Ollscoil na hÉireann, Corcaigh

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The Glycaemic Index of Fresh and Processed Potatoes

Thesis presented by

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Table of Contents

Declaration

I hereby declare that the work submitted is entirely my own and has not been submitted to any other university or higher education institute, or for any other academic award in this university

Signature:

Aine Muldoon 12th October 2020

_____ Date _____

Aine Muldoon

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Abstract

Potatoes are an important staple food, which provide vital nutrition to millions of people globally every year. However, potatoes have a relatively high carbohydrate content as well as being generally considered as a high Glycaemic Index (GI) food. Research suggests that the consumption of potatoes or high GI foods can contribute to the onset of certain chronic conditions such as cardiovascular disease and type II diabetes. The supposed link between potatoes and negative health outcomes has led to a decline in their consumption in the developed world.

The aim of this thesis was to assess the GI, glycaemic load (GL), and carbohydrate parameters of selected potato cultivars using an *in-vitro* method. The main objectives of the research were to employ various interventions in an attempt to reduce the GI and GL of the potatoes. Five potato cultivars commonly consumed in Ireland (Cultra, Gemson, Kerr's Pink, Maris Piper, and Rooster) were tested. All varieties were found to have a medium or high GI. Evidence suggests that certain types of processing can reduce GI therefore each of the potato cultivars were then subjected to high pressure processing (HPP) at either 400Mpa or 600Mpa. In the potatoes which had the highest GI a decline in the GI was observed as the pressure was increased, however this decrease in GI was not seen in the potato varieties with a lower GI.

Roosters were selected for further testing as they are the most widely produced and consumed potato variety in Ireland. To determine the effect of added fat on GI, a dose response was conducted by adding 10%, 15%, 20% or 25% (w/w) of rapeseed oil to potato and measuring GI. The GI of potato was also assessed following addition of butter, coconut oil, or olive oil at a concentration of 10% (w/w). No significant changes in GI or GL of the potato were observed following the addition of fat at any concentration; nor did the degree of saturation of the added fat impact GI or GL.

The impact of combining Roosters with either cheese, peas, beans, or tuna on the GI of the resultant meal was investigated. Each meal consisted of 50g of available carbohydrates.

Roosters alone had a medium GL, this was reduced to a low GL when they were included as part of a meal. The GI was also reduced for every meal in comparison to Rooster alone, apart from the potato-tuna meal. The greatest decrease in the GI was observed when Rooster were combined with beans which are a rich source of fibre.

Consequently, the addition of three fibres; pectin, HPMC (hydroxypropylmethylcellulose), or inulin was investigated as a means of reducing the GI of Rooster potatoes. The fibres were tested at three concentrations 5%, 7% or 10% (w/w). The viscosity of the digesta as well as the carbohydrate parameters were measured. Pectin induced the greatest reduction to the GI out of all three fibres, whilst causing the highest increase in viscosity. HPMC had a similar but less pronounced effect, whilst inulin did not affect the GI. Finally, the impact of HPMC on carbohydrate parameters in sweet potatoes as well as the antioxidant potential of both Roosters and sweet potatoes was investigated. Ferric reducing antioxidant potential (FRAP), Oxygen radical absorbance capacity (ORAC), total phenol content (TPC), as well as ascorbic acid content were quantified. The addition of the fibre caused reductions in the FRAP and ascorbic acid content of the tubers however, ORAC and TPC values remained unchanged.

Overall, our findings have identified methods to potentially reduce the GI of potatoes which could be useful for the food industry and have also demonstrated that *in-vitro* methods can be a convenient tool for the determination of GI and GL in potato and potato-based meals.

List of Abbreviations

4T1	Breast Cancer Cell Line
AGEs	Advanced Glycated End-products
AMD	Age-Related Macular Degeneration
ANOVA	Analysis of Variance
AOAC	Association of Analytical Chemists
AREDS	Age-Related Eye Disease Study
BB	Barley β-glucan
BMI	Body Mass Index
Ca	Calcium
CCK	Cholecystokinin
CDR	Cognitive Drug Research
cm	Centimetres
CVD	Cardiovascular Disease
d.d.	Distilled Deionized
DNA	Deoxyribonucleic acid
DW	Dry Weight
EPIC	European Prospective Investigation into Cancer and Nutrition
FFQ	Food Frequency Questionnaire
FG	Free Glucose
FRAP	Ferric Reducing Antioxidant Power
FSAI	Food Safety Authority of Ireland
FW	Fresh Weight
g	Grams
ẏ	Shear Rate
GE	Gastric Emptying
GG	Glucagel
GI	Glycaemic Index
GL	Glycaemic Load
GLP-1	Glucagon-like Peptide 1
GR	Glucose Response

ha	Hectare
HDL	High Density Lipoprotein
HPMC	Hydroxypropylmethylcellulose
HPP	High Pressure Processing
HSE	Health Service Executive
HT-29	Human Colorectal Adenocarcinoma Cell Line
HV	High Viscosity
IAUC	Incremental Area Under the Curve
JB6t	Mouse Epithelial Cells
Kg	Kilograms
LDL	Low Density Lipoprotein
M	Molar Concentration
mg	Milligram
ml	Millilitre
mm	Millimetres
mM	Millimolar
MPa	Megapascal
mPas	Millipascal-seconds
mRNA	Messenger Ribonucleic Acid
nm	Nanometres
ORAC	Oxygen Radical Antioxidant Capacity
pH	Scale of acidity/ basicity of a solution
PI2	Protease Inhibitor II
PYY	Peptide Tyrosine Tyrosine
RAG	Rapidly Available Glucose
RDA	Recommended Dietary Allowances
RDS	Rapidly Digested Starch
RS	Resistance Starch
RS1	Resistant Starch – physically entrapped starch
RS2	Resistant Starch- ungelatinized starch granules, impervious to digestive amylases

RS3	Resistant Starch- retrograded starch resulting from cooling after gelatinization
RS4	Resistant Starch- starches that have been chemically modified in a way that reduces digestibility
SAG	Slowly Available Glucose
SDS	Slowly Digested Starch
SES	Socioeconomic Status
T1D	Type 1 Diabetes
T2DM	Type 2 Diabetes Mellitus
TAC	Total Antioxidant Capacity
TE	Trolox Equivalents
TG	Total Glucose
TPC	Total Phenolic Content
UK	United Kingdom
UN	United Nations
USA	United States of America
UV	Ultra-violet
VEGF	Vascular Endothelial Growth Factor
VFB	Viscous Fibre Blend
W/W	Weight for Weight
WC	Waist Circumference
η	Viscosity
µL	Microliter

List of Tables and Figures

Chapter 1

Literature Review

1.1 Introduction

Potatoes first originated in the Andes near the border of Bolivia and Peru in South America over 10,000 years ago (Harris, 1992). In the 16th century potatoes were first introduced to Spain followed by the UK. Over the next couple of centuries, they then began being widely cultivated across Europe, Asia, and Africa. They are now cultivated in over 160 countries worldwide (Camire et al., 2009). Potatoes are the fourth most important crop worldwide after rice, maize, and wheat (Hancock et al., 2014). It is the largest of the non-cereal crops with approximately 377 million tons being produced annually. China, India, Russia, Ukraine, and the United States are the largest potato producers (Zhang et al., 2017). China alone is responsible for 22% of all potatoes produced (Jansky et al., 2009). While global potato consumption is increasing, the consumption of fresh tuber in developed nations such as the US is declining, relative to the intake of processed potato products (Furrer et al., 2018). In the Irish retail sector potatoes are purchased every 2 seconds with 1.65 million households purchasing them every year (Teagasc, 2012). The average Irish person consumes 85 kg of potatoes per year, this is two and a half times higher than the world average. However, in the 1990s the average Irish potato intake was 140 kg annually (Teagasc, 2017). Roosters are the most widely produced and consumed potato in Ireland (Bord Bia, n.d.).

The aetiologies of chronic health conditions discussed in this literature review are multifactorial, however diet is often singled out as one of the biggest contributing factors to their development. The expenditure associated with the treatment of such conditions can put a major financial burden on society, costing the taxpayers' millions of euro annually. The supposed link between potatoes and poor health is one of the reasons for this trend of decreasing consumption of unprocessed potatoes (Fernqvist et al., 2015).

The link between a high intake of fruits and vegetables and a reduction in the prevalence of non-communicable diseases such as particular types of cancers and cardiovascular disease has been identified (Heidemann et al., 2008). One of the reasons pertaining to these health benefits of fruits and vegetables could be their nutritional profile. In their native state potatoes are a very nutritious food item rich in antioxidants, vitamin C, potassium and low in

fat. Vitamin C and vitamin E have both been shown to counteract the activities of oxidant species thus preventing cell damage (Ali et al., 2020; Soto-Vaca et al., 2012). They are also a low fat (0.1g per 100g boiled potato) and low in sugar (0.8g per 100g of boiled potato) (McCance and Widdowson, 2019). Potato's secondary metabolites or phytochemicals also contribute to antioxidant activities. The increase in the consumption of processed forms of potatoes is often criticized, as processing is often thought to have a negative impact on their nutritional value whilst potentially increasing the fat and salt contents. French fries are the most consumed source of potatoes in the USA (USDA, ERS, 2019). Due to the cooking temperature used, French fries lose some of their nutritive value (Zaheer and Akhtar, 2014). Potatoes are an essential agricultural commodity as well as a staple food, they are a valuable crop due to their nutritional content as well as their high-volume crop yield and have many industrial uses (Zhang et al., 2017).

1.2 Energy

The energy provided by potatoes varies from 68kcals/100g for new/ salad potatoes boiled in unsalted water to 74kcals/100g for old potatoes boiled in unsalted water, which is considerably lower than other staple foods such as rice which has 129kcal/100g when cooked in a similar way (McCance and Widdowson, 2019). De Haan et al. (2019) reported a higher calorie content of 96.33 to 123.17kcal/100g for potatoes which is not dissimilar to that of rice (De Haan et al., 2019). Whilst potatoes have a low energy density when boiled; the preparation, cooking and serving of the tubers can greatly alter this. Potato crisps cooked in sunflower oil have an energy content of 493kcal/100g, frozen potato chips fried have 365kcal/100g, potato cakes cooked in rapeseed oil contain 210kcal/100g, to mention a few (McCance and Widdowson, 2019). The FSAI Healthy Eating Guidelines state that a portion of potatoes is two medium or four small potatoes and recommend only consuming processed varieties such as potato crisps a maximum of once or twice a week (FSAI, 2019). However, in countries where potatoes are considered a staple food, intake is considerably higher, for example in Huancavelica, a city in Peru, the consumption of potatoes is considerably higher with the average woman consuming 840g potato/ day when they are plentiful and 645g potato/day when they are is short supply. In this region potatoes tend to be prepared by boiling, they contribute considerably to the dietary needs of the population as well as

providing 28%-38% of the energy requirements of the female population, where as in the UK potatoes only contribute about 7% to dietary energy with an average intake of 85g/day (De Haan et al., 2009; Gibson and Kurilich, 2013).

1.3 Carbohydrates

1.3.1 Starch

Starch is the most abundant form of carbohydrate present in potatoes (Campos and Ortiz, 2020). It contributes upwards of 25% of calories in the diet (DeMartino and Cockburn, 2020).Depending on the cultivar the starch content may vary; Liu et al 2007 noted starch values of 16.5 to 20.0g/100g, whereas McCance and Widdowson reported slightly lower values with new potatoes (boiled unsalted water) reported to contain 13.8g/100g and old potatoes (boiled in unsalted water containing 16.7g/100g (Liu et al., 2007; McCance and Widdowson, 2019). The available starch in potatoes is principally composed of amylopectin and amylose in a ratio of 3:1, however some of the literature has reported up to 70-80% of potato starch to be composed of amylopectin (Zeeman et al., 2010; McGill et al., 2013; Camire et al., 2009). Both are composed of 1,4-α-D-glucan, however their structures differ amylose has a linear structure with a small number of long branches, whereas amylopectin has many more branches and a higher molecular weight (Lemos et al., 2019, Tortoe et al., 2017). There are major variations in digestibility of amylose and amylopectin, amylose is more resistant to digestion compared to amylopectin which can be broken down much quicker (Bach et al., 2013).

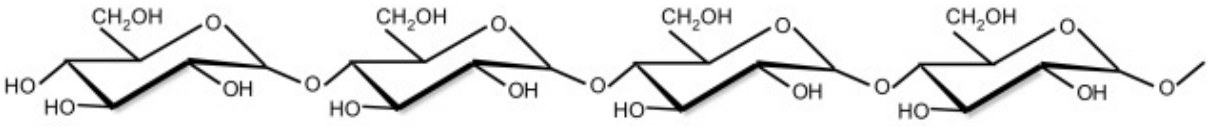


Figure 1.1. Structure of amylose (Liu, 2016)

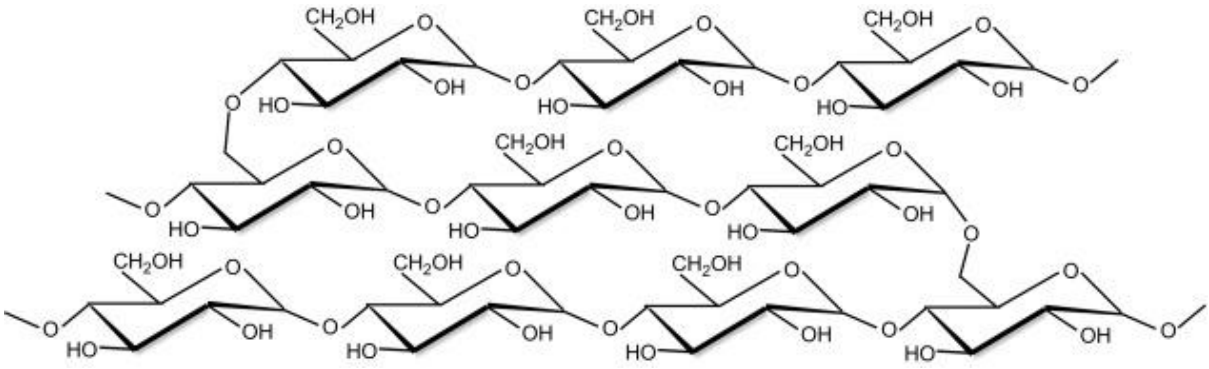


Figure 1.2. Structure of amylopectin (Liu, 2016)

Starch can be divided into three categories – rapidly digested starch (RDS), slowly digested starch (SDS) (both RDS and SDS are predominantly composed of amylopectin) and resistant starch (RS) (consists of mainly amylose) (Englyst et al., 1992). RDS is digested within the first 20 minutes post ingestion, SDS is broken down between 21-120 minutes following the consumption of the food item. RS remains undigested it includes the starch and starch by products which cannot be absorbed in the small intestine (Camire et al., 2009), it is fermented in the large intestine (Raigond et al., 2014). The amount of each of these types of starches present in the fresh weight (FW) of potato tubers varies, RDS 90-150mg/100g FW, SDS 0-17.2mg/100g FW, RS 5.8-10.5mg/100g FW (Monro et al., 2009). SDS and RS increase significantly when cooked carbohydrates are cooled, a study investigating RS levels in pasta found that RS levels were 40% and 44% higher in the meals that were cooked, cooled and reheated compared to the same meal freshly cooked (Alzaabi et al., 2020). The cooling of the potatoes causes the starch to retrograde, this is a process which occurs when the molecular motion of starch slows down due to a decrease in temperature, causing the amylose and amylopectin to rearrange into crystalline state due to the formation of hydrogen bonds (Zhu et al., 2020). Higher levels of SDS and RS have been to have been shown to be beneficial to health. The literature indicates RS and SDS increases satiety, increases gut barrier function, increases in the proliferation of gut epithelia cells, improved glucose tolerance, helps reduced blood lipid level in individuals with hyperlipidemia, reduces inflammation in the gut, as well as having beneficial effects for individuals who suffer from chronic kidney disease. (Alzaabi et al., 2020; DeMartino and Cockburn, 2020). Within the gut RS is the preferred substrate for *Ruminococcus bromii*, which promotes the growth of probiotic species (Ze et al. 2012). Potato

starch has been associated with an increase in the growth of *Lactobacillus* and *Bifidobacteria* species (Rodríguez-Cabezas et al., 2010). These ferment the RS and excrete short chain fatty acids which are linked with positive effects on colonocytes and colonic health (Conlon et al., 2012; den Besten et al., 2013)

1.3.2 Sugar

The sugars present in potatoes are primarily glucose and fructose (monosaccharides) and sucrose (disaccharide) (Camire et al., 2009; Duarte-Delgado et al., 2016). Depending on the variety of the potato the sugar content can vary greatly (Campos and Ortiz, 2020). Duarte-Delgado et al. (2016) noted levels of glucose, fructose and sucrose in four commonly Colombian potatoes (Criolla Colombia, Criolla Latina, Criolla Galeras and Criolla Guaneña) and found the sugars ranged from 6.39 to 29.42 g kg^{-1} dry weight (DW) for sucrose, from 0.29 to 27.23 g kg^{-1} DW, for fructose and from 0.46 to 28.04 g kg^{-1} DW, for glucose (Duarte-Delgado et al., 2016), in contrast Rodríguez et al. (2010), found lower sucrose (1.82–7.96 g kg^{-1} DW), fructose (0.10–1.08 g kg^{-1}) and glucose (0.13–2.39 g kg^{-1}) present in ten different cultivars from the Canary Islands (Rodríguez et al., 2010). McCance and Widdowson (2019) observed relatively low amounts of each of the sugars to be present in old potatoes (cooked in unsalted water); 0.3g/100g of glucose, 0.3g/100g of fructose and 0.2g/100g of sucrose.

Glucose, fructose, and the amino acid asparagine are all precursors in the formation of acrylamide which is formed during the Maillard pathway when food is cooked at high temperatures using methods such as baking, frying, and roasting (Foot et al., 2007). Cold-induced sweetening is a process in which there is an increase in the accumulation of sugars in potatoes stored in conditions of less than 10 °C, thus increasing the potential of acrylamides forming once tuber is cooked (Rosen et al., 2018, Lin et al., 2019). Acrylamide formation can be reduced by controlling the following parameters: tuber variety, storage temperature, pre-processing, final preparation/ processing, and colouring (Foot et al., 2007). The WHO acknowledges that acrylamide in food is of concern to humans, as it has been shown to cause cancer and heritable mutations in laboratory animals (WHO, 2002). It is estimated that nearly 70% of acrylamide intake in Canada was from French fries and potato chips (Zaheer and Akhtar, 2014).

1.3.3 Fibre

Fibre is an umbrella term which includes a broad spectrum of substances which can be classed as dietary fibre. In short, fibre is composed of the edible parts of plants which is impervious to both digestion and absorption in the small intestine but can be fermented within the large intestine (Campos and Ortiz, 2020). Fibre can be either soluble or insoluble both of which are beneficial to human health. Soluble fibre has been shown to lower serum cholesterol levels, increase viscosity within the gut, delay gastric emptying, regulate blood glucose levels, and promote colonic fermentation. Insoluble fibre increases stool weight, thus shortening bowel transit time, it also promotes the growth of the intestinal microflora and probiotic species (Slavin 2008; Chawla and Patil, 2010; Dai and Chau, 2016). One study found that fibre extracted from potato peel was composed of 92% dietary fibre, of which 73% was soluble, and 19% was insoluble, 6% moisture, 2% ash and <1% protein fat and carbohydrates (Curti et al., 2016). The fibre content of potatoes varies. Depending on if the skin is served on the potato, McCance and Widdowson reported fibre content to be 0.9g/100g in new potatoes and 1.0g/100g in old potatoes (McCance and Widdowson, 2019). Whilst potato is clearly not a high fibre food, it does contain more fibre than white rice which has 0.3g/100g and the same amount as brown rice 0.9g/100g, while other staples such as cassava 1.4g/100g and yams 1.4g/100g both contain more fibre than potatoes (McCance and Widdowson, 2019).

The dietary recommendations for fibre in Ireland are the child's age plus 5g per day, thus a 6-year-old child should consume 11g fibre/day, and for adults ≥25g fibre per day is recommended (FSAI, 2011). However, according to data collected in the UK National Diet and Nutrition Survey 2015-2016, potato accounted for 11% of AOAC Fibre in adults (approximately 2g), compared to bread which accounts for 20% of AOAC Fibre, but potato is still a bigger contributor to fibre in the diet than other starch foods such as pasta, pizza, rice and other "miscellaneous cereals" at 8% (Robertson et al., 2018).

1.4 Protein

Slight variations in the protein content of potatoes can be seen in the literature 1.8g/100g boiled, 2-3g/100g fresh weight or 1-1.5% of tuber fresh weight (McCance and Widdowson, 2019; Robertson et al., 2018; Camire et al., 2009). Whilst the protein content of potatoes is lower than that of other staple foods such as soya beans which contain 35.9g/100g (raw, dried soya beans) (McCance and Widdowson, 2019), its biological value is similar to that of egg protein which has a biological value of 100. Potato protein has a biological value of 90-100, whereas soya bean has a biological value of 84 (Camire et al., 2009). Potatoes contain 18 amino acids, including the 9 essential amino acids which cannot be synthesized by the body: arginine, histidine, isoleucine, lysine, methionine, phenylalanine, threonine, tyrosine, and valine (Bártová et al., 2015). Each of these amino acids plays an essential role in maintaining a healthy body for example methionine is needed for DNA and protein methylation in cells, arginine is required in gene expression and a deficiency in it can cause a reduction of in the vitality and sperm in males, and in pregnant females' insufficient arginine can affect fetal growth and survival (Wu 2013). The lysine levels of potato exceed those of other staples such as rice and cornmeal, but their sulphur-containing amino acids; cysteine and methionine are much lower (Camire et al., 2009). Lysine deficiency is common in some cereal based diets in developing countries and fortification with lysine in these affected areas is important to lower diarrheal prevalence and morbidity. In Syria and Eastern Europe fortification with lysine has also been associated with lowering stress and anxiety levels (Ghosh et al., 2010). Storage also influences amino acid content; Pęksa et al., (2018) noted that the amino acid content of potato decreased after both three and six months of storage. They also found that potatoes stored at a slightly cooler temperature (2 ºC) retained more amino acids than those stored at a warmer temperature (5 ºC) (Pęksa et al., 2018).

1.5 Lipids

Potatoes are known to contain low amounts of lipids; approximately 0.1g/100g boiled potato (McCance and Widdowson, 2019). The storage of tubers can cause a slight increase in total fatty acid content, however Dobson et al. (2004) found that prolonged storage resulted in total fatty acid content returning to initial values (Dobson et al., 2004). The majority of lipids found in potatoes are phospholipids, glycol and galactolipids which are found within the cell

membrane of the plant, with only trace amounts of triacylglycerols being present (Klaus et al., 2004; Ramadan and Oraby, 2016). The two most prevalent fatty acids found in potatoes are linoleic acid (C18:2 cis-9,12, an *n*-6 fatty acid) and linolenic acid (C18:3 cis-9,12,15, an *n*-3 fatty acid), however seventeen fatty acids have been identified to be present in raw potato tubers (Dobson et al., 2004). According to Gibson and Kurilich (2013), potato consumption accounts for 10-13% of dietary intake of linoleic acid and linolenic acid in Britain. Yet potatoes account for 7% of dietary fat intake in the UK (Gibson and Kurilich, 2013). The preparation, cooking and serving of the potato also greatly impacts the lipid profile of the food. Gonçalves Albuquerque et al. (2011), examined the difference in fat content of 18 different brands of potato crisp, they found that the fat content varied from 20.0g/100g to 42.8g/100g. It was also noted that the most abundant fatty acids present were palmitic acid (C16:0), oleic acid (C18:1) and linoleic acid (C18:2). Due to the popularity of fried and oven chips within the UK, it is estimated that potatoes account for 4% of saturated fatty acid intake (Gibson and Kurilich, 2013).

1.6 Micronutrients

1.6.1 Vitamins

Potatoes tend to be a relatively good source of B_6, Lui (2013) reported that one medium sized potato could contribute 0.54 mg/173g of the vitamin to the diet, providing approximately 27% of an individual's daily needs (Liu, 2013), however the levels reported by McCance and Widdowson (2019) were much lower reporting levels of 0.04mg/100g for baked potato flesh, with processed potatoes products such as potato rings or potato snacks (such as Pringles) have the highest B_6 levels. Vitamin B_6 is a water-soluble vitamin, and a key cofactor in many enzymatic reactions (Hellmann and Mooney, 2010). Vitamin B_6 is also is involved in reactions associated with lipid and carbohydrate metabolism (Mooney et al., 2013). B_6 additionally plays a role in normal nerve functioning and in the production of important neurotransmitters such as dopamine and serotonin (Percudani and Peracchi, 2009). It is also a recognised for its antioxidant activity. The average consumption of B_6 for adults in Ireland is 4.9mg/day (FSAI, 2020).

Potatoes are a rich source of ascorbic acid (vitamin C). Raw potatoes contain 14mg vitamin C/100g. In the USA potatoes contribute up to 50% of daily ascorbic acid consumption (Camire et al., 2009). However, depending on the cooking method vitamin C decreases, roasted potatoes contain 5-13mg vitamin C/100g, while boiled potatoes contain 7-14mg vitamin C/100g (McCance and Widdowson, 2019), aside from the cooking method, factors such as storage and genotype also affect the micronutrient content (McGill et al., 2013). Vitamin C has many important functions within the body, it acts as a cofactor in the production of collagen needed to maintain cardiovascular functions, wound healing, maintenance of healthy cartilage, bones, teeth as well as increasing the bioavailability of non-haem iron (Naidu, 2003; Teucher et al., 2004). Vitamin C is also a free radical scavenger of reactive oxygen species which can damage tissue, which may be linked to a number of non-communicable diseases (Yamdeu Galani, et al., 2017). The RDI of vitamin C for Irish adults is 80mg/day, however, the average intake from all sources (from food and supplements) is 114 mg/day for adult (18-64 years) males and 141mg/day for females (FSAI, 2020).

Vitamin E is an umbrella term for eight associated tocopherols and tocotrienols, which can be identified by their hydrophobic tail or hydrophilic tail (Campos and Ortiz, 2020). Vitamin E is found in the cell membrane of both plants and animals and it protects the polyunsaturated fatty acids in the phospholipid bilayer from being damaged by free radicals (Campos and Ortiz, 2020). A-Tocopherol is the main form of vitamin E found in potatoes with only trace amounts of β-tocopherol (Bramley et al., 2000; Andre et al., 2007b). Depending on the cooking method used and the maturity of the tuber, the levels of vitamin E changes. New potatoes boiled contain 0.11mg vitamin E/100g, old boiled potatoes contain 0.01mg vitamin E/100g, microwaved potatoes 0.13mg vitamin E/100g, and roasted potatoes contain 1.14-0.98mg vitamin E/100g (McCance and Widdowson, 2019). The average intake of vitamin E is 17.2mg/day (FSAI, 2020), which is slightly above the RDA of 15mg/day (Traber, 2012). The consumption of white potatoes, French fries and oven baked fries was found to contribute 5-10% of vitamin E intakes (Freedman and Keast, 2011). Vitamin E deficiency is often associated with degenerative diseases such as atherosclerosis. It is believed that increasing the intake of α-tocopherol may reduce the risk of certain chronic illnesses (Andre et al., 2010).

Table 1.1. Vitamin composition of potato and potato products

Potato products	Vitamin E (mg/100g)	Thiamin (mg/100g)	Riboflavin (mg/100g)	Niacin (mg/100g)	Vitamin B6 (mg/100g)	Folate (µg/100g)	Vitamin C (mg/100g)
Potato Rings	7.64	0.05	0.27	1.1	0.40	5	N
Potato snacks, (pringle-type, fried in vegetable oil)	10.80	0.09	0.16	3.9	0.31	62	N
Potatoes, new and salad, (boiled in unsalted water, flesh and skin)	0.11	0.13	0.01	0.7	0.13	21	7
Potatoes, old, (baked, flesh only)	0.06	0.21	0.01	0.6	0.06	10	3
Potatoes, old, (boiled in unsalted water, flesh only)	0.01	0.21	Tr	0.5	0.06	18	9
Potatoes, old, (roasted in rapeseed oil)	N	0.22	0.01	0.4	0.14	8	13
Potatoes, old, (raw, flesh only)	0.01	0.20	0.01	0.3	0.14	0.14	14

Compositional information obtained from McCance and Widdowson 2019.

1.6.2 Minerals

Potassium is the most abundant mineral present in potato tubers, with new potatoes (boiled in unsalted water) containing 377mg/100g and old potatoes (boiled in unsalted water) containing 365mg/100g (McCance and Widdowson 2019), slightly higher levels of 564mg/100g were noted by Camire et al (2009). Nassar et al. (2012) found that the levels of potassium in potatoes may be as high as 1386mg/100g FW (Nassar et al., 2012). The consumption of high levels of potassium can help reduce the risk of stroke and control high blood pressure. Potassium counteracts the effect of sodium and thus protects against hypertension (Camrie et al., 2009). It also acts as an electrolyte within the nervous system (Bethke and Jansky, 2008). The average level of potassium consumed daily by the Irish population is 4886mg (FSAI, 2020). The consumption of just 200g of new potatoes could contribute 15% of the average potassium intake of the population (McCance and Widdowson 2019).

Modest amounts of both magnesium and phosphorus are present in potatoes. Potatoes contain 18mg magnesium/100g, and 44mg phosphorus/100g, and old potatoes 31mg phosphorus/100g (McCance and Widdowson, 2019). The average adult intake of magnesium is 467mg and phosphorus is 2228mg per day (FSAI, 2020).

The RDA for calcium is 1300 mg for children aged 14-18, and 1000mg for adults aged 19-50 (FSAI, 2011). The level of calcium occurring in potatoes is relatively low, new potatoes contain 11mg Ca/100g, and old potatoes have 6mg Ca /100g (McCance and Widdowson, 2019). Therefore, potatoes are not a good source of calcium.

The iron content of potatoes varies from 0.61mg/100g to 0.34mg/100g, minor amounts of zinc are also present 0.2mg/100g (McCance and Widdowson, 2019). The RDA for iron in Ireland is 7mg for men (age 19-50) and 10mg for women (aged 19-50) and the average consumption of zinc in Ireland is 15.9mg/day (FSAI, 2011; FSAI, 2020). When comparing the

amount of iron and zinc present in potatoes to the RDA or the average intake, it is clear that potato is not a good source of either micronutrient. However, the bioaccessability of iron and zinc from potatoes is high due to the level of ascorbic acid which facilitates the absorption of iron (Camire et al., 2009). In addition, potatoes have low levels of phytic acid compared to other vegetables which acts as an inhibitor of both iron and zinc (Camire et al., 2009). Andre et al. (2015), found that up to 79% of the iron present in the potato matrix was released during an *in-vitro* digestion and thus available for absorption (Andre et al., 2015).

Table 1.2. Mineral composition of potato and potato products

Potato Products	Sodium (mg/100g)	Potassium (mg/100g)	Calcium (mg/100g)	Magnesium (mg/100g)	Phosphorus (mg/100g)	Iron (mg/100g)	Zinc (mg/100g)
Potato Rings	845	781	26	34	108	0.80	0.6
Potato snacks, pringle-type, fried in vegetable oil	599	706	35	40	103	1.10	0.7
Potatoes, new and salad, boiled in unsalted water, flesh and skin	3	377	11	18	44	0.61	0.2
Potatoes, old, baked, flesh only	1	360	7	18	40	0.40	0.3
Potatoes, old, boiled in unsalted water, flesh only	1	365	6	18	31	0.34	0.2
Potatoes, old, roasted in rapeseed oil	3	597	9	28	46	0.43	0.4
Potatoes, old, raw, flesh only	443	7	21	34	0.32	0.30	0.1

Compositional information obtained from McCance and Widdowson 2019.

1.6.3 Loss of Vitamins and Minerals in Potato on Processing

The levels of the micronutrients present in potato can vary greatly due to the cooking method used, variety of potato and type and length of storage. Cooking results in a decrease in vitamins and minerals due to the leaching into cooking liquid, destruction due to high temperatures, and oxidation. The boiling of peeled and cubed potatoes could result in up to a 50% loss of potassium and even greater amounts (70-75%) if potatoes are shredded; sizeable losses of other minerals such as magnesium, phosphorus, iron, and zinc on boiling were also noted (Bethke and Jansky, 2008). Cooking potatoes with the skin on can help prevent leaching of vitamin and minerals, unpeeled potatoes retained 10% more vitamin C and phenolic compounds on cooking than peeled potatoes (Camrie et al 2009; Woolfe and Poats, 1987). Han et al. (2004), found that cooking methods which do not use water maintain higher levels of water-soluble vitamins. Vitamin C loss was lower when using methods such as: microwaving, baking, and sautéing compared to boiling (Han et al., 2004).

During storage, the levels of vitamin C tend to decrease; Öhrvik et al. (2010) found that vitamin C levels were reduced by 60% after five months of storage (Öhrvik et al., 2010). Cold storage of potato tubers for several months resulted in a mean decrease of 52% of vitamin C (Külen et al., 2012). Vitamin B_6 is relatively stable during storage, slight increases may occur due to germination (Öhrvik et al., 2010). Factors such as pH, heat and light all influence micronutrient levels.

1.7 Phytochemical Content and Activity of Potatoes

Potatoes are composed of macronutrients, micronutrients, and phytochemicals. Phytochemicals is the collective term given to metabolites present in the flesh and skin of potato tuber. They are non-nutritive components of the diet and are not essential for short term health (Campos and Ortiz, 2020). They can be broken down into five groups, three of which are present in potatoes: phenolic compounds, carotenoids and alkaloids (Liu, 2004). As already mentioned, some micronutrients such as vitamin C and vitamin E have also been shown to have antioxidant effects by counteracting oxidative stress, which could damage structural aspects of the cell and biomolecules.

The antioxidant capacity of foods can be assessed *in-vitro* and has shown similar results to that of human plasma antioxidant content (Rautiainen et al., 2008). Potatoes contain both hydrophilic antioxidants (polyphenols, ascorbic acid, anthocyanins, and flavanols) as well as lipophilic antioxidants (carotenoids and vitamin E) (Reyes et al., 2005). Depending on the skin and flesh colour, some antioxidants are more abundant than others. Chlorogenic acid, gallic acid, caffeic acid and catechin are the more prominent in white, light yellow and yellow flesh tubers compared to anthocyanins and chlorogenic acid being the more prominent antioxidant in purple/red potatoes (Brown, 2005; Camire et al., 2009). Whilst the levels of antioxidants vary between genotype, ultimately the contribution of potato to antioxidant status to the diet is based on its consumption (Chu et al., 2002).

Table 1.3. Typical Phytochemical Content in a Variety of Potato Cultivars

	Polyphenols (μg/g DW)				Glycoalkaloids (μg/g DW)		Carotenoids (μg/g DW)
Potato Variety	**Total Phenolic Content**	**Chlorogenic Acid**	**Caffeic Acid**	**Rutin**	**α-Chaconine**	**α-Solanine**	**Total Carotenoids**
Rooster	796	119.21	0.47	2.68	1.52	3.99	4.54
Saxon	1098	106.72	1.23	0.31	1.05	1.48	0.89
Kerr's Pink	830	139.53	0.04	1.79	7.36	3.28	0.79

Phytochemical and glycoalkaloids information sourced from Tsikrika et al., 2019, total carotenoids information sourced from Valcarcel et al., 2014.

1.7.1 Polyphenols

One of the biggest groups of antioxidants are polyphenols, over 8000 of these substances have been identified. They are considered to be the most plentiful source on antioxidants in the diet (Ross and Kasum, 2002; Manach et al., 2004). They are a common secondary metabolite found in plants. Polyphenols have an aromatic ring structure which contains one or more hydroxyl groups. Within the plant they are believed to aid in defence mechanisms against pests and pathogens by aiding in the sealing and healing process of an injured surface of the plant. Flavonoids, another polyphenolic compound, provide plants such as potatoes with protection against radiation. Flavonoid pigments also attract pollinators and thus aid seed dispersion; some even have the ability to act as feeding deterrents (Beckman et al*.,* 2000; Parr and Bolwell, 2000).

Polyphenols can be subcategorised into multiple classes such as phenolic acids, lignans, stilbenes and flavonoids (Manach et al., 2004). They are considered to be beneficial to health as they have been shown to have antibacterial, antiviral, anti-inflammatory, anticarcinogenic effects as well as beneficial effects on cardiometabolic health, cognitive functioning and reducing the risk of diabetes (Greenberg, 2015; Larsson et al., 2016; Fraga et al., 2005).
An *in-vivo* study found that both yellow flesh and purple flesh potatoes cause reductions in the oxidative levels as well as inflammation biomarkers in blood plasma of men after 150g/day of potato was consumed (Kaspar et al., 2011). An increase in the antioxidant capacity of plasma and urine, and a reduction in blood pressure were noted post ingestion of purple potatoes (Vinson et al., 2012). A rat study conducted by Camire et al. (2009) found that the ingestion of white potatoes lowered serum urate levels, and the consumption of red and purple potatoes resulted in reduced oxidation levels in both the liver and serum (Camire et al. 2009).

The phase II metabolites of quercetin have been shown to impede the growth and spread of lung cancer cells. The metabolites of quercetin have also been shown to have anti-inflammatory effects, and to prevent the production of molecules which are involved in the onset of atherosclerosis. Studies involving mice and rats have indicated that catechin may

prevent the production of reactive oxygen species which in turn has been shown to delay the onset of tumours (Koga et al., 2001; Ebeler et al., 2002).

A study conducted by Chun et al. (2005), rated potatoes as the third most important source of phenolic compounds in the diet after apple and oranges. This study found potatoes had a higher total phenol content than tomatoes, lettuce and carrots and other commonly consumed vegetables (Chun et al., 2005). Some of the factors influencing the importance of potatoes as a source of polyphenols include their availability year-round, and the high consumption of potatoes (Mattila and Hellström, 2007).

The average potato contains 53.0-166.5mg/kg of polyphenols to fresh matter. These levels can vary and are often influenced by the location of the study, the flesh colour and cultivar type. White, light yellow and yellow flesh are the most popular genotype of potatoes in Europe. Within potatoes, chlorogenic acid accounts for greater than 80% of the phenolic acids, (Brown, 2005). Other phenolic acids present but in lesser amounts include *p*-coumaric, protocatechuic, gallic, ferulic, salicylic and vanillic acids (Brown, 2005; Reddivari et al., 2007a; Andre et al., 2008; Lewis et al*.,* 1998). Depending on the variety of potato, varying amounts of flavonoids may be present, including flavonols quercetin and kaempferol as the cognate glycosides quercetin-3-O-rutinoside (rutin) or kaempferol-3-O-rutinoside. Coloured varieties of potatoes have been noted to contain higher levels of anthocyanins (Reddivari et al., 2007a; Andre et al., 2008).

Anthocyanins are a water-soluble form of flavonoids. They are red, blue, and purple pigments found in the flesh and skin of tubers (Camire et al., 2009). They tend to be most abundant in the flesh and skin of red and purple potatoes (Brown et al., 2003). The concentration of anthocyanins present is largely dependent on the cultivar, location, and growth conditions, however the intensity of the colour of the flesh is believed to correlate with the volume of anthocyanins present (Ieri et al*.*, 2011, Brown 2005). Common anthocyanins found in the red potatoes include; peonidin, cyanidin, and pelargonidin. Purple potatoes also contain the same anthocyanins found in red potatoes along with petunidin, and malvidin grown in the Andes (Giusti et al., 2014). Potato tubers contain 9.5mg to 38mg/100g FW (McGill et al., 2013).

Anthocyanins found in potatoes appeared to be active against a range of cancers including stomach, breast, and prostate *in-vitro* (Camire et al., 2009) as well as having anti-obesogenic effects (Kim et al., 2020). A study examining the benefits of anthocyanins found that, following the breakdown of anthocyanin within the colon, their by-products were shown to have an apoptotic effect against gastric adenocarcinoma cells whilst protecting normal cells within humans. Anthocyanins also displayed anti-tumour activity (Lu et al. 2010; Lin et al. 2017). The anti-inflammatory effects of potato anthocyanins have been linked with reducing the risk of atherosclerosis as well as aiding in preventing complications which arise from diabetes (Del Rio et al., 2012). In mice models, the ingestion of anthocyanins has been shown to exert anti-obesogenic effects. They have the potential to inhibit fat accumulation in adipocytes, as well as improve serum and hepatic lipid levels and reduce oxidative stress and inflammation (Kim et al.,2020; Wu et al., 2018)

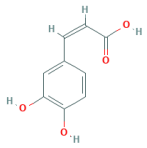
Anthocyanins may also be useful within the food industry as a colourant. Their colour stability is dependent on structure and concentration, temperature, pH, and the presence of certain metals and phenols (Bakowska et al., 2003). Their sensitivity to pH makes them useful in smart packaging, as a visible colour change would indicate spoiling of the product (Singh et al., 2018). Anthocyanins at a pH of 2 or lower are usually present in the form of red flavylium cations, if this low pH increases to pH 6 the red flavylium cation is converted into purple quinonoidal bases (Bakowska et al., 2003).

Chlorogenic acid is the most abundant polyphenol present in potatoes. The amounts present in potatoes vary greatly depending on the genotype, flesh colour, if they are peeled or not, and the cooking method. Yellow potatoes contain between 18.49mg/kg and 46.73mg/kg (Musilova et al., 2015). Piñeros-Niño et al. (2016), found that the chlorogenic acid content of 113 potato genotypes varied depending on the area in which it was grown (Piñeros-Niño et al., 2016). Isomers of chlorogenic acid are also found in tubers, including neo-chlorogenic acid, and crypto-chlorogenic acid, as well as caffeic acid (Andre et al., 2007b). Like all nutrients and phytochemicals, chlorogenic acid is affected by the cooking method used to process found boiled potatoes retained higher levels of chlorogenic acid compared to baked and microwaved tubers (Lachman et al., 2013). In contrast Tudela et al. 2002 noted that steam cooking was the best method for retaining chlorogenic acid levels and frying followed by

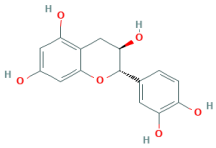
boiling had the caused the greatest decrease (Tudela et al. 2002). Whilst Andlauer et al. (2003) noted that the volume of water the potatoes were boiled in did not influence the leaching of chlorogenic acid (Andlauer et al., 2003).

Within the plant the primary function of chlorogenic acid is defence against pathogens. When consumed they carry out a similar role within the body, defending it against reactive oxygen species, which have been linked with the onset of multiple degenerative diseases (Tajik et al., 2017). Chlorogenic acid and its isomers, have been linked with a reduction in the oxidation of LDL, which is a key component in the development of plaques in atherosclerosis, improving atrial stiffness and lowering blood pressure in cell and animal studies (Natella et al., 2007; Suzuki et al., 2019; Loader et al., 2017). The colonic degradation products of chlorogenic acid have been seen to have a positive impact on hyper-reactive platelets which are produced due to hormonal and oxidative stress, both of which are linked to diabetes and heart disease (Del Rio et al., 2012). Mills et al. (2015), also suggested that the consumption of foods rich in chlorogenic acid such as coffee may promote a healthy gut microbiome and by promoting the growth of Bifidobacterium bacteria which may be valuable for gut health. Healthy gut microbiota has been linked with potentially preventing conditions such as obesity, irritable bowel syndrome and traveller's diarrhoea (Mills et al., 2015).

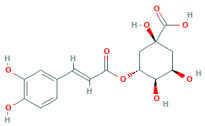
Caffeic Acid



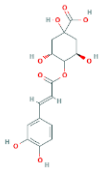
Catechin



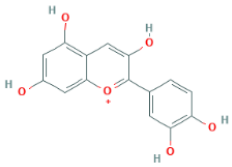
Chlorogenic Acid



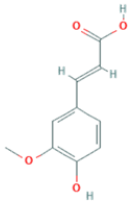
Crypto-Chlorogenic Acid



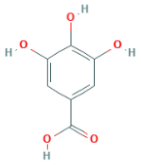
Cyanidin



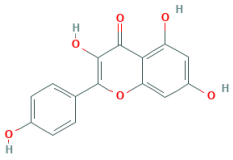
Ferulic Acid



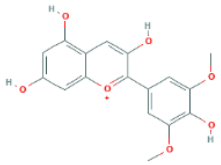
Gallic Acid



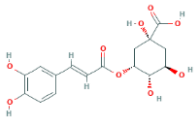
Kaempferol



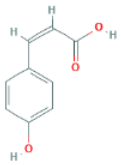
Malvidin



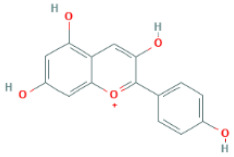
Neo-Chlorogenic Acid



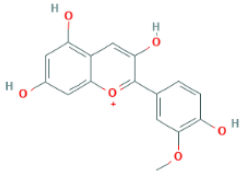
P-Coumaric



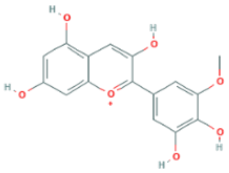
Pelargonidin



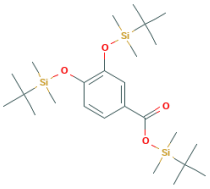
Peonidin



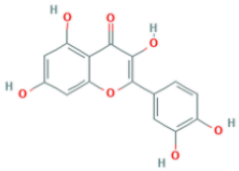
Petunidin



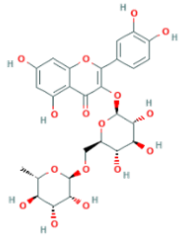
Protocatechuic



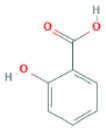
Quercetin



Rutin



Salicylic Acid



Vanillic Acids

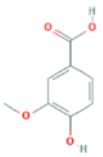


Figure 1.3 Structure of Polyphenols Commonly Found in Potatoes (National Library of Medicine, n.d)

1.7.3 Carotenoids

Carotenoids are a form of lipophilic phytonutrient found in potatoes, they are pigments which are found in the chloroplasts and chromoplasts of photosynthetic organisms. There are believed to be more than 600 carotenoid species. Within plants and photosynthetic organisms such as algae and fungi, carotenoids have two main roles, to absorb light and protect the chlorophyll from photodamage (Rao and Rao, 2007). Within the literature the quantities of carotenoids vary greatly between varieties, 38-1435 mg 100 g^{-1}FW (Morris et al., 2004; Fernandez-Orozco et al., 2013; Burgos et al., 2009; Andre et al., 2007b). Like anthocyanins, carotenoid concentration is related to the flesh colour of the potato cultivar, potatoes high in anthocyanins tend to be lower in carotenoids and vice versa (Brown, 2008). Potatoes with deep yellow flesh have been shown to have up to ten times the number of carotenoids of white fleshed varieties (Brown et al., 2005).

Xanthophylls are the most abundant carotenoids found in potatoes (Brown, 2008). Carotenoids found within different coloured varieties of potato include zeaxanthin, antheraxanthin (predominantly found in deep yellow-fleshed cultivars); violaxanthin, antheraxanthin, lutein, and zeaxanthin (found in yellow varieties) and violaxanthin, lutein, and β-carotene (present in paler flesh cream potatoes) (Burgos et al., 2009). Lutein and zeaxanthin are important pigments – they are selectively taken up by the macular of the eye where they help protect the eye from cataracts and age-related macular degeneration. There is also some evidence that they may protect the retina from damage by oxidizing species and blue light (Ahmed et al., 2005; Wu et al., 2015). High serum carotenoid levels have been shown to be associated with a reduced incidence of other conditions including breast and lung cancer, as well as heart disease and osteoporosis (Rock et al., 2009; Gallicchio et al., 2008; Koh et al., 2011; Sugiura et al., 2012).

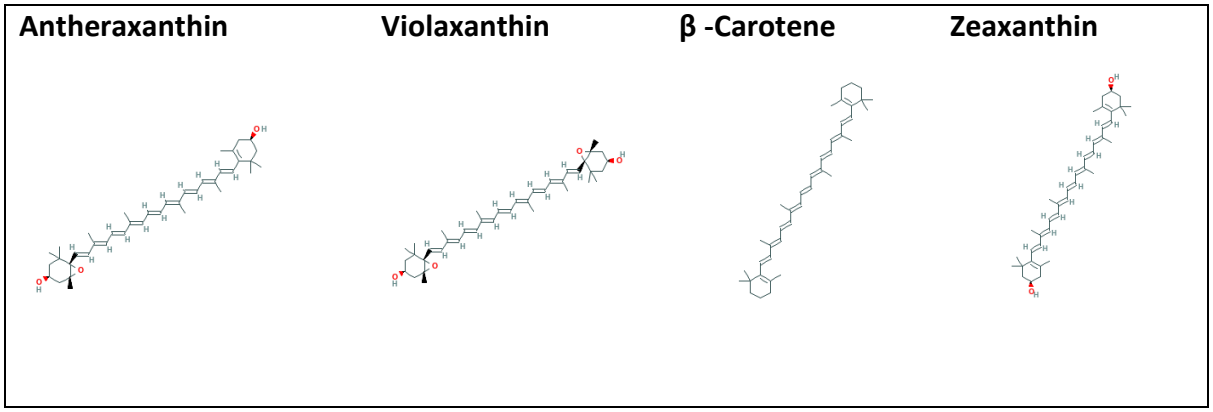


Figure 1.4. Structure of Carotinoids Commonly Found in Potatoes (National Library of Medicine, n.d)

1.7.5 Glycoalkaloids

Glycoalkaloids are secondary metabolites produced by the Solanaceae family, which includes tobacco, potato, tomato, eggplant and peppers. They act as a natural defence mechanism in these plants against insects, fungi, and viruses (Friedman, 2004). They can be identified due to their two distinctive chemical components: a hydrophilic oligosaccharide and a hydrophobic aglycone consisting of a steroidal molecule (Nema et al., 2008).

More than 90 varieties of glycoalkaloid have been identified in plants. In potatoes, higher concentrations of glycoalkaloids are found in the leaves of the plant which helps prevent pests from feeding on them. In the tuber 95% of the glycoalkaloids present in potato is either α-chaconine or α-solanine (Friedman et al., 1997; Milner et al., 2011).

The levels of glycoalkaloids present in the tuber (75mg/Kg FW), stem (30mg/Kg FW), leaves (400-1000 mg/Kg FW), sprouts (2000-4000 mg/Kg FW) and flower (3000-5000 mg/Kg FW) of the potato plant all vary (Abu Bakar Siddique and Brunton,2019). In high concentrations glycoalkaloids (>20mg/100g) can be harmful or even potentially lethal at a concentration of 3-6mg kg^{-1} body mass (Ruprich et al., 2009; Koleva et al., 2012). If present at a level of ≥14mg/100g of potato, they are said to give a bitter taste to the potato; if consumed at high concentrations, mild/severe burning sensation can be felt in the mouth/throat (Friedman,

2006). However, in potatoes α-solanine is present in greater quantities and has less of a toxic effect than α-chaconine (Campos and Ortiz, 2020; Visvanathan et al., 2016).

The preparation and cooking method of the potato tuber can influence the levels of glycoalkaloids present. As the majority of the glycoalkaloids present in potatoes are found in the skin, the removal of the skin as well as slicing reduces the total glycoalkaloids content significantly (Camire et al., 2009). Peeling can reduce levels of α-solanine to 43% and α-chaconine to 31% (Lachman et al., 2013). Cooking seems to have little effect on the glycoalkaloids levels (Tajner-Czopek et al., 2012). Other extrinsic factors such as heat, light, cutting, sprouting and growing conditions can influence levels of glycoalkaloid present in the tuber (Friedman, 2006; Andre et al., 2009).

Adverse effects of consuming high quantities of glycoalkaloids could include the onset of spina bifida, anencephaly, embryotoxicity, and other teratogenicity (Friedman, 2006; Friedman et al., 2003). In animal studies, high consumption was linked to intestinal damage, and it has been hypothesised that they may be linked to the higher incidences of bowel conditions in western countries (Langkilde et al., 2009; Iablokov et al., 2010; El-Tawil, 2008). However, glycoalkaloids have been shown to have an anticarcinoma effect on certain types of cancerous cells *in-vitro* (Yang et al., 2006; Friedman, 2015). Glycoalkaloids and their by-products may also have cholesterol lowering effects and anti-inflammatory effects (Friedman et al., 2003; Kenny et al., 2013).

Figure1.5. Structure of Glycoalkaloids Commonly Found in Potatoes (National Library of Medicine, n.d)

1.8 Potatoes and Non-Communicable Disease

High intakes of fruits and vegetables are recommended to obtain and maintain a healthy and balanced diet. The FSAI (2019) advised that meals should be based around this food group, "more is better". Five to seven portions are advised per day, making it the largest and base portion of the Irish food pyramid. Fruits and vegetables help control body weight in addition to their antioxidant and anti-inflammatory effects which help protect the body from disease and cell damage (FSAI, 2019). Epidemiological studies have also noted that high intakes of fruit and vegetables have a protective effect against non-communicable diseases such as cancer, diabetes, and cardiovascular disease, it is also estimated that one third of cancer deaths could be prevented through diet modification (Liu, 2013).

Potatoes are often considered to be less healthy than other vegetables, due to their high carbohydrate content, high-GI status, and limited evidence that they may contribute to the onset of certain chronic conditions such as diabetes and obesity (Campos and Ortiz, 2020). On the other hand, the nutrients and anti-nutrients present in potatoes have also been shown to have beneficial effects against some common chronic illnesses.

1.8.1 Cancer

The antioxidant activity of the phenolic acids as well as the presence of glycoalkaloids and fibre in potatoes, has been linked to the induction of apoptosis in cancerous cells as well as being linked with the repression of cancer cell proliferation (Campos and Ortiz, 2020; Visvanathan et al., 2016).

The two most prominent glycoalkaloids present in potatoes, α-Solanine and α-chaconine, have shown positive results against certain types of cancerous cells including cervical, liver, colon, lymphoma and stomach (Lee et al., 2004; Friedman et al., 2005; Ji et al., 2008). A-Chaconine was found to be more bioactive than α-solanine in the suppression of cancer cell proliferation (Friedman et al., 2005). Within the colon α-chaconine has been shown to induce apoptosis in human cancer cells HT-29, via the blocking of cell signals regulated by kinase

phosphorylation as well as the activation of caspase-3, which plays a role in the destruction of the cell and the fragmentation of DNA (Yang et al., 2006). Pancreatic cancer is the fourth biggest cause of cancer related mortality globally. It is often diagnosed late into the disease and resistant to drug treatment (Lv et al., 2014). The use of α-Solanine *in-vivo* and *in-vitro* has shown promising results in preventing the proliferation of pancreatic cancer cells by, decreasing the expression of proteins related to the growth of a secondary tumour in pancreatic cancer cells and also reducing the tumour weight in a xenograft model however possible side effects from the use of solanine need to be investigated (Sun et al., 2014; Lv et al., 2014).

Anthocyanins have been shown to play a role in the suppression of the growth of stomach cancer in mice, as well as being cytotoxic to prostate cancer cells, and increasing cell apoptosis in colon cancer cells (Reddivari et al., 2007b; Charepalli et al., 2015). Anthocyanins cause the mitochondria to release different proteins which induce cell apoptosis (Camrie et al., 2009). Red and purple potatoes are richest in anthocyanins, a study in which mice were fed steamed purple/red potatoes found that it inhibited the growth of stomach cancer (Hayashi et al., 2006). Li et al. (2018) found that the anthocyanins in purple sweet potato exerted an antitumor effect on bladder cancer cells *in-vitro*, by causing cell apoptosis at the start of the cell cycle (Li et al., 2018).

Many *in-vitro* studies have reported that chlorogenic acid, the main phenolic acid in potato, has antiproliferation and cytotoxic activity against many forms of human cancer cells: breast, bone, colon, kidney, and lung (Santana-Gálvez et al., 2020). Chlorogenic acid has been seen to have a significant inhibitory effect on the proliferation of liver cancer cells *in-vitro* (Wang et al., 2011). The inhibitory effects of chlorogenic acid were demonstrated in a study using the mouse epithelial cell line JB6t and the chlorogenic acid also repressed the proliferation of human lung cancer cells A549 (Feng et al. 2005). Chlorogenic acid also blocked the activation of certain inflammatory mediators linked with cancer (Feng et al., 2005). Changizi et al. (2020) found that chlorogenic acid played a role in the apoptosis of 4T1 breast cancer cells, through the regulation of 4T1 apoptotic proteins (Changizi et al., 2020). In a recent study, dihydrocaffeic acid, one of the main metabolites of chlorogenic acid, was shown to be more cytotoxic to several cancer cell lines including breast, prostate, colon, and liver than healthy

cells. It was noted by the authors that further research was required to determine the specific anticancer mechanisms of dihydrocaffeic acid (Santana-Gálvez et al., 2020).

1.8.2 Cardiovascular Health

Cardiovascular health can be affected by many different factors, a possible link between potatoes consumption and adverse cardiovascular health outcome has been investigated in many studies. Results from a number of large cohort studies have found no relationship between potato consumption and cardiovascular disease (CVD). A large Swedish prospective study, with a 13 year follow up found that there was no relationship between potato intake and an increased risk of the development of myocardial infarction, heart failure, stroke, or mortality from CVD (Larsson and Wolk 2016). The Swedish subjects were consuming potatoes on average 4.5-5.5 times a week with approximately 3.5 of these portions being boiled potatoes. No association between boiled or fried (French fries) potatoes and CVD was reported (Larsson and Wolk 2016). Joshipura et al. (1999) also found no relationship between potato intake and CVD, however the method of cooking was not investigated (Joshipura et al., 1999). In both animal and human studies, the consumption of boiled potatoes has been linked to improvements in lipid profiles, lower blood pressure and a reduction in inflammation biomarkers (McGill et al., 2013). When consumed, unpeeled potato as well as a diet rich in fruit and vegetables can reduce the risk of CVD significantly due to the presence of the fibre in the skin, which is sometimes lacking in western diets (Campos and Ortiz, 2020; Liu, 2013).

The inclusion of potassium rich foods (such as potatoes) in the diet has been linked with protective effects against hypertension, stroke, and cardiovascular disease (Camire et al., 2009; WHO, 2012; Adebamowo et al., 2015). Potassium helps to counterbalance the effects of a high sodium diet, thus reducing the risk of cardiovascular events such as stroke or hypertension (Camire et al., 2009). The micro-nutrient profile of potatoes which consists of high potassium and low sodium levels could therefore be beneficial in reducing the risk of CVD. Aburto et al. (2013) found that the consumption of potassium rich foods reduced the blood pressure of hypertensive patients (Aburto et al., 2013). D'Elia et al. (2011) concluded that the higher potassium intake was related to a reduced risk of coronary heart disease and CVD through a metanalysis of cohort studies (D'Elia et al., 2011).

Anthocyanins (6.5mg/g DW) are the predominant phenolic compound in Purple "Majesty" potatoes followed by chlorogenic acid (2.72mg/g DW) (Vinson et al., 2012). Due to this unique property this potato variety has been used in multiple studies to investigate potential benefits. The ingestion of these purple potatoes was linked to lower systolic blood pressure (5mmHg) as well as significantly lower diastolic blood pressure (4mmHg) ($p<0.001$) in hypertensive subjects, compared with those consuming no potato. The control consisted of a biscuit containing a comparable dose of starch similar to that found in the portion of potatoes provided. Both blood and urine samples were taken from the subjects and those who had ingested the potatoes had an increase in plasma and urine antioxidant capacity, whilst the control group had a decrease (Vinson et al., 2012). Another study using the Purple Majesty potatoes found that consuming 200g per day for 14 days was linked with reduced arterial stiffness compared with the ingestion of white potatoes, which had no effect. The authors reported no changes in other blood parameters such as total cholesterol (HDL or LDL), blood triglycerides, fasted glucose, insulin levels or blood pressure (Tsang et al., 2018). This suggests that the inclusion of anthocyanins in the diet may reduce arterial stiffness, which is a risk factor for the development of CVD (Safar, 2001).

Conflicting results are seen the literature when the relationship between potatoes and hypertension is investigated. Data from the China Health and Nutrition Survey 1989–2011, indicated that low or moderate consumption of potatoes (fried/non-fried) was inversely related to mortality, whereas high intakes of potatoes, fried and non-fried were not associated with any cause of mortality (Chen et al., 2020). Another study using these same data found that those within the cohort who consumed greater amounts of fried potatoes, non-fried potatoes and total potatoes had a higher risk of hypertension. However, when they excluded non-consumers of total or fried potato, participants with higher intakes had a lower risk of hypertension. A positive association between sweet potato intake and hypertension was seen, but only in an urban population. The authors noted that urbanization and transition to a more western style diet and lifestyle is becoming more popular and is linked with the consumption of more processed potato which could potentially be a risk factor for hypertension (Huang et al., 2019a). Borgi et al. (2016), also reported an increased risk of hypertension in association with potato consumption. They noted that ≥1 serving/day of

potato (all types) was related to a greater risk of hypertension, compared to <1 serving/month. They reported that ≥4 servings/week of potato (mashed, boiled, or baked) was also associated with a larger risk of hypertension in women but not men, when compared with consuming <1 serving/month. With regards to French fries, ≥4 servings/week was associated with an increased risk of hypertension, when compared to <1 serving/month (Borgi et al., 2016). On the other hand, based on the data collected in the HUNT3 survey, no link between potato consumption and changes in blood pressure was identified (Moholdt et al., 2019). Evidence from two Spanish cohort studies also indicated no link between total potato consumption and high blood pressure or risk of hypertension (Hu et al., 2017).

A study looking at the link between potatoes and chronic conditions, found that even at the highest dose of potatoes there was no increase in the risk of developing coronary heart disease or suffering from stroke. However, when French fries were consumed at the same level, an increase in the risk of hypertension was noted. No other cooking or preparation methods were linked with similar risks (Schwingshackl et al., 2018). The consumption of fried potato products two or three times per week was linked to an increased risk of morbidity (Veronese et al., 2017).

It is recommended that when preparing and cooking potatoes that the skins remain on the tuber and minimum amounts of fats are used in the cooking (Camire et al., 2009). Potato products such as French fries and potato crisps, are recommended to be consumed no more than twice a week according to the FSAI; they also note these foods may be linked to heart disease and a higher body mass (FSAI, 2019).

1.8.3 Hyperlipidemia

The cholesterol lowering properties of potatoes can be attributed to the fibre content, antioxidant activities of phenolic compounds as well as glycoalkaloids and resistant and phosphorylated starch (Campos and Ortiz, 2020). Robert et al. (2008) compared three diets consisting of potato, starch and sucrose, and found that rats fed the potato diet over the

course of three weeks had lower triglyceride, cholesterol and improved antioxidant status and short chained fatty acids compared to the rats on the starch or sucrose diet (Robert et al., 2008). Two rat feeding studies, showed improvements in lipid metabolism, lower plasma cholesterol and lower cholesterol levels in the liver following the consumption of potatoes. Both authors hypothesized that the retrograded starch present in the potato samples promotes the excretion of bile resulting in the decrease in serum cholesterol and the inhibition of the production of fatty acids at mRNA levels (Robert et al., 2006; Hashimoto et al., 2006).

Kanazawa et al. (2008) credited reductions in serum lipids to gelatinized potato starch. The high levels of phosphate found in gelatinized starch was associated with lower levels of serum lipids, free fatty acids, as well as hepatic triglycerides. The slower digestion in the small intestine of this gelatinized starch leads to lipid lowering effects (Kanazawa et al., 2008). Raw potato starch is reported in some studies to be more resistant to digestion than gelatinized starch. It can act like fibre in the digestive tract and has been linked with having a positive effect on liver and plasma lipids (Younes et al., 1995; Younes et al., 2001).

1.8.4 Obesity

Overweight and obesity are conditions which may develop gradually over a number of years, they are influenced by a number of factors including education, physical activity, social norms, genetic make-up, and access to healthy and affordable food (A Healthy Weight for Ireland – Obesity Policy and Action Plan 2016-2025).

Mixed results are seen in the literature, in relation to potatoes and obesity (Campos and Ortiz, 2020). The two main indicators of weight gain used are waist circumference (WC) and body mass index (BMI). The intake of calories from any food source resulting in a calorie surplus will result in weight gain. Whilst potato (excluding processed crisps and fries) are generally considered a low energy dense food, they do contain more calories than a lot of other

vegetables, on the other hand they are lower in energy than other popular staples such as rice or pasta (McCance and Widdowson 2019).

The data collected in the Nurse's Health II survey indicated that the western diet which included potatoes was linked with weight gain whilst a more restricted diet without potatoes was associated with weight maintenance (Schulze et al., 2006). However, the cooking and preparation method for the potatoes in this study was not disclosed. A follow up cohort study was completed which monitored weight gain over 4-year intervals between 1986 and 2006, it was noted that weight gain was associated with an increase intake of certain potatoes, potato crisps, as well as sugar sweetened beverages and processed meats and unprocessed red meats. French fries, boiled, baked and mashed potato were all linked to an increase in weight (Mozaffarian et al., 2011). Another study found that the consumption of potatoes resulted in an increase in waist circumference over a five-year period for women but not men (Halkjaer et al., 2009). French fry intake was linked to being overweight and obese in both children and adults (Receveur et al., 2008; Paradis et al., 2009). One cohort study found that the fibre present in potato, nuts and legumes was not significantly associated with changes in weight or waist circumference (Du et al., 2010). According to Camire et al. (2009), the inclusion of fried potato products in the diet plays the biggest role in the rise of obesity (Camire et al., 2009). Plain boiled potatoes are unlikely to contribute to obesity due to their high-water and low-fat content (Anderson et al., 2013). A systematic review conducted by Borch et al. (2016) concluded that there was no evidence linking potato consumption and obesity, on the other hand the data may indicate a correlation between French fry consumption and obesity (Borch et al., 2016).

Whilst the data from these studies are valid, few studies have accounted for socioeconomic status (SES). SES related factors such as consumption of fruit and vegetable, smoking, alcohol intake was included in most of the studies, however, the cost and availability of food was not. Food frequency questionnaires (FFQ) completed by large cohorts can be limited by the amount of information which can be obtained from them (Robertson et al., 2018), also some of the studies classed multiple types of potatoes (French fries, crisps, mashed, boiled, etc.) as

just potato (Schulze et al., 2006). The generalization of the term “French fries” can cause issues as it does not indicate the thickness of the French fry (Robertson et al., 2018). The thicker the baton the less oil absorbed during frying and thus a higher energy content, this is well illustrated by McCance and Widdowson, (2019) potato chips, fine cut, fried in corn oil contained 21.3g/100g of fat and 364Kcals, potato chips, straight cut, fried in corn oil has 13.5g/100g of fat and 273Kcals and potato chips, thick cut, fried in corn oil contained 10.2g/100g of fat and 234Kcals.

McGill et al. (2013) points out that studies need to be carried out looking at the direct relationship between French fries and increase in WC, controlling for confounding factors such as physical activity and total calorie intake. They also highlighted the lack of clinical trials investigating the direct relationship between potatoes and weight gain/increase in WC (McGill et al., 2013). Camire et al. (2009) stated that satiety can contribute to weight management by delaying the subsequent meals and reducing overall calorie intake (Camire et al., 2009).

Multiple gastrointestinal hormones play a role in satiety, including leptin, ghrelin, cholecystokinin (CCK) or glucagon-like peptide 1 (GLP-1), they therefore could be a useful therapeutic tool in weight control (Huda et al., 2006; Acosta et al., 2014). However, these peptides cannot be delivered orally due to enzymatic degradation occurring in the gastrointestinal tract. The use of protease inhibitors can be useful in slowing down the pace of this degradation (Peikin, 1989; Hill et al., 1990). Protease inhibitor II (PI2) found in potatoes has been shown to slow down the activity of proteases such as trypsin and chymotrypsin, thus enhancing the activity of satiety-inducing hormones (Bryant et al., 1976; Pearce et al., 1982). PI2 has been reported to enhance the release of CCK which plays a role in satiety (Nakajima et al., 2011; Komarnytsky et al., 2010). The ingestion of a meal is linked with the synthesis of the hormone form of CCK by endocrine cells and high circulating level of CCK are associated with a reduction in food intake (Louie et al., 1986; Smith et al., 1987). A dose of 1.5g of PI2 before a meal has been shown to reduce energy intake in healthy subjects (Hill et al., 1990), whilst a dose as little as 150mg before a meal has been shown to increase circulating CCK

during the course of a dietary intervention study in overweight and obese subjects thereby influencing appetite sensation and satiety (Flechtner-Mors et al., 2020).

Eating isoenergetic portions of potatoes, in isolation, especially boiled potatoes appear to be more satiating than other starchy carbohydrates. When the satiating effects of potatoes were examined, boiled potatoes were found to be more satiating than 38 other food items, even more so than portions of other staple carbohydrates such as pasta, rice and white bread with similar energy level, and protein and fat rich food items (Holt et al., 1996; Geliebter et al., 2013). However, it is important to note that boiled potatoes have a relatively low-energy density, so when energy values are matched (isoenergetic) a bigger volume of potato per portion is provided compared to more energy-dense foods. This is apparent in a study which identifies a potato meal to be more satiating than a rice or pasta meal. Each of the three meals were composed of carbohydrates, 200g of meat and 200g of mixed vegetables was examined. It was hypothesized that the potato meal was the most satiating as 337g of potato was required to provide the 45g of carbohydrates needed for the meal, compared to 142g rice and 138g pasta (Zhang et al., 2018b). Similarly, mashed potato was identified as being more satiating than French fries when portions are created based on energy content, however no significant difference in satiety was found when the portions were based on carbohydrate content (Leeman et al., 2008). Studies have shown that the consumption of boiled potatoes as part of a meal with meat have shown lower energy intakes postprandial, as well as an increase in hormones which regulate feeding, compared to other carbohydrate meals which included meat (Akilen et al., 2016; Erdmann et al., 2007).

Many components of a typical western diet have been linked with increased risk of disease, obesity, increase in BMI or WC. Weight gain and increase in WC is a slow process, many of the studies conducted were carried out over numerous years (A Healthy Weight for Ireland – Obesity Policy and Action Plan 2016-2025). It is therefore difficult to isolate one single component of the diet such as potatoes as a factor linked directly to the development of obesity.

1.8.5 Type 2 Diabetes Mellitus (T2DM)

The link between diabetes and potatoes has been widely attributed to the high Glycaemic Index (GI) of some potato cultivars, as well as the high saturated and trans-fat content of processed potato products. In some studies, the western lifestyle and diet is characterised by a high intake of potato and potato products, refined grains, red and processed meats, fried foods and sugar, is acknowledged as a factor in increasing the risk of developing diabetes and other chronic conditions (Schulze et al., 2006; Pastorino et al. 2016; Bidel et al., 2018).

When the data from three prospective cohort studies was examined, Muraki et al. (2016) found that the consumption of 3 servings/week of boiled, baked, or mashed potato was associated with a greater risk of developing T2DM, and an even greater risk was associated with the intake of French fries (Muraki et al., 2016). Bidel et al. (2018) found similar results, in that a positive correlation was observed between potato consumption and T2DM, as well as a significantly increased risk of 20% when servings/day was increased. However, Bidel et al. (2018) noted that the studies examined were all carried out in developed countries, and that health policy could play a role in preventing diabetes (Bidel et al., 2018). Again Zhang et al. (2018a) found similar results and singled out French fries as the food group associated with the highest risk, but baked, mashed, and boiled potatoes were also reported to be associated with the development of diabetes (Zhang et al., 2018a).

The Glycaemic Index (GI), has been suggested as a possible factor influencing the onset of T2DM. Barclay et al. (2008) found low GI and low Glycaemic Load (GL) diets to be independently associated with a reduced risk of T2DM (Barclay et al., 2008). Foods are categorized as low GI if they have a value of ≤55, medium if they fall into the range of 55 to 69, and high if they are ≥70 (Jenkins et al., 1981; Alkaabi et al., 2011). The GI of potatoes is dependent on the cultivar, storage conditions, maturity of the tuber as well as the cooking method (Nayak et al., 2014). Thus, many GI values have been reported for potatoes. Henry et al. (2005) reported values of 56 to 94, in a range of commercially available potatoes from Britain. Ek et al. (2014) found the GI values of seven Australian potatoes to vary from 53 to 103 (Ek et al., 2014). Others have reported values as high as 144 and as low as 23-41 (Foster-

Powell et al., 2002; Soh et al., 1999). In general, potatoes are considered to be a high GI food. French fries have a greater association with T2DM in comparison to other forms of cooked potato. However, the International Tables of Glycaemic Index and Glycaemic Load reported that boiled potatoes have a GI of 78±4, instant mash potatoes have a GI of 87±3, whilst French fries have a reported GI of 63±6 (Atkinson et al., 2008). This suggests that the link between French fries and T2DM is not related its GI, and other factors such as its high fat content or poor diet and lifestyle choices of subjects should be considered. Furthermore, potatoes are rarely consumed in isolation. Studies have showed that the components of a meal such as dairy, fibre, fat and protein, and vinegar can all influence the overall GI of a meal (Henry et al., 2008; Hätönen et al., 2011; Henry et al., 2006; Sugiyama et al., 2003; Pelletier et al., 1998; Östman et al., 2003).

In contrast, Farhadnejad et al. (2018) found that a moderate intake of boiled, but not fried potatoes was associated with a lower risk of developing T2DM (Farhadnejad et al., 2018). Borch et al. (2016) could not find convincing evidence that potatoes were positively associated with diabetes after carrying out a systematic review of 13 observational studies and clinical interventions. Again, a possible association between the consumption of French fries and diabetes was considered, however Borch et al. (2016) stated that cofounding factors should be considered (Borch et al., 2016). The 8 year follow up study to the EPIC-Potsdam study, investigated diet and its risk to chronic conditions, potatoes were not mentioned as a risk factor whist low fat dairy, red meat and fruit juices were all listed (Von Ruesten et al., 2013). Numerous studies found no association between potatoes and diabetes (Hodge et al., 2004; Liu et al., 2004). Whilst other studies found an inverse relationship between potato consumption and T2DM (Panagiotakos et al., 2007; Morimoto et al., 2012; Villegas et al., 2007).

The inconclusive findings reported in studies to date identify the need for long-term randomized control trials to establish definitively if there is an association between potato consumption and the onset of T2DM.

1.9 Glycaemic Index

The concept of Glycaemic Index (GI) was first introduced in the 1981. It was devised as a means of classifying different carbohydrate rich foods and sources based on their effect on postprandial glycaemia. It was applied to foods that had an energy content > 80% coming from carbohydrates such as potatoes, cereals, rice, etc. (Brouns et al., 2005). It was also initially used as a method of controlling blood sugar levels in type 1 diabetes (T1D) patients and later on, dyslipidemia (Jenkins et al., 1981; Jenkins et al., 1983; Jenkins et al., 1985).

GI is defined as the incremental area under the curve (IAUC) of blood glucose response over a two-hour period, in response to the ingestion of a carbohydrate portion of a test food compared to a reference food consumed by the same subject under standard conditions on separate occasions. GI is expressed as a percentage of IAUC of the reference food (Wolever et al., 1991; Australian Standards Organisation, 2007).

There are three classifications for the GI of foods: high-GI foods, medium-GI foods, or low-GI foods. Foods are categorized as low GI if they have a value of ≤55, medium if they fall into the range of 55 to 69, and high if they are ≥70. Low-GI foods are digested and absorbed more slowly than high or medium GI foods, and therefore, elicit a lower glycaemic response therefore, low-GI foods are considered beneficial to diabetics (Jenkins et al., 1981; Alkaabi et al., 2011, Atkinson et al., 2008).

Table 1.3. The Glycaemic Index of Common Foods

Food Item	GI	Food Item	GI
Potato, boiled	78±4	Apple, raw	36±2
Potato, instant mash	87±3	Orange, raw	43±3
Potato, French fries	63±5	Banana, raw	51±3
Potato crisps	56±3	Watermelon, raw	76±4
Kidney beans	24±4	Cornflakes	81±6
Soya beans	16±1	Porridge, rolled oats	55±2
Carrots, boiled	39±4	Instant oat porridge	79±3
Vegetable soup	48±5	Chocolate	40±3

Information from Atkinson et al., 2008

The GI concept has been broadened to account for the effect of the total amount of carbohydrate eaten. Hence, glycaemic load (GL), a product of GI and the amount of carbohydrate consumed in a carbohydrate meal was developed. It provides information on glucose available for energy or storage following the ingestion of a meal containing carbohydrates (Venn and Green, 2007).

1.9.1 The Rise of the High GI diet

An increase in carbohydrate consumption has been seen in the USA since the 1970s and since the 1980s the rates of obesity have risen by 75% with one in three men and women now being classed as obese (Ludwig, 2000). In Ireland 60% of adults are either overweight or obese (HSE Health and Wellbeing "Key Facts"). The consumption of protein for most individuals falls within a narrow range, therefore, it is hypothesized that a reduction in dietary fat intake has led to an increase in the consumption of carbohydrates (Ludwig, 2000).

Carbohydrate rich fruits, vegetables, wholegrains, and legumes can be extremely beneficial to health. They provide important sources of vitamins and minerals and have been linked to reduced incidences of certain conditions including cardiovascular disease, colon cancer, pancreatic disorders as well as depression (Angelino et al., 2019). However, the increase in carbohydrate consumption does not correlate with an increase in fruit and vegetable intakes.

In Ireland rates of vegetable consumption are low with only 9% of adults age 18-64 consuming the recommend portion per day, and the average intake being under half that recommended (National Adult Nutrition Survey, 2011). The consumption of potatoes has also decreased from 140Kgs per person in the 1990s to 85Kgs per person in 2017 (Teagasc, 2017) which suggests that the Irish population has changed both the compositional and nutritional aspects of their diet.

Improvements in technology and food processing have given rise to a dependence on novel, convenient, precooked and instant foods (Brand-Miller et al., 2009) Ultra-processed foods have been described by NOVA, the food classification system for processed foods used by the UN, as not modified food items, but products produced using cheap industrial sources of energy nutrients and additives and produced using a series of processes (Monteiro et al., 2018). Foods such as industrialized packaged breads, breakfast cereals, confectionary, sausage and reconstituted meats are just some of the commonly consumed ultra-processed foods in the UK diet (Rauber et al., 2018). These foods are often low in fibre and are digested more rapidly. For example, instant mash potato has a GI of 87 compared to boiled potato which has a GI of 78 (Atkinson et al., 2008). Many common staple food items now have a high GI. The National Children's Food Survey II found that white bread and ready-to-eat breakfast cereals were both more abundant in the diets of children compared to wholegrain bread or porridge (National Children's Food Survey, 2019).

The GI of barley-based porridge varies from 55-68, compared with other popular cereals such as Cheerios (Canada) which has a GI of 74, Cornflakes with a GI of 81, and Coco Pops with a GI of 77 (Foster-Powell, et al 2002). All these cereals fall into the high GI classification. Looking at the breakfast foods alone, it is clear that a high GI diet is becoming more prevalent. Dietary surveys in countries such as Canada, Brazil and the USA show consistently high consumption of these ultra-processed foods (Moubarac et al., 2017; Monteiro, et al., 2018; Louzada et al., 2018; Martínez Steele et al., 2017). In the UK, two separate surveys showed that approximately 51.3%-63.4% of total dietary energy was coming from highly processed foods (Moubarac et al., 2013; Adams and White, 2015).

1.9.2 Implications of a High GI Diet

1.9.2.1 Cognitive Decline

Cognitive function is an umbrella term used to describe the processing of information, utilisation of knowledge, and expanding and altering mental representations formed on past experiences. When investigating cognitive decline, multiple aspects of cognitive function are examined such as memory, learning, the processing of information and the management of day to day actions. Studying cognitive function is based on the responses to positive and negative stimuli, multiple areas of information processing are usually examined such as attention, memory, and language (Sunram-Lea, 2019).

Cognitive decline increases with age (Carneiro and Leloup, 2020). A number of preventative lifestyle measures are currently being studied to help slow down the onset of cognitive decline as there is currently no treatment. Diet is a major lifestyle factor which has been investigated, nutritional interventions can provide data on how to optimize cognitive development from early infancy, throughout childhood and into adulthood. In multiple studies, breakfast has been recognised to improve cognition, the type of carbohydrate consumed at breakfast can play a vital role in maximising cognitive performance (Rampersaud et al., 2005). GI is thought to be a significant factor in cognitive function, and evidence indicates that postprandial glycaemia may be linked with cognitive performance in adults and children (Sunram-Lea, 2019; Sunram-Lea and Owen, 2017).

The brain uses approximately 20% of the body's energy. The increase in glucose following food consumption reaches the brain via an increase in blood glucose levels which supplies the brain with a constant level of glucose needed to carry out regular cognitive functions (Álvarez-Bueno et al., 2019).

Ingwersen et al. (2007) examined the consumption of a low GI or a high GI breakfast cereal in children aged 6-11 years, and tested for attention and memory using the Cognitive Drug

Research (CDR) Computerised Assessment Battery test over the course of two hours post consumption. GI did not appear to influence the speed or accuracy of attention but significantly impacted on secondary memory and assessment time, indicating that consuming low GI breakfasts enhances cognitive performance of children (Ingwersen et al., 2007). Similarly, Micha et al. (2010) found that children who consumed a low GI/high GL breakfast had better results for speed of information processing and a series of seven tasks, the high GI breakfast was found to improve immediate word recall (Micha et al., 2010). Numerous other studies also indicate that the consumption of a low GI breakfast improves cognitive function (Copper et al., 2012; Micha et al., 2011), however, many of these studies are conducted over a short period of time and the longevity of the effects of consuming a low GI breakfast is not examined (Carneiro and Leloup, 2020). In contrast, Brindal et al. (2012) found no improvements in speed of processing, working memory, short-term memory, perceptual speed, or cognitive performance. The study was conducted with children aged 10 to 12 years, the test meals provided varied in GL, macronutrient composition and dairy content (high GL/no dairy, medium GL/medium dairy and low GL/high dairy) all meals had the same calorie content. The authors concluded that reducing the GL by replacing the carbohydrate with protein did not improve cognition or satiety over a three-hour period (Brindal et al., 2012).

As aging occurs the brain becomes more susceptible to cognitive decline, memory and learning are particularly vulnerable to metabolic brain disorders and abnormalities in glucoregulation. A study conducted in an elderly population, found that subjects consuming a strict, more prudent diet had scored higher in the cognitive function test compared with those following a typical high GI western diet. The data indicated that the GL of the diet was inversely related to cognitive performance (Power et al., 2015). It has been hypothesized that glucoregulation may play a role in the onset of neurodegenerative diseases in older adults (Rolandsson et al., 2008). Data from the Brain Motion Study found that age correlated with lower cognitive performance regardless of glucose control. The GL of meals did not impact those with normal glucose control, however in those with poorer glucoregulation the GL of the meal had a statistically significant impact on cognitive performance. In those with poorer glucoregulation, lower GL was associated with better figural memory and better cognitive function (Garber et al., 2018). Seetharaman et al. (2015) examined the influence of blood

glucose and dietary GL on cognitive aging in adults, aged ≥50 years, who did not have dementia and were in good cognitive health (Seetharaman et al., 2015). The findings of the study showed that high blood glucose levels were linked with declines in verbal ability and spatial abilities.

High blood glucose levels associated with the consumption of a western diet can cause deterioration in the hippocampus the part of the brain responsible for long-term and spatial memory (Kanoski and Davidson, 2011). Therefore, a low-GL diet may contribute to the prevention of neurodegenerative diseases in older adults and elevated blood glucose levels could speed up specific detrimental effects on cognitive function in older adults.

1.9.2.2 Age-Related Macular Degeneration (AMD)

AMD is a disease of the central retina known as the macula which causes a progressive decline in vision (Schleicher et al., 2013). If damage occurs to photo receptors in the macula, it affects the central field of vision. It is estimated that AMD affects 14% of the elderly population (Rowan et al. 2020). Rates of AMD are expected to rise from 196 million in 2020 to 288 million by 2040 (Jalbert et al., 2020). Healthy dietary patterns are believed to be protective against the development of the disease (Chapman et al., 2019).

Treatment options for the disease are limited and there is no treatment to reverse the disease (Al-Zamil et al., 2017). Treatments such as anti-vascular endothelial growth factor (VEGF) injections help to slow down the progression of AMD through reducing the pace of the development of lesions (Weikel et al., 2012). The use of supplements containing vitamin C, vitamin E, zinc, lutein, and zeaxanthin have shown to reduce the progression of intermediate and advanced AMD (Age-Related Eye Disease Study 2 Research Group, 2013). Laser treatments have been examined and shown to have beneficial outcomes however, they are associated with reoccurring symptoms (Al-Zamil et al., 2017).

A population-based prospective study using data from the Blue Mountain Eye Study population examined the incidence of AMD over a decade. Subjects who had diets highest in GI had a 77% heightened risk of developing early AMD compared with the subjects who had the lowest GI diet. The authors also noted that other modifications to an individual's lifestyle such as not smoking, and a high fruit and vegetable intake may have a similar effect to that of a low GI diet (Kaushik et al., 2008; Chapman et al., 2019). Chiu et al. (2007a) used data collected from AREDS (Age-Related Eye Disease Study) to observe the influence of dietary GI on the advancement of AMD in subjects without diabetes. The findings indicated that the risk of advancement of AMD was significantly larger in subjects with a high GI diet compared with those with a lower GI. The authors suggest that individuals at risk of AMD progression may benefit from consuming smaller quantities of refined carbohydrates. The data indicates that 7.8% of the new cases of advanced AMD could be prevented in the next 5 year by those at risk consuming a low GI diet (Chiu et al., 2007a).

Multiple animal studies have identified a high GI diet as a risk factor in the development of AMD, whilst a low GI diet appears to have a protective effect (Uchiki et al., 2012; Weikel et al., 2012). More recent studies hypothesized a possible link between AMD and the composition of the gut microbiota. The literature indicates that mice fed a high GI diet had a microbiome that was high in Firmicutes and Clostridia both of which have been associated with retinal damage. In contrast, the mice on a low GI diet showed an abundance of Bacteroidales and Eysipelotrichi classes that are protective against AMD (Rowan et al 2017; Rowan and Taylor, 2018). Similarly, mice fed a high-fat diet also had increased levels of Firmicutes and Clostridia compared to mice fed a regular diet (Andriessen et al., 2016). However, as this is an emerging area of research further studies are required on the use dietary interventions to regulate the gut microbiota and the development and progression of AMD (Rinninella et al., 2018).

1.9.3 How Starch can Influence the GI

Starch can be classed as resistant starch (RS), slowly digestible starch (SDS) and rapidly digestible starch (RDS) based on their rate of digestion. RS can be further sub-categorized into

RS1- physically entrapped starch; RS2- ungelatinized starch granules that are impervious to digestive amylases; RS3- retrograded starch resulting from cooling after gelatinization; and RS4- starches that have been chemically modified in a way that reduces digestibility (Englyst et al., 1992; Sajilata et al., 2006). RDS is associated with the rapid rise in blood glucose levels within the first 20 mins of consumption and is strongly correlated with GI. SDS takes much longer to digest, roughly the same length of time as it takes the food to pass through the small intestine (Eelderink et al., 2012). It causes a more sustained level of blood glucose, even in high GI foods. The SDS present in cooked potatoes can vary from 1-10% (Englyst et al. 1992). High RDS foods are associated with a higher Glycaemic Index (GI) response compared to lower levels of RDS which correlate to medium/ low GI responses (Lynch et al., 2007). Whilst the consumption of low GI foods is often associated with health benefits, a diet consisting primarily of high GI foods has been linked with multiple chronic illnesses (McGill et al., 2013).

Upon heating, starch gelatinizes and is converted into RDS and after boiling RDS can account for much as 53-86% of the total starch (Englyst et al., 1992; Dupuis et al., 2016). Amylose accounts for only 20-30% of the starch in potato (Hoover, 2001). However, during heating of the starch, it acts as a restraint to swelling and upon cooling forms retrograded starch more easily than amylopectin. As the potato cools down, the starch granules gel, retrograde and then form a semi-crystalline substance, which eventually forms into SDS or RS3 (Raigond et al., 2014). This reduces the GI of the potato as it is impervious to enzymatic digestion, and it acts like dietary fibre (Nugent, 2005).

There has been an increased interest in exploring methods of controlling starch digestion rates and the formation of SDS and RS3. The levels of SDS starch can rise to as much as 45% in potatoes where the starch has been retrograded (Mishra et al., 2008). The extent of this formation is dependent on the processing method used as well as the way the potato is consumed (Brand et al., 1985; Thed and Phillips, 1995; Englyst and Cummings, 1987). It is believed that the initial gelatinization is the most important, as it can help preserve some of the naturally occurring RS2, thus boosting the final potatoes' RS content. In their raw native

form, potatoes can have up to 75% RS which then diminishes to 5-10% during cooking and will remain low unless cooling strategies are used (Englyst et al., 1992).

Multiple studies have shown that heating followed by cooling of potatoes lowers GI. Starch retrogradation has been linked with higher levels of RS and SDS in both starch gels and potatoes (Xie et al., 2014a; Xie et al., 2014b; Dupuis et al., 2016). Reductions in the GI following retrogradation are still observed upon reheating (Tahvonen et al., 2006). Pinhero et al. (2020) observed that the potatoes with the lowest levels of RDS also had the lowest GI. Data from Fernandes et al. (2005) showed cold, boiled red potatoes had the lowest GI (56.2), and hot, boiled red potatoes had the highest (89.4) (Fernandes et al., 2005).

Colussi et al. (2017) determined the impact of high-pressure processing (HPP), with or without retrogradation (by storing at 4°C for one week), on the hydrolysis of potato starch throughout *in-vitro* gastro-intestinal digestion. Hydrolysis of the HPP-treated starch did not differ significantly from the untreated starch, however HPP treated samples with retrogradation had slightly lower values for starch hydrolysis and hence glucose release. The authors claimed that HPP in combination with retrogradation could be applied for the preparation of starch based low glycaemic foods.

1.9.4 Effect of Cooking Methods and Processing on the GI of Potatoes

Studies investigating the impact of cooking methods such as boiling, baking, and microwaving on GI have yielded differing results. Selected studies indicate lower glycaemic responses to baked potatoes compared to boiled potatoes. García-Alonso and Goni (2000) also found that boiled potatoes were the most digestible compared to potatoes cooked by other methods (García-Alonso and Goni, 2000). Higher levels of RS were reported in potatoes which had been baked compared to those that were boiled (Yang et al., 2016; Raatz et al., 2016). Singh et al. (2020) noted a slight difference in the RS of boiled and microwaved potatoes, with the microwaved potatoes having slightly less than the boiled, however, there was no notable difference in the GI (Singh et al., 2020). Pinhero et al. (2020) observed no notable difference

in the GI of baked or microwaved potatoes but did find differences depending on the cultivar. With that said, it was found that baked potatoes had a significantly higher GL than other cooked potatoes (Pinhero et al., 2020). However, other studies found no difference between the potatoes cooked by different methods and their glycaemic impact (Soh and Brand-Miller, 1999). Tahvonen et al. (2006) noted only minor differences in the GIs of potatoes which had been cubed, sliced, mashed, peeled, or cooked by steam boiling or baked with water (Tahvonen et al., 2006). The variability in the findings for the impact of the food/cooking on the GI of foods could be attributed to the time between cooking and testing the food and naturally occurring variations between the tubers.

Fried foods are associated with lower glycaemic responses. French fries have a medium GI of 63±6 (Atkinson et al., 2008); interestingly, French fries only contain approximately 7% RS (García-Alonso and Goni 2000). Li et al. (2019) found that French fries elicited a lower glycaemic response than that of home fries or hashbrowns (Li et al., 2019). Singh et al. (2020) also reported a significant reduction in the GI of the tubers when fried into potato chips compared with other cooking methods (Singh et al., 2020). Likewise, Tian et al. (2016) noted lower Gis for fried (74 ± 6) and stir-fried potatoes (69 ± 5) compared to boiled potatoes (103 ± 6). Insoluble amylose-lipid complexes were formed when amylose was heated with fatty acids (Mercier et al., 2013). Amylose-lipid complexes can be classed as a form of RS, RS3 which is the same form of RS formed when starch is retrograded (Panyoo and Emmambux, 2017). It is hypothesized that the increase in the fat content of fried potato products may also play a role in their lower GI status, as dietary fat reduces gastric emptying and prolongs digestion. The use of an *in-vitro* model would be suitable in confirming if the lower GI is due to a delay in gastric emptying rather than the formation of amylose-lipid complexes.

Emerging processing technologies such as High-Pressure Processing are being explored in relation to food processing. The use of HPP to reduce GI has been seen in the literature. However, its effects on the GI of whole potatoes has not yet been explored.

1.9.5 The Effect of Maturity and Cultivar on the GI of the potato Cultivar

In addition to the starch content and characteristics of the potato, the potato maturity also affects the GI. Early maturing potatoes (65-90 days) have a higher moisture content and lower dry matter than mid-season (90 – 120 days) and main crop varieties (>120 days) (Nayak et al., 2014). Higher moisture content is associated with a lower GL (Lynch et al., 2007). Pinhero et al. (2020) also observed that the potato with the highest moisture content also had the lowest GI (Pinhero et al., 2020). Pinhero et al. (2016) found that eleven out of fourteen early potato varieties had a low GI. In line with the literature, a positive correlation was seen between RDS and GI, whilst a negative correlation was observed between GI and RS (Pinhero et al., 2016).

The variety of potato influences the GI. Henry et al. (2005) reported values of 56-94 for the GI of a selection of British potatoes, whilst Ek et al. (2014) found a range of 53-103 when investigating the GI of Australian tubers. Foster-Powell et al. (2002) reported values as low as 23 and 24 for two non-specified varieties, and GI of 111 for another unspecified variety (Foster-Powell et al., 2002). GI can also differ greatly within a single potato variety; Desiree had three different reported Gis 77 (Henry et al., 2005), 74 (Ek et al., 2014) and 101±15 (Foster-Powell et al., 2002). Such differences could be accounted for by the various cooking times. Ek et al. (2014) cooked the potato for 8-9 minutes, Henry et al. (2005) for 15 minutes and Foster-Powell et al. (2002) for 35 minutes.

The polyphenol content of potatoes may also influence the GI and anthocyanins have demonstrated an inhibitory effect on intestinal α-glucosidase (Ramdath et al., 2014). Liu et al. (2018) noted that phenolic acids bound to starch molecules could have an inhibitory effect on starch hydrolysis (Li et al., 2018). Kalita et al. (2018) found that phenolic acid and anthocyanins were again identified as having inhibitory effects on digestive enzymes such as α-glucosidase, α-amylase, and aldose reductase. They surmised that the consumption of potatoes rich in phenolics could delay or interfere with the carbohydrate digestion and absorption, thus leading to a suppression in postprandial blood glucose (Kalita et al., 2018).

Aims and Objectives

The aim of this thesis was to evaluate the Glycaemic Index and Glycaemic Load of a range of potato varieties *in-vitro*. Each chapter looked at two different mechanisms for potentially reducing the GI of potato. The GI was assessed by *in-vitro* methods.

Chapter 2 investigated the GI of a variety of boiled potatoes commonly consumed in Ireland using an *in-vitro* gastro-intestinal model. Each potato variety was also subjected to a novel high-pressure processing treatment as a possible mechanism for reducing the GI. Also, in this chapter the influence of fat on GI was examined. A range of four fats of varying saturation from polyunsaturated to saturated were studied.

In Chapter 3, firstly we examined the GI of a range of potato-based meals. Each meal varied in macronutrient content, however the amount of available carbohydrates remained the same. The second part of this chapter looked at the addition of fibre to potatoes and the potential impact on GI. The viscosity as well as the antioxidant potential of the potatoes was also investigated. In addition to this, the impact of a selected fibres on carbohydrate parameters and antioxidant potential in sweet potatoes was also studied.

Chapter 2

In-Vitro Determination of Glycaemic Index of Different Varieties of Irish Potato – Effect of High-Pressure Processing or Fat Addition.

Abstract

Potatoes are a popular staple crop providing nutrition to individuals globally. However, potatoes are generally classified as having a high Glycaemic Index (GI), which is associated negatively with health. In this study two possible methods of reducing the GI were investigated. Changes in the GI would be observed if alterations in the carbohydrate parameters of the potato occurred, an *in-vitro* model was used to assess this. Firstly, high-pressure processing (HPP) was examined to see the impact it would have on the GI. Six potato cultivars were selected due to their popularity and how readily available they are in Ireland. Roosters and Kerr's Pinks are the two most widely consumed and grown varieties in Ireland (Bord Bia, n.d.). The potatoes underwent high-pressure processing (HPP) at either 400Mpa or 600Mpa, the carbohydrate parameters of tested and when compared to those of control samples, no significant changes in the GI or other carbohydrate parameters was found. Then fat was investigated to see the effect it would have on the GI. Rooster potatoes were combined with four fats of varying saturation. Following this procedure, no alterations in the carbohydrate parameters of the Rooster potatoes occurred, meaning that the GI remained the same as the control. This study provides useful information on the carbohydrate parameters of six commonly consumed potato cultivars in Ireland as well as the potential use of HPP in relation to improving the glycaemic profile of a food item, it also highlights the value of using an *in-vitro* laboratory method as well as the shortcomings of the method when compared to *in-vivo.*

2.1 Introduction

Elevated postprandial glycaemia and hyperinsulinemia contribute to the development of chronic diseases such as diabetes and cardiovascular disease (CVD) suggesting a link between impaired glucose tolerance and risk of death from CVD. (The DECODE Group, 1999; Ludwig et al., 2002).

The GI of food has traditionally been measured as the increase in blood glucose in a subject following the ingestion of a food containing 50g carbohydrate equivalents. The increase in

blood glucose is then compared to a reference food, usually glucose, however sometimes white bread is used as the reference food too (Wolever et al., 1991). Foods are categorized as low GI if they have a value of ≤55, medium if they fall into the range of 55 to 69, and high if they are ≥70 (Jenkins et al., 1981; Alkaabi et al., 2011).

The first *in-vitro* digestion model to measure GI of a food was developed in 1969 (Southgate et al., 1969). Since then, an array of *in-vitro* models has been developed, each model having been subjected to some criticism. Because of this there is no standard method for measuring GI of a food using an *in-vitro* system. Digestion occurs in several phases; oral, gastric, and intestinal and these need to be mimicked by an *in-vitro* model. In the oral phase, the mechanical breakdown of a food and an initial hydrolyzation of starch by α-amylase occurs, food is then formed into a bolus before it is swallowed. The digestion of protein commences in the stomach during the gastric phase before the chyme is emptied into the duodenum. The rate of this emptying can vary depending on the composition of the meal. In the small intestine phase, hydrolyzation of starch is completed, fats are emulsified and absorbed as fatty acids, and monosaccharides are absorbed causing a glycaemic effect. In some studies, the oral phase is completed *in-vivo* and the food is chewed and then the bolus is removed from the mouth and testing is continued *in-vitro* (Akerberg et al., 1998). Other studies omit a phase such as gastric (Araya et al., 2002; Urooj et al., 1999) or oral (Hudson et al., 1976; Heaton et al., 1988). In the present study, the Englyst et al. (2000) method was used where food was broken down mechanically by sieving and mixing was achieved by use of the glass balls. Pepsin was added to breakdown any protein-starch bonds followed by incubation in amylolytic enzymes. Conditions such as temperature, pH and mechanical mixing were all controlled. The Englyst et al. (2000) *in-vitro* method is widely used to assess GI of various foods, including in a study on digestibility and GI of boiled and processed potatoes (Haase, 2015).

Whilst potato is generally classed as a high GI food (Atkinson et al., 2008), studies have shown the GI of potato to vary considerably from 71 to 106 (Atkinson et al., 2008; Tahvonen et al., 2006) and from 67 to 119 (Jenkins et al., 1988). Variations occur between different cultivars, levels of maturity, cooking method and if the potato has undergone processing (Henry et al.,

2005). However, potato is a popular staple, and is the third most consumed food in the world (Kyriakidou et al., 2020). Potatoes are extremely nutritious as they are low in fat and a good source of vitamin C, as well as a range of B vitamins and minerals such as potassium, magnesium, and phosphorus (Camire et al., 2009). Potatoes are also a good source of phenols and are considered the third most important source of these phytochemicals (Chun et al., 2005) However, like the GI, the phenolic content differs between varieties. The phenolic profile of white-skinned tubers contained half the concentration of phenolic acids as red/purple-skinned tubers (Ezekiel et al., 2013).

Methods of modifying the GI of foods have been widely researched. Cooking and reheating of potato can cause a reduction in the GI compared with consuming a freshly cooked potato (Fernandes et al., 2005). Vinegar can be used to slow down the rate of gastric emptying (GE), the combination of consuming cold potatoes with a vinaigrette dressing was shown to reduce GI by 43% (Leeman et al., 2005). Mixed meals with a high fibre food source, or a high protein food, have both been shown to reduce the GI (Schäfer et al., 2003; Henry et al., 2006). The addition of fat to high GI foods has been shown to delay GE which reduces the glycaemic response (Hätönen et al., 2011). However, there are conflicting findings regarding the degree of saturation of fat and if this impacts the GI and which is the most beneficial fat at reducing the glycaemic response. The use of HPP has been used in the food industry for years in relation to microbial spoiling. It is only recently that it is being explored as a mechanism to reduce the GI of food stuffs, and literature published in the area so far is promising (Huang et al., 2019b).

HPP is a non-thermal preservation method which can be used to inactivate pathogens present in the food. HPP causes minor losses in colour, nutrients and flavour in vegetables (Elizondo-Montemayor et al., 2015). One study found that HPP treatment could reduce the absorption of starch in the small intestine by 10-15% (Chou et al., 2020). *In-vivo* studies carried out on other food items such as mango and atemoya showed promising results when treated with HPP (Elizondo-Montemayor et al., 2015, Chou et al., 2020). HPP was therefore selected to be investigated to see if GI lowering results found in these studies could be replicated in an *in-vitro* model. For cold pasteurization a pressure of up to 600MPa can be used without affecting the sensory qualities of the food item. Research has shown that the application of 600MPa for 2-5minutes can have an effect on the starch granule (Dourado et al., 2019). Other studies

investigating the effect of HPP on various characteristics of potatoes also used raw tubers (Oliveira et al., 2015).

The aim of the present study was, firstly, to determine the GI of fresh potatoes including Rooster, Cultra, Kerr's Pink, Maris Piper and Gemson, following boiling. The GI of the aforementioned varieties were then compared to the same cultivars which had undergone HPP treatment. Secondly, the addition of fat, at doses ranging from 5%-25%, was investigated in a single potato variety, Rooster. Additionally, fats with different degrees of saturations were investigated. A key aspect of this study was the use of an *in-vitro* model to determine GI of potato, effects of HPP and addition of fat on these GI values and assess if results generated were in line with those found in the literature, particularly results derived from human *in-vivo* studies.

2.2 Study Overview

The study is composed of two parts. The first part investigated the GI of a variety of Irish potato cultivars and the impact of HPP on- these GI values. The second part examined the effect of addition of increasing amounts of fat and fat with different degrees of saturation on GI of Rooster potatoes. Ireland has approximately 600 commercial potato producers nationwide. Rooster and Kerr's Pink are considered the two most important varieties as they take up 59% of the 12,000ha used in potato production, 38% for Roosters whilst Kerr's Pink account for 21% (Bord Bia, n.d.). Roosters were selected to investigate the effect of the addition of fat on the GI of potato due to their popularity and availability year-round.

In addition to GI, both parts of the study investigate the same carbohydrate parameters: rapidly available glucose (RAG), slowly available glucose (SAG), total glucose (TG), GI, Glycaemic Load (GL), and free glucose (FG). RAG and SAG subsamples are taken after 20 and 120 minutes of digestion, respectively. They reflect the rate that glucose is likely to be absorbed by the small intestine. TG indicates the total amount of glucose present in the food sample. GI is a measurement of how quickly a food is broken down and glucose absorbed into the blood stream. GL is a similar concept but considers the total amount of carbohydrate in a food and how the food as a whole, impacts the blood sugar levels.

2.3 Materials and Methods

2.3.1 Materials

All chemicals were purchased from Sigma Chemical Co (Dublin, Ireland) or Megazyme Ltd. (Bray, Co. Wicklow, Ireland), unless stated otherwise. Rooster potatoes for HPP were provided by Country Crest Ltd., Lusk, Co. Dublin. All other cultivars that underwent the high-pressure treatment were purchased in a local market. These varieties included Cultra, Maris Piper, Kerr's Pink, Gemson, and Maris Piper (baby variety). The Rooster potatoes used in the study to assess effects of added fat were purchased locally in Cork (Ireland).

2.3.2 Preparation of HPP Treated Potatoes

The HPP was carried out on the cultivars in HPP Tolling, St. Margaret's, Co. Dublin, using a Hiperbaric 55HT, Hiperbaric USA, Miami, FL, which is a commercial scale high pressure press. The whole unpeeled, washed potatoes were first separated by cultivar and then vacuum packed in polyethylene/polyamide pouches. Potatoes were subjected to 400MPa (4000 bar pressure) or 600MPa (6000 bar pressure) for a total of three minutes reaching a maximum temperature of 10.6°C. Only one batch of each potato cultivar was obtained for this testing, thus following HPP treatment, potatoes were stored at -18°C until needed for testing. Prior to testing samples were defrosted overnight at 4°C. Samples were boiled in unsalted water until tender and passed through a 5mm sieve. Samples which had undergone HPP treatment were compared with a corresponding control of the same cultivar not subjected to the treatment.

2.3.3 Preparation of Potatoes with the Addition of Fat

Rooster was the selected cultivar to test whether addition of fat modified the GI of this potato. Following the cooking of the potato, it had been pasted through the 5mm sieve, the fat was uniformly mixed into the sample and then pasted through the sieve for a second time. An initial dose response study was carried out. Cooked Rooster samples were combined with increasing concentrations of rapeseed oil, 10%, 15%, 20% or 25%. The potential impact of varying degrees in fat saturation was examined by the additions of olive oil, butter or coconut

oil at 10% (w/w) in addition to 10% rapeseed oil. The potato with coconut oil would be the most saturated, followed by potato and butter, potato and olive oil, with potato and rapeseed oil being the least saturated.

2.3.4 *In-Vitro* Method for the Determination of GI and Glucose Analysis

An *in-vitro* digestion procedure was used to obtain the RAG, SAG and TG values. A separate *in-vitro* digestion procedure was used to quantify the FG content of the samples. The RAG, SAG, TG and FG values were then used to calculate the GI, GL and total starch of the potato, HPP potato or potato with added fat.

2.3.4.1 *In-vitro* Method - RAG, SAG, TG, FG

The enzyme mixture (0.5g pepsin [Sigma, P7000], 0.5g Guar gum [Sigma, G4129], 100ml 0.05mol-1 HCl) was prepared and allowed to come to room temperature. Sample (1g) was added to 10ml of pepsin-guar gum mixture and was incubated in a shaking water bath at 37°C for 30 minutes. Then five glass balls (0.5 cm diameter) were added with 10ml of $0.25mol^{-1}$ sodium acetate, along with 5ml of the second enzyme mixture which contained Invertase (2,000 units/mL), amyloglucosidase and pancreatin. The samples were then incubated again in a 37°C water bath and after 20 minutes and 120 minutes, 0.2ml sub-sample was removed and added to 10ml of 66% ethanol for glucose analysis (these were the samples which were used to calculate the RAG and SAG). The remaining mixture was vortexed in the 100ml beakers before it was incubated for 30 minutes at 100°C. Mixture was cooled for 20 minutes in ice bath before 10mls of $7mol^{-1}$ KOH was added. It was incubated at 0°C in a shaking ice bath for 30minutes. The mixture was vortexed again before 1ml of solution was transferred into heat resistant beaker which contained 10ml $0.5mol^{-1}$ acetic acid and 0.2ml amyloglucosidase. It was incubated for 30minutes at 70°C in shaking water bath, removed from the water bath and 40ml of distilled H_2O was added and mixture was then vortex. This was the sample which was used to determine total glucose which was tested immediately (Englyst et al., 2000.)

2.3.4.2 Glucose Analysis

Glucose was measured using the Megazyme D-glucose assay procedure kit (GOPOD-format). In glass heat resistant tubes 3ml of the GOPOD reagent was added to 0.1ml of the sample solution (RAG, SAG, TG, and FG). The sample was then incubated for 20 minutes at 50°C and absorbance was read at 510nm on the spectrophotometer. (Megazyme D-Glucose Assay Procedure Booklet)

$$D-Glucose\ \left(\frac{\mu g}{0.1\mathrm{ml}}\right)=\frac{\Delta A\ Sample}{\Delta AD-Glucose\ Standard(100\mu g)}*100$$

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2.3.5 Data Analysis

The batches of potatoes were divided into three and the experiment was repeated a total of three times. Each sample was tested in duplicate.

Equations as described by Englyst et al. (2000) were used to calculate the GI, GL, starch, RAG, SAG

$$RAG_{rel}=\frac{RAG*100}{TG}$$

$$SAG_{rel=}\frac{SAG*100}{TG}$$

$$TS=0.9*(TG-FG)$$

$$GI=17.7+77.9(\frac{RAG}{TS+2FG})$$

$$GL=\left(\frac{GI(TS+2FG)}{100}\right)$$

Statistical analysis was carried out using a One-way ANOVA followed by Dunnett's multiple comparisons test using GraphPad Prism version 6.00 for Windows, GraphPad Software, La Jolla California USA, www.graphpad.com.

2.4 Results and Discussion

The proportion of total glucose which is released rapidly (RAG) is one of the key determinants of the GI of the food. The highest RAG values, relative to total glucose, and GI values were found in the coloured-skin potato varieties Rooster and Kerr's pink, with GI values of 97.79 and 81.60, respectively (Table 2.2.). Cultra potatoes, a white-skin potato variety, also had a high GI value at 79.10 (Table 2.1.). The remaining two varieties, Gemson and Maris Piper had GI values which would be classed as medium, at 69.21 (Table 2.3.) and 66.71 (Table 2.1.), respectively. The GL is calculated based on the total content of carbohydrate in the food thus, the Kerr pink potatoes which had a high GI but a low total glucose content compared to the other potato varieties, also had a lower GL compared to the other potatoes (Table 2.2.). Gemson "baby" potatoes had the lowest GL at 8.39 (Table 2.3.) and the highest GL was seen in the Cultra potatoes at 14.1 (Table 2.1.). There was no clear relationship between the results obtained for the GI and the GL for the different potato varieties.

There are limited published data on GI values for the potato varieties investigated in this study. However, Henry et al. (2005) reported the GI of Maris Piper as 85 in a study where they investigated the GI values of eight British potato cultivars, other varieties ranged from 56 to 94 (Henry et al., 2005). According to Henry et al. (2005) the Maris Piper is classed as a high GI food, however in the present study it was found to have a medium GI. Henry et al. (2005) used an *in-vivo* approach where human subjects ingested boiled potato and the increase in blood glucose was monitored. Ek et al. (2014) reported that GI values varied from 53 to 103 for seven Australian potato cultivars. It is not unusual for differences in GI value of the same cultivar to be reported in the literature, for example, the cultivar Desiree with a GI of 74 in one study (Ek et al., 2014) and 101 in an earlier study (Soh et al., 1999). Likewise, the cultivar, Russet Burrbank, was reported to have a GI of 82.0 (Ek et al., 2014) and 76.5 in another study (Fernandes et al., 2005), as well as with the cultivar Nicola (Ek et al., 2014; Tahvonen et al., 2006). However, it should be noted that many external factors pre-harvest and post-harvest can influence the sugar content of the tuber (Kumar et al., 2005). These changes in the carbohydrate composition may influence the GI and may result differences in GI and GL among the cultivars. Potato starch also differs in morphology between cultivars. The structure is often described as being an oval or ellipsoidal in shape, however the actual size of the starch

granule is 5 to 100 µm, this is considered to be a relatively broad range. The mean diameter of 23–30 µm (Alvani et al., 2011). This may be a factor attributing to the difference in GI amongst potatoes.

It could be hypothesised that the variation in the GI of potato cultivars could be due to differences in the starch structure of the crop. Both *in-vitro* and *in-vivo* studies investigating the differences in GI of rice, found that higher levels of resistant starch and the amylose in the crop reduced the GI (Kumar et al., 2018; Panlasigui et al., 1991).

The HPP was completed on six varieties, however no control sample was provided for the Maris Piper baby potato. The averages for these were then compared there was no significant difference in the data for the potatoes that had been subjected to the HPP treatment compared to the control, however interesting trends can be seen in the data for some but not all varieties. An incremental decrease was seen in the coloured skin cultivars, Roosters and Kerr's Pink (Table 2.2) as the pressure increased the GI decreased. There was a 13.71% decrease between the Kerr's Pink control and the Kerr's Pink treated at 400MPa, a 36.57% decrease in GI between the Kerr's Pink control and the Kerr's Pink treated with 600MPa. Only 3.5% decrease was seen between the Rooster control and the Rooster 400MPa, a 23.98% decrease was seen between the Rooster control and the 600MPa sample (Table 2.2). However, this pattern of HPP reducing the GI was not seen in all cultivars. Cultra (Table 2.1.), reported having a GI of 79.10, the initial HPP treatment of 400MPa reduced the GI to 68.36, however the higher pressure of 600MPa caused a slight increase with a GI of 80.97. The GI of the Maris Piper was increased from 66.71 to 73.20 with the 400MPa treatment, meaning that it would now be classes as a high GI food. The 600MPa treatment only caused a slight increase to 67.74 (Table 2.1.). Gemson's GI was also increased by 400MPa from 69.21 to 74.95. Maris Piper baby variety 400MPa had the highest GI out of all potatoes tested 117.70, however the 600MPa treated potato of the same cultivar was 44.34% less (Table 2.3.). As previously stated, it is important to note that these figures were not statistically significant.

Previously published literature suggests that treatments such as HPP may result in a decrease in GI. An *in-vivo* study conducted on the effects of HPP on the GI of fresh mango puree showed a significant decrease in the postprandial glycaemic response. The mean GI of the control (unprocessed) mango puree was 42.7 as compared to the processed mango puree (32.7), the authors also noted an increase in the viscosity of the processed product compared to the control (Elizondo-Montemayor, 2015). Huang et al. (2019b) examined the impact of high-pressure pasteurization of atemoya puree. Rats were fed the treated puree which had been subjected to pressures of 100MPa, 300 MPa and 600 MPa. The GI of untreated puree and puree treated at pressures of 100 MPa, 300 MPa, and 600 MPa were 65.4, 66.3, 55.4, and 49.8, respectively which suggests that GI decreases with an increase in treatment pressure (Huang et al., 2019b).

It could be hypothesised that changes in the starch present in these foods following HPP caused the decrease in the GI *in-vivo*. Starch is categorized into rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) based on the degree of digestion *in-vivo*. SDS is considered to be the most influential type of dietary starch in reducing the rate of starch digestion in the small intestine and lowers postprandial blood glucose levels (Huang et al., 2019b). Colussi et al. (2017), examined the effect of HPP on isolated potato starch and also included a retrogradation step. The starch treated with six 10-minute cycles of 600MPa and the retrogradation step, had lower starch hydrolysis during an *in-vitro* gastro-small intestine digestion and a slower release of glucose (Colussi et al., 2017).

In the present study an incremental decrease was observed in the GI of both coloured skin potato cultivars tested. The GI of Kerr's pink control and samples treated with 400MPa and 600 MPa were 97.74, 85.00, and 62.14, whereas Rooster were 81.60, 78.74 and 62.03 (Table 2.2.). These decreases were not statistically significant. However, this trend was not observed in the other cultivars tested. HPP treatment resulted in inconsistencies in the GI's of the Maris Piper, Cultra, Gemson and baby Maris Piper (Table 2.1.) (Table 2.3.). None of the cultivars had a statistically significant difference compare to the control.

The GI of foods, in particular fruits, reported in the literature were ready to eat requiring no further processing post HPP, whilst the potatoes required cooking post HPP, prior to the digestion. Therefore, the GI of these foods was not directly comparable to potato (Elizondo-Montemayor et al., 2015; Huang et al., 2019b). Nasehi et al. (2012) suggested that due to its high-water content, potato starch is more resistant to pressure than the starch found in rice, corn or tapioca, which may be a reason why some of our varieties were not affected by the HPP treatment (Nasehi et al., 2012). However, modifications could be made to the present study, which may yield more interesting and significant results. The use of cycles of HPP and longer periods of time, or a long continuous HPP treatment, rather than the single treatment for three minutes may cause the desired changes in the starch structure, which in turn slow down the release of glucose and the hydrolysis of the starch (Deng et al., 2014; Colussi et al., 2017). Starch retrogradation is applied in the production of some processed foods including dehydrated mash potatoes, par-boiled rice, and breakfast cereals as it causes desirable changes to structural and sensory properties (Ek et al., 2014). It could be hypothesized that the cooking of the potatoes reduced or cancelled out any possible effect that the HPP treatment may have had on the GI. Therefore, further research into a ready to eat potato product would be interesting to see if similar results to that of the literature for other starchy foods would be found.

Table 2.1. Rapidly Available Glucose, Total Glucose, Glycaemic Index and Glycaemic Load of Cultra and Maris Piper Potato Cultivars which have Been Subjected to High Pressure Treatment at 400MPa and 600MPa

	Rapidly Available Glucose* (g/100g)	**Total Glucose (g/100g)**	**Glycaemic Index**	**Glycaemic Load**
Cultra Ctrl	14.38 ± 0.59	19.31 ± 0.45	79.10 ± 3.92	14.09 ± 0.62
Cultra 400MPa	11.73 ± 0.23	18.92 ± 1.31	68.36 ± 6.08	11.73 ± 0.41
Cultra 600MPa	14.93 ± 0.43	20.18 ± 1.98	80.97 ± 4.46	14.92 ± 0.62
Maris Piper Ctrl	10.97 ± 0.38	17.87 ± 1.73	66.71 ± 7.54	11.06 ± 0.72
Maris Piper 400MPa	11.39 ± 0.01	17.69 ± 1.76	73.20 ± 5.49	11.76 ± 0.26
Maris Piper 600MPa	12.65 ± 1.94	18.21 ± 1.48	67.74 ± 1.74	11.61 ± 0.72

Mean± Standard Error. N=3 *Rapidly Available Glucose- refers to glucose released from the potato sample after 20mins of digestion.

Table 2.2. Rapidly Available Glucose, Total Glucose, Glycaemic Index and Glycaemic Load of Kerr's Pink and Rooster Potato Cultivars which have Been Subjected to High Pressure Treatment at 400MPa and 600MPa

	Rapidly Available Glucose* (g/100g)	Total Glucose (g/100g)	Glycaemic Index	Glycaemic Load
Kerr's Pink Control	10.77 ± 0.11	11.39 ± 0.29	97.79 ± 1.98	10.24 ± 0.10
Kerr's Pink 400MPa	9.23 ± 0.74	12.06 ± 2.39	85.00 ± 6.96	9.16 ± 0.98
Kerr's Pink 600MPa	9.37 ± 1.13	17.33 ± 1.21	62.14 ± 6.15	9.86 ± 1.02
Rooster Control	12.38 ± 1.60	16.38 ± 1.83	81.60 ± 3.10	12.31 ± 1.53
Rooster 400MPa	12.23 ± 0.92	17.30 ± 1.02	78.74 ± 6.64	12.32 ± 0.68
Rooster 600MPa	12.04 ± 0.48	20.97 ± 1.94	62.03 ± 5.17	11.75 ± 0.43

Mean± Standard Error. N=3 *Rapidly Available Glucose- refers to glucose released from the potato sample after 20mins of digestion.

Table2.3. Rapidly Available Glucose, Total Glucose, Glycaemic Index and Glycaemic Load of Gem and Maris Piper Potato Cultivars which have Been Subjected to High Pressure Treatment at 400MPa and 600MPa

	Rapidly Available Glucose* (g/100g)	Total Glucose (g/100g)	Glycaemic Index	Glycaemic Load
Gem Control	7.96 ± 0.87	13.46 ± 2.31	69.21 ± 6.67	8.39 ± 0.98
Gem 400MPa	8.42 ± 2.00	15.60 ± 7.29	74.95 ± 6.52	8.92 ± 2.83
Maris Piper baby 400MPa	12.18 ± 3.31	9.41 ± 1.72	117.70 ± 3.35	10.43 ± 2.26
Maris Piper baby 600MPa	11.40 ± 0.59	17.78 ± 0.87	65.52 ± 0.01	10.77 ± 0.55

Mean± Standard Error. N=3 *Rapidly Available Glucose- refers to glucose released from the potato sample after 20mins of digestion.

In the second approach employed to reduce GI of potato, we investigated the addition of fat and examined effects using an *in-vitro* model. An initial dose response was carried (Table 2.4.). The 10% dose of rapeseed meant that the oil-potato sample consisted of 10% rapeseed oil and 90% was potato, the 15% dose consisted of 15% rapeseed oil and 85% potato, the 20% dose was 20% rapeseed oil and 80% potato, and the 25% does was 25% rapeseed oil and 75% potato. The control Rooster had a GI of 83.27, when the dose of rapeseed oil was added at 10%, 15%, 20% and 25%, the GI was altered to 92.82, 83.12, 90.38, 87.48 respectfully. These values were not significantly different to the control. Following the initial dose response (Table 2.4.) a concentration of 10% was selected for investigation of fats/oils with different levels of saturation (Table 2.5.).

The impact of fats with various degrees of saturation on GI values was also assessed. Butter, coconut oil, olive oil and rapeseed oil were all added to the hot rooster potato. Each fat caused a slight increase in the GI, butter 86.24, coconut oil 88.17, olive oil 85.72, rapeseed oil 87.31, when compared to the control which had a GI of 83.27. However, these values were not significantly different to control.

Previous studies have reported that glycaemic response of potato is reduced with the addition of lipids (Henry et al., 2008, Hätönen et al., 2011). An *in-vivo* study conducted by Hätönen et al. (2011) showed that addition of 30g of rapeseed oil to 362g of potato resulted in a 34% reduction in the GI however this was not a statistically significant reduction (P = 0·06). The control mash potato had a GI of 108, compared to mashed potato with added oil with a GI of 71. Henry et al. (2006) in an *in-vivo* study also reported that addition of cheese to potatoes resulted in a decrease in the GI from 93 to 39. When cheese was added to pasta a reduction in the GI from 61 to 27 was seen and when added to white toast a reduction in GI from 50 to 35 was reported (Henry et al., 2006). Therefore, the addition of cheese as a source of fat lowered each of the carbohydrate dense foods' GI by 58%, 56% and 30%, respectively. While the results from this study are interesting they are not statistically significant. In the present study no statistically significant differences was found between control and samples containing fat. The main effect of fat during digestion is to slow GE. The *in-vitro* model used

in the present study is unable to simulate the rate of GE and hence may explain why GI results generated differ to those derived from human studies.

An additional aim of this study was to examine the effect of four different fats with varying degrees of saturation on the GI and other markers of glucose response (GR) of Rooster potato. No significant difference was seen between the control and the fats of varying degrees of saturation, rapeseed oil being the least saturated, then olive oil, butter and coconut oil being the most saturated. A study conducted by Henry et al. (2008) examined the effect of addition of different fats with varying levels of saturation (butter, olive oil and grapeseed oil) on the GI of white bread. Whilst the addition of the fats lowered the glycaemic response (which would be the equivalent of the RAG in the present study) of bread, no significant differences in GI were observed between the fats when they were ingested with bread. (Henry et al., 2008). Gatti et al. (1992) reported that the consumption of 35g olive oil or corn oil resulted in a 70% reduction in the GR compared with white bread alone, but the addition of 35g of saturated fat (butter) to a white bread meal had no effect on the postprandial blood GR, but. In contrast to both studies, Rasmussen et al. (1996) found that when volunteers with non-insulin-dependent diabetes mellitus consumed potato with 100g butter, their blood GR area was suppressed and significantly lower than potatoes alone, potatoes with 50g butter, potatoes with 40g olive oil, and potatoes with 80g olive oil. Joannic et al. (1997) found that glucose and insulin response was significantly lower when carbohydrates were consumed with polyunsaturated fatty acids compared to being consumed with monounsaturated fatty acids (Joannic et al. 1997), however these findings are contradicted by Clegg et al. (2012) who reported that monounsaturated fats reduced the GR to the greatest extent.

The evidence from the literature suggests that the change in GR with the addition of different fats may be facilitated by processes such as delayed GE and the timing of fat consumption. A study investigating the effect of the timing of fat consumption found that the effect of olive oil on the GR was greater when the oil was consumed 30 mins prior to a high GI meal, rather than when the meal and fat were consumed simultaneously. This would suggest that it is the fatty acids, released following digestion, rather than the triglycerides, present in the oil prior

to digestion, that cause the hormonal response that results in delayed GE and postprandial rise in blood glucose. (Lilly et al. 2019)

However, as the present studies were conducted *in-vitro*, the normal physiological effects such as delayed GE did not come into play hence results obtained were not reflective of those in the literature. The saturation of each fat used in the experiment was also not explicitly measured which may have contributed to a lack of effect due to their additions. The evidence on fat saturation affecting the GI/GR varied greatly and is clearly an area requiring more investigation.

The addition of fat did not alter the RAG, GI or other carbohydrate parameters in this study, and it is likely that the *in-vitro* method employed may not be suitable for assessing the impact of fat on the glycaemic index of potato. A model as described by Li et al. (2019) using a Caco-2 cell for assessing the impact of various processed potato products and the influence they have on glycaemic performance could be employed as an alternative *in-vitro* method to the one used in this study.

Table 2.4. Rapidly Available Glucose, Total Glucose, Glycaemic Index and Glycaemic Load of Mashed Rooster Potatoes Consisting of 10%,15%, 20% and 25% Rapeseed Oil.

Potato cultivar	Rapidly Available Glucose*(g/100g)	Total Glucose (g/100g)	Glycaemic Index	Glycaemic Load
Rooster Control	10.25±0.49	12.40±0.17	83.27±3.64	10.15±0.36
10% Rapeseed Oil	11.22±0.09	11.95±0.77	92.82±3.58	10.81±0.03
15% Rapeseed Oil	10.66±0.23	13.14±0.82	83.12±2.08	10.55±0.28
20% Rapeseed Oil	11.08±0.41	12.45±0.58	90.38±1.54	10.73±0.39
25% Rapeseed Oil	10.73±0.12	12.44±0.41	87.48±1.17	10.48±0.13

Mean± Standard Error. N=3 *Rapidly Available Glucose- refers to glucose released from the potato sample after 20mins of digestion.

Table 2.5. Rapidly Available Glucose, Total Glucose, Glycaemic Index and Glycaemic Load of Mashed Rooster Potatoes Consisting of 10% Butter, 10% Coconut Oil, 10% Olive Oil or 10% Rapeseed Oil.

Potato cultivar	Rapidly Available Glucose*(g/100g)	Total Glucose (g/100g)	Glycaemic Index	Glycaemic Load
Rooster Control	10.25±0.49	12.40±0.17	83.27±3.64	10.15±0.36
10% Butter	09.75±0.23	11.19±0.71	86.24±2.60	09.56±0.26
10% Coconut Oil	11.04±0.52	12.24±0.22	88.17±1.45	10.76±0.46
10% Olive Oil	11.15±0.10	13.21±0.60	85.72±2.60	10.95±0.16
10% Rapeseed Oil	10.60±0.44	11.95±0.53	87.31±0.32	10.36±0.43

Mean± Standard Error. N=3 *Rapidly Available Glucose- refers to glucose released from the potato sample after 20mins of digestion.

2.5 Conclusion

The *in-vitro* method used in the present study was useful for identifying varieties of potatoes which had the greatest GI, as well as the identifying other carbohydrate parameters such as the RAG and GL. From these results it could be seen that the cultivars investigated fell into what would be considered a normal range for potatoes.

No significant differences were seen between the control and HPP treated samples. Interestingly, for certain cultivars such as Kerr's Pink and Roosters non-significant reductions in GI were seen suggesting that the treatment may have had an influence on the carbohydrate parameters of these potato varieties. However, this was not seen for every cultivar, so it is difficult to determine to what extent the HPP treatment affected and attributed to changes in the carbohydrate parameters of the crop. This contrasted with the literature which found that HPP did in fact reduce the GI of certain pureed food items when tested *in-vivo.* HPP was effective for increasing SDS in isolated starch samples which could potentially decrease the GI of the product. If alterations to the HPP treatment were included such as using intervals of the treatment or continuous treatment over a longer period, it may cause the necessary changes in the starch fraction of all of the potatoes cultivars, therefore, resulting in a lower GI.

The addition of fat to Rooster potatoes resulted in no changes in the carbohydrate parameters regardless of dose or degree of saturation of the fat. Usually, fat would supress the GI of high GI foods. However, the *in-vitro* model used may not have been suitable for detecting this and a more appropriate method may have yielded similar results to that of the literature.

Chapter 3

Reducing the Glycaemic Index of Potato Through Combination with Selected Foods or Fibres.

Abstract

In this study potatoes with a high GI (Roosters) were combined with foods commonly consumed with potatoes such as cheese, tuna, peas, and baked beans. An *in-vitro* model was used to assess the carbohydrate parameters and any difference which may have occurred between the different potato-food combinations. The addition of cheese with its high fat, high protein content resulted in the greatest decrease to the GI of potato in comparison to beans, peas, and tuna. All foods did cause a significant reduction in the Glycaemic Load (GL) of the potato compared to it on its own.

Following on from this, three fibres- inulin, pectin and hydroxypropylmethylcellulose (HPMC) were investigated to see if they would all have a similar effect on the carbohydrate parameters, antioxidant level as well as viscosity. HPMC was also added to a medium GI food (sweet potato) to see if it caused a similar reaction as seen in Rooster potatoes. The addition of both the HPMC caused a slight decrease in the GI of sweet potato and a more pronounced decrease in the GI of Rooster potato. The decrease in the GI of the Rooster potato due to the addition of HPMC and pectin correlated with an increase in the viscosity of the potato, whilst inulin had little to no effect. The addition of fibre to the Rooster potato and sweet potato also decreased certain antioxidant activities following digestion.

3.1 Introduction

The Glycaemic Index (GI) was devised as a tool to assist patients with diabetes in the selection of appropriate foods for their condition (Jenkins et al., 1981). GI is a predictor of glycaemic response (GR) which is the rate of glucose entry into the circulatory system in humans. High GI carbohydrates cause a rapid rise and fall in blood glucose concentrations however, the consumption of low GI carbohydrates results in a slow and steady increase to a much lower peak glucose concentration followed by a steady decline (Triplitt, 2012). Studies which examine GI in relation to GR, provide subject participants with a fixed amount of 50g available carbohydrates (Hätönen et al., 2011; Sugiyama, 2003). However, the portion size also can influence the GR so a smaller portion of a high GI food may elicit a lower response than

expected, in a similar way a large portion of a low GI food could cause a greater response than anticipated (Vega-López et al., 2018). Glycaemic Load (GL) is another method used to predict the GR, it considers not only the GI but also the quantity of available carbohydrates in a food (Vega-López et al., 2018). Whilst neither GI nor GL considers the nutritive value of the food being consumed, they are both deemed useful tools for predicting GR.

Foods such as bread, rice, breakfast cereals and potatoes have a high nutritive value however they generally fall into the high GI category (Foster-Powell et al., 2002). Healthy eating guidelines recommend 3-5 portions of these foods should be consumed per day as part of a balanced diet (FSAI, 2019). These foods are frequently consumed along with other foods or as part of a meal. The inclusion of high GI foods as part of a meal has been shown to elicit a lower glycaemic response than if consumed on their own. High fat, high protein and high fibre foods have all been shown to reduce GI and GR when co-ingested with a high GI food (Henry et al., 2008; Hätönen et al., 2011; Lilly et al., 2019; Schäfer et al., 2003). Björck et al. (2000) suggested that the addition of viscous fibres could be used as a tool to lower the GI of foods (Björck et al., 2000).

The aim of this study was to investigate the effects of added foods on the GI and GL of a potato cultivar (Rooster) using an *in-vitro* method previously detailed in Englyst et al. (2000). The first objective of this study was to investigate if the macronutrient content of the meal affected the carbohydrate parameters. Each meal was composed of 50g of available carbohydrate, however, fibre, fat and protein contents varied between the meals. A second objective of the study was to examine the impact of fibre on GI of Rooster potatoes. Three fibres were used for this portion of the study; hydroxypropylmethylcellulose (HPMC), pectin, and inulin. A dose response was used to assess if the quantity of fibre added was linked to changes in the carbohydrate parameters for potatoes. The effect of HPMC on the GI of sweet potatoes was also studied. Antioxidant activity in digested potatoes with added fibre was also measured.

3.2 Materials and Methods

3.2.1 Materials

All chemicals were purchased from Sigma Chemical Co (Dublin, Ireland) and Megazyme Ltd. (Bray, Co. Wicklow, Ireland) unless stated otherwise. Rooster potatoes were purchased locally in Cork. HPMC and pectin were both purchased from Sigma-Aldrich, and inulin was purchased from Megazyme Ltd. Sweet potatoes, cheddar cheese (Tesco Mature Irish White Cheddar 400G), tuna, (Tesco tuna chunks in brine 80g tin), beans (Tesco Baked Beans in tomato sauce 420g tin), frozen peas (Tesco Garden Peas 1kg) were all purchased from local supermarket in Cork.

3.2.2 Preparation of Potato-Based Meals

The meals investigated in this study (Table 3.1.) were based on research conducted by Henry et al. (2006), in which a number of toppings traditionally consumed with baked potatoes were investigated for their impact on GI. The nutritional composition of each of the meals (Table 2.1.) was determined by using the nutritional values on the food packaging of the cheese, beans, peas and tuna, the values used for potato were proximate values from the McCance and Widdowson's composition of foods integrated 2019 (McCance and Widdowson, 2019). Whilst, obtaining the macronutrient content for the potatoes analytically would have been more accurate, proximate values for the potato macronutrients were used instead. McCance and Widdowson's Composition of Foods, is a robust source for food compositional data as it uses analytic data, data from the literature as well as manufactures data.

The rooster potatoes were peeled, quartered, and cooked in 500ml of boiling water, until tender when pierced with a fork. The potato was passed through a 0.5mm mesh sieve. The potato was then combined with the "topping" (see Table 3.1.) and blended until uniformly combined. Both peas and baked beans were cooked prior to blending. Peas were boiled in unsalted water for two minutes and the baked beans were microwaved for three minutes until piping hot. The cheese was grated prior to addition to the potato for blending. The

tinned tuna was drained and then added directly to the potato for blending. This process was repeated on three separate occasions and each sample was tested in duplicate.

3.2.3 Sample Preparation for the Addition of Fibre

The rooster potatoes were cooked as described above and combined with the fibre (5%, 7% or 10% w/w) until fully and uniformly incorporated. This procedure was carried out for all three fibres (pectin, inulin and HPMC). Sweet potato sample was prepared in the same manner as the Rooster potato and combined with HPMC at a concentration of 7% (w/w) only.

Table 3.1. Proximate Nutritional Composition of Potato-Based Meals Consisting of Rooster Potatoes and Cheddar Cheese, Baked Beans, Tuna, or Peas. Each Meal Containing 50g of Available Carbohydrate.

Meal number	Meal components	Weight (g)	Carbohydrate (g)	Non-starch polysaccharide (g)	AOAC Fibre (g)	Protein (g)	Fat (g)	Water (g)	Energy kJ	Energy Kcals
1	Potato	285	49.9	2.9	4.6	5.1	0.3	224.9	897.8	210.9
	Cheddar Cheese	120	0.1	0.0	0.0	30.5	41.9	43.9	2070.0	499.0
2	Potato	195	34.1	2.0	3.1	3.5	0.2	153.9	614.3	144.3
	Baked Beans	106	15.9	4.0	5.2	5.3	0.5	77.2	363.6	85.9
3	Potato	286	50.0	2.9	4.6	5.1	0.3	225.6	900.9	211.6
	Tuna (in brine) (Drained weight)	120	0.0	0.0	0.0	29.9	1.2	89.2	552.0	130.8
4	Potato	215	37.6	2.2	3.4	3.9	0.2	169.6	677.3	159.1
	Peas	125	12.5	5.6	7.0	8.4	2.0	94.5	411.3	98.8

3.2.4 *In-vitro* Method for Determining RAG, SAG and TG

The enzyme mixture (0.5g pepsin (Sigma, P7000), 0.5g Guar gum (Sigma, G4129), 100ml 0.05mol/L HCl, and was prepared and allowed to come to room temperature. Sample (1g) was added to 10ml of pepsin-guar gum mixture and was incubated in a shaking water bath at 37°C for 30 minutes. Then five glass balls (0.5 cm ⌀) were added with 10ml of 0.25mol/L sodium acetate, along with 5ml of the second enzyme mixture which contained invertase (2,000 units/mL), amyloglucosidase and pancreatin. The samples were then incubated again in the 37°C water bath and after 20 minutes and 120 minutes a sub-sample (0.2ml) was removed and added to 10ml of 66% ethanol for glucose analysis. These samples were used to measure RAG and SAG, respectively. The remaining mixture was vortexed in the 100ml beakers before it was incubated for 30 minutes at 100°C. The mixture was cooled for 20 minutes in an ice bath before 10mls of 7 mol/L KOH was added. It was incubated at 0°C in a shaking ice bath for 30minutes. The mixture was vortexed again before 1ml of solution was transferred into heat resistant beaker which contained 10ml 0.5 mol/L acetic acid and 0.2ml amyloglucosidase. It was incubated for 30minutes at 70°C in shaking water bath. 40ml of distilled H_2O was added and the mixture was vortexed and analyzed for total glucose (Englyst et al., 2000).

Equations as described by Englyst et al. 2000 were used to calculate the GI, GL, starch, reg RAG, reg SAG

$$RAG_{rel} = \frac{RAG * 100}{TG}$$

$$SAG_{rel=} \frac{SAG * 100}{TG}$$

$$TS = 0.9 * (TG - FG)$$

$$GI = 17.7 + 77.9(\frac{RAG}{TS + 2FG})$$

$$GL = \left(\frac{GI(TS + 2FG)}{100}\right)$$

3.2.5 Glucose Analysis

Glucose was measured using the Megazyme D-glucose assay procedure kit (GOPOD-format). In glass heat resistant tubes, 3ml of the GOPOD reagent was added to 0.1ml of the sample solution (RAG, SAG, TG, and FG). The sample was then incubated for 20 minutes at 50°C and absorbance was read at 510nm on the spectrophotometer.

3.2.6 Viscosity

The viscosity of the Rooster potato alone as well as in combination with inulin, pectin or HPMC at concentrations of 5%, 7% and 10% was tested. Samples were subjected to *in-vitro* digestion as described for SAG above. The viscosity was measured using a HAAKE RotoVisco 1 Rational Rheometer (Thermo Scientific). The Z38 rotor was used with the Z43 cup. The sample was brought to 25°C, there was a two-minute ramp as the shear rate reached 500 ẏ in 1⁄s, sample was then held at this rate for two minutes, followed by a two-minute ramp of the shear rate returning to zero. This procedure was repeated for each of the samples.

3.2.7 Antioxidant Testing

3.2.7.1 Sample Preparation

Rooster potatoes or sweet potatoes were peeled, quartered, and boiled in unsalted water. 4g of potato was weighed and uniformly combined with 0.28g of HPMC (7% dose). Samples were diluted to 10mls using 80% MeOH and used for analysis of FRAP, ORAC and TPC. Samples required for ascorbic acid testing were diluted with 10mls 75% MeOH diethyl (75ml methanol, 25ml deionized H_2O). Samples were then vortexed incubated at room temperature for 30 minutes and vortexed again. The samples were then centrifuged at 3000rpm for 5 minutes at room temperature.

3.2.7.2 Ferric Reducing Antioxidant Power (FRAP) Assay

The FRAP assay was carried out using the method of Benzie et al. (1996). The two stock solutions required were 300mM Acetate buffer and 40mM HCl. TPTZ (10mM) (0.031g TPTZ / 10 mL 40mM HCl) and $FeCl_3.6H_2O$ (20mM) (0.054g/ 10 mL distilled deionised H_2O) were added

to acetate buffer (1:1:10) to prepare FRAP sample reagent. The FRAP standard curve reagent was prepared using $FeSO_4.7H_2O$ (0.001M).

Table 3.2. Chemical Composition of FRAP Reagent for the Standard Curve and FRAP Reagent for Samples

	FRAP Reagent for Standard Curve	**FRAP Reagent for Samples**
Acetate Buffer	20ml	20ml
TPTZ	2ml	2ml
$FeCl_3$	-----	2ml
H_2O	2ml	-----

H_2O, $FeSO_4$ standard and FRAP standard curve reagent (20ml of acetate buffer, 2ml of TPTZ and 2ml of H_2O) were used to produce the standard curve. The standard curve was prepared as shown in Table 3.3.

Table 3.3. Concentration of Water, $FeSO_4$ standard and FRAP Standard Curve Reagent used to Produce the Ferric Reducing Antioxidant Power (FRAP) Standard Curve

	Concentration(µM)							
	0	**5**	**10**	**20**	**40**	**60**	**80**	**100**
H_2O (µL)	1000	985	970	940	880	820	760	700
$FeSO_4$ Std (µL)	0	15	30	60	120	180	240	300
FRAP (mL)	2	2	2	2	2	2	2	2

The potato samples were prepared to a concentration of 0.4g/ml and 100µL of each potato sample was mixed with 900µL of distilled water and 2mL of FRAP sample solution. Samples were incubated in the dark for 30 minutes. Samples were measured at 593nm using a spectrometer. Data was expressed as mM Fe+2/100g (Benzie et al., 1996). FRAP were just read off the standard curves and adjusted for any dilutions and the weight of potato used in the assay.

3.2.7.3 Oxygen Radical Absorbance Capacity (ORAC) Assay

The Phosphate buffer was first prepared using by making two solutions. Then 14mLs solution 2 (0.0276g of NaH2PO4 in 20mLs distilled water) were added to 40mLs solution 1 (0.0712g of Na_2HPO_4 in 40mLs distilled water) for pH 7.4. Next the Trolox standard curve was created. 0.01g Trolox was dissolved in 10mLs phosphate buffer; this then required bath sonication for approximately 1hr to dissolve (1mg/mL). 1mL of trolox solution was dissolved in 9mLs distilled water (100µg/mL). The standard curve was prepared as shown in Table 3.4.

Table 3.4. Concentration of Water and Trolox used to Produce the Oxygen Radical Absorbance Capacity (ORAC) Standard Curve

	Concentration µg/mL									
	0	**5**	**10**	**15**	**20**	**25**	**40**	**60**	**80**	**100**
Trolox (µL)	0	5	10	15	20	25	40	60	80	100
H_2O (µL)	100	95	90	85	80	75	60	40	20	0

Fluorescein stock was made by weighing 0.006g Fluorescein into an eppendorf and dissolved in 1mL phosphate buffer (~16mM). The16mM was diluted to 40µM (25µL in 10mL deionised H_2O); and aliquoted to 50µL stock and frozen. Fluorescein aliquot was prepared to a concentration of 40nm (10µL in 10mLs deionised water). 2,2 Azobis dihydrochloride (0.6g) was dissolved in 10mL of deionised H_20.

25µL of the potato samples were prepared as stated under sample preparation. They were then pipetted into a black Nunc 96 well plate. Fluorescein (50µL) was added to the samples. The samples were incubated in the plate reader for 20 minutes at 37°C. AAPH (100mL) was added. The fluorescence was measured at excitation 485nm and emission 535nm. 12 Readings were taken with 5-minute intervals between each reading. The following equations were used to calculate the AUC and Net AUC:

$$\text{AUC} = \left(\frac{R1}{R1}\right) + \left(\frac{R2}{R1}\right) + \left(\frac{R3}{R1}\right) + \cdots + \left(\frac{Rn}{R1}\right)$$

Where R_1 is the florescence at the start of the reaction and R_n is the last reading of the reaction.

Net AUC = AUC_{Sample}-AUC_{Blank}

The standard curve was obtained by plotting the Net AUC of different Trolox equivalents concentrations against the concentrations. ORAC values were obtained by reading the sample AUC from the standard curve. Data were expressed as µM TE/100g (Brescia, 2012).

3.2.7.4 Total Phenolic Content (TPC)

Na_2CO_3 (2g) was dissolved in 10mL of deionised H_2O. Gallic acid (0.05g) was dissolved in 100mL distilled deionised H_2O. To prepare the standard curve the Gallic Acid stock was diluted as indicated in Table 3.5.

Table 3.5. Concentration of Gallic Acid and Water used to Create the Standard Curve for Total Phenolic Content

	Concentration mg/100mL					
	0	**10**	**20**	**30**	**40**	**50**
Gallic acid stock (µL)	0	200	400	600	800	1000
d.d. H_2O (µL)	1000	800	600	400	200	0

50µL of the potato sample or standard (Gallic acid for curve) or distilled deionised H_2O (blank) was added to 250µL of Folin-Ciocalteau reagent and incubated for 4 minutes. Na_2CO_3 (500µL) was then added to stop the reaction. Samples were adjusted to a final volume of 5mL by adding distilled deionised H_2O. Samples were incubated in the dark for 2 hours at room temperature. Samples were measured at absorbance of 765nm. A graph was plotted of Abs. (Y) vs. Gallic acid concentration (X). The sample absorbance readings were read from the graph (X) using the trend line equation. Adjustments were made for dilutions and the weight of potato used in the assay. The data was expressed as mg gallic acid equivalents (GAE)/per 100g (Singleton et al., 1965).

Ascorbic Acid- Plate Reader Method

The stock solutions were first prepared. Sodium acetate buffer (2M) was prepared (108.8g of sodium diacetate trihydrate in 400mL of deionised H_2O), with a pH of 5.5 (adjusted using acetic acid). Methanol-Water was prepared by combining 75mL methanol and 25mL water

and 0.01g diethylenetriaminepentaacetic acid was added. The reagents needed for the experiment were made. 2.32 mM 4-Hydroy-TEMPO in sodium acetate buffer (0.01g was weighted and 25mL sodium acetate buffer was added). 5.5mM o-Phenylenediamine dihydrochloride in sodium acetate buffer (0.01g was weighted and 10mL sodium acetate buffer was added). The Ascorbic Acid standard solution (100mM) was prepared by weighing 0.7g and dissolving it in 40mL ddi H_20. It was then aliquoted into 1ml and frozen.

Table 3.6. Concentration of Ascorbic Acid Standard and Water used to Create the Standard Curve for Ascorbic Acid Assay.

	Concentration µM							
	0	**5**	**10**	**25**	**50**	**100**	**150**	**200**
Ascorbic Acid Standard (µL)	0	5	10	25	50	100	150	200
ddi H_20 (µL)	1000	995	990	975	950	900	850	800

Standard or potato samples (100µL) were added to a black Nunc 96 well plate. 100µL 4-Hydroy-TEMPO (2.23mM) was added to each of the samples and standards and the plate was incubated in the dark at room temperature for 10 minutes. 42µL of freshly prepared o-Phenylenediamine dihydrochloride (5.5mM) was added to the wells. The fluorescence was measured at 345nm (excitation), 425nm (emission), and samples were read a total of 12 times (every 30 seconds for 5 minutes). The slope (fluorescence V's time) was determined for each vitamin C standard and sample. A standard curve of vitamin C [concentration] V's vitamin C [slope] was then prepared. The slope of the samples was used to calculate the vitamin C content in the sample from the standard curve. Data were expressed as mg/100g (Vislisel et al.,2007).

3.2.8 Data Analysis

A fresh batch of each potato-based meal was made up on three separate occasions for testing. A fresh batch of potato-fibre combination was also made up on three separate occasions for testing. Each sample was tested in duplicate.

Statistical analysis was carried out using a One-way ANOVA followed by Dunnett's multiple comparisons test using GraphPad Prism version 6.00 for Windows, GraphPad Software, La Jolla California USA, www.graphpad.com.

3.3 Results and Discussion

The macronutrients for each meal was calculated using proximate values from the McCance and Widdowson's composition of foods integrated 2019 (McCance and Widdowson, 2019) as well as using the manufacturer's information. The combination of Rooster potatoes with either cheese, beans, peas, or tuna resulted in meals with variable contents of fat, protein, or fibre (Table 3.1.). However, all meals had the same available carbohydrate content of 50g. The addition of cheese reduced the RAG to a level 47% lower than that of the control (Table 3.2). The other three foods (bean, pea, and tuna) all had a similar RAG value which was approximately 60% that of the control. The meal with lowest GI was the cheese/potato meal, followed by the bean/, pea/ and finally the tuna/ potato meal which had a GI similar to that of the control (Table 3.7.). The addition of cheese to potato and the addition of beans to potato both resulted in a significant ($P<0.05$) reduction in the GI of the Rooster potato compared to the potato alone and moved these meals into the category of medium GI (55-69) foods. A significant ($P<0.05$) difference was also seen between the GI of the pea potato meal compared to the control, however the meal still remained high GI item. It is interesting to compare this data with the data collected from Henry et al., 2006. The study was carried out *in-vivo* using forty normal healthy subjects, and the results were as follows; potato alone had a GI of 93, potato with cheddar cheese had a GI of 39, potato and baked beans had a GI of 62 and potato and tuna had a GI of 76. this suggests that using an *in-vitro* method may not be as optimal as an-vivo method when comparing GIs.

Previous *in-vivo* studies have demonstrated that consuming high fat foods with potato, pasta and bread can reduce the GI (Henry et al., 2008; Hätönen et al., 2011). The consumption of dairy products such as cheese, milk, yogurt, or fermented milk products as part of a meal has also been shown to reduce the glycaemic response in healthy subjects (Sugiyama et al., 2003;

Pelletier et al., 1998; Östman et al., 2003). The addition of cheese to white toasted bread reduced the GI from 50 to 35; cheese also reduced the GI of potatoes from 93 to 39 and the GI of pasta from 61 to 27 in 40 healthy subjects (Henry et al., 2006). The evidence from the literature suggests that the change in glycaemic response with the addition of fat may be facilitated by processes such as delayed gastric emptying *in-vivo* and may also be influenced by the timing of fat consumption (Lilly et al., 2019). In the present study the cheese/potato meal, which had the highest fat content (42.2g), resulted in the lowest GI (Table 3.2.). However, as this study was conducted *in-vitro*, gastric emptying cannot have been a factor in the lowering of the GI of the potato-based meal. The cheese used in the present study was also rich in protein (35.6g) which may also influence digestion. Collier et al. (1983) demonstrated that the consumption of protein and fat together lengthened the digestion and absorption period and reduced gastric emptying *in-vivo* (Collier et al., 1983). In addition, the consumption of fat and protein with mashed potato significantly decreased the GI value, as well as the glycaemic response in subjects, which is consistent with the results of the present study (Hätönen et al.,2011).

Co-ingesting bread with either soy milk or dairy milk reduced the GI of white bread, from 100 to 77.2 or 74.6, respectively and the reduction in GI was greater when the soy milk or dairy milk was consumed 30 minutes before the bread (Sun et al., 2017). Milk and milk proteins have been shown to elicit an insulinotropic response *in-vivo* which controls the rate at which the glucose present in the meal enters the circulatory system and prevents spikes in blood glucose levels (Manullang et al., 2020). The consumption of dairy also triggers the release of gut hormones such as glucagon-like peptide-1 (GLP-1), peptide tyrosine tyrosine (PYY) and cholecystokinin (CCK) which influence blood glucose and delay gastric emptying (Panahi et al., 2014). In a study comparing whole milk with a simulated milk (protein, fat, lactose), Panahi et al. (2014) concluded that both insulin and non-insulin factors, such as interactions between macronutrients, were responsible for postprandial glycaemic control *in-vivo*, as whole milk caused lower postprandial glycaemia in comparison to the predicted value based on the sum of its components (fat, lactose, protein in a simulated milk). It could be hypothesized that in the present study interactions between the macronutrients in the cheese and potatoes were

responsible for the significant decrease in the GI, GL and RAG rather than its high protein content or high fat content.

The tuna/potato meal contained a similar quantity of protein to the cheese/potato meal; however, no significant difference was observed between the GI of the tuna/potato compared with potato alone. Henry 2006, observed that cheese had a greater impact on GI than tuna when added to potatoes, reporting a reduction of the GI from 93 for potato alone to 39 with cheese/potato compared to 76 with tuna/potato (Henry et al., 2006). Hätönen et al. (2011), also found that co-ingestion of a high protein food (chicken) with potato also decreased the GI considerably when compared to mash potato consumed independently, 108 compared with 64. Hätönen et al. (2011), found that the chicken potato meal had the highest incremental peak of insulin compared to all the other meals tested (Hätönen et al., 2011) which could account for the reduced GI. Interestingly, the tuna meal had the highest GI of all the test meals, but had the lowest GL. The GL considers the overall carbohydrate content of the food while the GI measures the rate of glucose release without regard to the overall carbohydrate content (Lenner et al., 2004). Similar to GI, foods can be classed as having a high (≥20), medium (11-19) or low (≤10) GL (Manullang et al., 2020). The addition of all four toppings caused the potato-based meal to fall into the low-GL range. Low-GL meals have been shown to be more satiating than high-GL meals and could potentially be used as a tool to decrease food consumption (Chang et al., 2012). Mehrabani et al. (2012) examined the benefits of using high-protein, low-GL, hypocaloric diets in overweight and obese women with polycystic ovarian syndrome and found the subjects lost a significant amount of body weight and had significantly increased insulin sensitivity (Mehrabani et al., 2012).

The GI of potato was reduced by the addition of baked beans (Table 2.2.) similar to the finding reported for an *in-vivo* study (Henry et al., 2006). A study conducted in Japan found that serving white rice with roast-ground soya bean, fermented soya bean, and miso soup (made with soya bean paste), reduced the GI of the rice from 100 to 68, 68 and 74, respectively *in-vivo* (Sugiyama et al., 2003). The addition of peas was also seen to reduce the GI of the potato (Table 3.7.). Schäfer et al. (2003) also showed a reduced GI when high GI foods were served

with peas as part of a meal. The pea-based meal was high in fibre, with 7.8g of non-starch polysaccharide, closely followed by the baked beans meal which had 6g of non-starch polysaccharide. It was observed that the glycaemia after consuming 36g of carbohydrates from dried peas was two thirds lower than if the same amount of carbohydrates was consumed from potatoes (Schäfer et al., 2003). Schäfer used cooked dry beans in the study, these may have had a more significant effect on glycaemia than the bean and peas used in the present study, as cooked dry beans have a higher energy content per gram and provide more fibre than canned versions (Mudryj et al., 2014). The presence of fibre such as phytate in a food may be a factor in GI reduction. Phytate is an antinutrient which impedes α-amylase and in turn inhibits starch hydrolysis (Kumar et al., 2019). Dietary fibres such as guar and tragacanth, present in leguminous foods, are known to help flatten the blood glucose curve (Jenkins et al., 1981). It may be the combination of soluble fibre and antinutrients found in bean, lentils and pulses that contribute to their low GI.

From the present study it was observed that the amount of carbohydrate in the meal was not a factor in the GI and GL of the meal as all meals in this study contained 50g of available carbohydrate, yet the carbohydrate parameters varied significantly based on the proximate composition of the meal (Table 3.1).

Table 3.7. Rapidly Available Glucose, Total Glucose, Glycaemic Index and Glycaemic Load of Potatoes Based Meals Containing Cheese, Beans, Tuna or Peas

	Rapidly Available Glucose* (g/100g)	Total Glucose (g/100g)	Glycaemic Index	Glycaemic Load
Rooster	14.15±3.45	18.14±4.12	83.15±10.63	14.01±3.17
Rooster + Cheese	6.69±1.62	10.98±1.44	66.77±5.44	8.38±1.50
Rooster + Beans	8.36±0.88	12.82±1.88	67.96±8.22	8.38±0.34
Rooster + Tuna	8.65±1.25	10.03±2.12	83.59±10.52	7.62±1.17
Rooster + Peas	8.65±0.91	12.13±0.98	76.57±11.22	8.79±0.57

Mean± Standard Deviation. N=3. *Rapidly Available Glucose- refers to glucose released from the potato sample after 20mins of digestion.

Impact of Fibre on the GI of Potato

Three different varieties of fibre (HPMC, pectin and inulin) were investigated for their potential to reduce the GI of cooked Rooster potatoes. Both HPMC and inulin caused a dose dependent decrease in the RAG, GI and GL of the Rooster potato (Table 3.8 and 3.10). A 73% reduction in the RAG was observed after 10% (w/w) HPMC was added, whilst 10% pectin resulted in the RAG being nearly 86% lower than the control sample. The 7% concentration caused a significant (P<0.05) decrease in RAG of 81% for pectin and 71% for HPMC. The biggest decrease in GI was seen at the 7% concentration of HPMC which caused a 40% reduction in the GI of Rooster potatoes (Table 3.8.), resulting in a product which would be classed as “low GI”. Both 5% HPMC and 10% HPMC reduced the potato’s GI to that of a “medium-GI” food. Each dose of pectin caused a significant (P<0.05) decrease in GI, reducing the samples to “low-GI” food category. Similar to the results of the RAG, 10% pectin was shown to cause the greatest decrease of 60% when compared with the control. The addition of inulin did not alter the RAG (Table 3.10.). As RAG is the main determent of the GI of a food, it is unsurprising that in the potato with varying concentrations of added inulin the GI was unchanged.

Several studies have demonstrated that the addition of fibre to a meal can reduce the glycaemic response *in-vivo*. The consumption of mashed potato with different doses (1%, 2% and 4%) high viscosity (HV)-HPMC caused a statistically significant reduction in the glycaemic response at all concentrations compared to the control samples (Lightowler et al., 2009). It was noted that addition of the lowest concentration (1%) HV-HPMC was sufficient to elicit a positive response when compared to the control. The authors hypothesized that the addition of HPMC may have influenced the starch digestibility via interactions with the amylose fraction (Lightowler et al., 2009). HPMC and amylose have been shown to form hydrogen bonds in the presence of heat and water which can result in the retrogration of the starch granule (Wang et al., 2015). A dough model study noted that the dough containing both HMPC and potato starch had the fasted starch retrogradation rate (Zhang et al., 2017). The digestibility of starch enzymatically is mainly determined by the degree of disruption to the starch granule from gelatinization and retrogradation (Wang et al., 2015). In subjects that consumed a beverage containing either 4g or 8g HPMC along with a carbohydrate dense meal, peak glucose levels were reduced in comparison to the control (Maki et al., 2007). Similarly, the consumption of a hot chocolate beverage containing 4g of HPMC together with a high carbohydrate breakfast reduced the AUC (Area under Curve) for serum glucose levels by 30% in comparison with the breakfast alone (Dow et al., 2012).

The effect of the addition of HPMC on the GI of Rooster potato was compared with the effect in sweet potato (Table 3.11.). Each of the potato varieties were combined with 7% HPMC. The GI of the sweet potato was lower than that of the Rooster potato and sweet potato would be classified as a "medium-GI" food. A modest decrease (8%) was seen in the GI of sweet potato following the addition of fibre in contrast to the decrease of 40% which was observed in the GI of the Rooster potato when combined with HPMC. The effect of various fibres on GI and GR has been found to vary depending on the type of fibre added and the food to which it is added. Chillo et al. (2011) found that semolina spaghetti enriched with barley β-glucan (BB) had a lower GR and GI in comparison to the control. However, another type of β-glucan, glucagel (GG), had no effect. The changes in GR and GI caused by BB were attributed to its molecular structure and ability to hold water. Unripe banana flour had the greatest effect on the glycaemic response with a 43% reduction in the incremental area under the blood glucose

curve when added to a “shake” (Galvão Cândido et al., 2014). In contrast, oat bran, flaxseed and flour phaseolamin (white bean extract) were found to have no effect on the glycaemic response.

The method of addition of the fibre to a meal may also influence the GI. Jenkins et al. (2014) found that ingesting a viscous fibre blend (VFB) within a margarine spread on bread reduced the postprandial glucose response more than if the VFB was consumed in a capsule or baked into a bread (Jenkins et al., 2014). The consumption of resistant maltodextrin as a shake along with starchy foods resulted in an almost 20% reduction in the postprandial blood glucose responses, compared to a 10% reduction when the fibre was incorporated into the food (Livesey et al., 2009). The lowering of the glycaemic response caused by added fibre has been linked to altered viscosity, bulk, solubility in water, adsorption, binding of molecules and fermentability of the food, which slows the digestion of lipids, carbohydrates and some nutrients (Schneeman, 1999).

Table 3.8. Rapidly Available Glucose, Total Glucose, Glycaemic Index and Glycaemic Load of Rooster Potatoes Containing 5%, 7% or 10% of HPMC.

	Rapidly Available Glucose* (g/100g)	**Total Glucose (g/100g)**	**Glycaemic Index**	**Glycaemic Load**
Control	14.15±3.45	18.14±4.12	83.15±10.63	14.01±3.17
HPMC 5%	6.07±0.90	11.75±0.50	61.07±5.08	6.66±0.79
HPMC 7%	4.08±0.68	12.24±1.16	52.90±4.96	4.77±0.63
HPMC 10%	3.73±0.64	7.96±1.16	56.28±4.96	4.24±0.63

Mean ± Standard Deviation. N=3. *Rapidly Available Glucose - refers to glucose released from the potato sample after 20mins of digestion.

Table 3.9. Rapidly Available Glucose, Total Glucose, Glycaemic Index and Glycaemic Load of Rooster Potatoes Containing 5%, 7% or 10% Pectin.

	Rapidly Available Glucose* (g/100g)	Total Glucose (g/100g)	Glycaemic Index	Glycaemic Load
Control	14.15±3.45	18.14±4.12	83.15±10.63	14.01±3.17
Pectin 5%	4.12±0.65	13.72±2.93	43.35±4.79	5.46±0.47
Pectin 7%	2.65±0.38	13.13±2.30	34.72±1.32	4.22±0.37
Pectin 10%	2.00±0.75	10.45±0.10	33.42±6.45	3.33±0.53

Mean ± Standard Deviation. N=3. *Rapidly Available Glucose - refers to glucose released from the potato sample after 20mins of digestion.

Table 3.10. Rapidly Available Glucose, Total Glucose, Glycaemic Index and Glycaemic Load of Rooster Potatoes Containing 5%, 7% or 10% Inulin.

	Rapidly Available Glucose* (g/100g)	Total Glucose (g/100g)	Glycaemic Index	Glycaemic Load
Control	14.15±3.45	18.14±4.12	83.15±10.63	14.01±3.17
Inulin 5%	15.78±1.74	17.23±4.82	92.47±8.42	16.79±3.80
Inulin 7%	14.11±0.90	15.52±1.32	87.39±2.68	13.79±0.95
Inulin 10%	20.68±1.78	21.23±2.88	93.72±6.19	19.65±1.76

Mean± Standard Deviation. N=3. *Rapidly Available Glucose - refers to glucose released from the potato sample after 20mins of digestion

Impact of Fibre on Viscosity and GI of Potato

Increases to the viscosity of the digesta caused by added fibre such as β-glucan can result in delayed gastric emptying and absorption of nutrients and hence reduce the glycaemic response (Chillo et al., 2011). The viscosity of the Rooster potatoes with added fibre was measured to determine if changes in viscosity could be correlated with the changes in GI. The control Rooster potato had a viscosity of 10.51η in mPas when it was held at a shear rate of $500s^{-1}$. The addition of the HPMC fibre resulted in an increase in viscosity to 11.74, 15.33, 13.16 η in mPas at 5%, 7% and 10% (w/w), respectively (Figure 2.1). A similar increase was seen upon the addition of pectin to the Rooster potatoes; at 5% the viscosity was 14.31η in mPas at 7%, it was 15.51η in mPas, and 16.38 η in mPas at 10% pectin (Figure 2.2). It is unsurprising that the HPMC caused an increase in viscosity, as HPMC is regularly used in the food industry as a gelling and thickening agent (Dow et al., 2012). Pectin had the greatest impact on viscosity of the three fibres investigated with a 55% increase compared to the control at the 10% (w/w) concentration. A negative correlation between viscosity and GI was observed. HV-HPMC is known to produce a viscous gel in the gastrointestinal tract (Lightowler et al., 2009) and it could be hypothesised that other viscous fibres such as pectin may act in a similar way.

In contrast, inulin caused no notable difference in the viscosity at 5% and 10% (w/w), however, an increase in the viscosity was seen at 7% (Figure 2.3). Inulin is typically non-viscous in water (Schneeman, 1999) and it is unsurprising that there was no significant difference in the viscosity of potato with inulin at 5% and 10% (w/w). There was also no observable difference in the GI of potato with added inulin. Inulin is technically not a type of fibre but a linear fructan (Roberfroid, 2005) and whilst it has similar properties to fibre such as creating bulk in the diet, and being non-digestible in the small intestine, it is also soluble in water, and highly fermentable (Schneeman, 1999).

The *in-vitro* method used does not replicate gut motility such as delayed gastric emptying. However, the addition of fibre can affect the enzymatic digestion of the food which can be seen using an *in-vitro* model. Lightowler et al. 2009, hypothesises that the addition of HV-HPMC may have contributed to lowering the GR by forming a mucilaginous layer around the

starch molecules making them impervious to enzymatic digestion and hence resulting in a lower GR (Lightowler et al., 2009). A study conducted by Brennan et al 1996, concluded that an increase in viscosity as well as the formation of a physical barrier to starch digestion reduces the hydrolystaion of starch *in-vitro* and lowers post-prandial glycemia *in-vivo*. (Brennan et al., 1996)

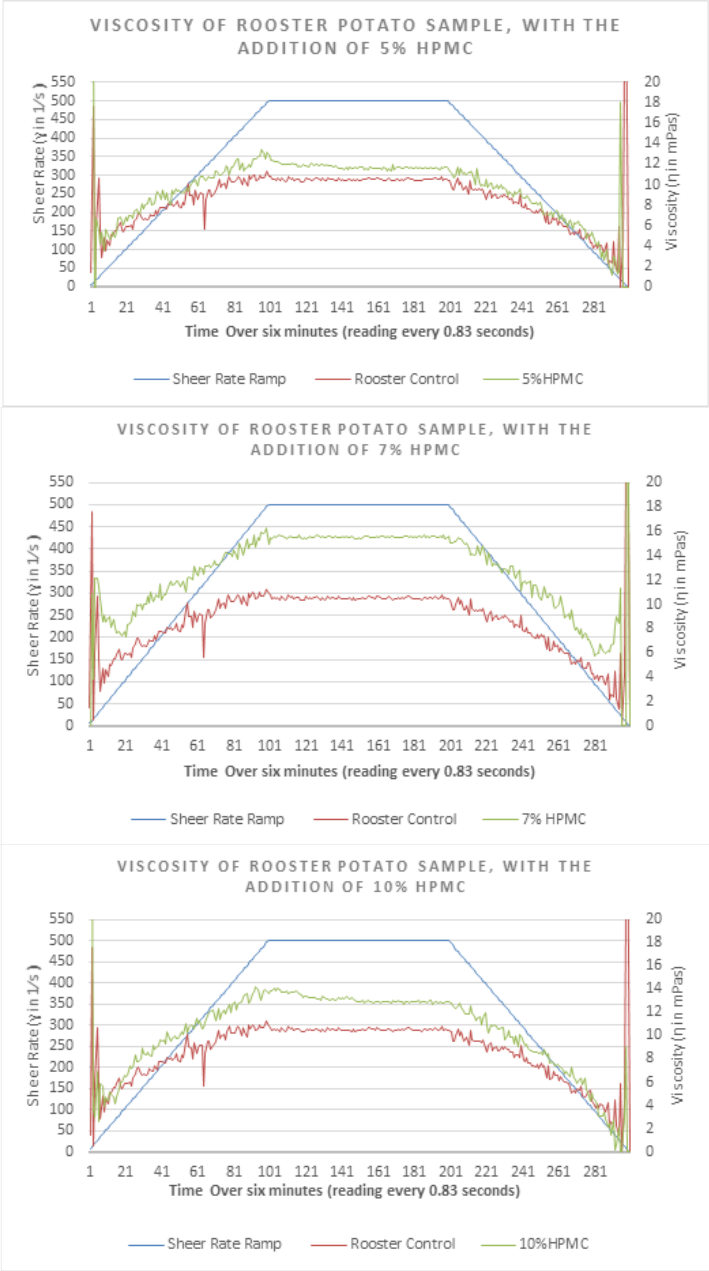

Figure 2.1. Viscosity of Rooster Control Digestate and Rooster Digestate Containing 5%, 7% or 10% HPMC – Rooster Digestate, at 25 °C for 2mins with a Shear Rate of 500 ẏ in 1⁄s

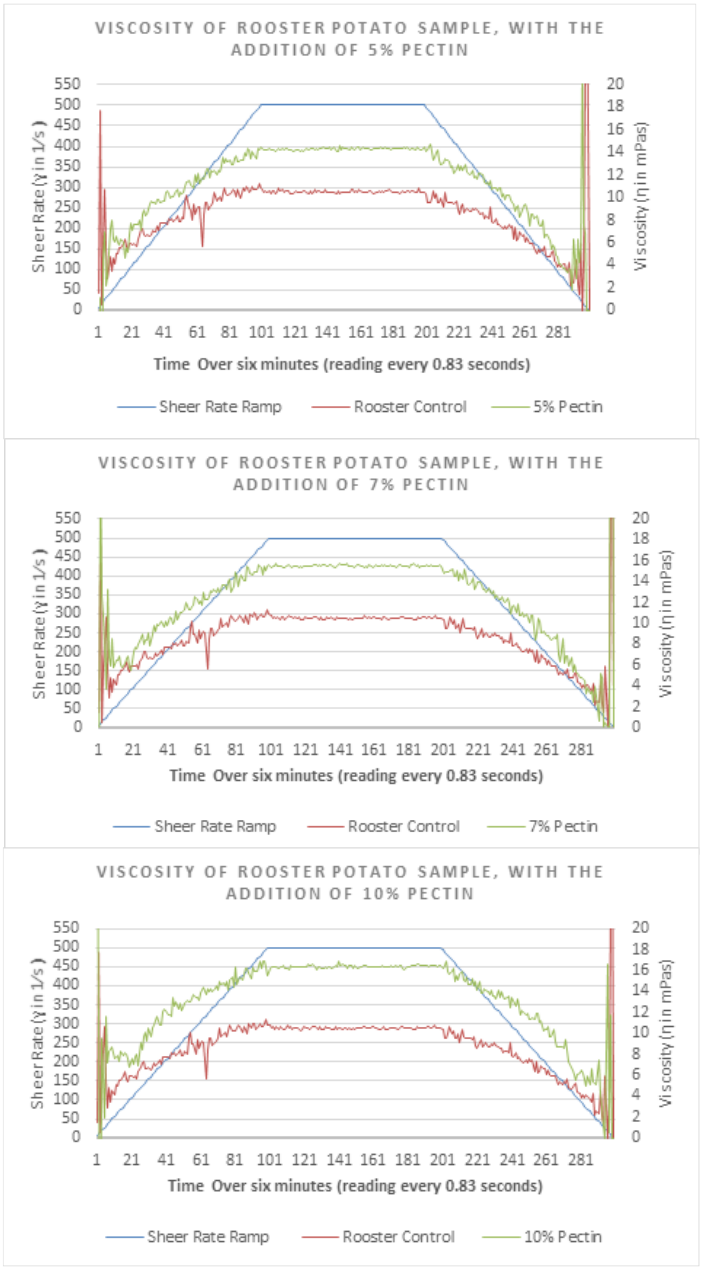

Figure 2.2. Viscosity of Rooster Control Digestate and Rooster Digestate Containing 5%, 7% or 10% Pectin– Rooster Digestate, at 25 °C for 2mins with a Shear Rate of 500 $\dot{\gamma}$ in 1⁄s

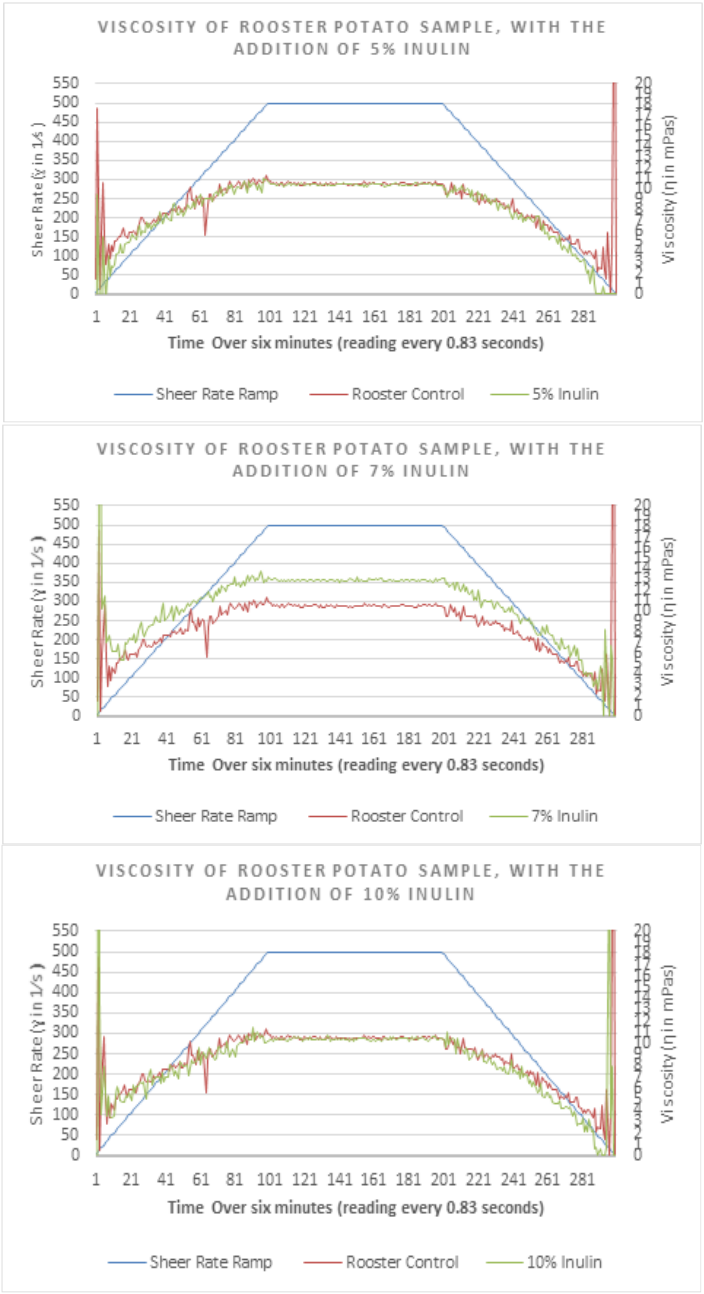

Figure 2.3. Viscosity of Rooster Control Digestate and Rooster Digestate Containing 5%, 7% or 10% Inulin– Rooster Digestate, at 25 °C for 2mins with a Shear Rate of 500 ẏ in 1⁄s

Table 3.11. Rapidly Available Glucose, Total Glucose, Glycaemic Index and Glycaemic Load of Rooster Potatoes and Sweet Potatoes Control Samples and Samples Containing 7% HPMC.

	Rapidly Available Glucose*(g/100g)	Total Glucose (g/100g)	Glycaemic Index	Glycaemic Load
Rooster	14.15±3.45	18.14±4.12	83.15±10.63	14.01±3.17
HPMC 7%	4.08±0.68	12.24±1.16	52.90±4.96	4.77±0.63
Sweet Potato	8.71±2.61	9.58±0.51	67.07±5.92	7.47±1.01
HPMC 7%	5.43±1.26	7.49±0.22	61.69±8.69	5.93±1.04

Mean ± Standard Deviation. N=3. *Rapidly Available Glucose - refers to glucose released from the potato sample after 20mins of digestion.

The effect of Fibre on the Presence of Potato Antioxidants

Potato contributes the third highest amount of TPC to the diet, after apples and oranges, due to the widespread and frequent consumption of potatoes (Tian et al., 2016). The metabolism of antioxidants depends on factors such as microbiota and digestive enzymes but may also be affected by the composition of the food matrix (Palafox-Carlos et al., 2011). Therefore, the addition of fibre may have an impact on the antioxidant compounds present in potatoes. To test this hypothesis the antioxidant content of the digestates of Rooster and sweet potato with added HPMC (7%; w/w) was assessed. The FRAP value of the sweet potato (72.77 mM Fe^{++}/100g) was more than twice that of the Rooster potato (28.67 mM Fe^{++}/100g). It was found that the FRAP values for Rooster potatoes was slightly lower than that reported in the literature, however the sweet potato FRAP fell within the reported range. Gumul et al., 2011, found that the FRAP content of five polish potato cultivars varied from 6.2 to 14.35 mM Fe^{++}/Kg DM (Gumul et al., 2011). The addition of HPMC reduced the FRAP value by 72% in the Rooster potatoes and 60% in the sweet potato (Figure 2.4). In contrast, the TPC and ORAC values were similar in the Rooster and sweet potato and the addition of HPMC had no significant effect on TPC and ORAC values of the digested potatoes (Figure 2.5 and 2.6). The digested Rooster control sample had a vitamin C content of 1.96mg/100g which was significantly reduced to 0.26mg/100g with added HPMC (Figure 7). Similarly, the vitamin C content of the sweet potato control sample was reduced from 2.27mg/100g to 0.11mg/100g

following the addition of the 7% dose of HPMC (Figure 2.7). Vitamin C has been described as an indicator of antioxidant activity in fruit and vegetables, however it only contributes a small percentage of antioxidant levels in certain fruits and vegetables.

TPC levels vary greatly in the literature from 1.05-11.91 g GAE/100 g (Fidrianny at al., 2017) to 4.15 -16.8 mg GAE/g (FW) (Tang et al., 2015), and a third study reporting levels as great as 70.67mg GAE/100 g (Ru et al., 2019). Chun et al., 2005 reported levels 35.28mg GAE/100 g being present in potato tubers this value is similar to the results of this experiment which found that 28.24 mg GAE/100 g was present in Roosters and 36.83mg GAE/100g was present in sweet potatoes (Chun et al., 2005). Apart from variance between cultivars, the levels of phenols present in the potato can differ, as chlorogenic acid is the most abundant phenol in potatoes its levels dictate the TPC of the tuber (Brown, 2005).

Vitamin C levels of 69.0 mg/100 g dried weight, DW (Thomas et al., 2021) were found in within the literature, which is considerably higher than the values reported in this experiment. A variety of factors are such as the variety of potato. The vitamin C levels in the tested cultivars were considerably lower than those reported in the literature. Vitamin C levels in a cultivar are strongly influenced by multiple factors such as genotype, climate and cooking method (Thomas et al., 2021).Cooking the potatoes peeled may also have contributed to a greater loss of the vitamin during cooking as it may have leached into the cooking water.

ORAC values published within the literature for potatoes also varies from 27umol TE/ 100g (Fidrianny at al., 2017), 7.6-14.27 umol TE/ 100g (Brown, 2005, Xu et al., 2009). A study investigating potatoes as a source of antioxidants and micronutrients found that the ORAC values was 28.25-250.67 µmol of TE g^{-1} of DW (Andre et al., 2007a). Variance in skin and flesh colour are the attributers to these differences (Andre et al., 2007a).

Previous studies have shown that the addition of fibre affects some antioxidant parameters whilst others remain unchanged. TPC was unaffected by the addition of fibre derived from apples, lemons, wheat or wheat bran to a food product however an increase in total

antioxidant capacity (TAC) was observed (Bilgicli et al., 2007). A slight, not statistically significant, increase in TPC was seen when brewers' spent grain, a rich source of fibre, was incorporated into an extruded snack, no difference was seen in TAC (Ainsworth et al., 2007). There is evidence indicating that some fibres may directly interact with antioxidants and interfere with their absorption. The addition of 7% HPMC adversely affected both the FRAP and vitamin C content of both the digested Rooster and sweet potato cultivars (Figure 2.4 and 2.7). Both the concentration of the fibre in the matrix as well as the type of link between the antioxidant and the fibre can influence the release of antioxidants from the matrix (Adam et al., 2002). Similarly, Palafox-Carlos et al. (2011) stated that the chemical and physical interactions formed between the antioxidants and limit bioavailability.

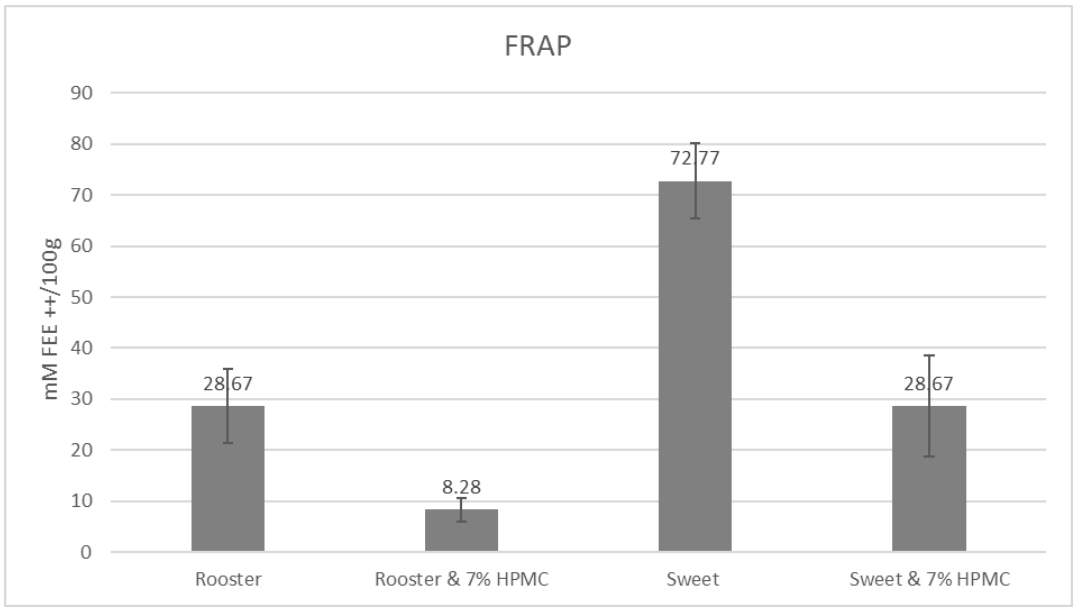

Figure 2.4. Ferric Reducing Antioxidant Power of Control Rooster and Sweet Potato Control Samples and Samples Containing 7% HPMC (mM Fe++/100g). N=3.

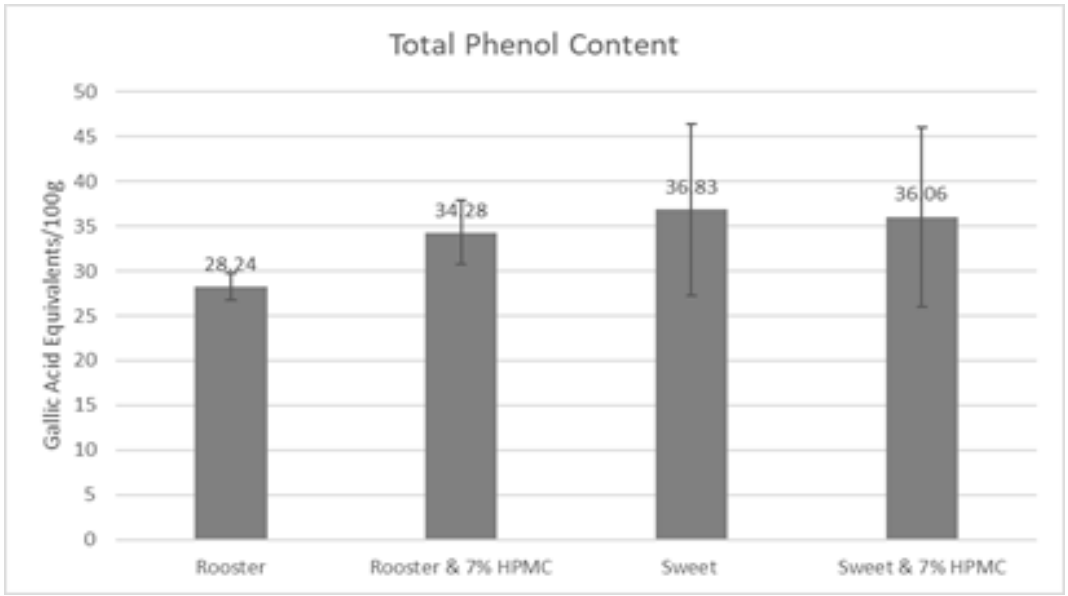

Figure 2.5. The Total Phenol Content of Control Rooster and Sweet Potato Control Samples and Samples Containing 7% HPMC (gallic acid equivalents/ per 100g). N=3.

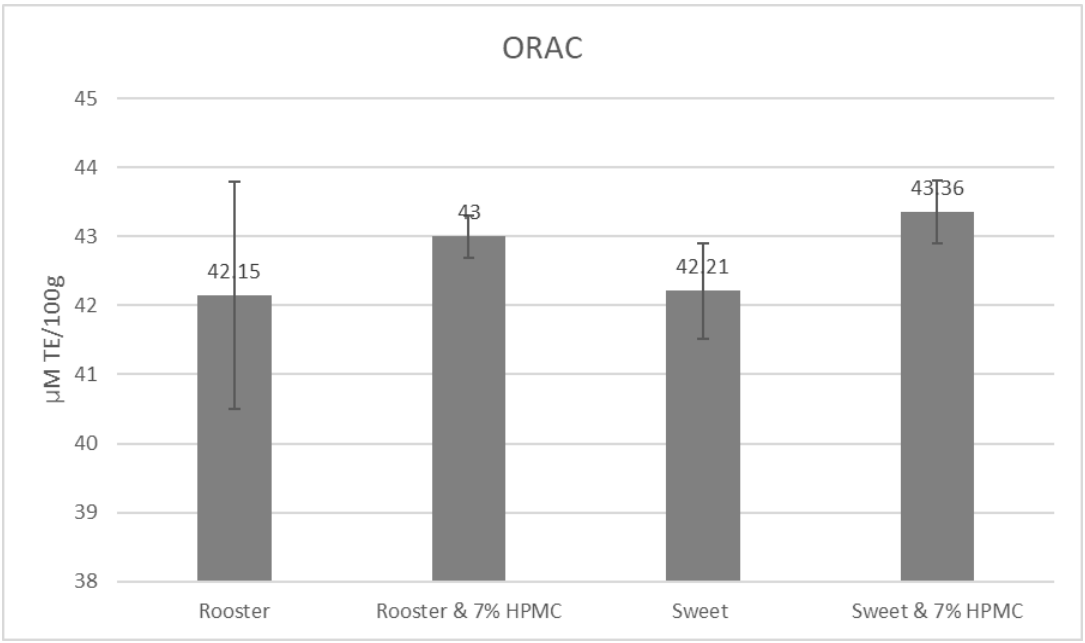

Figure 2.6. The Oxygen Radical Absorbance Capacity of Control Rooster and Sweet Potato Control Samples and Samples Containing 7% HPMC (uM TE/100g). N=3.

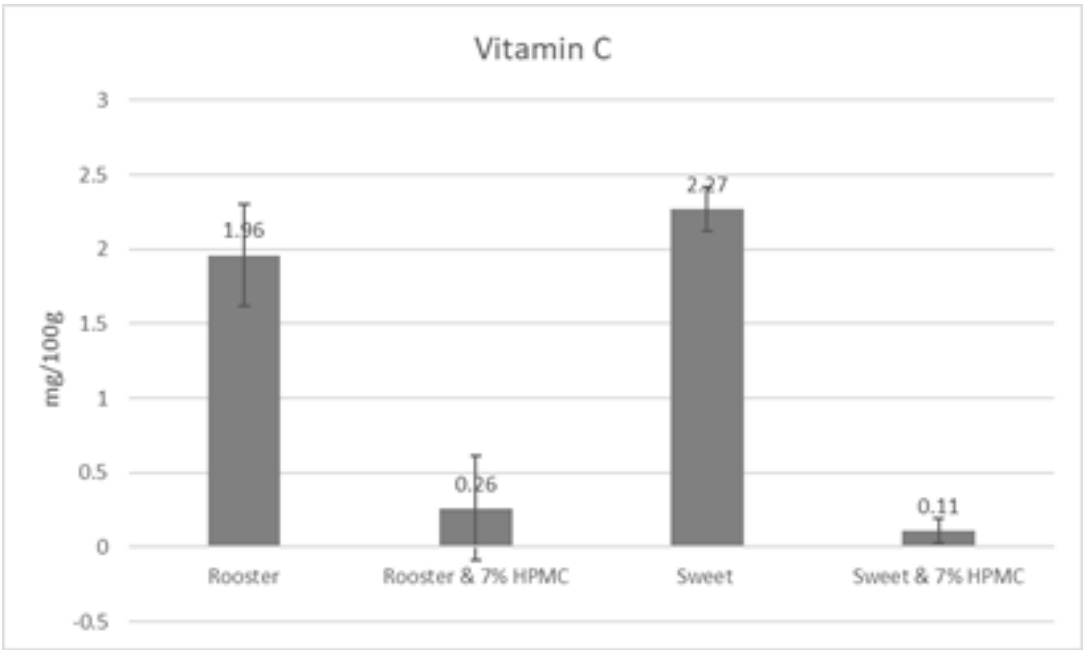

Figure 2.7: Vitamin C Concentrations of Control Rooster and Sweet Potato Control Samples and Samples Containing 7% HPMC (mg/100g). N=3.

3.4 Conclusion

The addition of cheese with its high fat, high protein content resulted in the greatest decrease to the GI of potato in comparison to beans, pea, and tuna. The data obtained were broadly similar to the results obtained using as *in-vivo* method (Henry *et al.*, 2006) however, the *in-vitro* model used in this study should be validated in human subjects to confirm the precision of this method.

The addition of both the HPMC and pectin caused a slight decrease in the GI of sweet potato and a more pronounced decrease in the GI of Rooster potato. The decrease in the GI of the Rooster potato on the addition of HPMC and pectin correlated with an increase in the viscosity of the potato. The addition of fibre to the Rooster potato and sweet potato also decreased certain antioxidant activities following digestion. The inclusion of fortified foods with high viscosity fibres could potentially be used as a tool to reduce the GI of high GI foods as well as a means of increasing total dietary fibre within the diet. However, more research would have to be carried out in this area to avoid issues that are associated with excessive dietary fibre such as palatability, texture, gastrointestinal issues and decreased nutrient bioavailability.

Chapter 4
General Discussion

Potatoes are an important source of carbohydrates, potassium, vitamin C and E whilst being low in fat, sodium and having variable quantities of phenolic compounds, carotenoids, and glycoalkaloids depending on the variety (McCance and Widdowson, 2019; Lovat et al. 2016). The nutrient content and antioxidant profile of potato is believed to be beneficial in reducing the onset of certain chronic conditions including cancers (Lee et al. 2004; Friedman et al. 2005; Ji et al. 2008). Potato consumption has also been associated with a positive impact on hyperlipidaemia (Liyanage et al. 2009; Kanazawa et al. 2008) and cardiovascular health (Camire et al. 2009). However, potatoes are generally considered a high GI food. High GI diets have been linked with the onset of cognitive decline (Sunram-Lea 2019; Sünram-Lea and Owen 2017) as well as age-related macular degeneration (AMD) (Chiu et al. 2007a) and type-2 diabetes (T2DM) (Pastorino et al. 2016; Bidel et al., 2018). The consumption of French fries is often identified as possible risk factor in the development of chronic conditions including obesity (Receveur et al.,2008; Paradis et al., 2009). French fries are the most consumed potato product in developed countries such as the USA (USDA, ERS, 2019). Due to the negative association between health and potatoes a decline in their consumption has been seen in the developed world (Fernqvist et al. 2015). Many of the negative associations between potatoes and health centre around their high carbohydrate content and GI. A method to reduce the GI of potato would be beneficial to those who have abnormal glucoregulation and improve the image of potato and potato products to consumers.

In Chapter II of this thesis an *in-vitro* method was developed to investigate the GI, GL and carbohydrate parameters of five potatoes cultivars commonly consumed in Ireland (Cultra, Kerr's Pink, Rooster, Maris Piper and Gemson). It was shown that the GI of these varieties varied from medium: Maris Piper (66.71) and Gemson (69.21) to high Rooster (81.60), Cultra (79.10) and Kerr's Pink (97.79), with none of the varieties having a low GI. The published values for the GI of potatoes vary substantially, but in general they are classed as a high GI food (Henry et al. 2005; Ek et al., 2014; Foster-Powell et al., 2002). The data from this experiment did agree with what is reported in the literature. Differences in the GI can be caused by variance within genotypes, growing conditions, maturity of the tuber, storage and processing conditions.

High-pressure processing (HPP) was investigated as a mechanism for lowering the GI of the cultivars, this was completed on the same five cultivars as well as a Maris Piper baby variety. HPP is often used in the food industry as a means of food preservation. More recently HPP has been explored as a mechanism for potentially reducing the GI of foods. The limited research completed on the impact of HPP on GI has yielded encouraging results (Elizondo-Montemayor, 2015; Huang et al., 2019b). The potatoes used in this study were subjected a pressure of 400MPa or 600MPa for 3 minutes (Chapter 2). The HPP treatment did cause changes in the GI. The two potatoes with the highest GIs, Rooster and Kerr's Pink, had the most promising results. A 13.71% decrease was observed in the GI of the Kerr's Pink potato after it was subjected to 400MPa of pressure and a 36.57% reduction in GI after 600MPa. There was a small decrease of 3.5% between the Rooster control and Rooster exposed to 400MPa however, a 23.98% decrease was seen between the Rooster control and the 600MPa treated sample. This trend was not observed for the other cultivars. The initial HPP treatment of 400MPa reduced the GI of Cultra potatoes from 79.10 to 68.36, however the higher pressure of 600MPa caused a slight increase with a GI of 80.97. The GI of the Maris Piper was increased from 66.71 to 73.20 with the 400MPa treatment; the 600MPa treatment only caused a slight increase to 67.74. The GI of Gemson potatoes was also increased following the 400MPa treatment from 69.21 to 74.95. Maris Piper baby variety treated at 400MPa had the highest GI out of all potatoes tested at 117.70, however the 600MPa treated potato of the same cultivar was 65.52. The HPP treatment did cause desirable changes in some of the cultivars. This suggests that the extreme pressure of the HPP treatment did result is some changes in the structure of the starch. However, the cooking of the potato post treatment may have dulled the effect of the HPP treatment. Therefore, the results from this experiment not as significant as was expected based on the published literature in this area.

The addition of fat to a high GI food has been shown to positively influence the GI according to the literature (Henry et al., 2008, Hätönen et al., 2011). The addition of fat at different doses as well as of different degrees of saturation was investigated to determine the impact on GI in Rooster potatoes (Chapter 2). An initial dose response was conducted using the most unsaturated fat, rapeseed oil. Doses of 10%, 15%, 20% and 25% were added to a sample of Rooster potato. No significant difference between the GI of the control and the Roosters with

added rapeseed oil was noted. A concentration of 10% was selected for further investigation of the remaining fats (butter, coconut oil and olive oil). None of the fats employed for this study were observed to impact the GI of the Rooster potato. The Rooster control GI was 83.27, compared to the potato with the addition of fat: butter (86.24), coconut oil (88.17), olive oil (85.72) and rapeseed oil (87.31). Whilst this data contradicts what is commonly found within the literature (fat reduces the GI of a food item), it can be theorised that the *in-vitro* method employed may not have been the most suitable for this study as fat causes reductions in the rate of gastric emptying *in-vivo*, which could not be replicated in the *in-vitro* model.

In Chapter III the GI of meals incorporating Rooster potatoes was studied. Each meal was designed to contain 50g of available carbohydrates. The meals consisted of Rooster potatoes and an additional food item commonly consumed with potato, cheese, baked beans, peas, or tuna. Again, this investigation was carried out using the same *in-vitro* method developed in Chapter II. Research indicated that consuming potato with another food item may reduce the GI compared to eating the potato in isolation (Henry et al., 2008, Hätönen et al., 2011). As potatoes are rarely consumed alone and are usually consumed as part of a meal, measuring the GI in a meal gives a more realistic insight into the impact of potatoes' GI in the diet. Rooster potato alone had a GI of 83.15, however when combined with beans the GI was reduced to 66.77, a similar reduction was seen with cheese (67.96), and slightly less of a decrease with peas (76.57) but the GI remained virtually the same when combined with tuna (83.59). Interestingly, the potato's GL (14.01) was reduced from a medium GL to a low GL for all food combinations: beans (8.38), cheese (8.38), tuna (7.62) and peas (8.79). The decrease in GI observed for the potato combined with beans or potato combined with peas meal may be due to the higher fibre content. In particular, the fibre from bean, pulses and legumes may reduce the GI and/or GL by inhibiting carbohydrate digesting enzymes (Kumar et al., 2019). Dairy products, protein rich and high fat foods have all been linked with having a lowering effect on GI (Mehrabani et al., 2012; Sugiyama et al., 2003; Hätönen et al 2011). The mechanism involved in the decrease of GI/GL observed in this study are uncertain due to the absence of normal physiological functions owing to the *in-vitro* model employed. Therefore, it may be hypothesized that the interactions between the macronutrients, soluble fibre and antinutrients found in the potatoes and the other food item in the meal. Thus, it can be

concluded that the amount of available carbohydrate is not the only factor influencing the glycaemic effect of a meal.

Three fibres were investigated (pectin, inulin, and HPMC) to determine the impact of their addition to Rooster potato on the GI. Each fibre was tested at three different levels 5%, 7%, and 10%. Once more the carbohydrate parameters were calculated. The addition of HPMC resulted in a decline of the GI from 83.15 to 61.07 at 5%, 52.90 at 7% and 56.28 at 10%. The similarity between the GI at 7% and 10% suggested that 7% is the optimal concentration and a higher concentration would have little to no additional effect. A similar trend was seen with the GL which reduced from 14.01 in the control to 6.66 at 5%, 4.77 at 7% and 4.24 at 10% HPMC. Pectin also positively impacted the GI and GL of the potato. The addition of pectin resulted in an even greater decrease in GI to 43.35, 34.72 and 33.42 and GL 5.46, 4.22 and 3.33 at 5%, 7% and 10%, respectively. Similar to HPMC a minor difference in GI/GL between the 7% and 10% concentration of added pectin was observed. In contrast to HPMC and pectin, inulin did not decrease the GI or GL. Viscosity testing was also completed, HMPC caused an increase in the viscosity from 10.51η in mPas in the control to 11.74, 15.33, 13.16 η in mPas at 5%, 7% and 10% (w/w), respectively. Pectin which had the greatest impact on the GI also had the greatest impact on the viscosity causing a 55% increase in the viscosity at 10%. No notable difference was seen at 5% or 10% inulin, however a small increase in viscosity was observed at the 7% concentration. The addition of the HPMC and the pectin may have resulted in the formation of a viscous gel thereby impeding the digestive enzymes from breaking down the carbohydrates and slowing down the rate of digestion. Inulin may not have had an impact on the GI of the potato as it did not have the ability to increase the viscosity of the digesta (Schneeman 1999).

Additionally, the impact of a 7% dose of HPMC was examined in sweet potatoes. The effect of adding 7% HPMC caused a less pronounced reduction in the GI (67.07 to 61.69) and GL (7.47 to 5.93) in sweet potato compared to Rooster potato. Sweet potato is a medium GI food and a low GL food whilst Rooster is a high GI and a medium GL food, differences in the microstructure of these two tubers may be the reason why the HPMC did not have the same

effect in sweet potato as that observed for Rooster potato. No viscosity test was completed on the sweet potato digesta so it in unclear to what degree if any the HPMC impacted the viscosity of the sweet potato.

The impact of added HPMC (7%) on the antioxidant potential of both sweet potatoes and Rooster potatoes was also investigated. The antioxidant potential of both tubers was assessed using four tests, ferric reducing antioxidant power (FRAP), oxygen radical absorbance capacity (ORAC), ascorbic acid content and total phenol content (TPC). Sweet potato (72.77 mM Fe^{++}/100g) had a much higher FRAP content compared to the Rooster (28.67 mM Fe^{++}/100g); the addition of the 7% HPMC resulted in a 60% and 72% reduction in FRAP in the sweet potato and Rooster, respectively. Interestingly, the ORAC and TPC of both tubers was similar and the addition of HPMC had no notable impact on either. However, results from the ascorbic acid assay, showed that the HPMC caused a reduction in vitamin C contents in both tubers. The digested Rooster control sample had a vitamin C content of 1.96mg/100g which was significantly reduced to 0.26mg/100g on addition of HPMC likewise, the vitamin C content of the sweet potato control sample was reduced from 2.27mg/100g to 0.11mg/100g following the addition of the 7% dose of HPMC. Literature suggests that the addition of fibre may directly interfere with some antioxidant compounds whilst having no impact on other antioxidant compounds (Bilgiçli et al., 2007). This may explain why some changes were noticed for some of the antioxidant tests but not for others.

4.1 Further Research

The information from this thesis highlights gaps in the current literature and areas where follow up work could be conducted:

1. Further research into the effects of HPP on GI should be investigated. The exposure of test foods to the high-pressure multiple times for long intervals may yield a greater impact on GI., Exploring the use on premade potato products could also be an interesting avenue to explore.

2. Incorporating fibres such as HPMC and pectin into readymade potato products and testing the effect *in-vivo* to determine if the results obtained *in-vitro* (Chapter 3) can be replicated.
3. Evaluating the effects of different meal combinations on GI, with validation of the findings in an *in-vivo* model should be examined.

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Appendix

In-vitro Method - FG

2g of each sample was weighed and placed into heat resistant beakers. 25mL 0.1mol^{-1} sodium acetate buffer was added to the samples along with 5 glass balls and vortexed vigorously. Beakers were then placed into a boiling water bath for 30 minutes. They were then cooled to room temperature for 20 minutes. 0.2 mL of invertase (2,000 Units/mL) was then added. The samples were incubated at 37°C shaken bath for 30 minutes. Then 0.5ml of sample were placed into 10ml of 66% ethanol for glucose analysis (Englyst *et al.,* 2000).

Table 4.1. Slowly Available Glucose, Free Glucose and Total Starch Content of a Variety of Potatoes Cultivars Subjected to the HPP Treatment at 400MPa or 600MPa

Potato Cultivar	Slowly Available Glucose**(g/100g)	Free Glucose (g/100g)	Total Starch (g/100g)
Cultra Ctrl	17.18 ± 0.68	0.41 ± 0.04	17.01 ± 0.43
Cultra 400MPa	16.53 ± 0.46	0.30 ± 0.03	16.76 ± 1.20
Cultra 600MPa	19.77 ± 2.03	0.41 ± 0.02	17.80 ± 1.80
Maris Piper Ctrl	16.23 ± 1.67	0.73 ± 0.03	15.42 ± 1.53
Maris Piper 400MPa	18.48 ± 1.73	0.33 ± 0.05	15.62 ± 1.62
Maris Piper 600MPa	19.31 ± 0.78	0.40 ± 0.08	16.44 ± 1.60
Kerr's Pink Control	12.27 ± 0.83	0.22 ± 0.05	10.05 ± 0.28
Kerr's Pink 400MPa	13.06 ± 1.75	0.26 ± 0.08	10.62 ± 2.08
Kerr's Pink 600MPa	15.81 ± 1.34	0.32 ± 0.03	15.31 ± 1.08
Rooster Control	14.25 ± 0.80	0.28 ± 0.07	14.48 ± 1.60
Rooster 400MPa	14.26 ± 0.79	0.18 ± 0.05	15.41 ± 0.89
Rooster 600MPa	16.60 ± 0.94	0.34 ± 0.15	18.57 ± 1.66
Gem Control	12.50 ± 0.14	0.27 ± 0.08	11.87 ± 2.02
Gem 400MPa	10.42 ± 0.71	0.34 ± 0.07	6.52 ± 11.29
Maris Piper baby 400MPa	9.58 ± 1.09	0.29 ± 0.11	8.21 ± 1.45
Maris Piper baby 600MPa	14.60 ± 0.92	0.39 ± 0.05	15.65 ± 0.74

Mean± Standard Error. N=3 **Slowly Available Glucose - refers to glucose released from the potato sample after 120mins of digestion.

Table 4.2. Slowly Available Glucose, Free Glucose and Total Starch content of Rooster Potatoes Containing 10%, 15%, 20%, 25% Rapeseed Oil, 10% Butter, 10% Coconut Oil, or 10% Olive Oil

Potato cultivar	Slowly Available Glucose**(g/100g)	Free Glucose (g/100g)	Total Starch (g/100g)
Rooster Control	18.44±2.02	0.96±0.11	10.30±0.21
10% Rapeseed Oil	16.00±0.56	0.84±0.25	10.01±0.90
15% Rapeseed Oil	18.27±2.32	0.82±0.16	11.09±0.84
20% Rapeseed Oil	17.58±3.66	0.61±0.05	10.65±0.57
25% Rapeseed Oil	19.64±4.60	0.72±0.15	10.55±0.49
10% Butter	14.20±1.52	0.95±0.18	09.22±0.75
10% Coconut Oil	14.56±0.62	1.07±0.15	10.06±0.16
10% Olive Oil	14.20±1.05	0.84±0.09	11.13±0.53
10% Rapeseed Oil	15.10±0.72	1.01±0.03	9.85±0.48

Mean± Standard Error. N=3. **Slowly Available Glucose - refers to glucose released from the potato sample after 120mins of digestion.

Table 4.3. Slowly Available Glucose, Free Glucose and Total Starch of Potato Based Meals Containing Cheese, Beans, Tuna and Peas.

Potato Based Meal	Slowly Available Glucose**(g/100g)	Free Glucose (g/100g)	Total Starch (g/100g)
Rooster Control	15.54±3.03	0.48±0.42	15.89±4.06
Rooster - Cheese Meal	11.55±3.91	0.58±0.09	9.36±1.28
Rooster -Beans Meal	10.85±2.36	00.85±0.15	10.78±1.56
Rooster -Tuna Meal	11.48±1.47	0.16±0.11	8.88±1.99
Rooster - Peas Meal	10.79±2.40	00.59±0.13	10.38±0.82

Mean± Standard Error. N=3. **Slowly Available Glucose - refers to glucose released from the potato sample after 120mins of digestion.

Table 4.4. Slowly Available Glucose, Free Glucose and Total Starch of Rooster Potato Samples with Additional 5%, 7%, 10% HPMC, 5%, 7%, 10% Pectin and 5%, 7%, 10% Inulin, and Sweet Potato Control with 7% HPMC.

Potato cultivar	Slowly Available Glucose**(g/100g)	Free Glucose (g/100g)	Total Starch (g/100g)
Rooster Control	15.54±3.03	0.48±0.42	15.89±4.06
HPMC 5%	10.14±2.22	0.29±0.17	10.31±0.30
HPMC 7%	8.08±2.16	0.18±0.10	8.67±1.11
HPMC 10%	7.40±2.16	0.38±0.10	6.82±1.11
Pectin 5%	29.52±2.77	0.32±0.11	12.05±2.73
Pectin 7%	7.33±2.30	0.32±0.14	11.53±2.12
Pectin 10%	7.33±3.00	0.55±0.28	8.91±0.26
Inulin 5%	20.64±6.57	1.01±0.79	14.60±5.05
Inulin 7%	13.20±2.87	1.67±0.26	12.47±0.98
Inulin 10%	23.29±2.97	0.80±0.41	18.39±3.01
Sweet Potato	9.83±0.92	3.03±0.53	5.51±1.52
HPMC 7% (Sweet Potato)	7.50±1.70	2.58±0.50	4.42±0.64

Mean± Standard Error. N=3. **Slowly Available Glucose - refers to glucose released from the potato sample after 120mins of digestion.