Starch and Antioxidant Properties of Quebec-bred Potato Genotypes

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Abstract

This thesis describes an investigation of the nutritional characteristics of potatoes *(Solanum tuberosum* L.*)* that included starch quality, glycemic index (GI), glycemic load (GL), protein content, antioxidant capacity, ascorbic acid, and phenolics. In the first study, nineteen genotypes from the Quebec breeding programs at Les Buissons and McGill were examined and three top performing genotypes were identified including Kalmia, QP01009.05JP, and MS1406, which were selected based on lower GL, superior starch characteristics, greater antioxidant capacity, and phenolic content. Multivariate regression models were developed to define various explanatory variables that can predict GL. In the second study, four somatic lines derived from Russet Burbank were compared to Russet Burbank (control), while inter-seasonal differences for starch quality and stability, antioxidant capacity, ascorbic acid and phenolic profile were also assessed. No notable differences between seasons were observed, although the MS1406 line (season two) had a lower GI, a greater antioxidant capacity measured by the Folin-Ciocalteu assay, and greater rutin content compared to the control. This somaclone shows potential as an alternative to Russet Burbank, with the same yield and fry quality but improved antioxidant value, which is of increasing interest to consumers. In the final study, a hormetic field trial was conducted to determine if yield, fry quality, and tuber phytonutrient content, were affected in field plants exposed to H_2O_2 sprays. There were some minor cultivar differences, but no impact on yield or nutritive quality was observed, in direct contrast to literature reports. Information in this thesis has immediate benefits; superior genotypes were identified for the Les Buissons breeding program. For example, cv. Kalmia and the advanced line QP01009.05JP can be promoted for their phytonutrient quality. Of the McGill somaclones, MS1406 appears to have better phytonutrient content than cv. Russet Burbank. The models that were developed using extensive data collected from 19 genotypes can now be applied more generally towards identification of genotypes with lower GL. Information in this thesis indicated, but did not explain, the ineffective use of H_2O_2 as a hormetic agent in the field. These results were in contrast to the hormetic effects seen in our lab in in vitro trials and by others, in field trials. In conclusion, Quebec genotypes with superior nutritional features were identified and modeling techniques were developed to more readily identify potato genotypes with lower GL.

Résumé

Cette thèse présente les résultats d'une étude des caractéristiques nutritionnelles de pommes de terre *(Solanum tuberosum* L.*)* notamment le type d'amidon, l'index glycémique, la charge glycémique, le contenu en protéine, le potentiel antioxydant, l'acide ascorbique, et les polyphénols. Dans la première étude, dix-neuf génotypes issus du programme de culture Québécois Les Buissons et de McGill, furent étudiés. De tous les génotypes, trois se sont distingués, Kalmia, QP01009.05JP, MS1406, en se basant sur les facteurs suivants: une charge glycémique plus basse, une meilleure qualité d'amidon, le potentiel antioxydant, et la teneur en polyphénols. Des modèles de régression multiple ont été développés pour identifier les caractéristiques permettant de prédire la charge glycémique. Dans la seconde, on a étudié quatre lignes somatiques dérivées de la pomme de terre Russet Burbank, pour en vérifier la stabilité d'une saison à l'autre et comparer avec l'échantillon de contrôle Russet Burbank, en se basant sur les facteurs suivants: une charge glycémique plus basse, une meilleure qualité d'amidon, le potentiel antioxydant, l'acide ascorbique et la teneur en polyphénols. Aucune différence saisonnière n'a été observée. Cependant, la ligne MS1406 (deuxième saison) présentait un index glycémique plus bas, un meilleure potentiel antioxydant, et taux plus élevé de rutine comparé avec l'échantillon de contrôle. Ce somaclone pourrait remplacer avantageusement Russet Burbank en vertu de ces meilleures caractéristiques nutritionnelles, sans compromettre le rendement et le potentiel de transformation industrielle. Pour la dernière étude, nous avons exposé les plantes, déjà en culture dans les champs, à H_2O_2 comme agent hormétique, afin d'en déterminer l'effet sur le rendement, le potentiel de transformation industrielle et les caractéristiques nutritionnelles. Contrairement à ce qu'on retrouve dans la littérature, nous n'avons pas observé d'amélioration des caractéristiques nutritionnelles, ou du rendement, suite à l'application de H_2O_2. L'importance de cette recherche est qu'elle démontre les avantages qu'il y a, pour Les Buissons, à promouvoir Kalmia et QP01009.05JP, en regard de leurs meilleures caractéristiques nutritionnelles. La ligne MS1406, de McGill, présentait des meilleures caractéristiques nutritionnelles comparées avec l'échantillon de contrôle (Russet Burbank). Les modèles de régression multiple développés à partir des données de dix-neuf génotypes, peuvent maintenant être utilisés pour prédire la charge glycémique de toute autre pomme de terre. Nous savons maintenant que l'utilisation de H_2O_2 comme agent hormétique ne fonctionne pas,

contrairement à ce qu'on a observé dans nos laboratoires invitro, et ce qui a été observé dans les cultures dans les champs selon la littérature.

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Contribution of Authors

This thesis has been prepared according to "Thesis preparation and submission guidelines" recommended and approved by Graduate and Postdoctoral Studies (GPS). This thesis consists of five chapters with Chapter 1 being General Introduction and Chapter 2 a Literature Review. Chapters 3, 4, and 5 describe three different experimental studies.

Specifically, Chapter 3 of this thesis is part of an on-going investigation of potato nutrients for the breeders at Les Buissons and for somaclonal lines from the McGill somatic breeding program, in the laboratories of Dr. Danielle J. Donnelly and Dr. Stan Kubow. All the assays for this study were conducted by me under the supervision of Dr. Danielle Donnelly. Additionally, Dr. Kebba Sabally and Ms. Hélène Lalande helped me to complete the HPLC analyses, and determining the starch phosphorus contents respectively. Chapter 4 was an inter-seasonal assessment of five genotypes; four somaclonal lines and the Russet Burbank control. All the assays for this study were conducted by me. Chapter 5 was a field study with the field experimental set-up developed by Dr. Atef Nassar and Dr. Danielle Donnelly. Field data was gathered by Emily Snowden. Starch grain photomicroscopy was done by Dr. Amir Al-Weshahy in Dr. Xiu-Qing Li's lab at AAFC-Fredericton, and the analysis of the photos was done with the help of Dr. Doaa Elkassas, at McGill. All further assessments and directions to determine treatment effect were developed and conducted by me.

For all chapters, the experimental work and statistical analyses were conducted by me. The choice of statistical methods and models as well as implementation of statistical programs were conducted by me with guidance from Dr. Amir Al-Weshahy, Christie Lovat and Dr. Jon Sakata. All the chapters in this thesis were prepared by me with extensive editorial help by Dr. Danielle Donnelly and Dr. Stan Kubow.

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List of Abbreviations

~	Approximately
%	Percentage
µg	Microgram
µl	Microliter
AA	Ascorbic acid
AAS	Atomic absorption spectroscopy
ABA	Abscisic acid
ABTS	(2,2-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid))
ANOVA	Analysis of variance
AMG	Amyloglucosidase
AOC	Antioxidant capacity
C-3	Carbon number 3
C-6	Carbon number 6
CFF	Caffeic acid
CGA	Chlorogenic acid
Con A	Concanavalin A
Cv.	Cultivar(s)
DDW	Double distilled water
DM	Dry mass
DMSO	Dimethylsulfoxide
DPPH	2,2 Diphenyl-1-Picryl hydrazyl
DS	Digestible starch
EDTA	Ethylenediaminetetraacetic acid

Eq.	Equivalents
FAO	Food and Agriculture Organization
F-C	Folin-Ciocalteu
FER	Ferulic acid
FG	Furostanol glycosides
FM	Fresh mass
g	Gram
GBSS	Granule-bound starch synthase
GI	Glycemic index
GL	Glycemic load
GOPOD	Glucose oxidase-peroxidase
GR	Goldrush; potato cultivar
Gy	Gray: the SI unit of energy absorbed from ionizing radiation
GWD	α-Glucan water dikinase
HPLC	High performance liquid chromatography
h	Hour(s)
IN	Innovator; potato cultivar
mg	Milligram
ml	Milliliter
mM	Millimole
NB	New Brunswick
nm	Nanometer
pGI	Predicted glycemic index
ppm	Parts per million
P- starch	Phosphorylated starch

r	Pearson Correlation Coefficient
RB	Russet Burbank; potato cultivar
RDS	Rapidly digestible starch
ROS	Reactive Oxygen Species
RS	Resistant starch
RT	Retention time
RUT	Rutin
SEM	Standard error of the mean
s	Second(s)
SDS	Slowly digestible starch
SET	Singlet Electron Transfer
SOD	Superoxide dismutase
T-CCFR	Sum of CGA, CFF, FER and RUT
T-CFR	Sum of CFF, FER and RUT
T-ACCFR	Sum of ascorbic acid and all polyphenols(CGA, CFF, FER, RUT)
TP	Total phenolics
TSP	Total soluble protein
Trt	Treatment
wk	Week (s)
yr	Year(s)
YG	Yukon Gold; potato cultivar

Chapter 1: General Introduction

1.1 Introduction

The potato (*Solanum tubersum* L.) has become a staple food in most parts of the world and is the fourth most significant food crop and the most important vegetable grown globally (Ek, Brand-Miller, & Copeland, 2012; Ezekiel, Singh, Sharma, & Kaur, 2013). Potato consumption continues to increase in popularity, particularly in developing countries (Ek et al., 2012; FAO, 2012). The potato was recognized to be such an important crop in terms of global nutrition that the United Nations General Assembly declared 2008 the year of the potato. Globally, approximately 365 million MT of potatoes were harvest in 2012, where China contributed 24 % of this amount and in Canada approx. 4.6 million MT were produced in 2012 on 147,000 ha of land area (FAO, 2012). In 2013, it was estimated that 80 % of consumers in the United States ate potatoes 3.6 times every 2 weeks (Nayak, Berrios, & Tang, 2014).

While potatoes are a major source of dietary carbohydrate, vitamin C, certain B vitamins, and minerals, different potato genotypes vary in nutritional content and in physical features (Ek et al., 2012). A major constituent of the potato is starch, which is a type of dietary carbohydrate. In the human diet, 40-75 % of our energy comes from carbohydrates, making it the principal source of food energy (Blennow, Nielsen, Baunsgaard, Mikkelsen, & Engelsen, 2002; Ek et al., 2012). The energy for 1 g of carbohydrate released is 17 kJ energy (4cal/g) (Ek et al., 2012). The degree to which starch is digested has an effect on how much glucose is released into the blood stream for energy uptake. There are many factors that determine the digestive properties of the starch granule including type of starch, degree of phosphorylation, protein content, and polyphenolic content.

Based on the potential of a carbohydrate to release glucose into the blood stream, carbohydrates can be classified by an experimental value called the *glycemic index* (GI) (Ek et al., 2012). Although GI is regularly used, glycemic load (GL) is another way to represent the glucose release of a food (Aziz, Dumais, & Barber, 2013). This value is determined by using the GI, the moisture content, and multiplying by the amount of carbohydrate in the serving size then dividing by 100 (Esfahani, Wong, Mirrahimi, Villa, & Kendall, 2011). The difference between

using GI or GL is substantial (Aziz et al., 2013). For example, potatoes are typically considered to have a high GI, but when the GI value *and* the portion size (150 g FM) are taken into account, the glycemic effect is actually medium to low. Typically, high GI foods are not recommended, and this is the primary reason why people have been advised to limit potato consumption. Now that it is understood that some genotypes could have a moderate to low GL, potato with a relatively low GL should be identified and promoted.

Although the range of antioxidant capacity in potato can vary and is generally lower than some other food crops, as a frequently consumed (staple) crop, the benefits of its phenolic antioxidant compounds can become significant (Brown, 2005; Ezekiel et al., 2013; Nzaramba, Scheuring, Koym, & Miller, 2013). Antioxidants quench free radicals and contribute health benefits, which include preventing DNA damage and affecting starch digestion (Blokhina, Virolainen, & Fagerstedt, 2003; Friedman, 1997; McDougall & Stewart, 2005). The polyphenols found in potatoes vary between cultivars and can be affected by growing conditions (Andre et al., 2007; Brown, 2005). In both plants and animals, these antioxidants scavenge and convert reactive oxygen species (ROS) into stable forms that cannot cause cell damage (Blokhina et al., 2003). The concept of hormesis involves placing an organism, such as the potato plant, under stress, such that a response is elicited (Shama & Alderson, 2005). Applications of low dose hormetic agents have been proposed to increase the nutraceutical content of fresh fruits and vegetables (Cisneros-Zevallos, 2003). Previous studies have already investigated some hormetic stressors, such as UV which can influence a plant's metabolic system and provide an increase in pathogen defense or other desired benefits such as increasing the nutritional value of the crop to humans (Kuźniak & Urbanek, 2000; Shama & Alderson, 2005). A field trial was conducted where the potato cv. Alpha, was treated with H_2O_2 such that tuber starch and lignins were significantly increased following treatment (López-Delgado et al., 2005).

1.2 Hypothesis

1. A predictive model of glycemic load can be based on the qualitative and quantitative differences in starch, protein, antioxidant capacity, ascorbic acid, and polyphenolics in potato genotypes.

2. Characteristics of starch, protein, antioxidant capacity, ascorbic acid and polyphenolics of new genotypes created from somaclone technology are stable between seasons.
3. Mild stress (hormetic effect) resulting from peroxide sprays can be observed in field-grown potato cultivars by an increase in antioxidant capacity and improved starch quality.

1.3 Objectives

1. To develop a predictive model of glycemic load that can be used to identify healthier genotypes.
2. To determine if the relative starch, protein content, antioxidant status, ascorbic acid content, and polyphenolics of somaclones change from one season to the next and relative to the control genotype.
3. To evaluate the hormetic effect of foliar peroxide sprays on starch, antioxidants, ascorbic acid and phenolics of tubers in a field trial.

1.4 Scope

This study enables informed nutritional recommendations for breeders and consumers. It identifies potato genotypes with superior starch quality, protein content, antioxidant capacity, ascorbic acid, and polyphenolics from the breeding program at Les Buissons and the somatic breeding program at McGill University. Additionally, a model for cultivars with a low glycemic load was created, and so potential screening tools can be suggested to identify healthier genotypes for both research, breeding, and industrial use. Inter-seasonal stability and somaclonal variation of starch and antioxidant qualities of McGill bred Russet Burbank somaclones is also important to assess as they go through the registration process. Consumers are becoming more aware of the health impacts that a balanced diet can have. Nutritionally superior genotypes from highly consumed crops such as potato can impact both short and long term human health (Camire, Kubow, & Donnelly, 2009). This study also contributes to the understanding of physiological processes in field-grown potato that are sensitive to hormetic stress. Overall, this work enables better genotype selection for improved phytonutrient content of field-grown potato tubers that can be included in a healthy human diet.

Chapter 2: Literature Review

2.1 Starch Composition

Starch is a polysaccharide, in which monomers of D-glucose are held together by glycosidic linkages (Blennow et al., 2002). The total starch content in foods indicates the available carbohydrate content. There are two main types of starch; amylose and amylopectin. Both types have α1–4 glycosidic bonds but only amylopectin has α1–6 bonds (Bergthaller & Hollmann, 2007). The two types of starch are structurally different; amylose is a linear polysaccharide whereas amylopectin has multiple branches. The typical three-dimensional structure of amylose is a linear left-handed helical configuration, where for one turn of the helix there are six glucose monomers.

Starch is broken down to its glucose components through enzymatic action at the non-reducing ends. Amylose, being a liner molecule, has only one non-reducing end for digestion (Bergthaller & Hollmann, 2007). Amylopectin molecules coil around into a double helical shape where the α1–6 bonds form branches that stick out from the main section (Ottenhof & Farhat, 2004). These branches are the non-reducing ends of the molecule and are available for digestion (Bergthaller & Hollmann, 2007). Starch is composed of 20-30 % amylose (Bach, Yada, Bizimungu, Fan, & Sullivan, 2013). As starch is created through enzymatic actions, granule-bound starch synthase (GBSS) is accountable for the formation of amylose. At least six other enzymes, such as four starch synthases and GBSS, are responsible for the production of amylopectin. This latter type of starch is more abundant.

Another method of classifying starch is by its degree of digestibility. These classes include: rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) (Bach et al., 2013). As the names suggest, RDS is digested first and is largely amorphous starch. SDS is also completely digested in the small intestine, although more slowly for reasons not yet fully understood (Nayak et al., 2014). Finally, RS is not digested by human enzymes, and for this reason is considered *resistant*. On a dry mass basis, the total starch in potato can be between 70-90 % depending on genotype (Nayak et al., 2014). Starch concentrations are mainly determined by the genotype of the potato but can also be influenced by environmental factors such as

growing conditions (Bach et al., 2013; Wolfgang Bergthaller, 2004). For example, Bach et al., (2013) found that a climate with a higher temperature increased the amount of SDS and consequently decreased the RDS.

Starch is stored in amyloplasts as starch granules. (Blennow et al., 2002; Ek et al., 2012). These granules range in size from microscopic (0.1-1 nm) to macroscopic (2-100 μm). These granules can vary by size, shape, and surface topography and this is where, for the most part, carbon is stored (Bergthaller & Hollmann, 2007; Ek et al., 2012). Starch digestibility properties and crop productivity of potatoes can depend on the starch granule size (Li et al., 2011). The "cluster" model for starch was proposed, which depicted alternating regions of crystalline and amorphous parts of a starch granule (Ottenhof & Farhat, 2004). Amylopectin causes starch to have its para-crystalline structure, whereas amylose is found in the amorphous sections (Nugent, 2005) (Fig.1).

The starch in potato is considered to have fewer impurities compared to starches from cereals. Potato starch and its level of lipid purity contribute to the neutral taste and the potato can be stored for long periods of time without developing an *off* taste (Wolfgang Bergthaller, 2004). The occurrence of these impurities is affected by cultivation and storage, where the granules themselves can be affected by genetic variability, climatic and edaphic conditions during plant growth (Vasanthan, Bergthaller, Driedger, Yeung, & Sporns, 1999). An enzymatic system comprised of ADP-glucose pyrophosphorylase, starch branching enzyme, starch synthase, and a debranching enzyme are involved in starch granule synthesis (Blennow, Bay-Smidt, Wischmann, Olsen, & Møller, 1998).

2.3 Resistant Starch

There are four different subclasses of resistant starch; RS_1, RS_2, RS_3, RS_4 (Sajilata, Singhal, & Kulkarni, 2006; Sha et al., 2012). The first class of RS is defined as being resistant because its form is physically inaccessible. In most cooking conditions, RS_1 is heat stable. Unprocessed whole grains are an example of RS_1. The second subclass is RS_2 which is defined as starch that resists digestion because of its granular form. These include starch found in raw (uncooked) potato, high amylose corn and bananas. This type of starch is in a compact structure such that the

accessibility of digestive enzyme is limited. The third type of resistant starch is retrograded amylose (Ek et al., 2012; Sajilata et al., 2006). This type of starch is of particular interest because it occurs in multiple types of starch-containing foods such as cooked pasta and potatoes. The development of this retrograded amylose will be explored and explained further on. The final type of resistant starch (RS_4) occurs because of chemical modification such that the starches have bonds other than α1–4 and α1–6 glycosidic bonds (Sha et al., 2012).

Resistant starch can be defined as the starch portion that cannot be digested by enzymes in the small intestine. Potato starch in its raw form is inedible, but is converted into a form digestible to humans by cooking (Ek et al., 2012). Potatoes can be cooked by a variety of methods and techniques including: baked, boiled, fried, or mashed. When a potato is cooked and then cooled, the starch granule structure undergoes chemical and physical reactions that change the arrangement of the molecules. These processes are called gelatinization (due to heating) and then retrogradation during the cooling that follows (Morris, 1990) (Fig. 2). This type of resistant starch is RD_3.

The process of gelatinization, also called crystal melting, is when the hydrogen bonds that interact between amylose and amylopectin in the starch granule are broken when sufficient heat and water are applied; disrupting the starch granules (Ek et al., 2012; Morris, 1990). The water molecules become bonded to the exposed hydroxyl groups of amylose and amylopectin. These new bonds enable the starch granules to take up more water, swell, and as a result the starch grains and organization of the starch structures are disrupted, increasing the solubility of the starch. Potato starch usually gelatinizes between 64-72 °C (Ek et al., 2012). Impaired starch synthesis and starch granule size can be a result of a higher gelatinization temperature with a greater amylose content (Wolfgang Bergthaller, 2004; Svihus, Uhlen, & Harstad, 2005).

When there is a cooling period, the starch molecules re-associate together slowly, but not with the same pre-heating level of organization. This process is called retrogradation, or crystallization (Morris, 1990). The molecules come together to create a gel that retrogrades into a new form that is distinct when compared to the native starch (Ek et al., 2012; Karlsson & Eliasson, 2003). The basis of re-association during retrogradation for amylose and amylopectin is essentially the same, although for amylopectin there are more branches to consider. Generally,

retrograded starch is more resistant to digestion than hot starch and amylose retrogradation is faster, due to its lack of branches, compared to amylopectin (Ek et al., 2012; Ottenhof & Farhat, 2004). Immediately after gelatinization, the amylose molecules are in a random coil conformation (Nugent, 2005; Ottenhof & Farhat, 2004). During retrogradation the formation of helices occurs, which eventually creates a gel network. The amylose molecules continue to aggregate together forming a complex amorphous layer of a starch granule (Nugent, 2005; Ottenhof & Farhat, 2004). The peripheral amylopectin chains crystallize during retrogradation. To conceptualize the retrogradation of amylopectin, a "fringed micelle" model was used. The model depicts the amorphous starch section of amylopectin in the middle, surrounded by the amylopectin crystals. For this reason, the name para-crystalline is often used. Molecules of amylopectin aggregate together, just as amylose does, but in contrast create an outer para-crystalline "shell" (Ottenhof & Farhat, 2004).

2.3.3 Factors Affecting Resistant Starch Digestibility

Resistant starch is not digested before it gets to the large intestine where it is fermented. For this reason, it is classified as an insoluble dietary fiber (Karlsson, Leeman, Björck, & Eliasson, 2007). Both genetic and environmental factors, such as growing conditions, can help determine the starch digestibility of a potato genotype (Bach et al., 2013; Wolfgang Bergthaller, 2004). Also, the ratio of digestible to non-digestible starch can change such that digestible starch content is greatly increased by cooking. Raw fresh potatoes have a lesser amount of digestible starch (10 %), whereas cooked potatoes can have more than 78 % digestible starch.

Englyst et al. (1992) established an *in vivo* technique using digestive enzymes to measure the digestibility of starch and the rate of glucose release (Englyst, Kingman, & Cummings, 1992). When compared to larger granules, smaller ones are digested more quickly when subjected to enzymatic hydrolysis because smaller granules have larger surface-to-volume ratios (Ek et al., 2012). Others have found that a lower rate of hydrolysis in raw potato starch was associated with a larger granule size (Li et al., 2011). Granule size does not appear to have an effect on amylose content (Noda et al., 2005). The type of starch is also thought to affect digestibility as a higher amylose content contributes to more RS (Ek et al., 2012). It is still unclear how the presence of amylose reduces the digestibility of the starch granule as the relationship, distribution, and forms

of amylose and amylopectin in the granule after retrogradation have not been fully established (Ek et al., 2012). In general, higher RS content is primarily associated with higher amylose content, which could be because amylose has a longer chain length and fewer branch points for digestion. Longer chain length plays a role in starch structural stability as it becomes less easily hydrolyzed and degraded by amylases (Ek et al., 2012). In contrast, recent literature found no correlation between starch digestibility and the amylose as well as total starch content of potatoes (Ek, Wang, Copeland, & Brand-Miller, 2014). It is therefore unclear if amylose content affects the amount of resistant starch found in potatoes, and so this aspect needs further investigation. This was examined in this thesis.

The choice of potatoes for food production and industrial applications can depend on nutritional characteristics. For instance some potato genotypes were genetically modified to have higher amylose content (Gupta, 2011; Schwall et al., 2000). These potatoes were engineered by inhibiting two branching enzymes for starch. The resulting potatoes had altered starch granule composition, such that more amylose was present, and had a decreased number of short chain starch molecules compared to the wild-type (Schwall et al., 2000). Coincidently, in these same potatoes, the starch also had more than a five-fold increase in phosphorous concentration in the amylopectin molecules. These overall changes increased the viscosity of the starch, which has practical applications for the potato industry (Gupta, 2011; Schwall et al., 2000).

2.4 Measuring Starch

The method of measuring the digestible, resistant and total starch is based on the original method of Englyst et al (1982) that was further refined (Megazyme, 2011). The Megazyme kit can provide consistent and reliable results. The procedure mimics human digestion, which is directed by adding key enzymes, and controlling temperature and pH. The samples are originally digested by the pancreatic enzyme α-amylase and amyloglucosidase (AMG), for 16 h at 37 ºC. The soluble starch is digested into D-glucose during this time. The enzymatic activity is stopped and the starch fractions extracted using ethanol through centrifugation. This result in two subsamples: the RS and DS. The RS is the pellet, as this starch was not degraded when simulating human digestion. To measure the starch content of the RS portion; it is eventually broken down to D-glucose using 2 M KOH, which is neutralised with acetate buffer, and finally

hydrolysed to glucose by AMG. The D-glucose of the DS and RS are measured using a glucose oxidase/peroxidase reagent (GOPOD), using a spectrophotometer at 510 nm. This solution changes colour in the presence of D-glucose.

2.5 Phosphorylated Starch

The amount of starch phosphorylation in potatoes and cereals depends on a variety of factors, mainly genetic variability but also includes ecological factors (Blennow et al., 1998). For example, potatoes have a higher level of phosphorylated starch if they were originally grown under climatic conditions where lower temperatures are typical. The phosphorous concentration can vary from 36-116 mg/100 g of potato starch (Ek et al., 2012). Another way of classifying phosphorylated starch content is based on a scale, where any value lower than 500 ppm is considered as having a low phosphorous content, 500-800 ppm is regarded as medium, and anything over 800 ppm is considered a high phosphorus content (Absar et al., 2009).

An enzymatic system comprised of ADP-glucose pyrophosphorylase, starch branching enzyme, starch synthase, and a debranching enzyme are involved in starch synthesis (Blennow et al., 1998). It is during the assembly of starch granules that the phosphate is incorporated by a protein called α-glucan water dikinase (GWD) (Blennow et al., 1998; Blennow et al., 2002). Phosphate esters bind to the C-3 and C-6 carbons of the glucose residues of amylopectin but not amylose, where nearly one third of the phosphorylation events happen on C-3 (Blennow et al., 1998; Blennow et al., 2002; Muhrbeck, Svensson, & Eliasson, 1991). The preference for phosphate binding at certain carbon positions depends on the type of starch and is most probably due to the different configurations of phosphates in the starch helical structure. Essentially, the C-3 phosphates are oriented out of the helices and the C-6 phosphates stick into the grooves of the helix (Blennow et al., 2002).

X-ray diffraction studies showed a higher degree of phosphorylation when the double helical starch structures were less densely packed. A helical structure that is less packed can accommodate the phosphate more easily and as a result has a higher phosphate concentration (Blennow et al., 1998). In addition, the high solubility of potato starch is due in part to the electrostatic repulsive forces between adjacent phosphate groups from nearby amylopectin

molecules. As a result, the chain strength weakens in this para-crystalline layer, and more water molecules can enter making this part more soluble (Ek et al., 2012).

Others have found, that the greater the number of phosphate groups, the less the digestibility in corn, rice and potato starches (Sitohy & Ramadan, 2001). This could be due to several reasons. One explanation is that the phosphorous molecules on C3 and C6 sterically hinder α-amylase from degrading starch. It is also theorized that the electrostatic environment between the phosphate group and the α-amylase enzyme is not favorable. This is because the active site of α-amylase is comprised of basic amino acids, and the phosphate groups on the starch are also negatively charged. For this reason, a strong interaction is unlikely. Consequently, when there is more phosphorylated starch, the activity of α-amylase would be decreased leading to less starch digestion.

It has been known since the 1950's, that the size of potato starch granules is associated with the concentration of phosphate ester groups (Wolfgang Bergthaller, 2004). There is a positive relationship between the chain length of amylopectin starch and the degree of phosphorylation (Blennow et al., 1998). So, the longer the amylopectin molecules, the more phosphorylation events can occur. It should be noted, however, that the content of amylose or the amylopectin: amylose ratio inside the starch granules have not been considered (Blennow et al., 1998).

Characteristics to guide breeding programs towards better industrial starch properties can involve selection for greater amounts of phosphorous and longer amylopectin chain length that can lead to improved potato starch quality (Wolfgang Bergthaller, 2004; Blennow et al., 1998). For example, the phosphate groups increase the viscosity of native potato starch used as a thickening agent for sauces, soups, and coatings for many baked snacks (Wolfgang Bergthaller, 2004).

2.7 Glycemic Index and Glycemic Load

The GI of a food is established by feeding subjects a standard amount of carbohydrate of a particular test food, as well as a control food such as white bread, on separate occasions (Ek et al., 2012). After consumption, the glucose levels in the blood stream are measured over a 2-hour period. The GI is then determined by dividing the incremental area under the glucose curve for

the test food by the area of the control (Dodd, Williams, Brown, & Venn, 2011; Ludwig, 2002). Generally, less starchy foods have a lower GI compared to foods that are starchy (Ludwig, 2002). Typically, potatoes are reported to have a high GI; that being above 70. A medium GI is between 56-69 and a low GI is below 55 (Atkinson, Brand-Miller, & Foster-Powell, 2008; Ek et al., 2012) (Fig. 3).

Mishra et al. (2012) noted that the glycemic response of potatoes was considered moderate when contrasted to other starchy foods as they took into consideration the moisture found in those potato cultivars and the serving size (Mishra, Monro, & Neilson, 2012). When using GL rather than GI, the weight of the water in the potato is accounted for. Therefore a potato with more moisture would have a lower GL. Other factors, such as storing temperatures and cooling after cooking can reduce the GI of various potato cultivars, due to the increased RS that occurs as a result of increased retrogradation (Nayak et al., 2014). Usually potatoes that are smaller and less mature have a lower GI, which could be the result of a lower degree of amylopectin branching leading to greater resistance to gelatinization and a slower rate of starch hydrolysis in the intestine.

Cooling after cooking has a significant effect on starch digestibility and GI. Overall, the digestibility of starch is reduced after a period of cooling as RS and SDS increase while RDS decreases (Mishra, Monro, & Hedderley, 2008). A rise in SDS can also occur during cooling that contributes to the formation of RS, which is caused by partial retrogradation of amylopectin rather than amylose molecules. This partial retrogradation occurs since amylopectin is comprised of branched amylose chains. For example, upon cooling, the digestible starch portion of potatoes was reduced from 96 % to 64 % (Monro & Mishra, 2009). Thus, consumption of cold potatoes, such as in potato salad, would be associated with a greater amount of RS and lower GI as compared to hot potato meals. Amylopectin creates a structure that slows down digestion, and partially resists hydrolysis, as compared to RS that resists hydrolysis completely. Therefore, selecting cultivars on the basis of a higher SDS, which would not cause a sudden increase in blood glucose levels as seen with RDS, would be a desirable characteristic for potato genotype selection.

Although a greater amylose content in potatoes would likely lead to a greater content of RS and a lower GI (Ek et al., 2012; Nayak et al., 2014), a recent study showed no correlation between either amylose or total starch content with starch digestibility and GI (Ek et al., 2014). In the latter study, the GI values were positively correlated with the percent starch hydrolysis as measured using an in vitro estimation of GI. Thus, the relationships among starch content, amylose content, starch digestibility and GI in potatoes has yet to be fully elucidated and requires further investigation. These aspects were examined in this thesis.

Using the GI of each food, the blood glucose response of a whole meal can be estimated as ingestion of higher GI foods and meals gives rise to higher blood glucose and insulin levels for 24 h in individuals, whether they are diabetic or not (Ludwig, 2002). The use of a GI system was originally created as a food guide for individuals with diabetes (Venn & Green, 2007). The convenience and effectiveness of using this dietary system has been questioned, specifically because it only considers blood glucose levels and not insulin response. The calculated GI of a meal can be overestimated when compared to the measured value of the GI for that meal (Dodd et al., 2011). For example, protein or fats when added to a carbohydrate can decrease the GI, therefore changing the apparent blood glucose response. Although the GI may be favorable for diabetics, the energy density and the type of dietary fat may not necessarily be a healthy choice. In addition, some foods with a low GI have properties that are undesirable for diabetes, such as high overall sugar content. Moreover, GI might not be a practical method of comparing the blood glucose effect between foods, as the servings generally do not have the equivalent amount of available carbohydrate (Monro & Mishra, 2009). For example, the GI of the potatoes was calculated to be high (72) but the measured potato-containing meal GI was low (55). Therefore, the debate as to whether the glycemic impact of a meal can be accurately calculated from glycemic indices of individual foods is still ongoing (Dodd et al., 2011; Ludwig, 2002). Consequently, the link between a low GI diet and its health benefits, such as reduced heart disease, are ambiguous and needs further investigation (Hatönen et al., 2006; Zhang, Mu, & Sun, 2012).

Early studies in the 1970's regarding the glycemic effects of foods showed that the type, as well as the amount of carbohydrate, were vital factors that determined the glycemic responses (Aziz

et al., 2013). Starches with a greater proportion of amylose, and more RS were linked with a lower GI due to the reduced starch digestibility. Glycemic load (GL) is determined by using the percent moisture, the GI and multiplying by the amount of carbohydrate in the serving size then dividing by 100 (Esfahani et al., 2011). GL is used due to the effects of quantity and quality (i.e., GI) of the carbohydrate on glucose release (Aziz et al., 2013). GL involves the global insulin demand exerted by diet. Depending on the size and variety, potatoes can have either a low or medium GL (Lynch et al., 2007) as GL takes into consideration the GI value, the moisture content, and the serving size of the carbohydrate. In particular, RS influences the glycemic effect of potatoes (Nugent, 2005). GL therefore represents a more accurate assessment of the glycemic response of some high GI foods, which can have a moderate or low GL (Aziz et al., 2013; Lynch et al., 2007). In that regard, a statistical model can be made to help define predictor values for GL, so that the relationships between variables (starch quality, polyphenols, etc.) and GL can be better defined.

2.8 Measuring Glycemic Index and Load Using an In Vitro Method

To avoid the use of human test subjects, many in vitro methods have been created and adapted to estimate the GI/GL of a test food, such as potatoes (Nayak et al., 2014). These in vitro approaches aim to replicate digestion processes, typically in the small intestine, and measure the rate of starch digestibility by measuring glucose content. Using an in vitro method has many advantages: it allows for increasing the screening capacity, efficient use of time, controlled conditions, presents less variability compared to human subjects, and is more cost effective. The complete replacement of the in vivo method cannot be done at this time, as few foods have been compared using both methods. In addition, there is no standardized in vitro method and many published procedures vary in their breakdown procedure, the amount and types of enzymes used, as well as the incubation times used (Germaine et al., 2008). For this reason, published results can often vary and conflict.

Extensive studies have been done regarding the glycemic indices of potatoes and the correlation was found to be high between the rate of starch digestibility in vivo and in vitro methods (Ek et al., 2014). On the other hand, in vivo methods of glycemic assessment have shown problems of

replication and variability. For example, an inter-laboratory study found that the measured GI of potato from a single source had a mean GI of 65.2, but values ranged from 44.6-98.5 (Monro & Mishra, 2009).

For cooked potato samples, a pepsin solution is initially added to disrupt starch-protein complexes, which are thought to interfere with starch digestibility and therefore subsequent glucose measurements. The solution and samples are incubated and the reaction stopped. After addition of pancreatic α-amylase, digestion is simulated, and timed aliquots are taken (30, 90, and 120 min) over a 2 h period. Each time point represents a different state of starch digestibility. Each aliquot is inactivated, and the glucose content measured with GOPOD. This glucose is then converted to a starch measurement.

Blood glucose concentrations for potato varieties are usually found to be at their greatest after 30 min and drop dramatically until 120 min (Ek et al., 2014). Using an adapted and modified procedure of Goñi et al. (1997), RDS is measured at 30 min and SDS at 120 min (Odenigbo, Rahimi, Ngadi, Amer, & Mustafa, 2012). Most in vitro methods use the 90 min time point to calculate the GI content because the starch hydrolysis content at 90 min typically has the highest positive correlation with GI values (Germaine et al., 2008; Goñi, Bravo, Larrauri, & Calixto, 1997). Recent research, however, has suggested that the 120 min time point is also correlated with GI and so can also be used (Ek et al., 2014).

2.8 Health Concerns of Starch

The consumption of foods with a higher RS content has many potential health benefits. For instance, overall colonic health can be improved by increasing the consumption of RS, which will reduce the chance of developing colorectal cancer during a lifetime (Nugent, 2005). More RS means that satiety is increased, therefore less food needs to be consumed to achieve a feeling of fullness, contributing to control of overweight and obesity (Nugent, 2005). In contrast, consumption of high GI foods may increase the risk of type-2 diabetes, cardiovascular disease, and obesity, although they may also increase satiety (Ek et al., 2012; Ludwig, 2002). On the other hand, the correlation between GI and the risk of type 2 diabetes is controversial with some studies showing a positive relationship and others a negative one (Dodd et al., 2011; Liu, Serdula, Sok-Ja, Cook, & et al., 2004; Villegas, Liu, Gao, & et al., 2007).

The idea that all potatoes have a high GI and therefore contribute to obesity is a recurring theme in the popular press, although current literature suggests that potatoes should be recommended in the diet because they have a low energy density compared to other frequently eaten carbohydrates (Monro & Mishra, 2009). Labeling the potato as a high GI food can limit its consumption, even though the potato carries many important nutritional components, including vitamins and minerals (Ek et al., 2012). Some authors have suggested that previous conclusions about the correlation between diabetes and a high GI associated with potato intake may be misleading as the studies were published before the issues regarding the GI of potatoes were fully understood (Nayak et al., 2014). The general consensus is that the glycemic impact of potatoes can vary depending on variety. For example, a potato genotype that contains a higher content of phosphorylated amylopectin would decrease starch digestibility and potentially also decrease the GL (Sitohy & Ramadan, 2001). Likewise, consuming potatoes with a relatively higher amylose to amylopectin ratio could be associated with a lower GL, compared to that of potatoes with relatively more amylopectin (Ek et al., 2012; Nugent, 2005).

The use and accuracy of GI measurements have been recently put into question when regulatory issues were evaluated regarding labelling food products based on their GI value (Aziz et al., 2013). A recent review noted three issues of concern related to the use of GI on food labelling:

1. The inconsistent and imprecise measurements of GI reported in the research literature. Measured GI values show wide variation and inconsistencies; a high GI carbohydrate measured in one case can be considered low in another (Aziz et al., 2013; Dodd et al., 2011; Ludwig, 2002). For example, GI changed when the ethnicity of the test subjects was changed due to genetic variation in digestion capabilities (Aziz et al., 2013).

2. One of the greatest limitations of GI is that it does not consider portion size (Aziz et al., 2013; Lynch et al., 2007). For example, a breakfast cereal with a GI value of 50 will be the same, no matter if 0.75 cups or 1.5 cups is eaten (Aziz et al., 2013). Choosing products based on GI value is only useful if the portion size is considered, so GL is preferable as a means of measuring glycemic response. To better illustrate this point, spaghetti (180 g, GI=49 [low]) supplies 48 g of accessible carbohydrate and has a GL of

24 (high) (Table 2.1). In comparison, a boiled potato (148 g, GI=82 [high]) has 25 g of accessible carbohydrate and therefore a high GL of 21. Therefore, the differences in the GI values were large where spaghetti had a low GI (49) and potato a high GI (82). However, their GLs were similar with the lower GI food (spaghetti) even having a slightly higher GL (Aziz et al., 2013). See Table 2.1 for a comprehensive summary.

3. Another issue was how labelling the GI value will coincide and conform to nutritional polices already in place. The rules and logistics of supporting a product based on GI might mislead consumers and may lead to unhealthy dietary choices. Likewise, with a general misunderstanding of the limitations of GI, and lack of emphasis on GL, consumption of nutritious foods could be limited and even discouraged (Aziz et al., 2013; Dodd et al., 2011). Use of alternative sweeteners, like fructose, could be used to decrease the overall GI value but can lead to negative health impacts. Consumers could thus be given conflicting and sometimes misleading dietary advice. Thus, the GI method must be questioned due to its inconsistency and misinterpreted values that can be corrected by use of the GL measurement (Aziz et al., 2013).

Determining potato genotypes for a selective breeding program for desired starch characteristics (e.g., higher RS in the form of higher retrograded amylose and phosphorylated starch) as well as better antioxidant capacity and protein content is important because the potato is already a highly-consumed food (Ek et al., 2012). It is not known yet how all the above phytochemical factors integrate towards generating a more nutritious potato, but by screening for desired individual characteristics, the best potato genotypes can be identified for a breeding program for consumers and industry.

2.9 Use of Freeze-Dried Material for Starch Analysis

Many studies report phytonutrient results such as polyphenolic content and antioxidant capacity on either a fresh mass or a dry mass basis, but typically use freeze-dried material and correct for the moisture content in their calculations (Navarre, Pillai, Shakya, & Holden, 2011; Pillai, Navarre, & Bamberg, 2013). This approach is used because the longer shelf life, more efficient storage capacity, and lower costs are usually associated with freeze dried samples (Zhang et al.,

2014). The use of freeze-dried material has become common for starch quality assessment for both research and industry (Zhang et al., 2014). Lyophilized samples must be adjusted for their original tuber moisture content, which influences glucose release measurements (Mishra et al., 2012). Mishra et al. (2008), concluded that "freeze-drying raw samples does not have a major impact on the proportions of starch fractions of differing digestibility when the potato powder is subsequently cooked and cooled". However, their results did show increased RS and SDS in some freeze-dried samples. It has been suggested that cooking and cooling the potatoes before freeze-drying would not affect the starch analysis (Mishra et al., 2008; Zhang et al., 2014). Assays kits, such as Megazyme, use the dehydrated food powder or fresh food samples and then adjust for the moisture content afterwards in the calculations (Megazyme, 2011), as indicated by Mishra (2012). Although, the Megazyme kit never specifies whether sample cooking should be done before or after the freeze-drying process. In contrast, others found that freeze-drying raw potato starch increases its digestibility when compared with other methods of drying such as oven or ethanol (Zhang et al., 2014). So, as freeze-drying raw potato starch affects starch structure and digestion, it would appear that rehydrating lyophilized potato starch for cooking, and subsequent testing for starch quality, is an inaccurate approach.

2.10 Protein Content

A range of total soluble protein from 6.8 - 8.6 % (fresh mass basis) has been found in various potato cultivars, but the scope can vary (Ortiz-Medina, Sosle, Raghavan, & Donnelly, 2009). There have been efforts to create transgenic potatoes with improved protein composition for increased nutritional value (Chakraborty et al., 2010). The amount of protein present in food has been linked to its starch digestibility. The interaction and relationship between protein, starch and GI is not completely understood. There is a high degree of variability in the measurement of GI, which appears to be dependent on the form (cooked or raw), the digestibility of starch, and the protein content (Thorne, Thompson, & Jenkins, 1983). There is evidence that protein - starch interactions affect starch digestibility and GI. In general, by eliminating the protein in wheat, carbohydrate malabsorption decreased (Anderson, Levine, & Levitt, 1990; Thorne et al., 1983). For example, when the protein was removed from wheat starch, there was a 20 % increase in starch digestibility, which by extension can increase GI and GL (Anderson et al., 1990). The processes in which protein, or fiber play a role in GI is not fully understood, but there is a strong

association (Allen, 2012; Thorne et al., 1983). Protein interacts with starch in a charge-charge manner; positively charged proteins are attracted to negatively charged starch molecules (Takeuchi, 1969). A decrease in glycemic response occurred when protein was added to a meal of mashed potatoes (Hätönen et al., 2011).The rationale was that the protein-starch complex was digested more slowly compared to starch alone. Insulin may play a role as protein consumption can aid insulin secretion to lower the glycemic effect. However, others have found that the protein-starch complexes cause a decrease in RS in potato, although the total profile of starch subtypes was not investigated (Escarpa, González, Morales, & Saura-Calixto, 1997).

2.11 Measuring Protein

The spectrophotometric test to measure the protein content is based on a method originally developed by Bradford (Bradford, 1976). The Bradford assay can develop a blue colour within 2 min and is stable for over 1 h. It is fast, reliable, and relatively easy to conduct and can be used to measure the protein from a vast range of potato genotypes, which can vary in their amino acid profiles (Snyder & Desborough, 1978). The assay uses the coloured reagent, Coomassie Blue, which creates a protein-dye complex in the presence of proteins and changes its absorbance at 595 nm (blue) (Bradford, 1976; Snyder & Desborough, 1978). Unlike other methods, Coomassie Blue does not measure interfering compounds such as free amino acids.

2.12 Ascorbic Acid, Phenolics, and Antioxidant Capacity

In potatoes, phenolic compounds are mainly found in the cortex and the periderm (peel), although the total range of polyphenols can differ based on genotype (Ezekiel et al., 2013; Friedman, 1997; Payyavula, Navarre, Kuhl, Pantoja, & Pillai, 2012). Phenolics are secondary metabolites that are synthesized in plants as a protective mechanism against ultraviolet radiation (Andre et al., 2007; Blokhina et al., 2003). Phenolics are also produced when plants are under oxidative stress (Blokhina et al., 2003). In comparison, humans cannot synthesize these compounds and must therefore acquire them through their diet (Andre et al., 2007; Blokhina et al., 2003). Phenolics can quench free radicals, which gives them value as antioxidants. Ascorbic acid (vitamin C), phenolic compounds such as chlorogenic, caffeic, and ferulic acids, and the flavonoid rutin, contribute a large fraction of the antioxidant capacity found in potato (Andre et al., 2007; Nassar, Kubow, Leclerc, & Donnelly, 2014). Chlorogenic acid is typically the phenolic

found in the greatest concentration in potatoes, sometimes representing about 80 % of the phenolic content (Andre et al., 2007; Brown, 2005). Although the range of antioxidant capacity in potato can vary and is lower than that of some other food crops, it is an often consumed (staple) in our diet and the benefits of its antioxidant compounds can therefore become significant (Brown, 2005; Ezekiel et al., 2013; Nzaramba et al., 2013). In that regard, some studies have found that potatoes, among common food staples, contain a relatively large quantity of phenolics (μg/g) as only apples and oranges had more phenolic content (Ezekiel et al., 2013).

There is considerable controversy concerning the effect that cooking has on the total antioxidant capacity, ascorbic acid, and phenolic content of potatoes. The literature relating to the differences between raw and cooked potato samples are limited and results are conflicting. In some cases, the phenolic content of cooked potatoes was greater than in raw potatoes (Burgos et al., 2013) whereas others reported no change (Blessington et al., 2010) or decreased antioxidant capacity in cooked material (Faller & Fialho, 2009). Typical screening and assessment methods for antioxidant capacity have routinely been done on raw freeze-dried potato samples. However, since potato is consumed after cooking, nutritional analysis concerning antioxidant and polyphenolics should also be done on cooked samples (Perla, Holm, & Jayanty, 2012). It is also thought that using raw samples as a screening tool for nutritional value can sometimes overestimate or underestimate the phenolics that are available in a cooked state and may therefore present false conclusions (Faller & Fialho, 2009). Genotype appears to be the single most important variable that determines if potato samples will have a significant difference in their antioxidant or phenolic content in raw and cooked samples (Blessington et al., 2010; Burgos et al., 2013). For example, using the same optimized extraction methods, cv. Guincho showed a significant increase in chlorogenic acid content after cooking whereas all other cv. (Leona, Challina and Boloña) did not (Blessington et al., 2010; Burgos et al., 2013).

Some research has shown that cooking decreases the contribution of phenolics by up to 52 %, although this varied with cooking method (Blessington et al., 2010; Faller & Fialho, 2009; Perla et al., 2012). In fact, cooking methods were shown to affect 95 % of all the measured phenolic acids which included; chlorogenic, caffeic, vanillic, and p-coumaric acids, and these compounds also differed in their concentrations depending on the cooking method used (Blessington et al.,

2010). Boiling caused the least amount of antioxidant and phenolic loss. The peel may act as a barrier, decreasing the leaching of polyphenols into the water resulting in no change in chlorogenic acid, ferulic acid, and rutin content after boiling (Deußer, Guignard, Hoffmann, & Evers, 2012). However, in the same boiled samples, a 33-fold increase in caffeic acid content was seen.

The method of extraction for raw and cooked samples may influence the quantification of total phenolics measured by the FC test (Burgos et al., 2013). This method might not be the most efficient for all genotypes, which may explain observed differences between phenolics in those samples. There is no apparent standardized phenolic extraction method for cooked potato samples. The most efficient extraction buffer for raw samples was found to be acidified 80 % methanol, but boiled samples required only 60 % methanol (Burgos et al., 2013).

Antioxidants are thought to decrease DNA damage in living organisms, including humans (Andre et al., 2007; McDougall & Stewart, 2005). Antioxidants prevent DNA damage by chelating Fe^{2+} ions and scavenging hydroxyl radicals, which are a subset of reactive oxygen species (ROS). These ROS are molecules that can be induced by oxidative stress, and contain oxygen, which is released by respiring cells (Blokhina et al., 2003 ; Hätönen et al., 2011). There are two main groups: free radicals (composed of superoxide and hydroxyl radicals) and non-radical oxygen species (such as hydrogen peroxide). All are toxic to cells if present in large quantities and can cause gene mutations, but are also continually produced in cells as products of cellular reactions; for example, the iron-catalyzed Fenton reaction (Blokhina et al., 2003; Hätönen et al., 2011). In addition to DNA damage, ROS species can negatively affect lipids and proteins by denaturing their functional conformation (Blokhina et al., 2003).

In the presence of ROS, a defense mechanism of ROS-scavenging is induced. This scavenging system includes a variety of enzymes, such as superoxide dismutase (SOD), catalase, and ascorbate peroxidase (Kuźniak & Urbanek, 2000). SOD coverts the superoxide anion radical into hydrogen peroxide which is considered less harmful (Blokhina et al., 2003). Peroxide is then converted to water and oxygen by catalase or peroxidase (Blokhina et al., 2003; Willekens, Inzé, Montagu, & Camp, 1995).

2.13 Measurement of Antioxidant Capacity and Polyphenols

For estimation of the antioxidant capacity of a sample, three spectrophotometric tests that are commonly used are: DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2,2-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid)), and the Folin-Ciocalteu (F-C) test. These screening tools cannot be used to identify and quantify specific phenolics; HPLC is used to investigate the phenolic proportions and quantities that can contribute to the total antioxidant capacity.

2.13.1 DPPH

DPPH (2,2-diphenyl-1-picrylhydrazyl) is a reliable, easy and applicable assay used to assess the reducing ability of antioxidants (Sánchez-Moreno, 2002). The convenience of this test is that the reduced compound is stable and can be measured using a spectrophotometer at 517-528 nm (Prior, Wu, & Schaich, 2005; Sánchez-Moreno, 2002). DPPH is originally a dark purple colour and becomes lighter as it is reduced by antioxidants (Prior et al., 2005; Sánchez-Moreno, 2002). The scavenging properties of the phenolics in a sample are based on the reduction of the DPPH compound (Prior et al., 2005) leading to a decrease in absorbance proportional to the antioxidant capacity. The results are expressed relative to a Trolox standard (Prior et al., 2005; Sánchez-Moreno, 2002). This assay cannot distinguish from other side reactions, such as H-transfer, that can contribute to the decrease in absorbance. Also, structural inaccessibility of the antioxidant to reduce DPPH may prevent or impede the reaction. For these reasons, it is common to supplement DPPH with additional antioxidant capacity assays.

2.13.2 ABTS

The ABTS (2,2-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid)) assay is a widely used spectrophotometric test that is not influenced by ionic strength, can work over a wide pH range, and is mechanistically simple (Prior et al., 2005). The absorbance of $ABTS^{\bullet+}$ is inhibited by antioxidants in the sample (Sánchez-Moreno, 2002). The $ABTS^{\bullet+}$ radical is made by an oxidation reaction using ABTS and potassium persulphate (Prior et al., 2005; Sánchez-Moreno, 2002). The antioxidant capacity of a sample is based on its ability to decrease the absorbance of $ABTS^{\bullet+}$ at 734 nm. Like DPPH, ABTS results are expressed relative to a Trolox standard (Sánchez-Moreno, 2002).

2.13.3 Folin-Ciocalteu

Another antioxidant capacity test is the F-C assay that works by an oxidation/reduction reaction (Prior et al., 2005). It was originally developed in 1927 where molybdo-tungstate reagent caused phenols to oxidize, yielding a coloured product that can be measured using a spectrophotometer at 745-750 nm. Since then, there have been improvements to the method, and a molybdotungstophosphoric heteropolyanion reagent can be used to obtain the coloured product that appears at 765 nm. The more coloured product that appears, the higher the antioxidant capacity during oxidation of the solution. Although this is assumed to be an accurate measure of phenolics in a sample, this is not always the case. Previously, gallic acid was used to create a calibration curve, although other acid equivalents, such as caffeic, ferulic, and chlorogenic acid standards are currently used (Prior et al., 2005) and chlorogenic acid is most appropriate for potato, as it is the major phenolic present (Nassar et al., 2014). Other compounds found within a sample extract can interfere with the reaction, which include sugars, aromatic compounds, ascorbic acid, and organic acids.

2.13.4 HPLC

There are numerous chromatographic techniques to separate sample mixtures (Harris, 2008; Weston & Brown, 1997). High Performance Liquid Chromatography (HPLC) involves a column containing micrometer-sized particles through which a solvent is passed under pressure to reliably and accurately identify and quantify many compounds (Harris, 2008). Typical HPLC equipment includes a sample injector, a solvent inlet system, a variable wavelength detector, and a system to control and read results (Harris, 2008; Weston & Brown, 1997). HPLC is a versatile tool that can be used to distinguish carbohydrates, nucleic acids, polyphenolics, and can even be used for protein sequencing (Navarre et al., 2011; Voet & Voet, 2004).

There are two main types of HPLC; normal- and reverse-phase chromatography (Harris, 2008). Normal-phase uses a less polar solvent for its mobile phase compared to its stationary phase (column). This means that less polar compounds would be eluted first. Reverse-phase uses a polar solvent and a non-polar stationary phase. Essentially, a solvent (mobile phase) containing the sample compounds (analytes) are run through the column containing a stationary phase (Harris, 2008; Weston & Brown, 1997). As the solvent flows through, the analytes become

attracted to the stationary phase and temporarily bind. Depending on the strength of the interaction, the compounds from the sample move within the column, elute out at different times and appear on the chromatogram (the digital output). The time it takes for the analyte to come out is called the retention time (RT) and appears as a peak. Analytes that have a high affinity for the mobile phase move through the column more quickly. The RT of each compound is unique and can be verified with a standard by comparing the RT. A typical stationary phase uses silica particles (solid support) inside the column. When using reverse phase chromatography, the pH of the mobile phase should be between 2-8 otherwise the silica support can become unstable (Weston & Brown, 1997).

The column is usually equipped with a guard column right above the solvent entry point, to avoid damaging the stationary phase, seeing as columns are easily ruined and very expensive (Harris, 2008). A guard column can be easily replaced and is made of the same stationary phase used for the main separating column. Compounds that would usually bind irreversibly, or damage the stationary phase of the column, bind to the guard column and stay there. To avoid other contaminants or damaging the HPLC system, samples are typically passed through a 0.5-2 μm filter before injection.

Sharper peaks are obtained when the particle size in the column is decreased, which affects the precision of the results (Harris, 2008). Different columns and HPLC conditions are used and tailored for the compounds to be separated. By changing the size or the pressure of the system, the RT can be affected (Harris, 2008; Weston & Brown, 1997). Other factors that affect elution order are the polarity of the solvents and the charges associated with the analyte and phases.

2.14 Starch Digestion, Antioxidants and Polyphenols

The inhibition of enzymes reduces starch digestibility, and limits the potential glucose release (Friedman, 1997). In that regard, phenolics have been proposed to reduce the amount of starch digested and decrease the glycemic response (McDougall & Stewart, 2005). Phenolics inhibit metabolic enzymes involved in the hydrolysis of starch to glucose. For example, chlorogenic acid, flavonols, and flavones can inhibit the enzymatic activity of α-amylase. Also, caffeic acid derivatives inhibit α-glucosidase maltase activity. Furthermore, gallic acid inhibits sucrase/isomaltase/trehalase, which are brush border disaccharidases in the mammalian intestine

(McDougall & Stewart, 2005). Previous research concerning the digestive effects of phenolics has focused on fruit species, particularly berries. However, as potato is a food staple, the effects of phenolic content on potato starch digestion is of major interest.

2.15 Hormesis

Hormesis, or hormetic effect comes from the Greek word "to excite" (Calabrese, Baldwin, & Holland, 1999). Low-dose of a potentially stressful or harmful agent causes the system, for example a plant or human, to be stimulated and elicit a response (Calabrese, 2008; Calabrese et al., 1999; Shama & Alderson, 2005). Some hormetic stressors are known, which can influence plants to increase in nutritional value (Shama & Alderson, 2005). The stressful agents affect the system in a dose-dependent manner and can be toxic at high doses (Calabrese et al., 1999).

Hormetic agents can vary and include: heavy metals, herbicides, radiation (UV), and oxidizing agents (such as H_2O_2), which can be used to stimulate plant growth or the production of secondary metabolites (Calabrese et al., 1999; Kuźniak & Urbanek, 2000; Shama & Alderson, 2005). Hormetic studies with potato have involved post-harvest irradiation. A study of the effects of storage duration and γ- irradiation on total antioxidant capacity and phenolic content on cv. Atlantic showed that the interaction between gamma irradiation and storage time had a positive effect on the measured antioxidants and phenolics (Blessington et al., 2007). Various doses of γ-irradiation (0-200 Gy) were delivered to post harvest potatoes. After storage, the antioxidant capacity originally decreased, but then increased with storage time. This was attributed to initial loss of moisture during storage followed by the subsequent hormetic stress effects of γ-irradiation.

Hormetic agents that could increase the nutritional value of potatoes would have significant economic and health benefits. Studies with lettuce have investigated water stress as a way to increase the antioxidant capacity of the crop (Oh, Carey, & Rajashekar, 2010). A one-time water stress at 6 weeks did not affect plant growth compared to the samples that had water withheld multiple times. The antioxidant capacity at harvest was significantly greater than the control, indicating that the application of a mild stress to a crop can improve its phytonutrient content without harming growth and yield (Oh et al., 2010).

2.16 Hormetic Trials with Potato

Pre-harvest studies have been done on potato plants, where they used an in vitro-to-greenhouse system using microplants to evaluate the effects of the plant hormone abscisic acid (ABA) on the cv. Atlantic and Alpha, on their tolerance to cold soil temperatures (Mora-Herrera & López-Delgado, 2007). ABA treatment increased the survival rate two-fold, but the stem growth was inhibited by 91 %. Potato plants that had time to recover from the ABA stress had an increased growth rate (6.9-10.4-fold compared to control). Interestingly, the activity of peroxidase, ascorbate peroxidase, and H_2O_2 significantly increased in plants exposed to ABA. When the stress of ABA was removed by transferring the microplants to an ABA-free medium, the activity of the enzymes and the level of H_2O_2 decreased back to control levels (Mora-Herrera & López-Delgado, 2007). No phenolic assays were completed to see if the ABA-induced increase of ROS enzymatic activity affected antioxidant capacity. Also, cultured potato cells that were put under oxidative stress with low doses of furostanol glycosides (FG) showed that certain enzymes involved with antioxidant capacity could be affected (Volkova, Maevskaya, Burgutin, & Nosov, 2007). There was an increase in antioxidant capacity and peroxidases, one of the main enzymes that work in the ROS-scavenging cycle, although the activity of SOD and catalase were unaffected. This study demonstrated that low doses of a mild stimulus, such as peroxide, can potentially improve the phytonutrient content of potatoes.

2.16.1 Hydrogen Peroxide as a Hormetic agent

Hormetic methods are of interest for potato plants because of the potential for increasing the market value of produce (potato tubers) through improved phytonutrients. For example, peroxide-treated plants resulted in tubers with increased antioxidant capacity and improved starch profile (López-Delgado, Sánchez-Rojo, Mora-Herrera, & Martínez-Gutierrez, 2012; López-Delgado et al., 2005). Hydrogen peroxide (H_2O_2) is an example of an interesting hormetic agent; it is an important signalling molecule that can stimulate the production of many secondary metabolites. However, as a ROS, it can be considered toxic at high doses (Kuźniak & Urbanek, 2000). Compared to other ROS molecules, H_2O_2 is the most stable; it carries no net charge and can easily cross cell membranes. It eventually breaks down to non-harmful water molecules. For these reasons, peroxide is potentially an ideal choice as a hormetic agent. As a defense

mechanism to environmental stress, plants synthesize antioxidants to scavenge ROS molecules (Andre et al., 2007). Hydrogen peroxide is believed to play a significant role in activating genes that encode proteins and enzymes involved in protection from oxidative stress. Potatoes can contain significant quantities of antioxidants that include ascorbic acid and phenolics (primarily chlorogenic acid).

2.16.2 Hydrogen Peroxide as a Hormetic Agent for Potatoes

Hydrogen peroxide had value in promoting in vitro yield (greater microtuber weight and numbers) in cv. Atlantic (López-Delgado et al., 2012). The procedure involved micropropagation on MS medium for 30 d. Single-node cuttings were incubated for 60 min with H_2O_2 at 0, 1, 5, or 50 mM and then transferred to microtuberization medium at 20 or 8 °C for 60 d. At 20 °C, the average weight/microtuber in the 1 mM treatment was significantly greater ($P \leq 0.05$) than at 0 mM peroxide (143.02±3.03 and 122.36±2.86 mg, respectively). At 8 °C, average weight/microtuber was significantly greater in the 1, 5 and 50 mM treatments (246.38±3.14, 251.57±3.30, 246.18±4.32 respectively) compared to 0 mM levels (235.20±3.59). The number of microtubers/plant was similar for all H_2O_2 treatment levels at 20 °C. Only at 8 °C and in the 50 mM treatment was the number of microtubers/plant significantly greater than the control (1.44±0.01 and 1.23±0.03, respectively). This relatively small but statistically greater yield (microtuber weight) was attributed to increased starch accumulation, although this was not measured.

Microtubers have been frequently used as a model system for field-grown tubers (Donnelly, Coleman, & Coleman, 2003). In vitro studies were conducted in the Donnelly lab with peroxide (2 mM and 4 mM) as a hormetic agent to manipulate the microtuber polyphenolic content of four potato cultivars; Goldrush, Onaway, Yukon Gold, and Russet Burbank (Nassar, Vunnam, Larder, Kubow, & Donnelly, unpublished; Vunnam, 2010). The total phenolics, including chlorogenic, caffeic, and ferulic acids, appeared to increase in cultivars with a lower total phenolic baseline. In Onaway, phenolic content increased at 2 and 4 mM by 13 and 14 % respectively, and in Yukon Gold phenolics increased by 22 and 21 %. The total antioxidant capacity comprised of: ascorbic acid, total phenolics, rutin, and quercetin, was also increased in Onaway at 2 and 4 mM by 14 and 19 %, as well as in Goldrush by 5 and 10 %, respectively. Hydrogen peroxide was

used at a dose that did not affect growth rate and yield. This study demonstrated a promising hormetic treatment to increase potato nutritional value. The results from this study lead to the current hormetic studies on field-grown potato (Chapter 3).

Yields and antioxidant enzyme activities have also been shown to increase with the use of a hormetic agent. Hydrogen peroxide (1 mM) spray application to greenhouse-grown cv. Alpha significantly increased minituber weight (g/plant) compared to a control (no exact values given) (Martínez-Gutiérrez, Mora-Herrera, & López-Delgado, 2012). Minituber total starch content (mg/g), estimated using the anthrone method, increased by 15 % in uninfected plants with the application of 1 mM H_2O_2, while plants infected with phytoplasma showed a 20 % increase (in contrast to typically decreased starch content that occurs with phytoplasma). The number of tubers/plant showed no significant difference between the 0 mM application of H_2O_2 and 1 mM in uninfected potato plants. Peroxide treatment did not affect the activity of the antioxidant enzyme catalase whereas the activity of peroxidase increased (exact numbers were not reported). A direct measurement of phenolic composition or antioxidant capacity was not done. This study suggested that peroxide treatment could increase not only yield and antioxidant capacity, but also protein content in cv. Alpha

There is only one report of hormetic agents applied to field-grown potato. López-Delgado et al. (2005) sprayed H_2O_2 at 0, 5, or 50 mM twice a week, 21-90 days after planting, on field-grown cv. Alpha. Total starch content (mg/g) per tuber, estimated using the anthrone method, was significantly increased in the 5 and 50 mM treatments by 30 and 28 %, respectively compared to the control. Total starch, estimated by specific gravity, also showed significant increase, although less than that suggested by the anthrone method. In the 5 and 50 mM treatments, the starch content was increased by 6.7 and 11 %, respectively. The starch profile was not investigated. Greenhouse-grown potatoes that received the same peroxide treatment as in the field (0, 5, or 50 mM) had a 62 % increase in lignin area (exact numbers not reported). Lignins are recognized to have antioxidant properties (Dizhbite, Telysheva, Jurkjane, & Viesturs, 2004), although this was not investigated by López-Delgado et al. (2005). Further starch and phenolic quality and quantity assessments were not done. This report gave a clear impression that starch parameters and antioxidant capacity could be affected by peroxide treatment in the field. Although seemingly very important, no other such field studies have been reported in the intervening years. An

agronomic tool like hydrogen peroxide sprays presents a promising opportunity for potato growers to increase the nutritional and production value of their field crops and requires further investigation.

Table 2.1. Comparison of spaghetti and boiled potato portions in terms of glycemic index (GI) and glycemic load (GL).

Produce type	Portion Weight (g)	GI	Available Carbohydrate (g)	GL
Spaghetti	180	49 (low)	48	24 (high)
Boiled Potato	150	82 (high)	25	21 (high)

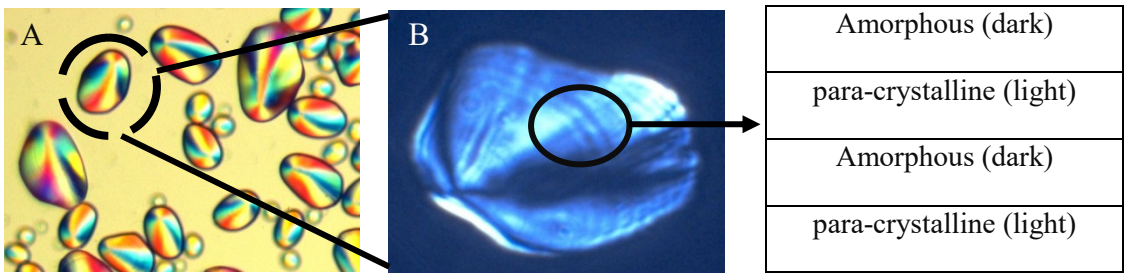

Figure 2.1. A) Field of view (200 X) showing starch granules under polarized light. B) Portion of one granule showing the concentric layers of amylose (amorphous) and amylopectin (para-crystalline) found in the granules, although relative composition can vary for different genotypes. This variation in ratio of amylose: amylopectin gives granules different characteristics that can have important nutritional and industrial implications.

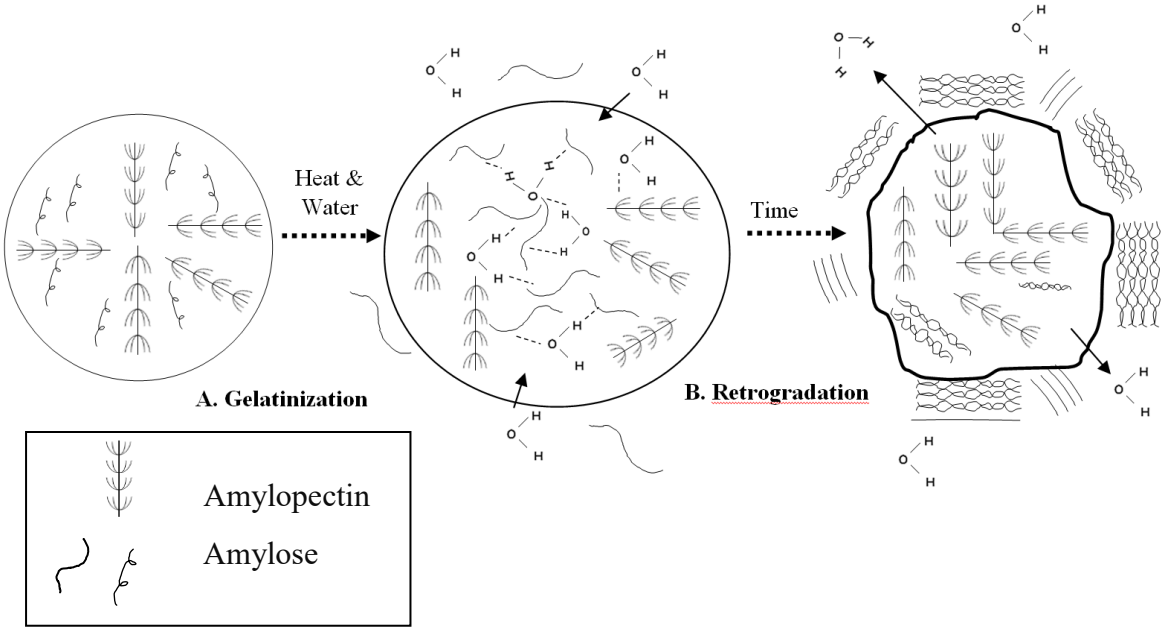

Figure 2.2. Starch (amylose and amylopectin) undergoing gelatinization and retrogradation.

A) During gelatinization hydrogen bonds between amylose and amylopectin in the starch granule are broken when sufficient heat and water are applied. Water molecules become bonded to the exposed hydroxyl groups of the starch molecules. Heat causes the crystalline amylopectin regions to become diffuse and some amylose leaches out of the granule. More water is absorbed into the granule; it begins to swell and as a result the starch grains and organization of the starch structures are disrupted. B) Retrogradation occurs during a cooling period. The starch molecules re-associate together slowly and continue to aggregate together forming many complexes. Parallel chains are stabilized with hydrogen bonds. Water is expelled from the network and the starch granule integrity is broken. During retrogradation, the formation of helices of amylose occurs and eventually creates a gel network. The peripheral amylopectin chains crystallize during retrogradation. Figure modified from http://www.food-info.net/uk/carbs/starch.htm

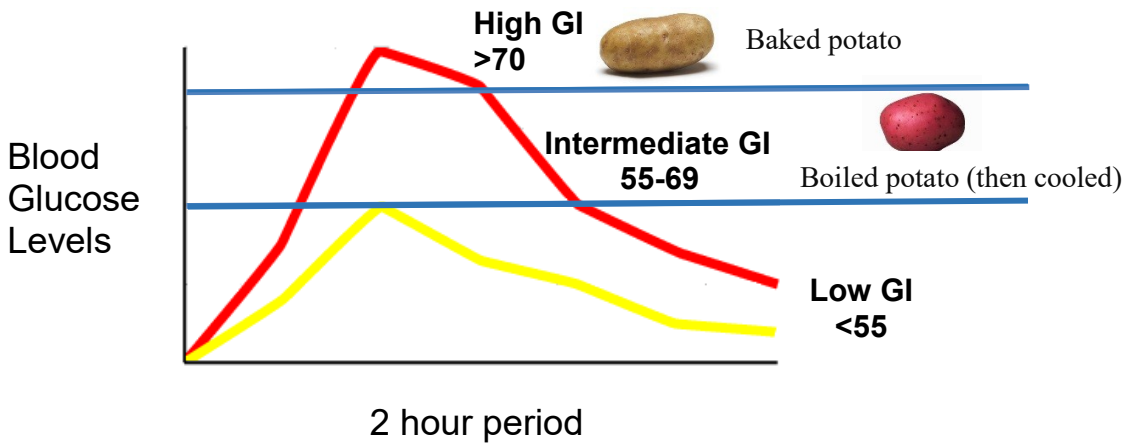

Figure 2.3. Glycemic index (GI) is an experimental value assigned to a particular food that indicates the total rise in blood glucose levels following consumption. The GI is measured over a 2 h period. The area indicated by the yellow line shows the blood glucose levels for a product that has a low GI (under 55). A boiled potato that has been completely cooled (or refrigerated) after cooking has an intermediate GI (55-69). A high GI (above 70), as indicated by the red line, is assigned to foods such as a hot baked potato. Figure adapted from http://sanjosefuncmed.com/gluten-summit-recap/

Connecting Statement

Chapter 3, entitled “Phytonutrient Evaluation of Quebec Potato Genotypes”, reports the nutritional qualities of parental lines and cultivars from the Quebec potato breeding program at les Buissons and advanced somatic lines of ‘Russet Burbank’ from the McGill somatic breeding program. This was done to identify superior lines for breeding purposes, and eventually, for a consumer market. Superior cultivars already on the market were also identified. For this study, models were developed to explain variables that can affect glycemic load.

Chapter 3: Phytonutrient Evaluation of Quebec Potato Genotypes

3.1 Introduction

Previous research has indicated that there appear to be several variables that can effect potato starch digestion including moisture content, starch quality (RS, RDS, SDS) and the content of amylose, phosphorylated amylopectin, protein, and phenolics (Ek et al., 2012; Ek et al., 2014; McDougall & Stewart, 2005; Sitohy & Ramadan, 2001; Thorne et al., 1983). A wide range of results have been reported, with some conflicting reports, concerning the phytonutrient factors that can affect GI and GL. For example, amylose content was positively correlated to RS content, which both decreased GI and GL response (Ek et al., 2012; Nayak et al., 2014) whereas others found no correlation between amylose content and GI (Ek et al., 2014). Another factor that is starch related, and can affect starch digestibility, is the phosphate groups bound to amylopectin. It has been proposed that a higher phosphorylated starch content in potatoes would decrease starch digestibility and consequently lower GL (Sitohy & Ramadan, 2001). The interaction and relationship between protein, starch, GI, and GL in potatoes is not completely understood although some reports have shown that higher protein content in wheat can decrease the glycemic response (Allen, 2012; Anderson et al., 1990; Hätönen et al., 2011). Additionally, higher phenolic content in some plant foods has been associated with reduced starch digestibility and a decreased glycemic response by direct inhibition of intestinal enzymatic action (Friedman, 1997; McDougall & Stewart, 2005).

A statistical model can help define predictor values for GL, so that the relationships between the above phytonutrient variables and starch digestibility can be further defined. In that regard, a multivariate linear regression model can be built using various continuous explanatory variables that define GL (Gotelli & Ellison, 2013). This type of modelling strategy requires that the final model be as simple as possible and creates a linear equation in multidimensional space (Crawley, 2013; Gotelli & Ellison, 2013). All redundant parameters are excluded and clear variables can be shown to explain the variance and predict GL. Multivariate regression models have been widely used in disciplines such as bioinformatics, genomics, and especially in ecological research (Martella, Vicari, & Vichi, 2015). There are three main strategies for completing a regression model using continuous variables: forward selection, backward elimination, and a stepwise

method (Gotelli & Ellison, 2013). Depending on the strategy used, the final linear equation can differ. In the case of forward selection, the type 1 error can be significantly reduced and overestimation of explained variance can be corrected for (Blanchet, Legendre, & Borcard, 2008). There are many tests that can be used to create a numerical value that can be used to determine if a continuous variable should be added to a model. These tests include; the Akaike information criterion (AIC), the use of an F-statistic, p values, and finally correlation values. Although the use of one test can be considered sufficient, using all can help validate the inclusion of an explanatory variable into a predictive multivariate regression model (Gotelli & Ellison, 2013; Murray & Conner, 2009). Explanatory variables can also be highly correlated (multicollinearity) and therefore make it difficult to complete and model without bias and then identify the unique contribution(s) of each predictor variable in the final model (Murray & Conner, 2009). Additionally, the data and its residuals are assumed to be normally distributed (Gotelli & Ellison, 2013). Transforming data can change the form of the data to become more normally distributed.

In the present study, following conventional agronomic selection for yield and processing, a variety of phytonutrient predictor variables were used in the statistical model to identify genotypes with lower GL. In that regard, the percentage of phosphorylated starch, content of amylose, protein, ascorbic acid, phenolics, and antioxidant capacity were measured among advanced breeding lines as well as current market potatoes to determine how these parameters could affect GI and GL within the statistical model. Such an approach was developed as a potential screening tool to enable breeders to select healthier genotypes in terms of starch quality (Bach et al., 2013).

This study was initiated through a collaboration with Dr. Pierre Turcotte, Quebec's foremost potato breeder, who has released many important, primarily table stock cultivars grown in the province and elsewhere. Although Dr. Turcotte predeceased the completion of this study, he was interested in the relative nutritional composition of his cultivars and advanced breeding lines and the McGill somatic lines. The objective was to determine the genotypes with superior starch quality, including: higher content of RS, as well as an optimal total digestible starch (DS) profile which is subdivided into RDS and SDS fractions. A ratio where the RDS is smaller is preferred because SDS is then less likely to cause a sudden large increase in blood glucose levels that

contribute to greater GI and GL. The percentage of phosphorylated starch, content of amylose, antioxidant capacity, and polyphenols were also investigated to determine if, as the literature suggests, these could be used to screen for starch quality. The GI and GL were evaluated to help select more nutritious genotypes. The relationship between GL to starch quality, total soluble protein, antioxidant capacity, and polyphenolic profile was investigated to create a model system to help define the characteristics that contribute the most to GL.

3.2 Materials and Methods

3.2.1 Source Material

For the first part of this study, we examined 14 genotypes (3 parental lines and 11 named cultivars) from the Quebec potato breeding program at Les Buissons (supplied by Quebec potato breeder Pierre Turcotte) and four advanced somatic lines and one control from the McGill somatic breeding program. The complete list of genotypes is shown in Table 3.1. From Les Buissons, tubers from the three parental lines were small due to a short season length in the breeder's plots whereas tubers from all other genotypes were grown to maturity. The Les Buissons and McGill lines were grown at Progest2001 near Quebec City.

3.2.2 Sample Preparation

Each potato genotype was separated into two treatment groups: raw and cooked. Each of these groups had three replicates, where each replicate contained ~2-5 tubers. Each replicate was washed under tap water and left to air dry under paper toweling to limit light exposure. The cooked group was boiled in a stainless steel pot for 20 min. Cooked potato material was used to measure the starch profile including RS, DS content and fractions, GI and GL.

Both cooked and raw samples were first chopped using a standard kitchen knife, then by a food processor (FP5050SC, Black and Decker, Canada), and then thoroughly mixed for a representative sample. Samples (~100 g) were taken and the wet weights were recorded. The samples were frozen under liquid nitrogen and subsequently freeze-dried (FTS Systems, NY, USA) for 2-5 days. After freeze-drying, the dried samples were reweighed for a dry weight,

ground using a grinder (CBG100SC, Black and Decker, Canada), and stored at -80 ºC until analysis. The percent moisture lost during the freeze-drying process was calculated. All subsequent analyses were completed on freeze-dried material (Navarre et al., 2011; Pillai et al., 2013).

3.2.3 Percent Moisture

Sample fresh (before freeze-drying) and dry mass (after freeze-drying) were measured using an electronic balance (950241, Adam Equip., Canada). From the weight of water lost from the fresh sample, the percent moisture was calculated using the following equation (mean of 3 replicates):

$$\frac{\text{Fresh mass (g) - Dry mass (g)}}{\text{Fresh mass (g)}} \times 100 = \% \text{ moisture}$$

3.2.4 Resistant, Digestible and Total Starch

For each genotype, the starch profile was determined. The Megazyme Resistant Starch assay kit (K-RSTAR; Megazyme 2011) was used, which follows a modified *in vitro* method based on the procedure of Goñi et al. (1997). For this procedure, 100 mg of rehydrated cooked sample was placed into a 50 ml Falcon tube and 4 ml of pancreatic α-amylase solution (10 mg/ml sodium maleate buffer) containing amyloglucosidase (10 µl/ml) was added, and the tube vortexed for 30 s. The samples were then incubated for 16 h at 37 ºC with constant shaking (Versa bath model S 224, Fisher Scientific, Waltham, MA, USA), for enzymatic digestion of the starch into glucose. After incubation, 4 ml of ethanol (99 % v/v) was added followed by vigorous vortex mixing. Sample tubes were then centrifuged at 1500 x g for 10 min. The supernatant was decanted and the pellet re-suspended in 2 ml of 50 % ethanol following which 6 ml of ethanol was added, the tube centrifuged, and the supernatant decanted and set aside. This step was repeated, and the supernatants combined. Finally, the tubes were inverted onto paper towels to drain the pellet. The supernatant was used to characterize the digestible starch, and the pellet was used for resistant starch measurement.

3.2.4.1 Digestible Starch Measurement

From the initial supernatant that was obtained from the ethanol washing, the volume was adjusted to 100 ml with 100 mM sodium acetate buffer (pH 4.5). From this, 0.1 ml aliquots were pipetted into glass test tubes for measurement of glucose.

3.2.4.2 Resistant Starch Measurement

A small magnetic stirrer was added to each tube containing a pellet and the tube placed into an ice water bath. Into each tube, 2 ml of 2 M KOH was added with vigorous stirring and then left to incubate for 20 min after which 8 ml of 1.2 M sodium acetate buffer (pH 3.8) was added with continued stirring. As the tubes continued to stir, 100 µl of AMG (3,300 U/ml) was added, and the mixture was incubated at 50 ^{o}C for 30 min. The samples were then centrifuged for 10 min at 1500 x g. After centrifugation, 0.1 ml aliquots of the test samples were put into glass test tubes and used for measurement of glucose.

3.2.4.3 Glucose Measurement

The glucose content of the digestible and resistant starch was measured at 510 nm using a spectrophotometer (DU640, Beckman, USA). Standards were made by transferring 0.1 ml D-glucose (1 mg/ml) into glass test tubes, and the blank was performed with 0.1 ml aliquot of 100 mM sodium acetate buffer (pH 4.5). To each sample, standard, and blank, 3 ml of GODPOD was added and incubated for 20 min at 50 ^{o}C and the absorbance was measured. The glucose content of the collected supernatant and digested pellet was mathematically converted to digestible starch (DS) and resistant starch (RS) content that were summed (DS + RS) to calculate total starch (TS) content.

3.2.5 Isolation of Starch and Phosphorous Content

The procedure for the isolation of starch for quantifying phosphorylated starch was adapted from Nielsen, Wischmann, Enevoldsen, & Møller (1994). Briefly, 2 g of freeze-dried sample was weighed in a 50 ml Falcon tube and 8 ml of ice-cold double distilled water was added and the mixture vortexed for 3 min. The samples were filtered through 2 layers of cheese cloth and washed three times with 10 ml double distilled water. The filtrate was centrifuged at 1500 x g for

10 min at room temperature. The supernatant was discarded and the pellet was washed three times with 10 ml double distilled water. The samples were centrifuged once again and the supernatant discarded. The pellet was then washed three times with 10 ml acetone and centrifuged as before. The supernatant was discarded and the pellets were left to dry overnight under the fume hood. The pellets were then kept at -20 ^{o}C until analysis for phosphorylated starch content.

Percent phosphorylated starch was measured after the starch was isolated as described above using a method adapted from Parkinson and Allen (1975) described by Lachat Instruments (QuickChem method number 13-115-01-1-B) (Parkinson & Allen, 1975). Briefly, freeze-dried samples (0.160 g) were digested in 4.4 ml of digestion mixture (420 ml sulfuric acid, 350 ml peroxide (30 %), 14 g lithium, and 0.42 g selenium) at 340 ºC for 3 h. The digest was diluted to 100 ml and analysed calorimetrically for phosphorus content at 880 nm in a flow injection instrument (QuickChem series 8000, Lachat Instruments, CO, USA).

3.2.6 Amylose Content

To measure amylose, the Megazyme Amylose/Amylopectin assay kit was used (K-AMYL; Megazyme 2011). The kit follows the modified method of Yun and Matheson (1990) that uses concanavalin A (Con A). A 20-25 mg sample is weighed followed by a starch pre-treatment involving addition of 1 ml DMSO with vigorous mixing on a vortex mixer. The samples were then placed into a boiling water bath for 1 min, vortexed again, and then placed back into the boiling water bath for 15 min with intermittent vortexing to ensure no gelatinous lumps remained. The samples were then left to cool for 5 min at room temperature. After cooling, 2 ml of ethanol (99 %) was added, vortexed, and another 4 ml of ethanol was added and vortexed once again. The tubes were then left to stand overnight. The next day, the samples were centrifuged at 2,000 x g for 5 min and the supernatant discarded. The tubes were inverted and left to drain for 10 min. After draining, 2 ml of DMSO were added to the pellet, followed by vortexing. The tube was placed into a boiling water bath for 15 min with intermittent mixing, and then 4 ml of Con A solvent was added to the hot, dissolved sample. The solution was then diluted to volume with Con A in a 25 ml volumetric flask. This solution was referred to as Solution A. Using this pre-treated starch fraction, the ratios of amylose and amylopectin were

measured. A 1 ml aliquot of solution A was added to an Eppendorf, and 0.5 ml of Con A was added, before the sample was vortexed and left to stand for 1 h at room temperature then centrifuged at 14,000 x g for 10 min. After the centrifugation step, 1 ml of the supernatant was transferred to a 15 ml Falcon tube, where 3 ml of 100 mM sodium acetate buffer (pH 4.5) was added and the tube placed into a boiling water for 5 min to denature Con A and then 0.1 ml of amyloglucosidase/α amylase was added and incubation continued for 30 min. The tubes were centrifuged at 2,000 x g for 5 min and a 1 ml aliquot of the supernatant added to 4 ml of the GOPOD reagent and incubated for 20 min in the 40 ºC water bath (182, Precision Scientific Group, USA). A blank and control were added, which consisted of solutions of 1 ml of 100 mM sodium acetate buffer and 0.1 ml of buffered D-glucose, respectively as well as 4 ml of GOPOD. Finally, the glucose content was measured using a spectrophotometer at 510 nm, and the amylose content was obtained. The total starch content was measured by taking 0.5 ml of solution A and 4 ml of 100 mM sodium acetate buffer and adding 0.1 ml of amyloglucosidase/α amylase, which were incubated at 40 ºC for 10 min. Later, an aliquot was added to GOPOD and treated the same as the samples mentioned above. Using the absorbance for the amylose content, and total starch, the percent amylose was calculated. The amylopectin content was estimated from total starch by subtracting the amylose content.

3.2.7 In Vitro Digestibility of Starch, Predicted Glycemic Index and Load

To measure starch digestibility, a modified *in vitro* method based on the procedure of Goñi et al. (1997) was used (Odenigbo et al., 2012). A volume of 10 ml of HCl-KCl buffer (pH 1.5) was added to a 50 ml Falcon tube containing 50 mg cooked potato, which was followed by addition of 200 µl of pepsin (Sigma P-7012) solution (1 mg /1 ml HCl-KCl buffer). The samples were incubated at 40 °C for 1 h in a shaking water bath after which 200 µl of pancreatic α-amylase solution (1.5 mg /10 ml phosphate buffer) was added and the samples incubated at 37 °C for 45 min. The enzyme reaction was stopped with the addition of 70 µl Na_2CO_3 and diluted to 25 ml with tris-maleate buffer (pH 6.9). To each tube, 5 ml pancreatic α-amylase solution (3 mg /5 ml tris-maleate buffer) was added and samples were subsequently incubated at 37 °C in a shaking water bath for 2 h. At time intervals (30, 90, and 120 min), 1 ml aliquots were removed and placed into boiling water for about 5 min to inactivate the enzyme. After the timed aliquots were extracted and inactivated, each sample was refrigerated until all the timed aliquots were

extracted. Each aliquot was treated with 3 ml of 0.4 M sodium acetate buffer (pH 4.75) and 60 µl of AMG (3,300 U/ml) then incubated in a shaking water bath at 60 °C for 45 min. The volume was adjusted to 10 ml with distilled water, mixed, and a 0.1 ml aliquot was transferred to a glass test tube. The glucose content was measured spectrophotometrically at 510 nm against a blank. The blank was made with distilled water and standards were generated by transferring 0.1 ml D-glucose (1 mg/ml) into glass test tubes. To each blank, sample, and standard, 3 ml of GOPOD was added and incubated for 20 min at 50 ^{o}C and then the absorbance measured. The glucose released was determined and converted into starch by multiplying by 0.9 (Odenigbo et al., 2012).

3.2.8 Total Soluble Protein

The TSP was determined spectrophotometrically by measuring the samples at 595 nm using a standard curve of Bradford reagent, where a known concentration and the absorbance were related. The extraction method for the protein was adapted from Jones et al. (1989) and the measurement from Bradford (Bradford, 1976; Jones, Daniel Hare, & Compton, 1989). To extract the TSP, ~20 mg of freeze-dried sample was placed into 1.5 ml of 0.1 M sodium phosphate buffer (pH 7.5) in an Eppendorf tube. The samples were vortexed for 30 s and kept at 4 ºC for 2 h. The samples were then vortexed again for 30 s and centrifuged at 4100 x g for 10 min at 4 ºC. The supernatant was collected for TSP analysis and 20 µl of each sample was added (in triplicate) into a 96 well plate to which 235 µl of Bradford reagent was added and mixed using the micropipette. The plate was incubated at room temperature for 10 min in the dark and the absorbance was read at 595 nm.

3.2.9 Methanolic Crude Extracts

Methanolic crude extracts were used to measure ABTS, DPPH, and F-C antioxidant capacity. Approximately 100 mg of freeze-dried potato sample was placed into an Eppendorf tube and 900 µl of 90 % methanol was added. The samples were vortexed for 60 s, sonicated for 30 min, and vortexed again. They were then centrifuged at 1500 x g for 10 min at 4 ºC. The supernatant was extracted into a new Eppendorf tube and the pellet was re-extracted with 600 µl of 90 % methanol and the supernatants were combined.

3.2.10 ABTS

The ABTS antioxidant capacity was calculated by comparing the measured absorbance of the samples to a Trolox standard curve using an adapted method by Re et al. (1999). An ABTS (7 mM) stock solution was prepared in 18Ω cm^{-1} water. The ABTS solution was combined with potassium persulfate (2.45 mM) and left to incubate in the dark for 12 h at room temperature before use so that the radical cation of ABTS ($ABTS^{\bullet+}$) could be produced. The ABTS radical solution was diluted with 95 % ethanol and the absorbance read at 734 nm, with the spectrophotometer blanked to air. Continuous dilutions followed until the absorbance of the working solution read 0.7 ($\pm$ 0.05) at 734 nm. A 1.2 ml volume of the ABTS working solution was added to 100 µl of methanolic potato extract in disposable plastic cuvettes. The absorbance at 734 nm was measured and recorded within 1-5 min after combining the methanolic extract and the ABTS working solution.

3.2.11 DPPH

The DPPH antioxidant capacity was determined by comparing the measured absorbance of the samples read at 517 nm, to a Trolox standard curve. A standard curve was created, which related a known concentration of Trolox to absorbance. The method was adapted from Martinez-Valverda et al. (2002) (Schlesier, Harwat, Böhm, & Bitsch, 2002) and is based on the reduction of the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH). The DPPH solution (1 mM) was made using methanol and subsequently diluted to an absorbance between 0.9 - 0.5. From this working solution, 1.5 ml of DPPH stock was added to a glass tube containing 100 µl of the methanolic sample extract. The tubes were vortexed and then left to incubate at room temperature for 30 min in the dark. The samples were poured into disposable cuvettes and read at 517 nm. The absorbance was determined using a spectrophotometer, blanked to air and the absorbance of the DPPH stock solution was read.

3.2.12 Folin-Ciocalteau

Total phenolics were calculated by measuring the absorbance of the samples using a standard curve of chlorogenic acid dilutions with related absorbances. The methanolic extract (100 µl) was added into 2 ml of water. Next, 200 µl of Folin-Ciocalteau Reagent (2N) was added,

vortexed for 30 s and left to incubate at room temperature for 30 min in the dark. Then, 1 ml of aqueous sodium carbonate solution (7.5 %) was added, vortexed again, and incubated at RT in the dark for 1 h. Finally, the absorbance was read at 765 nm using a spectrophotometer against a methanol blank.

3.2.13 Ascorbic Acid and Polyphenolic Profile

The polyphenolic profiles (total chlorogenic acid, caffeic acid, ferulic acid, rutin) as well as ascorbic acid were measured for each cultivar using HPLC (Varian 9012, Varian Chromatography Systems, Walnut Creek, CA) adapted from the method of Shakya and Navarre (2006). The HPLC had a refrigerated auto-sampler, a single variable wavelength detector, and two solvent pumps. The samples were run at 280 nm and each replicate was run twice. To prepare the samples, each freeze dried replicate was weighed (50 mg) into a 1.5 ml Eppendorf tube and 900 µl of 90 % methanol was added with 0.5 mM meta-phosphoric acid and 0.02 mM EDTA. The samples were sonicated for 30 min at 4 ºC, then vortexed for 1 min and centrifuged at 1500 x g for 15 min at 4 ºC. The supernatant was collected and the pellet was re-extracted with 600 µl of 90 % methanol. The supernatant was extracted again and the supernatants combined and mixed. These samples were the filtered through a 0.2 µl Whatman Nylon filter into a clean glass HPLC vial using a 1 ml plastic syringe. Finally, the samples were sealed using a rubber-topped metal lid and were processed using HPLC. Two mobile phases were used: buffer A was 10 mM formic acid and buffer B was 5 mM ammonium formate. Individual standards for each polyphenol and ascorbic acid with various dilutions and known concentrations were used to create standard curves that related peak areas to concentrations. The phenolics as well as ascorbic acid were identified by comparing the peaks and retention times to that of a prepared standard mix containing the compounds of interest. From this curve, the concentration of each compound in the samples could be found by using the peak areas.

3.2.14 Statistical Analysis

Statistical processing was performed using R script. All datasets were analysed first by ANOVA to determine overall significance. Duncan's Multiple Comparison post hoc test was used to compare between potato cultivars for percent moisture content, starch characteristics, and protein content. Two-way ANOVA considering both genotypes and treatments, following by Tukey's

HSD test were done on all antioxidant data (ABTS, DPPH, and F-C) as well as HPLC results. Data are presented as mean ± standard error of the mean (SEM) and differences were considered significant at $P \leq 0.05$. Pearson's correlation was used to relate nutrient variables with starch digestibility. Correlations were also used to determine the relationship between phenolics, ascorbic acid, and antioxidant capacity. A multiple regression model was created to find one or more predictor values for GL. Different multiple regression methods such as forward, backwards, step-wise selection, AIC, and ANOVA, were used to create various models; the most significant and best performing models were then identified (Crawley, 2013; Gotelli & Ellison, 2013).

3.3 Results

3.3.1 Moisture Content

There were some genotypic differences in percent moisture; Kalmia (69.76 ± 0.473) had a greater moisture content than Fjord (62.58 ± 0.855) and FP3405 (58.72 ± 1.181) (Table 3.2). The RB somaclone FP3405 had a lower moisture content compared to approximately half of the other genotypes screened.

3.3.2 Starch Profile

When all the genotypes are compared with one another, the RB somaclone FP3405 (5.1847±0.200) had a greater RS content (g/150 g FM) compared to all other genotypes except for the Russet Burbank control (RBP), Kalmia, and Altitude (4.569±0.636, 3.726±0.304, and 3.631±0.441, respectively) (Table 3.3). The RBP control also had greater RS as compared to the other genotypes apart from Kalmia, Altitude, MP18405, Roselys, Péribonka, and MS1406. Kalmia had greater RS content than Fjord, Abeille, and Rebound (1.759±0.157, 0.562±0.426, and 1.244±0.214, respectively). Although there were some genotypic differences in RS content between the cultivars from Les Buissons, no difference in RS content between the breeding lines was observed. Similarly, no differences in RS content was observed among the RB somaclones.

Overall, differences in the DS content (g/150 g FM) was observed among genotypes (Table 3.3). The cultivar Rebound had more DS (98.756 ± 0.214) compared with over half of the genotypes screened. Abeille had a greater DS content (98.438±0.426) than all the genotypes except for

Roselys, MP18405, Altitude, Kalmia, RBP and FP3405 (96.620±0.217, 96.598±0.660, 96.370±0.441, 96.274±0.304, 95.431±0.944, and 94.815±0.304, respectively). The RB somaclone FP3405 had a lower DS content to all other genotypes except RBP, Kalmia, and Altitude. When specifically looking at the cultivars from Les Buissons; there were some differences in DS content, although there were no differences among the parental lines. Among the somaclones, FC2006 had a greater DS content (97.327±0.247) than RBP and FP3405. Also, MP18405 and MS1406 had a greater content than FP3405 although not in comparison to RBP.

There were differences between genotypes regarding the percent of rapidly digestible starch (Table 3.3). Overall, when all the genotypes were considered, the RB somaclone MS1406 (20.227±0.692) had a lower RDS content than RBP, Fjord, FC2006, Altitude and Envol (22.616±0.750, 27.122±0.544, 26.671±1.673, 26.034±2.064, and 25.999±1.185, respectively). Primevère, Péribonka, and Kalmia (21.148±2.127, 21.258±0.254, 21.425±0.266, respectively) all had a smaller RDS content than FC2006, Fjord, and RBP. The genotype with the largest RDS content was RBP, which was greater than FP3405, Roselys, MP18405, Kalmia, Péribonka, Primevère, and MS1406. Fjord was also greater than Kalmia, Péribonka, Primevère, and MS1406. Among the named cultivars from Les Buissons, there were no differences in RDS content, as well as among the breeding lines. Somatic differences were apparent as MS1406 had significantly less RDS than FC2006.

The RB somaclone FC2006 had a greater SDS percent content than over half of the other genotypes (Table 3.3). Envol and Fjord (26.233±0.880 and 25.976±0.792, respectively) had greater SDS percent content than the somaclones MP18405 and MS1406, and cv. Primevère (23.974±0.208, 21.702±2.083, and 20.4787±2.663, respectively). In terms of cultivars from Les Buissons; the only observed difference pertained to Envol and Fjord that had a greater percent SDS than Primevère. There were no differences among the breeding lines. Somatic differences were observed as FC2006 (27.770±0.602) a greater percent SDS than all other RB somaclones except for RBP (24.631±0.963).

3.3.3 Glycemic Index

Genotypes ranged between the “medium” and “high” GI classification (Table 3.3). Primevère had a lower GI (65.346±2.951) than Belle D'Août and Envol (74.859±3.623 and 78.275±2.380,

respectively). Also, MS1406, QP010090.05JP, and Roselys had a lower GI (67.190±1.373, 67.430±0.554, and 68.343±1.743, respectively) than Envol.

3.3.4 Glycemic Load

The genotypes were mainly classified as being high (more than 20), although two were considered medium (between 10 and 20), which were Kalmia and QP010090.05JP (Table 3.3). The genotypes Kalmia, QP010090.05JP, MS1406, MP18405, Altitude, and Primevère (18.836+0.214, 19.808+4.2.468, 20.1971+0.583, 20.884+0.692, 20.894+2.753, and 20.956+0.311, respectively) had a lower GL compared to Aquilon, Envol, FP3405, and Fjord (27.470+1.127, 28.969+0.207, 29.263+0.461, and 29.372+0.904, respectively).

3.3.3 Phosphorylated Starch

There was some genotypic difference regarding percent phosphorylated starch content (Table 3.2). Specifically, Fjord (0.065±0.006) was greater than FC2006, Envol, Roselys, MP18405, Rebound, Abeille, and Primevère (0.054±0.002, 0.053±0.002, 0.053±0.002, 0.052±0.001, 0.051±0.001, 0.051±0.002, and 0.047±0.001, respectively). The parental line QP010090.05JP (0.062±0.003) was also greater than MP18405, Rebound, Abeille, and Primevère. Regarding the named cultivars from Les Buissons; Primevère had lower percent phosphorylated starch content than Altitude, Aquilon, Belle D'Août, and Fjord. The parental lines were not different. There were no differences among the somaclones.

3.3.6 Amylose

Overall, when all the genotypes are compared for percent amylose content, the RB somaclone MS1406 (25.694±0.763) showed greater content than FC2006, Roselys, QP99165.81RF, and Kalmia (19.194±1.174, 16.055±1.502, 15.860±1.706, and 15.295±0.991, respectively) (Table 3.2). Also, FP3405, MP18405, Fjord, and Abeille all had greater amylose content than Roselys, QP99165.81RF, and Kalmia. When only cultivars were considered, the differences were the same as those mentioned above (Fjord and Abeille had more percent amylose than Roselys and Kalmia). There were no differences among the parental lines as well as no difference in percent amylose content among the somaclones.

3.3.7 Protein Content

Overall, FC2006, Fjord, and MP18405 (9.173±0.407, 9.079±0.053, and 8.639±0.905, respectively) had a greater protein content (g/150 g FM) than all other genotypes except for RBP and MS1406 (8.444±0.301 and 8.187±0.282, respectively) (Table 3.2). Fjord was clearly greatest in protein content among the Les Buissons genotypes, which were similar to RBP and the somaclones that had a relatively high protein content per serving.

3.3.8 Antioxidant Capacity Tests

3.3.8.2 ABTS

Overall, the ABTS values (mM Trolox Eq/100 g DM) for the raw samples showed that MP18405 and RBP (218.65±3.553 and 218.35±3.491, respectively) were greater than 8 genotypes: Aquilon, QP010090.05JP, Belle D'Août, Altitude, FP3405, Kalmia, Primevère, and Roselys (Table 3.4).

For the cooked samples, the ABTS value of Kalmia (200.80±4.490) was greater than MP18405, Rebound, Aquilon, FP3405, Fjord and Roselys (161.55±6.531, 156.32±1.545, 155.87±13.67, 149.12±6.178, 138.37±4.652, and 121.05±4.516, respectively) (Table 3.4). Also, the RB somaclone MS1406 had a greater ABTS value than the same genotypes listed above except for MP18405.

Within each of the 19 genotypes, there were no significant differences between the cooked and raw ABTS values except for the following; RBP, MP18405, FC2006, QP02258.03, Rebound, Kalmia, and Fjord (Table 3.4). Each genotype listed had a significantly larger ABTS value in raw compared with cooked samples, except for Kalmia. Kalmia was the only genotype with a significantly greater ABTS value in cooked compared to raw tissue samples.

3.3.8.1 DPPH

The raw DPPH values (mM Trolox Eq/100 g DM) of MP18405 and Rebound (334.00±20.91 and 315.37±22.14, respectively), were greater than all other genotypes except for QP99165.81RF, Envol, RBP, MS1406, and Aquilon (Table 3.4). Notably, Roselys had a small DPPH value

(27.79±2.162), which was lower than all genotypes expect for Altitude, FP3405, Primevère, and Kalmia.

Overall, the DPPH values of the cooked samples showed genotypic differences; Kalmia had a greater value (328.82±13.61) than Abeille, Roselys, MP18405, Rebound, FP3405, and Fjord. Additionally, Altitude and Belle D'Août (314.87±20.18 and 308.33±18.17, respectively) had a greater DPPH value than all the genotypes listed above except for Abeille.

When considering sample genotype and treatment as separate variables, eight of the genotypes had a significant difference between cooked and raw DPPH values (Table 3.4). Only two genotypes (Rebound and MP18405), showed a greater DPPH value in raw than in cooked samples. However, six of the genotypes (Altitude, Belle D'Août, Kalmia, Primevère, Roselys, and the parental line QP010090.05JP) had a DPPH value that was significantly higher in cooked than raw tissue.

3.3.8.3 Folin-Ciocalteau

The F-C test (mg chlorogenic acid Eq/100 g DM) done on raw samples showed genotype differences; the RB somaclone MS1406 (549.64±34.04) had a greater value than all other genotypes except for Envol, and QP99165.81RF (131.62±21.97 and 251.12±50.01, respectively) (Table 3.4). Furthermore, Envol, QP99165.81RF, and RBP had a greater F-C value than FP3405, Primevère, Altitude, and Roselys.

Overall, the F-C values in cooked samples also showed that the RB somaclone MS1406 had a greater antioxidant content compared to other genotypes (QP99165.81RF, FC2006, Primevère, RBP, Rebound, Altitude, FP3405, Fjord, Envol, Roselys, and Abeille). Kalmia also had a greater F-C value than the same genotypes in the above list except for QP99165.81RF and FC2006. Abeille had a small F-C value compared to the genotypes MS1406, Kalmia,Péribonka, and QP02258.03.

There were only 4 genotypes where there was a significant difference between the F-C cooked and raw values (Table 3.4). Envol, QP99165, RBP, and the RB somaclone MS1406 all had a notably larger F-C value in raw compared to cooked samples.

3.3. 9 Ascorbic Acid and Polyphenolic Profile Using HPLC

The somaclone FP3405 had the largest raw ascorbic acid content (141.142 ± 20.5700 mg/150 g FM) which was greater than over half of the genotypes screened (Table 4.5). Additionally, RBP, Péribonka, MS1406 (130.840±3.560,123.647±5.139, and 121.533±9.048, respectively) had a greater raw ascorbic acid content than Roselys, Rebound, Kalmia, and Primevère (72.157±0.192, 70.760±9.119, 68.231±1.781, and 67.263±9.289, respectively).The breeding line QP33165.81RF (1116.523±1.788) was also greater than Primevère.

The cooked ascorbic acid content showed differences between genotypes. The RB somaclone MS1406 (152.858±4.037) was greater than all other genotypes except for FC2006, QP010090.05JP, QP99165.81RF, Belle D'Août and Aquilon. The somaclone FC2006 (114.914±8.249) was greater than Primevère, Rebound, Abeille and Kalmia. There were no significant differences in ascorbic acid content between cooked and raw samples

A wide range of variation was observed when comparing the raw values for chlorogenic acid (mg/150 g FM) profiles between genotypes (Table 3.5). The parental line, QP99165.81RF (79.9781±2.023) had a greater raw content of chlorogenic acid than Altitude, Belle D'Août, Kalmia, Primevère, and Roselys (47.882±7.162, 46.958±2.973, 35.742±2.204, 33.692±11.98, and 25.123±.617, respectively). Also, the RB somaclone FP3405, MS1406, RBP and the cv. Péribonka (72.993±7.695, 72.094±4.584, 72.001±0.271, and 69.990±7.959, respectively) had greater content of chlorogenic acid than the same genotypes listed above, although not including Altitude, and Belle D'Août. There were no differences between the cooked content of chlorogenic acid between genotypes. The chlorogenic acid content in raw potatoes was significantly higher than cooked samples for the following genotypes: Abeille, Aquilon, Envol, Fjord, Péribonka Rebound, QP01009.05JP, QP02258.03, QP99165.81 and the somaclones FC2006, FP3405, MS1406, as well as RBP.

There were no differences among genotypes regarding raw content of caffeic acid (mg/150 g FM) (Table 3.6). The cooked samples did show some genotypic differences; Roselys (14.129+0.310) had a greater content than half of the genotypes. The breeding line QP99165.81RF (13.875±5.022) had greater content of caffeic acid than MS1406, Primevère, Péribonka, Abeille, QP02258.03, Rebound, and Kalmia (5.474±0.680, 5.251±1.731,

4.903±0.094, 4.320±0.010, 4.024±0.038, 3.975±0.096, and 3.712±0.158, respectively). Furthermore, the RB somaclone FC2006, Aqulion and Altitude all had a greater cooked content of caffeic acid than QP02258.03, Rebound, and Kalmia. The only genotype to show a difference between the cooked and raw treatments was Roselys. In this case the cooked sample was significantly higher in caffeic acid content than the raw sample.

The raw samples of ferulic content did not differ between genotypes, unlike with the cooked samples (Table 3.6). Specifically, the breeding line QP99165.81RF (23.263±6.028) had a greater cooked ferulic acid content than all other genotypes except for Belle D'Août, Fjord, Altitude, Roselys, QP010090.05JP, Envol and Primevère. Belle D'Août (21.822±5.907) was also greater than all other genotypes except for those listed above, although including Kalmia (10.229±0.670). Fjord was only greater than RBP and Péribonka (4.305±0.984 and 1.696±0.456). Only the cultivars Belle D'Août and Fjord had significantly lower raw content of ferulic acid compared to cooked samples.

The raw content of rutin did not differ among genotypes (Table 3.6). The cooked values for rutin did show difference among the genotypes; QP99165.81RF was greater than Aquilon, Envol, QP010090.05JP, Abeille and Primevère (5.052±0.355, 4.423±1.512, 3.925±2.629, 2.341±0.976, and 1.044±0.338, respectively). The only other difference was that Rebound (13.444±0.042) had greater content of rutin than Primevère. The cooked content for rutin was significantly larger than raw sample type for the breeding line QP99165.81, but no other genotypes showed any differences.

3.3.11 Antioxidant and HPLC Correlations for Raw and Cooked Potato Samples

There were significant correlations among the content of phenolics as measured using HPLC for the raw potato samples (Table 3.7). Ascorbic acid (AA) content was highly correlated to chlorogenic acid (CGA) content (r=0.7532, p=2.776e-10) and to the sum of CGA, caffeic (CFF), ferulic (FER) and rutin (RUT) (T-CCFR) (r=0.6483, p=2.692e-07). AA was the only phytonutrient that was significantly correlated (negatively) to the F-C method (r=-0.2942, p=0.0472). The CGA content was significantly correlated to CFF (r=0.3257, p=0.0210). The CGA content was also correlated to the T-CCFR, the sum of CFF, FER and RUT (T-CFR) and the sum of AA, CGA, CFF, FER, and RUT (T-ACCFR), most likely because CGA was included

in the calculations and/or CF was also included which already showed a significant correlation. The CGA content showed no significant correlations to any of the antioxidant spectrophotometric tests. The CFF content showed a significant correlation with two of the phytonutrients measured; CFF was highly correlated to FER (r=0.8182, p=2.181e-12) and RUT (r=0.5873, p =7.348e-06). FER was also significantly correlated to RUT (r=0.4962, p = 0.0005). The antioxidant capacity test of DPPH was only correlated significantly to RUT (r =0.2791, p=0.0497). The antioxidant spectrophotometric ABTS test showed no significant correlation to any individual polyphenols or combined sums such as T-CCFR, T-CFR, and T-ACCFR.

As with the raw potato samples, the total AA content for cooked potatoes showed a significant correlation to CGA (r=0.6313, p= 3.311e-06) and T-CCFR (r=0.4551, p=0.0012) (Table 3.8). Additionally, AA was correlated to T-ACCFR, most likely due to the inclusion of AA itself and CGA, which was already shown to be highly correlated. As with the raw samples, the CGA content in cooked potato samples showed no significant correlation with any of the antioxidant capacity tests or with any of the other individual phenolics; only with AA. As with the raw potato samples, the CFF content of cooked samples was significantly correlated to FER (r=0.3148, p =0.0230) and RUT (r=0.3641, p=0.0080), but not the other phenolics. Like CFF, FER was correlated to T-CCFR and T-CFR although not T-ACCFR.

The antioxidant capacity tests, DPPH, ABTS and F-C, showed no correlations to any individual polyphenols or to the sums T-CCFR, T-CFR and T-ACCFR. Unlike in the raw potato samples, DPPH was not related to rutin content. However, DPPH and F-C were negatively correlated with one another (r= -0.3033, p=0.0361).

3.3.12 Starch Correlations

GL showed no correlations to percent moisture or content of protein, amylose and phosphorylated starch, RS, DS, RDS, and SDS, although it was highly correlated to GI (r=0.4753, p=0.0003) (Table 3.9). The GI also showed no correlations to the above, except that it was correlated negatively to protein content (r=-0.3269, p=0.0169). There were no correlations within the following; % moisture, protein, amylose, and phosphorylated starch content, RS, DS, RDS, and SDS, except that RDS and SDS were correlated together (r=0.6947, p=1.456e-09).

GL was negatively correlated to the ascorbic acid content of cooked potato samples (r= -0.3770, p= 0.0098) and also negatively related to the T-ACCFR (r=-0.3314, p=0.0154), although the sum correlation is most likely due to the inclusion of the ascorbic acid content (Table 3.10). The GL was not correlated to any individual phenolics, phenolic sums (T-CCFR, T-CFR, T-ACCFR), or antioxidant capacity tests (DPPH, ABTS, F-C) for cooked samples. GI was correlated to the antioxidant capacity test ABTS (r=0.2794, p=0.0321) when cooked samples were used. GL was not correlated to any individual phenolics, phenolic sums (T-CCFR, T-CFR, T-ACCFR) or antioxidant capacity tests (DPPH, ABTS, F-C) regarding raw sample types. The GI was only correlated with the raw CGA content (r=0.3352, p=0.0228).

3.3.13 Predictive Models for GL

Out of the many possible models that were created, 3 were selected that best defined GL (Table 3.11).

Model 1 → GL = % moisture + GI + RS + FER (cooked) + DPPH (cooked)

The first model, created using stepwise forward selection and ANOVA comparisons, defines GL as a linear combination of: percent moisture content, GI, resistant starch, ferulic acid content (cooked) as well as DPPH results (cooked samples only). These variables can be used to best predict and explain values of GL. The model explains 96 % of the variance observed with a p value of 8.581 x 10^-9 and F- statistic of 66.39. Additionally, when the model is tested under criticism, notably the normal Q-Q plot and residual VS fitted plot indicate that the model can be accepted.

Model 2 → GL = % moisture + GI + FER (cooked)

The second model that was selected, which was created using backward selection and an updating model function, was that GL is defined as a linear combination of: percent moisture content, GI, and ferulic acid (cooked) content. This model explained up to 91 % of the variance in GL with a p value of 4.564 x 10^-8 and F- statistic of 50.39. This model is very similar to model 1, although it does not include DPPH results (cooked samples), and RS. When model 2 is tested, the normal Q-Q plot, residual VS fitted plot and ANOVA analysis indicate that the model can be accepted and that all explanatory factors are relevant.

Model 3 → GL = % moisture + GI +Amylose + RDS + SDS + AA (raw) + CGA (raw) + CFF (cooked) + FER (cooked) + F-C (cooked)

The third model which was designed using AIC showed that GL can be predicted by the following explanatory variables: % moisture, GI, amylose content, RDS, SDS, the ascorbic acid and chlorogenic acid contents (raw samples), ferulic acid content (cooked), as well as the F-C result (cooked samples). This model explains 98 % of the variance in GL and has a p value of 2.152 x 10^-5 and F statistic of 32.52. When model 3 is tested the model can be accepted, although it is not as justifiable as the first two models. This is because the normal Q-Q plot, residual VS fitted plot and ANOVA analysis indicate that the model results do not behave as ideally as the above models.

3.3.9 Discussion

Selecting superior potato genotypes based on phytonutrient status can promote human health both in the short and long term (Bach et al., 2013; Camire et al., 2009; Vunnam, 2010). The optimal profile would include a desirable starch quality (high RS, low DS [where the ratio of SDS:RDS has a greater SDS content], low GI and GL, greater amount of phosphorylated starch and amylose content), as well as a greater quantity of ascorbic acid, greater antioxidant capacity and optimal phenolic profiles.

Between the registered cultivars from Les Buissons, Kalmia was the most nutritive genotype. This is largely due to having one of the lowest GL (18.836$\pm$0.214) and highest RS content (3.7258$\pm$0.3038 g/150 g FM), but also because it has both relatively high phenolic content and antioxidant capacity (Table 3.3, 3.4 & 3.6). Specifically, it had one of the highest ferulic acid contents as well as a greater ABTS, DPPH and F-C (for cooked samples) compared to the other cultivars. Kalmia could be commercially promoted as a relatively healthier cultivar. Consumers can be made more aware of the impact that starch quality and antioxidant capacity can have and its potential to provide significant health benefits. Kalmia does have a lesser protein content (2.784$\pm$0.112 g/150 g FM) than other cultivars, but it was not the least, nor is it significantly less than the range for potatoes in the literature (Ortiz-Medina et al., 2009). The superior characteristics determined for this cultivar are supported by the results of regression model 1 (GL

= % moisture + GI + RS + FER (cooked) + DPPH (cooked)). In summary, this cultivar had a low GL, which can be explained by its high RS, ferulic acid (cooked) and DPPH (cooked) content. The characteristics also support model 2.

Regarding the breeding lines from Les Buissons, QP010090.05JP was the best performing genotype overall. This was because it had a lower GL (19.808±2.468) and GI (67.430±0.554) compared to other breeding lines and cultivars, as well as a higher quantity of phosphorylated starch, which has previously been shown to decreased starch digestion, although this variable was not critical in any of our three proposed models (Ek et al., 2012; Sitohy & Ramadan, 2001) (Table 3.3). Cooked samples from the breeding line QP010090.05JP also showed a significant amount of ascorbic acid and phenolics (chlorogenic acid, caffeic acid, and rutin). This line therefore showed potential for further breeding activities because of its lower glycemic impact and greater phenolic content that can improve the nutritional value of future breeding lines of potatoes. This breeding line also illustrates the importance of GI in our 3 models; clearly, a lower GI predicts lower GL.

The somaclone that performed the best was MS1406. Regarding starch quality, this genotype had lower GL (20.197±0.583) and GI (67.189±1.373) compared to other genotypes, and more phosphorylated starch, lower RDS, and one of the highest amylose contents (25.694±0.763 %) (Tables 3.2 & 3.3). Also, this genotype had relatively high ascorbic and chlorogenic acid content (for both raw and cooked samples), and a fair quantity of rutin (cooked). The antioxidant capacity tests also showed that it had among the highest values for ABTS and F-C tests for both raw and cooked samples. This somaclone presents many possibilities; it can go directly to the market and be sold as a superior cultivar, it can be used as the starting material for continued somatic breeding, and could be suggested as an alternate to Russet Burbank from which it was originally derived for industrial use. The best performing characteristics of MS1406 support linear model 3 (GL= % moisture + GI + amylose + RDS + SDS + AA (raw) + CGA (raw) + CFF (cooked) + FER (cooked) + F-C (cooked)). Specifically, the low GL of MS1406 can be partly explained by the genotype's low GI, high amylose content, low RDS, its relatively high AA and CGA content and low F-C value. The models that were created used a complete database of results from 19 genotypes. It is therefore encouraging that the top 3 best performing genotypes

confirmed and supported the resulting models that were selected. This data provides support that the models will have future merit, although this remains to be further investigated.

For the most part, there appears to be no general trend or specific genotypes that conform to a pattern, regarding differences in ascorbic acid, phenolic content, or antioxidant capacity between raw and cooked samples (Table 3.4, 3.5, & 3.6). Of the few genotypes that did show some difference, the majority of genotypes showed greater antioxidant capacity and polyphenolic content, in raw compared with cooked samples. There was no difference between raw and cooked sample values for ascorbic acid content for all genotypes. Of the genotypes that showed any difference, 13 genotypes showed greater chlorogenic acid content in raw compared with cooked samples, the caffeic acid content was greater in cooked samples (although only for one cultivar; Roselys), ferulic acid was also greater in cooked samples (for only 2 genotypes; Belle D'Août and Fjord) and for rutin, a raw sample was greater than the cooked value only for one genotype (QP99165.81). The DPPH and F-C tests showed that the values for cooked samples were greater than raw samples, but for only 2 and 6 genotypes, respectively. ABTS was the exception; 6 genotypes had greater raw values, whereas only 1 genotype had greater cooked values.

The above results clearly show that the relationship between the antioxidant capacity and phenolic contents of raw versus cooked samples has not been fully established, which is supported by similar contradictory results in the literature. In some cases, the phenolic content of cooked potatoes was greater than in raw potatoes (Burgos et al., 2013) whereas others reported no change (Blessington et al., 2010) or decreased antioxidant capacity (Faller & Fialho, 2009). The lack of difference between sample type clearly showed that type essentially depends on genotype.

Interestingly, for both raw and cooked samples, ascorbic acid was correlated to chlorogenic acid ($r=0.7532$, $p=2.77e-10$, and $r=0.6313$, $p=3.311e-06$ respectively), although neither were correlated to any of the antioxidant capacity tests except for the raw content of ascorbic acid, which was negatively correlated to F-C ($r=-0.2942$, $p=0.0472$) (Table 3.7 & 3.8). Regarding the genotypes that were used for this trial, we did not see any significant trends regarding ascorbic acid and phenolics (individually or summatively). No correlations were found for any of the

antioxidant capacity tests except that rutin was correlated to DPPH for raw samples (r=0.2791, p=0.0497). This questions the reliability of using ABTS, DPPH, or F-C as a method of screening for individual phenolics, which our previous publications, and that of others, have suggested. The present findings imply that such correlations could also be genotype specific, and that perhaps side reactions of different sample sets cause significant changes regarding correlation results. In other words, due to the difference in genotypes, the degree of side reactions can change the final antioxidant capacity outcomes, and therefore affect the correlations between individual polyphenolics and antioxidant tests.

Regarding starch correlations, there were none to be found with amylose, RS, and phosphorylated starch in terms of GI and GL. Apart from phosphorylated starch, the above variables were included in the statistical models to predict GL. All of the predictive models that were selected are significant and can, for the most part, explain some of the results of the best performing genotypes. Notably, the inclusion of GI and percent moisture in every model suggests that these factors are important in predicting GL. In model 1, RS, DPPH (cooked) and ferulic acid (cooked) are also included. This latter observation is supported by previous results, which suggest that the more RS content, the less starch digestibility and therefore a lower GI and GL (Bach et al., 2013; Ek et al., 2012). Additionally, phenolics are thought to inhibit α-amylase, which can explain why ferulic acid, and therefore DPPH, is included into the model (McDougall & Stewart, 2005). The second model, which only included percent moisture, GI, and ferulic acid was also valid, due to similar explanations as above.

Model 3 (GL= % moisture + GI + amylose + RDS + SDS + AA (raw) + CGA (raw) + CFF (cooked) + FER (cooked) + F-C (cooked)), which includes a large number of explanatory variables, is also significant and is supported by previous literature results. Retrograded amylose forms RS, which in turn is not digested and hence does not contribute towards increasing GL. Previous reports had shown that there is a strong positive correlation between RS and amylose content (Ek et al., 2012; Nayak et al., 2014), although some findings showed no relationship to GI (Ek et al., 2014). It is important to note that correlations do not indicate causation, nor can they act as predictor values. The regression model allows elucidation of explanatory variables, such as amylose content, for GL. Correlations between predictor variables and the dependant variable such as GL is undesirable (multicollinearity). It is therefore difficult to complete the

model without bias and identify the unique contribution(s) of each predictor variable in the final model (Murray & Conner, 2009). We found that amylose did not correlate with GI or GL, but can still be used as an indicator of GL. This model also included AA (raw), CGA (raw), CFF (cooked) and FER (cooked). The inclusion of phenolics has been previously explained; however, there is a lack of evidence regarding the affect(s) of ascorbic acid on starch digestion, which needs further investigation. All three models thus present the possibility of using other characteristics to help select healthier genotypes in terms of glycemic impact either during a screening process, determining superior breeding lines or also for market sales. For example, a genotype that showed high percent moisture content, as well as high ferulic and DPPH content, has the potential to have one of the lowest GL, without having to complete all the necessary lab work to elucidate a GL value.

Overall, from all three models, it is clear that using cooked samples as the basis for GL predictions is merited and use of raw data is less accurate in predicting starch quality so that using cooked samples is important.

Further confirmation of these models is now the next logical step before one or more of these models are suggested for routine use. Using a new dataset for the phytonutrients tested in this thesis (preferably on a large enough population of potatoes, for at least 2 seasons), a prediction of genotypes with lower GL could be done with the use of the model systems and then validated. Additionally, the current predictive model(s) for GL could be confirmed and refined, with models that are developed from the new dataset.

Table 3.1. List of genotypes, including 11 named cultivars and 3 parental lines (QP02258.03, QP01009.05JP, and QP99165.81RF) from the Quebec Potato Breeding Program at Les Buissons and 4 advanced somatic lines (FC2006, MS1406, MP18405, and FP3405) and control (RBP) from McGill's somatic breeding program.

Genotypes	Les Buissons Breeding Program	McGill Somatic Breeding Program
Abeille	√	
Altitude	√	
Aquilon	√	
Belle D'Août	√	
Envol	√	
Fjord	√	
Kalmia	√	
Péribonka	√	
Primevère	√	
Rebound	√	
Roselys	√	
QP02258.03	√	
QP01009.05JP	√	
QP99165.81RF	√	
RBP		√
FC2006		√
FP3405		√
MP18405		√
MS1406		√

Table 3.2. Moisture content (%), phosphorylated starch (%), amylose (%) and protein content (g/150 g FM) of genotypes screened.

Genotypes	Moisture (%)	Phosphorylated starch (%)	Amylose (%)	Protein (g/150 g FM)
Abeille	65.006 ± 0.602^{ab}	0.051 ± 0.002^{ef}	22.246 ± 1.132^{ab}	5.396 ± 0.690^{de}
Altitude	68.809 ± 3.614^{ab}	0.060 ± 0.000^{abcde}	20.256 ± 1.534^{abcde}	4.486 ± 0.448^{efg}
Aquilon	63.001 ± 2.550^{abc}	0.059 ± 0.003^{abcde}	20.170 ± 0.608^{abcde}	6.190 ± 0.382^{cd}
Belle D'Août	64.913 ± 0.939^{abc}	0.057 ± 0.002^{abcde}	20.619 ± 0.196^{abcde}	5.183 ± 0.423^{def}
Envol	64.967 ± 0.273^{abc}	0.053 ± 0.002^{bcdef}	21.627 ± 0.550^{abcd}	3.524 ± 0.316^{gh}
Fjord	62.580 ± 0.855^{bc}	0.065 ± 0.006^{abcde}	22.516 ± 0.778^{ab}	9.079 ± 0.053^{a}
Kalmia	69.736 ± 0.473^{a}	0.055 ± 0.003^{abcdef}	15.295 ± 0.991^{e}	2.784 ± 0.112^{h}
Péribonka	67.432 ± 0.807^{ab}	0.056 ± 0.004^{abcdef}	21.275 ± 1.314^{abcd}	0.581 ± 0.043^{i}
Primevère	64.747 ± 4.536^{abc}	0.047 ± 0.001^{f}	21.821 ± 2.083^{abc}	3.798 ± 0.839^{fgh}
Rebound	66.022 ± 0.581^{ab}	0.051 ± 0.001^{def}	21.413 ± 1.025^{abcd}	6.315 ± 0.502^{cd}
Roselys	64.471 ± 2.003^{abc}	0.053 ± 0.002^{bcdef}	16.055 ± 1.502^{cde}	4.918 ± 0.428^{defg}
QP010090.05JP	65.163 ± 5.051^{abc}	0.062 ± 0.003^{ab}	20.556 ± 2.575^{abcde}	5.580 ± 0.328^{de}
QP02258.03	65.974 ± 0.319^{ab}	0.058 ± 0.002^{abcde}	19.990 ± 0.562^{abcde}	3.639 ± 0.691^{gh}
QP99165.81RF	64.394 ± 1.704^{abc}	0.061 ± 0.002^{abcd}	15.860 ± 1.706^{de}	5.656 ± 0.257^{de}
RBP	67.643 ± 0.570^{ab}	0.060 ± 0.001^{abcde}	21.088 ± 1.684^{abcd}	8.444 ± 0.301^{ab}
FC2006	66.745 ± 0.110^{ab}	0.054 ± 0.002^{bcdef}	19.194 ± 1.174^{bcde}	9.173 ± 0.407^{a}
FP3405	58.716 ± 1.181^{c}	0.062 ± 0.003^{abc}	23.937 ± 1.702^{ab}	7.147 ± 0.454^{bc}
MP18405	68.730 ± 0.903^{ab}	0.052 ± 0.001^{cdef}	23.051 ± 2.755^{ab}	8.639 ± 0.905^{a}
MS1406	68.400 ± 0.494^{ab}	0.062 ± 0.004^{abc}	25.694 ± 0.763^{a}	8.187 ± 0.282^{ab}

Values expressed as means ± SEM. Data arranged first by cultivars and breeding lines from Les Buissons, then somatic lines from McGill. Within each category, data was arranged by alphabetical order. Means with same superscript letter in the same column are not significantly different. Means were compared using Duncan's Multiple Comparison ($P \leq 0.05$).

Table 3.3. The content and percent of RS, DS, RDS, and SDS and the GI and GL values of genotypes screened.

Genotypes	RS (g/150 g FM)	DS (g/150 g FM)	RDS (%)	SDS (%)	GI	GL
Abeille	1.5623±0.4263^{ef}	98.4377±0.4263^{ab}	22.8110±0.1560^{abcd}	23.6228±0.098^{bcdef}	69.373±0.223^{abc}	24.946±0.233^{abcd}
Altitude	3.6307±0.4410^{abc}	96.3694±0.4410^{def}	26.0336±2.064^{abc}	23.1010±0.4737^{cdef}	71.324±2.035^{abc}	20.894±2.753^{d}
Aquilon	2.2947±0.5039^{cdef}	97.7053±0.5039^{abcd}	24.7537±1.731^{abcd}	25.1647±1.008^{abcde}	71.654±1.175^{abc}	27.470±1.127^{abc}
Belle D'Août	2.5107±0.2008^{cdef}	97.4893±0.2008^{abcd}	25.4030±0.5191^{abcd}	24.6203±0.9293^{abcde}	74.859±3.624^{ab}	24.962±1.351^{abcd}
Envol	2.1138±0.372^{cdef}	97.8862±0.3792^{abcd}	25.9985±1.185^{abc}	26.2329±0.8802^{abc}	78.275±2.380^{a}	28.969±0.207^{ab}
Fjord	1.7591±0.1565^{def}	98.2409±0.1917^{abc}	27.1215±0.5441^{ab}	25.9760±0.7923^{abc}	73.662±0.430^{abc}	29.372±0.904^{a}
Kalmia	3.7258±0.3038^{abc}	96.2742±0.3038^{def}	21.4245±0.2658^{cd}	24.1619±0.9313^{abcdef}	70.546±2.577^{abc}	18.836±0.214^{d}
Péribonka	3.1453±0.5593^{bcde}	96.8547±0.5593^{bcde}	21.2577±0.2537^{cd}	22.3226±0.0990^{cdef}	69.117±1.185^{abc}	22.067±0.311^{cd}
Primevère	2.4069±0.5421^{cdef}	97.5931±0.5421^{abcd}	21.1480±2.127^{cd}	20.4787±2.663^{f}	65.346±2.951^{c}	20.956±1.744^{d}
Rebound	1.2437±0.2139^{f}	98.7563±0.2139^{a}	24.4699±0.9572^{abcd}	25.0789±1.004^{abcde}	69.767±1.165^{abc}	24.871±0.278^{abcd}
Roselys	3.3801±0.2166^{bcd}	96.6199±0.2166^{cde}	22.2092±0.9474^{bcd}	22.4389±0.5974^{cdef}	68.343±1.743^{bc}	23.767±0.526^{abcd}
QP010090.05JP	2.5495±0.1211^{cdef}	97.4505±0.1211^{abcd}	24.5547±2.087^{abcd}	23.5173±0.4924^{bcdef}	67.430±0.554^{bc}	19.808±2.468^{d}
QP02258.03	2.3269±0.4363^{cdef}	97.6731±0.4363^{abcd}	24.2567±0.5602^{abcd}	25.8224±0.5389^{abcd}	73.662±1.683^{abc}	24.733±0.516^{abcd}
QP99165.81RF	2.6305±0.1248^{cdef}	97.3695±0.1248^{sbcd}	23.6821±1.993^{abcd}	23.0477±1.443^{cdef}	72.252±0.870^{abc}	24.553±0.865^{abcd}
RBP	4.5686±0.6363^{ab}	95.4314±0.9438^{ef}	22.6157±0.7497^{abcd}	24.6307±0.9627^{abcde}	70.212±0.390^{abc}	22.112±0.582^{bcd}
FC2006	2.6734±0.268^{cdef}	97.3266±0.2468^{abcd}	26.6713±1.673^{ab}	27.7693±0.6202^{a}	70.475±1.512^{abc}	22.987±1.005^{abcd}
FP3405	5.1847±0.1995^{a}	94.8153±0.3038^{f}	22.3088±0.5734^{bcd}	23.5398±0.4663^{bcdef}	70.190±0.109^{abc}	29.263±0.461^{ab}
MP18405	3.4017±0.6598^{bed}	96.5983±0.6598^{cde}	22.1154±0.4847^{bcd}	21.9735±0.2081^{def}	69.268±1.146^{abc}	20.884±0.692^{d}
MS1406	3.0971±0.2533^{bcde}	96.9029±0.2533^{bcde}	20.2269±0.6918^{d}	21.7019±2.083^{ef}	67.189±1.373^{bc}	20.197±0.583^{d}

Values expressed as means ± SEM. Data arranged first by Les Buissons cultivars and breeding lines then McGill control and somatic lines. Within each category, data was arranged in alphabetical order. Means with the same superscript letter in the same column are not significantly different. Means were compared using Duncan's Multiple Comparison ($P \leq 0.05$).

Table 3.4. Antioxidant capacity values for all genotypes.

	ABTS		DPPH		F-C	
Genotypes	Raw	Cooked	Raw	Cooked	Raw	Cooked
Abeille	189.62±14.95^{abcdefg}	174.66 ±0.8733^{bcdefghij}	205.53±26.42^{cdefg}	213.59±25.94^{bcdefg}	89.89±4.662^{cdefghi}	338.39 ± 40.38^{i}
Altitude	158.87±7.394^{ghijk}	171.07± 18.24^{cdefghij}	128.64±22.56^{ghi}	314.87±20.18^{ab}	182.13±16.21^{fghi}	236.19±34.33^{ghi}
Aquilon	182.90±10.53^{bcdefghi}	155.87± 13.67^{ghijk}	240.66±14.87^{abcdefg}	255.86±29.50^{abcdef}	258.32±19.79^{bcde}	538.16±28.76^{defghi}
Belle D'Août	161.70±2.902^{fghijk}	184.26 ± 14.51^{abcdefgh}	136.28±16.24^{gh}	308.33±18.17^{abc}	380.77±59.57^{cdefghi}	296.84±33.29^{bcdefghi}
Envol	200.53±4.663^{abcde}	163.85± 3.910^{efghijk}	274.64±5.338^{abcd}	267.39±16.66^{abcde}	131.62±21.97^{ab}	649.24±60.17^{hi}
Fjord	187.57±3.543^{abcdefg}	138.37± 4.652^{ijk}	141.23±12.98^{gh}	88.34±3.900^{hi}	133.91±8.374^{bcdefgh}	440.80±39.78^{hi}
Kalmia	150.52±0.9467^{ghijk}	200.80± 4.490^{abcde}	64.96±11.22^{hi}	328.82±13.61^{a}	537.23±59.19^{bcdefgh}	441.58±44.24^{bcde}
Péribonka	204.21±7.905^{abcd}	189.88± 6.906^{abcdefg}	166.78±11.66^{efgh}	258.85±14.81^{abcde}	453.51±42.82^{bcdefgh}	430.38±0.7406^{bcdefg}
Primevère	144.48±5.677^{hijk}	184.15±2.459^{abcdefghi}	67.66±3.844^{hi}	288.54±2.234^{abcd}	235.43±29.65^{defghi}	252.12±54.06^{fghi}
Rebound	207.96±4.302^{abcd}	156.32±1.545^{ghijk}	315.37±22.14^{a}	159.16±7.015^{efgh}	219.79±27.35^{bcdef}	479.08±64.54^{fghi}
Roselys	140.72±2.694^{ijk}	121.05±4.516^{k}	27.79±2.162^{i}	202.81±38.04^{defg}	124.96±25.69^{hi}	158.09±48.40^{hi}
QP010090.05JP	181.80±2.931^{bcdefghi}	170.02±3.030^{defghij}	146.83±18.13^{fgh}	269.33±19.08^{abcde}	309.18±28.84b^{cdefgh}	417.78±25.34^{cdefghi}
QP02258.03	208.97±9.307^{abc}	166.20±5.478^{efghijk}	204.81±14.25^{cdefg}	286.99±15.54^{abcd}	444.74±41.49^{bcde}	542.65±18.50^{bcdefgh}
QP99165.81RF	210.59±4.429^{ab}	188.55±3.615^{abcdefg}	282.64±7.366^{abcd}	281.27±6.107^{abcd}	251.12±50.01^{ab}	633.49±48.50^{efghi}
RBP	218.35±3.491^{a}	167.36±4.318^{efghij}	273.48±7.828^{abcd}	249.51±10.21^{abcdef}	234.55±42.70^{b}	582.69±54.92^{fghi}
FC2006	210.98±5.732^{ab}	172.52±1.629^{cdefghij}	222.82±6.580^{bcdefg}	293.62±5.277^{abcd}	237.83±8.366^{bcdefg}	450.53±19.60^{efghi}
FP3405	153.20±3.864^{ghijk}	149.12±6.178^{ghijk}	81.31±5.541^{hi}	96.29±3.522^{hi}	158.14±18.59^{defghi}	256.63±49.39^{ghi}
MP18405	218.65 ± 3.553^{a}	161.55±6.531^{fghijk}	334.00±20.91^{a}	165.92±1.241^{efgh}	311.44±53.66^{bc}	580.73±69.18^{cdefghi}
MS1406	213.41±0.7407^{ab}	196.20±2.931^{abcdef}	244.37±14.34^{abcdef}	241.33±10.70^{abcdefg}	549.64±34.03^{a}	835.48±57.32^{bcd}

Values expressed as means ± SEM. Data arranged first by Les Buisson cultivars and breeding lines then McGill control and somatic lines. Within each category, data was arranged by alphabetical order. Means with same superscript in the same column are not significantly different. Means were compared using Tukey HSD ($P \leq 0.05$). Units expressed as mM Trolox Eq/100 g DM (DPPH, ABTS) and mg chlorogenic acid Eq/100 g DM (F-C).

Table 3.5 High Performance Liquid Chromatography (HPLC) values for ascorbic acid and chlorogenic acid for all genotypes.

	Ascorbic Acid		Chlorogenic Acid	
Genotypes	Raw	Cooked	Raw	Cooked
Abeille	89.428±0.326^{cdefg}	40.642±7.468^{gh}	63.929±6.869^{abcd}	6.776±0.970^{h}
Altitude	88.574±2.662^{cdefg}	95.154±3.210^{bcdefg}	47.882±7.162^{bcdef}	16.646±5.322^{fgh}
Aquilon	107.811±3.486^{abcdef}	110.019±15.172^{abcdef}	61.607±11.823^{abcd}	9.468±0.193^{h}
Belle D'Août	93.835±2.583^{bcdefg}	110.321±4.776^{abcdef}	46.958±2.973^{bcdefg}	31.262±1.348^{defgh}
Envol	106.773±35.25^{abcdef}	93.588±6.135^{bcdefg}	53.699±18.291^{abcde}	7.970±1.657^{h}
Fjord	91.277±0.422^{bcdefg}	88.242±5.481^{cdefg}	68.694±1.434^{abc}	25.093±0.067^{efgh}
Kalmia	68.231±1.781^{defgh}	32.665±1.243^{h}	35.742±2.204^{cdefgh}	17.065±4.550^{fgh}
Péribonka	123.647±5.139^{abc}	72.716±4.696^{cdefgh}	69.990±7.959^{ab}	10.714±1.822^{h}
Primevère	67.263±9.289^{efgh}	59.270±10.875^{fgh}	33.692±11.98^{defgh}	17.243±1.822^{efgh}
Rebound	70.760±9.119^{defgh}	57.393±14.109^{fgh}	61.945±4.064^{abcd}	7.944±1.415^{h}
Roselys	72.157±0.192^{defgh}	66.591±4.723^{efgh}	25.123±1.617^{efgh}	7.439±0.379^{h}
QP010090.05JP	90.843±4.549^{cdefg}	111.658±3.308^{abcdef}	49.571±2.365^{abcde}	14.272±6.108^{h}
QP02258.03	90.741±4.141^{cdefg}	63.998±10.748^{efgh}	56.966±4.301^{abcd}	11.358±0.409^{h}
QP99165.81RF	116.523±1.783^{abcd}	111.261±4.575^{abcdef}	79.981±2.023^{a}	17.125±0.414^{efgh}
RBP	130.840±3.560^{abc}	94.851±7.070^{bcdefg}	72.001±0.271^{ab}	25.527±5.499^{efgh}
FC2006	116.294±2.878^{abcd}	114.914±8.249^{abcde}	54.352±1.074^{abcde}	16.115±4.988^{gh}
FP3405	141.153±20.57^{ab}	95.206±1.968^{bcdefg}	72.993±7.695^{ab}	29.160±1.136^{defgh}
MP18405	87.532±5.399^{cdefg}	89.700±12.312^{cdefg}	54.914±1.754^{abcde}	24.348±2.033^{efgh}
MS1406	121.533±9.048^{abc}	152.858±4.037^{a}	72.094±4.584^{ab}	36.372±5.771^{cdefgh}

Data arranged first by Les Buissons cultivars and breeding lines then McGill control and somatic lines. Values expressed as means ± SEM in mg/100 g FM. Means with the same superscript letter in the same column are not significantly different. Means were compared using Tukey HSD ($P \leq 0.05$).

Table 3.6 High Performance Liquid Chromatography (HPLC) results for caffeic, ferulic acid and rutin for all genotypes.

	Caffeic Acid		Ferulic Acid		Rutin	
Genotypes	Raw	Cooked	Raw	Cooked	Raw	Cooked
Abeille	5.254±0.500^{cd}	4.320±0.010^{cd}	2.508±0.315^{def}	9.299±5.053^{cdef}	11.628±0.305^{bc}	2.341±0.979^{abc}
Altitude	7.974±1.786^{abcd}	11.619±2.560^{abc}	5.438±0.766^{cdef}	14.294±1.887^{abcd}	11.292±6.989^{abc}	6.465±0.325^{abc}
Aquilon	6.022±0.116^{bcd}	11.633±3.298^{abc}	1.642±0.499^{ef}	5.719±1.556^{cdef}	4.851±0.143^{bc}	5.052±0.355^{bc}
Belle D'Août	6.584±0.108^{bcd}	6.966±0.886^{abcd}	1.053±0.032^{ef}	21.822±5.907^{ab}	8.109±0.263^{abc}	7.122±0.129^{abc}
Envol	5.414±1.186^{cd}	10.056±2.291^{abcd}	1.801±0.649^{def}	10.897±1.911^{abcdef}	2.946±0.814^{bc}	4.423±1.512^{bc}
Fjord	5.461±0.771^{cd}	7.078±0.370^{abcd}	0.262±0.016^{f}	16.813±1.343^{abc}	9.410±0.274^{abc}	6.057±0.743^{abc}
Kalmia	5.423±0.289^{cd}	3.712±0.158^{d}	1.413±0.214^{ef}	10.229±0.670^{bcdef}	7.062±0.442^{abc}	8.806±1.573^{abc}
Péribonka	8.896±1.608^{abcd}	4.903±0.094^{cd}	7.012±0.300^{cdef}	1.696±0.456^{ef}	12.930±4.670^{ab}	5.655±0.281^{abc}
Primevère	5.223±0.409^{cd}	5.251±1.731^{cd}	2.160±0.364^{def}	10.311±4.784^{abcdef}	5.170±0.406^{bc}	1.044±0.338^{c}
Rebound	5.055±1.004^{cd}	3.975±0.096^{d}	2.072±0.678^{def}	6.802±1.290^{cdef}	6.923±0.928^{abc}	13.444±0.042^{ab}
Roselys	4.962±0.500^{cd}	14.129±0.310^{a}	1.950±0.502^{def}	13.636±0.892^{abcde}	6.633±0.043^{abc}	10.435±1.584^{abc}
QP010090.05JP	6.861±0.212^{abcd}	7.253±1.166^{abcd}	2.323±0.517^{def}	11.608±4.198^{abcdef}	9.659±1.495^{abc}	3.925±2.629^{bc}
QP02258.03	5.842±0.359^{bcd}	4.024±0.038^{d}	2.674±0.517^{def}	9.307±1.900^{cdef}	5.864±1.453^{abc}	6.080±2.339^{abc}
QP99165.81RF	5.614±0.022^{bcd}	13.875±5.022^{ab}	2.172±0.156^{def}	23.236±6.028^{a}	4.900±0.145^{bc}	16.519±2.486^{a}
RBP	5.022±0.830^{cd}	6.498±0.495^{bcd}	2.287±0.048^{def}	4.305±0.984^{def}	2.890±0.166^{bc}	8.198±1.377^{abc}
FC2006	5.472±0.338^{cd}	11.846±2.580^{abc}	1.772±0.489^{ef}	8.990±2.181^{cdef}	4.244±1.597^{bc}	6.671±0.342^{abc}
FP3405	10.162±2.185^{abcd}	8.934±0.369^{abcd}	5.497±1.016^{cdef}	7.490±1.424^{cdef}	9.314±1.425^{abc}	9.120±1.084^{abc}
MP18405	5.327±1.192^{cd}	5.617±0.636^{bcd}	3.362±0.916^{def}	9.314±2.677^{cdef}	2.684±0.295^{bc}	8.160±1.500^{abc}
MS1406	5.984±0.095^{bcd}	5.474±0.680^{cd}	2.317±0.124^{def}	8.740±0.186^{cdef}	9.323±0.774^{abc}	6.956±2.089^{abc}

Data arranged first by Les Buissons cultivars and breeding lines then McGill control and somatic lines. Values expressed as means ± SEM in mg/100 g FM. Means with the same superscript letter in the same column are not significantly different. Means were compared using Tukey HSD ($P \leq 0.05$).

Table 3.7. Pearson Correlation Coefficients (r) and p values between raw spectrophotometric antioxidant results (ABTS, DPPH, and F-C) and raw HPLC results.

	AA	CGA	CFF	FER	RUT	T-CCFR	T-CFR	T-ACCFR	ABTS	DPPH	F-C
AA											
CGA	r=0.7532 p=2.776e-10										
CFF	r=0.2743 p=0.0514	r=0.3257 p=0.021									
FER	r=0.3055 p=0.0390	r=0.2331 p=0.1233	r= 0.8182 p=2.181e-12								
RUT	r= -0.0595 p=0.6845	r=0.1350 p=0.3605	r=0.5873 p=7.348e-06	r=0.4962 p=0.0005							
T-CCFR	r=0.6483 p=2.692e-07	r=0.9514 p=<2.2e-16	r=0.6700 p=1.412e-09	r=0.4273 p=0.0027	r=0.3398 p=0.0158						
T-CFR	r=0.0918 p=0.5216	r=0.2051 p=0.1531	r=0.8613 p= <2.2e-16	r=0.7628 p=4.626e-10	r=0.9176 p=<2.2e-16	r=0.6234 p=2.225e-07					
T-ACCFR	r=0.9184 p=<2.2e-16	r=0.9149 p=<2.2e-16	r=0.6538 p=3.513e-08	r=0.4254 p=0.0029	r=0.1206 p=0.4040	r= 0.9462 p=<2.2e-16	r=0.5322 p=2.041e-05				
ABTS	r= -0.0556 p=0.6986	r= -0.0071 p=0.9610	r= -0.1812 p=0.1774	r= -0.1930 p=0.1937	r= -0.2312 p=0.1063	r= -0.1675 p=0.2131	r= -0.2161 p=0.1063	r= -0.1515 p=0.2605			
DPPH	r= -0.1523 p=0.2861	r= -0.1587 p=0.2709	r=0.1097 p=0.4168	r=0.0368 p=0.8061	r=0.2791 p=0.0497	r= -0.0107 p=0.9372	r=0.2268 p=0.0898	r= -0.0601 p=0.6567	r= -0.3243 p=0.0065		
F-C	r= -0.2942 p=0.0472	r= -0.2916 p=0.0520	r= -0.0485 p=0.7380	r=0.0452 p=0.7762	r= -0.1977 p=0.1929	r= -0.1724 p=0.2260	r= -0.0929 p=0.5212	r= -0.1676 p=0.2447	r=0.1530 p=0.2888	r= -0.2183 p=0.1278	

Abbreviations used; AA= ascorbic acid, CGA= chlorogenic acid, CFF= caffeic acid, FER= ferulic acid, Rut= Rutin, T-CCFR= sum of CGA, CFF, FER and RUT, T-CFR= sum of CFF, FER, RUT, and T-ACCFR= sum ascorbic acid and all polyphenols. Boxes highlighted in red are significant.

Table 3.8. Pearson Correlation Coefficients (r) and p values between cooked spectrophotometric antioxidant results (ABTS, DPPH, and F-C) and cooked HPLC results.

	AA	CGA	CFF	FER	RUT	T-CCFR	T-CFR	T-ACCFR	ABTS	DPPH	F-C
AA											
CGA	r=0.6313 p=3.311e-06										
CFF	r=0.1374 p=0.3625	r=-0.2488 p=0.0881									
FER	r= 0.1313 p=0.3737	r=0.1641 p=0.2547	r=0.3148 p=0.0230								
RUT	r= 0.1117 p=0.4498	r=0.1997 p=0.1735	r=0.1296 p=0.3649	r=0.3641 p=0.0080							
T-CCFR	r= 0.4551 p=0.0012	r=0.7703 p=6.105E-11	r=0.3133 p=0.0223	r=0.6302 p=3.28e-07	r=0.5164 p=7.559e-05						
T-CFR	r=0.1650 p=0.2624	r=0.1086 p=0.4528	r=0.6136 p=1.031e-06	r=0.8541 p=2.22e-16	r= 0.6607 p=7.287e-08	r=0.7220 p=4.935e-10					
T-ACCFR	r=0.9469 p=<2.2e-16	r=0.7054 p=1.067e-08	r=0.2153 p=0.1216	r=0.2567 p=0.0610	r=0.3035 p=0.0272	r=0.7021 p=2.336e-09	r=0.3970 p=0.0027				
ABTS	r=0.2450 p=0.0920	r= -0.0172 p=0.9055	r=0.0458 p=0.7446	r=-0.2372 p=0.0842	r=0.0144 p=0.9183	r= -0.0419 p=0.7612	r= -0.1420 p=0.3009	r=0.1918 p=0.1606			
DPPH	r=0.2050 p=0.1621	r=-0.0527 p=0.7162	r= -0.0009 p=0.9947	r= -0.0314 p=0.8217	r= -0.0221 p=0.8750	r= -0.1220 p=0.3751	r= -0.0536 p=0.6975	r=0.1322 p=0.3359	r=0.0439 p=0.7389		
F-C	r= -0.0555 p=0.7272	r=0.0592 p=0.706	r= -0.1105 p=0.4648	r= -0.0778 p=0.6034	r=0.1645 p=0.2691	r=0.0122 p=0.9343	r= -0.0006 p=0.9970	r=0.0060 p=0.9679	r=-0.0962 p=0.5152	r= -0.3033 p=0.0361	

Acronyms used; AA= ascorbic acid, CGA= chlorogenic acid, CFF= caffeic acid, FER= ferulic acid, Rut= Rutin, T-CCFR= sum of CGA, CFF, FER and RUT, T-CFR= sum of CFF, FER, RUT, and T-ACCFR= sum of ascorbic acid and all polyphenols. Boxes highlighted in red are significant.

Table 3.9. Pearson Correlation Coefficients (r) and p values between starch characteristics.

	GL	GI	Moisture	Protein	Amylose	P-starch	RS	DS	RDS	SDS
GL										
GI	r=0.4753 p=0.0003									
Moisture	r=-0.1558 p=0.2653	r= -0.1211 p=0.9314								
Protein	r=-0.1559 p=0.2649	r=-0.3269 p=0.0169	r=-0.1293 p=0.3378							
Amylose	r=-0.0253 p=0.8574	r=0.1137 p=0.4178	r=-0.0188 p=0.8850	r=-0.1293 p=0.3378						
P- starch	r=0.1808 p=0.1951	r=0.2459 p=0.0760	r=-0.1542 p=0.2202	r=-0.0232 p=0.8642	r=0.2035 p=0.1127					
RS	r=0.1090 p=0.4374	r=0.0010 p=0.9434	r=-0.1066 p=0.3120	r=-0.001 p=0.9991	r=0.1129 p=0.3823	r=0.0835 p=0.5085				
DS	r=-0.1150 p=0.4120	r=-0.1324 p=0.3445	r=-0.1512 p=0.1525	r=-0.0125 p=0.9265	r=-0.1894 p=0.1404	r=0.1762 p=0.1603	r=0.1707 p=0.1057			
RDS	r=-0.1382 p=0.3284	r=-0.0258 p=0.8559	r=0.0572 p=0.6696	r=0.1089 p=0.4243	r=0.0154 p=0.9085	r=0.0113 p=0.9328	r=-0.2141 p=0.1066	r=-0.1625 p=0.2231		
SDS	r=-0.0211 p=0.8805	r=0.04310 p=0.7546	r=0.1819 p=0.1680	r=0.0499 p=0.7124	r=0.1007 p=0.4479	r=-0.0592 p=0.6561	r=-0.0459 p=0.7302	r=-0.2450 p=0.0614	r=0.6947 p=1.456e-09	

P-starch stands for phosphorylated starch. Boxes highlighted in red are significant.

Table 3.10. Pearson Correlation Coefficients (r) and p values between starch characteristics (GI and GL), spectrophotometric antioxidant results (ABTS, DPPH, and F-C) and HPLC results. Acronyms used; AA= ascorbic acid, CGA= chlorogenic acid, CFF= caffeic acid, FER= ferulic acid, Rut= Rutin, T=CCFR= sum of CGA, CFF, FER and RUT, T-CFR= sum of CFF, FER, RUT, and T-ACCFR= sum of all polyphenols. Boxes highlighted in red are significant.

	AA	CGA	CFF	FER	RUT	T-CCFR	T-CFR	T-ACCFR	ABTS	DPPH	F-C
Cooked											
GL	r=-0.3770 p=0.0098	r=-0.2587 p=0.0759	r=0.0797 p=0.5784	r=-0.0218 p=0.8781	r=-0.1927 p=0.1754	r=-0.1825 p=0.1909	r=-0.0720 p=0.6083	r=-0.3314 p=0.0154	r=-0.0612 p=0.6631	r=0.0027 p=0.9847	r=0.0317 p=0.8307
GI	r=-0.1914 p= 0.2025	r=-0.1530 p=0.2992	r=0.1505 p=0.2019	r=0.2440 p=0.0813	r=0.0182 p=0.8991	r=0.0024 p=0.9866	r=0.1782 p=0.2018	r=-0.1464 p=0.2955	r=0.2900 p=0.0352	r=0.0222 p=0.8747	r=-0.0802 p=0.5855
Raw											
GL	r=-0.1193 p=0.4246	r=-0.1651 p=0.2729	r=0.1357 p=0.3327	r=0.1523 p=0.3297	r=0.2261 p=0.1307	r=-0.1112 P=0.4246	r=0.1627 p=0.2444	r=-0.0945 p=0.5007	r=0.1194 p=0.3945	r=0.0994 p=0.4788	r=0.0421 p=0.7716
GI	r=-0.2620 p=0.0753	r=-0.3352 p=0.0228	r=0.0736 p=0.6008	r=0.0578 p=0.7130	r=0.1578 p=0.2949	r=-0.1668 p=0.2326	r=0.0715 p=0.6110	r=0.1534 p=0.2728	r=-0.01852 p=0.8953	r=-0.0038 p=0.9786	r=0.0416 p=0.7744

Table 3.11. Multivariate regression model coefficients that define GL

Coefficients	β(SE)	$P \leq 0.05$
Model 1		
Intercept	3.23e01 (7.52)	8.71e-04 ***
% Moisture	-0.733 (0.085)	9.85e-07 ***
GI	0.632 (0.065)	2.38e-07 ***
RS	-0.423 (0.184)	0.039 *
FER (cooked)	-0.100 (0.036)	0.016 *
DPPH (cooked)	-0.011 (0.003)	3.55e-03 **
Model 2		
Intercept	4.04e01 (9.66)	7.96e-04 ***
% Moisture	0.908 (0.099)	1.59e-07 ***
GI	0.625 (0.091)	5.55e-06 ***
FER (cooked)	-0.117 (0.051)	0.035 *
Model 3		
Intercept	2.52e01(8.46)	0.018 *
% Moisture	-0.760 (0.092)	3.42e-05 ***
GI	0.643 (0.096)	1.57e-04 ***
Amylose	0.306 (0.120)	0.034 *
RDS	-0.556 (0.242)	0.050 *
SDS	0.495 (0.258)	0.019 *
AA (raw)	-0.065 (0.020)	0.012 *
CGA(raw)	0.070 (0.0222)	0.013 *
CFF (cooked)	0.282 (0.114)	0.038 *
FER(cooked)	-0.093 (0.055)	0.013 *
F-C (cooked)	-2.452e-03 (2.06e-03)	0.027 *

Signifiant codes: 0 '***' 0.001 '**' 0.01 '*' 0.05
Model 1 explains 96 % of the variance observed with a p value of 8.581e-09 and F- statistic of 66.39. Model 2 describes up to 91 % of the variance in GL with a p value of 4.564e-08and F-statistic of 50.39. Model 3 explains 98 % of the variance in GL and has a p value of 2.152e-05 and F statistic of 32.52.

Connecting Statement

The work in Chapter 3 showed that Kalmia, QP010090.05JP, and MS1406 were nutritionally superior genotypes because of their superior starch quality, polyphenolic profiles, and antioxidant capacity. Furthermore, three models were selected that defined various characteristics that can be used as predictive variables to select genotypes with lower GL. Chapter 4, entitled “Somaclone Starch Quality, Antioxidant Capacity, Ascorbic Acid, and Phenolic Profile Over Two Growing Seasons”, investigates the inter-seasonal stability of starch and antioxidant capacity of four advanced somatic lines while at the same time comparing these to the original cultivar, Russet Burbank.

Chapter 4: Somaclone Starch Quality, Antioxidant Capacity, Ascorbic Acid, and Phenolic Profile Over Two Growing Seasons

4.1 Introduction

Somaclones that are grown in vitro in tissue culture can exhibit traits that differ from the original source genotype (Thieme & Griess, 2005). Compared to typical breeding methods for potato, somaclonal variation allows for another method of creating new genotypes especially from cultivars that have limited fertility, such as Russet Burbank (Nassar, Abdulnour, Leclerc, Li, & Donnelly, 2011; Nassar, Kubow, & Donnelly, 2015). When promoting nutritional characteristics of a particular potato cultivar for a consumer market, it is essential that the qualities advertised are as accurate as possible (Thieme & Griess, 2005). Therefore, the stability of the starch and its subtypes (RS, DS, SDS, and RDS), its digestibility (GI, GL), antioxidant capacity, and phenolic composition from one season to the next is important to establish, especially in potatoes developed through somaclonal methods. These nutritional assessments are completed after the selection process has already chosen superior lines based on yield, type, specific gravity, fry quality (sugars) after long storage intervals, and other factors such as disease resistance (Bach et al., 2013; De Meulenaer et al., 2008; Kumar, Singh, & Kumar, 2004). This allows for desirable agronomic characteristics to be selected as well as healthier potatoes to be promoted on the mass market. Furthermore, inter-seasonal variability needs to be determined due to the numerous environmental factors that can affect a crop (Bach et al., 2013) and these distinguished, if possible, from issues of somaclonal stability.

In some cases, when starch content was estimated or defined by specific gravity, it was found that inter-seasonal differences between individual genotypes were greater than differences among the varieties grown and tested each season (Stevenson, Akeley, & Cunningham, 1964). Similarly, sugar quality varied among genotypes from one season to the next (De Meulenaer et al., 2008). Specifically, in a 2-year study, reducing sugars were greater in one year for cv. Bintje, Ramos, and Saturna, although not for Lady Rosetta. This phenomenon was attributed to differences in temperature and rainfall between seasons. On the same set of potato genotypes, there were no differences in sucrose or crude protein content between seasons. Differences in temperature can significantly affect sugar production in potatoes during tuber formation and

growth (Kumar et al., 2004). For example, tubers grown below 8-12 °C or above 25-30 °C, showed an increased sugar content compared with tubers grown between these two extremes.

It has been shown recently that the degree to which environmental and seasonal conditions effect potato cultivars depends on genotype (Valcarcel, Reilly, Gaffney, & O'Brien, 2014). For example, one class of secondary metabolites (glycoalkaloids) showed genotype-dependence, and the glycoalkaloid content was affected by growing temperature, storage conditions, and light exposure. Interestingly, the site of cultivation had no effect on the glycoalkaloid concentration. Furthermore, there was an interaction between season and genotype. In other words, the extent of the environmental effects were different depending on the variety.

Genotype can also affect the degree to which seasonal conditions can influence starch content and quality. When 12 genotypes were compared over two growing seasons in six different field environments, some increased in RDS while others decreased (Bach et al., 2013). Even though the RDS content changed from one season to the next, two genotypes (CV96044 and Goldrush) ranked the lowest over 2 years. When genotype effects were excluded, only a small seasonal interaction effect was observed for all 12 genotypes (Bach et al., 2013). Another starch fraction, SDS, measured in raw potatoes, was stable from year to year in some genotypes but demonstrated great variability in other genotypes. For example, the genotype CV96044 was stable for SDS, with 1.7 % and 1.6 % in the two growing seasons respectively. In contrast, F06035 had an SDS of 2.1 % in the first year and 0.7 % in the second year (Bach et al., 2013). Potato somaclones can vary in starch content (Thieme & Griess, 2005). These authors investigated somaclones derived from 17 potato cultivars over 3 field generations, and found after initial screening and choosing potentially superior somaclones, 5.7 % had lower starch content, and 86.4 % did not differ compared with the controls.

In the field, many growing conditions can be regulated such as soil nutrient and irrigation levels, pesticides, and cultural practices. However, weather is impossible to control. The production and levels of secondary metabolites are mainly affected by genotype, but some varieties respond to stress differently (Valcarcel et al., 2014). Sometimes, lack of observed genotype-related responses can be due to the small number of varieties assessed that all happen (by chance) to have similar responses (De Meulenaer et al., 2008).

To identify healthier genotypes in evaluation trials, screening and selection should be done with data from more than one season. This is because environmental factors may have influenced the production of secondary metabolites or starch synthesis, and the stability of the genotype and its response to stress can affect the desirable nutritional characterises under evaluation (Bach et al., 2013; Valcarcel et al., 2014). Selecting potato genotypes on the basis of nutritional characteristics that can be used both for fresh market sale, and for the processing industry, is still a relatively new concept. Our McGill group and others (Bach et al., 2013) are advocating that this be done. The objective of this study was to compare Russet Burbank (control) with its four advanced somatic lines (RB somaclones) over two growing seasons for starch quality (including RS, DS, SDS, RDS), GI, and GL, as well as for some features that had been previously assessed, including antioxidant capacity, ascorbic acid, and polyphenolic profile (Nassar et al., 2014).

4.2 Materials and Methods

4.2.1 Source Material

We examined Russet Burbank and four RB somaclones from the McGill somatic breeding program from two growing seasons (2013, 2014). The tubers were grown from plantlets in field plots at Progest2001 near Quebec City, QC in 2013. The tubers were grown from seed tubers obtained from McCain test plots at Foreston, NB and grown in pre-registration trials in New Maryland, NB in 2014. The tubers for both seasons were allowed to grow to full maturity, and then were shipped to McGill for analyses.

4.2.2 Sample Preparation

Each potato genotype was separated into two treatment groups: raw and cooked. Each of these groups had three replicates, where each replicate contained ~5 tubers. Each replicate was washed under tap water and left to air dry, and light exposure was limited by covering with paper towels and closing overhead lights. Boiling was performed in a stainless steel pot. The samples were chopped first with a standard kitchen knife and then with a food processor (FP5050SC, Black and Decker, Canada) and then thoroughly mixed for two representative sub-samples. Sub-samples were weighed (FM; fresh mass), frozen under liquid nitrogen and subsequently freeze-dried (FTS Systems, NY, USA) for 2-5 days. After freeze-drying, the samples were reweighed

(DM; dry mass), ground in a grinder (CBG100SC, Black and Decker, Canada), and stored in screw-topped 20 ml vials at -80 ºC until analysis.

The protocols for compositional analysis of tubers are the same ones used for the Quebec-bred potatoes (Chapter 3). All of the assays were done on freeze-dried samples only. See section 3.2 for details of the following procedures: percent moisture (% moisture), resistant starch (RS), total digestible starch (DS) and their fractions (rapidly (RDS) and slowly (SDS) digestible starch), GI and GL, methanolic crude extracts, antioxidant scavenging assays (ABTS, DPPH, F-C), ascorbic acid, as well as polyphenolic profile. Total starch (TS) is the sum of two components (RS and DS).

4.2.3 Statistical Analysis

Statistical processing was performed using 2-way ANOVA with R script. A post hoc Tukey's HSD test was used to compare between somaclones and seasons, while factoring in different locations and starting material. Data are presented as mean ± standard error of the mean (SEM) and differences were considered significant at $P \leq 0.05$.

4.3 Results

4.3.1 Moisture Content

In season one (but not season two) only one RB somaclone (FP3405) had less % moisture than the other RB somaclones and the Russet Burbank control (Table 4.1). The % moisture content of this same somaclone FP3405 was less in season one (59.277 $\pm$1.286 %) than season two (70.919 $\pm$ 1.662 %). There were no other differences in moisture content for both seasons individually or between seasons for these genotypes.

4.3.2 Starch Profile

There were no differences in total starch (by summation), digestible, and slowly digestible starch, between genotypes in either season or between seasons (Table. 4.2). In season one (but not season two) RB somaclone FC2006 had a lesser RS (g/150 g FW) content (2.034$\pm$0.205) than FP3405 (4.690$\pm$0.359), but not compared to the other RB somaclones or the Russet

Burbank control. The RB somaclone FP3405 was the only somaclone that showed a seasonal effect for RS content; it had more RS in the first than in the second season (1.806±0.636).

In season one there were no differences between genotypes for RDS. In season two, RDS was similar between the Russet Burbank control and the RB somaclones. However, there were some differences solely among the RB somaclones; FC2006 and MP18405 (13.902±0.432 % and 13.030±1.120 %, respectively) were similar but had more RDS than MS1406 (6.975±1.433 %). A seasonal effect was noted for RDS content; this was greater in the first compared to the second season for all genotypes.

4.3.3 Glycemic Index and Load

There were no GI differences among genotypes in season one. However, more variation in GI occurred in season two; the RB somaclones FC2006 (98.578±6.921) and MP18405 (89.135±5.928) and the Russet Burbank control (84.370±3.690) were all greater than MS1406 (56.536±3.626) (Table 4.3). The GI value for MS1406 in season two (56.536 ± 3.626) (but not season one; 95.314± 1.909) could be classified as "intermediate", whereas all other genotypes had GI values above 70, in both seasons, therefore falling into the "high" category.

The GL for all genotypes, in both seasons, was above 20, so classified as "high". There were no seasonal differences, and no differences between the GL of the RB somaclones and the Russet Burbank control.

4.3.5 Antioxidant Capacity Tests

In season one, DPPH activity (mM Trolox Eq/100 g DM) was the least in somaclone FP3405 (81.306±5.541) and the greatest in MP18405 (334.004±20.906) but only FP3405 varied from the Russet Burbank control (273.478±7.828) (Table 4.4). In season two, DPPH values did not vary between genotypes. The DPPH values for most genotypes (FC2006, FP3405, MS1406, and Russet Burbank) were less in season one compared with season two.

In season one, the ABTS value (mM Trolox Eq/100 g DM) of the same somaclone FP3405 (153.200±3.864) was less than all other genotypes (Table 4.4). In season two, ABTS values did

not vary between genotypes. Only FP3405 showed an increased ABTS value in season two (234.446 ± 5.210) compared with season one.

In season one, F-C value (mg chlorogenic acid Eq/100 g DM) for RB somaclone MS1406 was greater (835.484±57.323) than all other genotypes (Table 4.4). The F-C values for MP18405 (580.728±37.161) and the Russet Burbank control (582.694±54.921) were similar and greater than FP3405 (256.633±49.388). In season 2, the F-C values did not vary among genotypes. For all RB somaclones except FP3405, the F-C values for season one were much larger than for season two.

4.3.6 HPLC Analysis of Ascorbic Acid Content and Phenolic Profiles

Ascorbic acid content (mg/100 g DM) did not vary among genotypes in season one (Table 4.5). In season two, MS1406 (372.46±36.35) had greater ascorbic acid content than FP3405 (234.41±22.55) but not more than the control or other RB somaclones. There were no differences in ascorbic acid content between seasons for any genotype. Chlorogenic and ferulic acid content did not vary with genotype within or between seasons.

Caffeic acid did not vary with genotype within each season. However, comparing between seasons, the caffeic acid content was greater in season two for FC2006, MS1406, and Russet Burbank but not FP3405 and MP18405.

Rutin content (mg/100 g DM) was affected by genotype in season one. The RB somaclone MS1406 had much more rutin (19.52±1.92) than FC2006, MP18405, and Russet Burbank (9.00±3.41, 6.02±0.43, and 5.96±0.15, respectively). Rutin content was not affected by genotype in season two and no differences occurred in rutin content between seasons.

4.4 Discussion and Conclusions

Seasonal variation is common among genotypes, because some varieties respond to stress differently (Valcarcel et al., 2014). In the field, it is impossible to control all the parameters, which can ultimately effect changes in phytonutrient content. Changes in moisture content between cultivars over seasons have often been observed, although in our study only one RB somaclone (FP3405) showed any change in moisture content between seasons. Considering the

different planting material used (plantlets vs seed tubers), and the different planting sites used (Quebec, QC vs New Maryland, NB), Russet Burbank and somaclones derived from it seem quite stable for this characteristic.

In previous cases, when starch content was estimated or defined by specific gravity, inter-seasonal differences between genotypes were greater than differences among the varieties tested (Stevenson et al., 1964). We found very little inter-seasonal effect on starch quality which showed that the starch characteristics of these somaclones are stable; even considering that the samples were grown at two different locations. Only the RDS content of all genotypes was higher in season one compared to season two. Change in RDS content was reported by Bach et al. (2013) who showed that some genotypes, when compared over two growing seasons in six different field environments, increased in RDS while others decreased. The somaclones FC2006 and MP18405 had a greater RDS than MS1406, but no differences were apparent compared to Russet Burbank. We observed no changes in SDS between seasons. This partly agrees with Bach et al. (2013) who showed that SDS in some genotypes was stable from year to year but greatly variable in others. In our study, all other starch subtypes (RS, DS) were unaffected by season. When the greater importance that is placed on RS as a starch quality is considered, we can conclude that the starch profile of our RB somaclones from one season to the next did not change the overall nutritional quality of these genotypes. This RS and DS starch stability was impressive considering that the original planting materials were so different. When comparing the RB somaclones with the Russet Burbank control, there was no difference in starch profile components (RS, DS, Total, RDS, and SDS) (Table 4.2). Our results are similar to Thieme and Griess (2005) who found that from 17 potato cultivars over 3 field seasons, 86.4 % of the somaclones they investigated did not differ compared to the control in regards to total starch content. Individually the fractions that make up total starch in our study showed no difference to the control. We went further and also investigated subtypes of digestible starch, which again showed no difference to the control. Thieme and Griess (2005) did find that a small percentage (5.7 %) of their somaclones had lower starch content. This can be attributed to different breeding materials compared to ours as well as having a larger number of somaclones that they screened, which increased the chances of finding a significant difference.

The GI value for MS1406 in season two was interesting because the value was “intermediate” which has potential nutritional interest. The seasonal difference between GI was minor, where only MS1406 was greater in season one than season two. More importantly, there was no seasonal change in GL among genotypes, which is an experimental value that is more important than GI. This starch profile stability was suggested because of the consistent specific gravity results that were obtained during the somaclone selection process (data not provided) (Nassar et al., 2011; Nassar et al., 2014).

The glycemic index and load did not vary between RB somaclones and the Russet Burbank control. This makes sense, as the starch profiles which we know can affect the glycemic impact did not change, (Nayak et al., 2014). The one difference in GI noted was a lower value than the Russet Burbank control in GI for RB somaclone MS1406 in season two. This indicates that this somaclone has potentially lower starch quality and needs further investigation. However, and more importantly, the GL was not affected. The difference in GI might be partly explained by the lower RDS content in MS1406. The GL of all the somaclones was considered high (above 20), which is less desirable for market potatoes in terms of health. These results were not unexpected since the RB somaclones were derived from Russet Burbank, which has known high GI and GL (Aziz et al., 2013; Nayak et al., 2014).

The production of secondary metabolites can be affected by seasonal differences and location (De Meulenaer et al., 2008; Valcarcel et al., 2014). Phenolics are a type of secondary metabolite that are created naturally in plants as a protection mechanism (Andre et al., 2007; Blokhina et al., 2003). The antioxidant capacity of the McGill RB somaclones differed between growing seasons, and was affected by method of quantifying antioxidant capacity. For example, in season one all the RB somaclones (except MP18405) had lesser DPPH levels than in season two, but all the somaclones had greater F-C values in season one compared with season two (Table 4.4). ABTS values were similar in both seasons, except for FP3405 which was greater in season two. These discrepancies between seasons and tests can be partly explained by the different reactions that take place for each test. The DPPH assay cannot distinguish from other side reactions, such as H-transfer and does not react with oxidative chain reaction products or with free radical intermediates (Prior et al., 2005). Structural inaccessibility of the antioxidants to reduce the DPPH may prevent or impede the reaction as well. For these reasons, it is common to

complement DPPH with additional antioxidant assays (Nair et al., 2007; Prior et al., 2005). In contrast, the F-C assay has been assumed to be an accurate measure of phenolics in a sample, but this is not always the case. Other compounds found within a sample extract can interfere with the reaction, and inflate the antioxidant capacity results. These interfering compounds include sugars, aromatic compounds, ascorbic acid and organic acids (Prior et al., 2005). This further supports the notion that multiple antioxidant capacity screening tests must be completed to fully understand, screen and select superior somaclone lines.

The possibility of side reactions that help contribute to inflating the antioxidant capacity of the samples is also probable, since the HPLC results showed no seasonal difference for any phenolic except for caffeic acid; such that season two levels were greater than in season one for RB somaclones FC2006, MS1406, and Russet Burbank. Additionally, the HPLC method only measured ascorbic acid and key phenolics, whereas other compounds that can contribute to total antioxidant capacity, such as anthocyanins and proteins and amino acids, were not measured. This can also explain the difference that was seen between the antioxidant capacity results, ascorbic acid, and phenolics.

In each season individually, there was some significant difference in antioxidant capacity of the somaclones compared to the control, depending on the test used. The control showed a significantly greater DPPH content in season one compared to FP3405, although no difference was seen in season two. As described previously, this difference between seasons can be attributed to environment changes caused by different locations and possibly because of the different starting material. The ABTS results showed no significant difference compared to the controls for both seasons but this time FP3405 had a greater antioxidant capacity than the somaclone FC2006. Most importantly, the results from the F-C test showed that MS1406 had a greater antioxidant capacity than the control as well as all the other somaclones. Differences between somaclone antioxidant capacity and the controls have been previously reported in the literature (Nassar et al., 2011; Nassar et al., 2014). Interestingly, the RBP somaclone FP3405 always had a lower antioxidant capacity, regardless of the test used. No difference was found between the control and the somaclones for all of the measured phenolics for both seasons except that in season one, whereby MS1406 had a greater rutin content compared to FC2006, MP18405, and Russet Burbank.

The somaclones evaluated in this trial were originally selected for yield, sugar quality and differences in antioxidant capacity (Nassar et al., 2011; Nassar et al., 2014). Combining the results of Nassar et al., 2014 with the current results, a decision-making table was created to determine the strengths and weaknesses of the four RB somaclones (Table 4.6).

Overall, the MS1406 line (season two) has been shown to have a lower GI, a greater antioxidant capacity measured by F-C, and greater rutin content compared to the control. This somaclone shows improved nutritional quality compared to Russet Burbank, with the same yield and fry quality but improved antioxidant value, which has become of more and more interest for consumers. Identification of a somaclone with lesser glycemic index has commercial value, which underlines the importance of this new technology for industry and consumers.

Table 4.1. Moisture content of four RB somaclones and the Russet Burbank control over two growing seasons. Values expressed as means ± SEM. Data arranged based on alphabetical order. Means with same are not significantly different; differences between genotypes are within each column, and inter-seasonal differences are by rows. Means were compared using Tukey HSD ($P \leq 0.05$).

	Moisture (%)	
Genotype	Season one (2013)	Season two (2014)
FC2006	67.609±0.559[a]	69.321±1.824[a]
FP3405	59.277±1.286[b]	70.919±1.662[a]
MP18405	69.245±0.552[a]	72.387±0.841[a]
MS1406	68.231±0.343[a]	70.720±2.101[a]
Russet Burbank	67.807±0.366[a]	72.660±0.406[a]

Table 4.2. The starch profile for Russet Burbank and four RB somaclones over two growing seasons. Values expressed as means ± SEM. Means were compared using Tukey HSD ($P \leq 0.05$). Means with the same superscript are not significantly different; differences between genotypes are within each column, and inter-seasonal differences are by rows.

	Resistant starch (g/150 FW)		Digestible starch (g/150 FW)		Rapidly digestible starch (%)		Slowly digestible starch (%)	
Genotype	Season one (2013)	Season two (2014)	Season one (2013)	Season two (2014)	Season one (2013)	Season two (2014)	Season one (2013)	Season two (2014)
FC2006	2.034±0.205[b]	2.081±0.169[b]	74.747±5.246[a]	76.018±3.430[a]	26.671±1.673[a]	13.902±0.432[b]	27.769±0.620[a]	22.333±2.770[ab]
FP3405	4.690±0.359[a]	1.806±0.636[b]	76.018±6.987[a]	68.351±2.755[a]	22.309±0.573[a]	10.579±1.088[bc]	23.540±0.506[ab]	22.752±0.485[ab]
MP18405	2.661±0.378[ab]	1.797±0.218[b]	78.890±8.805[a]	69.677±0.752[a]	22.115±0.485[a]	13.030±1.120[b]	21.974±0.208[ab]	19.213±1.637[ab]
MS1406	2.136±0.096[ab]	2.163±0.183[b]	68.211±4.848[a]	70.522±7.008[a]	20.227±0.692[a]	6.975±1.433[c]	21.702±2.083[ab]	13.143±2.148[b]
Russet Burbank	3.400±0.443[ab]	3.047±0.663[ab]	74.289±3.861[a]	70.139±4.127[a]	25.087±0.649[a]	10.436±0.355[bc]	25.992±0.963[ab]	19.670±2.060[ab]

Table 4.3. Glycemic index and load results for Russet Burbank and four RB somaclones over two growing seasons. Values expressed as means ± SEM. Means were compared using Tukey HSD ($P \leq 0.05$). Means with the same superscript are not significantly different; differences between genotypes are within each column, and inter-seasonal differences are by rows.

	Glycemic Index		Glycemic Load	
Genotype	Season one (2013)	Season two (2014)	Season one (2013)	Season two (2014)
FC2006	100.367±2.931^{a}	98.578±6.921^{a}	32.749±1.265^{a}	27.510±2.010^{a}
FP3405	85.695±2.497^{a}	72.968±6.176^{ab}	36.686±0.127^{a}	23.988±1.066^{a}
MP18405	99.252±2.507^{a}	89.135±5.928^{a}	30.646±0.798^{a}	28.521±3.020^{a}
MS1406	95.314±1.909^{a}	56.536±3.626^{b}	28.651±0.801^{a}	28.745±1.220^{a}
Russet Burbank	99.603±1.348^{a}	84.370±3.690^{a}	31.745±0.950^{a}	24.986±1.032^{a}

Table 4.4. Antioxidant results for Russet Burbank and four RB somaclones over two growing seasons. Values expressed as means ± SEM. Means were compared using Tukey HSD ($P \leq 0.05$). Means with the same superscript are not significantly different; differences between genotypes are within each column, and inter-seasonal differences are by rows. Units expressed as mM Trolox Eq/100 g DM (DPPH, ABTS) and mg chlorogenic acid Eq/100 g DM (F-C).

	DPPH		ABTS		F-C	
Genotype	Season one (2013)	Season two (2014)	Season one (2013)	Season two (2014)	Season one (2013)	Season two (2014)
FC2006	222.818±6.580^{d}	355.373±24.283^{ab}	210.980±5.732^{b}	223.439±2.868^{ab}	450.525±19.603^{bc}	107.915±12.159^{d}
FP3405	81.306±5.541^{e}	367.608±16.033^{ab}	153.200±3.864^{c}	234.446±5.209^{a}	256.633±49.388^{cd}	122.037±9.561^{d}
MP18405	334.004±20.906^{abc}	393.923±12.067^{a}	218.651±3.553^{ab}	218.162±2.823^{ab}	580.728±39.161^{b}	121.485±10.212^{d}
MS1406	244.370±14.342^{cd}	414.383±7.239^{a}	213.414±0.741^{b}	222.497±3.079^{ab}	835.484±57.323^{a}	130.443±10.565^{d}
Russet Burbank	273.478±7.828^{bcd}	391.655±9.726^{a}	218.354±3.491^{ab}	218.324±2.360^{ab}	582.694±54.921^{b}	128.331±6.041^{d}

Table 4.5 High Performance Liquid Chromatography (HPLC) results over two growing seasons. Values expressed as means ± SEM in mg/100 g DM. Means were compared using Tukey HSD ($P \leq 0.05$). Means with the same superscript are not significantly different; differences between genotypes are within each column, and inter-seasonal differences are by rows. Units are expressed as mg/100 g DM.

	Ascorbic Acid		**Chlorogenic Acid**		**Caffeic Acid**		**Ferulic Acid**		**Rutin**	
Genotype	Season one (2013)	Season two (2014)	Season one (2013)	Season two (2014)	Season one (2013)	Season two (2014)	Season one (2013)	Season two (2014)	Season one (2013)	Season two (2014)
FC2006	246.45 ±10.81^{ab}	247.21 ±22.27^{ab}	115.03 ±2.41^{a}	108.31 ±9.56^{a}	11.549 ±0.392^{de}	24.504 ±1.164^{ab}	3.701 ±0.968^{a}	6.237 ±0.457^{a}	8.997 ±3.405^{b}	12.947 ±1.732^{ab}
FP3405	207.30 ±33.13^{b}	234.41 ±22.55^{b}	99.10 ±22.88^{a}	112.65 ±5.86^{a}	14.720 ±3.131^{bcde}	24.490 ±1.259^{ab}	7.885 ±1.124^{a}	6.700 ±0.505^{a}	13.343 ±0.528^{ab}	14.144 ±1.498^{ab}
MP18405	196.87 ±15.90^{b}	304.92 ±14.23^{ab}	123.41 ±6.31^{a}	136.01 ±13.56^{a}	11.79 ±2.674^{de}	22.436 ±4.860^{abcd}	7.488 ±1.912^{a}	4.015 ±1.309^{a}	6.022 ±0.425^{b}	13.805 ±2.532^{ab}
MS1406	253.23 ±14.72^{ab}	372.46 ±36.35^{a}	150.40 ±8.56^{a}	138.53 ±24.98^{a}	12.505 ±0.409^{cde}	26.494 ±1.289^{a}	4.847 ±0.338^{a}	5.923 ±0.185^{a}	19.524 ±1.919^{a}	11.754 ±0.001^{ab}
RBP	270.12 ±1.40^{ab}	338.03 ±45.20^{ab}	148.76 ±2.72^{a}	120.34 ±20.36^{a}	10.432 ±1.673^{e}	23.141 ±1.547^{abc}	4.723 ±0.005^{a}	6.717 ±0.789^{a}	5.963 ±0.149^{b}	8.697 ±0.569^{b}

Table 4.6 Combined spectrophotometric antioxidant results for somaclonal evaluation including current thesis results and those from Nassar et al., 2014, which includes averaged field data from 2005-2007. Each data set was analysed separately, although both using Tukey HSD ($P \leq 0.05$). Values expressed as means ± SEM. Means with the same superscripts within the same column are not significantly different, and were kept as is from Nassar et al. (2014). Units used in Nassar et al. (2014) were: DPPH (mg gallic acid equivalent/150 g FM), ABTS (µM trolox equivalent /150 g FM), and F-C (mg chlorogenic acid equivalent /150 g FM).

	DPPH			ABTS			F-C		
Genotype	2005-2007	2013	2014	2005-2007	2013	2014	2005-2007	2013	2014
FC2006	130.86^{bcd}	222.818 ± 6.580^{d}	355.373 ± 24.283^{ab}	2451.18^{jkl}	210.980 ± 5.732^{b}	223.439 ± 2.868^{ab}	700.96^{a}	450.525 ± 19.603^{bc}	107.915 ± 12.159^{d}
FP3405	116.64^{j}	81.306 ± 5.541^{e}	367.608 ± 16.033^{ab}	$4006.43^{g–k}$	153.200 ± 3.864^{c}	234.446 ± 5.209^{a}	320.98^{de}	256.633 ± 49.388^{cd}	122.037 ± 9.561^{d}
MP18405	$128.43^{b–f}$	334.004 ± 20.906^{abc}	393.923 ± 12.067^{a}	8278.17^{bcd}	218.651 ± 3.553^{ab}	218.162 ± 2.823^{ab}	274.47^{e}	580.728 ± 39.161^{b}	121.485 ± 10.212^{d}
MS1406	118.25^{ij}	244.370 ± 14.342^{cd}	414.383 ± 7.239^{a}	11163.07^{a}	213.414 ± 0.741^{b}	222.497 ± 3.079^{ab}	336.58^{de}	835.484 ± 57.323^{a}	130.443 ± 10.565^{d}
Russet Burbank	132.51^{bc}	273.478 ± 7.828^{bcd}	391.655 ± 9.726^{a}	5113.90^{fgh}	218.354 ± 3.491^{ab}	218.324 ± 2.360^{ab}	663.15^{ab}	582.694 ± 54.921^{b}	128.331 ± 6.041^{d}

Connecting Statement

Phytonutrient composition of four advanced somatic lines in relation to the source cultivar Russet Burbank was compared over two growing seasons in Chapter 4. Other factors that can affect the nutritional qualities of potato cultivars involve cultural practices, for example, hormetic agents such as hydrogen peroxide as described in Chapter 5. For this experiment, four cultivars (Goldrush (GR), Innovator (IN), Russet Burbank (RB), and Yukon Gold (YG)) were sprayed with peroxide solutions in the field, and the hormetic effects assessed.

Chapter 5: Hormetic Field Trial Using H_2O_2

5.1 Introduction

Plant scientists, nutritionists and consumers are becoming more aware of the health impact that a balanced diet that includes potato can have on human health (Bach et al., 2013; Camire et al., 2009). Hormesis is a biological response to potentially harmful stressors that act in a dose-dependent manner. Hormetic agents applied at low levels can stimulate the production of favorable secondary metabolites but these same agents may be toxic when applied at higher concentrations (Calabrese, 2008). Hormetic methods are of interest for potato plants because of the potential for increasing the market value of produce (potato tubers) through improved phytonutrient content. For example, peroxide treated plants resulted in tubers with increased antioxidant capacity and improved starch profile (López-Delgado et al., 2012; López-Delgado et al., 2005).

Hydrogen peroxide (H_2O_2) is an example of an interesting hormetic agent; it is an important signalling molecule that can stimulate the production of many secondary metabolites. However, as a reactive oxygen species (ROS), it can be considered toxic at high doses (Kuźniak & Urbanek, 2000). Compared to other ROS molecules, H_2O_2 is the most stable; it carries no net charge and can easily cross cell membranes. It eventually breaks down to non-harmful water molecules. For these reasons, peroxide is potentially an ideal choice as a hormetic agent. As a defense mechanism to environmental stress, plants synthesize antioxidants to scavenge ROS molecules (Andre et al., 2007). Hydrogen peroxide is believed to play a significant role in activating genes that encode proteins and enzymes involved in protection from oxidative stress. Potatoes can contain significant quantities of antioxidants that include ascorbic acid and phenolics (primarily chlorogenic acid).

Some pre-harvest studies were done on potato plants, where they used an in vitro-to-greenhouse system of microplants to evaluate the effects of the hormone abscisic acid (ABA) on the tolerance of cvs. Atlantic and Alpha to cold soil temperatures (Mora-Herrera & López-Delgado, 2007). The activity of peroxidase, ascorbate peroxidase, and the concentration of H_2O_2 significantly increased in plants exposed to ABA. When the stress of ABA was removed by

transferring the microplants to an ABA-free medium, the activity of the enzymes and the level of H_2O_2 decreased back to control levels. Unfortunately, phenolic assays were not done by this group to see if the ABA-induced increase in ROS enzymatic activity affected antioxidant capacity.

Hydrogen peroxide application has been indicated to have value in promoting in vitro yield (greater microtuber weight and numbers) in cv. Atlantic (López-Delgado et al., 2012). The whole procedure involved micropropagation on MS medium for 30 d. Single-node cuttings were incubated for 60 min with H_2O_2 at 0, 1, 5, or 50 mM and then transferred to microtuberization medium at 20 or 8 °C for 60 d. At 20 °C, the average weight/microtuber in the 1 mM treatment was significantly greater ($P \leq 0.05$) than at 0 mM peroxide (143.02±3.03 and 122.36±2.86 mg, respectively). At 8 °C, average weight/microtuber was significantly greater in the 1, 5, and 50 mM treatments (246.38±3.14, 251.57±3.30, and 246.18±4.32, respectively) compared to 0 mM levels (235.20±3.59). The number of microtubers/plant was similar for all H_2O_2 treatment levels at 20 °C. Only at 8 °C and in the 50 mM treatment was the number of microtubers/plant significantly greater than the control (1.44±0.01 and 1.23±0.03, respectively). This relatively small but statistically greater yield (microtuber weight) was attributed to increased starch accumulation, although this was not measured. The above suggests that the effects of a peroxide hormetic agent can be temperature dependent.

In vitro studies were conducted in the Donnelly lab with peroxide (2 mM and 4 mM) as a hormetic agent to manipulate the polyphenolic content of microtubers in four potato cultivars; Goldrush, Onaway, Yukon Gold, and Russet Burbank (Nassar et al., unpublished; Vunnam, 2010). The peroxide levels were pre-selected to not affect microtuber yield. The total phenolics which included chlorogenic, caffeic, and ferulic acids, increased in cultivars with a lower total phenolic baseline. Onaway showed increased phenolic content at 2 and 4 mM by 13 and 14 %, respectively, and Yukon Gold phenolics increased by 22 and 21 %, respectively. The total antioxidant capacity comprised of: ascorbic acid, total polyphenolics, rutin, and quercetin, was again increased in Onaway at 2 and 4 mM by 14 and 19 %, respectively as well as in Goldrush by 5 and 10 %, respectively. The hydrogen peroxide treatments did not affect growth rate and yield. This study demonstrated a promising hormetic treatment to increase potato nutritional value, and preceded this current study. The results from the in vitro study showed hormetic effect

of peroxide on several cultivars, and influence on several phenolics at doses that did not depress (or increase) microtuber yield.

Yield and antioxidant enzyme activities have also been shown to increase with the use of a hormetic agent in the greenhouse (Martínez-Gutiérrez et al., 2012). Hydrogen peroxide (1 mM) spray application to greenhouse-grown cv. Alpha significantly increased minituber yield (weight (g) but not number per plant) compared to a control (exact numbers were not reported). Minituber total starch content (mg/g), estimated using the anthrone method, increased by 15 % in uninfected plants with the application of 1 mM H_2O_2, while plants infected with phytoplasma, showed a 20 % increase (in contrast to typically decreased starch content that occurs with phytoplasma). Peroxide treatment increased peroxidase but not catalase activity (exact numbers were not reported). A direct measure of phenolic composition or antioxidant capacity was not done. This study suggested that peroxide treatment could increase not only yield and antioxidant capacity, but also protein content in cv. Alpha.

There have been very few reports of hormetic agents applied to field-grown potato. López-Delgado et al. (2005) sprayed H_2O_2 at 0, 5, or 50 mM twice a week, 21-90 days after planting, on field-grown cv. Alpha. Total starch content (mg/g) per tuber, estimated using the anthrone method, was significantly increased in the 5 and 50 mM treatments by 30 and 28 %, respectively compared to the control. Total starch, estimated by specific gravity also showed significant increase, although less than that suggested by the anthrone method. In the 5 and 50 mM treatments, the starch content was increased by 6.7 and 11 %, respectively. The starch profile was not investigated. Greenhouse-grown potatoes that received the same peroxide treatment as in the field (0, 5, or 50 mM) had a 62 % increase in lignin content (exact numbers not reported). Lignins are recognized to have antioxidant properties (Dizhbite et al., 2004), although this was not investigated by López-Delgado et al. (2005). Further starch and phenolic quality and quantity assessments were not done. This report gave a clear impression that starch parameters and antioxidant capacity could be affected by peroxide treatment in the field.

Although seemingly very important, no other such field studies have been reported in the intervening years. An agronomic tool like hydrogen peroxide sprays presents a promising opportunity for potato growers to increase the nutritional and production value of their field

crops. It is also desirable to develop a reliable method that can improve tuber antioxidant capacity towards promotion of greater consumer health. The aim of this study was to evaluate the impact of foliar peroxide sprays on field-grown potato to assess the agronomic value of this technique. Hormetic treatments were applied to four potato cultivars in a field trial, and evaluated for effects on yield, processing quality, and functional food properties.

5.2 Materials and Methods

5.2.1 Source Material, Field Plan, Hormetic Spray Program, and Analysis

Seed tubers of four cultivars (Goldrush, GR; Innovator, IN; Russet Burbank, RB; and Yukon Gold, YG) were obtained from the McCain seed repository and grown at Greenfield, the McCain test plot in Florenceville, NB. The field design was done by Dr. Atef Nassar (McGill PDF) in association with McCain agronomist Dr. Yves Leclerc and his field crew. The layout of the field, which included cultivar and treatment arrangement are represented in Table 5.1. The in-row spacing followed the NB provincial recommendations for each cultivar. These were 12" (30.48 cm) for GR, 10" (25.4 cm) for IN and 15" (38.1 cm) for both RB and YG. Numbers after the cultivar names indicate replicates, where each cultivar had four replicates/treatment. The dates of the pesticide and the treatment sprays are shown in Tables 5.2 and 5.3, respectively. The peroxide sprays were done with a hand-held CO_2 sprayer using 2 L of peroxide per 480-ft (146 m) of row. The treatment sprays began 2 mo after planting (at the time of tuber initiation) and 0 mM (control), 1, 10, and 100 mM of hydrogen peroxide were used.

The potatoes were harvest on October 1st 2013, and graded (≥ 5 cm) by Emily Snowden (field manager) and her team at the McCain Foods Research Farm facility. Yield was measured for each plot, by counting the total number of tubers before and after grading and taking the cumulative fresh weight measurements before and after grading. Tubers that were "graded out" of a 5 cm grading ring were counted as large (> 5 cm diameter means they are suitable for fry processing), anything less was considered small (used for other processing purposes), and very tiny, injured, or diseased tubers were discarded. From the large tubers, a subsample of at least 10 tubers was used to calculate the specific gravity (weight in air and water). Tubers that were not sent to McGill for analysis were stored at the NB provincial station at Wicklow for 5 mo. Following removal from storage, some tuber samples were tested for sugars (YSI 2700 select

Biochemistry Analyser, YSI Ltd., Hampshire, UK) by Emily Snowdon at Wicklow and some were sent to McGill University for further evaluation.

5.2.2 Sample Preparation

Once the tubers were received at McGill University, each replicate composed of ~10 tubers was washed under tap water and air-dried. The samples were chopped using a food processor (FP5050SC, Black and Decker, Canada) and two subsamples were weighed to obtain the fresh mass (FM). These were frozen under liquid nitrogen and subsequently freeze-dried (FTS Systems, NY, USA) for 5 d. Freeze-dried samples were weighed to obtain a dry mass (DM) and the % moisture in the original sample was calculated. These were ground (CBG100SC grinder, Black and Decker, Canada), and stored at -80 ºC until analysis.

For methods related to: % moisture, starch analysis including resistant, digestible (rapidly and slowly digestible starch), predicted glycemic index, total soluble protein, methodology for methanolic crude extracts, antioxidant scavenging assays (ABTS, DPPH, F-C), ascorbic acid, and polyphenolic profile see Chapter 3, section 3.2. Assays were done on raw freeze-dried potato samples as used in current literature (Bach et al., 2013; Mishra et al., 2012; Zhang et al., 2014). However, if starch results had been more promising, starch assays would have been completed on rehydrated cooked samples as recommended by Mishra et al. (2008).

5.2.3 Measuring Starch Grains

Tuber samples were collected by Emily Snowden in Florenceville and sent to Dr. Xiu-Qing Li's lab at The Center for Potato Research (AAFC-Fredericton, NB). Dr. Amir El-Weshahy (McGill PDF) worked with Dr. Li's team to prepare and photograph the samples for starch grain analysis.

Three tubers from each of four replicates per cultivar were randomly selected for a composite sample. The 3 tubers were used to prepare samples for microscopy (1 tuber per slide and ~4 photos/ slide; 12 photos/treatment/cv.). Potato tubers were washed under running tap water and dried using a soft cloth. A 2 cm horizontal slice was cut from the middle of the tuber and then a 2 cm^2 subsample was cut from the center of the slice. The rectangle of tissue (1 x 1 x 2 cm) was

transferred to a garlic press and the juice squeezed into an Eppendorf tube. A 20 µl aliquot of juice was mixed with 80 µl of water in a new Eppendorf tube. Slides were prepared ahead of time for photo-microscopy. To prepare the slides, a strip of tape, containing three holes made with a hole punch, was affixed to each slide. One drop of diluted solution was placed into each of the 3 holes on the slide. A Carl Zeiss light microscope equipped with a polarizing filter and a 20X objective was used to take pictures. Four photos/ slide were taken at 200X.

The starch grains were measured from digital photographs by Dr. Doaa Elkassas (McGill PDF). The largest starch grains were measured, seeing as they compose the majority of the starch biomass found in a tuber (Fajardo, Haynes, & Jansky, 2013). The length and width for the 10 largest starch grains per photograph were measured using AxioVision Rel 4.7 software and exported into Microsoft Excel.

5.2.4 Statistical Analysis

Statistical analysis was performed using a combination of SAS 9.3 and R script. SAS was used to compare the means between: yield components, specific gravity, starch granule measurements, and sugars. R Studio was used to analyse the results from: starch quality, vitamin C and polyphenolic profiles, total soluble protein, and antioxidant capacity. An ANOVA was completed to determine if there was an overall treatment effect, while disregarding cultivar as a variable. A Tukey's HSD post hoc test followed to determine if there were significant differences between the hydrogen peroxide treatments for each cultivar. If there was no treatment effect, then an ANOVA was completed on all data to determine if there were any differences among cultivars. If there was a difference in treatment, then only the control values were used to determine if there were any differences between cultivars. Again, a Tukey's HSD post hoc test followed. A two-way ANOVA was completed on each result section, with both cultivar and treatment as a variable. A Tukey's HSD post hoc test was done to determine if there were significant differences between the hydrogen peroxide treatments and cultivars.

5.3 Results

5.3.1 Yield

5.3.1.1 Ungraded Tuber Yield

There was no effect of treatment on total ungraded tuber yield (total FM, tuber number, and mean tuber FM) when cultivar was discounted as a variable (Table 5.4). There were some cultivar effects on total ungraded tuber FM (kg); IN (18.139±1.346) was 28.32 % greater than RB (14.136±0.828) (Table 5.4). There were also cultivar effects on ungraded tuber number; IN had the greatest mean tuber number (115.063±4.300) which was more than GR and YG (97.000±5.261 and 92.188±4.411, respectively) but not RB (99.625±4.681). There was no overall cultivar effect on ungraded mean tuber FM (kg/tuber). Within cultivars, there were no effects on total ungraded FM, tuber number and mean tuber FM when both cultivar and treatment were considered (Table 5.5).

5.3.1.2 Graded Tuber Yield; Small Tubers

There was no effect of treatment on graded small tuber FM, number of small tubers, or mean small tuber FM, when cultivar was discounted as a variable (Table 5.4). Cultivar effects were apparent for FM (kg); RB had the greatest graded small tuber FM (2.287±0.200) compared with all other cultivars. Also, IN (1.516±0.135) had 97.4 % greater small tuber FM than YG (0.768±0.087). There was some cultivar effect concerning graded small tuber number which followed the same trend as above. Specifically, RB had the most small tubers (35.750±2.967 tubers) compared with all other cultivars. Again, IN had more small tubers (24.250±1.815) than YG (15.563±1.552). There was a cultivar effect on mean small tuber FM (kg/tuber), where RB (0.064±0.001) was greater than all cultivars except for IN (0.062±0.002). Additionally, small tuber FM for IN and GR (0.056±0.002) were greater than YG (0.049±0.001) which was the least. Within cultivars there were no effects of treatment on small tuber FM, small tuber number, and mean small tuber FM when both cultivar and treatment were considered as variables (Table 5.5).

5.3.1.3 Graded Tuber Yield; Large Tubers

There was no effect of treatment on graded large tuber FM, large tuber number, and mean large tuber FM, when cultivar was discounted as a variable, as well as no cultivar effect on mean tuber FM (Table 5.4). There were some effects of cultivar regarding graded large tuber FM (Table5.4); IN (10.118±0.588 kg) had 30.07 % greater large tuber FM than GR (7.779±0.662) and 63.17 % greater than RB (6.201±0.672). Also, IN had greater large tuber numbers (90.813±3.806) than YG (76.625±3.728) and RB (63.875±3.806). Within cultivars, there were no effects of treatment on large graded tuber FM, number or mean tuber FM when both cultivar and treatment were considered as variables (Table 5.5).

5.3.2 Specific Gravity

There was no effect of hormetic treatment on specific gravity when cultivar was discounted as a variable (Table 5.6). However, cultivar effects were apparent; YG and RB (1.092±0.001 and 1.091±0.001, respectively) had a greater specific gravity than IN and GR (1.085±0.001 and 1.083±0.001, respectively). Within cultivars, treatment had no effect on specific gravity when both cultivar and treatment were considered as variables (Table 5.7).

5.3.3 Sugars

There was no effect of hormetic treatment when the effect of cultivar was disregarded (Table 5.6). Some cultivar affects were apparent; GR had a greater glucose content (0.125±0.015) than all other cultivars except YG (0.093±0.008). Also, YG and RB (0.073±0.017) had 257.69 % and 180.77 % greater glucose content, respectively than IN (0.026±0.003). Within cultivars, there was no effect of peroxide treatment on % glucose when both cultivar and treatment were considered as variables.

There was no effect of treatment on sucrose content (mg/g), when cultivar was discounted as a variable and within cultivars when it was included (Table 5.6 and 5.7, respectively). Cultivar effects were apparent; the sucrose content of YG and RB (1.002±0.063 and 0.899±0.040, respectively), were greater than IN and GR (0.679±0.053 and 0.592±0.037, respectively) (Table 5.6).

5.3.4 Starch Granule Area

There was no effect of treatment on starch granule area (μm^2), when cultivar was discounted as a variable (Table 5.6). Cultivar differences were observed; RB and IN (2.752±0.145 and 2.561±0.098, respectively) had 44.16 % and 34.15 % greater starch grain size than YG (1.909±0.212). The cultivar GR (2.457±0.131) had similar starch grain size to all other cultivars. When cultivar and treatment were both considered as factors, the starch granule area (as well as granule length and width parameters) was not affected (Table 5.8).

5.3.5 Starch Profile and Glycemic Index

There was no treatment effect on RS, DS, RDS, and SDS, when cultivar was not considered a variable and no treatment effect within a cultivar when cultivar was included (Table 5.9 and 5.10 respectively). There was a measurable cultivar effect. Regarding RS content (g/100 g DM), IN (75.084±1.054) had a 7.42 % and 13.82 % greater RS value than GR and RB (69.895±1.344 and 65.970±0.963, respectively) (Table 5.9). Additionally, YG (73.948±1.558) had 12.09 % more RS than RB.

There was a cultivar effect concerning DS content; RB had a 30.62 % and 36.58 % greater DS (g/100 g DM) value (34.030±0.963) than YG and IN respectively, although was not greater than GR. Also, GR had 20.83 % more DS than IN. There was a difference in RDS (%) by cultivar. YG (23.776±0.367) had 14.27 % greater RDS content than GR (20.806±0.570). Again, the cultivar YG (24.514±0.449) had 17.49 % more SDS than GR (20.560±0.522).

There was no effect of treatment on GI, when cultivar was discounted as a variable (Table 5.9). Cultivar differences were apparent; YG had an 8.61 % greater GI (86.229±0.871) compared to IN (79.394±1.779). All observed GI values can be classified as “high” GI, because they are over 70 (Table 5.9 and 5.10). Within a cultivar, there was no treatment effect on GI when both cultivar and treatment were considered as variables (Table 5.10).

5.3.7 Total Soluble Protein Content

There was no effect of hormetic treatment on total soluble protein content when cultivar was discounted as a variable (Table 5.6). There was also no cultivar effect. Within cultivars, the

hormetic treatment did not affect protein content (Table 5.7). Total soluble protein values ranged from 9.136 ± 0.909 g/150 FM (IN at 1 mM) to 12.128 ± 0.730 g/150 g FM (GR at 1 mM).

5.3.8 Antioxidant Capacity Tests

There was no effect of treatment on antioxidant capacity (ABTS, DPPH and F-C) when cultivar was discounted as a variable (Table 5.11). However, cultivar differences were apparent for each test (Table 5.12). RB had 17.42 % and 25.79 % greater ABTS content (211.266±5.861 mM Trolox Eq/100 g DM) than YG and GR (179.921±5.987 and 167.946±11.680, respectively) (Table 5.11). Regarding the DPPH content (mg Trolox Eq/100 g DM), GR (232.409±9.607) was 28.18 % greater than YG (181.321±7.687). The cultivar differences for the F-C values (mg chlorogenic acid Eq/100 g DM) were more diverse; GR had the largest content (943.892±56.605) and IN the smallest (523.656±18.317). Both RB and YG had lesser F-C values than GR but greater values than IN.

Within cultivars, the peroxide treatments did not affect ABTS or DPPH values (Table 5.12). Regarding F-C (mg chlorogenic acid Eq/100 g DM), when both treatment and cultivar were considered as variables, GR at 1 mM (597.053±22.016) had 36.31 % lesser F-C value than the control (937.471±33.749) and was also less than in the other treatments (10, 100 mM). For all other cultivars, antioxidant capacity was not affected by hormetic treatments.

5.3.9 Ascorbic Acid Content and Phenolic Profile

There was no treatment effect on content of ascorbic acid, chlorogenic acid, caffeic acid, ferulic acid, or rutin, when cultivar was not considered a variable (Table 5.11). However, cultivar effects were apparent. The ascorbic acid (mg/100 g DM) content of GR and RB (78.585±6.330 and 72.731±4.138) were 47.92 % and 36.91 % greater, respectively than IN (53.125±5.148). GR had a greater chlorogenic acid content (122.977±7.258 mg/100 g DM) than all other cultivars except for IN (108.557±7.452). IN had 30.4 % more chlorogenic acid than YG (83.251±2.390). Caffeic acid (mg/100g DM) also showed a cultivar effect; RB (7.573±0.248) had 23.21 % more caffeic acid than IN (6.146±0.555). RB also had more rutin than all other cultivars. Within cultivars, there were no effects of treatment on ascorbic acid, chlorogenic acid, caffeic acid, ferulic acid, and rutin content, when both cultivar and treatment were considered (Table 5.13).

5.4 Discussion

There were no effects of hormetic treatment on yield (total FM, tuber number, and mean tuber FM) for either ungraded or graded (small and large) tubers for the four cultivars (Table 5.4). These findings do not support previous results reported in the literature on cv. Alpha (Martínez-Gutiérrez et al., 2012). When cv. Alpha received a similar hydrogen peroxide (1 mM) spray, minituber total weight (g/plant) but not number was increased relative to the control (exact numbers were not reported). Hydrogen peroxide had value in promoting in vitro yield (greater microtuber weight and numbers) in cv. Atlantic (López-Delgado et al., 2012). There appeared to be a trend that depended on temperature; at 8 ºC, all levels of hydrogen peroxide treatment (1, 5 and 50 mM) promoted an increase in microtuber weight, and increased numbers, although only at 1 mM. The results at 20 ºC only showed greater microtuber weight at 1 mM, and no increase in numbers for all treatment levels. Most importantly though, unpublished results from the Donnelly lab indicate that growth and microtuber yield were unaffected for four cultivars (GoldRush, Onaway, Russet Burbank, and Yukon Gold) under treatment conditions that increased polyphenolic content (Nassar et al., unpublished; Vunnam, 2010). These were the same cultivars used in the current field trial (except Onaway was replaced by Innovator).

The discrepancy between the reported results for Atlantic, an important chipper, showing possible yield increases and our data can be partly explained by the use of different cultivars, as well as their greenhouse conditions compared to our field one. We used four important cultivars, rather than one; including Russet Burbank and Innovator (important in the French fry processing industry), and Yukon Gold and Goldrush, (important table stock cultivars), therefore providing a wider range of results that are relevant to potato growers in Canada and elsewhere. Our study also represents a more realistic approach regarding how potato growers could apply a hydrogen peroxide hormetic agent; in the field rather than in the greenhouse. Yield results did vary by cultivar, but this was expected (Table 5.4).

As a defense mechanism to environmental stress, plants synthesize antioxidants to scavenge ROS molecules (Andre et al., 2007). Hydrogen peroxide is believed to play a significant role in activating genes that encode proteins and enzymes involved in protection from oxidative stress. We did not observe an increase in any of the three antioxidant capacity assays, or the

components that most affect these, including ascorbic acid and phenolics, with the exception of cv. Goldrush in the 1 mM treatment, which was less that all other treatment levels including the control when measured using F-C. Previous reports showed that enzymatic activity that contributes to phenolics can be affected by the application of a hormetic agent (Martínez-Gutiérrez et al., 2012). Specifically, peroxide treatment (1 mM) increased peroxidase (exact numbers not reported) but not catalase activity in cv. Alpha in a greenhouse trial. A direct measure of phenolic composition or antioxidant capacity was not done. In our study, we did not observe increased total soluble protein content, so it is unlikely that any enzymes were increased significantly.

Prior to our field trial, in vitro studies were conducted in the Donnelly lab with peroxide (2 mM and 4 mM) as a hormetic agent to manipulate the polyphenolic content of four microtuber potato cultivars; Goldrush, Onaway, Yukon Gold, and Russet Burbank (Nassar et al., unpublished; Vunnam, 2010). The total phenolics which included chlorogenic, caffeic and ferulic acids, appeared to increase in cultivars with a lower total polyphenolic baseline. Onaway total phenolic content was increased in the 2 and 4 mM treatments by 13 and 16 %, respectively with a baseline of 143.41 mg/100 g DM and Yukon Gold phenolics were increased by 22 and 21 %, respectively with a baseline of 108.36 mg/100 g DM. The total antioxidant capacity was also increased in Onaway in the 2 and 4 mM treatments by 14 and 19 %, respectively, as well as in Goldrush by 5 and 10 %, respectively. The results from this preliminary study lead to the current hormetic studies on field-grown potatoes, which were ineffective in altering phenolic content or antioxidant capacity. This can partly be explained by the significant amount of stress potatoes are already exposed to under field conditions, compared to in vitro studies. Clearly, the application of a hormetic agent in the field to boost nutritional value was ineffective.

Some pre-harvest studies were done on potato plants on the cvs. Atlantic and Alpha, where they used an in vitro-to-greenhouse system using microplants; they observed a significant increase in activity of peroxidase, ascorbate peroxidase, and H_2O_2 in plants exposed to ABA (Mora-Herrera & López-Delgado, 2007). Unfortunately, phenolic assays were not done by this group to see if the increase in enzymatic activity directly affected antioxidant capacity. Less control is available in a field trial compared to the vitro-to-greenhouse system these authors described. Greenhouse-

grown cv. Alpha that received peroxide treatments (0, 5, or 50 mM) had a 62 % increase in lignin content after the application of a peroxide treatment (exact numbers for lignin were not reported) (López-Delgado et al., 2005). Lignins are recognized to have antioxidant properties (Dizhbite et al., 2004), although the effect of lignin on starch was not investigated by López-Delgado et al. (2005). This suggests that hydrogen peroxide treatments can affect the antioxidant capacity of potatoes, although our field results do not support this claim.

The current trial, which reflects the cultivation practices that New Brunswick farmers use to grow potatoes in the field is a more comprehensive hormetic study than the previous report in the literature. We observed some cultivar differences, which were not unexpected. It is possible that plants growing in the field under conventional treatments in NB exhibit maximum stress responses already, that can't be further increased by hormetic treatment. Plants in the reported field trial, in the greenhouse, or in vitro exhibit may have experienced less stress (or a different type of stress) and may have therefore been more responsive to hormetic treatment.

Cultivar differences were observed regarding starch content (RS, DS, RDS, and SDS) and GI (Bach et al., 2013; Ek et al., 2012). These differences were not unexpected. Previous reports have shown that starch quality is affected by both genotype and environment (See Chapter 4). Raw potatoes typically have between 70-80 % RS, but can even be as low as 66.5 % (Bach et al., 2013; Megazyme, 2011). The percent RS content was within the range found in the reported literature. Our percent RDS and SDS were significantly more than observed in reports by Bach et al.(2013) for 12 genotypes (CV96044-3, FV12272-3, WV5475-1, F03031, F05035, F04037, F05081, F05090, Atlantic, Goldrush, Norland, and Russet Burbank), which typically ranged from ~8-10 % and 1-3 % respectively, whereas our results showed 20-24 % for both. Our results are similar to the values reported by Mishra et al., (2008) who worked with composite samples of cvs. Draga, Nadine, Frisia, Desirée, Karaka, Moonlight, Agria, Fronkia, and White Delight, as well as results from Mishra et al. (2012) who used the genotypes Almera, "Crop 17", Agria, Moonlight, and Nadine.

No differences for RS, DS, RDS, and SDS content or GI resulted from hormetic treatments. This shows that the application of a foliar hydrogen peroxide spray for cultivars GR, IN, RB, and YG is ineffective at changing (increase or decrease) starch content, or modifying the profile meaning

that the overall starch quantity would be the same, but the quality would be affected. These results do not reflect previous findings; one such example was that total starch content (mg/g), of cv. Alpha, increased by 15 % in tubers of plants that received an application of 1 mM H_2O_2, (Martínez-Gutiérrez et al., 2012). The difference between our results and those above may be due to different cultivar use and different experimental set-up (minituber system VS field application), although our trial represents a more realistic field approach for farmers and industry. Hydrogen peroxide was found to have value in promoting in vitro yield (greater microtuber weight) in cv. Atlantic which was attributed to increased starch accumulation, although this was not measured (López-Delgado et al., 2012). Our results showed that the GI values did not change after treatment, and all could be classified as having a "high" GI (Aziz et al., 2013; Bach et al., 2013).

In addition to measuring the starch content and quality, we measured the starch granule length, width and calculated the overall area, to further assess whether starch had been affected after treatment. Our results showed that there was no treatment effect within a cultivar for every variable (length, width, and area) (Table 5.8). Cultivar differences were observed, and were expected seeing as previous results have reported that genotypes differ in granule mean length (Li et al., 2011). Li et al. (2011) just measured mean length (labeled width in our study). When we compare our results, which measured the top ten largest granules, to theirs which included values from the largest 10 % of granules, we obtained similar results. For example the average width for Yukon Gold starch granules in our study was 42.39$\pm$2.53 µm, while Li et al. (2011) reported a similar 38.40$\pm$8.75 µm for the same cultivar, even though the field conditions and season were different.

There have been very few reports of hormetic agents applied onto field-grown potato and only one example has been found to date; López-Delgado et al. (2005). They reported that after a peroxide treatment, total starch content (mg/g) per tuber could be increased up to 30 %. The type of starch was not investigated. Different cultivar and sampling methods may explain the contrasting results between our study and López-Delgado et al., (2005). The current study measured starch using Megazyme kits, which report consistent and reliable starch results that are nutritionally relevant (Megazyme, 2011). The starch results reported by López-Delgado et al., (2005) showed a huge difference in starch content even when they compared between anthrone

(30 and 28 % starch content increase) and specific gravity (6.7 and 11 % increase) measurements; so determining which method is more accurate and closest to the "true" starch value is impossible in their study. Starch contents determined in the current study were similar to those previously reported in the literature, using the same or similar method (Megazyme, 2011; Mishra et al., 2008).

Although seemingly very important, no other such field studies have been reported in the intervening years. The positive results reported by López-Delgado et al. (2005) was published 10 years ago, and only used one cultivar (cv. Alpha) in their study. In comparison, we used four cultivars that are important and relevant to Canadian industry (Russet Burbank and Innovator; French fry cultivars) as well as Goldrush and Yukon Gold (table stock cultivars). We also included a wider treatment range to more thoroughly explore any change in phytonutrient content. Many reports have suggested that hydrogen peroxide presents a potential new chemical tool to promote starch quantity or antioxidant capacity but our results though do not support this claim (López-Delgado et al., 2005). Overall, there were not effects on the yield or phytonutrient content of potatoes of four important cultivars subjected to hormetic stress caused by hydrogen peroxide in the field. There was no suggestive evidence that further investment or scientific resources should be applied to this area of research. While it is possible that other hormetic agents could be more successful in this regard, it is also likely that the overall stress under conventional planting situations exceeds any baseline that might be increased through hormetic stress in the greenhouse or in vitro.

Table 5.1 Layout of the field plots for the hormetic study indicating cultivars, peroxide treatments and replicates. Abbreviations used: Goldrush (GR), Russet Burbank (RB), Innovator (IN), Yukon Gold (YG). Peroxide treatments are indicated for each column (0, 1, 10, and 100 mM). Numbers after the cultivar indicate replicates, where each cultivar had four replicates/treatment.

H_2O_2 Treatments (mM)			
0	1	10	100
GR1	RB4	IN2	YG3
YG2	YG2	RB2	IN1
IN4	RB1	IN1	IN2
IN1	YG3	YG1	GR4
YG3	RB3	YG3	GR1
GR2	GR3	GR4	RB3
RB4	GR1	GR2	GR2
RB2	GR2	GR3	RB1
GR4	RB2	RB3	RB2
YG4	IN3	IN3	YG2
YG1	IN2	YG4	YG4
GR3	YG1	RB1	IN4
RB3	IN4	YG2	GR3
IN2	GR4	IN4	RB4
IN3	YG4	RB4	IN3
RB1	IN1	GR1	YG1

Table 5.2 The field plots were sprayed (dates indicated) with the following fungicides/pesticides at the concentrations indicated. * indicates a change in unit.

Date (2013)	Fungicide/Pesticide	Concentration (L/ha) or *(Kg/ha)
01-Aug	Bravo	2.40
01-Aug	Quadris Top	1.00
07-Aug	Tattoo C	2.70
12-Aug	Bravo	2.40
12-Aug	Coragen	0.50
19-Aug	Ranman	0.20
25-Aug	Polyram	2.25 *
30-Aug	Ranman	0.20
09-Sep	Royal MH	12.60
10-Sep	Bravo	2.40

Table 5.3 Field plots were sprayed (0. 1, 10, 100 mM) on the treatment dates indicated.

Date (2013)
04-July
11-July
18-July
25-July
01-August
08- August
15- August
22- August
29- August

Table 5.4 Yield (mean ± SEM) showing overall treatment effects of peroxide spray concentrations, disregarding cultivar as a variable for ungraded and graded (small and large) total fresh mass, number and mean tuber mass. The second section indicates yield (mean ± SEM) showing overall cultivar effects, disregarding treatment as a variable.

Treatment Effect									
	Ungraded			**Graded**					
Trt H_2O_2 (mM)	Total fresh mass (Kg)	Total tuber number	Tuber mass (Kg/tuber)	Total small tuber mass (Kg)	Total small tuber number	Small tuber mass (Kg/tuber)	Total large tuber mass (Kg)	Total large tuber number	Large tuber mass (Kg/tuber)
0	14.314±0.906^{a}	96.750±4.646^{a}	0.147±0.005^{a}	1.688±0.229^{a}	27.563±2.924^{a}	0.058±0.003^{a}	7.490±0.578^{a}	71.400±3.831^{a}	0.105±0.007^{a}
1	14.875±1.253^{a}	103.750±5.344^{a}	0.145±0.010^{a}	1.529±0.197^{a}	25.438±2.890^{a}a	0.058±0.002^{a}	7.751±0.705^{a}	78.313±4.552^{a}	0.095±0.007^{a}
10	16.244±0.953^{a}	103.000±4.216^{a}	0.157±0.005^{a}	1.340±0.164^{a}	23.625±2.575^{a}	0.056±0.002^{a}	8.515±0.753^{a}	79.375±4.490^{a}	0.107±0.007^{a}
100	16.588±0.957^{a}	100.375±4.646^{a}	0.167±0.005^{a}	1.253±0.172^{a}	21.375±2.566^{a}	0.058±0.002^{a}	8.464+0.668^{a}	79.000±4.531^{a}	0.105±0.005^{a}
Cultivar Effect									
	Ungraded			**Graded**					
Cv	Total fresh mass (Kg)	Total tuber number	Tuber mass (Kg/tuber)	Total small tuber mass (Kg)	Total small tuber number	Small tuber mass (Kg/tuber)	Total large tuber mass (Kg)	Total large tuber number	Large tuber mass (Kg/tuber)
GR	15.063±0.971^{ab}	97.000±5.261^{b}	0.155±0.004^{a}	1.238±0.093^{bc}	22.438±1.715^{bc}	0.056±0.002^{b}	7.779±0.662^{b}	77.133±3.656^{ab}	0.098±0.005^{a}
IN	18.139±1.346^{a}	115.063±4.300^{a}	0.159±0.011^{a}	1.516±0.135^{b}	24.250±1.815^{b}	0.062±0.002^{ab}	10.118±0.588^{a}	90.813±3.806^{a}	0.111±0.004^{a}
RB	14.136±0.828^{b}	99.625±4.681^{ab}	0.142±0.006^{a}	2.287±0.200^{a}	35.750±2.967^{a}	0.064±0.001^{a}	6.201±0.672^{b}	63.875±3.572^{b}	0.095±0.010^{a}
YG	14.683±0.604^{ab}	92.188±4.411^{b}	0.161±0.005^{a}	0.768±0.087^{c}	15.563±1.552^{c}	0.049±0.001^{c}	8.140±0.417^{ab}	76.625±3.728^{b}	0.107±0.005^{a}

All means were compared using Tukey HSD ($P \leq 0.05$) and means with same superscript in the same column in each section are not significantly different. Trt indicates treatment (peroxide at 0, 1, 10, and 100 mM). Cv indicates cultivar (Goldrush, GR; Innovator, IN; Russet Burbank, RB and Yukon Gold, YG).

Table 5.5 Yield (mean ± SEM) showing treatment and cultivar effects of foliar hydrogen peroxide sprays, for ungraded and graded (small and large) total fresh mass, number and mean tuber mass.

		Ungraded			Graded					
Cv	**Trt**	**Total fresh mass (Kg)**	**Total number of tubers**	**Mean tuber mass (kg/tuber)**	**Small tuber mass (Kg)**	**Number of small tubers**	**Mean small tuber mass (kg/tuber)**	**Large tuber mass (kg)**	**Number of large tubers**	**Mean large tuber mass (kg/tuber)**
GR	0	12.478± 1.716^{a}	90.250± 7.878^{a}	0.1361± 0.0090^{a}	1.308± 0.132^{bc}	24.750± 2.689^{abc}	0.0529± 0.0016^{abc}	7.297± 0.484^{ab}	75.333± 2.082^{ab}	0.0967± 0.0578^{a}
	1	16.383± 1.161^{a}	103.000± 6.311^{a}	0.1591± 0.0054^{a}	1.453± 0.167^{bc}	23.500± 3.227^{abc}	0.0623± 0.0016^{ab}	8.128± 0.728^{ab}	79.500± 3.948^{ab}	0.1021± 0.0067^{a}
	10	16.723± 2.480^{a}	99.750± 12.122^{a}	0.1659± 0.0051^{a}	1.068± 0.199^{bc}	21.750± 3.326^{bc}	0.0584± 0.0076^{bc}	8.293± 1.916^{ab}	78.000± 9.336^{ab}	0.1019± 0.0143^{a}
	100	14.668± 2.151^{a}	95.000± 16.325^{a}	0.1580± 0.0083^{a}	1.125± 0.243^{bc}	19.750± 5.039^{bc}	0.0598± 0.0047^{abc}	7.280± 1.744^{ab}	75.250± 11.821^{ab}	0.0909± 0.0135^{a}
IN	0	16.573± 2.122^{a}	100.500± 8.391^{a}	0.1636± 0.0086^{a}	1.695± 0.165^{bc}	25.250± 2.213^{abc}	0.0671± 0.0023^{ab}	8.250± 1.592^{ab}	75.250± 8.740^{ab}	0.1073± 0.0079^{a}
	1	15.575± 4.579^{a}	125.500± 1.190^{a}	0.1244± 0.0365^{a}	1.653± 0.116^{bc}	26.250± 1.377^{abc}	0.0629± 0.0029^{ab}	10.358± 0.152^{ab}	99.250± 1.031^{a}	0.1044± 0.0008^{a}
	10	20.040± 1.255^{a}	116.500± 5.752^{a}	0.1729± 0.0127^{a}	1.180± 0.261^{bc}	20.750± 4.289^{bc}	0.0564± 0.0025^{abc}	11.323± 1.318^{a}	95.750± 5.648^{ab}	0.1186± 0.0129^{a}
	100	20.370± 1.819^{a}	117.750± 12.439^{a}	0.1748± 0.0093^{a}	1.538± 0.449^{bc}	24.750± 5.907^{abc}	0.0603± 0.0041^{abc}	10.543± 0.948^{ab}	93.000± 8.134^{ab}	0.1139± 0.0060^{a}
RB	0	15.335± 1.522^{a}	111.750± 5.391^{a}	0.1367± 0.0089^{a}	3.043± 0.160^{a}	44.500± 3.304^{a}	0.0689± 0.0032^{a}	6.778± 1.049^{ab}	67.250± 7.663^{ab}	0.1026± 0.0166^{a}
	1	12.953± 2.493^{a}	96.000± 14.939^{a}	0.1338± 0.0137^{a}	2.320± 0.484^{ab}	37.000± 7.517^{ab}	0.0626± 0.0010^{ab}	4.675± 1.943^{b}	59.000± 9.635^{b}	0.0692± 0.0225^{a}
	10	12.735± 0.307^{a}	91.500± 3.122^{a}	0.1398± 0.0065^{a}	2.100± 0.313^{ab}	33.250± 6.060^{abc}	0.0644± 0.0037^{ab}	6.063± 0.944^{ab}	58.250± 3.224^{b}	0.1079± 0.0244^{a}
	100	15.520± 1.685^{a}	99.250± 10.036^{a}	0.1573± 0.0136^{a}	1.685± 0.346^{bc}	28.250± 5.006^{abc}	0.0589± 0.0014^{abc}	7.290± 1.409^{ab}	71.000± 7.348^{ab}	0.0996± 0.0134^{a}
YG	0	12.870 ±1.669^{a}	84.500± 11.244^{a}	0.1526± 0.0070^{a}	0.705± 0.039^{c}	15.750± 1.181^{bc}	0.0451± 0.0021^{c}	7.588± 1.441^{ab}	68.750± 10.912^{ab}	0.1118± 0.0200^{a}
	1	14.590± 0.820^{a}	90.500± 8.395^{a}	0.1634± 0.0082^{a}	0.690± 0.221^{c}	15.000± 3.979^{c}	0.0440± 0.0028^{c}	7.845± 0.379^{ab}	75.500± 5.605^{ab}	0.1047± 0.0038^{a}
	10	15.478± 1.073^{a}	104.250± 7.052^{a}	0.1489± 0.0075^{a}	1.013± 0.270^{bc}	18.750± 4.871^{bc}	0.0536± 0.0019^{abc}	8.383± 0.631^{ab}	85.500± 4.735^{ab}	0.0978± 0.0029^{a}
	100	15.795± 0.976^{a}	89.500± 8.431^{a}	0.1783± 0.0086^{a}	0.663± 0.052^{c}	12.750± 1.031^{c}	0.0520± 0.0004^{bc}	8.745± 0.771^{ab}	76.750± 7.598^{ab}	0.1144± 0.0034^{a}

Means were compared using Tukey HSD ($P \leq 0.05$). Means with same superscript in the same column are not significantly different. Trt indicates treatment (peroxide at 0, 1, 10, and 100 mM). Cv indicates cultivar (Goldrush, GR; Innovator, IN; Russet Burbank, RB and Yukon Gold, YG).

Table 5.6. Overall treatment effect, disregarding cultivar as a variable, for specific gravity, glucose content, sucrose content, starch grain area and protein content. The second section shows cultivar effect, disregarding treatment as a variable.

	Specific Gravity	Glucose (%)	Sucrose (mg/g)	Starch Grain Area (μm^2)	Protein
Treatment Effect					
0	1.091 ± 0.005^{a}	0.075 ± 0.012^{a}	0.750 ± 0.063^{a}	2343.595 ± 362.059^{a}	10.027 ± 0.379^{a}
1	1.086 ± 0.005^{a}	0.075 ± 0.017^{a}	0.801 ± 0.059^{a}	2315.384 ± 186.703^{a}	10.157 ± 0.526^{a}
10	1.087 ± 0.005^{a}	0.065 ± 0.013^{a}	0.810 ± 0.088^{a}	2429.190 ± 191.957^{a}	10.028 ± 0.341^{a}
100	1.088 ± 0.005^{a}	0.075 ± 0.012^{a}	0.750 ± 0.063^{a}	2590.438 ± 73.332^{a}	10.178 ± 0.324^{a}
Cultivar Effect					
GR	1.083 ± 0.001^{b}	0.125 ± 0.015^{a}	0.592 ± 0.037^{b}	$2457.164 \pm 131.073^{ab}$	10.761 ± 0.376^{a}
IN	1.085 ± 0.001^{b}	0.026 ± 0.003^{c}	0.679 ± 0.053^{b}	2560.824 ± 97.937^{a}	9.448 ± 0.271^{a}
RB	1.091 ± 0.001^{a}	0.073 ± 0.017^{b}	0.899 ± 0.040^{a}	2751.541 ± 145.068^{a}	10.423 ± 0.212^{a}
YG	1.092 ± 0.001^{a}	0.093 ± 0.008^{ab}	1.002 ± 0.063^{a}	1909.078 ± 211.978^{b}	9.740 ± 0.568^{a}

All values expressed as means ± SEM. Means were compared using Tukey HSD ($P \leq 0.05$) and means with same superscript in the same column are not significantly different. Four levels of treatments with peroxide (0, 1, 10, and 100 mM). Cultivars included Goldrush, GR; Innovator, IN; Russet Burbank, RB and Yukon Gold, YG.

Table 5.7 Results for specific gravity, glucose, sucrose content, and protein content with both treatment and cultivar considered.

Cultivar	Treatment	Specific Gravity	Glucose (%)	Sucrose (mg/g)	Protein (g/150g FM)
GR	0	1.0859 ± 0.0021^{ab}	0.107 ± 0.012^{ab}	0.662 ± 0.047^{ab}	9.988 ± 0.855^{a}
	1	1.0810 ± 0.0026^{b}	0.091 ± 0.021^{ab}	0.615 ± 0.097^{ab}	12.128 ± 0.729^{a}
	10	1.0817 ± 0.0008^{b}	0.126 ± 0.005^{ab}	0.458 ± 0.074^{b}	10.275 ± 0.654^{a}
	100	1.0832 ± 0.0027^{ab}	0.175 ± 0.046^{a}	0.566 ± 0.063^{ab}	10.652 ± 0.483^{a}
IN	0	1.0882 ± 0.0029^{ab}	0.029 ± 0.005^{b}	0.613 ± 0.080^{ab}	9.226 ± 0.490^{a}
	1	1.0834 ± 0.0013^{ab}	0.023 ± 0.004^{b}	0.670 ± 0.037^{ab}	9.136 ± 0.909^{a}
	10	1.0817 ± 0.0023^{b}	0.016 ± 0.005^{b}	0.588 ± 0.057^{ab}	9.549 ± 0.316^{a}
	100	1.0851 ± 0.0033^{ab}	0.035 ± 0.010^{b}	0.803 ± 0.178^{ab}	9.882 ± 0.434^{a}
RB	0	1.0934 ± 0.0030^{a}	0.048 ± 0.002^{ab}	1.025 ± 0.045^{ab}	11.029 ± 0.500^{a}
	1	1.0878 ± 0.0012^{ab}	0.110 ± 0.054^{ab}	0.833 ± 0.056^{ab}	9.994 ± 0.541^{a}
	10	1.0923 ± 0.0006^{ab}	0.062 ± 0.014^{ab}	1.024 ± 0.109^{ab}	10.238 ± 0.166^{a}
	100	1.0896 ± 0.0029^{ab}	0.059 ± 0.010^{ab}	0.807 ± 0.032^{ab}	10.431 ± 0.366^{a}
YG	0	1.0945 ± 0.0018^{a}	0.112 ± 0.010^{ab}	0.844 ± 0.201^{ab}	9.813 ± 1.161^{a}
	1	1.0906 ± 0.0033^{ab}	0.074 ± 0.013^{ab}	1.114 ± 0.091^{a}	9.369 ± 1.424^{a}
	10	1.0918 ± 0.0010^{ab}	0.076 ± 0.012^{ab}	1.051 ± 0.078^{ab}	10.050 ± 1.289^{a}
	100	1.0924 ± 0.0008^{ab}	0.104 ± 0.020^{ab}	1.001 ± 0.122^{ab}	9.748 ± 1.163^{a}

Values expressed as means ± SEM for 4 cultivars and 4 treatment levels of foliar hydrogen peroxide sprays. Means were compared using Tukey HSD ($P \leq 0.05$) and means with same superscript in the same column are not significantly different.

Table 5.8. The results for starch granule length, width and area with both treatment and cultivar considered.

Cultivar	Treatment	Length (µm)	Width (µm)	Area (μm^2)
GR	0	66.42 ± 3.11^{ab}	46.64 ± 2.54^{a}	2436.36 ± 221.17^{abc}
	1	61.45 ± 3.11^{abc}	44.35 ± 2.54^{ab}	2146.16 ± 221.17^{abc}
	10	65.43 ± 3.81^{abc}	47.87 ± 3.11^{ab}	2458.82 ± 270.88^{abc}
	100	69.56 ± 3.11^{ab}	51.02 ± 2.54^{a}	2787.32 ± 221.17^{ab}
IN	0	63.17 ± 2.69^{abc}	46.99 ± 2.20^{ab}	2368.00 ± 191.54^{abc}
	1	65.97 ± 3.81^{ab}	48.42 ± 3.11^{ab}	2507.33 ± 270.88^{abc}
	10	69.11 ± 3.11^{ab}	51.81 ± 2.54^{a}	2833.51 ± 221.17^{ab}
	100	66.18 ± 3.11^{ab}	48.69 ± 2.54^{ab}	2534.46 ± 221.17^{abc}
RB	0	73.80 ± 3.11^{a}	54.61 ± 2.54^{a}	3167.33 ± 221.17^{a}
	1	68.92 ± 3.11^{ab}	50.23 ± 2.54^{a}	2723.88 ± 221.17^{ab}
	10	66.71 ± 2.69^{ab}	48.01 ± 2.20^{ab}	2515.25 ± 191.54^{abc}
	100	66.52 ± 3.11^{ab}	49.52 ± 2.54^{a}	2599.70 ± 221.17^{ab}
YG	0	47.47 ± 3.11^{c}	35.93 ± 2.54^{b}	1402.69 ± 221.17^{c}
	1	55.72 ± 3.11^{bc}	42.95 ± 2.54^{ab}	1884.17 ± 221.17^{bc}
	10	57.33 ± 3.11^{bc}	42.38 ± 2.54^{ab}	1909.18 ± 221.17^{bc}
	100	64.29 ± 3.11^{abc}	48.29 ± 2.54^{ab}	2440.27 ± 221.17^{abc}

Values expressed as means ± SEM for 4 cultivars and 4 treatment levels of foliar hydrogen peroxide sprays. Data arranged based on alphabetical order of cultivars. Means were compared using Tukey HSD ($P \leq 0.05$). Means with same superscript in the same column are not significantly different.

Table 5.9 The starch profile including RS, DS, RDS, SDS, pGI concerning overall treatment effect, disregarding cultivar as a variable. The second section shows cultivar effect for the starch profile.

	RS (g/150 DM)	DS (f/150 g DM)	RDS (%)	SDS (%)	pGI
Treatment Effect					
0	73.270±1.757^{a}	26.730±7.027^{a}	22.078±0.598^{a}	22.102±0.836^{a}	82.063±1.629^{a}
1	71.120±1.060^{a}	28.880±4.105^{a}	21.724±0.799^{a}	22.183±0.688^{a}	81.568±1.796^{a}
10	70.506±1.524^{a}	29.494±6.097^{a}	23.569±0.361^{a}	23.498±0.601^{a}	82.859±2.244^{a}
100	70.077±1.640^{a}	29.923±6.559^{a}	21.804±0.598^{a}	22.355±0.559^{a}	82.038±1.085^{a}
Cultivar Effect					
GR	69.895±1.344^{bc}	30.105±5.206^{ab}	20.806±0.570^{b}	20.560±0.522^{b}	81.037±1.714^{ab}
IN	75.084±1.054^{a}	24.916±4.215^{c}	22.219±0.553^{ab}	22.740±0.498^{ab}	79.394±1.779^{b}
RB	65.970±0.963^{c}	34.030±3.851^{a}	22.444±0.698^{ab}	22.361±0.839^{ab}	81.637±1.975^{ab}
YG	73.948±1.558^{ab}	26.052±6.230^{bc}	23.776±0.367^{a}	24.514±0.449^{a}	86.299±0.871^{a}

Values expressed as means ± SEM for 4 treatment levels of foliar hydrogen peroxide sprays. Means were compared using Tukey HSD ($P \leq 0.05$). Means with same superscript in the same column are not significantly different.

Table 5.10. Results for starch quality (RS, DS, RDS, SDS, and pGI) considering both treatment and cultivar as variables.

Cultivar	Treatment	RS (g/150 DM)	DS (g/150 g DM)	RDS (%)	SDS (%)	pGI
GR	0	72.999±1.729^{abc}	27.001±1.729^{abc}	20.067±1.368^{ab}	19.023±1.473^{b}	83.700±3.038^{a}
	1	71.886±2.487^{abc}	28.114±2.487^{abc}	19.549±1.399^{b}	21.087±0.954^{ab}	81.230±3.781^{a}
	10	67.098±1.353^{bc}	32.902±1.353^{ab}	22.967±0.369^{ab}	20.876±1.063^{ab}	78.375±5.228^{a}
	100	68.093±3.977^{abc}	31.907±3.977^{abc}	20.640±0.511^{ab}	21.252±0.402^{ab}	80.842±1.817^{a}
IN	0	76.952±2.908^{a}	23.048±2.908^{bc}	21.494±0.923^{ab}	22.494±1.383^{ab}	79.651±3.166^{a}
	1	75.122±1.166^{abc}	24.878±1.166^{abc}	21.259±1.133^{ab}	22.449±0.718^{ab}	79.663±2.753^{a}
	10	73.252±1.263^{abc}	26.748±1.263^{abc}	24.057±1.197^{ab}	23.991±1.028^{ab}	78.542±5.618^{a}
	100	75.012±2.917^{abc}	24.988±2.917^{abc}	22.016±0.499^{ab}	21.789±0.359^{ab}	79.829±2.965^{a}
RB	0	63.761±1.180^{c}	36.239±1.180^{a}	23.283±1.185^{ab}	22.218±1.778^{ab}	78.043±2.328^{a}
	1	68.346±1.333^{abc}	31.654±1.333^{abc}	20.572±1.445^{ab}	19.679±1.882^{ab}	76.350±3.436^{a}
	10	66.717±2.598^{bc}	33.283±2.598^{ab}	24.069±0.681^{ab}	25.030±1.258^{a}	89.649±3.330^{a}
	100	65.055±2.155^{bc}	34.945±2.155^{ab}	21.383±1.704^{ab}	21.845±1.243^{ab}	81.185±1.383^{a}
YG	0	79.369±1.343^{a}	20.631±1.343^{c}	23.468±0.655^{ab}	24.674±1.150^{ab}	86.858±1.846^{a}
	1	69.318±2.073^{abc}	30.682±2.073^{abc}	25.226±0.692^{a}	24.891±0.809^{a}	87.724±2.457^{a}
	10	74.957±4.304^{abc}	25.043±4.304^{abc}	23.182±0.492^{ab}	24.096±0.439^{ab}	84.868±1.062^{a}
	100	72.150±2.353^{abc}	27.850±2.353^{abc}	23.230±0.768^{ab}	24.393±1.318^{ab}	85.746±1.737^{a}

Values expressed as means ± SEM and means were compared using Tukey HSD ($P \leq 0.05$). Means with same superscript in the same column are not significantly different.

Table 5.11. Antioxidant capacity using ABTS (mM Trolox Eq/ 100 g DM), DPPH (mg Trolox Eq /100 g DM) and F-C (mg chlorogenic acid Eq /100 g DM) and High Performance Liquid Chromatography (HPLC) results (mg/100 g DM) which include ascorbic acid, chlorogenic acid, caffeic acid, ferulic acid, and rutin.

	ABTS	DPPH	F-C	Ascorbic Acid	Chlorogenic acid	Caffeic Acid	Ferulic Acid	Rutin
Treatment Effect								
0	195.247±7.509^{a}	199.051±11.135^{a}	709.808±42.809^{a}	67.400±7.003^{a}	107.313±8.393^{a}	7.025±0.375^{a}	4.210±0.506^{a}	19.645±1.624^{a}
1	178.618±10.350^{a}	187.252±15.879^{a}	631.537±23.836^{a}	73.699±5.181^{a}	106.784±8.155^{a}	7.353±0.480^{a}	2.953±0.414^{a}	20.022±2.618^{a}
10	183.827±10.984^{a}	22.742±8.399^{a}	746.583±53.467^{a}	64.129±3.249^{a}	102.102±5.120^{a}	6.559±0.410^{a}	3.674±0.335^{a}	21.863±0.908^{a}
100	186.997±5.960^{a}	207.018±11.350^{a}	708.717±67.729^{a}	74.371±4.266^{a}	95.800±5.973^{a}	6.853±0.178^{a}	4.333±0.434^{a}	19.072±1.344^{a}
Cultivar Effect								
GR	167.946±11.680^{b}	232.409±9.607^{a}	943.892±56.605^{a}	78.585±6.330^{a}	122.977±7.258^{a}	7.183±0.412^{ab}	3.606±0.623^{a}	18.387±1.761^{b}
IN	184.857±7.189^{ab}	217.814±9.639^{ab}	523.656±18.317^{c}	53.125±5.148^{b}	108.557±7.452^{ab}	6.146±0.555^{b}	3.988±0.285^{a}	19.126±1.734^{b}
RB	211.266±5.861^{a}	190.544±16.341^{ab}	704.901±23.184^{b}	72.731±4.138^{a}	98.397±5.776^{bc}	7.573±0.248^{a}	2.646±0.637^{a}	25.753±1.865^{a}
YG	179.921±5.987^{b}	181.321±7.687^{b}	704.137±23.029^{b}	68.975±2.474^{ab}	83.251±2.390^{c}	6.814±0.179^{ab}	4.422±0.377^{a}	17.578±0.508^{b}

Means were compared using Tukey HSD ($P \leq 0.05$). Means with same superscript in the same column for each section are not significantly different.

Table 5.12 Antioxidant capacity using ABTS (mM Trolox Eq/ 100 g DM), DPPH (mg Trolox Eq /100 g DM) and F-C (mg chlorogenic acid Eq /100 g DM) considering both cultivar and treatment as variables.

Cultivar	Treatment	ABTS	DPPH	F-C
GR	0	170.647±11.484^{a}	240.175±5.660^{a}	937.471±33.749^{ab}
	1	140.998±22.717^{a}	196.381±28.826^{a}	597.053±22.016^{cdef}
	10	167.575±20.803^{a}	240.175±0.793^{a}	1090.224±20.806^{a}
	100	183.581±11.617^{a}	246.095±0.333^{a}	1035.205±31.569^{a}
IN	0	184.780±15.146^{a}	210.405±18.888^{a}	499.737±17.380^{ef}
	1	188.475±23.817^{a}	205.381±28.160^{a}	549.164±31.530^{def}
	10	188.409±9.080^{a}	230.698±1.788^{a}	571.911±25.155^{cdef}
	100	176.607±10.202^{a}	231.952±2.828^{a}	473.814±52.623^{f}
RB	0	216.687±10.895^{a}	194.893±25.173^{a}	762.589±26.832^{bc}
	1	208.034±4.372^{a}	176.500±22.957^{a}	699.723±49.652^{cde}
	10	226.940±2.691^{a}	211.548±21.499^{a}	689.917±51.963^{cde}
	100	200.431±14.842^{a}	189.738±28.573^{a}	629.853±20.102^{cdef}
YG	0	205.217±8.946^{a}	161.012±14.287^{a}	696.351±40.411^{cde}
	1	167.974±4.148^{a}	177.560±16.397^{a}	662.964±25.634^{cdef}
	10	165.226±14.514^{a}	204.191±17.918^{a}	720.190±73.560^{bcd}
	100	183.918±8.113^{a}	182.524±8.404^{a}	748.011±25.317^{bcd}

Values expressed as means ± SEM and were compared using Tukey HSD ($P \leq 0.05$). Means with same superscript in the same column are not significantly different.

Table 5.13 High Performance Liquid Chromatography (HPLC) results. Values expressed as means ± SEM of ascorbic acid, chlorogenic acid, caffeic acid, ferulic acid, and rutin for 4 cultivars and 4 treatment levels of foliar hydrogen peroxide sprays.

Cultivar	Treatment	Ascorbic Acid	Chlorogenic Acid	Caffeic Acid	Ferulic Acid	Rutin
GR	0	96.408±12.398^{a}	144.252±3.409^{a}	8.020±0.517^{a}	1.689±0.288^{a}	17.709±0.431^{ab}
	1	78.948±13.883^{ab}	142.279±11.621^{a}	8.002±0.517^{a}	3.370±0.129^{a}	12.791±4.796^{b}
	10	59.193±6.671^{ab}	104.165±13.377^{ab}	6.034±1.131^{a}	3.136±0.076^{a}	24.215±1.104^{ab}
	100	80.196±15.999^{ab}	101.209±13.307^{ab}	6.677±0.282^{a}	5.667±1.887^{a}	18.758±1.062^{ab}
IN	0	37.874±8.642^{b}	118.288±15.085^{ab}	5.151±0.181^{a}	4.421±0.396^{a}	17.793±1.168^{ab}
	1	59.215±6.511^{ab}	93.806±22.200^{ab}	7.056±1.703^{a}	2.900±0.665^{a}	21.841±6.128^{ab}
	10	61.515±5.881^{ab}	116.673±6.182^{ab}	6.213±1.059^{a}	4.643±0.165^{a}	19.651±1.062^{ab}
	100	68.396±6.146^{ab}	94.990±16.864^{ab}	6.181±0.143^{a}	3.445±0.780^{a}	15.314±0.229^{ab}
RB	0	68.014±12.186^{ab}	78.408±15.552^{b}	7.976±0.315^{a}	5.722±1.183^{a}	24.159±5.905^{ab}
	1	76.538±10.596^{ab}	96.266±6.292^{ab}	7.459±0.751^{a}	1.445±0.168^{a}	31.194±3.014^{a}
	10	73.329±8.541^{ab}	109.330±3.251^{ab}	7.565±0.639^{a}	1.870±0.169^{a}	24.464±2.406^{ab}
	100	74.115±5.259^{ab}	109.050±9.985^{ab}	7.263±0.317^{a}	1.936±0.263^{a}	24.555±2.208^{ab}
YG	0	67.457±4.770^{ab}	88.305±5.812^{b}	6.954±0.300^{a}	4.600±0.826^{a}	18.434±0.677^{ab}
	1	74.272±5.580^{ab}	88.910±3.340^{b}	6.922±0.503^{a}	3.539±0.906^{a}	17.056±0.796^{ab}
	10	62.481±5.543^{ab}	78.241±4.140^{b}	6.425±0.287^{a}	4.595±0.644^{a}	19.122±0.351^{ab}
	100	71.689±3.342^{ab}	77.547±3.714^{b}	6.954±0.382^{a}	5.189±0.556^{a}	15.701±1.285^{ab}

Results are reported in mg/100 g DM. Data arranged based on alphabetical order of cultivars. Means were compared using Tukey HSD ($P \leq 0.05$). Means with same superscript in the same column are not significantly different.

Chapter 6: Summary, Conclusions, and Suggestions for Future Research

6.1 General Summary and Conclusions

The potato (*Solanum tubersum* L.) has become a staple food in most parts of the world as it is the fourth most significant food crop and the most important vegetable grown globally (Ek et al., 2012; Ezekiel et al., 2013). Although the range of nutritional values in potato can vary depending on genotype and environment, as a frequently consumed (staple) crop, the benefits of a superior starch profile, lower glycemic impact, higher protein content, and higher antioxidant capacity are dietarily significant (Bach et al., 2013; Brown, 2005; Ezekiel et al., 2013; Nzaramba et al., 2013).

This thesis was divided into three main studies. In the first study (Chapter 3), potatoes from two Quebec breeding programs were evaluated and the best genotypes in terms of human nutrition were identified. Additionally, a model system was created to reveal various explanatory and predictive factors for GL. In the second study (Chapter 4), inter-seasonal differences regarding nutritional profiles were assessed for the cv. Russet Burbank (control) and its four advanced somatic lines (RB somaclones; FC2006, FP3405, MS1406 and MP18405) which are currently in registration trials. At the same time, differences between the original breeding material (RBP) and the somaclones were examined to identify select superior lines that could be used in the market or as future somatic breeding material. The final study (Chapter 5) involved investigating the use of hydrogen peroxide as a hormetic agent on field grown potatoes. This thesis forms an important contribution towards on-going projects by our research group (Drs. Donnelly & Kubow).

In Chapter 3, 14 genotypes (3 parental lines and 11 named cultivars) from the Quebec potato breeding program (MAPAQ at Les Buissons), as well as four advanced somatic lines and one control from the McGill somatic breeding program were screened and the best genotypes in terms of human nutrition, were selected. These nutritional characteristics included an optimal starch profile (high RS, lower DS [where SDS>RDS]), lower GI and GL, as well as a high

amylose, phosphorylated starch, protein, antioxidant capacity, ascorbic acid and polyphenolic profile. The relationship between GL and the above variables was done to create a model system to help define the characteristics that contribute the most to GL and to determine which can be used as predictor variables.

Three superior genotypes were identified, based on their potential to provide significant health benefits. The cultivar Kalmia, the breeding line QP010090.05JP, and the somaclone MS1406 were the best, overall. Kalmia had one of the lowest GL (18.836±0.214) and highest RS content (3.7258±0.3038 g/150 g FM), and also a greater polyphenolic and antioxidant capacity for all three tests (ABTS, DPPH, and F-C) compared to other genotypes (Table 3.3, 3.4, & 3.6). The breeding line QP010090.05JP also had a lower GL (19.808±2.468) but also a lesser GI (67.430±0.554) compared to other breeding lines and cultivars, as well as a higher quantity of phosphorylated starch, which has previously been shown to decreased starch digestion (Ek et al., 2012; Sitohy & Ramadan, 2001), although this variable was not included in any of our models (Table 3.3). Additionally, this genotype had a significant content of ascorbic acid and phenolics (chlorogenic acid, caffeic acid, and rutin) for cooked samples. This line therefore showed great potential for further breeding activities because of its lower glycemic impact and greater phenolic composition that can improve the nutritional value of future lines of potatoes. The somaclone MS1406 had a smaller GL (20.197±0.583) and GI (67.189±1.373) and also a high amount of phosphorylated starch, low RDS, and one of the highest amylose contents (25.694±0.763 %) (Table 3.2 & 3.3). Also, this genotype had a high ascorbic and chlorogenic acid content (for both raw and cooked samples), and it was one of the highest genotypes using the ABTS and F-C tests for both raw and cooked samples. Consumers can be made more aware of the impact that starch quality and antioxidant capacity can have towards providing significant health benefits. Kalmia and MS1406 can be promoted on the fresh market as being a healthier choice, where the breeding line as well as the somaclone MS1406 can be used as breeding materials (using traditional or somaclone technology) to create superior genotypes in the future. Also, the somaclone can be used as an alternative to Russet Burbank in the table stock market.

There appears to be no general trend or pattern regarding differences in phenolic, ascorbic acid content, or antioxidant capacity between raw and cooked sample types, or any specific genotype showing consistent sample type differences. Also no significant starch correlations within the

characteristics or when compared to GI and GL were noteworthy. That being said, the models were then easier to compute, manipulate and determine. Out of the many possible models, 3 were selected due to their high significance;

Model 1 → GL = % moisture + GI + RS + FER (cooked) + DPPH (cooked)

Model 1 explains 96 % of the variance observed ($p=8.581 \times 10^{-9}$, F- statistic = 66.39).

Model 2 → GL = % moisture + GI + FER (cooked)

Model 2 explains up to 91 % of the variance in GL ($p=4.564 \times 10^{-8}$, F- statistic = 50.39). Model 2 is simpler than Model 1, as it does not include RS or DPPH (cooked).

Model 3 → GL = % moisture + GI+ amylose + RDS + SDS + AA (raw) + CGA (raw) + CFF (cooked) + FER (cooked) + F-C (cooked)

Model 3 explains 98 % of the variance in GL ($p= 2.152 \times 10^{-5}$, F statistic of 32.52)

These models present an interesting opportunity for breeding programs; they can complete a quick screening of their cultivars for characteristics that help predict a lower GL, then select their top parental lines. This can save both time, resources, and in the end help growers and industry to more easily select healthier cultivars, without having to screen all possible genotypes for all characteristics. The models that were created were done so using a complete database of results from 19 genotypes. It is therefore encouraging that the top three best performing genotypes (Kalmia, QP010090.05JP, and MS1406), conformed and validated the resulting models that were selected. This thesis data provides support that the models will have future merit, although this remains to be tested.

Our models also help support results found in the literature. Overall, reports have suggested that the more RS content, the less starch digestion occurs and therefore a lower GI and GL is expected (Bach et al., 2013; Ek et al., 2012). It is retrograded amylose that forms resistant starch, which in turn is not digested and hence does not contribute to GL. Previous reports have shown that there is a high positive correlation between RS and amylose content (Ek et al., 2012; Nayak et al., 2014), although some findings have indicated that there is no relationship to GI (Ek et al.,

2014). Additionally, phenolics are thought to inhibit α-amylase, which can explain why ferulic acid, and also DPPH, are included into the models (McDougall & Stewart, 2005).

Further confirmation regarding these models is necessary. Possibilities include completing another set of evaluations on other genotypes for the same characteristics, determining top models and then comparing. Also, after screening is completed, the models from this thesis could be validated, such that the genotypes with high moisture, lower GI, higher RS and amylose, as well as a greater phenolic and antioxidant values (specifically DPPH and ferulic acid) are tested to confirm, if in fact, the GL is lower compared to other genotypes.

In Chapter 4, the cv. Russet Burbank (control) and its four advanced somatic lines (RB somaclones; FC2006, FP3405, MS1406, and MP18405) which are currently in registration trials, were evaluated for starch quality (including RS, DS, SDS, RDS), GI and GL, as well as antioxidant capacity, ascorbic acid, and polyphenolic profile over two growing seasons. This was done to assess seasonal differences to determine if the characteristics listed above were stable and to identify relatively healthier genotypes that could be promoted for market consumption and as new breeding material. Environmental factors between seasons may influence the production of secondary metabolites or starch synthesis, and the stability of the genotypes and their response to stress can affect the desirable nutritional characterizes under evaluation (Bach et al., 2013; Valcarcel et al., 2014).

All starch characteristics (RS, DS, SDS, RDS) did not change between seasons, except that the RDS content of all the genotypes were higher in season one compared to season two (Table 4.2). Also, there were no differences in starch profile components, when comparing the RB somaclones with the Russet Burbank control. Therefore, for the most part, starch synthesis and fractions were stable regardless of the season and somaclone line. There were no seasonal difference between GI and GL and among genotypes. The GI value for the somaclones in season one (except for FC2006) and season two (except for RBP) were interesting because the values were “intermediate” which has potential nutritional interest, although they were very close to the cut-off point that defines GI as high (70). The glycemic index and load did not significantly vary between RB somaclones and the Russet Burbank control (although all somaclones in season two were considered to have an “intermediate” GI, whereas the Russet Burbank control was “high”).

This lack of statistical significance between the control materials and the somaclones makes sense, as the starch profiles which we know can affect the glycemic impact did not change, (Nayak et al., 2014). Our data thus further supports evidence that the starch profiles of the somaclones do not vary between somaclones and seasons.

The antioxidant capacity of the McGill RB somaclones differed between growing seasons, and was affected by method of quantifying antioxidant capacity (Table 4.4). The ABTS values were similar in both seasons, except for FP3405 which was greater in season two. The somaclones showed no difference in ABTS compared to the controls for both seasons individually. The control (273.478$\pm$7.828) showed a significantly greater DPPH content (mM Trolox Eq/100 g DM) in season one compared to FP3405 (81.306$\pm$5.541), although no difference was seen in season two. All the somaclones had greater F-C values in season one compared with season two. Most importantly, the results from the F-C test showed that MS1046 had a greater antioxidant capacity than the control as well as all the other somaclones. Interestingly, the RBP somaclone FP3405 always had a lower antioxidant capacity, regardless of the test used. No difference was found between the control and the somaclones for all phenolics (chlorogenic, caffeic, and ferulic acid) and ascorbic acid for both seasons, except that in season one, MS1406 had a greater rutin (mg/100 g DM) content (19.524$\pm$1.919) compared to FC2006, MP18405, and Russet Burbank (6.022$\pm$0.425, and 5.963$\pm$0.149, respectively) (Table 4.5).

Overall, the MS1406 line (season two) has been shown to have an “intermediate” GI, a greater antioxidant capacity measured by F-C, and greater rutin content compared to the control. This somaclone shows potential as an alternative to Russet Burbank, with the same yield and fry quality but improved antioxidant value, which has become of greater interest for consumers. Additionally, a somaclone with lower GI can benefit from this effective marketing tool to encourage consumers concerned regarding the impact of the glycemic response to foods and human health (Aziz et al., 2013). This somaclone has merit, and could possibly be used for somatic breeding material to test whether its promising nutritional value can be even further improved.

In Chapter five, a hormetic agent (hydrogen peroxide) was applied as a foliar spray on field-grown potatoes (Goldrush, GR; Innovator, IN; Russet Burbank, RB; and Yukon Gold, YG) to

assess the agronomic value of this technique. The treatment sprays began 2 mo after planting (at the time of tuber initiation) and 0 mM (control), 1, 10, and 100 mM of hydrogen peroxide were used. To determine the benefit of using this technique, effect on yield, processing quality, and functional food properties were investigated. The functional food properties examined were; starch quality (RS, DS, RDS, and SDS), GI and GL, as well as protein content, antioxidant capacity, ascorbic acid, and polyphenolic profile, which allowed us to determine if the hormetic agent caused any changes in nutritional value for these cultivars. Cultivar differences in nutritional value were apparent and expected, although the effect of the hormetic agent was the main focus of this chapter.

There were no effects of hormetic treatment on yield (total FM, tuber number and mean tuber FM) for either ungraded or graded (small and large) tubers for all four cultivars (Tables 5.4 and 5.5). The hormetic treatment was also ineffective at altering specific gravity, glucose, sucrose, and protein content (Table 5.6 and 5.8). This means that it is unlikely that any enzymes in general or those specifically involved with polyphenols were affected by the use of hydrogen peroxide in the field. No differences for RS, DS, RDS, and SDS content, or GI resulted from hormetic treatments (Table 5.9 and 5.10). This shows that the application of a foliar hydrogen peroxide spray for cultivars GR, IN, RB, and YG is ineffective at changing (increase or decrease) starch content, or modifying the profile meaning that the overall starch quantity would be the same, but the quality would be affected. In addition to measuring the starch content and quality, we measured the starch granule length, width and calculated the overall area, to further confirm that starch was not affected after treatment. Our results showed that there was no treatment effect within a cultivar for every variable (length, width and area) (Table 5.6 and 5.8). Results from a preliminary study in the Donnelly lab, showed that hydrogen peroxide applied to microtubers could increase phenolic and antioxidant capacity and lead to the current hormetic studies on field-grown potatoes. It was found that in the field, the quality and profile of antioxidants, ascorbic acid and polyphenolics were unaffected after treatment (Table 5.11, 5.12, and 5.13). There was one exception; using F-C (mg chlorogenic acid Eq/100 g DM), the cv. Goldrush at 1 mM treatment (597.053$\pm$22.016) showed lower antioxidant capacity than all other treatment levels including the control (937.471$\pm$33.749). It can still be concluded though that the total antioxidant capacity, ascorbic acid, and polyphenols did not significantly increase after treatment.

Although seemingly very important, no other such field studies have been reported in the intervening years. The positive results reported by López-Delgado et al. (2005) were published 10 years ago, and only used one cultivar (cv. Alpha) in their study. In comparison, we used 4 cultivars that are important and relevant to Canadian industry (Russet Burbank and Innovator; French fry cultivars) as well as Goldrush and Yukon Gold (table stock cultivars). We also included a wider treatment range to more thoroughly explore any change in phytonutrient content. We can conclude that there is no suggestion that further investment or scientific resources should be applied to this area of research. While it is possible that other hormetic agents could be more successful in this regard, it is also likely that the overall stress under conventional planting situations exceeds any baseline that might be increased through hormetic stress in the greenhouse or in vitro.

6.2 Suggestions for Future Research and Techniques

- While many labs use freeze dried samples for starch analysis, the literature is often ambiguous and no standard method has been suggested. A comprehensive comparison is lacking in the literature and should be done to confirm the best method of sample preparation. The different sample types, where starch analysis would immediately follow, could include;
    - Cooked fresh potato samples (no freeze-drying)
    - Raw potatoes which are freeze dried into a powder. Before analysis they are rehydrated (based on the % moisture lost during the freeze-drying process). This paste is then subsequently cooked and used (Mishra et al., 2008).
    - Potatoes that have been cooked, and then freeze-dried right away (the % moisture is taken into consideration during the calculations, after the experiment). The powder is used directly. This method was used in the current thesis.
    - Cooked potatoes that are freeze dried, but then rehydrated and then used immediately (would assess the impact of freezing on analysis).
- Other hormetic agents could be tested with the potential of being above the natural baseline of stress, and applied to potatoes to determine if they can affect the nutritional value of field-grown cultivars.
- Testing the GL models derived in this thesis. This would include;

    - Using a new dataset for the phytonutrients tested in this thesis (preferably on a large enough population of potatoes, for at least 2 seasons) so that the current predictive model(s) for GL should be validated and refined.
    - On this same alternate dataset mentioned above, the model(s) that defined predictive variables for GL should be used and then confirmed.
- Human feeding trials should be carried out to determine whether major differences in predictive variables could significantly impact their GL values.

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