Relationship between phenolic content of potato and digestion of carbohydrate *in vitro* and *in vivo*

Zida Muhammad Karim

Submitted in accordance with the requirements for the degree of
Doctoral of Philosophy

The University of Leeds
School of Food Science and Nutrition

September, 2016

The candidate confirms that the work submitted is her own and that appropriate credit has been given where reference has been made to the work of others.

Publications

Part of this thesis based on this publications:

Zida Karim, Melvin Holmes and Caroline Orfila. 2017. Inhibitory effect of chlorogenic acid on digestion of potato starch*. Food Chemistry*, 217,498–504

Z. Karim, M. Holmes and C. Orfila. 2015. Inhibition of pancreatic alpha amylase digestion of potato starch by chlorogenic acid in vitro. Proceedings of the Nutrition Society (2015), 74 (OCE1), E64. Summer Meeting, 14–17 July 2014, Carbohydrates in health: friends or foes

Manuscript in preparation:

Zida Karim, Amin Sadeghpour, Melvin Holmes and Caroline Orfila. 2016. Relationship between starch structure and digestibility of potato *in vitro* and *in vivo*. In preparation.

Conference Presentation:

Z. Karim , M. Holmes and C. Orfila. Poster presentation. Inhibition of pancreatic alpha amylase digestion of potato starch by chlorogenic acid in vitro. 4th International Conference of Food Digestion, Naples, Italy, 17-19 March 2015.

Acknowledgements

Undertaking PhD has been truly a life-exchange experience for me with the support and guidance I received from many people.

First of all I would like to thanks my supervisors Dr. Caroline Orfila and Dr. Melvin Holmes for their patient guidance, encouragement during the period of this study. I have been extremely lucky to have a supervisors who cared so much about my work, without their guidance and constant feedback this PhD would not have been achievable.

I gratefully acknowledge the funding received towards my PhD from the Ministry of Higher Education and Scientific Research of the Kurdistan Region Government (KRG) KRG-HCDP scholarship program. I greatly appreciate the support received from all academic staff at the Department of Food Technology, Salahaddin University-Erbil.

Many thanks go out to graduate and postgraduate students at Leeds university for their support during human study. I am especially grateful to Amin Sadeghpour Dilmaghani and Denise Li for the assistance I received during physical analysis of starches. I am also very grateful to Lucia Hernandez she was always so helpful and provided me with their assistance throughout analysis of phenolic acids. I would like thanks Lilynorfesha Mohd Shah for their assistance during statistical analysis of data.

A very special thank you to my friends Aras Saber and Dr. Heero Abdullah for their accompaniment, inspiration and support.

And Finally, I would also like to say a heartfelt thank you to my family especially my father Muhammad Karim and my mother Sewa for always believing in me and encouraging me to follow my dreams.

Abstract

Potato (*Solanum tuberosum*) is the third most important crop in the world after rice and wheat. Potato tubers are rich in starch, but also contain minor amounts of phenolic compounds. The chlorogenic acid isomer 5-O-caffeoylquinic acid (5-CQA) is the predominant phenolic acid in potato. The main aim of this study was to investigate the relationship between phenolic content and starch digestibility *in vitro* and *in vivo*. Firstly, the effect of 5-CQA on digestion of commercial potato starch by porcine pancreatic alpha amylase (PPAA) was investigated. The results showed that co- and pre-incubation of PPAA with 5-CQA significantly reduced PPAA activity in a dose dependent manner with an IC50 value of about 2 mg mL^{-1}. Lineweaver-Burk plots indicated that 5-CQA exerts a mixed type inhibition as k_m increased and V_{max} decreased.

Secondly, *in vitro* starch digestion was performed on steam cooked tubers from five varieties of potato (Desiree, Mozart, Rooster, Maris Piper and Maris Peer) with varying phenolic content. 5-CQA content of peeled tuber tissue ranged from 10.36 to 29.46 mg $100g^{-1}$ dry weight (DW) in raw tubers and 6.51 to 21.24 mg $100g^{-1}$ DW in cooked tubers. With the exception of Desiree, 5-CQA levels decreased after cooking. The composition of the tubers in term of dry matter (DM), total starch (TS), free sugars, starch properties (amylose and starch granule structure) were also determined. Significant differences in digestibility by PPAA alone were observed between potato varieties when measured as area under curve (AUC) of glucose released *in vitro* ($p \leq 0.05$). Rooster had the highest and Maris Piper the lowest AUC. Similar results were obtained when an *in vitro* digestion that simulated gastric (including pepsin)

and intestinal (including amyloglucosidase AMG) digestion was used to calculate hydrolysis index (HI) and estimated glycaemic index (eGI).

Finally, the digestibility of potato was measured *in vivo* using steamed tubers from Desiree, Rooster and Maris Piper. Standard glycaemic index (GI) methodology was followed. The results showed that Rooster has the highest GI (120), followed by Maris Piper (109) and Desiree (98). The differences were not statistically significant. Strong and positive correlations were observed between *in vivo* GI and eGI ($p \leq 0.01$).

It was observed that estimated glycaemic index (eGI) of cooked potato negatively correlated with 5-CQA ($r=-0.91$, $p \leq 0.05$) and *in vivo* glycaemic response (GI) negatively correlated with TPC and 5-CQA ($r =-0.82,-0.91$) ($p \leq 0.05$) respectively. Principle component analysis indicated that starch digestibility is affected by multiple factors including phenolic, dry matter, starch content, starch granule size and crystalline lamella structure. It appears that the same single factor does not strongly determine digestibility in all varieties.

Table of Contents

Table of Contents

List of Tables

List of Figures

Abbreviations

AMG…….Amyloglucosidase

AUC…….Area under curve

BMI……...Body mass index

CA……Caffeic acid

DCCT……Diabetes Control and Complication Trail

DM…….Dry matter

DNS……..3,5-Dinitrosalicylic acid

DS…….....Digestible starch

DW……Dry weight

eGI ……Estimated glycaemic index

FW…….Fresh weight

GI…….....Glycaemic index

HI…….....Hydrolysis index

HPAEC-PAD……High performance anion exchange chromatography with pulsed amperometric detection

HPLC-DAD-MS……High performance liquid chromatography with diode array detector – mass spectrometry

iAUC……..Incremental area under curve

NDNS……National Diet and Nutrition Survey

PCA……Principle component analysis

PPA……Pancreatic alpha amylase

PPAA…….Porcine pancreatic alpha amylase

QA……..Quinic acid

RAG……...Rapidly available glucose

RDS…….....Rapidly digestible starch

RS ……Resistant starch

SACN…….Scientific Advisory Committee on Nutrition.

SAXS…….Small-angle X-ray scattering

SD……..Standard deviation

SDS……...Slowly digestible starch

TPC…….....Total phenolic content

TS……..Total starch

UKPDS…….United Kingdom Prospective Diabetes Study

5-CQA……...5-caffeoylquinic acid

1 Introduction

1.1 The origin of potato

The cultivated potato (*Solanum tuberosum)* has been used as food for over 10,000 years, and is originally from South America (Camire *et al*., 2009). Potato is the third most consumed food crop by humans behind rice and wheat (Birch *et al*., 2012, Ek *et al*., 2012, Camire *et al*., 2009). It is the only major food crop that is a tuber, and provides 5% to 15% of dietary calories for various populations around the world (Ezekiel *et al*., 2013, Thompson *et al*., 2009). According to the National Diet and Nutrition Survey (NDNS) (2008-2011), the average intake of various forms of potato (chips and roasted potato, potato salad, potato dishes, potato products and other potato) among UK adults was just under 85g/day (Gibson and Kurilich, 2013).

1.2 Nutritional composition of potatoes

Potatoes have a high nutritional value compared to wheat and rice. It contains around 80% water and 20% dry matter (DM). Carbohydrates represent 66-80% of DM in potatoes mainly in the form of starch. Starch content ranges between 16-20% wet weight of fresh potato (Camire *et al*., 2009, FAO,2008), while protein content ranges between 2-2.5% and fibre around 1-2%. While the protein content of potatoes is small, it has high biological value that is comparable to that of whole egg and soybean. Potatoes contain some minor components, including minerals (potassium 564 mg g^{-1} FW, phosphorus 30-60 mg/g FW, calcium 6-18 mg/g FW), and vitamins like ascorbic acid (vitamin C) with content ranging from 11.5-30.8 mg $100g^{-1}$ FW in raw potato (Camire

et al., 2009, Dale *et al*., 2003, Love *et al*., 2004). Phosphorus content in the potato is within starch in the form of mono-phosphate esters that are covalently bound to amylopectin (Hoover, 2001, Jane *et al*., 1996).

Potatoes also contain plant secondary metabolites (phytochemicals) which are highly desirable in the human diet because they are associated with beneficial effects on health (Ezekiel *et al*., 2013, Lemos and Sivaramareddy, 2013). Potatoes by their content of ascorbic acid, polyphenols, tocopherols, carotenoids and selenium are considered one of the richest sources of antioxidant in the human diet (Ezekiel *et al*., 2013).

1.3 Starch structure and composition

Carbohydrates are the most abundant organic compounds on earth and widely distributed in animal and plant tissues. Common carbohydrates include soluble sugars, starch, cellulose and glycogen. They are composed of carbon, hydrogen and oxygen usually in a ratio 1:2:1 and have a general molecular formula is $(CH_2O)_n$ where n is three or more. Carbohydrates can be classified according to the number of individual simple sugar units into monosaccharide (such as glucose, galactose, fructose and arabinose), disaccharides (such sucrose, maltose and lactose) and polysaccharides (such as starch, cellulose and glycogen) (Zakrzewska *et al*., 2010).

Starch is comprised of two types of glucose polymers; amylose which is a linear polymer of α-1,4 linked glucose and amylopectin with linear α-1,4 linked glucose chains branched through α-1,6 linked glucose linkages (figure 1.1) (Butterworth *et al*., 2011, Coultate, 2002, Tester *et al*., 2004, Imberty *et al*., 1991, Buleon *et al*., 1998).

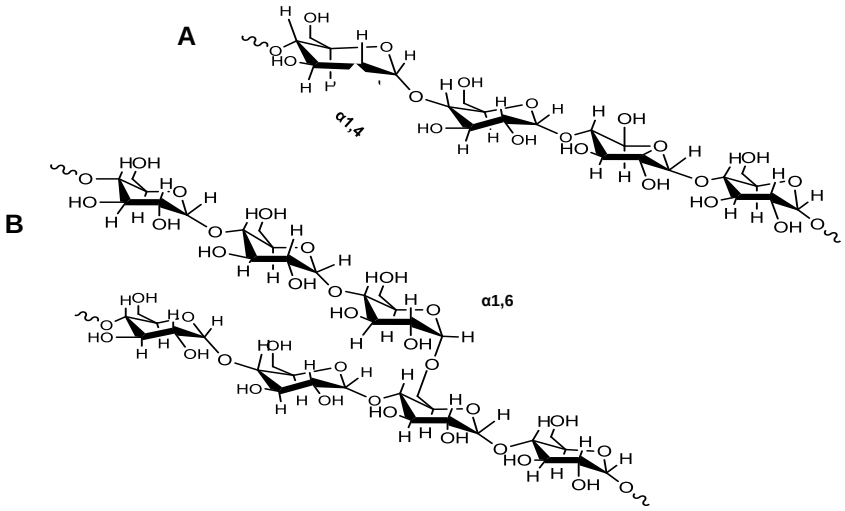


Figure 1.1 Representative partial structure of A) amylose B) amylopectin.

The main site of starch synthesis is plastids and amyloplasts responsible for storing the starch granules particularly in stroma cells within tubers and roots such as potato (Preiss, 2004). A starch granule has an organised internal structure composed of semi-crystalline concentric layers with a thickness 100-400 nm (figure 1.2). Each layer is called a growth ring. The crystallinity of starch varies from 15% relative crystallinity for high amylose starch to 45% for high amylopectin (waxy) starch (Perez and Bertoft 2010, Copeland *et al*., 2009). Within the growth ring, each growth ring has a lamellar structure with a repeat distance 9-11 nm that is interpreted to be due to alternating crystalline and amorphous layers (figure 1.2B) (Donald, 2004, Yuryev *et al*., 2004). Both amylopectin chains and amylose chains have the ability to form double helices, however, in most granules starch amylopectin is the main crystalline component (Cyras *et al*., 2006). Amylose helices may contribute to the crystallinity of granules only in high-amylose starches. The external (outer) and internal (inner) chains of amylopectin are classified into A, B and C- chains (Butterworth *et al*., 2011, Buleon *et al*., 1998). Three different types of starch

crystal structure have been also identified, cereal starches contain mainly A type, tuber starches are B type while legume starches have mainly a C- type arrangement. The A type starch has low water content in internal structure while, B type intra structure is more open with hydrated helical core (figure 1.3) (Butterworth *et al*., 2011, Tester *et al*., 2004, Donald, 2004). The purpose of this study will be mainly concerned with the properties of potatoes starch.

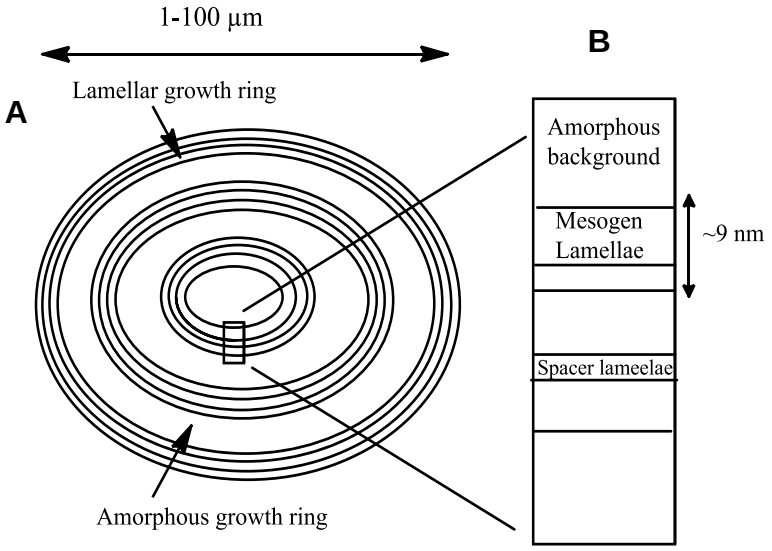


Figure 1.2 schematic representation of starch structure A) polarized micrograph showing typical lamellar growth ring. B) diagram representing the organisation of the amorphous and crystalline region within each lamella. (adapted from Donald, 2004).

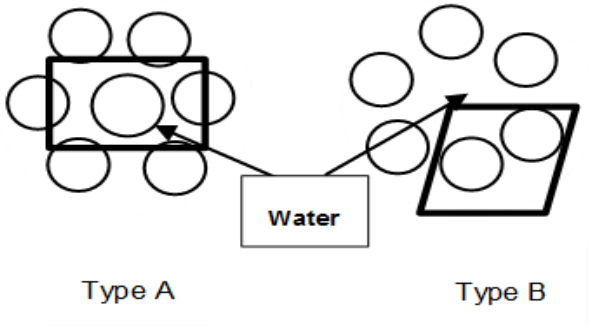


Figure 1.3 A- and B-type features of chain polymorphs and the location of water.

1.3.1 Potato starch properties

Potato contains starch in the range between 66% and 80% based on dry weight (DW) according to cultivar varieties and plant growth stage (Chung *et al*., 2014). Physical properties of potato starch are unique amongst other source of starches and this is attributed to their shape, size and crystal structure. Potato starch granules are lenticular, oval or spherical shaped with granule size ranging from 5-100 µm, showing a B- type crystalline structure. Potato starch has a high phosphorus content ranging from 36 to116 mg/100 g the amylose content of potato starch ranges between 21% and 33% (Alvani *et al*., 2011, Kaur *et al*., 2007, Liu *et al*., 2003 and Liu *et al*., 2007). Potato amylopectin side chains are long with a degree of polymerization (DP) of 19 to 56 glucose residues and show the ability to form a thick visco-elastic gels following gelatinisation (Alvani *et al*., 2011, Yusuph *et al*., 2003).

Potato starch in its raw form is largely enzyme resistant and insoluble in water. Most uses of potato starch involve heating in the presence of excess water (gelatinisation) also it has valuable ability to form clear gels when gelatinised due to the high phosphoester and low lipid content (Chung *et al*., 2014, Alvani *et al*., 2011). When potato tubers are steam cooked or baked, starch is gelatinised using the high water content within the tuber.

There are many publications about factors affecting the composition, structure and properties of potato starch and most of them conclude that the genotype of potato as well as environmental growth conditions have an influence on the properties of potato starch. Recently it has been shown that potato DM and starch content varies amongst cultivars and growth location (table 1.1) (Chung *et al*., 2014). On the other hand, a recent study reported that different potato

cultivars grown under the same condition show less variation in their starch physicochemical properties than might be expected (Alvani *et al*., 2011).

Table 1.1 Comparison of carbohydrate content of potato grown in different regions of North America. (Chung *et al*., 2014)

Region	Total starch*	Dietary fiber*	Free glucose*	Resistant starch**	Apparent Amylose**	Relative crystallinity**
Manitoba		Higher	Higher		Higher	
New Brunswick	Higher			Higher		Higher

*in dry matter content. **in starch content

1.4 Analysing starch structure (physio-chemical properties)

The structure of starch amylose and amylopectin and molecular organization within starch granules has been studied using many approaches and techniques. Amylose content in starch granules can be measured using a colorimetric test with iodine as amylose helices giving a blue colour with polyiodide. The size of amylose and amylopectin have been studied using size exclusion chromatography. Differential scanning calorimetry (DSC) has been used to study the thermal behaviour of starch, including gelatinisation as well as glass transition temperature of starch and its products (Yu and Christie, 2001, Zeleznak and Hoseney 1997, Shogren 1991). To study the starch molecular organisation and thickness of crystalline regions, microscopy and X-ray diffraction have been used. Small-angle X-ray scattering (SAXS) diffraction provides information about the lamellar structure of starch within the granules. Experimentally measuring hydrated starch by SAXS provides a peak at a value of 0.062-0.66$Å^{-1}$ (Perez and Bertoft, 2010, Pikus, 2005). Light and electron microscopy also have been used for several decades to study

the morphology (appearance, shape and size of the granules) and structure of native and modified starch (Cappa *et al*., 2016, Li *et al*., 2016, Polesi *et al*., 2016, Mishra and Rai, 2006).

1.5 Starch digestion

Starch digestion, metabolism begins by the endo-hydrolysis of α-1,4-glucosidic linkages by salivary α-amylases mainly producing maltose, maltotriose and limited dextrins, which are branched oligosaccharides that are formed at the end of the digestion of amylopectin (Hanhineva *et al*., 2010). After this partial digestion, the bolus is swallowed and enters the stomach. Here, there is no further starch enzymatic digestion, as stomach produced acid reduces the pH to 1 and amylase is inactivated . The majority of carbohydrate digestion takes place in the small intestine, first by the activity of pancreatic α-amylase that produces disaccharides and small dextrins and then to release glucose. These can be absorbed through the intestinal epithelium into the bloodstream by glucose transporters with co-transport of a sodium ion. Raw starch granules from different botanical origins vary in the rate and extent of susceptibility to amylolytic hydrolysis. Potato starch granules in native form are considered the most resistant to digestion compared to other native starch granules such as maize and wheat starch. Cooking alters the availability of starch for enzymatic digestion. Gelatinised potato starch is more easily digested than native starch and is more easily digested than cooked maize or wheat starch.

1.5.1 Alpha amylase structure and catalytic characteristics

Alpha amylase (α-1,4-glucan-4-glucanohydrolayses; E.C. 3.2.1.1) is one of the digestive enzymes that has a significant role in carbohydrate digestion. It

is found in bacteria, plants and other higher organisms. In humans, it is produced by the pancreas and salivary glands as major secretory products. Alpha amylase is a glycoprotein composed of 496 amino acids in a single polypeptide chain with a molecular weight 57.6 kDa (Brayer et al., 1995). The tertiary structure of the enzyme alpha amylase from human pancreas, saliva and porcine pancreas was obtained by X-ray crystallography and are all very closely related (Brayer *et al*., 1995, Gilles *et al*., 1996). The protein structure of mammalian amylases is comprised of three domains A, B and C as well as a calcium and a chloride ion (Kandra *et al*., 2005). Domain A which is the largest one, contains about 280-300 residues and constructed from two segments of a polypeptide chain (residues 1-99 and 169-404). It is a catalytic domain and located in the central part, the most important active residues are two aspartate and one glutamate residues (Asp197, Asp 300, Glu 233). Chloride ion Cl^- also found in this vicinity (de Sales *et al*., 2012, Brayer *et al*., 1995). Domain B (residues 100-168) which is the smallest domain and complex loop serves to bind calcium (calcium binding site) and located at the wall of the β-barrel of domain A. (Kandra *et al*., 2005, de Sales *et al*., 2012, Brayer et al., 1995). Domain C (residues 405-496) folds into an antiparallel β-barrel type structure and forms a compact unit on the opposite side of domain A from domain B. It is improbable for this domain to plays a direct role in the catalytic mechanism (Kandra *et al*., 2005, de Sales *et al*., 2012). The substrate binding site of pancreatic amylase is located in a cleft between domain A and B contains five sub-sites for binding with glucose residues (Butterworth *et al*., 2011). The stability of active site cleft is related to binding the residues of A and B domains with calcium (Whitcomb and Lowe, 2007).

1.5.2 Determination of alpha amylase activity

In general, for assaying alpha amylase activity, soluble starch or modified starch is used as a substrate. Alpha amylase catalyses hydrolysis of α-1,4 glucan linkages in starch components (amylose, amylopectin) producing maltose and maltotriose as the main products. There are various methods available for monitoring and determination of alpha amylase activity based on increasing reducing sugar content, decreasing iodine binding, decreasing starch viscosity and degradation of colour complex substrates. Some methods of measuring the increase in reducing sugars are described below.

1.5.2.1 Increase in reducing sugar using the dinitrosalicyclic acid (DNS) method

This method is based on measuring the amount of reducing sugar, which is produced as a result of starch hydrolysis by alpha amylase (Xiao *et al*., 2006, Brenfeld, 1955). DNS methods have some disadvantages including the slow loss of the colour produced and breakdown of glucose by constituents of the DNS reagent.

The limitations were overcome by developing a modified method for estimation of reducing sugar (Miller, 1959). Modifications include the addition of 0.05% sodium sulphate to prevent the oxidation of reagent. The modified method since then has been used widely to measure reducing sugar without any further modification (Miller, 1959, Gupta *et al*., 2003).

1.5.2.2 Chromatographic method

Chromatographic methods are the most powerful analytical techniques for the analysis of the quantity and quality of monosaccharides and oligosaccharides

in foods. Ion exchange chromatography with a sodium hydroxide eluent has been used to separate carbohydrates such as sugar alcohols, monosaccharides, disaccharides, and other oligosaccharides as anions (Rocklin and Pohl, 1983). The detection of carbohydrates after elution is complicated because they do not absorb UV light. For this reason, pulsed amperometric detection (PAD) is widely used for detecting carbohydrates.

1.5.3 Factors affecting enzymatic digestion of starch

The first step in enzymatic catalysis reaction is the formation of an enzyme-substrate complex. Adsorption of an enzyme to its substrate is controlled by the availability of the binding site. The interaction of amylase with substrate within granules is complicated by the necessity of the enzyme to bind and adsorb to the starch granules (Warren *et al*., 2012).

There are some primary factors that may affect adsorption of amylase enzyme to starch within granules, including granule size, presence of the surface pores, the crystalline structure of the carbohydrate chains at the granule surface (Warren *et al*., 2012). Small granules are digested more rapidly than large granules suggesting that enzyme binding is dependent on the surface area or that small granules have a more accessible internal structure. The amorphous regions of the lamella are more accessible to amylolytic enzymes than crystalline regions.

The type of starch granules also affects the susceptibility to amylase digestion. Raw cereal starches are more favourable substrates for amylase compared to potato starches as A type starch amylopectin, have a larger proportion of short amylopectin branch chains (Sujka and Jamroz, 2007). The granules show pinholes on the surface and channels inside the granules compared to

potato starch granules (Tester *et al*., 2004). However, upon gelatinisation, the B-type starch present in potato allows a higher degree of hydration and the granule structure is disrupted. Crystalline lamellas were resistant to enzymatic action whereas hydrolysis occurred mainly in the more amorphous zones (Sujka and Jamroz, 2007).Therefore, cooked potato starch tends to be more digestible than cooked cereal starch.

There are also some chemicals and physical techniques used for alteration of starch properties. Physical methods are more suitable for commercial use and are widely used in starch modification than chemical modification due to safety, simplicity and cost effectiveness (Xie *et al.,* 2014). Retrograded starch is resistant to hydrolysis by amylase (Butterworth *et al*., 2011, Warren *et al*., 2012). Recently Xie *et al*. (2014) investigated that repeated retrogradation of waxy potato starch produced a high slowly digestible starch (SDS) content of 40.41% and alter their crystalline form B-type to C-type which in turn have a significant impact on waxy potato starch digestibility. Another *in vitro* study indicated that manipulation of rice flours and starches, by adding high amylose and high resistant starch maize starch resulted in a reduction in starch digestion (Mir *et al*., 2013).

1.6 Hyperglycaemia

Postprandial hyperglycaemia and hyperinsulinemia are induced by the consumption of food that contains digestible carbohydrate that is digested to glucose and absorbed into the blood circulatory system, resulting in an increase in blood glucose after ingestion (Hanhineva *et al*., 2010, Adisakwattana *et al*., 2012). The absorption is initiated at the apical membrane when Na ions bind to sodium-dependent transporter SGLT1 and allows

glucose binding and subsequent transport into the enterocyte. Glucose is released from the enterocyte through glucose transporter GLUT2 at the basolateral membrane to enter the portal circulation.

The increase in blood glucose in the postprandial stage is determined by the difference between the amount of the glucose entering and leaving the circulation. In healthy, non-diabetic individuals, glucose levels are maintained at homeostatic levels (4-6 mmol L^{-1}) by pancreatic hormonal control (glucagon, insulin, adrenaline). After oral ingestion, blood glucose starts to rise (>6 mmol L^{-1}) and secretion of insulin by pancreatic cells increases, promoting glucose uptake by the liver, kidney, muscle, and adipose tissue. Secretion of insulin as a response to the rise in blood glucose is in two phases; in the first phase insulin release within 10 minutes after a sudden increase in plasma glucose then, beta cells pause and if the blood glucose not back under 5.5 mmol L^{-1} 10-20 minute later another small insulin response take place. In a healthy person this lowers the blood glucose to the starting level. The chief determinant of postprandial glucose level is associated with secretion of insulin in the first phase, loss of this ability result in substantial elevation of glucose and characterises type-2 diabetes and impaired glucose tolerance (Rendell and Jovanovic, 2006). Therefore, in diabetic patients, a carbohydrate-rich diet can have detrimental effects on glycaemic control. Furthermore, measurement of fasting plasma glucose is recommended for diagnosis of diabetes mellitus. WHO guidelines define diabetes mellitus based on fasting plasma glucose levels > 126 mg dL^{-1} (7.0 mmol L^{-1}), 2 hours postprandial glucose loads > 200 mg dL^{-1} (11.1 mmol L^{-1}) or both (Alberti *et al*., 1998).

Despite of increasing plasma glucose and insulin with a high-carbohydrate diet, triacylglycerol concentrations also tend to increase along with other cardiovascular disease risk factors (Riccardil *et al*., 2008). Controlling postprandial hyperglycaemia and insulinemia are thought to be important factors in the prevention and treatment of type-2 diabetes, cardiovascular disease and obesity (Butterworth *et al*., 2011). According to the evidence from the Diabetes Control and Complication Trail (DCCT) (involving patients with type 1 diabetes) and the United Kingdom Prospective Diabetes Study (UKPDS), that performed the largest study of type 2 diabetes in the United Kingdom; by controlling of blood glucose near to normal as possible, incidence and progression of microvascular complication are reduced which in turn reduce the morbidity and mortality in patients with diabetes (DCCT, 1993, UKPDS, 1998). It is therefore important to control carbohydrate digestion and absorption of glucose following high-carbohydrate meals.

Abnormal elevation of glucose after a meal (referred to as postprandial dysmetabolism) can be controlled by diet, exercise and pharmacologic intervention (Rendell and Jovanovic, 2006, O'Keefe and Bell, 2007). The type of food consumed has a great impact on how quickly the level of glucose increase after a meal (Smolin, 1997). Effects of different diets are shown in Table 1.2. After consumption of gumdrop that is mostly sucrose, blood glucose rises after a minute because it is digested and absorbed rapidly in the small intestine. The consumption of food that have a mixture of fat, protein and carbohydrate and foods that are high in fibre take a longer time for blood glucose levels to increase as they remain for a longer time in the stomach and small intestine before glucose is absorbed (Smolin, 1997). The saturated and trans fat has been reported to be involved in insulin resistance (Zhao *et al*.,

2016). In potato, the main digestible carbohydrate is starch at 16-20 g $100g^{-1}$ fresh weight, and a smaller contribution from free sugars, including glucose, fructose and sucrose at 0.6-1.1g $100g^{-1}$ fresh weight (McCance and Widdowson, 2002).

Table 1.2 Effects of different type of foods on postprandial hyperglycaemia. (Ratner, 2001)

Foods	Effects
Fruit and vegetables (broccoli, spinach, berries, tomatoes avocadoes, etc.	Improves
Lean protein (egg, whites, whey protein, fish, non-fat dairy, chicken breast, etc.)	Improves
Omega-3 (fish, nut, etc.)	Improves
Processed carbohydrates (sugar, white flour, mashed potatoes, etc.)	Worsen
Saturated fat (fatty meats, full-fat dairy, etc.)	Worsen
Trans fat (French fries, commercial baked goods)	Worsen

1.7 Classification of dietary carbohydrate

Both *in vivo* and *in vitro* methods have been used to measure starch digestibility and classification of carbohydrate.

1.7.1 Glycaemic index (GI)

It has been shown that different starchy foods have different effect on postprandial blood glucose in healthy and diabetic subjects. Glycaemic index (GI) is one of the most popular measures and was developed by Jenkins et al (1981) for *in vivo* measurement of the availability of carbohydrates in foods and their ability to raise blood glucose. GI is defined as the ratio of the

incremental area under the blood glucose response curve (iAUC) for a test food over a reference food (glucose solution or white bread), both containing 50 g of available carbohydrate (starch or free sugars) (Jenkins *et al*., 1981). The normal range of blood glucose concentrations in healthy people is between 4 and 10 mmol L^{-1}.

Carbohydrates are important in the human diet. Carbohydrate-rich foods are classified according to their postprandial glycaemic effect using GI classification on a scale 0-100. The first systematic classification of food according to GI response was undertaken by Otto and Niklas (1980) for the purpose of informing diabetics on how to keep their blood glucose as constant as possible. For practical reasons, GI value foods are grouped into three categories according to their GI into low ≤55, medium 56-69 and high ≥70 where glucose =100 (Soh and Brand-Miller, 1999).

In some research laboratories white bread was used as the reference food for measuring GI values, whereas others use glucose (dextrose). When glucose is used as the reference food the GI value for glucose = 100 and the GI value of white bread = 70. When white bread used as the reference food the GI value for white bread = 100; and hence the GI value of glucose = 143. Therefore, when bread was the reference food used in the original study, the GI value for the food was multiplied by 0.7 to obtain the GI value with glucose as the reference food (Foster-Powell *et al*., 2002).

In 1998, the Food and Agriculture Organization (FAO) developed a standard protocol for testing GI (FAO, 1998). Basically the *in vivo* GI testing method include measuring blood glucose after fasting for 12 hours and then feeding subjects 50 g available carbohydrate food (test food or reference) and

measuring blood glucose over 2-3 hours at 15-30 min intervals. According to the FAO/WHO, (1998) 6 subjects are enough for GI testing while, Brouns *et al*, (2005) suggested that 10 subjects is more reasonable. In order to estimate the intra-individual variation Brouns *et al* (2005) recommended to test the reference food at least two times for each subject.

According to the FAO/WHO (1998) the incremental area under glucose response curve (iAUC) should be calculated geometrically by applying the trapezoid and triangle rule ignoring area beneath baseline fasting glucose concentration figure 1.4.

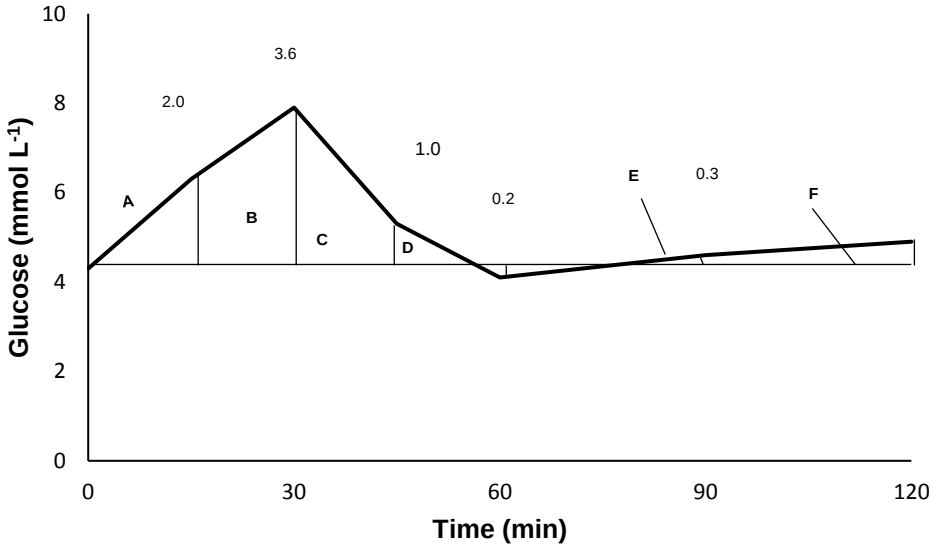


Figure 1.4 Sample calculation of incremental area under curve iAUC (adapted from FAO/WHO 1998). The area of triangle A = 2x 15/2. The area of trapezoid B= (2+3.6)x15/2. The area of trapezoid D= 1xt'/2, t'/15=1/(1+2). Therefore the iAUC = SUM area of triangles and trapezoids (A+B+C+D+E+F).

There are many factors that affect the glycaemic response, including food factors and human factors. The food factors include food thermal processing, food particle size, length of fasting, the macronutrient composition of the food, and the ratio of amylose and amylopectin content (Arvidsson-Lenner *et al*., 2004, Bjorck *et al*., 1994). Human factors also affect the blood glucose

response. Factors documented include age, ethnicity, physical activity, smoking, alcohol consumption, body mass index, gastrointestinal disease.

Due to so many factors affecting the blood glucose response, scientists have tried to use *in vivo* methods to estimate GI based on food factors alone.

1.7.2 *In vitro* starch digestion to estimate GI

Estimation of GI *in vivo* is time consuming and costly as human subjects are needed to be recruited and reference and test foods to be consumed on different days. *In vitro* methods have been introduced as an alternative to estimate the starch digestion *in vivo*. *In vitro* method simulate enzymatic digestion under controlled condition. According to the rate and extent of digestion *in vitro,* starch can be classified into three types; rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS) (Englyst *et al*., 1992). Values for RDS , SDS and RS obtained by the method reflect the rate of starch digestion *in vivo*. RDS such as fully gelatinised waxy starch digested rapidly within 20 minutes, while SDS digested slowly with glucose released after 20-120 min. RS is resistant to digestion and is the total starch not digested within 120 minutes (Yu *et al*., 2014, Zhang *et al*., 2014, Lehmann and Robin, 2007).

Granfeldt, *et al*. (1992) derived the hydrolysis index (HI) by calculating the relation between the area under curve for food and reference as a percentage. Goni *et al.* (1997) described an *in vitro* procedure for estimation of GI through an equation by using HI (GI=39.7+(0.6xHI).

Moreover, it is also reported that there are a good correlation between *in vitro* and *in vivo* digestibility of starch (Jenkins *et al*., 2002, Chung *et al*., 2008).

1.7.3 Potato and GI

Cooked potato has been labelled as a high GI food that causes a sharp increase of blood glucose concentration, however, there is great variation in the GI amongst potato and potato products (Weichselbaum, 2010). The glycaemic index of potatoes has been determined in many studies using *in vitro* or *in vivo* testing. There are only limited studies that subjected potatoes to both *in vitro* and *in vivo* testing for comparison.

Table 1.3 includes the GI of potato varieties determined in many *in vivo* studies. Soh and Brand Miller (1999) have shown that there is no significant difference in GI between varieties (Sebago, Pontica and Desiree) and among cooking methods (boiling, oven-baking, microwaving or mashing). All potatoes tested had a high GI value >78. However, Henry *et al*. (2005), who studied the glycaemic index values for commercially available potatoes in Great Britain demonstrated that the varieties cooked using the same cooking method(boiling for 15 min) show a wide range of GI values from 56-94 . The differences were explained by differences in textural characteristics; potatoes with waxy texture showed medium GI whiles floury potato showed a high GI (Henry *et al*., 2005). Ramdath *et al*. (2014) mentioned that the mean GI for pigmented potatoes (red, purple, yellow and brown) varied from 77 to 93 and were not significantly different. Therefore, there is still no conclusive evidence explaining differences in GI amongst potato products. One of the hypotheses of this study is that chlorogenic acid content (a colourless polyphenol abundant in the skin and flesh of the potato) may influence starch digestibility *in vivo* and *in vitro*.

Table 1.3 glycaemic index of potato varieties

Varieties	GI	Reference food	No. of subject	Preparation method	serving
Carisma[d]	53±7	glucose	10	Peeled and boiled 8-9min	Hot
Marfona[b]	56±3	glucose	10	Peeled, cut into quarters and boiled for 15 min	Hot
Nicola[b]	57±7	glucose	10	Peeled, cut into quarters and boiled for 15 min	Hot
Nicola[d]	69±5	glucose	10	Peeled and boiled 8-9min	Hot
Estima[b]	66±5	glucose	10	Peeled, cut into quarters and boiled for 15 min	Hot
Charlotte[b]	66±5	glucose	10	Peeled, cut into quarters and boiled for 15 min	Hot
Desiree[d]	74±8	glucose	10	Peeled and boiled 8-9min	Hot
Desiree[b]	77±17	glucose	10	Peeled, cut into quarters and boiled for 15 min	Hot
Desiree[a]	101 ±15*	White bread	10	peeled and boiled 35 min.	Hot
King Edward[b]	75±10	glucose	10	Peeled, cut into quarters and boiled for 15 min	Hot
Russet Burbank[c]	76.5±8.7*	White bread	12	baked	Hot
Russet Burbank[d]	82±2	glucose	10	Peeled and boiled 8-9min	Hot
Purple Majesty[e]	77±9	glucose	9	cut into cubes 2 cm^3 and baked in oven for 40 min	Hot with skin
Red potato (Y38)[e]	78±14	glucose	9	cut into cubes 2 cm^3 and baked in oven for 40 min	Hot with skin

Varieties	GI	Reference food	No. of subject	Preparation method	serving
Yellow potato (Yukon Gold)[e]	81±16	glucose	9	cut into cubes 2 cm3 and baked in oven for 40 min	Hot with skin
Maris Piper[b]	85±4	glucose	10	Peeled, cut into quarters and boiled for 15 min	Hot
Sebago[a]	87±7*	White bread	10	peeled and boiled 35 min.	Hot
Pontiaca[a]	88±9*	White bread	10	peeled and boiled 35 min	Hot
Red potato[c]	89.4±7.2*	White bread	12	Cut into cubes 2.5 to 3 cm3 ,Boiled for 12 minute in salted water	Hot
Virginia Rose[d]	93±10	glucose	10	Peeled and boiled 8-9min	Hot
White potato (Snowden)[e]	93±17	glucose	9	cut into cubes 2 cm3 and baked in oven for 40 min	Hot with skin
Bintje[d]	94±8	glucose	10	Peeled and boiled 8-9min	Hot
Maris Peer[b]	94±16	glucose	10	Peeled, cut into quarters and boiled for 15 min	Hot
Maiflower[d]	103±8	glucose	10	Peeled and boiled 8-9min	Hot

[a] Soh and Brand-Miller 1999. [b]Henry *et al.,* 2005.[c] Fernandes *et al*., 2005. [d] Ek *et al*., 2014a. [e] Ramdath *et al*., 2014

*values converted from white bread to glucose reference by multiplied by 0.7 (Foster-Powell & Brand Miller, 1995).

Kingman and Englyst, (1994) investigated the effect of variety and preparation method for the *in vitro* digestibility of potato varieties. Maris Piper, Belle de Fontenany and Desiree were subjected to *in vitro* digestibility in hot and cooked form. The starch digestion rate index which is calculated as the relationship between the rapidly digestible starch (RDS) and total starch (TS) as percentage were 92, 96 and 96% respectively in hot potato. Upon cooling, values were reduced to 87, 80 and 91% respectively.

Glycaemic index of potato has also been estimated from *in vitro* kinetic of starch digestion and compared to GI values referenced in the literature. Garcia-Alonso and Goni (2000) analysed the effect of processing on starch digestibility *in vitro* and eGI for boiled, mashed, oven baked crisps and French-fries. The eGI ranged from 56.6 in French-fries to 107.5 in mashed potato and this was comparable with the reported GI value for potatoes prepared in the same way. Moreover, recently Pinhero *et al*. (2016) estimated the GI of mature potatoes from 14 varieties and reported that eGI was higher in cooked potato than in retrograded potato (boiled and cooled for 48 h at 4˚C) due to increase in RS and SDS in cooled samples.

Only one study is available that tested *in vitro* digestibility of potato and compared to *in vivo* blood glucose response of same potatoes. Ek *et al*. (2014a) reported the range of GI from 53 to 103 for potato varieties prepared by boiling for 8-9 min. When the same potato varieties were subjected to *in vitro* starch digestion, all GI values were positively and significantly correlated with percentage of hydrolysis during *in vitro* starch digestion at each time point (15, 20, 30, 45, 60, 90, 120 min). The strongest positive correlation with GI values exhibited at 90 and 120 min ($r = 0{\cdot}91$ and $P < 0{\cdot}01$).

1.8 Phenolics in potato

In addition to starch content, potato tubers contain secondary metabolites such as polyphenols that differ according to the variety of potato and condition of cultivation (Lachman *et al*., 2003). It was suggested that potato shows carcinogenesis inhibitory effects and greater effects were shown in cultivars with higher content of chlorogenic acid and anthocyanin (Thompson *et al*., 2009). Therefore, potatoes can be considered as a functional food and are receiving increasing attention as a source of phytochemicals, antioxidant and nutrients (Lemos and Sivaramareddy, 2013, Thompson *et al*., 2009). In the American diet, potatoes were considered the third most important source of phenolic compounds after apples and oranges (Chun *et al*., 2005).

Phenolics present in potato include; phenolic acids, tannins, lignin, flavons, coumarins, monohydric phenols, polyhydric phenols and anthocyanins (Ezekiel *et al*., 2013, Reyes *et al*., 2004). Chlorogenic acid, cinnamic acid, caffeic acid, ferulic acid and *p*-coumaric acids are the most common phenolic acids in potato. It was found that potatoes were among the best vegetable sources of total phenolic acids with contents ranging from 7.9 in cooked peeled potato to 52 mg per 100 g in only cooked peel (Mattila and Hellström, 2007). Caffeic acid derivatives are present in the greatest proportion with chlorogenic acid being the most abundant (Deusser *et al*., 2012, Manach *et al*., 2004).

The polyphenols in potato are mostly distributed between their cortex and skin (figure 1.5). About 50% of the polyphenolic compounds are located in the skin and adjoining tissues while the concentration decreases from the outside towards the centre of the potato tuber (Friedman, 1997).

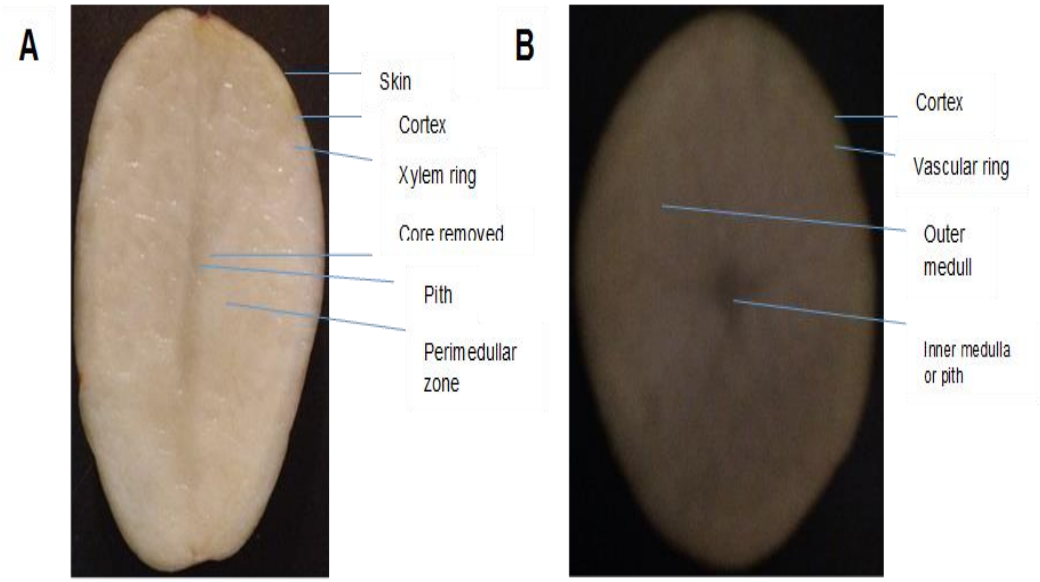


Figure 1.5 (A) Longitudinal and (B) Cross section of potato tuber.

1.8.1 Chlorogenic acid

About 90% of the phenolics of potato content comprise of chlorogenic acid, therefore it is considered as the predominant phenolic acid in potato tubers. Chlorogenic acid is mainly found in skin amounting to 1000–4000 mg/kg (Ezekiel *et al*., 2013). Chlorogenic acid is formed by an ester bond between quinic acid and cinniamic acid (figure 1.6). The main known subclasses of phenolic acids are caffeoylquinic acids (CQA), feruloylquinic acids (FQA) and dicaffeoylquinic acids (Narita and Inouye, 2009). The only commercial available form of chlorogenic acid is 5-O-caffeoylquinic acid (5-CQA) (Clifford, 2000).

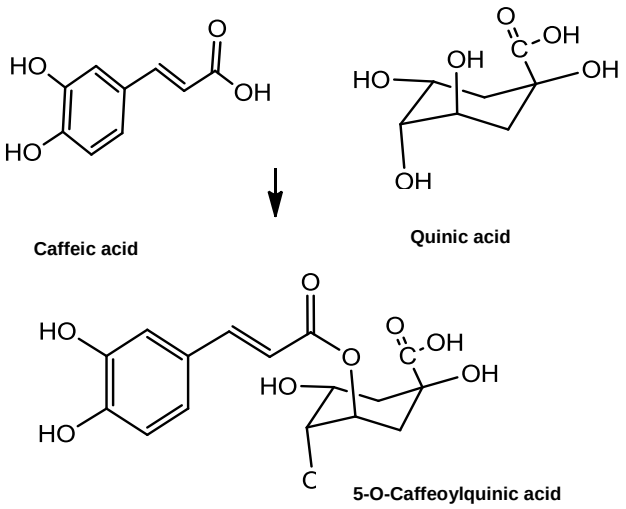


Figure 1.6 Precursors and structure of chlorogenic acid.

CQA also widely distributed in other plants such as green coffee. One cup of roast and ground coffee (weak brew, very pale roast robusta) supply 675 mg CQA, fruits like apples, cherries, blueberries, plums, kiwis, pears and prunes and vegetables like chicory and artichoke (Rocha *et al*., 2012, Clifford, 2000) are also good sources.

1.8.2 Factors affecting phenolic content of potatoes

There are some factors that are known to affect the polyphenol content of plant and plant-based foods such as environmental growing conditions, genetic and technological factors such as industrial food processing, storage and culinary processing. These factors must be taken into consideration to avoid nutrient loss and to optimise the phenolic content of food (El Gharras, 2009).

1.8.2.1 Variety of potato

There are many studies about the relation between potato genotype and polyphenol content. A recent study by Pinhero *et al*. (2016) on 14 potato

varieties indicated that there are significant differences between fresh potato varieties (skin + flesh) in total phenolic content (TPC). It was reported that TPC also varied significantly from 2.5 to 5.8 g/g FW between four fresh potato varieties directly after harvesting (Andersen *et al.*, 2002). A study by Onyeneho and Hettiarachchy (1993) indicated that total polyphenols content of red peel potatoes (24.5-32.2%) by weight of the freeze-dried extracts was higher than those varieties with brown peels (15.8-22.4%). Chlorogenic acid has been shown to be the predominant phenolic acid, with more than 83% of the total phenolic compounds in both red and brown peel potatoes at 753mg and 821.3mg per 100g potato peel extract respectively. Furthermore Navarre *et al.* (2011) investigated the potato phenolics profile in 50 diverse potato genotype and indicated that the chlorogenic acids were the most abundant phenolic acids in all potato genotypes. The range of chlorogenic acid concentration is presented in (Table 1.4).

Table 1.4 Range of chlorogenic acid in tubers of potato genotype varying in flesh colour. (Navarre *et al*., 2011)

Type	Chlorogenic acid (mg/100g dry matter)
Yellow	22.9 -211.0
White	31.0-170.0
White/purple	21.9-231.0
Red/Purple	80.4 -473.0

1.8.2.2 Cooking and processing techniques

According to several studies, most processing and cooking techniques change the phenolic content of food mostly resulting in their loss due to thermal degradation, leaching into water, isomerisation and oxidation by polyphenol oxidase (Ezekiel *et al*., 2013). The study by Perla *et al*. (2012)

investigated the effect of cooking methods, including boiling, microwaving and baking on the total polyphenol content of some varieties of potato. It was indicated that all cooking methods led to the reduction of the total polyphenol of most varieties by 44, 52 and 53% respectively. However, the total polyphenol of two red fleshed potato remained high or even increased after cooking (Perla *et al*., 2012). It is known that frying has an influence on decreasing the polyphenol content because of thermal degradation, on the other hand, oils which are used for frying can contain their own phenolic compounds and can lead to an increase in phenolic content because of absorption of oil by the potatoes (Lemos and Sivaramareddy, 2013). Recently, a study on the effect of cooking on the phenolic content of potato observed that the total phenolic content of potato increased after cooking. Separate phenolic acids such as chlorogenic acid, vanilic, caffeic, p-coumaric acid and epicatechin were found to increase in baked, fried and/or microwaved potato in comparison to uncooked potato samples (Blessington *et al*., 2010, Bembem and Sadana, 2012). Furthermore, it has recently been assessed that cooking techniques including boiling, steaming, microwaving and baking ranked in order of greatest effect on reducing the total phenolics content on *purple majesty* potatoes (Lemos and Sivaramareddy, 2013).

Some other minor processing which is required during processing of potato such as washing, handling and cutting have an impact on phenolic content. Cutting and damage of potato during processing lead to activation of some enzymes and oxidation of phenolic compounds. Phenylalanine ammonia-lyase is the main enzyme involved in the production of phenolic compounds, activation of this enzyme by damage of potato results in more phenolic products being available as substrates for peroxidase and polyphenol oxidase

enzymes which are responsible for the enzymatic browning reaction and oxidation (Ezekiel *et al*., 2013).

1.8.2.3 Agronomic factors

Andre *et al*. (2009) evaluated the influence of environmental growing conditions on the phenolic content of potato tubers and indicated that the growing environment effected the quantity of the polyphenol content of 13 native Andean potato cultivars while the proportion of individual polyphenols remain the same in all cultivars. The total phenolic content of purple- and red-flesh potato was 1.4 times higher when grown in a cooler temperature and longer days (Reyes *et al*., 2004). The stages of plant development, also have an effect on phenolic content. In potatoes, chlorogenic acid concentration was highest when harvested at a young development stage compared to mature tubers. It was observed that mature potatoes also contain the lowest level of phytonutrients than potatoes harvested before maturation. In regards with fertilizers which make N, P, K available for growth, there is no evidence to indicate their influence on phenols content of potatoes (Ezekiel *et al*., 2013).

1.8.2.3.1 Post-harvest storage

The effect of post-harvest storage on potato phytochemicals was reviewed by Ezekiel *et al*. (2013) and reported that potato phenolics generally increased with storage time while, some studies reported little change or a decrease in potato phenols after storage. It was found that storage of potato tubers at 6 ºC up to 271 days led to an increase of total phenolic content of potato tubers while, potato phenols decreased after 220 days of storage at 20 ºC (Ezekiel *et al*., 2000, Ezekiel *et al*., 2013). After analysing the total phenolic content of 8 potato genotypes that were stored at 5 ºC for 112 days, four genotypes

showed a small decrease while two genotypes showed no change after storage (Stushnoff *et al*., 2008). This shows that changes in polyphenol content during storage are dependent on cultivar type and storage conditions. Another study observed that there were no changes in the total phenolic content, chlorogenic acid, rutin, caffeic acid, p-coumaric acid, vanillic acid of potatoes after storage at 4 or 20ºC for 110 days, however, reconditioning of potatoes stored 10 days at 20ºC lead to significant increase in all of the phenolic compounds mentioned above (Blessington *et al*., 2010).

1.9 Inhibitory impact of polyphenols on alpha-amylase

It has been shown that inhibition of alpha amylase reduces the bioavailability of glucose (Forester *et al*., 2012). Controlling blood glucose level (hyperglycaemia) by alpha amylase inhibitors has a role in controlling diabetes mellitus (Alagesan *et al*., 2012). Alpha amylase inhibitors are synthetic molecules or natural plant components. The evidence from a number of in *vitro* and in *vivo* studies indicates the possibility of inhibitory effects of polyphenols on digestive enzymes such as α-amylase and α-glycosidase.

A recent study observed that a methanolic extract from three traditionally used antidiabetic native medicinal plants of Mauritius, *Erythoroxylum laurifolium* (EL), *Elaeodendro orientale* (EO) and *Antidesma madagascariensis* (AM) have a significant ($p < 0.05$) inhibitory effect on alpha amylase activity *in vitro* and the effect was comparable to acarbose (Picot *et al*., 2014). It was proposed by the authors that polyphenols present in the methanolic extract were responsible for the inhibitory effects.

There is not sufficient information about the mode of the interaction of alpha amylase with the substrate and polyphenols as inhibitors. An *in vitro* study by

Akkarachiyasit *et al*. (2011) provided the first evidence for the effect of cyanidin3-rutinoside (C3R), a flavonoid, in inhibition of pancreatic alpha amylase (PAA). Kinetic analysis indicated non-competitive inhibitory effects of C3R against pancreatic alpha amylase. C3R can bind either to active site of free enzyme or to the enzyme-substrate complex without competing with the substrate (Akkarachiyasit *et al*., 2011). Another study reported the competitive inhibitory mode effect of three flavonoid compounds from Tartary buckwheat bran, namely, quercetin (Que), isoquercetin (Iso) and rutinb (Rut) on PAA activity (Li *et al*., 2009).

The inhibitory effect of red kidney bean lectin and tannin on PAA activity was investigated in an *in vitro* study. Soluble potato starch was used as substrate. The study indicated that pre-incubation of lectin alone with enzyme had a greater inhibitory effect on amylase activity (down to 11.58% of control) than tannin alone (down to 57.69%) and lectin-tannin in combination (down to 84.31%). In comparison to co-incubation of the enzyme with substrate, tannin, lectin alone or in a combination showed limited inhibition. This indicates that polyphenols are weak inhibitors that are able to interact with the enzyme directly, but do not compete with the substrate (Fish and Thompson, 1991).

Furthermore, according to a recent study, citrus flavonoids (naringin, hesperidin, nobiletin and neohesperidin) showed a weak inhibitory effect on the digestive enzymes PAA and α-glucosidase *in vitro* but citrus flavonoids could bind to amylose therefore delaying starch digestion. The better inhibitory effect was shown on α-amylase rather than α-glucosidase for all four flavonoids. The study also reported that glucose consumption, glycogen concentration, and glucokinase activity were significantly elevated, and

glucose-6-phosphatase activity was markedly decreased by citrus flavonoids. Thus, it has been suggested that citrus flavonoids could control the postprandial rises in blood glucose and prevent postprandial hyperglycemia (Shen *et al*., 2012).

An investigation indicated that (5-CQA), quinic acid (QA) and caffeic acid (CA) have an inhibitory effect against porcine PAA isomers I and II *in vitro* and in the sequence of 5-CQA > CA>> QA (Narita and Inouye, 2009). In an animal study, oral intake of 5-CQA acid solution at 3.5mg /kg body weight (bw) by rats during a glucose tolerance test lowered the height of glycemia peak at 10 and 15min (Bassoli *et al*., 2008). Moreover, 5-CQA slowed down the absorption of glucose in the gut of obese, hyperlipidemic, insulin resistant (fa/fa) Zucker rats and consequently reduced the peak of the postprandial response to a glucose challenge (Camire *et al*., 2009). It was estimated that 95% of cafeic acid and one third of chlorogenic acid, out of 2.8 mmol, were absorbed in humans small intestine (Olthof *et al*., 2001). The inhibitors are removed from the intestine with time, inhbition happens before absorbance therfore, removal may indicate transient inhibition.

1.10 Aims of the study

Considering that chlorogenic acid has been shown to inhibit PAA and it is the predominant phenolic acid in potato, the main aim of this study was to investigate the inhibitory effect of chlorogenic acid on potato starch digestion *in vitro* and *in vivo*. The study was also aimed to investigate the role of potato starch physico-chemical characteristics such as amylose content, starch granule size and crystallinity in determining potato starch digestibility *in vitro* and *in vivo*.

1.11 Objectives

The research objectives are

1. To measure the rate of hydrolysis of commercial potato starch by porcine PAA in the absence and presence of 5-CQA *in vitro*.
2. To calculate enzymatic kinetic parameters of potato starch digestion by PAA in the presence and absence of 5-CQA
3. To determine the mechanism of inhibition of 5-CQA against PAA using potato starch as substrate by analysis of kinetic parameters derived from Linweaver-Burk plots.
4. To measure the chemical components of 5 varieties of potatoes (Desiree, Mozart, Rooster, Maris Piper and Maris Peer) such as total starch, dry matter, free sugar, total phenolic and 5-CQA content.
5. To investigate the physio-chemical properties of starch granules extracted from the same 5 varieties of potato such as amylose content, granule size and crystallinity.
6. To compare the rate of starch degradation of the same 5 varieties of potato (peeled and cooked) by PAA and PAA+amyloglucosidase (AMG) *in vitro,* and derive HI values.
7. To estimate GI of five potato varieties from HI.
8. To perform *In vivo* GI measurements of cooked potato from three varieties (Rooster, Maris Piper and Desiree).
9. To perform statistical analysis to investigate the correlation between phenolics content and starch digestibility of cooked potato *in vitro* and *in vivo*

10. To perform statistical analysis to investigate the correlation between starch properties and starch digestibility of cooked potato *in vitro* and *in vivo*.

1.12 Hypothesis

The main hypothesis tested was that potato varieties with higher phenolic content will be digested to a lesser extent than varieties with lower phenolic content.

The second hypothesis is that starches with a higher amylose content and degree of crystallinity will be digested to a lesser extent than those with low amylose and crystallinity.

2 Materials and methods

2.1 Potato samples

Five different varieties of potato that varied with regards to flesh and skin colour were purchased from food markets in Leeds, UK. Table 2.1 shows the properties, place and date of purchase of five potato varieties. These varieties represent a diverse range of commercial potatoes varieties commonly fresh in the UK.

Table 2.1 Information of five potato varieties

Potato varieties	Skin color	Flesh color	Place of purchase	Date of purchase
Desiree	red	Light yellow	Sainsbury's	November 2014
Mozart	red	Yellow	Morrison's	October 2013
Rooster	red	Yellow	Morrison's	October 2013
Maris Piper	creamy	white	Sainsbury's	October 2013
Maris Peer	creamy	Bright white	Morrison's	October 2013

2.2 Preparation of potato samples

Three tubers of each were rinsed with water and dried with a paper towel then, potatoes were peeled using potato peeler (Wilko vertical vegetable peeler) and cut into cubes 1 cm^3, then mixed well and 100 gm batches were weighted, quick frozen and stored at -80^oC until needed. Traditional home steam cooking method was performed for preparation of potatoes. Potato cubes were placed into a metal steam pan containing approximately 500 ml water and steam cooked for 30 min at boiling temperature. To avoid retrogradation

the cooked potatoes were immediately used for enzymatic digestion *in vitro* or *in vivo*.

2.3 Analytical methods for quantification and qualification of carbohydrates

General reagents were of analytical grade and were purchased from Fisher Scientific unless otherwise stated.

2.1.1 3, 5 Dinitrosalicylic acid assay (DNS)

2.1.1.1 DNS solution

DNS reagent was prepared by dissolving 5g of 3,5 dinitrosalicyclic acid (Sigma Aldrich) in 250 mL of distilled water preheated to 80°C. The solution was cooled to room temperature and 100 mL of 2 N NaOH was added followed by 150g of potassium sodium tartrate-4-hydrate (Sigma Aldrich). The solution was mixed well using magnetic stirrer then volume was completed with de-ionized water to 500 mL.

2.1.1.2 Protocol

Total reducing sugar was measured according to the method by Miller (Miller, 1959, Fei *et al.*, 2014) with some modification. A glucose or maltose standard (Sigma Aldrich) stock solution was prepared at 200 mg mL^{-1} and then serial dilutions were prepared (0.1, 0.4, 0.8, 1.2, 1.6, 2 and 4 mg mL^{-1}). Two mL of either sample or standard was placed in a tube and one mL of DNS reagent was added, tubes were shaken using a vortex and placed in a boiling water bath (Grant SBB 14) for 15 min to allow the reducing sugars to react with DNS. Tubes were allowed to cool down to room temperature and 9 mL of Millipore water was added. Absorbance was read at 540 nm using Cecil Aquarius CE

7200 double beam spectrophotometer. A blank sample was prepared by substituting the sample or standard with distilled water. A standard curve was used for calculating the amount of either maltose or glucose released during digestion.

ΔA_{540nm} Standard = A_{540nm} Standards - A_{540nm} Blank

ΔA_{540nm} Sample = A_{540nm} sample - A_{540nm} Blank

2.1.2 High performance anion exchange chromatography with pulsed amperometric detector (HPAEC-PAD).

Standard stock solutions of mixed sugars; (glucose, fructose, sucrose, maltose and maltotriose at 1 mg mL^{-1} Sigma Aldrich) were used to prepare serial dilutions containing 0, 2, 4, 6, 8 and 10 ug mL^{-1} of each sugar. For carbohydrate analysis of potato samples, one mL aliquot of sample was diluted in Millipore water (1:100 for digestible carbohydrate in cooked potato and 1:10 for soluble sugar). Fucose (5 ug mL^{-1}) was added to samples and standards as external standard. Solutions were filtered through Polytetrafluorethylene (PTFE) 0.2 µm membrane filters (Chromacol Ltd, 100X17-SF-02(T),UK,) before injection. The HPAEC-PAD instrument (Dionex DX 500) was equipped with a GP40 gradient pump, PAD system composed of ED 40 electrochemical detectors including gold working and silver reference electrodes, and LC20 chromatography enclosure column oven. The analytical column used was Carbopac P20 (Dionex, 3x150 mm) with guard column (3x30mm) with anion exchange capacities of 65 µeq/column. The mobile phase was 200 mM NaOH and flow rate was 0.4 mL/min. Injections (10µL) were made by using an AS 500 auto sampler, and the maximum operation pressure was 3500 psi.

Before injection of each sample, the column was washed with 60mM NaOH *(*Fisher Scientific*)* for 8 min. After injection, the concentration was increased from 60mM to 200mM NaOH in 17 min then eluted with 200mM for the next 6 min. Therefore the run time was 30 min for separation of sugars.

2.4 Determination of potato composition

2.4.1 Total starch (TS)

TS was determined enzymatically according to the method of Goni *et al.* (1997) with some modifications. Raw potato (50 mg) was homogenized in 6 mL of 2M KOH and then agitated using a shaking vortex at room temperature for 30 min. The agitation step was very important to ensure complete solubility of the starch. 3 mL of 0.4 M sodium acetate buffer (pH=4.75) was added to the suspension and pH was adjusted to 4.75 using 3 M acetic acid. 60 µl of amyloglucosidase (AMG) from *Aspergillus niger* (70 U/mg, Sigma Aldrich) was added to the solubilised starch and hydrolysed for 45 min at 60 ˚C in a shaking water bath. The digestion mixture was centrifuged for 5 min and pH was neutralised with 0.2 M NaOH. Glucose in the supernatant was measured using the DNS method. Glucose amount was converted to starch content by multiplying by a factor 0.9.

2.4.2 Free sugars

Free sugar content was determined in order to correct the TS value in potato samples. Potato samples, 200 mg of raw or cooked tuber, were homogenized in 6 mL 0.4 M sodium acetate buffer (pH=4.75) and then centrifuged for 10 min. Soluble sugars in supernatants were determined using the DNS method and HPAEC-PAD.

2.4.3 Moisture content

Potato samples fresh and cooked (in triplicate) were weighed and frozen at -80˚C then freeze dried for 48 hours using a Free zone freeze dryer system (LABCONCO Corporation). The moisture content was calculated as percentage of weight loss of the samples.

2.4.4 Phenolic composition

Phenolic acids were purchased from Sigma Aldrich. 5-caffeoylquinic acid (5-CQA) that is the dominant phenolic acid in potato was selected for this study. Sinapic acid was used as external standard. The stock solution of phenolic acids were prepared at concentration 1 mg mL^{-1} in acetonitrile HPLC grade purchased from VWR and dilution was made using Milli-Q purified water. An external calibration curve was used for both Folin-Ciocalteau method at a range of 5-CQA standard of 0, 10, 50, 100, 150, 200, 250 and 300 µg mL^{-1} and 5, 10, 30, 50, 100, 150 and 200 µg mL^{-1} for LC-MS. A concentration of 100 µg mL^{-1} was used for the external standard sinapic acid. Standards used for LC-MS analysis were filtered using 0.2 µm PTFE filter prior to analysis.

2.4.4.1 Extraction of phenolic compounds

The method of extraction was adapted from Shakya and Navarre (2006). Phenolic compounds were extracted in four replicates taken from freeze dried raw and cooked potatoes. Freeze-dried powder (200 mg) was mixed with 1.5 mL of extraction buffer (50% MeOH, 2.5% metaphosphoric acid, 1 mM EDTA (Sigma Aldrich), chilled to 4ºC) and 500 mg of glass beads (1.0 mm in diameter; Fisher Scientific). Tubes were shaken with a vortex for 10 min at room temperature and then sonicated at 10 °C for 10 min. After sonication,

tubes were shaken again with a vortex for 10 min. Tubes were centrifuged at 4000 rpm at 4^{o}C for 10 minutes and the supernatant was transferred to a clean tube. Extractions were repeated three times and supernatants combined. Samples were kept chilled at all times and not exposed to light.

2.4.4.2 Total phenolic content (TPC) assay

TPC of potato was measured by Folin-Ciocalteau method (Singleton and Rossi, 1965). Potato extract (50µL) was mixed with Folin-Ciocalteau reagent (50 µL, 1 N) prepared by diluting 2 N Folin-Ciocalteau reagent purchased from Sigma Aldrich, sodium carbonate (150 µL, 20% w/v) and distilled water (750 µL). After vortexing, the mixture was incubated in the dark at room temperature for 45 min. Absorbance was measured at 765 nm using a spectrophotometer (Cecil, CE 7200 Double Beam UV/VIS Spectrophotometer). Different concentrations of 5-CQA (10- 300 µg mL^{-1}) were used to generate a standard curve. The phenolic content was expressed as mg chlorogenic acid per 100 g freeze dried potato using the linear equation based on the calibration curve.

2.4.4.3 Quantification of phenolic acids using high performance liquid chromatography with diode array detector – mass spectrometry (HPLC-DAD-MS).

The HPLC-DAD-MS system consisted of a micro vacuum degasser (Prominence Degasser LC-20 A5, Shimadzu), a liquid chromatograph (Prominence Liquid Chromatograph LC-30 AD, Shimadzu), an auto sampler (Prominence Auto Sampler SIL-30 AC, Shimadzu), a diode array detector (Prominence Diode Array Detector system SPD-M20A, Shimadzu) detects the separated compounds by molecular weight, hydrophobicity (reverse-phase)

or ionic charge and records the ultraviolet and visible (UV-vis) absorption spectra of samples that are passing through a high-pressure liquid chromatograph, a column oven (Prominence Column Oven CTO-20 AC, Shimadzu), a controller (Prominence Controller CBM-20 A, Shimadzu), and an MS detector with electrospray ion source and quadrupole analyser (Liquid Chromatograph Mass Spectrometer LC-MS-2020, Shimadzu). The greatest advantage in using an MS as the LC detector is that it easily provides mass information on each peak at the same time as the respective retention times. The Labs solutions (Shimadzu) software was used to control the LC–MS system and for data processing. The column used for chromatographic separation was an Agilent Zorbax Eclipse plus C18 column 4.6 mm × 150 mm, 5 µm internal diameter. A gradient elution program of solvent A (0.1 % formic acid, 5% acetonitrile and 94% water) and solvent B (0.1% formic acid, 5% water and 94% acetonitrile) was set as follows: 61-min; linear gradient from 0-51 min from 0% to 100% solvent B, isocratic elution from 51.1-56 min with 100% solvent B, linear gradient from 56-56.1 min to 0% solvent B and isocratic elution from 56.1-61 min with 0% solvent B. The column temperature was 35˚C and flow rate of 0.5 mL min^{-1} and injection volume 10 µL. The diode array detection spectra was recorded at wavelengths of 280, 290, 315, 320 and 330 nm.

2.4.5 Statistical analysis

Differences between potato composition and the effect of cooking on total phenolic content were evaluated using T-test and one way ANOVA using IBM SPSS statistic 2007 for Windows.

2.5 Determination physio-chemical properties of starch from different potato varieties

2.5.1 Extraction of starch

2.5.1.1 Extraction buffer 50 mM Tris pH 7.5/ 10 mM EDTA/0.5 g L^{-1} Na-metabisulfate

One litre of 10X buffer solution was prepared by weighing: 60.57 g of TRIS (500 mM), 29.22 g of EDTA (100 mM) and 5 g Na metabisulfate. Compounds were mixed in 800 mL of MilliQ water and dissolve by using a stirring plate and a stirring magnet. pH was adjusted to 7.5 with 2 M HCl. The mixture was transferred to a 1000 mL volumetric flask and the volume adjusted to 1000 mL with MilliQ water. To obtain a 50 mM TRIS (pH = 7.5) / 10 mM EDTA/ 0.5 g/ litre Na metabisulfate buffer, a 1:10 dilution was made of the 10X buffer with MilliQ water. The buffer, was stored overnight at 4 °C until use.

2.5.1.2 Protocol

Starch in fresh tubers was extracted as described by Bustos *et al*. (2004). Potatoes were washed, peeled and homogenized in cold extraction buffer (5 g of potatoes in 250 mL buffer). Buffer consisted of 50 mM Tris, 10 mM EDTA, 0.5 g L^{-1} sodium metabisulfate, pH 7.5, pre-cooled to 4 °C. The homogenised sample was filtered through four layers of cheesecloth. The filtrate was centrifuged at 20,000 x g and 4 ˚C for 15 min. The pellet was washed with extraction buffer by suspension three times and supernatant was discarded. The purified starch were then rinsed twice with acetone and centrifuged as above. Then starch was spread on glass plate and dried in air.

2.5.2 Composition of starch

2.5.2.1 Amylose content

The amylose content of extracted potato starch was determined according to the colorimetric method of Morrison and Laignelet (1983). A range of standards containing amylose from 0 to 100% was prepared from combination of potato amylose and corn amylopectin (both from Sigma Aldrich). Starch was defatted by dispersing 2 mg of starch in 1 mL of 85% methanol and heating at 60 °C for 1 hour with occasionally vortex. Solution was centrifuged at 13 000 x g for 5 min, the supernatant was removed and extraction of lipid was repeated. After drying at 60 °C for 1 hour, starch was dissolved in 1 mL of urea dimethyl sulphoxide (UDMSO) solution (0.6 M urea in 90% dimethyl sulphoxide) by using immediate vigorous vortex and then incubated at 95 °C in a water bath for 1 hour. The mixture was intermediately vortexed for complete dissolution of the starch. 50 µl of aliquot of the starch and UDMSO solution was treated with 20 µl of I_2-KI solution (1:10 w/w in water) and made up to 1 mL with water. Absorbance was read at 620 nm.

Percentage of amylose from potato samples starch was calculated (alpha glucan basis) using a regression equation derived from the standard samples.

2.5.3 Physical analysis of starch

2.5.3.1 Particle size distribution of potato starches granules

Granule size dimensions and distributions of potato starches was determined by using laser diffraction particle size analyser Malvern Mastersizer 3000 (Malvern Instruments Limited, UK). The instrument uses laser light scattering and is capable of measuring particle size between 0.01 to 3500 µm. The

Mastersizer is comprised of the main optical unit that measures the sample using red laser and blue light detection, one or more dispersion units and a measurement cell. Commonly, a dispersant such as de-ionised water is also connected directly to the dispersion unit. The Mastersizer application software was used to control the optical unit and dispersion unit hardware, and also processes the raw data gathered by the system, providing flexible data analysis and reporting features. The refractive index of 1.31 for water and 1.52 for starch were used as a standard. The particle size distribution of potato starches granule was expressed as volume of equivalent spheres. The criterions $Dv_{0.1}$, $Dv_{0.5}$ and $Dv_{0.9}$ were the particle size at which 10%, 50% and 90% of all particles by volume were smaller, respectively. The $D_{4,3}$ was the volume mean diameter and the $D_{3,2}$ was the surface mean diameter and D_{50} was the median volume diameter. The specific surface area of potato starches was also performed.

2.5.3.2 Small-angle X-ray scattering (SAXS) characterisation

The crystallographic properties of potato starch samples were examined by using SAXS.

2.5.3.2.1 Preparation of potato starch slurry

Potato starch and water slurries with 45% w/v concentration were prepared for all samples. According to Cameron *and Donal* (1992), this concentration of starch is enough to prevent the sedimentation within the capillary cell. Each starch slurry was mixed using a vibrating stirrer before the capillary cell was filled as to ensure the slurry was homogenous. All samples were loaded into the same vacuum-tight, reusable 1 mm quartz capillary (Anton Paar, Graz, Austria) to attain exactly the same scattering volume using a 1mL syringe.

2.5.3.2.2 SAXS

The X-ray scattering experiment was performed using Anton Paar's modular nanostructure analyzer SAXSpace system (Anton Paar, Graz, Austria). The X-ray radiation Cu-Kα (wavelength λ=0.154 nm) used was generated form a sealed-tube Cu anode X-ray generating equipment ISO-DEBYEFLEX3003 (GE Inspection Technologies GmbH) which was operated at 40 kV voltage and a tube current 50 mA. The X-ray tube is chilled by a closed water circuit (Chilly 35, HYFRA, Germany). To achieve a high resolution mode with a minimum accessible scattering vector, q min, of 0.04 nm^{-1} ($q = (4\pi/\lambda) \sin\theta$, where 2θ is the scattering angle) the SAXSpace collimation block converts the divergent polychromatic X-ray beam into a focused line shaped beam. The experiment was started by placing the sample packed capillary into sample holder equipped with Peltier elements (TCStage 150, Anton Paar, Graz, Austria), which is connected to a water cooling thermostat (Julabo GmbH, Seelbach, Germany) to get rid of the excess of heat and controlling the experiment condition at 25 °C with a temperature stability of 0.1°C. To avoid the undesirable background produced by air scattering the sample chamber was evacuated by vacuum pump (Vacuubrand GmbH, Wertheim, Germany) achieving a minimum pressure of ~1 mbar. After passing the sample the 1D scattering patterns and primary beam were recorded by a single photon counting detector which was Mythen micro-strip X-ray detector (Dectris Ltd, Baden, Switzerland) has a sensitive area of 64×8 mm^2 comprising of 1280 channels each with a channel size of 0.05 × 8 mm (v × h). The primary beam was attenuated by semitransparent beam stop for the exact determination of the zero scattering vector and transmission correction. The distance between

detector and sample was 317.09 mm. Each sample was exposed three times for 1800 s, and their integrated scattering profiles were averaged.

The primary data analysis correction regarding primary beam position was carried out using the SAXStreat software (Anton Paar, Graz, Austria). The SAXS data was further transmission-corrected by setting the attenuated scattering intensity at q = 0 to unity and background subtracted using the SAXSQuant software (Anton Paar, Graz, Austria). SAXS curve was plotted from the relative intensity I, versus scattering vector q. The parameters of SAXS peaks (peak position ($q_{max}=4\pi Sin\theta/\lambda$) and intensity I_{max}) were determined by the graphical method as described by Yuryev *et al*. (2004). The average interlamellar distance, Bragg spacing was calculated according to the Bragg equation ($d=2\pi/q_{max}$).The peak area was determined manually.

2.5.4 Statistical analysis

Statistical analysis was conducted to determine the difference between potato starches amylose content as well as granule size distribution and crystallinity using T-test and one way ANOVA using IBM SPSS statistic 2007 for window.

2.6 *In vitro* digestion of commercial potato starch

2.6.1 Reagents

2.6.1.1 Sodium phosphate buffer solution

A stock solution of 100 mM sodium phosphate buffer was prepared by mixing 550 mL of 100 mM Na_2HPO_4 (di-basic) and 450 mL of 100 mM NaH_2PO_4 (mono-basic) then stock solution diluted to obtain 20 mM of sodium phosphate buffer containing 6.7 mM sodium chloride adjusting pH to 6.9 with NaoH. All chemical used were purchased from Sigma Aldrich.

2.6.1.2 Standard starch solution

Soluble potato starch 1% (W/V) prepared by addition 1g of native potato starch (Sigma Aldrich) to 100 mL 20 mM sodium phosphate buffer pH 6.9 containing 6.7 mM sodium chloride. Starch suspension was gelatinised by heating in a glass beaker directly on a heating stir plate with constant stirring until boiling and this temperature was maintained for 15 minutes. Then starch solution was cooled to room temperature with stirring. Solution was adjusted to original volume of 100 mL by adding de-ionized water.

2.6.1.3 Porcine pancreatic alpha amylase solution

The enzyme stock solution was prepared by dissolving 100 mg of porcine pancreatic alpha amylase (PPAA) (16 U/mg, Sigma Aldrich) in 16 mL 20 mM phosphate buffer pH 6.9 containing 6.7 mM sodium chloride at 20 °C then, stock solution was diluted by 1:100 using 20 mM sodium phosphate buffer pH 6.9 to provide 1 unit enzyme per mL of solution.

2.6.1.4 Chlorogenic acid 5-CQA solution

5-CQA (Sigma Aldrich; PubChem CID 12310830) was prepared at different concentrations (3, 6, 9, 12 mg mL^{-1}). First 5-CQA was dissolved in 5 mL absolute ethanol and volume was adjusted to 100 mL by adding 20 mM sodium phosphate buffer pH 6.9. To avoid destruction of 5-CQA, solution was stored in an amber bottle and low temperature.

2.6.2 Protocols

2.6.2.1 Alpha amylase activity assay

Enzymatic assay was adopted from Sigma Aldrich (itself adopted from Bernfield (1951)) with some modification. A range of pancreatic alpha amylase (16 unit/mg, Sigma Aldrich) concentrations from 1 to 10 unit mL^{-1} was prepared then, 1 ml of enzyme solution was added to 1 mL of 1% (W/V) potato starch solution. Separate screw-top plastic tubes were used for each enzyme concentrations. Final volume of reaction mixture was 6 mL. The tubes were incubated in water bath (Grant GLS Aqua 12 plus) for 3 min at 37 °C. Two mL of the incubation mixture was removed and reducing sugar produced measured using DNS method described previously. For zero time (min) reaction a separate set of tubes were also prepared and DNS solution was added to reaction mixture directly after addition of enzyme at zero time. Control was conducted which contain DNS colour reagent solution and enzyme but not starch. According to the unit definition, one unit of enzyme will liberate 1 mg maltose from starch at 3 min and 37ºC.

2.6.2.2 Optimisation of reaction time

The enzymatic reaction time optimisation was performed using a fixed amount of enzyme (2 unit mL^{-1}) and 1 mL of 1% gelatinised potato starch as substrate then, incubated at different period of time from 0 to 60 min. A separate set of tubes were conducted for each period of hydrolysis. The rest of experiment was carried out as mentioned in alpha amylase amylase assay (2.6.2.1) and released sugars were measured by the DNS method (2.1.1).

2.6.2.3 Inhibitory effect of chlorogenic acid on digestion of commercial potato starch by porcine pancreatic amylase *in vitro*

The activity of PPAA (16 unit/mg, Sigma Aldrich) enzyme on potato starch (Sigma Aldrich) in the presence and absence of 5-CQA (Sigma Aldrich; PubChem CID 12310830) were examined by the method of Brenfeld (1955). Reactions were carried out with PPAA at a concentration of 0.33 unit mL^{-1} using potato starch (1%) in 20 mM sodium phosphate buffer pH 6.9 containing 6.7 mM NaCl at 37^{o}C for up to 20 minutes. 5-CQA (final concentration 1.5 mg mL^{-1}) was either added to the enzyme-substrate mixture at the start of the reaction or was pre-incubated for 10 minutes with the enzyme prior to addition of the substrate. All reactions were carried out in four replicates. Reducing sugar released was measured at two reaction times (5 and 20 min) using the DNS colorimetric assay (Miller, 1959, Fei *et al.*, 2014). An enzymatic kit was not used due the inhibition of enzymes in the kit by chlorogenic acid. Brayer has shown that maltose is the preferred leaving group for PPAA (Brayer et al., 2000), and therefore maltose was used for generating standard curve to quantify the reducing sugar released. The enzymatic activity of PPAA was also determined in the presence of various concentrations of 5-CQA (0.08 - 2

mg mL^{-1}). IC_{50} was calculated as the concentration of 5-CQA required to inhibit 50% of enzyme activity.

$$\%\ Inhibition = \frac{\mathrm{A} - \mathrm{B}}{\mathrm{A}} \times 100$$

Where A was amount of maltose produced in absence of 5-CQA and B was that produced in presence of 5-CQA.

2.6.2.4 Enzymatic kinetics and mode of inhibition

Michaelis-Menten kinetic parameters and mode of inhibition of PPAA by 5-CQA was determined from a Lineweaver-Burk plot. One mL of 5-CQA at concentrations ranging from 0 to 2 mg mL^{-1} was added to a mixture containing 1 mL of starch solution at concentrations from 0 to 6.6 mg mL^{1} in the same buffer solution as described in the previous section. The reaction was initiated by addition of a fixed concentration of PPAA (0.33 unit mL^{-1}). The solution mixture was incubated for 5 min at 37 °C. The reducing sugar produced was determined using the DNS colorimetric method as described previously (2.1.1).

2.6.3 Statistical analysis

An independent sample t-test was used to compare amylase activity in presence or absence of chlorogenic acid using IBM SPSS statistic 2007 for Windows.

2.7 *In vitro* starch digestion of steam cooked potatoes

2.7.1 *In vitro* digestion using PPAA

In vitro digestion was performed for different varieties of steam cooked potatoes (Desiree, Mozart, Rooster, Maris Piper and Maris Peer) using PPAA activity assays with some modifications.

2.7.1.1 Modification of substrate concentration and incubation time

To determine effect of digestion time and substrate concentration, different substrate concentrations of Maris Piper potato were used. Steam cooked potato 6 gm was homogenized in 100 mL of 20 mM sodium phosphate buffer pH 6.9 containing 6.7 mM sodium chloride. One mL was taken out (representing 10 mg mL^{-1} starch) and added to test tube containing 4 mL buffer then digestion started by adding 1 mL of 0.33 unit mL^{-1} enzyme solution as final concentration. The mixture was incubated at different time first from 0 to 90 min and then 0 to 150 min. The amount of reducing sugar released measured as maltose using the DNS method (2.1.1). For each concentrations the experiment were obtained in triplicate.

2.7.1.2 Modification of enzyme dosage

The above experiment was conducted at using 6 g of potato diluted 100 times as substrate and enzyme concentration was increased to 0.66 mg mL^{1} as final concentration. The rest of experiment was done as described above. Zero time measurement and non-enzyme controls were conducted by replacing enzyme solution with buffer solution. Each experiment was measured in triplicate.

2.7.1.3 Applying all modification

After condition optimisation, the rate of digestion of all five steam cooked potato varieties was measured. Substrate concentration was 6 g potato in 100 mL of buffer and enzyme concentration was 0.66 unit mL^{-1}.Released reducing sugars were measured at 0, 3, 5, 10, 20, 40, 60, 90, 120, and 150 minutes by DNS method (2.1.1). 1 mL of reaction mixture was taken at each time point and boiled for 5 min to inactivated enzyme then used for further analysis of sugar profile using HPAEC-PAD.

2.7.2 *In vitro* digestion of potatoes using gastric phase and intestinal phase

2.7.2.1 Reagents

2.7.2.1.1 HCl-KCl buffer (0.2 M each) pH 1.5

Stock solutions (0.2 M) were prepared separately for KCl and HCl. Buffer solution was prepared by mixing 50 mL KCl solution with HCl until pH was adjusted to 1.5. The mixture was diluted to a total 200 mL by deionized water.

2.7.2.1.2 Pepsin solution

Pepsin solution was prepared by dissolving 1 g pepsin from porcine gastric mucosa (≥250 U mg^{-1} solid Sigma Aldrich) in 10 mL KCl-HCl buffer pH 1.5.

2.7.2.1.3 Tris-Maleate buffer pH 6.9

Tris acid maleate stock solution (0.2 M) was prepared by dissolving 24.2 g of Tris-(hydroxymethyl) aminomethane and 32.2 g of maleic acid or 19.6 g of maleic anhydride in 1 L of deionized water. Then 0.2 M NaOH stock solution was prepared. Buffer solution was prepared by mixing 50 mL of Tris-Maleate

0.2 M with 0.2 M NaOH until pH adjust to 6.9 then mixture was diluted to a total 200 mL by deionized water.

2.7.2.1.4 PPAA 2.6 unit/ 5 mL

PPAA 2.6 unit in 5 mL Tris-Maleate buffer pH 6.9 was prepared from PPAA (16 unit/mg, Sigma Aldrich).

2.7.2.1.5 Sodium acetate buffer 0.4 M pH 4.75

Equal volumes of 0.4 mol L^{-1} sodium acetate and 0.4 mol L^{-1} acetic acid was mixed. The pH was adjusted to 4.75 adding either 0.4 mol L^{-1} sodium acetate or 0.4 mol L^{-1} acetic acid.

2.7.2.1.6 Amyloglucosidase solution 60 *µ*l, 184 U

Amyloglucosidase (AMG) from *Aspergillus niger* (70 unit/mg, Sigma Aldrich) was prepared by dissolving enzyme powder 3080 unit in 1 mL sodium acetate buffer pH 4.75.

2.7.2.2 Protocol

The potato starch was digested according to the method of Goni *et* al, (1997) with some modifications. Potato samples were prepared as mentioned earlier and 250 mg of potato sample was homogenized in 10 mL of HCl-KCl buffer pH 1.5. Pepsin solution (0.2 mL) was added and sample incubated in 40 ˚C shaking water bath for 1 hour. Volume was adjusted to 25 mL with Tris-Maleate buffer pH 6.9. PPAA 2.6 unit in 5 mL of Tris-Maleate buffer was added and pH was adjusted to 6.9 by NaOH 0.2 M. Tubes were incubated in shaking water bath at 37 ˚C. One mL of solutions were taken from each tubes every 15 and 30 min interval from 0 to 3 hours. Enzyme was inactivated by placing aliquots in boiling water bath at 100 ˚C for 5 min and then centrifuged for 10

min and refrigerated until the end of the digestion time. Three mL of sodium acetate buffer pH 4.75 were added to each aliquot and pH was adjusted to 4.75 by HCl 0.2 M and 60 µl of AMG 184 unit were added to hydrolyse the digested starch into glucose for 45 min at 60 ˚C in shaking water bath. Two mL of aliquots were taken for analyse released glucose by DNS. Glucose was converted to starch by multiplying for 0.9.

2.7.3 Statistical analysis

One way-ANOVA was used to compare the starch digestibility between potato varieties. Pearson correlation were applied to analyse the correlation between *in vitro* digestion of potatoes and each of the total phenolic content and starch composition and properties using IBM SPSS statistic 2007 for window.

Principle component analysis (PCA) was conducted to analyse the relationship between starch hydrolysed and TS, DM, TPC and 5-CQA content of potatoes. PCA was performed using MATLAB software R2015a (MathWorks, Inc.).

2.8 *In vivo* determination of glycaemic index (GI)

A human study was performed to determine the blood glucose response in human volunteers to ingestion of steam cooked potato varieties with different skin colour and different levels of phenolic content.

2.8.1 Requirements

2.8.1.1 Ethical approval

The study was reviewed and approved by the Ethics Committee of the Faculty of Mathematics and Physical Sciences, University of Leeds, United Kingdom. Ethical reference number [MEEC 15-006] (Appendix A).

2.8.1.2 Subjects

Eleven healthy volunteers participated in this study (9 female and 2 male) with mean age of 20 to 36 years of age (28.88±4.72) after advertisements were put on the School of Food Science and University and different places of University of Leeds (Appendix E). According to FAO/WHO 1998 to determine the GI tests should be carried in six or more subjects.

Selection of subjects were made based the inclusion criteria; no known allergic or intolerance to any food, not taking any medication that affect the blood glucose, not diagnosed with chronic disease such as diabetes and not pregnant or lactating.

On initial presentation, volunteers who showed an interested were given a participation information form (Appendix D). Written informed consent was obtained from each participant. Volunteers had the opportunity to ask about the detail of test protocol and information on the risks involved in participation and they have two weeks to decide whether to take part. A health

questionnaire form was completed by interested volunteer to further assess their eligibility (Appendix B).

2.8.1.3 Testing facility

The food preparation area should separate from that in which blood was taken. The preparation of potato samples was carried out in Food Technology Laboratory, School of Food Science and Nutrition, University of Leeds. The blood tests were carried out in the human study room.

2.8.2 Study protocol

2.8.2.1 Reference and test food

In this study four types of foods were selected (1 reference and 3 test foods). White bread (3 slices containing about 50 g available carbohydrate) was used as reference food as recommended by ISO (2010) and WHO/FAO (1998). Tested foods were three types of potato Desiree, Maris piper and Rooster. The tested potatoes were prepared prior to study. They were washed, peeled, cut into cubes 1 cm^3 in size, weighted into plastic zipped bags in a portion containing 50 grams of available carbohydrate and then frozen by using fast freezer and stored in freezer until use. On the day of study frozen potatoes were steam cooked using electrical steam cooker (Russel Hobbs) and served hot. White bread (Morrisons medium white loaf 3 slices) was used as reference food and served fresh without toasting.

2.8.2.2 Experimental procedure

Subjects arrived fasted at the School of Food Science and Nutrition. They were asked to fast overnight (10-14 h) before the study and to avoid excessive physical exercises and alcohol consumption in the evening before the

experimental day. On the morning of experimental day, upon arriving subjects were made comfortable. Weight and height measurements were obtained from the subjects also they were asked to complete a pre-study health questionnaire form.

After ensuring the subjects were comfortable and warm, they were prepared for taking blood samples in fasting state (0 time). Their middle or ring finger was wiped by alcohol swab (Universal ALCOTIP PRE-INJECTION SWABS) and alcohol allowed to dry. The side of the chosen finger was pricked by a disposable lancet (Accu-Chek Safe-T-Pro Plus lancet, Roche diagnostics GmbH). For blood glucose measurement, the first drop of blood was wiped and then blood droplet was placed into test strip (Accu-Chek Aviva test strip, Roche) which was previously inserted into a glucometer (Accu-Check). Blood glucose concentration was measured in mmol L^{-1}.

To avoid retrogradation of starch, tested foods (potato or bread) were prepared in the morning of experimental day as described previously and subjects consumed randomly one of four foods (test or reference) within 15 minutes with 250 ml of water. Further blood glucose measurement were obtained at 15, 30, 45, 60, 90 and 120 minutes after consuming foods. Subjects were asked to repeat the procedure above another 3 times, one week between, to reproduce the analysis with each foods (3 potato samples, 1 white bread).

2.8.2.3 Calculation of GI

The incremental are under the blood glucose curve (iAUC) ignoring the area beneath baseline, was measured geometrically by applying trapezoid and triangle rule according to FAO/WHO (1998). The GI for each tested food was

calculated as percentage of the mean of iAUC of the tested food over the mean iAUC for the reference food taken by same subject.

2.8.2.4 Statistical analysis

Pearson correlation was used to investigate the relationship between the potato phenolic component, potato starch physio-chemical properties and GI of potato samples using IBM SPSS statistics 2007 for Windows.

Principle component analysis (PCA) was conducted to analyse the relationship between GI and TS, DM, TPC , 5-CQA content of potatoes, amylose content, granules size distributions and crystallinity. PCA was performed using MATLAB software R2015a (MathWorks, Inc.).

3 Inhibitory effect of chlorogenic acid on digestion of commercial potato starch by porcine pancreatic amylase *in vitro*

3.1 Introduction

Evidence from a number of *in vitro* and *in vivo* studies indicates inhibitory effects of plant polyphenols on enzymes involved in carbohydrate digestion (Meng *et al*., 2013, Trinh *et al*., 2016). 5-CQA has been shown to inhibit PPAA by mixed type mechanisms (both competitive and non-competitive) as the inhibitor might bind to the surface of the enzyme, in addition to the substrate binding site (Narita and Inouye 2011).

However, the potential effect and mode of inhibition on digestion of native potato starch has not been shown.

This chapter describes the optimisation of assay conditions for PPAA activity using commercial potato starch as the substrate and the determination of enzymatic kinetic parameters in the presence and absence of 5-CQA.

3.2 Aims

The aim of the present study was to characterise the effect of 5-CQA on PPAA activity using commercial potato starch as substrate *in vitro*.

3.3 Hypothesis

The hypothesis is that 5-CQA will reduce the activity of PPAA catalysed hydrolysis of potato starch competitively and non-competitively.

3.4 Objectives

The objectives of this study are:

- To optimise a protocol for digestion of gelatinised potato starch by PPAA *in vitro* by testing different conditions such as enzyme dosage and incubation period.
- To hydrolyse potato starch in presence of 5-CQA at various concentrations.
- To calculate enzymatic kinetic parameters of potato starch digestion by PPAA in the presence and absence of 5-CQA
- To determine the inhibition mechanism by analysis of enzyme kinetic parameters derived from Linweaver-Burk plots.

3.5 Results

3.5.1 Optimisation of enzyme concentration

The method used for potato starch digestion by PPAA was adapted from Bernfield (1951) with some modification. DNS method was used for measuring reducing sugar released after *in vitro* digestion. A range of PPAA enzyme concentrations from 0 to 2.5 unit mL^{-1} were used in order to hydrolyse 10 mg of the gelatinised potato starch over 3 min. Figure 3.1 shows the percentage of total starch hydrolysed against enzyme unit concentration after 3 min of digestion. At 0 concentration of enzyme, it was found that potato starch contained about 1.5% of free sugar which was subtracted as a blank.

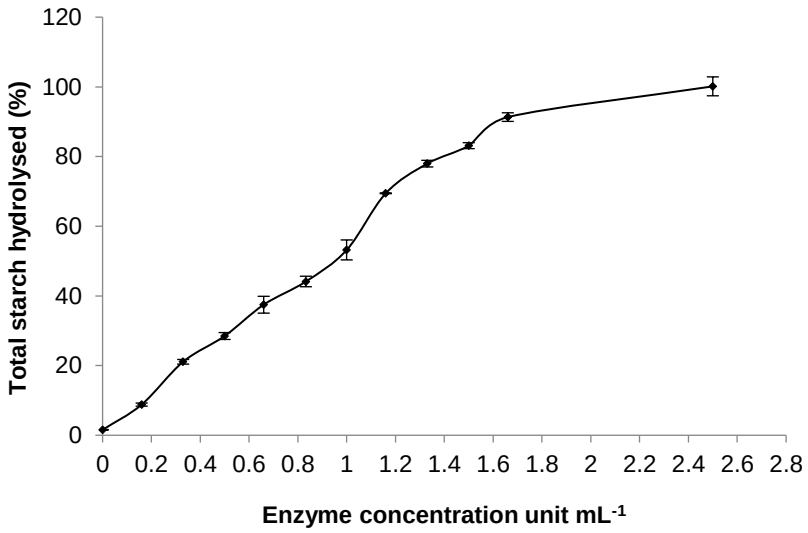


Figure 3.1 Effect of PPAA concentration on digestion of potato starch at 3 min incubation. Data expressed as mean of % of total starch hydrolysed (n=6). Error bars represent standard error of mean.

It was found that by adding enzyme, the amount of reducing sugar released increased until reach it is optimum by 1.66 unit of enzyme. 91.3% of total starch was hydrolysed in 3 min with 1.66 units mL^{-1}. 100% of starch was hydrolysed by 2.5 units mL^{-1} of enzyme.

3.5.2 Optimisation of substrate concentration and incubation time

Figure 3.2 shows hydrolyses curve for 1.66 mg mL^{-1} (equivalent to 10 mg starch in volume of 6 mL) gelatinised potato starch by 0.33 unit mL^{-1} PPAA over 50 minutes. At initial time of incubation (3, 5, 10 min) the enzymatic digestion of potato starch was fast digesting 21, 37 and 60% of total starch respectively. By extending incubation time to 15, 20 minutes, hydrolysis were increased to 70 and 72% of total starch respectively. It was seen that after 20 min of incubation the percentage of reducing sugars producing was not as fast as at beginning of the reaction reaching 78% at 30 and 80% at 50 minute of reaction.

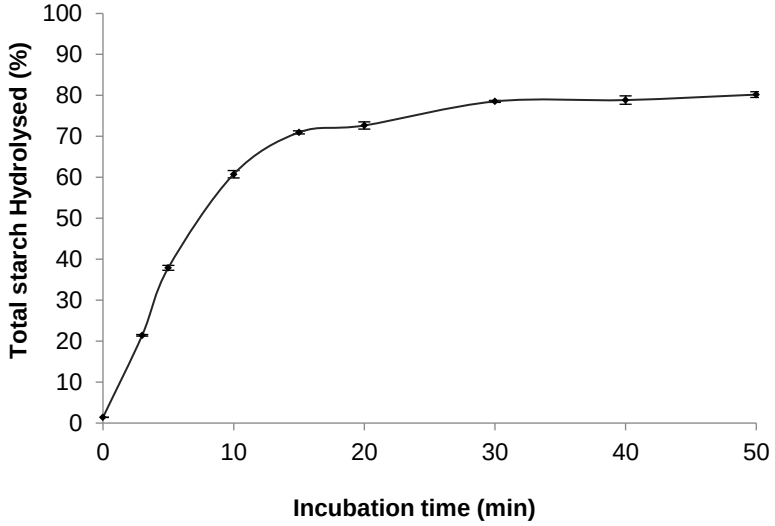


Figure 3.2 Hydrolysis curve for 10 mg gelatinised potato starch by 0.33 units mL^{-1} PPAA over 50 min. Data expressed as average % of hydrolysis of total potato starch (n=6). Error bars represent standard error of mean.

Figure 3.3 shows the enzyme activity as a function of substrate amount using a constant enzyme concentration (0.33 units mL^{-1}) at 5 min of incubation. It was observed that small amount of starch hydrolysed by enzyme using low amount of substrate 0.83 and 1.25 mg mL^{-1} was 38 and 44% respectively at

5 minute and increasing of substrate amount to 1.66 mg mL^{-1} was resulted in increasing amount of starch hydrolysed by PPAA 52% While, this increase was not continuous with increasing amount of substrate. At substrate amounts above 1.66 mg mL^{-1}, less hydrolysis of potato starch was occurring as amount of reducing sugars released becomes constant as enzyme reaches its maximum access to the substrate (figure 3.3).

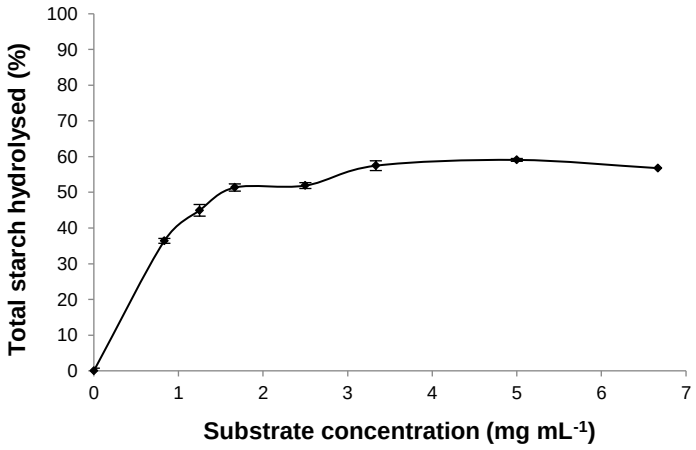


Figure 3.3 Effect of different substrate concentration ranges from 0.83-6.6 mg mL^{-1} (5-40 mg final amount) on activity of 0.33 unit pancreatic alpha amylase at 5 min. Data expressed as average % of hydrolysis of total potato starch (n=9). Error bars represent standard error of mean.

3.5.3 *In vitro* hydrolysis of potato starch in presence of chlorogenic acid

The reaction conditions chosen to analyse the inhibition were an enzyme concentration of 0.33 units mL^{-1} of PPAA, digestion time of 5 or 20 minutes, using a starch concentration of >1 mg mL^{-1}. These conditions were chosen because they are in the dynamic range for the reaction.

The inhibitory effect of the 5-CQA on PPAA activity using potato starch as substrate was evaluated at two incubation time periods of 5 and 20 min (Figure 3.4 A). A significant difference ($p \leq 0.05$) was observed in the amount of

reducing sugar released in the presence and absence of 5-CQA. Results were showed that less sugar released when 5-CQA added to enzymatic mixture indicating an inhibitory effect. The inhibition was more pronounced at 5 min (25.5% inhibition) compared to 20 min (1.5% inhibition). Pre-incubation of enzyme with 5-CQA for 10 min inhibited the enzyme by 53.8 and 28.3% at 5 and 20 min respectively. In order to calculate the IC_{50}, PPAA activity was measured in the presence of increasing concentrations of 5-CQA (Figure. 3.4 B). The IC_{50} was found to be around 2 mg mL^{-1} 5-CQA which was the maximum concentration tested.

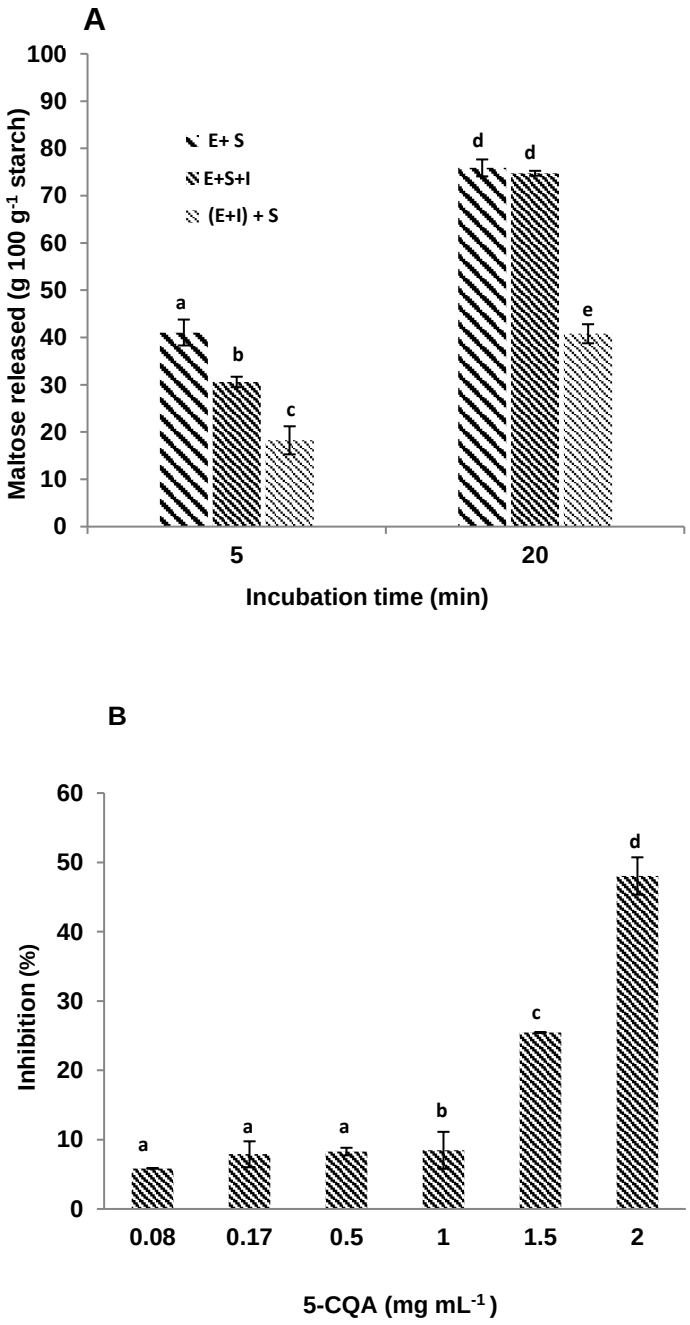


Figure 3.4 Effect of 5-caffeoylquinic acid (5-CQA) on the activity of porcine pancreatic alpha amylase (PPAA) activity using 10 mg potato starch as substrate. A) Effect of pre-incubation and co-incubation of 5-CQA (1.5 mg mL^{-1}) on the digestion of 1% starch by PPAA (0.33 unit mL^{-1}) at 5 and 20 minutes incubation. E = enzyme, S = substrate and I = inhibitor. B) Effect of increasing concentration of 5-CQA on inhibition of PPAA relative to control (no inhibitor) at 5 min incubation. Each bar represents mean of four measurements ± standard deviation. Bars with different letters have significant difference at $p<0.05$.

3.5.3.1 Enzymatic Kinetic and mode of inhibition

The enzymatic kinetic properties were examined by applying Michaelis-Menten assumptions. The initial velocity of the reaction (*V*) was determined at various concentrations [S] of gelatinised potato starch in the absence and presence of various concentrations of the 5-CQA and a fixed concentration of PPAA. Figure 3.5 shows effect of the substrate concentration on PPAA activity in the presence and absent of different concentration of 5-CQA. It was observed that at low substrate concentrations initial velocity of the reaction was directly related to substrate concentration while, increasing the amount of substrate reduce the proportional response of initial velocity of enzyme.

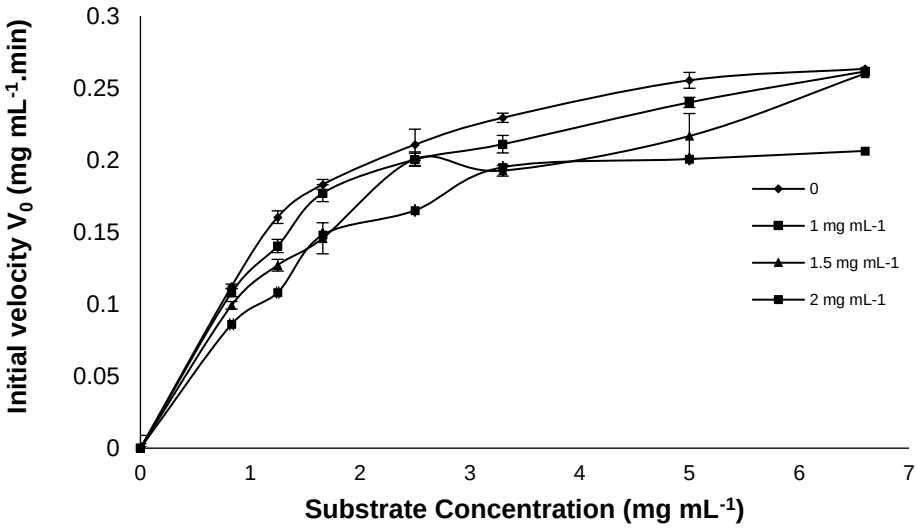


Figure 3.5 Effect of substrate concentration on enzyme activity (0.33 unit mL^{-1}) in the presence and absent of 5-CQA (1, 1.5 and 2 mg mL^{-1}). Michaelis-Menten plot. Data expressed as mean of the initial velocity V_0 of enzyme at 5 min (n=6). Error bars represent standard error of mean.

A Lineweaver-Burk plot produced linear relationships for 1/*V* against 1/[S] at various 5-CQA concentrations (Figure 3.6). Increasing inhibitor concentrations led to an increase in slope and y-intercept on the vertical axis. The intersection of lines in the second quadrant suggests a mixed type of

inhibition of 5-CQA against PPAA. Kinetic parameter V_{max} and k_m were obtained from Lineweaver-Burk plots (Table 3.1); k_m values ranged from 1.66 to 2.08 mg mL^{-1} starch with increasing inhibitor concentrations and maximum velocity (V_{max}) values from 0.35 to 0.29 mg mL^{-1} min^{-1} maltose.

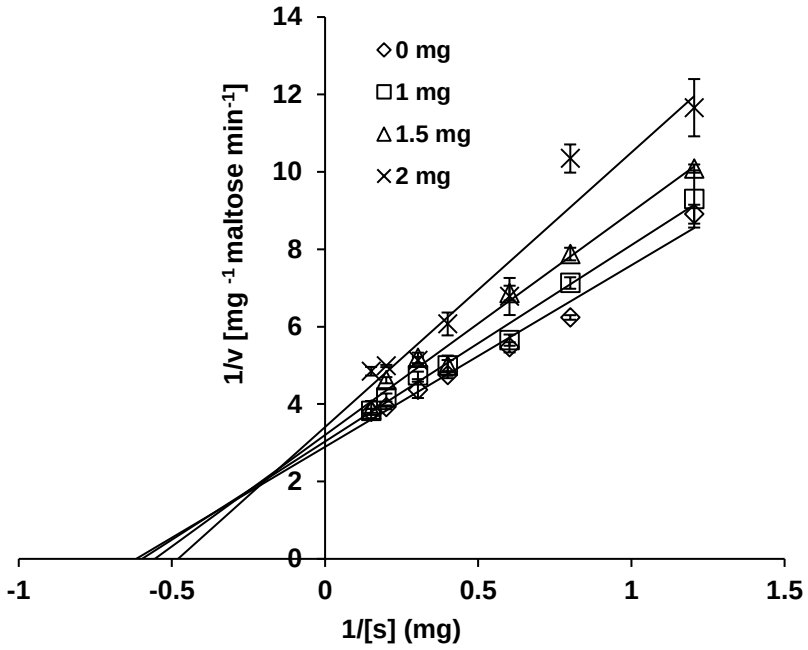


Figure 3.6 Lineweaver-Burk plot for porcine pancreatic alpha amylase (PPAA) catalysed hydrolysis of potato starch in the presence of 5-CQA (0, 1, 1.5 and 2 mg mL^{-1}). Each point represents mean of four measurements ± standard deviation.

Table 3.1 Kinetic parameters K_m and V_{max} of porcine pancreatic alpha amylase (PPAA) for increasing concentrations of 5-CQA.

[5-CQA] (mg mL^{-1})	k_m (mg mL^{-1})	V_{max} (mg mL^{-1} min^{-1})
0	1.66	0.35
1	1.69	0.32
1.5	1.85	0.31
2	2.08	0.29

3.6 Discussions

We evaluated the inhibitory effect of 5-CQA on PPAA activity using potato starch as substrate at two incubation time periods of 5 and 20 min (figure 3.4). The results indicate that 5-CQA significantly ($p \leq 0.05$) inhibited PPAA at both incubation times, the inhibition was more pronounced at 5 min (25.5% inhibition) compared to 20 min (1.5% inhibition). The difference in inhibition at the two reaction times indicates that 5-CQA is most efficient during early stages of hydrolysis, most probably by interacting with the enzyme and the high molecular weight substrate. Pre-incubation of enzyme with 5-CQA for 10 min inhibited the enzyme by 53.8 and 28.3% at 5 and 20 min respectively indicating that pre-incubation (without substrate) leads to the inactivation of the enzyme non competitively. IC50, was found to be around 2 mg mL^{-1} 5-CQA and this in agreement with results of Sun *et al*. (2016) who reported IC50 of 1.96 mg mL^{-1} for chlorogenic acid against PPAA hydrolysis of maize starch.

The enzymatic kinetic properties were examined by applying Michaelis-Menten assumptions. Type of inhibition was determined from Lineweaver-Burk plots. k_m values increased from 1.66 to 2.08 mg mL^{-1} starch with increasing inhibitor concentrations while maximum velocity (V_{max}) values decreased from 0.35 to 0.29 mg mL^{-1} min^{-1} maltose. These results also suggest that 5-CQA has a mixed type inhibition (competitive and non-competitive) against PPAA when potato starch is used as a substrate. This mixed type inhibition behaviour was previously reported for 5-CQA using p-nitrophenyl-α-D-maltoside as a substrate (Narita and Inouye, 2009). Recently, Sun *et al*. (2016) reported that chlorogenic and caffeic acid have a mixed type inhibitory effect on PPAA hydrolysis of maize starch. These two inhibitors

decrease the V_{max}, by interacting more strongly with the enzyme-substrate complex than with the free enzyme (Sun *et al*., 2016, Narita and Inouye, 2011). Rohn *et al*. (2002) reported that the incubation of digestive enzymes (PPAA, trypsin, lysozyme) with simple phenolic compounds (CQA, caffeic acid, gallic acid and ferulic acid) for 24 hours resulted in covalent attachment of the phenolic compounds to the free amino groups of the enzymes and in consequence, decreased their activity irreversibly and non-competitively. The non-competitive inhibition of PPAA was reported for millet seed coat extract using potato starch as substrate (Shobana *et al*., 2009).

The inhibitory activity of phenolic acids is enhanced with increasing the number of phenolic sub-structures. The inhibitory effect of caffeic acid was enhanced 5-fold by combining with quinic acid to form chlorogenic acid(Narita & Inouye, 2011). We show that 5-CQA is able to inhibit PPAA activity on potato starch in both competitive and non-competitive manners.

3.7 Conclusions

In conclusion, alpha amylase inhibitory activity of 5-CQA were measured *in vitro*. This study is the first to examine the mechanism of inhibition of PPAA by 5-CQA using potato starch as a substrate. Kinetics analyses showed a mixed-type inhibition, with stronger inhibition at earlier incubation times when more substrate is present.

In consequence, future work will test the *in vitro* digestibility of varieties of potato with different levels of phenolic content TPC and 5-CQA, applying the optimised digestions conditions described in this chapter.

4 *In vitro* starch digestion of five steam cooked potatoes by PPAA

4.1 Introduction

Results of the chapter 3 suggest that the chlorogenic acid isomer, 5-CQA inhibits PPAA hydrolysis of potato starch by mixed type inhibition mechanism. It has been shown that inhibitors of α-amylase reduce bioavailability of glucose (Bozzetto *et al*., 2015). Controlling blood glucose level by α-amylase inhibitors may play a role in preventing hyperglycaemia in patients with diabetes mellitus. Some α-amylase inhibitors are naturally present in foods. Potatoes are valued globally as a readily assimilated source of carbohydrate. The GI of potato products has been reported generally to vary by several studies. It has been suggested that the glycaemic impact of potatoes is affected by structure and phosphorylation of starch, content of dietary fibre and cooking method (Absar *et al*., 2009, Lynch *et al*., 2007, Fernandes *et al*., 2005).

Phenolics present in potatoes include phenolic acids, tannins, lignin, flavonoids, coumarins and anthocyanins (Ryes and Cisneros-Zevallos, 2003). 5-O-caffeoylquinic acid (5-CQA) is an isomer of chlorogenic acid which makes up 90% of the phenolic content of potato (Malenberg and Theander, 1985). The concentration of 5-CQA is higher in the skin than in the medulla. Analysis has shown that cooked unpeeled potato contains between 9.1 to 12 mg 5-CQA per 100 g fresh weight compared to 0.86 to 6.6 mg for equivalent peeled samples (Mattila and Hellstrom, 2007). The variation in 5-CQA content suggests that different potatoes may inhibit pancreatic amylase to different extents. The hypothesis tested in this chapter is that the content of 5-CQA has an influence on starch digestibility. Five varieties of potatoes with different

flesh and skin colours were selected for the experiment. *In vitro* hydrolysis of starch from steam cooked potatoes was conducted to the rate of starch hydrolysis.

4.2 Aims

The aim of this study is to determine the *in vitro* digestibility of steam cooked potatoes from five varieties which are expected to vary in their phenolic content.

4.3 Objectives

- To measure the dry mater (DM), total starch (TS), total phenolic content (TPC) and 5-CQA content of raw and cooked potatoes.
- To determine the *in vitro* digestibility of steam cooked potatoes by measuring the sugar content released during digestion with porcine pancreatic alpha amylase.

4.4 Results

4.4.1 Sugar standards and ranges used for detection of carbohydrate in potato varieties

Two different methods (DNS and HPAEC-PAD) were investigated to measure the carbohydrate content in five potato cultivars. For colorimetric method of DNS, a range of maltose and glucose standards were used and the colour intensity depended on the reaction between DNS reagent solution and each of maltose and glucose. Higher concentrations of maltose and glucose produced darker colour (Figure 4.1) and consequently higher absorbance when measured by the spectrophotometer.

In HPAEC-PAD different concentrations of mixed sugars plus 50 µl fucose as external standard were prepared and injected for preparation of the calibration curve. Figure 4.2 show the retention time for each sugar; 2.41 min for fucose, 3.96 min glucose, 4.31 min fructose, 6.73 min sucrose, 14.86 min maltose and 21.56 min maltotriose.

Figure 4.3 show the representative maltose and glucose standard curve which is obtained by DNS and HPAEC-PAD that have been used to measure total and digestible starch and soluble sugar content in potato samples used in this study.

Figure 4.1 DNS colourimetric reaction using maltose standards in the range from 0 to 4 mg mL^{-1}; the reaction was measured at 540 nm

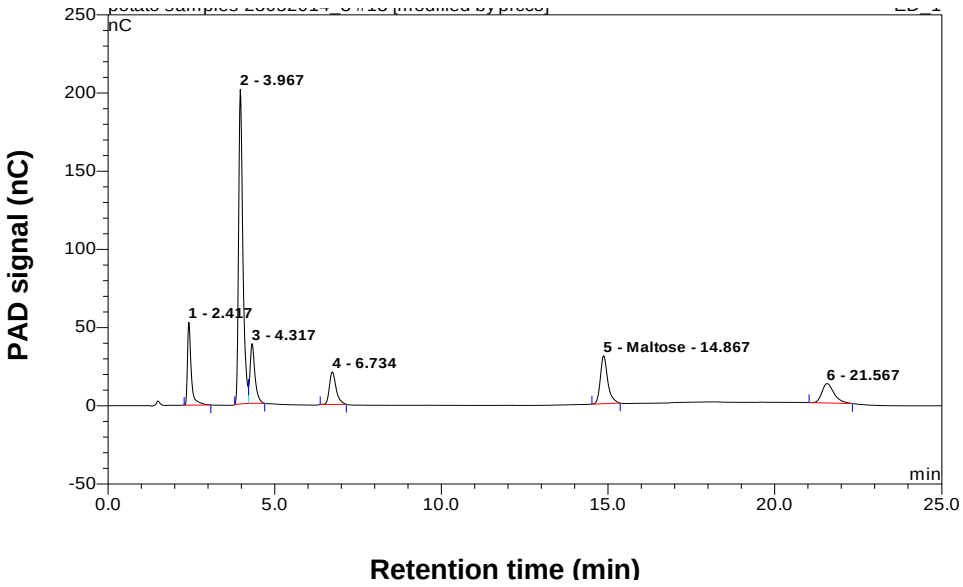


Figure 4.2 Chromatogram of sugar standards 0.1 µg in 10 µl injection volume. 1) Fucose (external standard). 2) Glucose. 3) Fructose. 4) Sucrose. 5) Maltose. 6) Maltotriose.

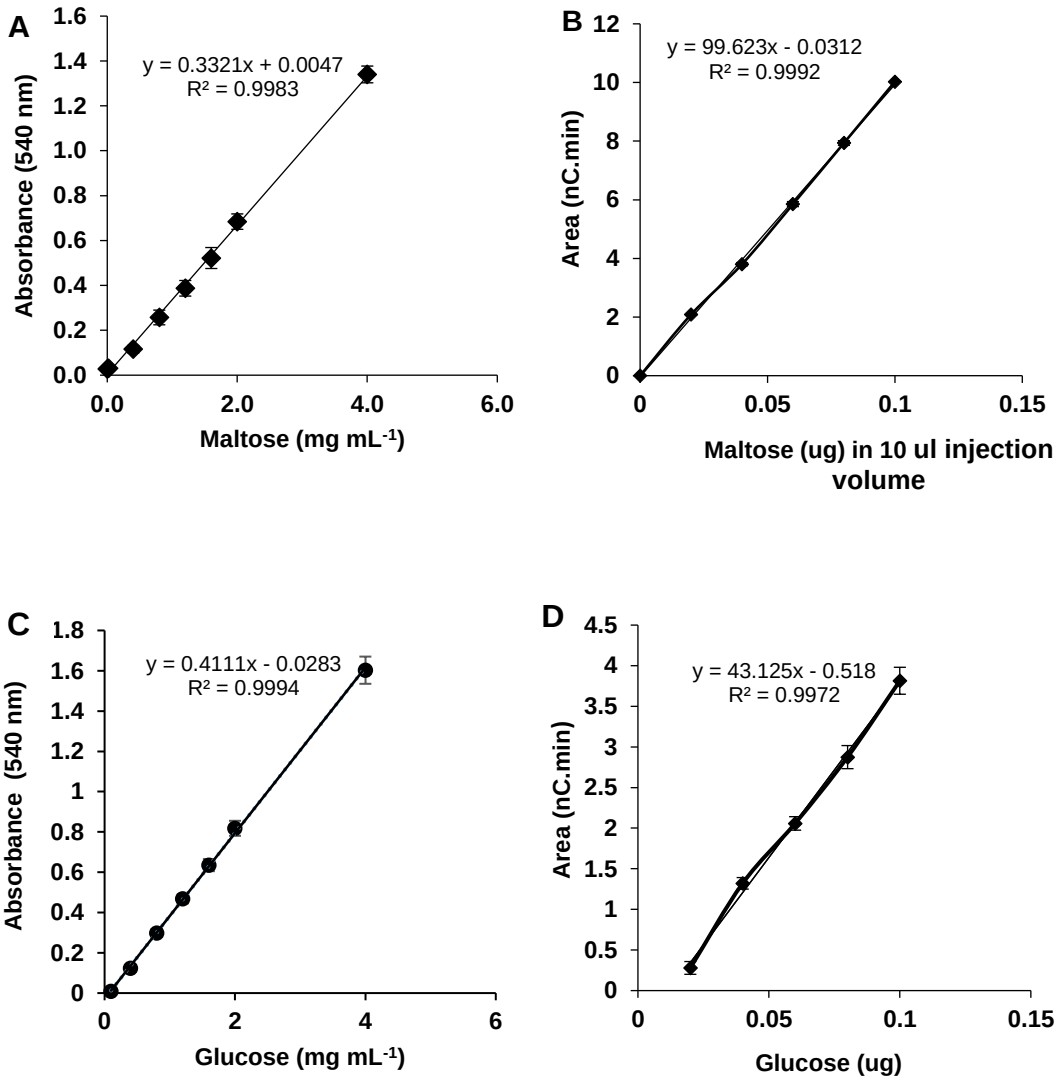


Figure 4.3 Standard curve for measuring carbohydrate and soluble sugar content of potatoes using maltose and glucose solution as standard. A) Maltose detected by DNS (n=21), B) Maltose detected by HPAEC-PAD (n=6), C) Glucose standard detected by DNS (n=9) and D) Glucose detected by HPAEC-PAD (n=6). Data represent as mean ± SD.

4.4.2 Chemical composition of five potato varieties

4.4.2.1 Dry matter content

Table 4.1 represent the percentage of dry matter (DM) content of five raw and cooked potato cultivars based on wet weight. There were significant difference between percentages of DM content of five potato cultivars in both raw and cooked forms ($p \leq 0.05$). The DM content in cooked potatoes were ranged from highest in Maris piper potato 21.47±0.61% to lowest in Mozart potato 19.10±1.13%. In raw potato the DM content was ranged from 16.12±0.81% to 22.12±3.13%. Mozart in both raw and cooked form showed lowest DM content compared to other potato cultivars. In cooked form, with the exclusion of Rooster and Desiree potato, all other potato cultivars DM content was slightly higher than raw form.

Table 4.1 Dry matter (DM) content of potato cultivars. (Mean values with their SD, n=4).

Potato Cultivars	DM content %	
	Raw	Cooked
Desiree^{R}	21.04±0.10^{ac}	20.08±0.11^{abc}
Mozart^{R}	16.12±0.81^{b}	19.10±1.13^{a}
Rooster^{R}	22.12±3.13^{a}	19.52±0.10^{abc}
Maris Piper^{Y}	20.67±0.10^{ac}	21.47±0.61^{b}
Maris Peer^{Y}	18.90±0.47^{acd}	19.66±0.22^{ac}

^{R}= red skin, ^{Y}= yellow skin; Mean value within a column with different subscript letter ^{a,b,c,d} indicate significant differences (one-way ANOVA, $P \leq 0.05$).

4.4.2.2 Total starch content

Total starch (TS) was measured in five potato cultivars using the method of Goni *et al*. (1997) with some modification. DNS was used rather than glucose assay kit by glucose oxidase peroxidase GOD-PAP for measuring glucose.

Table 4.2 shown the TS content of different varieties of potato based on fresh weight (FW) and as percentage of DM content. It was found that TS content in fresh potato varied significantly ($p \leq 0.05$) from 13.40±1.44 to 17.48±1.22 g $100g^{-1}$ FW representing 65 to 91% of DM content of potatoes. The lowest TS content were observed in Desiree and Rooster while, the highest were observed in Mozart and Maris Piper.

Table 4.2 Total starch content of five potato varieties (mean values with their SD, n=9).

Potato cultivars	TS (g $100g^{-1}$ FW)[*1]	TS (% DM content)
Desiree	13.40±1.44[b]	65.27±7.17
Mozart	17.48±1.22[a]	91.55±6.40
Rooster	13.61±0.80[b]	69.74±0.80
Maris Piper	16.97±3.62[ab]	79.01±16.87
Maris Peer	15.76±2.44[b]	80.17±12.42

[*] Free sugar corrected. [1] Factor converting glucose to starch was 0.9. Mean value within a column with different subscript letter [a,b,c,d] indicate significant differences (one-way ANOVA, $P \leq 0.05$).

4.4.2.3 Free sugar content

Free sugar content was determined in potato samples in order to correct the TS value using both DNS and HPAEC-PAD for detection. Free sugar content was extracted using water and shown in table 4.3. Free sugar contents were varied between potato samples and the range obtained was from 0.64 to 3.04 g 100 g^{-1} and 0.98 to 2.64 g $100g^{-1}$ potato for DNS and HPAEC-PAD respectively. The highest free sugar content was detected in Desiree potato in both method used for detection compared to all other potato samples while, Rooster and Maris Piper had the lowest free sugar content using DNS and HPAEC-PAD respectively. The value of free sugar detected by HPAEC-PAD in Desiree, Maris Piper and Maris Peer were slightly lower than DNS while,

the value detected in Mozart and Rooster value was slightly lower in DNS compared to HPAEC-PAD method.

Table 4.4 shows the free sugar profile for potato varieties provided by HPAEC-PAD. Glucose, fructose and sucrose were detected as free sugars in all potato samples (Figure 4.4) and the value of each sugars for all potato varieties are shown in table 4.4.

Table 4.3 Amount of free sugar content in potato using two method for detection. (Mean values with their SD, DNS (n=15) and HPAEC-PAD (n=6).

Potato varieties	Free sugar content (g 100 g^{-1})	
	DNS	HPAEC-PAD
Desiree	3.04±0.34	2.64±0.06
Mozart	0.82±0.11	1.06±0.02
Rooster	0.64±0.14	1.15±0.01
Maris Piper	1.02±0.59	0.98±0.06
Maris Peer	1.25±0.49	1.09±0.02

Table 4.4 Free sugars profile in five potato varieties obtained by HPAEC-PAD. Data are mean value expressed as g 100g^{-1} potato (n=6).

Potato varieties	Free sugar content profile (g 100 g^{-1})		
	Glucose	Fructose	Sucrose
Desiree	0.48	0.78	1.38
Mozart	0.43	0.51	0.14
Rooster	0.38	0.62	0.15
Maris Piper	0.20	0.48	0.31
Maris Peer	0.36	0.55	0.14

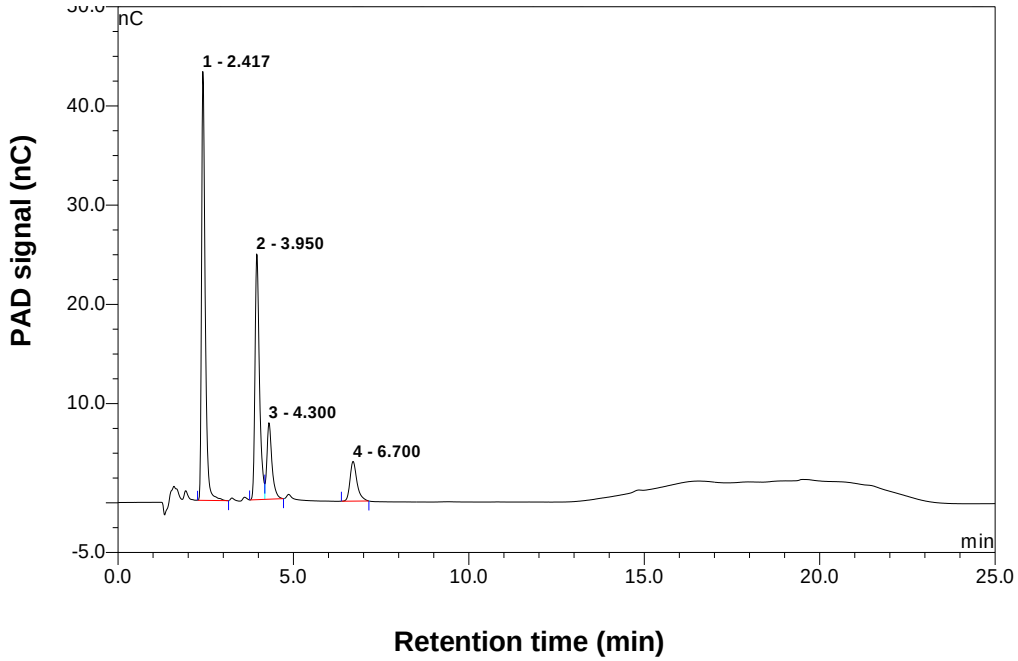


Figure 4.4 HPAEC-PAD Chromatogram showing free sugars extracted from a potato sample. 1) Fucose (external standard), 2) Glucose, 3) Fructose and 4) Sucrose.

4.4.2.4 Determination of polyphenols

4.4.2.4.1 Total phenolic content

The total phenolic content (TPC) of raw and cooked potato samples was measured using the Folin-Ciocalteu colorimetric method (Singleton and Rossi, 1965). A range of 5-CQA standards was used and the colour intensity depended on the reaction between Folin reagent solution and 5-CQA. Higher concentrations of 5-CQA produced darker colour. Figure 4.5 shown the typical 5-CQA standard curve obtained by Folin–Ciocalteu that was used for measuring TPC of potato samples.

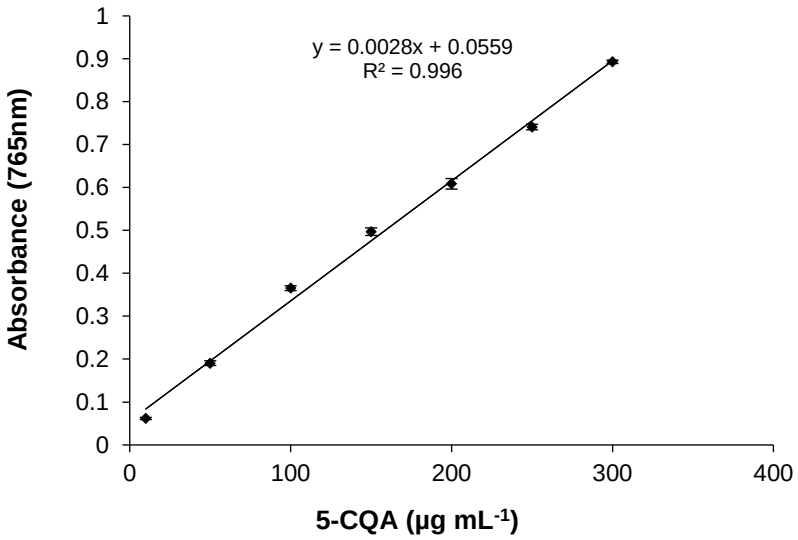


Figure 4.5 5-CQA standard curve for measuring total phenolic content TPC of potato cultivars. Data represent as mean ±SD (n=6).

The TPC of raw and cooked potato samples is shown in table 4.5. TPC in fresh peeled potatoes varied from 320.59 mg 100 g^{-1} DW (5-CQA equivalent) in Rooster to 528.94 mg 100 g^{-1} DW in Maris Peer. TPC content was in the order Maris Peer > Mozart > Desiree > Maris Piper > Rooster from highest to lowest content. Variety affected TPC content significantly ($p \leq 0.05$).

In general, steam cooking affected the TPC in all potato samples. Cooking reduced the TPC in Maris Peer (-35%) and Mozart (-19%) significantly. Smaller non-significant decreases were observed in Maris Piper (-7%) and Rooster (-12%) ($p = 0.26$ and 0.20 respectively). However, the TPC content increased in Desiree potatoes by 22% ($p=0.04$). This variation in the response to cooking has been reported in the literature (Tian *et al*., 2016).

Table 4.5 Total phenolic content TPC in raw and cooked potato samples. Data are mean value (n=4).

Potato varieties	TPC (mg 100 g^{-1} DW)*	
	Raw	Cooked
Desiree[R]	445.14±32.89[b]	543.96±20.68[a]
Mozart[R]	524.64±27.10[a]	425.53±17.36[b]
Rooster[R]	320.59±15.34[c]	282.03±5.07[d]
Maris Piper[Y]	375.69±29.06[d]	349.18±13.58[c]
Maris Peer[Y]	528.94±15.72[a]	343.83±11.57[c]

[R]= red skin, [Y]= yellow skin. *Chlorogenic acid equivalent. . [a,b,c,d,e] Mean values within a column with different subscript are significantly different ($p \leq 0.05$; one-way ANOVA).

4.4.2.4.2 Separation and quantification of 5-CQA by HPLC-DAD-MS

To address the non-specificity of the Folin–Ciocalteu assay, the individual phenolic acids in potato extracts were identified using HPLC-DAD-MS (typical chromatogram shown in figure 4.7). Different concentration of 5-CQA plus 100 µg mL^{-1} sinapic acid as external standard were prepared and injected to obtain a calibration curve. The peak area was proportional to concentration. The order of elution for each phenolic acid were 5-CQA 12.2 min and sipanic acid 18.2 min. Figure 4.6 shows the typical standard curve obtained by HPLC-DAD-MS for measuring phenolic acids in potato samples.

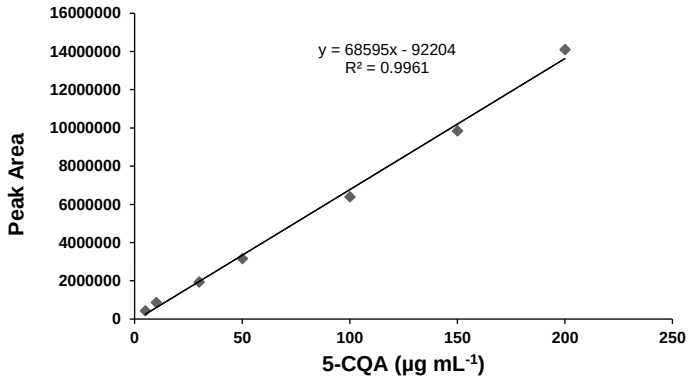


Figure 4.6 5-CQA standard curve obtained by HPLC-DAD-MS for measuring phenolic acids in potato samples. Data are means± SD (n=4)

5-CQA was the predominant phenolic acid in the flesh of raw and cooked potatoes. Other minor peaks were also observed but not quantified (figure 4.7). Table 4.6 shows 5-CQA content of the potato samples. It was observed that 5-CQA level varied between potato samples significantly in both raw and cooked form ($p \leq 0.05$). The content of 5-CQA in raw potato ranged from the lowest in Desiree (10.36 mg 100 g^{-1} DW) to highest in Maris Piper (29.46 mg 100 g^{-1} DW). In cooked tuber, the 5-CQA content was lowest in Rooster (6.51 mg 100 g^{-1} DW) and highest in Maris Piper (21.24 mg 100 g^{-1} DW). Similar to the effects of cooking on TPC, steam cooking significantly decreased the 5-CQA level of all potatoes tested ($p \leq 0.05$), except Desiree which saw an increase. 5-CQA in cooked Desiree was 1.7 time higher than in raw potato ($p \leq 0.05$).

Table 4.6 5-CQA content of raw and cooked potato extract. Value are mean ± SD (n=4).

Potato varieties	5-CQA (mg 100 g^{-1} DW)	
	Raw	Cooked
Desiree^{R}	10.36±0.16^{d}	17.83±0.40^{b}
Mozart^{R}	16.84±0.59^{b}	11.12±0.31^{c}
Rooster^{R}	13.07±0.70^{c}	6.51±0.23^{e}
Maris Piper^{Y}	29.46±1.19^{a}	21.24±0.42^{a}
Maris Peer^{Y}	11.12±1.86^{cd}	7.40±0.35^{d}

^{R}= red skin, ^{Y}= yellow skin. ^{a,b,c,d,e} Mean values within a column with different subscript are significantly different ($p \leq 0.05$; one-way ANOVA).

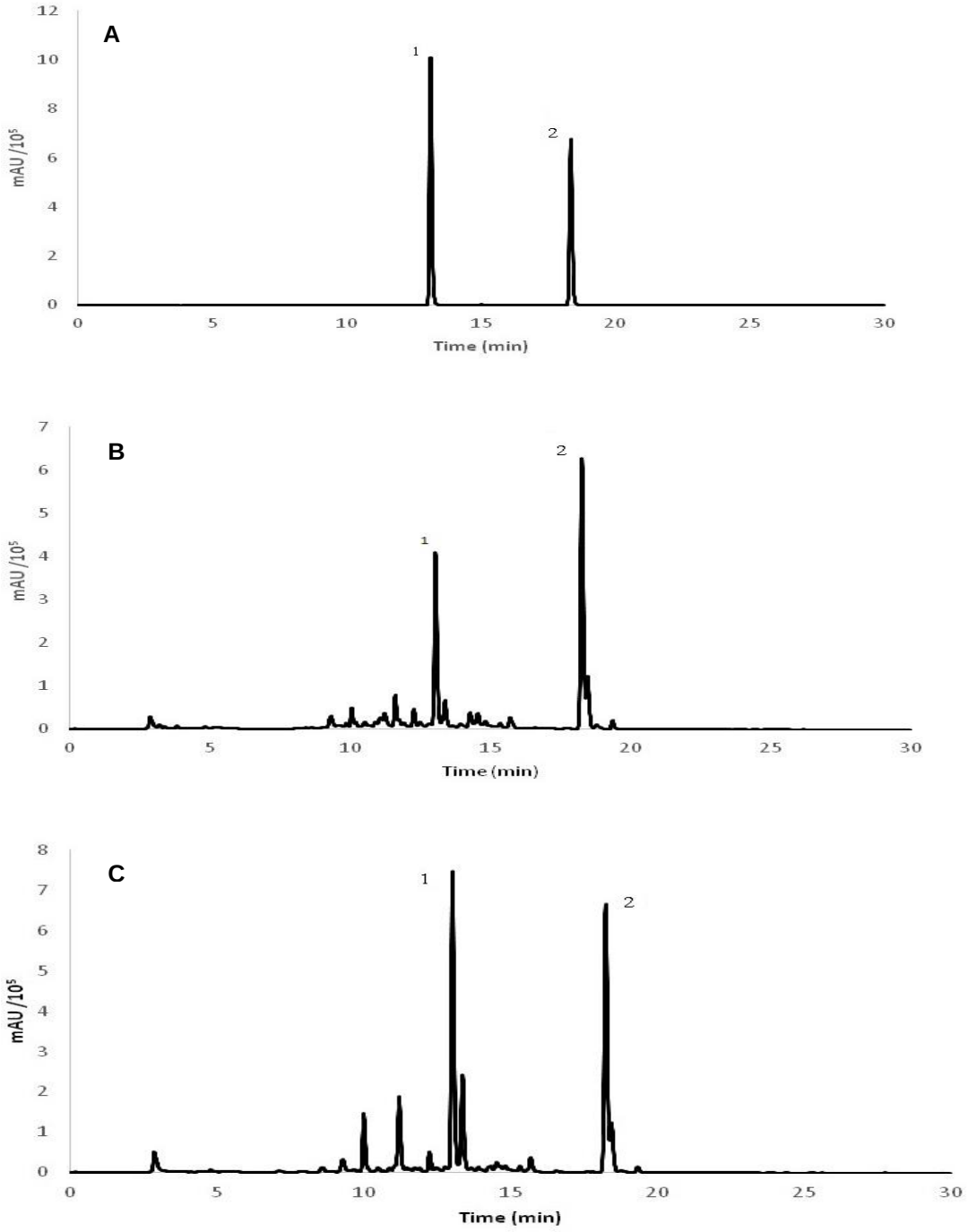


Figure 4.7 HPLC-DAD chromatogram of A) standard compounds, B) raw potato sample (Desiree) and C) cooked potato sample (Desiree). Peaks were detected at wavelength 330 nm. Peaks; (1) 5-caffeoylquinic (5-CQA) (2) sinapic acid (external standard).

4.4.3 *In vitro* starch hydrolysis of steam cooked potato

4.4.3.1 Optimisation of enzyme conditions

The conditions at which porcine pancreatic alpha amylase (PPAA) showed the maximal activity on hydrolysis of pure potato starch was investigated in chapter 3 using DNS for sugar measurement. It might be different conditions are required for digestion of cooked potato than commercial available potato starch as potato contains starch, fibre and protein. Therefore, to optimise potato digestion a series of modification were done. For optimisation, DNS was selected as a fast and non-expensive method to measure hydrolysed sugar in potato samples.

4.4.3.1.1 Incubation time and enzyme dosage

The mean TS content of five potato samples used in this study was 15 g 100 g^{-1} potato. The substrate concentration selected in chapter 3 at which that PPAA showed maximum activity was 1.66 mg mL^{-1} (10 mg final concentration) gelatinised potato starch therefore the cooked potato concentration selected as substrate was about 1.66 mg ml^{-1} (10 mg final concentration) starch.

Figure 4.8 shows the hydrolysis curve of steam cooked potato (Maris Piper) by PPAA over 90 min. It was shown that at 20 min of incubation 33.7% of starch was rapidly hydrolysed and starch was hydrolysed more slowly until 60 min at which 52.8% of TS hydrolysed. A sharp increase was observed to 77% starch hydrolysed at 90 minutes. Thus, 90 min was shown not enough for maximum hydrolysis of cooked potato.

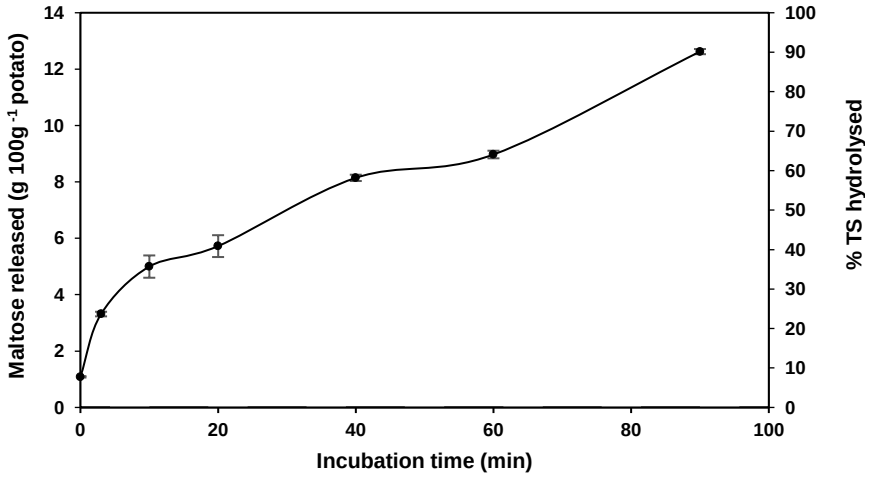


Figure 4.8 Starch hydrolysis curve after *in vitro* hydrolysis of 10 mg Maris Piper potato by 0.33 units PPAA over 90 minute. Data expressed as % of total starch hydrolysed and g of maltose released. Error bars are standard error of mean (n=3).

Further modification was conducted by providing longer incubation time and increasing enzyme dosage. Figure 4.9 shows the hydrolysis curve for *in vitro* digestion of steam cooked potato starch over 150 minute for both 0.33 and 0.66 unit of enzyme concentration. It was observed from curve of hydrolysis that after 120 minute of incubation, no more hydrolysis of starch takes place, 150 minute is enough for digestion of cooked potato starch using both enzyme concentrations 0.33 and 0.66 units. Although in initial time of incubation 0 to 90 min no differences observed between two different dosages 0.33 and 0.66 unit in hydrolysis of potato starch (1.08 to 12.62 and 1.08 to 11.23 g maltose 100 g^{-1} potato) respectively however, % TS hydrolysed by 0.66 unit over 15 min was higher compared to % hydrolysed by 0.33 unit (88.5 and 74.37%) respectively. Therefore, 0.66 unit enzyme and 150 minute incubation time was selected as ideal concentration and incubation time.

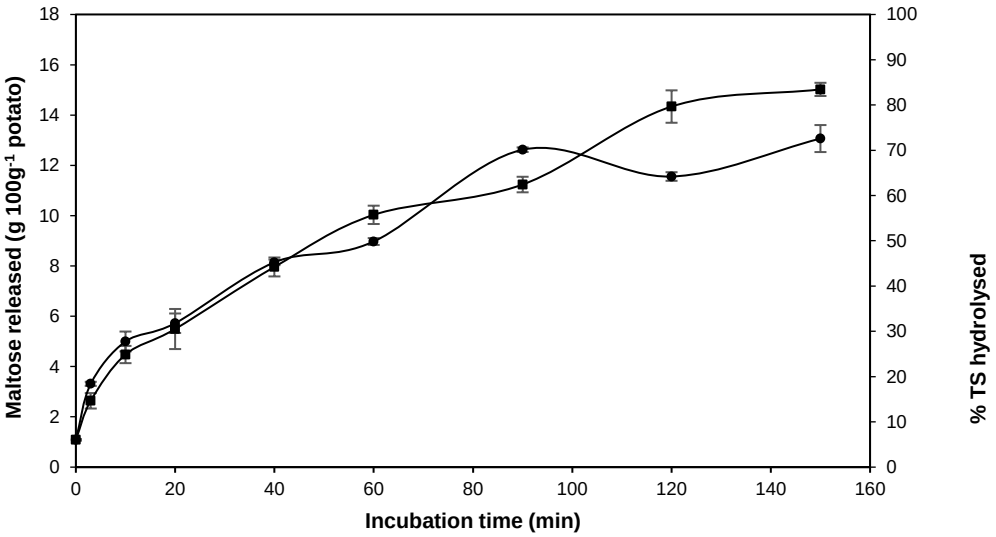


Figure 4.9 Starch hydrolysis curve after *in vitro* hydrolysis of 10 mg Maris Piper potato over 150 minute. ● 0.33 and ■ 0.66 units PPAA. Data expressed as % of total starch hydrolysed and g of maltose released. Error bars are standard error of mean (n=3).

4.4.3.2 *In vitro* hydrolysis of five potato samples applying all modifications

Starch hydrolysis curves of different steam-cooked potato samples by PPAA are shown in figure 4.10 A to E. Hydrolysis of potato starch was monitored by measuring the reducing sugar produced at different times using the DNS colorimetric method and the maltose content by HPAE-PAD (typical chromatogram shown in figure 4.10 F). Using HPAEC-PAD, free soluble sugars including glucose, fructose and sucrose were detected at low levels at time 0 (figure 4.4). While after hydrolysis by PPAA, maltose and maltotriose were detected as products of digestion (Figure 4.10 F).

Table 4.7 and 4.8 represent the digestible starch (DS) and area under curve (AUC) for *in vitro* digestion of each varieties over 150 min. In all cases, the amount of sugar detected was around 1.5 times higher using the DNS method than using HPAEC-PAD. Using DNS, apart from Rooster there were no

significant differences between other potato varieties DS ($p \geq 0.05$) while, using HPAEC-PAD significantly highest DS was detected in Rooster and Maris Peer potato compared to the other three potato varieties ($p \leq 0.05$).

There were significant ($p \leq 0.05$) differences in the AUC_{PPAA} between the varieties using HPAEC-PAD for sugar measurement. Rooster showed consistently the highest extent of digestion. Meanwhile, Mozart and Maris Peer showed lower levels of digestibility than Desiree and Maris Piper. There were no significance different in the AUC_{PPAA} between varieties when DNS used for sugar measurement ($p \geq 0.05$).

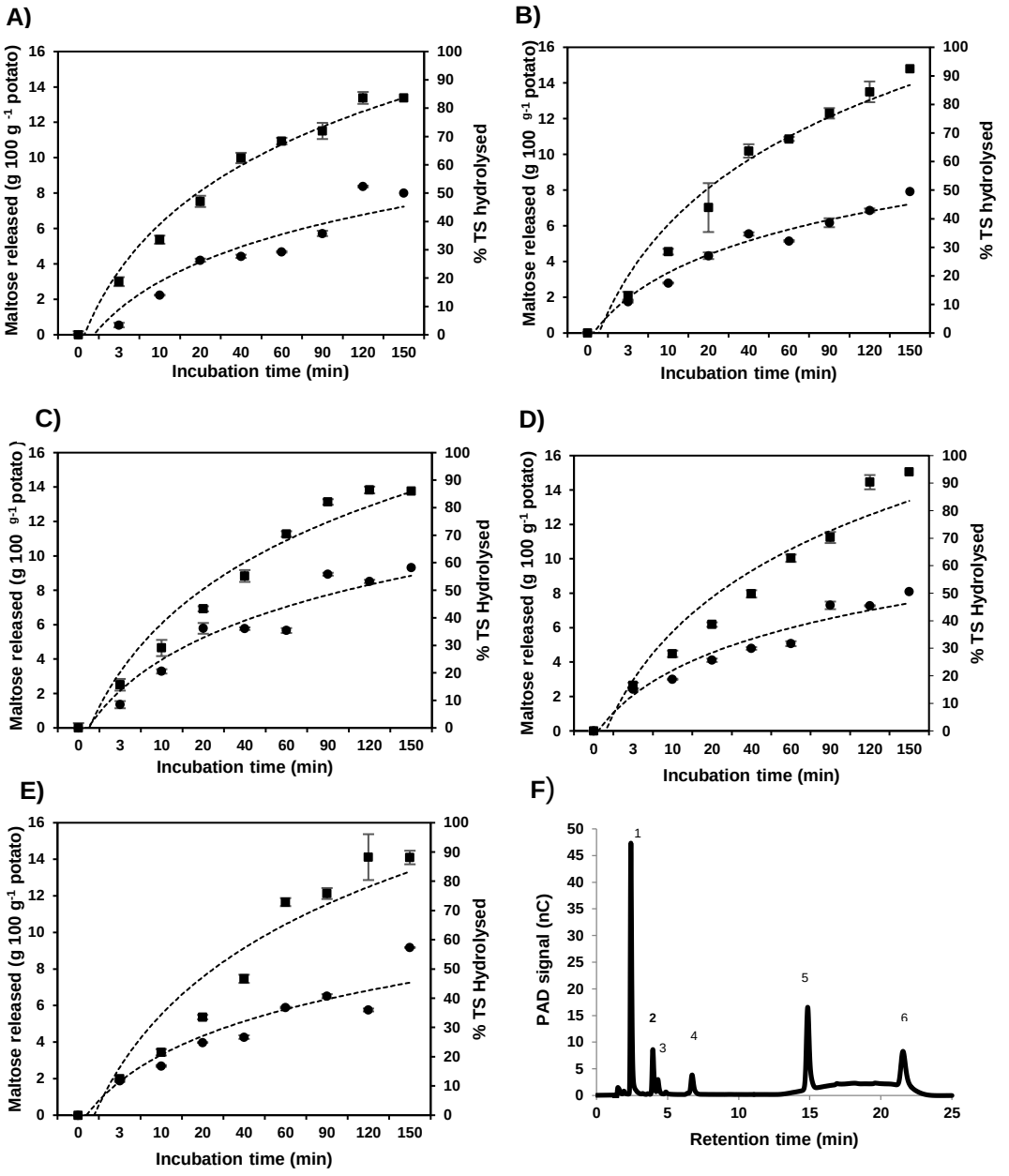


Figure 4.10 Starch hydrolysis curves of five steam cooked potato samples using 0.66 unit alpha amylase over 150 min. A) Desiree B) Mozart. C) Rooster. D) Maris Piper. E) Maris Peer. Data expressed as amount of maltose released and as % of total starch (TS) after digestion at each time point as measured by DNS (■) (n=9) and HPAEC-PAD (●) (n=3). Error bars represent standard deviation of mean. F) HPAEC-PAD chromatogram of total sugars present after digestion of a potato sample (Maris Piper) for 120 min. Peaks; (1) fucose (external standard), (2) glucose, 3) fructose, 4) sucrose, 5) maltose, 6) maltotriose. Glucose, fructose and sucrose were already present at time 0 (Figure 4.4).

Table 4.7 Digestible starch (DS) and area under curve AUC of five potato varieties. Sugar detected by HPAEC-PAD method. Data expressed as mean value ±SD (n=3).

Potato varieties	HPAEC-PAD	
	DS*	AUC
Desiree	8.00±0.08[b]	517.35±12.82[b]
Mozart	7.91±0.01[b]	484.05±5.21[c]
Rooster	9.31±0.16[a]	599.25±1.31[a]
Maris Piper	8.08±0.21[b]	507.00±11.51[b]
Maris Peer	9.17±0.01[a]	486.27±5.30[c]

*g per 100 g potato. [a,b] Mean values within a column with different subscript are significantly different ($p \leq 0.05$; one-way ANOVA).

Table 4.8 Digestible starch (DS) and area under curve of five potato varieties. Sugar detected by DNS. Data expressed as mean value ±SD (n=9).

Potato varieties	DNS	
	DS*	AUC
Desiree	13.83±0.46[ab]	908.64±38.30[a]
Mozart	14.79±1.11[a]	920.07±14.15[a]
Rooster	13.71±0.56[b]	920.83±16.39[a]
Maris Piper	15.02±0.45[a]	892.15±17.94[a]
Maris Peer	14.09±0.90[a]	888.97±37.34[a]

*g per 100 g potato. [a,b,c,d,e] Mean values within a column with different subscript are significantly different ($p \leq 0.05$; one-way ANOVA).

4.4.4 Relationship between potato polyphenol content and their *in vitro* starch digestibility

In order to evaluate the relationship between potato polyphenols and starch digestibility *in vitro* by PPAA, Pearson correlation analysing was conducted. The correlation coefficient was determined for potato TPC and 5-CQA and *in vitro* starch digestibility (table 4.9).

There was trend but non-significant correlation between *in vitro* starch digestibility (AUC_{DNS} , $AUC_{HPAEC-PAD}$) and 5-CQA or TPC of potato varieties ($p \geq 0.05$).

Table 4.9 Correlation between 5-CQA content of potato and their *in vitro* starch digestibility.

Characteristics		AUC_{PPAA} DNS	AUC_{PPAA} HPAEC-PAD
TPC	r	0.10	-0.43
	p	0.81	0.61
5-CQA	r	-0.32	-0.31
	p	0.58	0.31

4.4.5 Principle component analysis

Principle component analysis (PCA) was conducted in order to determine the relationship between potato components; TS, DM, TPC, 5-CQA and AUC of starch hydrolysis. Figure 4.11 and 4.12 showing the loading and score of the characteristics of five potato cultivars with the first two PCs explaining 64% and 66.3% of the total variance respectively. In figure 4.11 PC1 explaining 40.61% and PC2 explained 24.67% of total variance and in figure 4.12 PC1 explained 39.75% and PC2 explained 26.68% of total variance. In both figures

(4.11, 4.12) for PC1, TS, DM, 5-CQA and TPC had positive loading and AUC of sugar detected by HPAEC-PAD and DNS had negative loading. TS and DM had positive loading for PC2 and the other potato characteristic variable had negative loading for PC2.

By comparing the score and loading plot the relation between potato cultivars and variables was identified. In both first and second PCs Maris Piper potato had higher positive score for TS, DM, 5-CQA and Desiree had higher score for TPC than other potato varieties used in this study. Rooster, Maris Peer and Mozart showed the opposite characteristics.

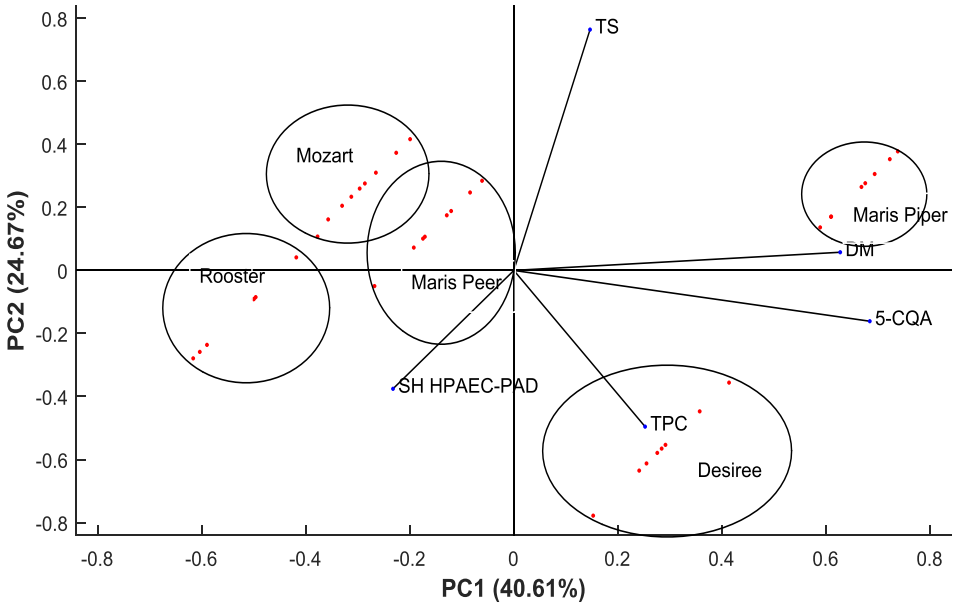


Figure 4.11 PCs of potato characteristics. A) Loading of first and second principle components (64%); TS, DM, TPC, 5-CQA, SH (HPAEC-PAD). B) First and second components score of potato cultivars. PC1 (40%) andPC2 (24%).

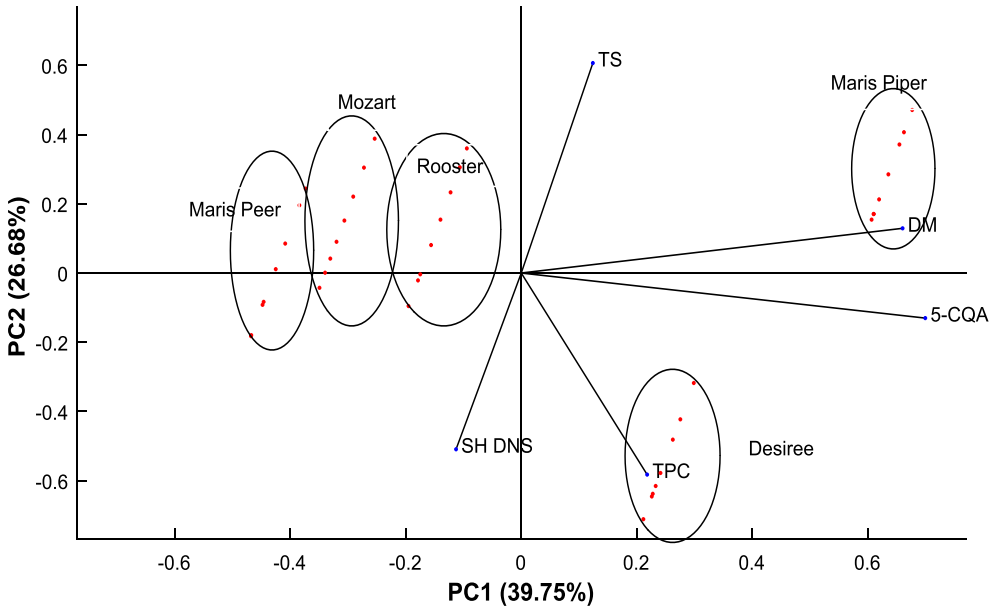


Figure 4.12 PCs of potato characteristics. A) loading of first and second principle components; TS, DM, TPC, 5-CQA, SH (DNS). B) First and second components score of potato cultivars. PC1 (39.75%) andPC2 (26.68%).

4.5 Discussions

4.5.1 Chemical composition of five potato varieties

4.5.1.1 Carbohydrates and dry matter content

The compositions of potato were determined. Potato DM content ranges were between 16-22%. Thus, DM content measured in potato varieties in present study was in agreement of ranges that has been previously reported (Ek *et al*., (2014a, Ramdath *et al*., (2014). The TS content of raw potato reported elsewhere for eight potato varieties was in the range 65 to 73% in DM content (Pinhero *et al*., 2016) and 9.10 to 16 g 100 g^{-1} potato (Ek *et al*., 2014a). The TS of Mozart potatoes in present study higher than the range reported by Pinhero *et al*. (2016) and Ek *et al*. (2014a) by 20 to 29% and 8.4 to 48.6% respectively. Soluble sugars of five potato samples were extracted and measured by DNS and HPAEC-PAD (Table 4.3). Apart from Desiree potato,

all other potatoes free sugar content measured by both method was in the range reported in McCance and Widowsons the composition of foods dataset (0.6-1.1 g 100 g^{-1} for boiled in unsalted water potato).

In general potato composition values were in agreement with reported values but, variations was observed between potato cultivars in their DM, TS and free sugar content. According to Thybo *et al*. (2006), cultivars contributed significantly to the variation in the chemical components in the raw potatoes.

4.5.1.2 Polyphenols content

There are many reports of TPC in different genotypes of potato in peeled, unpeeled, fresh and cooked form. The TPC varies according to genotype, agricultural practices and method of extraction. Ah-Hen *et al*. (2012) examined the TPC in some native coloured potatoes from Chiloe Island (Southern Chile) and found that TPC in peeled potato samples varied from 192 to 1864 mg $100g^{-1}$ DW (ferulic acid equivalent). Lachman *et al*. (2008) reported TPC in red and yellow-fleshed potato to range from 296 mg 100 g^{-1} DW in yellow-fleshed potato to 468 mg 100 g^{-1} DW (gallic acid equivalent) in purple-fleshed potato. These levels are consistent with the results obtained in this study. Recently, Tierno *et al*. (2015) reported that boiling peeled potato for 30 min reduced the TPC of tubers by about 50% and similar results also were obtained by Lemos *et al*. (2015). Meanwhile, Blessington *et al*. (2010) reported an increase in TPC after baking and microwaving. Bembem and Sadan (2013) and Burgos *et al.* (2003) observed an increase in TPC after boiling, steam cooking, microwaving and pressure cooking. Therefore, an increase in level of TPC of Desiree potato in our study after steam cooking is in accordance with some

published reports. The increased levels could be due to the release by cooking of phenolic or other compounds (Tian *et al*., 2016).

One of the disadvantages of the TPC assay using Folin-Ciocalteau reagent is the low level of specificity. For instance, the reagent reacts with ascorbic acid and tyrosine, both abundant in potatoes. It has been reported that the ascorbic acid response to the Folin-Ciocalteau reagent is two times higher than gallic acid (Lachman *et al*., 2008). Therefore, results using TPC values need to be interpreted with caution. To address the non-specificity, the individual phenolic acids in potato extracts were identified using HPLC-DAD-MS.

The level of chlorogenic acid in 50 unpeeled potato genotypes was reported by Navarre *et al*. (2011) to be in the range 21.9 to 473.0 mg $100g^{-1}$ DW. Mattila and Hellstrom (2007) reported that cooked and peeled potato contain 4.13 to 31.2 mg 100 g^{-1} DW, in agreement with the current study.

The colour of the skin has been suggested to be associated with TPC (Tierno *et al*., 2015) However, in this study, this was not found to be the case. Observed colour was not a good indicator of TPC and 5-CQA content in flesh. Rooster has red skin but its flesh showed one of the lowest TPC and 5-CQA contents amongst the varieties investigated. Conversely, Maris Piper has yellow skin and had the highest 5-CQA in cooked flesh. The HPLC results indicate that Folin-Ciocalteau method over estimates potato phenolic content by several orders of magnitude. Nevertheless, the varieties do differ in 5-CQA content and the potential impact on starch digestion was tested.

4.5.2 *In vitro* starch hydrolysis of steam cooked potato

The digestible starch content (DS) of five steam cooked potato cultivars was measured using DNS method and HPAEC-PAD for sugar detection. Amount of sugar detected in all cases, was around 1.5 times higher using the DNS method than using HPAEC-PAD this can be explained by DNS reacting with oligosaccharides produced by amylase digestion that can react with DNS (Van der Mareel *et al*., 2002, Nigam and Singh, 1995, Robyt and Whelan, 1972)**.** Using HPAEC-PAD, free soluble sugars including glucose, fructose and sucrose were detected at low levels at time 0 (Figure 4.4). While, after hydrolysis by PPAA, maltose and maltotriose were detected as products of digestion (Figure 4.10 F). The HPAEC-PAD also showed that the PPAA preparation contains sucrose, most probably as a stabiliser and this needs to be corrected for if needed. Sucrose does react with DNS and therefore it is important to undertake a blank correction of the enzyme preparation. The value of 13.1 g 100 g^{-1} reported for DS in boiled potato by Goni *et al*. (1997) this value was higher than value of DS detected by HPAEC-PAD in all potato cultivars while, the DS detected by DNS close to reported value.

4.5.3 Relationship between potato phenolic content and their *in vitro* starch digestibility

The correlation between TPC and 5-CQA and starch digestibility was determined at different times of digestion. There was trend but not significant correlation between TPC and 5-CQA of potatoes and starch digestion as measured using DNS and HPAEC-PAD. In this study, the amount of steam cooked potato used as substrate contained about 0.08 mg mL^{-1} TPC. According to results of chapter 3 (figure 3.4 B), this is a low concentration of

5-CQA, enough to inhibit up to 5.8% of the alpha amylase activity. There is a non-significant correlation between the TPC level and potato digestibility because there would not be enough chlorogenic acid to cause inhibition.

According to the PCA scores, potato varieties segregated according to their components: Maris Piper showed high DM, high 5-CQA content and low digestibility. Rooster, Maris Peer and Mozart potato appeared in one cluster and showed the opposite characteristics. The digestibility of Desiree appeared to be heavily influenced by TPC, in contrast to the other varieties. It appears that the same single factor does not strongly determine digestibility in all varieties.

4.6 Conclusion

This study showed that skin colour was not a good predictor of TPC, 5-CQA and digestibility in peeled tubers. This study also demonstrated that the Folin-Ciocalteau TPC assay over estimates phenolic content in potato, while the DNS assay for reducing sugar may over estimate sugar release during amylase digestion. We suggest that alternative methods such as HPLC must be used to identify and quantity of the phenolic compounds and the sugars.

The results presented in this study also advance our understanding of varietal differences in digestibility and suggest that multiple factors affect potato digestibility, and the effects may be variety specific. Ultimately testing the digestibility of the varieties *in vitro* using PPAA+AMG and *in vivo* will also confirm whether these observations have biological significance.

5 Simulated digestion from five steam cooked potato varieties to estimate glycaemic index *in vitro*

5.1 Introduction

It has been previously described that pancreatic amylase has a main role in enzymatic degradation of starch and anything that affects the contact of this enzyme to substrate will affect the rate of starch digestion. In chapter 4 cooked potatoes subjected to starch digestion with PPAA however, did not lead to the ultimate breakdown of starch to glucose. Englyst *et al*. (1992) investigated the rate and extend of glucose released from *in vitro* enzymatic digestion of carbohydrate foods under controlled conditions in order to determine the physio-chemical breakdown of starch. This *in vitro* method classified starch based on digestibility into: rapidly digestible starch (RDS) (rapid digestion in the small intestine within 20 min), slowly digestible starch (SDS) (slow but complete digestion in the small intestine) and resistant starch (RS) (resistant to digestion in the small intestine). Moreover, Englyst *et al*. (1996) used an *in vitro* classification of starch fraction to provide the rapidly available glucose (RAG) which is free glucose and sugar from starch digestion likely to be available for rapid absorption in the human intestine. The *in vitro* starch digestibility has been shown to have a good correlation with *in vivo* glycaemic response and *in vitro* measurement of RAG has been significantly correlated to glycaemic response *in vivo*. Thus, *in vitro* method has been proposed as an alternative method for predicting *in vivo* glycaemic response of carbohydrate foods (Ek *et al*., 2014a, Englyst *et al*., 1999, Goni *et al*., 1997, Englyst *et al*., 1996).

It has been reported that the proportion of RDS, SDS and RS in the single source of starch food such as potatoes varies according to the method by which it is processed. Boiled potato contains 0.7-1.1 g RS while cooled, canned potatoes provide 3 g RS (Kingman and Englyst 1994). Recently Pinhero *et al.* (2016) evaluated the estimated glycaemic index (eGI) of potatoes with respect to starch profile digestibility (RDS, SDS and RS) in cooked and retrograded potatoes and observed lower eGI of retrograded potatoes than cooked potato due to increase in RS and SDS.

To our knowledge there are no data available on the relation of phenolic content of potatoes prepared under the same condition with the starch digestibility profile (RDS, SDS,RS and RAG). This study for first time investigated the relationship between potato phenolic content (TPC and 5-CQA) with eGI and starch digestibility profile (RDS, SDS, RS and RAG).

The chemical compositions (DM, TS, free sugar, TPC, 5-CQA) of five potato varieties were determined in previous chapter 4. Pearson's correlation indicated non-significant correlation between starch digestibility using PPAA and TPC or 5-CQA. However, depending on PCA results it appears that the same single factor does not strongly determine digestibility in all varieties. Thus, in order to estimate the glycaemic index of potatoes with respect to TPC and 5-CQA, standardised method for determining the rate of starch hydrolysis *in vitro* to mimic gastrointestinal tract digestion will be required (Goni *et al*., 1997).

5.2 Aims

The aim of present study was to use standardised *in vitro* method to estimate the glycaemic index of five potato varieties varied in their phenolic content.

5.3 Objectives

- To determine the rate of starch digestion using PPAA+AMG
- To determine the starch digestibility profile RAG, RDS, SDS and RS
- To calculate the hydrolysis index (HI) from AUC of glucose released over 180 min *in vitro* digestion
- To estimate glycaemic index (eGI) from HI
- To correlate the eGI and HI with TPC and 5-CQA measured in previous chapter.
- To evaluate the correlations between starch digestibility profile and phenolic content (TPC and 5-CQA)

5.4 Results

5.4.1 *In vitro* starch digestion

The rate of starch digestion was measured by method adapted from Goni *et al*. (1997) with some modification. Starch was hydrolysed using PPAA and AMG each after other to mimic the digestion in the gastrointestinal tract. The tested foods used in this study were five potato varieties (Desiree, Mozart, Rooster, Maris Piper and Maris Peer) and reference food white bread. Total starch and free sugar of each foods were determined experimentally in chapter 4. The amount of starch hydrolysed was determined by measuring glucose released using DNS. Glucose converted to starch by multiplying by 0.9 conversion factor.

5.4.1.1 Starch hydrolysis rate

Figure 5.1 displays the hydrolysis rate of starch digestion for different potato varieties compared to white bread as a reference food. Rate of starch digestion was expressed as the percent of total starch (TS) hydrolysed and amount of starch hydrolysed from 100 g tested foods at different time from 0 to 180 min. In general, the hydrolysis rate of starch from all tested food was similar, the amount of glucose released increased gradually with time and reach plateau at 60-90 min, however, by increasing digestion time hydrolysis rate decreased gradually.

Table 5.1 represents the rate of starch digested at different times and area under glucose concentration curve $AUC_{PPAA+AMG}$ of five potatoes. There were significant differences ($p \leq 0.05$) in the percent of the starch hydrolysed at different times and $AUC_{PPAA+AMG}$ between varieties. Rooster showed

consistently highest extent of digestion. Meanwhile Maris Piper and Desiree is showing lower level of digestibility compared to Mozart and Maris Peer. In comparison with reference food (white bread) for carbohydrate digestibility (figure 5.1) Rooster and Maris Peer potato with AUC 1046.43 and 1044.82 respectively were showed higher percent of starch hydrolysis while, Maris Piper had significantly lower starch digestibility than reference food and other potato varieties ($p \leq 0.05$).

Carbohydrate profile based on it is digestibility was determined according to Englyst *et al*. (1992) and Englyst *et al*. (1996). Table 5.2 presents the calculated amount of DS over 180 min, RDS (starch digested at 30 min), SDS (between 30 to 120 min), RS calculated from the difference between TS and DS and rapidly available glucose RAG (sum of free sugar and RDS). The value of DS, RDS, SDS, RS and RAG were varied significantly among some potato varieties ($p \leq 0.05$). Rooster showed highest DS and the lowest level of RS, Maris piper potato had the lower RDS and RAG and higher SDS and RS than other potato varieties while, Mozart potato showed highest RDS among all potatoes and significantly higher RAG and lower SDS than Rooster and Maris Piper.

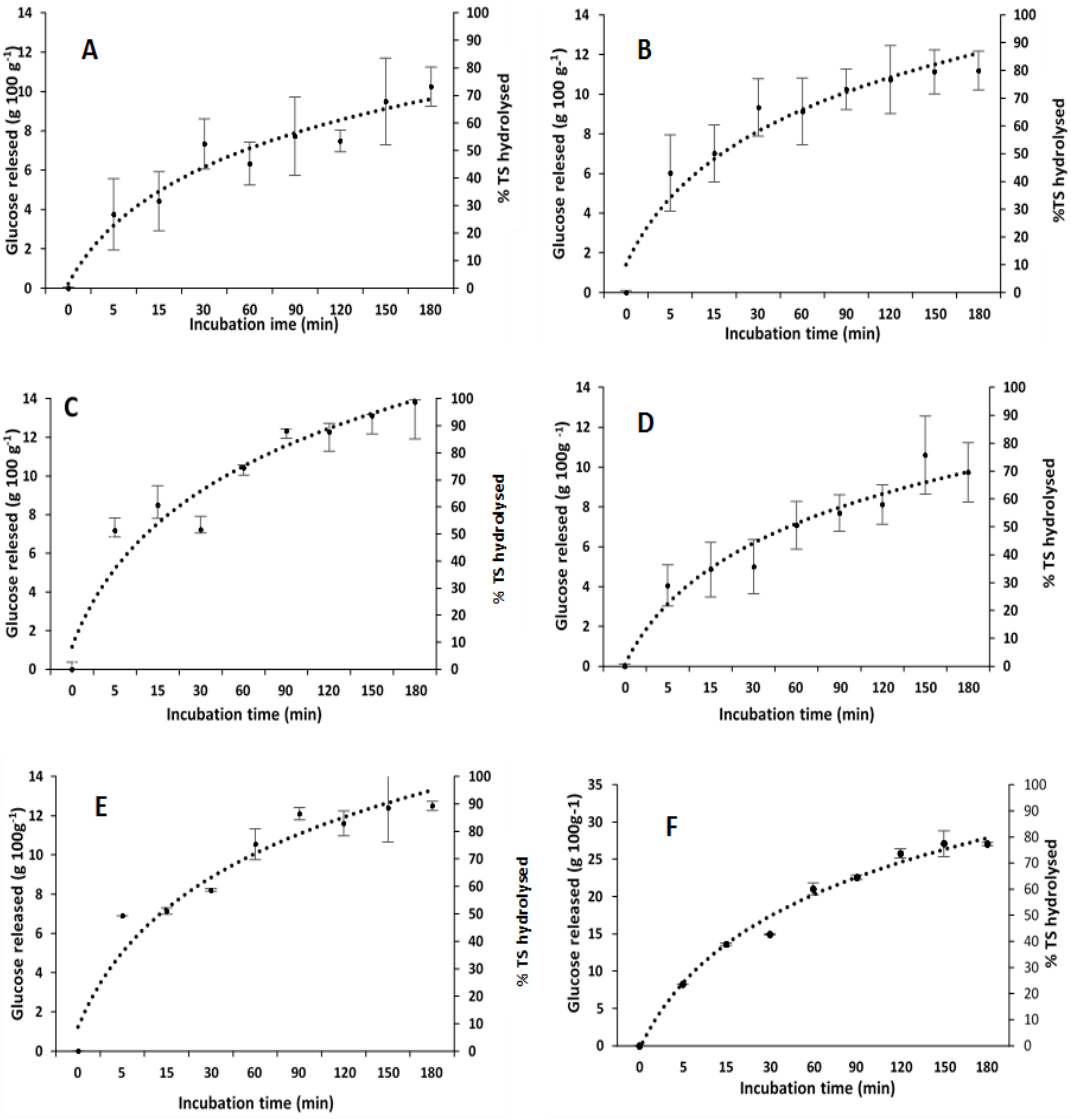


Figure 5.1 Starch hydrolysis curves of steam cooked potato samples digested with porcine pancreatic alpha amylase (PPAA) followed by amyloglucosidase (AMG). A) Desiree B) Mozart. C) Rooster. D) Maris Piper. E) Maris Peer. F) White bread. Data expressed as amount of glucose released and as % of total starch (TS) at each time point as measured by DNS (n=4). Error bars represent standard deviation of mean.

Table 5.1 *In vitro* kinetics of starch digestion in five steam cooked potatoes and incremental area under glucose curve (AUC). Data expressed as % of total starch hydrolysed at different times. Mean value ±SD (n=4).

Potato cultivars	5 min	15 min	30 min	60 min	90 min	120 min	150 min	180 min*	% AUC
Desiree	28.0^{c}	33.0^{c}	54.7^{a}	47.2^{c}	57.7^{c}	55.8^{d}	70.8^{c}	76.5^{c}	752.5±29.1^{b}
Mozart	34.5^{c}	40.1^{b}	53.3^{b}	53.3^{c}	60.6^{c}	62.8^{c}	63.6^{d}	64.0^{d}	970.4±38.8^{a}
Rooster	53.1^{a}	61.9^{a}	53.5^{b}	76.5^{a}	90.5^{a}	90.2^{a}	96.3^{a}	101.5^{a}	1046.4±48.5^{a}
Maris Piper	23.8^{c}	28.6^{d}	29.4^{c}	41.7^{d}	45.3^{d}	47.8^{e}	62.4^{d}	57.3^{e}	648.2±2^{b}
Maris Peer	43.7^{b}	45.3^{b}	52.0^{b}	66.9^{b}	76.8^{b}	73.7^{b}	78.7^{b}	79.3^{b}	1044.8±41.3^{a}

* Equilibrium concentration of starch hydrolysed after 180 min C∞ . Mean value within a column with different subscript letter ^{a,b,c,d,e} indicate significant differences (one-way ANOVA, $P \leq 0.05$).

Table 5.2 Carbohydrate profile of five potato varieties including total starch (TS), free sugars, digestible starch (DS), rapidly digestible starch (RDS), slowly digestible starch (SDS) , resistant starch (RS) and rapidly available glucose (RAG). Data expressed as mean value of g 100 g^{-1} ± SD (n=4).

Potato cultivars	TS[1]	Free sugars[1]	DS[2]	RDS[3]	SDS[4]	RS[5]	RAG[6]
Desiree	13.4±1.4^{b}	3.0±0.3^{a}	10.6±0.1^{b}	7.3±0.3^{b}	0.3±0.0^{b}	3.1±0.2^{b}	10.3±0.3^{a}
Mozart	17.4±1.2^{a}	0.8±0.1^{c}	10.4±0.3^{b}	9.3±0.5^{a}	0.5±0.2^{b}	6.2±0.3^{a}	10.1±0.5^{a}
Rooster	13.6±0.8^{b}	0.6±0.1^{c}	13.8±0.1^{a}	7.2±0.1^{b}	1.7±0.2^{a}	0.2±0.1^{c}	7.9±0.1^{b}
Maris Piper	16.9±3.6^{ab}	1.0±0.5^{b}	11.6±0.1^{b}	5.0±0.0^{c}	2.0±0.2^{a}	6.3±0.3^{a}	6.0±0.0^{c}
Maris Peer	15.7±2.4^{b}	1.2±0.4^{b}	11.5±0.3^{b}	8.2±0.7^{b}	2.1±0.8^{a}	3.2±0.3^{b}	9.4±0.7^{a}

[1] TS and free sugars measured in chapter 4

[2] amount of digestible starch over 180 min

[3] amount of digestible starch at 30 min

[4]Obtained from amount of starch digested at 120 min-RAG.

[5]Calculated from TS-DS

[6]Sum of RDS and free sugar.

Mean value within a column with different subscript letter ^{a,b,c} indicate significant differences (one-way ANOVA, $P \leq 0.05$).

5.4.1.2 Hydrolysis index (HI) and estimated glycaemic index (eGI)

The percentage of starch hydrolysed over 180 min was plotted and the area under the curve (AUC) was calculated. Hydrolysis index was calculated per individual potato variety by dividing the area under the hydrolysis curve of the potato samples with the area for a reference food as reported by Goni *et al*. (1997). White bread with HI=100 was used as reference food and the HI value of potato samples is represented in table 5.3. The estimated glycaemic index eGI was calculated from HI using the equation created by Goni *et al*. (1997) eGI= 39.71+ (0.549 x HI).

Table 5.3 shows the HI and eGI value of five potato varieties. There was significant variation between potato varieties in HI and eGI value ($p \leq 0.05$). Rooster potato exposed highest value of HI and eGI and Maris Piper had the lowest value among all potato samples tested. No significant difference was observed between Desiree and Mozart potato in HI and eGI values ($p \geq 0.05$).

Table 5.3 Hydrolysis index and estimated glyceamic index of five steam cooked potato varities. Data expressed as mean ± SD (n=4).

Potato cultivars	HI*	eGI
Desiree	97.76±4.16^{c}	92.95±2.43^{c}
Mozart	98.46±3.99^{c}	93.76±2.19^{c}
Rooster	128.73±2.37^{a}	110.39±1.30^{a}
Maris Piper	77.71±1.19^{d}	82.37±0.65^{d}
Maris Peer	121.55±4.44^{b}	106.44±2.44^{b}

*Hydrolysis index (White bread=100). Mean value within a column with different subscript letter ^{a,b,c,d} indicate significant differences (one-way ANOVA, $P \leq 0.05$).

5.4.2 Relationship between starch digestibility profile and HI

The Pearson's correlation was conducted to study the relationship between the HI and RDS, SDS and RAG calculated from *in vitro* kinetic of starch

digestion. Table 5.4 summarise the coefficient of variation *r.* The summary of correlations indicated that was no significant correlation between HI and each of RDS, SDS, RAG and RS expressed as g 100 g^{-1} *r*= 0.48, 0.20, 0.30 and -0.83 reactively. Any *p* value ≥ 0.05 indicates there is no correlation.

Table 5.4 Pearson correlation showing relationship between HI and starch digestibility profile RDS, SDS, RS and RAG (n=4).

Factors		HI
RDS	r	0.48
	p	0.40
SDS	r	0.20
	p	0.74
RS	r	-0.83
	p	0.07
RAG	r	0.30
	p	0.61

5.4.3 Relationship of AUC, HI and eGI with TPC and 5-CQA content of five potato varieties

Total phenolic content TPC and 5-CQA of five potato varieties are shown in table 4.5 and 4.6 respectively. Person correlation was conducted in order to study the relationship between *in vitro* starch digestibility and phenolic content of five potato varieties. Table 5.5 summarised the relationship between AUC, HI, eGI and potato phenolic content (TPC and 5-CQA) and any *p* value ≥ 0.05 indicates there is no significant correlation. TPC showed no significantly correlation with each of AUC, HI and eGI (*r*=-0.43, -0.44, -0.29 and *p*=0.46, 0.45, 0.63) respectively while, 5-CQA were inversely and significantly

correlated with each of AUC, HI and eGI and (*r*=-0.91, -0.91, -0.97 and *p*=5.2×10^{-8}, $5.2\times10^{-8}$and 4.0×10^{-13}) respectively.

Table 5.5 Pearson correlation (r) showing relationship between HI, eGI and phenolic content of five potato varieties.(n=20)

Factors		HI	eGI	AUC $_{PPAA+AMG}$
TPC	r	-0.43	-0.44	-0.29
	p	0.46	0.45	0.63
5-CQA	r	-0.91	-0.91	-0.97
	p	5.2×10^{-8}	5.2×10^{-8}	4.0×10^{-13}

5.4.4 Relationship of TPC and 5-CQA content with starch digestibility profiles of five potato varieties

Further correlation using Pearson correlations were conducted in order to investigate the relationships of TPC and 5-CQA with starch digestibility profile of five steam cooked potato varieties. Table 5.6 summarised the relationships and any p value ≤ 0.05 indicating there is correlations. RS positively correlated with TPC and 5-CQA while, inverse correlations were observed between 5-CQA and RAG, RDS and SDS however they were not significant statistically.

Table 5.6 Pearson correlation (r) showing relationship between starch digestibility profiles and phenolic content of potato varieties.

Factors		RAG	RDS	SDS	RS
TPC	r	-0.83	0.64	0.20	0.72
	p	0.07	0.24	0.22	0.16
5-CQA	r	-0.23	-0.33	-0.66	0.41
	p	0.69	0.58	0.73	0.48

5.4.5 Principle component analysis

Principle component analysis (PCA) was conducted in order to determine the relationship between potato composition (TS, DM, TPC, 5-CQA) and percent of hydrolysed starch (as measured using $AUC_{PPAA+AMG}$). Figure 5.2 shows the loading and scores of the characteristics of the five potato cultivars with the first two PCs explaining 72% of the total variance respectively. PC1 explained 47.76% and PC2 explained 24.38% of total variance. For PC1, TS, DM, 5-CQA and TPC had positive loading and starch hydrolysis had negative loading. TS and DM had positive loading for PC2 while 5-CQA and TPC had negative loading for PC2. According to the PCA scores, potato varieties segregated according to their components: Maris Piper showed high DM, high 5-CQA content and low digestibility. Rooster, Maris Peer and Mozart potato appeared in one cluster and showed the opposite characteristics.

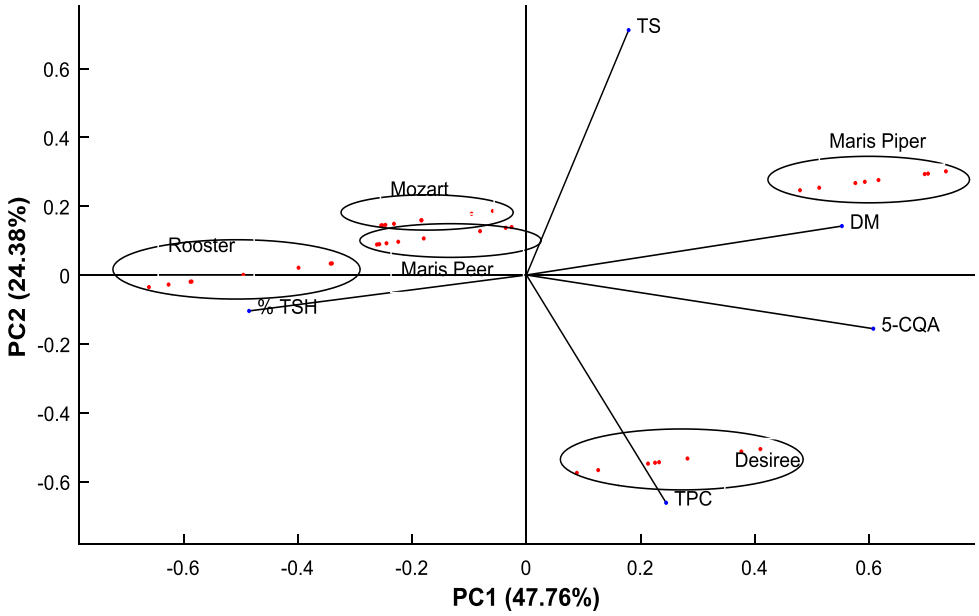


Figure 5.2 Principle component analysis (PCA) using composition and digestibility data from five potato varieties. Graph shows loading of potato components; total starch (TS), dry matter (DM), total polyphenol content (TPC), 5-caffeoylquinic acid (5-CQA) content and percentage total starch hydrolysed (%TSH) and scores of potato varieties according to PCA 1 and 2.

5.5 Discussion

5.5.1 *In vitro* starch digestion

It has been suggested that *in vitro* starch digestibility can predict the *in vivo* glycaemic response (Ek *et al*., 2014a, Goni *et a*l., 1997, Granfeldt *et al*., 1992). In present study starch of potato varieties was digested *in vitro*. Pepsin was used as protease to avoid protein, starch interaction (Jenkins *et al*., 1987).

The differences in the rate of starch digestion in starchy food provide a plausible mechanism for differences in glycaemic responses to these foods (Englyst *et al*., 1996). Boiled potatoes considered to have a high susceptibility to alpha amylase than other plant foods such as lentil, chickpeas and beans (Goni *et al*., 1997). Significant differences were observes between cultivars in HI. HI value ranged from 77 to 128. HI of 112 and 103 has been reported for boiled potato (Goni *et al*., 1997, Rosin *et al*., 2002).Thus, steam cooked Rooster and Maris Peer potato had higher and Maris Piper had lower HI value than reported for boiled potato. The differences between studies could be due to botanical variation among potatoes, or the effect of processing (boiled vs steamed).

The HI values were applied to equation to estimate GI value. eGI ranged from 92.95 to 110.39 thus, according to classification, all potato varieties in this study can be considered as high GI because their eGI value is higher than 70 (Brand-Miller *et al*., 2003).

5.5.2 Relationships between phenolic content and *in vitro* starch digestibility

As shown in chapter 3, 5-CQA is an inhibitor of amylolytic enzymes. According to Pearson's correlation, *in vitro* starch digestibility with PPAA+AMG over 20 min (AUC, HI, eGI) in this study was significantly ($p < 0.001$) and inversely correlated with 5-CQA and TPC. This supports the results of chapter 3 on the digestion of a commercial starch over 20 min. In this study, the amount of steam cooked potato used as substrate contained 10 mg mL^{-1} of starch (similar to the first experiments) and around 0.08 mg mL^{-1} 5-CQA. The level of 5-CQA is low compared to the levels used in chapter 3, enough to inhibit around 5.8% of PPAA activity.

Interestingly, there was not a significant correlation between RAG, RDS and SDS of cooked potato and phenolic content (TPC and 5-CQA) content. This suggests that the effect of 5-CQA on digestion of native starch may occur over the duration of the reaction, rather than at particular time points.

Furthermore, According to the PCA scores, potato varieties segregated according to their components: Maris Piper showed high DM, high 5-CQA content and low digestibility. Rooster, Maris Peer and Mozart potato appeared in one cluster and showed the opposite characteristics. The digestibility of Desiree appeared to be heavily influenced by TPC, in contrast to the other varieties. It appears that the same single factor does not strongly determine digestibility in all varieties. This is similar to the results obtained in the previous chapter.

According to Englyst *et al*. (1996) RAG measurement *in vitro* can be an approximation of glucose likely to be absorbed and accounts for 61%

glycaemic response *in vivo*. Recently Pinhero *et al*. (2016) identified that RDS and RS contributed to eGI, former positively correlated and latter negatively correlated with eGI. In the present study, SDS did not correlate with HI while, RS negatively r=-0.83 correlated with HI. This result was in contrast to results obtained by Pinhero *et a*l. (2016) and Moreira and Wolever 2011. They reported that either SDS and RS affect the eGI by lowering the blood glucose level either by slowing down the digestion or the starch not being digested at all in the small intestine.

No significant positive correlation was observed between HI and RAG or RDS in this study. These results are not in agreement with results obtained by Pinhero *et al*. (2016). The present study evaluated only 5 potato varieties while, Pinhero *et al*. (2016) evaluated 14 varieties and compared different cooking methods. Therefore the higher number of varieties may be required to improve the observations.

5.6 Conclusion

In vitro kinetic of five potato varieties was measured for estimation of GI and calculation of RAG, RDS, SDS and RS. This study for the first time examined the correlation of potatoes TPC and 5-CQA with eGI and starch digestibility profile RAG RDS, SDS and RS . Pearson correlation indicated that TPC and 5-CQA level negatively and significantly affected cooked potato starch digestibility with PPAA+AMG, but PCA analysis showed that not a single factor determines starch digestibility in all varieties. These results suggest that potatoes with higher 5-CQA and TPC level could have lower rates of starch digestibility *in vivo*.

6 Physical and chemical properties of potato starch from different varieties of potatoes

6.1 Introduction

Up to now, the focus of the thesis was on determining whether the phenolic content of potato would affect digestibility of starch in different varieties. However, intrinsic starch factors may also influence digestibility. According to Singh *et al*. (2003) morphological, thermal and rheological properties of starches from different botanical sources differ. Potato varieties may differ in their starch structure and properties consequently, this may affect the rate of starch hydrolysis and glucose response after consumption, digestion and absorption. Recently, differences in functional properties (gelatinisation, hydrolysis and *in vitro* digestion) of starches from 10 rice varieties have been reported to be attributed to the diversity in starch molecular components and crystalline structure (Cai *et al.*, 2015).

Potato starch is different compared to cereal starches (corn, wheat, rice, etc), because of their larger granule size and their smooth and oval shape, longer amylose and amylopectin chain lengths and having the presence of phosphate ester groups on amylopectin (Alvani *et al*., 2011, Kaur *et al*., 2007, Wischmann *et a*l., 2007, Yusuph *et al*., 2003, Singh *et al*., 2003, Hoover, 2001).

Many published studies have shown that starch structure (physical and chemical properties) influenced starch degradation and digestion. For example amylose content of starch has a significant inverse effect on starch hydrolysis, where high amylose starches showed low hydrolysis and may delay carbohydrate absorption (Goda *et al*., 1994, Rendleman 2000, Hu *et al*.,

2004, Tester *et al*., 2006). Panlasigui *et al*. (1991) determined that rice with similar amylose content 26 and 27% showed different rates of starch digestibility and glycaemic response. Therefore, it is important in order to predict starch digestibility and glycaemic response not only rely on amylose content.

Starch granule size is also important for controlling the rate and extent of starch digestion. Different sized potato starch granules vary in their functional properties, therefore granule size distribution has been suggested as an important factor that can influence the behaviour of potato starch during processing (Singh and Kaur, 2004). Dhital *et al.* (2010) reported that the digestion rate of smaller granule size of maize (< 10 µm) and potato starch (< 20 µm) showed higher rate of digestion when compared with those of medium and larger granules of the same starches. This difference indicated that in smaller granules there is more surface area available for enzyme activity and consequently, an increased hydrolysis rate.

Starch is a semi-crystalline polymer and varies in crystallinity depending on the source of the starch. There is a possibility that variations in internal structure of starch granules are likely to have an effect on starch digestibility. The proportions and distribution of crystallinity control the rate of starch hydrolyses, Lamellar distance varies significantly between rice starches from different cultivars negatively and significantly correlated with *in vitro* starch digestion (Cai *et al*., 2015). In regards to potato, no changes appeared in the amount of crystalline and lamella thickness of starch extracted from single potato grown at different temperatures (Protserov *et al*., 2002) and different potato varieties grown under the same condition (Yusuph *et al*., 2003). Small

angle X-ray scattering is one powerful technique for the study of the structure of starch as it enables structural information in the range 1-100 nm.

6.2 Aims

The aim was to investigate the physical and chemical properties of starch isolated from the same varieties described in chapters 4 and 5. The study also aimed to analyse the correlation between physio-chemical properties of starch from different potato varieties and *in vitro* digestion.

6.3 Hypothesis

The hypothesis is that potato with higher amount of amylose, higher crystallinity and larger granule size may show higher resistance to enzymatic hydrolysis.

6.4 Objectives

- To measure amylose content of potato starch by colorimetric method.
- To determine starch granule dimensions and distribution by static light scattering analysis using Malvern Mastersizer, 3000.
- To examine crystallographic properties of potato starch by small angle X-ray scattering (SAXS) using Anton Paar SASXpace.
- Determine the correlation between potato varieties starch physiochemical properties and *in vitro* digestion determined in the chapters 4 and 5.

6.5 Results

6.5.1 Amylose standard and amylose content

The amylose content of defatted potato starches was determined by iodine based colorimetry using urea dimethyl sulphoxide UDMSO and referenced to standards (α-glucan basis). Mixtures of amylose/amylopectin were used for preparation of a standard curve as previously shown by Juliano *et al*. (1981). Amylose/amylopectin mixtures showed a better response rather than amylose alone at a wavelength of 620 nm. Figure 6.1 represents the amylose/amylopectin standard curve that has been used in this study for calculation of the amylose of content of potato starches. The colour (blue) intensity depended on the reaction of either amylose or amylopectin with iodine. More bluish colour indicating more amylose detected (figure 6.2).

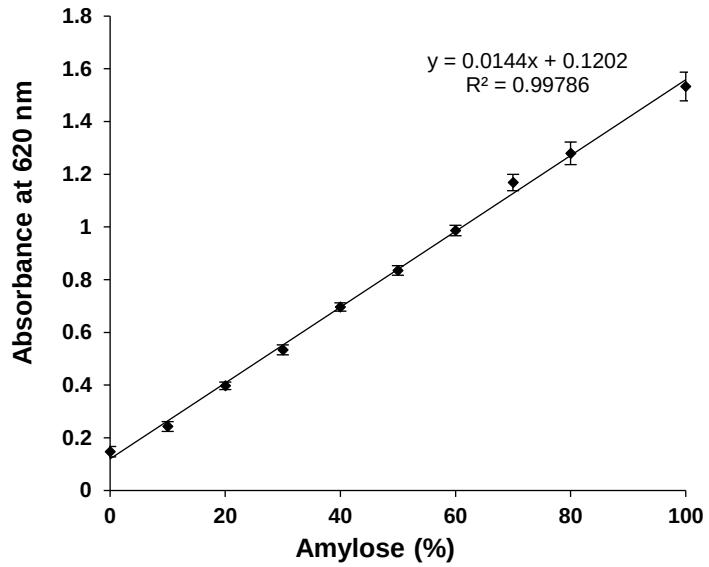


Figure 6.1 Standard curve for measuring amylose content in potato starches using amylose/amylopectin mixture. n= (9) Error bars represent standard error of mean.

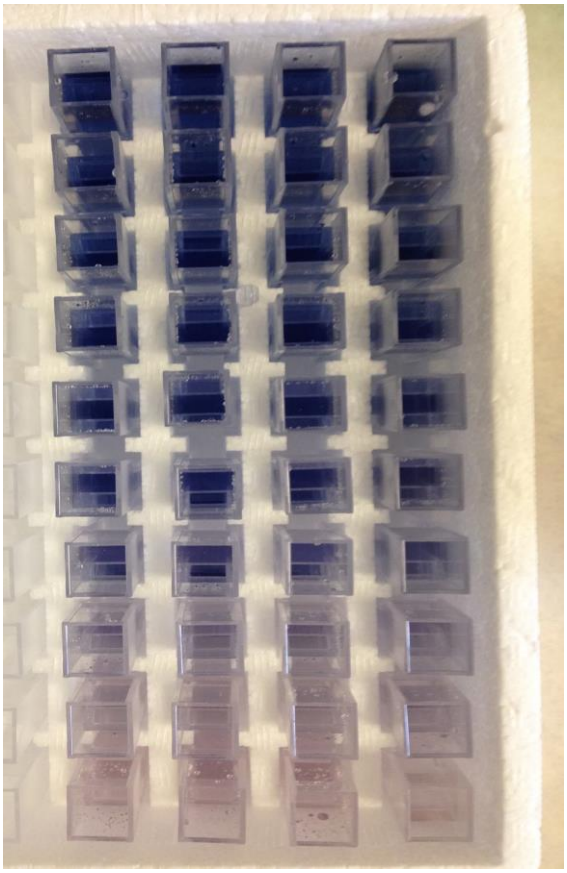


Figure 6.2 Iodine colorimetry using amylose standard ranging from 0 to 100% measured at 620 nm (n=4).

Table 6.1 shows the average percentage of amylose content in potato starches. The amylose content of potato starches ranged from the lowest level in Maris Piper 26.2% to the highest level in Maris Peer 31.6% and the average was 28.8%. Significant differences were found in amylose content among some potato starches ($p \leq 0.05$). Maris Peer starch showed a significantly higher amount of amylose content when compared with Maris Piper and Rooster potato but it was not statistically different from the amylose content of Mozart and Desiree potato starch ($p \geq 0.05$). There were also no significant differences between Maris Piper, Rooster and Desiree potato starches in amount of amylose content.

Table 6.1 Amylose content of potato starches. (mean with their SD, n=8)

Potato samples	Amylose content %
Desiree	28.68^{abc} ±5.11
Mozart	29.49^{ac} ± 4.46
Rooster	28.06^{bc} ± 5.28
Maris Piper	26.20^{b} ± 2.35
Maris Peer	31.63^{a} ± 3.05
Average	28.81±1.98

Amylose content is expressed as α-glucan content of potato starch (average mean = 87%) (Alvani *et al*., 2011).value with same letter ^{a,b,c} are not different significantly ($p > 0.05$)

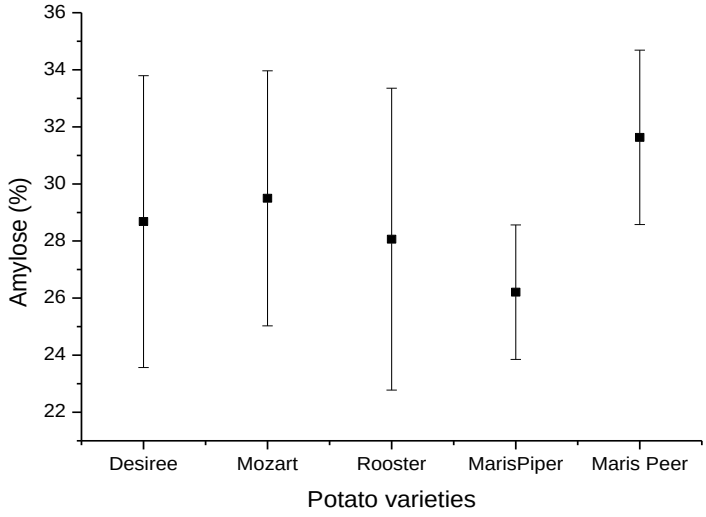


Figure 6.3 Variability in amylose content of different potato varieties starch (n= 8).

The amylose content of five potato varieties in this study was consistent with the range of amylose content reported for potatoes of 24.4 to 30.9% (Cottrell *et al*., 1995) 25.8 to 31.2% (Yusuph *et al*., 2003), 23.4 to 31.0% (Weisenborn *et al*., 1994) and 25.2 to 31.2% (Kuar *et al*., 2002). On the other hand, results were slightly higher than the range reported in 10 UK potato starches (grown under same condition) by Alvani *et al* (2011) (25.5 to 29.1% with average 26.9%) two varieties (Desiree and Maris Piper) used this study were also

tested, while the results were considerably higher than range previously reported by Kaur *et al* (2007) of 15 to 23.1% the potato varieties tested were not similar to varieties tested in present study.

Some potato varieties in this study showed different amounts of amylose content compared to reported amounts of same potato elsewhere and some others showed a similar value. Although, amylose content of Maris Piper potato 26.2% were consistent with the amount detected in Maris Piper elsewhere by Alvani *et al*. (2011) and Yusuph *et al*. (2003). Desiree potato amylose content was 28.6% and these value was higher than those reported by Alvani *et al*. (2011) (25.5%) and less than the amount reported by Yusuph *et al*. (2003) (31.2%).

6.5.2 Granule size distribution

Typical volume and surface particle size distributions of potato starch granules are shown in figure 6.4. Granules size from 10 to 100 µm comprised a large proportion of potato granules. The range was 9.86 to 98.1 µm and this is consistent with the results of microscopy images shown in figure 6.5. The narrow range of particle size 9.8 to 66.9 µm was observed in Mozart potato among all the potato varieties. Rooster and Maris Piper potato had the widest range of granule size distribution 11.2 to 98.1 and 14.5 to 98.1 µm respectively. Maris Peer and Desiree potato on the other hand showed similar granule size distribution 12.7 to 86.4 and 14.5 to 86.4 µm respectively this was also consistent with the microscopy images of these Desiree and Rooster varieties (figure 6.5). This result agreed well with results obtained by Vasanthan *et al*. (1999) of Alberta potato starches.

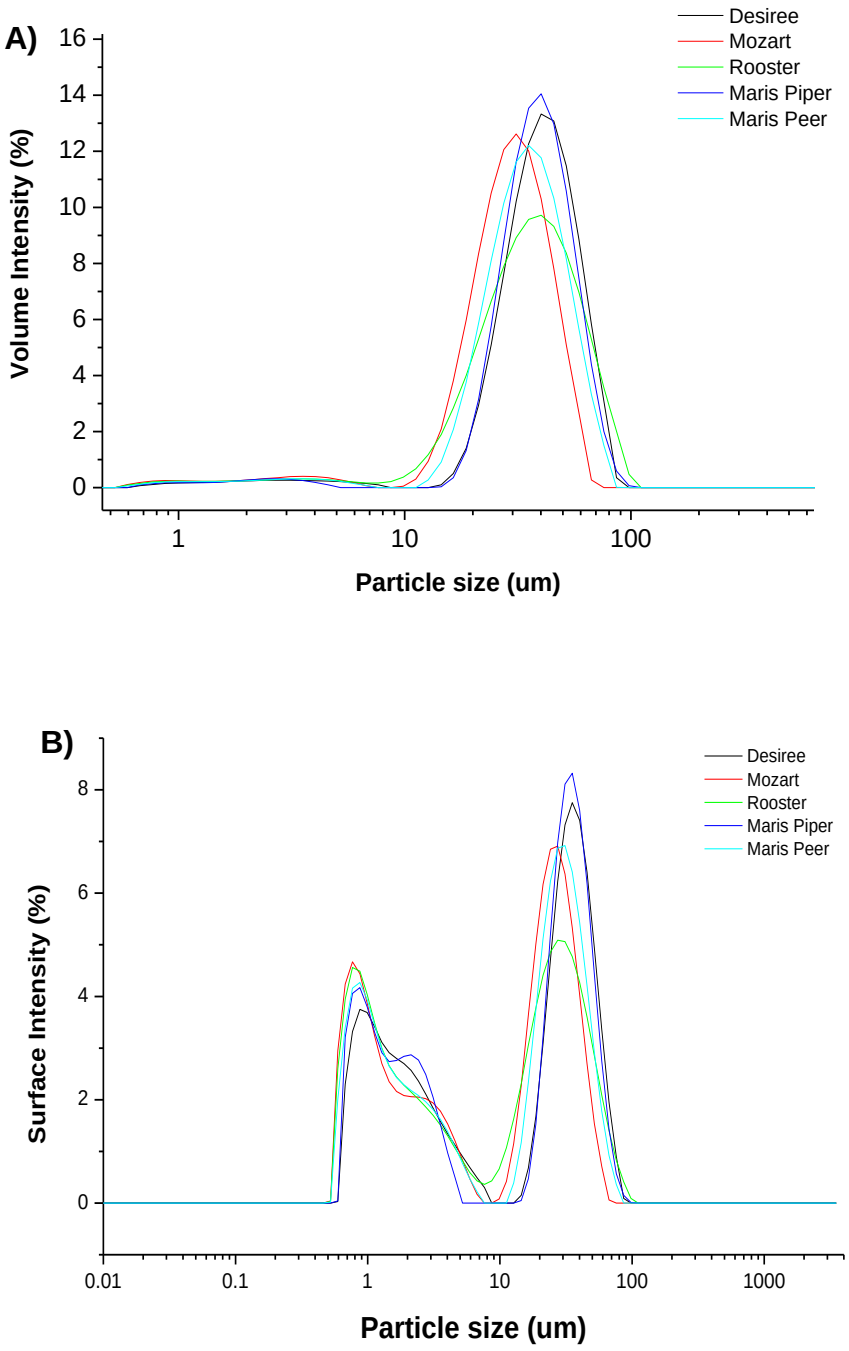


Figure 6.4 granule size distributions of potato varieties starch. A) Volume based distribution. B) Surface based distribution. (n=5)

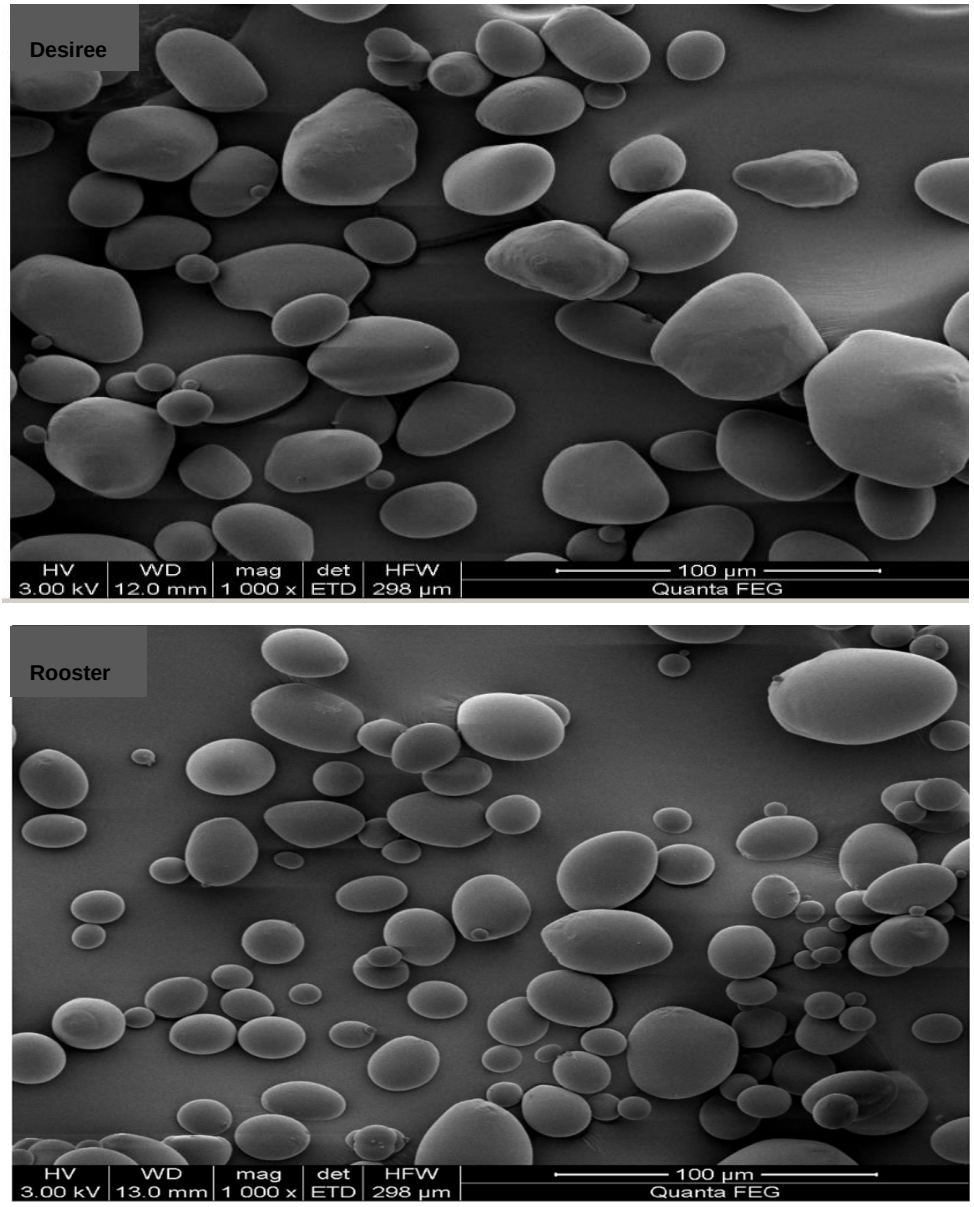


Figure 6.5 Microscopy images of native potato starch. 1000X magnification.

The parameters of size distribution based on volume namely the 10th ($Dv_{0.1}$), 50th ($Dv_{0.5}$) or median and 90th ($Dv_{0.9}$) percentiles as well as volume weighted mean $D_{4,3}$ and surface weighted mean $D_{3,2}$ are presented in table 6.2.

The median granule size value $Dv_{0.5}$ is defined as the size in micron where half of the particle resides above this value and half below this value. The $Dv_{0.5}$ values ranged from 31.30 to 42.92 µm for potato starches granules. Mozart significantly showed the lowest value of median granule size, among other varieties and Desiree was the highest ($p \leq 0.05$). The $Dv_{0.9}$ and $Dv_{0.1}$ value indicated that as 90% and 10% of granules are above and below these values respectively. The $Dv_{0.9}$ and $Dv_{0.1}$ for potato starches granules ranged from 50.12 to 69.18 and 17.10 to 25.10 µm respectively. Significant differences in the diameters of granule size distributions between the potato starches were clearly detected from the results presented in table 6.2 ($p \leq 0.05$) and this was consistent with shift of the position of the peaks observed in figure 6.4 (A and B).

Table 6.2 Diameters of potato starch granules. (Mean values ± SD, n=5).

Potato samples	$Dv_{0.1}$ µm	$Dv_{0.5}$ µm	$Dv_{0.9}$ µm	$D_{3,2}$ µm	$D_{4,3}$ µm	Specific surface area m^2/g
Desiree	24.54±0.24^{b}	42.92±0.40^{a}	69.18±2.80^{a}	24.02±0.21^{a}	44.56±0.90^{a}	0.249
Mozart	17.30±0.16^{d}	31.30±0.16^{e}	50.12±1.16^{d}	16.85±0.23^{e}	32.17±0.41^{e}	0.3565
Rooster	17.10±0.00^{e}	37.91±0.04^{c}	69.11±0.07^{a}	18.80±0.00^{d}	40.51±0.04^{c}	0.3194
Maris Piper	25.10±0.00^{a}	40.80±0.14^{b}	63.06±0.58^{b}	23.02±0.08^{b}	42.08±0.24^{b}	0.2612
Maris Peer	20.32±0.17^{c}	36.70±0.24^{d}	60.84±1.65^{c}	20.04±0.23^{c}	38.44±0.52^{d}	0.2991

Mean value within a column with different subscript letter ^{a,b,c,d,e} indicate significant differences (one-way ANOVA, $P \leq 0.05$).

The results of median granule size distributions in this study were consistent with those obtained elsewhere. According to Noda *et al*. (2005) the median granule sizes for large, small and extremely small potato starch are 39.9-43.7, 20.3-23.4 and 13.2-14 µm respectively. Similar results have been obtained by Dhital *et al.* (2010) with a median granule size distribution for fractioned potato starch granules from very small (<20 µm) to very large (> 55 µm) with a range between 16.86 to 69.86 µm.

Surface mean $D_{3,2}$ also called Sauter mean is the weighted average surface area, based on the assumption that the spherical particles are of the same surface area as the actual particles. The $D_{3,2}$ value of starches significantly varied between potato varieties ($p \leq 0.05$) and ranged from the lowest value in Mozart (16.85 µm) to the highest value in Desiree (24.02 µm) with a mean of 20.56 µm. These values are similar to results obtained by Ibrahim and Achudan (2011) with a $D_{3,2}$ for native potato starch of 16.16 µm. The results are also consistent with results of diffraction potato starch from large to small granules with $D_{3,2}$ value 15.96 and 67.74 µm respectively. While, the results were lower than of that obtained from potato starch 30.47 µm (Dhital *et al*., 2010).

The mean volume diameter $D_{4,3}$ is the weighted average volume, assuming spherical particles have the same volume as the actual particles. The $D_{4,3}$ value also significantly ($p \leq 0.05$) differed among potato varieties and range were about 32.17 to 44.56 µm from lowest in Mozart and highest Desiree potato and with a mean of 38.55 µm for all potato starches. With the exception of Mozart, all other potato starch results were consistent with that obtained elsewhere by Weisenborn *et al*. (1994) reporting that the mean granule

diameter of starches from 44 potato samples ranges between 38.7 to 53.8 µm.

The specific surface area is defined as the total area of particles divided by the total weight, based on the assumption that spherical granules have uniform density. Potato starch specific surface areas in this study were ranged from 0.249 to 0.356 $\mu m^2 g^{-1}$. Mozart potato had the highest value of specific surface area and Desiree had the lowest amongst all starches. The specific area of Mozart, Rooster and Maris Peer potato starch was consistent with Molenda *et al*. (2006) reporting 0.315 $m^2 g^{-1}$ for potato starch using Mastersizer 2000. However, many researchers using Coulter counter Multisizer, nitrogen adsorption and Malvern Mastersizer Hydro 2000 MU [1] (Dihital *et al*., 2010, Yusuph *et al*., 2003, Alvani *et al*., 2011) reported the specific area of potato starch between 0.130 to 0.160 $\mu m^2 g^{-1}$. This range is lower than the calculated value of specific surface of potato starches in this study using Malvern Mastersizer 3000 UM.

The correlation coefficient was determined for potato starch granule size diameters and specific surface area. Any *p* value ≤ 0.05 indicates that there is significant correlation between potato starch granules size diameters. Table 6.3 shows the correlation between the specific surface area of potato starches and median Dv_{50}, $D_{3,2}$ and $D_{4,3}$ of starch granules size distributions. Significant negative correlations were found between the specific surface area of starches and each of Dv_{50}, $D_{3,2}$ and $D_{4,3}$ values $p = 8*10^{-3}$, $3*10^{-4}$ and 0.02 respectively.

Table 6.3 Pearson Correlation (r) showing simple linear relationship between specific surface area and granules size parameters Dv_{50}, $D_{3,2}$ and $D_{4,3}$

		Specific surface area
Dv_{50}	r	-0.96
	p	8×10^{-3}
$D_{3,2}$	r	-0.99
	p	3×10^{-4}
$D_{4,3}$	r	-0.92
	p	0.02

6.5.3 Crystallinity

Small angle X-ray scattering (SAXS) was used to investigate the semi-crystallinity structure of potato starch. The SAXS peak parameters: peak position q_{max}, maximum intensity I_{max} and peak area were obtained by fitting the scattering profile to a Lorentzian function. The average inter lamellar distance (d) also called Bragg's spacing, was calculated according to Bragg's equation $d=2\pi/q$. The values of SAXS peak parameters are shown in table 6.4.

Table 6-4 Potato varieties starch structure parameters obtained from SAXS.

Potato varieties	$q_{\text{first peak}}$ (nm^{-1})	Peak area	I_{max}	d (nm)
Desiree	0.67	0.14845[d]	0.56	9.377
Mozart	0.66	0.14799[d]	0.57	9.519
Rooster	0.65	0.18291[a]	0.69	9.666
Maris Piper	0.65	0.17287[a]	0.70	9.666
Maris Peer	0.66	0.16621[c]	0.63	9.519

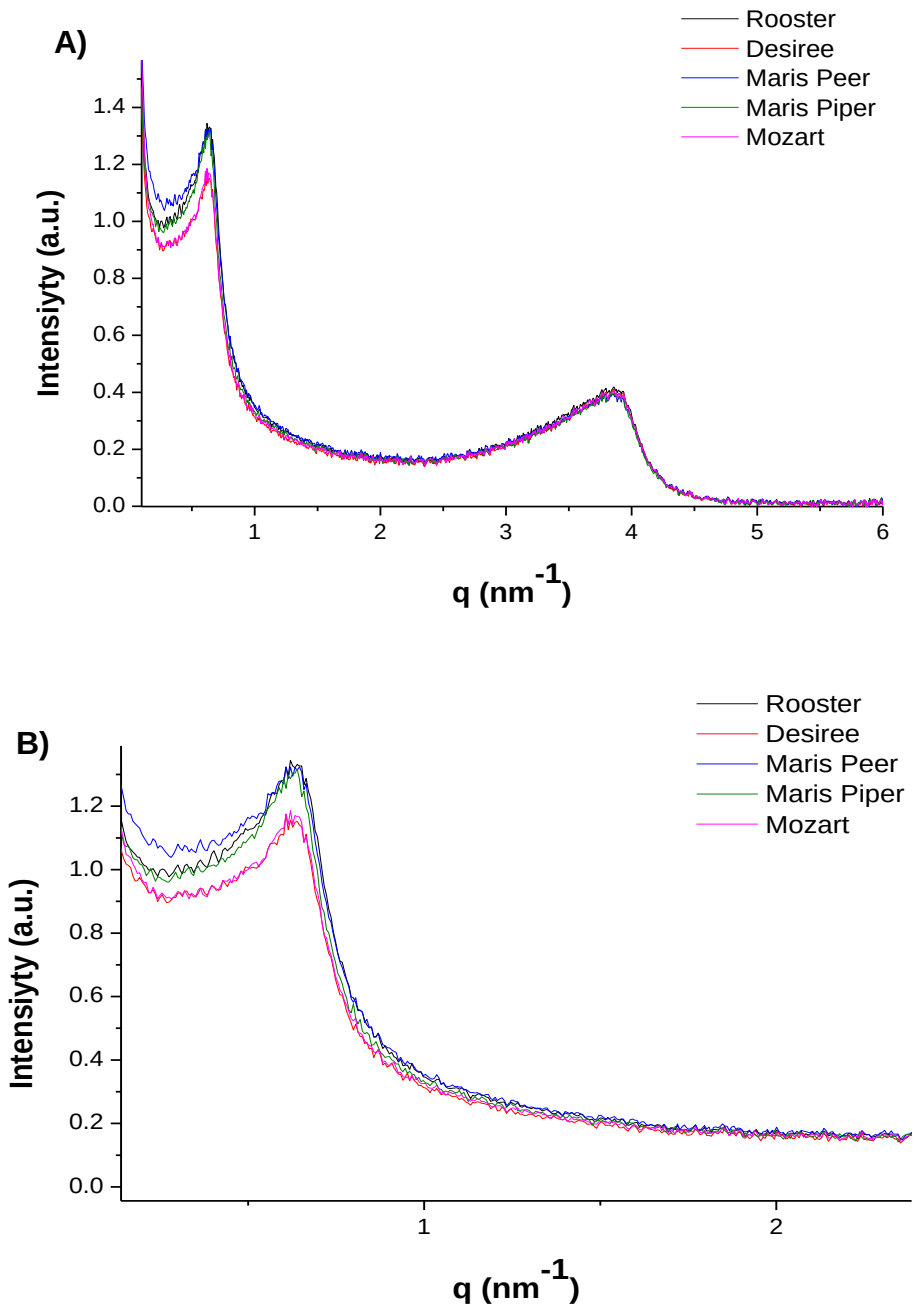


Figure 6.6 The SAXS patterns of potato starch isolated from 5 varieties. A) Show two peaks B) show first peak only. (n=5).

Figure 6.6 shows the potato starches SAXS curves, which show well-defined peaks by SAXS pattern. The first peak was observed at position ranging from to q= 0.65 to 0.67nm^{-1} and corresponds to the lamellar repeat distance or Bragg spacing ranging between 9.37 to 9.66 nm. In native starch this peak is thought to arise from the lamella of amylopectin. According to Blazek and

Gilber (2011) the location of the peak depends on the size of lamella. The second peak position about 3.90 nm^{-1} is specific for B-type starch. Thus, this result was in agreement with the values of peak positions q obtained by Zhu *et al*. (2012) for the first peak (0.68 nm^{-1}) of native potato starch and corresponds to 9.25 nm Bragg's distance. The scattering peak intensity I_{max} of potato starch was also determined and ranged from lowest value in Desiree potato 0.56 to highest in Maris Piper potato 0.70.

The SAXS peak area varied between potato starch. The variability in potato starch SAXS peak areas is shown in figure 6.7. Rooster potato had sharp peak with the largest area of 0.182 amongst all the potato starches. Maris Piper and Maris Peer, also showed similar peaks below Rooster peak 0.172 and 0.162 respectively, while, Desiree and Mozart had similarly boarder and smallest peaks at 0.148 and 0.147 respectively in contrast to other potato starches.

Correlations were obtained to analysis relationship between SAXS parameters and measured amylose content of potato varieties starch used in this study and the results are summarised in table 6.5. Amylose content showed trend but non-significant correlation with peak area, I_{max}, lamella spacing (d) and peak position (q).

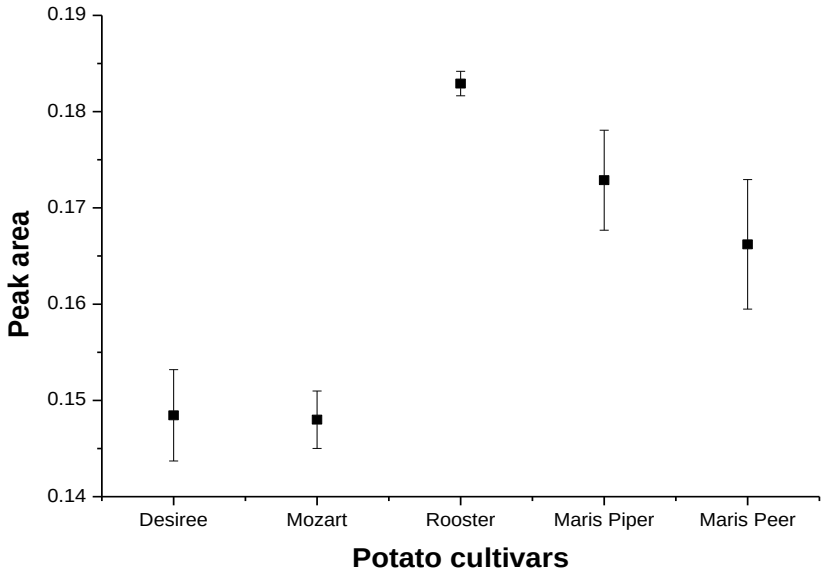


Figure 6.7 Variability in peak area for potato varieties starch. Error bars representing standard deviations of three replicates.

Table 6.5 Correlation between amylose content and peak position (q) and lamella distance (d)

		Amylose content
Peak position (q)	r	0.48
	p	0.40
Peak area	r	-0.32
	p	0.59
Lamella spacing (d)	r	-0.49
	p	0.39
Peak Intensity I_{max}	r	-0.49
	p	0.39

6.5.4 Correlation between physical and chemical properties of potato starch and *in vitro* digestibility

The results of *in vitro* hydrolysis of different potato varieties are presented in chapter 4 and results of potato starch amylose content and granule size distribution are shown in table 6.1 and 6.2. In order to evaluate the relationship between structural properties (physical and chemical) of potato starch and hydrolysis of potato varieties by PPAA and PPAA+AMG, Pearson correlation coefficients were determined for area under *in vitro* starch digestion curve (AUC) of potato varieties, released sugar was measured using DNS and HPAEC-PAD methods. The correlation coefficient was calculated for all potato starch properties with p value ≤ 0.05 indicated a statistically significant correlation.

Table 6.6 shows the correlations between eGI, AUC (PPAA+AMG), AUC (PPAA) and amylose content of starch, granule size distributions and SAXS parameters. Estimated glycaemic index eGI and AUC (PPAA+AMG) showed non-significant positive correlation with amylose content r= 0.59 and 0.79 respectively, while AUC (PPAA) as sugar measured by DNS and HPAEC-PAD showed non-significant negative correlation with amylose r=-0.11 and -0.38 respectively. There were no significant correlation between eGI and AUC of potato starch digestion using both PPAA+AMG and PPAA (DNS) and each of granule size distribution $Dv_{0.5}$, $D_{3,2}$ and $D_{4,3}$, specific surface area and SAXS parameters of starch crystallinity ($P \geq 0.05$).

Table 6.6 Pearson correlation (r) showing linear relationship between predicted GI and amylose content, granule size diameters and SAXS parameters of potatoes varieties. (n=5)

		Correlations of potato starch component, size distributions and SAXS parameters with *in vitro* starch digestion			
		PPAA+AMG		AUC (PPAA)	
		eGI[1]	AUC	DNS*	HPAEC-PAD*
Amylose	r	0.59	0.70	-0.11	-0.38
	p	0.29	0.18	0.85	0.52
Granule size distribution					
$Dv_{0.5}$	r	-0.27	-0.65	-0.39	0.24
	p	0.65	0.22	0.51	0.68
$D_{3,2}$	r	-0.48	-0.79	-0.52	-0.05
	p	0.40	0.11	0.35	0.93
$D_{4,3}$	r	-0.12	-0.54	-0.33	0.34
	p	0.83	0.34	0.57	0.57
Specific surface area	r	0.42	0.75	0.57	0.01
	p	0.47	0.14	0.31	0.97
SAXS peaks parameter					
q_{max}	r	-0.06	-0.08	0.03	-0.44
	p	0.91	0.89	0.95	0.45
Peak area	r	0.37	0.18	-0.18	0.66
	p	0.53	0.77	0.79	0.21
d spacing	r	0.06	0.07	-0.03	0.45
	p	0.91	0.90	0.95	0.44

GI[1] estimated from HI using Goni *at al*., 1997 equation.*sugar detection method.

6.5.5 Principle component analysis (PCA)

Principle component analysis was conducted in order to determine the relationship between potato physiochemical properties (amylose content, granule size, crystallinity) and percent of hydrolysed starch (as measured using AUCPPAA+AMG and PPAA). Figure 6.8 show the loading and scores of the characteristics of the five potato cultivars with the first two PCs explaining 84.73%, 81.96% and 82.11% of the total variance respectively.

According to figure 6.8 A, B granule size distribution parameter ($Dv_{0.5}$, $D_{3,2}$ and $D_{4,3}$) and SAXS parameters (peak area, peak position and Imax) had positive loading for PC1 while, starch hydrolysis by PPAA+AMG and PPAA sugar measured by DNS, amylose content and specific surface area had negative loading. While, in figure 6.8.C starch hydrolysis by PPAA sugar measured by HPAEC-PAD had positive score for PC1.

According to PCA in all three cases, Maris Piper and Desiree potato had highest granule size peak area, peak position and I_{max} while, Maris Peer, Mozart and Rooster had higher starch digestibility, amylose, specific surface area. This was consistent with results in table 6.1 and 6.2.

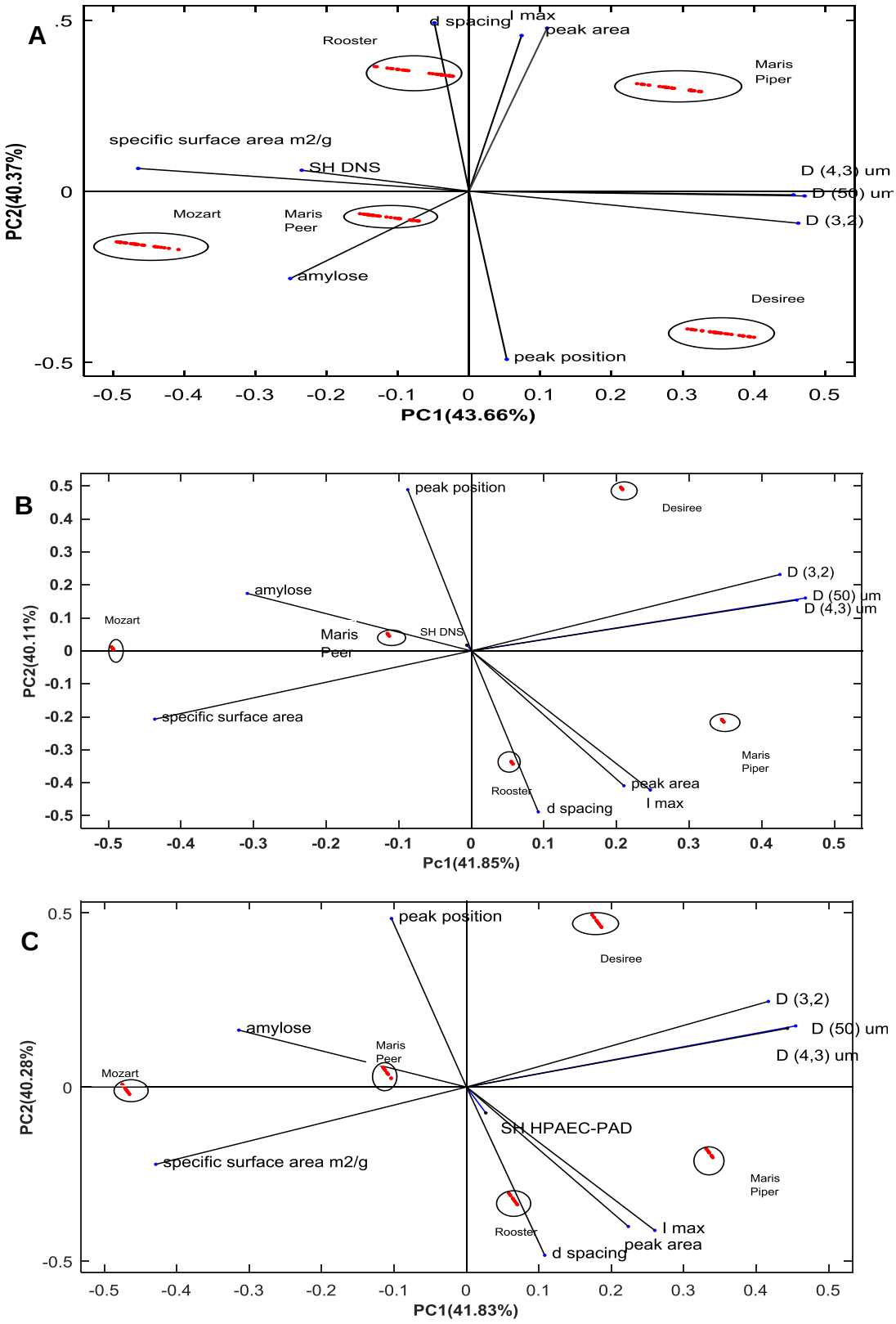


Figure 6.8 Loading of potato varieties characteristics (physio-chemical properties and *in vitro* digestibility) and scores of on first and second principle components. A) Starch hydrolysed by PPAA+AMG. B). Starch hydrolysed by PPAA and sugar detected by DNS.C) Starch hydrolysed by PPAA and sugar detected by HPAEC-PAD

6.6 Discussion

6.6.1 Amylose content

In general the range of amylose content of five potato varieties in this study was in range with those reported by other investigators. The difference in amylose content of same potato cultivars may be due to harvest dates, environmental conditions such as temperature, canopy coverage, storage, as well as differences in starch isolation and analytical methods used for measuring the amylose (Jansky and Fajardo 2014, Kaur *et al*., 2007, Noda *et al*., 2004, Kim *et al*., 1995, Weisenborn *et al*., 1994, Cottrell *et al*., 1995). Recently, differences in amylose content and *in vitro* digestibility of separated heterogeneous starch granules (polygonal, aggregate and hollow starch), from different regions of high amylose rice starch (60%) have been reported (Huang *et al*., 2016, Man *et al*., 2014). Furthermore, amylose content of large starch granule size (40 - 85µm) separated from four potato cultivar was higher than the smaller granule size (5- 20 µm) (Singh and Kaur, 2004). Thus, the other possibility of the difference within same potato starch amylose content in this study may be attributed to the fact that heterogeneous starch granules from different regions of potato and different starch granules size may vary in amylose content and other structural properties.

Although amylose content varied between the five potato varieties in this study, the variation was small (±1.98%) values were close to the amount of amylose in potato starch as indicated in other studies. Therefore, with the aim of this study amylose content of potato starch possibility would not be an indicator of starch digestibility in the cooked potato as the values were close in five potato varieties.

6.6.2 Granule size distribution

The granule sizes are classified into very small granules (< 5 µm), small (5-10 µm), medium granules (10-25 µm) and large granules (> 25 µm) (Lindeboom *et al*., 2004). Distribution of granule size and proportion of large and small granules differed among the sources of starches. Regarding potato starch, it has been reported that starch size distribution is bimodal as it exhibits the large granule size with 50-60 µm in diameter and a small proportion of a fine fraction with a mean diameter of 10 µm (Molenda *et al*., 2006, Garrido *et al*., 2011).The results of potato starch granule size distribution in this study were shown in figure 6.4 consistent with other findings and microscopy observations (figure 6.5) show that potato starch granules are composed of a major portion of large granule size and a minor portion of small granule size and consequently shows a bimodal distribution. Also the microscopy images in this study are in agreement with that reported there are no pores available on potato starch granules as discovered on corn, maize, sorghum and millet starch (Fannon *et al*., 2004).

The values of particle size diameters ($Dv_{0.5}$, $D_{3,2}$ and $D_{4,3}$) in this study showed that although different varieties were investigated the levels obtained were in the range of detecting values of other investigators. According to Kaur *et al.* (2007) the difference in granule size diameters may be related to the temperature during tuber growth, they revealed that potato starch with higher granule size resulted from low temperatures during crop growth. Similar results also found by Alvani *et al.* (2011) and Yusuph *et a.l* (2003) determined a small variation in the physio- chemical properties of potato cultivars when they are grown under the same growing conditions. This could be the cause

in variation between potato starches granules size in present study however, potato varieties used in present study were purchased from local supermarket the information about growing information not available.

The negative significant relation between specific surface area and other granule size diameter ($Dv_{0.5}$, $D_{4,3}$ and $D_{3,2}$) indicating that potato starch with smaller granule size have more specific area than the larger granule size and more digestibility would be expected.

6.6.3 Crystallinity

Pikus (2005) reviewed that SAXS peak at q value 0.06 A˚ arise from the alternating crystalline and amorphous regions and the location of peaks depend on lamella size, whereas the peak area arise mainly from degree of ordering in semi-crystalline regions. The small difference in peak position q of potato starches in the present study may be due to diversity in lamella thickness, the lower value of peak position q representing the thickness of the lamella and larger repeat distance (Salman *et al*., 2009). The effect of amylose content of starch in semi-crystalline structures of starch have been reported by Cameron and Donald (1995) and Blazek *et al*. (2009) they proposed that a significant correlation between amylose content and organisation of semi-crystalline lamella within starch granules thus, increasing in amylose content increases the repeat spacing in lamellas within crystalline lamellae as amylose is shown to accumulate in both crystalline and amorphous part of lamella. In this study it was observed that Desiree potato had higher q value and lower repeated distance and amylose content while, Rooster potato had lower q value and higher amylose and d value. This observation consistent with reported elsewhere that increasing amylose being linked with the disrupting

packaging of amylopectin chains within crystalline lamellae in crystalline section and increase the repeat spacing present in crystalline lamellae (Cameron and Donald (1995).

Intensity mainly depends on the differences in electron density between amorphous and crystalline region of lamella. Yuryev *et al*. (2004) reported that increasing amylose content decreases the scattering peak intensity. In this study amylose content of potato starches showed negative non-significant correlation with d spacing, peak area and I_{max}, these results to some extent agreed with previous study and the non-significant correlation, possibly thought to be due to the narrow range of amylose content of potato varieties starch investigated in this study. It may be suggested that the difference in crystalline structure of the potato varieties of starch in this study may affect gelatinisation as well as *in vitro* digestion and blood glucose response properties as more crystalline starch (higher lamellar thickness) require higher temperature for gelatinisation and produce more resistant starch.

6.6.4 Correlation between physical and chemical properties of potato varieties starch and *in vitro* digestibility

It is important to determine the amylose content of starch as many properties of starch, for instance, gelatinisation, chain length, crystallinity and pasting properties influenced by its amylose content (Zhou *et al*., 2015). The gelatinisation and re-crystallinity of amylose differ from that of amylopectin. The recrystallized amylose produces resistant starch that affects the bioavailability and digestibility of starch in the gastro intestinal tract. In potato it has been reported that high amylose content potato is more resistant to degradation by alpha amylase (Cottrell *et al*., 1995). Genetically modified

potatoes with high amylose content have faster re-crystallinity, higher gelatinisation peak, higher resistant starch and lower hydrolysis rate *in vitro* and lower predicted GI compared to potato (unmodified) with normal amylose content 22, 23% (Karlsson *et al*., 2007). Furthermore Hu *et al*. (2004) determined that amylose content of starch has a significant impact on the estimated glycaemic score value. They indicated that rice with higher amylose content 26.8% showed lower glycaemic value and less starch hydrolysis compared to low 1.1% and intermediate 13.6% amylose content rice.

The relationship between amylose and starch digestibility of potato varieties was investigated in the present study by principle component analysis (PCA). Negative correlation between amylose content and amount of starch hydrolysed from steam-cooked potato by PPAA was only observed when sugar released after digestion measured by HPAEC-PAD. This revealed that amylose content to some extent may have reverse influence on starch digestibility and supports that higher amylose starch content decreases the rate of starch hydrolysis by amylolysis.

On the other hand, some potato samples (Maris Peer, Mozart and Desiree) did not differ statistically in amylose content while, a difference was observed in the distribution of granule size (table 6.2). Therefore, the results are consistent with that hypothesis, namely that amylose content is not solely sufficient for predicting starch digestibility (Panlasigui *et al*., 1991). The impact of starch granule size on their degradation and digestion by enzymes has been reported by (Kainuma *et al*., 1978, Fujita, *et al*., 1983, Dhital *et al*., 2010, Mahasukhonthachat *e al*., 2010, Lin *et al*., 2015, Noda *et al.,* 2005). They revealed that hydrolysis rate of starch are increased as granule size

decreases. It is proposed by Kainuma *et al.* (1978) that this seems to be attributed to the fact that smaller granule size have larger surface area in comparison with large granule sizes of starch. The impact of potato starch surface area on rate of digestibility have been emphasised by Dihital *et al*. (2010).

In the present study PCA results reveal that Desiree and Maris Piper had higher scores of granules size parameters ($Dv_{0.5}$, $D_{3,2}$ and $D_{4,3}$) which is negatively correlated with starch hydrolysis. Thus the results of this study support the hypothesis that the larger the starch granule the lower hydrolysis. Furthermore, the results also support that the surface area of starch granule size plays an important role in the initial stage of amylolytic digestion, the initial contact between the enzyme and granule is greater in small size starch granule with large surface area compared to large granules with small surface area (Kim and Huber 2008, Salman *et al*., 2009).

In respect with crystallinity, Hoover and Sosulksi (1985) reported that difference in crystallinity of starch have an effect on digestibility. Increases in crystallinity reduce the bioavailability of starch for digestion (Lin *et al*., 2015) and lamellar distance positively correlated with resistant starch (RS) (Cai *et al*., 2015). According to PCA results in this study Rooster and Maris Piper potato had the highest score for d spacing (Braggs distance) meaning highest lamellar distance. Whereas Rooster showed high AUC. Therefore, in this study increasing in crystalline lamella thickness due to increase in amylose content is not related with *in vitro* digestibility of potato varieties.

6.7 Conclusions

In conclusion, this study showed the comparable value of amylose content, granule size distribution and SAXS patterns of potato starch to be similar to reported values. Extracted starch from five potato varieties showed similar shapes while, different in their chemical component (amylose), granule size distribution and crystallinity structure this could be affected by varieties and or growing condition.

Principle component analysis (PCA) revealed that chemical components (amylose), granules size distribution and crystalline structure of potato starches used in this study to some extend had an influence on starch hydrolysis *in vitro*. The results also support that no single physical or chemical properties were predominate to determine the effect of starch properties on the digestibility and predicted glycaemic response. Potato samples that did not differ statistically on their amylose content had different granule size distributions.

Finally, *in vivo* measurements of GI for potato varieties are essential to determine the effect of potato phenolic and starch properties on digestibility, this will be described in the following chapter.

7 Determination of glycaemic index of three varieties of potato

7.1 Introduction

In previous chapters, the *in-vitro* digestion of five potato varieties was investigated and the glycaemic index (GI) was calculated from the hydrolysis index (HI). In this chapter, the same varieties were used to evaluate the *in vivo* glycaemic response to the same batch of potatoes from five varieties. The GI concept has been used for classification of starchy food on the basis of their postprandial blood glucose response in healthy individuals. Potatoes are assumed to have a high GI food that causes a sharp increase in postprandial blood glucose response. Soh and Brand-Miller (1999) showed this to be the case for instant mashed potato. However, this may not be observed in potatoes cooked by different means. The GI of commonly consumed potato varieties in Canada, UK, the USA and Australia have been determined (Ramdath *et al*., 2014, Ek *et al*., 2014a, Henry *et al*., 2005, Fernandes *et al*., 2005) and potatoes have been reported to have low to high GI depending on varieties (Ek *et al*., 2014, Henry *et al*., 2005, Fernandes *et al*., 2005), texture (Henry *et al*., 2005), cooking method (Ek *et al*., 2012, Fernandes *et al*., 2005, Garcia-Alonso and Goni 2000) and processing and meal consumption (Henry *et al*., 2006). The Desiree potato has been shown to have GI ranging from 74 to 101 depending on boiling duration from 9 min to 35 min (table 1.2).

Although potato is one of the important sources of carbohydrates, it is a good source of phytochemicals such as phenolics. To our knowledge, there is a lack of studies regarding the relationship between phenolic acids and GI in potatoes. Only one study recently described a significant inverse relationship between total anthocyanin, total phenol content; and glycaemic index *in-vivo*

(Ramdath *et al*., 2014). One study reported that potatoes with similar amylose content have different GI and *in vitro* digestibility (Ek *et al*., 2014a) and another study by the same author examined the characteristics of potato starch (relative crystallinity, granule size distribution, amylopectin chain length, and thermal and pasting properties) on selected potatoes with identified GI ranging from 56 to 103 (Ek *et al*., 2014b). To our knowledge this is the first study to investigate the effect of potato components (TPC, 5-CQA, total starch) on *in vivo* GI of different varieties of potato.

7.2 Aims

The aim of this study was to measure the GI of three potato varieties; Maris Piper, Desiree and Rooster which vary in their polyphenol level and starch characteristics. Validity of predicted GI from HI was also assessed against *in vivo* GI.

7.3 Hypothesis

The hypothesis is that TPC, 5-CQA content, amylose content, granule size and crystallinity of potatoes affect the glycaemic response in healthy individuals.

7.4 Objectives

- To measure the blood glucose response of healthy human subjects after consuming a portion of potato or white bread containing 50 g available carbohydrates. Four samples were tested by each subject: three peeled and steam cooked potato samples from the three different varieties. The fourth sample was white bread which was used as the GI reference sample.

- To explore the effect of dietary (carbohydrate intake) and non-dietary factors such as demographic characteristics and lifestyle factors on blood glucose response using Pearson correlation.
- To examine the correlation between GI and TPC and 5-CQA
- To examine the correlation between GI and amylose content, granule size distribution and crystallinity structure
- To examine the correlation between predicted GI from HI and GI *in vivo*

7.5 Results

7.5.1 Ethics

The study was reviewed and approved by the Ethics Committee of the Faculty of Mathematics and Physical Science, University of Leeds, United Kingdom. Ethical reference number [MEEC 15-006] (Appendix A).

7.5.2 Data collection

Demographics, dietary information was collected in this study using a questionnaire adapted from National Health and Nutrition Examination Survey (NHANES) (Appendix B).

7.5.2.1 Demographic characteristics

Table 7.1 shows the demographic information. A total of eleven healthy subjects (2 male and 9 female) were recruited among students and staff at University of Leeds in this study (n=1 European, n=1 African, n= 2 South-American and n=7 Asian). The mean age was 28.82± 4.72 years and ranged from 24-36 years. The mean body mass index (BMI) was 24.34 ± 3.42 kg/m^2 and this was within normal range 18.5 – 24.5 kg/m^2. One subject was obese and 3 were overweight. Seven (63%) of subjects reported that they have daily physical activity such as walking, running, dancing and bicycling. Among 11 subjects 3 (27%) were alcohol consumer and only one smoker. The subjects were all generally healthy according to inclusion criteria for subjects in this study; none were diabetic, had reported any chronic disease and were not allergic to gluten.

Table 7.1 Demographic characteristics of subjects (n=11)

#	Age (Years)	Gender	BMI[1] (Kg/m^2)	Ethnicity	Physical activity	Alcohol consumption	smoking
1	36	Female	25.8	Asian	Yes	NO	NO
2	29	Female	19.3	Asian	Yes	NO	NO
3	24	Female	22.6	Europe	Yes	Yes	NO
5	30	Female	24.3	Mexican	Yes	NO	NO
6	30	Female	25.5	Mexican	Yes	Yes	Yes
7	34	Female	**31.3**	Asian	Yes	NO	NO
8	31	Male	28.8	Asian	NO	NO	NO
9	23	Male	23.2	Asian	NO	NO	NO
10	20	Female	23.7	Asian	NO	NO	NO
				Excluded subjects			
4	30	Female	22.4	African	Yes	Yes	NO
11	30	Female	20.9	Asian	NO	NO	NO

[1] Body mass index ranges: 18.5 or below underweight, 18.5 – 24.9 normal, 25 – 29.9 overweight, 30 and over obese.

7.5.2.2 Dietary assessment

A 24- hour dietary recall was conducted before each test in order to determine the average daily energy and carbohydrate intake of subjects. Rice and wheat were the common staple carbohydrate food among subjects and rice was the staple food of majority of subjects 72% (Table 7.2). The average daily energy and carbohydrate intake of subjects was analysed using WinDiets.

The average energy and total carbohydrate intake of subjects were below the estimated average requirements (2772, 2749 kcal/day for male age 19-24 and

25-34 respectively and 2175 kcal/day for female age 19-24 and 25-34 (SACN 2011). The average calories consumed by males in this study was1688±489.7 kcal/day while female consumed 1192.3±171.8 kcal/day. The mean daily intake of total carbohydrates by subjects was 165.3±36.5 g/day and this value was lower than the daily recommended amount for male and females (age 19-64) of 224 g/day (SACN 2015). The average percent of energy obtained from carbohydrates was 44.1±6.1% and this was slightly below the recommended 50% (SACN 2015).

Both the highest daily energy and carbohydrate intake were associated with subject #9 (Male and Asian) at 2034.3±853.9 kcal/day and 234.7±110.0 g/day respectively and the lowest daily energy intake was 799±251.8 kcal/day was subject #4 (Female and African) while, the lowest total carbohydrate intake and lowest percent of daily energy from carbohydrate was observed with subject #2 (Female and Asian) 122.2±42.8 g/day and 42.8±16.6% respectively.

Table 7.2 Staple food, total daily energy and carbohydrates intake of subjects over four days. Data expressed as mean ± SD (n=11).

#	Staple food	Total Energy (Kcal/day)	Total carbohydrates (g/day)	Calories from carbohydrate (%)
1	Wheat, rice	1011.7±387.7	153.6±18.8	60.6±12.4
2	Rice	1160±465.1	122.2± 42.8	42.8±16.6
3	Wheat	1251±110.6	152.7±16.9	47.0±9.9
5	Wheat	1528±249.4	199.8±32.7	49.9±6.3
6	Rice	1077.5 ±268.5	136.4±46.1	49.1±18.0
7	Rice	1082±266.9	129.4±81.5	42.4±20.7
8	Rice	1341.7±375.5	182.1±31.5	53.3±11.5
9	Rice	2034.3±853.9	234.7±110.0	43.4±11.2
10	Rice	1236.5 ±479.7	177.3±98.2	52.0±13.7
		Excluded subjects		
4	Rice	799±251.8	131.1±41.8	61.9±4.6
11	Wheat, rice	983±0.0	108.5±0.0	43.9±0.0

7.5.2.3 Exclusions

In vivo GI was measured in 9 subjects out of 11. Two subjects were excluded in this study. One subject (participant # 4) showed a very low iAUC after consuming white bread (standard food) and the other one (participant #11) took more than 15 minutes to complete portion of potato sample.

7.5.2.4 Food Samples

In order for subjects to consume a portion of food containing 50 g available carbohydrate, total starch and available carbohydrate in either potato samples or white bread (reference food) was measured experimentally using DNS method chapter 4. Table 7.3 describe the size of the portions and figure 7.1 shows a portion of potato served to subjects. Table 7.4 presents total phenolic content (TPC) and 5-CQA of potato varieties tested. TPC and 5-CQA were determined using Folin–Ciocalteu and HPLC method respectively as described in chapter 4.

Figure 7.1 Left panel shows homogenous portions of raw potato sample representing 50 g available carbohydrate, right panel shows steamed portion as it was served to subjects. Foods were steamed just before serving.

Table 7.3 Test portion and available carbohydrate portion of sample and reference food used in this study.

	Desiree	Rooster	Maris Piper	White bread
Available carbohydrate portion	50 g	50 g	50 g	50 g
Test portion (g)	366 g + 200 mL water	346 g + 200 mL water	395 g + 200 mL water	3 slices + 200 mL water

Table 7.4 Total phenolic and 5-CQA content of 50 g available carbohydrate test portion of steam cooked potato samples used in this study on a fresh weight basis (FW) (n=4).

Potato samples	TPC[*1]	5-CQA[*1]
Desiree	413.97±50.09	13.60±0.31
Rooster	190.51±8.59	4.40±0.15
Maris Piper	291.10±9.31	17.88±0.62

[*]chlorogenic acid equivalent. [1] mg per portion of FW

7.5.3 GI study protocol

The *in vivo* GI study protocol that used in this study was based on the method adapted from FAO/WHO, (1988) and described by Wolever *et al*. (1991).

7.5.3.1 Blood glucose response to bread, the reference food

The mean fasting blood glucose (FBG) and incremental area under curve (iAUC) toward 3 slices of white bread (reference food) containing 50 g available carbohydrate after 10-12 hour of fasting are shown in table 7. 5. The average fasting blood glucose was 4.85 ±0.35 (mmol L^{-1}) this was within the normal range of fasting blood glucose 3.9 to 5.5 (mmol L^{-1}) reported by Diabetes UK. The average iAUC was 97.90 ±34.68 (mmol. min L^{-1}) the highest value of iAUC was 144.675 (mmol. min L^{-1}) associated with participant 1 and the lowest was 31.13 associated with participant 4.

Table 7.5 Mean fasting blood glucose response and blood glucose response for ingestion 50 g available carbohydrate from the reference food (white bread).

#	Fasting Blood glucose (mmol L^{-1})	iAUC (mmol . min L^{-1}) 0-120 min
1	5.2	**144.675**
2	4.2	138
3	4.9	137.75
5	4.4	77.35
6	5.2	63.85
7	4.8	67.75
8	4.7	87.85
9	5	64.85
10	5	75.9
	Excluded subject	
4	5.1	31.137
11	ND	ND

ND= none determined

7.5.3.2 Blood glucose response to potatoes

Mean change in blood glucose response of 9 subjects after ingestion of portion 50 g available carbohydrate from potato or bread over 120 min time period is represented in figure 7.2. Blood glucose peak of white bread, Desiree, Rooster and Maris Piper potato were 2.4±0.94, 2.94±0.70, 3.02±1.03 and 3.22±1.01 mmol L^{-1} respectively. All potatoes showed higher blood glucose response than white bread (reference food). Rooster potato like white bread, showed a maximum glucose peak at 45 min whereas Maris Piper and Desiree have similar glucose maximum peak at 30 min. One way ANOVA single factor was used to determine the difference in blood glucose response toward three potato samples between subjects at different time point and revealed that the blood glucose response between subjects towards potato

samples was similar ($p \geq 0.05$) while, only differed from white bread at 120 min ($p \leq 0.05$). The blood glucose curve in figure 7.2 show that 120 min was enough for glucose response of potatoes to reach back to baseline while, white bread showed significantly higher blood glucose response than potato varieties at this time point indicating that white bread may require more than 120 min to go back baseline level again.

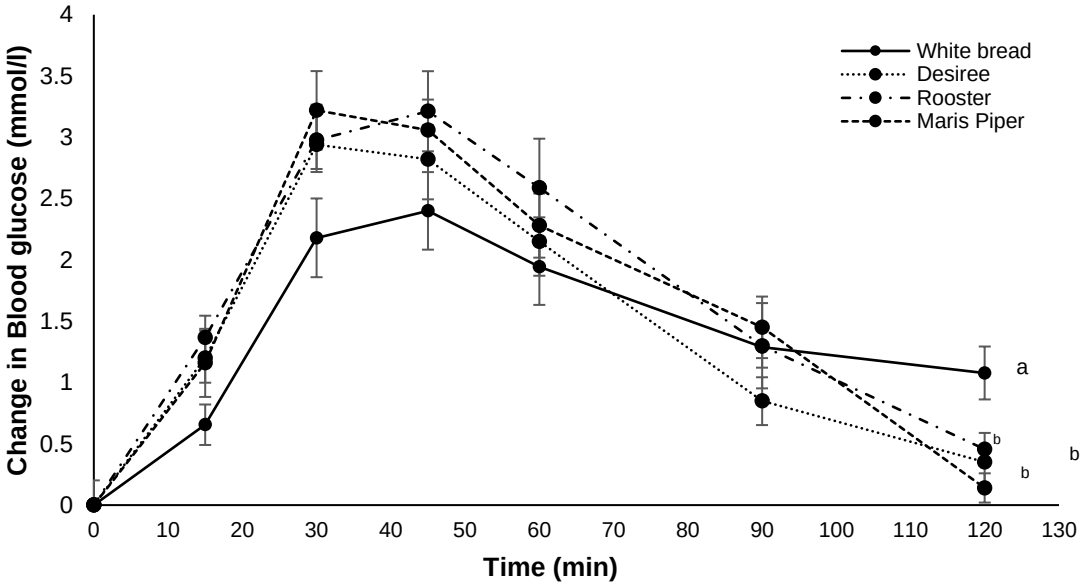


Figure 7.2 Blood glucose response elicited by 50 g available carbohydrate portion of Desiree, Rooster and Maris Piper potatoes compared to white bread. Values at each time point are mean for 9 subjects with standard errors represented by vertical bars. Different subscript letter ^{a,b} at 120 min meaning significant differences (One –way ANOVA $p \leq 0.05$).

7.5.3.3 Calculation of glycaemic index GI

The glycaemic index was calculated for each potato samples from the iAUC of each potato samples eaten by each subject and expressed as percentage of the iAUC for the reference food eaten by the same subject equation (1). The *in vivo* GI of each potato was taken as the mean of the whole group (9 subjects).

$$\mathbf{GI} = \frac{\text{iAUC of test food}}{\text{iAUC of the refernce food}} \times 100 \qquad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

The iAUC and GI values of potato varieties used in this study accordance to colour are represented in table 7.6. The mean GI and iAUC for Desiree, Maris Piper and Rooster potato were 108.72, 113.51, 132.2 and 98.82, 109.29 and 120.96 (mmol .min L^{-1}) respectively. Neither GI nor iAUC of potato samples were significantly different ($p \geq 0.05$), using one-way ANOVA single factor for assessment. The GI for all potato samples in this study classified as high GI.

Table 7.6 Incremental area under curve (iAUC) and GI values of potato varieties with different skin colour. Data expressed as mean of the iAUC and GI of the nine subjects for each potato samples with their standard error.

	iAUC (mmol .min L^{-1})		GI Value		GI classification
	Mean	SEM	Mean	SEM	
Desiree[R]	98.82	10.44	108.72	12.65	High
Rooster[R]	120.96	13.61	132.21	17.03	High
Maris Piper[Y]	109.29	10.40	113.51	9.07	High

[R]=red and [Y]=yellow skin. GI value classification ≥ 70 high, 56 to 69 medium, ≤ 55 low (Brand-Miller *et al*., 2003)

7.5.4 Inter-individual variation in blood glucose response and effect of dietary and non-dietary factors on blood glucose response and GI

7.5.4.1 Non dietary factors

Table 7.1 shows the recorded non-dietary information of subjects such as demographic and lifestyle.

7.5.4.1.1 Demographic characteristics and blood glucose response

The differences in iAUC and GI of reference and potato samples between gender groups as well as ethnicity were investigated (Appendix H). The number of female and male recruited in this study was 7 and 2 respectively. Four ethnicity groups were recruited in this study: Asian, Mexican, European and African. Only the Asian and Mexican groups were used in statistical analysis because there was only one European subject and the African subject was excluded.

The results were indicated that there were non-significant differences ($p \geq 0.05$) in the mean iAUC of the reference and tested potato samples and GI of potato varieties between two gender subjects and between two ethnicity subjects.

7.5.4.1.2 Lifestyle and blood glucose response

Subject # 2 that had the lowest BMI 19.3 kg/m^2 and iAUC of 138 (mmol. min L^{-1}) for white bread this value was 49% higher than the iUAC 67.75 (mmol. min L^{-1}) of subject #7 with highest BMI of 31.1 kg/m^2 (obese). Highest and lowest iAUC 144.67 and 31.13 (mmol. min L^{-1}) of reference food were subjects 1 and 4 respectively were both non-smoker and attending physical activities.

Table 7.7 Shows the mean iAUC observed in each different weight group. The calculated GI for potato samples were shown in table 7.8. The glucose response of overweight subjects was slightly higher than normal weight subjects 7% iAUC = 106.50±40.59 and 98.77±36.02 (mmol. Min L^{-1}) respectively. Rooster potato showed highest blood glucose response iAUC 147±57.20 (mmol. min L^{-1}) compared to all tested foods among both weight group of subjects. However, there were no significant differences between both iAUC and GI of tested food samples for normal and overweight BMI. The differences also between glucose responses and GI for all tested foods within same BMI group were not significant ($p \geq 0.05$). No correlation was found between iAUC of reference food and both BMI and physical activity r=-0.09, -0.07 respectively.

Table 7.7 iAUC (mmol.min/L) observed in the normal and overweight BMI subjects after consumption a set of food sample. Data expressed as mean of iAUC ±SD.

Food Samples	**Normal BMI kg/m^2 (n=5)**	**Overweight BMI kg/m^2(n=3)**	***P*^{1}**
White bread (reference food)	98.77±36.02	106.50±40.59	0.78
Desiree	108.39±39.72	81.2±7.77	0.29
Rooster	104.46±29.08	147±57.20	0.20
Maris Piper	120.07±28.77	93.75±39.52	0.31
***P*^{2}**	0.78	0.28	

Table 7.8 Calculated GI for the normal and Overweight BMI subjects after consumption a set of food sample. Data expressed as mean of iAUC ±SD.

Food samples	Normal BMI (kg/m^2) (n=5)	Overweight BMI (kg/m^2) (n=3)	*P*[1]
Desiree	113.15±34.26	86.50±40.47	0.35
Rooster	109.66±17.57	108.81±42.78	0.27
Maris Piper	116.09±21.05	114.30±44.08	0.93
P[2]	0.78	0.54	

7.5.4.2 Dietary factors

Total energy and total carbohydrates intake of subjects 24 hours prior to study are presented in table 7.2. Subject #9 had the highest daily energy and carbohydrate intake 2034.3±853.9 Kcal/day and 234.7±110.0 g/day respectively and had blood glucose response iAUC 64.85 toward reference food this value 52% higher than iAUC subject #4 with lowest energy intake while, 53% lower than subject #2 who the lowest total carbohydrate intake.

Pearson correlation was conducted for analysis of correlation between iAUC, total energy and total carbohydrate intake. No correlation was observed neither between iAUC and total energy intake nor between iAUC and total carbohydrate intake during 24 hours before study. r= -0.38, -0.37 and *p*= 0.30 and 0.32 respectively.

7.5.5 Variation between individuals

Figure 7.3 represents the individual variations in the iAUC toward 50g available carbohydrate reference and potato samples. The mean coefficient of variation CV% between the subjects for the reference food was 35%.

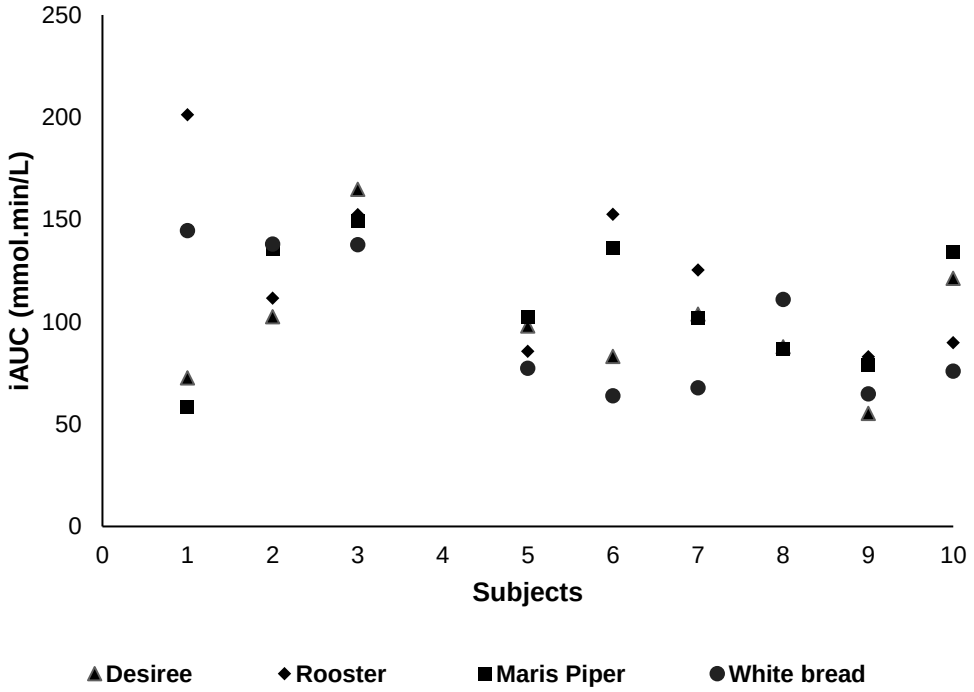


Figure 7.3 Between subject iAUC variation elicit by consuming 50 g available carbohydrate from tested foods on different occasions. Data expressed as iAUC mmol.mi/L (n=9).

Table 7.9 represents the inter individuals variation in the blood glucose response coefficient of variation CV% for each tested food (white bread and potatoes) in each group of the subjects, according to BMI, gender and ethnicity. In general within individual variation CV of reference food 35% was higher than the potatoes. For Desiree potato between individual variation CV for overweight group was highest among other groups in all tested foods (CV= 38, 38 and 42%). Mixed-South American Subjects showed lower between individual CV for white bread, Desiree and Rooster potato among other groups (CV= 13, 11and 20%) respectively.

Table 7.9 Coefficient of variation (CV) % observed in the subjects from different gender, weight and different ethnicities consumption a set of portion food samples.

Tested foods	All (n=9)	Weight		Gender		Ethnicity	
		Normal (n=5)	Overweight (n=3)	Male (n=2)	Female (n=7)	Asian (n=6)	Mixed- South America (n=2)
White bread	35%	36%	38%	37%	36%	35%	13%
Desiree	31%	36%	9%	32%	28%	26%	11%
Rooster	33%	27%	38%	3%	31%	38%	39%
Maris Piper	28%	23%	42%	6%	26%	31%	20%

7.5.6 Correlation between *in vivo* GI and *in vitro* starch digestibility

In chapter 5 *in vitro* digestibility of five potato varieties was conducted using controlled enzymatic condition in order to estimate the GI from starch hydrolysis (HI) index. Table 7.10 represent correlation between *in-vivo* GI of three out of five potato varieties used in this study and starch hydrolysis at different time point (5-120 min) *in vitro*. The positive correlation was observed between GI values with their percentage of starch hydrolysed *in-vitro* at all time point. The strongest positive correlation was exhibited at 5, 15 and 120 minutes r= 0.99, 0.99 and 0.99 respectively (figure 7.4) whereas weak correlations were found at 30 minute r= 0.30. This indicates that percentage of starch hydrolysed over 30 min is not a good time indicator *for measuring* glycaemic response of potato.

Table 7.10 Correlation coefficient (r) between glycaemic index (GI) and the percentage of starch hydrolysed *in vitro* at different time.

Time (min)	r	*p*
5	0.99	0.05
15	0.99	0.01
30	0.30	0.81
60	0.98	0.01
90	0.97	0.01
120	0.99	0.01

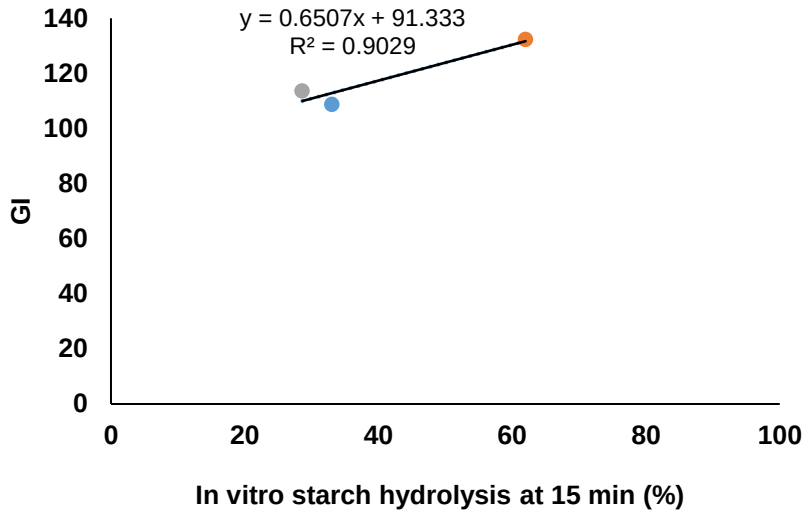


Figure 7.4 Correlation between rate of *in vitro* starch hydrolysis at 15 min (r=0.95) and glycaemic index of potatoes. Orange dot Rooster, Blue dot Desiree and Grey dot Maris Piper.

A strong positive correlation was obtained for the parameters of *in vitro* HI and *in vivo* GI values r= 0.82. For enabling calculation of GI from the HI values a relationship was also obtained for three potato varieties analysed in this study Y=0.4X+ 77.594 (Figure 7. 5).

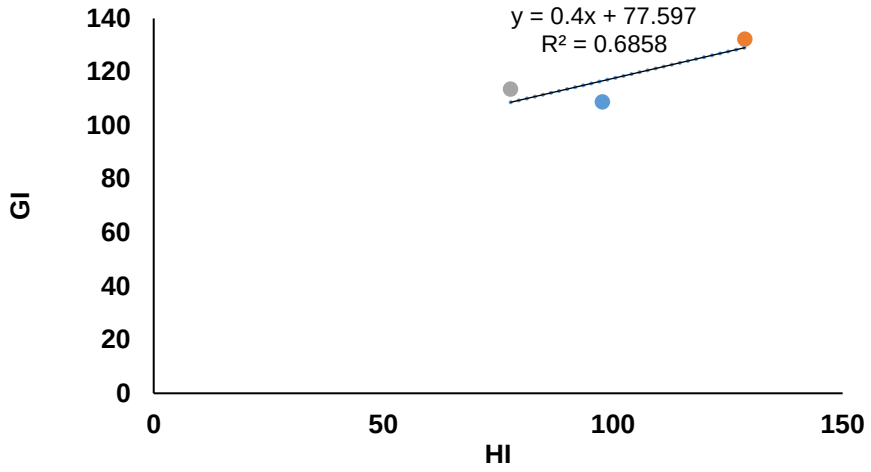


Figure 7.5 Correlation between *in vitro* HI and *in vivo* GI values of Desiree, Rooster and Maris Piper potato. Orange dot Rooster, Blue dot Desiree and Grey dot Maris Piper.

Table 7.11 shows the validation of *in vitro* GI with *in vivo* GI. There was no significant difference between *in vivo* GI of tested potatoes in this study (P=0.43) using one-way ANOVA. The GI of Maris Piper potato obtained *in vivo* was significantly (p=0.04) higher than eGI value obtained *in vitro*, however a good correlation was also found between *in vivo* and *in vitro* GI for all varieties (r=0.83).

Table 7.11 Comparison between GI obtained *in vitro* and *in vivo*.

Potato Varieties	GI[1]		GI[2]	
	Mean	SEM	Mean	SEM
Desiree	108.72[a]	12.65	92.95[a]	1.13
Rooster	132.21[a]	17.03	110.39[a]	0.65
Maris Piper	113.51[a]	9.07	82.37[b]	0.32

[1] in-vivo GI (n=9). [2] GI estimated *in vitro* from starch hydrolysis index chapter 5. Mean value within a raw with different subscript letter [a,b] indicate significant differences (one-way ANOVA, P ≤ 0.05).

7.5.7 Correlation between *in-vivo* GI and phenolic content of potato

A Pearson correlation was conducted to analyse the correlation between *in vivo* GI of three varieties of potato and their phenolic content level prior and after steam cooking. Table 7.12 shows the correlation matrix between each of GI, iAUC and total phenolic and 5-CQA of potato samples. It shows that there is a strong negative relationship between TPC of raw and cooked potato with GI and iAUC r= -0.92, -0.99 and -0.82, -0.95 respectively. 5-CQA level after steam cooking also had a strong and significant inverse relationship with GI r= -0.91, and inverse while non-significant with iAUC r= -0.75 whereas, 5-CQA level in raw potatoes had not correlated with GI and iAUC r= 0.01, 0.30 respectively.

Table 7.12 Pearson Correlation (r) showing simple linear relationship between each of GI, iAUC and TPC and 5-CQA of raw and steam cooked potato varieties.

	GI		iAUC (mmol . min L^{-1})	
	r	p	r	p
TPC				
Raw	-0.92	0.01	-0.99	0.00
Cooked	-0.82	0.01	-0.95	0.01
5-CQA				
Raw	0.01	0.99	0.30	0.80
Cooked	-0.91	0.01	-0.75	0.45

A further analysis of relationship between potato composition (TS, DM, TPC, 5-CQA) and iAUC of glucose response was conducted by principle component analyses (PCA). Figure 7.5 show the loading and scores of the characteristics

of the three potato cultivars with the first two PCs explaining 83.37% of the total variance. PC1 explained 53.22% and PC2 explained 30.15% of total variance. For PC1, TS, DM, 5-CQA and TPC had positive loading and iAUC had negative loading. TS and DM had negative loading for PC2 while 5-CQA and TPC had positive loading for PC2. According to the PCA scores, potato varieties segregated according to their components: Maris Piper showed high DM, high 5-CQA content and low iAUC, Desiree had high TPC and low iAUC and Rooster, showed the opposite characteristics.

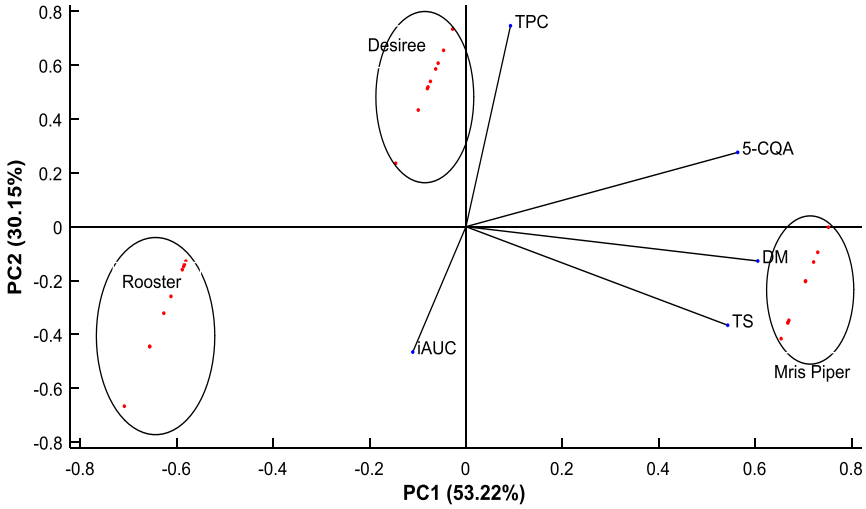


Figure 7.6 Principle component analysis (PCA) using composition and digestibility data from five potato varieties. Graph shows loading of potato components; total starch (TS), dry matter (DM), total polyphenol content (TPC), 5-caffeoylquinic acid (5-CQA) content and iAUC of glucose released after starch digestion and scores of potato varieties according to PCA 1 and 2.

7.5.8 Correlation between *in-vivo* GI and physio-chemical properties of potato varieties starch

In order to determine the relationships between potato varieties *in-vivo* GI and potato starch composition (amylose content) and structure (granule size and crystalline) correlation analysis were conducted. Pearson's correlation using

the coefficient of determination *r* and *P*-values are summarised in table 7.13. The correlation results indicated that there were no significant correlation ($p \geq 0.05$) between GI *in-vivo* and iAUC with amylose content of potato starches. GI and iAUC showed a significant negative correlation with granules size distribution parameters Dv_{50}, $D_{3,2}$ and $D_{4,3}$ and significant positive correlation ($p \leq 0.05$) with specific surface area. There was a trend, but no significant correlation between SAXS parameters for crystalline and *in- vivo* GI and iAUC (($p \geq 0.05$).

Table 7.13 Pearson Correlation (r) showing simple linear relationship between each of GI, iAUC of potato varieties and amylose content, granules size and SAXS pattern of potato starches.

Starch characteristics	GI		iAUC (mmol . min L^{-1})	
	r	p	r	p
Amylose content	0.08	0.94	-0.20	0.86
Granules size distribution				
D_{v50}	-0.97	0.13	-0.99	0.05*
$D_{3,2}$	-0.99	0.002**	-0.95	0.18
$D_{4,3}$	-0.85	0.34	-0.96	0.15
Specific surface area	0.99	0.02*	0.94	0.20
SAXS patterns				
peak position	-0.65	0.54	-0.85	0.35
peak area	0.84	0.36	0.96	0.17
d spacing	0.65	0.54	0.85	0.35
I max	0.60	0.58	0.81	0.39

*Correlation is significant at 0.05 level (2-tailed). ** Correlation is significant at 0.01 level (2-tailed).

A further analysis of relationship between potato starch physio-chemichal characteristics (amylose content, granules size, and crystallinity) and iAUC of glucose response was conducted by principle component analyses (PCA). Figure 7.6 show the loading and scores of the physical and chemicals characteristics of the three potato cultivars with the first two PCs explaining 90.85% of the total variance. PC1 explained 72.91% and PC2 explained 17.94% of total variance. For PC1, specific surface area, peak area, I max, d spacing and iAUC had positive loading and amylose, peak position and granule size distribution parameters ($D_{3,2}$, D_{50} and $D_{4,3}$) had negative loading. According to the PCA scores, potato varieties segregated according to their components: Rooster showed high specific surface area and high iAUC, Desiree had high amylose and big granule size and low iAUC, and Maris Piper, showed the low amylose, low specific surface area. The digestibility of Desiree appeared to be influenced by amylose and granule size.

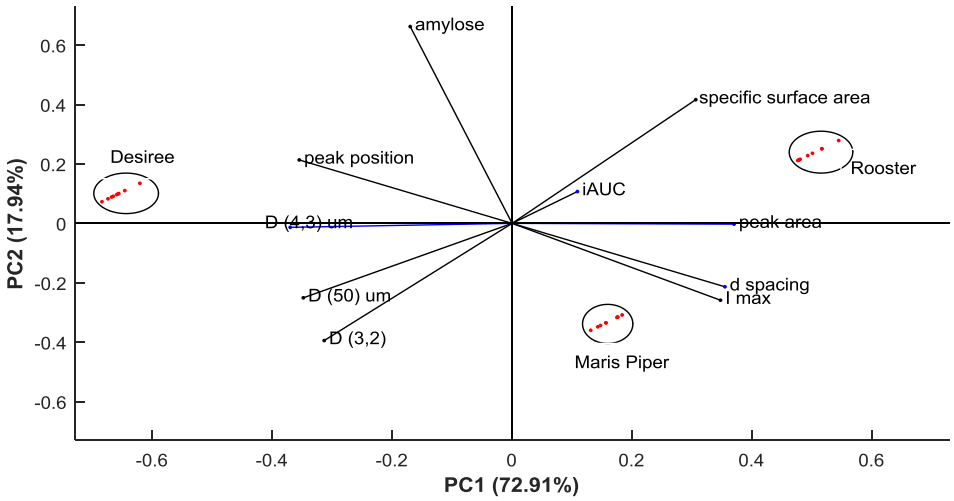


Figure 7.7 Principle component analysis (PCA) using composition and digestibility data from five potato varieties. Graph shows loading of potato components; total starch (TS), dry matter (DM), total polyphenol content (TPC), 5-caffeoylquinic acid (5-CQA) content and iAUC of glucose released after starch digestion and scores of potato varieties according to PCA 1 and 2.

7.6 Discussion

Set portions of four food samples with the same available carbohydrate content were used in this study to evaluate whether there were differences in glycaemic response. The study protocol used in this study in glycaemic response was based on standardised methods described by WHO/FAO (1998). According to Brouns *et al*. (2005) selecting healthy subject will improve the GI results and reduce the variation within the subjects. Thus, in this study healthy subjects with no long-term health problems such as diabetes or cardiovascular disease were selected using a health screen questionnaire. However, the inter-subjects variation was not determined in this study as the reference and tested food consumption was repeated only one time by each subject.

The reliability in the study was also improved by adhering to standardised protocol. For example, regardless being prinked several times, finger-prick capillary blood sample was selected for sampling blood to measure blood glucose in order to remove potential variations of measured GI. Foster-Powel *et al.* (2002) reported that similar GI value and less inter-individual variation observed for the same foods tested by 5 laboratories used the finger-prick capillary blood samples. Capillary blood glucose has been shown to have lower coefficient of variation (CV), greater degree of glucose concentration change and higher iAUC than venous blood (Hatonen *et al*., 2006). Capillary blood is also the preferred method of blood sampling (WHO/FAO 1998, Wolever, 2006). Moreover, blood sample also taken in standardised schedules of blood sampling recommended by WHO/FAO 1998 fasting state and at 15, 30, 60, 90 and 120 min after consuming tested foods by healthy

subjects regarding frequency and duration of blood sampling important for obtaining valid and consistent GI. Wolever *et al.* (2003) reported that GI values, not significantly related to the subjects's characteristics (age, gender, ethnicity, BMI and physical activity) therefore, in this study no specific subject's characteristics selected for recruiting subjects.

7.6.1 Glucose response and glycaemic index

As mentioned earlier potato is one of the largest sources of carbohydrate in the world. Potatoes also have long been regarded as one of the highest GI values of any foods. While, potato GI values reported in literature (International table of glycaemic index and load) include a wide range of variations in GI values from 33 to 144 using white bread as a reference food according to Foster-Powel *et a*l. (2002). The calculated GI for three steam cooked potato varieties in this study classified as high GI ≥ 70.

In the present study, consuming the 50 g available carbohydrate potato varieties with different TPC and 5-CQA levels resulted in non-significantly different postprandial blood glucose iAUC ($p \leq 0.05$). This was similar to those reported by Ek *et al*. (2014a), Henry *et al*. (2005) and Fernandes *et al*. (2005). The iAUC of Maris Piper and Desire potato (109.29, 98.82 mmol. $minL^{-1}$) respectively, were below what was reported by Henry *et al*. (2005) (167, 133 mmol. min L^{-1}) for same potatoes respectively.

All potato varieties showed higher blood glucose response than white bread, but the difference was not statistically significant. This was also observed in results obtained by Fernandes *et al*. (2005) for measuring the GI of potatoes. The glucose response maximum peak for white bread was at 45 min and this was similar to those reported by Wolever *et al.* (2008).

At 120 minutes white bread (reference food) showed higher blood glucose response than three potato varieties indicating that potatoes were digested faster than white bread. This was comparable to what was observed in studies by Fernandes *et al.* (2005) using 50 g available carbohydrate foods while, potato prepared with different cooking method (baked, boiled, roasted, instant mashed, and French fries).

In general the GI values of three potatoes in this study were described as high and in the range of GI values of potato reported elsewhere (79.2- 125.9) using white bread as a reference food (Fernandes *et al.,* 2005) and higher than GI of potatoes reported (Henry *et al*., 2005, Ek *et al*., 2014a, Ramdath *et al*., 2014, Fernandes *et al*., 2005), where glucose used as the reference food (59-94), (53-103), (77-93) and (56.2-89.4) respectively. Wolever *et al.* (1991) reviewed important of the choice of the standard food and reported white bread has higher GI value than glucose by a factor of 100/73 = 1.73

The GI values of Desiree and Maris Piper potato in this study (108.72±12.65, 113.51±9.07) are higher than the GI value reported by Henry *et al*. (2005) for same potatoes in Great Britain (table 7.18). While, a GI of Desiree lower than reported for Desiree potato in literature (144±22) (Foster-Powel *et al*., 2002). The variations in GI value for same food may be raised from differences in processing method such as cooking, physiochemical characteristics of food and differences in GI testing method (portion size, reference food and blood sampling) (Foster-Powel *et al*., 2002, Wolever *et al*., 1991). Table 7.14 shows the differences in GI values of Desiree and Maris Piper obtained by various investigators. Potatoes in present study steam cooked for 30 minutes and available carbohydrate was measured experimentally while, in Henry *et al*.

(2005) and Ek *et al*. (2014a) boiled in water for 15 and 8-9 minute respectively. The portion size of food tested has a major effect on GI value, Wolever *et al*. (1991) accounted the variability in the GI value of potato obtained by various investigators for the differences in the weight of potato used. Ek *et al*. (2014a) used smaller portion size of potato compare to this study and Henry *et al*. (2005) obtained available carbohydrate from published values. Thus, variation in GI values of Desiree and Maris Piper potato was accounted for using different cooking method and time as well as different method for measuring available carbohydrate and portion size.

Table 7.14 Comparison between GI values of Desiree and Maris Piper potato obtained by different investigators.

	Desiree				**Maris Piper**	
	1	2	3	4	1	3
GI	108.72±12	144±22	77±17	74±80	113.51±9	85±4
No. of subjects	9 healthy	10, healthy	10, healthy	10, healthy	9 healthy	10, healthy
Reference food	White bread	White bread	glucose	glucose	White bread	glucose
Weight of available carbohydrate. Balance g	366	466	NA	197	395	NA
Cooking method	Peeled, steam cooking 30 min	peeled, boiled 35 min	Peeled, boiled 15 min	Peeled, boiled 8-9 min	Peeled, steam cooking 30 min	Peeled, boiled 15 min
Available carbohydrate (g/serving)	50	50	50	25	50	50
Blood sampling	finger-prick	Capillary blood samples	finger-prick	finger-prick capil-lary blood sample	finger-prick	finger-prick

1 GI measured in vivo in this study. 2 GI published in International table of glycemic index and glycemic load values: 2002 by Foster-Powel *et al*.(2002). 3 GI obtained by Henry *et al*. (2005). 4 GI obtained by Ek *et al*. (2014a). NA=none available

7.6.2 Inter-individual variation in glucose response and glycaemic index

7.6.2.1 None-dietary factors affecting glucose response and glycaemic index

7.6.2.1.1 Demographics factors

It has been shown that females have lower postprandial blood glucose response after consuming a meal with rye bran than males (Ulmius *et al*., 2009). In present study non-significant correlation was found between glucose response iAUC in female and male consuming different potato varieties. Indicating that gender has no effect on glucose response and GI values. This is possible due to the small size of male group in this study (2 male) compared to female (7) subjects.

Ethnicity has been suggested which might affect the GI (Aziz *et al*., 2013). In this study the blood glucose response of South-American subjects were not significantly higher than Asian of reference and potatoes, and this may accounted for the small size of South-American only two subjects. Thus, ethnicity in the present study did not affect the blood glucose response and GI values and this was well agreed with the finding in the study obtained by Wolever *et al.* (2015).

7.6.2.1.2 Lifestyle factors

According to Wolever (2006) inverse correlation has been shown between BMI and glucose response. In this study, the blood glucose response iAUC of white bread and Rooster potato in overweight BMI subjects were slightly higher than normal BMI subjects however, the differences was not significant.

Overall, in present study, BMI was not associated with glucose response compared to what observed in a study performed by Perala *et al*. (2011).

7.6.2.2 Dietary factor affecting glucose response and glycaemic index

7.6.2.2.1 Daily energy and carbohydrate intake

It has been shown that consumption of high carbohydrate food has adverse effect on glucose metabolism and insulin sensitivity. According to Ble-Castillo *et al*. (2012) high carbohydrate and high fat diets are more important factors for metabolic disturbance and insulin resistance than energy intake. It has been shown that consumption of low glycaemic diet reduces fasting blood glucose and fasting insulin concentration (Livesey *et al*., 2008).

Therefore, the effect of energy and carbohydrate on blood glucose response was investigated in this study and in contrary to studies elsewhere energy intake nor daily carbohydrate intake were significantly correlated with blood glucose response iAUC and GI. This possibly due to the small size of the subjects.

7.6.3 Between individuals variation

In the present study coefficient of variation (CV%) for 50 g available carbohydrates white bread, Desiree, Rooster and Maris Piper potato were 35, 31, 33 and 28% respectively. The CV% for all tested food samples were around or slightly higher than the recommended value by Wolever *et al*. (2008) (CV% ≤30). The accuracy could have been improved if the reference and/ or test food samples had been repeated at least two time, therefore, within-individual variation CV could be measured. The low average AUC subjects

have a higher CV and higher CV leads to bias and imprecision of GI result (Wolever *et al*., 2008).

7.6.4 Correlation between *in-vivo* GI and *in-vitro* starch digestibility

According to Goni *et al.* (1997) it is possible to determine the GI from the percentage of total starch hydrolysed *in vitro*. The GI of basic and mixed meal of South Asian origin was predicted from the hydrolysis indices (HI) and a significant positive correlation was obtained between *in vitro* HI and *in vivo* GI of the mentioned foods (Hettiaratchi *et al*., 2012). More recently Ek *et al*. (2014a) obtained a significant correlation between *in-vitro* hydrolysis and *in-vivo* GI values of potatoes.

In the present study a positive correlation between *in vitro* rate of starch hydrolysis and GI of potato was observed. The strongest correlation between HI at 5, 15 and 120 min and GI indicates that this time is the best for estimating the GI of potatoes and GI value largely depend on percentage of starch hydrolysis measured *in vitro* by mentioning time points. This may account for accessing of enzyme to rapidly digestible starch at the earlier time of digestion that cause the fast increase in blood glucose after potato consumption and 120 min also enough to complete digestion of potato starch and reach the fasting glucose level again.

Thus, the results of this study were in agreement with that an *in vitro* method might be used to predict and classify the GI of food particularly simple food such as potato.

7.6.5 Correlation between GI and phenolic content of potatoes

More recently coloured potatoes gained extra interest as they contain significant amount of polyphenols. Extracted anthocyanin from pigmented potato showed a moderate inhibitory effect against intestinal alpha glucosidase *in-vitro* and negative correlation between potato anthocyanins and *in-vivo* GI have been reported (Ramdath *et al*., 2014). An *in vivo* study also indicated that feeding diabetic rats with 5 and 10% dietary potato peel powder markedly reduced their blood glucose compared to the control group (Singh *et al*., 2005). The author attributed this hyperglycaemic effect of potato peel powder to the high polyphenol and dietary fibre content of the potato peel. However, effect of chlorogenic acid 5-CQA that is predominant phenolic acid in potato and glucose response of potato has not been explored *in vivo*.

Results from literature and chapter 3 showed that 5-CQA exhibits an *in vitro* inhibitory effect against PPAA and mechanism of inhibition was shown to be a mixed type inhibition. Significant inverse correlation was found between 5-CQA and predicted GI from the hydrolysis index of potatoes *in vitro*. The *in vivo* GI assessed the validity of the correlation between 5-CQA and estimated GI calculated from HI.

The present study data of Pearson's correlation and PCA were showed that *in vivo* GI and iAUC was inversely related to both TPC of potato (r=-0.82, -0.95) similar to results obtained by Ramadath *et al*. (2014) and 5-CQA and (r=-0.91, -0.75). The digestibility of Desiree appeared to be heavily influenced by TPC, and digestibility of Maris Piper influenced by 5-CQA in contrast to the Rooster potato that had lowest 5-CQA. It appears that the same single factor does not strongly determine digestibility in all varieties.

In contrast to Ramdath *et al*. (2014), the present study used peeled potatoes, and calculated 5-CQA and TPC content of cooked, rather than raw potato.

7.6.6 Correlation between *in vivo* GI and physio-chemical properties of potatoes starch

Although all potatoes in this study were prepared under the same cooking condition, it was observed that potatoes used in this study differed in the TPC, starch composition (amylose content) and inner structure (crystalline lamella). It has been reviewed by Thorne *et al*. (1983) that starch composition affects digestibility of starch and consequently affecting blood glucose response after consumption. The degree of gelatinisation of starch is proportional to the starch amylose content, the less amylose there is the greater degree of gelatinisation, digestibility and glycaemic index and vice-versa (Karlson *et al*., 2007). In this study the amylose content of three potatoes Desiree, Rooster and Maris Piper were 28.68±5.11%, 28.06±5.28% and 26.20±2.35% respectively Chapter 6 and did not differ statistically ($p \geq 0.05$). Potatoes blood glucose response iAUC and GI were not correlated with their starch amylose content r= -0.20 and 0.08 respectively. These results are in agreement with Ek *et al*. (2014b) that found potatoes with similar amylose content and cooked under similar cooking condition differed in both rate of starch digestibility *in vitro* and *in vivo* blood glucose response. Similar results also reported in uncooked, cooked and retrograded potatoes that significantly differed in their amylose content (Pinhero *et al*., 2016).

The results of this study showed that Rooster potato varied from their characteristics (crystalline structure, granules size and phenolics content) compared to the other two varieties Desiree and Maris Piper. Rooster showed

the lowest peak position, highest d-spacing and highest peak area. The low position of peak meaning thickness and larger size of crystalline lamella which may arise an from increase of amylose content of starch (Cameron and Donald, 1995), while according to PCA data the digestibility of Rooster is not influenced by amylose content and crystalline structure while affected by granule size. Rooster showed smaller granule size chapter 6 and lowest TPC and 5-CQA (chapter 5). A significant and negative correlation was found between iAUC, GI and potatoes starch granule size parameters (D_{V50}, $D_{3,2}$ and $D_{4,3}$) and a positive correlation with specific surface area. The PCA data also revealed that the iAUC of Rooster potato was influenced by granule size and specific surface area as Rooster potato had a positive score for iAUC and specific surface area and negative score for granules size. Thus, the smaller starch granule size has a more specific surface area and higher digestibility than larger granule size this in agreement with Franco *et al*. (1992).

7.7 Limitations

Although this study evaluated for the first time the effect of 5-CQA level in potato on their glucose response, only three potato varieties were used. In order to minimise the large variation of the glycaemic response, the control and test samples should be repeated at least twice preferably three times. However, this represents a high burden for the participants.

7.8 Conclusion

The glycaemic index of three potatoes used in this study was classified as high GI. The demographics and dietary factors had no influence on blood glucose response and GI values.

On the other hand characteristics of potatoes affected the blood glucose response. A negative relationship was found between both iAUC and GI and TPC and 5-CQA content of potatoes. Amylose content did not influence the GI values of potatoes whereas granule size significantly and inversely affected the GI values. It appears that the same single factor does not strongly determine digestibility in all varieties.

This study also successfully validated *in vitro* GI with *in vivo* GI as the rate of *in vitro* starch hydrolysis in steam-cooked potatoes was strongly associated with the *in vivo* GI values.

Moreover, this study for first time suggests that 5-CQA levels in potato could influence the digestibility of potatoes and blood glucose response. Further analysis requires recruiting more human subjects and testing more potato varieties with wide range of TPC and 5-CQA in cooked form to get more reliable and reproducible results.

8 General discussion

Potato consumption is increasing worldwide, especially in developing countries. At the same time, the incidence of diabetes is also increasing. Potatoes are often referred to a ‘high glycaemic index (GI)’ foods, leading to sharp increases in blood glucose concentration following consumption. However, potatoes contain natural phytochemicals which may inhibit starch digesting enzymes. One of these phytochemicals in chlorogenic acid, which is the most abundant phenolic compound in potato. Therefore, the main hypothesis tested in this thesis is that potatoes containing different levels of phenolic compounds will display different starch digestion characteristics and glycaemic responses.

The inhibitory effect and mixed-type inhibitory behaviour of 5-CQA on porcine pancreatic alpha amylase (PPAA) hydrolysis of artificial substrate p-nitrophenyl-α-D-maltoside was previously reported (Sun *et al*., 2016, Narita and Inouye, 2009). This study for first time evaluated the inhibitory effect of the 5-CQA on PPAA activity using potato starch as substrate and determined enzymatic kinetic parameters both in the presence and absence of pure 5-CQA.The results indicate that 5-CQA significantly ($p \leq 0.05$) inhibited PPAA at two incubation times of 5 and 20 min. The inhibition was more pronounced at 5 min compared to 20 min. The difference in inhibition at the two reaction times indicates that 5-CQA is most efficient during early stages of hydrolysis. It has been suggested that 5-CQA interacts with the enzyme-substrate complex more effectively than with free enzyme (Narita and Inouye, 2009), and decreases the affinity for the substrate. At early stages of the reaction, it is expected that more substrate is available to interact with the enzyme,

making the interaction with 5-CQA more likely than when the substrate has been depleted. Pre-incubation of the enzyme with 5-CQA for 10 min in the absence of substrate increased the inhibition of the enzyme at both time points. Rohn *et al*. (2002) reported that the incubation of digestive enzymes (PPAA, trypsin, lysozyme) with simple phenolic compounds (CQA, caffeic acid, gallic acid and ferulic acid) for 24 hours resulted in the covalent attachment of the phenolic compounds to the free amino groups of the enzymes and in consequence, decreased their activity irreversibly and non-competitively. The enzymatic kinetic properties were examined by applying Michaelis-Menten assumptions. Kinetic parameters V_{max} and k_m were obtained from Lineweaver-Burk plots; k_m values increased with increasing inhibitor concentrations while maximum velocity (V_{max}) values decreased. These results are similar to previous reports that also suggest that 5-CQA has a mixed-type inhibition (competitive and non-competitive) against PPAA when potato starch is used as a substrate. The non-competitive inhibition of PPAA was reported for millet seed coat extract using potato starch as substrate (Shobana *et al*., 2009).

Since the results showed that 5-CQA inhibited PPAA activity, the next step was to investigate whether endogenous 5-CQA had a similar effect on native potato starch. For this approach, five UK potato varieties (Desiree, Mozart, Rooster, Maris Piper and Maris Peer) were selected because they had different skin colours and expected to have levels of phenolic compounds. Firstly, the composition of the tubers was determined. The phenolic compound (total phenolic content (TPC) and 5-CQA), dry matter (DM) and carbohydrate content (Total starch (TS) and free sugars) were measured.

Significant differences were observed between potato varieties in their chemical components TPC, 5-CQA, TS and DM. Desiree had the highest level of free sugars and TPC. Maris Piper had the highest level of TS and 5-CQA. Rooster showed the lowest level of TPC and 5-CQA. The lowest TS content were observed in Desiree and Rooster. According to Thybo *et al*. (2006), genetic variation between varieties significantly contributed to variation in the chemical components in the raw tubers. The TPC and 5-CQA varied amongst varieties and the colour of the skin did not appear to reflect the phenolic acid content in the medulla, as has been suggested by Tierno *et al*. (2015). As an example, Rooster has red skin, but its flesh showed one of the lowest TPC and 5-CQA contents amongst the varieties investigated. Conversely, Maris Piper has yellow skin and had the highest 5-CQA in cooked flesh. During cooking, phenolic content decreased in most varieties, except in Desiree where phenolic content actually increased. Steaming was chosen as the processing method to reduce leaching of 5-CQA. The losses are likely to be due to heat degradation. The reason for the increase in Desiree not known, but it could be due to the solubilisation of phenolics from the matrix during cooking (Tian *et al*., 2016). The level of 5-CQA was at the lower end of the range tested in the *in vitro* assay using commercial starch, therefore a high level of inhibition of PPAA was not expected. Nevertheless, a significant change in digestibility of native starch was observed amongst varieties, and a significant correlation between 5-CQA and *in vitro* digestibility was observed. This suggests the endogenous 5-CQA could be having an inhibitory effect on the digestion of native starch by PPAA. It would have been interesting to add exogenous 5-CQA to the native potato starch preparations to evaluate the levels of 5-CQA that would be required for more pronounced inhibitory effects.

In addition, testing more varieties would be beneficial to increase the confidence of the associations. Furthermore, the potatoes were subjected to simulated *in vitro* digestion consisting of a ‘gastric phase’ for pepsin digestion, followed by an ‘intestinal phase’ with PPAA and amyloglucosidase (AMG). An oral phase was not carried out, even though up to 10% of starch can be digested in a typical mouthful. The simulated digestion method has been suggested by several authors as a good predictor of *in vivo* GI (Argyri *et al*., 2016, Goni *et al*., 1997). It was observed that adding AMG increased the AUC values of digestion. This was expected as AMG is able to degrade dextrins (not detected with HPAEC-PAD or DNS) into simple sugars. For this reason, adding AMG could give a better representation of starch digestion compared to PPAA alone. However, adding the two enzymes together made it difficult to interpret whether 5-CQA inhibited AMG, as well as PPAA.

The key results from the simulated digestion experiments were that there were significant differences in the AUC for starch digestibility amongst varieties. Statistical analysis using Pearson’s correlations indicated that there are significant inverse correlations between *in vitro* starch digestion with PPAA+AMG and *in vivo* glycaemic response with potato TPC and 5-CQA, while, *in vitro* starch digestion by PPAA was not correlated with potato phenolic content. In this study, the amount of steam cooked potato used as substrate contained 10 mg of starch (similar to the first experiments) and around 0.08 mg 5-CQA. According to figure 3.4B, this is a low concentration of 5-CQA, enough to inhibit up to 5.8% of the alpha amylase activity.

Table 8.1 and 8.2 clearly illustrates that different results are obtained depending on the digestion or the detection method chosen. While efforts

have been made by the scientific community to harmonise the digestion protocols (and even then there is variation in amount and kind of enzymes used), there is no harmonised method for carbohydrate detection. The study by Goni *et al*. (1997) which is widely referenced in many *in vitro* digestion studies, uses the glucose oxidase assay for glucose detection. In our experience, glucose oxidase is inhibited by phenolic acids, and therefore is not reliable for carbohydrate analysis of foods containing phenolics, including potatoes.

Table 8.1 variation between total digestible starch DS in potato varieties obtained by different methods.

Potato Varietie	**DS_{PPAA} g maltose. min***	**DS_{PPAA} g maltose. min****	**$DS_{PPAA+AMG}$ g glucose. min***	***P*[1]**
Desiree	13.83±0.46[a]	8.00±0.08[c]	10.62 ± 0.13[b]	3.1×10^{-5}
Mozart	14.79±1.11[a]	7.91±0.01[c]	10.44 ± 0.33[b]	1.2×10^{-5}
Rooster	13.71±0.56[a]	9.31±0.16[b]	13.81 ± 0.11[a]	2.1×10^{-3}
Maris Piper	15.02±0.45[a]	8.08±0.21[c]	11.63 ± 0.13[b]	4.0×10^{-5}
Maris Peer	14.09±0.90[a]	9.17±0.01[c]	11.51 ± 0.31[b]	5.9×10^{-7}

*Sugars measured using DNS.

**sugar measured using HPAEC-PAD. Mean value within a raw with different subscript letter [a,b,c] indicate significant differences.

[1] significant value (one-way ANOVA, $P \leq 0.05$).

Table 8.2 variation between *in vitro* starch hydrolysis AUC of five potatoes with different method

Potato Varieties	$AUC_{PPAA(DNS)}$ g maltose. min*	$AUC_{PPAA(HPAEC-PAD)}$ g maltose. min**	$AUC_{PPAA+AMG}$ g glucose. min*	*P*[1]
Desiree	908.64±38.30[a]	517.35±12.82[c]	752.59±29.19[b]	2.2×10^{-6}
Mozart	920.07±14.15[a]	484.05±5.21[b]	970.41±38.82[a]	1.3×10^{-7}
Rooster	920.83±16.39[b]	599.25±1.31[c]	1046.43±48.58[a]	1.3×10^{-6}
Maris Piper	892.15±17.94[a]	507.00±11.51[c]	648.22±22[b]	7.7×10^{-7}
Maris Peer	888.97±37.34[b]	486.27±5.30[c]	1044.82±41.32[a]	3.4×10^{-7}

*Sugars measured using DNS.

**sugar measured using HPAEC-PAD. Mean value within a raw with different subscript letter [a,b,c] indicate significant differences.

[1]significant value (one-way ANOVA, P ≤ 0.05).

Using the *in vitro* digestion data, the hydrolysis index (HI) was calculated and from that estimated GI (eGI) was calculated, using the HI of white bread as a reference. The calculations indicated that all varieties tested should have a high GI value >70.

This was confirmed by *in vivo* GI testing of three varieties (Maris Piper, Desiree and Rooster). These were selected because Maris Piper had the highest 5-CQA level, Desiree the highest TPC and Rooster with lowest 5-CQA and TPC among all varieties. More varieties were not tested to limit the burden of *in vivo* GI testing on human volunteers. Variation between eGI *in vitro* (GI^1) and measured *in vivo* (GI^2) in this study and reported by Henry *et al*. (2005) (GI^3) are shown in table 8.3. Although, the eGI in all potato varieties was less than *in vivo* GI, the result of this study revealed a positive significant correlation ($p \leq 0.01$) between the rate of starch hydrolysis *in vitro* and *in vivo*

GI (table 7.14) thus, *in vitro* starch hydrolysis could be used for estimating GI. The value of eGI and *in vivo* GI obtained from Desiree in this study was higher than reported by Henry *et al*. (2005) while, eGI of Maris Piper was comparable to GI value reported for same potato in the mentioned study.

Table 8.3 Variation between GI obtained *in vitro* and *in vivo*

Potato Varieties	GI[1]		eGI[2]		GI[3]	
	Mean	SEM	Mean	SEM	Mean	SEM
Desiree	108.72	12.65	92.95	1.13	77	17
Rooster	132.21	17.03	110.39	0.65	NA	NA
Maris Piper	113.51	9.07	82.37	0.32	85	4

[1] *in-vivo* GI (n=9). [2] GI estimated in vitro from starch hydrolysis index chapter (4). [3] *in vivo* GI from literature (Henry et al 2011).NA= none available

It was considered that the phenolic acid content could be just one factor leading to the differences between varieties. The composition and structure of the substrate (i.e. native starch) could also play an important role. For this reason, the amylose content and starch granule size and crystallinity was measured.

The amylose content of starch has been reported irreversibly correlated with starch digestibility. It was reported that amylose level of starch affects their crystalline structure by defecting the crystalline region within amylopectin crystalline lamella therefore, the thickness of crystalline lamella within the crystalline region of starch attributed to higher amylose content. More amylose content in starch also produces higher retrograded starch known as resistant starch after starch gelatinisation which is resistant to digestion by amylolytic

enzyme. Native starch granule size also inversely correlated with starch hydrolysis. According to Singh and Kuar (2004) the transition temperatures and gelatinisation temperature range of the increased with decreasing granule size. Amylose content positively correlated with d-spacing and negatively with peak area. In other words, low peak area and peak position arise from higher levels of amylose which leads to thickness of crystalline lamella d-spacing.

Furthermore, PCA was conducted to better understand the correlations between all potato components and starch digestibility. The PCA shown in figure 8.1 explains the correlation between all potato characteristics in term of starch digestibility *in vitro* and blood glucose response *in vivo*, a chemicals component of potato and physical and chemical properties of potato starch. According to PCA figure 8.1 A, B the iAUC and percentage of starch hydrolysis *in vitro* by PPAA+AMG in Desiree potato were influenced by their higher TPC, biggest starch granule size and high peak position of SAXS that arise from less d-spacing of lamella structure, in Maris Piper starch digestibility was influenced by high 5-CQA, large granules size and higher DM.

Although amylose content varied between the five potato varieties in this study, the variation was small (±1.98%) and values were close to the amount of amylose in potato starch as indicated in other studies (Jansky and Fajardo 2014, Kaur *et al*., 2007, Noda *et al*., 2004, Kim *et al*., 1995, Weisenborn *et al*., 1994, Cottrell *et al*., 1995). There were also small differences in lamellar structure between the varieties, and these probably arise from their small differences in amylose content. Increasing amylose has been linked with the disruption of the packaging of amylopectin chains within crystalline lamellae in crystalline layers and an increase in the repeat spacing

present in crystalline lamellae (Cameron and Donald 1995). The low value of scattering vector q representing the thickness of the lamella and larger repeat distance (Salman *et al*., 2009). In this study it was observed that Rooster potato had lower q value and higher amylose content and d value while, Desiree potato had higher q value and lower repeated distance and amylose content among all varieties. On the other hand, starch granule size distributions, greatly varied between varietal sources. Granule size may be affected by temperature during tuber growth, a study revealed that potato starch with higher granule size resulted from low temperatures during crop growth (Kaur *et al*., 2007). Since only one batch of each variety was tested, it is not possible to confirm whether the differences observed are due to genetic or environmental conditions.

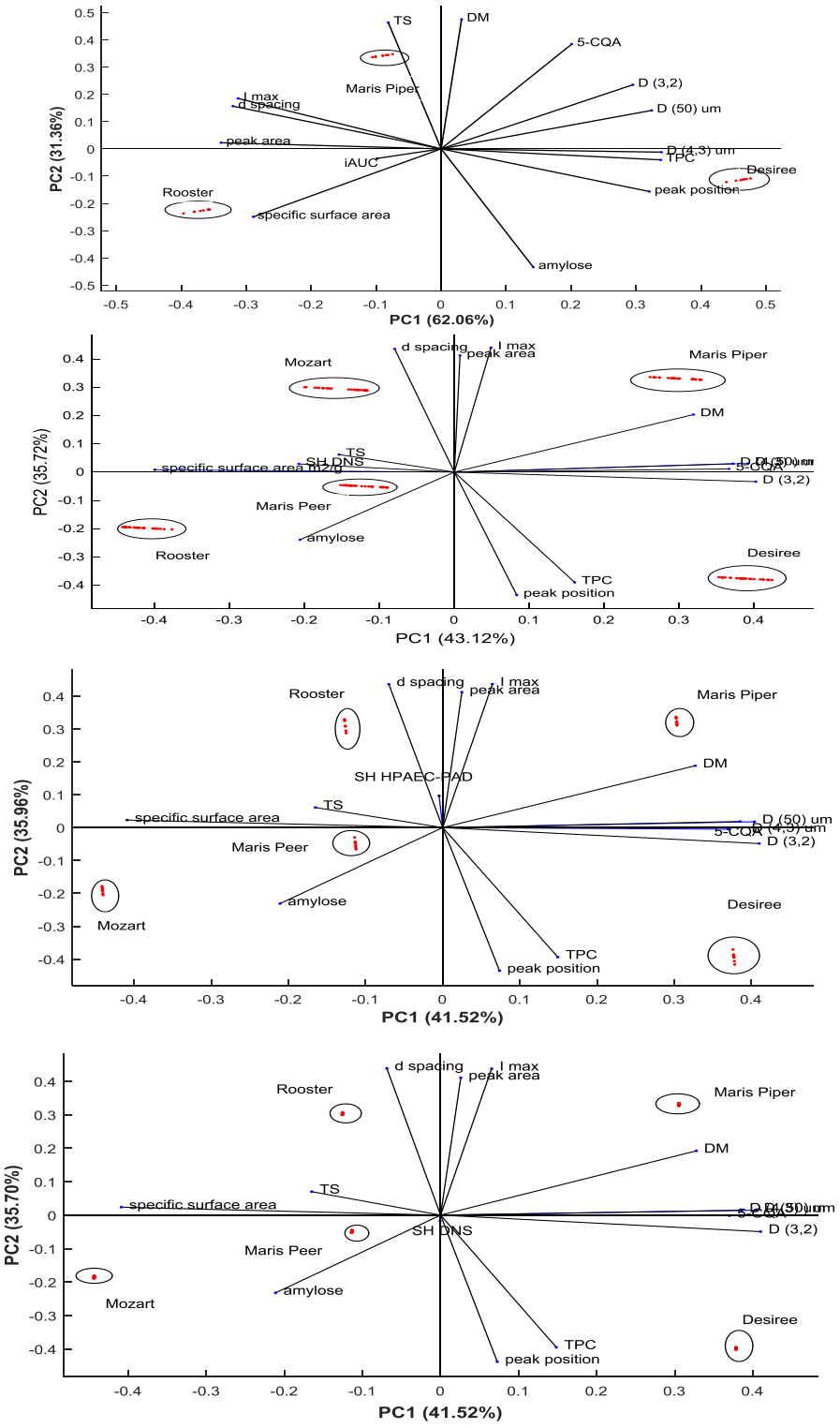


Figure 8.1 Principle component analysis (PCA) using composition and digestibility data from five potato varieties. Graph shows loading of potato components; total starch (TS), dry matter (DM), total polyphenol content (TPC), 5-caffeoylquinic acid (5-CQA), granules size ($D_{3,2}$, D_{50}, $D_{4,3}$, Specific surface area), SAXS parameters and A) iAUC of glucose released after starch digestion, B) Starch digestion *in vitro* PPAA+AMG$_{(DNS)}$, C) starch digestion *in vitro* PPAA$_{(HPAEC-PAD)}$, D) starch digestion *in vitro* PPAA$_{(DNS)}$, and scores of potato varieties according to PCA 1 and 2.

8.1 Limitation

In the present study because of the limited time small number of potato varieties and human subjects were used to investigate the effect of potato 5-CQA content on starch digestibility. Also, only one processing method was tested. Several relevant parameters were also not investigated in this study that affect the starch digestibility because of limited time and some of them beyond the scope of this study such as gelatinisation temperature, swelling power of potato starch, viscosity, protein and phosphorus content.

8.2 Conclusions

This study is the first to examine the mechanism of inhibition of pancreatic alpha amylase by 5-CQA using potato starch as a substrate. Kinetic analyses showed a mixed-type inhibition. *In vitro* digestion of steam cooked potato tubers showed that the 5-CQA content in tubers is probably too low to affect PPAA digestion, but a significant effect was observed when AMG was also used. The inhibitory effect of 5-CQA on multiple carbohydrate digestive enzymes needs further investigation. *In vivo*, high 5-CQA content in Maris Piper and Desiree was associated with lower GI values compared to Rooster.

The results presented in this study suggest that multiple factors affect potato digestibility, and the effects may be variety specific. Increasing 5-CQA in potatoes may be a suitable strategy to decrease digestibility of starch.

List of References

Absar, N., Zaidul, I.S.M., Takigawa, S., Hashimoto, N., Matsuura-Endo, C., Yamauchi, H. & Noda, T. 2009. Enzymatic hydrolysis of potato starches containing different amounts of phosphorus. *Food Chemistry,* 112(1), 57–62.

Ah-Hen, K., Fuenzalida, C., Hess, S., Contreras, A., Vega-Galvez, A. & Lemus-Mondaca, R. 2012. Antioxidant capacity and total phenolic compounds of tweleve selected potato landrace clones grown in southernchile. *Chilean Journal of Agricultural Research,* 72 (1), 3-9.

Adisakwattana, S., Ruengsamran, T., Kampa, P. & Sompong, W. 2012. In vitro inhibitory effects of plant-based foods and their combinations on intestinal alpha-glucosidase and pancreatic alpha-amylase. *Bmc Complementary and Alternative Medicine,* 12.

Akkarachiyasit, S., Yibchok-Anun, S., Wacharasindhu, S. & Adisakwattana, S. 2011. In vitro Inhibitory effects of cyandin-3-rutinoside on pancreatic α-amylase and its combined effect with acarbose. *Molecules*, 16, 2075-2083.

Alagesan, K., Raghupathi, P. K., and Sankarnarayanan, S. 2012. Amylase inhibitors: Potential source of anti-diabetic drug discovery from medicinal plants. 3(2), *International Journal Of Pharmacy & Life Sciences,* 3(2), 1407-1412.

Alberti, K., Zimmet, P. Z. & Consultation, W. H. O. 1998. Definition, diagnosis and classification of diabetes mellitus and its complications part 1: Diagnosis and classification of diabetes mellitus - Provisional report of a WHO consultation. *Diabetic Medicine,* 15, 539-553.

Alvani, K., Qi, X., Tester, R. F., & Snape, C. E. 2011. Physico-chemical properties of potato starches. *Food Chemistry*, 125(3), 958–965.

Andersen, A.W., Tong, C.B.S., Kruege, D.E., 2002. Comparison of periderm color and anthocyanins of four red potato varieties. *American Journal of Potato Research* 79, 249–253.

Andre, C. M., Oufir, M., Hoffmann, L., Hausman, J. F., Rogez, H., Larondelle, Y. & Evers, D. 2009. Influence of environment and genotype on polyphenol compounds and in vitro antioxidant capacity of native Andean potatoes (Solanum tuberosum L.). Journal of Food Composition and Analysis, 22, 517-524.

Argyri, K., Athanasatou, A., Bouga, M., & Kapsokefalou, M. 2016. The Potential of an in Vitro Digestion Method for Predicting Glycemic Response of Foods and Meals. *Nutrients,* 8(4), 209.

Arvidsson-Lenner, R., Asp, N.-G., Axelsen, M., Bryngelsson, S., Haapa, E., Jarvi, A., Karlstrom, B., Raben, A., Sohlstrom, A. & Thorsdottir, I. 2004. Glycaemic index. Relevance for health, dietary recommendations and food labelling. *Food & Nutrition Research*, 48, 84-94.

Aziz, A., Dumais, L. & Barber, J. 2013. Health Canada's evaluation of the use of glycemic index claims on food labels. *American Journal of Clinical Nutrition,* 98**,** 269-274.

Bassoli, B.K., Cassolla, P., Borba-Murad, G.R., Constantin, J., Salgueiro-Pagadigorria, C.L., Bazotte, R.B., da Silva, Rui S. dos S. & de Souza, H.M. 2008. Chlorogenic acid reduces the plasma glucose peak in the oral glucose tolerance test: effects on hepatic glucose release and glycaemia. Cell Biochemistry and Function, 26(3), 320–328.

Bembem, K., Sadana, B. 2012. Effect of cooking methods on the nutritional composition antioxidant activity of potato tubers. *Nt. J. Food Nutr. Sci,* 2, (4), 26-30.

Bembem, K. & Sadana, B. 2013. Effect of cooking methods on the nutritional composition and antioxidant activity of potato tubers. *International Journal of Food and Nutritional Sciences,* 2(4), 26-30.

Birch, P. R. J., Brayan, G., Fenton, B., Gilroy, E. M., Hein, I., Jones, J. T., Prashar, A., Taylor, M. A., Torrance, L., Toth, I. 2012. Crops that feed the world 8: Potato: are the trends of increased global production sustainable? *Food Sec*, 4, 477-508.

Bjorck, I., Granfeldt, Y., Liljeberg, H., Tovar, J., & Asp, N.G.1994. Food properties affecting the digestion and absorption of carbohy-drates. *Am. J. Clin. Nutr.* 59, 699S–705S

Blazek, J. & Gilbert, E. P. 2011. Carbohydr. Polym. 85, 281–293.

Blazek, J., Salman, H., Lopez-Rubio, A., Gilbert, E. P., Hanley, T., & Copeland, L. 2009. Structural parameters of wheat starches granules differing in amylose content and functional characteristics studied by small-angle x-ray scattering. *Carbohydrate Polymers*, 75(4), 705-711.

Ble-Castillo, J. L., Aparicio-Trapala, M. A., Juarez-Rojop, I. E., Torres-Lopez, J. E., Mendez, J. D., Aguilar-Mariscal, H., Olvera-Hernandez, V., Palma-Cordova, L. C. & Diaz-Zagoya, J. C. 2012. Differential Effects of High-Carbohydrate and High-Fat Diet Composition on Metabolic Control and Insulin Resistance in Normal Rats. *International Journal of Environmental Research and Public Health,* 9**,** 1663-1676.

Blessington, T., Nzaramba, M. N., Scheuring, D. C., Hale, A. L., Reddivari, L. & Miller, J. C., JR. 2010. Cooking Methods and Storage Treatments of

Potato: Effects on Carotenoids, Antioxidant Activity, and Phenolics. *American Journal of Potato Research,* 87(6), 479-491.

Bozzetto, L., Annuzzi, G., Pacini, G., Costabile, G., Vetrani, C., Vitale, M., et al. 2015. Polyphenol-rich diets improve glucose metabolism in people at high cardiometabolic risk: a controlled randomised intervention trial. *Diabetologia,* 58 (7), 1551-1560.

Brand-Miller, J., Wolever, T.M., Foster-Powell, K., & Colagiuri, S, 2003. The New Glucose Revolution. Marlowe & Company: New York

Brayer, G. D., Luo, Y. G. & Withers, S. G. 1995. The structure of human pancreatic alpha-amylase 1.8 angstrom resolution and comparision with related enzyme. *Protein Science*, 4, 1730-1742.

Brenfeld, P. 1955. Amylases, α and β. *Methods in Enzymology,* 1**,** 149-158.

Brouns, F., Bjorck, I., Frayn, K. N., Gibbs, A. L., Lang, V., Slama, G. & Wolever, T. M. S. 2005. Glycaemic index methodology. *Nutrition Research Reviews,* 18**,** 145-171.

Buleon, A., Colonna, P., Planchot, V. & Ball, S. 1998. Starch granules: structure and biosynthesis. *International Journal of Biological Macromolecules*, 23, 85-112.

Burgos, G., Amoros, W., Munoa, L., Sosa, P., Cayhualla, E., Sanchez, C., Diaz, C. & Bonierbale, M. 2013. Total phenolic, total anthocyanin and phenolic acid concentrations and antioxidant activity of purple-fleshed potatoes as affected by boiling. *Journal of Food Composition and Analysis,* 30(1), 6-12.

Bustos, R., Fahy, B., Hylton, C.M., Seale, R., Nebane, N.M., Edwards, A., Martin, C., Smith, A.M. 2004. Starch granule initiation is controlled by a heteromultimeric isoamylase in potato tubers. *Proc Natl Acad Sci U S A*, 101(7) 2215-20.

Butterworth, P. J., Fredrick, J., Warren & Ellis, P. R. 2011. Human a-amylase and starch digestion: An interesting marriage. *Starch*, 63, 395-405.

Cai, J., Mana, J .,Huanga, J., Liu, Q., Wei, W., & Wei, C. 2015. Relationship between structure and functional properties of normal rice starches with different amylose contents. *Carbohydrate Polymers*, 125, 35–44.

Cameron, R.E. and Donald, A.M. 1992. A small-angle X-ray scattering study of the annealing and gelatinization of starch. *Polymer*, 33, 2628–2635.

Camire, M. E., Kubow, S. & Donnelly, D. J. 2009. Potatoes and Human Health. *Crit Rev Food Sci Nutr*, 49(10), 823-840.

Cappa, C., Lucisano, M., Barbosa-Cánovasb, G. V., & Mariottia M. 2016. Physical and structural changes induced by high pressure on corn

starch, rice flour and waxy rice flour. *Food Research Internationa*l, 85, 95–103.

Chun, O. K., Kim, D. O., Smith, N., Schroeder, D., Han, J. T. & Lee, C. Y. 2005. Daily consumption of phenolics and total antioxidant capacity from fruit and vegetables in the American diet. *Journal of the Science of Food and Agriculture*, 85, 1715-1724.

Chung, H. J., Shin, D. H. & Lim, S. T. 2008. In vitro starch digestibility and estimated glycemic index of chemically modified corn starches. *Food Research International*, 41, 579-585.

Chung, H.-J., Li, X.-Q., Kalinga, D., Lim, S.-T., Yada, R. & Liu, Q. 2014. Physicochemical properties of dry matter and isolated starch from potatoes grown in different locations in Canada. *Food Research International*, 57, 89-94.

Clifford, M. N. 2000. Chlorogenic acids and other cinnamates - nature, occurrence, dietary burden, absorption and metabolism. *Journal of the Science of Food and Agriculture*, 80, 1033-1043.

Copeland, L., Blazek, J., Salman,H., &Tang M. C.2009. Form and functionality of starch. *Food Hydrocolloids*, 23 (6), 1527–1534.

Cottrell, J. E., Duffus,C.M., Paterson, L., & Mackay G.R. 1995. Properties of potato starch: Effects of genotype and growing conditions. *Phytochemistry*, 40, 1057–1064.

Coultate, T. P. 2002. FOOD The Chemistry of Its Components, Thomas Graham House, Science Park, Milton Road, Cambridge CB4 0WF,UK, The Royal Society of Chemistry.

Cyras, V. P., Zenklusen, M. C. T. & Vazquez, A. 2006. Relationship between structure and properties of modified potato starch biodegradable films. *Journal of Applied Polymer Science*, 101, 4313-4319.

Dale, M. F., Griffiths, D. W., Todd, D.T., 2003. Effects of genotype, environment, and postharvest storage on the total ascorbate content of potato (Solanum tuberosum) tubers. *J Agric Food Chem*, 51(1), 244-8.

DCCT, T. D. C. A. C. T. R. G.1993. The Effect of Intensive Treatment of Diabetes on the Development and Progression of Long-Term Complications in Insulin-Dependent Diabetes Mellitus. *New England Journal of Medicine*, 329, 977-986.

de Sales, P. M., de Souza, P. M., Simeoni, L. A., Magalhaes, P. D. & Silveira, D. 2012. Alpha-amylase Inhibitors: A Review of Raw Material and Isolated Compounds from Plant Source. *Journal of Pharmacy and Pharmaceutical Sciences*, 15, 141-183.

Deusser, H., Guignard, C., Hoffmann, L. & Evers, D. 2012. Polyphenol and glycoalkaloid contents in potato cultivars grown in Luxembourg. *Food Chemistry*, 135, 2814-2824.

Dhital, S., Shrestha, A. K., & Gidley M. J. 2010. Relationship between granule size and in vitro digestibility of maize and potato starches. *Carbohydrate Polymers*, 82(2), 480-488.

Donald, A. M. 2004. Understanding starch structure and functionality. In A.-C. Eliasson (Ed), *Starch in food: Structure, function and applications* (pp. 156-184). Cambridge/Boca Raton: Woodhead Publishing Limited/CRC Press LLC.

Ek, K. L., Brand-Miller, J. & Copeland, L. 2012. Glycemic effect of potatoes. *Food Chemistry,* 133(4)**,** 1230-1240.

Ek, K. L., Wang, S., Brand-Miller, J. C., Copeland, L. 2014b. Properties of starch from potatoes differing in glycemic index. *Food & Function,* 5 (10), 2509-2515.

Ek, K. L., Wang, S., Copeland, L. & Brand-Miller, J. C. 2014a. Discovery of a low-glycaemic index potato and relationship with starch digestion in vitro. *British Journal of Nutrition,* 111**,** 699-705.

El Gharras, H. (2009). Polyphenols: food sources, properties and applications – a review. *International Journal of Food Science & Technology,* 44, 2512-2518.

Englyst, K. N., Englyst, H. N., Hudson,G.J., Cole,T. J. & Cummings, J. H. 1999. Rapidly available glucose in foods: an in vitro measurement that reflects the glycemic response. *AM J Clin Nutr,* 69, 448-54.

Englyst, H.N., Veenstra, J.& Hudson G.J.1996. Measurement of rapidly available glucose (RAG) in plant foods: a potential in vitro predictor of the glycaemic response. *British Journal of Nutrition,*75 (3), 327-337.

Englyst, H.N., Kingman SM, Cummings JH. 1992. Classification and measurement of nutritionally important starch fractions. *European Journal of Clinical Nutrition*, 46, S33-S50.

Ezekiel, R., Paul, V., Singh, B., Peshin, A. & Shekhawat, G. S. 2000. Effect of low temperature, desprouting and gibberellic acid treatment on little tuber formation on potatoes during storage. *Journal of the Indian Potato Association*, 27 No.1/2, pp. 13-23.

Ezekiel, R., Singh, N., Sharma, S. & Kaur, A. 2013. Beneficial phytochemicals in potato - a review. *Food Research International,* 50(2)**,** 487-496.

Fannon, J. E., Gray,J. A., Gunawan, N., Huber, K. C., & BeMiller, J. N. 2004. Heterogeneity of starch granules and the effect of granule channelization on Starch modification. *Cellulose*, 11, 247-254.

FAO Trade and Markets Division. 2008. International Year of the Potato: Economy.International Year of the Potato: FAO Factsheets.Available from:http://www.fao.org/potato2008/en/potato/economy.html.Accessed 2014Sept 21.

FAO/WHO 1998. Carbohydrates in human nutrition. Report of a Joint FAO/WHO Expert Consultation. *FAO food and nutrition paper,* 66**,** 1-140.

Fei, Q., Gao, Y., Zhang, X., Yi, S., Hu, B., Zhou, L., et al. (2014). Effects of Oolong tea polyphenols, EGCG, and EGCG300Me on pancreatic a-amylase activity in vitro. *Journal of Agriculture and Food Chemistry*, 62, 9507–9514.

Fernandes, G., Velangi, A. & Wolever, T. M. S. 2005. Glycemic index of potatoes commonly consumed in North America. *Journal of the American Dietetic Association,* 105**,** 557-562.

Fish, B. C. & Thompson, L. U. 1991. Lectin tannin interactions and their influence on pancreatic amylase activity and starch digestibility. *Journal of Agricultural and Food Chemistry*, 39, 727-731.

Forester, S. C., Gu, Y., & Lambert, J. D. 2012. Inhibition of starch digestion by the green tea polyphenol, (−)-epigallocatechin-3-gallate. *Mol Nutr Food Res*, 56(11), 1647–1654.

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

Foster-Powell, K., Miller J. 1995. International tables of glycemic index. *Am J Clin Nutr*, 62, 871S–90S.

Franco, C. M. L., Preto, S. J. R. & Ciacco, C. F. 1992. Factors that Affect the Enzymatic Degradation of Natural Starch Granules -Effect of the Size of the Granules. Starch – Stärke, 44(11) 422–426.

Friedman, M. 1997. Chemistry, biochemistry, and dietary role of potato polyphenols. A review. *Journal of Agricultural and Food Chemistry*, 45, 1523-1540.

Fujita, S., Sugimoto, Y., & Fuwa. H. 1983. Some characteristics of air-classified potato starch-granules and their effects on digestive functions of rats. *Journal of Japanese Society for Nutrition Food Science (Nippon Eiyo Shokuryo Gakkaishi)*, 36, 453–459 (in Japanese with English summary).

García-Alonso, A.,& Goñi, I.2000. Effect of processing on potato starch: in vitro availability and glycaemic index. *Nahrung*, 44(1), 19-22.

Gibson, S., & Kurilich, A.C. 2013. The nutritional value of potatoes and potato Mcproducts in the UK diet. *Nutrition Bulletin*, 38, 389–399

Gilles, C., Astier, J. P., Marchis-Mouren, G., Cambillau, C. & Payan, F. 1996. Crystal structure of pig pancreatic α-amylase isoenzyme II, in complex with the carbohydrate inhibitor acarbose. European journal of biochemistry, 238, 561-569.

Goda, T., Urakawa,T., Watanabe, M., & Takase S. 1994. Effect of high-amylose starch on carbohydrate digestive capability and lipogenesis in epididymal adipose tissue and liver of rats. *The Journal of Nutritional Biochemistry*, 5(5), 256-260.

Goni, I., Garciaalonso, A. & Sauracalixto, F. 1997. A starch hydrolysis procedure to estimate glycemic index. *Nutrition Research,* 17**,** 427-437.

Granfeldt, Y., Bjorck, I., Drews, A., & Tovar, J. 1992. An in vitro procedure based on chewing to predict metabolic response to starch in cereal and legume products. *European Journal of Clinical Nutrition,* 46, 649-660.

Gupta, R., Gigras, P., Mohapatra, H., Goswami, V. K. & Chauhan, B. 2003. Microbial α-amylases: a biotechnological perspective. *Process Biochemistry*, 38, 1599-1616.

Hanhineva, K., Torronen, R., Bondia-Pons, I., Pekkinen, J., Kolehmainen, M., Mykkanan, H. & Poutanen, K. 2010. Impact of Dietary Polyphenols on Carbohydrate Metabolism. *International Journal of Molecular Sciences*, 11, 1365-1402.

Hatonen, K. A., Simila, M. E., Virtamo, J. R., Eriksson, J. G., Hannila, M. L., Sinkko, H. K., Sundvall, J. E., Mykkanen, H. M. & Valsta, L. M. 2006. Methodologic considerations in the measurement of glycemic index: glycemic response to rye bread, oatmeal porridge, and mashed potato. *American Journal of Clinical Nutrition,* 84**,** 1055-1061.

Henry, C. J. K., Lightowler, H. J., Kendall, F. L. & Storey, M. 2006. The impact of the addition of toppings/fillings on the glycaemic response to commonly consumed carbohydrate foods. *European Journal of Clinical Nutrition,* 60**,** 763-769.

Henry, C. J. K., Lightowler, H. J., Strik, C. M. & Storey, M. 2005. Glycaemic index values for commercially available potatoes in Great Britain. *British journal of nutrition*, 94, 917-921.

Hettiaratchi, U.P.K., Ekanayake, S. & Welihinda, J. 2012. Prediction of glycaemic indices (GI) of meals by starch hydrolysis indices. *International Food Research Journal,* 19(3), 1153-1159.

Hoover, R. 2001. Composition, molecular structure, and physicochem-ical properties of tuber and root starches: A review. *Carbohydrate Polymers*, 45, 253 – 267.

Hoover, R., & Sosulski, F.1985. Studies on the functional characteristics and digestibility of starches from Phaseolus vulgaris biotypes. *Starch – Stärke*, 37 (6), 181–191.

Hu, P., Zhao, H., Duan, Z., Linlin, Z., & Wu D. 2004. Starch digestibility and the estimated glycemic score of different types of rice differing in amylose contents. *Journal of Cereal Science.* 40(3), 231–237.

Huang, J., Lin, L., Wang, J., Wang, Z., Liu, Q., & Wei, C. 2016. In vitro digestion properties of heterogeneous starch granules from high-amylose rice. *Food Hydrocolloids*, 54, 10–22.

Ibrahim, N. H. and Achudan S. N. 2011. Physical properties and stability of emulsions as affected by native and modified yam starches. *International Journal of Biological, Biomolecular, Agricultural, Food and Biotechnical Engineering,* 5 (9), 539-543.

Imberty, A., Buleon, A., Tran, V. & Peerez, S. 1991. Recent advances in knowledge of starch structure. *Starch-Starke*, 43, 375-384.

International Standards Organisation ISO (2010) Food Products – Determination of the Glycaemic Index (GI) and Recommendation for Food Classification.SO 26642-2010. Geneva: International Organisation for Standardisation.

Jane, J., Kasemsuwan, T., & Chen, J. F. 61996). Phosphorus in rice and other starches. *Cereal Foods World*, 41(11), 827-832.

Jansky, S. H. & Fajardo, D. A. 2014. Tuber starch amylose content is associated with cold-induced sweetening in potato. *Food Science & Nutrition,* 2 (6), 628-633.

Jenkins, D. J. A., Kendall, C. W. C., Augustin, L. S. A., Franceschi, S., Hamidi, M., Marchie, A., , A. L. & Axelsen, M. 2002. Glycemic index: overview of implications in health and disease. *American Journal of Clinical Nutrition*, 76, 266S-273S.

Jenkins, D. J. A., Wolever, T. M. S., Taylor, R. H., Barker, H., Fielden, H., Baldwin, J. M., Bowling, A. C., Newman, H. C., , A. L. & Goff, D. V. 1981. Glycemic index of soods: a phsyological basis for carbohydrate exchange. *American Journal of Clinical Nutrition,* 34, 362-366.

Jenkins, D.J., Thorne, M.J., Wolever, T.M., , A.L., Rao, A.V. & Thompson, L.U. 1987. The effect of starch-protein interaction in wheat on the glycemic response and rate of in vitro digestion. *Am J Clin Nutr*, 45 (5), 946-51.

Jenkins, P.J. & Donald A.M. 1995.The influence of amylose on starch granule structure. *International Journal of Biological Macromolecules*, 17 (6), 315–321.

Juliano, B.O., Perez, C.M., Blakeney, A.B., Castillo, T., Kongseree, N., & Laignelet, B. 1981. International cooperative testing on the amylose content of milled rice. *Starch – Stärke*, 33 (5), 157–162.

Kainuma, K., Yamamoto, K., Suzuki, S., Takaya, T.,& Fuwa, H. 1978. Studies on structure & physio-chemical properties of starch. Part IV. Structural, chemical & rheological properties of air-classified small and large granules potato starch. *Journal of Japanese Society for Starch Science* (Denpun Kagaku), 25, 3–11

Kandra, L., Zajacz, A., Remenyik, J. & Gyemant, G. 2005. kienetic investigation of a new inhibitor for human salivary α-amylase. *Biochemical and biophysical research communications*, 334, 824-828.

Karlsson, M. E., Leeman, A.M., & Björck, I.M.E. Eliasson, A.-C. 2007. Some physical and nutritional characteristics of genetically modified potatoes varying in amylose/amylopectin ratios. *Food Chemistry*, 100 (1) 136–146.

Kaur, A., Singh, N., Ezekiel, R., & Guraya, H. S. 2007. Physico-chemical, thermal and pasting properties of starches separated from different potato cultivars grown at different locations. *Food Chemistry*, 101, 643–651.

Kaur, L., Singh, N., Sodhi, N. S., & Gujral, H. S. 2002. Some properties of potatoes and their starches I. Cooking, textural and rheological properties of potatoes. *Food Chemistry*, 79 (2), 177–181.

Kim, H.S., & Huber, K.C. 2008. Channels within soft wheat starch A- and B-type granules. *Journal of Cereal Science*, 48 (1), 159–172.

Kim,Y.S., Wiesenborn, D.P., Orr, P.H.,& Grant, L.A. 1995. Screening potato starch for novel properties using differential calorimetry. *Journal of Food Science*, 60, 1060–1065.

Kingman, S.M & Englyst H. N. 1994. The influence of food preparation methods on the in-vitro digestibility of starch in potatoes. *Food Chemistry*, 49(2), 181-186.

Lachman, J., Hamouz, K., Sulc, M., Orsak, M. & Dvorak, P. 2008. Differences in phenolic content and antioxidant activity in yellow and purple-fleshed

potatoes grown in the Czech Republic. *Plant Soil and Environment,* 54(1)**,** 1-6.

Lachman, J., Hamouz, K., Hejtmankova, A., Dudjak, J., Orsak, M. & Pivec, V. 2003. Effect of white fleece on the selected quality parameters of early potato (Solanum tuberosum L.) tubers*. Plant Soil and Environment*, 49, 370-377.

Lehman, U. & Robin, F. 2007. Slowly digestible starch - its structure and health implications: a review. *Trends in Food Science & Technology,* 18, 346-355.

Lemos, M. A., Aliyu, M.M. & Hungerford, G. 2015. Influence of cooking on the levels of bioactive compounds in Purple Majesty potato observed via chemical and spectroscopic means. *Food Chemistry,* 173, 462-467.

Lemos, M. A. & SivaramareddyI, A. 2013. Effect of oil quality on the levels of total phenolics, total anthocyanins and antioxidant activity of French fries.

Li, W., Gao,J., Wu,G., Zheng, J., Ouyang,S., Luo, Q. & Zhang, G., 2016. Physicochemical and structural properties of A- and B-starch isolated from normal and waxy wheat: Effects of lipids removal. *Food Hydrocolloids*, 60, 364–373.

Li, Y., Gao F., Shan, F., Bian, J. & Zhao, C. 2009. Study on the Interaction between 3 Flavonoid Compounds and α-Amylase by Fluorescence Spectroscopy and Enzymatic Kinetics. Journal of Food Science, 74, C199-C203.

Lin, L., Huang, J., Zhao, L., Wang, J., Wang, Z.,& Wei, C. 2015. Effect of granule size on the properties of lotus rhizome C-type starch. *Carbohydrate Polymers*, 134, 448–457.

Lindeboom, N., Chang, P.R., & Tyler. R.T. 2004. Analytical, biochemical and physicochemical aspects of starch granule size, with emphasis on small granule starches: A review. *Starch*, 56 (3), 89–99.

Liu, Q., Tarn, R., Lynch, D., Skjodt, N. M. 2007. Physicochemical properties of dry matter and starch from potatoes grown in Canada. *Food Chemistry.* 105(3), 897–907.

Liu, Q., Weber, E., Currie, V., & Yada, R. 2003. Physicochemical properties of starches during potato growth. *Carbohydrate Polymers*, 51(2), 213–221.

Livesey, G., Taylor, R., Hulshof, T. & Howlett, J. 2008. Glycemic response and health - a systematic review and meta-analysis: the database, study characteristics, and macronutrient intakes. *American Journal of Clinical Nutrition,* 87**,** 223S-236S.

Love. S. L. S., T.; Shafii, B., Price, W. J., Mosley. A. R.. & Thornton. R. E. (2004). Stability of expression and concentration of ascorbic acid in North American potato germplasm. *HortScience* 39, 156-160.

Lynch, D. R., Liu, Q., Tarn, T. R., Bizimungu, B., Chen, Q., Harris, P., Chik , C. L. & Skjodt N. M. 2007. Glycemie index — a review and implications for the Potato industry. *American Journal of Potato Research,* 84 (2), 179-190.

Mahasukhonthachat, K., Sopade, P.A., Gidley, M.J. 2010. Kinetics of starch digestion in sorghum as affected by particle size. *Journal of Food Engineering*, 96, 18–28.

Malenberg, A.G. & Theander O. 1985. Determination of chlorogenic acid in potato tubers *Journal of Agricultural and Food Chemistry*, 33, 549–551

Man, J., Lin,L., Wang, Z., Wang,Y., Liu, Q., & Wei, C. 2014. Different Structures of Heterogeneous Starch Granules from High-Amylose Rice. *Journal of Agricultural and Food Chemistry,* 62, 11254−11263.

Manach, C., Scalbert, A., Morand, C., Remesy, C. & Jimenez, L. 2004. Polyphenols: food sources and bioavailability. *American Journal of Clinical Nutrition*, 79, 727-747.

Mattila, P. & Hellstrom, J. 2007. Phenolic acids in potatoes, vegetables, and some of their products. *Journal of Food Composition and Analysis*, 20, 152-160.

Mccance, R. A. & Widdowson, E. M. 2002. The composition of foods, Cambridge, London Royal Society of Chemistry and Food Standards Agency.

Meng, S., Cao, J., Feng, Q., Peng, J. & Hu, Y. 2013. Roles of chlorogenic acid on regulating glucose and lipids metabolism: a review. Evidence Based Complementary and Alternative Medicine,

Miller, G. L. 1959. Use of Dinitrosalicylic acid reagent for determination of reducing sugar. *Analytical Chemistry,* 31(3), 426-428.

Mir, J., Srikaeo, K. & Garcia, J. 2013. Effects of amylose and resistant starch on starch digestibility of rice flours and starches. *International Food Research Journal*, 20, 1329-1335.

Mishra, S., & Rai T. 2006. Morphology and functional properties of corn, potato and tapioca starches. *Food Hydrocolloids,* 20(5), 557–566.

Molenda, M., Stasiak, M., Horabik, J., Fornal,J., Błaszczak, W., Błaszczak, W., Błaszczak,W., & Ornowski, A. 2006. Microstructure and mechanical parameters of five types of starch. *Pol. J. Food Nutr. Sci*., 15/56 (2), 161–168.

Moreira, T.S., & Wolver, T.S. 2011. The Role of Starch Physicochemical Properties in Determining the Glycaemic Index of Novel Potato Varieties. *The FASEB Journal* (251b1196).

Morrison, W. R., & Laignelet, B. 1983. An improved colorometric procedure for determining apparent and total amylose in cereal and other starches. *J. Cereal Sci*., 1(1),9-20

Narita, Y. & Inouye, K. 2009. Kinetic analysis and mechanism on the inhibition of chlorogenic acid and its components against porcine pancreas alpha-amylase isozymes I and II. *Journal of Agricultural and Food Chemistry*, 57(19), 9218-9225.

Narita, Y. & Inouye, K. 2011. Inhibitory effects of chlorogenic acids from green coffee beans and cinnamate derivatives on the activity of porcine pancreas α-amylase isozyme I. *Journal of Food Chemistry*. 127, 1532-1539.

Navarre, D. A., Pillai, S. S., Shakya, R. & Holden, M. J. 2011. HPLC profiling of phenolics in diverse potato genotypes. *Food Chemistry*, 127, 34-41.

Nigam, P., Singh, D. 1995. Enzymes and microbial systems involved in starch processing. *Enzyme and Microbial Technology,* 17(9), 770-778.

Noda, T., Tsuda, S., Mori, M., Takigawa, S., Matsuura-Endo, C., Saito, K., Mangalika, W. H. A., Hanaoka, A., Suzuki, Y., Yamauchi, H. 2004. The effect of harvest dates on the starch properties of various potato cultivars. *Food Chemistry*, 86 (1), 119–125.

Noda, T., Takigawa, S., Matsuura-Endo, C., Kim, S., Hashimoto, N., Yamauchi,H., Hanashiro, I., & Takeda Y. 2005. Physicochemical properties and amylopectin structures of large, small, and extremely small potato starch granules. *Carbohydrate Polymers,* 60 (2), 245–251.

O'keefe, J. H. & Bell, D. S. H. 2007. Postprandial Hyperglycemia/Hyperlipidemia (Postprandial dysmetabolism) is a cardiovascular risk factor. *American Journal of Cardiology*, 100, 899-904.

Olthof, M. R., Hollman, P. C. H. & Katan, M. B. 2001. Chlorogenic acid and caffeic acid are absorbed in humans. *Journal of Nutrition*, 131, 66-71.

Onyeneho, S. N. & Hettiarachchy, N. S. 1993. Antioxidant activity, Fatty-acids and phenolic-acids composition of potato peels. *Journal of the Science of Food and Agriculture*, 62, 345-350.

Otto, H., & Niklas, L. 1980. Different glycemic responses to carbohydrate-containing foods. Implications for the dietary treatment of diabetes mellitus. *Hyg (Geneve),* 38, 3424-3429, (in French).

Panlasigui, L.N., Thompson,L.U., Juliano,B.O., Perez,C.M., Yiu, S.H., & Greenberg G.R. 1991. Rice varieties with similar amylose content differ in starch digestibility and glycemic response in humans. *American Journal of Clinical Nutrition*, 54, 871–877

Perala, M.-M., Hantonen, K. A., Virtamo, J., ErikssonR, J. G., Sinkko, H. K., Sundvall, J. & Valsta, L. M. 2011. Impact of overweight and glucose tolerance on postprandial responses to high- and low-glycaemic index meals. *British Journal of Nutrition,* 105**,** 1627-1634.

Perez, S., & Bertoft, E. 2010. The molecular structures of starch components and their contribution to the architecture of starch granules: A comprehensive review. *Starch/Starke*, 62, 389–420.

Perla, V., Holm, D. G. & Jayanty, S. S. 2012. Effects of cooking methods on polyphenols, pigments and antioxidant activity in potato tubers. *Lwt-Food Science and Technology,* 45, 161-171.

Picot, C., Subratty, A. H. & Mahomoodally, M. F. 2014. Inhibitory Potential of Five Traditionally Used Native Antidiabetic Medicinal Plants on α-Amylase, α-Glucosidase, Glucose Entrapment, and Amylolysis Kinetics In Vitro. *Advances in Pharmacological Sciences*, 2014.

Pikus, S. 2005. Small-angle X-ray scattering (SAXS) studies of the structure of starch and starch products. *Fibers & Textiles in Eastern Europe*, 5 (53), 82—86.

Pinhero, R.G., Waduge, R.N., Liu, Q., Sullivan, J.A., Tsao, T., Bizimungu,B. & Yada, R.Y. 2016. Evaluation of nutritional profiles of starch and dry matter from early potato varieties and its estimated glycemic impact. *Food Chemistry*, 203, 356-366.

Polesi, L. F., Sarmento,S. B., de Moraes, J., Franco,C. M. L., Canniatti-Brazaca, S. G. 2016. Physicochemical and structural characteristics of rice starch modified by irradiation. *Food Chemistry*, 191, 59–66.

Protserov, V.A., Wasserman, L.A., Tester, R.F., Debon, S.J.J., Ezernitskaja, M.G.,& Yuryev, V.P. 2002. Thermodynamic and structural properties of starches extracted from potatoes grown at different environmental temperatures. *Carbohydrate Polymers,* 49, 271–279.

Ramdath, D. D., Padhi, E., Hawke, A., Sivaramalingam, T. & Tsao, R. 2014. The glycemic index of pigmented potatoes is related to their polyphenol content. *Food & Function,* 5**,** 909-915.

Ratner, R. E. 2001. Controlling postprandial hyperglycemia. *American Journal of Cardiology*, 88, 26H-31H.

Rendell, M. S. & Jovanovic, L. 2006. Targeting postprandial hyperglycemia. *Metabolism-Clinical and Experimental*, 55, 1263-1281.

Rendleman, J. A. 2000. Hydrolytic action of alpha- amylase on high-amylose starch of low molecular mass. *Biotechnology and Applied Biochemistry*, 31, 171-178.

Reyes, L. F., Miller, J. C. & Cisneros-Zevallos, L. 2004. Environmental conditions influence the content and yield of anthocyanins and total phenolics in purple- and red-flesh potatoes during tuber development. *American Journal of Potato Research* 81, pp. 187-193.

Riccardil, G., Rivellese, A. A. & Giacco, R. 2008. Role of glycemic index and glycemic load in the healthy state, in prediabetes, and in diabetes. *American Journal of Clinical Nutrition*, 87, 269S-274S.

Robyt, J.F. & Whelan, W.J. 1972. Reducing value methods for maltodextrins: chain length dependence of alkaline 3, 5-dinitrosalisylate and chain length independence of alkalinecopper. *Analytical Biochemistry*, 45(2), 510-516.

Rocha, L. D., Monteiro, M. C. & Teodoro, A. J. 2012. Anticancer properties of hydroxycinnamic acids-A Review. Cancer and clinical oncology, 1, p109.

Rocklin, R. D. & Pohl, C. A. 1983. Determination of Carbohydrates by Anion Exchange Chromatography with Pulsed Amperometric Detection. *Journal of Liquid Chromatography*, 6 (9), 1577-1590.

Rohn, S., Rawel, H. M. & Kroll, J. 2002. Inhibitory effects of plant phenols on the activity of selected enzymes. Journal of Agricultural and Food Chemistry, 50(12), 3566-3571.

Rosin, P. M., Lajolo, F. M.,& Menezes, E. W. 2002. Measurement and Characterization of Dietary Starches. *Journal of Food Composition and Analysis,* 15 (4), 367-377.

SACN Scientific Advisory Committee on Nutrition, 2011 Dietary Reference Values for Energy. London, TSO.

SACN Scientific Advisory Committee on Nutrition, 2015. Carbohydrates and Health. London, TSO.

Salman, H., Blazek, J., Lopez-Rubio, A., Gilbert, E. P., Hanley,T., & Copeland, L. 2009. Structure–function relationships in A and B granules from wheat starches of similar amylose content. *Carbohydrate Polymers*, 75(3), 420–427.

Shakya, R. & Navarre, D. A. 2006. Rapid screening of ascorbic acid, glycoalkaloids, and phenolics in potato using high-performance liquid chromatography. *Journal of Agricultural and Food Chemistry*, 54(15), 5253-5260.

Shen, W., Xu, Y. & Lu, Y. H. 2012. Inhibitory effects of citrus flavonoids on starch digestion and antihyperglycemic effects in HepG2 cells. *Journal of Agricultural and Food Chemistry*, 60, 9609-9619.

Shobana, S., Sreerama, Y. N. & Malleshi, N. G. 2009. Composition and enzyme inhibitory properties of finger millet (Eleusine coracana L.) seed coat phenolics: Mode of inhibition of α-glucosidase and pancreatic amylase. *Food Chemistry*, 115(4), 1268-1273.

Shogren, R.L. 1992. Effect of moisture content on the melting and subsequent physical aging of corn starch. *Carbohydrate Polymers,* 19, 83–90.

Singh, N., & Kaur, L. 2004. Morphological, thermal, rheological and retrogradation properties of potato starch fractions varying in granule size. *Journal of the Science of Food and Agriculture*, 84, 1241–1252.

Singh, N., Singh, J., Kaur,L., Sodhi,N. S., & Gill B. S. 2003. Morphological, thermal and rheological properties of starches from different botanical sources. *Food Chemistry*, 81(2), 219–231.

Singh, N., Kamath, V. & Rajini, P. 2005. Protective effect of potato peel powder in ameliorating oxidative stress in streptozotocin diabetic rats. *Plant Foods for Human Nutrition,* 60(7-8)**,** 49-54.

Singleton, V.L. & Rossi J.A. 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enoogy and Viticulture,* 16, 144-158.

Smolin, L. A., Marry, B., & Grosvenor. 1997. Nutrition: science and applications, Fort Worth philadephela Sand deigo New York orlando Austin, san antonio toronto, Monteral london, sydney, tokyo, Saunders College Pub. : Harcourt Brace College Publishers.

Soh, N. L. & Brand-Miller, J. 1999. The glycaemic index of potatoes: the effect of variety, cooking method and maturity. *European Journal of Clinical Nutrition*, 53(4), 249-254.

Sujka, M. and Jamroz,J. 2007. Starch granule porosity and its changes by means of amylolysis. *Int. Agrophysics,* 21, 107-113

Stushnoff, C., Holm, D., Thompson, M. D., Jiang, W., Thompson, H. J., Joyce, N. I. & Wilson, P. 2008. Antioxidant properties of cultivars and selections from the Colorado potato breeding program. *American Journal of Potato Research*, 85, 267-276.

Sun, L., Chen, W., Meng,Y., Yang, X., Yuan, L. & Guo, Y. 2016. Interactions between polyphenols in thinned young apples and porcine pancreatic a -amylase: Inhibition, detailed kinetics and fluorescence quenching. *Food Chemistry,* 208, 51–60

Tester, R. F., Qi, X., Karkalas, J. 2006. Hydrolysis of native starches with amylases Animal Feed. *Science and Technology*, 130(1–2), 39–54.

Tester, R. F., Karkalas, J., & Qi, X. 2004. Starch - Composition, fine structure and architecture. *Journal of Cereal Science*, 39(2), 151-165.

Tian, J., Chen, J., Ye, X., & Chen, S. 2016. Health benefits of the potato affected by domestic cooking: A review. *Food Chemistry*, 202, 165–175

Thompson, M. D., Thompson, H. J., Mcginley, J. N., Neil, E. S., Rush, D. K., Holm, D. G. & Stushnoff, C. 2009. Functional food characteristics of potato cultivars (Solanum tuberosum L.): Phytochemical composition and inhibition of 1-methyl-1-nitrosourea induced breast cancer in rats. *Journal of Food Composition and Analysis*, 22, 571-576.

Thorne, M. J., Thompson, L. U. & , D. J. A. 1983. Factors affecting starch digestibility and the glycemic repsonse with special reference to legumes. A *American Journal of Clinical Nutrition,* 38**,** 481-488.

Thybo, A.K., Christiansen, J., Kaak, K. & Petersen, M.A. 2006. Effect of cultivars, wound healing and storage on sensory quality and chemical components in pre-peeled potatoes. *LWT*, 39, 166-176.

Tierno, R., Hornero-Mendez, D., Gallardo-Guerrero, L., Lopez-Pardo, R. & Ignacio Ruiz de Galarreta, J. 2015. Effect of boiling on the total phenolic, anthocyanin and carotenoid concentrations of potato tubers from selected cultivars and introgressed breeding lines from native potato species. *Journal of Food Composition and Analysis,* 41**,** 58-65.

Trinh, B.T.D., Staerk, D. & Jäger. A.K. 2016. Screening for potentialα-glucosidase andα-amylase inhibitoryconstituents from selected Vietnamese plants used to treat type2 diabetes. *Journal of Ethnopharmacology*, 186, 189–195.

Ulmius, M., Johansson, A. & Onning, G. 2009. The influence of dietary fibre source and gender on the postprandial glucose and lipid response in healthy subjects. *European Journal of Nutrition,* 48**,** 395-402.

UK Prospective Diabetes Study Group. 1998. Efficacy of atenolol and captopril in reducing risk of macrovascular and microvasvular complications in type 2 diabetes: UKPDS 39. *Br Med J*., 317, 713–720.

Van der Mareel, M. j. e.c., Van der Veen, B., Uitdehaag, J. c. m., Leemhuis, H. & Dijkhuizen, L. 2002. Properties and applications of starch-converting enzymes of the α-amylase family. *Journal of Biotechnology,* 94(2), 133-135.

Vasanthan, T., Bergthaller, W., Driedger, D., Yeung, J., & Sporns, P.1999. Starch from Alberta potatoes: wet isolation and some phy- sicochemical properties. *Food Research International*, 32, 355–365.

Warren, F. J., Butterworth, P. J. & Ellis, P. R. 2012. Studies of the effect of maltose on the direct binding of porcine pancreatic alpha-amylase to maize starch. *Carbohydrate Research*, 358, 67-71.

Weichselbaum, E. 2010. An overview of the role of potatoes in the UK diet. *Nutrition Bulletin*, 35, 195-206.

Whitcomb, D. C. & Lowe, M. E. 2007. Human pancreatic digestive enzymes. *Digestive Diseases and Sciences,* 52, 1-17.

Wiesenborn, D.P., Orr, P.H, Casper, H.H., & Tacke, B.K. 1994. Relationship between potato starch paste behavior and selected physical /chemical properties. *Journal of Food Science*, 58. 644–648.

Wischmann, B., Ahmt, T., Bandsholm, O., Blennow, A., Young, N., Jeppesen, L., & Thomsen L. 2007. Testing properties of potato starch from different scales of isolations—A ring test. *Journal of Food Engineering* 79, 970–978.

Wolever, T. M. 2006. The glycaemic index a phsyiological classification of dietery carbohydrate. Wallingford, UK: CABI Publishing,

Wolever, T. M. S., , D. J. A., , A. L., & Josse, R. G. 1991. The glycemic index: methodology and clinical implications. *Am J Clin Nutr*, 54, 846-854.

Wolever, T. M. S., Brand-Miller, J. C., Abernethy, J., Astrup, A., Atkinson, F., Axelsen, M., Bjorck, I., Brighenti, F., Brown, R., Brynes, A., Casiraghi, M. C., Cazaubiel, M., Dahlqvist, L., Delport, E., Denyer, G. S., Erba, D., Frost, G., Granfeldt, Y., Hampton, S., Hart, V. A., Hantonen, K. A., Henry, C. J., Hertzler, S., Hull, S., Jerling, J., Johnston, K. L., Lightowler, H., Mann, N., Morgan, L., Panlasigui, L. N., Pelkman, C., Perry, T., Pfeiffer, A. F. H., Pieters, M., Ramdath, D. D., Ramsingh, R. T., Robert, S. D., Robinson, C., Sarkkinen, E., Scazzina, F., Sison, D. C. D., Sloth, B., Staniforth, J., Tapola, N., Valsta, L. M., Verkooijen, I., Weickert, M. O., Weseler, A. R., Wilkie, P. & Zhang, J. 2008. Measuring the glycemic index of foods: interlaboratory study. *American Journal of Clinical Nutrition,* 87**,** 247S-257S.

Wolever, T. M. S., Giddens, J. L. & Sievenpiper, J. L. 2015. Effect of ethnicity on glycaemic index: a systematic review and meta-analysis. *Nutrition & Diabetes,* 5.

Wolever,T. M. S., Vorster,H. H., Björck, I., Brand-Miller, J., Brighenti, F. J., Mann, I., Ramdath, D. D., Granfeldt, Y., Holt, S., Perry,T. L., Venter, C., & Wu X. 2003. Determination of the glycaemic index of foods: interlaboratory study. *European Journal of Clinical Nutrition*, 57, 475–482.

Xiao, Z. Z., Storms, R. & Tsang, A. 2006. A quantitative starch-iodine method for measuring alpha-amylase and glucoamylase activities. *Analytical Biochemistry*, 351(1), 146-148.

Xie, Y.-Y., Hu, X.-P., Jin, Z.-Y., Xu, X.-M. & Chen, H.-Q. 2014. Effect of repeated retrogradation on structural characteristics and in vitro digestibility of waxy potato starch. *Food Chemistry*, 163, 219-225.

Yu, K., Ke, M.-Y., Li, W.-H., Zhang, S.-Q. & Fang, X.-C. 2014. The impact of soluble dietary fibre on gastric emptying, postprandial blood glucose and insulin in patients with type 2 diabetes. *Asia Pacific journal of clinical nutrition,* 23, 210.

Yu, L., & Christie, G. 2001. Measurement of starch thermal transitions using differential scanning calorimetry. Carbohydrate Polymers, 46 (2), 179–184.

Yuryev, V.P., Krivandin, A. V., Kiseleva, V. I., Wasserman, L. A., Genkina N.K., Fornal, J., Blaszczak, W., & and Schiraldi, A. 2004. Structural parameters of amylopectin clusters and semi-crystalline growth rings in wheat starches with different amylose content. *Carbohydrate Research*, 339 (16), 2683–2691.

Yusupha,M., Testera,R. F., , Ansella,R.,& Snapeb, C. E. 2003. Composition and properties of starches extracted from tubers of different potato varieties grown under the same environmental conditions. *Food Chemistry,* 82(2), 283–289.

Zakrzewska, M. E., Bogel-Lukasik, E. & Bogel-Lukasik, R. 2010. Solubility of Carbohydrates in Ionic Liquids. *Energy & Fuels*, 24, 737-745.

Zeleznak, K.L. & Hoseney R.C. 1997. The glass transition in starch. *Cereal Chemistry*, 64 (2), 121–124.

Zhang, B., Zhao, Y., Li, X., Zhang, P., Li, L., Xie, F. & Chen, L. 2014. Effects of amylose and phosphate monoester on aggregation structures of heat-moisture treated potato starches. *Carbohydrate Polymers,* 103, 228-233.

Zhao, X., Shen, C., Zhu, H., Wang, C., Liu, X., Sun, X., Han, S., Wang, P., Dong, Z., Ma, X., Hu, K., Sun, A.,& Ge, J. 2016. Trans-Fatty Acids Aggravate Obesity, Insulin Resistance and Hepatic Steatosis in C57BL/6 Mice, Possibly by Suppressing the IRS1 Dependent Pathway. *Molecules*, 21(6), 705.

Zhou, W., Yang, J., Hong, Y., Liu, G., Zheng, J., Gu, Z.,& Zhang, P. 2015. Impact of amylose content on starch physicochemical properties in transgenic sweet potato. *Carbohydrate Polymers,* 122, 417–427

Zhu, J., Li, L., Chen, L., & Li, X. 2012. Study on supramolecular structural changes of ultrasonic treated potato starch granules. *Food Hydrocolloids*, 29 (1), 116–122.

Appendix A
Ethical approval

Performance, Governance and Operations
Research & Innovation Service
Charles Thackrah Building
101 Clarendon Road
Leeds LS2 9LJ Tel: 0113 343 4873
Email: ResearchEthics@leeds.ac.uk

UNIVERSITY OF LEEDS

Zida Karim
Food Science and Nutrition
University of Leeds
Leeds, LS2 9JT

MaPS and Engineering joint Faculty Research Ethics Committee (MEEC FREC)
University of Leeds

18 November 2016

Dear Zida

Title of study	**Comparing the glycemic index of potatoes with different level of total phenolic content in vivo**
Ethics reference	**MEEC 15-006**

I am pleased to inform you that the application listed above has been reviewed by the MaPS and Engineering joint Faculty Research Ethics Committee (MEEC FREC) and I can confirm a favourable ethical opinion as of the date of this letter. The following documentation was considered:

Document	*Version*	*Date*
MEEC 15-006 Ethical Form Zida KArim 22092015.docx	1	29/09/15
MEEC 15-006 Appendixes ALL CO (2).docx	1	29/09/15
MEEC 15-006 risk_assessment_form_20092015 (1).pdf	1	29/09/15

Please notify the committee if you intend to make any amendments to the original research as submitted at date of this approval, including changes to recruitment methodology. All changes must receive ethical approval prior to implementation. The amendment form is available at http://ris.leeds.ac.uk/EthicsAmendment.

Please note: You are expected to keep a record of all your approved documentation, as well as documents such as sample consent forms, and other documents relating to the study. This should be kept in your study file, which should be readily available for audit purposes. You will be given a two week notice period if your project is to be audited. There is a checklist listing examples of documents to be kept which is available at http://ris.leeds.ac.uk/EthicsAudits.

We welcome feedback on your experience of the ethical review process and suggestions for improvement. Please email any comments to ResearchEthics@leeds.ac.uk.

Yours sincerely

Jennifer Blaikie
Senior Research Ethics Administrator, Research & Innovation Service
On behalf of Professor Gary Williamson, Chair, MEEC FREC

CC: Student's supervisor(s)

Appendix B
Health screening questionnaire

Date: / / **Participant code……**

Please provide brief information about yourself by ticking the appropriate answer(s) or provide additional information where necessary. These questions are designed to determine whether you are eligible to participate in the study. If you have any queries, please do not hesitate to ask the researcher.

1. Would you consider yourself to be in good health?

   □Yes □No

   If the answer is 'no', please explain the reasons for your choice:

2. Have you ever been told by a doctor or other health professional that you have any of the following?

□Type I diabetes	□Yes	□No	□ don't know
□ Type II diabetes	□Yes	□No	□ don't know
□ Gestational diabetes (Diabetes during pregnancy)	□Yes	□No	□ don't know
□ Prediabetes	□Yes	□No	□ don't know
□ Impaired fasting glucose	□Yes	□No	□ don't know
□ Impaired glucose tolerance	□Yes	□No	□ don't know
□ Borderline diabetes	□Yes	□No	□ don't know

3. Do you suffer from any health problems associated with your digestive system (stomach, intestine), your pancreas, your liver or your kidneys?
   □Yes □No □ don't know

If the answer is 'yes', then please provide details of your condition.

4. Are you taking any type of medication?

   □Yes □ No

   If the answer is 'yes', please list below the name of all the medications that you are currently taking:

5. Do you have any history of allergy (food or non-food related)?

□Yes □No

If yes, which kind of allergy do you have?

8. Are you pregnant? □Yes □No □ don‘t know

9. Are you breastfeeding? □Yes □No

Thank you for your time, the answers will allow us to determine whether you are eligible for the trial. A researcher will contact you soon to explain what the next steps are.

Appendix C
pre-study questionnaire and blood measurement record

Date: / / **Participant code……**

Please provide brief information about yourself by tick at the appropriate answer (s) or provide additional information where necessary. These questions are designed to gather information about demographic, diet and lifestyle factors that may affect how you respond to food.

Nationality: □ United kingdom □ EU □Other…….	Weight:
Age (years):	Height:
Gender: Male □ Female □	Waist circumference

1. Ethnic background :

   □ White - British
   □ White - Irish
   □ White – Scottish
   □ Irish Traveller
   □ Other White Background
   □ Black or Black British– Caribbean
   □ Black or Black British – African
   □ Other Black Background
   □ Asian or Asian British - Indian
   □ Asian or Asian British - Pakistani
   □ Asian or Asian British – Bangladeshi
   □ Chinese
   □ Asian Other
   □ Mixed – White and Black Caribbean
   □ Mixed – White and Black African
   □ Mixed – White and Asian
   □ Other Mixed Background
   □ Other Ethnic Background
   □ Information Refused

2. Do you consider yourself to be in good health today?
   □Yes □No

   If the answer is ‘no’, please explain the reasons for your choice:

3. Do you drink alcohol?

□Yes □No

If the answer is 'yes', how many units do you consume every week? (1 unit is ½ glass of wine, ½ pint of beer, 1 measure of spirits)

4. What is your usual staple food?

□Rice □wheat □corn □potato □other (please specify)……..

5. Are you currently on a special of diet?

□Yes □No

If the answer is 'yes', please specify what kind of diet you are on:

6. Are you vegetarian or vegan?

□Yes □No

7. Do you smoke cigarettes/cigars/pipe regularly?

□Yes □No

If the answer is 'yes', how many do you smoke in our day?

8. Do you do any vigorous-intensity sports, fitness, or recreational activities that cause large increases in breathing or heart rate like running or basketball for at least 10 minutes continuously at least 3 days a week?

□Yes □No

If the answer is 'yes', please specify type of exercise, duration and frequency per week.

9. Do you do any moderate-intensity sports, fitness, or recreational activities that cause a small increase in breathing or heart rate such as yoga, brisk walking, bicycling, swimming, or golf for at least 10 minutes continuously per day?

□Yes □No

If the answer is ‘yes’, please specify type of exercise, duration and frequency per week.

10. How did you come to school today?

□ by foot □by bicycle
□by any other transportation (please specify……)

11. Do you feel nervous or stressed today?

□Yes □No

If the answer is ‘yes’, please explain why you think you may be feeling this way.

12. 24- Hours food consumption recall. Please write down all the foods and drinks that you consumed in the last 24 hours

Food intake	Time	Description	Amount
Break fast			
Snack			
Lunch			
Snack			
Dinner			
Snack			

Timetable for the blood glucose measurement record

Time	0 Min	15 Min	30 Min	45 Min	60 Min	90 Min	120 Min
Blood glucose							

Thank you for your time

Appendix D
Participation information sheet

The title of the research project: Comparing the glycaemic index of potatoes with different level of total phenolic content *in vivo*

You are being invited to take part in a research project. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask the researcher if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

What is the purpose of the project?

Glycaemic index (GI) is a way to measuring the post-prandial glycaemic response to carbohydrate-rich food compared to a reference food such as white bread. In diabetic patients, a carbohydrate-rich diet can have detrimental effects on glycaemic control. Controlling postprandial hyperglycaemia are thought to be important factors in the prevention and treatment of type-2 diabetes.

Potato has been labelled as a high glycaemic index (GI) food that causes a sharp increase of blood glucose concentration following consumption; however there is great variation in the GI of potato ranged from 56-94. Preliminary data obtained in the present and published study suggests that chlorogenic acid is the most abundant phenolic in potato which can inhibit potato starch digestion in vitro. The level of the total phenolic content of varieties of potato in present study ranging from 282 – 543 mg per 100 g Dw measured as chlorogenic acid equivalent. These levels of total phenolic may be enough to inhibit alpha amylase in vivo.

The aim of this study is to compare the GI of three varieties of potato with different levels of chlorogenic acid.

Why have I been chosen?

We are looking for a healthy individuals (males and females) aged 18-60 years, living in the Leeds area, who are pregnant or lactating women, not allergic to any food (potato), not diagnosed with other chronic disease such as diabetes, cardiovascular disease, cancer and not taking any medication that affect digestion or the glucose response.

Do I have to take part?

Participation is voluntary and if you do decide to take part you will be given this information sheet to kneepad be asked to sign a consent form. You can still withdraw at any time. You do not have to give a reason.

What do I have to do? / What will happen to me if I take part?

If you decide to take part in this study, you will be invited to attend a briefing session. During the briefing session the researcher will explain the details of study and will answer any questions that you may have. If you decide to take part, you will be given a consent form to sign and you will be given a copy to keep. You will then complete a health screening questionnaire which will establish if you are suitable to enrol in the study. If you are eligible, you will be asked to visit the research centre on 4 occasions for around 3 hours.

On the day before each visit, you will be asked to restrict your physical activity, refrain from drinking alcohol, smoking for 24 hours and consume high fibre foods at dinner. You will be asked not to consume any foods or drinks (apart from water) until you arrive at the research centre. On the morning of the visit, you will be asked to arrive in fasted. We will then measure your blood glucose using a finger prick glucose testing strip. Then you will be asked to consume a portion of steam cooked potato or white bread as provided by researcher, consumed with a glass of water. Your blood glucose will measured again by finger prick over 2 hrs at 15, 30, 45, 60, 90 and 120 minutes. You will be asked to avoid eating or drinking any foods during these two hours. Following each study visit, you will be provided with a light breakfast. After completion of the study, a £20 retail voucher will be offered as compensation for your time.

What are the possible disadvantages and risks of taking part?

You may experience minor discomfort from fasting and blood taking by finger pricking. If you are concerned or feel that you are experiencing high levels of discomfort, please let the researcher know.

What are the possible benefits of taking part?

There are no direct benefits to participants taking part in this study. We will let you know the results of your blood glucose test. If we find any abnormal results, we will encourage you to discuss them with the GP.

Knowledge from this study will allow us to refine the knowledge that we have on the GI of potatoes, and may open dietary strategies for people who need to manage their blood glucose.

Will my taking part in this project be kept confidential? / What will happen to the result of the research project?

Yes, any information you provide will be treated confidentially and stored according to the data protection Act (1998). You will not be identified as having taking part in this study as all completed food diaries will be number coded and stored securely in a locked cabinet at the school of Food Science and

Nutrition, University of Leeds. Only researcher and the principal investigator will have access to the cabinet.

The anonymised data may be published through scientific journals and through data repositories.

Withdrawing

You can withdraw at any stage of during the study without having to give a reason. You can also withdraw your data from the study up until the data is published. However, we kindly request that you let the research team know by e-mail.

Contact for further information

If you would like more information or have any questions please contact:

Zida Karim

PhD research student

School of Food Science and Nutrition

Faculty of Mathematics & physical Sciences

University of Leeds

LS2 9JT

TEL:

E-mail: fszmk@leeds.ac.uk

Or

If you require further information, you may also contact the principal investigators:

Dr Caroline Orfila by E-mail: C.Orfila@leeds.ac.uk

Dr Melvin Holmes by E-mail: prcmjh@leeds.ac.uk

This study has been reviewed by The Faculty of Mathematics and Physical Sciences and Engineering Ethics Committee, University of Leeds. [**MEEC 15-006**]

Thank you for you to making the time to read this information

Appendix E
Information on food samples

Type pf meal	information
Reference Food	white bread (3 Slices) containing 50g of available carbohydrate+ water
Test food	1. Peeled and steam cooked (30 min) Desiree potato (350 g) + water. The amount of total phenolic content of cooked potato measured by Folin-ciocalteu method is 546.96 mg/100 g DW chlorogenic acid equivalent. 2. Peeled and steam cooked (30 min) Rooster potato (350 g) + water. The amount of total phenolic content of cooked potato measured by Folin-ciocalteu method is 282.03 mg/100 g DW chlorogenic acid equivalent. 3. Peeled and steam cooked (30 min) Maris piper potato (350 g) + water. The amount of total phenolic content of cooked potato measured by Folin-ciocalteu method is 349.81 mg/100 g DW chlorogenic acid equivalent.

The volunteers should eat one of the foods reported above in one day, in the amount mentioned in the protocol.

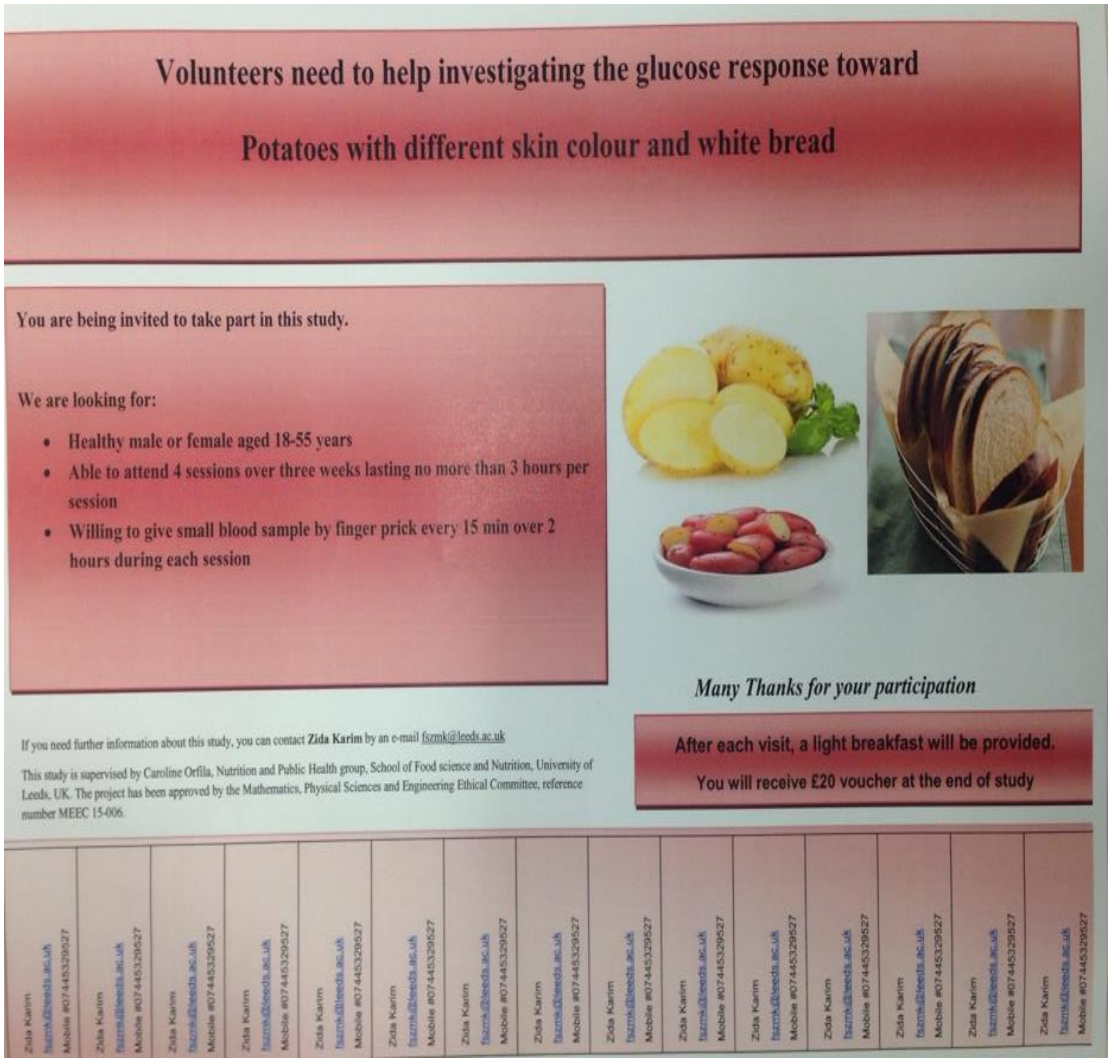
Table of test meal randomization

Test food for each study visit is randomized using Latin-squares design.

1= White bread + water
2= Steam cooked Desiree potato + water
3= Steam cooked Rooster potato + water
4= Steam cooked Maris Piper potato+ water

15 participants	**1st visit**	**2nd visit**	**3rd visit**	**4th visit**
1	4	2	3	1
2	3	4	1	2
3	1	3	2	4
4	2	1	4	3
5	4	1	2	3
6	3	2	1	4
7	1	3	4	2
8	2	4	3	1
9	1	2	4	3
10	4	1	3	2
11	2	3	1	4
12	3	4	2	1
13	2	4	1	3
14	3	3	2	1
15	3	1	4	2

Appendix E
Invitation sheet



Appendix F
Consent form

Consent to take part in [Comparing the glycaemic index of potatoes with different level of total phenolic content *in vivo*]

	Please confirm the statements below
I confirm that I have read and understand the information sheet explaining the above research project and I have had the opportunity to ask questions about the project.	
I agree for the anonymised data collected from me to be stored and used in relevant future research.	
I understand that any information I provide, including personal details, will be confidential, stored securely and only accessed by those carrying out the study.	
I agree to take part in the above research project and will inform the lead researcher should my contact details change.	

Name of participant	
Participant's signature	
Date	
Name of lead researcher	
Signature	
Date*	

* To be signed and dated in the present of the participant

When completed: one copy for participant, one copy to secure research file.

Appendix G
Standard operating procedure (sop) measurement of blood glucose

Objective:
The objectives of this sop is to provide general guideline on how to carry out blood glucose measuring in order to provide consistency of the procedure as specified in the trial protocol while, ensuring the safety of subjects and staff involved in this study.

Equipment

- Couch or chair for the patient
- Meter
- Test Strips
- Single Use Safety Lancets
- Cotton Wool
- Disposable gloves
- Sharps bin
- Orange plastic disposable bag
- Documentation

Procedures
Subject prerequisites

1. The subject should be fasted for a least 10 hours, and not more than 14 hours, prior to each visit.
2. The subject should refrain from consuming alcohol during the evening before the study visit.
3. The subject should refrain from strenuous exercising in the evening before and in the morning before each study visit.

Obtaining Blood glucose measurement:

1. Explain the procedure to the research participant and obtain informed consent.
2. Ensure that the participant is comfortable and warm.
3. Prepare the blood glucose meter and test strip (instruction for this depends upon the type of glucose meter used). Ensure the blood glucose meter is clean, working and that the test strips are in date.
4. Wash hands and put on gloves.
5. The participant should be asked to wash the area to be used to obtain the sample with warm water and soap, rinse thoroughly and dry. This

is to remove any potential interfering substances and to improve the flow of blood.

6. Prepare the blood glucose meter and test strip.
7. Insert the test strip to the analyser.
8. Wipe the subject hand by alcohol swap, let the alcohol to dry out before finger pricking.
9. The area to be used is the side of the finger; the tips are to be avoided. The chosen puncture site should continually be rotated.

10. Use a disposable lancet to prick the side of finger.
11. Wipe off the first drop of blood, and then allow the second drop of blood to elapse.
12. For blood glucose measurement, then touch the drop of blood to the front edge of the yellow window of the test strip, ensure the yellow window is full of blood. Once the result is obtained, record immediately.
13. Apply cotton wool to the puncture site and apply pressure.
14. The result must be recorded in the relevant study source documentation.
15. After completion, dispose the lancets and strips in clinical waste bin.
16. In the event the subject becomes unwell, the investigator will stop the blood measurement and inform the first aid personnel to assist the subject.

Quality control testing

Quality control refers to the routine testing of the blood glucose and triglyceride testing meters with control solution. This will ensure the device is working correctly and assure the investigator of the reliability of results. Quality control test must be completed prior to use on the study visit.

A quality control must be performed:

- Before using meter for the first time
- Each time a new vial of strips is opened
- If the batteries in the meter have been replaced
- If an improbable result is obtained

The quality control standards should be purchased and used according to the manufacturer’s instruction. A log of quality control tests should be kept including the date, the lot number of reagents used, the date the reagent first opened and their expiry date.

Expected values

For the first glucose and triglyceride measurement, Glucose levels showing diabetes or hypoglycemia will be notified to the participant and participant will not be included in the study.

The expected value for fasting blood glucose

Normal range: 4.1-5.9 mmol/L.

The high fasting level: >6.1 mmol/L.

References:

Buyers Guide, blood glucose systems NHS (2008)

Blood Glucose Monitoring NHS (2013), SOP CRF.C116 Version 3.0

Blood glucose monitoring. Scottish Diabetes Research Network (SDRN) (2013). Clinical S.O.P. No. 23 version 1.1

Appendix H
Demographic characteristics and blood glucose response

Table 1. iAUC (mmol.min/L) observed in the subjects with different gender after consumption of food samples. Data expressed as the mean of iAUC ±SD.

Tested foods	Male (n=2)	Female (n=7)	p^1
White bread	87.92±32.63	100.75±37.19	0.67
Desiree	71.56±23.02	106.60±30.02	0.17
Rooster	85.05±3.04	131.22±40.86	0.17
Maris Piper	82.67±5.62	116.89±31.46	0.18
p^2	0.85	0.40	

Table 2. calculated for the subjects with different gender after consumption of food samples. Data expressed as the mean of GI ±SD.

Tested foods	Male (n=2)	Female (n=7)	p^1
Desiree	82.19±4.32	116.30±40.21	0.29
Rooster	103.19±34.84	140.51±54.03	0.39
Maris Piper	120.49±30.90	111.52±28.41	0.70
p^2	0.46	0.40	

Table 3. iAUC (mmol.min/L) observed in the subjects from different background after consumption of food samples. Data expressed as mean of iAUC ±SD.

Food samples	Asian (n=6)	Mexican (n=2)	p^1
White bread (reference food)	100.36±45.18	70.60±9.54	0.31
Desiree	90.58±23.82	90.55±10.53	0.99
Rooster	116.34±44.66	119.1 ±47.37	0.94
Maris Piper	99.27±31.07	119.42±23.93	0.44
p^2	0.63	0.34	

Table 4. GI calculated for the subjects from different background after consumption of food samples. Data expressed as mean of GI±SD.

Food samples	Asian (n=6)	Mexican (n=2)	P^1
Desiree	100.33±45.18	128.42±2.44	0.43
Rooster	121.61±39.71	174.83±90.74	0.25
Maris Piper	110.41±23.25	134.33±42.05	0.32
P^2	0.62	0.35	