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THE CHARACTERISTICS OF POTATOES DIFFERING IN GLYCEMIC INDEX

Kai Lin Ek

A thesis submitted in fulfilment of the requirement for the degree of Doctor of Philosophy (Food Science and Nutrition)

Faculty of Agriculture and Environment, The University of Sydney
January 2014
STATEMENT OF ORIGINALITY

This thesis is submitted to the University of Sydney in fulfilment of the requirement for the degree of Doctor of Philosophy.

The work presented in this thesis is, to the best of my knowledge and belief, original except as acknowledged in the text. I hereby declare that I have not submitted this material, either in full or in part for a degree at this or any other institution.

28th January 2014
To David, Matthew and Oliver
ACKNOWLEDGEMENTS

“DO NOT DESPISE THESE SMALL BEGINNINGS, FOR THE LORD REJOICES TO SEE THE WORK BEGIN”
Zechariah 4:10, The Holy Bible

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<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AM</td>
<td>Amylose</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
</tr>
<tr>
<td>AP</td>
<td>Amylopectin</td>
</tr>
<tr>
<td>APTS</td>
<td>3-aminopropyl-trimethoxysilane</td>
</tr>
<tr>
<td>BMI</td>
<td>Body mass index</td>
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<tr>
<td>BV</td>
<td>Breakdown viscosity</td>
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<tr>
<td>CAA</td>
<td>Carisma potato cultivar grown in Pinaroo</td>
</tr>
<tr>
<td>CAB90</td>
<td>Carisma potato cultivar grown in Bant (harvested after 90 days)</td>
</tr>
<tr>
<td>CAB120</td>
<td>Carisma potato cultivar grown in Bant (harvested after 120 days)</td>
</tr>
<tr>
<td>CFIA</td>
<td>Canadian food inspection agency</td>
</tr>
<tr>
<td>CIP</td>
<td>International potato centre</td>
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<tr>
<td>CLSM</td>
<td>Confocal laser scanning microscopy</td>
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<td>CV</td>
<td>Coefficient of variation</td>
</tr>
<tr>
<td>DMSO</td>
<td>Dimethyl sulphoxide</td>
</tr>
<tr>
<td>DP</td>
<td>Degree of polymerization</td>
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<tr>
<td>DSC</td>
<td>Differential scanning calorimetry</td>
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<td>ESEM</td>
<td>Environmental scanning electron microscopy</td>
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<tr>
<td>FITC</td>
<td>Fluorescein isothiocyanate</td>
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<tr>
<td>FV</td>
<td>Final viscosity</td>
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<td>GBSS</td>
<td>Granule-bound starch synthase</td>
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<td>GI</td>
<td>Glycemic index</td>
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<tr>
<td>GL</td>
<td>Glycemic load</td>
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<tr>
<td>GSA</td>
<td>Granule surface area</td>
</tr>
<tr>
<td>HI</td>
<td>Hydrolysis index</td>
</tr>
<tr>
<td>HPAEC-PAD</td>
<td>High Performance Anion Exchange Chromatography with Pulsed Amperometric Detection</td>
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HPLC  High performance liquid chromatography
IAUC  Incremental area under the curve
ICQC  International Carbohydrate Quality Consortium
ISO  International Standards Organization
K  Potassium
LM  Light microscopy
MES/TRIS  2(N-Morpholino) ethanesulfonic acid/tris(hydroxymethyl) aminomethane
N  Nitrogen
NIB90  Nicola potato cultivar grown in Bant (harvested after 90 days)
NIB120  Nicola potato cultivar grown in Bant (harvested after 120 days)
P  Phosphorus
PT  Pasting temperature
PV  Peak viscosity
RBB90  Russet Burbank potato cultivar grown in Bant (harvested after 90 days)
RBB120  Russet Burbank potato cultivar grown in Bant (harvested after 120 days)
RC  Relative crystallinity
RDS  Rapidly digestible starch
RS  Resistant starch
RVA  Rapid Visco Analyser
SB  Setback viscosity
SDS  Slowly digestible starch
TV  Trough viscosity
VR  Virginia Rose potato cultivar
XRD  X-ray diffraction


REFERENCE FORMATTING

Citations in the text and listing of references in this thesis are according to the Food Chemistry Journal published by Elsevier. Citations containing six or more authors in the text are abbreviated by the first author’s name followed by et al. and the year of publication. Groups of references are listed first alphabetically, then chronologically.
PRESENTATIONS AND AWARDS

15-20th September 2013 “Discovery Of A Low Glycemic Index Potato And Relation Between In Vitro Starch Digestion And Glycemic Response”. 20th International Congress of Nutrition (IUNS) conference, Granada, Spain.

31st August 2013 “3-minute thesis” competition, 1st runner-up, University of Sydney, Sydney, Australia.

22nd July 2013 “3 minute thesis” competition, winner of the academic and public choice awards, Faculty of Agriculture and Environment, Sydney Australia.

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8th July 2011 "A closer look into the potato myth” research Outreach award, Faculty of Agriculture and Environment research symposium, Sydney, Australia

10th June 2011, “Glycemic properties of potatoes”. Plant and Soil Science Microscopy Group meeting, Australian Centre for Microscopy and Microanalysis (ACMM), Sydney, Australia.
ABSTRACT

This thesis describes studies on seven potato cultivars with the objective of identifying a potato cultivar with a low glycemic index (GI), and to describe its tuber and starch properties. The potato cultivars were selected in consultation with potato breeders from Agrico Holland and sourced from growers in South Australia (The Mitolo Group) and Tasmania (Agronico) Australia and consisted of well established (Bintje, Desiree, Nicola, Russet Burbank) and newly introduced commercial cultivars (Carisma, Maiflower, Virginia Rose). The potato cultivars were tested for their GI according to International Standard Organisation (ISO) guidelines. In vitro enzymatic starch hydrolysis and chemical analyses were performed for each potato cultivar and correlations sought with the respective GI values. Different imaging techniques were used to study and compare cell structure and native starch granule morphology, and the effect of cooking on cell wall structure and starch gelatinization. Physicochemical and functional properties of starch from the seven potato cultivars were analyzed for amylose content, amylopectin chain length distribution, relative crystallinity, phosphorus content, granule size distribution, thermal properties and starch pasting profiles. Physicochemical, thermal and pasting properties of starch from the same cultivars of potatoes grown in the Netherlands under very different conditions were also examined.

This study showed that potato cultivars have a wide range of GI values (53 - 103), and this variability in the GI values of potatoes was due to genotype. The cultivar Carisma was classified as low GI (GI = 53), Nicola (GI = 69) as medium GI and the other five cultivars were classified as high GI according to ISO guidelines. The GI values were found to be strongly and positively correlated with the percentage in vitro enzymatic hydrolysis of starch from the cooked potatoes, particularly by the hydrolysis percentage at 120 min ($r = 0.91, p<0.01$). Content of amylose, dietary fibre and total starch did not correspond to rankings in either in vitro starch digestibility or GI values.

Native starch granules from the low GI potato Carisma appeared to have more tightly packed growth rings and a stronger fluorescence at the hilum. Internal imperfections, which were apparent in the starch from the higher GI potato cultivars were not evident in starch from Carisma potatoes. No differences were observed in cell sizes, starch granule size and granule morphology amongst the seven cultivars. On cooking, Carisma potatoes showed markedly less cell swelling compared to the high GI cultivar Russet Burbank, but it was unclear whether this was due to lower starch content, stronger cell wall structure, lesser
degree of granule gelatinization or combinations of these. Microscopy images showed that starch from Carisma potato appeared to be more resistant to gelatinization during cooking. Properties that could confer this resistance are the absence of imperfections in the native starch and an apparent tighter packing of the starch granules.

Most of the physicochemical properties of starch analyzed from the seven potato cultivars did not correspond to in vitro starch digestibility or GI ranking. However, starch from the Carisma cultivar had significantly higher DSC thermal transition temperatures and pasting temperature compared to the other cultivars. This indicated that Carisma starch had greater thermal stability and resistance to gelatinization. Starch from Carisma potatoes also had significantly higher trough and final starch paste viscosity indicating a greater resistance to breakdown and a more viscous retrograded starch paste. This has implications in the rate of starch hydrolysis as a high level of viscosity in the food matrix slows down the digestion process.

Starch from potato cultivars grown in the Netherlands had significantly different properties compared to the starch from the Carisma cultivar grown in Australia. Thermal transition temperatures and starch pasting temperature, trough and final viscosity of starch from the Carisma potatoes grown in The Netherlands were significantly lower than the corresponding values of the starch from the Carisma potatoes grown in Australia. This suggests that the starch from Carisma potatoes grown in The Netherlands is less resistant to gelatinization and subsequent enzymatic digestion. For quarantine reasons, it was not possible to test the GI values of the Carisma potatoes grown in the Netherlands and to ascertain the physiological significance of these differences.

Potatoes are generally considered as a high GI food but are an important and popular source of carbohydrates in human nutrition. Developing a screening method for finding more low GI cultivars is both a health and agricultural priority. The findings from this study suggest that low GI potato cultivars can be identified by screening using high-throughput in vitro digestion procedure, and thermal analysis and starch pasting properties may be useful indicators for preliminary identification of potato cultivars that have a low GI.
# TABLE OF CONTENTS

Statement of originality ............................................................................................................... iii
Acknowledgements ........................................................................................................................ vi
Abbreviations ............................................................................................................................... viii
Publications ........................................................................................................................................ x
Reference formatting ....................................................................................................................... x
Presentations and Awards ............................................................................................................... xi
Abstract ........................................................................................................................................... xii
Table of contents ............................................................................................................................. xiv

## CHAPTER 1: LITERATURE REVIEW ......................................................................................... 1

1.1 Introduction .............................................................................................................................. 2

1.2 Potatoes as a food crop ........................................................................................................... 4

1.2.1 Background of potatoes ................................................................................................. 4

1.2.2 Production and consumption ........................................................................................ 5

1.2.3 Taxonomy and genetics .................................................................................................... 5

1.2.4 Potato plant growth ......................................................................................................... 7

1.2.5 Dry matter and starch accumulation during growth ..................................................... 9

1.2.6 Influence of environmental conditions on growth ......................................................... 9

1.3 Potato starch ............................................................................................................................ 11

1.3.1 Physicochemical properties .......................................................................................... 13

1.3.2 Functional properties ....................................................................................................... 14

1.3.3 Enzymatic hydrolysis ..................................................................................................... 17

1.4 Effect of cooking and processing .......................................................................................... 19

1.5 Glycemic effect of potatoes .................................................................................................... 24

1.5.1 Glycemic index and glycemic load.................................................................................. 25

1.5.2 Health implication of GI and GL..................................................................................... 26

1.5.3 Measuring the GI of foods ............................................................................................... 26

1.5.4 Potato GI values ............................................................................................................. 27

1.5.5 Effect of processing on GI values .................................................................................. 31
### TABLE OF CONTENTS

2.2.14 *In vitro* enzymatic digestibility procedure ............................................ 50
2.2.15 Microscopy .............................................................................................. 51

2.2.15.1 Light microscopy of raw and cooked potato tissue .................... 51
2.2.15.2 Micro-CT of raw and cooked potato tissue ................................. 51
2.2.15.3 Environmental scanning electron microscopy (ESEM) ........... 51
2.2.15.4 Particle size analysis using LM and ImageJ software .......... 52
2.2.15.5 Confocal laser scanning microscopy (CLSM) ......................... 54

2.2.16 Statistical analysis ................................................................................ 54

CHAPTER 3: DISCOVERY OF A LOW GLYCEMIC INDEX POTATO AND
RELATION BETWEEN *IN VITRO* STARCH DIGESTION AND GLYCEMIC RESPONSE ................................. 57

3.1 Introduction ........................................................................................................ 58
3.2 Results .................................................................................................................. 59

3.2.1 Storage study .................................................................................................. 59
3.2.2 Chemical composition and GI testing portions ...................................... 62
3.2.3 *In vivo* glycemic response ......................................................................... 62
3.2.4 *In vitro* starch digestibility ......................................................................... 65
3.2.5 Correlation between *in vivo* glycemic response and
*in vitro* starch digestibility ............................................................................. 67

3.3 Discussion ............................................................................................................ 68
3.4 Conclusion .......................................................................................................... 71

CHAPTER 4: IMAGING OF TISSUE AND STARCH FROM POTATOES DIFFERING IN GI .................. 73

4.1 Introduction ........................................................................................................ 74
4.2 Results ................................................................................................................. 77

4.2.1 ESEM .............................................................................................................. 77
4.2.2 LM and CLSM imaging of potato starch granules .............................. 78
4.2.3 Effect of heat-treatment on potato tissue and isolated potato starch 79
4.2.4 Effect of cooking on microstructure as observed by micro-CT .......... 84
<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.3</td>
<td>Discussion</td>
<td>87</td>
</tr>
<tr>
<td>4.3.1</td>
<td>Raw potato tissue and starch granules</td>
<td>87</td>
</tr>
<tr>
<td>4.3.2</td>
<td>Effects of cooking on potato tissue and starch</td>
<td>90</td>
</tr>
<tr>
<td>4.3.3</td>
<td>Micro-CT examination of raw and cooked potato</td>
<td>91</td>
</tr>
<tr>
<td>4.4</td>
<td>Conclusion</td>
<td>93</td>
</tr>
<tr>
<td>5.1</td>
<td>Introduction</td>
<td>96</td>
</tr>
<tr>
<td>5.2</td>
<td>Results</td>
<td>97</td>
</tr>
<tr>
<td>5.2.1</td>
<td>Physicochemical properties</td>
<td>97</td>
</tr>
<tr>
<td>5.2.2</td>
<td>Thermal properties</td>
<td>98</td>
</tr>
<tr>
<td>5.2.3</td>
<td>Starch pasting properties</td>
<td>98</td>
</tr>
<tr>
<td>5.3</td>
<td>Discussion</td>
<td>104</td>
</tr>
<tr>
<td>5.4</td>
<td>Conclusion</td>
<td>107</td>
</tr>
<tr>
<td>6.1</td>
<td>Introduction</td>
<td>110</td>
</tr>
<tr>
<td>6.2</td>
<td>Results</td>
<td>111</td>
</tr>
<tr>
<td>6.2.1</td>
<td>Physicochemical properties</td>
<td>113</td>
</tr>
<tr>
<td>6.3.2</td>
<td>Thermal properties</td>
<td>118</td>
</tr>
<tr>
<td>6.3.3</td>
<td>Starch pasting properties</td>
<td>118</td>
</tr>
<tr>
<td>6.4</td>
<td>Discussion</td>
<td>121</td>
</tr>
<tr>
<td>6.5</td>
<td>Conclusion</td>
<td>123</td>
</tr>
<tr>
<td>7.1</td>
<td>General Discussion</td>
<td>125</td>
</tr>
<tr>
<td>7.2</td>
<td>Bibliography</td>
<td>133</td>
</tr>
<tr>
<td>7.3</td>
<td>Appendix</td>
<td>159</td>
</tr>
</tbody>
</table>
CHAPTER 1

LITERATURE REVIEW
1.1 Introduction

Would you like fries with that? Potatoes are the most extensively consumed root vegetable and the third largest food crop worldwide. They are the number one non-grain food commodity produced globally, with production in 2012 reaching 368 million tonnes (FAO, 2012). For various reasons, potatoes have received much attention in recent years from nutritionists and agriculturalists (FAO, 2008).

Carbohydrates are the principal energy source in the human diet, contributing more than that of fat, protein or alcohol. Carbohydrates have an energy value of 17 kJ/g (4 kcal/g) and account for 40% to over 80% of energy intake depending on social and economic factors (FAO/WHO, 1998). Carbohydrates that release glucose affect blood glucose and insulin levels, cholesterol and triglyceride metabolism, as well as influencing satiety, and exerting prebiotic effects in the large intestine (Eckel, Grundy & Zimmet, 2005; Englyst, Liu & Englyst, 2007; Holt, Miller, Petocz & Farmakalidis, 1995; Saltiel & Kahn, 2001; Venn & Green, 2007).

Besides being a carbohydrate staple and energy source, they are also rich in micronutrients, for example, vitamin C, B vitamins and potassium, as well as carotenoids and antioxidant phenolics. Potatoes may also contain a toxic glycoalkaloid, solanine (Burlingame, Mouille, & Charrondiere, 2009). The nutrient composition of different varieties of potatoes are described in recent reviews (Burlingame et al., 2009; Camire, Kubow, & Donnelly, 2009), and will not be considered further here.

The major nutrient in potatoes is starch, which is the largest source of carbohydrates in the human diet. The importance and complexity of this plant polysaccharide has been the subject of extensive research, as indicated by the following selected examples, which cover biosynthesis (Zeeman, Kossman, & Smith, 2010); structure (Zobel, 1988; Perez & Bertoft, 2010); physico–chemical, morphological and functional characteristics (Tester, Karkalas, & Qi, 2004; Copeland, Blazek, Salman, & Tang, 2009; Delcour & Hoseney, 2010); digestion (Singh, Dartois, & Kaur, 2010); and nutritional qualities (Englyst et al., 2007).

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1 A modified version of this literature review has been published in Food Chemistry.
The main focus of this review is concerned with the glycemic effect of potatoes. The term glycemic index (GI) was first introduced in 1981 by Jenkins et al. as a physiological (in vivo) way of classifying foods rich in carbohydrates based on their blood glucose-raising potential (Jenkins et al., 1981; carbohydrates with blood-glucose raising potential will be referred to hereafter in this review as available carbohydrates). Foods with a GI value of above 70 are classified as high GI, foods with a GI of 56–69 as medium GI, and foods that have a GI of 55 and less are classified as low GI (ISO Standard 26642:2010).

An in vitro method for measuring digestibility of starch and classifying nutritionally important fractions was introduced by Englyst et al. in 1992, who measured the rate of glucose released from starch using digestive enzymes (Englyst, Kingman, & Cummings, 1992). Both in vivo and in vitro approaches are used to classify digestibility of carbohydrates and starch from a nutritional perspective. Consumption of available carbohydrates that are digested and absorbed rapidly results in high postprandial blood glucose levels, which over the long term are associated with increased risks of obesity, and diet-related diseases including type-2 diabetes, cardiovascular disease and certain types of cancers (Liu et al., 2000; Ludwig, 2002; Mozaffarian, Hao, Rimm, Willett, & Hu, 2011, Willett, Manson, & Liu, 2002). Studies on different cultivars of potatoes using in vivo and in vitro methods indicate that cooked potatoes have mostly rapidly digested starch (Leeman, Barstrom, Bjorck, 2005) and a high GI (Atkinson, Foster-Powell, & Brand-Miller, 2008). Hence, many nutritionists advise that potatoes should be substituted with a low GI carbohydrate to reduce the risk of chronic disease (Brand-Miller, McMillan-Price, Steinbeck, & Caterson, 2009; Willett et al., 2002). However, as discussed subsequently in this review, this advice may not apply to all potatoes.

This review provides a background on potatoes as a food crop, considers the properties of potato starch, particularly in relation to glycemic index (GI) and digestibility, and explores areas for future research.
1.2 Potatoes as a food crop

1.2.1 Background of potatoes

Potatoes were first domesticated more than 6000 years ago near Lake Titicaca in the Andes mountains of South America, on the border between Bolivia and Peru. The greatest diversity of wild species of potatoes is still currently found in this region (CIP, 2008; Reader, 2008). The Spanish Conquistadores first encountered the potato when they arrived in Peru in 1532 in search of gold. Potatoes were brought back to Europe in the 16th Century as a novelty, but due to their association with the poisonous “nightshade” family were initially considered as food fit only for livestock and the poor. This started to change in the 18th Century when early maturing varieties were bred and grown, and found to perform well in northern growing conditions. Famine plagued much of Europe during this period and the potato became recognized as a food security crop. However the potato crops cultivated were limited to a few genetically similar varieties, and when the disease late blight struck the Irish potato crop in 1845, it caused the tragic “Irish potato famine”. This famine led to the death of at least one million people and due to subsequent emigration the population of Ireland decreased from 8 to 3 million people. “Expeditions” were mounted to import new germplasm from Latin America for breeding of higher yielding and disease-resistant varieties. Research on improving potato germplasm continues worldwide and in particular at the International Potato Centre (CIP) in Peru, where more nutrient-rich varieties are being bred. A recent example is the selective breeding of varieties that are rich in bioavailable iron, to address deficiencies of this micronutrient in pregnant women and pre-school children in the developing world (CIP, 2011).
1.2.2 Production and consumption

The importance of the potato was recognized by the Food and Agriculture Organization (FAO) of the United Nations with its declaration of 2008 as the “The International Year of the Potato”. This initiative sought to focus world attention on the importance of the potato in food security and alleviating poverty, and resulted in the collation of much valuable information on potatoes (www.potato2008.org; FAO, 2008a). Potatoes are the number one non-grain food commodity produced globally with an annual production of 300 to 320 million tons in 2005, accounting for almost 50% of the total production of food roots and tubers (FAO, 2008b). This number has grown to 368 million tonnes in 2012 (FAO, 2012). Much of the recent growth in world potato production (from 268 million tons in 1991) has occurred in developing countries where more than 50% of the world’s harvested crop is now produced (FAO, 2008b).

Western countries produce and consume the highest amount of potatoes but consumption is increasing rapidly in developing countries. Per capita potato consumption in 2005 was estimated to be 93 kg in Europe, 60 kg in North America, 55 kg in Australia and 22 kg in China (FAO, 2008b). In 2009, annual global consumption continued to exceed 200 million tonnes, with a decreased consumption of 74 kg consumed per capita in Europe, 55 kg in North America, 53 kg in Australia and an increase to 37 kg in China (FAOSTAT, 2009).

1.2.3 Taxonomy and genetics

Potatoes exhibit great genetic and morphological variability, which has resulted in much debate about the taxonomic classification of wild and cultivated varieties. This debate is concerned with the number of recognized potato species and their interrelationships, as well as methods of classification by morphological and physiological characteristics, ecogeography, ploidy or a combination of these properties.
While most potatoes cultivated currently are designated collectively under the single species name of *Solanum tuberosum*, there are about 10 other *Solanum* species that have been cultivated and some 200 other wild species recognized (FAO, 2008c). However, taxonomic studies suggest that there are only four cultivated potato species (Spooner, Nunez, Trujillo, Herrera, Guzman & Ghislain, 2007). A review by Ovchinnikova *et al* (2011) describes the history and the different taxonomic classifications, and includes images of lectotype specimens.

*Solanum tuberosum* belongs to the *Solanaceae* family, the nightshade family of flowering plants. Potatoes share the genus *Solanum* with other economic plants like tomato, eggplants, capsicum, tobacco and petunias. The sequencing of the potato genome was recently completed by the Potato Genome Sequencing Consortium and the published findings provide a platform for future genetic improvement of the crop (The Potato Genome Sequencing Consortium, 2011). The potato genome is a medium sized plant genome with 12 chromosomes and a haploid length of approximately 840 million base pairs (Visser *et al*., 2009). The most commonly cultivated potato, *Solanum tuberosum* L. (*2n = 4x=48*) is an autotetraploid species that is reproduced by asexual propagation. Most potato genotypes are genetically heterogeneous regardless of cultivar or genetic stock (Razdan & Mattoo, 2005; The Potato Genome Sequencing Consortium, 2011).

Two major difficulties in the genetic analysis of potatoes are the lack of pure lines and polyploidy. Hence, many qualitative and quantitative agronomic characteristics are still not well understood. Quality attributes used agriculturally and commercially include high yields in different environments, broad-spectrum resistance to diseases, number of days to maturity, good storage characteristics, tuber and flesh colour, and traits suited to cooking and processing methods. Many potato growing countries have their own potato variety database of details about cultivated varieties in their local conditions. Examples are the British Potato Council (Potato Council), the Canadian Food Inspection Agency (CFIA), the Potato Association of America (PAA) and the European Cultivated Potatoes database (ECPD).
1.2.4 Potato plant growth

The modern cultivated potato *Solanum tuberosum* is an annual plant that grows up to 100 cm tall with branching stems (Figure 1.1). It has odd pinnate leaves with three or four pairs of ovate leaflets, with smaller ones in between. The potato plants produce small (2.5 cm) white to purplish flowers and the fruit is a tomato-like green or yellowish berry about 2 cm wide. The plant possesses fibrous roots and many rhizomes, which can swell to form edible tubers (Harrison, Masefield & Wallis, 1985). The potato plant can be distinguished into three parts: the haulm (foliage of leaves and stems), tubers and stolons, and the roots.

Potatoes are mainly propagated vegetatively from seed potatoes instead of true seed. The "seed piece" is obtained from a previously harvested tuber as a whole or cut pieces and the main stem grows to form the haulm. Products from photosynthesis are transported and stored in the stolon and over time this enlarged stolon becomes a tuber. There are three main periods in the growth cycle of the plant: pre-emergence/emergence, haulm growth and tuber growth. The pre-emergence/emergence stage is when the seed tuber develops sprouts and roots. This happens after planting and sometimes even before planting. After emergence, the haulm and roots develop concurrently. Tuber growth occurs at 2-4 weeks after emergence and continues at constant rate. Tuber and haulm growth then proceed together and are interrelated. Excessive haulm development results in late tuber growth and less haulm growth results in early tuber growth (Beukema & Van der Zaag, 1990).
Figure 1.1: Diagram of a potato plant (Image adapted from Manitoba Agriculture Food and Rural Initiatives)
1.2.5 Dry matter and starch accumulation during growth

After tuber initiation, dry matter increases exponentially followed by a period of nearly linear growth, then growth rate decreases and ends when the shoot senesces. The rate of tuber growth is an almost linear process when growing conditions are favourable and this phase is called the bulking period. The bulking rate is the daily increase in fresh tuber weight per hectare, which under favourable conditions, can be as high as 800-1000 kg/ha per day. The rates of dry matter and starch accumulation in potato tubers are similar and generally reach maximal values between 90-110 days after emergence. Thereafter, the starch content decreases slightly until maturity (Kolbe & Stephan-Beckmann, 1997). Liu et al. (2003) observed the highest level of dry matter (19.2–24.2%) at 64–71 days of growth, then a decrease. Starch content was observed to be lowest in early harvest (48–55 days) at 66–71% dry matter then increased to a maximum of 78–80% dry matter by 84–112 days of growth followed by slight decrease (Liu, Weber, Currie & Yada, 2003). The final total starch content and dry matter in potatoes at maturity vary depending on cultivar and species (Burlingame et al., 2009; Liu et al., 2003; Lu et al., 2011). Different potato varieties have different lengths of time required for plant maturity. For agricultural purposes it is useful to classify varieties by the number of days to maturity following planting of whole or cut seed tubers. The Canadian Food Inspection Agency classification of maturing varieties is as follows: very early (65–70 days); early (70–90 days); mid-season (90–100 days); late (110–130 days) and very late (> 130 days) (CFIA, 2004).

1.2.6 Influence of environmental conditions on growth

The stages in the growth cycle of potatoes are influenced by many factors including atmospheric and soil temperature, day length, light intensity, physiological age of the seed, plant density, and nutrient and water supply (Beukema et al., 1990; Gopal & Khurana, 2006; Taiz & Zeiger, 2010). These factors affect plant growth individually, and through a combination of interactions between the factors. Different potato varieties respond in different ways to each of the various factors (Lambers, Chapin & Pons, 2008), but some general observations are discussed in the following paragraphs.
Day length and temperature affect tuber initiation but have less effect once tuber growth has been established. Sunlight provides a source of energy for photosynthesis and regulatory signals that control growth and development. Photoperiod is pivotal in triggering tuberization in potatoes, with shorter day lengths required for the initiation of tuberization (Martinez-Garcia, Virgos-Soler & Prat, 2002). It has been suggested that each potato variety has its own critical day length. This would mean that tuber formation takes place only if the day length is shorter or equal to that critical day length (Martinez-Garcia et al., 2002). Short day length (12 hrs) and low night temperatures (8°C) were observed to stimulate tuber initiation. Ambient temperature affects certain temperature sensitive reactions in the photosynthetic process, with 20–25°C depending on light intensity and 15–18°C for soil temperature, generally observed as optimum for potato growth (Beukema et al., 1990). The intensity of light reaching the crop affects the photosynthetic process and depends on the angle of incidence of the sun’s rays on the foliage (time of day, latitude and leaf angle distribution of the foliage) and the clearness of the sky (cloud cover and pollution). It was estimated that on an overcast day the daily gross assimilation of the potato crop under a closed canopy with adequate water supply is approximately half of that on a clear day. Likewise, on a long day more light can be intercepted by the foliage compared to short day and consequently produce more tuber yield (Beukema et al., 1990).

In potato crops, the mineral nutrient uptake ranges from 2.28 kg to 3.57 kg of nitrogen (N), 0.04–0.12 kg of phosphorus (P), and 3.7 to 5.4 kg of potassium (K) per tonne of fresh tuber, as well as high quantities of micronutrients such as zinc, iron and copper being required (Gopal et al., 2006). When nutrients such as N and P are in short supply, plants allocate relatively less biomass to leaves and more to their roots (Lambers et al., 2008). The amount of nutrients available in the soil and the timing of application of fertilisers have a significant influence on potato yield. High N early in the growing season delays tuberization and canopy senescence, and results in prolonged tuber bulking and a larger yield. Increased amounts of P were observed to decrease the number of large and medium sized tubers and increase the proportion of small sized tubers, while the converse occurred when K was applied (Gopal et al., 2006).
Potatoes are sensitive to drought stress and the shortage of water reduces tuber yield by shortening the duration of the growing period and reducing the net photosynthetic assimilation rate (Beukema et al., 1990; Gopal et al., 2006). Water supply is crucial to dry matter and starch deposition in potatoes, with at least 50% soil moisture required to be maintained at all stages of plant growth to obtain high yields in potatoes (Singh, 1969). Soil types can affect water availability, with clay soils having the greatest water holding capacity, followed by silt, loam, and sandy soil. Irregular water supply results in irregular tuber growth, promoting misshapen tubers and cracks. Soil tillage, seed bed preparation, planting and ridging are other factors that are important in potato growth but will not be discussed further here.

1.3 Potato starch

Starch is ubiquitous in plants as the main storage reserve of carbon. It is deposited in semi-crystalline granules, which differ in size (from <1 to 100 μm in diameter); shape (oval, polygonal, spherical, lenticular, irregular); size distribution (unimodal or bimodal); and surface topography (grooves, ridges and pores) depending on botanical origin (Fannon, Hauber, & Bemiller, 1992; Jane, Kasemsuwan, Leas, Zobel, & Robyt, 1994; Perez et al., 2010; Sujka & Jamroz, 2009; Tester et al., 2004).

Starch granules consist of two polymers of D-glucose joined together by glycosidic bonds: amylose, an essentially linear polysaccharide of α(1→4) linked D-glucoses and amylopectin, a highly branched polysaccharide which consists of α(1→4) linked D-glucoses with α(1→6) branching links (Smith, 2001; Zeeman et al., 2010). Amylose is synthesized by granule bound starch synthase (GBSS) in particular the isoform GBSS1, whereas soluble starch synthase isoforms are responsible for synthesizing amylopectin chains (Denyer, Clarke, Hylton, Tatge & Smith, 1996; Martin & Smith, 1995; Zeeman et al., 2010). The organization of the amylose and amylopectin chains into starch granules can be considered at five hierarchical scales, from the molecular (0.1-1.0 nm) to the macroscopic (2-100 μm). These are the arrangement of amylose and amylopectin into amorphous and crystalline lamellae.
(with a repeat interval of 9–10 nm), the aggregation of amorphous and crystalline regions into blocklets (20–500 nm), growth rings (120–500 nm) and finally the intact starch granule (2–100 μm) (Perez et al., 2010; Vandeputte & Delcour, 2004).

The central region of the starch granule is proposed to be a bulk amorphous region consisting mainly of amylose and disordered reducing ends of amylopectin (Wang & Copeland, 2013). The assembly of amylose and amylopectin results in two distinct regions known as the crystalline and amorphous lamellae and a semi-crystalline growth ring consists of repeats of alternating amorphous and crystalline lamellae, with repeat distances of amorphous and crystalline lamellae about 9–11 nm regardless of botanical origin (Cameron & Donald, 1992; Perez et al., 2010; Vandeputte et al., 2004). The amorphous growth rings within starch granules are considered to be made up of interspersed amylose chains and the branch points of amylopectin side chains, whereas the crystalline regions contain double helices formed by unbranched or singly branched amylopectin chains with ten or more glucose units (Wang et al., 2013; Copeland et al., 2009; Oostergetel & Vanbruggen, 1993).

The amylopectin double helices pack into different polymorph structures which are classified as A-type, B-type or C-type according to their X-ray diffraction patterns. Cereal starches like rice, wheat and maize have mostly the A-type polymorph; tuber and root starches have mostly B-type; some legume starches have the C-type polymorph, which may be a combination of A-type and B-type polymorphs (Buleon, Colonna, Planchot & Ball, 1998; Perez et al., 2010). The A-type polymorph is more compact and has lower water content than the B-type polymorph which has an open structure with a hydrated core (Gallant, Bouchet & Baldwin, 1997; Perez et al., 2010 Tester, et al., 2004; Zobel, 1988). The overall structure, with crystalline and amorphous regions arranged in concentric growth rings (Figure 1.2A), gives intact mature starch granules their characteristic property of birefringence when viewed under polarized light (Figure 1.2B).
Progress towards understanding enzymatic regulation of starch metabolism (Kotting, Kossman, Zeeman, & Lloyd, 2010) and how amylose and amylopectin are assembled to form starch granules (Wang, et al., 2013; Perez et al., 2010) are discussed elsewhere. For the purpose of this review, the discussion will be concerned mainly with properties of potato starch.

1.3.1 Physicochemical properties

Total starch content of different potato varieties can vary greatly, from about 9% to 23% of the fresh weight (Burlingame, et al., 2009). These values represent 66–80% of potato dry matter as starch (Liu, Tarn, Lynch, & Skjodt, 2007; Liu et al., 2003). Potato starch granules are large, lenticular, oval or spherical shaped, with a mean diameter of 23–30 μm, and a broad size range of 5–100 μm. The granules have a unimodal size distribution (Hoover, 2001; Tester, et al., 2004). Native potato starch granules are generally smooth, although scanning electron microscopy (SEM) and atomic force microscopy (AFM) have revealed ridges and grooves on the surface (Baldwin, Davies, & Melia, 1997; Sujka et al., 2009; Szymonska & Krok, 2003).
The moisture content of native starches from roots and tubers range from 14 to 18%, whereas the moisture content of native cereal starches range from 10 to 12% (Tester, et al., 2004). The amylose content of potato starch is between 25–33% (Alvani, Qi, Tester, & Snape, 2011; Kaur, Singh, McCarthy, & Singh, 2007; Liu et al., 2007; Liu et al., 2003) and the molecules are relatively long compared to starches from other plant sources. Amylose chains from potato starch contain approximately 670 glucose residues compared to maize (305), rice (250–370) and wheat (270) starches (Tester et al., 2004). Likewise, average chain lengths of amylopectin from potato starches (29–34 glucose units) are longer than those of wheat (23), corn (24) and rice (23) starches (Hoover, 2001; Srichuwong & Jane, 2007; Tester et al., 2004).

Potato starch has very little lipid and protein compared with cereal starches (Hoover, 2001; Alvani et al., 2011; Tester et al., 2004), but contains much higher quantities of phosphorus compared to other starches (Blennow, Bay-Smidt, Wischmann, Olsen, & Moller, 1998; Hizukuri, Tabata, & Nikuni, 1970; Hoover, 2001; Srichuwong et al., 2007). The phosphorus content of potato starch can range between 36 mg/100g and 116 mg/100g with a median of 60–80 mg/100g (Alvani et al., 2011; Noda et al., 2007; Yusuph, Tester, Ansell, & Snape, 2003). Phosphorus in potato starch is in the form of phosphate monoesters attached to amylopectin, with one phosphate group per approximately 317 glucose residues (Takeda & Hizukuri, 1982). Phosphorus was found to be more abundant in the core of the starch granule with smaller granules observed to have higher phosphorus content compared to large granules (Jane & Shen, 1993).

1.3.2 Functional properties

Potatoes need to be cooked prior to consumption. Hence, an examination of the functional properties of potato starch, such as starch solubility and swelling power, gelatinization and retrogradation, and thermal characteristics, are important in understanding the glycemic effect of potatoes. When starch granules are heated in excess water, intra and inter chain hydrogen bonds between amylose and amylopectin are broken and water molecules bond to exposed hydroxyl groups. This process, termed gelatinization, disrupts the crystalline
structure of the starch granules, resulting in increased solubility of glucan chains (mostly amylose) and granule swelling (Hoover, 2010; Wang et al., 2013). The extent of swelling and solubility reflect the strength of interactions between starch chains. As the temperature increases, starch undergoes an irreversible phase transition where the native crystallinity, structural organization and birefringence are lost (Jenkins & Donald, 1998).

The start of gelatinization is the entry of water into the amorphous growth rings, followed by their rapid expansion. Starch swelling is initially concentrated in the amorphous regions while the semicrystalline layers are essentially unchanged. Disruption of the crystalline order occurs when sufficient pressure builds up from swelling in the amorphous regions. The loss of crystallinity, leaching of amylose and granule breakdown occur in the later stages of gelatinization (Hoover, 2010; Jenkins et al., 1998; Wang et al., 2013). The unusually high solubility and swelling power of potato starch, compared to starches from other botanical sources, are considered to be due at least in part to repulsive forces between phosphate groups on adjacent amyllopectin chains weakening the bonds in the crystalline domain, allowing water molecules to more readily disrupt the structure (Hoover, 2001).

Differential scanning calorimetry (DSC) is widely used to measure the heat energy required for starch gelatinization. The enthalpy change (∆H) measures the loss of molecular order due to hydrogen bonds breaking within the granule; this parameter is considered to be an indicator of the quantity and quality of the starch crystalline structure (Tester & Morrison, 1990). However, a recent study with pea starch observed that ∆H was influenced by the water:starch ratio in the DSC pan, and an increase in ∆H was observed with an increase in water availability until a water:starch ratio of more than 10:1 to 15:1 was reached (Wang & Copeland, 2012). Most starches gelatinize in the temperature range of 60 to 80°C, depending on the botanical sources and heating conditions. Potato starches generally gelatinize between 64 and 72°C (Alvani et al., 2011; Kaur et al., 2007; Liu et al., 2007; Liu et al., 2003; Singh, Singh, Kaur, Sodhi, & Gill, 2003; Yusuph et al., 2003).

If the starch concentration is above about 5%, the hydrated and disaggregated amylose and amyllopectin molecules in the disrupted granules reassociate to form a gel that retrogrades gradually on cooling into a semi-crystalline form different from that of native starch. Further cooling causes an increase in gel firmness and the loss of water through
Syneresis. Amylose molecules retrograde more quickly (minutes to hours) than amylopectin molecules, which take days to weeks to retrograde (Ring et al., 1987, Sievert & Wursch, 1993). The reassociation of linear amylose chains is restricted by the presence of amylopectin (Sievert et al., 1993) and the long chain length of potato amylose may also restrict chain reassociation (Chung & Liu, 2009). Nutritionally, retrograded starch is more resistant to digestion (Englyst et al., 1992) and its effect on GI is discussed later in this review.

The Rapid Visco Analyser (RVA), is a heating and cooling viscometer that is commonly used to study starch paste viscosity, or its viscoelasticity. Starch paste viscosity is measured by applying mechanical shearing during gelatinization. It is meant to simulate food processing and is used to relate functionality to starch structural properties. Five characteristic parameters are usually measured from the pasting curve from the RVA and are described as follows (Figure 1.3).

![RVA pasting profile of potato starch](image)

**Figure 1.3:** RVA pasting profile of potato starch
Starch paste formed during heating and shearing increases in viscosity until it finally reaches a peak (measured as peak viscosity) and is followed by a decrease in paste viscosity, as the granules are ruptured, starch components are leached and molecules become dispersed (measured as the breakdown). Then as temperature is decreased, viscosity increases from the minimum value at breakdown to a final value (measured as the setback). The peak time and peak viscosity show the water-binding capacity of the starch and loss of crystalline structure. The breakdown viscosity is regarded as a measure of paste stability (Newport Scientific, 1995). During breakdown, swollen granules are disrupted, and amylose molecules leach out into the solution. The setback viscosity is the viscosity increase resulting from the rearrangement of amylose molecules that have leached from swollen starch granules during cooling, and is generally regarded as the retrogradation tendency of starch (Karim, Norziah, & Seow, 2000). Like gelatinization, the RVA displays pasting profiles which are characteristic for the botanical origin of the starch.

Potato starches develop clear starch pastes with an extremely large peak viscosity and large breakdown attributed to the high phosphate monoester content (Alvani et al., 2011; Hoover, 2001; Mishra & Rai, 2006; Noda et al., 2007). Potato starches with higher phosphorus content showed significantly higher peak viscosity and swelling power (Noda et al., 2007). Higher amylose potato starches showed lower peak viscosity and breakdown but higher setback viscosity (Zaidul, Yamauchi, Takigawa, Matsuura-Endo, Suzuki, & Noda, 2007). Starches with higher peak viscosity and breakdown were more easily digested by enzymes (Noda et al., 2007).

1.3.3 Enzymatic hydrolysis

The rate of breakdown of starch granules into glucose units by amylolytic enzymes has been investigated extensively. Native starch granules from different botanical origins have different susceptibility to enzymatic breakdown, with potato starch granules among the most resistant to digestion (Gallant, Bouchet, Buleon, & Perez, 1992; Hoover, 2001; Kimura & Robyt, 1995). Small granules are digested more rapidly than large granules (Dhital, Shrestha, & Gidley, 2010; Kaur et al., 2007; Noda et al., 2005). The larger surface area to volume ratio of small granules allows greater access for enzymes, and hence more rapid breakdown. The surface area to
volume ratio of potato starches ranges from 0.06 to 1.2 given the large variation in granule size (Tester, Qi, & Karkalas, 2006).

Dhital et al. (2010) observed that native maize starch was digested at a 30-fold faster rate than potato starch. They postulated that the presence of enzyme-accessible channels on the surface of maize granules was the main reason for such a large difference in rates of hydrolysis between maize and potato granules. Native potato starch granules incubated with enzymes showed minor surface pitting, which suggests that digestion of potato starch is by exocorrosion of the granule (Gallant et al., 1992; Kimura et al., 1995). The exocorrosion of the granule has been proposed to be slowed down by the presence of hard crystalline layers under the granule surface made from large blocklets (Gallant, et al., 1992).

High amylose content has been associated with a greater resistance to enzymatic digestion in rice (Hu, Zhao, Duan, Zhang, & Wu, 2004) and maize starch in both raw and processed states (Htoon et al., 2009). How amylose reduces digestibility is unclear, as the location and distribution of amylose in relation to amylopectin in the structural matrix of the granule is still not well established. Amylose chains are thought to be abundant in the core of the granules and distributed in a radial fashion amongst amylopectin (Wang et al., 2013). Larger amylose molecules may be located more in the centre of the granule, whereas shorter amylose chains located at the periphery of the starch granule may be more easily leached during gelatinization (Jane et al., 1993). Potato amylose has a very long chain length, which could have a structure-stabilising effect and contribute to the resistance of the potato starch granule to enzymatic digestion. In maize, barley and pea starch, amylose is thought to disrupt the structural order within the crystalline arrangement of amylopectin (Jenkins & Donald, 1995).

The high resistance to enzymatic digestion of raw potato starch is completely lost when potato starch is gelatinized (Farhat, Protzmann, Becker, Valles-Pamies, Neale, & Hill, 2001; Noda et al., 2008). Factors such as median granule size and amylose content, which influence the rate of enzymatic digestion of raw potato starch, no longer apply when the starch is gelatinized (Noda et al., 2008). Absar et al. (2009) observed that phosphorus content, which had no effect on digestion rate of raw potato starch, conferred resistance to enzymatic digestion for gelatinized potato starch. This effect was also observed by Noda et al. (2008) for mixtures of potato and other starches, but there was no correlation between phosphorus content and the rate of enzymatic digestion of gelatinized potato starch alone. Liu et al. (2007) suggest that amylose content, phosphorus content, the proportion of
specific chain length fractions and morphology are all factors that influence digestibility of gelatinized potato starch. Rapid enzymatic breakdown after gelatinization was also observed with wheat starch, but no clear correlation was found with chemical and functional properties (Blazek & Copeland, 2010).

Recent work has shown that the fine structural features of both amylose and amylopectin significantly influence the in vitro digestion rate of starch in cooked rice grains. Longer chain lengths of amylose branches, a smaller relative amount of long to short amylopectin branches and a smaller ratio of longer amylose branches to short amylopectin branches increased in vitro digestion rate (Syahariza, Sar, Hasjim, Tizzotti, & Gilbert, 2013).

1.4 Effect of cooking and processing

Potatoes are prepared domestically by various methods prior to consumption, including boiling, baking, microwaving or frying. This section describes cellular changes and starch gelatinization in situ when different methods of heating are used to cook potatoes. Potato tissue consists of large (200 x 340 μm) and small (80 x 90 μm) cells, with each cell observed to contain six to ten large starch granules (10–70 μm in diameter) and hundreds of very small starch granules (0.5–1.0 μm) (Singh, Kaur, Ezekiel, & Guraya, 2005). The cells in raw potato tubers have been reported to have an isodiametric polyhedral outline, with variation in cell size, cell shape, cell wall thickness and starch granule sizes between cultivars (Singh et al., 2005; Thybo, Martens, & Lysheide, 1998). Figure 1.4 shows an environmental SEM image of a section of a Russet Burbank potato in which some of these features are evident.
Isolated potato starch granules do not gelatinize simultaneously, with larger granules tending to gelatinize before smaller ones (Parada & Aguiler, 2009; Singh & Kaur, 2004). This property has not been studied extensively for starch granules in situ. As digestibility in the gut is likely to be dependent on the proportion of gelatinized granules after cooking, it therefore is important to understand starch gelatinization in situ. Published microscopy studies on the effects of cooking on potato microstructure and starch gelatinization have been summarised in Table 1.1.
Table 1.1. Morphological changes in potato tissue and starch granules following different cooking methods

<table>
<thead>
<tr>
<th>Cooking method</th>
<th>Imaging method</th>
<th>Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole potatoes heated in water (5 min, 100°C); potato centre, 46°C</td>
<td>SEM</td>
<td>Small individual granules at the centre of the potato. Small granules were clumped together at potato periphery. Cell walls were intact.</td>
</tr>
<tr>
<td>Whole potatoes heated in water (10 min at 100°C); potato centre, 65°C</td>
<td>SEM</td>
<td>Small and medium granules clumped together at the centre of the potato. Large swollen granules at the potato periphery. Cell walls were intact.</td>
</tr>
<tr>
<td>Whole potatoes heated in water (20 min at 100°C); potato centre, 90°C</td>
<td>SEM</td>
<td>Swollen granules were seen in all areas of the potato. Adjacent cell walls remained unseparated.</td>
</tr>
<tr>
<td>Potato discs (16 mm x 4 mm) heated in water (5 min at 100°C)</td>
<td>TEM</td>
<td>Cell walls expanded with reducing intercellular contact.</td>
</tr>
<tr>
<td>Potato discs (16 mm x 4 mm) heated in water (15 min at 100°C)</td>
<td>TEM</td>
<td>Cell walls intact but disrupted middle lamellae.</td>
</tr>
<tr>
<td>Potato discs (10 mm diameter x 10 mm length) heated in water (5–30 min at 60–100°C)</td>
<td>LM</td>
<td>Partly crystalline granules at 60°C. At ≥70°C granules swelled dramatically after 5 mins, followed by loss of birefringence on continued heating. Swollen granules occupied most of the cell volume.</td>
</tr>
<tr>
<td>Potato (68% amylose) discs (10 mm diameter x 10 mm length) heated in water (5–30 min at 60–100°C)</td>
<td>LM</td>
<td>Limited granule swelling after heating at 100°C for 5min. Birefringence apparent at 70°C but lost at 100°C.</td>
</tr>
<tr>
<td>Isolated potato cells fried in oil (30°C increasing at 40°C/min until 180°C and held for 3 min)</td>
<td>Hot-stage LM and video microscopy</td>
<td>Granules began to swell at 72°C and changed shape at 85°C. At 100°C granules were swollen and merged, but boundaries between granules were still visible.</td>
</tr>
<tr>
<td>Cooking method</td>
<td>Imaging method</td>
<td>Observations</td>
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<tr>
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<tr>
<td>Isolated potato cells fried in oil (2–3 min at 180°C)</td>
<td>Hot-stage LM and video microscopy</td>
<td>Granules started to swell after 3–5 secs of immersion. Complete gelatinization observed in &lt; 2 secs after initiation of swelling. Cells were filled by swollen granules.</td>
</tr>
<tr>
<td>Whole potatoes, microwave-heated (0.5 min, with temperature reaching 38°C, followed by 1 min, with temperature reaching 66°C and 2 min, with temperature reaching 80°C)</td>
<td>SEM</td>
<td>After 0.5 min, small individual granules were seen at the centre of the potato and small granules were clumped at the potato periphery. After 1 min, clustered large granules filled cells were at the centre of the potato, no individual granules were visible at potato periphery. Inter cellular spaces started to form. After 2 min, large swollen granules were visible in all parts of the potato.</td>
</tr>
</tbody>
</table>

b. vanMarle, Stolle-Smits, Donkers, Dijk, Voragen, & Recourt, 1997  
c. Ormerod, Ralfs, Jobling, & Gidley, 2002  

As indicated in these studies, the two main changes that occurred concurrently during boiling are breakdown of the cell walls and middle lamellae, and intracellular starch swelling and gelatinization. When potatoes are boiled for 5 min, starch granules hydrate, swell and fill the cell lumen. This continues throughout the cooking period as the cells start to separate at the middle lamella. After 10 min of cooking, swollen starch granules were observed to have formed dense clusters in and did not undergo further expansion on continued heating. Cells are separated completely after 20–30 min, and after 50 min the cell structure is collapsed (Thybo et al., 1998). Differences observed between varieties include cell wall density and rate of breakdown of the middle lamellae (vanMarle et al., 1997).

When potatoes are fried, the high temperature and the existing water within cells lead to starch gelatinization. Water inside the cells is quickly absorbed by the starch granules before dehydration of the cells occurs. Confocal laser scanning microscopy was used to optically section the potato crust after frying and revealed that frying oil was located in the
interior in pockets or surrounding intact potato cells (Bouchon & Aguilera, 2001). Isolated starch granules on frying gelatinized at 6–7°C lower than starch granules located inside the potato cells (Aguilera et al., 2001).

The changes that occur in baked potatoes have not been studied as extensively as fried or boiled potatoes. Baking uses dry heat to cook foods and the process to reach cooking temperature occurs much more slowly than other forms of cooking. In a study by Wilson et al. (2002a), whole potatoes were baked for 60 min in a conventional fan-forced oven. The temperature profiles within the potatoes were determined using direct measurement. The centre of the potato required 30 min to reach a temperature of 100°C, and 60 min were required before the texture at the centre of the potatoes reached a degree of softness acceptable for consumption. The authors concluded that the rate of heat transfer into the potato was limited by evaporative cooling at the potato surface and that the dried potato skin acted as a barrier to heat transfer.

Microwave cooking is increasingly popular due to its speed and convenience. In microwave cooking, heat generated by molecular vibration of water molecules in foods is suggested to have a greater penetrating depth than conventional cooking methods. Microwave cooking of potatoes was found to occur in two phases: in the first phase the internal temperature of the potatoes increased to 100°C with little water loss, whereas in the second phase further energy absorption was used to evaporate water (Wilson, MacKinnon, & Jarvis, 2002b). In this study, whole potatoes were cooked in an 800 W microwave oven and the gelatinization temperature of the potato starch was assumed to be 65°C. This temperature was reached in 2–2.5 min of cooking. The second phase of rapid water loss was observed after 4 min of cooking, and after 7 min the potatoes were cooked enough texturally for consumption. Microwave cooking of potatoes was found to be faster than other conventional methods, and while the potato was deemed as cooked texturally, there was no closer examination of the extent of starch gelatinization in situ in this study. As observed by SEM in another microwave cooking study, the starch granules gelatinized evenly in situ throughout the whole potato with increasing cooking time and temperature (Huang et al., 1990).

The direct effect of microwave irradiation on isolated potato starch granules were studied using light microscopy. At 68°C, the approximate gelatinization temperature of potato starch, there was no change in starch appearance, and the initial phase of gelatinization and amylase leakage were only observed when 90°C was reached.
Post irradiation, the mainly B-type crystalline polymorph structure of potato starch had changed to mainly A-type (Lewandowicz, Fornal, & Walkowski, 1997). When AFM was used to study the effect of microwave heating on the nanostructure of the potato starch, the granules were observed to be incompletely gelatinized. The authors explained their observations on the basis that there was very rapid water transfer and non-uniform heating caused by microwave radiation (An, Yang, Liu, & Zhang, 2008).

Microscopy techniques are valuable in examining changes to potato microstructure and starch gelatinization in situ. However, a standardized quantitative method for measuring such changes has not been developed, but would be useful to evaluate these changes when different forms of heat are used to cook potatoes in relation to digestibility.

### 1.5 Glycemic effect of potatoes

Blood glucose concentration is tightly controlled in healthy people in a narrow range between ~4 mmol/L and ~10 mmol/L by homeostatic regulatory systems in the human body. Hyperglycemia stimulates insulin secretion from the β-cells in the pancreas, promoting glucose uptake by muscle and fat cells and inhibiting hepatic glucose production. Hypoglycemia, on the other hand results in the release of glucagon and other hormones, which have opposite actions to insulin to restore normal glucose levels. Besides acting as the primary regulator of blood glucose, insulin also stimulates lipogenesis and inhibits lipolysis for storage in fat, liver and muscle cells (Ludwig, 2002; Saltiel et al., 2001). When a large meal of rapidly-digested available carbohydrates is consumed, it induces an initial period of high blood glucose and elevated insulin levels, which is followed by “reactive hypoglycemia” and elevated serum free fatty acid concentrations. In some individuals, the frequent consumption of high glycemic impact meals over time is associated with insulin resistance, β-cell dysfunction, dyslipidemia and endothelial dysfunction, all of which increase risk for obesity, metabolic syndrome, type-2 diabetes and cardiovascular disease (Eckel et al., 2005; Ludwig, 2002; Venn et al., 2007).
1.5.1 Glycemic index and glycemic load

The GI is a relative measure of the postprandial glycemia evoked by foods that are significant sources of carbohydrate (Jenkins et al., 1981). The GI of a food is determined as the incremental area under the curve (IAUC) for blood glucose response after the consumption of a test food containing a known carbohydrate portion expressed as a percent of the IAUC for the same amount of carbohydrate from a reference food (Standards Australia, 2007; ISO, 2010). The GI value also reflects the rate of glucose absorption after a meal, which has significant effect on postprandial hormonal and metabolic responses (Ludwig, 2002). Thus, the GI is a physiologically based measure that includes the digestion of the carbohydrate, absorption of glucose from the gut, and its clearance from the bloodstream during the test period. The Australian Standard “Glycemic Index of Foods” was published in 2007 to establish a recognized scientific method for determining the GI of foods (Standards Australia, 2007). In 2010, the International Standards Organisation published its definition of GI, along with the GI test method and the GI classification of foods as a new international standard (ISO standard 26642:2010).

As both quantity and quality of available carbohydrate foods affect the glycemic response, the concept of glycemic load (GL) was introduced in 1997 by nutritionists at Harvard University to quantify the overall glycemic effect, factoring in the portion size of the carbohydrate food (Salmeron, Manson, Stampfer, Colditz, Wing, & Willett, 1997). The GL of a portion of the food was therefore defined as the product of the amount of available carbohydrate in the food and the GI of the food divided by 100. By adding up the GL contributed by individual carbohydrate foods in the meal, the overall dietary GL can be calculated. The greater the GL, the higher is the increase in blood glucose and insulinogenic effect of the food (Bao, Atkinson, Petocz, Willett, & Brand-Miller, 2011).
1.5.2. Health implication of GI and GL

The use of GI in making carbohydrate food choices in conjunction with information on food composition was endorsed by the FAO in 1998 (FAO/WHO, 1998). In their 2007 update on “Carbohydrates in Human Nutrition”, GI was deemed most appropriately used to guide food choices when comparing similar types of carbohydrate foods (Mann et al., 2007). However, there is also controversy surrounding the GI concept and the use of GI claims for food labelling. In a position statement from Health Canada, the inclusion of the GI value on the label of eligible food products was stated to be potentially misleading and would not add value to nutrition labelling and dietary guidelines in assisting consumers to make healthier food choices (Aziz, Dumais, & Barber, 2013). The International Carbohydrate Quality Consortium (ICQC) responded with a letter refuting the arguments in Health Canada’s position statement based on a lack of understanding of the evidence base for current information on food labels and of the GI concept (Jenkins et al., 2014). The ICQC provided evidence for the inclusion of low GI and low GI dietary patterns in nutrition recommendations by various international diabetes associations, for example, the American Diabetes Association and The International Diabetes Federation and advised that every effort should be made to assist consumers in choosing carbohydrate foods that will not exacerbate post-prandial glycemia.

In light of these arguments the main concern with using only the GI to make carbohydrate food choices is that some low GI foods may be high in energy and contain high amounts of sugars or fat that could suppress the glycemic response (Mann et al., 2007). For our purposes, as fresh potatoes are naturally low in fat and sugars (Burlingame et al., 2009) and are a food choice that is minimally changed in composition by processing prior to consumption, the GI of potatoes should serve as a useful tool for ranking potato varieties.

1.5.3 Measuring the GI of foods

GI testing is an in vivo method involving the determination of blood glucose responses over a period of two hours in human volunteers after consuming either a test food or a reference food. Testing should be conducted in 10 or more volunteers of mixed gender and ethnicity without known food allergies or intolerances and not taking medications that affect glucose tolerance. The most accepted reference food is anhydrous glucose powder dissolved in
250 mL of water. White bread or rice is also used as reference foods but the final result must be converted to the glucose reference equivalent. Volunteers arrive in a fasting state and duplicate capillary (finger prick) blood samples are taken as an average baseline blood glucose concentration. Volunteers are then served the test food or reference food and asked to consume the food at an even pace within 12 min. Further blood samples are taken at 15, 30, 45, 60, 90 and 120 min. Either whole blood or plasma can be used for analysis of blood glucose concentration according to established enzymatic methods (Brouns et al., 2005; ISO, 2010). Data are graphed using either absolute blood glucose values or the change in blood glucose values from fasting value and calculated geometrically as described in the Australian Standard “Glycemic Index of Foods” (Standards Australia, 2007). Foods with a GI of above 70 are classified as high GI, foods with a GI of 56-69 as medium GI and foods that have a GI of 55 and less are classified as low GI according to the ISO standard (ISO, 2010).

1.5.4 Potato GI values

Potatoes were included in the listed values in the first GI paper published in 1981. They were described as “potato (new)” and had a high GI value of 70 (Jenkins et al., 1981). Subsequent studies on potatoes have resulted in a wide range of values from as low as 56 (Henry, Lightowler, Strik, & Storey, 2005) to high as 101 (Soh & Brand-Miller, 1999). Some values were determined in only a small number of healthy subjects (<10) or in subjects with impaired glucose tolerance. These values are all listed in the updated “International Tables of Glycemic index and Glycemic Load values: 2008” (Atkinson et al., 2008). The GI values of potatoes extend over a large range and vary depending on cooking method, processing and the meal composition (Atkinson et al., 2008; Fernandes, Velangi, & Wolever, 2005; Henry, Lightowler, Kendall, & Storey, 2006). For the purpose of this review, the list has been condensed into Table 1.2, which includes only those studies where the potato cultivar was specified and at least 10 healthy subjects were tested. Studies in which potato cultivars were not specified or were eaten with additional ingredients have not been included.
Table 1.2. GI of potato varieties

<table>
<thead>
<tr>
<th>Variety</th>
<th>GI</th>
<th>Preparation method</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Low GI (55 and under)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carisma&lt;sup&gt;a&lt;/sup&gt;</td>
<td>53 ± 7</td>
<td>Boiled pieces (8 mins)&lt;sup&gt;**&lt;/sup&gt;, served hot.</td>
</tr>
<tr>
<td><strong>Medium GI (56–70)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Marfona&lt;sup&gt;b&lt;/sup&gt;</td>
<td>56 ± 3</td>
<td>Boiled quarters (15 mins)&lt;sup&gt;**&lt;/sup&gt;, served hot.</td>
</tr>
<tr>
<td>Nicola&lt;sup&gt;c&lt;/sup&gt;</td>
<td>58 ± 3</td>
<td>Boiled whole (15 mins)&lt;sup&gt;**&lt;/sup&gt;, served hot.</td>
</tr>
<tr>
<td>Nicola&lt;sup&gt;b&lt;/sup&gt;</td>
<td>59 ± 7</td>
<td>Boiled quarters (15 mins)&lt;sup&gt;**&lt;/sup&gt;, served hot.</td>
</tr>
<tr>
<td>Estima&lt;sup&gt;b&lt;/sup&gt;</td>
<td>66 ± 5</td>
<td>Boiled quarters (15 mins)&lt;sup&gt;**&lt;/sup&gt;, served hot.</td>
</tr>
<tr>
<td>Charlotte&lt;sup&gt;b&lt;/sup&gt;</td>
<td>66 ± 5</td>
<td>Boiled quarters (15 mins)&lt;sup&gt;**&lt;/sup&gt;, served hot.</td>
</tr>
<tr>
<td><strong>High GI (above 70)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Russet Burbank&lt;sup&gt;e&lt;/sup&gt;</td>
<td>72 ± 9*</td>
<td>Baked whole (220°C for 55-60 mins). Stored cold for 1-5 days, microwave reheated and served.</td>
</tr>
<tr>
<td>Prince Edward Island&lt;sup&gt;a&lt;/sup&gt;</td>
<td>73 ± 5*</td>
<td>Microwaved whole (18 mins), rested for 5 mins, served hot.</td>
</tr>
<tr>
<td>King Edward&lt;sup&gt;d&lt;/sup&gt;</td>
<td>75 ± 10</td>
<td>Boiled quarters (15 mins)&lt;sup&gt;**&lt;/sup&gt;, served hot.</td>
</tr>
<tr>
<td>Russet Norkotah&lt;sup&gt;h&lt;/sup&gt;</td>
<td>77 ± 9*</td>
<td>Microwaved whole (18 mins), rested for 5 mins, served hot.</td>
</tr>
<tr>
<td>Desiree&lt;sup&gt;b&lt;/sup&gt;</td>
<td>77 ± 17</td>
<td>Boiled quarters (15 mins)&lt;sup&gt;**&lt;/sup&gt;, served hot.</td>
</tr>
<tr>
<td>Russet Burbank&lt;sup&gt;e&lt;/sup&gt;</td>
<td>79 ± 9*</td>
<td>Microwaved whole (18 mins), rested for 5 mins. Stored cold for 1-5 days, microwave reheated and served.</td>
</tr>
<tr>
<td>Pontiac&lt;sup&gt;d&lt;/sup&gt;</td>
<td>79 ± 9*</td>
<td>Microwaved whole (6–7.5 mins), served hot.</td>
</tr>
<tr>
<td>Asterix&lt;sup&gt;e&lt;/sup&gt;</td>
<td>79 ± 10*</td>
<td>Boiled whole (30 mins), served hot.</td>
</tr>
<tr>
<td>Maris Piper&lt;sup&gt;b&lt;/sup&gt;</td>
<td>85 ± 4</td>
<td>Boiled quarters (15 mins)&lt;sup&gt;**&lt;/sup&gt;, served hot.</td>
</tr>
<tr>
<td>Variety</td>
<td>Glucose Equivalent</td>
<td>Cooking Method</td>
</tr>
<tr>
<td>--------------</td>
<td>--------------------</td>
<td>--------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Sebago^d</td>
<td>87 ± 7*</td>
<td>Boiled whole or halved (35 mins)***, served hot.</td>
</tr>
<tr>
<td>Pontiac^d</td>
<td>88 ± 9*</td>
<td>Boiled whole or halved (35 mins)***, served hot.</td>
</tr>
<tr>
<td>Sava^h</td>
<td>89 ± 12*</td>
<td>Boiled whole (21-30 mins)**, stored cold for 24 hrs, served cold.</td>
</tr>
<tr>
<td>Pontiac^d</td>
<td>91 ± 9*</td>
<td>Boiled pieces (15 mins)***, mashed and served hot.</td>
</tr>
<tr>
<td>Estima^f</td>
<td>93 ± 8</td>
<td>Microwaved (6 mins) then oven baked at 200°C (10 mins), served hot.</td>
</tr>
<tr>
<td>Pontiac^d</td>
<td>93 ± 11*</td>
<td>Baked whole (190°C for 25 mins), served hot.</td>
</tr>
<tr>
<td>Maris Peer^b</td>
<td>94 ± 16</td>
<td>Boiled quarters (15 mins)***, served hot.</td>
</tr>
<tr>
<td>Russet Burbank^e</td>
<td>98 ± 8*</td>
<td>Microwaved whole (18 mins), rested for 5 mins, served hot.</td>
</tr>
<tr>
<td>Russet Burbank^a</td>
<td>101±13</td>
<td>Boiled pieces (8 min)**, served hot.</td>
</tr>
<tr>
<td>Desiree^d</td>
<td>101 ± 15*</td>
<td>Boiled whole or halved (35 mins)***, served hot.</td>
</tr>
<tr>
<td>Russet Burbank^e</td>
<td>104 ± 11*</td>
<td>Baked whole (220°C for 55-60 mins), served hot.</td>
</tr>
</tbody>
</table>

b. Henry et al., 2005.
c. Atkinson et al., 2008.
e. Fernandes et al., 2005.
f. Henry et al., 2006.
g. Leeman, Ostman, & Bjorck, 2008.
h. Leeman, Ostman, & Bjorck, 2005.

* Values converted from white bread reference to glucose reference by division by 1.4 (Wolever et al., 2003).
** Total cooking time includes time taken for water to reach boiling temperature.
*** Total cooking time excludes time taken for water to reach boiling temperature.
Most cultivars of potatoes fall into the medium or high GI range, with a few cultivars classified as low GI. When different cooking methods are compared, it is apparent that all medium and low GI values listed in Table 1.2 were obtained from potatoes boiled for 15 min or less, although some potato cultivars cooked in this way had a high GI. For example, a GI value of 56 for Marfona cultivar can be compared to a GI of 85 for Maris Piper variety. This large variation in GI values, which was observed in the same study (Henry et al., 2005), indicates inherent variance in GI values between potato cultivars.

As noted previously, potatoes are generally considered a high GI food, but because they have high water content (Burlingame, et al., 2009), they provide physical bulk to a meal. Holt et al. (1995) found potatoes to be one of the most satiating foods per 1000 kJ of energy among 40 common foods. In a comparison of potatoes with other carbohydrate staples, potatoes have lower available carbohydrate content per nominal serving than pasta and low GI rice, resulting in a similar glycemic load to pasta and low GI rice. While potatoes have a similar glycemic load to pasta and low GI rice based on serving size, on an isoenergetic basis (per 1000 kJ), potatoes have a higher GL (Table 1.3).

**Table 1.3.** Comparison of carbohydrate intake and glycemic load between staple carbohydrate foods

<table>
<thead>
<tr>
<th>Food</th>
<th>Glycemic index (GI)</th>
<th>Average serving size (g)</th>
<th>Available carbohydrate per serving (g)</th>
<th>Glycemic load per serving</th>
<th>Glycemic load per 1000 kJ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potatoes</td>
<td>56-102</td>
<td>150</td>
<td>26-32</td>
<td>18-26</td>
<td>44-64</td>
</tr>
<tr>
<td>Low GI breads</td>
<td>43-55</td>
<td>30</td>
<td>10-14</td>
<td>5-8</td>
<td>18-29</td>
</tr>
<tr>
<td>Pasta</td>
<td>52-55</td>
<td>180</td>
<td>45-46</td>
<td>23-25</td>
<td>27-29</td>
</tr>
<tr>
<td>Low GI rice</td>
<td>43-54</td>
<td>150</td>
<td>39-43</td>
<td>18-21</td>
<td>24-28</td>
</tr>
</tbody>
</table>

*Compiled from tables by Atkinson et al., 2008 and Holt et al., 1995.*
1.5.5 Effect of processing on GI values

The two main food factors that lower postprandial glycemia act by affecting the rate of gastric emptying and/or the rate of carbohydrate digestion and absorption. These factors can be a natural quality of the food, such as the high beta-glucan content of barley (Granfeldt, Liljeberg, Drews, Newman, & Bjorck, 1994) or the high amylose nature of legume starches (Guillon & Champ, 2002). Food processing, for example the extent of milling of grains and legumes, can increase the access of enzymes and water for gelatinization and in turn, the rate of starch digestion (Berg, Singh, Hardacre, & Boland, 2011). The effect on GI of meal preparation and way in which potatoes are consumed are also relevant and are described subsequently.

There are many variations in the methods used to cook potatoes. They may be cut into pieces or cooked whole, using methods that employ boiling in water, baking in the oven, frying in oil or microwave radiation and cooking times that range from 10 to 60 min. These cooking factors are important in determining the extent of starch gelatinization, and hence digestion. Soh et al. (1999) investigated the effect of cultivar and cooking method on the GI of potatoes and found all of the studied cultivars to have high GI values. One of the cultivars (Pontiac) was tested after boiling, baking, mashing or microwave cooking, and all of the GI values were in the high range. There was little difference in the GI value when the same variety of potatoes was boiled and mashed versus boiled and cut into large pieces (Soh et al., 1999). Likewise, in another study using Nicola potatoes, different industrial processes were used to cook and process the potatoes, high GI values were recorded regardless of processing method (Tahvonen, Hietanen, Sihvonen, & Salminen, 2006).

One preparation method that has been shown to significantly reduce the GI of potatoes is to serve them cold. Refrigeration at 8°C for 24 hours reduced the GI value by 26% compared to the same potatoes served freshly cooked and warm (Leeman et al., 2005). In this study, resistant starch content increased only from 3.3% to 5.2% (starch basis), and from this relatively small increase, it could be inferred that cold storage converts some of the rapidly digested starch into slowly digested starch. Tahvonen et al. (2006) also found
that the GI of potatoes after cold storage and reheating was lowered about 25%. A study by Fernandes et al. (2005) showed that reheating cooled Russet Burbank potatoes before serving resulted in a lower GI value (70–80) compared to a GI of about 100 for the freshly cooked, hot potatoes. This effect was observed for both baked and microwave-cooked potatoes.

The presence of some additives, for example acetic acid (vinegar), in the test meal reduced postprandial glycemia by reducing the rate of gastric emptying (Liljeberg, & Bjorck, 1998). The addition of vinegar and olive oil in the form of a vinaigrette dressing to cooked and cooled Sava potatoes (for example a potato salad) reduced the GI by 43% compared to the boiled potatoes served hot, whereas refrigeration alone reduced the GI by 26% (Leeman et al., 2005). A UK study using common toppings on baked potatoes found that the co-ingestion of fat lowered the GI of potatoes (Estima) by 58%, changing the GI classification from high GI to low GI whereas co-ingestion of protein only lowered the GI of potatoes by 18% with the classification remaining as high GI (Henry et al., 2006).

1.6 In vitro methods for starch digestion

*In vitro* methods to measure the rate of starch digestion were introduced as an alternative to *in vivo* testing of the glycemic response to carbohydrate foods. GI testing is labour and time intensive as human subjects need to be recruited and screened. The reference and test foods have to be consumed on different days, requiring several days for the test to be completed. This results in higher costs and a delay for results to be available. With the introduction of *in vitro* testing, a new classification of starch was proposed for nutritional purposes: rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS). Most starchy foods contain these fractions in different proportions (Englyst et al., 2007) and *in vitro* methods are designed to measure them accordingly.
1.6.1 Principles and techniques

In vitro studies are designed to simulate human digestion. The physico-chemical properties of a carbohydrate food are described by measuring the rate and extent of glucose release by enzymatic digestion under controlled conditions (Englyst, Englyst, Hudson, Cole, & Cummings, 1999). Further development of in vitro techniques include chewing of test foods by subjects rather than mechanical homogenisation of foods (Akerberg, Liljeberg, Granfeldt, Drews, & Bjorck, 1998; Granfeldt, Bjorck, Drews, & Tovar, 1992), the use proteolytic enzymes in addition to amylases (Goni, Garcia-Alonso, & Saur-Calixto, 1997) and dialysis tubing to imitate the small intestine (Granfeldt et al., 1992). Granfeldt et al. (1992) derived a hydrolysis index (HI) by calculating the area under a hydrolysis curve from plotting the rate of glucose released over a period of 180 min using white wheat bread as a reference. HI was then compared to GI values determined by the in vivo method for the same foods and a predicted GI equation was formulated. Researchers found a correlation of $r^2 = 0.769$ for 17 of 21 food samples tested, which consisted mainly of legumes and cereal products. Subsequent studies have also used HI and other equations to predict GI for in vitro digestion rates (Garcia-Alonso & Goni, 2000; Goni et al., 1997; Leeman et al., 2005).

A recent development in GI prediction was the creation of an artificial neural network designed to predict the GI of unknown food samples. The method used foods with known GI values and tested them using an in vitro method simulating human digestion under both stomach and small intestine conditions. The digestate was analyzed for glucose, fructose, sucrose, lactose, galactose, and maltitol using HPLC (high performance liquid chromatography). These results were combined with nutritional information (protein, fat and total dietary fibre content) of the test food and reported or tested in vivo GI values were used as the calibration set of data. The sample set consisted of 72 food types and a correlation of $r^2 = 0.93$ was obtained, indicating a good predictive ability of the method (Magaletta, DiCataldo, Liu, Li, Borwankar, & Martini, 2010).
1.6.2 *In vitro* potato digestion studies

Potato starch in its raw form is highly resistant to enzymatic hydrolysis but is rendered rapidly digestible by conventional cooking methods. On cooking, raw potato starch fractions change from a high RS content of about 75% of total starch to only 1% on cooking, while RDS changes from less than 10% to almost 100%. RS levels have been found to increase on cooling but after reheating the potato becomes readily digestible again (Englyst, Trowell, Southgate, & Cummings, 1987; Kingman & Englyst, 1994). Monro *et al.* (2009) measured RDS, SDS and RS content in nine New Zealand cultivars (Draga, Frisia, Nadine, Desiree, Karaka, Moonlight, Agria, White Delight and Fronkia) immediately after 30 min of boiling, and after the cooked potatoes were stored at 4°C for 44 hours. Immediately after boiling, the potatoes had a profile of 62–73% RDS, 0–9% SDS and 3–7% RS whereas after cool storage, the profile changed to 33–53% RDS, 15–34% SDS and 5–11% RS. The increase in RS formed on cooling was attributed to retrograded amylose.

In the literature, studies reporting a low GI value for potatoes using *in vitro* testing methods were not found and most reported results for potatoes were a high predicted GI or HI. Leeman *et al.* (2005) used the HI method of Granfeldt *et al.* (1992) to test the predicted GI of six different potato cultivars (Asterix, Bintje, King Edward, Frieslander, Platina and Rocket), which were boiled. They found that the predicted GI was high for all of the cultivars irrespective of tuber size. One of the cultivars (Asterix) was also tested under conditions favouring amylose retrogradation (24 hours at 6°C followed by 24 hours at 70°C) and increase RS content. The HI and predicted GI were high, irrespective of the RS content. Other potato cultivars such as Marfona, Maris Piper, Belle de Fontenay and Desiree potatoes were analyzed using the technique of Englyst *et al.* (1992). Digestion rates after processing by different cooking methods (boiling, frying, oven & microwave baking) showed no differences between cultivars and between processing methods. All samples had a high starch digestion rate (Kingman *et al.*, 1994). In another *in vitro* study a variety of potato products, fresh potatoes, instant mash, crisps and potato flour were tested and all the samples had a high estimate for the GI value (Garcia-Alonso *et al.*, 2000).
1.6.3 Comparisons with in vivo studies

The in vitro method has been proposed as an alternative method for classifying carbohydrates with some studies showing correlation of results with in vivo GI testing (Araya, Contreras, Alvina, Vera, & Pak, 2002; Englyst et al., 1999; Granfeldt et al., 1992). However, only a few foods have been subjected to both in vitro and in vivo testing for comparison. In vitro methods are still being developed; there is no standard protocol for testing, and different models are used to predict GI (Garcia-Alonso et al., 2000; Granfeldt et al., 1992; Leeman et al., 2005). Table 1.4 gives a comparisons between in vivo and in vitro methods.

Table 1.4. Comparison of GI testing and in vitro digestion studies.

<table>
<thead>
<tr>
<th>Testing method</th>
<th>GI</th>
<th>in vitro digestion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time required</td>
<td>Slow (days to weeks).</td>
<td>Rapid (hours)</td>
</tr>
<tr>
<td>Labour</td>
<td>Higha</td>
<td>Low</td>
</tr>
<tr>
<td>Costs</td>
<td>Higha</td>
<td>Low</td>
</tr>
<tr>
<td>Variability</td>
<td>High inter and intra individual variabilityb</td>
<td>Controlled test conditions, easily replicated.</td>
</tr>
<tr>
<td>Representative of physiology</td>
<td>Good</td>
<td>Does not consider gastric emptying and the heterogeneous environment of the gutc.</td>
</tr>
<tr>
<td>Standard for testing</td>
<td>Established ISO methodd and Australian standarde</td>
<td>No standard testing protocol established.</td>
</tr>
<tr>
<td>Other factors</td>
<td>Comprehensive database of GI values availablef.</td>
<td>Can be used to test novel and genetically modified foods.</td>
</tr>
</tbody>
</table>

a. Brouns et al., 2005
b. Pi-Sunyer, 2002
c. Araya et al., 2002
d. ISO, 2010
e. Standards Australia, 2007
f. Atkinson et al., 2008
It is noteworthy that in the *in vitro* studies, all potatoes tested had a high HI, and consequently the predicted GI values were high, regardless of cultivar, preparation and cold storage time. This is in contrast to *in vivo* testing where cultivars tested in the same study were classified into different GI rankings. This may relate to the difference between *in vivo* methods, which reflect a physiological process that includes starch breakdown, glucose absorption into and clearance from the bloodstream, and *in vitro* methods, which measure physico-chemical processes of starch comminution and hydrolysis. *In vivo* and *in vitro* studies of starch digestion are complementary rather than alternatives, as sometimes implied.

Although *in vitro* studies may not be a replacement for *in vivo* studies, they offer considerable benefits in the speed of testing, the potential to use controlled conditions and the freedom to test novel foods and ingredients. *In vitro* tests should prove valuable in the search for low GI potato varieties by affording high-throughput means to screen germplasm. The complementary application of these two approaches should provide a clearer understanding of potato digestion.

1.7 Conclusions

Potatoes are a popular carbohydrate food in many parts of the world, particularly the western world and increasingly in developing countries. The genetic pool of potatoes is vast and with four species and thousands of varieties there is much untapped potential. The main nutrient in potatoes is starch. Potato starch has low digestibility in the raw form but is rapidly digested once gelatinized. *In vitro* starch digestion methods with whole potato tissue show that in the raw form potatoes are high in RS and low in RDS. This is reversed when potatoes are cooked, with most of potato starch converted to RDS. Physiological studies show that most potatoes are high GI regardless of cooking method. Cold storage and the addition of ingredients may lower the GI of potatoes but restrict the form in which they can be consumed. However, a summary table of tested cultivars show that there may be exceptions. The importance and popularity of potatoes as a food crop indicates a need to develop cultivars that are digested slowly and have a low GI, regardless of preparation method. As shown from the wide range of GI values, the development of potato cultivars with low GI is not an unrealistic proposition.
1.8 Aims of the project

The aim of this project is to identify a low GI cultivar of potato amongst a selection of well-established and new commercial potato cultivars. More specifically, the objectives of the project were:

i. To investigate the cell structure and starch granule morphology of the low GI potato cultivar in comparison to high GI cultivars of potatoes and to examine the effect of cooking on these features;

ii. To characterize the chemical composition and functional properties of starch from a low GI potato compared to starch from high GI cultivars;

iii. To study changes to starch properties when the low GI cultivar is grown under different environmental conditions.

iv. To propose selection criteria for the identification of more low GI cultivars based on the results from this research project.
CHAPTER 2

MATERIALS AND METHODS
2.1 Materials

2.1.1 Potato samples

Seven potato cultivars comprising a mix of well-established and newly developed commercial cultivars were used in the study. The selection of the cultivars was made in consultation with potato breeders from Agrico Holland. The Carisma, Desiree and Virginia Rose cultivars were sourced from The Mitolo group in South Australia while Bintje, Maiflower, Nicola and Russet Burbank cultivars were sourced from Agronico in Tasmania. Potato starch from the Carisma, Nicola and Russet Burbank cultivar grown in The Netherlands was obtained from Agrico Holland.

2.1.2 Chemicals and Reagents

Chemical, reagents, solvents and solutions used in this study were all analytical grade. Fluorescent dye Rhodamine-B was purchased from Life Technologies (Carlsbad, CA, USA). Potato amylose (A0512), potato amilopectin (A8515), pancreatic α-amylase (150 U/mg, Sigma A3176) and porcine heparin sodium salt (180 U/mg, Sigma H3393) were purchased from Sigma Chemical Co. (St Louis, MO, USA). Total starch, maltose, sucrose, D-glucose and total dietary fibre assay kits, amylglucosidase (3,260 U/mL), isoamylase (280 U/mg) and glucose oxidase-peroxidase reagent were purchased from Megazyme International Ireland, Ltd. (Bray Co., Wicklow, Ireland).

2.1.3 Water used for solutions

Water purified by an Arium® 611UF Ultrapure Water Purification System (Sartorius, Goettingen, Germany) with a resistivity up to 18.2 μS·cm⁻¹ was used to make all solutions.
2.1.4 Buffers

Sodium acetate buffer (100 mM, pH 5.0) was prepared by dissolving 5.8 mL of glacial acetic acid in 900 mL of deionized water. This solution was adjusted to pH 5.0 by the addition of 1 M NaOH solution. Calcium chloride dihydrate (0.74 g) was added for preservation and the volume was adjusted to 1 litre.

MES/TRIS buffer (0.05 M, pH 8.2) was prepared by dissolving 19.52 g 2(N-morpholino) ethanesulfonic acid (MES) (Sigma M8250), and 14.2 g tris (hydroxymethyl) aminomethane (TRIS) (Sigma T1503) in 1.7 L of deionised water. This solution was adjusted to pH 8.2 by the addition of 6.0 M sodium hydroxide solution. This was then diluted to a volume of 2 L with the addition of deonized water.

2.2 Methods

2.2.1 Potato dry matter content

Fresh potatoes were peeled, cut into small pieces and homogenised in a blender. The homogenate was frozen at -80°C for at least 24 hours and freeze-dried for 48 hours. Duplicate samples were prepared and weighed prior to and after freeze-drying for dry matter content determination. Freeze-dried material was ground into a powder and stored in desiccators for total starch and total dietary fibre determination. The dry matter content was calculated as follows:

\[
\% \text{ Dry matter} = \frac{B - A}{B} \times 100,
\]

where A = moisture loss in grams, B = original weight of sample.
2.2.2 Total starch content

Total starch content was determined using the Total Starch Megazyme Assay Kit according to the method described in the procedure manual by Megazyme International Ireland, Ltd. (Bray Co., Wicklow, Ireland). Duplicate samples of ground potato dry matter (100 mg) from each cultivar were dispersed in aqueous ethanol (80% v/v) and incubated with thermostable α-amylase followed by amyloglucosidase. The glucose concentration was estimated from the absorbance after addition of GOPOD reagent at 510 nm, using a D-glucose standard solution (1 mg/mL). Percentage total starch was calculated on a dry weight basis and converted to percentage starch in fresh potatoes.

2.2.3 Reducing sugar content

Reducing sugar content was assayed in potato juice extracted from peeled and homogenised fresh potatoes. The juice was filtered and diluted 1 in 10 with deionized water, then frozen and stored at -20°C prior to the assay. Reducing sugars were measured on triplicate samples using the Maltose, Sucrose and D-glucose Megazyme assay kit according to the method described in the accompanying manual (Megazyme International Ireland Ltd. Bray Co., Wicklow, Ireland). Samples were thawed immediately prior to analysis and the addition of α-glucosidase, β-fructosidase was used for maltose and sucrose content determination respectively. Hexokinase and glucose-6-phosphate dehydrogenase were added to form NADPH and gluconate-6-phosphate. Absorbance at 340 nm was measured to estimate NADPH concentration, with NADPH content in proportion to sucrose and D-glucose content and half the maltose content.

2.2.4 Total dietary fibre content

Total dietary fibre content was determined using the Total Dietary Fibre Megazyme Assay Kit using the method described in the procedure manual by Megazyme International Ireland, Ltd. (Bray Co., Wicklow, Ireland). Duplicate samples of ground potato dry matter from each cultivar were dispersed in MES-TRIS buffer solution and incubated with thermo-stable
α-amylase at 95–100°C for 35 min. This was followed by incubation with protease at 60°C for 30 min. The pH of the digestate was lowered to 4.1–4.8 by the addition of 0.6 M HCl before incubation with amylglucosidase at 60°C for 30 min. Soluble fibre was precipitated at room temperature for 60 min after the addition of hot (60°C) 95% ethanol. The residue was filtered under vacuum through a crucible containing celite and washed with 78% ethanol, 95% ethanol, and acetone. The crucible containing the residue was dried overnight in a 103°C oven, cooled and weighed. Total dietary fibre content was calculated as % w/w dry weight basis, and converted to fresh weight basis.

2.2.5 Potato starch extraction

Starch was extracted from each potato cultivar according to the method of Noda et al. (2004) with modifications, as follows. Tubers (1 kg) were peeled and homogenized at room temperature (20–22°C) in a blender with 1400 mL of distilled water. The slurry was filtered successively through muslin and 250 μm and 100 μm sieves. The filtrate was allowed to settle overnight at 4°C and the supernatant removed by decantation. The sedimented starch was washed three times with deionised water by re-suspension and centrifugation (1000 g, 5 min). The granules were collected on filter paper with suction, washed with ethanol, dried under a gentle air stream and stored in sealed containers at room temperature until used for analyses.

2.2.6 Starch moisture content determination

The moisture content of extracted starch was determined using the AACC-I method 44–15A (American Association of Cereal Chemists–International, 2000). Duplicate samples of approximately 2 g of starch from each cultivar were placed in pre-weighed aluminium dishes and dried at 135°C in an oven for 2 hours. The dishes were reweighed after drying and the moisture content was calculated as follows:

\[
\% \text{ Moisture} = \frac{A}{B} \times 100,
\]

in which \(A\) = moisture loss in g, \(B\) = original weight of sample.
2.2.7 Amylose content of starch by iodine-binding method

Total amylose content of the potato starches was determined by the iodine binding method of Chrastil (1987) with modifications, as follows. Starch (20 mg) was solubilised overnight in 1.0 M NaOH solution (2.0 mL). Deionised water (4.0 mL) was added and the samples were heated at 95°C for 30 min with frequent vortexing. An aliquot (0.1 mL) of each solution was added to 5 mL of 0.5% trichloroacetic acid in a separate test-tube, the tubes were vortexed, followed by the addition of 0.05 mL of iodine solution (1.27 g I₂ + 3.0 g KI made up to a litre). The absorbance was read at 620 nm after 30 min of incubation at 25°C. A standard curve of 10, 20, 40, 50 and 60% potato amylose mixed with potato amyllopectin (A0512 and A8515 from Sigma Chemical Co. St. Louis, MO, USA) was constructed for the estimation of potato amylose content.

2.2.8 Phosphorus content determination

Phosphorus content was determined according to the method of Morrison (1964). Starch samples (20 mg) were weighed into 30 mL micro-Kjeldahl flasks and digested with 2.2 mL of concentrated H₂SO₄. The flasks were heated gently over a micro burner flame until charring was completed. Hydrogen peroxide (30% w/v) solution was added drop-wise to the hot acid, with thorough shaking between the additions of each drop until the solution was clear. After further heating for 1 min, the flasks were cooled and the digests transferred to 100 mL beakers with several rinses of water. Sodium sulphite solution (1 mL; 33% w/v) was added and mixed, followed by 10 mL of ammonium paramolybdate (2% w/v) solution and 1 g of ascorbic acid. The beakers were gently swirled to dissolve the ascorbic acid and the solution boiled for 1 min. On cooling, the solutions were transferred to 50 mL volumetric flasks and adjusted to volume with deionised water. The absorbance was read at 822 nm and a standard curve consisting of 0, 0.1, 0.2, 0.3 and 0.4 μg phosphorus/mL was constructed using a phosphorus standard solution (NaH₂PO₄·H₂O).
2.2.9 Amylopectin chain length distribution (HPAEC-PAD)

The amylopectin chain length profile was determined using high performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD), according to the method of Liu et al. (2007). Starch samples (10 mg) were dispersed in 2 mL of 90% DMSO with stirring in a boiling water bath for 20 min. The samples were cooled, 6 mL of methanol was added with vortexing and placed in an ice bath for 30 min. Samples were centrifuged at 1000 g for 12 min and the pellet was dispersed in 2 mL of 50 mM sodium acetate buffer (pH 3.5) and placed a boiling water bath for 20 min with stirring. Samples were cooled to 37°C before the addition of 5 μL of isoamylase (280 U/mg, Megazyme International Ireland Ltd. Bray Co., Wicklow, Ireland) and further incubated at 37°C for 24 hours in a shaking water-bath (100 strokes/min). Enzymes were inactivated by placing samples in a boiling water bath for 10 min and an aliquot (200 μL) from the de-branched samples was diluted with 2 mL of 150 mM NaOH, filtered (0.45 μm nylon syringe filter) and injected into the HPAEC-PAD system (5 μL sample loop) (Dionex Corporation, Sunnyvale, CA, USA). The HPAEC-PAD system consisted of a Dionex HPLC equipped with an ED50 electrochemical detector with a gold working electrode, P680 HPLC pump, TCC-100 column oven, and ASI-100 automated sampler (Dionex Corporation, Sunnyvale, CA, USA). The standard triple potential waveform was employed, with the following periods and pulse potentials: T1=040 s, with 0.20 s sampling time, E1 = 0.05 V; T2 = 0.20 s, E2 = 0.75 V; T3 = 0.40 s, E3 = -0.15 V. The Dionex CarboPac™ PA1 column with gradient elution (-5 to 0 min, 40% A; 5 min, 60% A; 45 min, 80% A) at a column temperature of 26°C and a flow rate of 1 mL/min (0.5 Hz) was used. Data were collected using Chromeleon software, version 6.80 (Dionex Corporation, Sunnyvale, CA). The weight fractions of DP 6-13, 14-18, 19-37, 38-60 were quantified based on the area of peaks. Standards were prepared by dissolving 0.5-1.0 mg from a Shodex STANDARD P–82 kit (Showa Denko K.K. Shodex Group, Kawasaki, Kanagawa, Japan) in distilled water to make 0.1–0.5% solutions.
2.2.10 Starch pasting properties

Starch pasting properties were analyzed using a Rapid Visco Analyser RVA-4 (Newport Scientific) according to the AACC-I method 76-21 (American Association of Cereal Chemists-International, 2000a). Starch samples and deionised water (8% dry starch basis, total weight of 28 g) were weighed directly into a test canister and the mixture was agitated by stirring using the plastic paddle before the canister was inserted into the instrument. The starch suspension was stirred at 960 rpm for the first 10 s then decreased to 160 rpm for the remainder of the experiment. Samples were equilibrated at 50°C for 1 min then heated at 6°C/min to 95°C, held at 95°C for 5 min before cooling at 6°C/min back to 50°C and held for 2 min. Peak viscosity, trough viscosity and final viscosity were recorded, breakdown (peak minus trough viscosity) and setback (final minus trough viscosity) were calculated using Thermocline software.

2.2.11 Differential scanning calorimetry (DSC)

Thermal measurements were made using a Modulated Differential Scanning Calorimeter MDSC 2920 instrument (TA Instruments Inc., Delaware, USA) equipped with a thermal analysis data station and data recording software. The procedures for DSC measurements and analysis of the thermal transition parameters were according to the method by Wang et al. (2011). Approximately 3 mg of starch from each cultivar was weighed accurately into an aluminium sample pan. Water was added to the starch sample with a microsyringe to obtain a starch:water ratio of 1:2 (w/w) and the pan was hermetically sealed. The sample pans were reweighed and allowed to stand for 24 h prior to analysis. The pans were heated from 30 to 95°C at a rate of 10°C/min with the sample chamber flushed with dry nitrogen to avoid moisture condensation. An empty pan was used as a reference. The instrument was calibrated using indium as a standard. Thermal transition temperatures, onset \( T_o \), peak \( T_p \) and conclusion \( T_c \) temperatures were determined from the thermograms by means of the Universal Analysis 2000 software. The enthalpy change of the thermal transition \( \Delta H \) was determined by integrating the area between the thermogram and a base line under the peak and expressed as Joules per gram dry starch (Figure 2.1). The average values of the thermodynamic parameters were determined from duplicate measurements.
Figure 2.1: DSC thermal transition profile potato starch-water system (water-starch = 2:1) with a heating rate of 10°C/min. The thermal transition temperatures are indicated as $T_o$, $T_p$, $T_c$.

2.2.12 Starch crystallinity

Relative crystallinity of starch was measured using a DiffracTech Mini Materials Analyser X-ray diffractometer (GBC Scientific Equipment Pty. Ltd.) according to the method by Wang et al. (2009). The X-ray generator was equipped with a cobalt anode ($\lambda = 1.78897$ Å) operating at 1 kW and 3.36 mA. All starch samples were kept at constant humidity (75%) in a desiccator over a saturated NaCl solution for a week prior to analyses. X-ray diffractograms were acquired at room temperature ($20 \pm 1^\circ$C), the scattering intensity was measured from 4° to 30° as a function of 2$\Theta$ and at a scanning speed of 0.5°/min and a step size of 0.02°. Traces software v. 6.7.13 (GBC Scientific Equipment Pty. Ltd.) was used to subtract the background representing the amorphous portion of diffractograms. Relative crystallinity was calculated as a ratio of the crystalline area to the total area between 4 to 30°(2$\Theta$) (Nara & Komiya, 1983) (Figure 2.2).
Figure 2.2: X-ray diffractogram (XRD) of potato starch. $\alpha_c$ and $\alpha_a$ indicate the crystalline and amorphous portions of the XRD respectively.

2.2.13 Glycemic Index (GI) determination

2.2.13.1 GI testing subjects

Healthy subjects were recruited among students at the University of Sydney. A total of 27 subjects (12 males and 15 females) were recruited over a 1 y period and potato cultivars were tested in sub-groups of 10 subjects. The mean age was 27.0 ± 5.1 (range 18–38), body mass index (BMI; in kg/m$^2$) was 22.3 ± 2.0, and fasting plasma glucose was 5.32 ± 0.31 mmol/L. The inclusion criteria were as follows: non-smoking, 18–40 y of age, stable body weight, BMI of 19 to 25, normal glucose tolerance, no food allergy or intolerance and not taking medications known to affect glucose tolerance. On average, each subject tested 4 cultivars in random order separated by 3–5 days. This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the Human Research Ethics Committee of the University of Sydney. Written informed consent was obtained from all subjects.
2.2.13.2 Test foods

The seven potato cultivars were prepared for GI testing according to the method provided by the producers of the cultivars. Potatoes were peeled, cut into 5 cm by 5 cm slices, at a thickness of 0.5 cm, added to excess water at room temperature and brought to boil. The potatoes were cooked in boiling water for a further 4 min, drained, cooled by rinsing in tap water and served immediately along with 250 mL of water (total cooking time 8–9 min). The testing volunteers provided feedback that Carisma, Nicola and Virginia Rose cultivars were considered cooked and firm, whereas the other four cultivars were regarded as softer. Glucose solution was used as the reference food and tested on 3 separate occasions; each potato cultivar was tested once according to the ISO standard (ISO, 2010). The test and reference foods were fed as a portion providing 25 g of carbohydrate determined as the sum of starch plus sugars by direct measurement.

2.2.13.3 GI testing procedures

The study protocol used was in accordance to the ISO standard for the determination of glycemic index (GI) of foods (ISO, 2010). Subjects were instructed to refrain from unusual physical activity, consumption of alcohol and legumes, and to eat a high-carbohydrate, low-fat dinner meal on the day before a test. On the test morning, subjects arrived at the metabolic kitchen after a 10–12 h overnight fast. After warming a hand in hot water, 2 baseline finger-prick blood samples (0.5 mL each) were collected 5 min apart. Subjects then consumed the reference or test food at a comfortable pace within 12 min. The portion of potatoes fed was adjusted so that each contained 25 g of glycemic carbohydrate. Additional finger-prick capillary blood was collected 15, 30, 45, 60, 90, and 120 min after eating commenced. The subjects remained seated throughout and were not permitted to eat or drink until the end of the session. Blood samples were collected into anticoagulant-coated tubes (Eppendorf tubes, grade II; Sigma Chemical Company, Castle Hill, Australia) containing 10 IU heparin sodium salt and centrifuged immediately (10,000 x g for 1 min at room temperature). The plasma layer was pipetted into a labelled tube and stored at -20°C.
until analyzed. Plasma glucose was analysed with the glucose hexokinase enzymatic assay on a centrifugal analyzer (model HITACHI 912; Hitachi, Tokyo, Japan). The mean within-assay and between-assay precisions (CVs) were both < 6%.

2.2.13.4 GI calculations

The incremental area under the curve (IAUC) was calculated using the trapezoidal method (ISO, 2010). The GI of each potato cultivar was defined as the IAUC of the blood glucose response curve of a 25 g glycemic carbohydrate portion expressed as a percentage of the response to the same amount of carbohydrate from the reference food. The GI was calculated from the two incremental glucose areas using glucose as a reference (i.e., GI of 100). One-way ANOVA was applied to compare the final mean GI of the different cultivars.

2.2.14 In vitro enzymatic digestibility procedure

In vitro digestion of starch from cooked potatoes was determined in duplicate samples by a modified Englyst procedure (Englyst et al., 1992). Enzyme solution was prepared by suspending 0.45 g of porcine pancreatic α-amylase (150 U/mg, Sigma A3176) in 16 mL of water with magnetic stirring for 10 min at 37 °C. The mixture was centrifuged (1,500 × g for 10 min) and 2 mL of amyloglucosidase (3,260 U/mL, Megazyme) was added to 10.8 mL of the above enzyme supernatant. Preparation and cooking procedures of potatoes were the same as used in GI testing. After cooking, the potato pieces were mashed evenly with a spatula, and an amount of mash containing 100 mg starch (dry weight) was dispersed in 4 mL of 0.1 M sodium acetate buffer (pH 5.2). After adding 1 mL of the freshly prepared enzyme solution, the mixture was incubated in a shaking water bath (37°C, 160 strokes/min). Aliquots (0.1 mL) were taken at the same time intervals as for GI testing and mixed with 1 mL of 95% ethanol. The glucose released was measured by the glucose oxidase-peroxidase reagent according to the supplier instructions (Megazyme International Ireland Ltd. Bray Co., Wicklow, Ireland).
2.2.15 Microscopy

2.2.15.1 Light microscopy of raw and cooked potato tissue

Images were acquired with a Leica DM 2500M light microscope. Raw potato samples were sliced using the Leica RM 2255 microtome (Leica, Germany) into 100 μm sections, mounted on glass slides and imaged. Potato samples were cooked using the same method as for GI testing, sliced using a razor, mounted on a glass slide and imaged. Multiple micrographs of each sample under the given conditions were collected and examined.

2.2.15.2 Micro-CT of raw and cooked potato tissue

Images of raw and cooked potato samples were acquired with an Xradia MicroXCT-400. Potato samples were cooked using the same method as for GI testing. Excised raw and cooked potato pieces were submerged in 5% Lugol’s solution for 3 hrs, enclosed in a 0.5 mL centrifuge tube and placed in the MicroXCT. The following settings were used for imaging: 70 kV, 7 W, 4x magnification detector, 10 sec exposure, 911 images, slow readout, detector 18.4 mm from sample, source 115 mm from sample, pixel size 5.7 μm, reference image taken every 200 images with 10 averages, total scan time 5 hrs. The resulting data files were reconstructed using Xradia XMReconstructor 7.0 software and exported as a series of raw axial .tif files using the Xradia XM Controller 7.0 software. 3-D datasets were visualised and analysed using VGStudio MAX 2.0 software.

2.2.15.3 Environmental scanning electron microscopy (ESEM)

Images of potato cells and starch granules were acquired using a FEI Quanta 200 3D scanning electron microscope in ESEM mode with a beam voltage of 20 kV, a working distance of 5.0–6.9 mm, at a temperature of 5°C and a gaseous vapour pressure of 3.5 to 5.0 Torr. A thin section of potato tissue from each cultivar was sliced from fresh potatoes using a razor, mounted on a stub with a drop of distilled water and placed in the microscopy chamber. Multiple micrographs of each sample under the given conditions were collected and examined.
2.2.15.4 Particle size analysis using LM and ImageJ software

Particle size of starch granules was quantified as starch surface area. Starch granules (20 mg) were dispersed in 1 mL of deionised water and a few drops of the suspension were placed on a microscope slide with a coverslip and sealed using nail varnish. Images were obtained using a Leica DM 2500M light microscope (Leica, Germany). Five slides were prepared of starch from each potato cultivar and three micrographs were obtained from each slide. Three micrographs were selected randomly from the total of 15 micrographs collected per cultivar giving a triplicate measurement of starch surface area. The micrographs were converted into binary images, a scale in μm was set and granule surface area was measured using ImageJ 1.43u (Rasband, W.S., ImageJ. U. S. National Institutes of Health, Bethesda, Maryland, USA. http://imagej.nih.gov/ij/, 1997-2012) (Figure 2.3). The output was copied into Excel spreadsheets for data analysis. Classification of the size of starch granules according to surface area was: small (< 500 mm²), medium (500-1000 mm²) and large (> 1000 mm²).

MATERIALS AND METHODS
Figure 2.3: Particle size determination of starch granules by image analysis. Light micrographs of starch granules of cultivar Russet Burbank (A) were converted in binary images (B) and the surface area of the granules was estimated using ImageJ software (C). The scale bars represent 100 μm.
2.2.15.5  Confocal laser scanning microscopy (CLSM)

Images of potato starch granules were acquired using a Leica 5-D TCS SP II confocal laser scanning microscope with an inverted microscope (Leica, Germany) was used in a single-photon mode with an Ar/Kr laser. The Leica objective lens used was HCX PL APO CS 63.0 x 1.30 GLYC 21°C UV. A stock solution of 20% concentration rhodamine-B fluorescent staining agent was prepared by dissolving the appropriate amount in deionised water. Starch granules (2mg) were dispersed in 200 μL of rhodamine-B and stored for 24 h at room temperature before analysis. Samples were rinsed three times using deionised water, 20% glycerol in PBS was added and a drop was placed on a glass slide, covered with a microslip and sealed with nail varnish. A 561 nm laser line was used for excitation and an emission bandwidth 569 – 653 nm was detected. A laser power of 10% was maintained during acquisition of all images, and the gain was varied to prevent saturation of the detector and to ensure comparable fluorescence intensities in all the images. Digital images were saved in multiple .tif formats and in 1024 x 1024 pixel resolution. Multiple images of each sample under the given conditions were collected and examined.

2.2.16  Statistical analysis

Statistical analysis of the data in this thesis was performed using the SPSS V. 20 software (SPSS Inc., Chicago, IL, USA). All analyses were performed on duplicate samples except relative crystallinity determination, which was done as a single test, and granule size analysis and reducing sugars content which was performed in triplicate. One-way ANOVA by Duncan’s test (p<0.05) was used to compare differences between cultivars. Inter- and intra-subject variation of three standard (glucose) tests were assessed by determining the CV. Correlation analyses were performed using the PASW statistical package (version 18.0; SPSS Inc., Chicago, IL).
MATERIALS AND METHODS
CHAPTER 3

DISCOVERY OF A LOW GLYCEMIC INDEX POTATO AND RELATION BETWEEN IN VITRO STARCH DIGESTION AND GLYCEMIC RESPONSE
3.1 Introduction

Potatoes are the world’s third largest food crop and most extensively consumed root vegetable. Consumption is currently greatest in the Western world but potatoes are rapidly becoming a staple in developing countries. Compared with bread and rice, potatoes are more satiating (Holt et al., 1995), a source of vitamin C (up to 42 mg/100 g) and magnesium (Burlingame et al., 2009).

However, nutritional studies on different cultivars of potatoes using in vivo and in vitro approaches indicate that cooked potatoes contain mostly rapidly digested starch (Leeman et al., 2005) and have a high glycemic index (GI) (Atkinson et al., 2008). In prospective observational studies, potato consumption has been linked to higher weight gain (Mozaffarian et al., 2011) and increased risk of type 2 diabetes (Ludwig, 2002). Hence, some health professionals advise that potatoes should be substituted with a low GI carbohydrate to reduce the risk of chronic disease (Brand-Miller et al., 2009; Willet et al., 2002). However, given the importance of potatoes as a food, the discovery and development of low GI potatoes would be desirable from consumer, agricultural, food industry and health perspectives.

Postharvest storage has been shown to affect the cooking quality (Nourian, Ramaswamy, & Kushalappa, 2003), sugars content (Sowokinos, Orr, Knoper, & Varns, 1987), physicochemical and functional properties of starch (Kaur, Singh, Ezekiel, & Sodhi, 2009) in potatoes. The changes reported in these studies were observed over months of storage and it is not known whether a shorter period of storage will result in any significant changes to the tuber and the starch.

In vitro studies measure the physicochemical breakdown of starch in carbohydrate foods and have been proposed as an alternative approach for classifying carbohydrates, with the results from some studies showing correlation with in vivo GI testing (Englyst et al., 1999; Araya et al., 2002; Granfeldt et al., 1992). However, the number of foods that have been subjected to both in vitro and in vivo testing for direct comparison is very limited.

2 Results from this chapter have been published in the British Journal of Nutrition.
The aim of this chapter was to use standardised GI testing methodology to screen established and newly introduced commercial cultivars of potatoes, to identify a low GI potato. In vitro enzymatic starch hydrolysis and chemical analyses were performed for each potato cultivar and correlations sought with the respective GI values. It was also determined whether amylose content was a significant predictor of potato GI as has been suggested for cereals and legumes (Bjorck, Granfeldt, Liljeberg, Tovar, & Asp, 1994; Hu et al., 2004). Emphasis was placed on direct determination of the available carbohydrate content (as starch + sugars) of the potatoes studied, as opposed to relying on food composition tables. Prior to the GI testing of potatoes, a storage study was conducted to observe whether there were any changes to the tuber and starch that could affect test results from the time the potatoes were received until the completion of GI testing.

3.2 Results

Seven potato cultivars as described in Materials section 2.1.1 were used in this study. For the storage study, Carisma and Russet Burbank cultivars were stored in the dark in cupboard boxes for 20 days in a temperature controlled fridge at 18°C, similar to the storage conditions of the potatoes prior to GI testing. Potatoes were tested at 0, 5, 10, 15, 20 days after receipt from the supplier. The analyses were performed as described in respective Methods sections in Chapter 2.

3.2.1 Storage study

Physicochemical properties of the potato tubers from both Carisma and Russet Burbank cultivars did not change significantly during the period of storage (Table 3.1). For both cultivars, there were significant differences in the pasting properties of the starch from tubers stored for different periods (Table 3.2). Though there were some statistically significant differences observed in starch properties over the period of storage these differences were unlikely to have any consequence to the GI values.
Table 3.1. Physicochemical properties of potato cultivars stored over 20 days at 18°C

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Day</th>
<th>Dry matter (% dry matter)</th>
<th>Amylose content (%)</th>
<th>Starch (g/100g)</th>
<th>Total sugars (g/100g)</th>
<th>Reducing sugars (g/100g)</th>
<th>Total starch (g/100g)</th>
<th>Density (g/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carisma</td>
<td>1</td>
<td>1.43 ± 0.2</td>
<td>24.9 ± 3.3</td>
<td>0.95 ± 0.0</td>
<td>0.56 ± 0.02</td>
<td>0.57 ± 0.02</td>
<td>11.0 ± 0.02</td>
<td>0.85 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>1.48 ± 0.2</td>
<td>24.2 ± 0.4</td>
<td>0.91 ± 0.0</td>
<td>0.55 ± 0.02</td>
<td>0.57 ± 0.02</td>
<td>11.0 ± 0.02</td>
<td>0.85 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>1.43 ± 0.2</td>
<td>22.6 ± 0.6</td>
<td>0.89 ± 0.0</td>
<td>0.52 ± 0.02</td>
<td>0.57 ± 0.02</td>
<td>11.0 ± 0.02</td>
<td>0.85 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>1.43 ± 0.2</td>
<td>21.9 ± 0.3</td>
<td>0.87 ± 0.0</td>
<td>0.51 ± 0.02</td>
<td>0.57 ± 0.02</td>
<td>11.0 ± 0.02</td>
<td>0.85 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>1.43 ± 0.2</td>
<td>21.5 ± 0.4</td>
<td>0.85 ± 0.0</td>
<td>0.50 ± 0.02</td>
<td>0.57 ± 0.02</td>
<td>11.0 ± 0.02</td>
<td>0.85 ± 0.2</td>
</tr>
<tr>
<td>Russet Burbank</td>
<td>0</td>
<td>1.43 ± 0.2</td>
<td>22.2 ± 2.2</td>
<td>0.92 ± 0.0</td>
<td>0.56 ± 0.02</td>
<td>0.57 ± 0.02</td>
<td>11.0 ± 0.02</td>
<td>0.85 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>1.48 ± 0.2</td>
<td>24.5 ± 3.2</td>
<td>0.94 ± 0.0</td>
<td>0.57 ± 0.02</td>
<td>0.57 ± 0.02</td>
<td>11.0 ± 0.02</td>
<td>0.85 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>1.43 ± 0.2</td>
<td>22.5 ± 0.0</td>
<td>0.93 ± 0.0</td>
<td>0.57 ± 0.02</td>
<td>0.57 ± 0.02</td>
<td>11.0 ± 0.02</td>
<td>0.85 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>1.43 ± 0.2</td>
<td>21.9 ± 0.1</td>
<td>0.91 ± 0.0</td>
<td>0.57 ± 0.02</td>
<td>0.57 ± 0.02</td>
<td>11.0 ± 0.02</td>
<td>0.85 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>1.43 ± 0.2</td>
<td>21.6 ± 0.3</td>
<td>0.90 ± 0.0</td>
<td>0.57 ± 0.02</td>
<td>0.57 ± 0.02</td>
<td>11.0 ± 0.02</td>
<td>0.85 ± 0.2</td>
</tr>
</tbody>
</table>

Values are means ± SD, n=2. One-way ANOVA was used to compare the means between different cultivars. Means with the same superscript in a column do not differ significantly (p>0.05).

DISCOVERY OF A LOW GLYCEMIC INDEX POTATO AND RELATION BETWEEN IN VITRO STARCH DIGESTION AND GLYCEMIC RESPONSE
Table 3.2. Pasting properties of potato starch from cultivars stored over 20 days at 18°C (n=2)  

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Day</th>
<th>Pasting Temperature (°C)</th>
<th>Peak viscosity (cP)</th>
<th>Trough viscosity  (cP)</th>
<th>Breakdown viscosity (cP)</th>
<th>Final viscosity (cP)</th>
<th>Setback viscosity (cP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carisma</td>
<td>0</td>
<td>69.3 ± 0.6a</td>
<td>12494 ± 147a</td>
<td>5778 ± 107bc</td>
<td>6716 ± 40b</td>
<td>6974 ± 54c</td>
<td>1196 ± 53c</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>69.1 ± 0.6a</td>
<td>12915 ± 86bc</td>
<td>5476 ± 94a</td>
<td>7440 ± 8c</td>
<td>6559 ± 76b</td>
<td>1083 ± 170ab</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>67.9 ± 0.5a</td>
<td>12307 ± 195a</td>
<td>6798 ± 52c</td>
<td>5509 ± 247a</td>
<td>7925 ± 110c</td>
<td>1127 ± 58ab</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>69.1 ± 0.6a</td>
<td>13038 ± 69a</td>
<td>5381 ± 83a</td>
<td>7658 ± 152c</td>
<td>6296 ± 164a</td>
<td>915 ± 99a</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>68.7 ± 0.6a</td>
<td>12622 ± 187bc</td>
<td>6042 ± 54c</td>
<td>6580 ± 133b</td>
<td>7316 ± 71f</td>
<td>1274 ± 17b</td>
</tr>
<tr>
<td>Russet Burbank</td>
<td>0</td>
<td>63.0 ± 0.3b</td>
<td>8332 ± 111d</td>
<td>3073 ± 66a</td>
<td>5259 ± 45e</td>
<td>3832 ± 40f</td>
<td>759 ± 26d</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>63.6 ± 0.1bc</td>
<td>8511 ± 100de</td>
<td>43.33 ± 22a</td>
<td>4178 ± 122d</td>
<td>5122 ± 14h</td>
<td>790 ± 8d</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>63.8 ± 0.2c</td>
<td>9427 ± 296f</td>
<td>4175 ± 44d</td>
<td>5253 ± 340e</td>
<td>5077 ± 35g</td>
<td>902 ± 8e</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>63.4 ± 0.4bc</td>
<td>10811 ± 113f</td>
<td>3793 ± 15f</td>
<td>7019 ± 128f</td>
<td>4555 ± 68f</td>
<td>763 ± 53d</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>63.2 ± 0.1bc</td>
<td>8954 ± 161e</td>
<td>4412 ± 16v</td>
<td>4542 ± 177d</td>
<td>5294 ± 141</td>
<td>882 ± 2e</td>
</tr>
</tbody>
</table>

Values are means ± SD, n=2. One-way ANOVA was used to compare the means between different cultivars, means with the same superscript in a column do not differ significantly (p> 0.05).
3.2.2 Chemical composition and GI testing portions

The potato cultivars had different chemical compositions (Table 3.3). Virginia Rose had the lowest total starch content (9.1%), the highest reducing sugar content (0.68%), the highest amylose content of starch (27.7%), and the lowest glycemic carbohydrate (9.8 g/100 g). In contrast, Russet Burbank had the highest total starch content (16.0%), the lowest reducing sugar content (0.16%), and the highest glycemic carbohydrate (16.2 g/100 g). Accordingly, the GI testing portions differed considerably with the smallest portion for Russet Burbank (154 g) and the largest portion for Virginia Rose (255 g).

3.2.3 In vivo glycemic response

The plasma glucose concentration increased to the maximum value at 30 min for all the potato cultivars, and then decreased rapidly until 120 min. The postprandial response to the Carisma cultivar was lower than the other 6 potato cultivars at all time points (Figure 3.1). When GI values were calculated (Table 3.4), there were significant differences among the cultivars. The majority had a high GI of more than 70, with the Nicola cultivar just falling into the medium GI classification (GI = 69). Only the Carisma cultivar fell into the low GI category (GI = 53), according to the ISO recommended classification (ISO, 2010).
Table 3.3 Carbohydrate content and GI testing portion of seven potato cultivars

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Dry matter content (g/100g)</th>
<th>Total starch (g/100g)</th>
<th>Reducing sugars (g/100g)</th>
<th>Total dietary fibre (g/100g)</th>
<th>Amylose content (% starch)</th>
<th>Available carbohydrate (g/100g)</th>
<th>GI testing portion (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carisma</td>
<td>15.2±0.6^a</td>
<td>9.90 ±0.4^a</td>
<td>0.65±0.0^a</td>
<td>1.64±0.0^a</td>
<td>25.2±1.7^a</td>
<td>10.5</td>
<td>238</td>
</tr>
<tr>
<td>Nicola</td>
<td>19.1±0.4^b</td>
<td>12.1±0.6^b</td>
<td>0.69±0.0^a</td>
<td>1.96±0.0^cd</td>
<td>25.6±1.7^b</td>
<td>12.8</td>
<td>195</td>
</tr>
<tr>
<td>Desiree</td>
<td>20.8±0.7^cd</td>
<td>12.0±0.9^b</td>
<td>0.65±0.0^a</td>
<td>1.85±0.0^bc</td>
<td>23.1±0.9^a</td>
<td>12.7</td>
<td>197</td>
</tr>
<tr>
<td>Russet Burbank</td>
<td>22.1±0.3^d</td>
<td>16.0±0.8^c</td>
<td>0.16±0.0^c</td>
<td>1.70±0.1^de</td>
<td>24.4±0.8^a</td>
<td>16.2</td>
<td>154</td>
</tr>
<tr>
<td>Virginia Rose</td>
<td>15.0±0.2^a</td>
<td>9.10±0.4^a</td>
<td>0.68±0.0^a</td>
<td>1.74±0.1^bc</td>
<td>27.7±0.8^a</td>
<td>9.8</td>
<td>255</td>
</tr>
<tr>
<td>Bintje</td>
<td>19.7±0.1^bc</td>
<td>12.7±0.6^b</td>
<td>0.47±0.0^b</td>
<td>1.94±0.0^cd</td>
<td>24.7±1.3^a</td>
<td>13.2</td>
<td>189</td>
</tr>
<tr>
<td>Maiflower</td>
<td>21.1±0.2^d</td>
<td>12.9±0.3^b</td>
<td>0.34±0.1^d</td>
<td>2.07±0.1^d</td>
<td>24.1±1.9^a</td>
<td>13.2</td>
<td>189</td>
</tr>
</tbody>
</table>

1 Values are means ± SD, n=2 with the exception of reducing sugars where n=3. One-way ANOVA was used to compare the means between different cultivars, means with the same superscript in a column do not differ significantly (p> 0.05).

2 Percent-wet basis except amylose content, which was percent-starch basis.
Figure 3.1. Plasma glucose response curves for seven potato cultivars: Carisma, Nicola, Desiree, Russet Burbank, Virginia Rose, Bintje, Maiflower. ◊, Glucose, dotted lines indicate glucose reference. Values are the mean change in plasma glucose (PG) with their standard errors represented by vertical bars (n=10).
Table 3.4. GI and classification of seven potato cultivars (n=10)

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>GI</th>
<th>Classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carisma</td>
<td>53±7a</td>
<td>Low</td>
</tr>
<tr>
<td>Nicola</td>
<td>69±5ab</td>
<td>Medium</td>
</tr>
<tr>
<td>Desiree</td>
<td>74±8abc</td>
<td>High</td>
</tr>
<tr>
<td>Russet Burbank</td>
<td>82±3bcd</td>
<td>High</td>
</tr>
<tr>
<td>Virginia Rose</td>
<td>93±10cd</td>
<td>High</td>
</tr>
<tr>
<td>Bintje</td>
<td>94±8cd</td>
<td>High</td>
</tr>
<tr>
<td>Maiflower</td>
<td>103±8d</td>
<td>High</td>
</tr>
</tbody>
</table>

GI values with the same superscript in a column do not differ significantly (p> 0.05). One way ANOVA was used to compare the mean GI of the different cultivars.

3.2.4 In vitro starch digestibility

The amount of glucose released from cooked potatoes as a percentage of the initial 100 mg of starch increased progressively with time during in vitro digestion, reaching a plateau at 45–60 min, while the hydrolysis rate decreased gradually (Figure 3.2). The percentage of starch hydrolysed at different time points is shown in Table 3.5. Carisma and Nicola showed significantly lower percentages of carbohydrate hydrolysed at all time points, for example Carisma (24.0%) and Nicola (24.5%) compared to Bintje (34.5%) and Maiflower (36.5%) after 15 min of hydrolysis. Likewise, by the end of 120 min, Carisma and Nicola showed a hydrolysis percentage of 50.5% and 52.2%, respectively, compared to Bintje at 67.0% and Maiflower at 76.8%.
Figure 3.2. *In vitro* starch hydrolysis (%) of cooked potatoes from different cultivars. Values are the mean hydrolysis percentage with their standard errors represented by vertical bars.

Table 3.5. *In vitro* hydrolysis of starch in seven potato cultivars ($n=2$)

<table>
<thead>
<tr>
<th>Potato cultivar</th>
<th>15 min</th>
<th>20 min</th>
<th>30 min</th>
<th>60 min</th>
<th>120 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carisma</td>
<td>24.0^a</td>
<td>31.7^a</td>
<td>39.6^a</td>
<td>48.0^ab</td>
<td>50.5^a</td>
</tr>
<tr>
<td>Nicola</td>
<td>24.5^a</td>
<td>32.5^a</td>
<td>38.0^a</td>
<td>44.2^a</td>
<td>52.2^a</td>
</tr>
<tr>
<td>Desiree</td>
<td>33.1^b</td>
<td>46.3^bc</td>
<td>50.6^b</td>
<td>54.1^bc</td>
<td>59.2^b</td>
</tr>
<tr>
<td>Russet Burbank</td>
<td>34.9^b</td>
<td>49.3^cd</td>
<td>51.3^b</td>
<td>56.5^c</td>
<td>60.1^b</td>
</tr>
<tr>
<td>Virginia Rose</td>
<td>32.6^b</td>
<td>43.5^b</td>
<td>51.5^b</td>
<td>60.5^c</td>
<td>60.8^b</td>
</tr>
<tr>
<td>Bintje</td>
<td>34.5^b</td>
<td>54.2^d</td>
<td>65.4^c</td>
<td>69.9^d</td>
<td>67.0^c</td>
</tr>
<tr>
<td>Maiflower</td>
<td>36.5^b</td>
<td>53.5^d</td>
<td>75.5^c</td>
<td>72.9^d</td>
<td>76.8^d</td>
</tr>
</tbody>
</table>

1 Means with the same superscript in a column do not differ significantly ($p>0.05$).

2 Values are % starch hydrolysed at each time point.
3.2.5 Correlation between *in vivo* glycemic response and *in vitro* starch digestibility

![Graph A](image1)

*Figure A.* Correlation between ratio of *in vitro* starch digestibility at 90 min (A) and GI values of cooked potatoes. The correlation coefficient is $r = 0.91$, $p = 0.00393$.

![Graph B](image2)

*Figure B.* Correlation between ratio of *in vitro* starch digestibility at 120 min (B) and GI values of cooked potatoes. The correlation coefficient is $r = 0.91$, $p = 0.00493$.

**Figure 3.3.** Correlation between ratio of *in vitro* starch digestibility at 90 min (A) and 120 min (B) and GI values of cooked potatoes.
The GI values of cooked potatoes were all significantly and positively correlated with hydrolysis percentage during in vitro starch digestion at each time point (Figure 3.3). In vitro percent hydrolysis at 90 and 120 min showed the strongest positive correlation with GI values \( r = 0.91, p < 0.01 \), Figure 3.3. No significant correlations were found between GI values and amylose content, dietary fibre content or total starch content. Similarly, there were no correlations between in vitro starch digestibility and amylose content, dietary fibre content or total starch content.

### 3.3 Discussion

The present study compared the in vivo and in vitro digestibility of a series of potato cultivars that had been prepared, cooked and consumed under identical conditions. Differences could therefore be attributed to the characteristics of the potatoes rather than differences in processing or post–harvest changes during storage. Of the seven potato cultivars tested, Carisma, \( \text{GI} = 53 \) was identified as low GI, Nicola \( \text{GI} = 69 \) as medium GI and the other five cultivars as high GI (e.g. Russet Burbank, \( \text{GI} = 82 \)). The low GI property of Carisma was unrelated to amylose or total starch content, but could be predicted from in vitro measures of rate of starch digestion. The amount of non-starch carbohydrate was too small (<1 g/100 g) to account for the large differences in blood glucose response. Previous studies on potatoes have shown a wide range of GI values from as low as 23 to as high as 118 (Atkinson et al., 2008). However, much of this variability in GI values may be due to non-standard variations in methodology, inaccurate estimation of glycemic carbohydrate content of the portion fed, differences in cooking method or processing before consumption, and the overall meal composition (Atkinson et al., 2008; Ek, Brand-Miller & Copeland, 2012; Fernandes et al., 2005; Henry et al., 2006).

For the purpose of comparison, Table 1.2 (Chapter 1) shows that GI values of potatoes ranged from 56 to 104 in studies where the cultivar was specified, tested with no additional ingredients, and when at least 10 healthy subjects were used. The Marfona cultivar had a GI value of 56 compared to a value of 85 for Maris Piper, when both the cultivars were boiled for 15 min prior to consumption (Henry et al., 2005). These findings, like those of the present study, show that there is considerable variability in the GI values of potatoes due solely to botanical characteristics.
Another pertinent observation is that the two lowest GI cultivars did not produce an undershoot in postprandial glucose below baseline levels as observed for the high GI potatoes (Figure 3.1). This undershoot in plasma glucose curves was generally observed for potatoes (Brand-Miller, Stockmann, Atkinson, Petocz, & Denyer, 2009) and has negative implications in appetite and weight control through decreased satiety or increased hunger and subsequent food intake (Campfield & Smith, 2003).

During the storage of potatoes for 20 days at 15-17°C, there were no significant changes observed in tuber and starch properties that could have affected GI values. Cold storage (4°C) post harvest has been reported to result in cold induced-sweetening in potato tubers, where starch is converted to reducing sugars (Sowokinos, 2001). Storage at higher temperatures (20°C) showed increased amyllose content and higher starch peak viscosity compared to starch isolated from potatoes stored at lower temperatures (4-16°C) (Kaur et al., 2009). In the present study the period from receipt of potato samples to the completion of GI testing was considerably shorter than those reported in other studies and essentially no changes were observed.

Another important finding of the present study was the significant positive correlation between the rate of in vitro digestion and the in vivo glycemic response. The blood glucose response curves of each of the seven potato cultivars show a similar rate of rise in blood glucose (Figure 3.1) to the rate of carbohydrate hydrolysis (Figure 3.2). The strong correlation between the hydrolysis percentage at 120 min and GI values indicates that the glycemic response to the potatoes was largely dependent on the percentage of starch hydrolysis by 120 min measured by the in vitro method. As much as 75% of the total starch in raw potatoes is highly resistant to enzymatic attack (Englyst et al., 1987; Kingman et al., 1994). As the cooking time used in this study was relatively short (as specified by the potato growers), starch gelatinization may not have been complete (Hoover, 2010; Wang et al., 2013) and the potatoes consumed would have contained a mixture of granules with partially disrupted structure. Under the same cooking conditions, however, differences between the cultivars in starch fine structure would have resulted in the conversion of different amounts of the enzymatically-resistant starch in raw potatoes into available starch for hydrolysis.
There appears to be no reports in the literature that in vitro digestibility of starch has reliably predicted GI classifications for the same food item prepared in the same way but varying only in genotype. This has important practical implications in that simple in vitro digestion methods might be used to screen foods and as a predictor of GI classification, i.e., high or low GI, although the actual GI value of the food would require in vivo testing. However, it is likely that the in vitro method is more suitable for screening simple foods such as potato, rather than complex foods with a mixture of ingredients. High throughput in vitro methods would have great benefit in plant breeding programs that seek to improve the glycemic response of carbohydrates in food crops.

Starch-containing foods that are digested slowly and result in low glycemic response have been considered to be more beneficial to health and in the prevention and management of diabetes and hyperlipidemia than starchy foods that are digested rapidly and result in high glycemic responses (Ludwig, 2002; Eckel et al., 2005; Venn et al., 2007). Hence, the identification of foods with a low GI and the factors that influence GI should be of continuing research interest in the future.

The present chapter has shown that under identical cooking conditions, potato cultivars with similar amylose content differed significantly in both in vitro digestion and in vivo blood glucose responses. The composition and state of starch in foods are the major determinants of the rate at which foods are digested and at which they elicit postprandial blood glucose and insulin responses. In general, native starches with high amylose content are considered to be more resistant to the enzymatic digestion, whereas high amylopectin starches are more susceptible to digestion. High amylose native starches are thought to be more difficult to swell and gelatinize under typical cooking conditions, and therefore digested more slowly, eliciting lower blood glucose and insulin responses than those with low amylose content (Goddard, Young, & Marcus, 1984; Thorne, Thompson, & Jenkins, 1983). However, this interpretation needs refinement as the fine structure of amylose and amyllopectin have also been shown to be important in determining the digestibility of starch (Blazek et al., 2010; Syahariza et al., 2013). A further discussion of these points will be explored in Chapter 5.
3.4 Conclusion

This study identified the first commercially grown low GI cultivar of potato (Carisma, GI = 53) amongst a group of established and newly introduced cultivars. The Carisma potatoes were also commercially tested numerous times using tubers from field trials in Australia, differing in growth locations and harvest seasons, and showed consistently low GI values. The rate of in vitro digestion of starch, in particular the percentage available (glycemic) carbohydrate hydrolysed by 120 min in the cooked potato product was demonstrated to be a possible predictor of the GI classification. The importance and popularity of potatoes as a food crop dictate the need to identify and develop cultivars that are digested slowly and have a low GI, regardless of preparation method. In the following chapter, tuber and starch morphology are studied to find characteristics which would help in the identification of more low GI potato cultivars.
CHAPTER 4

IMAGING OF TISSUE AND STARCH
FROM POTATOES DIFFERING IN GI
4.1 Introduction

In the previous chapter, a low GI potato cultivar (Carisma) was identified. In addition to Carisma (GI=53), the cultivar Nicola (GI = 69) was classified as medium GI and the five other cultivars were classified as high GI according to ISO guidelines (ISO, 2010). The lower GI cultivars Carisma and Nicola had significantly lower percentages of starch hydrolysed at all time points during in vitro amylolysis tests. The GI values were found to be strongly and positively correlated with the percentage in vitro enzymatic hydrolysis of starch from the cooked potatoes. The results indicated that the differences in both in vivo and in vitro digestibility amongst the seven cultivars are due to variability in botanical characteristics as the potatoes were prepared for testing under the same cooking conditions.

Potato tubers are made up of zones of different cell types with most of the starch granules found in the storage parenchyma cells (Figure 4.1). Parenchyma tissue consists of large (200 x 340 μm) and small (80 x 90 μm) cells adhered together by pectin in the middle lamellae pectin, with the presence of small intercellular spaces. Variations occur in cell size, shape and arrangement amongst different potato cultivars (Bordoloi, Kaur, & Singh, 2012; Fedec, Ooraikul, & Hadziyev, 1997; Singh et al., 2005; Sun & Li, 2003).
Figure 4.1: Potato tuber cross section with zone and tissue designations (Adapted from Fedec et al., 1977).
Potatoes need to be cooked before consumption and the two main changes that occur concurrently during boiling are breakdown of the cell walls and middle lamellae, and swelling and gelatinization of intracellular starch (Huang et al., 1990; Ormerod et al., 2002; vanMarle et al., 1997). Plant cell wall structures have been suggested as a limiting factor for starch hydrolysis in foods (Colonna, Leloup, & Buleon, 1992). They can act as a physical barrier to heat conductance during cooking and block enzyme access during digestion and thereby reduce the rate of starch hydrolysis. During digestion, the breakdown of plant wall structures and the ease of separation of the potato cells separate are dependent on the extent of starch swelling, cell wall density and the strength of the middle lamellar constituents, which vary amongst potato cultivars (Bordoloi et al., 2012; Fedec et al., 1977; Thybo et al., 1998; vanMarle et al., 1997). The digestibility of cooked potatoes under in vivo and in vitro conditions is likely to be dependent on: 1) heat conductance through the tissue to the starch granules, 2) the extent of starch gelatinization, and 3) the breakdown of the cell structures affecting enzyme access to gelatinized starch after cooking.

Imaging techniques can provide valuable information on starch morphology and cell structures in potatoes, cellular changes and starch gelatinization following cooking (Bordoloi et al., 2012; Ormerod et al., 2002; Schirmer, Hochstotter, Jekle, Arendt, & Becker, 2013), and relationships between starch granule morphology and digestibility (Apinan et al., 2007; Dhital et al., 2010). Powerful new microscopy techniques permit high resolution imaging of native structures in situ with minimal preparation to reduce the presence of artefacts.

One such technique is environmental scanning electron microscopy (ESEM), where samples are imaged at atmospheric pressure. This allows sample preparation prior to imaging to bypass fixation, dehydration and coating with a conducting material, which are requirements for conventional scanning electron microscopy (SEM) (Danilatos, 1993). Confocal laser scanning microscopy (CLSM) has been used to examine the internal structure of protein and starch networks in food (Durrenberger, Handschin, Conde-Petit, & Escher, 2001), for characterization of starch granule morphology (Schirmer et al., 2013), detailed examination of the growth ring structure of native and gelatinized starch granules (van de Velde, van Riel, & Tromp, 2002) and to reveal internal granular structures (Blennow, Hansen, Schulz, Jorfensen, Donald, & Sanderson, 2003).
Another new technique is micro-computed tomography (micro-CT), which uses X-rays to visualize the surface and internal structures of a specimen in three dimensions. Samples are imaged at atmospheric pressure without the need for extensive or destructive specimen preparation. The use of X-ray micro-computed tomography (micro-CT) has been reported to be a useful tool for the study of the internal 3D structure of a variety of cellular food materials (Lim & Barigou, 2004) and food microstructure (Mousavi, Miri, Cox, & Fryer, 2007).

This chapter describes the use of imaging techniques to study and compare cell structure and native starch granule morphology of the low GI cultivar Carisma with the medium GI and the five high GI potato cultivars. LM and micro-CT were used to compare Carisma (GI = 53) and Russet Burbank (GI = 82) cultivars and look for changes to potato tissue and potato starch granules in the raw and cooked states. The aim was to identify cell wall structure and starch granule morphology characteristics that may influence the GI values of potatoes.

4.2 Results

Seven potato cultivars as described in 2.1.1 were used in this study. Starch granules were isolated from the potatoes as described in 2.2.5, and cooked potato tissue prepared as described in 2.2.13.2. ESEM, LM, Micro-CT and CLSM micrographs were acquired as described in section 2.2.15.

4.2.1 ESEM

The cells in potato tissue from all seven cultivars had an isodiametric polyhedral outline, with some variation in cell size, cell shape, cell wall thickness and starch granule sizes between cultivars (Figure 4.2). The raw potato tuber parenchyma from all of the cultivars showed clear cell wall outlines, with multiple starch granules inside each cell. Parenchyma cells of the seven cultivars were estimated to have an average size of 100 x 250 μm and observed to be filled with a range of starch granules of different shapes and sizes, similar to results reported by others (Bordoloi et al., 2012; Singh et al., 2005; Thybo et al., 1998).
4.2.2 LM and CLSM imaging of potato starch granules

Starch granule morphology, size distribution and internal granular structure were characterised by LM and CLSM (Figure 4.3). Measurement of granule size and the discussion of those results are presented in Chapter 5. Growth rings were clearly evident in all potato starch samples with the dark rings appearing thinner than the bright rings. Rings were ellipsoid-shaped and deposited around the eccentric hilum. Carisma starch granules seemed to present the sharpest and most intensive growth ring structures compared to the other starches (Figure 4.3B).

Internal heterogeneities (indicated by the arrow in Figure 4.3C and hereafter referred to as imperfections) were observed in starch from Nicola and all of the high GI potato cultivars studied. The internal imperfections were less evident and hard to detect in Carisma starch compared to starch from the other cultivars. Optical cross-section images of Nicola and Russet Burbank native starch granules indicate that these imperfections appear to extend from the centre/hilum of the granule and were not external or surface cracks (Figure 4.4). Carisma starch appeared to have a more compact granule structure, darker growth rings and had an intense fluorescence in the hilum which decreased toward the granule surface (Figure 4.4A). This was different from the other starches, which had the brightest fluorescent areas located in the growth rings at the periphery of the starch granules.
4.2.3 Effect of heat-treatment on potato tissue and isolated potato starch

Carisma and Russet Burbank potatoes were cooked in the same way as for GI testing to assess the state of potato tissue and starch in the material consumed by volunteers for GI testing. Cooking of potatoes affected the cell contents and the extent of starch swelling varied between the two cultivars. In raw samples, Russet Burbank tuber cells were a larger, had more large granules and a greater number of starch granules compared to Carisma (Figure 4.5). After cooking for 8 min, Russet Burbank cells appeared to have swollen to a greater extent than Carisma cells, and the parenchyma had a largely disintegrated cell structure. In comparison, Carisma cells appeared more intact (Figure 4.5). In both cultivars, distinct starch granules within the cells were no longer visible by LM.

Isolated starch granules from Carisma and Russet Burbank potatoes showed different resistance to swelling and disintegration (Figure 4.6). After boiling for six min, granular forms were more clearly evident in Carisma starch, whereas Russet Burbank starch showed a greater degree of granule swelling and disintegration. After eight min, Russet Burbank starch appeared to have lost its structure completely, whereas outlines of swollen granules were still evident in Carisma starch.
Figure 4.2A: ESEM images of fresh tissue from the potato cultivars shown at two different magnifications. Scale bars represent 100 μm. The square box indicates the tuber tissue area used for imaging.

Carisma
GI = 53

Virginia Rose
GI = 93

Bintje
GI = 94

Maiflower
GI = 103

Fresh Potato Section

Nicola
GI = 69

Desiree
GI = 74

Russel Burbank
GI = 82

Carisma
GI = 55
Figure 4.2B: ESEM images of fresh tissue from the potato cultivars shown at two different magnifications. Scale bars represent 50 μm.
Figure 4.3: Micrographs of potato tissue and starch granules from potato cultivars with different GI values. (A) LM of starch, (B) CLSM of starch, and (C) CLSM optical sections of starch. The arrow highlights internal imperfections observed in the starch granules. Scale bars represent 100 μm (A), 25 μm (B), and 10 μm (C).

GI: Carisma = 103, Bintje = 94, Virginia Rose = 93, Russet Burbank = 82, Desiree = 74, Nicola = 69, Maiflower = 53.
Figure 4.4: CLSM images of cross sections of Carisma, Nicola and Russet Burbank starch granules (colouring: rhodamine-B; objective 63x in glycerol). Scale bars represent 10 μm. Distance between cross-sections 2.5 μm.

- Carisma
  GI = 53

- Nicola
  GI = 69

- Russet Burbank
  GI = 82
Figure 4.5: LM micrographs of raw potato and potato cooked in boiling water for 8 min Carisma, Russet Burbank. The scale bars represent 100 μm.

4.2.4 Effect of cooking on microstructure as observed by micro-CT

Potato sections (size: 0.5 cm x 0.5 cm x 1 cm) Carisma and Russet Burbank were excised from raw and cooked tissue, stained in iodine solution and imaged as depicted in Figure 4.7. Micrographs at the midway point of the each scan direction are presented in Figure 4.8. Both the images of raw Carisma and Russet Burbank tissue showed a dense and packed cell distribution with similar cell sizes compared to each other (Figure 4.8). Cooked Russet Burbank potato samples showed larger swollen cells in micrographs from both scanning directions compared to Carisma (Figure 4.8). Russet Burbank potato also showed more extensive dark regions in the centre of the potato pieces compared to Carisma. The dark regions were areas where the specimen did not take up the iodine stain.
Figure 4.6: LM micrographs of native starch granules after heating in boiling water for 6 min and 8 min, respectively Carisma, Russet Burbank. Scale bars represent 100 μm.

Figure 4.7: Sample set-up for micro-CT imaging: Arrows indicate X-ray scan direction: transverse (A) and longitudinal (B).
Figure 4.8: Micro-CT images of raw and cooked Carisma and Russet Burbank potato. Transverse and longitudinal cross sections are shown. Scale bar represents 0.1 cm.
4.3 Discussion

4.3.1 Raw potato tissue and starch granules

Native starch granules from the low GI potato Carisma appeared to have more tightly packed growth rings and a stronger fluorescence at the hilum. Starch imperfections which were observed in the starch granules from the other potato cultivars, were not apparent in Carisma starch. Cell sizes and starch granule size appeared to be similar amongst the seven cultivars. Starch granules had a smooth surface and were lenticular, oval or spherical with a broad size range, which is typical of native potato starch (Hoover, 2001; Tester et al., 2004).

An intense fluorescence in the hilum has been suggested to indicate a high concentration of amylose (Blennow et al., 2003; Glaring, Koch, and Blennow, 2006) and the average thickness and number of growth rings are considered to be dependent on the amylose content of the starch (Yuryev et al., 2004). Accordingly, the CLSM images of Carisma starch are consistent with a high concentration of amylose at the hilum, although chemical analyses showed no difference in amylose content of Carisma starch compared to the other six cultivars. It is possible that the location and distribution of amylose branch lengths are different in Carisma starch despite similar amylose content to starches from the other potato cultivars. On the other hand, others have reported no direct relationship between the amylose and amylpectin contents and the internal growth ring structures (Schirmer et al., 2013).

The imperfections observed in the present study appear similar to the fissures, cavities or cracks detected in native potato, mung bean and corn starch granules by others (Blennow et al., 2003; Glaring et al., 2006; van de Velde et al., 2002). Severe cracks in the starch granules were also detected in granules with suppressed starch branching enzyme (SBE) and small fissures were detected in the granules of antisense mutants for the starch phosphorylating enzyme (Blennow et al., 2003). These are examples of altered starch enzyme metabolism and indicate sub-optimal packing of starch molecules in the starch granules (Blennow et al., 2003; Glaring et al., 2006).
Rhodamine-B was used as the fluorophore for CLSM in the present study. Rhodamine-B is a member of the family of rhodamine fluorescence dyes with widespread applications in environmental and biological studies (Sun, Liu, Lv, Liu, Zhao, & Guo, 2012). Rhodamine dyes are derivatives of benzoic acid and rhodamine-B has the chemical structure as shown in Figure 4.9. Different fluorophores have been used for visualisation of starch structures, from general dyes like FITC (fluorescein isothiocyanate) and safranin (van de Velde et al., 2002) to APTS (3-aminopropyl-trimethoxysilane) which specifically labels the reducing ends of starch fragments (Blennow et al., 2003).

![Figure 4.9: Structure of the fluorophore Rhodamine–B (Adapted from National Institutes and Technology)](image)

Van de Velde et al. (2002) tested the behaviour of different fluorescent probes, including rhodamine-B, in the study of starch granules and starch gelatinization behaviour. They proposed that the ability of rhodamine-B to visualise growth rings in starch granules may be a result of hydrophobic interaction with the semicrystalline domains of the starch granules (van de Velde et al., 2002). A proposed model of a starch granule is presented in Figure 4.10, showing internal growth ring structure and chain distribution of amylose and amylopectin.
Figure 4.10: Model of starch granule and its hierarchical structure from Wang et al., 2013. The depiction of amylopectin arrangement in the granule is stylized. The amyllose and amylopectin chains are likely to be packed more densely *in situ* than depicted.

According to this model, and the observation that rhodamine-B interacts with semicrystalline regions, the dark areas or imperfections observed in the starch from the higher GI potato cultivars indicate a bulk amorphous region (Figure 4.3C) which do not interact strongly with the dye. Another possible explanation is that the internal imperfection is a cavity, and there is the absence of material for the dye to interact with, hence the lack
of fluorescence. The affinity of rhodamine-B for semicrystalline material also suggest that the bright rings observed in the starch granules are the crystalline areas, and the dark rings indicate the amorphous lamella. Based on the above observations, Carisma starch appears to have the most compact and regular granule structure of the cultivars studied, consistent with Carisma starch being more thermally stable.

4.3.2 Effects of cooking on potato tissue and starch

The potato slices used in the study were prepared in the same way as for GI testing described in Methods 2.2.5.2 with a total cooking time of 8 min. In the raw state, the cell sizes of both Carisma and Russet Burbank appeared similar, but after cooking a difference in size was observed. Carisma potato showed less cell swelling and extent of starch granule gelatinization compared to Russet Burbank after cooking. Russet Burbank potatoes have a higher total starch content (16% fresh weight) (Table 3.3) and a higher percentage of large granules (11.4%) (Figure 5.2), in comparison to Carisma potatoes which have a total starch content of 10% (Table 3.3) and a lower percentage of large granules (3.9%) (Figure 5.2). The greater extent of cell swelling in Russet Burbank could be attributed to the combination of higher starch concentration, larger granule sizes and greater extent of granule swelling.

Weakening of potato tissue on cooking has been suggested to be controlled by the thermal degradation of the middle lamellae (Ormerod et al., 2002). Cultivars with a higher dry matter and starch content were observed to retain cell wall outline with gelatinized starch filling cells, whereas a cultivar with lower dry matter and starch content the cell wall was completely disintegrated after boiling (Bordoloi et al., 2012). After cooking, swelling and disintegration were observed in starch from both Carisma and Russet Burbank cultivars, but there was no separation of the middle lamella or breakdown in parenchyma. This suggests that was no difference between the two cultivars in terms of the resilience of the cell wall structure to cooking, however the cooking time used in the study may not have been long enough to induce such a differentiation.
Isolated starch granules from Carisma showed less swelling and disintegration than Russet Burbank starch, with outlines of swollen starch granule still visual after boiling for 6 min and even after 8 min. Van de Velde et al. (2002) used CLSM to examine the gelatinization of starch heated at 115°C at different heating times and reported the appearance of a large central cavity at the hilum which expanded as the heating time increased. Others also observed that starch granule gelatinization starts in the hilum and proceeds toward the periphery (Singh et al., 2003). The presence of internal imperfections (indicated in Figure 4.3) in the higher GI starches of the present study could be points of weakness for water and heat penetration during cooking that make the initiation of starch gelatinization more easy. In Carisma starch, the absence of these imperfections at the hilum could increase resistance of the onset of starch gelatinization.

4.3.3 Micro-CT examination of raw and cooked potato

Micro-CT was chosen as a novel non-invasive technique for the study of cooked potato tissue. There were no reports found where micro-CT was used in the study of starch gelatinization but was considered as a promising technique to use in this study. Most other microscopy techniques can be used to look at the surface or a transmission image through a thin section, which means that the internal 3D structure can only be investigated invasively. Images obtained may not be reliable due to potential artefacts created from the destructive effect of physical cutting on the microstructure of the specimen. Potato tissue softens and starch gelatinizes after cooking, resulting in difficulty in obtaining precise thin cuts and clear images due to starch gel smearing across sample sections.

Cooked Russet Burbank potato samples showed a much larger dark area in the centre of the pieces in both transverse and longitudinal cross section images compared to Carisma. The dark areas are an indication that these central regions did not take up the iodine stain. There was no dark area in the centre of the uncooked potato pieces from both cultivars, indicating that the penetration of the iodine stain was complete. This suggests that cooking the potato pieces brought about a change that resulted in less iodine staining, particularly in the Russet Burbank pieces.
A possible explanation could be that swollen engorged potato cells slow down the rate of iodine stain penetration resulting in the central regions remaining unstained. Starch gelation, which occurs after gelatinization, is the slow reassociation of molecules in a semi-dry state leading to the formation of a turbid viscoelastic paste, or at sufficiently high starch concentrations (>5%, w/w), an opaque elastic gel (Wang et al., 2013). After cooking, a starch gel consisting of swollen granules surrounded by leached amylose molecules would have formed within potato cells. Retrogradation of starch is enhanced by low temperature, high starch concentration and long chains of amylose and amylopectin (Colonna et al., 1992). Russet Burbank potatoes had a starch concentration that was about 50% more than that of Carisma and retrogradation would have occurred to a larger extent than in Carisma potatoes. This retrograded starch would thus present a greater barrier to iodine stain movement in the tissue. In addition, Russet Burbank potato had a greater extent of cell swelling and engorgement compared to Carisma potato after cooking, the increase in physical size could result in slower and lesser stain penetration and a greater size of the central dark regions during imaging.

On hindsight, a more suitable high GI cultivar that could have been used to compare the effects of cooking on potato tissue and starch on a high GI and low GI cultivar is the Virginia Rose cultivar, which had similar total starch content, amylose content and granule size to the Carisma cultivar. However, as the micro-CT technique required long hours and technical assistance it was not possible to analyze another potato cultivar given the limited access to the MicroXCT.
4.4 Conclusion

Starch from the low GI cultivar Carisma potato appeared to be more resistant to gelatinization during cooking. Properties that could confer this resistance are the absence of internal imperfections in the native starch, a higher concentration of amylose at the hilum and a tighter packing of starch molecules in the starch granules. Raw Carisma potato did not show differences in cell size and starch granule size distribution in the raw samples compared to the other cultivars. On cooking, Carisma showed markedly less cell swelling compared to the high GI cultivar Russet Burbank, but it is unclear whether this property is attributable to lower starch content, stronger cell wall structure or a lesser extent of granule gelatinization or combinations of these. The observed differences in starch swelling and gelatinization are likely to be influenced by the physico-chemical properties of starch, and will be examined in the following chapter.
CHAPTER 5

PROPERTIES OF STARCH FROM POTATOES
DIFFERING IN GLYCEMIC INDEX
5.1 Introduction

Starch is the main available carbohydrate in potatoes. The digestibility of native starch has been proposed to be influenced by various factors, including amyllose content (Liu et al., 2007), phosphorus content (Liu et al., 2007; Lu et al., 2011), granule size and starch morphology (Dhital et al., 2010), amyllopectin chain length profile (Liu et al., 2007) and the fine structures of branch chains in both amyllose and amyllopectin (Syahariza et al., 2013). In contrast, the physical state of starch in processed or cooked foods is the main determinant of the rate at which it is digested and elicits postprandial blood glucose responses (Singh et al., 2010; Wang et al., 2013).

In the preceding chapter, cell structure and native starch granule morphology of the low GI cultivar Carisma was studied and compared with the medium GI and the five high GI potato cultivars using imaging techniques. Changes to potato tissue and potato starch granules in the raw and cooked states in Carisma were compared to a high GI cultivar, Russet Burbank. While raw Carisma potato did not show differences in cell size and starch granule size distribution in the raw samples compared to the other cultivars, the Carisma potato appeared to be more resistant to gelatinization during cooking and showed markedly less cell swelling compared to Russet Burbank. These observed differences are likely to be influenced by potato starch characteristics. In the present chapter, the properties of starch from Carisma and the medium and high GI potatoes were examined to identify characteristics of potato starch that influence their GI values.

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3 A modified version of this chapter has been published in Food and Function
5.2 Results

Starch isolated from the seven potato cultivars (Carisma, Nicola, Desiree, Russet Burbank, Virginia Rose, Bintje and Maiflower) was analyzed as described in the respective sections in Chapter 2.

5.2.1 Physicochemical properties

No significant differences were noted among the starches from the seven potato cultivars in their amylose and phosphorus contents and relative crystallinity (Table 5.1). The chain length profile of amylopectin from all of the potato cultivars had the degree of polymerization (DP) 13-24 fraction as the highest percentage (47-51%) and DP 6-12 fraction as the lowest (7-9%). However, there were only minor differences in amylopectin chain length distributions among the cultivars, which did not fall into a pattern that corresponded to the GI rankings (Table 5.1).

Russet Burbank starch had the largest mean granule size, whereas the mean granule size of Maiflower was significantly smaller than that of the other cultivars (Table 5.1). Maiflower had the highest percentage of small starch granules (90%) and the lowest percentage of large granules (3%). In comparison, Russet Burbank had the lowest percentage of small granules (57%) and highest percentage of large granules (11%). The percentage of small starch granules (82%) and large granules (4%) in Carisma was significantly different from the respective values for Russet Burbank, but did not differ significantly from Maiflower (Figure 5.1). Although there were significant differences in granule size distributions between the cultivars, these did not correspond to differences in GI values.
5.2.2 Thermal properties

The potato starches presented well-defined single differential scanning calorimetry (DSC) endotherms (Figure 5.2). The thermal transition temperatures $T_0$, $T_p$, and $T_c$ ranged from 60.0 to 66.2°C, 62.8 to 69.3°C and 67.6 to 75.8°C, respectively, and the gelatinization enthalpies ranged from 18.5 to 19.5 Jg$^{-1}$ (Table 5.2). Carisma starch had the highest values $T_0$ (66.2°C), $T_p$ (69.3°C) and $T_c$ (75.8°C), whereas the lowest respective values were observed for Russet Burbank. The average gelatinization temperature range ($T_c - T_0$) was 10.0°C. The thermal transition temperatures varied significantly between some of the cultivars, but there were no significant differences in gelatinization enthalpies ($p > 0.05$).

5.2.3 Starch pasting properties

All seven starches displayed similar pasting profiles, which were typical of potato starch. Pasting temperature ranged from 60.8°C to 70.2°C (Table 5.3 and Figure 5.3). Carisma starch had the highest pasting temperature (70.2°C), whereas Russet Burbank starch had the lowest (60.8°C), consistent with the ranking of DSC thermal transition temperatures. The starches had similar peak viscosities with the exception of Russet Burbank starch, which was significantly lower than those of the others. Final paste viscosity was lowest for Russet Burbank (3869 cP) and the highest for Carisma (9009 cP). Carisma starch also had the highest trough (7595 cP) and final viscosity (9009 cP).
Table 5.1. Properties of starches from seven potato cultivars

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>AM (%)</th>
<th>P (%)</th>
<th>RC (%)</th>
<th>Mean GSA (µm²)</th>
<th>DP 6 – 12</th>
<th>DP 13–24</th>
<th>DP 25–36</th>
<th>DP 37–54</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carisma</td>
<td>25.2 ± 1.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.054 ± 0.005&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>24</td>
<td>314 ± 31&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.0 ± 0.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>49.2 ± 0.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>26.8 ± 0.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>17.1 ± 0.7&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Nicola</td>
<td>25.6 ± 1.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.061 ± 0.007&lt;sup&gt;de&lt;/sup&gt;</td>
<td>25</td>
<td>288 ± 53&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.4 ± 0.4&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>50.5 ± 4.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>22.8 ± 3.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>18.3 ± 0.7&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Desiree</td>
<td>23.1 ± 0.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.032 ± 0.004&lt;sup&gt;a&lt;/sup&gt;</td>
<td>26</td>
<td>438 ± 86&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7.2 ± 0.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>46.7 ± 0.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>27.6 ± 0.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>18.4 ± 0.6&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Russet Burbank</td>
<td>24.4 ± 0.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.047 ± 0.001&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>27</td>
<td>649 ± 74&lt;sup&gt;d&lt;/sup&gt;</td>
<td>8.4 ± 0.6&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>46.6 ± 0.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>27.5 ± 1.3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>18.5 ± 1.2&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Virginia Rose</td>
<td>27.7 ± 0.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.040 ± 0.000&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>24</td>
<td>259 ± 22&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>7.0 ± 0.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>47.0 ± 0.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>27.9 ± 0.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>18.7 ± 0.2&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Bintje</td>
<td>24.7 ± 1.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.068 ± 0.004&lt;sup&gt;a&lt;/sup&gt;</td>
<td>23</td>
<td>362 ± 54&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>7.2 ± 0.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>47.3 ± 1.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>27.6 ± 0.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>18.0 ± 0.2&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Maiflower</td>
<td>24.1 ± 1.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.042 ± 0.009&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>30</td>
<td>196 ± 58&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.3 ± 1.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>47.7 ± 0.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>26.8 ± 0.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>16.3 ± 0.6&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>1</sup> Values in a column with the same superscript do not differ significantly (p > 0.05).

<sup>2</sup> Abbreviations: AM, amylose; AP, amylopectin; P, phosphorus; RC, relative crystallinity; GSA, granule surface area.
Figure 5.1: Percentage particle size distribution of starch granules from seven different potato cultivars

Table 5.2. Thermal properties of starch from seven potato cultivars

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Transition temperature (°C)</th>
<th>∆H (J/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$T_o$</td>
<td>$T_p$</td>
</tr>
<tr>
<td>Carisma</td>
<td>66.2 ± 0.1$a$</td>
<td>69.3 ± 0.0$a$</td>
</tr>
<tr>
<td>Nicola</td>
<td>60.4 ± 0.1$a$</td>
<td>64.0 ± 0.2$bc$</td>
</tr>
<tr>
<td>Desiree</td>
<td>63.7 ± 0.2$d$</td>
<td>67.1 ± 0.3$d$</td>
</tr>
<tr>
<td>Russet Burbank</td>
<td>60.0 ± 0.2$a$</td>
<td>62.8 ± 0.2$a$</td>
</tr>
<tr>
<td>Virginia Rose</td>
<td>61.4 ± 0.0$bc$</td>
<td>65.4 ± 0.0$c$</td>
</tr>
<tr>
<td>Bintje</td>
<td>61.8 ± 0.4$c$</td>
<td>66.2 ± 0.1$c$</td>
</tr>
<tr>
<td>Maiflower</td>
<td>61.2 ± 0.0$b$</td>
<td>66.2 ± 0.1$c$</td>
</tr>
</tbody>
</table>

1 Values in a column with the same superscript do not differ significantly ($p > 0.05$).
2 Abbreviations: $T_o$ = onset temperature; $T_p$ = peak temperature; $T_c$ = conclusion temperature; $\Delta H$ = enthalpy change.
Figure 5.2: DSC thermograms of starch from the seven potato cultivars.
Table 5.3. Pasting properties of starch from seven potato cultivars1, 2

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>PT (°C)</th>
<th>PV (cP)</th>
<th>TV (cP)</th>
<th>BV (cP)</th>
<th>FV (cP)</th>
<th>SB (cP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carisma</td>
<td>70.2 ± 0.4f</td>
<td>13128 ± 69d</td>
<td>7595 ± 322d</td>
<td>5533 ± 253a</td>
<td>9009 ± 301b</td>
<td>1415 ± 21e</td>
</tr>
<tr>
<td>Nicola</td>
<td>62.8 ± 0.0b</td>
<td>12847 ± 28c</td>
<td>4088 ± 9b</td>
<td>8759 ± 19b</td>
<td>4876 ± 11b</td>
<td>788 ± 2d</td>
</tr>
<tr>
<td>Desiree</td>
<td>66.0 ± 0.0a</td>
<td>10812 ± 370b</td>
<td>5186 ± 70c</td>
<td>5626 ± 440a</td>
<td>6304 ± 31b</td>
<td>1119 ± 39c</td>
</tr>
<tr>
<td>Russet Burbank</td>
<td>60.8 ± 0.1a</td>
<td>8521 ± 83a</td>
<td>3215 ± 20a</td>
<td>5306 ± 64a</td>
<td>3869 ± 13a</td>
<td>654 ± 7a</td>
</tr>
<tr>
<td>Virginia Rose</td>
<td>64.2 ± 0.2d</td>
<td>13723 ± 141a</td>
<td>4934 ± 199c</td>
<td>8789 ± 59b</td>
<td>5881 ± 214c</td>
<td>947 ± 15c</td>
</tr>
<tr>
<td>Bintje</td>
<td>63.4 ± 0.4c</td>
<td>13262 ± 292a</td>
<td>3849 ± 24b</td>
<td>9413 ± 268c</td>
<td>4543 ± 106b</td>
<td>694 ± 82b</td>
</tr>
<tr>
<td>Maiflower</td>
<td>63.4 ± 0.3c</td>
<td>12478 ± 100c</td>
<td>3907 ± 52b</td>
<td>8571 ± 48b</td>
<td>4671 ± 105b</td>
<td>764 ± 52b</td>
</tr>
</tbody>
</table>

1 Values in a column with the same superscript do not differ significantly (p > 0.05).
2 Abbreviations: PT, pasting temperature; PV, peak viscosity; TV, trough viscosity; BV, breakdown viscosity; FV, final viscosity; SB, setback.
**Figure 5.3:** RVA profiles of starch from the seven potato cultivars
5.3 Discussion

The present study has shown that starch from the low GI potato cultivar Carisma had significantly higher thermal transition temperatures ($T_o$, $T_p$, and $T_c$) and starch paste temperature compared to starches from high GI cultivars. Trough and final viscosities, and hence setback viscosity, were also significantly different for Carisma compared to the other cultivars. Granule size of starch from Russet Burbank was significantly larger compared to the other six potato varieties but no relationship between granule size and GI was observed. While there were some differences between the cultivars with respect to amylose content, phosphorus content, relative crystallinity and amylopectin chain length profiles, none of the trends were consistent with the GI ranking of the potatoes.

Starch isolated from cultivar Nicola, which has a GI value (69) intermediate between Carisma and the other cultivars, had thermal and pasting properties that were similar to those of starch from the high GI cultivars. It is possible that physical properties of the tuber may also influence the GI and in vitro digestibility of starch in cooked potatoes. Potato cultivars differ in tuber cell size, shape and strength of cell wall structures (Bordoloi et al., 2012; Singh et al., 2005). Cell walls are considered to be a limiting factor for starch hydrolysis in foods (Colonna et al., 1992; Singh et al., 2010). Cell walls could act as a physical barrier for heat conductance during cooking and thereby reduce the extent of starch gelatinization, and they can affect the rate of starch hydrolysis by restricting enzyme access.

Higher DSC transition temperatures are thought to result from a higher degree of crystallinity, or more ordered crystalline regions, which impart greater structural stability and make the granules more resistant to gelatinization (Barichello, Yada, Coffin, & Stanley, 1990; Leszkowiat, Yada, Coffin, & Stanley, 1990). Potato starch with less crystalline order was observed to gelatinize at a lower temperature and reach a greater degree of gelatinization at the same temperature than more crystalline potato starch (Parada & Aguilera, 2012). The same study showed that glycemic response increased with a greater degree of starch gelatinization. The higher gelatinization temperature of Carisma starch suggests that the crystalline regions of Carisma starch are more stable than those of the other cultivars. Hence, under the same cooking conditions, Carisma starch could be gelatinized to a lesser extent and therefore elicit a lower glycemic response.
The parameter $\Delta H$, which measures the energy change due to loss of molecular order when hydrogen bonds break within the granule, is considered to be an indicator of the quantity and quality of the starch crystalline structure (Tester et al., 1990; Cooke and Gidley, 1992). Recent work examining the influence of water on the phase transition of starch during gelatinization in the DSC pan found that at a water:starch ratio of 1.5:1 or 2:1, the swelling of starch granules was incomplete and there was considerable residual crystallinity and lamellar structure at the end of the DSC gelatinization transition (Wang et al., 2012). Therefore in the present study, the DSC endotherm obtained at a water/starch ratio of 2:1, was water-limited and the gelatinization of starch granules was incomplete. The enthalpy change measured would have corresponded to the energy taken up until water became limiting, and was not indicative of the quantity or quality of the starch crystalline structure of the seven potato cultivars.

The pasting profile of Carisma starch was clearly different from that of the other six cultivars (Figure 5.3), with a significantly higher pasting temperature, higher trough and final viscosities. RVA pasting temperature provides an indication of the temperature at which granule disruption commences (Perten, 2011; Newport Scientific 1995). A higher pasting temperature indicates that Carisma starch required more heat for the onset of starch gelatinization during cooking. Continued heating past the temperature of peak viscosity results in the breakdown of swollen granules and realignment of starch polymer molecules, causing a decrease in paste viscosity. Carisma starch had greater resistance to breakdown as indicated by the significantly higher trough viscosity compared to the other starches (Figure 5.3). The setback viscosity is thought to result from the rearrangement of amylose molecules that have leached from swollen starch granules during cooling, and is indicative of the retrogradation tendency of starch (Karim, Toon, Lee, Ong, Fazilah, & Noda, 2007). Carisma starch had significantly higher final and setback viscosities compared to the other starches indicating a more viscous retrograded starch paste, which could confer resistance to enzymatic digestion. Food matrix viscosity has been observed to affect the enzymatic digestibility of starch and glycemic response (Singh et al., 2010). A high level of viscosity slows down propulsive and mixing effects generated by peristalsis, reducing interactions between substrates and digestive enzymes and also the absorption of hydrolysis products thus lowering postprandial glycemia (Singh et al., 2010).
There was no trend observed between many of the measured physico-chemical properties of the starches from the seven potato cultivars with their respective GI values, and with their DSC and RVA properties. These observations are similar to those of others, who found no significant relationship between amylose and phosphorus content with gelatinization temperature and enthalpy (Karim et al., 2007; Liu et al., 2007). Smaller granule sizes have been reported to be related to increased DSC transition temperatures and decreased enthalpy of gelatinization (Kaur, Singh, Sodhi, & Gujral, 2002; Singh et al., 2004). However, in the present study, Maiflower starch had a significantly smaller mean granule size compared to Carisma starch but did not show higher transition temperatures. Higher amylose content, fewer short amylopectin branch chains and smaller granule size were reported to be associated with higher pasting temperature, higher setback viscosity and higher peak viscosity temperature (Kaur et al., 2007; Schirmer, et al., 2013; Zaidul et al., 2007), however these associations were not observed in the present study. The presence of starch granule proteins, especially granule-bound starch synthase (GBSS) has been observed to affect starch swelling power, the integrity of the swollen granule and consequently starch paste rheology through playing a structural role in maintaining the gelatinized ghost structure (Han and Hamaker, 2002a). GBSS proteins are responsible for amylose production and were found to be concentrated internally in discrete concentric spheres in potato starch granules (Denyer et al., 1996; Han and Hamaker, 2002b; Zeeman et al., 2010). However, as the amylose content of the potato starches in this study did not differ significantly, a possible role for GBSS was not explored. Some studies have shown that the fine structural features of both amylose and amylopectin significantly influence the in vitro digestion rate of starch in cooked rice grains (Benmoussa, Moldenhauer, and Hamaker, 2007; Syahariza et al., 2013). Longer chain lengths of amylose branches, a smaller relative amount of long to short amylopectin branches and a smaller ratio of longer amylose branches to short amylopectin branches increased in vitro digestion rate (Syahariza et al., 2013). In the present study, no relationship was found between amylose content, amylopectin chain length profile and GI value, but the fine structures of branch chains in amylose and amylopectin were not investigated and could be possible factors that influence starch granule resistance to gelatinization during cooking, starch digestibility and consequently the GI value.
5.4 Conclusion

Starch from the low GI potato cultivar Carisma had higher DSC thermal transition temperatures and higher RVA starch paste temperature. These properties indicate that Carisma starch is more heat resistant, more resistant to shear and breakdown, and forms a stronger retrograded starch paste than the starches from the high GI potatoes. The combination of these functional properties could confer resistance of the starch in Carisma potatoes to enzymatic hydrolysis and hence eliciting a lower postprandial blood glucose response. The importance of dietary glycemic load and the popularity of potatoes as a food crop dictate the need to identify and develop cultivars that are digested slowly and have a low GI. This study suggests that thermal analysis and starch paste properties could be used as an aid in identifying and developing cultivars that are digested slowly and have a low GI.
CHAPTER 6

THE EFFECT OF GROWTH CONDITIONS
ON STARCH PROPERTIES
THE EFFECT OF GROWTH CONDITIONS
ON STARCH PROPERTIES

6.1 Introduction

The process of starch accumulation in potatoes is dependent on the genotype and environmental conditions as well as genotype by environmental interaction effects. There is increasing interest in the genotype by environment interaction and the effect on starch properties, particularly starch digestibility and the glycemic index of potatoes (Bach, Yada, Bizimungu, Fan, & Sullivan, 2013; Lu et al., 2011).

There is much evidence in the literature that environmental factors have significant effects on the synthesis and properties of potato starch (Haase & Plate, 1996; Kaur, et al., 2007; Noda et al., 2004; Tester et al., 1999; Yusuph et al., 2003). For some cultivars, particular tuber and starch characteristics were found to be more heritable while for other cultivars growth environment was a significant contributing factor to variability. For example, the total starch content of the Russet Burbank cultivar was not changed significantly across six different growing environments compared to the total starch content of the Norland cultivar which was found to be highly variable (Bach et al., 2013).

On the other hand, some tuber and starch characteristics were reported to be more genetically stable across cultivars whereas some are more controlled by the growth conditions. Morrison et al. (2001) found that different potato cultivars grown in the same environment showed significantly different starch granule size distribution and starch phosphorus content. Conversely, others reported that starch granule size, starch phosphorus as well as amylose content and gelatinization parameters of starches from different potato cultivars showed little variation when grown under the same environment conditions (Alvani et al., 2011; Yusuph et al., 2002). Some studies found that the same cultivars grown under different growing conditions had significant variations in total starch content, starch phosphorus contents, starch granule size distributions and gelatinization parameters (Cottrell, Duffus, Paterson, & Mackay, 1995; Haase et al., 1995; Tester et al., 1999). Haase et al. (1996) reported that amylose contents were consistently heritable but others found that amylose content was different between the same cultivars grown under different conditions (Cottrell et al., 1995; Tester et al., 1999).

The interplay between different potato genotypes and environmental conditions is complex and not clear. In Chapter 5, starch from the Australian grown Carisma cultivar had higher DSC thermal transition temperatures and RVA starch paste temperature,
higher trough and final starch paste viscosities compared to the higher GI cultivars. These properties indicated that Carisma starch was more resistant to gelatinization, and enzymatic hydrolysis compared to the starches from the high GI potatoes and were possibly factors influencing the GI value. It is not known how genetically stable these starch characteristics are if the Carisma cultivar is grown in a different environment. The studies in this chapter describe the physicochemical, thermal and starch paste properties of starch isolated from potato cultivars grown in the Netherlands under very different growing conditions.

6.2 Results

Carisma (low GI), Nicola (medium GI) and Russet Burbank (high GI) potato cultivars described in Chapter 3 were used in this study. According to advice from the growers in the Netherlands, the genotypes for the cultivars were the same as the cultivars grown in Australia but the genotypes were not tested with genomics. The potatoes were grown from April 2012 in clay soil in Bant, the Netherlands and tubers were harvested in two batches, the first batch was after 90 days of growth and the second batch after 120 days of growth. Starch was isolated from the potatoes shortly after harvest by a researcher from Agrico Holland using the method as described in Methods 2.2.10 as it was not possible to import the potatoes into Australia because of quarantine restrictions. The starch samples were then sealed and sent to Sydney for analysis. Starch isolated from Carisma potatoes grown in Australia and characterised in Chapter 5 was used as a control sample. The Australian grown Carisma potatoes were planted in April 2011 in sandy soil in the Pinaroo, South Australia for a growth period of 110-130 days. The meteorological conditions corresponding to the periods of growth in both locations are detailed in Table 6.1.

The following abbreviations will be used to refer to the starch samples analyzed in this chapter: CAB90, Carisma Bant (harvested after 90 days); CAB120, Carisma Bant (harvested after 120 days); NIB90, Nicola Bant (harvested after 90 days); NIB120, Nicola Bant (harvested after 120 days); RBB90, Russet Burbank Bant (harvested after 90 days); RBB120, Russet Burbank Bant (harvested after 120 days); CAA, Carisma Australia.

All analyses were performed as described in the respective sections in Chapter 2.
Table 6.1 Meteorological conditions in Bant, Flevoland, the Netherlands in 2012 and in Pinaroo, South Australia, Australia in 2011.

<table>
<thead>
<tr>
<th>Location</th>
<th>Month</th>
<th>Average Maximum daily temperature (°C)</th>
<th>Average Minimum daily temperature (°C)</th>
<th>Total Precipitation (mm)</th>
<th>Mean daily irradiation (MJ/m²)</th>
<th>Relative humidity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bant</td>
<td>April</td>
<td>8.6</td>
<td>29.8</td>
<td>5.2</td>
<td>15.5</td>
<td>78</td>
</tr>
<tr>
<td></td>
<td>May</td>
<td>9.6</td>
<td>26.7</td>
<td>5.5</td>
<td>16.6</td>
<td>77</td>
</tr>
<tr>
<td></td>
<td>June</td>
<td>10.4</td>
<td>24.3</td>
<td>7.4</td>
<td>17.7</td>
<td>75</td>
</tr>
<tr>
<td></td>
<td>July</td>
<td>11.4</td>
<td>22.8</td>
<td>9.3</td>
<td>18.9</td>
<td>83</td>
</tr>
<tr>
<td>Pinaroo</td>
<td>April</td>
<td>12.5</td>
<td>23.3</td>
<td>9.3</td>
<td>23.3</td>
<td>88</td>
</tr>
<tr>
<td></td>
<td>May</td>
<td>12.4</td>
<td>21.6</td>
<td>9.3</td>
<td>21.0</td>
<td>85</td>
</tr>
<tr>
<td></td>
<td>June</td>
<td>12.3</td>
<td>19.5</td>
<td>7.4</td>
<td>18.1</td>
<td>87</td>
</tr>
<tr>
<td></td>
<td>July</td>
<td>11.9</td>
<td>17.9</td>
<td>6.6</td>
<td>17.9</td>
<td>85</td>
</tr>
</tbody>
</table>

Weather data for Bant and Pinaroo was obtained from the Marknesse (KNMI) and Lameroo (BOM) weather stations respectively.

Humidity data for Pinaroo in 2011 was unavailable and based on average data from 1981-2010.
6.2.1 Physicochemical properties

Starch moisture content was significantly different amongst all seven starch samples (Table 6.2). CAB90, NIB90 and RBB90 had significantly higher starch moisture content compared to the CAB120, NIB120, RBB120 and CAA. CAB90 had the highest starch moisture content (19%) and CAB120 (14%) had the lowest. CAB90, NIB90 and RBB90 also had significantly higher amylose content (26–28%) compared to the respective 120-day starches (24–25%) and CAA (29%) had the highest amylose content while CAB120 (24%) had the lowest.

Mean granule size of the CAB90, NIB90 and RBB90 starches was significantly smaller than their 120-day counterparts (Figure 6.1 and Table 6.2). RB120 granules were the largest (mean size = 742 μm²) and CAB90 granules were the smallest (mean size = 176 μm²). while the granule size of CAA (mean size = 314 μm²) was similar to CAB120 (mean size = 335 μm²) and NIB120 (mean size = 345 μm²). CAB90 had the highest percentage of small starch granules (95%) and the lowest percentage of large granules (1%). In comparison, RBB120 had the lowest percentage of small granules (52%) and highest percentage of large granules (25%). The percentage of small starch granules in the CAB90, NIB90 and RBB90 starches were higher (85–95%) than the respective values for 120-day starches (52–82%), and higher than CAA (82%)(Figure 6.2).
Table 6.2: Properties of starch isolated from potato cultivars grown in different locations and periods of time.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Moisture content (%)</th>
<th>AM (%)</th>
<th>Mean GSA (µm²)</th>
<th>Transition temperature (°C)</th>
<th>DH (J/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAB90</td>
<td>19.0 ± 0.2</td>
<td>f</td>
<td>6.9 ± 0.5</td>
<td>74.9 ± 0.2</td>
<td>74 ± 2</td>
</tr>
<tr>
<td>CAB120</td>
<td>14.4 ± 0.1</td>
<td>a</td>
<td>24.1 ± 0.5</td>
<td>62.1 ± 0.5</td>
<td>63 ± 2</td>
</tr>
<tr>
<td>NIB90</td>
<td>16.9 ± 0.2</td>
<td>d</td>
<td>6.5 ± 0.5</td>
<td>74.5 ± 0.2</td>
<td>66 ± 2</td>
</tr>
<tr>
<td>NIB120</td>
<td>16.1 ± 0.1</td>
<td>c</td>
<td>24.1 ± 0.5</td>
<td>62.1 ± 0.5</td>
<td>63 ± 2</td>
</tr>
<tr>
<td>RBB90</td>
<td>18.9 ± 0.1</td>
<td>b</td>
<td>26.4 ± 0.4</td>
<td>74.1 ± 1.0</td>
<td>73 ± 2</td>
</tr>
<tr>
<td>RBB120</td>
<td>17.3 ± 0.1</td>
<td>e</td>
<td>25.2 ± 0.7</td>
<td>74.2 ± 1.0</td>
<td>73 ± 2</td>
</tr>
<tr>
<td>CAA</td>
<td>14.8 ± 0.0</td>
<td>a</td>
<td>28.6 ± 0.7</td>
<td>74.8 ± 2.0</td>
<td>77 ± 3</td>
</tr>
<tr>
<td>CAA20</td>
<td>17.3 ± 0.1</td>
<td>a</td>
<td>25.2 ± 0.7</td>
<td>74.2 ± 1.0</td>
<td>73 ± 2</td>
</tr>
<tr>
<td>CAA210</td>
<td>14.4 ± 0.1</td>
<td>f</td>
<td>6.9 ± 0.5</td>
<td>74.9 ± 0.2</td>
<td>74 ± 2</td>
</tr>
</tbody>
</table>

1 = peak temperature; 2 = conclusion temperature; DH = enthalpy change.

Values in a column with the same superscript do not differ significantly (p > 0.05).

Abbreviations: AM = amylose content; GSA = granule surface area; T = transition temperature.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Moisture content (%)</th>
<th>AM (%)</th>
<th>Mean GSA (µm²)</th>
<th>Transition temperature (°C)</th>
<th>DH (J/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAB90</td>
<td>19.0 ± 0.2</td>
<td>f</td>
<td>6.9 ± 0.5</td>
<td>74.9 ± 0.2</td>
<td>74 ± 2</td>
</tr>
<tr>
<td>CAB120</td>
<td>14.4 ± 0.1</td>
<td>a</td>
<td>24.1 ± 0.5</td>
<td>62.1 ± 0.5</td>
<td>63 ± 2</td>
</tr>
<tr>
<td>NIB90</td>
<td>16.9 ± 0.2</td>
<td>d</td>
<td>6.5 ± 0.5</td>
<td>74.5 ± 0.2</td>
<td>66 ± 2</td>
</tr>
<tr>
<td>NIB120</td>
<td>16.1 ± 0.1</td>
<td>c</td>
<td>24.1 ± 0.5</td>
<td>62.1 ± 0.5</td>
<td>63 ± 2</td>
</tr>
<tr>
<td>RBB90</td>
<td>18.9 ± 0.1</td>
<td>b</td>
<td>26.4 ± 0.4</td>
<td>74.1 ± 1.0</td>
<td>73 ± 2</td>
</tr>
<tr>
<td>RBB120</td>
<td>17.3 ± 0.1</td>
<td>e</td>
<td>25.2 ± 0.7</td>
<td>74.2 ± 1.0</td>
<td>73 ± 2</td>
</tr>
<tr>
<td>CAA</td>
<td>14.8 ± 0.0</td>
<td>a</td>
<td>28.6 ± 0.7</td>
<td>74.8 ± 2.0</td>
<td>77 ± 3</td>
</tr>
<tr>
<td>CAA20</td>
<td>17.3 ± 0.1</td>
<td>a</td>
<td>25.2 ± 0.7</td>
<td>74.2 ± 1.0</td>
<td>73 ± 2</td>
</tr>
<tr>
<td>CAA210</td>
<td>14.4 ± 0.1</td>
<td>f</td>
<td>6.9 ± 0.5</td>
<td>74.9 ± 0.2</td>
<td>74 ± 2</td>
</tr>
</tbody>
</table>

1 = peak temperature; 2 = conclusion temperature; DH = enthalpy change.

Values in a column with the same superscript do not differ significantly (p > 0.05).

Abbreviations: AM = amylose content; GSA = granule surface area; T = transition temperature.

Table 6.2: Properties of starch isolated from potato cultivars grown in different locations and periods of time.
Figure 6.1: LM images showing different sizes of starch granules from Carisma, Nicola and Russet Burbank potatoes grown for 90 days and 120 days in Bant, Netherlands. Scale bars represent 100 μm.
Figure 6.2: Percentage particle size distributions of starch from cultivars grown in different locations and growth periods.

Abbreviations: CAB90, Carisma Bant (harvested after 90 days); CAB120, Carisma Bant (harvested after 120 days); NIB90, Nicola Bant (harvested after 90 days); NIB120, Nicola Bant (harvested after 120 days); RBB90, Russet Burbank Bant (harvested after 90 days); RBB120, Russet Burbank Bant (harvested after 120 days); CAA, Carisma Australia.
Figure 6.3: DSC thermograms of starch from cultivars grown in different locations and growth periods.

Abbreviations: CAB90, Carisma Bant (harvested after 90 days); CAB120, Carisma Bant (harvested after 120 days); NIB90, Nicola Bant (harvested after 90 days); NIB120, Nicola Bant (harvested after 120 days); RBB90, Russet Burbank Bant (harvested after 90 days); RBB120, Russet Burbank Bant (harvested after 120 days); CAA, Carisma Australia.
6.3.2 Thermal properties

The potato starches presented well-defined single differential scanning calorimetry (DSC) endotherms (Figure 6.3). The thermal transition temperatures $T_o$, $T_p$, and $T_c$ ranged from 61.7 to 66.5°C, 64.5 to 69.9°C and 70.6 to 77.4°C, respectively, and the gelatinization enthalpies ranged from 18.9 to 21.2 Jg$^{-1}$ (Table 6.2). CAA starch had the highest values of $T_o$ (66.5°C), $T_p$ (69.9°C) and $T_c$ (77.4°C) followed by CAB90 and CAB120, whereas the lowest respective values were observed for RBB120. The DSC thermal transition temperatures $T_o$ and $T_c$ but not $T_p$ were also significantly higher than CAB120. Gelatinization enthalpies of CAA and CAB90 were significantly higher than NIB90 but there were no significant differences in gelatinization enthalpies between the other starch samples ($p > 0.05$).

6.3.3 Starch pasting properties

CAA, CAB90, NIB90 and RBB90 displayed RVA pasting profiles which were typical of potato starch but CAB120, NIB120 and RBB120 had different profiles with sharp peak viscosities and irregular breakdowns (Figure 6.4).

The main findings are summarised as follows:

- Pasting temperature ranged from 50.5°C to 69.2°C (Table 6.3 and Figure 6.4). CAA had the highest pasting temperature (69.2°C) followed by CAB90 and CAB120, whereas NIB90 had the lowest (50.5°C). CAA and CAB90, but not CAB120, had significantly higher pasting temperatures compared to the NIB90, NIB120, RBB90 and RBB120 consistent with DSC thermal transition temperatures.
- CAB120, NIB120 and RBB120 reached peak viscosity significantly faster (3.7–3.9 min) than the respective 90-day starches (5.6–5.9 min) and CAA (5.7 min) (Table 6.3).
- CAB90, NIB90 and RBB90 had significantly lower peak viscosities (8020 – 8133 cP) compared to their respective 120-day starches (9718 – 11053 cP) and CAA (10948 cP). Peak viscosity was lowest for NIB90 (8020 cP) and the highest for RBB120 (11053 cP).
- CAA had the highest trough (4425 cP) and final viscosity (4601 cP). Trough and final viscosities of the CAB90, NIB90 and RBB90 were all significantly higher than their corresponding 120-day starches.
**Figure 6.4:** Starch paste profile of starches from cultivars from different locations and growth periods.

Abbreviations: CAB90, Carisma Bant (harvested after 90 days); CAB120, Carisma Bant (harvested after 120 days); NIB90, Nicola Bant (harvested after 90 days); NIB120, Nicola Bant (harvested after 120 days); RBB90, Russet Burbank Bant (harvested after 90 days); RBB120, Russet Burbank Bant (harvested after 120 days); CAA, Carisma Australia.
Table 6.3: Pasting properties of starch isolated from potato cultivars grown in different locations and periods of time

<table>
<thead>
<tr>
<th>Sample</th>
<th>PT (°C)</th>
<th>PV (cP)</th>
<th>TV (cP)</th>
<th>BV (cP)</th>
<th>FV (cP)</th>
<th>SB (cP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAB90</td>
<td>65.8 ± 0.3</td>
<td>d</td>
<td>5.93 ± 0.0</td>
<td>d</td>
<td>8038 ± 74</td>
<td>a</td>
</tr>
<tr>
<td>CAB120</td>
<td>63.9 ± 1.0</td>
<td>e</td>
<td>5.71 ± 0.0</td>
<td>e</td>
<td>8995 ± 24</td>
<td>a</td>
</tr>
<tr>
<td>NIB90</td>
<td>50.5 ± 0.6</td>
<td>a</td>
<td>5.60 ± 0.1</td>
<td>c</td>
<td>8020 ± 18</td>
<td>a</td>
</tr>
<tr>
<td>NIB120</td>
<td>54.8 ± 1.6</td>
<td>b</td>
<td>3.70 ± 0.0</td>
<td>b</td>
<td>8082 ± 22</td>
<td>d</td>
</tr>
<tr>
<td>RBB90</td>
<td>69.2 ± 0.0</td>
<td>e</td>
<td>5.73 ± 0.2</td>
<td>cd</td>
<td>10948 ± 100</td>
<td>a</td>
</tr>
</tbody>
</table>

Values in a column with the same superscript do not differ significantly (p < 0.05).

Abbreviations: PT, pasting temperature; PV, peak viscosity; TV, trough viscosity; BV, breakdown viscosity; FV, final viscosity; SB, setback.

PROPERTIES OF STARCH FROM POTATOES DIFFERING IN GLYCEMIC INDEX
6.4 Discussion

Starches from Carisma potatoes grown in Bant, the Netherlands (CAB90 and CAB120) had significantly lower thermal transition temperatures ($T_o$, $T_p$ and $T_c$), pasting temperature, RVA trough and final viscosities compared to starch from Carisma potatoes (CAA) grown in Pinaroo, Australia. The starches from the potatoes grown for 90 days had significantly higher moisture content, higher amylose content, smaller mean granule size and higher percentage of small granules compared to their respective 120-day starches. The RVA starch pasting profile of starches from potatoes with a growth period of 120 days were significantly different from starches from potatoes grown for 90 days.

CAB120 was the most suitable starch sample for comparison with the Australian sample CAA as it has the same genotype and a similar growth period with only growth environment as a point of difference. CAB120 had a similar starch moisture content and mean granule size to CAA but significantly lower amylose content, lower DSC thermal transition temperatures, lower starch paste temperature and significantly lower trough and final viscosities. In Chapter 5, starch from the Australian Carisma cultivar had higher thermal transition temperatures suggesting that it was more thermally stable and hence more resistant to gelatinization compared to the higher GI cultivars. Starch from the Australian grown Carisma also had a greater resistance to breakdown and had a more viscous retrograded starch paste and hence more resistant to enzymatic digestion. The thermal and starch paste profiles of CAB120 was significantly different to CAA and showed no differences to starch from the medium GI cultivar (NIB120) and high GI cultivar (RBB120). The results suggest that CAB120 had significantly lower resistance to gelatinization and enzymatic digestion. Due to quarantine restrictions, it was not feasible to GI test the Bant grown Carisma potatoes. The possibility that Carisma potatoes grown in the Netherlands have a higher GI than Australian grown Carisma cannot be excluded.

CAB90 had significantly higher thermal transition temperatures, pasting temperatures and final and trough viscosities than the starches from the other cultivars, but for CAB120 these differences were not observed. This suggests that under the growing conditions in the Netherlands, when the Carisma cultivar is grown for a shorter growth period, the starch has greater resistance to starch gelatinization and enzymatic digestion, but these properties are lost as the tuber matures.
There were differences observed in the starches isolated from the same cultivar of potatoes but with different growth periods. CAB90, NIB90 and RBB90 had higher amylose contents, smaller mean granule sizes, higher DSC transition temperatures compared to the starches from their respective 120-day potatoes. CAB90, NIB90 and RBB90 also had lower peak viscosity, smaller breakdown and lower final viscosity compared to their respective 120-day starches. Starch paste temperatures of NIB90 and RBB90 but not CAB90 were significantly lower than their respective 120-day starches. The differences observed in the starches from potatoes with different growth periods were similar to those reported in other studies, with the exception of differences in starch pasting temperature (Christensen & Madsen, 1996; Liu et al., 2003; Noda et al., 2004).

CAB120, NIB120 and RBB120 starches had a significantly different starch pasting profile from their respective 90-day starches, reaching peak viscosity significantly faster with significantly higher peak viscosities, higher breakdown and lower final viscosities. These starch pasting profiles were different from the typical RVA profile for potato starch in that they displayed an unusually sharp peak and an uneven breakdown (Figure 2.1). These 120-day starch samples were tested 8-10 times with rewashing of the starch granules. The profiles continued to be atypical with the uneven breakdown, which is possibly due to polydispersity. It was concluded that the atypical starch profiles are a property of the starches and not an instrumental error as the 120-day samples were analyzed alongside the 90-day samples.

Australia generally has a dryer and warmer climate compared to the Netherlands. Though the meteorological data showed some similarities, the overall climate is different (Table 6.1). Generally, the temperatures from both growth locations, Bant (the Netherlands) and Pinaroo (Australia) were different during plant growth. The potatoes from Bant were grown from mid-spring to mid-summer, starting with colder conditions (4.3-11.9°C) during the initiation of tuber growth then harvested when it was warmer (12.1-21.0°C). Conversely, the potatoes from Pinaroo were grown from mid-autumn to mid-winter starting with warmer conditions (9.3-23.3°C) then harvested when the climate was colder at (5.2-15.5°C) (Table 6.1). Higher temperatures during tuber growth have been reported to result in smaller starch granule size, higher amylose content and higher pasting and transition temperatures (Cottrell et al., 1995; Kaur et al., 2007). In a study by Tester et al. (1999), the Maris Piper potato
cultivar was grown in similar conditions but at different temperatures of 10°C, 16°C, 20°C and 25°C. Increased gelatinization transition temperatures were observed with elevation of growth temperature independent of differences in granule size or starch composition (Tester, Debon, Davies, & Gidley, 1999). It may be possible that an overall warmer climate or higher temperatures at later stages of growth are factors for developing more thermally stable starch. However, other growing conditions could also contribute to these differences observed. The Bant location in the Netherlands had a different soil type, a wetter climate and more solar irradiation compared to the Pinaroo location in South Australia. To the best of knowledge found in the literature, soil type, water supply and amount of irradiation has not been shown to be directly linked to DSC thermal transitions and RVA starch paste characteristics. Genotype, environment and genotype by environmental interactions are complex and direct correlations to physiological properties are difficult to draw.

6.5 Conclusion

Properties of starch from the low GI cultivar Carisma are changed when the cultivar is grown under different environmental conditions. Starches from Carisma potatoes grown in the Netherlands were not as thermally stable and less resistant to gelatinization. Due to quarantine restrictions, the import of potatoes grown in the Netherlands was not possible. The GI testing of Carisma potatoes grown in The Netherlands would be ideal to observe the influence of starch thermal properties on GI values, and the performing of in vitro digestion tests to compare starch hydrolysis rates. This study has shown that for the Carisma genotype, low GI properties could be dependent on the growth environment of the potatoes.
In this thesis, seven potato cultivars consisting of well-established and newly introduced commercial cultivars of potatoes were studied to identify a low GI potato and to describe potato cell structures and starch properties of a low GI potato cultivar. This study showed that the potato cultivar Carisma was classified as low GI (GI = 53), Nicola (GI = 69) as medium GI and the other five cultivars were classified as high GI according to ISO guidelines. The GI values were strongly and positively correlated with the percentage in vitro enzymatic hydrolysis of starch from the cooked potatoes, particularly with the hydrolysis percentage at 120 min ($r = 0.91, p<0.01$). After cooking, the low GI cultivar Carisma showed markedly less cell swelling and isolated starch granules appeared to be more resistant to gelatinization compared to a high GI cultivar Russet Burbank. Native starch granules from Carisma potatoes appeared to have a more compact growth ring structure, and internal heterogeneities, which were observed in the starch from the higher GI cultivars, were not evident in Carisma starch. DSC studies showed that starch granules from Carisma were more thermally stable and hence more resistant to gelatinization. Carisma starch was also more resistant to shear and breakdown and formed a more viscous retrograded starch paste which could confer resistance to enzymatic digestion. However, thermal and starch paste properties from the low GI cultivar Carisma were changed when the same cultivar was grown in The Netherlands under different conditions.

The Carisma cultivar was an example of a low GI potato in which cell structure and starch properties was examined and compared with high GI potato cultivars. In addition to the GI testing in the present study, Carisma potatoes were also GI tested commercially numerous times using tubers from different field trials in Australia. The cultivar continued to show a consistently low GI classification. The characterization of properties of the Carisma cultivar in this research project allowed suggestions to be made toward criteria in the selection of other low GI cultivars.

The selection of low GI potato cultivars was discussed in a meeting with Agrico, a potato corporation based in the Netherlands that has research programs focused on breeding and developing new cultivars. Agrico are the breeders of the Carisma cultivar and the seed potatoes were grown by The Mitolo group in South Australia for sale into the fresh potato market in Australia. The whole breeding and selection process from the crossing of...
parental lines to the introduction of new cultivars to market requires at least 12–15 years of work. Firstly, the parent lines are selected for artificial cross-pollination between cultivars. After cross pollination, potato berries produce genetically unique seedlings that grow from true seeds and produce tubers that can be replanted as seed tubers. At this early stage, tens of thousands of genotypes are generated and go through various stages of screening. Tests are based on agronomical characteristics and yield, and taste and visual aesthetics of the cooked product, pest and disease resistance, suitability to target environments and the end use of cultivars (Haverkort & Grashoff, 2004). The Carisma cultivar was originally bred for the Mediterranean market, which prefers large, longish and smooth skinned potatoes. The cultivar performed well in the location trials in Egypt and other countries in that region. It is only after the mentioned criteria are fulfilled that the selection for nutritional properties such as the GI would be considered. A screening test for GI would be applicable when selection is narrowed down to 40–50 cultivars, coinciding with the 7th–9th year of the breeding cycle. These suggestions consider the stage of plant breeding that selection could be applied and the ease of conducting experimental techniques.

Results from this study suggests that thermal analysis, starch paste properties and in vitro starch hydrolysis could be used as an aid in identifying and developing cultivars that are digested slowly and have a low GI. Two approaches are suggested for the screening for low GI qualities. One approach is to use the in vitro starch hydrolysis method described in section 2.2.6. The use of this method would require the assessment of available carbohydrate content of all the cultivars tested, which could be performed by internal researchers or sent to commercial laboratories with such services. The second approach would be to examine the thermal qualities and starch paste properties of starch using a differential scanning calorimeter (DSC) and a rapid visco analyzer (RVA). Both the DSC and RVA are bench top instruments that are easy to operate.

The recommended approach would be the combination of both methods, the first step would be thermal and starch paste analysis of isolated potato starch from 20–30 cultivars. From these tests, a selection of 10–15 cultivars that show significantly higher thermal transition temperatures or starch paste temperature could be made. The in vitro starch digestion tests could then be performed on the cooked potato cultivars. Finally, breeders
could select 5-8 cultivars that show the lowest rate of starch hydrolysis as an indication of a lower GI classification. However, the confirmation of the actual GI value of the screened cultivars would still require in vivo testing according to the ISO standard (ISO, 2010). The in vitro starch digestion protocol described in this thesis is currently being implemented in South Australia in field trials for assessing the effect of growth conditions on the low GI property of the Carisma cultivar.

The extent of starch gelatinization is a key factor in digestibility and the rate of starch hydrolysis, and is inextricably linked to cooking time. Cooking increases the susceptibility of starch to enzymatic digestion, which is dependent on the degree of starch gelatinization, as shown in some in vitro (Tester et al., 2004; Monro et al., 2009) and in vivo studies (Parada et al., 2012). The cooking time of the GI tested potatoes in this project was considered short (9 min total cooking time) and it is highly likely that the starch granules in the potatoes were not completely gelatinized. The cooked product could include starch granules that are close to the raw state, granules in different stages of partial gelatinization, as well as completely gelatinized granules (Schirmer et al., 2013; Bordoloi et al., 2012). This cooking method was recommended by the growers and the testing volunteers provided feedback that Carisma, Nicola and Virginia Rose cultivars were considered cooked and firm, whereas the other four cultivars were regarded as softer. The seven potato cultivars were prepared using the same cooking method and the GI of the potatoes ranged from 53-103. The feedback from volunteers and range of GI results indicate that the potatoes in the study were “cooked” but the proportion gelatinized starch at the different stages is likely different, i.e. Carisma potatoes had more starch granules closer to the raw state compared to a high GI cultivar. In the list of published GI values (Table 1.2), medium and low GI values for potatoes were only obtained where potatoes were boiled for 15 min or less. This shows that cooking time is crucial for the differentiation of GI values between different potato cultivars. In studies where long cooking times were used, there was no differentiation in GI between potato cultivars (Fernandez et al., 2005; Soh et al., 1999). It is proposed that there is a point in cooking where cultivar specific properties such as a higher resistance to cell breakdown and/or thermal resistance to starch gelatinization no longer have an “advantage” when the potatoes are overcooked. Overcooking any potato may result in a high GI value regardless of cultivar or variety.
Other potato and starch properties examined in this research were not indicative of \textit{in vitro} starch digestibility or GI of potatoes namely: total starch content, reducing sugar content, dietary fibre content, amylose and phosphorus content of the starch, relative crystallinity, granule size distribution and amylopectin chain length distribution. The thermal resistance and starch paste quality of starch from Carisma potatoes is suggested to be attributed to the starch granule structure. Carisma starch granules appeared to have a more compact growth ring structure and internal imperfections which were found in starch from higher GI cultivars were not evident in Carisma starch. Fine structural features such as shorter chain lengths of amylose branches, a higher relative amount of long to short amylopectin branches and a higher ratio of longer amylose branches to short amylopectin branches were observed in cooked rice starch which was more resistant to \textit{in vitro} digestion (Syahariza \textit{et al.}, 2013). In this research project internal granular protein content, the fine structures of branch chains in amylose and amylopectin were not investigated and could be possible factors that influence starch granule resistance to gelatinization during cooking, starch digestibility and consequently the GI value.

Thermal transition temperatures and the pasting temperature of starch from Carisma potatoes grown in the Netherlands were significantly lower than the starch from Carisma potatoes grown in Australia. There were also no significant differences in the thermal and pasting properties of starch from the Carisma cultivar compared to medium and high GI cultivars that were grown in The Netherlands under the same conditions. It was not possible to GI test the Carisma potatoes grown in The Netherlands, but the possibility that they are not low GI cannot be excluded.

The use of micro-CT to study the effects of cooking on potatoes was a novel technique that has great promise. Possible future directions for research may include the analysis of the fine structural features of both amylose and amyllopectin and a more detailed study on the influence of potato cell structure on starch gelatinization. It would be pertinent to test the GI value of Carisma potatoes grown in the Netherlands to see if it would still be low GI and to use CLSM to examine the starch granules of Carisma potatoes grown in the Netherlands. Effect of environmental factors on tuber growth and starch properties is also an area where further research is required, given that the interplay of genotype and environmental factors is complex and multi-faceted.
Hyperglycaemia and the incidence of diabetes is rising globally (Danaei et al., 2011) and the prevalence of overweight or obesity among men and women has reached a level of over 65% and 50% respectively in Australia, the USA and England (IASO, 2013). It is generally agreed that diet can have a significant effect on human health outcomes but recommended diet composition has been a continuing source of debate in human nutrition. The Diet, Obesity and Genes (Diogenes) study, a pan-European, multicenter dietary intervention study involving more than 750 participants showed that the combination of a low GI and high protein diet was the most beneficial for the maintenance of weight loss compared to other diets (Larsen et al., 2013). The low GI, high protein combination diet was also the easiest to sustain. In that study, potatoes were estimated to have a GI of 85 and classified as a high GI food (Aston et al., 2010). However, potatoes are a carbohydrate staple globally and avoidance is not realistic. The importance of dietary load for health and popularity of potatoes as a food crop dictate the need to identify and develop cultivars that have a low GI. This thesis provides characterization of a low GI cultivar of potatoes and suggests methods to screen for more low GI cultivars.
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Glycemic effect of potatoes

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1. Introduction

Would you like fries with that? Potatoes, as the world’s third largest food crop and the most extensively consumed root vegetable, have received much attention in recent years by nutritionists and agriculturalists (FAO, 2008). This review provides a background on potatoes as a food crop, considers the properties of potato starch, particularly in relation to glycemic index (GI) and digestibility, and explores areas for future research.

Carbohydrates make up one of the four major sources of energy in the human diet, the other three being fat, protein and alcohol. Carbohydrates have an energy value of 17 kJ/g (4 kcal/g) and account for 40–75% of energy intake depending on social and economic factors (FAO/WHO, 1998). Carbohydrates that release glucose affect blood glucose and insulin levels, cholesterol and triglyceride metabolism, as well as influencing satiety, and exerting prebiotic effects in the large intestine (Eckel, Grundy, & Zimmet, 2005; Englyst, Liu, & Englyst, 2007; Holt, Miller, Petocz, & Farmaklidis, 1995; Saltiel & Kahn, 2001; Venn & Green, 2007).

Potatoes are a popular source of carbohydrates that are consumed globally. Consumption is currently greatest in the Western world, but potatoes are rapidly becoming a staple in developing countries. Besides being a carbohydrate and energy source, they are also rich in micronutrients, for example, vitamin C, B vitamins and potassium, as well as carotenoids and antioxidant phenols. Potatoes may also contain a toxic glycoalkaloid, solanine (Burlingame, Mouille, & Charrondiere, 2009). The nutrient composition of different varieties of potatoes are described in recent reviews (Burlingame et al., 2006; Camire, Kubow, & Donnelly, 2009), and will not be considered further here.

The major nutrient in potatoes is starch, which is the largest source of carbohydrates in the human diet. The importance and complexity of this plant polysaccharide has been the subject of extensive research and detailed reviews, as indicated by the following selected examples, which cover biosynthesis (Zeemann, Kossmann, & Smith, 2010); structure (Perez & Bertoft, 2010; Zobel, 1988); physico-chemical, morphological and functional characteristics (Copeland, Blazek, Salman, & Tang, 2009; Delcour & Hoseney, 2010; Tester, Karkalas, & Qi, 2004); digestion (Singh, Dartois, & Kaur, 2010); and nutritional qualities (Englyst et al., 2007).

The main focus of this review is concerned with the glycemic effect of potatoes. The term glycemic index (GI) was first introduced in 1981 by Jenkins et al. as a physiological (in vivo) way of classifying foods rich in carbohydrates based on their blood glucose-raising potential (Jenkins et al., 1981; carbohydrates with blood-glucose raising potential will be referred to hereafter in this review as available carbohydrates). Foods with a GI value of above 70 are classified as high GI, foods with a GI of 56–69 as medium GI, and foods that have a GI of 55 and less are classified as low GI (ISO Standard 26,642, 2010).

An in vitro method for measuring digestibility of starch and classifying nutritionally important fractions was introduced by Englyst et al. (1992), who measured the rate of glucose released from starch using digestive enzymes (Englyst et al., 1992). Both in vivo and in vitro approaches are used to classify digestibility of carbohydrates and starch from a nutritional perspective. Consumption of available carbohydrates that are digested and

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absorbed rapidly results in high postprandial blood glucose levels, which over the long term are associated with increased risks of obesity, and diet-related diseases including type-2 diabetes, cardiovascular disease and certain types of cancers (Liu et al., 2000; Ludwig, 2002; Mozaffarian, Hao, Rimm, Willett, & Hu, 2011; Willett, Manson, & Liu, 2002). Studies on different cultivars of potatoes using *in vivo* and *in vitro* methods indicate that cooked potatoes have mostly rapidly digested starch (Leeman, Barstrom, & Bjorck, 2005) and a high GI (Atkinson, Foster-Powell, & Brand-Miller, 2008). Hence, many nutritionists advise that potatoes should be substituted with a low GI carbohydrate to reduce the risk of chronic disease (Brand-Miller, McMillan-Price, Steinbeck, & Caterson, 2009; Willet et al., 2002). However, as discussed subsequently in this review, this advice may not apply to all potatoes.

### 2. Potatoes as a food crop

Potatoes were first domesticated more than 6000 years ago near Lake Titicaca in the Andes Mountains of South America, on the border between Bolivia and Peru. The greatest diversity of wild species of potatoes is still currently found in this region (CIP., 2008; Radder, 2008). The Spanish Conquistadores first encountered the potato when they arrived in Peru in 1532 in search of gold. Potatoes were brought back to Europe in the 16th century as a novelty, but due to their association with the poisonous “nightshade” plant family, were initially considered as food fit only for livestock and the poor. This started to change in the 18th century when early maturing varieties were bred and grown, and found to perform well in northern growing conditions. Famine plagued much of Europe during this period and the potato became recognised as a food security crop. However, the potato crops cultivated were limited to a few genetically similar varieties, and when the disease “late blight” struck the Irish potato crop in 1845, it caused the tragic Irish potato famine. This famine led to the death of at least one million people and due to subsequent emigration, the population of Ireland decreased from 8 to 3 million people. Expeditions were mounted to import new germplasm into Europe from Latin America for breeding of higher yielding and disease-resistant varieties. Research on improving potato germplasm continues worldwide and in particular at the International Potato Centre (CIP) in Peru, where varieties with enhanced nutrient content are being bred. A recent example is the selective breeding of varieties that are rich in bioavailable iron, to address deficiencies of this micronutrient in pregnant women and pre-school children in the developing world (CIP., 2011).

The importance of the potato as a human food was recognised by the Food and Agriculture Organization (FAO) of the United Nations with its declaration of 2008 as the “The International Year of the Potato”. This initiative sought to focus world attention on the importance of the potato in food security and alleviating poverty, and resulted in the collation of much valuable information on potatoes (www.potato2008.org; FAO, 2008a). Potatoes are the number one non-grain food commodity produced globally with an annual production quantity of 300–320 million tons, accounting for almost 50% of the total production of food roots and tubers (FAO, 2008b). Much of the recent growth in world potato production (from 268 million tons in 1991) has occurred in developing countries where more than 50% of the world’s harvested crop is now produced (FAO, 2008b). Per capita potato consumption in 2005 was estimated to be 93 kg in Europe, 60 kg in North America and 22 kg in China (FAO, 2008b).

Potatoes exhibit great genetic diversity and morphological variability, which has resulted in much debate about the taxonomic classification of wild and cultivated varieties. This debate is concerned with the number of recognised potato species and their interrelationships, as well as methods of classification by morphological and physiological characteristics, ecogeography, ploidy or a combination of these properties. Most potatoes cultivated currently are designated collectively under the single species name of *Solanum tuberosum* L., although taxonomic studies suggest that there are four cultivated potato species (Spooner et al., 2007). About 10 other *Solanum* species have been cultivated and some 200 additional wild species are recognised (FAO, 2008c). We refer readers to a review by Ovchinnikova et al. (2011) on the history and description of these different taxonomic classifications, as well as images of lectotype specimens.

*S. tuberosum* L. belongs to the *Solanaceae* family which is the nightshade family of flowering plants. Potatoes share the genus *Solanum* with other economic plants like tomato, eggplants, capsicum, tobacco and petunias. The potato genome is a medium sized plant genome with 12 chromosomes and a haploid length of approximately 840 million base pairs (Visser et al., 2009). *S. tuberosum* L. (2n = 4x = 48) is an autotetraploid species that is reproduced by asexual propagation. Most potato genotypes are genetically heterogeneous regardless of cultivar or genetic stock (Razdan & Mattoo, 2005).

Two major difficulties in the genetic analysis of potatoes are the lack of pure lines and polyploidy. Hence, many qualitative and quantitative agronomic characteristics are still not well understood. Quality attributes used agriculturally and commercially include high yields in different environments, broad-spectrum resistance to diseases, number of days to maturity, good storage characteristics, tuber and flesh colour, and traits suited to cooking and processing methods. Many potato growing countries have their own database cultivated potato varieties for the local conditions. Examples are the British Potato Council, the Canadian Food Inspection Agency, the Potato Association of America and the European Cultivated Potatoes Database.

### 3. Potato starch

Starch is ubiquitous in plants as the main storage reserve of carbon. It is deposited in semi-crystalline granules, which differ in size (from <1 to 100 μm in diameter); shape (oval, polygonal, spherical, lenticular, irregular); size distribution (unimodal or bimodal); and surface topography (grooves, ridges and pores) depending on botanical origin (Fannon, Hauber, & Bemiller, 1992; Jane, Kasemsuwan, Leas, Zobel, & Robyt, 1994; Sujka & Jamroz, 2009; Tester et al., 2004). Potato starch granules consist of two polymers of D-glucose joined together by glycosidic bonds: amylose, an essentially linear polysaccharide of α(1 → 4) linked D-glucoses and amylopectin, a highly branched polysaccharide which consists of α(1 → 4) linked D-glucoses with α(1 → 6) branching links (Smith, 2001). The organisation of the amylose and amylopectin chains into starch granules can be considered at several hierarchical size scales, from the molecular (0.1–1.0 nm) to the macroscopic (2–100 μm). These are the nanoscale arrangement of amylose and amylopectin into amorphous and crystalline lamellae, the aggregation of amorphous and crystalline regions into blocklets (20–500 nm), growth rings (120–500 nm) and finally the intact starch granule (2–100 μm) (Vandeputte & Delcour, 2004). The overall structure, with crystalline and amorphous regions arranged in concentric growth rings (Fig. 1a), gives intact mature starch granules their characteristic property of birefringence when viewed under polarised light (Fig. 1b). Progress towards an understanding of how amylose and amylopectin are assembled to form starch granules is discussed elsewhere (Copeland et al., 2009; Perez & Bertoft, 2010). For the purpose of this review, the discussion will be concerned mainly with properties of potato starch.
3.2. Functional properties of potato starch

Potatoes need to be cooked prior to consumption. Hence, an examination of the functional properties of potato starch, such as solubility and swelling power, gelatinisation and retrogradation, and thermal characteristics, are important in understanding the glycemic effect of potatoes. When starch granules are heated in excess water, intra and inter chain hydrogen bonds between amylose and amylopectin are broken and water molecules bond to exposed hydroxyl groups. This process, termed gelatinisation, disrupts the crystalline structure of the starch granules, resulting in increased solubility of glucan chains (mostly amylose) and granule swelling. The extent of swelling and solubility reflect the strength of interactions between starch chains. As the temperature increases, starch undergoes an irreversible phase transition where the native crystallinity, structural organization and birefringence are lost (Jenkins & Donald, 1998). The unusually high solubility and swelling power of potato starch, compared to starches from other botanical sources, are considered to be due at least in part to repulsive forces between phosphate groups on adjacent amylopectin chains weakening the bonds in the crystalline domain, allowing water molecules to more readily disrupt the structure (Hoover, 2001).

Differential scanning calorimetry (DSC) is widely used to measure the heat energy required for starch gelatinisation. The enthalpy change ($\Delta H$) measures the loss of molecular order due to hydrogen bonds breaking within the granule; this parameter is considered to be an indicator of the quantity and quality of the starch crystalline structure (Tester & Morrison, 1990). Most starches gelatinise in the temperature range of 60–80°C, depending on the botanical sources and heating conditions. Potato starches generally gelatinise between 64 and 72°C (Alvani et al., 2011; Kaur et al., 2007; Liu et al., 2003; Liu et al., 2007; Singh, Singh, Kaur, Sodhi, & Gill, 2003; Yusuph et al., 2003).

If the starch concentration is above about 5%, the hydrated and disaggregated amylose and amylopectin molecules in the disrupted granules reassociate to form a gel that retrogrades gradually on cooling into a semi-crystalline form different from that of native starch. Further cooling causes an increase in gel firmness and the loss of water binding through syneresis. Amylose molecules retrograde more quickly (minutes to hours) than amylopectin molecules, which take days to weeks to retrograde (Ring et al., 1987; Sievert & Wursch, 1993). The reassociation of linear amylose chains is restricted by the presence of amylopectin (Sievert & Wursch, 1993) and the long chain length of potato amylose may also restrict chain reassociation (Chung & Liu, 2009). Nutritionally, retrograded starch is more resistant to digestion (Englyst et al., 1992) and its effect on GI is discussed later in this review.

3.3. Enzymatic hydrolysis

The rate of breakdown of starch granules into glucose units by amylolytic enzymes has been investigated extensively. Native starch granules from different botanical origins have different susceptibility to enzymatic breakdown, with potato starch granules among the most resistant to digestion (Gallant, Bouchet, Buleon, & Perez, 1992; Hoover, 2001; Kimura & Robyt, 1995). Small granules are digested more rapidly than large granules (Dhital, Shrestha, & Gidley, 2010; Kaur et al., 2007; Noda et al., 2005). The larger surface area to volume ratio of small granules allows greater access for enzymes, and hence more rapid breakdown. The surface area to volume ratio of potato starches ranges from 0.06 to 1.2 given the large variation in granule size (Tester, Qi, & Karkalas, 2006).

Dhital et al. (2010) observed that native maize starch was digested at a 30-fold faster rate than potato starch. They postulated that the presence of enzyme-accessible channels on the surface of
maize granules was the main reason for such a large difference in rates of hydrolysis between maize and potato granules. Native potato starch granules incubated with enzymes showed minor surface pitting, which suggests that digestion of potato starch is by exocorrosion of the granule (Gallant et al., 1992; Kimura & Robyt, 1995). The exocorrosion of the granule has been proposed to be slowed down by the presence of hard crystalline layers under the granule surface made from large blocklets (Gallant et al., 1992).

High amylose content has been associated with a greater resistance to enzymatic digestion in rice (Hu, Zhao, Duan, Zhang, & Wu, 2004) and maize starch in both raw and processed states (Htoon et al., 2009). How amylose reduces digestibility is unclear, as the location and distribution of amylose in relation to amylopectin in the structural matrix of the granule is still not well established. Amylose chains are thought to be abundant in the core of the granules and distributed in a radial fashion amongst amylopectin. Larger amylose molecules may be located more in the centre of the granule, whereas shorter amylose chains located at the periphery of the starch granule may be more easily leached during gelatinisation (Jane & Shen, 1993). Potato amylose has a very long chain length, which could have a structure-stabilising effect and contribute to the resistance of the potato starch granule to enzymatic digestion. In maize, barley and pea starch, amylose is thought to disrupt the structural order within the crystalline arrangement of amylopectin (Jenkins & Donald, 1995).

The high resistance to enzymatic digestion of raw potato starch is completely lost on gelatinisation (Farhat et al., 2001; Noda et al., 2008). Factors such as median granule size and amylose content, which influence the rate of enzymatic digestion of raw potato starch, no longer apply when the starch is gelatinised (Noda et al., 2008). Absar et al. (2009) observed that higher phosphorus content, which had no effect on digestion rate of raw potato starch, conferred resistance to enzymatic digestion for gelatinised potato starch. This effect was also observed by Noda et al. (2008) for mixtures of potato and other starches, but there was no correlation between phosphorus content and the rate of enzymatic digestion of gelatinised potato starch alone. Liu et al. (2007) suggest that amylose content, phosphorus content, the proportion of specific chain length fractions and starch morphology are all factors that influence digestibility of gelatinised potato starch. Rapid enzymatic breakdown after gelatinisation was also observed with wheat starch, but no clear correlation was found with chemical and functional properties (Blazek & Copeland, 2010).

4. Effect of cooking and processing

Potatoes are prepared domestically by various methods prior to consumption, including boiling, baking, microwaving or frying. This section describes cellular changes and starch gelatinisation in situ when different methods of heating are used to cook potatoes. Potato tissue consists of large (200 × 340 μm) and small (80 × 90 μm) cells, with each cell observed to contain six to ten large starch granules (10–70 μm in diameter) and hundreds of very small starch granules (0.5–1.0 μm) (Singh, Kaur, Ezekiel, & Guraya, 2005). The cells in raw potato tubers have been reported to have an isodiametric polygonal outline, with variation in cell size, cell shape, cell wall thickness and starch granule sizes between cultivars (Singh et al., 2005; Thybo, Martens, & Lyshede, 1998). Fig. 2 shows an environmental SEM image of a section of a Russet Burbank potato in which some of these features are evident.

Isolated potato starch granules do not gelatinise simultaneously, with larger granules tending to gelatinise before smaller ones (Parada & Aguilera, 2009; Singh & Kaur, 2004). This property has not been studied extensively for starch granules in situ. As digestibility in the gut is likely to be dependent on the proportion of gelatinised granules after cooking, it therefore is important to understand starch gelatinisation in situ. Published microscopy studies on the effects of cooking on potato microstructure and starch gelatinisation are summarised in Table 1.

As indicated in these studies, the two main changes that occurred concurrently during boiling were breakdown of the cell walls and middle lamellae, and intracellular starch swelling and gelatinisation. When potatoes are boiled for 5 min, starch granules hydrate, swell and fill the cell lumen. This continues throughout the cooking period as the cells start to separate at the middle lamellum. After 10 min of cooking, swollen starch granules were observed to have formed dense clusters in which the starch granules did not undergo further expansion on continued heating. Cells are completely separated after 20–30 min and after 50 min the cell structure is completely collapsed (Thybo et al., 1998). Differences observed between potato varieties include cell wall density and rate of breakdown of the middle lamellae (van Marle et al., 1997).

When potatoes are fried, the high temperature and the existing water within cells lead to complete starch gelatinisation. Water inside the cells is quickly absorbed by the starch granules before dehydration of the cells occurs. Confocal laser scanning microscopy was used to optically section the potato crust after frying and revealed that frying oil was located in the interior in pockets or surrounding intact potato cells (Bouchon & Aguilera, 2001). Isolated starch granules on frying gelatinised at a 6–7 °C lower temperature than starch granules located inside the potato cells (Aguilera, Cadache, Lopez, & Gutierrez, 2001).

The changes that occur in baked potatoes have not been studied as extensively as fried or boiled potatoes. Baking uses dry heat to cook foods and for cooking temperature to be reached the process occurs much more slowly than other forms of cooking. In a study by Wilson, Mackinnon, and Jarvis (2002a), whole potatoes were baked for 60 min in a conventional fan-forced oven. The temperature profiles within the potatoes were determined using direct measurement. The centre of the potato required 30 min to reach a temperature of 100 °C, and 60 min were required before the texture at the centre of the potatoes reached a degree of softness acceptable for consumption. The authors concluded that the rate of heat transfer into the potato was limited by evaporative cooling at the potato surface and that the dried potato skin acted as a barrier to heat transfer.
Microwave cooking is increasingly popular due to its speed and convenience. In microwave cooking, heat generated by molecular vibration of water molecules in foods is suggested to have a greater convenience. In microwave cooking, heat generated by molecular vibration was found to occur in two phases: in the first phase, the starch granules gelatinised evenly in situ, but there was no closer examination of the extent of starch gelatinisation. In the second phase of rapid water absorption was used to evaporate water (Wilson, MacKinnon, & Jarvis, 2002b). In this study, whole potatoes were cooked in an 800 W microwave oven and the gelatinisation temperature of the potato starch was assumed to be 65 °C. This temperature was reached in 2–2.5 min of cooking. The second phase of rapid water loss was observed after 4 min of cooking, and after 7 min the potatoes were cooked enough texturally for consumption. Microwave cooking of potatoes was found to be faster than other conventional methods, and while the potato was deemed as cooked texturally, there was no closer examination of the extent of starch gelatinisation in situ in this study. As observed by SEM in another microwave cooking study, the starch granules gelatinised evenly in situ throughout the whole potato with increasing cooking time and temperature (Huang, Hess, Weber, Purcell, & Huber, 1990).

The direct effect of microwave irradiation on isolated potato starch granules was studied using light microscopy (LM). At 68 °C, the approximate gelatinisation temperature of potato starch, there was no change in starch appearance, and the initial phase of gelatinisation and amylose leakage were only observed when 90 °C was reached. Post irradiation, the mainly B-type crystalline poly-morph structure of potato starch had changed to mainly A-type (Lewandowicz, Fornal, & Walkowski, 1997). When AFM was used to study the effect of microwave heating on the nanostructure of the potato starch, the granules were observed to be incompletely gelatinised. The authors explained their observations on the basis that there was very rapid water transfer and non-uniform heating caused by microwave radiation (An, Yang, Liu, & Zhang, 2008).

Microscopy techniques are valuable in examining changes to potato microstructure and starch gelatinisation in situ. However, a standardised quantitative method for measuring such changes has not been developed, but would be useful to evaluate these changes when different forms of heat are used to cook potatoes in relation to digestibility.

## 5. Glycemic effect of potatoes

### 5.1. Glycemic index and glycemic load

Blood glucose concentration is tightly controlled in healthy people in a narrow range between 4 and 10 mmol/L by homeostatic regulatory systems in the human body. Hyperglycemia stimulates insulin secretion from the β-cells in the pancreas, promoting glucose uptake by muscle and fat cells and inhibiting hepatic glucose production. Hypoglycemia, on the other hand results in the release of glucagon and other hormones, which have opposite actions to insulin to restore normal glucose levels. Besides acting as the primary regulator of blood glucose, insulin also stimulates lipogenesis and inhibits lipolysis for storage in fat, liver and muscle cells (Ludwig, 2002; Saltiel & Kahn, 2001). When a large meal of rapidly-digested available carbohydrates is consumed, it induces an initial period of high blood glucose and elevated insulin levels, which is followed by “reactive hypoglycemia” and elevated serum free fatty acid concentrations. In some individuals, the frequent consumption of high glycemic impact meals over time is associated with insulin resistance, β-cell dysfunction, dyslipidemia and endothelial dysfunction, all of which increase risk for obesity, metabolic syndrome, type-2 diabetes and cardiovascular disease (Eckel et al., 2005; Ludwig, 2002; Venn & Green, 2007).

The GI is a relative measure of the postprandial glycaemia evoked by foods that are significant sources of carbohydrate (Jenkins et al., 1981). The GI of a food is determined as the incremental area under the curve (IAUC) for blood glucose response after the consumption of a test food containing a known carbohydrate portion expressed as a percent of the IAUC for the same food as the reference.
amount of carbohydrate from a reference food (Standards Australia, 2007; ISO, 2010). The GI value also reflects the rate of glucose absorption after a meal, which has significant effect on postprandial hormonal and metabolic responses (Ludwig, 2002).

Thus, the GI is a physiologically based measure that includes the digestion of the carbohydrate, absorption of glucose from the gut, and its clearance from the bloodstream during the test period. The Australian Standard “Glycemic Index of Foods” was published in 2007 to establish a recognised scientific method for determining the GI of foods (Standards Australia, 2007). In 2010, the International Standards Organisation published its definition of GI, along with the GI test method and the GI classification of foods as a new international standard (ISO Standard 26,642, 2010).

As both quantity and quality of available carbohydrate foods affect the glyemic response, the concept of glycemic load (GL) was introduced in 1997 by nutritionists at Harvard University to quantify the overall glyemic effect, factoring in the portion size of the carbohydrate food (Salmeron et al., 1997). The GI of a portion of the food was therefore defined as the product of the amount of available carbohydrate in the food and the GI of the food divided by 100. By adding up the GI contributed by individual carbohydrate foods in the meal, the overall dietary GL can be calculated. The greater the GI, the higher is the increase in blood glucose and insulinogenic effect of the food (Bao, Atkinson, Petocz, Willett, & Brand-Miller, 2011).

The use of GI in making carbohydrate food choices in conjunction with information on food composition was endorsed by the FAO in 1998 (FAO/WHO, 1998). In their 2007 update on “Carbohydrates in Human Nutrition”, GI was deemed most appropriately used to guide food choices when comparing similar types of carbohydrate foods. One concern with using only the GI to make carbohydrate food choices is that some low GI foods may be high in energy and contain high amounts of sugars or fat that could suppress the glyemic response (Mann et al., 2007). For our purposes, as fresh potatoes are a food that is minimally changed in composition by processing prior to consumption, the GI of potatoes should serve as a useful tool for ranking potato varieties.

5.2. Potato GI values

Potatoes were included in the listed values in the first GI paper published in 1981. They were described as “potato (new)” and had a high GI value of 70 (Jenkins et al., 1981). Subsequent studies on potatoes have resulted in a wide range of values from as low as 56 to as high as 104 (see Table 2). Some values were determined in only a small number of healthy subjects (<10) or in subjects with impaired glucose tolerance. These values are all listed in the updated “International Tables of Glycemic index and Glycemic Load values: 2008” (Atkinson et al., 2008). The GI values of potatoes extend over a large range and vary depending on cooking method, processing and the meal composition (Atkinson et al., 2008; Fernandes, Velangi, & Wolever, 2005; Henry, Lightowler, Kendall, & Storey, 2006). For the purpose of this review, the list has been condensed into Table 2, which includes only those studies where the cultivar was specified and where at least 10 healthy subjects were tested. Studies in which potato varieties were not specified or were eaten with additional ingredients have not been included.

Most cultivars of potatoes fall into the medium or high GI range, with a few varieties classified as low GI. When different cooking methods are compared, it is apparent that all medium and low GI values listed in Table 2 were obtained from potatoes boiled for 15 mins or less, although some potato varieties cooked in this way had a high GI. For example, a GI value of 56 for Marfona variety can be compared to a GI of 85 for Maris Piper variety. This large variation in GI values, which was observed in the same study (Henry, Lightowler, Strik, & Storey, 2005), indicates inherent variance in GI values between potato varieties.

As noted previously, potatoes are generally considered a high GI food, but because they have a high water content (Burlingame et al., 2009), they provide physical bulk to a meal. Holt et al. (1995) found potatoes to be one of the most satiating foods per 1000 kJ of energy among 40 common foods. In a comparison of potatoes with other carbohydrate staples, potatoes have lower available carbohydrate content per nominal serving than pasta and low GI rice, resulting in a similar glyemic load to pasta and low GI rice. While potatoes have a similar glyemic load to pasta and low GI rice based on serving size, on an isocaloric basis (per 1000 kJ), potatoes have a higher GI (Table 3).

5.3. Effect of processing on GI values

The main food factors that lower postprandial glycaemia act by the affecting the rate of gastric emptying and/or the rate of carbohydrate digestion and absorption. These factors can be an inherently natural quality of the food, such as the high beta-glucan content of barley (Granfeldt, Liljeborg, Drews, Newman, & Bjorck, 1994) or the high amylose nature of legume starches (Guillon & Champ, 2002). Food processing, for example the extent of milling of grains, can increase the access of enzymes and water for gelatinisation and in turn, the rate of starch digestion. The effect on GI of meal preparation and way in which potatoes are consumed are also relevant and are described subsequently.

There are many variations in the methods used to cook potatoes. They may be cut into pieces or cooked whole, using methods that employ boiling in water, baking in the oven, frying in oil or microwave radiation and cooking times that range from 10 to 60 min. These cooking factors are important in determining the extent of starch gelatinisation, and hence digestion. Soh and Brand-Miller (1999) investigated the effect of variety and cooking method on the GI of potatoes and found all of the studied varieties to have high GI values. One of the varieties (Pontiac) was tested after boiling, baking, mashing or microwave cooking, and all of the GI values were in the high range. There was little difference in the GI value when the same variety of potatoes was boiled and mashed versus boiled and cut into large pieces (Soh & Brand-Miller, 1999). Likewise, in another study on different industrial processes for cooking and making potato products, high GI values were recorded regardless of processing method (Tahvonen, Hietanen, Sihvonen, & Salmi-Nen, 2006).

One preparation method that has been shown to significantly the GI of potatoes is to serve them cold. Refrigeration at 8 °C for 24 h reduced the GI value by 26% compared to the same potatoes served hot (Leeman, Ostman, & Bjorck, 2005). In this study, resistant starch content increased only from 3.3% to 5.2% (starch basis), and from this relatively small increase, it could be inferred that cold storage converts some of the rapidly digested starch into slowly digested starch. Tahvonen et al. (2006) also found that the GI of potatoes after cold storage and reheating was lowered about 25%. A study by Fernandes et al. (2005) showed that reheating cooled Russet Burbank potatoes before serving resulted in a lower GI value (70–80) compared to a GI of about 100 for the freshly cooked, hot potatoes. This effect was observed for baked or microwave-cooked potatoes.

The presence of some additives, for example acetic acid (vinegar), in the test meal reduced postprandial glycaemia by reducing the rate of gastric emptying (Liljeborg & Bjorck, 1998). The addition of vinegar and olive oil in the form of a vinaigrette dressing to cooked and cooled Sava potatoes (for example a potato salad) reduced the GI by 43% compared to the boiled potatoes served hot, whereas refrigeration alone reduced the GI by 26% (Leeman et al., 2005). A UK study using common toppings on baked potatoes
Table 2
GI of potato varieties.

<table>
<thead>
<tr>
<th>Variety</th>
<th>GI</th>
<th>Preparation method</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Low GI (55 and under)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carisma</td>
<td>53 ± 7</td>
<td>Boiled pieces (8 mins)°°, served hot</td>
</tr>
<tr>
<td><strong>Medium GI (56–70)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Marfona</td>
<td>56 ± 3</td>
<td>Boiled quarters (15 mins)°°, served hot</td>
</tr>
<tr>
<td>Nicola</td>
<td>58 ± 3</td>
<td>Boiled whole (15 mins)°°, served hot</td>
</tr>
<tr>
<td>Nicola</td>
<td>59 ± 7</td>
<td>Boiled quarters (15 mins)°°, served hot</td>
</tr>
<tr>
<td>Estima</td>
<td>66 ± 5</td>
<td>Boiled quarters (15 mins)°°, served hot</td>
</tr>
<tr>
<td>Charlotte</td>
<td>66 ± 5</td>
<td>Boiled quarters (15 mins)°°, served hot</td>
</tr>
<tr>
<td><strong>High GI (above 70)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Russet Burbank</td>
<td>72 ± 9</td>
<td>Baked whole (220 °C for 55–60 mins). Stored cold for 1–5 days, microwave reheated and served</td>
</tr>
<tr>
<td>Prince Edward Island</td>
<td>73 ± 5</td>
<td>Microwaved whole (18 mins), rested for 5 mins, served hot</td>
</tr>
<tr>
<td>King Edward</td>
<td>75 ± 10</td>
<td>Boiled quarters (15 mins)°°, served hot</td>
</tr>
<tr>
<td>Russet Norkotah</td>
<td>77 ± 9</td>
<td>Microwaved whole (18 mins), rested for 5 mins, served hot</td>
</tr>
<tr>
<td>Desiree</td>
<td>77 ± 17</td>
<td>Boiled quarters (15 mins)°°, served hot</td>
</tr>
<tr>
<td>Russet Burbank</td>
<td>79 ± 9</td>
<td>Microwaved whole (18 mins), rested for 5 mins. Stored cold for 1–5 days, microwave reheated and served</td>
</tr>
<tr>
<td>Pontiac</td>
<td>79 ± 9</td>
<td>Microwaved whole (6–7.5 mins), served hot</td>
</tr>
<tr>
<td>Asterix</td>
<td>79 ± 10</td>
<td>Boiled whole (30 mins), served hot</td>
</tr>
<tr>
<td>Mars Piper</td>
<td>85 ± 4</td>
<td>Boiled quarters (15 mins)°°, served hot</td>
</tr>
<tr>
<td>Sebago</td>
<td>87 ± 7</td>
<td>Boiled whole or halved (35 mins)°°, served hot</td>
</tr>
<tr>
<td>Pontiac</td>
<td>88 ± 9</td>
<td>Boiled whole or halved (35 mins)°°, served hot</td>
</tr>
<tr>
<td>Sava</td>
<td>89 ± 12</td>
<td>Boiled whole (21–30 mins), stored cold for 24 h, served cold</td>
</tr>
<tr>
<td>Pontiac</td>
<td>91 ± 9</td>
<td>Boiled pieces (15 mins)°°, mashed and served hot</td>
</tr>
<tr>
<td>Estima</td>
<td>93 ± 8</td>
<td>Microwaved (6 mins) then oven baked at 200 °C (10 mins), served hot</td>
</tr>
<tr>
<td>Pontiac</td>
<td>93 ± 11</td>
<td>Baked whole (190 °C for 25 mins), served hot</td>
</tr>
<tr>
<td>Mars Peer</td>
<td>94 ± 16</td>
<td>Boiled quarters (15 mins)°°, served hot</td>
</tr>
<tr>
<td>Russet Burbank</td>
<td>98 ± 8</td>
<td>Microwaved whole (18 mins), rested for 5 mins, served hot</td>
</tr>
<tr>
<td>Russet Burbank</td>
<td>101 ± 13</td>
<td>Boiled pieces (8 min)°, served hot.</td>
</tr>
<tr>
<td>Desiree</td>
<td>101 ± 15</td>
<td>Boiled whole or halved (35 mins)°°, served hot</td>
</tr>
<tr>
<td>Russet Burbank</td>
<td>104 ± 11</td>
<td>Baked whole (220 °C for 55–60 mins), served hot</td>
</tr>
</tbody>
</table>

* K. EK, unpublished results.
* Henry et al. (2003).
* Atkinson et al. (2008).
* Solh and Brand-Miller (1999).
* Fernandes et al. (2005).
* Henry et al. (2006).
* Leeman, Ostman, & Bjorck (2008).
* Leeman et al. (2005).

Table 3
Comparison of carbohydrate intake and glycemic load between staple carbohydrate foods.

<table>
<thead>
<tr>
<th>Food</th>
<th>Glycemic index (GI)</th>
<th>Average serving size (g)</th>
<th>Available carbohydrate per serving (g)</th>
<th>Glycemic load per serving</th>
<th>Glycemic load per 1000 kJ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potatoes</td>
<td>56–102</td>
<td>150</td>
<td>26–32</td>
<td>18–26</td>
<td>44–64</td>
</tr>
<tr>
<td>Low GI breads</td>
<td>43–55</td>
<td>30</td>
<td>10–14</td>
<td>5–8</td>
<td>18–29</td>
</tr>
<tr>
<td>Low GI rice</td>
<td>43–54</td>
<td>150</td>
<td>39–43</td>
<td>18–21</td>
<td>24–28</td>
</tr>
</tbody>
</table>

Compiled from tables by Atkinson et al. (2008) and Holt et al. (1995).

found that the co-ingestion of fat lowered the GI of potatoes (Estima) by 58%, changing the GI classification from high GI to low GI whereas co-ingestion of protein only lowered the GI of potatoes by 18% with the classification remaining as high GI (Henry et al., 2006).

6. In vitro methods

In vitro methods to measure the rate of starch digestion were introduced as an alternative to in vivo testing of the glycemic response to carbohydrate foods. GI testing is labor and time intensive as human subjects need to be recruited and screened. The reference and test foods have to be consumed on different days, requiring several days for the test to be completed. This results in higher costs and a delay of a few weeks for results to be available. With the introduction of in vitro testing, a new classification of starch was proposed for nutritional purposes: rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS). Most starchy foods contain these fractions in different proportions (Englyst et al., 2007) and in vitro methods are designed to measure them accordingly.

6.1. Principles and techniques

In vitro studies are designed to simulate digestion in the small intestine. The physico-chemical properties of a carbohydrate food are described by measuring the rate and extent of glucose release by enzymatic digestion under controlled conditions (Englyst, Englyst, Hudson, Cole, & Cummings, 1999). Further development of in vitro techniques include chewing of test foods by subjects rather than mechanical homogenisation of foods (Akerberg, Liljeberg, Granfeldt, Drews, & Bjorck, 1998; Granfeldt, Bjorck,
Drews, & Tovar, 1992), the use proteolytic enzymes in addition to amylases (Goni, Garcia-Alonso, & Saur-Calixto, 1997) and dialysis tubing to imitate the small intestine (Granfeldt et al., 1992). Granfeldt et al. (1992) derived a hydrolysis index (HI) by calculating the area under a hydrolysis curve from the rate of glucose released over a period of 180 min using white-wheat bread as a reference. HI was then compared to GI values determined by the in vivo method for the same foods and an equation to predict GI was formulated. Researchers found a correlation of $r^2 = 0.769$ for 17 of 21 food samples tested, which consisted mainly of legumes and cereal products. Subsequent studies have also used HI and other equations to predict GI for in vitro digestion rates (Garcia-Alonso & Goni, 2000; Goni et al., 1997; Leeman et al., 2005).

A recent development in GI prediction was the creation of an artificial neural network designed to predict the GI of unknown food samples. The method uses foods with known GI values and tested them using an in vitro method simulating human digestion under both stomach and small intestine conditions. The digestate was analysed for glucose, fructose, sucrose, lactose, galactose, and maltitol using HPLC. These results were combined with nutritional information (protein, fat and total dietary fibre content) of the test food, and reported or tested in vivo GI values were used as the calibration set of data. The sample set consisted of 72 food types and a correlation of $r^2 = 0.93$ was obtained, indicating a good predictive ability of the method (Magaletta et al., 2010).

### 6.3. Comparisons with in vivo studies

The in vitro method has been proposed as an alternative method for classifying carbohydrates with some studies showing correlation of results with in vivo GI testing (Araya, Contreras, Alvina, Vera, & Pak, 2002; Englyst et al., 1999; Granfeldt et al., 1992). However, there are only a few foods that have been subjected to both in vitro and in vivo testing for comparison. In vitro methods are still being developed; there is no standard protocol for testing, and different models are used to predict GI (Garcia-Alonso & Goni, 2000; Granfeldt et al., 1992; Leeman et al., 2005). Table 4 illustrates comparisons between in vivo and in vitro methods.

It is noteworthy that in the in vitro studies mentioned above, all potatoes tested had a high HI, and consequently the predicted GI values were high, regardless of variety, preparation and cold storage age. This is in contrast to in vivo testing where varieties tested in the same study were classified into different GI rankings. This may relate to the difference between in vivo methods, which reflect a physiological process that includes starch breakdown, glucose absorption into and clearance from the bloodstream, and in vitro methods, which measure physico-chemical processes of starch commination and hydrolysis. In vivo and in vitro studies of starch digestion are complementary rather than alternatives, as sometimes implied.

Although in vitro studies may not be a replacement for in vivo studies, they offer considerable benefits in the speed of testing, the potential to use controlled conditions and the freedom to test novel foods and ingredients. In vitro tests should prove valuable in the search for low GI potato varieties by affording the means to screen germplasm. The complementary application of these two approaches should provide a clearer understanding of potato digestion.
7. Conclusions

Potatoes are a popular carbohydrate food, particularly in the western world and increasingly in developing countries. Physiological studies show that most potatoes are high GI regardless of cooking method. Cold storage and the addition of ingredients may lower the GI of potatoes but restrict the form in which they can be consumed. The importance and popularity of potatoes as a food crop indicates a need to develop cultivars that are digested slowly and have a low GI, regardless of preparation method. The germlasm and plant breeding lines of potatoes are vast and diverse, and with four species and thousands of varieties there is much untapped genetic potential for plant breeders. As shown by the wide range of GI values in Table 2, the development of potato cultivars with low GI is not an unrealistic proposition.

Acknowledgements

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References


Discovery of a low-glycaemic index potato and relationship with starch digestion in vitro

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Abstract

Potatoes are usually a high-glycaemic index (GI) food. Finding a low-GI potato and developing a screening method for finding low-GI cultivars are both health and agricultural priorities. The aims of the present study were to screen the commonly used and newly introduced cultivars of potatoes, in a bid to discover a low-GI potato, and to describe the relationship between in vitro starch digestibility of cooked potatoes and their in vivo glycaemic response. According to International Standard Organisation (ISO) guidelines, seven different potato cultivars were tested for their GI. In vitro enzymatic starch hydrolysis and chemical analyses, including amylose content analysis, were carried out for each potato cultivar, and correlations with the respective GI values were sought. The potato cultivars had a wide range of GI values (53–103). The Carisma cultivar was classified as low GI and the Nicola cultivar (GI = 69) as medium GI and the other five cultivars were classified as high GI according to ISO guidelines. The GI values were strongly and positively correlated with the percentage of in vitro enzymatic hydrolysis of starch in the cooked potatoes, particularly with the hydrolysis percentage at 120 min (r = 0.91 and P < 0.01). Amylose, dietary fibre and total starch content was not correlated with either in vitro starch digestibility or GI. The findings suggest that low-GI potato cultivars can be identified by screening using a high-throughput in vitro digestion procedure, while chemical composition, including amylose and fibre content, is not indicative.

Key words: Glycaemic index: Starch: In vitro digestion: Carbohydrates

Potatoes, as the world’s third largest food crop and most extensively consumed root vegetable, have received much attention in recent years from nutritionists and agriculturists(1). Consumption is currently greatest in the Western world, but potatoes are rapidly becoming a staple in developing countries. Annual per capita potato consumption in 2005 was estimated to be 95 kg in Europe, 60 kg in North America and 22 kg in China(2). Compared with bread and rice, potatoes are more satiating(3) and a source of vitamin C (up to 42 mg/100 g) and Mg(4). In prospective observational studies, however, potato consumption has been reported to be associated with higher weight gain(5) and increased risk of type 2 diabetes(6).

Nutritional studies on different cultivars of potatoes using in vivo and in vitro approaches to classify the digestibility and glycaemic properties of carbohydrates indicate that cooked potatoes contain mostly rapidly digested starch(7) and have a high glycaemic index (GI)(8). Hence, some health professionals advise that potatoes should be substituted with a low-GI carbohydrate to reduce the risk of chronic disease(9,10). However, given the importance of potatoes as a food, the discovery and development of low-GI potatoes would be desirable from consumer, agricultural, food industry and health perspectives.

In in vitro studies, the physico-chemical breakdown of starch in a carbohydrate food is measured by determining the rate and extent of glucose release from enzymatic digestion under controlled conditions(11). In vitro methods have been proposed as an alternative approach for classifying carbohydrates, with the results reported by some studies showing a correlation with in vivo GI testing(11–13). However, the number of foods that have been subjected to both in vitro and in vivo testing for direct comparison is very limited.

The aim of the present study was to use a standardised GI testing methodology to screen the established and newly introduced commercial cultivars of potatoes, in a bid to identify a low-GI potato. Emphasis was placed on the direct determination of the carbohydrate content (as starch + sugars) of the different potatoes studied, as opposed to

Abbreviation: GI, glycaemic index.

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reliance on food composition tables. We determined whether amylose content was a significant predictor of potato GI as has been suggested for cereals and legumes\(^{(14,15)}\). To our knowledge, this is the first study to test the practical value of \textit{in vitro} testing where the botanical cultivar is the only point of difference between the food samples.

**Experimental methods**

**Test foods**

A total of seven commercial potato cultivars comprising a mix of well-established cultivars and newly released cultivars were sourced from growers in Tasmania (Russet Burbank, Maiflower, Nicola and Bintje) and South Australia (Carisma, Desiree and Virginia Rose). The potatoes were prepared for testing according to the method specified by the producers of the cultivars. The potatoes were peeled, cut into 5 cm by 5 cm slices, at a thickness of 0.5 cm, added to excess water at room temperature and brought to boil. The potatoes were cooked in boiling water for a further 4 min, drained, cooled by rinsing in tap water and served immediately along with 250 ml of water (total cooking time 8–9 min). The testing volunteers provided feedback that the Carisma, Nicola and Virginia Rose cultivars were cooked and firm and the other four cultivars were softer. Glucose solution was used as the reference food and tested on three separate occasions; each potato cultivar was tested once according to the International Potato Association\(^{(16,17)}\). The test and reference foods were fed as a portion providing 25 g of carbohydrate determined as the sum of starch and sugars by direct measurement.

**Determination of starch and reducing sugar and total dietary fibre content**

Fresh potatoes were peeled and homogenised in a blender. The homogenate was frozen at \(-80^\circ\text{C}\) and freeze-dried, and the dried material was ground into a powder for total starch and total dietary fibre content determination using the respective Megazyme assay kits (Megazyme International Ireland Limited). Total starch and total dietary fibre content was determined using duplicate samples.

Reducing sugar content was assayed using potato juice extracted from peeled and homogenised fresh potatoes. The juice was filtered and diluted 1:10 with distilled water and then frozen and stored at \(-20^\circ\text{C}\) before the assay. Reducing sugar content was measured using triplicate samples with the Megazyme reducing sugar assay kit (Megazyme International Ireland Limited). The sum of total starch content and reducing sugar content was considered to be the glycaemic carbohydrate content.

**Determination of amylose content in potato starch**

Starch was extracted from each potato cultivar using a modified method of Noda \textit{et al.}\(^{(17)}\). Potato tubers (1 kg) were peeled and homogenised in a blender with distilled water. The slurry was filtered successively through muslin, and 250 and 100 \(\mu\text{m}\) sieves. The starch suspension was allowed to settle overnight at 4 \(^\circ\text{C}\), and the supernatant was removed by decantation. The sedimented starch was washed with deionised water, and the granules were collected on a filter paper with suction, washed with ethanol, dried under a gentle air stream and stored in sealed containers at room temperature for further analysis. Total amylose content of the potato starches was determined by the iodine binding method of Chrastil\(^{(18)}\) using a standard curve of 10, 20, 40, 50 and 60 \% potato amylose mixed with potato amylopectin (A0512 and A8515 from Sigma Chemical Company).

**Glycaemic index testing subjects**

Healthy subjects were recruited among students at the University of Sydney. A total of twenty-seven subjects (twelve males and fifteen females) were recruited over a 1-year period, and potato cultivars were tested in subgroups of ten subjects. Their mean age was 27.0 (SD 5.1) (range 18–38) years, BMI was 22.3 (SD 2.0) kg/m\(^2\), and fasting plasma glucose value was 5.32 (SD 0.31) mmol/l. The inclusion criteria were as follows:

- A total of seven commercial potato cultivars comprising a mix of well-established cultivars and newly released cultivars were sourced from growers in Tasmania (Russet Burbank, Maiflower, Nicola and Bintje) and South Australia (Carisma, Desiree and Virginia Rose).
- Glucose solution was used as the reference food and tested on three separate occasions; each potato cultivar was tested once according to the International Potato Association\(^{(16,17)}\). The test and reference foods were fed as a portion providing 25 g of carbohydrate determined as the sum of starch and sugars by direct measurement.
- Total starch and total dietary fibre content was determined using duplicate samples.
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### Table 1. Carbohydrate content and glycaemic index (GI) testing portion of the seven potato cultivars\(^*\)

<table>
<thead>
<tr>
<th>Cultivars</th>
<th>DM content (g/100 g)</th>
<th>Total starch content (g/100 g)</th>
<th>Sugar content (g/100 g)</th>
<th>Total dietary fibre content (g/100 g)</th>
<th>Amylose content (% starch)</th>
<th>Available (glycaemic) carbohydrate content (g/100 g)</th>
<th>GI testing portion (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carisma</td>
<td>15.2 (a)</td>
<td>6.0</td>
<td>9.90 (a)</td>
<td>0.85 (a)</td>
<td>0.00</td>
<td>25.2 (2)</td>
<td>10.5</td>
</tr>
<tr>
<td>Nicola</td>
<td>19.1 (b)</td>
<td>0.4</td>
<td>12.1 (b)</td>
<td>0.69 (a)</td>
<td>0.00</td>
<td>25.6 (2)</td>
<td>12.8</td>
</tr>
<tr>
<td>Desiree</td>
<td>20.8 (c,d)</td>
<td>0.7</td>
<td>12.0 (c)</td>
<td>0.65 (a)</td>
<td>0.00</td>
<td>23.1 (4)</td>
<td>12.7</td>
</tr>
<tr>
<td>Russet Burbank</td>
<td>22.1 (a,b)</td>
<td>0.3</td>
<td>16.0 (a)</td>
<td>0.16 (a)</td>
<td>0.00</td>
<td>24.4 (4)</td>
<td>16.2</td>
</tr>
<tr>
<td>Virginia Rose</td>
<td>15.0 (a)</td>
<td>0.2</td>
<td>9.10 (a)</td>
<td>0.68 (a)</td>
<td>0.00</td>
<td>27.7 (4)</td>
<td>9.8</td>
</tr>
<tr>
<td>Bintje</td>
<td>19.7 (c,d)</td>
<td>0.1</td>
<td>12.7 (c)</td>
<td>0.47 (a)</td>
<td>0.00</td>
<td>24.7 (4)</td>
<td>13.2</td>
</tr>
<tr>
<td>Maiflower</td>
<td>21.1 (d)</td>
<td>0.2</td>
<td>12.9 (d)</td>
<td>0.34 (d)</td>
<td>0.1</td>
<td>21.4 (1)</td>
<td>13.2</td>
</tr>
</tbody>
</table>

\(\text{a,b,c,d}\) Mean values within a column with unlike superscript letters are significantly different \((P < 0.05;\) one-way ANOVA).

* Percentage-wet basis, except for amylose content, which was percentage-starch basis.
non-smoking; 18–40 years of age; stable body weight; BMI of 19–25 kg/m²; normal glucose tolerance; no food allergy or intolerance; not taking medications known to affect glucose tolerance. On average, each subject tested four cultivars in a random order separated by 3–5 d. The present study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects were approved by the Human Research Ethics Committee of the University of Sydney. Written informed consent was obtained from all the subjects.

**Glycaemic index testing procedures**

The study protocol used was in accordance with the International Organisation for Standardisation standard for the determination of GI of foods (16). The subjects were instructed to refrain from unusual physical activity and alcohol and legume consumption and to eat a high-carbohydrate, low-fat dinner meal the day before the test. On the morning of the test day, the subjects arrived at the metabolic kitchen after a 10–12 h overnight fast. After warming the hand in hot water, two baseline finger-prick blood samples (0.5 ml each) were collected.
Table 2. Glycaemic index (GI) and classification of the seven potato cultivars (Mean values with their standard errors, n 10)

<table>
<thead>
<tr>
<th>Cultivars</th>
<th>GI</th>
<th>SEM</th>
<th>Classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carisma</td>
<td>53a,b</td>
<td>7</td>
<td>Low</td>
</tr>
<tr>
<td>Nicola</td>
<td>69a,b,c</td>
<td>5</td>
<td>Medium</td>
</tr>
<tr>
<td>Desiree</td>
<td>74b,c,d</td>
<td>8</td>
<td>High</td>
</tr>
<tr>
<td>Russet Burbank</td>
<td>83a,c,d</td>
<td>3</td>
<td>High</td>
</tr>
<tr>
<td>Virginia Rose</td>
<td>93a,c,d</td>
<td>10</td>
<td>High</td>
</tr>
<tr>
<td>Bintje</td>
<td>94a,c,d</td>
<td>8</td>
<td>High</td>
</tr>
<tr>
<td>Maiflower</td>
<td>103</td>
<td>8</td>
<td>High</td>
</tr>
</tbody>
</table>

Mean values within a column with unlike superscript letters are significantly different (P < 0.05; one-way ANOVA).

Statistical analyses

Statistical analyses were carried out using the PASW statistical package (version 18.0; SPSS, Inc.). Inter- and intra-individual variations of the three standard (glucose) tests were assessed by determining the CV. One-way ANOVA followed by Duncan’s test (P < 0.05) was used to compare all the data obtained for the seven potato cultivars using the SPSS 20 Statistical Software Program (IBM Corporation).

Results

Chemical composition and glycaemic index testing portions of the seven potato cultivars

The potato cultivars had different chemical compositions (Table 1). The Virginia Rose cultivar had the lowest total starch content (9.1%), the highest reducing sugar content (0.68%), the highest amylose content of starch (27.7%) and the lowest glycaemic carbohydrate (9.8 g/100 g). In contrast, the Russet Burbank cultivar had the highest total starch content (16.0%), the lowest reducing sugar content (0.16%) and the highest glycaemic carbohydrate (16.2 g/100 g). Accordingly, the GI testing portion differed considerably with the smallest portion for the Russet Burbank cultivar (154 g) and the largest portion for the Virginia Rose cultivar (255 g).

In vitro potato digestibility procedure

In vitro digestion of starch from the cooked potatoes was determined using duplicate samples with a modified Englyst procedure. Enzyme solution was prepared by suspending 0.45 g of porcine pancreatic α-amylase (150 U/mg; EC 3.2.1.1.; A3176, Sigma Chemical Company) in 16 ml of water at 37°C with magnetic stirring for 10 min. The mixture was centrifuged (1500 g for 10 min), and 2 ml of amyloglucosidase (3260 U/ml; EC 3.2.1.3.; Megazyme) were added to 10.8 ml of the enzyme supernatant. The preparation and cooking procedures of potatoes were the same as those used in GI testing. After cooking, the potato pieces were mashed evenly with a spatula, and an amount of mash containing 100 mg starch (dry weight) was dispersed into 4 ml of 0.1 M sodium acetate buffer (pH 5.2). After adding 1 ml of the freshly prepared enzyme solution, the mixture was incubated in a shaking water-bath (37°C, 160 strokes/min). Aliquots (0.1 ml) were taken at time intervals that were the same as those used in GI testing and mixed with 1 ml of 95% ethanol. Glucose that was released was measured using the glucose oxidase–peroxidase reagent according to the supplier’s instructions (Megazyme International Ireland Limited).

Glycaemic index calculations

The incremental AUC was calculated using the trapezoidal method. The GI of each potato cultivar was defined as the incremental AUC of the blood glucose response curve of a 25 g glycaemic carbohydrate portion expressed as a percentage of the response to the same amount of carbohydrate from the reference food. The GI was calculated from the two incremental glucose areas using glucose as a reference (i.e. GI of 100). One-way ANOVA was used to compare the final mean GI of the different cultivars.

Fig. 2. Percentage of in vitro starch hydrolysis of cooked potatoes from different cultivars. Values are the mean hydrolysis percentages, with their standard errors represented by vertical bars. Maiflower; Bintje; Russet Burbank; Virginia Rose; Desiree; Nicola; Carisma.
Similarly, there were no correlations between the glycaemic index (GI) and amylose, dietary fibre or total starch content. No significant correlations were found between GI digestibility and amylose, dietary fibre or total starch content. (Fig. 3). Correlation between GI values and GI with hydrolysis percentage at 120 min showed a strong positive correlation (r = 0.91 and P < 0.01; Fig. 5). No significant correlations were found between GI values and amylose, dietary fibre or total starch content. Similarly, there were no correlations between in vitro starch digestibility and amylose, dietary fibre or total starch content. In vitro starch digestibility
The amount of glucose released from the cooked potatoes as a percentage of the initial 100 mg of starch increased progressively with time during in vitro digestion, reaching a plateau at 45–60 min, while the hydrolysis rate decreased gradually (Fig. 2). The percentage of starch hydrolysed at different time points is given in Table 3. The Carisma and Nicola cultivars exhibited significantly lower percentages of carbohydrate hydrolysed at all time points, e.g. 24.0% by Carisma and 24.5% by Nicola compared with 34.5% by Bintje and 36.5% by Maiflower after 15 min of hydrolysis. Similarly, by the end of 120 min, the Carisma and Nicola cultivars exhibited hydrolysis percentages of 50.5 and 52.2%, respectively, compared with the Bintje and Maiflower cultivars exhibiting hydrolysis percentages of 67.0 and 76.8%, respectively.

Discussion
The present study compared the in vitro and in vivo digestibility of a series of potato cultivars that had been prepared, cooked and consumed under identical conditions. Therefore, differences could be attributed to the characteristics of the potatoes rather than to differences in processing. Of the seven potato cultivars tested, the Carisma cultivar (GI = 53) was identified as low GI and the Nicola cultivar (GI = 69) as medium GI and the other five cultivars were identified as high GI (e.g. Russet Burbank, GI = 82). The low-GI property of the Carisma cultivar was unrelated to amylose or total starch content, but it could be predicted from in vitro measures of the rate of starch digestion. The amount of non-starch carbohydrate was too small (<1 g/100 g) to account for the large differences in blood glucose response. Previous studies on potatoes have shown a wide range of GI values from as low as 23 to as high as 118. However, much of this variability in GI values may be due to non-standard variations in methodology, inaccurate estimation of glycaemic carbohydrate content of the portion fed, differences in cooking method or processing before consumption, and the overall meal composition.

For the purpose of comparison, we refer the reader to a condensed table of potato GI values listed in an earlier review. This table shows that the GI values of potatoes ranged from 56 to 104 in studies where the cultivar was specified and tested with no additional ingredients and when at least ten healthy subjects were used. The Maris Piper cultivar had a GI value of 56 compared with the Maris Piper cultivar having a value of 85, when both the cultivars were boiled for 15 min before consumption. These findings, similar to those of the present study, show that there is considerable variability in the GI values of potatoes solely due to botanical characteristics.

Another important finding of the present study was the significant positive correlation between the rate of in vitro digestion and the in vivo glycaemic response. The blood glucose response curves of each of the seven potato cultivars showed a similar rate of increase in blood glucose levels (Fig. 1) to the rate of carbohydrate hydrolysis (Fig. 2). The strong correlation between the hydrolysis percentage at 120 min and GI values indicates that the glycaemic response to the potatoes was largely dependent on the percentage of starch hydrolysed by 120 min measured by the in vitro method. As much as 75% of the total starch in raw potatoes was hydrolysed by 120 min.

In vivo glycaemic response
Blood glucose concentration increased to the maximum value at 30 min for all the potato cultivars and then decreased rapidly until 120 min. The postprandial response to the Carisma cultivar was lower than that to the other six potato cultivars at all time points (Fig. 1). When GI values were calculated (Table 2), there were significant differences among the cultivars. The majority had a high GI (70 or above), with the Nicola cultivar just falling into the low-GI category (GI = 53), according to the International Organisation for Standardisation-recommended classification.

In vitro starch digestibility
The amount of glucose released from the cooked potatoes as a percentage of the initial 100 mg of starch increased progressively with time during in vitro digestion, reaching a plateau at 45–60 min, while the hydrolysis rate decreased gradually (Fig. 2). The percentage of starch hydrolysed at different time points is given in Table 3. The Carisma and Nicola cultivars exhibited significantly lower percentages of carbohydrate hydrolysed at all time points, e.g. 24.0% by Carisma and 24.5% by Nicola compared with 34.5% by Bintje and 36.5% by Maiflower after 15 min of hydrolysis. Similarly, by the end of 120 min, the Carisma and Nicola cultivars exhibited hydrolysis percentages of 50.5 and 52.2%, respectively, compared with the Bintje and Maiflower cultivars exhibiting hydrolysis percentages of 67.0 and 76.8%, respectively.

Correlation between in vivo glycaemic response and in vitro starch digestibility
All the GI values of the cooked potatoes were significantly and positively correlated with hydrolysis percentage during in vitro starch digestion at each time point (Table 4). In vitro hydrolysis percentages at 90 and 120 min exhibited the strongest positive correlation with GI values (r = 0.91 and P < 0.01; Fig. 3). No significant correlations were found between GI values and amylose, dietary fibre or total starch content. Similarly, there were no correlations between in vitro starch digestibility and amylose, dietary fibre or total starch content.

Table 3. Values for in vitro hydrolysis of starch in the seven potato cultivars (n 2)*

<table>
<thead>
<tr>
<th>Potato cultivars</th>
<th>15 min</th>
<th>20 min</th>
<th>30 min</th>
<th>60 min</th>
<th>120 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carisma</td>
<td>24·0</td>
<td>31·7</td>
<td>39·6</td>
<td>48·0</td>
<td>50·5</td>
</tr>
<tr>
<td>Nicola</td>
<td>24·5</td>
<td>32·5</td>
<td>38·0</td>
<td>44·2</td>
<td>52·2</td>
</tr>
<tr>
<td>Desiree</td>
<td>33·1</td>
<td>46·3</td>
<td>50·6</td>
<td>54·1</td>
<td>59·2</td>
</tr>
<tr>
<td>Russet Burbank</td>
<td>34·9</td>
<td>49·3</td>
<td>51·3</td>
<td>56·5</td>
<td>60·1</td>
</tr>
<tr>
<td>Virginia Rose</td>
<td>32·6</td>
<td>43·5</td>
<td>51·5</td>
<td>60·5</td>
<td>60·8</td>
</tr>
<tr>
<td>Bintje</td>
<td>34·5</td>
<td>54·2</td>
<td>65·4</td>
<td>69·9</td>
<td>67·6</td>
</tr>
<tr>
<td>Maiflower</td>
<td>36·5</td>
<td>53·5</td>
<td>75·5</td>
<td>72·9</td>
<td>76·8</td>
</tr>
</tbody>
</table>

*a,b,c,d Mean values within a column with unlike superscript letters are significantly different (P < 0.05).

Table 4. Correlation between glycaemic index (GI) and starch digestion percentage at different time points

<table>
<thead>
<tr>
<th>In vitro and in vivo digestion correlation</th>
<th>r</th>
</tr>
</thead>
<tbody>
<tr>
<td>GI with hydrolysis percentage at 15 min</td>
<td>0·85*</td>
</tr>
<tr>
<td>GI with hydrolysis percentage at 20 min</td>
<td>0·84*</td>
</tr>
<tr>
<td>GI with hydrolysis percentage at 30 min</td>
<td>0·87*</td>
</tr>
<tr>
<td>GI with hydrolysis percentage at 45 min</td>
<td>0·86*</td>
</tr>
<tr>
<td>GI with hydrolysis percentage at 60 min</td>
<td>0·90**</td>
</tr>
<tr>
<td>GI with hydrolysis percentage at 90 min</td>
<td>0·91**</td>
</tr>
<tr>
<td>GI with hydrolysis percentage at 120 min</td>
<td>0·91**</td>
</tr>
</tbody>
</table>

*P < 0.05, **P < 0.01.
is highly resistant to enzymatic attack\(^{24,25}\). As the cooking time used in the present study was relatively short (as specified by the potato growers), starch gelatinisation may not have been complete\(^{26}\) and the potatoes consumed would have contained a mixture of granules with a partially disrupted structure. Under the same cooking conditions, however, differences between the cultivars with regard to the fine structure of starch would have resulted in the conversion of different amounts of the enzymatically resistant starch in raw potatoes into available starch for hydrolysis.

To the best of our knowledge, this is the first time that the \textit{in vitro} digestibility of starch has reliably predicted GI classifications for the same food item prepared in the same way but varying only in botanical origin. This has important practical implications in that simple \textit{in vitro} digestion methods might be used to screen foods and as a predictor of GI classification, i.e. high or low GI, although the actual GI value of the food would require \textit{in vivo} testing. However, it is likely that the \textit{in vitro} method is more suitable for screening simple foods such as potatoes, rather than for screening complex foods with a mixture of ingredients. High-throughput \textit{in vitro} methods would be greatly useful for plant breeding programmes that seek to improve the glycaemic response of carbohydrates in food crops.

The present study has shown that under identical cooking conditions, potato cultivars with similar amylose content differ significantly in both \textit{in vitro} digestion and \textit{in vivo} blood glucose responses. The composition and state of starch in foods are the major determinants of the rate at which foods are digested and at which they elicit postprandial blood glucose and insulin responses. In general, native starches with high amylose content are considered to be more resistant to enzymatic digestion, whereas high-amylopectin starches are more susceptible to digestion. High-amylose native starches are thought to be more difficult to swell and gelatinise under typical cooking conditions, and therefore digested more slowly, eliciting lower blood glucose and insulin responses than those with low amylose content\(^{27,28}\). However, this interpretation needs refinement as the fine structure of amylose and amylopectin has also been shown to be important in the determination of the digestibility of starch\(^{29,30}\).

Starch-containing foods that are digested slowly and elicit a low glycaemic response have been considered to be more beneficial to health and in the prevention and management of diabetes and hyperlipidaemia than starchy foods that are digested rapidly and elicit high glycaemic responses\(^{30,31,32}\). Hence, the identification of foods with a low GI and the factors that influence GI should be of continuing research interest in the future. It is pertinent that the two lowest-GI cultivars did not produce an undershoot in postprandial glucose values below baseline levels, as this has implications for appetite control through decreased satiety or increased hunger and subsequent food intake.

In summary, the present study identified the first commercially grown low-GI cultivar of potato (Carisma, GI = 53) among a group of commonly used and newly introduced cultivars. We demonstrated that the rate of \textit{in vitro} digestion of starch, in particular, the percentage of available (glycaemic) carbohydrate hydrolysed by 120 min in the cooked potato product, could be used as a predictor of the GI classification. The importance and popularity of potatoes as a food crop dictate the need to identify and develop cultivars that are digested slowly and have a low GI, regardless of the preparation method.

**Acknowledgements**

The authors thank the Mitolo Group and Agricono for supplying the potato cultivars used in the present study.

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The author's responsibilities were as follows: J. C. B.-M., L. C. and K. L. E. designed and conceived the study; K. L. E. selected the potato cultivars, conducted the chemical analyses and GI testing, and undertook statistical analyses; K. L. E. and S. W. conducted the starch extraction and in vitro experiment; S. W. analysed the in vitro digestibility data and carried out all correlation analyses; K. L. E. and S. W. interpreted the findings and wrote the manuscript; L. C. and J. C. B.-M. reviewed and edited the paper. All authors read and approved the final manuscript.

K. L. E., S. W. and L. C. have no conflicts of interest to declare.

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J. C. B.-M. is a co-author of The Low GI Handbook book and other books (DeCapo, New York, NY), President of the Glycaemic Index Foundation, and Director of a non-profit GI-based food endorsement programme in Australia and manages the University of Sydney GI testing service.

References

1. Introduction

Potatoes are the most important non-grain food commodity produced globally, with production in 2012 reaching 368 million tonnes.\(^1\) They are a major source of carbohydrate in the Western world and consumption is increasing rapidly in developing countries. Annual global consumption is more than 200 million tonnes, with an estimated 74 kg consumed per capita in Europe, 53 kg in Australia and 26 kg in Asia.\(^2\) Carbohydrates are the principal energy source in the human diet accounting for 40–80% of energy intake.\(^3\)

The terminology and classification of carbohydrates for translation into nutritional characteristics is complex. The FAO/WHO scientific update on carbohydrates in human nutrition suggested the glycemic index (GI) as one of the ways to guide food choices when considering similar carbohydrate-containing foods.\(^4\) The GI is a system of classifying carbohydrate-rich foods based on their blood glucose-raising potential.\(^5\) The carbohydrate in a high GI food is digested and absorbed rapidly and results in high postprandial blood glucose and insulin levels, which over the long term are associated with increased risks of diet-related diseases including type-2 diabetes and cardiovascular disease.\(^6,9\) According to the International Standards Organisation (ISO) Standard,\(^10\) foods that have a GI of greater than 70 are classified as high GI, foods with a GI that fall in the range of 56–69 are classified as medium GI, and foods that have a GI of 55 or less are classified as low GI.\(^10\)

Potatoes are generally considered a high GI food\(^11\) and some nutritionists have advised substitution with a low GI option.\(^9,12\) This advice may not apply to all potatoes, as there is considerable natural variability between cultivars in the GI values of potatoes that have been prepared for consumption by similar methods.\(^13,14\)

Starch is the main carbohydrate with blood glucose raising potential (i.e., available carbohydrate) in potatoes. The susceptibility of native starch to enzymic breakdown \textit{in vitro} is influenced by various factors, including amylose content,\(^13\) phosphorus content,\(^15,16\) granule size and starch morphology,\(^17\) amylopectin chain length profile\(^15\) and the fine structures of branch chains in both amylose and amylopectin.\(^18\) In contrast, the extent of gelatinization and retrogradation of starch in processed or cooked foods is the main determinant of the rate at which it is digested and elicits postprandial blood glucose responses.\(^19,20\)

In a previous study, a low GI potato cultivar, Carisma, with a GI of 53, was identified amongst seven potato cultivars. The other cultivars had GI values ranging from 69 to 103.\(^21\) The GI values were strongly and positively correlated with the extent of \textit{in vitro} enzymatic hydrolysis of starch in the cooked potatoes at 120 min (\(r = 0.91, p < 0.01\)), but not to dry matter, total starch or dietary fibre content of the potatoes.\(^22\) There were no significant differences in the amylose content among the starches isolated from the seven potato cultivars.\(^21\) In the present study, the properties of starch from Carisma and high GI potatoes were examined to identify characteristics of potato starch that influence their GI values.
2. Materials and methods

2.1 Potatoes

Potatoes were obtained from growers in South Australia (Carisma, Desiree, Virginia Rose) and Tasmania, Australia (Russet Burbank, Maiflower, Nicola, Bintje).

2.2 Starch extraction

Starch was extracted from potatoes according to the method of Noda et al.\textsuperscript{22} with modifications, as described by Ek et al.\textsuperscript{21}

2.3 Phosphorus content

Phosphorus content was determined spectrophotometrically according to the method of Morrison.\textsuperscript{23}

2.4 Particle size analysis

Particle size of starch granules was quantified as starch surface area using a method based on image analysis of light micrographs. Starch granules (20 mg) were dispersed in 1 mL of deionised water and a few drops of the suspension were placed on a microscope slide with a coverslip and sealed using nail varnish. Images were obtained using a Leica DM 2500M light microscope (Leica, Germany). Five slides were prepared of starch from each potato cultivar and three micrographs were obtained from each slide. Three micrographs were selected randomly from the total of 15 micrographs collected per cultivar giving a triplicate measurement of starch surface area. The micrographs were converted into binary images, a scale in μm was set and granule surface area was measured using ImageJ 1.43u (Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, http://imagej.nih.gov/ij/, 1997–2012) (Fig. 1). The output was copied into Excel spreadsheets for data analysis. Classification of the size of starch granules according to surface area was: small (<500 μm\textsuperscript{2}), medium (500–1000 μm\textsuperscript{2}) and large (>1000 μm\textsuperscript{2}).

2.5 Starch crystallinity

Relative crystallinity of starch was measured using a Difftech Mini Materials Analyser X-ray diffractometer (GBC Scientific Equipment Pty. Ltd.) according to the method by Wang et al.\textsuperscript{24} The X-ray generator was equipped with a cobalt anode (\(λ = 1.78897\) Å) operating at 1 kW and 3.36 mA. All starch samples were kept at constant humidity (75%) in a desiccator over a saturated NaCl solution for a week prior to analyses. X-ray diffractograms were acquired at room temperature (20 ± 1 °C), the scattering intensity was measured from 4° to 30° as a function of 2θ and at a scanning speed of 0.5° min\textsuperscript{−1} and a step size of 0.02°. Traces software v. 6.7.13 (GBC Scientific Equipment Pty. Ltd.) was used to subtract the background representing the amorphous portion of diffractograms. Relative crystallinity was calculated as a ratio of the crystalline area to the total area between 4 to 30° (2θ).\textsuperscript{25}

2.6 Amylopectin chain length profile

The amylopectin chain length profile was determined using high performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD), according to the method of Liu et al.\textsuperscript{15} using isomylase (280 U mg\textsuperscript{−1}, Megazyme International Ireland Ltd. Bray Co., Wicklow, Ireland) to de-branch the starch. Enzymes were inactivated by placing samples in a boiling water bath for 10 min and an aliquot (200 μL) from de-branched samples was diluted with 2 mL of 150 mM NaOH,
filtered (0.45 μm nylon syringe filter) and injected into the HPAEC-PAD system (5 μL sample loop) ( Dionex Corporation, Sunnyvale, CA, USA). The HPAEC-PAD system consisted of a Dionex HPLC equipped with an ED50 electrochemical detector with a gold working electrode, P680 HPLC pump, TCC-100 column oven, and ASI-100 automated sampler (Dionex Corporation, Sunnyvale, CA, USA). The standard triple potential waveform was employed, with the following periods and pulse potentials: $T_1 = 0.40$ s, with 0.20 s sampling time, $E_1 = 0.05$ V; $T_2 = 0.20$ s, $E_2 = 0.75$ V; $T_3 = 0.40$ s, $E_3 = -0.15$ V. A Dionex CarboPac™ PA1 column with gradient elution (−5 to 0 min, 40% A; 5 min, 60% A; 45 min, 80% A) at a column temperature of 26 °C and a flow rate of 1 mL min$^{-1}$ (0.5 Hz) was used. Data were collected using Chromeleon software, version 6.80 ( Dionex Corporation, Sunnyvale, CA). The weight fractions of chain lengths 6–13, 14–18, 19–37, 38–60 were quantified based on the area of peaks. Standards were prepared by dissolving 0.5–1.0 mg from a Shodex STANDARD P-82 kit ( Showa Denko K.K. Shodex Group, Kawasaki, Kanagawa, Japan) in distilled water to make 0.1–0.5% solutions.

2.7 Thermal analysis

DSC measurements were made using a Modulated Differential Scanning Calorimeter MDSC 2920 instrument (TA Instruments Inc., Delaware, USA) equipped with a thermal analysis data station and data recording software. Approximately 3 mg of starch from each cultivar was weighed accurately into an aluminium sample pan. Water was added to the starch sample with a microsyringe to obtain a starch–water ratio of 1:2 (w/w) and the pan was hermetically sealed. The detailed procedures for DSC measurements and analysis of the thermal transition parameters are described elsewhere.26

2.8 Starch pasting properties

Starch pasting properties were analyzed using a Rapid Visco Analyser RVA-4 (Newport Scientific, Warriewood, Australia). Starch samples and deionised water (8% dry starch basis, total weight of 28 g) were weighed directly into a test canister and the mixture was agitated by stirring using the plastic paddle before the canister was inserted into the instrument. The starch suspension was stirred at 960 rpm for the first 10 s then decreased to 160 rpm for the remainder of the experiment. Samples were equilibrated at 50 °C for 1 min then heated at 6 °C min$^{-1}$ to 95 °C, held at 95 °C for 5 min before cooling at 6 °C min$^{-1}$ back to 50 °C and held for 2 min. Peak viscosity, trough viscosity and final viscosity were recorded, and breakdown (peak minus trough viscosity) and setback (final minus trough viscosity) were calculated using the Thermocline software provided with the instrument.

2.9 Statistical analyses

All analyses were performed on duplicate starch samples except relative crystallinity determination, which was done as a single test, and granule size analysis which was performed in triplicate. One-way analysis of variance (ANOVA) by Duncan’s test ($p < 0.05$) was performed using SPSS V. 20 software (SPSS Inc., Chicago, IL).

3. Results

3.1 Physicochemical properties

The chain length profile of amylopectin from all of the potato cultivars had the chain length 13–24 fraction as the highest percentage (47–51%) and the chain length 6–12 fraction as the lowest (7–9%). Although small differences were noted among the starches from the seven potato cultivars in their amylopectin chain length distributions, and also in their phosphorus content and relative crystallinity (Table 1), these differences did not differentiate Carisma from the other, high GI cultivars (Table 1).

Russet Burbank starch had the largest mean granule size, whereas the mean granule size of Maiflower was significantly smaller than that of the other cultivars (Table 1). Maiflower had the highest percentage of small starch granules (90%) and the lowest percentage of large granules (3%). In comparison, Russet Burbank had the lowest percentage of small granules (57%) and highest percentage of large granules (11%). The percentage of small starch granules (82%) and large granules (4%) in Carisma was significantly different from the respective values for Russet Burbank, but did not differ significantly from Maiflower (Fig. 2). Although there were significant differences in granule size distributions between the cultivars (Table 1), these did not correspond to the differences in GI values.

3.2 Thermal properties

The potato starches presented well-defined single differential scanning calorimetry (DSC) endotherms (Fig. 3). The thermal transition temperatures $T_\alpha$, $T_p$ and $T_c$ ranged from 60.0 to 66.2 °C, 62.8 to 69.3 °C and 67.6 to 75.8 °C, respectively, and the gelatinization enthalpies ranged from 18.5 to 19.5 J g$^{-1}$ (Table 2). Carisma starch had the highest values of $T_\alpha$ (66.2 °C), $T_p$ (69.3 °C) and $T_c$ (75.8 °C), whereas the lowest respective values were observed for Russet Burbank. The average gelatinization temperature range ($T_c - T_\alpha$) was 10.0 °C. The thermal transition temperatures varied significantly between some of the cultivars, but there were no significant differences in gelatinization enthalpies ($p > 0.05$).

3.3 Starch pasting properties

All seven starches displayed similar pasting profiles, which were typical of potato starch. Pasting temperature ranged from 60.8 °C to 70.2 °C (Table 3). Carisma starch had the highest pasting temperature (70.2 °C), whereas Russet Burbank starch had the lowest (60.8 °C), consistent with the ranking of DSC thermal transition temperatures. The starches had similar peak viscosities with the exception of Russet Burbank starch, which was significantly lower than that of the others. Final paste viscosity was lowest for Russet Burbank (3869 cP) and the highest for Carisma (9009 cP). Carisma starch also had the highest trough (7595 cP) and final viscosities (9009 cP).

4. Discussion

The present study has shown that starch from the low GI potato cultivar Carisma was more resistant than starch from the high
GI cultivars to the effects of hydrothermal treatment in the DSC and RVA. Carisma starch had significantly higher thermal transition temperatures \(T_\text{on}, T_\text{p} \) and \(T_\text{c} \) and starch pasting temperature compared to starches from high GI cultivars. Trough and final viscosities, and hence setback viscosity, were also significantly different for Carisma compared to the other cultivars. While there were some differences between the cultivars with respect to starch granule size distribution, amylose content, phosphorus content, relative crystallinity and amylopectin chain length profiles, none of these trends differentiated Carisma from the high GI potatoes.

Higher DSC transition temperatures are thought to result from a higher degree of crystallinity, or more ordered crystalline regions, which impart greater structural stability and make the granules more resistant to gelatinization. Potato starch with less crystalline order was observed to gelatinize at a lower temperature and reach a greater degree of gelatinization at the same temperature than more crystalline potato starch. The same study showed that glycemic response increased with a greater degree of starch gelatinization. The higher gelatinization onset temperature of Carisma starch suggests that the crystalline regions of Carisma starch are more stable than those of the other cultivars. Hence, under the same cooking conditions, the lower glycemic response elicited by Carisma could be because its starch was gelatinized to a lesser extent than starch from the potatoes with a high GI value.

The parameter \(\Delta H\) measures the energy change due to loss of molecular order and melting of crystallites when hydrogen bonds break within the granule. The value of \(\Delta H\) has been considered to be an indicator of the quantity and quality of the
starch crystalline structure.\textsuperscript{28,30} However, more recent studies have indicated that the DSC endotherm obtained at a water–starch ratio of 2 : 1 does not represent complete starch gelatinization and corresponds to the energy taken up until the available water becomes limiting.\textsuperscript{31} Under these conditions, considerable residual crystallinity and lamellar structure remains at the end of the DSC endotherm.\textsuperscript{32} Therefore, in the present study, the onset and peak temperatures, but not $\Delta H$, in the DSC endotherm obtained at a water–starch ratio of 2 : 1 would have been indicative of the quality of the starch crystallinity of the seven potato cultivars.

The pasting profile of Carisma starch was clearly different from that of the other six cultivars, with a significantly higher pasting temperature, higher trough and final viscosities (Table 3). RVA pasting temperature provides an indication of the temperature at which granule disruption commences. A higher pasting temperature indicates that Carisma starch required more heat for the onset of starch gelatinization during cooking. Continued heating past the temperature of peak viscosity results in the breakdown of swollen granules and realignment of starch polymer molecules, causing a decrease in paste viscosity. Carisma starch had greater resistance to breakdown as indicated by the significantly higher trough viscosity compared to the other starches (Table 3). The setback viscosity is thought to result from the rearrangement of amylose molecules that have leached from swollen starch granules during cooling, and is indicative of the retrogradation tendency of starch.\textsuperscript{33} Carisma starch had significantly higher final and setback viscosities compared to the other starches indicating more viscous retrograded starch paste which could confer resistance to enzymatic digestion. Food matrix viscosity has been observed to affect the enzymatic digestibility of starch and glycemic response.\textsuperscript{19} A high level of viscosity slows down propulsive and mixing effects generated by peristalsis, reducing interactions between substrates and digestive enzymes and also the absorption of hydrolysis products thus lowering post-prandial glycaemia.\textsuperscript{19}

No significant correlations were observed between amylose content, phosphorus content and amylopectin chain length distributions of the seven starches with their respective DSC and RVA properties, nor with the GI values of the potatoes. The lack of such correlations was similar to the results of other studies, which found no significant relationship between amylose and phosphorus content with gelatinization temperature and enthalpy.\textsuperscript{15,16} Smaller granule sizes have been reported to be related to increased DSC transition temperatures and decreased enthalpy of gelatinization.\textsuperscript{35} However, in the present study, Maiflower starch had a significantly smaller mean granule size compared to Carisma starch but did not show higher transition temperatures. Higher amylose content, fewer short amylopectin branch chains and smaller granule size were reported to be associated with higher pasting temperature, higher setback viscosity and higher peak viscosity temperature.\textsuperscript{36–38} However these associations were not observed in the present study.

Recent work has shown that the fine structural features of both amylose and amylopectin significantly influence the \textit{in vitro} digestion rate of starch in cooked rice grains. Longer chain

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Table 2  Thermal properties of starch from seven potato cultivars\textsuperscript{\textdagger}

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>$T_o$ (°C)</th>
<th>$T_p$ (°C)</th>
<th>$T_c$ (°C)</th>
<th>$T_c - T_o$ (°C)</th>
<th>$\Delta H$ (J g\textsuperscript{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carisma</td>
<td>66.2 ± 0.1\textsuperscript{c}</td>
<td>69.3 ± 0.0\textsuperscript{c}</td>
<td>75.8 ± 0.1\textsuperscript{c}</td>
<td>9.6 ± 0.1\textsuperscript{b}</td>
<td>19.4 ± 0.6\textsuperscript{a,b}</td>
</tr>
<tr>
<td>Nicola</td>
<td>60.4 ± 0.1\textsuperscript{c}</td>
<td>64.0 ± 0.2\textsuperscript{b}</td>
<td>67.6 ± 0.2\textsuperscript{b}</td>
<td>10.6 ± 0.4\textsuperscript{c}</td>
<td>18.5 ± 0.5\textsuperscript{a}</td>
</tr>
<tr>
<td>Desiree</td>
<td>63.7 ± 0.2\textsuperscript{d}</td>
<td>67.1 ± 0.3\textsuperscript{d}</td>
<td>74.4 ± 0.1\textsuperscript{d}</td>
<td>10.7 ± 0.3\textsuperscript{c}</td>
<td>18.7 ± 0.4\textsuperscript{a,b}</td>
</tr>
<tr>
<td>Russet Burbank</td>
<td>60.9 ± 0.2\textsuperscript{c}</td>
<td>62.8 ± 0.2\textsuperscript{b}</td>
<td>67.6 ± 0.2\textsuperscript{c}</td>
<td>7.6 ± 0.0\textsuperscript{a}</td>
<td>18.7 ± 0.2\textsuperscript{a,b}</td>
</tr>
<tr>
<td>Virginia Rose</td>
<td>61.4 ± 0.4\textsuperscript{b,c}</td>
<td>65.4 ± 0.0\textsuperscript{c}</td>
<td>72.6 ± 0.5\textsuperscript{c}</td>
<td>11.2 ± 0.5\textsuperscript{c}</td>
<td>19.4 ± 0.3\textsuperscript{a,b}</td>
</tr>
<tr>
<td>Bintje</td>
<td>61.8 ± 0.4\textsuperscript{c}</td>
<td>66.2 ± 0.1\textsuperscript{c}</td>
<td>71.1 ± 1.2\textsuperscript{b}</td>
<td>9.3 ± 0.8\textsuperscript{b}</td>
<td>18.5 ± 0.3\textsuperscript{a}</td>
</tr>
<tr>
<td>Maiflower</td>
<td>61.2 ± 0.0\textsuperscript{b}</td>
<td>66.2 ± 0.1\textsuperscript{c}</td>
<td>72.1 ± 0.3\textsuperscript{b}</td>
<td>10.9 ± 0.3\textsuperscript{c}</td>
<td>19.5 ± 0.1\textsuperscript{b}</td>
</tr>
</tbody>
</table>

\textsuperscript{a} b c d e Values in a column with the same superscript do not differ significantly ($p > 0.05$). \textsuperscript{\textdagger} Abbreviations: $T_o$, onset temperature; $T_p$, peak temperature; $T_c$, conclusion temperature; $\Delta H$, enthalpy change.

Table 3  Pasting properties of starch from seven potato cultivars\textsuperscript{\textdagger}

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>PT (°C)</th>
<th>PV (cP)</th>
<th>TV (cP)</th>
<th>BV (cP)</th>
<th>FV (cP)</th>
<th>SB (cP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carisma</td>
<td>70.2 ± 0.4\textsuperscript{a}</td>
<td>13 128 ± 69\textsuperscript{c}</td>
<td>7595 ± 322\textsuperscript{d}</td>
<td>5533 ± 253\textsuperscript{a}</td>
<td>9009 ± 301\textsuperscript{c}</td>
<td>1415 ± 21\textsuperscript{c}</td>
</tr>
<tr>
<td>Nicola</td>
<td>62.8 ± 0.0\textsuperscript{b}</td>
<td>12 847 ± 28\textsuperscript{d}</td>
<td>4088 ± 9\textsuperscript{b}</td>
<td>8759 ± 19\textsuperscript{b}</td>
<td>4876 ± 11\textsuperscript{b}</td>
<td>788 ± 2\textsuperscript{b}</td>
</tr>
<tr>
<td>Desiree</td>
<td>66.0 ± 0.0\textsuperscript{a}</td>
<td>10 812 ± 370\textsuperscript{b}</td>
<td>5186 ± 70\textsuperscript{c}</td>
<td>5626 ± 440\textsuperscript{a}</td>
<td>6304 ± 31\textsuperscript{d}</td>
<td>1119 ± 39\textsuperscript{d}</td>
</tr>
<tr>
<td>Russet Burbank</td>
<td>60.8 ± 0.1\textsuperscript{c}</td>
<td>8521 ± 83\textsuperscript{a}</td>
<td>3215 ± 20\textsuperscript{a}</td>
<td>5306 ± 64\textsuperscript{a}</td>
<td>3869 ± 13\textsuperscript{a}</td>
<td>654 ± 7\textsuperscript{a}</td>
</tr>
<tr>
<td>Virginia Rose</td>
<td>64.2 ± 0.2\textsuperscript{d}</td>
<td>13 723 ± 141\textsuperscript{b}</td>
<td>4934 ± 199\textsuperscript{b}</td>
<td>8789 ± 59\textsuperscript{b}</td>
<td>5881 ± 214\textsuperscript{b}</td>
<td>947 ± 15\textsuperscript{b}</td>
</tr>
<tr>
<td>Bintje</td>
<td>63.4 ± 0.4\textsuperscript{c}</td>
<td>13 262 ± 292\textsuperscript{d}</td>
<td>3849 ± 24\textsuperscript{b}</td>
<td>9413 ± 268\textsuperscript{b}</td>
<td>4543 ± 106\textsuperscript{b}</td>
<td>694 ± 825\textsuperscript{a,b}</td>
</tr>
<tr>
<td>Maiflower</td>
<td>63.4 ± 0.3\textsuperscript{c}</td>
<td>12 478 ± 100\textsuperscript{d}</td>
<td>3907 ± 52\textsuperscript{b}</td>
<td>8571 ± 48\textsuperscript{b}</td>
<td>4671 ± 105\textsuperscript{b}</td>
<td>764 ± 52\textsuperscript{b}</td>
</tr>
</tbody>
</table>

\textsuperscript{a} b c d e Values in a column with the same superscript do not differ significantly ($p > 0.05$). \textsuperscript{\textdagger} Abbreviations: PT, pasting temperature; PV, peak viscosity; TV, trough viscosity; BV, breakdown viscosity; FV, final viscosity; SB, setback.
lengths of amylose branches, a smaller relative amount of long to short amylopectin branches and a smaller ratio of longer amylose branches to short amylopectin branches increased in vitro digestion rate. In the present study no relationship was found between amylose content, amylopectin chain length profile and GI value, but other aspects of the fine structures of branch chains in amylose and amylopectin (for example, spacing between branch points) were not investigated and could be possible factors that influence starch granule resistance to gelatinization during cooking, starch digestibility and consequently the GI value. It is also possible that due to fine structural differences the glucan chains of Carisma starch are less disordered and therefore less susceptible to amyolysis when hydrothermally treated in the potato tissue. 

Potato cultivars differ in the size and shape of tuber cells, and the strength of cell wall structures. Hence, the physical properties of the tuber may also influence the GI and in vitro digestibility of starch in cooked potatoes. Cell walls are considered to be a limiting factor for starch hydrolysis in foods. Cell walls could act as a physical barrier for heat conductance during cooking and thereby reduce the extent of starch gelatinisation. They can also limit the extent of starch swelling, and the rate of starch hydrolysis by restricting enzyme access. Nevertheless, the significantly different hydrothermal properties of isolated Carisma starch indicate that the characteristics of the starch are likely to be a major determinant of digestibility.

5. Conclusions

Starch from the low GI potato cultivar Carisma was more resistant to the effects of hydrothermal treatment in the DSC and RVA than starch from the high GI cultivars used for comparison in this study. Carisma starch was also more resistant to shear and breakdown, and formed a stronger retrograded starch paste than the starches from the high GI potatoes. Further examination of these properties, which could be associated with the fine structure of amylose and amylopectin and the way these molecules are organized in the granules, may provide insights into why the starch in cooked Carisma potatoes has greater resistance to enzymatic hydrolysis, and elicits a lower postprandial blood glucose response than other potatoes. The importance and popularity of potatoes as a food crop dictate the need to identify and develop cultivars that are digested slowly and have a low GI. This study suggests that thermal analysis and starch paste properties could be used as an aid in identifying and developing cultivars that are digested slowly and have a low GI.

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