples were then incubated for 1 h with 0.01ml of amyloglucosidase (180 min) to digest all remaining starch. The accumulated mass of starch up to the 180 min sample was defined as 100% of the potentially available starch (After homogenization denoted as dashed red line and "h*" in **Figure 5.3**) as all samples had been fully gelatinized.

The proportion of starch digested at 120 min for the >2.8 mm pea and wheat particles was 28% and 24% respectively of the potentially available starch. Almost 100% of the available carbohydrates were digested in the milled particles of peas and wheat once it was homogenized and digested with amyloglucosidase.

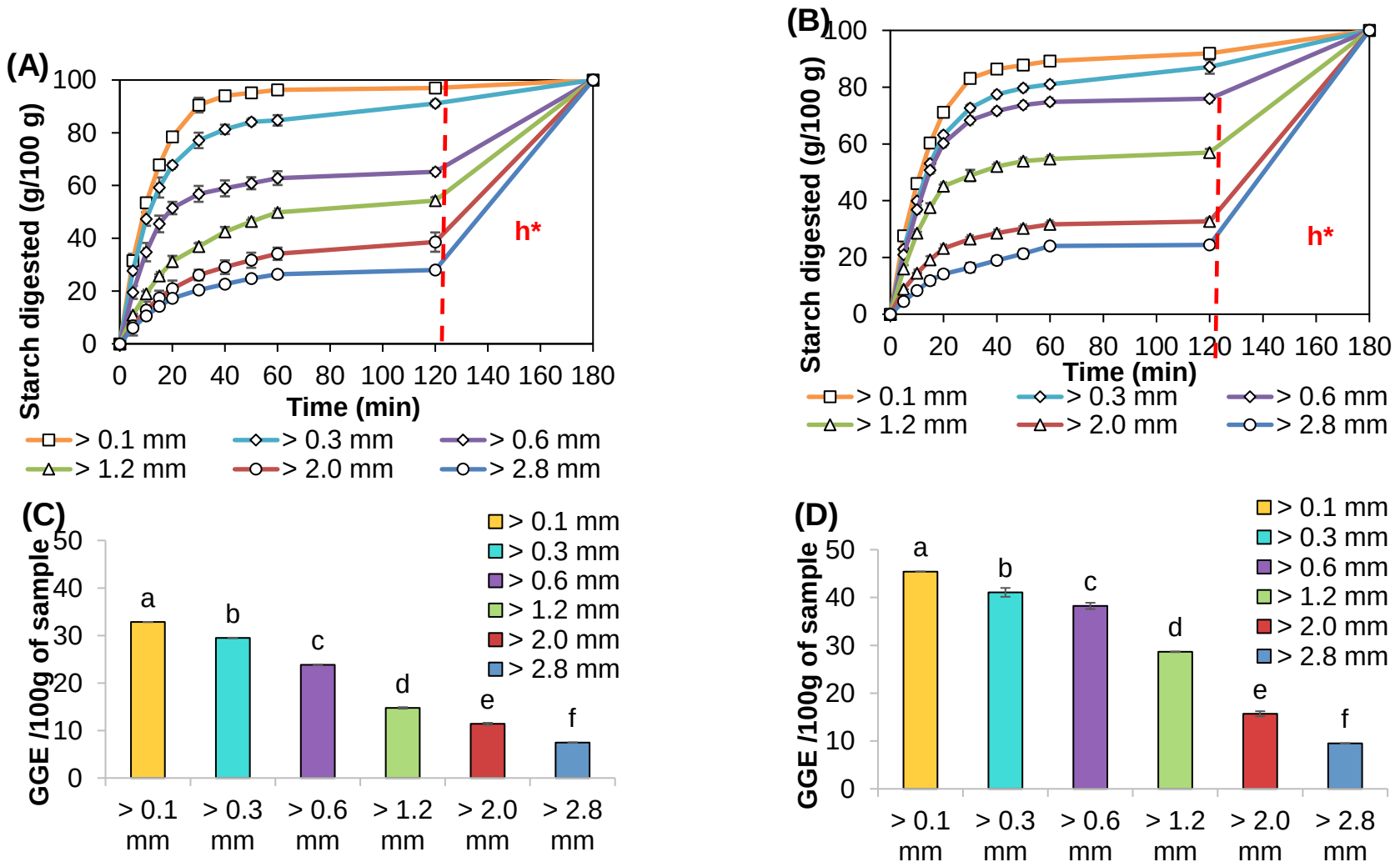


Figure 5.3 *In-vitro* starch digestibility curves of grains of various size fractions of (A) Peas and (B) Wheat samples digested by porcine pancreatin. The time point (120-180 min) (h*) near the graph shows the potentially available carbohydrates. Glycaemic glucose equivalents of grains based on 100 g of sample (C) Pea and (D) Purple wheat. Values are means of duplicate ± SD. Different letters indicate significant differences between the groups ($p < 0.05$). Note the vertical red dashed line denotes homogenization of the sample at this point.

Clearly the lower surface to volume ratio for these larger particles resulted in a much lower proportion of starch being available for digestion at 120 min. The particle volume is clearly reducing the rate of access of digestive enzymes and the rate of sugar release into the liquid phase.

The proportions of RDS, SDS and RS starch for the pea and wheat particles are shown in **Figure 5.4 A and B**, respectively. RDS was greater by 60% for the smaller particles (>0.1 mm) in comparison to the larger particles (>2.8 mm) while the RS was lower for the smaller particles of peas and wheat (**Figure 5.4 A and B)**. The starch that was undigested at 120 min (RS) was directly related to the particle size (**Figure 5.4 C**), i.e. larger particles had higher proportions of RS starch in comparison to the *in-vitro* particles.

5.3.1.2 Particle size (Low surface to volume ratio)

During *in-vitro* digestion of the six particle size fractions the mean volume of the particles was measured after 0, 20 and 120 min of digestion by a fluid displacement technique. Assuming that the particles were spherical, the diameter was calculated from the calculated density and volume using **Equation 3.1.** Interestingly, during digestion, the mean diameter of larger fractions (>2.0 mm and >2.8 mm) for pea and wheat particles did not change. As starch was digested it would have been expected that the diameter would reduce. However, it appears that the remaining cell wall structures were sufficient to retain particle integrity and reduce the loss of material from the surface of the particles. For the smaller pea and wheat particles between >0.1 mm and >1.2 mm particle size was reduced at least by 16% - 47% and 10% - 47% respectively at 20 min although the reduction was not significant between the smaller particles. For smaller particles, (between >0.1 mm and >1.2 mm) volumes decreased significantly by between 23%- 77% and 16%-70% with 120 min of digestion (**Figure 5.4 C).** This coupled with the much greater proportion of starch digested shows access by the digestion enzymes and egress of sugars resulting from the digestion of the starchy material was more rapid for smaller particles and was coupled with a loss in the structure of the particles.

The correlation between particle size and the proportion of starch digested for pea and purple wheat particles at 20 min and 120 min (**Figure 5.4 D)**, was negative with R^2 values between 0.88 and 0.97 respectively. From this, it is concluded that larger particles have a lower surface to volume ratio which reduces the access of digestive enzymes to the interior of the particles

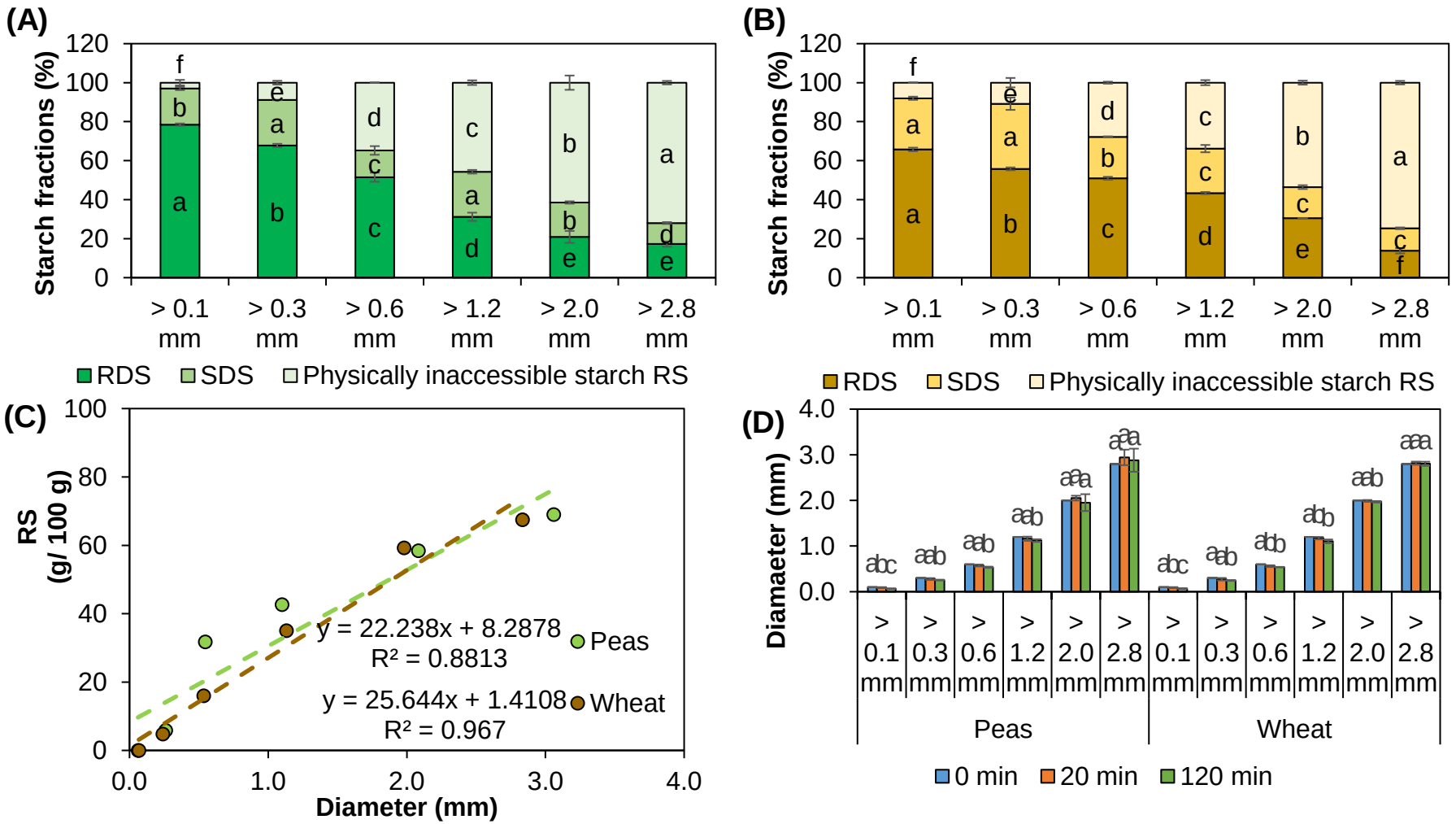


Figure 5.4 (A) Contribution of different starch fractions: RDS, SDS, RS to the total extent of starch digested at 120 min for (A) Peas and (B) Purple wheat of various size fractions. (C) Relationship of the proportion of RS (starch undigested at 120 min) with the diameters of the grains digested (D) Diameters of the grain particles before (0 min) and after digestion (20 and 120 min). Values are means of duplicate ± SD. Different letters indicate significant differences between the groups ($p < 0.05$).

due to the presence of cell walls and other structures, resulting in a much lower proportion of starch being available for digestion.

5.3.1.3 Kinetics of starch digested in milled particles.

5.3.1.3.1 LOS Plot

LOS plots were obtained from the *in-vitro* starch digestibility curves and were used to study the first-order reaction rate parameters during the first 120 min of digestion (**Figure 5.5 A-F**). Plots obtained from smaller (>0.1 mm to >0.6 mm) particle classes (**Figure 5.5 A-C**) for pea and (>0.1 mm to >1.2 mm) wheat particles (**Figure 5.6 A-D**) were characterised by a single linear phase, denoted by one rate constant (k). The rate constants were similar for > 0.1 mm pea and wheat particles (k = 0.086 and 0.073 min^{-1}). As expected from the digestion rates and particle size reduction, the rate constants for the first linear phase decreased as the particle size increased to >2.8 mm by 43% and 44% in peas and wheat particles respectively.

Plots obtained for larger pea (>1.2 to >2.8 mm) (**Figure 5.5 D-F**) and wheat particles (>2.0mm to >2.8 mm) (**Figure 5.6 E-F**) showed that starch digestion was biphasic, characterised by two linear phases over the 120 min digestion period, the second rate constant being 10 to 30% of the first. It is suggested that this represents digestion of 'easily accessed' starch near the surface of the particles followed by slower rates as diffusion processes dominate the movement of enzymes and products into and out of the larger particles. The total C_∞ represented the total extent of starch that was digested in the biphasic reaction. As expected, based on the *in-vitro* digestibility curves obtained, the total C_∞ values were reduced with increasing particle size and were more similar for milled fractions of peas and purple wheat for particle size below 0.6 mm. The total C_∞ values represent the sum of starch digested in two digestion phases, and thus, $C_{1\infty}$ and $C_{2\infty}$ values provide insight into the contribution of each reaction phase to total starch digestion. In both pea and wheat fractions, a higher proportion of starch digestion occurred during the rapid phase, than in the slow-phase, suggesting that both cereals contained starch that was equally susceptible to amylase hydrolysis. We conclude from this section of the work that for the larger grain particles, LOS plot was not a good method for comparing rates of starch digestion, probably due to different mechanisms dominating the rate of digestion with time for the larger particles.

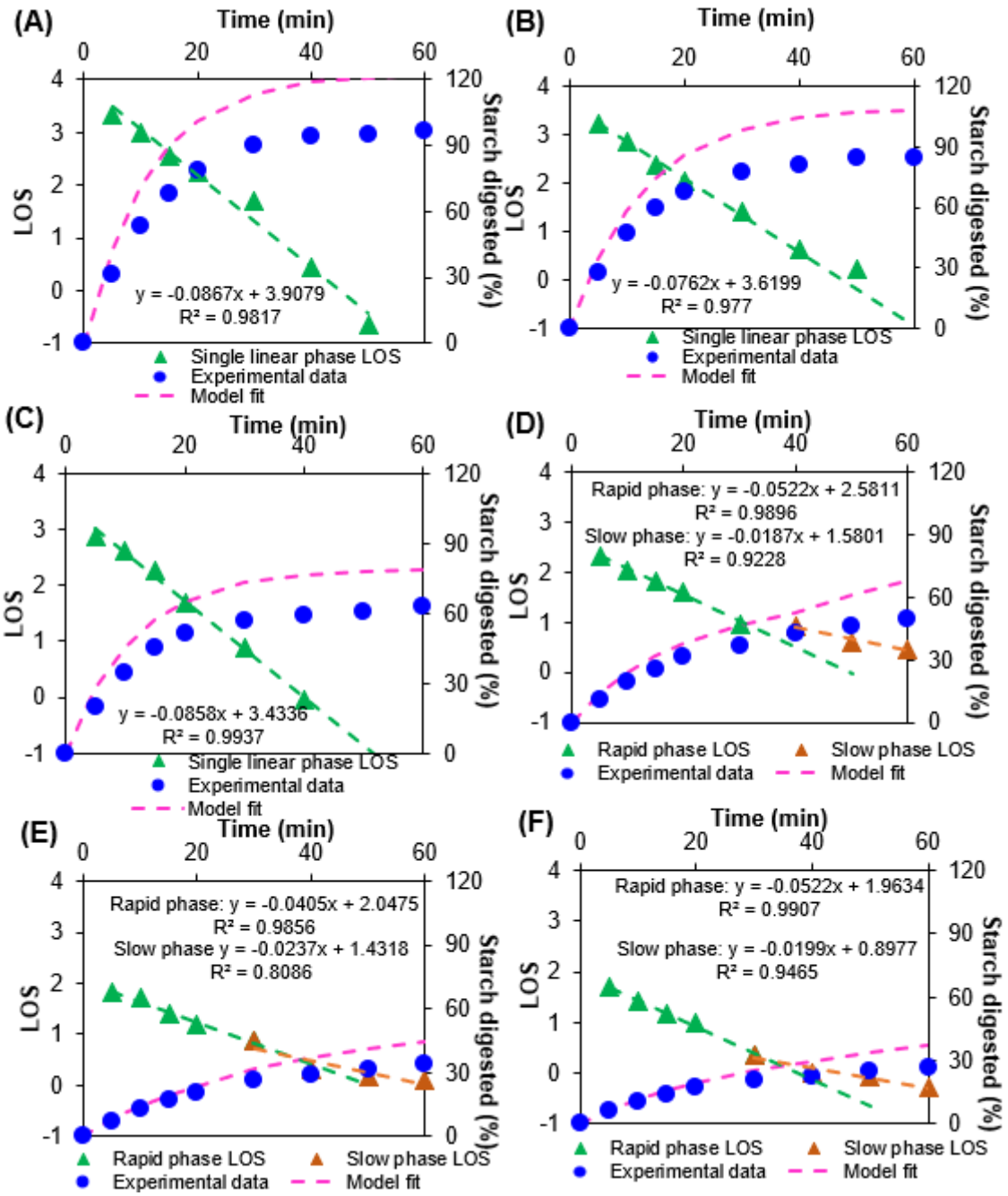


Figure 5.5 Logarithm of Slope (LOS) plots obtained from starch digestibility curves for pea particles (A)> 0.1 mm, (B)>0.3 mm, (C)> 0.6 mm, (D)>1.2 mm, (E)>2.0 mm, (F)>2.8 mm

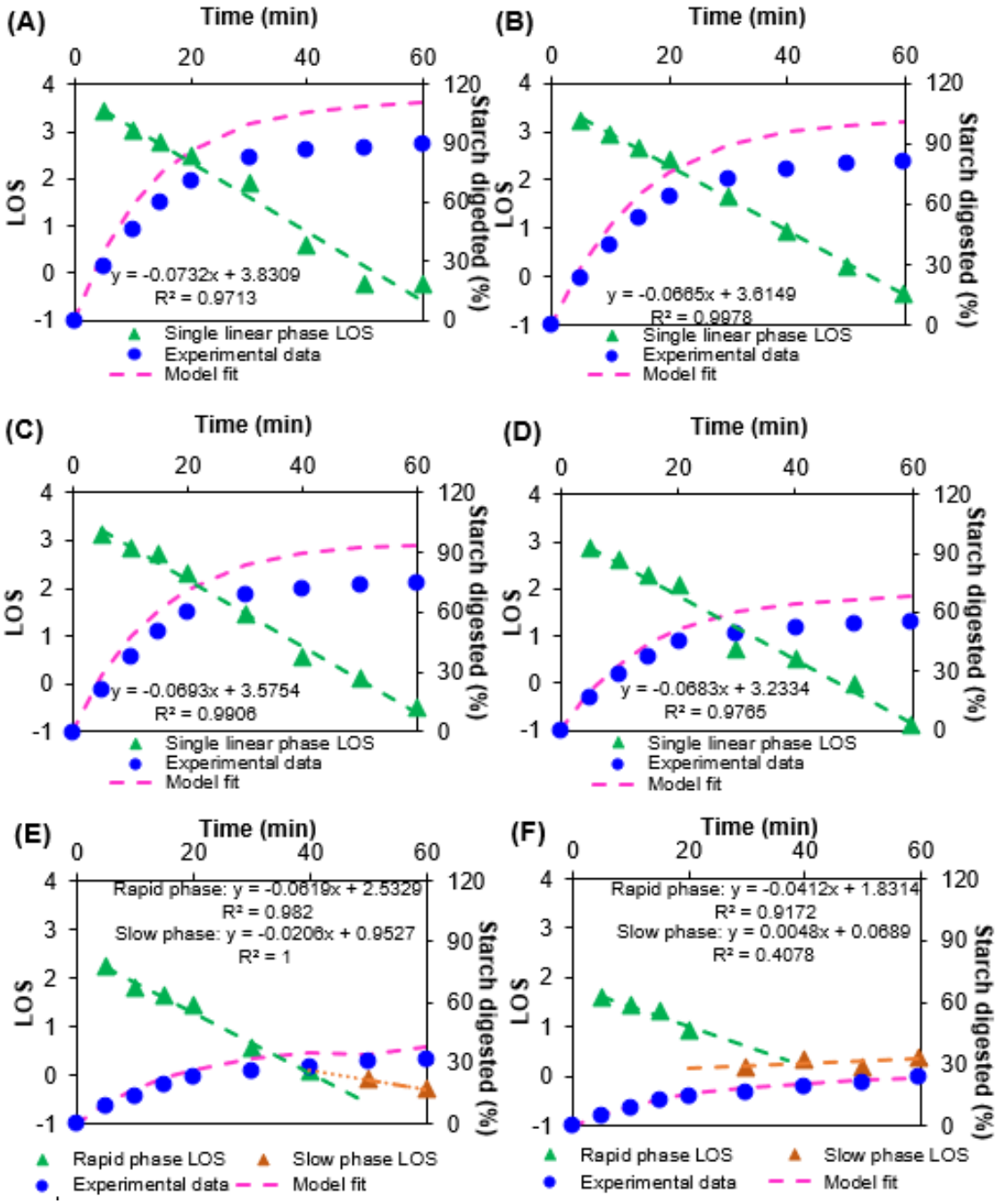


Figure 5.6 Logarithm of Slope (LOS) plots obtained for starch digestibility curves of purple wheat particles (A)> 0.1 mm, (B)>0.3 mm, (C)> 0.6 mm, (D)>1.2 mm, (E)>2.0 mm, (F)>2.8 mm.

5.3.1.3.2 Englyst method

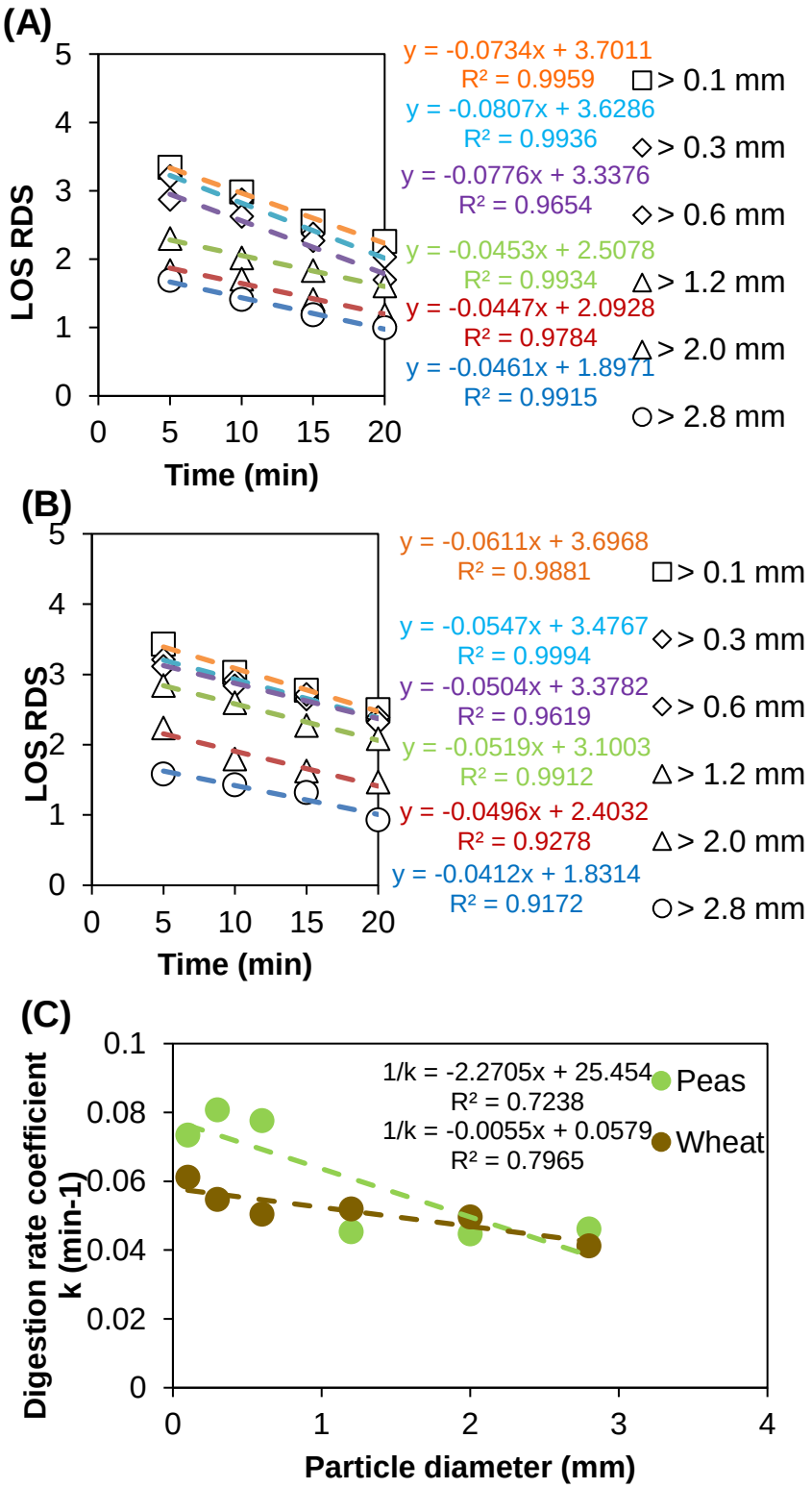


Figure 5.7 First order kinetics obtained for the RDS (0-20 min) from the *in-vitro* starch digestibility curves of (A) peas and (B) purple wheat of particles (> 0.1 mm, >0.3 mm, > 0.6 mm, >1.2 mm, >2.0 mm, >2.8 mm). (C)The relationship between the digestion rate constant and particle size of peas and purple wheat.

Since the LOS plot overestimated the proportion of starch digested after 20 min in many cases but modelled the rate of digestion well during the first 20 min of digestion, further comparisons of digestion rates in this study will only use the RDS proportion of starch for comparisons among treatments for k and the calculation of GE (Hardacre, Lentle, Yap, & Monro, 2016). From the first-order kinetics for RDS, digestion rate coefficients (k) were 0.073 mg/g/min and 0.61 mg/g/min in **Figure 5.7 A and B** for the> 0.1 mm pea and wheat particles. The digestion rate coefficients (k) decreased as the particle size increased (**Figure 5.7 C)**. The linear trend fitted well for all the sizes of pea and wheat particles, with R^2 values between 0.92 and 0.99.

The digestion rate constant k was similar for particles below 0.6 mm in diameter but decreased by between about 20% and 40% for particles 1.2 mm and larger. This was particularly obvious for the pea particles about less so for the wheat. This may suggest that during the milling process, smaller particles have more internal damage allowing better enzyme penetration during digestion. Similar trends of decreasing digestion rate coefficient with an increase in particle size were reported by several authors (Al-Rabadi et al., 2009; Dhital et al., 2010). The difference in the digestion rate of starch in the various particles suggests that the enzymes take longer to penetrate the larger grain particles and slowly digest the starch, whereas the diffusion of pancreatin is rapid for the >0.1 mm particles thereby quickly digesting the starch.

From the results obtained so far, we can see it is important to consider the digestion rate coefficient for the RDS as it is most likely to lead to glycemia. The results show that particle sizes above 1.2 mm in diameter are digested more slowly, probably due to limitations imposed by the rate of diffusion through the relatively dense larger grain particles. Thus, increasing the size of grain particles that are used to make the bread may be a useful method of reducing the rate of starch digestion and hence glycemia.

5.3.1.4 **Degree of gelatinization**

Pasting profiles for flours made from the particles used for these experiments and compared with uncooked control flours are shown in **Figure 5.8 A** and **B,** respectively. The uncooked flours were characterized with a high peak viscosity, a discernible breakdown viscosity and a final viscosity typical of ungelatinized starch (**Table 5.1**). The flours made from cooked >2.8 mm pea and wheat particles showed pasting curves of similar form to the uncooked flour suggesting that gelatinization of the starch in cooked >2.8 mm grain particles was incomplete.

The higher paste viscosity indicates that the starch from >2.8 mm peas and purple wheat have higher thermal stability and potential for swelling even after cooking, that indicates that the starch granules would have been entrapped within the structure of the >2.8 mm particles with less starch damage when the grains were intact (Al-Rabadi et al., 2011). Less water could be absorbed during gelatinization (cooking) because of the constraints imposed by the structure of the particles, and as a result, gelatinization was incomplete. For the smaller particles, the structure could distort to allow water absorption and therefore complete gelatinization.

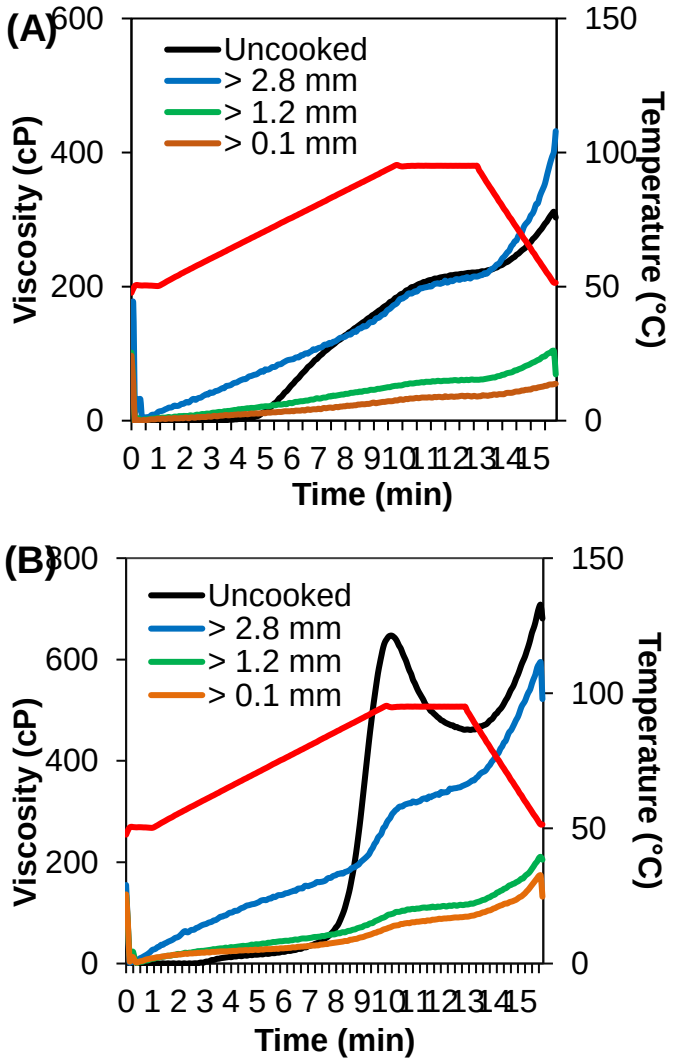


Figure 5.8 Viscograms for particles of (A) peas and (B) purple wheat (> 2.8 mm, >1.2 mm and >0.1 mm). Uncooked/untreated peas and purple wheat flour were used as a control. Curves are means of duplicate.

Cooked ground >1.2 mm and >0.1 mm pea particles showed a decrease in the peak viscosity, breakdown viscosity and final viscosity by 65%, 73% and 55% respectively (**Figure 5.8**). The cooked ground >1.2 mm and >0.1 mm pea particles showed a similar decrease as in the pea particles. This crudely shows that smaller particles from the grains were more easily hydrated,

which caused swelling and completed starch gelatinization during cooking. The pasting temperature of cooked particles showed a progressive increase as the particle size decreased; however, the increases were small, indicating that the starch in all particles was almost fully gelatinized. Determination of thermal properties including transition temperatures and enthalpies of uncooked and cooked flours from the various pea and wheat particles using the DSC are presented in **Table 5.1**. The mean peak transition temperature of the uncooked pea and wheat flour was 61.9° C to 61.8° C, respectively. There was a significant increase in the peak transition temperature for >2.8 mm for peas and wheat particles by 5.6° C and 2.9° C respectively. The peak transition temperatures were not found for the >1.2 mm and >0.1 mm peas and purple wheat particles (**Figure 5.9 A and B**). The transition temperatures (T_o, T_p, and T_c) of starch crystallites are controlled indirectly by the surrounding amorphous region of starch and the loss of order of crystallinity was due to the interaction of water with the starch (Gunaratne & Hoover, 2002).

The ΔH value, calculated based on the area of the endothermic peak, represents the number of double helices that unravel and become less organised during gelatinization (Cooke & Gidley, 1992). The uncooked flour of peas and purple wheat had ΔH of 4.6 and, 5.2 $Jg^{-1,}$ respectively, values similar to those reported by (Ma, Wang, Wang, Jane, & Du, 2017; Polesi, Sarmento, & Anjos, 2011). The ΔH of cooked >2.8 mm pea and wheat samples were significantly reduced compared with the uncooked samples, and no endotherms were found for the flours made from the cooked >1.2 mm and >0.1 mm pea and wheat particles. The absence of a ΔH for the smaller particles provides additional evidence that the starch was completely gelatinized (Martínez-Preciado et al., 2014; Rodríguez-Sandoval, Fernández-Quintero, Cuvelier, Relkin, & Bello-Pérez, 2008). The disappearance of ΔH suggested that the cooking of smaller particles caused complete disorganization in the crystalline and non-crystalline regions of starch granules during gelatinization (Pinto et al., 2012; Tan et al., 2017).

These results were in accordance with the crystallinity (%) of the uncooked and cooked grain particles measured by X-ray diffraction. The relative crystallinity significantly decreased from 30% and 28.1% (uncooked) to 17.5% and 12.6% for the cooked >2.8 mm pea and wheat particles respectively, confirming that gelatinization was incomplete after cooking of the large grain particles. There was no evidence of any crystalline structure in >1.2 mm and >0.1 mm pea and wheat particles after cooking (**Appendix F)**.

Table 5.1 Data obtained from Rapid visco analysis, Differential scanning calorimetry and X-ray diffraction.

Parameters	Uncooked	>2.8 mm	>1.2 mm	>0.1 mm	Uncooked	>2.8 mm	>1.2 mm	>0.1 mm
Rapid Visco Analysis								
Peak viscosity (cP)	215.5±6.4^{a}	209±1.4^{a}	76.5±2.1^{b}	41±5.7^{c}	648.5±14.8^{a}	316±22.6^{b}	129±11.3^{c}	132±28.3^{c}
Breakdown viscosity (cP)	9.5±0.7^{a}	7.5±2.1^{b}	2.5±0.7^{c}	2±0.02^{c}	188.5±2.1 ^{a}	65±41.01^{b}	10.6±6.4^{c}	13±1.4^{c}
Final viscosity (cP)	311.5±12.0^{a}	345.5±37.5^{a}	140.5±2.1^{b}	58±5.7^{c}	707.5±37.5^{a}	518±5.7^{b}	236±43.8^{c}	260.5±62.9^{c}
Pasting temperature (°C)	70.5±0.3^{c}	80.2±0.2^{b}	90.7±0.2^{a}	90.4±0.5^{a}	82.05±0.01^{b}	84.8±4.8^{ab}	88.63±4.5^{a}	87.2±2.9^{a}
Differential Scanning Calorimetry								
T_o **(°C)**	61.7±0.2^{b}	66.8±0.6^{a}	No data		57.1±0.1^{b}	58.6±0.8^{a}	No data	
T_p **(°C)**	61.9±0.2^{b}	67.5±0.5^{a}			61.8±0.1^{b}	64.7±0.1^{a}		
T_c **(°C)**	64.5±0.3^{b}	69.29±1.1^{a}			70.1±015^{a}	68.8±0.2^{b}		
ΔH **Jg^{-1}**	4.6±0.3^{a}	0.8±03^{b}			5.2±0.1^{a}	0.13±0.03^{b}		
X-Ray Diffraction								
Relative Crystallinity (%)	30.0±0.6^{a}	17.5±1.1^{b}	10.8±1.2^{c}	10.0±0.1^{c}	28.1±2.04^{a}	12.6±0.7^{b}	6.7±0.5^{c}	5.6±1.1^{c}

Onset temperature (T_o), peak temperature (T_p), conclusion temperature (T_c) denoted.
Uncooked/ untreated peas and purple wheat flour were used as a control.
Data are means of duplicate.

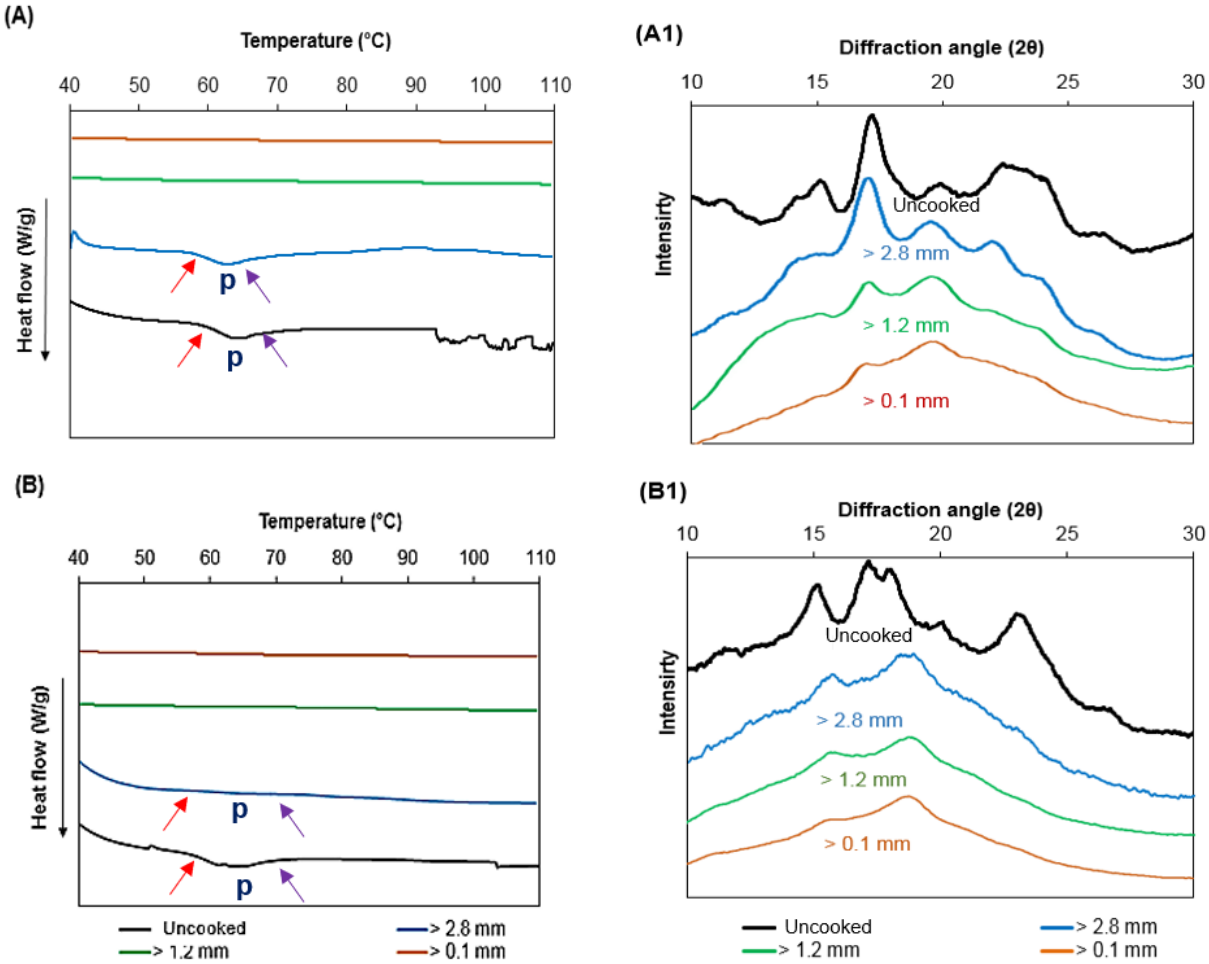


Figure 5.9 Thermograms for grain particles of (A) peas and (B) purple wheat (> 0.1 mm, >1.2 mm and >2.8 mm): Onset temperature (T_o) denoted with red arrow, peak temperature (T_p) denoted with letter "p", conclusion temperature (T_c) denoted with purple arrow and X-ray diffraction graphs for grain particles of (A1) Peas and (B1) purple wheat (> 0.1 mm, >1.2 mm and >2.8 mm). Uncooked/ untreated peas and purple wheat flour were used as a control. Plots are means of duplicate.

From the work reported to this point, it is clear that the structure retained in the larger seed particles and degree of incomplete gelatinization go hand in hand to reduce the digestibility of starch in cooked grain particles of above 1.2 mm in size. Particles of all the tested grains below 1.2 mm in size are completely gelatinized. However, it is unclear if the larger particles (>2.8mm) will be digested more slowly if they are completely gelatinized or after they have been chewed. In the second phase of the work carried out and reported in this chapter, 2.8 mm and >0.1 mm particles were completely gelatinized, and the rate of *in-vitro* starch digestion and the glycaemic potency calculated for and compared with untreated >2.8 mm and >0.1 mm particles of peas and wheat.

5.3.2 Phase 2: Will particle size reduce the rate of starch digestion when larger grain particles are completely gelatinized?

5.3.2.1 *In-vitro* starch digestibility and glycaemic potency

To confirm that pressure cooking had completely gelatinized the > 2.8 mm and >0.1 mm particles of the pea and wheat particles, the pasting properties of flours milled from these particles were measured using the RVA. Starch from the pressure-cooked particles (> 2.8 mm and >0.1 mm) showed no peak hot paste viscosity and no evidence of a pasting profile as is expected for fully gelatinised starch (**Appendix F**). The proportion of starch digested as g/100 g of potentially available starch digested for 120 min for uncooked, PC and PC- H (>2.8 and >0.1 mm) pea and wheat particles are presented in **Figure 5.11 (A) and (B)**, The proportion of starch digested for the >2.8 mm (PC) pea and wheat particles was about 60% and 75% greater than for the uncooked >2.8 mm particles (**Figure 5.11**) and about 25% and 35% greater than for the cooked but not pressure cooked particles (**Figure 5.3**).

The calculated glycaemic potency (GGE/100 g of the sample) for the (>2.8 mm PC) particles was as expected from the digestion rates, always significantly lower than the > 0.1 mm PC particles **(Figure 5.10)**. Also, when the rates for starch digested for first 20 min of *in-vitro* digestion was measured, it can be seen that plant structure was a major factor in slowing down the rates of starch digestion in the completely gelatinized large particles (>2.8 mm PC). The >2.8 mm (PC) pea and wheat particles have digestion rates about 42.5% and 43.5% lower than for the >0.1 mm (PC) particles, but this difference was lost when the larger particles were homogenized (>2.8 mm PC-H).

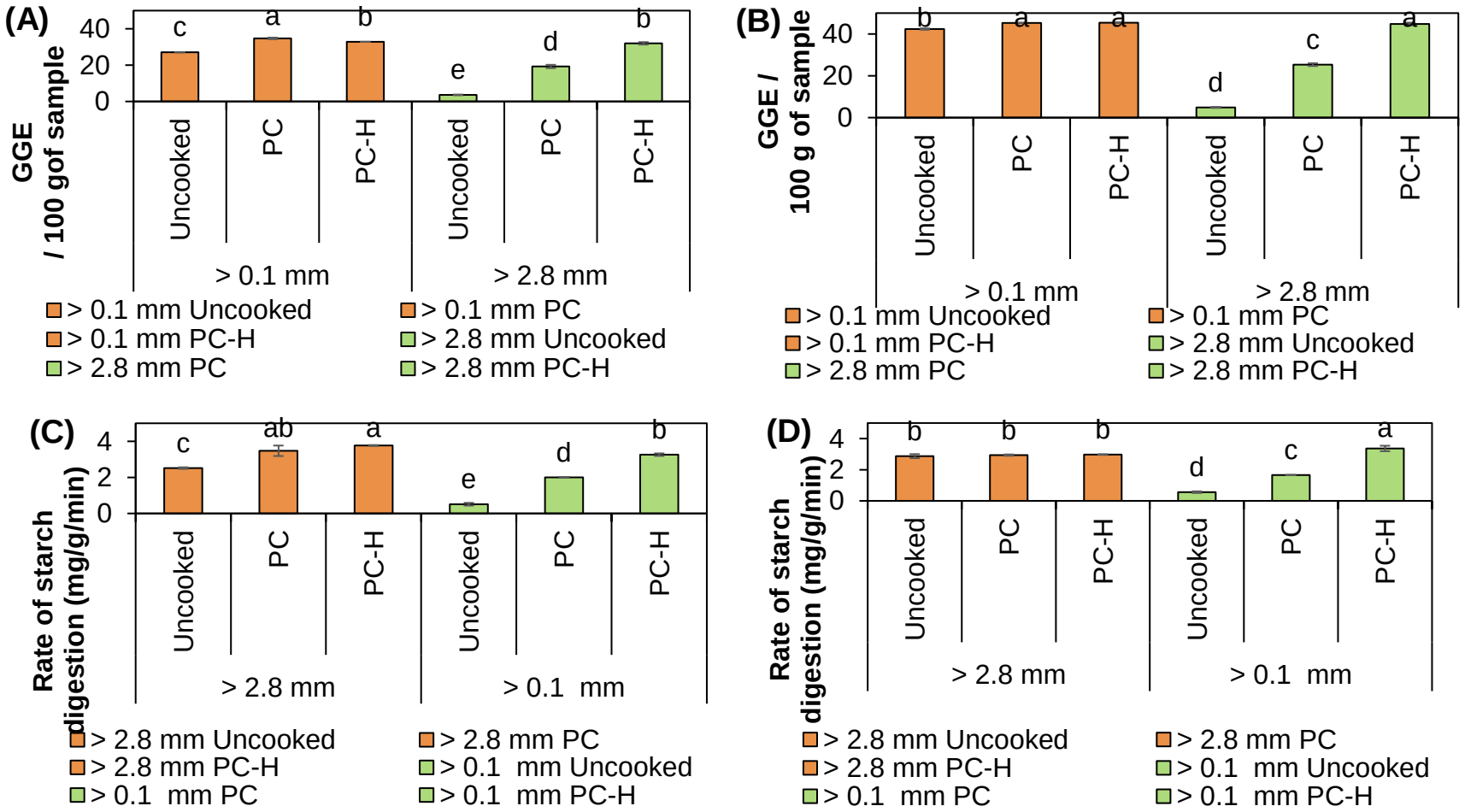


Figure 5.10 Glycaemic potency (calculated) of particles of > 2.8 mm and >0.1 mm size particles of Uncooked, Pressure cooked (PC) and Pressure cooked - Homogenized (PC-H) particles of (A) Peas and (B) Wheat. Rate of starch digestion for RDS phase for > 2.8 mm and >0.1 mm particles of Uncooked, Pressure cooked (PC) and Pressure cooked -Homogenized (PC-H) (C) Peas and (D) Purple wheat grain particles. Values are means of duplicate ± SD. Different letters indicate significant differences between the groups ($p < 0.05$).

This agrees with the hypothesis that plant structure-dependent reduction in the proportion of starch digestion is indicative of reduced starch bioaccessibility where the food matrix imposes a physical barrier to enzyme access even when it is completely hydrated and gelatinized.

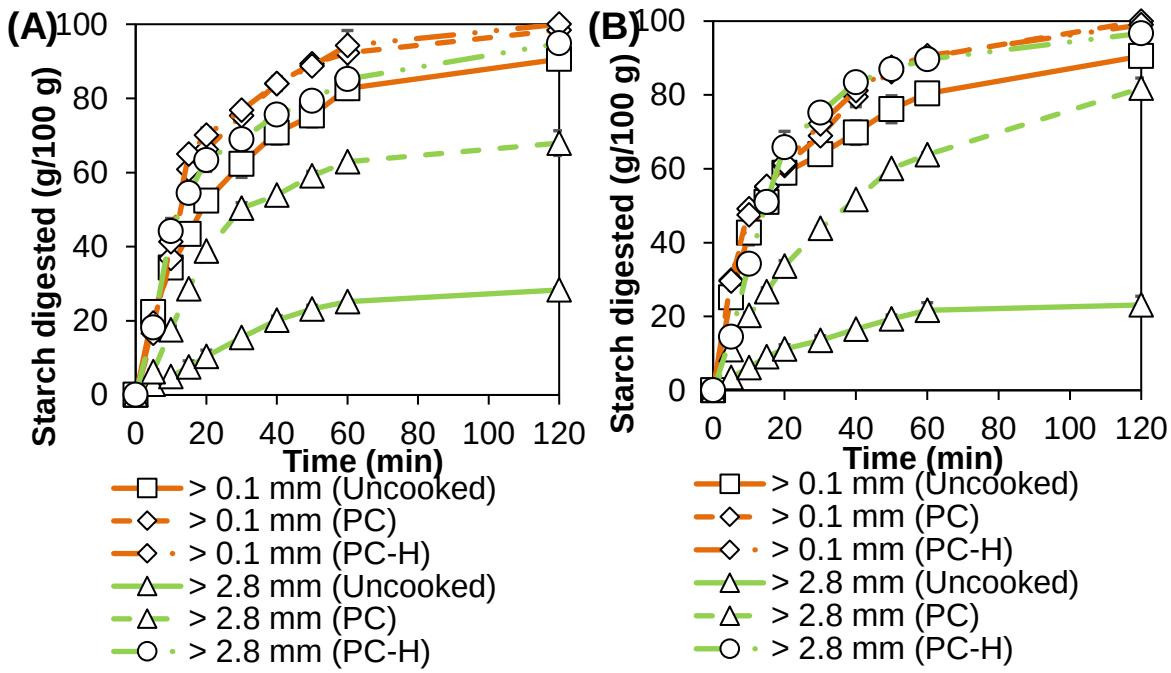


Figure 5.11 *In-vitro* starch digestibility curves for > 2.8 mm and 0.1 mm size particles of Untreated, Pressure cooked (PC) and Pressure cooked- Homogenized (PC-H) grains (A) Peas and (B) Purple wheat digested by porcine pancreatin. Values are means of duplicate ± SD.

5.4 Conclusion

This study aimed to determine how plant structure was critical in reducing the starch digestibility and glycaemic potency of grains when the particle size was increased in cereals. **Chapter 4** has provided evidence that the grains present in the breads measured less than 1 mm and they did not significantly differ in starch digestibility except for the pumpernickel. In addition to that from **Chapter 5**, enzyme kinetic studies of milled, cooked and pressure milled fractions of peas and wheat provided new insight into the characteristics responsible, including factors such as particle volume and degree of gelatinization in two different cereal types.

LOS analysis and Englyst method to determine the rate of starch digestion revealed that larger particles >2.8 mm have a proportion of starch that is completely resistant to α-amylase hydrolysis even after 120 min of *in-vitro* digestion. Larger particles of peas and wheat showed starch resistant to digestion even when completely gelatinized with pressure cooking. Once the larger particles are homogenized, the starch becomes accessible to the digestive enzyme and almost 100% of the starch was digested. In large particles >2.8 mm where this starch was

encapsulated within plant cell walls (i.e. as would be expected when eating whole cooked peas or wheat) the extent of starch digestion was markedly reduced in both botanical sources, indicating that intact plant cells impose limitations of starch bioaccessibility the grains. It is clear that by increasing the particle size of milled cereals starch digestibility and glycaemic potency can be limited by incomplete gelatinization of starch inside cooked intact kernels, which suggests limited swelling of granules trapped inside the cells. However, complete gelatinization of the starch inside large kernels may not be completely digested due to slow rates of diffusion of the enzyme into and sugars out of the particles when the grains are intact or kibbled.

Chapter 6

Chapter 6: Can processing treatments be exploited to reduce the rapidly digested starch content in cereal products?

6.1 Introduction

In **Chapter 5**, it was shown that large grain particles are digested more slowly than smaller particles, thereby decreasing the glycaemic potency of milled grains. However, large grain particles do not always meet functional criteria set by the industry and are not suitable for the manufacturing of certain foods (Dhen et al., 2016; Segundo, Román, Gómez, & Martínez, 2017). Therefore, finding alternative ways to decrease starch bioaccessibility other than increasing the particle size would provide the opportunity to develop novel ingredients with potential applications in the management and prevention of hyperglycemia-related diseases. Processing treatments such as hydration (soaking) and cooking of the starch/starch-based products can increase the digestibility of the starch (Mishra et al., 2008). Usually, starch-rich foods such as cereals are hydrated and cooked before consumption. Hydration has also been suggested for improving cooking quality (Rehman, Salariya, & Zafar, 2001) and soaking grains before cooking is a common practice to soften the texture and hasten the cooking process. Bakers tend to soak the grain at warm (≥30° C) temperatures before adding it to the dough of the bread. Soaking hydrates the grain and facilitates access by enzymes to the interior of the particles but this can be slow for large particles as the process is governed by diffusion and not mass transfer. If the hydrated grain is cooked a small proportion of the water required for gelatinization is already present which disrupts the starch structure and gelatinizes the starch completely, thereby making the starch rapidly digested (Eyaru et al., 2009) and highly glycaemic. This kind of behaviour can lead to acute glycaemic response and can lead to chronic diseases.

According to Martínez, Román, and Gómez (2018), limiting hydration in the flour of bread matrix caused slower rates of starch digestion. A hydration depletion down to 45%, which is close to the minimum hydration found in commercially available white bread, did not prevent the starch in the crumb from being completely gelatinized. Lower hydration caused the crumbs to be more resistant to physical breakdown during *in-vitro* digestion, resulting in a 96.81% increase of SDS from 75% to 45% dough hydration. The degree of gelatinization in crust samples was significantly reduced with a depletion in the dough hydration, which led to an

increase of SDS by 13% in breads. Accordingly, my research aimed to reduce the rate and extent of starch digestion with a focus on limiting hydration to reduce gelatinization of starch in cereals. The chapter will be reported in two stages (**Figure 6.1**). Firstly **(Phase 1)**, the chapter reports the effect of hydrating grain particles at various temperatures on starch digestibility as a function of particle size of grains. Secondly **(Phase 2)**, it reports the effects of limiting hydration during cooking on the starch digestibility as a function of particle size. The overall aim of the project was to reduce the glycaemic potency of cereals by reducing the rate of digestion of CHO, mainly starch, within the food matrix while maintaining organoleptic properties.

6.2 Methods and Materials

6.2.1 Materials

Pea and amethyst purple wheat particles were used as a test grain in this chapter. The grains were milled as mentioned in **Section 3.2.1,** to produce three sizes of particle denoted as >2.8 mm, > 1.2 mm, and > 0.1 mm according to the upper and exact sieve apertures.

6.2.2 Treatment

6.2.2.1 Hydration (Soaking)

The grains studied continued to absorb water during hydration for up to 7 h, after which measurable water absorption ceased (**Appendix G**). Consultation with several bakers around New Zealand showed that before adding dry kibbled or whole-grains into bakery products, they were soaked for at least 12 hours, typically overnight. Therefore, in this research, a common soaking time of 16 h was chosen before the grain was used to make breads.

6.2.2.2 Phase 1 - Effect of hydrating temperatures on starch digestibility of grain as a function of particle size.

Pea and Amethyst purple wheat particles >2.8 mm, and > 0.1 mm were weighed (0.2 g) into 10 ml falcon tubes containing 4 ml of deionised water and mixed thoroughly. The samples were hydrated for 16 h at temperatures of 20°C, 40°C, 60°C, 80°C and 100°C in water baths. The hydrated particles in sealed tubes were then cooked in a water bath (100°C) for 15 min. The samples were finally cooled to 37°C and rates of starch digestion measured.

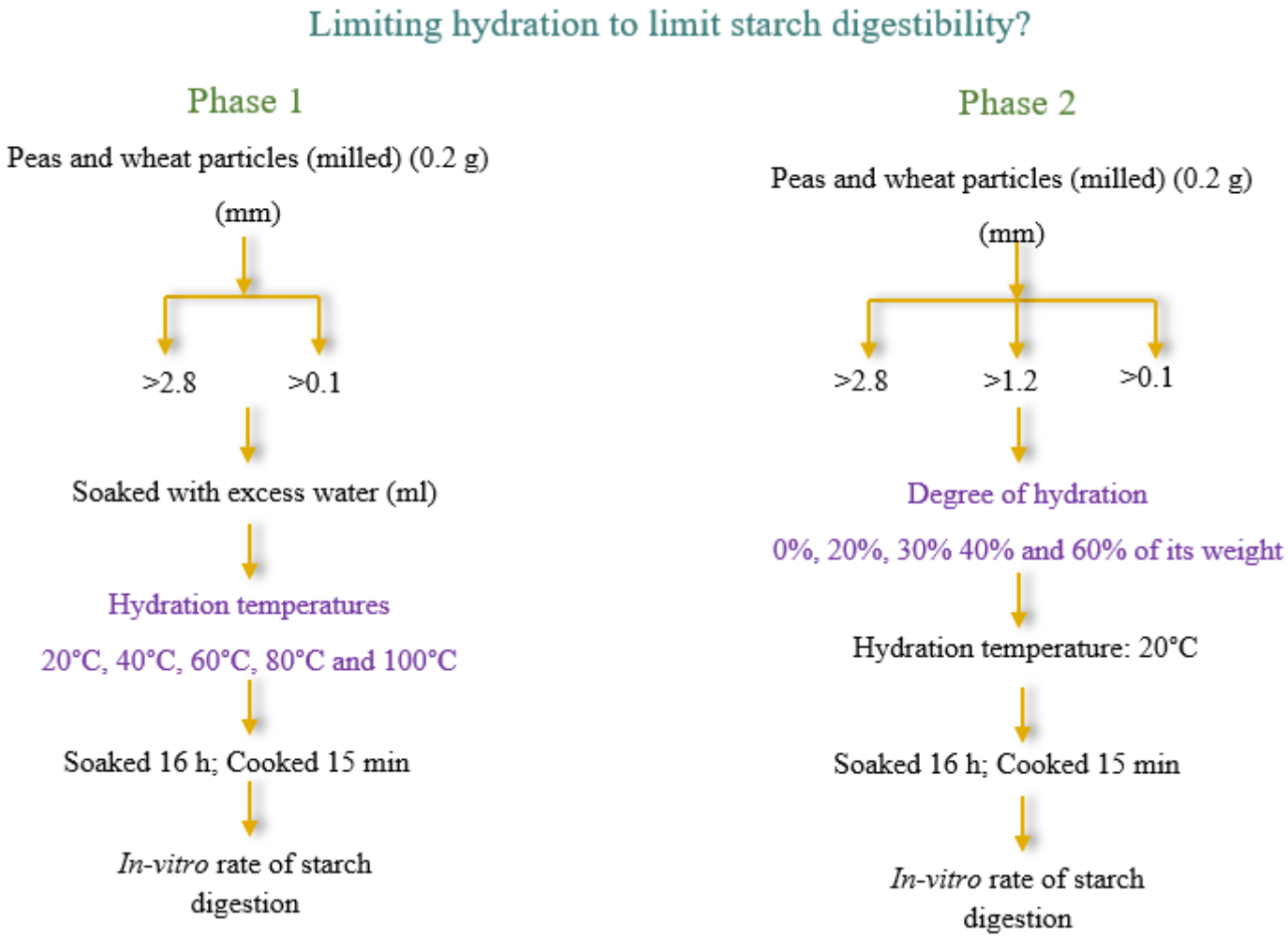


Figure 6.1 Schematic flow chart of Chapter 6

6.2.2.3 Phase 2 - Effect of limiting hydration on starch digestibility as a function of particle size.

Pea and Amethyst purple wheat particles >2.8 mm,>1.2 mm and > 0.1 mm were weighed (0.2 g) into 10 ml falcon tubes containing 0%, 20%, 30% 40% and 60% of deionised water (w/w) and mixed thoroughly. Moisture content was measured before and after hydration to ensure that the degree of hydration reached the expected levels.The samples were hydrated for 16 h at 20°C and were cooked in a water bath (100°C) with sealed tubes for 15 min. The samples were cooled to 37°C and rates of starch digestion measured.

6.2.3 *In-vitro* starch digestion and glycaemic potency

The rates of *in-vitro* starch amylolysis for the treated grains were performed using 0.05 ml of 5% pancreatin (Sigma P-7545) and 0.02 ml of amyloglucosidase in 10 ml of the digestion medium containing 2 ml of 0.1 M maleate buffer pH 6 (**Section 3.2.3)**. Digesta aliquots of 0.05 ml were removed before adding the pancreatin (0 min) and at 20 and 120 min, followed by the DNS assay (**Section 3.1.4**).

6.2.4 Data analysis

All data manipulations were conducted in a Microsoft Excel spreadsheet (Microsoft Corp., Redmond, WA, USA). All experiments were conducted in duplicate. Analysis of variance and Pearson correlation coefficients for the relationship between selected parameters was carried out using SPSS 24.0 software (IBM Corporation, Chicago, IL, USA). Comparisons of means were determined by Tukey's test to a 5% significance level.

6.3 Results and discussions

6.3.1 Phase 1: Effect of hydration temperature on starch digestibility of grains as a function of particle size.

The digestion of starch over time was plotted for peas and purple wheat particles of 2.8 mm and >0.1 mm that had been hydrated at 20°C, 40°C, 60°C, 80°C and 100°C (**Figure 6.2 A and B)**. Between 90- 100 % of the potentially available carbohydrates were digested from the >0.1 mm particles of peas and purple wheat after 120 min and the proportion of starch digested did

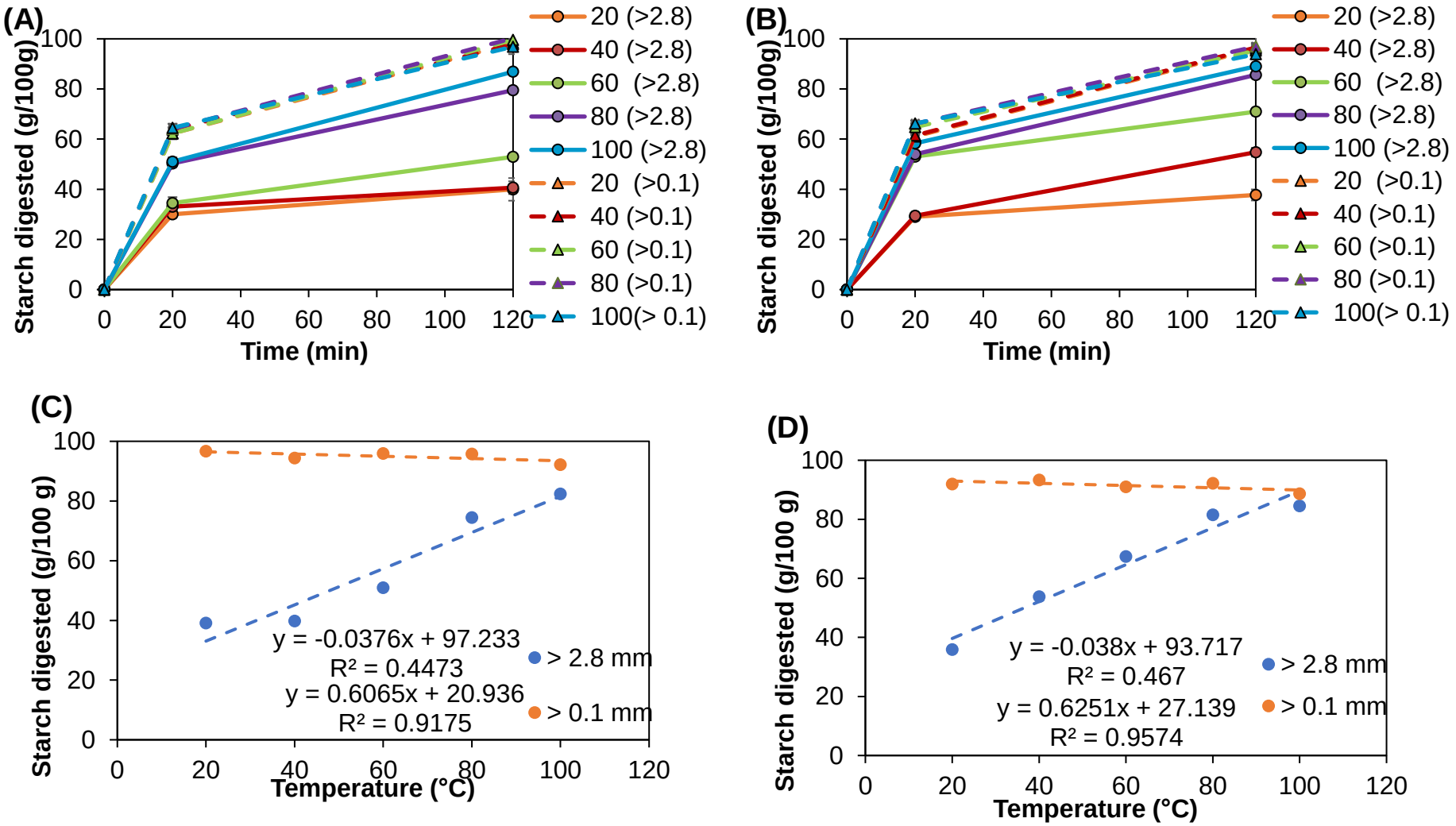


Figure 6.2 *In-vitro* starch digestion curves for > 2.8 mm and >0.1 mm size particles of (A) peas and (B) purple wheat particles with various hydration temperatures (20°C, 40°C, 60°C, 80°C, 100°C): Relationship of proportions of starch digested at 120 min for > 2.8 mm and >0.1 mm size particles of (C) peas and (D) purple wheat particles with various hydration temperatures (20°C, 40°C, 60°C, 80°C, 100°C): Values are means of duplicate ± SD.

not differ significantly with the temperature at which they were hydrated. The larger particles (>2.8 mm) of peas and wheat showed significant differences in the proportion of starch digested at the various temperatures at which they were hydrated. A very low proportion of starch was digested for the >2.8 mm pea particles hydrated at 20°C and 40°C and they did not differ significantly. However, for the >2.8 mm particles of wheat, a lower proportion of starch was digested at 20°C and 40°C over 20 min than for the >0.1 mm pea particles. At 120 min of digestion, the proportion of starch digested was significantly greater for wheat than for pea particles. Closer examination of digestion rates showed that hydration temperature has no effect for the smaller particles. However, as the degree of hydration increased, the starch digestion rate of the >2.8 mm particles increased. The trends of digestion at 120 min for peas and wheat (>2.8 mm and >0.1 mm) particles hydrated at different temperatures was shown in **Figure 6.2 C and D** respectively which are plotted for the temperature of hydration versus the proportion of starch digested. From the trend plots, it can also be noted that the critical region in which the temperature-induced an increase in digestibility of starch in larger particles (>2.8 mm) occurred between at 20°C and 100°C for both peas and wheat. Reducing the temperature of hydration reduced the extent of starch digestibility drastically for the >2.8 mm particles. The increase in starch digestibility occurred steadily between 40-100°C and 20°C-80°C for >2.8 mm peas and wheat particles, respectively. The linear regressions and derived coefficients have been calculated (**Figure 6.2 C and D**) and showed that for the >0.1 mm particles of peas and purple wheat showed correlation coefficients close to 0 which indicated that irrespective of the hydration temperature, the >0.1 mm particles tend to gelatinize completely during cooking resulting in similar digestion profiles for all hydration temperatures. It was also seen that as the hydration temperatures increased, the starch digestibility has also increased for the larger particle size (>2.8 mm) irrespective of the grain type, and this can help us create a prototype product which has lower starch digestibility.

6.3.2 Phase 2: Effect of limiting the degree of hydration on the starch digestibility of grains as a function of particle size

Initially, the *in-vitro* procedure was carried out for twelve different cereals (Broad beans, peas, rice, rye, soy, Bagherra barley, Streaker barley, Reliance wheat, Empress wheat, Amethyst wheat, Tyrian wheat and corn) and three particle sizes (>2.8 mm, >1.2 mm and >0.1 mm) (**Appendix H**). Large particles (>2.8 mm) from cereals that showed a greater difference in digestibility in comparison to >0.1 mm particles are subjected to detailed analysis – Peas and

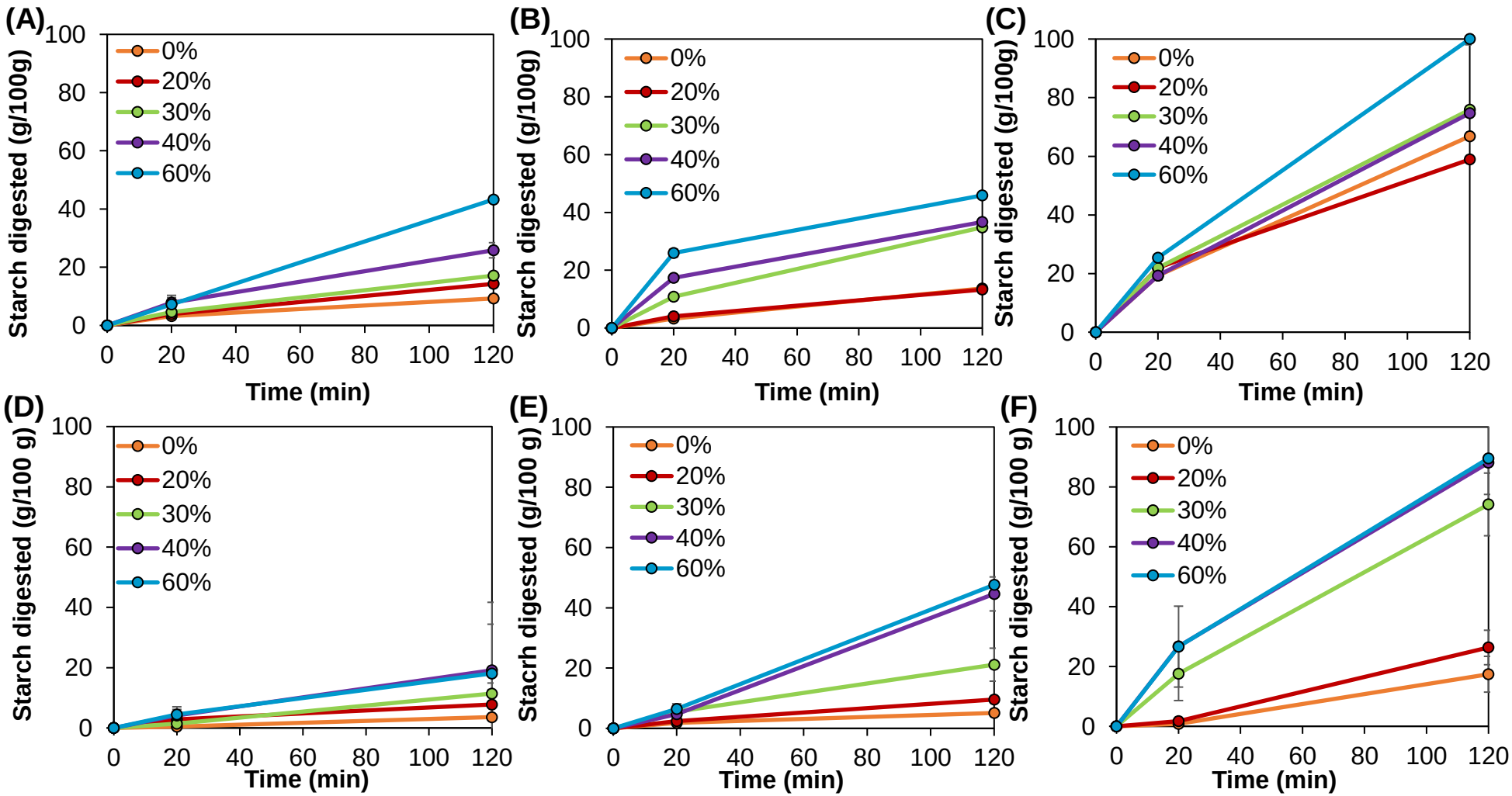


Figure 6.3 *In-vitro* starch digestion curves > 2.8 mm (A and D), >1.2 mm (B and E) and >0.1 mm (C and F) size particles of (A-C) peas and purple wheat particles (D-F) with various degree of hydration (0%, 20%, 30%, 40% and 60%). Values are means of duplicate ± SD.

Amethyst purple wheat. The effect of degree of hydration on starch digestibility of peas and purple wheat for three different particle sizes (>2.8 mm, >1.2 mm and >0.1 mm) and six different hydration levels (0%, 20%, 30%, 40% and 60%) are shown in **Figure 6.3 A-F.** From the graphs, it can be seen that the pancreatin digested 90- 100 % of the CHO present in the 60% (w/w) hydrated (excess water) >0.1 mm particles in peas and wheat at 120 min, whereas 60% hydrated >1.2 mm and >2.8 mm peas and purple wheat particles showed a very low amount of CHO digested. Closer examination of the *in-vitro* digestion curves shows that the moisture present at any given size of the particle affects the digestion of starch present in the grains.

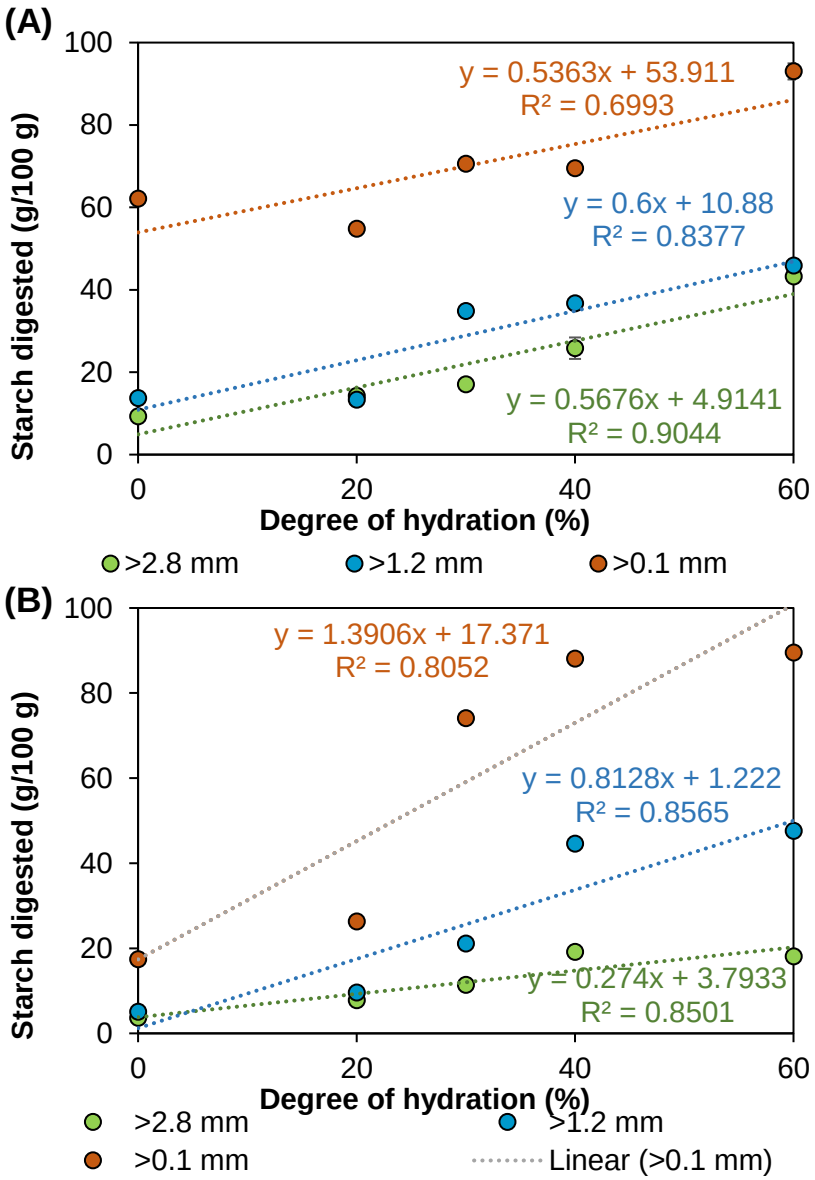


Figure 6.4 Relationship of proportions of starch digested at 120 min for > 2.8 mm, >1.2 mm and >0.1 mm size particles of (A) peas and (B) purple wheat with various degree of hydration (0%, 20%, 30%, 40% and 60%). Values are means of duplicate ± SD.

The trend of hydration for different peas and purple wheat cereals at 120 min is shown in **Figure 6.4 A and B,** respectively, which are plots of the degree of hydration versus the proportion of starch digested. From the trend curves, it can also be noted that the critical region in which the moisture-induced an increase in the digestibility of the starch occurred between 30 and 60% of hydration for both peas and wheat — limiting hydration reduced starch digestibility drastically in >0.1 mm and > 1.2 mm particles. Closer examination at the graphs from **Figure 6.4 A and B** shows the relationship between the moisture content and starch digestibility during cooking and revealed that most of the increase in starch digestibility occurred steadily between 20- 40% moisture content for >0.1 mm and >1.2 mm particles and between 30 -60% moisture content for the >2.8 mm particles of peas and wheat.

The regression coefficients for the relationship between starch digested and the degree of hydration for the three size particles were determined for peas and purple wheat, respectively (**Figure 6.4 A and B).** As the degree of hydration increased in the sample as a function of particle size, the starch digestibility has also increased. The >2.8 mm particles of peas and wheat have lower regression coefficient for the relationship between starch digested and hydration than for the other particle sizes. This behaviour shows us that the digestion rate for the larger particle size is not significantly controlled by the degree of hydration, and this can help us create a prototype product which has lower starch digestibility. Smaller particles are substantially affected by the degree of hydration but not the hydration temperature. Lowering starch digestibility has several health benefits, such as lowering postprandial blood glucose and weight management. From these results, it can be inferred that the starch digestibility of the grains could be manipulated by varying hydration over a large range of about 60% points. However, the organoleptic properties cannot be maintained by limiting hydration during cooking as the grains will be very hard to chew when the starch is not completely gelatinized.

6.4 Conclusion

This study aimed to determine how temperature and degree of hydration affect the digestibility of starch in cereals and serves as starting point for further strategies to modify the physical properties of starch with the aim of improving the nutritional quality of grains and breads. The results allow us to conclude that limiting heat and water uptake during cooking could have important benefits in reducing the rate and extension to which grains are digested.

From **Chapter 5**, it was clear that by increasing the particle size of the milled cereals above 1.2 mm the digestibility of starch and glycaemic potency can be reduced. Further, this chapter shows that decreasing temperature of hydration to 20 °C and degree of hydration to less than 40% limits gelatinization of the starch and hence digestibility. Bakery products such as breads can be made healthier by the addition of larger particles that are only partially hydrated, so they digest slowly, thereby reducing their glycaemic potency through the formation of limited RDS and SDS while retaining a significant amount of RS. This agrees with the hypothesis that was put forward and it clearly shows that manipulating hydration temperature and degree of hydration of cereals can limit the water uptake during the processing and the subsequent rate of *in-vitro* digestion and later glycaemia. However, the organoleptic properties cannot be maintained by limiting hydration during cooking as the grains will be very hard to chew when the starch is not completely gelatinized. Having this in mind, larger particles of > 2.8 mm that are hydrated at 20°C with excess water will be used in the development of breads.

Chapter 7

Chapter 7: The effect of grain structure on the *in-vitro* glycaemic potency of bread

7.1 Introduction

The glycaemic responses to breads made with wholemeal flour are much the same as to those made with white flour, suggesting a similar digestive process despite a higher percentage of fibre (Fardet, Leenhardt, Lioger, Scalbert, & Rémésy, 2006) When the porous physical structure of bread with its highly gelatinized starch is considered, it is obvious that accessibility of the pancreatic α-amylases in the small intestine to the starch is very easy, especially after the bread has been chewed and mixed with saliva; consequently, the GI of most commercial breads white or wholemeal is high (~70) and similar to white bread (Eelderink et al., 2012). Several authors have shown that incorporating grain particles into breads can reduce their glycaemic potency *in-vivo* (Jenkins et al., 1986; Liljeberg & Bjorck, 1994). Our research has shown that the quantity, size and type of whole-grain incorporated into commercial New Zealand breads do not confer low digestibility and glycaemic potency (Srv et al., 2019). In order to make low glycaemic breads, methods of reducing the accessibility of amylolytic enzymes to the starch need to be identified.

From current knowledge, there are **two ways** of reducing the accessibility of the amylases to the starchy grain components;

1. by increasing the particle size and keeping these particles intact during digestion and

2. by limiting the hydration of large grain particles during cooking so limiting the degree of gelatinization of the starch.

Limiting hydration and degree of gelatinization was highly effective in reducing glycaemic potency of grains, but breads made using this strategy will not have acceptable eating qualities (Hera, Rosell, & Gómez, 2014). **Chapter 4** has also shown that the proportion of large grain particles in the breads, unless incorporated more than 65%, were insufficient to reduce the glycaemic potency.

Therefore, in this chapter, we explored various methods of reducing starch digestibility and hence the glycaemic potency of breads with a focus on increasing particle size and proportion of intact grain particles incorporated into the breads (**Figure 7.1**). Therefore, the **two strategies** used in this study are given below.

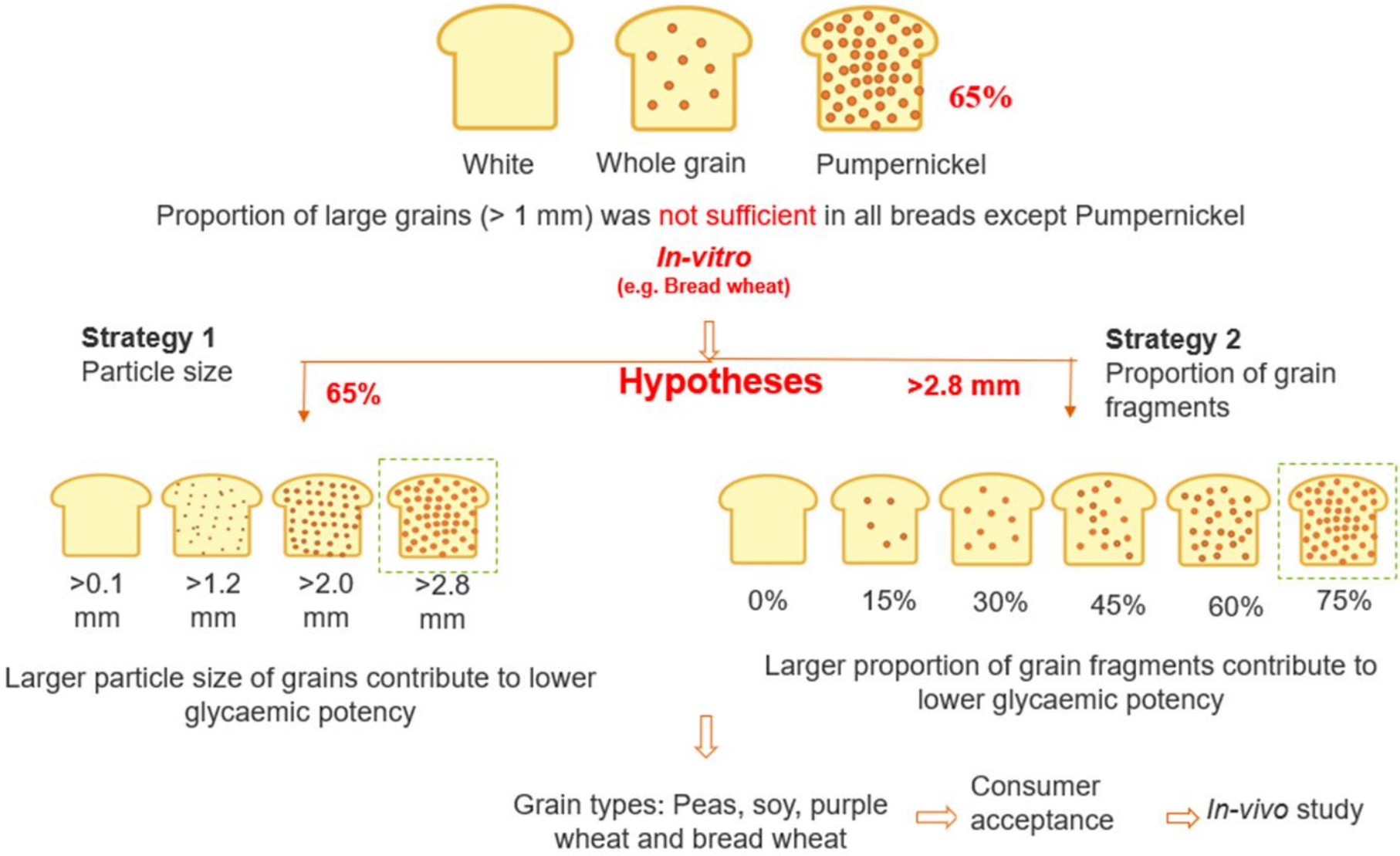


Figure 7.1 Schematic flow chart of Chapter 7

Strategy 1 Particle size

Incorporating kibbled grain particles (> 1.2 mm, >2.0 mm, > 2.8 mm) into a white bread matrix at the highest possible proportion (65%) of the bread dough, this level is comparable to the level of kibbled grain particles present in pumpernickel breads.

Strategy 2 Proportion of particles

Incorporating variable proportions (0%, 30%, 45%, 60% and 75%) of large grain particles (> 2.8 mm) into a white bread matrix.

Development of low glycaemic breads with the knowledge gained from the study

The development of low glycaemic breads was based on knowledge obtained from prior work in this thesis. In this chapter, grain particles from peas, soy, purple wheat, and bread wheat were used. Purple wheat produces a deep purple grain colour favoured by end-users for both kibbling and milling in the bread industries. To give a fair comparison of grains, bread wheat which is used in bread baking was added as a comparative test grain to white breads. Soy has similar properties to peas, both are legumes and soy flour is commonly used by the bread industry. In contrast to the small grain cereals, the cells of the endosperm are living tissue and the cell walls remain intact unless damaged by milling, for this reason, they may provide a useful barrier to enzymes during digestion.

7.2 Materials and Methods

7.2.1 Materials

Bread ingredients: Wheat flour (Champion Flour Milling Ltd, 12.2% moisture, 11.5% protein, 1.4% fat, 76.8% carbohydrates), salt (Woolworths, Home brand), sugar (Chelsea, NZ Sugar Company Ltd), wheat gluten (Cargill, Inc), vegetable oil (Harvest, Natural sugars New Zealand Ltd) and instant dry yeast (Edmonds, Goodman Fielder New Zealand Ltd) was purchased from the local supermarket. Country oven Dobrim 90 dough improver was purchased from Bakels, New Zealand. Breads incorporating kibbled grain particles (> 2.8 mm) from Peas, bread wheat, purple wheat and soy incorporated into a white bread matrix at 75% of the dough mass were made (**Table 7.1**) and the *in-vitro* digestibility was tested. Purple wheat, bread wheat and soy were obtained from the General Health Store, Auckland, New

Zealand. Peas were obtained for Davis Trading, Palmerston North, New Zealand. Tap Water used was from the Palmerston North domestic supply.

Digestion chemicals: Dry pepsin powder (Pepsin EC 3.4.23. from Porcine stomach mucosa, P 7000, 800-2500 U/ml), and (P7545, 8 x USP specifications) were purchased from Sigma-Aldrich, USA. Fungal amyloglucosidase (EC. 3.2.1.3. from Aspergillus niger, E-AMGDF; 3260 U/ml) was purchased from Megazyme International Ltd.

Table 7.1 Ingredients and Formulations for white (WB) and kibbled grain breads.

Ingredients (g)	White bread[1]	65% Kibbled grain bread[2]	75% Kibbled grain bread[3]	75% Kibbled 50:50 grain bread[3]
Flour	100	100	100	100
Sugar	1.89	1.89	1.89	1.89
Salt	1.89	1.89	1.89	1.89
Gluten	3.77	3.77	3.77	3.77
Yeast	3.21	3.21	3.21	3.21
Dough improver	0.57	0.57	0.57	0.57
Oil	3.40	3.40	3.40	3.40
Water	73.96	73.96	73.96	73.96
Kibbled grains	N/A	285.71	300	150/150

*NB, the numbers in the table of ingredients, are bakers' proportions where flour =100% and the other ingredients are a proportion of the flour. The proportion of kibbles is a proportion of the wet dough. Where two kibbled grains were used the proportions of the grains were equal.

7.2.2 Grain milling

Wheat grains were supplied as is and along with the peas and soy were de-hulled and milled, as reported in **Section 3.2.1**. Three particle sizes were separated by sieving >2.8 mm, >2 mm

and > 1.2 mm. To make the breads, kibbled grain were hydrated and added to white bread dough which is explained in detail at the later stages of this chapter.

7.2.3 Formulations

7.2.3.1 Strategy 1 Particle size:

Breads made with the kibbled grain particles (>2.8 mm, >2.0 mm, >1.2 mm) incorporated at 65% of the dough mass (d.w.b) and were formulated to have equal proportions of carbohydrates in 100 g of whole bread (40± 2% d.w.b). Moisture content and *in-vitro* starch digestibility were determined as reported in **Section 7.2.5** and Section **7.2.6,** respectively. White bread with a flour particle size of between 0.1 and 0.2 mm (denoted >0.1) was used as a control.

Test foods:

a) Breads made with 65% of kibbled (> 0.1 mm) bread wheat and 35% of white bread dough as a matrix (Reference white bread - WB).
b) Breads made with 65% of kibbled (> 1.2 mm) bread wheat and 35% of white bread matrix (B1.2).
c) Breads made with 65% of kibbled (> 2.0 mm) bread wheat and 35% of white bread matrix (B2.0).
d) Breads made with 65% of kibbled (> 2.8 mm) bread wheat and 35% of white bread matrix (B2.8).

7.2.3.2 Strategy 2 Proportion of large kibbles particles:

To determine the effect of kibbled particle size on the digestibility of the starch in the breads 0%, 15%, 30%, 45%, 60% and 75% of > 2.8 mm kibbled soft wheat grain particles were incorporated into the breads with white flour dough as the balance of the bread preparations. All breads were formulated to (40 ± 2% d.w.b) of carbohydrates in the cooked bread). Moisture content and *in-vitro* starch digestibility were performed as reported in **Section 7.2.5** and **Section 7.2.6,** respectively. White bread containing no kibbled grain was used as a control and is denoted 0% (to show that grain particles were not incorporated in white bread) in this section.

Test foods:

a) Breads made with 100% of white bread matrix (Reference white bread - WB).
b) Breads made with 15% of kibbled (> 2.8 mm) bread wheat and 85% of white bread matrix (B15).
c) Breads made with 30% of kibbled (> 2.8 mm) bread wheat and 70% of white bread matrix (B30).
d) Breads made with 45% of kibbled (> 2.8 mm) bread wheat and 55% of white bread matrix (B45).
e) Breads made with 60% of kibbled (> 2.8 mm) bread wheat and 40% of white bread matrix (B60).
f) Breads made with 75% of kibbled (> 2.8 mm) bread wheat and 25% of white bread matrix (B75).

7.2.3.3 Development of low glycaemic breads with the knowledge gained from the study

To determine the effects of starch digestibility on the breads made with 75% (d.w.b) of kibbled grains (> 2.8 mm) from soft wheat, hard (purple) wheat, peas and soy. Moisture content and *in-vitro* starch digestibility were performed as reported in **Sections 7.2.5** and **7.2.6,** respectively. In this section, kibbled particles of peas, soy, purple wheat, and bread wheat were used. Large pea and purple wheat particles (>2.8 mm) had a lower proportion of starch digested and slower rates of starch digestion in previous chapters **(Chapter 6)**.

Test foods:

a) Breads made with 75% of kibbled (> 2.8 mm) bread wheat and 25% of white bread matrix.
b) Breads made with 75% of kibbled (> 2.8 mm) purple wheat and 25% of white bread matrix.
c) Breads made with 75% of kibbled (> 2.8 mm) peas and 25% of white bread matrix.
d) Breads made with 75% of kibbled (> 2.8 mm) soy and 25% of white bread matrix.
e) Breads made with 37.5% kibbled (> 2.8 mm) peas, 37.5% kibbled (> 2.8 mm) purple wheat and 25% of white bread matrix.
f) Breads made with 37.5% kibbled (> 2.8 mm) soy, 37.5% kibbled (> 2.8 mm) purple wheat and 25% of white bread matrix.

7.2.4 Bread baking

The kibbled grain breads and reference white breads were prepared in The New Zealand Institute of Plant & Food Research Limited, food-safe laboratory. The standard white bread (reference) was formulated with 100% flour along with 1.9% salt, 1.9% sugar, 3.8% gluten, 3.2% yeast, 0.6% dough improver, 3.4% oil and 73.6% water, the latter as a proportion of the flour (bakers proportion). Variations to make the kibbled grain breads are described below.

To make the bread, yeast was suspended in warm water (33±2 °C) and left for 10 min to hydrate. The other dry ingredients were blended in the bowl of a KitchenAid® Mixer (Model 5KSM150PS, KitchenAid, USA) with a hook attachment at a speed of level 2. Fresh yeast suspension, the oil and water were then added and mixing continued for 12 min. The dough was divided into 400 g pieces, and shaped into loaves and placed into 27.6 x 14 x 6.5 cm silicone loaf pans and sealed tightly with foil wraps to reduce water loss allowing space for leavening of the bread. The loaves were then transferred to a standard bread proofer (Irinox MF 70.1) at 100% humidity and 30 °C for 60 min for the initial proofing, after which the dough was again kneaded for 5 minutes using the KitchenAid® Mixer. The dough was then shaped into loaves and placed in the loaf tins with tightly covered foil wraps allowing space for the leavening of the breads. The breads were finally proofed at 30 °C and 100%RH (Irinox MF 70.1) for 60 min. The loaves were baked in a conventional oven (Zanussi Professional) with the air vent shut to prevent moisture loss, at 215 °C until the internal temperature of the breads reached 90° C (Traceable® Food Thermometer). After baking, the loaves were cooled for 1 h until the internal temperature of the bread reached 30 °C, and then cut into slices, packed and labelled in polyethene Ziploc bags and stored at -20 °C until analysis.

The kibbled grain breads had the same bread-making process however the cooking time was adapted to obtain an internal bread temperature of 90° C. To make the breads, kibbled grain were preconditioned by soaking in tap water for 16 h at 20° C. After 16 h, the soaked grains were drained for 20 min and patted with paper towels to remove excess water at this point the grain particles contained about 50% by weight of water. The white bread matrix was prepared with all the wet and dry ingredients and kneaded for 10 min. After 10 min, the kibbled grains were added to the white dough mixture and kneaded for 2 min. The proportions of grains and dough used in the preparation of breads are given in **Table 7.1**. The dough was divided into 800 g pieces, shaped into loaves and placed into 27.6 x 14 x 6.5 cm silicone loaf pans and

tightly sealed with foil at baking. The dough weights of kibbled grain breads were twice that of the white breads to give similar dimensions when the loaves were baked. Initial, final dough proofing and baking of the breads were similar to that of the white breads. After baking, the loaves were cooled, packed and stored similar to the white bread. All the test breads were baked, sliced, packed and frozen before human intervention analysis.

7.2.5 Moisture content:

Moisture contents were determined as mentioned in **Section 3.1.1**.

7.2.6 *In-vitro* amylolysis

The rate of *in-vitro* amylolysis of the bread samples was measured as reported in **Section 3.1.3**. Briefly, samples (5g) of each of the breads, were either homogenized or left intact and were subjected to simulated gastric peptic digestion and a subsequent amylolytic phase at pH 6.5 that simulated small intestinal digestion using pancreatin (P7545, Sigma-Aldrich, USA; 8 x USP specifications) and amyloglucosidase (Megazyme, E-AMGDF; 3260 U/ml) as reported in **Section 3.1.3**.

Aliquots (0.5 ml) before 0 min and after the addition of pancreatin were taken at 10, 20, 40, 60 and 120 min. Potentially available carbohydrates for intact and homogenized breads were measured as reported in **Section 3.1.5**. The glucose released was quantified by the DNS method (**Section 3.1.4)**. The glycaemic potency of breads was derived from the glucose equivalents (GE) present in a solution after digestion (**Section 3.1.4)**. The rates of starch digestion over the first 20 min (RDS), was assessed (**Section 2.8)**.

7.2.6.1 Contribution of the proportion of starch in kibbled grains to overall starch digestibility of low glycaemic breads

To measure the contribution of starch from intact grains to the overall digestibility of the kibbled grain breads during *in-vitro* amylolysis, the following experiment was performed on breads specified in **Section 7.2.3.3**. The rate of *in-vitro* amylolysis of the bread samples was measured as reported in **Section 7.2.6**. Samples (5g) of each of the breads, were weighed into six 70 ml digestion pots containing 30 ml of deionized water. One of the 5 pots was sampled

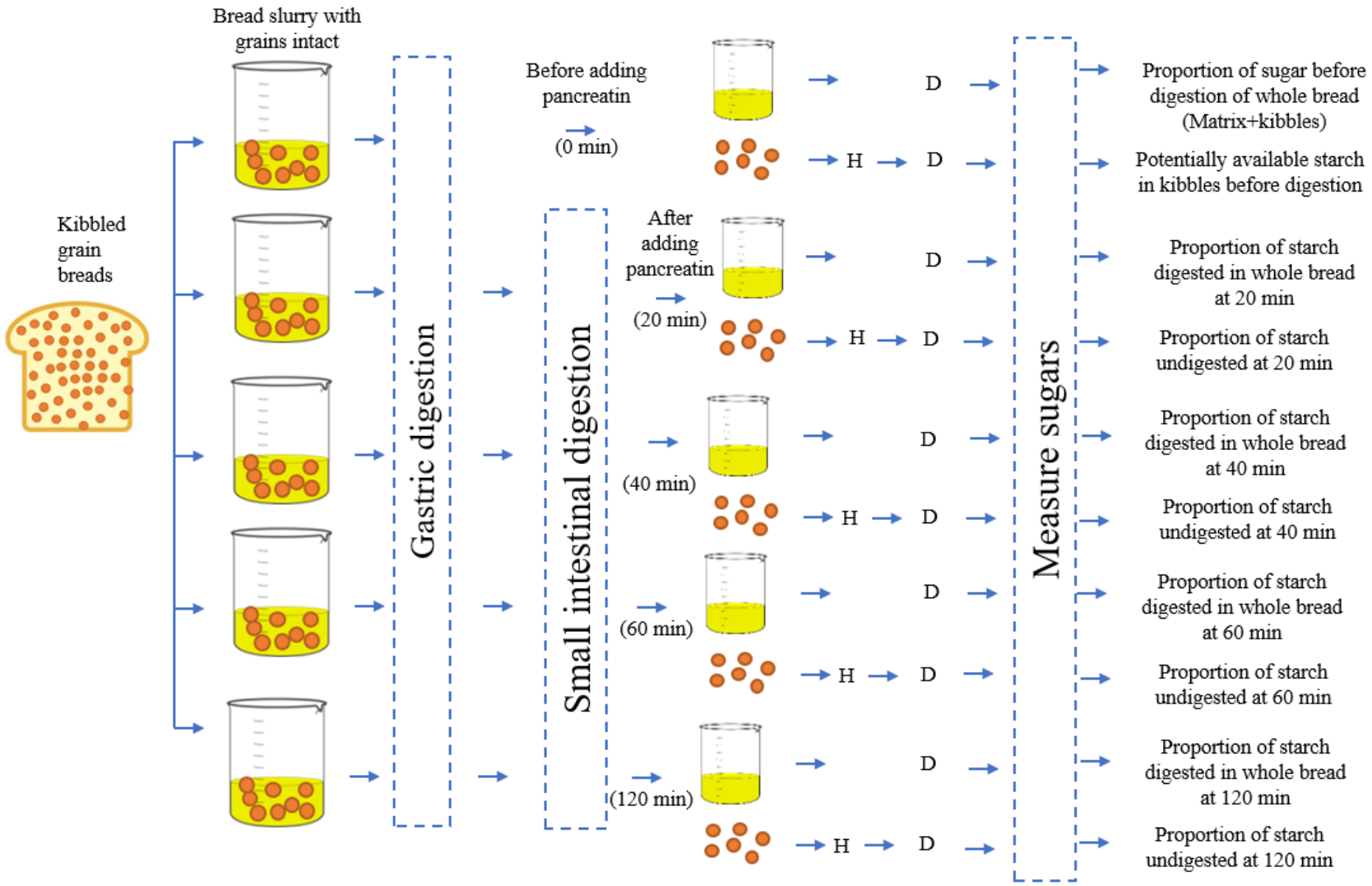


Figure 7.2 Schematic representation of measuring the proportion of starch contributed by intact grains to overall starch digestibility of whole breads. H – Homogenized; D- Digested.

before adding the pancreatin (0 min) and the other 4 pots 20, 40 60 and 120 min after adding the pancreatin as shown in **Figure 7.2**.

As for all experiments, digestion was conducted in two stages, one simulating gastric peptic digestion and a subsequent stage simulating small intestinal digestion (**Section 3.1.3**). To measure the proportion of starch digested from the kibbled grain particles baked in the breads, the particles were separated from the digesta at each time interval and immediately mixed with four volumes of absolute alcohol to inhibit amylase activity. A similar protocol using 2ml of absolute alcohol was used to inhibit the 0.5 ml samples of liquid digesta sampled at each time interval. At least 30 min after adding alcohol to the samples, the tubes were centrifuged for 10 min at 1000 *g* at 20°C (Centrifuge Omnifuge 2.0 RS Heraeus Sepatech) to clarify the supernatant before analysis of sugars released during digestion. The separated grains were maintained in alcohol for 10 min, before being drained and dried completely. To the grains, 30 ml of deionised water was added and the mixture homogenized (H) for 2 min with an UltraTurrax Homogenizer (IKA®-Werke, GmbH & Co.KG, Staufen, Germany) with an S18N-19G dispersing element. Homogenized samples were checked to ensure that all of the samples passed through a 1 mm sieve and hence that homogenizing was effective. These samples were subjected to *in-vitro* amylolysis, as reported in **Section 7.2.6,** and the undigested starch was measured in homogenized 120 min.

All samples were stored at 20°C until reducing sugar analysis by DNS method reported in **Section 3.1.4**. All experiments were conducted in duplicate. The total available starch was measured from grains obtained at 0 min. The proportion of starch that remained undigested in the kibbled grains at each sampling time was calculated by subtracting the sugar digested at each time point from the kibbled grain with the total available starch in grains. The proportion that was undigested was subtracted from 100 in order to obtain the percentage of starch digested by the kibbled grains in the breads.

7.2.6.2 **Informal Consumer acceptance**

The overall acceptability of the breads made using the kibbles from the different grain types was evaluated using a nine-point hedonic Likert rating scale (**Figure 7.3**). The breads were prepared in the Plant & Food, food-safe laboratory. Breads were baked the day before testing and were cut into 4 cm x 4 cm x 2 cm pieces and packed in labelled ziplock bags not more than 1 h before being served. Breads were given unique identity codes. Breads made with kibbled

bread wheat were given to each of the subjects as a "warm-up" sample and the results were not used. Warm-up samples were given to avoid any "first food" effect as the high proportion of kibbled grain in the breads rendered the test breads, unlike standard bread. White bread control was not used as a control in the informal consumer acceptance as the aim was to choose the kibbled breads that are highly liked by the subjects for the subsequent human trial and not to compare the liking of the kibbled grain breads with white breads. Water was provided after tasting each bread. Each test food was tested once in each subject.

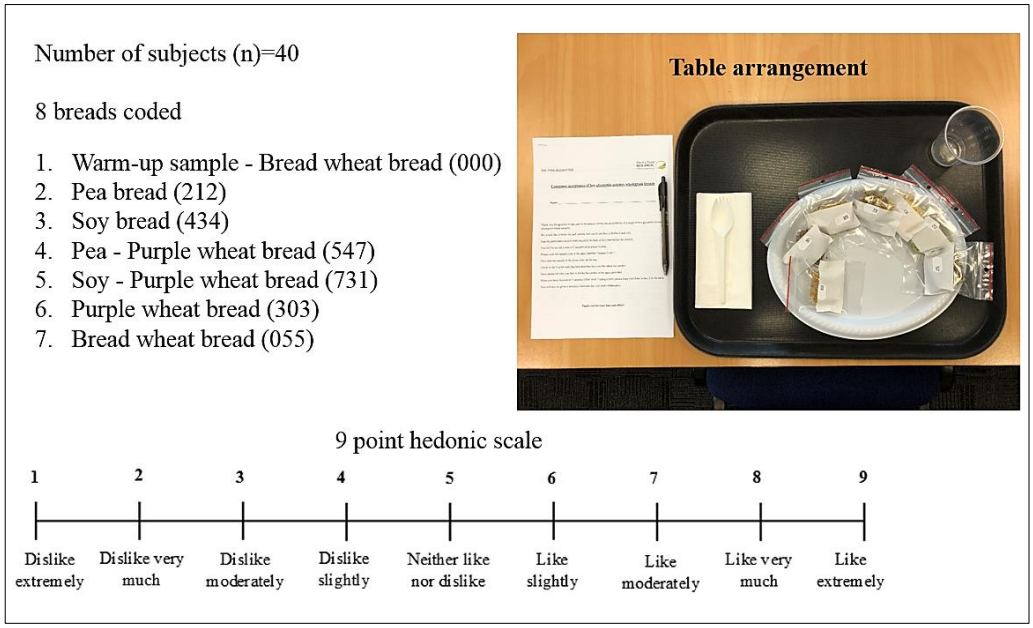


Figure 7.3 Informal consumer acceptance set up

The subject number (n = 40) is typical of studies involving comparisons of foods and was recruited from within Plant & Food Research for the study subject to the following inclusion criteria:

- Age: Aged between 18 and 65.
- Gender: Male or female.
- Gluten and glucose tolerance: No history of diabetes or gluten intolerance.

On the day of testing, breads and 200 ml of water were placed on the desks provided in the rooms. Subjects were asked to sign a consent form before tasting the foods. Subjects were given a questionnaire to be filled that contained the codes of all the breads. Subjects marked the overall liking after tasting each bread, the sheets were collected, and a token of appreciation was given. Statistical analysis was completed to describe the differences in the overall consumer acceptability of the breads.

7.2.7 Data analysis

All data manipulations were conducted in a Microsoft Excel spreadsheet (Microsoft Corp., Redmond, WA, USA). All experiments were conducted in duplicate. Analysis of variance and Pearson correlation coefficients for the relationship between different parameters was carried out using SPSS 24.0 software (IBM Corporation, Chicago, IL, USA). Comparisons of means were determined by Tukey's test to a 5% significance level.

7.3 Results and Discussion

7.3.1 Strategies to develop low glycaemic breads

7.3.1.1 Strategy 1: Particle size

The moisture content of the breads WB, B1.2, B2.0 and B2.8 were 46.50%, 47.07%, 46.92%, and 47.27%, respectively and were not significantly different at $p < 0.05$. The *in-vitro* rates of starch digestion were plotted as the proportion of starch digested with the time. Based on potentially available carbohydrates (sugar released after 180 min of digestion of the homogenized samples) (**Figure 7.4 A-D**), the proportion of starch digested for the homogenized samples containing kibbled particles at 120 min was between 90%- 98% and at least 16%-41% greater than for the intact bread samples. The proportion of starch digested for WB was similar for intact and homogenized WB at 120 min. At least 70% of the starch was digested for both intact and homogenized white bread at 20 min. It is suggested that structure remaining in the grain particles reduced the rate of digestion of the starchy components, in white bread there were no large particles and hence homogenisation had no effect on rates of digestion. In comparison to the homogenized breads, the proportion of starch that remained undigested at 20 min for intact B2.8, B2.0 and B1.2 were 57.1 %, 42.8% and 28.5% respectively. This suggests that such structural factors alone may have retarded starch digestion in the intact kibbled grain breads (Berg et al., 2012; Edwards et al., 2015; Johansson et al., 2018; Mishra et al., 2012).

When the proportion of starch digested over the first 20 minutes (**Figure 7.4 A-D**) was fitted to linear regressions, the slopes of the regressions estimated the rate of starch digestion (g/min). When the rates of digestion are compared for intact and homogenized breads, it is very clear

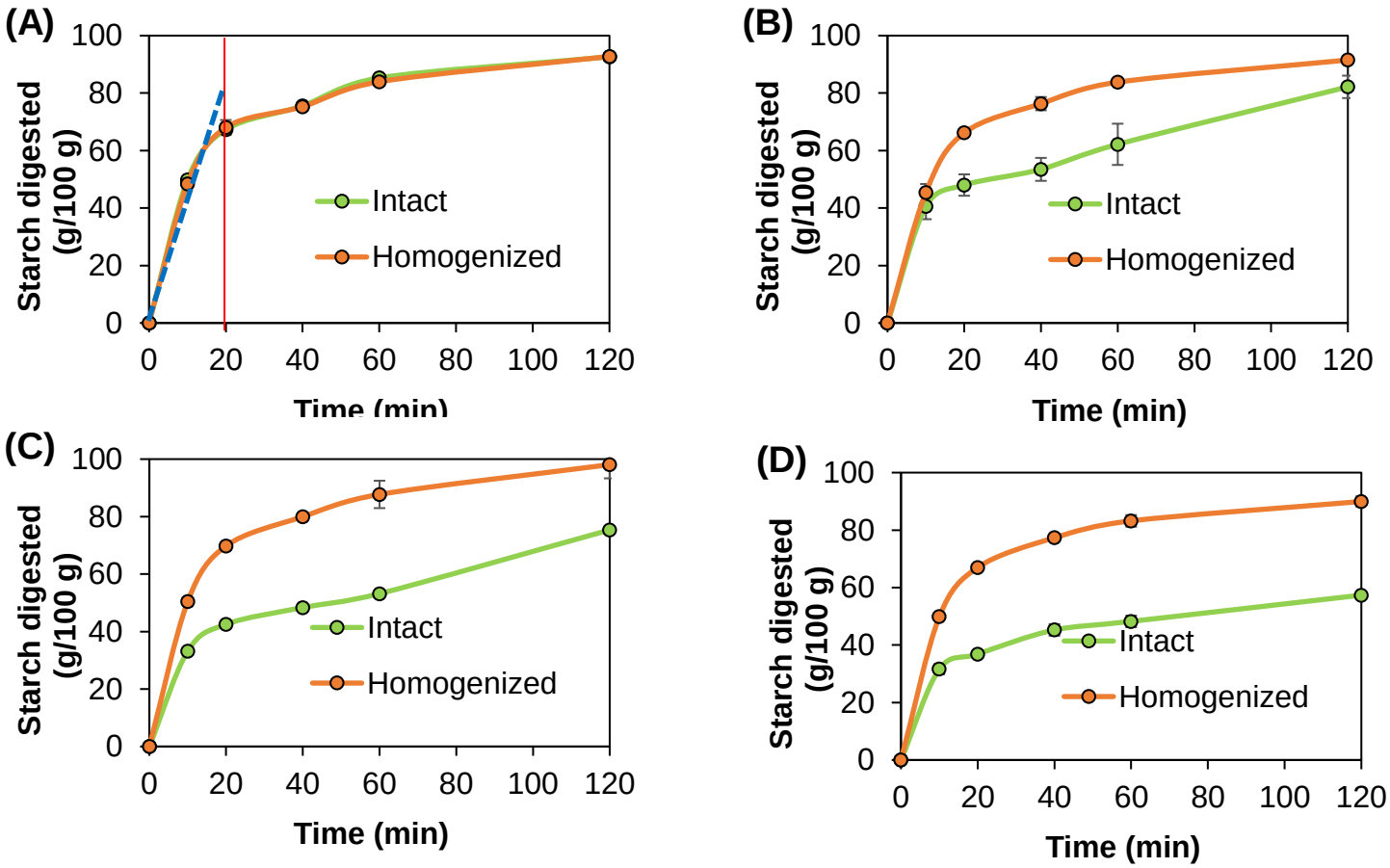


Figure 7.4 *In-vitro* starch digestion curves of breads made with 65% of bread wheat grain particles of various particle size (A) White bread (> 0.1 mm), (B) > 1.2 mm, (C) > 2.0 mm and (D) > 2.8 mm digested for 120 min with 0.2 ml of 5% pancreatin measured as intact and homogenized. Points are the average of 2 samples; intervals are (±) SD. Blue dashed line fitted data for the first 20 min of digestion.

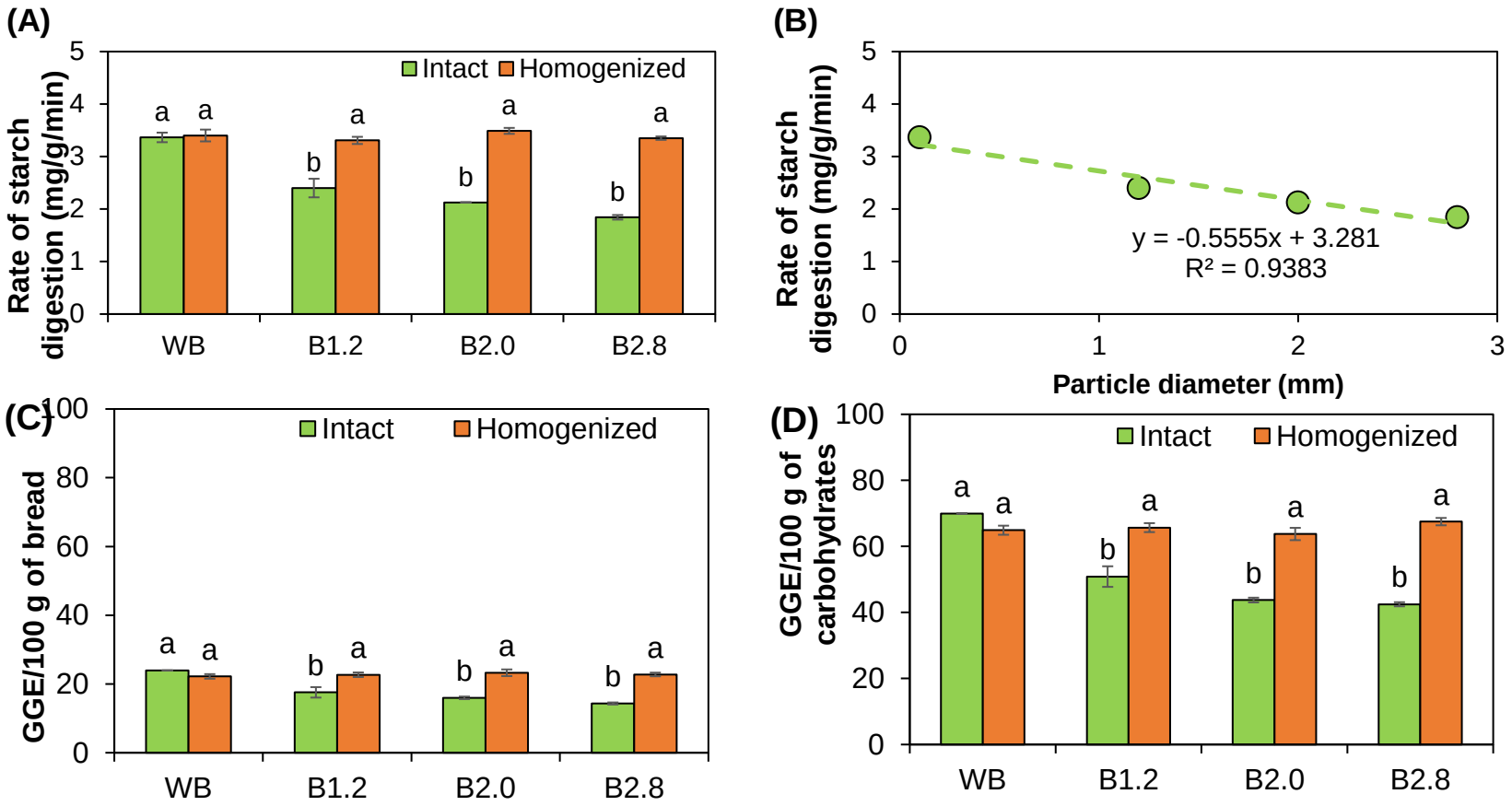


Figure 7.5 (A) Relative rate of starch digestion (20 min) of White bread (WB) and breads made with 65% of bread wheat grain particles of various particle size > 1.2 mm (B1.2), > 2.0 mm (B2.0) and > 2.8 mm (B2.8), measured as intact and homogenized. (B) Relationship between various particle size (0.1 mm (WB), > 1.2 mm, >2.0 mm >2.8 mm) of grains with the rate of starch digestion (mg glucose/g starch/min) in non-homogenised bread samples. Glycaemic glucose equivalents on the basis of (C) 100 g of bread (glycaemic potency) and (D) per 100 g of CHO (GI) and (C). Points are the average of 2 samples; intervals are (±) SD. Different letters indicate significant differences between the breads in each group ($p < 0.05$).

Table 7.2 Available carbohydrate (CHO) content and the contribution of homogenisation to the difference in net GE between white bread (WB) and breads containing kibbled grain.

Bread	Treatment[1]	Available CHO (%)	RGP[2] GGE (g/100g)	Net GE as a % of WB net GE[3]	Difference due to particle size
WB	I	35.40	24.8[a]	100.0[a]	-8.3
	H		22.7[a]	91.7[a]	
B1.2	I	34.54	17.9[b]	72.2[b]	20.2
	H		22.9[a]	92.4[a]	
B2.0	I	36.50	16.1[b]	65.1[b]	30.7
	H		23.7[a]	95.8[a]	
B2.8	I	33.73	14.6[b]	58.9[b]	33.9
	H		23.0	92.8[a]	

[1]I – Intact; H- Homogenized
[2]Relative glycaemic potency (RGP) (GGE (g)/100 g of food) = [(AUC bread/AUCWB (100 g))* 24.78)] White bread GGE = GI * % avail. carb. = 70 *35.40 = 24.78. (Monro, 2002)
[3]Used mean white bread (WB) GGE value (24.78 g)

that all breads except WB (3.3 mg/g/min) had slower rates of digestion over the first 20 min when digested intact (**Figure 7.5 A)**. The rate at which the starch digested in the intact B2.8 was about half that of the reference WB; however, the rate of digestion increased to that of the WB when homogenized. Destructuring the larger grain particles by homogenizing increased the rate of starch digestion in the kibbled grain breads (**Figure 7.5 A**). The rate of starch digestion for the first 20 min of *in-vitro* digestion and particle sizes of the kibbled grains was significantly and linearly negatively correlated (**Figure 7.5 B**). Therefore, it is proved that breads that contained a high proportion of large intact endosperm particles have lower rates of *in-vitro* starch digestion.

The estimated glycaemic potency (GGE/100 g of bread) of homogenized breads was significantly higher (~30%) than the non-homogenised samples for the kibbled grain breads **(Figure 7.5 C)**. However, B2.8 (**Figure 4.4 D**) had a low adjusted GE release in comparison to other breads due to slow rates of diffusion of the enzyme into and sugars out of the particles when the grains size becomes larger. The contribution of particle size to the difference in GGE/100 g of bread between WB and the kibbled grain breads was less than 34 % for all breads analysed. Compared to WB the largest reduction in glycaemic potency (~40%) was measured for the breads made with > 2.8 mm kibbled grains (B2.8) (**Table 7.2**). The *in-vitro* GI estimate

for intact WB was 71.6±3.3 GGE/100 g CHO. The GI (GGE/100g of CHO) for B2.8 was much lower than other intact kibbled grain breads, but maceration by homogenization, simulating chewing, of this bread increased the GI to 73.31 ± 0.26 GGE/100g of CHO which comparable with WB. This shows that our first strategy to include a high proportion (> 65%) of larger (> 2.8 mm) kibbled grains is likely to lower the glycaemic potency of breads by about 40% provided the kibbled particles remained intact. However, like homogenization it is expected that chewing will reduced particle size and the breads will become rapidly digestible. Behall et al. (1999) tested bread made with white flour, conventional whole grain flour and ultrafine whole grain flour, and found no difference between the breads in glycaemic response. Their result is consistent with our results showing that most of the effect of particle size occurs above 1.2 mm and explains the need to increase the particle size to lower GGE; however, it seems that this strategy seems unlikely to work when the breads are chewed.

7.3.1.2 Strategy 2: Glycaemic potency of breads made with various proportions of kibbled grains

The breads made with different proportions of intact (> 2.8 mm) kibbled grains (WB, B15, B30, B45, B60 and B75) had similar moisture contents (44.89%, 46.50%, 45.51%, 46.11%, 48.01% and 47.61%, respectively) and there were no significant differences among them ($p < 0.05$). As for the breads containing particles of different size, these breads were also digested either homogenized or intact. The data were plotted as the relative proportion of available starch digested at each time interval (mg/g/min). The sugar released after 120 min of digestion of the homogenized sample represented 100% of the digestible starch. (**Figure 7.6 A-F**). For the WB sample and breads containing 15% kibbled grain particles the proportion of starch digested was similar ($p<0.05$) for the homogenised and non-homogenised samples (**Figure 7.6**). For the B30 breads, the proportions of starch digested were significantly lower over the first 40 min for the non-homogenised compared with homogenized breads, after which the proportions of starch digested were similar. For the B45, B60 and B75 samples homogenisation significantly increased digestibility. The estimated glycaemic potency (GGE/100 g of bread) of homogenized breads was significantly higher than the non-homogenised samples for the kibbled grain breads. The breads B60 and B75 that showed the greatest increase in digestibility after homogenising had the highest content of grain particles > 2.8 mm in diameter. The glycaemic potency of non-homogenised B75 is 46.7% less than the glycaemic potency of WB. *In-vitro* GI estimates were high when the kibbled grain breads such as B45, B60 and B75 were

homogenized. The GI for intact and homogenized WB was 70.1 GGE/100 g of CHO which was not statistically different to the GI's of B15 and B30 suggesting that inclusion rates of larger grain particles between 15% and 30% by weight were ineffective in reducing GI when homogenised. GIs of B45 and B60 were lower than WB by 18 and 22 points. However, B75 had a low adjusted GI (39.78±0.83) in comparison to other breads due to the presence of larger proportion (75%) of kibbled grains (>2.8 mm) which retarded starch digestion. The contribution of graininess (proportion of particles in the bread) to the percentage difference in GGE/100 g of breads between WB and the other breads was less than 43.81 % for all breads analyzed (**Table 7.3**), with a much larger proportion of the difference in GGE could be attributed to B75. Furthermore, there was a negative association between the proportion of large grain particles in the breads and the glycaemic potency measured *in-vitro* (**Figure 7.7**).

Table 7.3 Available carbohydrate (CHO) content and the contribution of particle integrity (non-homogenised vs homogenized) to the difference in net GE between white bread (WB) and other breads.

Bread	Treatment[1]	CHO (%)	RGP[2] GGE (g/100g)	Net GE as a % of WB net GE[3]	Difference due to grain content (%)
WB	I	35.40	24.57	100.01	0.13
	H		24.60	100.14	
B15	I	32.59	22.41	91.20	5.85
	H		23.84	97.04	
B30	I	32.64	21.62	87.98	13.84
	H		25.02	101.82	
B45	I	35.70	19.33	78.66	27.51
	H		26.09	106.17	
B60	I	32.02	15.84	64.49	31.90
	H		23.68	96.39	
B75	I	32.69	13.11	53.36	43.81
	H		23.87	97.17	

[1]I – Intact; H- Homogenized
[2]Relative glycaemic potency (RGP) (GGE (g)/100 g of food) = [(AUC bread/AUCWB (100 g))* 24.78)] White bread GGE = GI * % avail. carb. = 70 *35.40 = 24.78. (Monro, 2002)
[3]Used mean white bread (WB) GGE value (24.78 g)

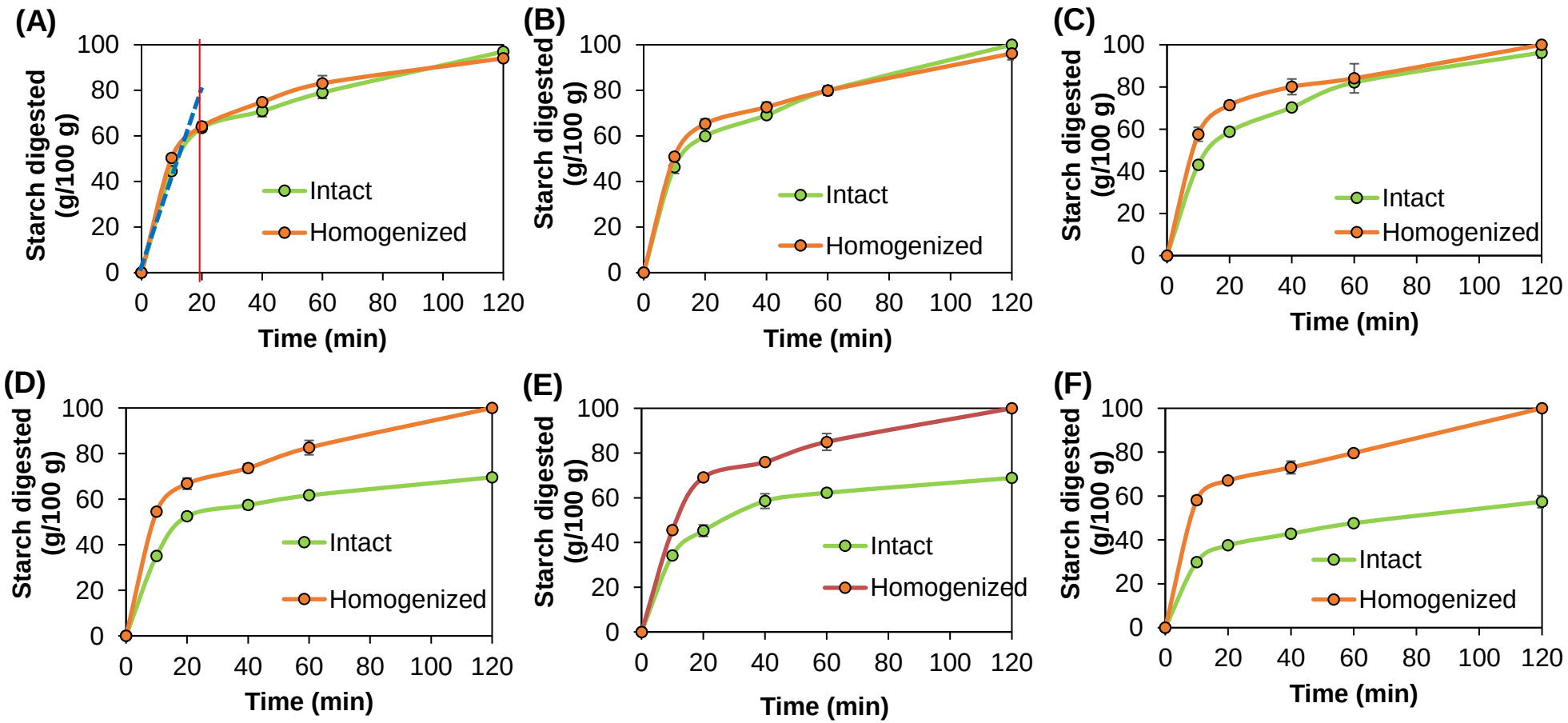


Figure 7.6 *In-vitro* starch digestion curves of breads made with different proportions of > 2.8 mm soft bread wheat grains (A) 0%, (B) 15%, (C) 30%, (D) 45%, (E) 60% and (F) 75% digested for 120 min with 0.2 ml of 5% pancreatin measured as intact and after homogenized. Points are the average of 2 samples; intervals are (±) SD. Blue dashed line fitted data for first 20 min of digestion.

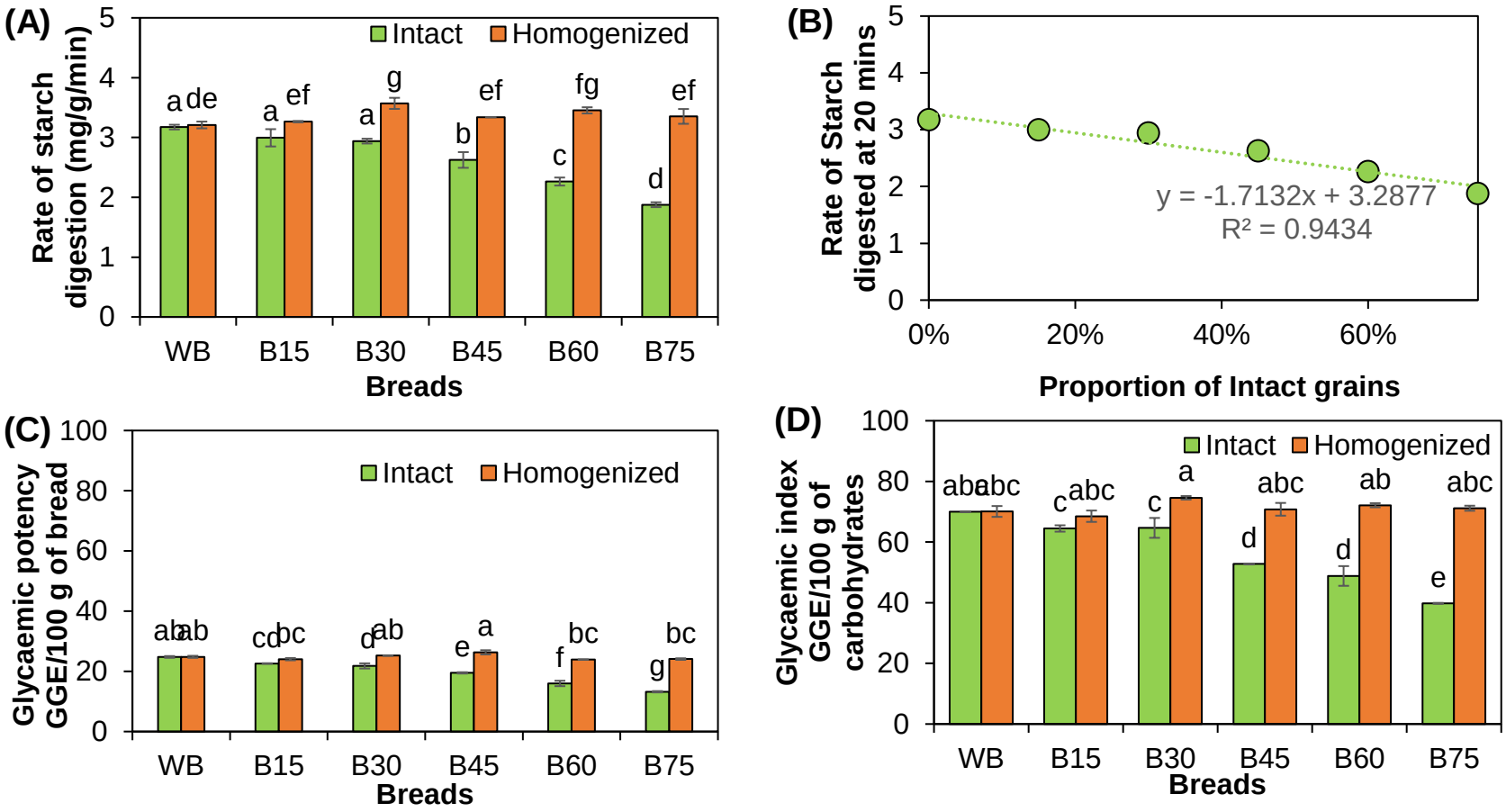


Figure 7.7 Glycaemic glucose equivalents on the basis of (A) 100 g of bread and (B) 100 g of CHO and (C) Rate of starch digestion (20 min) of breads made with different proportions of >2.8 mm bread wheat grains (A) 0%, (B) 15%, (C) 30%, (D) 45%, (E) 60% and (F) 75% measured as intact and after homogenized. (D) Relationship between different proportions of >2.8 mm bread wheat grains (A) 0%, (B) 15%, (C) 30%, (D) 45%, (E) 60% and (F) 75% with the rate of starch digestion (mg glucose/g starch/min) in intact bread samples. Points are the average of 2 samples; intervals are (±) SD.

Our results have shown that the *in-vitro* digestion procedure we used was highly sensitive to the effects of particle size on digestion and that the GGE/100 g of breads decreased as the content of large particles increased, however when the breads were homogenized these differences vanished. Therefore, the presence of large grain particles in high proportions was responsible for reduced *in-vitro* glycaemic potency. The results showed that while particle size is associated with reduced glycaemic potency in some breads, the proportion of these large particles (>2.8 mm at 75% inclusion) in the whole breads is a major factor that indicates reduced glycaemic potency. For instance, the glycaemic potency of bread B75 made with 75% of >2.8 mm kibbled grains, was 10.20% less than that of B65, made with 65% of > 2.8 mm kibbled grains.

When the data presented in **Figure 7.6 A-D** were fitted to linear regressions for the first 20 min, the slopes of the regressions estimated the relative rate of starch digestion. When the relative rates of digestion are compared for non-homogenized and homogenized breads, it is very clear that all breads except WB, B15 and B30 had slower rates of digestion over the first 20 min when digested intact (**Figure 7.7 A)**. The proportion of kibbled particles was significantly and negatively correlated with the relative rate of starch digestion. Increasing the particle content to 65% decreased the rate of starch digestion by 34.5%, and increasing it to 75% by 46.26%. This proves the conclusions from Chapter 4 that the current inclusion rates of about 30% in current New Zealand supermarket breads have no potential to lower the glycaemic potency and starch digestibility in these breads.

7.3.2 Development of low glycaemic breads with different grains

Strategies to develop low glycaemic breads showed that increasing the proportion of > 2.8 mm particles of bread wheat to 75% can halve glycaemic potency when consumed non-homogenized. In this part of the study, we evaluated the effects of inclusion of different types of kibbled grains (>2.8 mm) at 75% when digested non-homogenized or homogenized on the *in-vitro* starch digestibility and glycaemic potency. As expected, in comparison to homogenized breads, in all the non-homogenized kibbled grain breads starch digestion was 50%-70% lower, glycaemic potency 21%-39% lower, and rates of starch digestion 31%-61% lower due to the effects of particle size and the proportion of kibbled grains on digestion (**Figure 7.8**). However, homogenizing did make a large difference to the digestibility of the breads analyzed.

Based on the glycaemic potency (GGE/100 g of breads) (**Figure 7.9 A**), it can be seen that the GGEs were significantly lower for the intact and homogenized breads made with kibbled soy (Intact: 7 GGE/100 g of bread, Homogenized 11 GGE/100 g of bread) and pea (Intact:11 GGE/100 g of bread, Homogenized 16 GGE/100 g of bread) due to the lower proportion of available carbohydrate in the breads. This shows that the proportion of starch in the bread is the major factor determining its glycaemic potency, and it appears that formulating for a low carbohydrate content more effectively reduces the glycaemic potency of breads than changing particle size. In addition, this principle can be valid for a very wide range of bakery goods.

The *in-vitro* GI estimates of the homogenized breads were significantly higher by at least 22% in comparison to the intact breads and breads made with legumes (peas and soy). They also had lower GI's compared with the breads made with hard and soft wheat (**Figure 7.9 B**). The GI's of the homogenized kibbled soy-purple wheat bread and kibbled soy bread were quite low in comparison to the other homogenized breads because legumes such as soy contain little starch (<2%) and have robust cell walls, which may have survived homogenization better than the thin-walled partially degraded endosperm of the small grain cereals. The other legume used was pea which contains a much greater proportion of starch (~60%) than soy, but again cell walls are intact leading to lower glycaemic responses (Berg et al., 2012; Johansson et al., 2018; Mishra et al., 2012). The rates of starch digestion between 0-20 min followed the same trend as the GI for all the breads (**Figure 7.9 C**). Irrespective of the grain type, the GI's were similar to that of the WB when the grains were completely homogenized.

7.3.2.1 Contribution large grain particles to starch digestion of bread

Using *in-vitro* digestion, the proportions of starch digested in kibbled grains from the whole breads were established. From **Figure 7.10,** it can be seen that in comparison to the grain particles, the breads had faster rates of starch digestion, i.e. nearly 40% of the starch was converted into sugars within 20 min. Kibbled grains in the breads had contributed only 20% of the starch digested within 20 min, the bulk coming from the bread matrix. This behaviour revealed that the soft porous matrix digested rapidly in the whole breads while the kibbled grains are digested more slowly providing the samples are intact. It is expected that this would lead to a low to moderate postprandial glycaemic response due to the size of the particles acting as a barrier to digestive enzymes and restricting starch digestion. The gelatinised starch present in the bread matrix was digested quickly as in the case of WB were 70% of CHO is digested in 20 min while with a higher proportion of kibbled grains, the

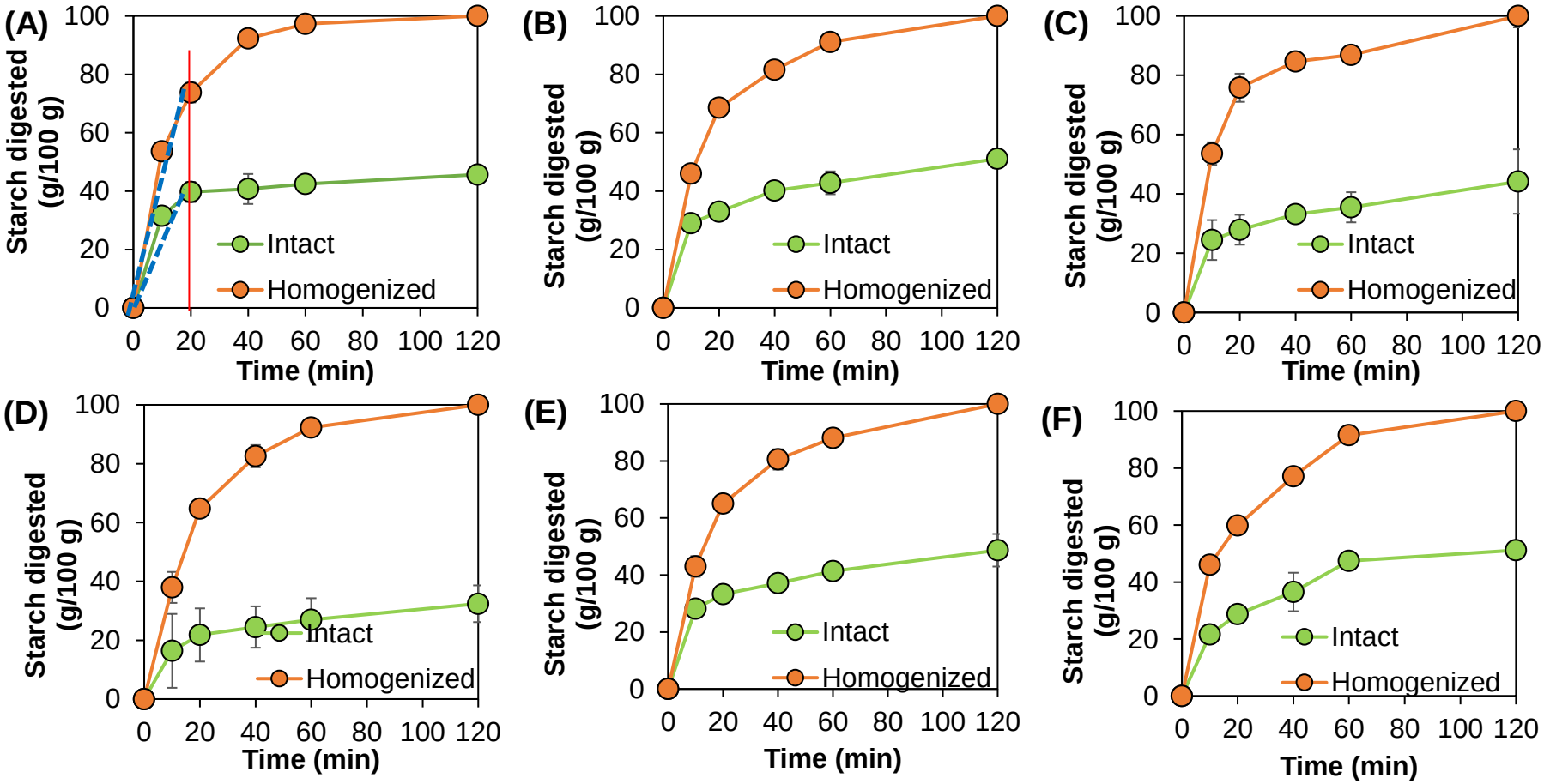


Figure 7.8 *In-vitro* starch digestion curves of breads made with different types of >2.8 mm grain particles (A)75% soft bread wheat, (B) 75% of purple wheat, (C) 75% of peas, (D) 75% of soy, (E)37.5% of peas and 37.5% of purple wheat and (F) 35% of soy and 35% of purple wheat digested for 120 min with 0.2 ml of 5% pancreatin measured as intact and after homogenized. Points are the average of 2 samples; intervals are (±) SD. Blue dashed line fitted data for first 20 min of digestion

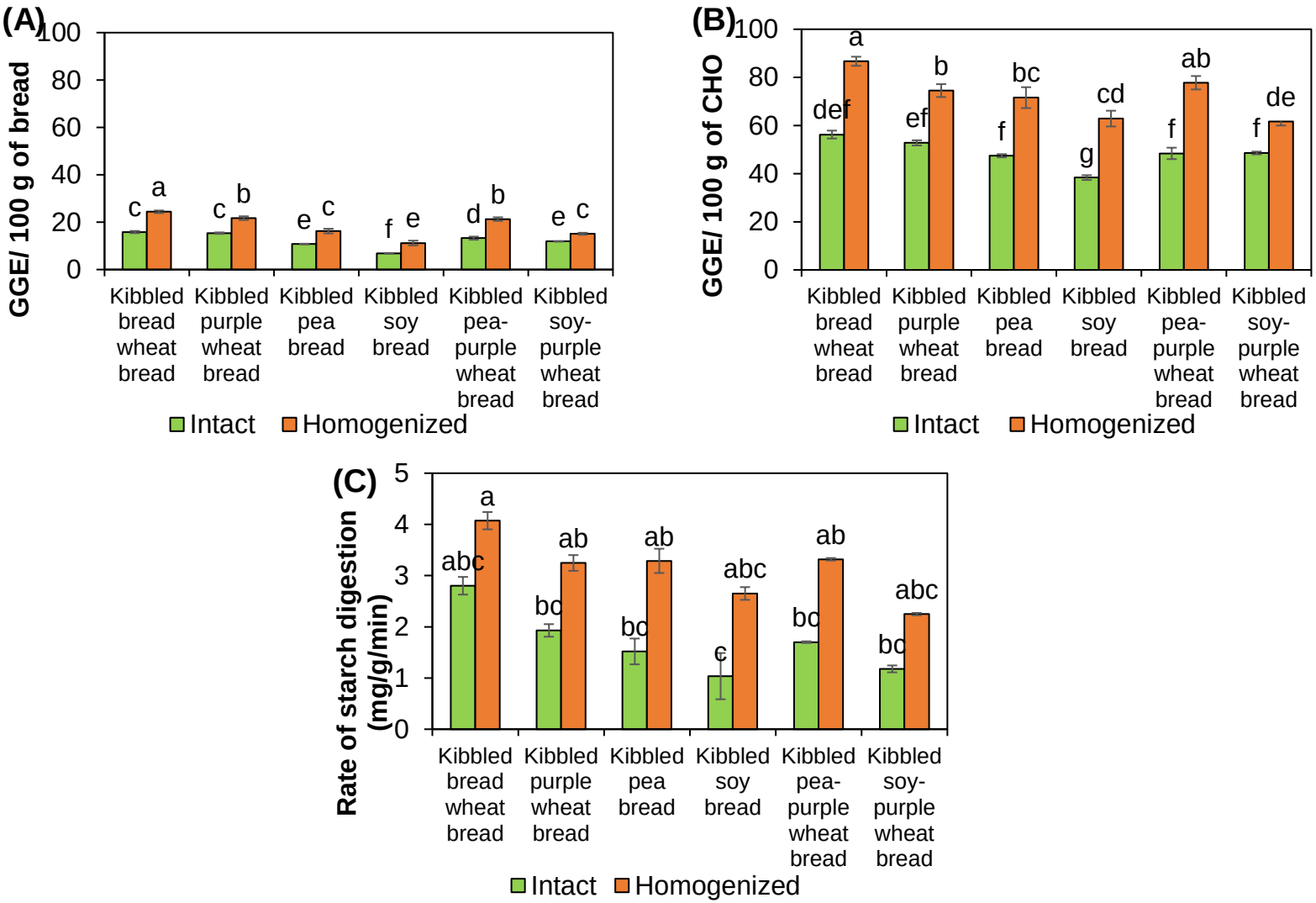


Figure 7.9. Glycaemic glucose equivalents on the basis of (A) 100 g of bread and (B) 100 g of CHO and (C) Rate of starch digestion (20 min) of breads made with different types of grains measured as intact and after homogenized. Points are the average of 2 samples; intervals are (±) SD

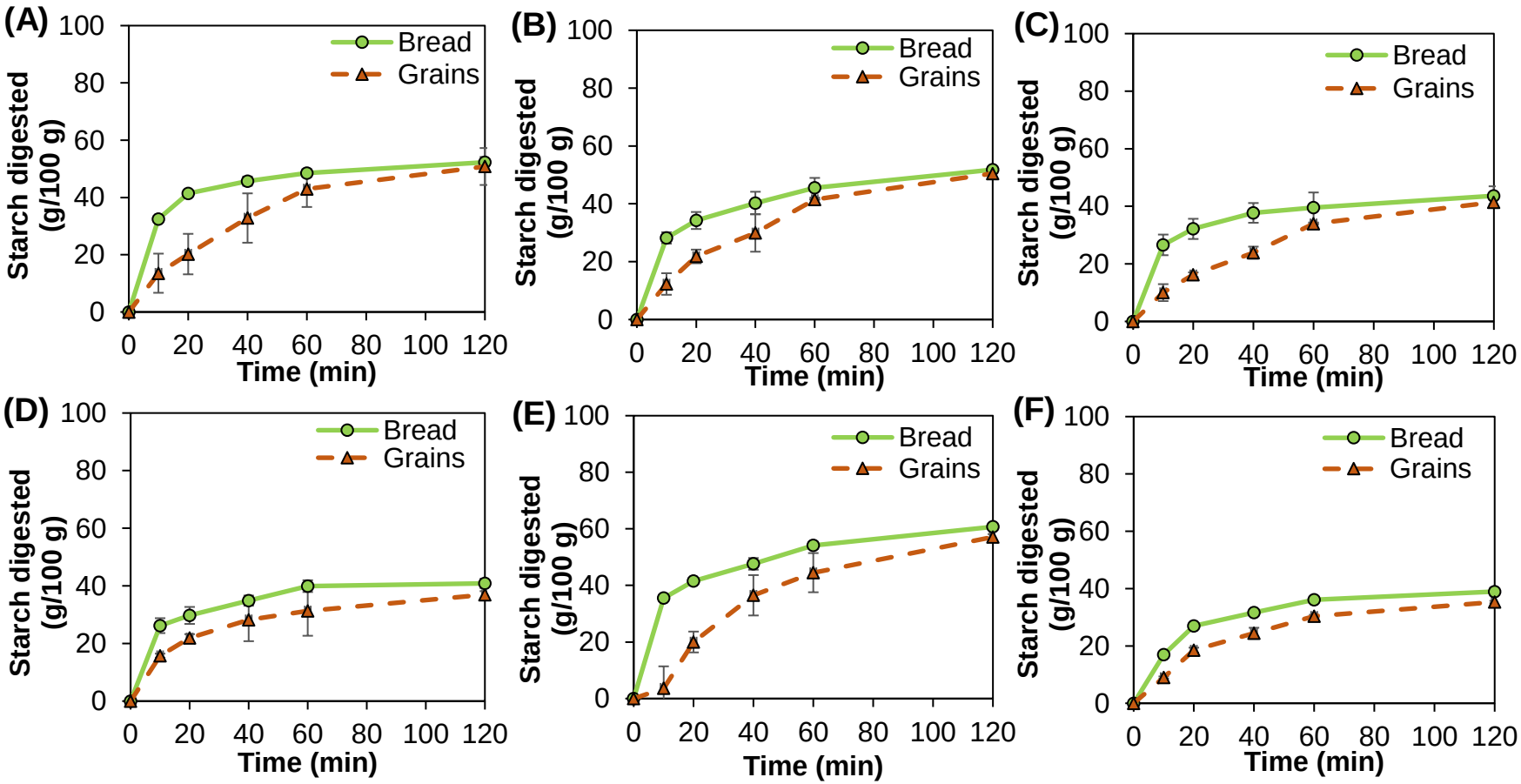


Figure 7.10 *In-vitro* starch digestion curves of breads made with different types of kibbled grains (entire line) and of the kibbled grains extracted from the breads (dashed line) (A)75% bread wheat, (B) 75% of purple wheat, (C) 75% of peas, (D) 75% of soy, (E)37.5% of peas and 37.5% of purple wheat and (F) 35% of soy and 35% of purple wheat digested for 120 min with 0.2 ml of 5% pancreatin measured. Points are the average of 2 samples; intervals are (±) SD.

proportion of free available starch is reduced by the of intact grain particles. Having 25%, white bread matrix in 75% kibbled grain breads results in 40% of the starch being digested at 20 min.

7.3.2.2 **Informal consumer acceptance**

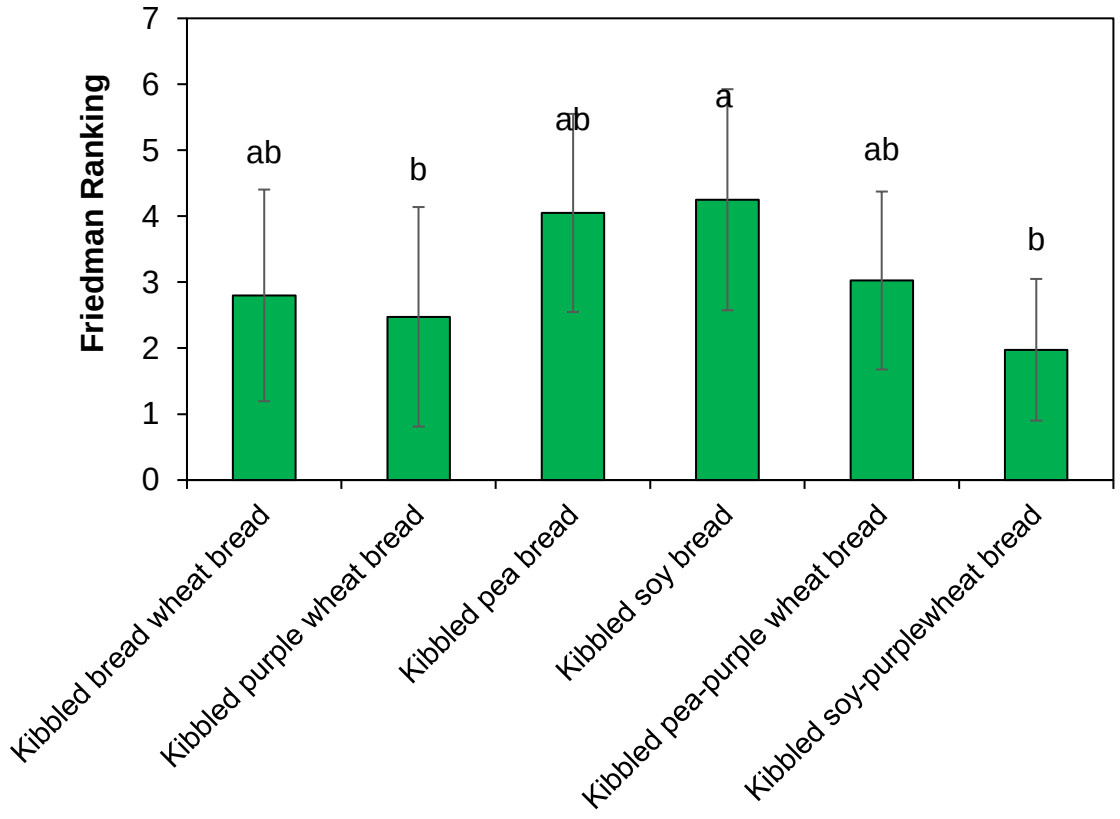


Figure 7.11 Friedman Test performed for acceptability rates form the hedonic scale. Points are the average of 40 subjects; intervals are (±) SD.

Though all the breads made with kibbled particles had similar and low glycaemic potency and starch digestibility, consumer acceptability was highly important before testing them for the human intervention study. As these breads contain a large proportion of partially intact grains, their texture and taste are not like store-bought breads. Before investigating our results with a human intervention study, informal consumer acceptance was needed to choose the breads that had overall acceptability by the consumers. All 40 of the subjects completed the consumer acceptance, and the results analysed. The between-subject variations in the acceptability of breads were large, as is typical of such studies, but no outliers were removed. The scores for each bread for the participants were processed to Friedman ranking using SPSS software. Kibbled purple wheat bread and kibbled soy-purple wheat bread had the highest ranking of 2.5±1.7 and 2.0±1.1, respectively (**Figure 7.11**) for scores between liked very much 0 and disliked 6. These breads were used for the *in-vivo* study (**Chapter 8**).

7.4 Conclusions

Grain content and particle size were highly important in making bread with low in-vitro glycaemic potency. In our study, the maximum proportion of intact particles > 2.8 mm was 75%, which when intact decreased the rates of starch digestion compared to the finely divided flours in white breads. The present study has, therefore, been about the potential effect that intact grain particles might have on the glycaemic potency of bread. However, in reality, when the effects of chewing are taken into account the role of grain particles in reducing the glycaemic potency of wholegrain breads is likely to be close to that of white bread.

Chapter 8

Research Article

Srv, A., Mishra, S., Hardacre, A., Matia-Merino, L., Goh, K., Warren, F., & Monro, J. (2019). The effects of chewing on kibbled-grain breads- An *in vivo* study. British Journal of Nutrition, (Under Review)

Chapter 8: Determination of glycaemic potency of kibbled grain breads- An *in vivo* study.

8.1 Introduction

From the knowledge gained in the previous chapters, a range of kibbled grain breads containing 75% of > 2.8 mm grain particles in the bread matrix were developed and the best in terms of flavour and texture identified through informal consumer acceptability. The following breads were selected and used for a human intervention study: (>2.8 mm) kibbled purple wheat bread (PB), (>2.8 mm) kibbled soy - purple wheat bread (SPB) and a reference white bread (WB) with the results being reported in this chapter. These breads were examined either with the grains intact (I) or homogenized (H), using the *in-vitro* digestive analysis with 5 ml of 0.2% wt/wt pancreatin as mentioned in **Section 7.2.6**.

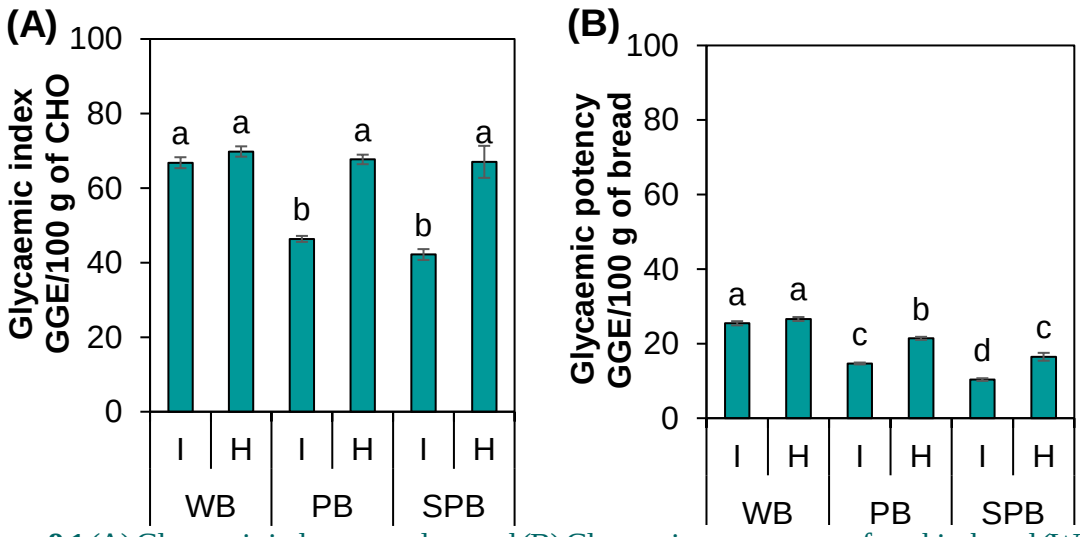


Figure 8.1 (A) Glycaemic index$_{in\text{-}vitro}$ values and (B) Glycaemic potency $_{in\text{-}vitro}$ for white bread (WB), and breads containing >2.8 mm kibbled purple wheat (PB) or >2.8 mm kibbled soy-purple wheat (SPB) with treatments: Intact (I) and homogenized (H) ($p < 0.05$). (Means ± SD). Results with a common letter in the data label do not differ significantly.

In **Chapter 7** it was reported that increasing the proportion of >2.8 mm grain particles in the bread matrix reduced the GGE/100 g CHO by 30% when the breads were digested *in-vitro* without homogenisation compared to the white breads (**Figure 8.1 A**). However, when the kibbled grain breads were completely homogenized and digested *in-vitro*, GGE/ 100 g CHO was similar to the white bread. As the *in-vitro* model measures extreme structural states of a bread (intact *vs* homogenized). It is of interest to investigate the effect of *in-vivo* processing as the bread is ingested by human subjects as chewing may not destroy grain particle structures

as effectively as homogenization. Therefore, during the intervention study presented here, three experimental breads were tested and consumed in three different ways: as usual, swallowed without chewing (intact) and swallowed after homogenization.

The primary aim of this study was to demonstrate the effect of kibbled grain and the role of grain structure for breads consumed by human subjects. The effects on postprandial blood glucose responses were compared to those after consuming a white bread meal. A realistic intake of sufficient bread to provide 40 g of potentially available carbohydrate in all samples consumed. The secondary aim was to determine the effect of chewing breads during consumption on the modulation of glycaemic response.

8.2 Materials and Methods:

8.2.1 Materials

Bread ingredients: Wheat flour (Champion Flour Milling Ltd, 12.2% moisture, 11.5% protein, 1.4% fat, 76.8% carbohydrates), salt (Woolworths, Home brand), sugar (Chelsea, NZ Sugar Company Ltd), wheat gluten (Cargill, Inc), vegetable oil (Harvest, Natural sugars New Zealand Ltd) and instant dry yeast **(**Edmonds, Goodman Fielder New Zealand Ltd**)** were purchased from the local supermarket. Country oven dobrim 90 dough improver was purchased at Bakels, New Zealand. Purple wheat and soy were obtained from the General Health Store, Auckland, New Zealand. Tap Water was used from the Palmerston North domestic supply.

Digestion chemicals: Dry pepsin powder (Pepsin EC 3.4.23. from Porcine stomach mucosa, P 7000, 800-2500 U/ml) and porcine pancreatin (P7545, 8 x USP specifications) were purchased from Sigma-Aldrich, USA. Fungal amyloglucosidase (EC. 3.2.1.3. from A Niger, E-AMGDF; 3260 U/ml) was purchased from Megazyme International Ltd.

8.2.2 Grain Milling

Purple wheat were purchased de-hulled while soybeans were de-hulled and milled after purchase, as mentioned in **Section 3.2.1**.

8.2.3 Baking

The kibbled grain breads and reference white breads were prepared in a food-safe laboratory at Plant and Food Research, as described in **Section 7.2.2** and **Section 7.2.4**. After baking, the loaves were cooled, packed and stored similar to the white bread. All the test breads were baked, sliced, packed and frozen before the human intervention analysis. Starch digestibility analysis was performed on the fresh (day 0) and frozen bread (day 1-7) as described in **Section 3.1.3** to confirm that freezing, storing and thawing the breads did not alter the starch digestibility of the breads (**Appendix I**). The following breads depicted in **Figure 8.2** were generated for this study:

a. White bread (WB) — Bread made with 0% kibbled grains and 100% white bread matrix.
b. Kibbled purple wheat bread (PB) — Breads made with 75% of kibbled (> 2.8 mm) purple wheat and 25% of white bread matrix.
c. Kibbled soy-purple wheat bread (SPB) — Breads made with 37.5% kibbled (> 2.8 mm) soy, 37.5% kibbled (> 2.8 mm) purple wheat and 25% of white bread matrix.

Table 8.1 Ingredients and formulations for white (WB) and kibbled grain breads.

Ingredients	WB[1]	75% PB[2]	75% SPB[3]
	(g)		
Flour	100	100	100
Sugar	1.89	1.89	1.89
Salt	1.89	1.89	1.89
Gluten	3.77	3.77	3.77
Yeast	3.21	3.21	3.21
Dough improver	0.57	0.57	0.57
Oil	3.40	3.40	3.40
Water	73.96	73.96	73.96
Kibbled grains	N/A	300	150/150

[1]WB = white bread, [2]PB = purple kibbled wheat bead, [3]SPB = kibbled soy/kibbled purple wheat bread.

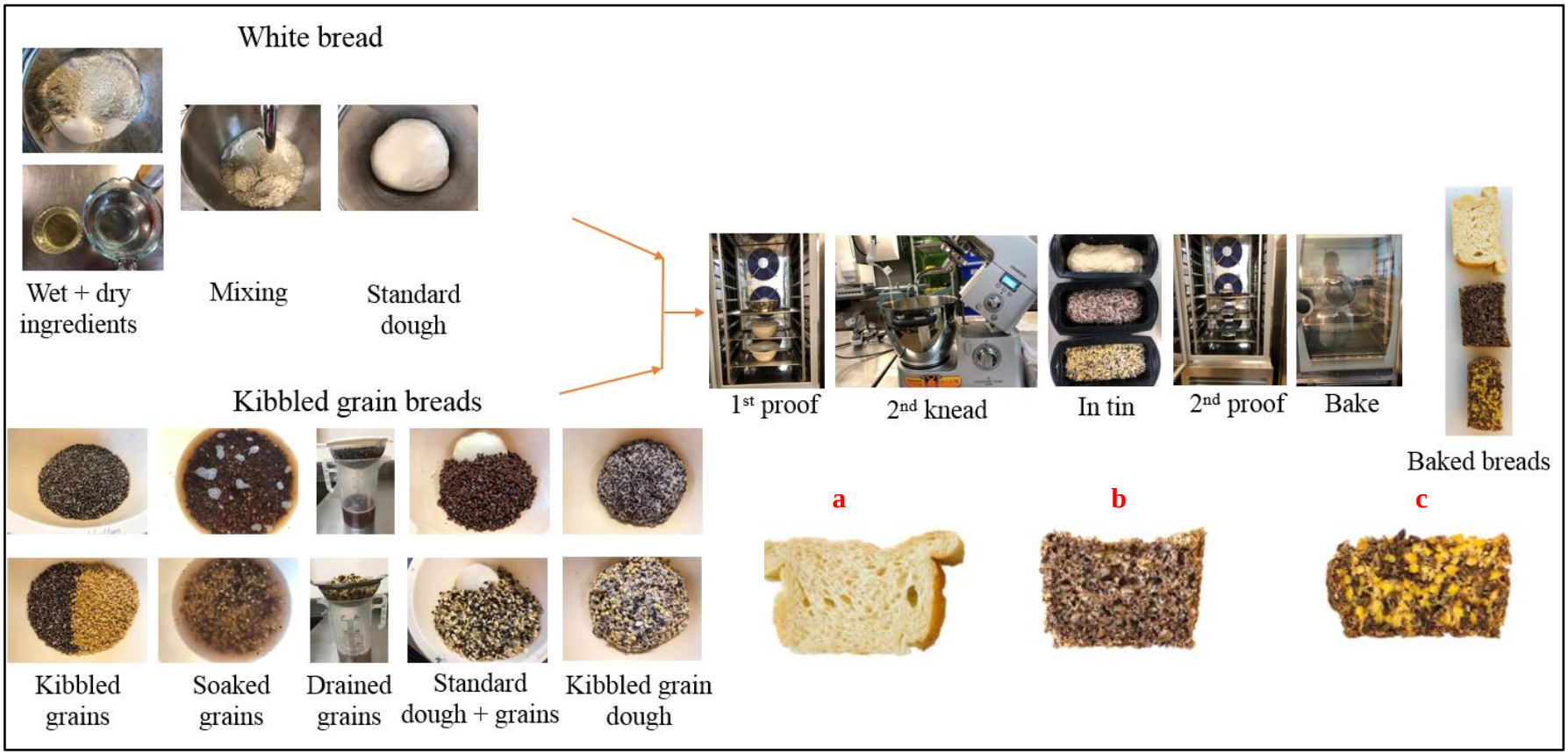


Figure 8.2 Flow chart of bread baking. a. WB = white bread, b. PB = purple kibbled wheat bead, c. SPB = kibbled soy/kibbled purple wheat bread.

8.2.4 Available carbohydrate analysis of breads

The CHO content of the breads was measured by a validated *in-vitro* digestion procedure, using 5 g of the homogenized bread in a volume of 50 ml (**Section 7.2.6**). The potentially available carbohydrate content of the digesta was measured as reducing sugar released into the digestion medium at 120 min after adding the digestive enzymes using the DNS method after an amyloglucosidase–invertase secondary digestion of an aliquot of the ethanolic samples (**Section 3.1.5**).

8.2.5 Blood glucose measurements

A clinical trial was conducted in which all participants ingested each of the three breads in each form – chewed (C), unchewed (U), homogenized (H) – so all participants consumed a total of nine breads (3 treatments). Ethical approval for the trial was obtained from the New Zealand Health and Disabilities Ethics Committee (HDEC, no. 18\NTA\160- **Appendix J**), and the trial was registered with the Australia New Zealand Clinical Trials Registry (no. ACTRN12618001826235 – **Appendix K**). Blood glucose concentrations were measured by finger-prick blood sampling using a HemoCue® blood glucose meter.

8.2.6 Experimental design for bread testing

The test breads were:

Standard chewed (C)

1. White bread (Reference): 105 g plus 188 ml of water
2. Purple wheat kibbled bread: 126 g plus 171 ml of water
3. Soy-purple wheat kibbled bread: 163 g plus 150 ml of water

Chewed (C)

Unchewed (U)

1. White bread (reference): 105 g plus 188 ml of water
2. Purple wheat kibbled bread: 126 g plus 171 ml of water
3. Soy-purple wheat kibbled bread: 163 g plus 150 ml of water

Unchewed (U)

Homogenized (H)

1. White bread (reference): 105 g homogenized with 188 ml of water
2. Purple wheat kibbled bread: 126 g homogenized with 171 ml of water
3. Soy-purple wheat kibbled bread: 163 g homogenized with 150 ml of water

} Homogenized (H)

A randomised repeated measures design was used in which each participant received all breads once, with the order of the breads and treatments randomised for each participant. A Latin square was used to ensure every subject had every combination of bread and treatment (chewed/unchewed/homogenised), and that every combination of bread and treatment was tested at least once per week. To fit the study into the available time (seven weeks), for each person there were two weeks when they came in for two tests that week, testing different combinations; this meant all combinations were tested at least once in a week and some were tested twice.

The human intervention study presented here used nine test breads, each formulated to contain 40 g CHO based on the digestive analysis of the homogenized sample described in **Section 8.2.2 and Table 8.1**. The moisture content of reference WB, PB and SPB was measured according to AOAC (1995) and was found to be 40.2%, 47.2% and 49.1 % respectively. Therefore, an allowance was made for the difference in the moisture content between the breads in all calculations and weight measurements.

The weights of the test breads for ingestion were carefully calculated to maintain equal intakes of CHO (40 g) and ensure the amount of water provided would keep the intake volume the same for all samples (**Table 8.2**). An additional 150 ml of water was provided to be consumed with all samples. *In-vitro* digestive analysis showed that WB, PB and SPM contained 38.11%, 31.65%, and 24.57% available carbohydrates, respectively. So, for instance, the amount of WB required to deliver 40 g available carbohydrate was: 40/38.11 x 100 = 104.96 g. Each bread was tested once by each participant.

Breads were baked once a week and divided into required portions and frozen until testing. All breads were thawed to room temperature overnight (14 h) and weighed before serving. Breads for treatment H were homogenized with 150 ml of water not more than 1 h before testing.

Table 8.2 Weights of test foods (g), available carbohydrate content and water used.

Test foods	Treatment[1]	CHO (g)	Weight of bread (g)	Water for drinking (ml)	Water for Homogenizing (ml)	Additional drinking water (ml)[2]
WB	C	40	105	300	_	38.18
	U	40	105	300	_	38.19
	H	40	105	150	150	38.20
PB	C	40	126	300	_	21
	U	40	126	300	_	21
	H	40	126	150	150	21
SPB	C	40	163	300	_	_
	U	40	163	300	_	_
	H	40	163	150	150	_

[1]Chewed (consumed as normal). U = unchewed. H = homogenized [2]To equalise water content of treatments. WB = white bread, PB = purple kibbled wheat bead, SPB = kibbled soy/kibbled purple wheat bread.

8.2.7 Subjects

Twenty-four volunteers were recruited for the initial screening using a flyer that briefly described the study. Volunteers were pre-screened at PFR, and asked initial recruitment questions in order to determine their suitability to take part in the study. The nature of the study and their involvement and responsibilities were described to them. Eligible volunteers who were willing to participate were invited to attend the study at PFR premises. Each volunteer was presented with an information sheet (**Appendix L**), containing study details, and an informed consent form. Their fasting blood glucose concentration and glycated haemoglobin (HbA1c) were measured to make sure that they were within the normal (non-diabetic) range and to familiarise them with the blood sampling procedure to be used in the study if they took part.

A total of twelve participants (five male and seven female) were recruited for the final study. The characteristics (mean±SD) of the study group were: age 33.3±11.7 years, BMI 23.6±3.3 kg/m^2, fasting glucose 4.4±SD 0·3 mmol/l and HbA1c 34.5± 4.5 mmol/mol. The participant number (n = 12) exceeds the minimum number (n=10) specified by the current ISO method

(ISO 26642:2010) for determination of glycaemic index and is typical of studies involving comparisons of foods. Twelve participants were recruited for the study from within PFR, Palmerston North, and Massey University, Palmerston North, subjected to the following exclusion criteria:

- Age: Aged below 18 or above 65.
- BMI: Volunteers do not have a body mass index below 18 or above 35 kg/m^2.
- Glucose intolerance: Any history of diabetes or evidence of glucose intolerance in a preliminary test.
- Gluten and soy intolerance: Any history of intolerance to gluten, soy or bread products.
- Non-fasting: Having consumed anything apart from water in the twelve hours before the test.
- Recent ill health

8.2.8 Glycaemic response testing

Participants were asked to come for the trial on weekdays. In preparation for each testing, session participants were requested to:

- Avoid strenuous physical activity and refrain from smoking or consuming alcohol the evening before a test and on the day of the test.
- Consume a similar carbohydrate-based meal the evening before each test session.
- Fast from 9.00 p.m the night before a test, with water consumption not restricted.
- Not have had a similar test in the previous 48 h (wash-out time).

On each test day the volunteers were seated and asked to remain so for the duration of the test, either as they continued work in their own office, or at the testing location, as long as any physical exertion was avoided. Once each subject was relaxed and comfortable for 10 min, a baseline blood sugar measurement was taken in duplicate for that day. Each subject was then given a test bread and instructed to consume the whole amount within 10 minutes. Blood glucose was tested by finger-prick blood sampling and was collected at 0 (baseline x 2), 15,

30, 45, 60, 90 and 120 min. Blood glucose was measured immediately using a HemoCue® blood glucose meter.

Figure 8.3 Clinical study setup

8.2.9 Data analysis

The blood glucose concentration changes from baseline were plotted against time to obtain blood glucose response curves. Each individual's baseline fasted blood glucose value was subtracted from subsequent measurements to obtain the incremental blood glucose response from which the incremental area under the blood glucose response curve (iAUC) was derived. The highest postprandial blood glucose peak for each individual, irrespective of the time of occurrence (nearly all were at either 30 or 45 min), was used to determine the mean peak height for each meal.

Glycaemic potency of the breads was calculated as GGE/ 100 g of bread based on a GI of 70 for the white bread (i.e. 70 GGE per 100 g of CHO). Data were entered into a Microsoft® Excel spreadsheet for preliminary analysis. For statistical comparison of means (ANOVA), GenStat software was used (version 11.1; VSNi Ltd). Data were analysed using analysis of variance (ANOVA) blocked by individual, testing differences between breads and treatments. The statistical analysis described the differences between the breads on their effects on blood glucose concentrations at different postprandial time points and allowed the precision of the glycaemic potency values to be determined. p values ≤ 0.05 were considered significant.

8.3 Results and Discussion

8.3.1 Blood glucose responses

All 12 of the subjects completed the trial, and no obvious outliers were detected, all results were included in the analysis. Between-subject variations in the blood glucose responses were large, as is typical of such studies (Mishra, Willis, Ansell, & Monro, 2016).

8.3.2 Response amplitude (peak height)

Plasma glucose concentrations reached peak values (**Figure 8.4 A, B and C**) between 30 and 45 min after the ingestion of test breads and decreased slowly thereafter as metabolism and storage removed glucose from the plasma. The WB control gave the highest postprandial blood glucose responses in all three treatments C, U and H. For both the PB and SPB breads, chewing and homogenization resulted in a peak amplitude similar to that of the WB. However, swallowing PB and SPB without chewing caused a substantial and statistically significant reduction in the peak amplitude of about 26% and 15% respectively compared to the WB treatments (**Figure 8.4 and Table 8.3**) (Mishra et al., 2012).

8.3.3 The area under the blood glucose response curve

The mean iAUC for the chewed white bread reference (182.13 mmol/L) and the unchewed and homogenized WB's were similar indicating that there was no structural impediment to starch digestion in the white bread that could be removed by homogenizing or chewing (**Table 8.3)**. The Chewed PB treatment also had a mean iAUC similar (194.5 mmol/L) to WB. However, the mean iAUC for the homogenized PB was 17% lower than that for the WB, indicating that chewing is more effective in promoting starch digestion than homogenizing alone.

The mean iAUC for the chewed and homogenized SPB were also statistically lower than for the WB by 12% and 23% respectively (**Table 8.3**). The lowest mean iAUC values occurred for the unchewed PB and SPB which were 45% and 31% respectively lower than the WB treatments. Swallowing WB unchewed had no effect on reducing the blood glucose response in comparison to chewed WB, clearly reflecting the fact that the white bread lacked any protective structures that chewing would eliminate (Burton et al., 2011; Eelderink et al., 2012).

8.3.4 Glycaemic index (GI *in vivo*)

All bread quantities consumed in the current study contained an equal quantity (40 g) of potentially available carbohydrate as determined by *in-vitro* digestion. Thus, the GGE's for all breads could be expressed as a percentage of the response compared to the WB (**Table 8.3**). The GIs (GGE per 100 g of CHO) for the breads, followed the same trends reported for peak amplitude and iAUC (**Figure 8.5** and **Table 8.3**). Only for the unchewed PB (41%) and SPB (30%) breads was GI significantly lower than the GI for WB, reflecting the protection of starch from digestion by kibbled grain structure (Edwards et al., 2015). It is notable that in all possible comparisons for PB and SPB the peak amplitude and iAUC and therefore GI of the homogenized samples was lower than that of the chewed samples; although the difference was not statistically significant it provides good circumstantial evidence that homogenization is less effective in preparing particulate foods for digestion than chewing. It is very likely that amylolysis by salivary amylase is an important component for the rapid digestion of starches (Butterworth et al., 2011; Woolnough, Bird, Monro, & Brennan, 2010) as demonstrated earlier.

8.3.5 Glycaemic potency*in-vivo*

The GI values were obtained from ingestion of 40 g of potentially available carbohydrate, whereas the standard quantity for the determination of GI is stipulated as 50 g of potentially available carbohydrates, or 25 g where large food volumes are involved (Mishra et al., 2016). However, the primary aim of the present study was to determine the relative glycaemic potency of the entire breads expressed as glucose equivalents, as people eat foods and not just the carbohydrates in them. The glycaemic potency is a more appropriate and practical estimate as it is based on the whole bread and on customary measures such as 100 g or a serving of bread (Monro, 2002; Monro & Shaw, 2008). When the glycaemic potency was calculated based on 100 g bread consumed, the values for the PB and SPB were substantially lower than those of WB (**Figure 8.5 B**), however, chewing considerably reduces these differences. Although adding 75% of kibbled grain (>2.8 mm in diameter) into the bread substantially reduced the expected glycaemic potency calculated using *in-vitro* data, it is clear that chewing the breads before swallowing sufficiently disrupts the structure to allow rapid access of amylolytic enzymes during digestion. When chewed, the glycaemic potency of the PB bread was even higher than of the homogenized bread, possibly because chewing would involve more significant exposure to salivary α-amylase than drinking a bread slurry.

Table 8.3 Characteristics of the blood glucose response curves and glycaemic indices during 0–120 min after ingestion of three breads[1] in three forms[2].

`	WB			PB			SPB			
	C	U	H	C	U	H	C	U	H	LSD
Amplitude (mmol/L)										
Mean	2.61^{abc}	2.76^{abc}	3.03^{ab}	3.24^{a}	1.94^{c}	2.72^{abc}	2.73^{abc}	2.23^{bc}	2.41^{abc}	0.78
SEM	0.20	0.23	0.29	0.42	0.21	0.31	0.26	0.31	0.24	
Diff WB (%)	0.00	5.75	16.09	24.14	-25.67	4.21	4.60	-14.56	-7.66	
iAUC (mmol/L x min)										
Mean	182.13^{ab}	173.44^{ab}	176.07^{ab}	194.50^{a}	99.90^{c}	151.94^{abc}	159.98^{ab}	124.88^{bc}	139.63^{abc}	53.98
SEM	13.26	18.23	24.71	26.85	12.79	22.87	16.51	18.25	14.99	
Diff WB (%)	0.00	-4.77	-3.33	6.79	-45.15	-16.58	-12.16	-31.43	-23.33	
GI (%)										
Mean	70.00^{ab}	69.46^{ab}	70.44^{ab}	76.65^{a}	41.54^{c}	60.87^{abc}	63.00^{abc}	49.04^{bc}	55.29^{abc}	20.51
SEM	0.00	7.13	9.78	10.05	6.49	8.78	5.71	7.12	5.55	
Diff WB (%)	0.00	-0.77	0.63	9.50	-40.66	-13.04	-10.00	-29.94	-21.01	

[1]WB = white bread, PB = kibbled purple wheat bread, SPB = kibbled soy-purple wheat bread. [2]C = Chewed, U = unchewed. H = homogenized. GI = GGE/ 100 g of CHO calculated from the response to 40 g carbohydrate in the consumed food and approximates GI ($p < 0.05$). Diff WB (%) = % difference from white bread ($p < 0.05$).

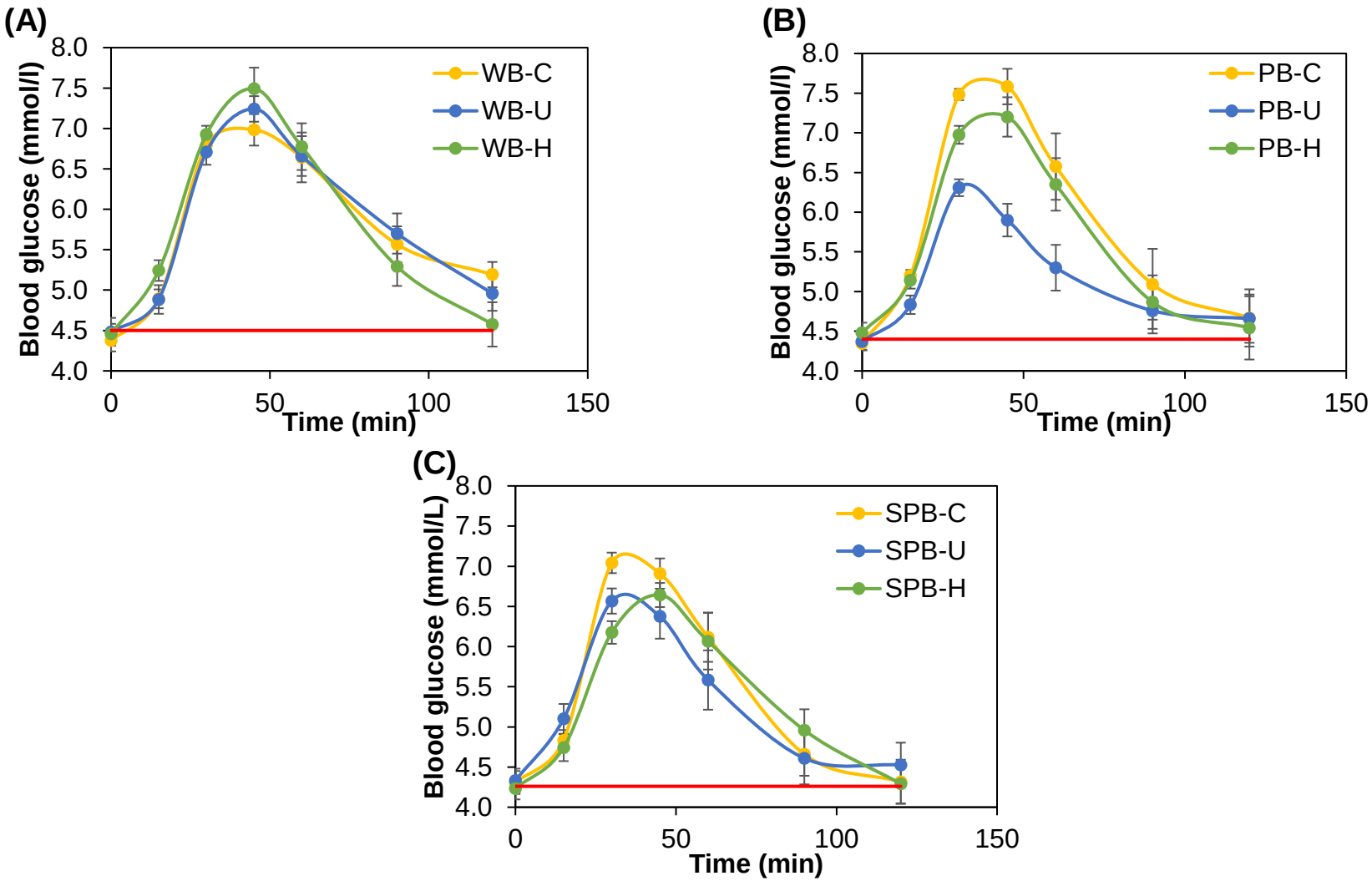


Figure 8.4 Blood glucose responses (means ± SEM) induced by equal carbohydrates (40 g) for the three breads: A white bread (WB), B kibbled purple wheat bread (PB) and C kibbled soy-purple wheat bread (SPB) with three treatments: Chewed (C), unchewed (U) and homogenized (H).

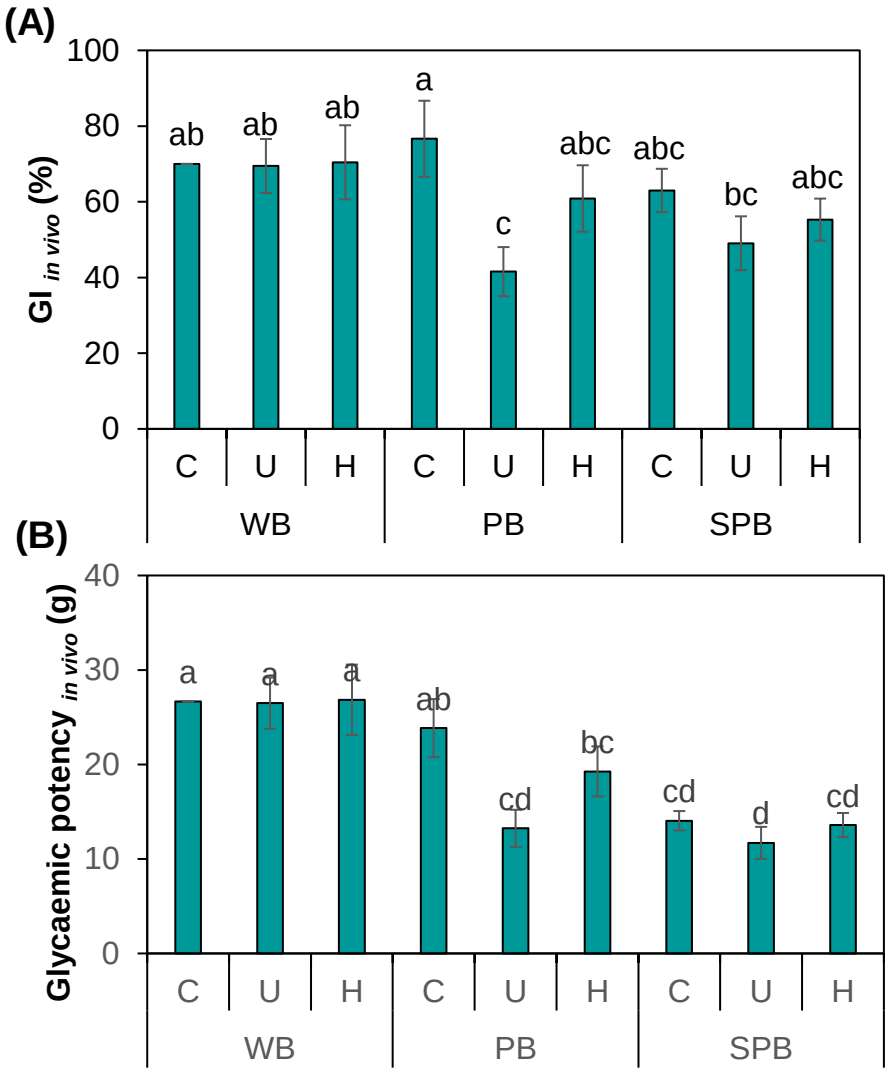


Figure 8.5 (A) Glycaemic index(GI *in vivo*) values (%) and (B) Glycaemic potency *in vivo* (GGE/100 g bread) for white bread (WB), kibbled purple wheat bread (PB) and kibbled soy-purple wheat (WB) with three different treatments: Chewed (C), unchewed (U) and homogenized (H). The GI values were based on each bread ingested to give a dose of 40 g of potentially available carbohydrate (measured in the homogenised sample) (LSD = 20.51, $p < 0.05$). The RGP values were based on each bread ingested to give a dose of 40 g of potentially available carbohydrate (measured in the homogenized sample) and then calculated to an equal 100 g intake of bread (LSD = 5.58, $p < 0.05$). (Means ± SEM). Results with a common letter in the data label do not differ significantly.

The formation of the bolus, the time in mouth and wetting by saliva are important factors for the oral digestion process (Hoebler, 2000) a bread slurry drink would have had less time in the mouth with a very little exposure to the salivary amylase and would be quickly dispersed in the acidic gastric phase, in which amylase activity would be inhibited. Therefore, the intact particles in the PB bread probably contributed little to reduce the GI of the bread ingested unchewed or homogenized. SPB bread containing high proportions of soy and purple wheat >2.8 mm, showed a low glycaemic potency relative to the WB in all forms (chewed, unchewed and

homogenized) in which it was ingested. However, the differences between the three forms in which SPB was consumed were small.

This finding should not be surprising since the dentition and chewing action involved in human mastication has evolved specifically to reduce food structure and convert potentially digestible food components to available nutrients. Reducing the proportion of glycaemic material in breads while maintaining good organoleptic properties seem to be the most effective and reliable strategy to decrease the glycaemic potency of breads and could be a focus of future research for the development of breads with reduced glycaemic potency.

8.3.6 *In-vitro* prediction of glycaemic response to kibbled grain breads.

Table 8.4 Relationship between glycaemic glucose equivalents (GGE) determined *in-vivo* (y) and GGE determined *in-vitro* (x) from the area between the *in-vitro* GGE release 100 g of food basis[1].

Samples	Treatments	Experiment	Glycaemic potency Correlation equation	
			y	R^2
All breads	Chewed (y) *vs.* Homogenized (x)	*In-vivo* (y) *vs. in-vitro* (x)	y=1.24x - 5.17	0.90
	Unchewed (y) *vs.* Intact (x)	*In-vivo* (y) *vs. in-vitro* (x)	y=0.76x + 5.39	0.98
	Homogenized (y) *vs.* Homogenized (x)	*In-vivo* (y) *vs. in-vitro* (x)	y=0.76x + 6.38	0.99

GGEs based on 100 g of breads (RGP) measured as the area under the blood glucose response curve from *in vivo* was correlated with *in-vitro* results as: (A) $Chewed_{in\text{-}vivo}$ *vs.* $homogenized_{in\text{-}vitro}$; (B) $Unchewed_{in\text{-}vivo}$ *vs.* $Intact_{in\text{-}vitro}$; (C) $Homogenized_{in\text{-}vivo}$ *vs.* $Homogenized_{in\text{-}vitro}$. $RGP_{in\text{-}vitro}$ that was calculated from *in-vitro* sugar release curves (taking into account the glucose disposal) accurately predicted *in-vivo* glycaemic potency over the range of bread intakes in the three different forms (**Table 8.4**). The correlation was very close and showed

that over the whole dataset the *in-vitro* and *in vivo* methods of estimating glycaemic potency were highly correlated (Monro, 2002; Monro & Mishra, 2010; Monro et al., 2010a).

8.4 Conclusion

Kernel structure played a significant role in reducing the glycaemic potency of the breads only when the grains were swallowed intact. This suggests that even with larger proportions of intact grain particles, the glycaemic potency of the breads cannot be greatly reduced due to the chewing factor, which tends to destroy much of the plant structure that was intended in the formulation to reduce the rate of starch digestion and hence the blood glucose response after consuming the breads. High proportions of whole-grain were only effective in reducing glycaemic potency when the particles were intact.

Chapter 9

Chapter 9: Can the addition of non-flour components, such as proteins, fibres and NSP's affect the glycaemic potency of the bread matrix?

9.1 Introduction

Nutritional recommendations for the management of postprandial hyperglycaemia include reducing carbohydrate intake per meal, controlling portion size and choosing foods in which carbohydrate digestion rate is relatively slow (Evert & Boucher, 2014; Forouhi, Misra, Mohan, Taylor, & Yancy, 2018). Recent evidence also suggests that co-ingestion of protein or carbohydrates such as fibres or NSP's with starch-based products will lower the glycaemic response to the carbohydrate (Gunnerud, Ostman, & Bjorck, 2013; Mishra, Edwards, Hedderley, Podd, & Monro, 2017; Roberts, Desbrow, Grant, Anoopkumar-Dukie, & Leveritt, 2013). In pastas based on high-protein durum wheat, a relatively slow rate of digestion and low glycaemic potency has been attributed to protein coating the starch granules, inhibiting both gelatinization and amylase access to starch (Jenkins et al., 1987). Microscopy has revealed that protein-starch conglomerates survive in cooked pasta (Kim et al., 2008). Because of the protein occlusion of starch, carbohydrate digestion in pasta may be enhanced by cooperative protease activity (Mishra et al., 2012). An *in- vitro* system was used to determine if the addition of insoluble (wheat fibre, wood and AllBran®) or soluble fibre (Xanthan gum) to aqueous suspensions of gelatinised starch affected the rate at which the starch was digested. The rates of starch digestion and suspension viscosity declined asymptotically and were unaffected by the addition of wheat fibre, but were considerably reduced by the addition of wood and AllBran® fibre and to a much greater extent (60%) by the addition of guar. The latter effect may be due to inhibition of amylase activity by NSP sequences.

Generally, in bakery products containing hydrating components other than starch, such as non-starch polysaccharides or sugars, these hydrocolloids compete with the starch for available water so modifying their pasting, thermal and rheological properties and therefore the final food product quality. Competition for water by NSP's may reduce the degree of gelatinization of starch during cooking so reducing subsequent rates of digestion. Therefore, the addition of NSP additives is a strategy to make low glycaemic potency breads. The addition of xanthan gum at concentrations ≥1% to the bread formula caused a significant decrease in the extent of

starch digestion in both the samples. The bread crumb containing xanthan gum had a dense structure and formed an agglomeration after grinding, resulting in delayed enzyme penetration into the crumb structure and thus delayed the enzymatic hydrolysis of starch (Sasaki, 2018). Soluble fibres such as guar gum and Xanthan gum slow starch digestion as they increase the viscosity of the digesta in the small intestine so reducing rates of digestion and absorption (Dartois, Singh, Kaur, & Singh, 2010; Hardacre, Yap, Lentle, & Monro, 2015; Hong et al., 2016). However, insoluble fibres such as cellulose and lignin have a small effect in influencing the rate of starch digestion (Sajilata et al., 2006).

On the other hand, guar galactomannan had a direct non-competitive inhibitory effect on alpha-amylase due to the direct binding of the enzyme to galactomannan which causes galactomannan-amylase complex being inactive (Slaughter et al., 2002). However, the effects depend greatly on the type of hydrocolloid (BeMiller, 2011) and several occasions, the addition of gums has increased digestibility; therefore no clear mechanism regarding the effect of added gums has been established. Therefore, the effects of protein, fibres and NSP's on postprandial responses have not been studied systematically, and their effects in mixed meals cannot be predicted reliably.

In this chapter, results of the glycaemic potency of breads made with partial substitution of flour with proteins, NSPs and fibres and their effect on the glucose release during *in-vitro* digestion are reported.

9.2 Materials and Methods

9.2.1 Samples

Wheat flour (Champion Flour Milling Ltd, 12.2% moisture, 11.5% protein, 1.4% fat, 76.8% carbohydrates), salt (Woolworths, Home brand), sugar (Chelsea, NZ Sugar Company Ltd), wheat gluten (Cargill, Inc), vegetable oil (Harvest, Natural sugars New Zealand Ltd) and instant dry yeast **(**Edmonds, Goodman Fielder New Zealand Ltd**)** were purchased from the local supermarket. Country oven dobrim 90 dough improver was purchased at Bakels, New Zealand. Tap water was used in bread-making. Guar Gum and Xanthan Gum were purchased from Davis Trading, New Zealand. Hi-Maize (high amylose starch) was purchase from Hawkin Watts Ltd. instant dry yeast **(**Edmonds, Goodman Fielder New Zealand Ltd**)** was purchased from the local supermarket. Country oven dobrim 90 dough improver was purchased at Bakels, New Zealand.

9.2.2 Bread Making

The reference white bread and non-flour components added breads were prepared in The New Zealand Institute of Plant and Food Research Limited (PFR), food safe laboratory as described in **Section 7.2.4**. After baking, all formulated loaves were cooled, packed and stored in ziplock bags similarly to the reference white bread. *In-vitro* amylolysis was performed on the day of baking the breads.

9.2.3 Bread Formulations

Table 9.1 shows the formulations of the breads for analysis. Breads were divided into low protein and high protein breads. High protein bread was formulated and processed to comply with food labelling requirements for the nutrient content claims that required ≥10 g/serving customarily consumed per eating occasion (Grafenauer & Curtain, 2018). The high protein breads formulated in this chapter contained approximately 20-23% of the protein in 100 g of bread (d.w.b). Only a maximum substitution of 5% flour was tested here due to time limitations. Furthermore, the addition of more than 5% of xanthan produced an unacceptably hard bread. The reason to use 5% protein, fibre or starch is to make a fair comparison of starch digestibility following the the substitution of 5% of the flour. Moreover, this is the maximum level substitution likely to be used in a commercial scenario while still making an acceptable bread. The breads were not formulated to have the same viscosities or texture, the main focus was only on formulating different breads with the same proportions of non-flour components and moisture content while still making a bread with acceptable texture. The texture of the baked breads would not therefore be a factor influencing the rate of starch digestion, firstly because the breads had a bread like texture and secondly because for the work in this chapter, the breads were completely homogenized before testing and analysis.

The following is the description of all tested samples.
Reference bread:

1. White bread: Reference bread (WB)

Low protein breads:

2. WB + P: Bread made with white bread matrix made with flour substituted 5% by soy protein isolate (WP)

Table 9.1 Ingredients and formulations for white (WB), low protein breads and high protein breads.

		Flour	Sugar	Salt	Gluten	Improver	Yeast	Oil	Water	P*	G*	X*	H*	F*
	White bread (WB)	53	1	1	2	0.3	1.7	1.8	39.2					
Low protein breads	WB+P(WP)	48	1	1	2	0.3	1.7	1.8	39.2	5				
	WB + G (WG)	48	1	1	2	0.3	1.7	1.8	39.2		5			
	WB + X (WX)	48	1	1	2	0.3	1.7	1.8	39.2			5		
	WB + H (WH)	48	1	1	2	0.3	1.7	1.8	39.2				5	
	WB + F (WF)	48	1	1	2	0.3	1.7	1.8	39.2					5
High protein breads	High protein bread (HP)	38	1	1	2	0.3	1.7	1.8	39.2	15				
	HPB + G (HPG)	33	1	1	2	0.3	1.7	1.8	39.2	15	5			
	HPB + X (HPX)	33	1	1	2	0.3	1.7	1.8	39.2	15		5		
	HPB + H (HPH)	33	1	1	2	0.3	1.7	1.8	39.2	15			5	
	HPB + F (HPF)	33	1	1	2	0.3	1.7	1.8	39.2	15				5

*P- protein, G- guar gum, X- xanthan gum, H- high amylose starch, F- wheat fibre.

3. WB + G: Bread made with white bread matrix made with flour substituted 5% by guar gum (WG)
4. WB + X: Bread made with white bread matrix made with flour substituted 5% by xanthan gum (WX)
5. WB + H: Bread made with white bread matrix that is substituted using 5% high amylose starch with flour (WH)
6. WB + F: Bread made with white bread matrix that is substituted using 5% wheat fibre with flour (WF)

High protein breads:

7. High Protein bread: Bread made with white bread matrix that is substituted using 15% soy protein isolate with flour (HP)
8. HP + G: Bread made with high protein matrix in which the flour is substituted 5% by guar gum (HPG)
9. HP + X: Bread made with high protein matrix that is substituted using 5% xanthan gum with flour (HPX)
10. HP + H: Bread made with high protein matrix that is substituted using 5% high amylose starch with flour (HPH)
11. HP + F: Bread made with high protein matrix that is substituted using 5% wheat fibre with flour (HPF)

9.2.4 *In-vitro* digestion

The rate of *in-vitro* amylolysis of the bread samples was measured, as mentioned in **Section 3.1.3**. Samples (5g) of each of the breads, were weighed into 70 ml digestion pots containing 30 ml of deionized water. The samples were homogenized and subjected first to simulated gastric peptic digestion followed by an amylolytic phase at pH 6.5 that simulated small intestinal digestion by adding a 1 ml aliquot of 5% pancreatin (P7545, Sigma-Aldrich, USA; 8 x USP specifications) and 0.1 ml of fungal amyloglucosidase (EC. 3.2.1.3. from A niger, Megazyme, E-AMGDF; 3260 U/ml) as described in **Section 3.1.3**.

Aliquots (0.5 ml) before adding the enzymes and after the addition of pancreatin were taken at 10, 20, 40, 60 and 120 min. and the reducing sugars were measured by DNS method as detailed in **Section 3.1.4**. Sugar released at 120 min was measured as total available carbohydrates in the breads. The glycaemic potency of breads was derived from the analytical glucose

equivalents (GE) measurements (**Section 3.1.4)**. The rates of starch digested to reducing sugars for the first 20 mins of *in-vitro* digestion were determined by linear regression. All experiments were conducted in duplicate.

9.2.5 Data analysis

All data calculations were conducted using Microsoft Excel (Microsoft Corp., Redmond, WA, USA). Analysis of variance and Pearson correlation coefficients for the relationship between different parameters was carried out using SPSS 24.0 software (IBM Corporation, Chicago, IL, USA). Comparisons of means were determined by Tukey's test to a 5% significance level.

9.3 Results and discussion

9.3.1 Simulated glucose release and blood glucose response during *in-vitro* digestion

The moisture content of all tested breads varied between 40.2% (HPX) and 43.2% (WG and WX) and were not statistically different ($p < 0.05$). The *in-vitro* digestion results show the glucose release during the small intestinal digestion phase, expressed on an equal weight basis (per 100 g of bread) are presented in **Figure 9.1 A and B.**

Breads made substituting 5% of the flour with non-flour components in the low protein category, yielded less available carbohydrates (CHO)—quantified as the glucose release at 120 min—compared to the reference WB (by 7% -16%). Similarly, all of the high protein breads such as HPG, HPX, HPH and HPF yielded less CHO than standard WB, with the HP bread showing 27% less available carbohydrates than standard white bread **(Table 9.2)**. These results suggest that the breads in the high protein category would have a much lower effect on blood glucose concentrations than the reference WB, most likely due to the overall decrease of starch content in these breads. Further analysis of the digestion curves distinguishing the different types of starches (rapidly digested, RDS, measured from the glucose release at 20 min), or slowly digested starch (SDS), is reported in **Table 9.2.** Substituting flour with xanthan gums reduced glucose release at 20 min for HPX and WX by 37.18% and 22.88% in comparison to WB respectively. The other high and low protein breads showed similar glucose release at 20 min in comparison to WB breads. Incorporating high amylose starch into the breads reduced the glucose release by 4.15% in comparison to WB at 20 min.

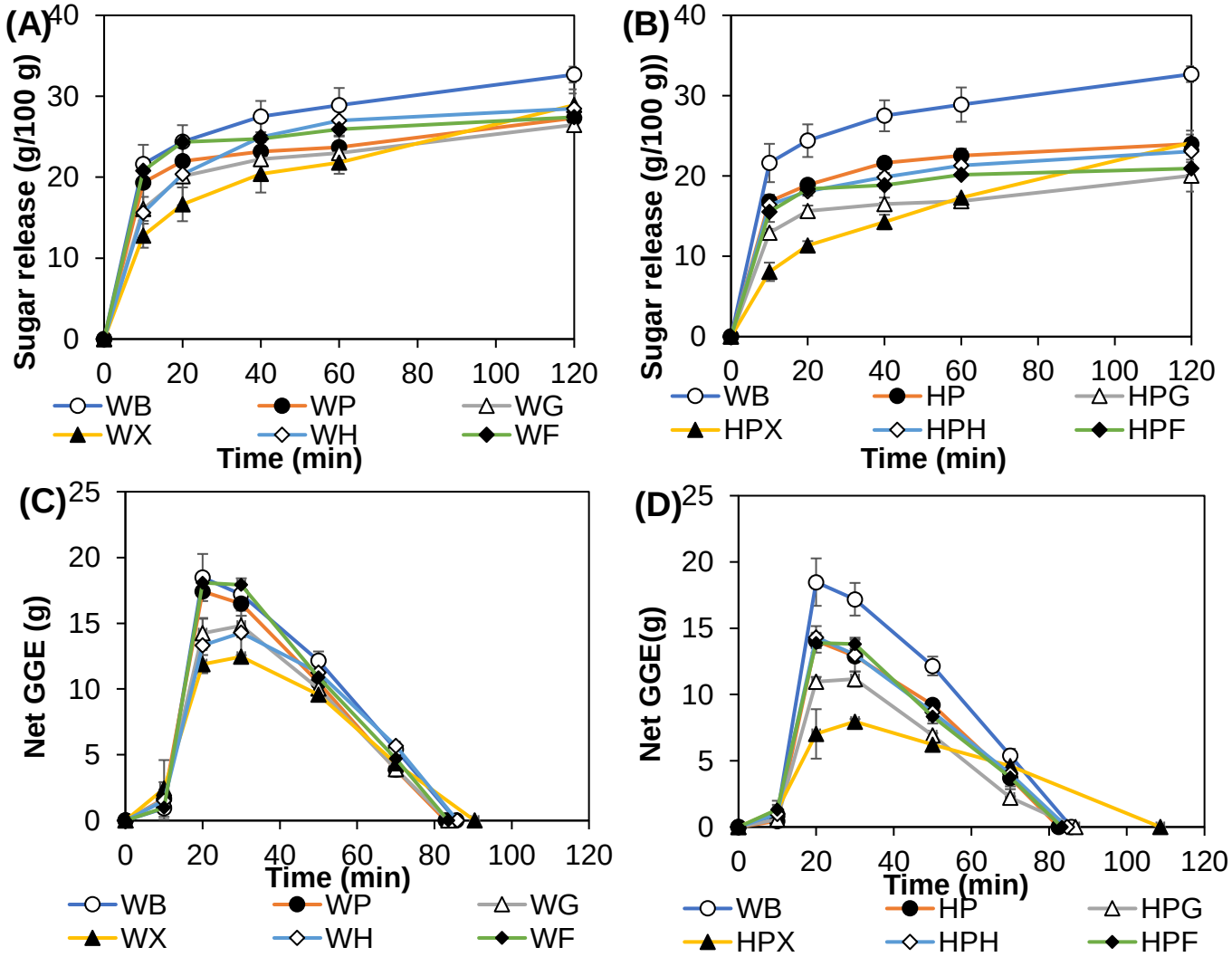


Figure 9.1 *In-vitro* digestive profile for (A) Low protein breads: WP, WG, WX, WH, WF and (B) High protein breads: HP, HPG, HPX, HPH, HPF based on 100 g of breads. Simulated blood glucose response curves for 100 g of bread based on the *in-vitro* digestion curves (C) Low protein breads: WP, WG, WX, WH, WF and (D) High protein breads: HP, HPG, HPX, HPH, HPF. WB - reference. Points are the average of 2 samples; intervals are (±) SD.

Table 9.2 Characteristics of starch digestibility curves during 0–120 min of *in-vitro* digestion.

Based on	Variables	Reference	Low protein					High protein				
	Breads	WB	WP	WG	WX	WH	WF	HP	HPG	HPX	HPH	HPF
100 g of bread	CHO at 120 min	32.68^{a}	27.31^{bc}	26.47^{bc}	28.91^{ab}	28.48^{ab}	27.42^{bc}	24^{bcd}	20.04^{d}	24.16^{bcd}	23.06^{cd}	20.93^{d}
	Diff WB (%)		9.83	17.59	31.76	16.46	0.15	22.58	35.82	53.56	25.78	24.62
100 g of CHO	RDS	74.66^{ab}	80.55^{a}	75.94^{a}	57.58^{bc}	71.56^{ab}	88.85^{a}	78.73^{a}	78.11^{a}	46.9^{c}	78.52^{a}	87.86^{a}
	Diff WB (%)		7.89	1.72	-22.88	-4.15	19.01	5.46	4.62	-37.18	5.18	17.68
100 g of CHO	SDS	25.34^{bc}	19.45^{c}	24.06^{c}	42.42^{ab}	28.44^{bc}	11.15^{c}	21.27^{c}	21.90^{c}	53.10^{a}	21.48^{c}	12.14^{c}
	Diff WB (%)		-23.25	-5.07	67.41	12.21	-55.99	-16.08	-13.60	109.52	-15.26	-52.10

CHO= Available carbohydrates, RDS= Rapidly digested starch, SDS- Slowly digested starch. Diff WB (%) = % difference from white bread ($p < 0.05$). Points are the average of two samples.

Estimates of the blood glucose-raising potential of the breads generated as simulated blood glucose response curves are given in **Figure 9.1 C and D.** These curves were used to estimate glycaemic potency (GGE per 100 g food) and glycaemic index (GGE per 100g CHO) values for the breads in the next section**.** The simulated blood glucose response curves for equal quantities of all of the breads show that the white bread reference had by far the greatest glycaemic potency (AUC = 748.80 GGE min) followed by the bread with 5% wheat fibre substitution (WF) (AUC = 724.35 GGE min). The AUC of all low protein breads did not differ statistically except for WX. All of the high protein breads fell within a narrow band and had the lowest areas under the curves, which were significantly lower than the low protein and reference breads. The AUC of all high protein breads did not differ statistically except for lower values for the HPG and HPX breads by 50% and 59% respectively.

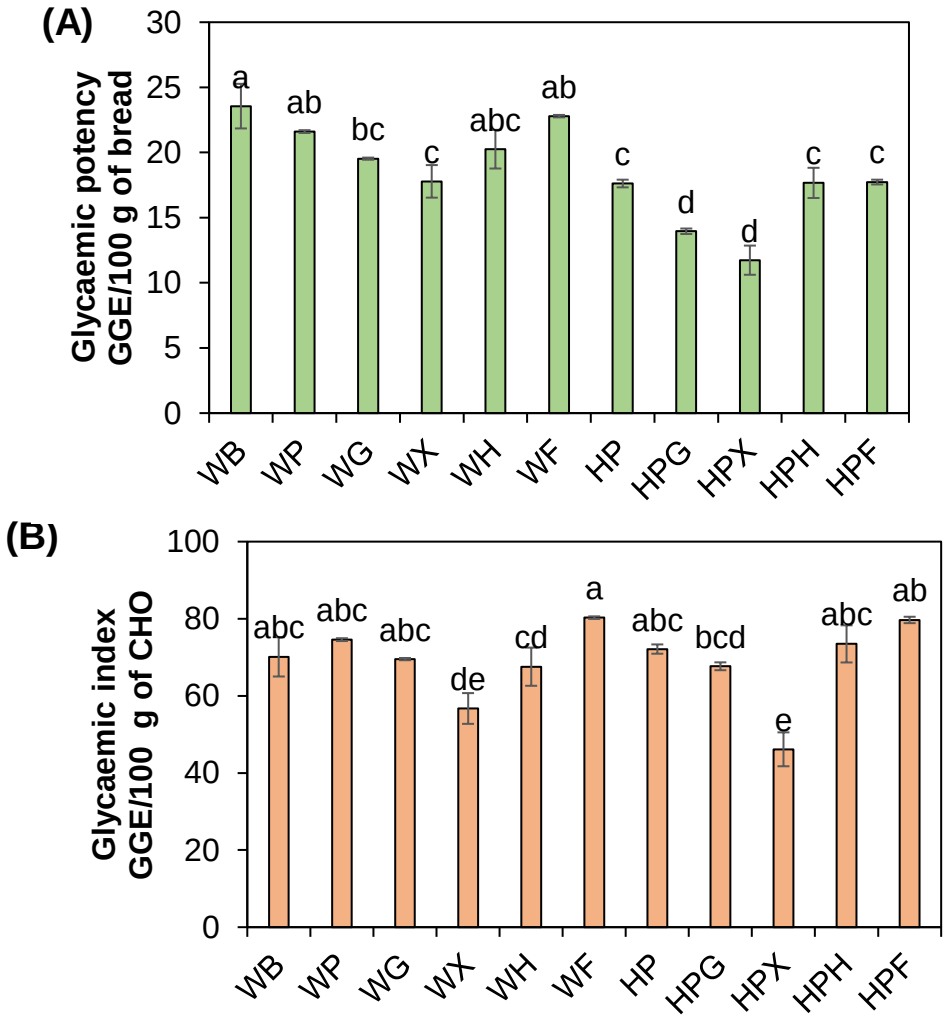


Figure 9.2 (A) Glycaemic potency and (B) GI of Low protein breads: WP, WG, WX, WH, WF and High protein breads: HP, HPG, HPX, HPH, HPF. The GI values were based on 100 g of carbohydrates ($p < 0.05$). WB – reference. The glycaemic potency values were based equally to 100 g of bread ($p < 0.05$). (Means ± SD). Results with a common letter in the data label do not differ significantly.

9.3.2 Estimation of glycaemic potency and glycaemic index

The *in-vitro* digestion study estimated glycaemic potency (GGE/100 g of bread) as the area under the net adjusted GE (adjusted GE minus glucose disposal at each time point) from time 0 to baseline. The glycaemic potencies of WP, WH and WF were lower than WB, but the differences were not statistically significant **(Figure 9.2 A)**. WG and WX had a significantly lower glycaemic potency in comparison to other low protein breads. The glycaemic potency of high protein breads tended to be lower in comparison to the low protein breads and again the breads containing xanthan and guar gum were significantly different from the rest. The GI (GGE/100 g of CHO) of the breads was calculated firstly by adjusting the areas to an equal CHO intake, and then adjusting all values relative to an assumed GI value of 70 for the area under the curve of the white bread reference. GI values lower than 55 are classified as "low", and above 70 are classified as "high" (Monro, 2002). The breads made with xanthan gum, WX and HPX, had a low or near to low GIs when compared with the white bread (70) (**Figure 9.2 B**).

9.3.3 Rates of starch digested *in-vitro*

Using the *in-vitro* digestion system as described in **Section 9.2.4**, 70-90% of the starch was converted into sugars within 20 min, except for WX and HPX, with the remainder of the starch being digested gradually between 20 and 120 min (**Figure 9.3 A and B**) for both low and high protein breads. The presence of xanthan in the WX and HPX breads caused a significant decrease in the extent of the starch being digested between 20 and 60 min. Expressed as a percentage of the total available carbohydrate in the samples, all the breads including high and low protein breads, except WX and HPX, showed similar ratios of RDS to SDS than the standard white bread (70 % RDS to 30 % SDS). For the WX and HPX, the ratio was about 58% RDS: 42% SDS and 47% RDS: 53% SDS respectively. The rates of digestion were therefore similar for all the breads except for WX and HPX. This clearly showed that substitution of proteins, fibres and guar gum for flour had very little to no effect on the rate and extent of starch digestibility, although all these ingredients lead to a reduction in glycaemic potency of the bread through the substitution starch for non-glycaemic materials.

For both low and high protein categories, Xanthan gum significantly reduced the starch digestibility and glycaemic potency. The effect of reduced starch digestibility and glycaemic potency is considered to be due to molecular interactions of xanthan gum in bread and not due

to the influence of bread structure or texture as the breads where completely homogenized before *in-vitro* starch digestion.

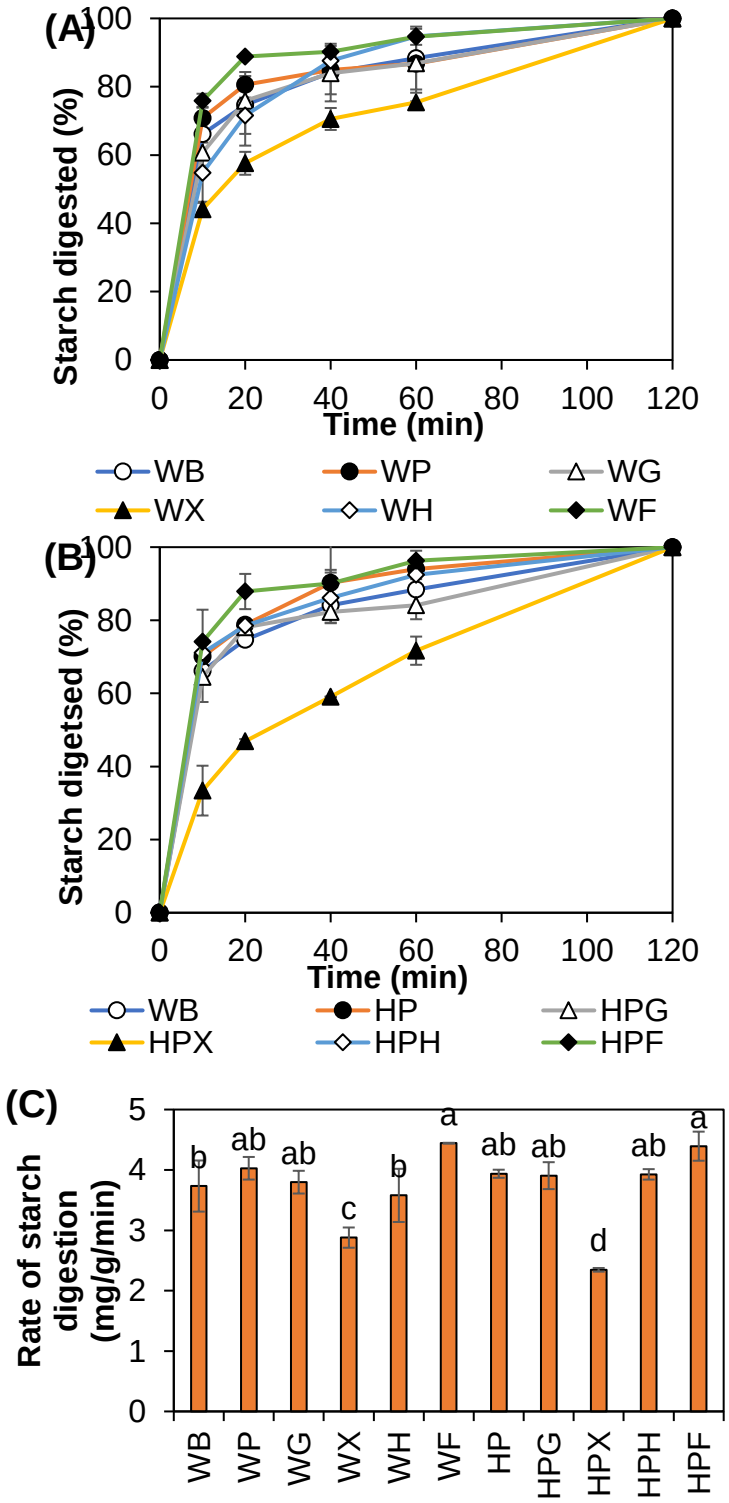


Figure 9.3 Starch digested (g/100 g) for (A) Low protein breads: WP, WG, WX, WH, WF and (B) High protein breads: HP, HPG, HPX, HPH, HPF based on 100 g of available carbohydrates ($p < 0.05$). (C) Rate of starch digestion for WP, WG, WX, WH, WF, HP, HPG, HPX, HPH and HPF. WB – reference. Results with a common letter in the data label do not differ significantly. Values are the average of 2 samples; intervals are (±) SD.

NSPs such as xanthan gum and guar gum are often used in gluten-free products combined with starches and gluten-free flours. The increase in viscosity they provide helps to retain the gas during fermentation of doughs similar to a viscoelastic gluten-like network. The effect of xanthan on starch gelatinization has been explained earlier by the ability of xanthan molecules to associate and coat the starch granule surface, restricting starch swelling and gelatinization (Gonera & Cornillon, 2002; Mandala & Bayas, 2004; Tester & Sommerville, 2003). Xanthan gum has been recently shown to substantially decrease the viscoelasticity and strength of native pure starch gels by probably restricting starch gelatinization by this coating effect and/or by affecting amylose interactions in the final network (Matia-Merino *et al*., 2019). It has also been reported that xanthan gum decreases starch digestibility by increasing viscosity of the starch suspension and gelatinized starch or by interacting with starch molecules (Brennan, 2005; Sasaki, 2018; Sasaki, Sotome, & Okadome, 2015). Viscosity data were not measured in the current study however future work standardising viscosities to compare various added gums into bread formulations will be useful to isolate the effects. Apart from the competition of water and increase in viscosity in the digesta medium, guar gum molecules have the ability to inhibit amylase activity which causes a reduction in starch digestibility (Slaughter et al., 2002).

9.4 Conclusion

The *in-vitro* starch digestibility of bread was determined for two types of bread samples: Low protein breads and high protein breads. The addition of gums was found to reduce the extent of glucose release of the bread for both samples. Xanthan gum showed notably lower GI, and glycaemic potency and the effect of guar gum on reducing starch digestibility was more pronounced in the high protein bread sample than in the low protein bread sample. These results implied that the molecular interactions of xanthan gum resulted in delayed enzyme penetration into the crumb structure and thus delayed enzymatic hydrolysis of starch. Addition of xanthan gum significantly reduced the sugar release during *in-vitro* digestion possibly because the non-starch polysaccharide tends to block the adsorption sites, thereby inhibiting enzyme binding capacity which may have caused the slower starch digestibility.

Overall, reducing available carbohydrates in high protein breads reduced the glycaemic potency of the breads (GGE/ 100 g of breads), and it is evident that formulating breads with a high protein/low carbohydrate content may be a more effective strategy for reducing the glycaemic potency of breads than the inclusion of semi-intact grain particles.

Chapter 10

Chapter 10: Overall conclusions and recommendations

10.1 Primary findings

This PhD thesis has shown the role of whole-grains in widely available wholegrain breads that are marketed for their grain-derived health attributes. The purpose of the research was to determine structural and processing factors for starches and flours that limit the rate of hydration and gelatinization of starch in grains and eventually breads. The focus was on the role of the physical structure of kernel in grains that affects the ingress of water during processing and the subsequent rate of *in-vitro* digestion and later glycaemia.

The overall aim of the project was to discover and apply methods to reduce the glycaemic potency of breads by reducing the rate of digestion of available carbohydrates, particularly starch, within the food matrix while maintaining organoleptic properties.

The study involved:

- Examination of role and contribution of whole grains in New Zealand supermarket breads.
- *In-vitro* measurement of the rate of digestion of milled grain particles and establishing the importance of kernel structure.
- Characterisation of milled particles and their rates of hydration, gelatinization and digestion.
- Measurement and controlling of hydration in milled particles.
- Selection of grain size and treatments that modulate digestion for inclusion in breads.
- Assessment of glycaemic potency of breads *in-vitro* and *in-vivo*.

My study found evidence that breads available in the New Zealand supermarket shelves had similar rates of starch digestion and glycaemic potency in comparison to the standard white bread. There was not enough intact/semi-intact grain proportion to lower starch digestibility and glycaemic potency. However, Pumpernickel (rye bread) had a lower rate of in-vitro starch digestion due to its high proportion of intact grain particles (65%). The results obtained showed that the proportion of CHO was the major factor to reduce glycaemic potency of the breads. My work provided evidence that the proportion, size and type of grain, whole grain used in commercial New Zealand whole grain breads do not confer low digestibility when they are chewed and hence the low glycaemic potency reported from the *in-vitro* experiments

incorporating up to 60% of kibbled grain particles does not transfer to normal consumption of breads.

By exploiting kernel structure, this research has presented that the intactness of kernel structure in cereals is the limiting factor that controls the rate and extent of amylolysis of starch entrapped inside the digestion resistant cell walls in the kernel structure. Reducing gelatinization by controlled hydration has demostrated that it could limit starch digestibility but organoleptic properties are compromised and the breads are commercially unacceptable. Larger grain particles result in low and slow digestion of starch in grain particles due to the diffusion of enzyme and product limitations.

After a careful exploration of several strategies which was aimed at reducing starch digestibility and glycaemic potency, a range of kibbled grain breads was made with a high proportion of large kibbled grains which were tested *in-vitro* and *in-vivo*. We developed a novel technique based on the type of eating the bread; chewed (eat as usual), unchewed and homogenized to study the effects on kernel structure on glycaemic potency *in vivo*.

The results showed that even with larger proportions of intact grain particles, the glycaemic potency of the breads could not be greatly reduced as chewing tends to destroy much of the plant structure that was intended to reduce the rate of digestion and the blood glucose response after consuming the breads as usual. Moreover, it is also clear that kernel structure can act as an efficient barrier to the ingress of the amylolytic enzyme, provided that mechanical damage during oral processing during digestion is prevented. If the passage of intact grains to the large intestine can be achieved, fermentation of dietary fibres and resistant starch present in these structures by the resident microbiota may have health benefits however, the introduction of significant amounts of starch into the colon may have other undesirable effects. The *in-vitro*/*in-vivo* determination of glycaemic potency of homogenised and non-homogenised breads allowed correlation of results from the two methods and extrapolation to predict GI and glycaemic potency. The correlations were high and showed that *in-vitro* and *in-vivo* methods corresponded well for the homogenised samples.

Since the product matrix, as opposed to kernel particles embedded in it makes a major contribution to glycaemic potency, the differences in the disassembly and digestion of starch in breads with the addition of various non-flour components such as proteins, fibres and NSPs was exploited. My work showed that reducing the proportion of CHO either by direct substitution or starch for other ingredients or as a result of incorporating grain particles

containing a greater proportion of fibre seemed to be the most effective and reliable strategy to decrease the glycaemic potency (GGE/100 g of bread) of breads. Interestingly, the addition of Xanthan gum significantly reduced the starch digestibility and glycaemic potency, this may be due increases in the viscosity of the digesta or inhibition of enzymes by xanthan similar the that which occurs for guar.

10.2 Limitations and future work

This research has presented that oral processing can affect the structure of the kernels added to the breads. It will be worth measuring the degree of damage to the kernels during chewing to have a clear understanding of their relative integrity during gastrointestinal transit.

My work has also provided evidence that substituting flour with 5% Xanthan gum significantly reduced starch digestibility and glycaemic potency in standard white bread. However, the limitation of Chapter 9 is that the breads were not formulated to have the same viscosities, as the main focus was on formulating breads with similar proportions of non-flour components and moisture content. It would be of value to extend this research to study the effects of viscosity on starch digestibility in detail. The following recommendations have been proposed as a future work following on from this study.

Breads with added proteins, fibres or NSPs can be formulated to have the same viscosities as that of the Xanthan breads and starch digestibility evaluated. It is hypothesised that when the viscosity of the bread in the digesta reaches a critical point, rates of digestion will be limited by viscosity and the alternative hypothesis is that some gums will inhibit amylolytic digestion.

10.3 Applications

The main objective of this study was to investigate the effects of incorporating kibbled or intact grain in breads on the glycaemic potency of the breads. The results provide an opportunity to communicate to bread companies that “graininess is healthiness” can be attributed to nutritional components of whole grains and their ability to substitute starch for fibre. Incorporating whole grains or large grain particles in breads does not reduce the rate of starch digestion. Also, this thesis provided evidence that formulating large proportions of protein into bread matrix is a straightforward and most effective strategy to reduce blood glucose responses in a serving which will be useful to cater to people with diabetes.

References

References

Ahlborn, G. J., Pike, O. A., Hendrix, S. B., Hess, W. M., & Huber, C. S. (2005). Sensory, Mechanical, and Microscopic Evaluation of Staling in Low-Protein and Gluten-Free Breads. *Cereal Chemistry, 82*(3), 328-335. doi: 10.1094/CC-82-0328

Al-Rabadi, G. J., Torley, P. J., Williams, B. A., Bryden, W. L., & Gidley, M. J. (2011). Particle size of milled barley and sorghum and physico-chemical properties of grain following extrusion. *Journal of Food Engineering, 103*(4), 464-472. doi: https://doi.org/10.1016/j.jfoodeng.2010.11.016

Al-Rabadi, G. J., Torley, P. J., Williams, B. A., Bryden, W. L., & Gidley, M. J. (2012). Particle size heterogeneity in milled barley and sorghum grains: Effects on physico-chemical properties and starch digestibility. *Journal of Cereal Science, 56*(2), 396-403. doi: https://doi.org/10.1016/j.jcs.2012.04.009

Al-Rabadi, G. J. S., Gilbert, R. G., & Gidley, M. J. (2009). Effect of particle size on kinetics of starch digestion in milled barley and sorghum grains by porcine alpha-amylase. *Journal of Cereal Science, 50*(2), 198-204. doi: http://dx.doi.org/10.1016/j.jcs.2009.05.001

Anderson, J. W. (2003). Whole grains protect against atherosclerotic cardiovascular disease. *Proc Nutr Soc, 62*(1), 135-142. doi: 10.1079/pns2002222

Anjum, F. M., & Walker, C. E. (1991). Review on the significance of starch and protein to wheat kernel hardness. *Journal of the Science of Food and Agriculture, 56*(1), 1-13. doi: 10.1002/jsfa.2740560102

Anklam, E. (2005). H.-D. Belitz, W. Grosch, P. Schieberle: Food Chemistry, Third Edition. *Analytical and Bioanalytical Chemistry, 382*(1), 10-11. doi: 10.1007/s00216-004-3036-9

ao, Z., Simsek, S., Zhang, G., Venkatachalam, M., Reuhs, B., & R Hamaker, B. (2007). Starch with a Slow Digestion Property Produced by Altering Its Chain Length, Branch Density, and Crystalline Structure. *Journal of agricultural and food chemistry, 55*, 4540-4547. doi: 10.1021/jf063123x

Ao, Z., Simsek, S., Zhang, G., Venkatachalam, M., Reuhs, B. L., & Hamaker, B. R. (2007). Starch with a Slow Digestion Property Produced by Altering Its Chain Length, Branch Density, and Crystalline Structure. *Journal of Agricultural and Food Chemistry, 55*(11), 4540-4547. doi: 10.1021/jf063123x

AOAC. (2005). Official Methods of Analysis. 18th edn. *Association of Official Analytical Chemists; Arlington, VA, USA: 2005.*

AOAC. (2006a). Official methods of analysis Proximate Analysis and Calculations Acid Insoluble Ash - item 66. *Association of Analytical Communities, Gaithersburg, MD, 17th edition, 2006. Reference data: Method Ba 5b-68; MIN; ASH.*

AOAC. (2006b). Official methods of analysis Proximate Analysis and Calculations Total, Soluble Dietary Fiber Foods - item 9. *Association of Analytical Communities, Gaithersburg, MD, 17th edition, 2006. Reference data: Method 991.43 (32.1.17); NFNAP; CHO; FIB.*

AOAC, I. (1995). Official methods of analysis of AOAC International. *Arlington, Va.: AOAC Intl. pv (loose-leaf).*

Aronoff, S. L., Berkowitz, K., Shreiner, B., & Want, L. (2004). Glucose Metabolism and Regulation: Beyond Insulin and Glucagon. *Diabetes Spectrum, 17*(3), 183-190. doi: 10.2337/diaspect.17.3.183

Barclay, A. W., Petocz, P., McMillan-Price, J., Flood, V. M., Prvan, T., Mitchell, P., & Brand-Miller, J. C. (2008). Glycemic index, glycemic load, and chronic disease risk—a meta-

analysis of observational studies. *The American Journal of Clinical Nutrition, 87*(3), 627-637.
Bart, J. C. J., Gucciardi, E., & Cavallaro, S. (2012). *Biolubricants: Science and Technology*: Elsevier Science.
Bauer, U. E., Briss, P. A., Goodman, R. A., & Bowman, B. A. (2014). Prevention of chronic disease in the 21st century: elimination of the leading preventable causes of premature death and disability in the USA. *The Lancet, 384*(9937), 45-52. doi: 10.1016/S0140-6736(14)60648-6
Bautista-Castaño, I., & Serra-Majem, L. (2012). Relationship between bread consumption, body weight, and abdominal fat distribution: evidence from epidemiological studies. *Nutrition Reviews, 70*(4), 218-233.
Behall, K. M., & Hallfrisch, J. (2002). Plasma glucose and insulin reduction after consumption of breads varying in amylose content. *Eur J Clin Nutr, 56*(9), 913-920. doi: 10.1038/sj.ejcn.1601411
Behall, K. M., Scholfield, D. J., & Hallfrisch, J. (1999). The effect of particle size of whole-grain flour on plasma glucose, insulin, glucagon and thyroid-stimulating hormone in humans. *J Am Coll Nutr, 18*(6), 591-597.
Belitz, H.-D., Grosch, W., & Schieberle, P. (2004). Food Chemistry. *eBook*, 245-341.
BeMiller, J. N. (2011). Pasting, paste, and gel properties of starch-hydrocolloid combinations. *Carbohydrate Polymers, 86*(2), 386-423. doi: 10.1016/j.carbpol.2011.05.064
Benmoussa, M., Suhendra, B., Aboubacar, A., & Hamaker, B. R. (2006). Distinctive Sorghum Starch Granule Morphologies Appear to Improve Raw Starch Digestibility. *Starch - Stärke, 58*(2), 92-99. doi: 10.1002/star.200400344
Berg, T., Singh, J., Hardacre, A., & Boland, M. J. (2012). The role of cotyledon cell structure during in vitro digestion of starch in navy beans. *Carbohydrate Polymers, 87*(2), 1678-1688. doi: http://dx.doi.org/10.1016/j.carbpol.2011.09.075
Bertoft, E., Piyachomkwan, K., Chatakanonda, P., & Sriroth, K. (2008). Internal unit chain composition in amylopectins. *Carbohydrate Polymers, 74*(3), 527-543. doi: https://doi.org/10.1016/j.carbpol.2008.04.011
Bhattarai, R. R., Dhital, S., Mense, A., Gidley, M. J., & Shi, Y.-C. (2018). Intact cellular structure in cereal endosperm limits starch digestion in vitro. *Food Hydrocolloids, 81*, 139-148. doi: https://doi.org/10.1016/j.foodhyd.2018.02.027
Birt, D. F., Boylston, T., Hendrich, S., Jane, J.-L., Hollis, J., Li, L., . . . Whitley, E. M. (2013). Resistant Starch: Promise for Improving Human Health. *Advances in Nutrition, 4*(6), 587-601. doi: 10.3945/an.113.004325
Black, J. L. (2001). Quality Feed Grains – Research highlights and opportunities. *Proceedings of 10th Australian barley technical symposium 2001: A barley odyssey*.
Blasel, H. M., Hoffman, P. C., & Shaver, R. D. (2006). Degree of starch access: An enzymatic method to determine starch degradation potential of corn grain and corn silage. *Animal Feed Science and Technology, 128*(1), 96-107. doi: http://dx.doi.org/10.1016/j.anifeedsci.2005.08.018
Borneo, R., & Leon, A. E. (2012). Whole grain cereals: functional components and health benefits. *Food Funct, 3*(2), 110-119. doi: 10.1039/c1fo10165j
Brand-Miller, J., Dickinson, S., Barclay, A., & Celermajer, D. (2007). The glycemic index and cardiovascular disease risk. *Curr Atheroscler Rep, 9*(6), 479-485.
Brand-Miller, J., Hayne, S., Petocz, P., & Colagiuri, S. (2003). Low-glycemic index diets in the management of diabetes: a meta-analysis of randomized controlled trials. *Diabetes Care, 26*. doi: 10.2337/diacare.26.8.2261
Brand-Miller, J. C. (2003). Glycemic Load and Chronic Disease. *Nutrition Reviews, 61*, S49-S55. doi: 10.1301/nr.2003.may.S49-S55

Brennan, C. S. (2005). Dietary fibre, glycaemic response, and diabetes. *Molecular Nutrition & Food Research, 49*(6), 560-570.
Buléon, A., Colonna, P., Planchot, V., & Ball, S. (1998). Starch granules: structure and biosynthesis. *International Journal of Biological Macromolecules, 23*(2), 85-112. doi: http://dx.doi.org/10.1016/S0141-8130(98)00040-3
Burton, P. M., Monro, J. A., Alvarez, L., & Gallagher, E. (2011). Glycemic impact and health: new horizons in white bread formulations. *Crit Rev Food Sci Nutr, 51*(10), 965-982. doi: 10.1080/10408398.2010.491584
Butterworth, P. J., Warren, F. J., & Ellis, P. R. (2011). Human α-amylase and starch digestion: An interesting marriage. *Starch - Stärke, 63*(7), 395-405. doi: 10.1002/star.201000150
Butterworth, P. J., Warren, F. J., Grassby, T., Patel, H., & Ellis, P. R. (2012). Analysis of starch amylolysis using plots for first-order kinetics. *Carbohydrate Polymers, 87*(3), 2189-2197. doi: http://dx.doi.org/10.1016/j.carbpol.2011.10.048
Carpita, N. C., & Gibeaut, D. M. (1993). Structural models of primary cell walls in flowering plants: consistency of molecular structure with the physical properties of the walls during growth. *Plant J, 3*(1), 1-30.
Cheraskin, E., Ringsdorf Jr, W., & Clark, J. W. (1995). *Diet and disease*: Keats Publishing Inc.
Chung, H.-J., Lim, H. S., & Lim, S.-T. (2006). Effect of partial gelatinization and retrogradation on the enzymatic digestion of waxy rice starch. *Journal of Cereal Science, 43*(3), 353-359. doi: 10.1016/j.jcs.2005.12.001
Chung, H.-J., Shin, D.-H., & Lim, S.-T. (2008). In vitro starch digestibility and estimated glycemic index of chemically modified corn starches. *Food Research International, 41*(6), 579-585. doi: http://dx.doi.org/10.1016/j.foodres.2008.04.006
Colonna, P., Leloup, V., & Buleon, A. (1992). Limiting factors of starch hydrolysis. *Eur J Clin Nutr, 46 Suppl 2*, S17-32.
Cooke, D., & Gidley, M. (1992). *Loss of crystalline and molecular order during starch gelatinisation: origin of the enthalpic transition* (Vol. 227).
Damager, I., Engelsen, S. B., Blennow, A., Lindberg Møller, B., & Motawia, M. S. (2010). First Principles Insight into the α-Glucan Structures of Starch: Their Synthesis, Conformation, and Hydration. *Chemical Reviews, 110*(4), 2049-2080. doi: 10.1021/cr900227t
Dankar, I., Haddarah, A., Omar, F. E. L., Pujolà, M., & Sepulcre, F. (2018). Characterization of food additive-potato starch complexes by FTIR and X-ray diffraction. *Food Chemistry, 260*, 7-12. doi: 10.1016/j.foodchem.2018.03.138
Dartois, A., Singh, J., Kaur, L., & Singh, H. (2010). *Influence of Guar Gum on the In Vitro Starch Digestibility—Rheological and Microstructural Characteristics* (Vol. 5).
Debet, M. R., & Gidley, M. J. (2006). Three classes of starch granule swelling: Influence of surface proteins and lipids. *Carbohydrate Polymers, 64*(3), 452-465.
Dhen, N., Román, L., Rejeb, I. B., Martínez, M. M., Garogouri, M., & Gómez, M. (2016). Particle size distribution of soy flour affecting the quality of enriched gluten-free cakes. *LWT-Food Science and Technology, 66*, 179-185.
Dhital, S., Dolan, G., Stokes, J. R., & Gidley, M. J. (2014). Enzymatic hydrolysis of starch in the presence of cereal soluble fibre polysaccharides. *Food & Function, 5*(3), 579-586. doi: 10.1039/C3FO60506J
Dhital, S., Shrestha, A. K., & Gidley, M. J. (2010). Relationship between granule size and in vitro digestibility of maize and potato starches. *Carbohydrate Polymers, 82*(2), 480-488. doi: http://dx.doi.org/10.1016/j.carbpol.2010.05.018
Donald, A. (2004). Understanding starch structure and functionality. *Starch in food: Structure, function and applications*, 156-184.
Eckelkamp, S. (2016). What's The Healthiest Bread For Your Sandwich? *Online*.

Edwards, C. H., Grundy, M. M., Grassby, T., Vasilopoulou, D., Frost, G. S., Butterworth, P. J., . . . Ellis, P. R. (2015). Manipulation of starch bioaccessibility in wheat endosperm to regulate starch digestion, postprandial glycemia, insulinemia, and gut hormone responses: a randomized controlled trial in healthy ileostomy participants. *Am J Clin Nutr, 102*(4), 791-800. doi: 10.3945/ajcn.114.106203

Edwards, C. H., Maillot, M., Parker, R., & Warren, F. J. (2018). A comparison of the kinetics of in vitro starch digestion in smooth and wrinkled peas by porcine pancreatic alpha-amylase. *Food Chemistry, 244*, 386-393. doi: https://doi.org/10.1016/j.foodchem.2017.10.042

Edwards, C. H., Warren, F. J., Milligan, P. J., Butterworth, P. J., & Ellis, P. R. (2014). A novel method for classifying starch digestion by modelling the amylolysis of plant foods using first-order enzyme kinetic principles. *Food & Function, 5*(11), 2751-2758. doi: 10.1039/C4FO00115J

Eelderink, C., Schepers, M., Preston, T., Vonk, R. J., Oudhuis, L., & Priebe, M. G. (2012). Slowly and rapidly digestible starchy foods can elicit a similar glycemic response because of differential tissue glucose uptake in healthy men. *The American Journal of Clinical Nutrition, 96*(5), 1017-1024. doi: 10.3945/ajcn.112.041947

Efsa Panel on Dietetic Products, N., & Allergies. (2010). Scientific Opinion on Dietary Reference Values for carbohydrates and dietary fibre. *EFSA Journal, 8*(3), 1462-n/a. doi: 10.2903/j.efsa.2010.1462

Englyst, H. N., Kingman, S. M., & Cummings, J. H. (1992). Classification and measurement of nutritionally important starch fractions. *Eur J Clin Nutr, 46 Suppl 2*, S33-50.

Englyst, K. N., Vinoy, S., Englyst, H. N., & Lang, V. (2003). Glycaemic index of cereal products explained by their content of rapidly and slowly available glucose. *The British Journal of Nutrition, 89*(3), 329-340. doi: http://dx.doi.org/10.1079/BJN2002786

Evert, A. B., & Boucher, J. L. (2014). New Diabetes Nutrition Therapy Recommendations: What You Need to Know. *Diabetes Spectrum, 27*(2), 121-130. doi: 10.2337/diaspect.27.2.121

Eyaru, R., Shrestha, A. K., & Arcot, J. (2009). Effect of various processing techniques on digestibility of starch in Red kidney bean (Phaseolus vulgaris) and two varieties of peas (Pisum sativum). *Food Research International, 42*(8), 956-962. doi: http://dx.doi.org/10.1016/j.foodres.2009.06.007

Fardet, A. (2010). New hypotheses for the health-protective mechanisms of whole-grain cereals: what is beyond fibre? *Nutrition Research Reviews, 23*(1), 65-134. doi: 10.1017/S0954422410000041

Fardet, A., Leenhardt, F., Lioger, D., Scalbert, A., & Remesy, C. (2006). Parameters controlling the glycaemic response to breads. *Nutrition Research Reviews, 19*(1), 18-25. doi: 10.1079/nrr2006118

Fardet, A., Leenhardt, F., Lioger, D., Scalbert, A., & Rémésy, C. (2006). Parameters controlling the glycaemic response to breads. *Nutrition Research Reviews, 19*(1), 18-25.

Forouhi, N. G., Misra, A., Mohan, V., Taylor, R., & Yancy, W. (2018). Dietary and nutritional approaches for prevention and management of type 2 diabetes. *BMJ, 361*, k2234. doi: 10.1136/bmj.k2234

Foster-Powell, K., Holt, S. H., & Brand-Miller, J. C. (2002). International table of glycemic index and glycemic load values: 2002. *The American Journal of Clinical Nutrition, 76*(1), 5-56.

Franco, C. M. L., & Ciacco, C. F. (1992). Factors that affect the enzymatic degradation of natural starch granules - effect of the size of the granules. *Starch-Starke, 44*(11), 422-426. doi: 10.1002/star.19920441106

Fuentes-Zaragoza, E., Riquelme-Navarrete, M. J., Sánchez-Zapata, E., & Pérez-Álvarez, J. A. (2010). Resistant starch as functional ingredient: A review. *Food Research International, 43*(4), 931-942. doi: http://dx.doi.org/10.1016/j.foodres.2010.02.004

Gallant, D. J., Bouchet, B., & Baldwin, P. M. (1997). Microscopy of starch: evidence of a new level of granule organization. *Carbohydrate Polymers, 32*(3), 177-191. doi: http://dx.doi.org/10.1016/S0144-8617(97)00008-8

Gonera, A., & Cornillon, P. (2002). Gelatinization of starch/gum/sugar systems studied by using DSC, NMR, and CSLM. *Starch-Starke, 54*(11), 508-516. doi: 10.1002/1521-379x(200211)54:11<508::aid-star508>3.0.co;2-k

Gous, P. W., Gilbert, R. G., & Fox, G. P. (2015). Drought-proofing barley (Hordeum vulgare) and its impact on grain quality: A review. *Journal of the Institute of Brewing, 121*(1), 19-27. doi: 10.1002/jib.187

Grafenauer, S., & Curtain, F. (2018). An Audit of Australian Bread with a Focus on Loaf Breads and Whole Grain. *Nutrients, 10*(8), 1106. doi: 10.3390/nu10081106

Grains & Legumes Nutrition Council. (2012). The leading independent voice for grains and legumes in health and nutrition. *Grains & Legumes Nutrition Council Ltd*.

Granfeldt, Y., & Björck, I. (1991). Glycemic response to starch in pasta: a study of mechanisms of limited enzyme availability. *Journal of Cereal Science, 14*(1), 47-61. doi: http://dx.doi.org/10.1016/S0733-5210(09)80017-9

Greenwell, P., & Schofield, J. D. (1986). A starch granule protein associated with endosperm softness in wheat. *Cereal Chemistry*(63), 397-380.

Grewal, A., & Jood, S. (2009). Chemical composition and digestibility (in vitro) of green gram as affected by processing and cooking methods. *British Food Journal, 111*(2-3), 235-242. doi: 10.1108/00070700910941444

Grundy, M. M., Edwards, C. H., Mackie, A. R., Gidley, M. J., Butterworth, P. J., & Ellis, P. R. (2016). Re-evaluation of the mechanisms of dietary fibre and implications for macronutrient bioaccessibility, digestion and postprandial metabolism. *Br J Nutr, 116*(5), 816-833. doi: 10.1017/s0007114516002610

Grundy, M. M., Lapsley, K., & Ellis, P. R. (2016). A review of the impact of processing on nutrient bioaccessibility and digestion of almonds. *51*(9), 1937-1946. doi: 10.1111/ijfs.13192

Gunaratne, A., & Hoover, R. (2002). *Effect of heat-moisture treatment on the structure and physicochemical properties of tuber and root starches* (Vol. 49).

Gunnerud, U. J., Ostman, E. M., & Bjorck, I. M. (2013). Effects of whey proteins on glycaemia and insulinaemia to an oral glucose load in healthy adults; a dose-response study. *Eur J Clin Nutr, 67*(7), 749-753. doi: 10.1038/ejcn.2013.88

Hager, A.-S., & Arendt, E. (2013). *Influence of hydroxypropylmethylcellulose (HPMC), xanthan gum and their combination on loaf specific volume, crumb hardness and crumb grain characteristics of gluten-free breads based on rice, maize, teff and buckwheat* (Vol. 32).

Hamaker, B. R., & Bugusu, B. A. (2003). *Overview: sorghum proteins and food quality*. Paper presented at the Workshop on the proteins of sorghum and millets: enhancing nutritional and functional properties for Africa [CD](Pretoria: South Africa).

Han, J.-A., & BeMiller, J. N. (2007). Preparation and physical characteristics of slowly digesting modified food starches. *Carbohydrate Polymers, 67*(3), 366-374. doi: http://dx.doi.org/10.1016/j.carbpol.2006.06.011

Han, X.-Z., & Hamaker, B. R. (2001). Amylopectin Fine Structure and Rice Starch Paste Breakdown. *Journal of Cereal Science, 34*(3), 279-284. doi: 10.1006/jcrs.2001.0374

Hardacre, A. K., Lentle, R. G., Yap, S.-Y., & Monro, J. A. (2016). Does viscosity or structure govern the rate at which starch granules are digested? *Carbohydrate Polymers, 136*, 667-675. doi: http://dx.doi.org/10.1016/j.carbpol.2015.08.060

Hardacre, A. K., Yap, S.-Y., Lentle, R. G., & Monro, J. A. (2015). The effect of fibre and gelatinised starch type on amylolysis and apparent viscosity during in vitro digestion at a physiological shear rate. *Carbohydrate Polymers, 123*, 80-88. doi: http://dx.doi.org/10.1016/j.carbpol.2015.01.013

Hasjim, J., Ai, Y., & Jane, J.-l. (2013). Novel Applications of Amylose-Lipid Complex as Resistant Starch Type 5 *Resistant Starch* (pp. 79-94): John Wiley and Sons Ltd.

Hawkins, A., & Johnson, S. K. (2005). In vitro carbohydrate digestibility of whole-chickpea and chickpea bread products. *International Journal of Food Sciences and Nutrition, 56*(3), 147-155. doi: 10.1080/09637480500103920

Hera, E., Rosell, C., & Gómez, M. (2014). Effect of water content and flour particle size on gluten-free bread quality and digestibility. *Food chemistry, 151*, 526-531. doi: 10.1016/j.foodchem.2013.11.115

Higley, J. S., Love, S. L., Price, W. J., Nelson, J. E., & Huber, K. C. (2003). The Rapid Visco Analyzer (RVA) as a tool for differentiating potato cultivars on the basis of flour pasting properties. *American Journal of Potato Research, 80*(3), 195. doi: 10.1007/bf02855691

Hoebler, M. F. D. A. K. C. B. J. L. B. C. (2000). Particle size of solid food after human mastication and in vitro simulation of oral breakdown. *International Journal of Food Sciences and Nutrition, 51*(5), 353-366. doi: 10.1080/096374800426948

Holt, S., & Miller, J. B. (1994). Particle size, satiety and the glycaemic response. *Eur J Clin Nutr, 48*(7), 496-502.

Hong, Y., Liu, G., Zhou, S., Gu, Z., Cheng, L., Li, Z., & Li, C. (2016). Influence of guar gum on the in vitro digestibility of tapioca starch. *Starch - Stärke, 68*(3-4), 339-347. doi: 10.1002/star.201500142

Hoover, R. (2001). Composition, molecular structure, and physicochemical properties of tuber and root starches: a review. *Carbohydrate Polymers, 45*(3), 253-267. doi: https://doi.org/10.1016/S0144-8617(00)00260-5

Horwitz, W., & Latimer, G. W. (2005). *Official methods of analysis of AOAC International*. Gaithersburg, Md.: AOAC International.

Huber, K., & BeMiller, J. (2000). Channels of maize and sorghum starch granules. *Carbohydrate Polymers, 41*(3), 269-276.

Ick Shin, S., Jin Choi, H., Min Chung, K., R. Hamaker, B., Hwa Park, K., & Wha Moon, T. (2004). *Slowly Digestible Starch from Debranched Waxy Sorghum Starch: Preparation and Properties* (Vol. 81).

Imberty, A., Chanzy, H., Pérez, S., Buléon, A., & Tran, V. (1988). The double-helical nature of the crystalline part of A-starch. *Journal of molecular biology, 201*(2), 365-378. doi: 10.1016/0022-2836(88)90144-1

Immel, S., & Lichtenthaler, F. W. (2000). The Hydrophobic Topographies of Amylose and its Blue Iodine Complex. *Starch - Stärke, 52*(1), 1-8. doi: 10.1002/(SICI)1521-379X(200001)52:1<1::AID-STAR1>3.0.CO;2-H

Irika binti Idril, N., Diana, A., & Firmansyah Wargahadibrata, A. (2013). *Preliminary Study: Glycemic Index of Brown and White Rice Variant IR64 in Healthy Adult Men* (Vol. 1).

Ishiguro, K., Noda, T., Kitahara, K., & Yamakawa, O. (2000). Retrogradation of Sweetpotato Starch. *Starch - Stärke, 52*(1), 13-17. doi: 10.1002/(SICI)1521-379X(200001)52:1<13::AID-STAR13>3.0.CO;2-E

Jacobs, D., Pereira, M., Slavin, J., & Marquart, L. (2000). Defining the impact of whole-grain intake on chronic disease. *Cereal Foods World, 45*, 51-53.

Jacobs Jr, D., Pereira, M., Slavin, J., & Marquart, L. (2000). Defining the impact of whole-grain intake on chronic disease. *Cereal Foods World, 45*(2), 51-53.

Jenkins, D. J., Thorne, M. J., Wolever, T. M., Jenkins, A. L., Rao, A. V., & Thompson, L. U. (1987). The effect of starch-protein interaction in wheat on the glycemic response and rate of in vitro digestion. *The American Journal of Clinical Nutrition, 45*(5), 946-951.

Jenkins, D. J., Wesson, V., Wolever, T. M., Jenkins, A. L., Kalmusky, J., Guidici, S., . . . Wong, G. S. (1988). Wholemeal versus wholegrain breads: proportion of whole or cracked grain and the glycaemic response. *BMJ, 297*(6654), 958-960.

Jenkins, D. J., Wolever, T. M., Jenkins, A., Giordano, C., Giudici, S., Thompson, L. U., . . . Wong, G. S. (1986). Low glycemic response to traditionally processed wheat and rye products: Bulgar and pumpernickel bread. *American Journal of Clinical Nutrition, 43*, 516-520.

Jenkins, D. J., Wolever, T. M., Taylor, R. H., Barker, H., Fielden, H., Baldwin, J. M., . . . Goff, D. V. (1981). Glycemic index of foods: a physiological basis for carbohydrate exchange. *Am J Clin Nutr, 34*(3), 362-366.

Johansson, D. P., Gutiérrez, J. L. V., Landberg, R., Alminger, M., & Langton, M. (2018). Impact of food processing on rye product properties and their in vitro digestion. *European journal of nutrition, 57*(4), 1651-1666. doi: 10.1007/s00394-017-1450-y

Juntunen, K. S., Niskanen, L. K., Liukkonen, K. H., Poutanen, K. S., Holst, J. J., & Mykkänen, H. M. (2002). Postprandial glucose, insulin, and incretin responses to grain products in healthy subjects. *The American Journal of Clinical Nutrition, 75*(2), 254-262.

Juszczak, L., Fortuna, T., & Krok, F. (2003). Non-contact Atomic Force Microscopy of Starch Granules Surface. Part II. Selected Cereal Starches. *Starch - Stärke, 55*(1), 8-16. doi: 10.1002/star.200390010

Kaur, B., Ariffin, F., Bhat, R., & Karim, A. A. (2012). Progress in starch modification in the last decade. *Food Hydrocolloids, 26*(2), 398-404. doi: http://dx.doi.org/10.1016/j.foodhyd.2011.02.016

Kim, E. H.-J., Petrie, J. R., Motoi, L., Morgenstern, M. P., Sutton, K. H., Mishra, S., & Simmons, L. D. (2008). Effect of structural and physicochemical characteristics of the protein matrix in pasta on in vitro starch digestibility. *Food Biophysics, 3*(2), 229-234.

Kim, H.-S., & Huber, K. (2008). *Channels within soft wheat starch A- and B-type granules* (Vol. 48).

Kim, H.-S., Kim, B.-Y., & Baik, M.-Y. (2012). *Application of Ultra High Pressure (UHP) in Starch Chemistry* (Vol. 52).

Lanzerstorfer, P., Rechenmacher, E., Lugmayr, O., Stadlbauer, V., Höglinger, O., Vollmar, A., & Weghuber, J. (2018). *Effects of Various Commercial Whole-Grain Breads on Postprandial Blood Glucose Response and Glycemic Index in Healthy Subjects* (Vol. 5).

Lehmann, U., & Robin, F. (2007). Slowly digestible starch – its structure and health implications: a review. *Trends in Food Science & Technology, 18*(7), 346-355. doi: http://dx.doi.org/10.1016/j.tifs.2007.02.009

Lemmens, L., Van Buggenhout, S., Van Loey, A. M., & Hendrickx, M. E. (2010). Particle size reduction leading to cell wall rupture is more important for the beta-carotene bioaccessibility of raw compared to thermally processed carrots. *J Agric Food Chem, 58*(24), 12769-12776. doi: 10.1021/jf102554h

Liljeberg, H., Åkerberg, A., & Björck, I. (1996). Resistant starch formation in bread as influenced by choice of ingredients or baking conditions. *Food Chemistry, 56*(4), 389-394. doi: https://doi.org/10.1016/0308-8146(95)00199-9

Liljeberg, H., & Bjorck, I. (1994). Bioavailability of starch in bread products. Postprandial glucose and insulin responses in healthy subjects and in vitro resistant starch content. *Eur J Clin Nutr, 48*(3), 151-163.
Liljeberg, H., Granfeldt, Y., & Bjorck, I. (1992). Metabolic responses to starch in bread containing intact kernels versus milled flour. *Eur J Clin Nutr, 46*(8), 561-575.
Liljeberg, H., Granfeldt, Y., & Björck, I. (1994). Metabolic responses to starch in bread containing intact kernels vs milled flour. *The American Journal of Clinical Nutrition, 59*(3), 779S-779S. doi: 10.1093/ajcn/59.3.779S
Liljeberg, H. G., Granfeldt, Y., & Bjork, I. (1992). Metabolic responses to starch in bread containing intact kernels versus milled flour. *European Journal of Clinical Nutrition, 52* 368-371.
Lincoln Taiz, Eduardo Zeiger, Ian M. Møller, & Murphy, A. (2015). Plant Physiology and Development. (Sixth Edition), Topic 8.13.
Livesey, G., Wilkinson, J. A., Roe, M., Faulks, R., Clark, S., Brown, J. C., . . . Elia, M. (1995). Influence of the physical form of barley grain on the digestion of its starch in the human small intestine and implications for health. *Am J Clin Nutr, 61*(1), 75-81.
Lopez-Rubio, A., Flanagan, B. M., Gilbert, E. P., & Gidley, M. J. (2008). A novel approach for calculating starch crystallinity and its correlation with double helix content: A combined XRD and NMR study. *Biopolymers, 89*(9), 761-768. doi: 10.1002/bip.21005
López, C. A., de Vries, A. H., & Marrink, S. J. (2012). Amylose folding under the influence of lipids. *Carbohydrate Research, 364*, 1-7.
Lovegrove, A., Edwards, C. H., De Noni, I., Patel, H., El, S. N., Grassby, T., . . . Shewry, P. R. (2017). Role of polysaccharides in food, digestion, and health. *Crit Rev Food Sci Nutr, 57*(2), 237-253. doi: 10.1080/10408398.2014.939263
Ma, M., Wang, Y., Wang, M., Jane, J.-l., & Du, S.-k. (2017). Physicochemical properties and in vitro digestibility of legume starches. *Food Hydrocolloids, 63*, 249-255. doi: https://doi.org/10.1016/j.foodhyd.2016.09.004
Mahasukhonthachat, K., Sopade, P. A., & Gidley, M. J. (2010). Kinetics of starch digestion in sorghum as affected by particle size. *Journal of Food Engineering, 96*(1), 18-28. doi: http://dx.doi.org/10.1016/j.jfoodeng.2009.06.051
Mandala, I. G., & Bayas, E. (2004). Xanthan effect on swelling, solubility and viscosity of wheat starch dispersions. *Food Hydrocolloids, 18*(2), 191-201. doi: 10.1016/s0268-005x(03)00064-x
Martens, B. M. J., Gerrits, W. J. J., Bruininx, E. M. A. M., & Schols, H. A. (2018). Amylopectin structure and crystallinity explains variation in digestion kinetics of starches across botanic sources in an in vitro pig model. *Journal of Animal Science and Biotechnology, 9*(1), 91. doi: 10.1186/s40104-018-0303-8
Martin, C., & Smith, A. M. (1995). Starch biosynthesis. *The plant cell, 7*(7), 971.
Martínez-Preciado, A. H., Estrada-Girón, Y., González-Álvarez, A., Fernández, V. V. A., Macías, E. R., & Soltero, J. F. A. (2014). Physicochemical, morphological and rheological properties of canned bean pastes "negro Queretaro" variety (Phaseolus vulgaris L.). *Journal of food science and technology, 51*(9), 1795-1805. doi: 10.1007/s13197-012-0737-5
Martínez, M. M., Román, L., & Gómez, M. (2018). Implications of hydration depletion in the in vitro starch digestibility of white bread crumb and crust. *Food Chemistry, 239*, 295-303. doi: https://doi.org/10.1016/j.foodchem.2017.06.122
Maughan, R. (2013). Carbohydrate metabolism. *Surgery (Oxford), 31*(6), 273-277. doi: https://doi.org/10.1016/j.mpsur.2013.04.008
McDougall, G. J., Morrison, I. M., Stewart, D., & Hillman, J. R. (1996). Plant Cell Walls as Dietary Fibre: Range, Structure, Processing and Function. *Journal of the Science of*

Food and Agriculture, 70(2), 133-150. doi: 10.1002/(SICI)1097-0010(199602)70:2<133::AID-JSFA495>3.0.CO;2-4
Mera, S. (1994). Diet and disease. *British journal of biomedical science, 51*(3), 189-206.
Miao, M., Jiang, B., Cui, S., Zhang, T., & Jin, Z. (2013). *Slowly Digestible Starch—A Review* (Vol. 55).
Miao, M., Jiang, B., Cui, S. W., Zhang, T., & Jin, Z. (2015). Slowly Digestible Starch—A Review. *Critical Reviews in Food Science and Nutrition, 55*(12), 1642-1657. doi: 10.1080/10408398.2012.704434
Miao, M., Jiang, B., & Zhang, T. (2009). Effect of pullulanase debranching and recrystallization on structure and digestibility of waxy maize starch. *Carbohydrate Polymers, 76*(2), 214-221. doi: https://doi.org/10.1016/j.carbpol.2008.10.007
Miao, M., Li, R., Jiang, B., Cui, S. W., Zhang, T., & Jin, Z. (2014). Structure and physicochemical properties of octenyl succinic esters of sugary maize soluble starch and waxy maize starch. *Food Chemistry, 151*, 154-160. doi: https://doi.org/10.1016/j.foodchem.2013.11.043
Ministry of Health. (2015). Eating and Activity Guidelines for New Zealand Adults. *Wellington: Ministry of Health*.
Mishra, S., Edwards, H., Hedderley, D., Podd, J., & Monro, J. (2017). Kiwifruit Non-Sugar Components Reduce Glycaemic Response to Co-Ingested Cereal in Humans. *Nutrients, 9*(11), 1195. doi: 10.3390/nu9111195
Mishra, S., Hardacre, A., & Monro, J. (2012). *Food structure and carbohydrate digestibility*: INTECH Open Access Publisher.
Mishra, S., Monro, J., & Hedderley, D. (2008). Effect of Processing on Slowly Digestible Starch and Resistant Starch in Potato. *Starch - Stärke, 60*(9), 500-507. doi: 10.1002/star.200800209
Mishra, S., Willis, J., Ansell, J., & Monro, J. A. (2016). Equicarbohydrate partial exchange of kiwifruit for wheaten cereal reduces postprandial glycaemia without decreasing satiety. *Journal of nutritional science, 5*, e37-e37. doi: 10.1017/jns.2016.30
Mofidi, A., Ferraro, Z. M., Stewart, K. A., Tulk, H. M. F., Robinson, L. E., Duncan, A. M., & Graham, T. E. (2012). The Acute Impact of Ingestion of Sourdough and Whole-Grain Breads on Blood Glucose, Insulin, and Incretins in Overweight and Obese Men. *Journal of Nutrition and Metabolism, 2012*, 184710. doi: 10.1155/2012/184710
Moghaddam, E., Vogt, J. A., & Wolever, T. M. S. (2006). The Effects of Fat and Protein on Glycemic Responses in Nondiabetic Humans Vary with Waist Circumference, Fasting Plasma Insulin, and Dietary Fiber Intake. *The Journal of nutrition, 136*(10), 2506-2511.
Monro, J. A. (2002). Glycaemic glucose equivalent: combining carbohydrate content, quantity and glycaemic index of foods for precision in glycaemia management. *Asia Pac J Clin Nutr, 11*(3), 217-225.
Monro, J. A., & Mishra, S. (2010). Glycemic Impact As a Property of Foods Is Accurately Measured By an Available Carbohydrate Method That Mimics the Glycemic Response. *The Journal of Nutrition, 140*(7), 1328-1334. doi: 10.3945/jn.110.121210
Monro, J. A., Mishra, S., & Venn, B. (2010a). Baselines representing blood glucose clearance improve in vitro prediction of the glycaemic impact of customarily consumed food quantities. *Br J Nutr, 103*(2), 295-305. doi: 10.1017/s0007114509991632
Monro, J. A., Mishra, S., & Venn, B. (2010b). Baselines representing blood glucose clearance improve in vitro prediction of the glycemic impact of customarily consumed food quantities. *British Journal of Nutrition, 103*, 295-305.
Monro, J. A., Mishra, S. S., & Hardacre, A. (2011). Glycaemic Impact Regulation Based on Progressive Geometric Changes in Solid Starch-Based Food Particles During Digestion. *Food Digestion, 2*(1), 1-12. doi: 10.1007/s13228-011-0009-2

Monro, J. A., & Shaw, M. (2008). Glycemic impact, glycemic glucose equivalents, glycemic index, and glycemic load: definitions, distinctions, and implications. *American Journal of Clinical Nutrition, 87*(1), 237S-243S.
Morrison, W. R. (1988). Lipids in cereal starches: A review. *Journal of Cereal Science, 8*(1), 1-15.
O'Connor, A. (2012). An overview of the role of bread in the UK diet. *Nutrition Bulletin, 37*(3), 193-212. doi: 10.1111/j.1467-3010.2012.01975.x
Oates, C. G. (1997). Towards an understanding of starch granule structure and hydrolysis. *Trends in Food Science & Technology, 8*(11), 375-382. doi: http://dx.doi.org/10.1016/S0924-2244(97)01090-X
Otago, U. o., & Health, M. o. (2011). A Focus on Nutrition: Key findings of the 2008/09 New Zealand Adult Nutrition Survey. *Wellington: Ministry of Health.*
Øverby, N. C., Sonestedt, E., Laaksonen, D. E., & Birgisdottir, B. E. (2013). Dietary fiber and the glycemic index: a background paper for the Nordic Nutrition Recommendations 2012. *Food & Nutrition Research, 57*, 10.3402/fnr.v3457i3400.20709. doi: 10.3402/fnr.v57i0.20709
Palou, A., Bonet, M. L., & Pico, C. (2009). On the role and fate of sugars in human nutrition and health. Introduction. *Obes Rev, 10 Suppl 1*, 1-8. doi: 10.1111/j.1467-789X.2008.00560.x
Panyoo, A. E., & Emmambux, M. N. (2017). Amylose–lipid complex production and potential health benefits: A mini-review. *Starch - Stärke*, n/a-n/a. doi: 10.1002/star.201600203
Park, E. Y., Baik, B.-K., & Lim, S.-T. (2009). Influences of temperature-cycled storage on retrogradation and in vitro digestibility of waxy maize starch gel. *Journal of Cereal Science, 50*(1), 43-48.
Park, J. T., & Rollings, J. E. (1994). Effects of substrate branching characteristics on kinetics of enzymatic depolymerizaion of mixed linear and branched polysaccharides: I. Amylose/amylopectin alpha-amylolysis. *Biotechnol Bioeng, 44*(7), 792-800. doi: 10.1002/bit.260440704
Parker, R., & Ring, S. G. (2001). Aspects of the Physical Chemistry of Starch. *Journal of Cereal Science, 34*(1), 1-17. doi: https://doi.org/10.1006/jcrs.2000.0402
Pedersen, B., Krudsen, K. E. B., & Eggum, B. O. (1989). Nutritive value of cereal products with emphasis on the effects of milling. *World Review of Nutrition and Dietetics, 60*, 1-91.
Perera, A., Meda, V., & Tyler, R. T. (2010). Resistant starch: A review of analytical protocols for determining resistant starch and of factors affecting the resistant starch content of foods. *Food Research International, 43*(8), 1959-1974. doi: http://dx.doi.org/10.1016/j.foodres.2010.06.003
Pinto, V. Z., Vanier, N. L., Klein, B., Zavareze, E. d. R., Elias, M. C., Gutkoski, L. C., . . . Dias, A. R. G. (2012). Physicochemical, crystallinity, pasting and thermal properties of heat-moisture-treated pinhão starch. *Starch - Stärke, 64*(11), 855-863. doi: doi:10.1002/star.201200040
Planchot, V., Colonna, P., Gallant, D. J., & Bouchet, B. (1995). Extensive degradation of native starch granules by alpha-amylase from aspergillus fumigatus. *Journal of Cereal Science, 21*(2), 163-171. doi: https://doi.org/10.1016/0733-5210(95)90032-2
Polesi, L., Sarmento, S., & Anjos, C. (2011). Composition and characterization of pea and chickpea starches. *Brazilian Journal of Food Technology, 14*(1), 74-81.
Raguin, A., & Ebenhöh, O. (2017). Design starch: stochastic modeling of starch granule biogenesis. *Biochemical Society Transactions, 45*(4), 885-893. doi: 10.1042/bst20160407

Rahman, S., Kosar-Hashemi, B., Samuel, M., Hill, A., Abbott, D., Skerritt, J., . . . Morell, M. (1995). The major proteins of wheat endosperm starch granules. *Functional Plant Biology, 22*(5), 793-803.

Raigond, P., Ezekiel, R., & Raigond, B. (2015). Resistant starch in food: a review. *Journal of the Science of Food and Agriculture, 95*(10), 1968-1978. doi: 10.1002/jsfa.6966

Ranawana, V., Monro, J. A., Mishra, S., & Henry, C. J. K. (2010). Degree of particle size breakdown during mastication may be a possible cause of interindividual glycemic variability. *Nutrition Research, 30*(4), 246-254. doi: https://doi.org/10.1016/j.nutres.2010.02.004

Rehman, Z.-u., Salariya, A. M., & Zafar, S. I. (2001). Effect of processing on available carbohydrate content and starch digestibility of kidney beans (Phaseolus vulgaris L.). *Food Chemistry, 73*(3), 351-355. doi: https://doi.org/10.1016/S0308-8146(00)00311-3

Rehman, Z.-u., & Shah, W. H. (2005). Thermal heat processing effects on antinutrients, protein and starch digestibility of food legumes. *Food chemistry, 91*(2), 327-331. doi: http://dx.doi.org/10.1016/j.foodchem.2004.06.019

Reynolds, R. C., Stockmann, K. S., Atkinson, F. S., Denyer, G. S., & Brand-Miller, J. C. (2009). Effect of the glycemic index of carbohydrates on day-long (10 h) profiles of plasma glucose, insulin, cholecystokinin and ghrelin. *Eur J Clin Nutr, 63*(7), 872-878. doi: 10.1038/ejcn.2008.52

Richardson, D. (2000). The grain, the wholegrain and nothing but the grain: the science behind wholegrain and the reduced risk of heart disease and cancer. *Nutrition Bulletin, 25*, 353-360.

Richardson, G., Kidman, S., Langton, M., & Hermansson, A.-M. (2004). Differences in amylose aggregation and starch gel formation with emulsifiers. *Carbohydrate Polymers, 58*(1), 7-13. doi: https://doi.org/10.1016/j.carbpol.2004.06.013

Ridout, M. J., Gunning, A. P., Parker, M. L., Wilson, R. H., & Morris, V. J. (2002). Using AFM to image the internal structure of starch granules. *Carbohydrate Polymers, 50*(2), 123-132. doi: http://dx.doi.org/10.1016/S0144-8617(02)00021-8

Roberts, S., Desbrow, B., Grant, G., Anoopkumar-Dukie, S., & Leveritt, M. (2013). Glycemic response to carbohydrate and the effects of exercise and protein. *Nutrition, 29*(6), 881-885. doi: 10.1016/j.nut.2012.12.022

Rodríguez-Sandoval, E., Fernández-Quintero, A., Cuvelier, G., Relkin, P., & Bello-Pérez, L. A. (2008). Starch retrogradation in cassava flour from cooked parenchyma. *Starch-Stärke, 60*(3-4), 174-180.

Roman, L., Gomez, M., Li, C., Hamaker, B. R., & Martinez, M. M. (2017). Biophysical features of cereal endosperm that decrease starch digestibility. *Carbohydrate Polymers, 165*, 180-188. doi: https://doi.org/10.1016/j.carbpol.2017.02.055

Sablani, S. (2009). Gelatinization of Starch *Food Properties Handbook, Second Edition*: CRC Press.

Sajilata, M. G., Singhal, R. S., & Kulkarni, P. R. (2006). Resistant Starch–A Review. *Comprehensive Reviews in Food Science and Food Safety, 5*(1), 1-17. doi: 10.1111/j.1541-4337.2006.tb00076.x

Sasaki, T. (2018). Effects of xanthan and guar gums on starch digestibility and texture of rice flour blend bread. *Cereal Chemistry, 95*(1), 177-184. doi: 10.1002/cche.10024

Sasaki, T., Sotome, I., & Okadome, H. (2015). In vitro starch digestibility and in vivo glucose response of gelatinized potato starch in the presence of non-starch polysaccharides. *Starch-Stärke, 67*(5-6), 415-423.

Segundo, C., Román, L., Gómez, M., & Martínez, M. M. (2017). Mechanically fractionated flour isolated from green bananas (M. cavendishii var. nanica) as a tool to increase the

dietary fiber and phytochemical bioactivity of layer and sponge cakes. *Food Chemistry, 219*, 240-248.
Shoemaker, E. (2015). Is Any Bread Actually Healthy? A Must-Read Before You Buy Your Next Loaf. *Online*.
Singh, J., Dartois, A., & Kaur, L. (2010). Starch digestibility in food matrix: a review. *Trends in Food Science & Technology, 21*(4), 168-180. doi: http://dx.doi.org/10.1016/j.tifs.2009.12.001
Slaughter, S. L., Ellis, P. R., Jackson, E. C., & Butterworth, P. J. (2002). The effect of guar galactomannan and water availability during hydrothermal processing on the hydrolysis of starch catalysed by pancreatic alpha-amylase. *Biochim Biophys Acta, 1571*(1), 55-63. doi: 10.1016/s0304-4165(02)00209-x
Slavin, J. (2004). Whole grains and health. *Nutrition Research Reviews, 17*, 99-110.
Smrčková, P., Saglamtas, M., Hofmanová, T., Koláček, J., Chena, D., & Šárka, E. (2014). Effect of process parameters on slowly digestible and resistant starch content in extrudates. *Czech Journal of Food Sciences, 32*(5), 503-508.
Srichuwong, S., Sunarti, T. C., Mishima, T., Isono, N., & Hisamatsu, M. (2005). Starches from different botanical sources I: Contribution of amylopectin fine structure to thermal properties and enzyme digestibility. *Carbohydrate Polymers, 60*(4), 529-538. doi: http://dx.doi.org/10.1016/j.carbpol.2005.03.004
Srv, A., Mishra, S., Hardacre, A., Matia-Merino, L., Goh, K., Warren, F., & Monro, J. (2019). Kernel structure in breads reduces in vitro starch digestion rate and estimated glycaemic potency only at high grain inclusion rates. *Food Structure, 21*, 100109. doi: https://doi.org/10.1016/j.foostr.2019.100109
Sujka, M., & J, J. (2007). *Starch granule porosity and its changes by means of amylolysis* (Vol. 21).
Taggart, P., & Mitchell, J. R. (2009). 5 - Starch* *Handbook of Hydrocolloids (Second edition)* (pp. 108-141): Woodhead Publishing.
Tai, M. M. (1994). A mathematical model for the determination of total area under glucose tolerance and other metabolic curves. *Diabetes Care, 17*(2), 152-154.
Tan, X., Li, X., Chen, L., Xie, F., Li, L., & Huang, J. (2017). Effect of heat-moisture treatment on multi-scale structures and physicochemical properties of breadfruit starch. *Carbohydr Polym, 161*, 286-294. doi: 10.1016/j.carbpol.2017.01.029
Tester, R., Qi, X., & Karkalas, J. (2006). Hydrolysis of native starches with amylases. *Animal Feed Science and Technology, 130*(1), 39-54.
Tester, R. F., & Sommerville, M. D. (2003). The effects of non-starch polysaccharides on the extent of gelatinisation, swelling and alpha-amylase hydrolysis of maize and wheat starches. *Food Hydrocolloids, 17*(1), 41-54. doi: 10.1016/s0268-005x(02)00032-2
Theander, O., & Åman, P. (1982). Studies on dietary fibre. A method for the analysis and chemical characterisation of total dietary fibre. *Journal of the Science of Food and Agriculture, 33*(4), 340-344.
Thiex, N. J., Anderson, S., & Gildemeister, B. (2003). Crude Fat, Hexanes Extraction, in Feed, Cereal Grain, and Forage (Randall/Soxtec/Submersion Method): Collaborative Study. *Journal of AOAC International, 86*(5), 899-908.
Thomas, D. J., & Atwell, W. A. (1999). Chapter 1- Starch Structure *Starches* (pp. 1-11): American Association of Cereal Chemists International.
Thompson, D. B. (2000). *On the non-random nature of amylopectin branching* (Vol. 43).
Thorne, M. J., Thompson, L. U., & Jenkins, D. J. (1983). Factors affecting starch digestibility and the glycemic response with special reference to legumes. *Am J Clin Nutr, 38*(3), 481-488.

Tian, Y., Li, Y., Manthey, F. A., Xu, X., Jin, Z., & Deng, L. (2009). Influence of β-cyclodextrin on the short-term retrogradation of rice starch. *Food chemistry, 116*(1), 54-58.

Tian, Y., Zhang, L., Xu, X., Xie, Z., Zhao, J., & Jin, Z. (2012). Effect of temperature-cycled retrogradation on slow digestibility of waxy rice starch. *International Journal of Biological Macromolecules, 51*(5), 1024-1027. doi: http://dx.doi.org/10.1016/j.ijbiomac.2012.08.024

Tydeman, E. A., Parker, M. L., Wickham, M. S., Rich, G. T., Faulks, R. M., Gidley, M. J., . . . Waldron, K. W. (2010). Effect of carrot (Daucus carota) microstructure on carotene bioaccessibilty in the upper gastrointestinal tract. 1. In vitro simulations of carrot digestion. *J Agric Food Chem, 58*(17), 9847-9854. doi: 10.1021/jf101034a

Varavinit, S., Shobsngob, S., Varanyanond, W., Chinachoti, P., & Naivikul, O. (2003). Effect of Amylose Content on Gelatinization, Retrogradation and Pasting Properties of Flours from Different Cultivars of Thai Rice. *Starch - Stärke, 55*(9), 410-415. doi: 10.1002/star.200300185

Wahlquist, A. (2010). The rise and fall of our daily bread (Article). *The Australian*.

Waigh, T. A., Gidley, M. J., Komanshek, B. U., & Donald, A. M. (2000). The phase transformations in starch during gelatinisation: a liquid crystalline approach. *Carbohydrate Research, 328*(2), 165-176. doi: https://doi.org/10.1016/S0008-6215(00)00098-7

Wang, S., & Copeland, L. (2013). Molecular disassembly of starch granules during gelatinization and its effect on starch digestibility: a review. *Food & Function, 4*(11), 1564-1580. doi: 10.1039/C3FO60258C

Wang, S., Li, C., Copeland, L., Niu, Q., & Wang, S. (2015). Starch retrogradation: A comprehensive review. *Comprehensive Reviews in Food Science and Food Safety, 14*(5), 568-585.

Wang, T. L., Bogracheva, T. Y., & Hedley, C. L. (1998). Starch: as simple as A, B, C? *Journal of Experimental Botany, 49*(320), 481-502. doi: 10.1093/jxb/49.320.481

Watson, E. (2014). 56% of US shoppers say they are cutting back on white bread, says Packaged Facts (Article). *William Reed-News and analysis on food & beverage development - North America, EU edition*(APAC Edition).

Welch, I. M., Bruce, C., Hill, S. E., & Read, N. W. (1987). Duodenal and ileal lipid suppresses postprandial blood glucose and insulin responses in man: Possible implications for the dietary management of diabetes mellitus. *Clinical Science, 72*(2), 209-216. doi: 10.1042/cs0720209

WHO. (2003). Diet, nutrition and the prevention of chronic diseases. *World Health Organ Tech Rep Ser, 916*(i-viii).

WHO. (2014). *Global status report on noncommunicable diseases 2014*: World Health Organization.

Wilde, P. J. (2009). Eating for life: designing foods for appetite control. *J Diabetes Sci Technol, 3*(2), 366-370. doi: 10.1177/193229680900300219

Wolever, T. M., & Jenkins, D. J. (1986). The use of the glycemic index in predicting the blood glucose response to mixed meals. *The American Journal of Clinical Nutrition, 43*(1), 167-172.

Wolever, T. M. S., & Bolognesi, C. (1996). Source and amount of carbohydrate affect postprandial glucose and insulin in normal subjects. *Journal of Nutrition, 126*(11), 2798-2806.

Woolnough, J. W., Bird, A. R., Monro, J. A., & Brennan, C. S. (2010). The Effect of a Brief Salivary α-Amylase Exposure During Chewing on Subsequent in Vitro Starch Digestion Curve Profiles. *International Journal of Molecular Sciences, 11*(8), 2780-2790. doi: 10.3390/ijms11082780

Wu, Y., Lin, Q., Chen, Z., Wu, W., & Xiao, H. (2012). Fractal analysis of the retrogradation of rice starch by digital image processing. *Journal of Food Engineering, 109*(1), 182-187. doi: https://doi.org/10.1016/j.jfoodeng.2011.10.006

Wursch, P., Del Vedovo, S., & Koellreutter, B. (1986). Cell structure and starch nature as key determinants of the digestion rate of starch in legume. *Am J Clin Nutr, 43*(1), 25-29.

Zavareze, E. d. R., & Dias, A. R. G. (2011). Impact of heat-moisture treatment and annealing in starches: A review. *Carbohydrate Polymers, 83*(2), 317-328. doi: 10.1016/j.carbpol.2010.08.064

Zhang, & Hamaker, B. R. (2009). Slowly Digestible Starch: Concept, Mechanism, and Proposed Extended Glycemic Index. *Critical Reviews in Food Science and Nutrition, 49*(10), 852-867. doi: 10.1080/10408390903372466

Zhang, G., Ao, Z., & Hamaker, B. R. (2006). Slow Digestion Property of Native Cereal Starches. *Biomacromolecules, 7*(11), 3252-3258. doi: 10.1021/bm060342i

Zhang, G., Sofyan, M., & Hamaker, B. R. (2008). Slowly digestible state of starch: mechanism of slow digestion property of gelatinized maize starch. *Journal of Agricultural and Food Chemistry, 56*(12), 4695-4702. doi: 10.1021/jf072823e

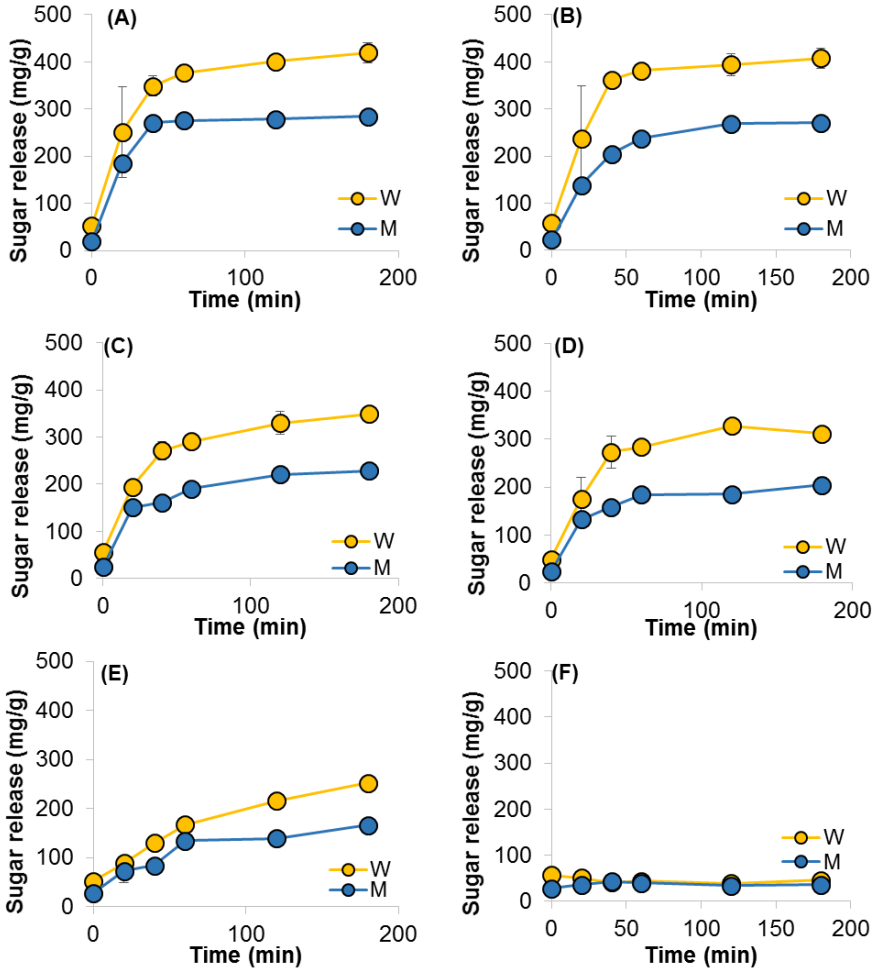
Zhang, G., Venkatachalam, M., & Hamaker, B. R. (2006). Structural basis for the slow digestion property of native cereal starches. *Biomacromolecules, 7*(11), 3259-3266. doi: 10.1021/bm060343a

Zhu, F., & Wang, Y.-J. (2012). Rheological and thermal properties of rice starch and rutin mixtures. *Food research international, 49*(2), 757-762.

Appendix

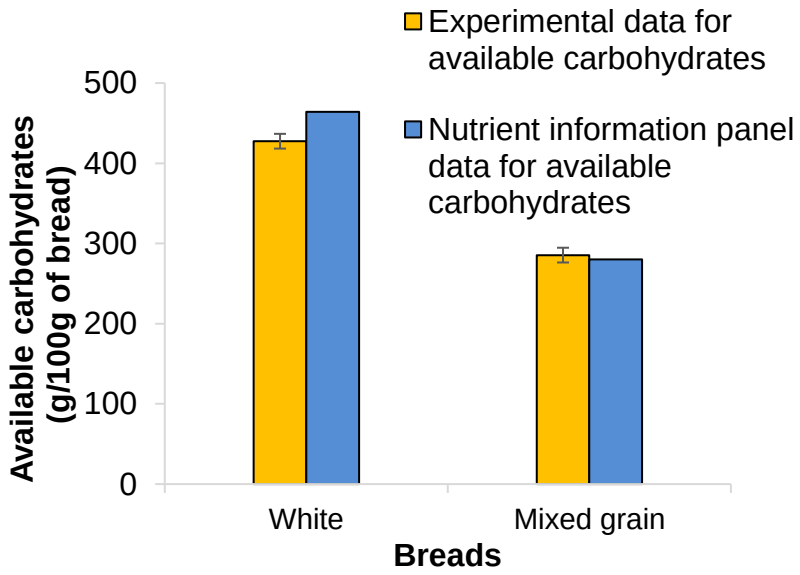
Appendices

A. Study on the optimal pancreatic concentration



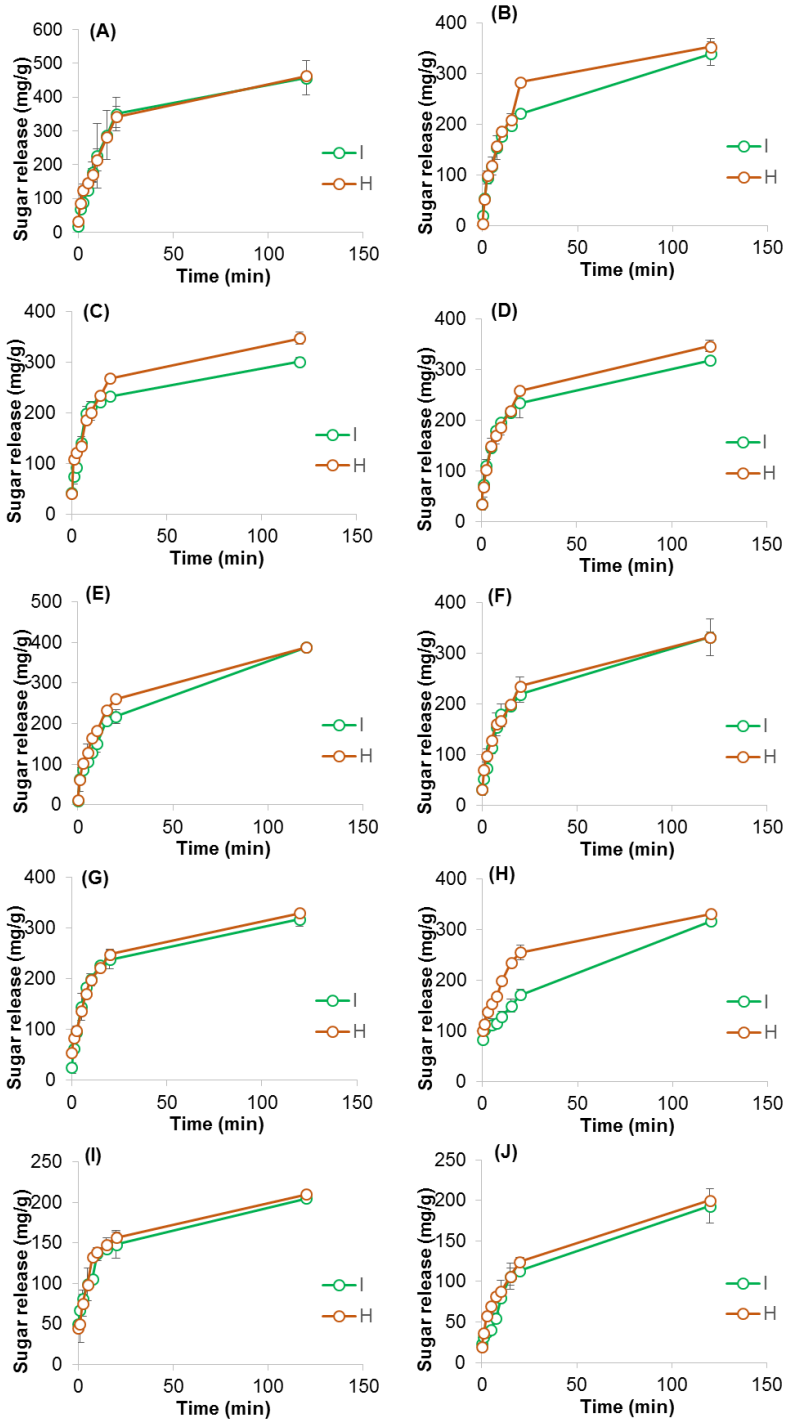
In-vitro digestive profile for white (W) and mixed grain (M) breads digested with different volumes of 5% pancreatin (A) 1 ml (B) 0.5 ml (C) 0.1 ml (D) 0.05 ml (E) 0.01 ml (F) 0 ml. Points are the average of 2 samples; intervals are (±) SD.

B. Precision of various amounts of 5% pancreatin used to digested white and mixed grain breads



Precision of experimentally determined Av. CHO in white and mixed grain breads digested by 1.0 ml of 5% pancreatin in comparison to the Av. CHO provided in the nutrient information panel. Points are the average of 2 samples; intervals are (±) SD.

C. Intrinsic initial starch digestibility in the NZ breads



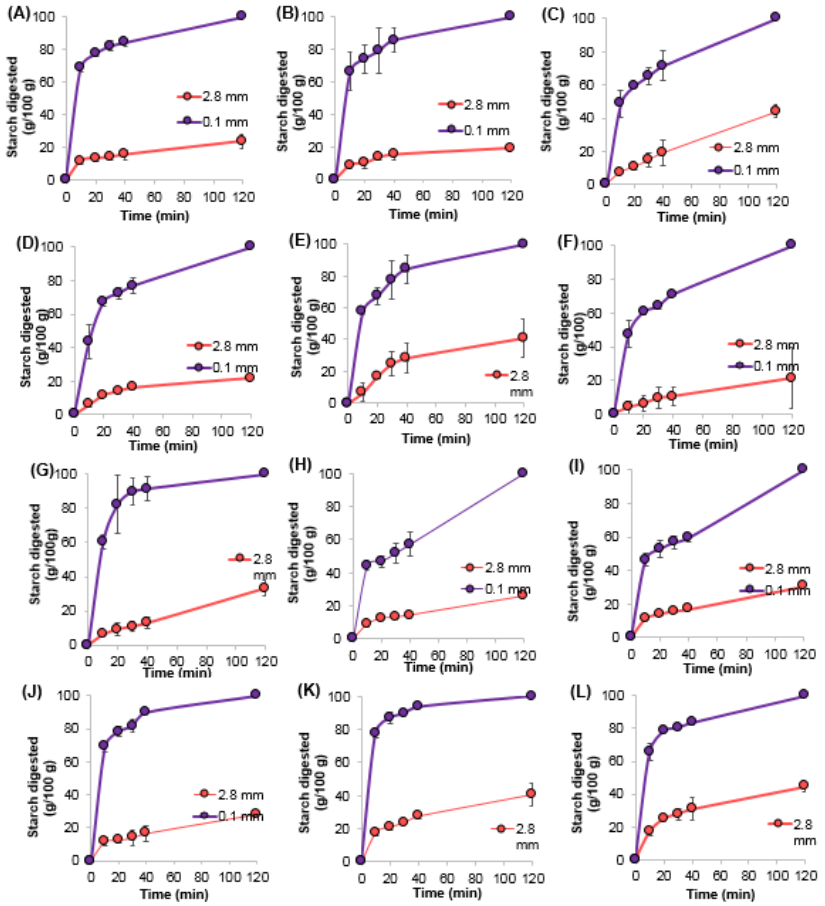
In-vitro digestive profile for A (White), B (Whole meal), C (Light grains), D (Super thick), E (Soy and linseed), F (Original mixed grain), G (Grain plus), H (Rye bread), I (Whole meal/gluten free), J (Soy and linseed/Low carb) breads digested with 0.1 ml of 5% pancreatin measure as intact ("I") and after homogenised ("H"). To measure the intrinsic starch digestion, high frequency initial sampling was performed. Points are the average of 2 samples; intervals are (±) SD.

D. Chemical composition of cereals

Chemical composition

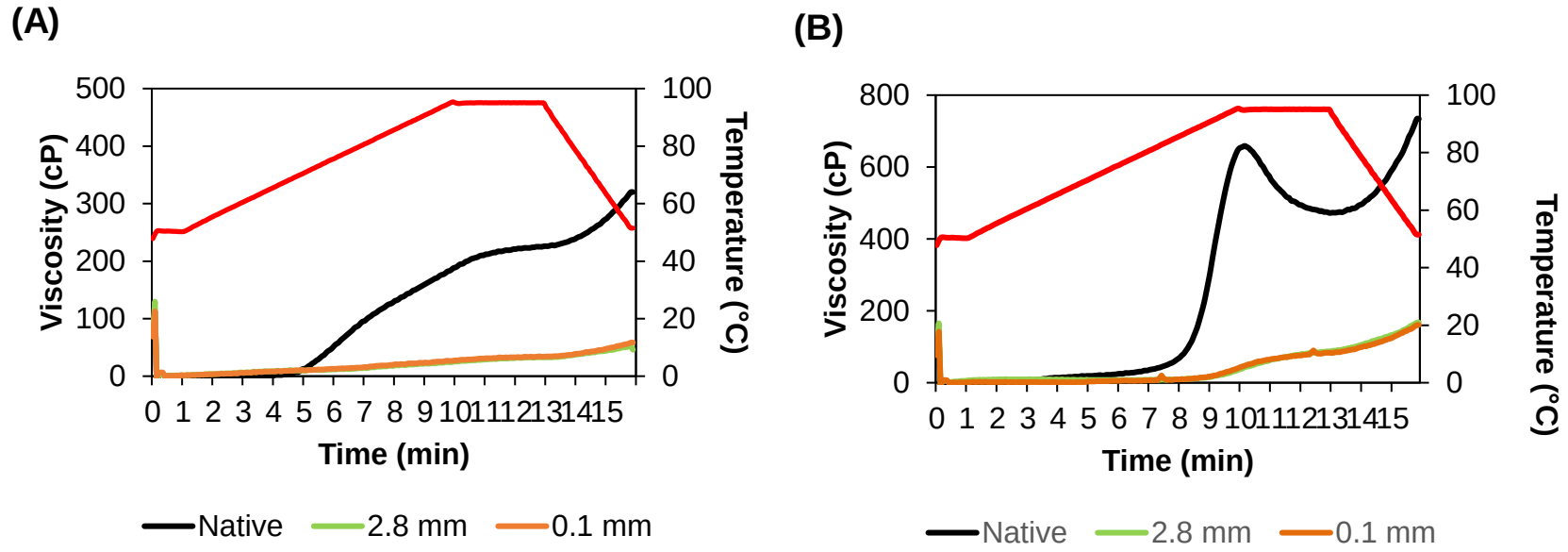
g/100 g of sample											
	Carbohydrates							Protein	Fat	Ash	Dry Matter
	By difference	By *in-vitro* digestion	Starch	Sugar (T=0)	Total fibre	Insoluble fibre	Soluble fibre				
Broad beans	63.10	45.56	37.76	7.80	7.90	4.00	3.90	22.60	2.90	1.20	89.80
Peas	66.10	50.97	40.78	10.19	8.72	6.24	2.48	20.50	2.80	1.30	90.70
Rice	77.70	51.80	42.22	9.57	5.10	3.66	1.43	7.50	1.20	2.20	88.60
Rye	76.50	59.93	49.36	10.57	13.14	10.24	2.90	11.10	1.50	0.90	90.00
Soy	30.40	17.42	4.79	12.63	11.80	7.38	4.43	35.80	4.90	21.60	92.70
Streaker barley	75.50	56.83	37.93	18.90	18.17	14.09	4.08	11.10	1.60	2.40	90.60
Bagherra barley	75.80	56.17	26.50	29.67	14.83	7.71	7.12	10.60	1.70	1.70	89.80
Reliance wheat	73.70	62.03	51.13	10.90	6.57	6.21	0.36	14.10	1.40	1.30	90.50
Empress wheat	75.60	73.01	64.26	8.75	4.26	3.52	0.74	10.60	1.50	1.50	89.20
Amethyst wheat	75.50	52.74	41.77	10.98	5.87	4.43	1.44	11.30	1.50	1.30	89.60
Tyrian wheat	73.30	47.17	36.07	11.10	7.83	5.45	2.38	12.50	1.50	1.80	89.10
Corn	80.40	64.42	55.18	9.24	3.79	1.64	2.15	7.10	0.30	0.50	88.30

E. Starch digestibility of twelve grains hydrated and cooked in excess water



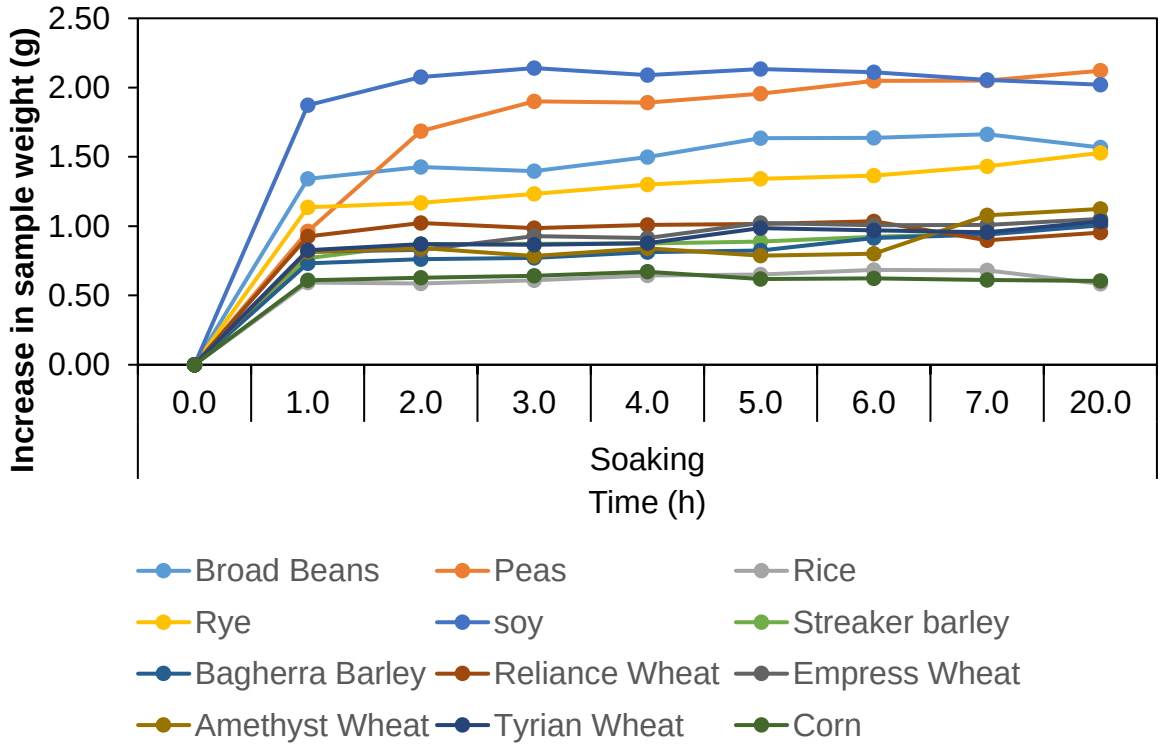
In-vitro digestion profile expressed between starch digested (g/100 g of starch) and time (0–120 min of pancreatic digestion) for different cereals with extreme particle sizes (A) Broad beans, (B) Peas, (C) Rice. (D) Rye, (E) Soy, (F) Streaker barley (G) Bagherra barley (H) Reliance wheat (I) Empress wheat (J) Amethyst wheat (K) Tyrian wheat and (L) Corn.

F. Viscograms of pressure cooked milled particles



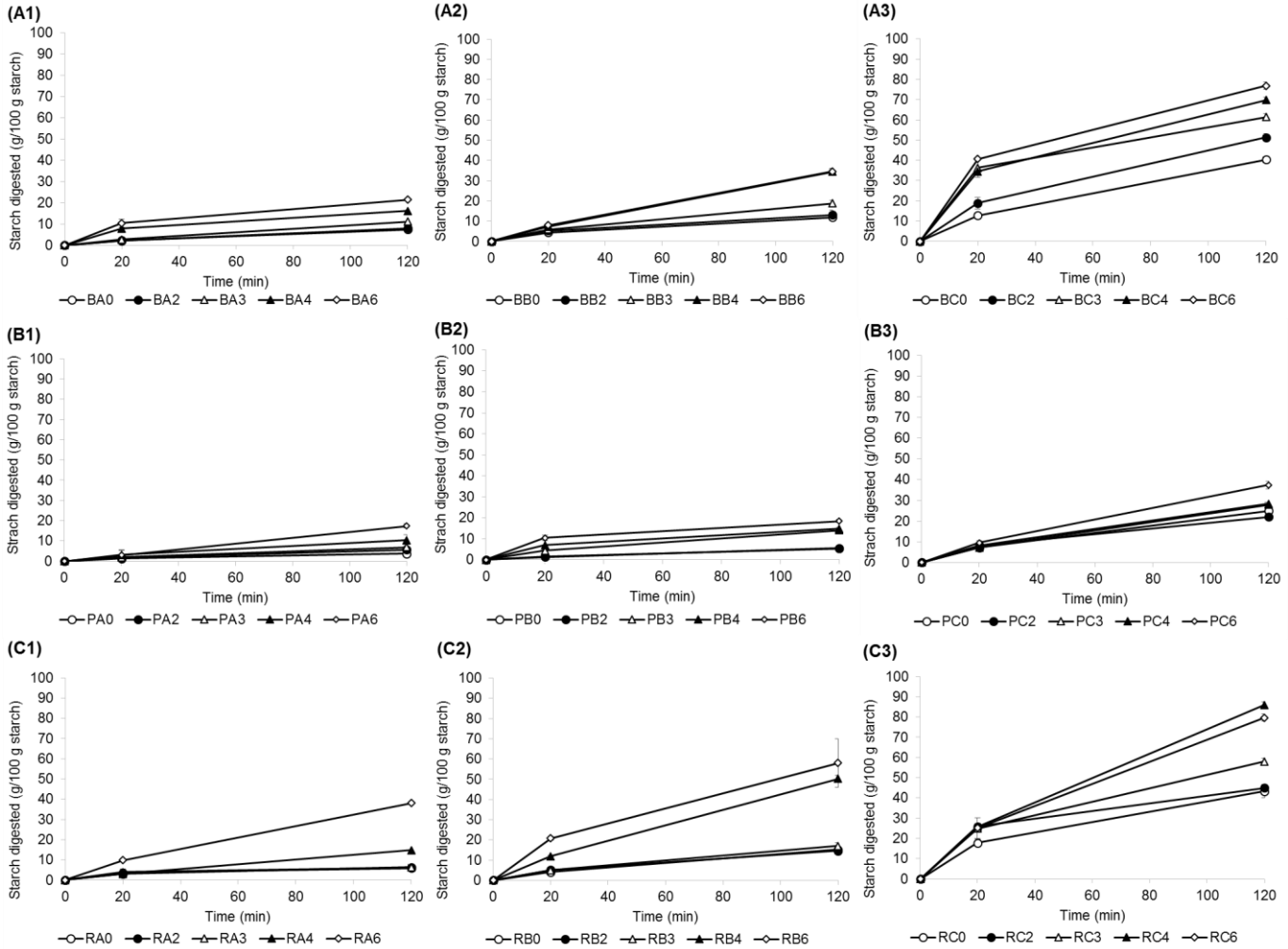
Viscograms for pressure cooked milled particles (PC) of (A) Peas and (B) Wheat > 0.1 mm, >1.2 mm and >2.8 mm.

G. Soaking times

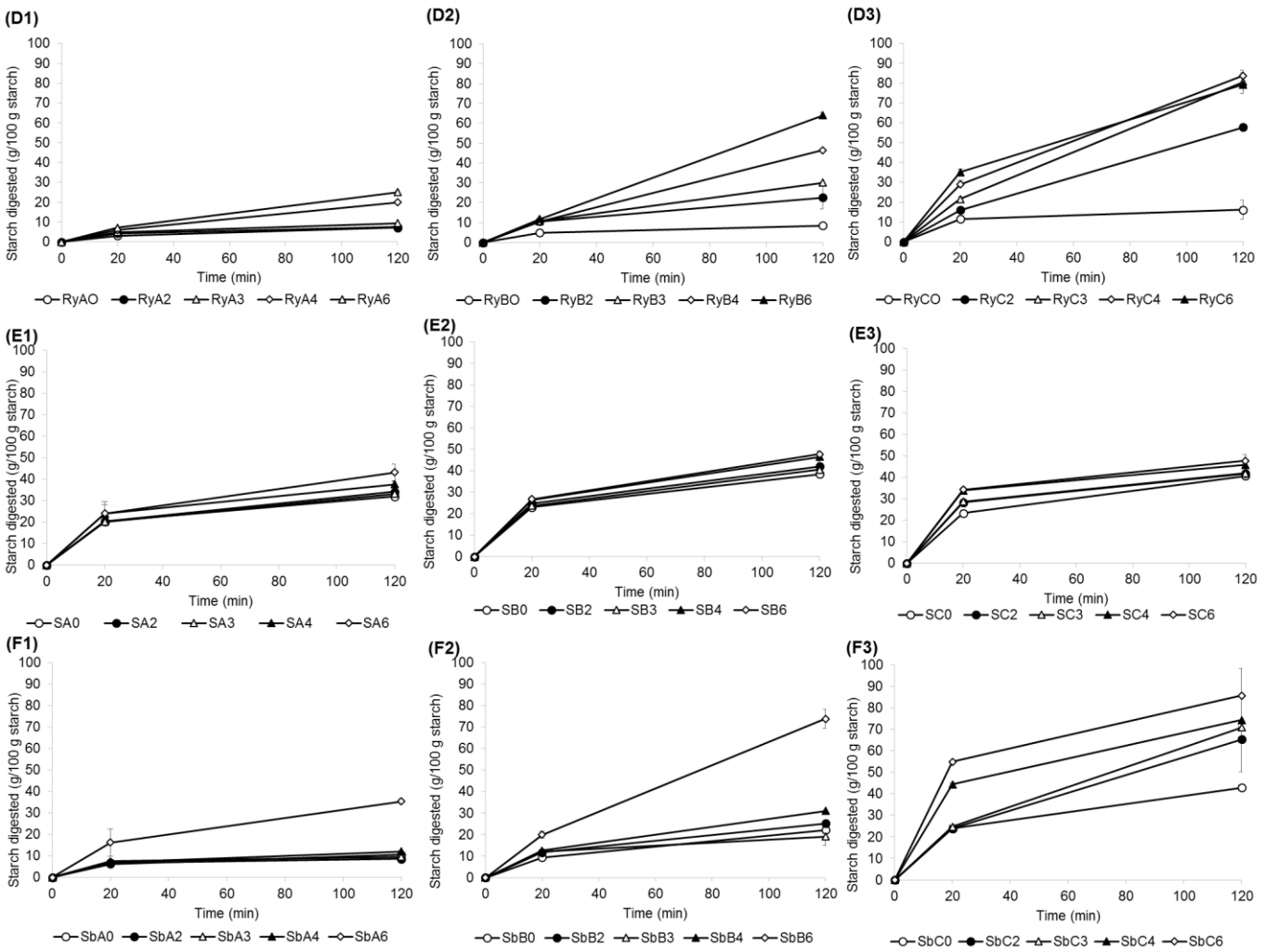


Soaking profiles of > 2.8 mm particles *of* (A) Broad beans, (B) Peas, (C) Rice. (D) Rye, (E) Soy, (F) Streaker barley (G) Bagherra barley (H) Reliance wheat (I) Empress wheat (J) Amethyst wheat (K) Tyrian wheat and (L) Corn.

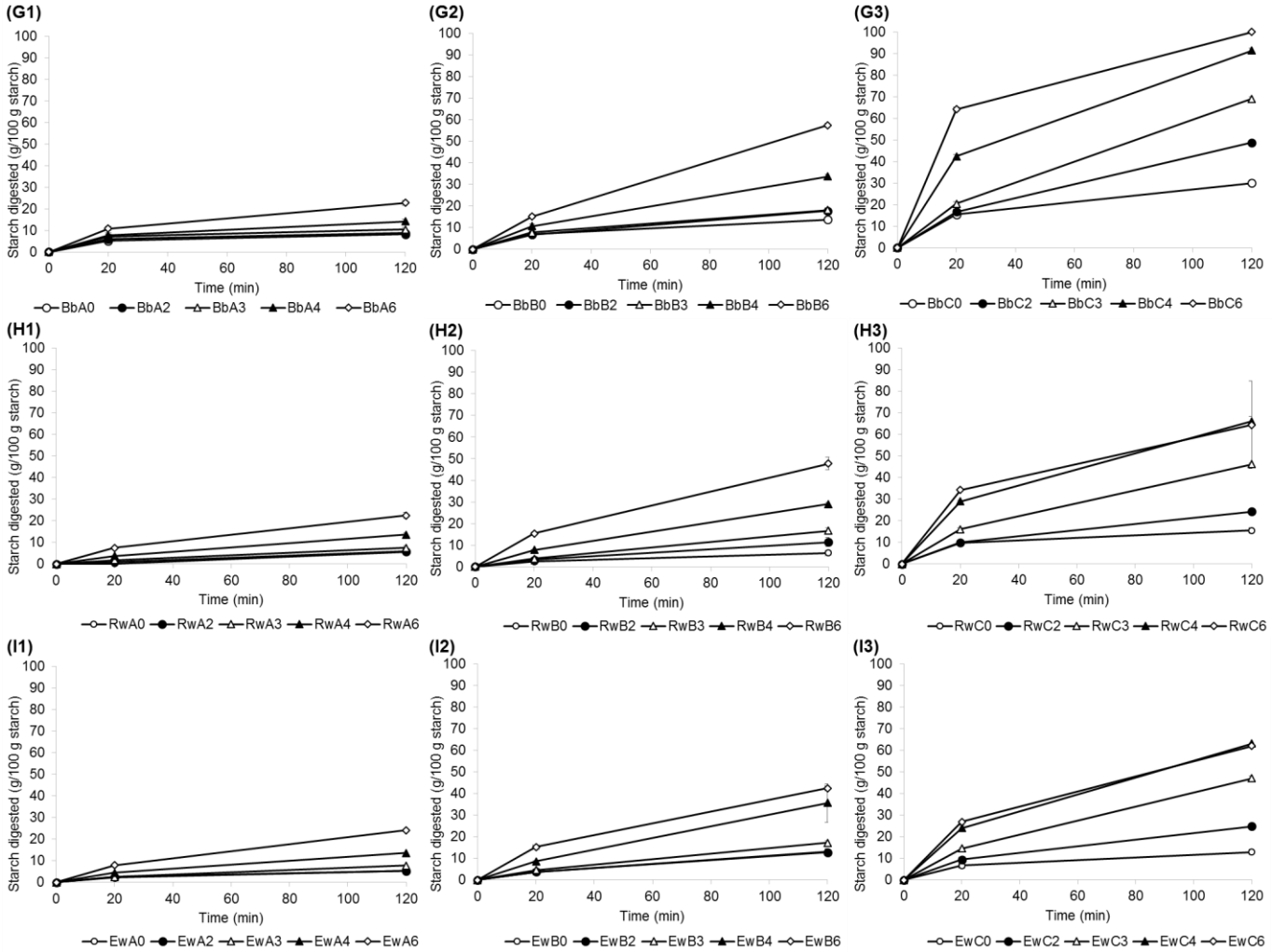
H. Starch digestibility of twelve different grains hydrated and cooked in various degrees of hydration



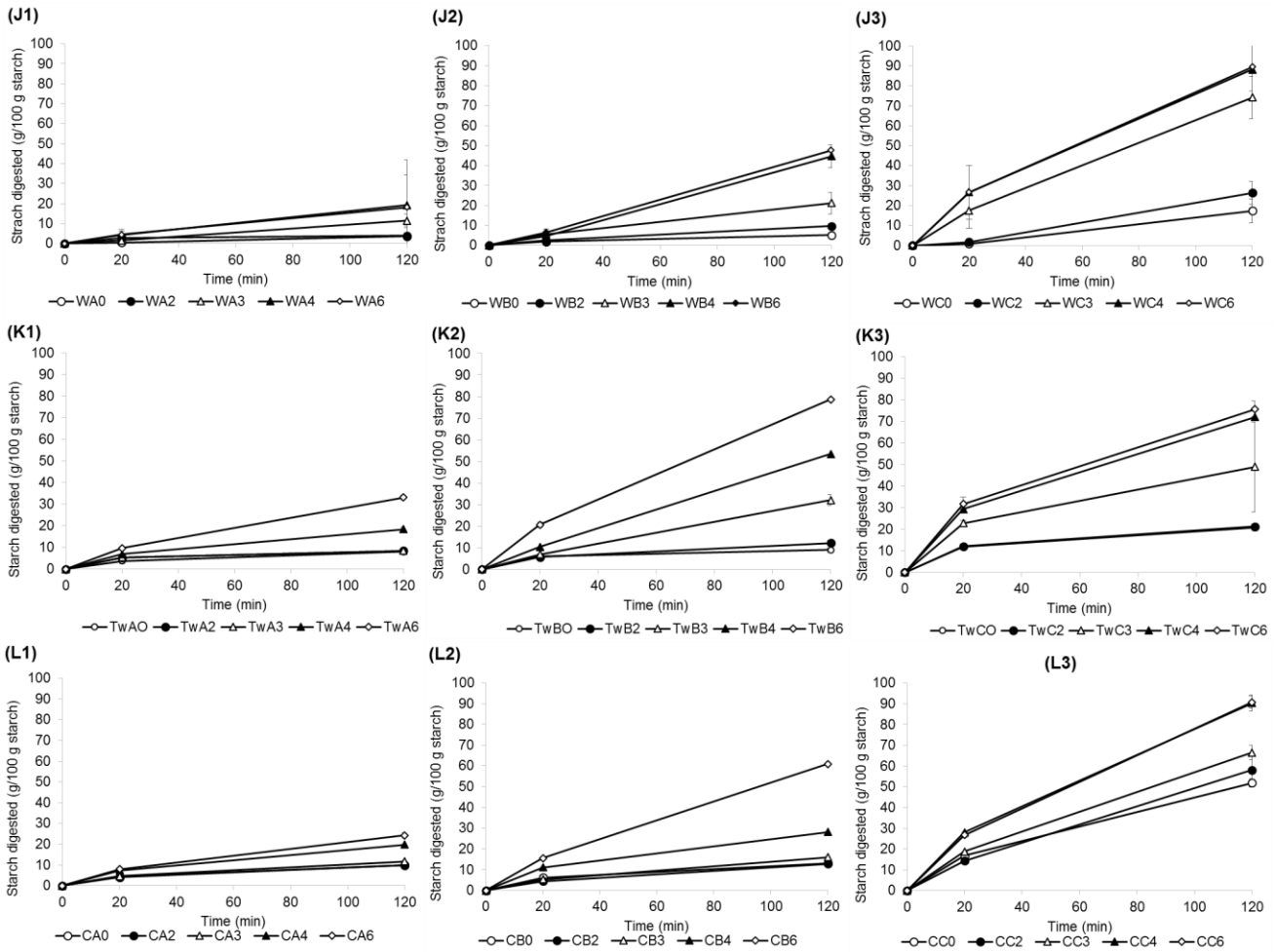
In-vitro digestion profile expressed between starch digested (g/100 g of starch) and time (0–120 min of simulated ileal digestion) for different cereals (A) Broad beans, (B) Peas, (C) Rice with different particle sizes 2.8 mm (A1-C1), 1.2 mm (A2-C2) and 0.1 mm (A3-C3) at various degree of hydration 0% (0) , 20% (2), 30% (3), 40% (4) and 60% (6).



In-vitro digestion profile expressed between starch digested (g/100 g of starch) and time (0–120 min of pancreatic digestion) for different cereals (D) Rye, (E) Soy, (F) Streaker barley (G) Bagherra barley (H) Reliance wheat (I) Empress wheat (J) Amethyst wheat (K) Tyrian wheat and (L) Corn with different particle sizes 2.8 mm (D1-F1), 1.2 mm (D2-F2) and 0.1 mm (D3-F3) at various degree of hydration 0% (0) , 20% (2), 30% (3), 40% (4) and 60% (6).



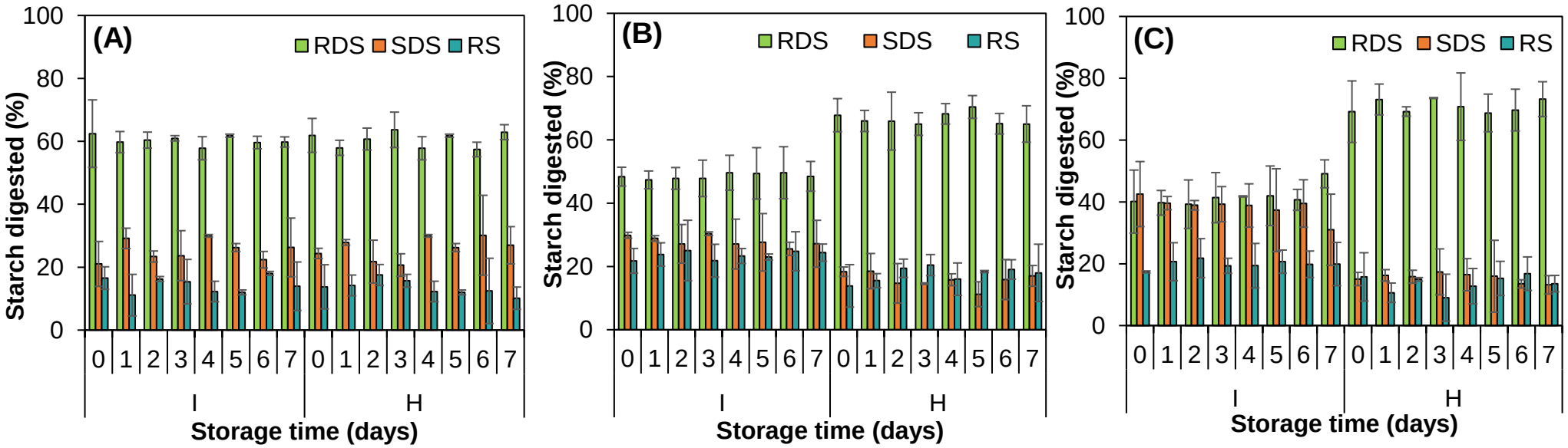
In-vitro digestion profile expressed between starch digested (g/100 g of starch) and time (0–120 min of pancreatic digestion) for different cereals (G) Bagherra barley (H) Reliance wheat (I) Empress wheat (J) Amethyst wheat (K) Tyrian wheat and (L) Corn with different particle sizes 2.8 mm (G1-I1), 1.2 mm (G2-I2) and 0.1 mm (G3-I3) at various degree of hydration 0% (0) , 20% (2), 30% (3), 40% (4) and 60% (6).



In-vitro digestion profile expressed between starch digested (g/100 g of starch) and time (0–120 min of pancreatic digestion) for different cereals (J) Amethyst wheat (K) Tyrian wheat and (L) Corn with different particle sizes 2.8 mm (J1-L1), 1.2 mm (J2-L2) and 0.1 mm (J3-L3) at various degree of hydration 0% (0) , 20% (2), 30% (3), 40% (4) and 60% (6).

I. Effect of freezing on the starch digestibility of white and kibbled grain breads.

Starch digestibility analysis was performed to breads that were stored at -20°C for seven days. After baking, the bread loaves were cooled, sliced, packed and stored in labelled airtight Ziplock bags. Before starch analysis, the breads were thawed overnight for 14 h and were subjected to *in-vitro* amylolysis as mentioned in **Section 7.2.6.** Breads that were tested on the day of baking with freezing were used as a control/reference. RDS, SDS and RS were measured to study the effects of freezing breads in the starch digestibility.



Proportion of starch particles RDS, SDS, RS to total extend of starch digested at 120 mins for (A) White bread and (B) Kibbled purple wheat bread (C) Kibbled soy- purple wheat bread during freezing storage for 7 days digested as intact ("I") and after homogenized ("H"). Values are means of duplicate ± SD. Results with no letter in the data label do not differ significantly.

J. Health and Disability Ethics Committees Approval

Health and Disability Ethics Committees

Ministry of Health
133 Molesworth Street
PO Box 5013
Wellington 6011
0800 4 ETHICS
hdecs@moh.govt.nz

17 October 2018

Dr John Monro
Plant and Food
Research Food
Industry Science
Centre Private Bag
116000
Palmerston North 4474
Dear Dr Monro

Re:	**Ethics ref:**	**18/NTA/160**
	Study title:	The role of kibbled grains in breads for blood glucose control - A randomised, repeated measures, human intervention study.

I am pleased to advise that this application has been *approved* by the Northern A Health and Disability Ethics Committee. This decision was made through the HDEC-Expedited Review pathway.

Conditions of HDEC approval

HDEC approval for this study is subject to the following conditions being met prior to the commencement of the study in New Zealand. It is your responsibility, and that of the study's sponsor, to ensure that these conditions are met. No further review by the Northern A Health and Disability Ethics Committee is required.

Standard conditions:

1. Before the study commences at *any* locality in New Zealand, all relevant regulatory approvals must be obtained.

2. Before the study commences at *any* locality in New Zealand, it must be registered in a clinical trials registry. This should be a WHO-approved registry (such as the Australia New Zealand Clinical Trials Registry, www.anzctr.org.au) or https://clinicaltrials.gov/.

3. Before the study commences at *each given* locality in New Zealand, it must be authorised by that locality in Online Forms. Locality authorisation confirms that the locality is suitable for the safe and effective conduct of the study, and that local research governance issues have been addressed.

Non-standard conditions:

4. In the Consent Form, please only keep yes/no tick boxes next to the statements that are truly optional (ie, if participant ticks no, they can still take part in the study).
5. Please consider that colleagues may feel pressure to take part. Please ensure they are provided sufficient time to consider taking part.

Non-standard conditions must be completed before commencing your study, however, they do not need to be submitted to or reviewed by HDEC.

If you would like an acknowledgement of completion of your non -standard conditions you may submit a post approval form amendment through Online Forms. Please clearly identify in the amendment form that the changes relate to non-standard conditions and ensure that supporting documents (if requested) are tracked/highlighted with changes.

For information on non-standard conditions please see section 128 and 129 of the *Standard Operating Procedures for Health and Disability Ethics Committees* (available on www.ethics.health.govt.nz)

After HDEC review

Please refer to the *Standard Operating Procedures for Health and Disability Ethics Committees* (available on www.ethics.health.govt.nz) for HDEC requirements relating to amendments and other post-approval processes.

Your next progress report is due by 16 October 2019.

Participant access to ACC

The Northern A Health and Disability Ethics Committee is satisfied that your study is not a clinical trial that is to be conducted principally for the benefit of the manufacturer or distributor of the medicine or item being trialled. Participants injur ed as a result of treatment received as part of your study may therefore be eligible for publicly-funded compensation through the Accident Compensation Corporation (ACC).

Please don't hesitate to contact the HDEC secretariat for further information. We wish you all the best for your study.

Yours sincerely,

Dr Brian Fergus Chairperson
Northern A Health and Disability Ethics Committee

K. Australian New Zealand Clinical Trials Registry.

DATA SHARING STATEMENT

The role of kibbled grains in breads for blood glucose control - A randomised, repeated measures, human intervention study.

Registration number:	ACTRN12618001826235
Date registered:	9/11/2018
Date this registration last updated:	9/11/2018
Type of registration:	Retrospectively registered
Date this document generated:	10/04/2019

Will individual participant data be available?	IPD is not available
Reason	No material that could personally identify the participant will be used in any reports on this study. All information collected in all parts of the study is confidential and will not be available to anyone other than the principal investigator and the co-investigator. The samples and data will be identified by study ID number only and will be stored in a lockable filing cabinet on a security-carded floor.
What additional, related documents will be available?	No other documents available

L. Participant information sheet

Study title:	**The glycaemic potency of kibbled grain breads** *(The effect of kibbled grain breads on blood glucose concentration)*	
Locality:	**Plant and Food Research, Palmerston North**	Ethics committee ref.:
Lead investigator:	**Dr John Monro**	Contact phone number: 06 3556137

You are invited to consider taking part in a study on the effect of kibbled grain breads on blood glucose. If you do want to take part now, but change your mind later, you can pull out of the study at any time.

This Participant Information Sheet will help you decide if you'd like to take part. It sets out why we are doing the study, what your participation would involve, what the benefits and risks to you might be, and what would happen after the study ends. We will go through this information with you and answer any questions you may have. You do not have to decide today whether or not you will participate in this study. Before you decide you may want to talk about the study with other people, such as family, whānau, friends, or healthcare providers. Feel free to do this.

If you agree to take part in this study, you will be asked to sign a Consent Form, which is a record that you joined the study voluntarily, but it is not binding. You will be given a copy of both the Participant Information Sheet and the Consent Form to keep. This document is five pages long. Please make sure you have read and understood all the pages.

WHAT IS THE PURPOSE OF THE STUDY?

Our research has shown that a serving of kibbled grain bread may have a small effect on blood glucose concentrations, compared with a serving of white bread. It is therefore possible that substituting kibbled grain breads for white breads in a meal, without altering the total available carbohydrate intake, may lower the blood glucose response to the meal. This may be beneficial for people with impaired glucose tolerance, such as those with Type 2 diabetes. This study will compare the effect of intact grain structure in kibbled grain breads on blood glucose response compared with standard white bread.

WHAT DOES THE STUDY INVOLVE?

The study is a randomized repeated measures design which means you will be asked to consume nine test foods, in a particular way, one per testing session, in random order.

The test foods will be as follows:

Standard chewed (C)

4. White bread (Reference): 105 g plus 188 ml of water
5. Purple wheat kibbled bread: 126 g plus 171 ml of water
6. Soy-purple wheat kibbled bread: 163 g plus 150 ml of water

Eaten as usual

Chewed

Unchewed (U)

4. White bread (reference): 105 g plus 188 ml 9of water
5. Purple wheat kibbled bread: 126 g plus 171 ml of water
6. Soy- purple wheat kibbled bread: 163 g plus 150 ml of water

Unchewed

Homogenized (H)

4. White bread (reference): 105 g homogenized with 188 ml of water
5. Purple wheat kibbled bread: 126 g homogenized with 171 ml of water
6. Soy-purple wheat kibbled bread: 163 g homogenized with 150 ml of water

Homogenized

An additional 200 ml of water will be consumed with all diets.Therefore you will need to be available for nine testing sessions, which will be separated by 3 days to a week. Individuals will be chosen to participate in the study if they are within the age bracket (18-65) and considered to be generally healthy. Health status will be determined initially by the use of a health questionnaire, which asks about your current and past health issues.

During the study, you will be required to refrain from eating after 9 pm, and avoid any strenuous physical activity on the night preceding testing. We ask that you consume a similar evening meal before each of the 9 test days. In the morning you will be asked to rest for 15 mins before 2 baseline blood samples (by finger prick) are taken. You will then be given one of the nine test foods to consume in a required way within 10 mins and small blood samples will be taken at 0 (2 x baselines), 15, 30, 45, 60, 90 and 120 min after consuming the food. Blood sampling involves the use of a finger prick device which allows a small bead of blood to be massaged from the finger and used to monitor your blood glucose levels over the two hours after consuming the food. During the two hours you will be able to continue with your normal work if it is not strenuous (e.g., office work), and drink a set amount of water if thirsty but consume no food until after the final blood test. Otherwise, you will be asked to rest and do some non-strenuous activity such as reading. You will be asked to fill out a visual analogue scale to assess your hunger at 0, 30, 60, 90 and 120 mins. An example of the scale is attached along with this information sheet for your information. Lunch will be provided free after the testing has been completed.

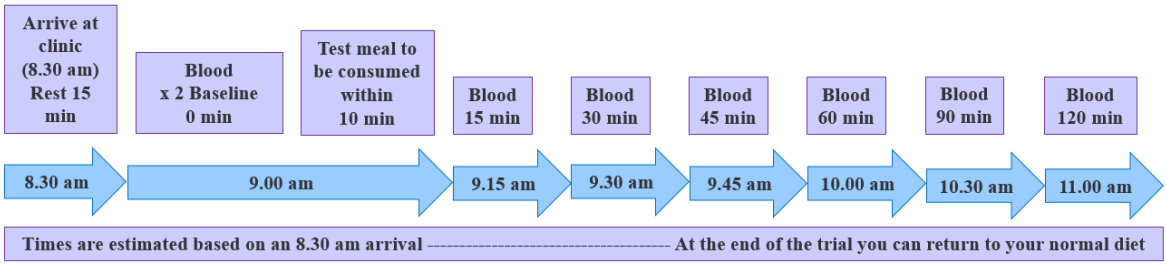
If you agree to take part in the study, we would like you to be available once or twice (Between Mondays - Fridays) a week, over seven weeks, with each visit lasting a maximum of three hours. A follow-up meeting will also be included to feedback the results of the study and answer any questions.

Participants: Men and women will be suitable for the study. The age range is 18 to 65 years and the BMI range is 18 to 35 kg/m^2 (BMI is the abbreviated term of body mass index which is used to estimate a healthy weight range for individuals based on weight and height. BMI is determined by your weight in kilograms divided by your height (in meters squared).

All participants will need to:

- Have no known allergy to, or intolerance of, glucose, bread, soy or wheat.
- Have a BMI between 18 and 35
- Not suffer from diabetes or prediabetes.
- Be healthy, as confirmed by a short medical questionnaire
- Be willing to fast from 9.00 pm the evening before a test.
- Be willing to only consume breakfast supplied and water until the last blood sample of the trial day.

Trial day (fasted from 9 pm the previous day)



The research in this project will be undertaken in a culturally sensitive manner with all aspects of the trial explained in full to you in a manner most suitable to you. We will be available to answer questions throughout the study and will seek advice from appropriate advisory groups should it be necessary. You will be given access to interpreters at any time in the study should you require them. The opportunity for whanau support is available at all times.

WHAT DO WE EXPECT FROM YOU?

Screening: If you agree to take part in this study, we will make an initial appointment for you to come into Plant & Food Research in Palmerston North. At this appointment, we will measure your height and weight and ask you some questions about your general health. This is so we can assess if you are eligible to participate in the study. If you are eligible we will ask you to have a quick blood glucose test to check that your blood glucose is in the normal range by measuring glycated haemoglobin (HbA1c). The test involves taking a small finger prick sample of blood and testing it, as is done by people with diabetes at home.

POSSIBLE BENEFITS AND RISKS FROM THIS STUDY

This study focuses on the effects of natural commonly consumed foods on blood glucose levels and does not use any pharmaceutical products, so the chance of risks and side-effects is minimal. Due to the initial exclusion of subjects with a history of breads, soy, wheat or glucose intolerance, it is unlikely that there will be any adverse reaction to the foods. If you think you are intolerant of glucose, wheat, soy or of bread products you would be advised not to take part in the study. You may experience a small discomfort from the finger prick to draw blood; however, the finger prick device is used daily by many people with diabetes. If any discomfort occurs, you will be given the opportunity to take a break, or if necessary stop participation in the study. You may feel hungry on trial days as some test meals may be less than you would normally consume for breakfast. However, you will be able to consume as much as you wish

for the lunch provided at the end of the 3 hours test period. A benefit from the study is that participants will receive feedback and gain some understanding of their own individual responsiveness to different types of carbohydrate foods. Published results from the study may be very useful for people with diabetes who wish to consume kibbled grain breads while managing blood glucose responses. They may also help in the education of consumers generally, on how best to incorporate kibbled grain breads into the diet for health benefits.

YOUR PARTICIPATION

Your participation in this study is completely voluntary. We are happy for you to bring along a support person to each of the clinic appointments if you would like. We will give you a $20 supermarket voucher for the initial screening visit and if you are accepted into the study you will receive a further $20 voucher each time you participate in a test to partially compensate you for your travel and time. This will make a total of **$200**. In addition, you will be able to select from a choice of lunches at the end of each session. If you decide to take part, but later change your mind, you are free to withdraw at any time without having to give a reason.

COMPENSATION

If you were injured as a result of treatment given as part of this study, which is unlikely, you **won't** be eligible for compensation from ACC. However, compensation would be available from the study's sponsor Plant & Food Research in line with industry guidelines. We can give you a copy of these guidelines if you wish. You would be able to take action through the courts if you disagreed with the amount of compensation provided.

This trial is being conducted by Plant & Food Research. If participants suffer physical harm from the breads being tested, liability for compensation is borne by Plant & Food Research. If participants suffer any harm from any other aspects of the trial, for example from blood sampling by the researchers (very unlikely), liability is borne by Plant & Food Research, in both instances subject to the appropriate application or legal processes. If you have private health or life insurance, you may wish to check with your insurer that taking part in this study won't affect your cover. You may obtain further information concerning medical treatment for injuries by contacting the Principal Investigator.

CONFIDENTIALITY

No material that could personally identify you will be used in any reports on this study. A code that identifies you to the research team will be used on all study documentation. During the

study, your file will be held in a locked cupboard or filing cabinet when not in use. At the end of the study, your files will be kept for 10 years in secure document storage, and then destroyed by shredding.

RIGHTS

Participation in this study is completely voluntary, and you have the right to decline to participate or to withdraw from the research at any stage, without the need to give a reason and also without experiencing any disadvantage. As some personal information is obtained from you during this study, you have the right to access the information that we have collected about you at any stage. You will also be informed of your own blood glucose readings as soon as they become available; we would tell you if there was the reason for concern. Information obtained, from the questionnaire and blood samples, will be kept completely confidential at all times. Only the investigators of the study will have access to these records.

If any new information arises about adverse or beneficial effects related to this study that may have an impact on health, then you will be informed straight away. If you have any queries or concerns about your rights as a participant in this research study you can contact an independent health and disability advocate. This is a free service provided under the Health and Disability Commissioner Act.

Telephone (NZ wide): 0800 555 050

Free Fax (NZ wide): 0800 2787 7678 (0800 2 SUPPORT)

Email (NZ wide): advocacy@hdc.org.nz

This study has been given approval by the New Zealand Health Ethics Committee.

CONTACT DETAILS

If you have any questions about the study at any time please do not hesitate to call:

Akila Srinivasagam (Trial coordinator) Contact numbers: 021 0491606

Dr John Monro (Principal investigator) Contact number: (06) 355 6137

Dr Suman Mishra (Co-Investigator) Contact number: (06) 3556146

M. Statement of contribution

DRC 16

MASSEY UNIVERSITY
GRADUATE RESEARCH SCHOOL

STATEMENT OF CONTRIBUTION

DOCTORATE WITH PUBLICATIONS/MANUSCRIPTS

We, the candidate and the candidate's Primary Supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the candidate's contribution as indicated below in the *Statement of Originality*.

Name of candidate:	Akila SRV
Name/title of Primary Supervisor:	Dr. Lara Matia-Merino
Name of Research Output and full reference:	
Srv, A., Mishra, S., Hardacre, A., Matia-Merino, L., Goh, K., Warren, F., & Monro, J. (2019). Kernel structure in breads reduces *in vitro* starch digestion rate and estimated glycaemic potency only at high grain inclusion rates. Food Structure, 100109.	
In which Chapter is the Manuscript /Published work:	Chapter 4
Please indicate:	
• The percentage of the manuscript/Published Work that was contributed by the candidate:	75%
and	
• Describe the contribution that the candidate has made to the Manuscript/Published Work:	
Carried out all the experimental work, data analysis, graph production and drafting of the first draft	
For manuscripts intended for publication please indicate target journal:	
Candidate's Signature:	Akila SRV
Date:	SRV Akila
Primary Supervisor's Signature:	
Date:	21/01/2020

MASSEY UNIVERSITY
GRADUATE RESEARCH SCHOOL

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Name of candidate:	Akila SRV
Name/title of Primary Supervisor:	Dr. Lara Matia-Merino
Name of Research Output and full reference:	
Srv, A., Mishra, S., Hardacre, A., Matia-Merino, L., Goh, K., Warren, F., & Monro, J. (2019). The effects of chewing on kibbled-grain breads- An in vivo study.	
In which Chapter is the Manuscript /Published work:	Chapter 8
Please indicate:	
• The percentage of the manuscript/Published Work that was contributed by the candidate:	75%
and	
• Describe the contribution that the candidate has made to the Manuscript/Published Work:	
Carried out all the experimental work, data analysis, graph production and drafting of the first draft	
For manuscripts intended for publication please indicate target journal:	
British Journal of Nutrition	
Candidate's Signature:	Akila SRV
Date:	SRV Akila
Primary Supervisor's Signature:	
Date:	21/01/2020