Impact of cooking method on the protein quality of Russet potatoes

By

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Abstract

Prior research has found that even though the amount of protein within potatoes is low (average of 2% w/w), due to the high consumption of potatoes in North America, it is estimated that they provide 2-4% of daily protein intake. Thus, potatoes are an important contributor for protein in diets. However, research is limited on the impact of cooking method on the quality of the protein in Russet potatoes, a major potato varietal. The current study was designed to address this knowledge gap. Russet potatoes were secured and subjected to the following cooking conditions (3 replicates per condition): raw, boiled, baked, microwaved and fried (3, 6 and 9 minutes). Following cooking, samples were analyzed for crude protein (CP) and total amino acids (AA; 3 hydrolysis conditions) by AOAC methods, *in vitro* protein digestibility (%IVPD) by pH-stat analysis, and *in vivo* protein digestibility by AOAC methods determining true fecal protein digestibility (TPD). *In vitro* protein digestibility-corrected amino acid score (PDCAAS) values were determined as the product of AAS and %IVPD. For %CP, on an as-consumed basis, with the exception of boiled, all cooking methods yielded higher ($p<0.0001$) values than the RAW samples. (RAW=1.85±0.04; BOIL=1.67±0.04); MICRO= 2.99±0.06; BAKE=2.44±0.03; FRIED3M=3.07±0.07; FRIED6M=3.87±0.03; FRIED9M=4.77±0.08). The AAS of raw potato was 0.66±0.01 with histidine as the first limiting AA. The AAS for the fried cooking methods (FRIED3M=0.474±0.009; FRIED6M=0.044±0.012; FRIED9M=0.36±0.015) as well as BAKE (0.57±0.045) were significantly lower than the RAW control ($p=<0.05$). The other AAS (BOIL=0.675±0.03; MICRO=0.589±0.008) were not significantly different from RAW. Based on 1-way ANOVA, there was a significant ($p<0.05$) main effect of cooking method on %IVPD except for BOIL and FRIED9M when compared to RAW (RAW=74.1±0.6; BOIL=74.3±0.5; FRIED9M=76.6±1.7; MICRO=78.1±2.3; BAKE=79.2±1.0; FRIED3M=78.4±0.6; and FREID6M=78.0±0.7). IVPDCAAS was lowest for FRIED9M=27.7±1.8, while BOIL=50.2±0.4 was the highest. The TPD was impacted by cooking methods, with values being: MICRO = 0.49; BAKE = 0.53; and BOIL = 0.56, which were all significantly higher than values observed for RAW (0.27) and FRIED6M (0.29). To summarize, cooking methods can significantly influence protein quality, with longer frying times leading to lower quality scores. Based on the limiting AA profile, potatoes are complementary protein sources to pulses and cereals, however frying can negatively impact potato protein quality.

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List of Abbreviations

AA - Amino Acid

AAS - Amino Acid Score

ALA – Alanine

ANFS – Anti-nutritional Factors

AOAC – American Association of Analytical Chemists

ARG – Arginine

ASP – Asparagine

BV – Biological Value

CNF – Canadian Nutritional Files

CP – Crude Protein

CTL – Central Testing Laboratory

CYS – Cysteine

DIAAS - Digestible Indispensable Amino Acid Score

DV - Daily Value

EAA – Essential Amino Acids

FAO - Food and Agriculture Organization

GI – Glycemic Index

GIT - Gastrointestinal Tract

GLU – Glutamine

GLY – Glycine

HCl - Hydrochloric acid

HIS – Histidine

HPMC - Hydroxypropyl Methylcellulose

ILE – Isoleucine

IVPD - *in vitro* Protein Digestibility

IVPDCAAS - *in vitro* protein digestibility-corrected amino acid score

LAA – Limiting Amino Acid

LUE – Leucine

MET – Methionine

NaOH – Sodium hydroxide

NPN – Non-Protein Nitrogen

NPU – Net Protein Utilization

OPA - o-Phthaldialdehyde

PDCAAS – Protein Digestibility Corrected Amino Acid Score

PER - Protein Efficiency Rating

PHD – pH-drop

PHDIVPD – pH-drop *in vitro* protein digestibility

PHDIVPQ - pH-drop *in vitro* Protein Quality

PHE – Phenylalanine

PHS – pH-static

PHSIVPD – pH-static *in vitro* protein digestibility

PHSIVPQ - pH-static *in vitro* Protein Quality

PRO – Proline

RACC - Reference Amount Customarily Consumed

RDA - Recommended Dietary Allowance

RPM - Revolutions Per Minutes

RS - Resistant Starch

RS2 - Resistant Starch 2

RS3 - Resistant Starch 3

SER – Serine

TCA – Trichloroacetic Acid

TPD - True Fecal Protein Digestibility

THR – Threonine

TIM-1 - TNO Gastro-Intestinal Model

TIMIVPD - TIM-1 *in vitro* protein digestibility

TIMIVPQ – TIM-1 *in vitro* Protein Quality

TRP – Tryptophan

TSIVPQ – Two-Step *in vitro* Protein Quality

TSIVPS - Two-Step *in vitro* protein digestibility

TYR – Tyrosine

VAL – Valine

WHO - World Health Organization

Chapter 1: Introduction

As consumers demand more plant-based protein sources, there is a need to search for alternative sources. The most common plant-based proteins currently used as protein ingredients are soybean and pea proteins. These sources are available in large quantities globally. The protein in these plant products is known to be in high quantities. In 100g of raw green peas there is an average of 5.42g of protein (1). The same amount of soybeans provides an average of 36g of protein (2). It is important to note that plant-based proteins can be poorer in terms of protein quality compared to animal proteins. Animal proteins are often complete proteins, meaning that all 9 essential (indispensable) amino acids (EAA) are available for utilization. The same cannot be said for all plant-based proteins, as they are often limiting in one or more amino acids, and may have intrinsic factors that impact the ability of consumers to fully digest and absorb the amino acids. As such, consumers may have to ingest more plant-based protein in order to match the amino acid supply provided by animal proteins.

Proteins contain 20 amino acids within their primary structure, thus forming the basis for the variety of proteins found in plant and animal tissues. These amino acids are the building blocks for proteins in the diets of both animals and humans. Of the 20 amino acids that are typically encoded for inclusion within proteins, only 9 are considered truly essential or indispensable, meaning that the human body is unable to produce them on its own and they must therefore by supplied in the diet. When considering plant proteins as sources of protein certain amino acids need additional consideration, including lysine, tryptophan and the sulphur amino acids, which tend to be limiting in plant-based proteins. The amino acids in purified potato protein provides a balance of all essential amino acids (3).

Global production of potatoes reached 379 883 663 tonnes in 2017 (4). These numbers put potatoes behind rice and wheat as a food crop. Consumption of potatoes globally in 2013 per capita was 32.6 kg (5). The world's largest consumer of potatoes is China with 99, 122, 420 tonnes consumed in 2017. India follows with 50% less consumption equal to 43,770,000 tonnes (4). In 2016, the United States was 5th for total consumption of potatoes at 19,990,950 tonnes (4). Consumption of potatoes in China per capita was 41.4kg in 2013 while, in Germany, per capita consumption was 61.5kg (5). Over half of all consumption of potatoes in the United States is in the form of frozen fries, making up 55% of all forms of potatoes (4). The Netherlands

export the highest dollar value of prepared/preserved potatoes at 2.2 US billion dollars, with the United States sitting third, exporting 1.2 US billion dollars (6).

Current research on potato protein isolates have found that all EAA are found in amounts that exceed the Food and Agriculture Organization's (FAO) EAA requirements for adults (7).

Positioning potatoes to hold a place in the plant-based protein market has been as uphill battle, as many consumers see potatoes as an unhealthy vegetable. Labeling potatoes as unhealthy couldn't be farther from the truth, as they provide a large variety of micronutrients and can make up 2-4% of one's daily protein intake.

Chapter 2: Literature Review

2.1 The Potato

Every continent except for Antarctica produces potatoes. Potatoes are consumed by over a billion people daily (8), making potatoes the third most important food crop behind only cereal rice and wheat (9). All potatoes belong to the family *Solanaceae*, with the Latin name of *Solanum tubersum.* There are over 5000 varieties of potatoes cultivated world-wide, with the majority arising in the Andes (10). Potatoes are indigenous to South America, where the Inca people cultivated them since 5000 B.C.E. Potatoes were introduced to Europe in the 16th century as a result of early exploration, and from Europe they were then introduced to North America. For all species of potatoes, there are 5 distinctive botanical features, including the overall plant, leaves, flowers, tubers and sprouts (11). The tubers of potatoes are the component that humans consume, and they can vary in macronutrient composition as a function of the specific variety that is cultivated.

There are over 200 varieties of potatoes sold in the United States within 7 different categories: russet, red, white, yellow, blue/purple. fingerling and petite. Each category has differing features which can be found in **Table 1**.

Table 1. Popular varieties of potatoes in North America

Category	Features	Best Uses
Russet	Thick skin Fluffy and light centre	Baked Pan fried Mashed fried
Red	Thin skin Firm after cooked	Baked Salad Soup Grilled Steamed
Yellow	Butter flavour Creamy texture	Baked Mashed Salad Soup grilled
White	Thin skin Nutty flavour Firm after cooking	Pan fried Salads Soup Fried Steamed
Purple	Medium skin Earthy flavour Vibrant colour	Baked Salads Steamed Microwaved
Fingerling	Nutty and butter flavour Firm texture	Baked Pan fried Steamed Microwaved
Petite	Similar to larger varieties	Baked Pan fried Steamed microwaved

Data comprised from Potatoes USA (12)

From a commercial perspective, Russet potatoes are popular due to the fact that they are high yielding potatoes that also have a long storage capacity. Russet potatoes were first bred in 1880 in Santa Rosa, California and were registered in Canada in 1923 (11). Prior to genetic diversity being added to the crops, the crops were left vulnerable to diseases such as late blight, which caused the 1845 Irish Famine (13). Potatoes are an important and affordable staple food and have been so for the past hundreds of years. Their role as an economical global food staple, with sustainable supply as well as the potential to reduce poverty and malnutrition were outlined in the FAO 'International Year of the Potato'(14). Compared to 38 other foods tested, boiled potatoes have the highest satiety index, even those with higher protein and fat levels (15,16). The use of potatoes extends past using raw materials for food processing, as potatoes are also used in the development of value-added components such as starch, for use in alcohol, food and feed ingredients.

2.1.1 Current Statistics on growth and production

Given the initial high start-up costs, it can be difficult for low-income farmers to enter into commercial potato production. In developing countries such as India, programs are in place, including one from McCain Foods Ltd, that support local farmers and facilitate increased crop production, as well as the ability to capture increased wealth (8). Global potato production increased 21% from 1991 to 2007, highlighting its importance as a staple food around the globe (9). As the need for sustainable food staples increases, it is important to note that potatoes, on a per hectare basis, produce more protein and dry matter than rice and wheat (9). In regard to sustainability, to generate a tonne of potatoes, 0.06ha of land is required. In comparison, to generate the same amount of rice and wheat, 0.24ha and 0.35ha are needed, respectively, with less water required, particularly in comparison to rice production (17).

2.1.2 Potatoes within the North American diet

There is a wide variety of health benefits of consuming potatoes as part of one's balanced diet. As stated, above potatoes provide a significant number of dietary vitamins and micronutrients. A medium sized baked potato for an adult male can provide half the daily intake of vitamin C and vitamin B6, 30% potassium, 28% folate, 24% iron and 18% magnesium. In 2008-2011, potatoes contributed to 7% of total energy intake in the United Kingdom, however this value has decreased by 8.8% from 2013-2016 (18,19). In 2007 the US consumption of potatoes was an average of 56kg/year, with fresh potatoes equating to 16kg in 2006 (20).The average Canadian

consumption of potatoes in 2007 was 76kg/year and 66.6 in 2015 (5,21). Regions of South America consumed upwards of 800g of potatoes per day (22). By geographic location, Asia is the largest consumer of potatoes at 94038000 tonnes, when the population is taken into account, the per capita consumption is 23.6 kg/year. Europe, at a total consumption of 64902000 tonnes, had a per capita consumption of 87.8kg/capita, making it the highest consumer of potatoes per capita globally in 2005. In 2017 the average global consumption per capita was 33.5kg (5). In terms of satiety, potatoes have been reported to be more satiating than bread, pasta and rice (16).

2.1.3 Nutritional Data on Potatoes

With respect to potassium, the established Adequate Intake values for potassium is 2600 mg for adult women and 3400 mg for adult men (23). Potassium is important for the regulation of muscle contractility, and thus linked to heartrate, and also in the conduction of nerve impulses. High levels of blood potassium known as hyperkalemia can aid in controlling high blood pressure as well as the potential to reduce the risk of stroke (24,25). There is no upper limit for potassium for daily intake, however blood potassium levels of 5.5 mmol/L results in a diagnosis of hyperkalemia, whereas the optimal range is 3.5-5.0 mmol/L (26). Given their potassium content and the extent to which they are consumed, potatoes represent an important dietary source of potassium (27). However, as potato consumption trends are shifting, these values are decreasing in first world countries, influenced by the incorrect reports that potatoes are an unhealthy food (16).

Table 2. Protein content (grams) of common potato varieties

Variety of Potato	Amount in 100 gram serving size	Contribution to DRI[1]
[1]**Russet**	2.14	3.8%
[2]**Red**	1.89	3.4%
[3]**Yellow**	1.18	2.1%
[4]**White**	2.03	3.6%
[5]**Purple**	1.74	3.1%

[1]DRI for protein based on 0.8grams/protein per kg of body weight. 70kg used for reference equaling 56gram protein/day

[2]Russet potato NDB number 11353 [3]Red NDB number: 11355 [4]Yellow FDC ID: 475348 [5]White NDB number: 11354 [6]PurpleFDC ID: 1144101

Table 3. Carbohydrate content (grams) of common potato varieties

Variety of Potato	Amount in 100 gram serving size	Contribution to DRI[1]
[1]**Russet**	18.07	13.9%
[2]**Red**	15.9	12%
[3]**Yellow**	14.12	10.9%
[4]**White**	17.57	13.5%
[5]**Purple**	17.39	13.3%

[1]DRI for carbohydrates 130gram/day for adults age >18
[2]Russet potato NDB number 11353 [3]Red NDB number: 11355 [4]Yellow FDC ID: 475348 [5]White NDB number: 11354 [6]PurpleFDC ID: 1144101

Table 4. Fiber content (grams) of common potato varieties

Variety of Potato	Amount in 100 gram serving size	Contribution to DRI Males[1]	Contribution to DRI Females[1]
[1]**Russet**	1.3	3.4%	5.3%
[2]**Red**	1.7	4.4%	6.8%
[3]**Yellow**	1.4	3.7%	5.6%
[4]**White**	2.4	6.3%	9.6%
[5]**Purple**	1.7	4.4%	6.8%

[1]DRI for Fiber 38g/day male, 25g/day female age 19-50years
[2]Russet potato NDB number 11353 [3]Red NDB number: 11355 [4]Yellow FDC ID: 475348 [5]White NDB number: 11354 [6]PurpleFDC ID: 1144101

Table 5. Potassium content (mg) of common potato varieties

Variety of Potato	Amount in 100 gram serving size	Contribution to DRI Males[1]	Contribution to DRI Females[1]
[1]**Russet**	417	12.2%	16.0%
[2]**Red**	455	13.3%	17.1%
[3]**Yellow**	419	12.3%	16.1%
[4]**White**	407	12.0%	15.7%
[5]**Purple**	419	12.3%	16.0%

[1]DRI for Potassium 3400mg/day male, 2600mg/day female age 19->70years
[2]Russet potato NDB number 11353 [3]Red NDB number: 11355 [4]Yellow FDC ID: 475348 [5]White NDB number: 11354 [6]PurpleFDC ID: 1144101

Table 6. Vitamin C (total ascorbic acid) content (mg) of common potato varieties

Variety of Potato	Amount in 100 gram serving size	Contribution to DRI Males[1]	Contribution to DRI Females[1]
[1]**Russet**	5.7	7.6%	9.5%
[2]**Red**	8.6	11.4%	14.3%
[3]**Yellow**	18.2	24.3%%	30.3%
[4]**White**	9.1	12.3%	15.1%
[5]**Purple**	N/A	N/A	N/A

[1]DRI for Vitamin C 75mg/day male, 60mg/day female, age 19 - >70 years
[2]Russet potato NDB number 11353 [3]Red NDB number: 11355 [4]Yellow FDC ID: 475348 [5]White NDB number: 11354 [6]PurpleFDC ID: 1144101

Potatoes have low amounts of fats and, similar to legumes, they have a low energy density (28). The carbohydrate in potatoes is predominately starch, comprised of amylose and amylopectin. It is predicted that cultivars of potatoes with higher amounts of amylose are more beneficial for those with glycemic issues such as Type 2 Diabetes; it is assumed that this is due to the relative speed potato starch with which it is digested relative to simple sugars (28). Cooking of potatoes causes the starch molecules to gelatinize and be readily available for digestion. However, a portion of the starch in potatoes has been shown to be resistant starch (RS). These resistant starches can be fermented by the microflora in the large intestine, which results in short chain fatty acids being produced, with subsequent lowering of the pH in the gastrointestinal tract (GIT) (12). The lowering of the pH in the GIT reduces toxic ammonia, and acts as a pre-biotic (12). These pre-biotics can allow for beneficial bacteria to grow in the GIT (12). Two types of resistant starches are found in potatoes: RS2, which is found in raw potatoes, and RS3 which forms from cooked then cooled potatoes. This cooling after cooking allows for the starch to gelatinize and become retrograded (29). In a study looking at the effects of cooking method and serving temperature, it was shown that chilled potatoes contained more RS3 (4.7±0.5g/100g baked and 3.8±0.2g/100g boiled) than freshly cooked ones (3.5±0.2g/100g baked and 2.6±0.4g/100g boiled). When potatoes were cooked, cooled then reheated RS3 was reduced but not to the level of the initial cooked (4.2±0.7g/100g baked and 3.6±0.6g/100g boiled) (30). Weaver et al. investigated the effects of frying on total sugars, as well as fructose, glucose and sucrose content of potatoes (31). It was found that different varieties produced non-uniform concentrations of sugars when cut root to stem length and then analyzed. Storage conditions were a large part of this research and, when varieties were analyzed, Russet Burbank potatoes exhibited a significant reduction in glucose after both 2 and 4 months of storage at 7°C (31). As potatoes are a high source of carbohydrate, focus is shifted to the effect that potato consumption has on glycemic index (GI).

According to the Glycemic Index Foundation, GI is a relative ranking of carbohydrate in foods, in accordance to how they affect one's blood glucose levels; low GI level foods digest slower, causing a lower and prolonged rise in blood glucose, and generally lower insulin levels (32). The GI of foods can provide consumers a metric to understand the effects that consuming them will have on insulin levels, as the lower the GI level of food is generally perceived to be better for those at risk of high blood glucose levels. To assist consumers, individual foods are generally

classified into low (55 or less), mid (56-69) and high (70+) GI levels. However, whole day GI levels are lower than those for individual foods, in order to aid in lowering GI overall: low (45 or less), mid (59) and high (60+) (32). Potatoes when consumed alone contribute to a high GI response, however GI should be considered for the entirety of the meal, not individual items.

Table 7. Glycemic Index of Grains and Starches[1]

Low (55 or less)	**Medium (56-69)**	**High (70 or more)**
Pulse Flour	Potato (red, white cooled)	Instant Mashed Potatoes
Peas	Corn	Potato (red, white, hot)
Apples	French Fries	Carrots
Popcorn	Wild Rice	Pretzels
Sweet Potato	Parsnip	Soda Crackers
Winter Squash	Instant Oats	Watermelon
Quinoa	Pineapple	Banana (brown, overripe)

[1]Data provided from the Glycemic Index Food Guide, Diabetes Canada (33)

2.2 Protein Nutrition

2.2.1 Protein Requirements

Proteins are organic compounds that contain nitrogen contained within the amino acid building blocks of these polymers. Proteins serve as the structural components of muscles and other body tissues. Amino acids can be utilized as energy source, although not a primary choice for the body. The Recommended Dietary Allowance (RDA) for protein is 0.8g/kg body weight adults aged 19 plus. Thus, a 70kg adult would require 56 grams of protein per day. Protein can be found in many different sources and forms, including those derived from animal and plant-based sources. While the protein content of foods is primarily based on the crude protein determination (nitrogen content x 6.25), the nutritional value of protein is primarily determined by the respective amino acid composition. The primary structure of all proteins is generally composed of 20 different amino acids, which are needed for protein synthesis by most organisms. However, not all of these amino acids need to be consumed in one's diet. In general, animals (including humans) have the metabolic pathways in place to synthesize 11 of the amino acids found in proteins, and these are generally referred to as the dispensable or non-essential amino acids. At various times within the life cycle or under certain circumstances, non-essential amino acids can be considered "conditionally essential or indispensable", owing to the fact that the metabolic pathways involved in synthesizing them are not able to meet the metabolic demand. However, in general the remaining nine amino acids must be provided in the diet and are therefore referred to as indispensable or essential amino acids. The EAA are histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine. The following section will discuss the specifics related to the protein and amino acid composition of potatoes, and how this impacts the overall quality of the dietary protein found in this specific food.

2.2.2 Overview of Protein Digestion

The process of digestion for all substances begins with mastication, which happens in the oral cavity. Mastication can be influenced by composition, bite force, condition of the teeth, amount of chewing, and level of hunger (34). In the presence of food, α-amylase is released, allowing for enzymatic breakdown of polysaccharides to take place within a timeframe of a few seconds to two minutes, at a pH of 5-7 (35). The breakdown of food via mastication affects the size, surface area and shape of the particles which factor into protein digestibility; however it is thought that the α-amylase in the oral cavity has little to no effect on protein digestibility (35). After the oral

cavity, food particles enter the stomach where, due to the low pH and activity of the enzyme pepsin, there is significant protein digestion (35,36). The stomach consists of three sections: 1) the fundus, for storage; 2) the antrum, for mixing; and 3) the pyloric sphincter, which separates the stomach and small intestine. Within the fundus, food is released very slowly, with controlled release to optimize digestion and absorption. Food particles within the antrum are mixed with digestive fluids to form chyme. Hydrochloric acid (HCl) in the stomach controls the pH level, allowing for chyme to solubilize. As the pH is reduced due to the action of HCl, proteolytic activity of pepsin increases to peak at a pH of 2.5 (34). Conditions that will affect the ability for protein digestion include, but are not limited to, the pH of the stomach, food composition, time of day, personal habits and the proteolytic effectiveness of pepsin (37). The impact the above factors have on gastric secretion and emptying rates can impact the enzyme kinetics of pepsin (37).

After the stomach, the food particles have been reduced to a size that will travel through the pylorus, where food then enters the duodenal section of the small intestine. The small intestine is comprised of three sections, namely the duodenum, jejunum and ileum. The primary function of the small intestine is to provide sufficient surface area for the continued digestion of food via the mixing with pancreatic enzymes, ultimately leading to nutrient and water absorption (34). Within the proximal region of the small intestine (duodenum), the pH is increased to ~6.5 by the addition of sodium bicarbonate, which has been released by the pancreas (35). Proteases released from the pancreas enter the small intestine leading to the further breakdown of proteins and amino acids. Pancreatic enzymes include mostly trypsin, chymotrypsin and other proteases, as well as amylase, lipase and nucleases (35). Brush border enzymes also aid in the breakdown of peptides in the small intestine, and the former are contained on the microvilli within the villi of the small intestine to increase the surface area for digestion (34,35). From the liver, bile is released in the duodenum, thus aiding in the breakdown of fatty acids via emulsification. Digestion of nutrients in the small intestine is determined by many factors including: enzyme activity, secreted components and residence time (34). Enzymes and substrates travel through the small intestine, where substrates change due to degradation and solubilization and enzymes also gradually breakdown (34). Absorption of amino acids takes place in the small intestine, particularly within the ileum section, and this happens via simple, passive, facilitated and active diffusion allowing for the absorption of hydrophilic, lipophilic molecules, as well as transporting

across membranes with carrier proteins. Additionally, specific amino acid transporters and activity are defined within the brush border membrane of enterocytes (34). There are ten known types of amino acid transporters in humans responsible for the uptake and release of amino acids within cells and tissues (38). Given that absorption of amino acids does not readily occur after the ileum, the FAO introduced the concept of Digestible Indispensable Amino Acid Score (DIAAS), as a method to determine protein quality of food proteins, which is based on a scoring system dependent on ileal amino acid absorption (39).

The large intestine is where undigested materials are broken-down by microorganisms, and water and sodium are absorbed. The small and large intestine are separated by the ileocecal valve. The large intestine is made up of 6 sections, including: caecum, ascending colon, transverse colon, descending colon, sigmoid colon, and the rectum. There are over 400 species of bacteria making up half the total dry matter, with ranges of 10^{10}/ml in the caecum and 10^{11}/ml in the feces (34). Bacteria found in the large intestine ferment undigested materials, allowing energy to be obtained via fermentation of dietary fiber, more specifically resistant starches, that are not digested in in the small intestine. In western culture this fermentation of fiber makes up 5-10% of total energy requirements (34).

Table 8. Phases of the human digestion tract, including pH, duration and components.

Digestive Phase	**pH**	**Transit Time**	**Components**
Oral Cavity	5-7	10-120 seconds	Salivary amylase and lingual lipase
Gastric	1-5	15-180 minutes	HCl, pepsin, and lipase
Small Intestine	6.5-7.5	2-5 hours	Pancreatic enzymes
Colon	5-7	12-24 hours	Microbiota

2.2.3 Protein Quality Determination

For the purposes of substantiating protein content claims, Canada relies primarily on the Protein Rating system, which is based on the Protein Efficiency Ratio (PER). More recently, Canada has allowed the Protein Digestibility-Corrected Amino Acid Score (PDCAAS) method to be used to calculate a Protein Rating, where the PER is calculated as PDCAAS x 2.5 (40). Each method requires the use of an animal assay with differing duration of feeding times. Protein Quality is defined as a function of the amino acid content of the protein relative to a reference requirement pattern, as well as the overall digestibility/availability of those amino acids (41).

The amino acid composition of food proteins is an important determinant of their respective ability to contribute to the amino acid needs of the consumer. Animals, including humans, need to consume sufficient dietary protein to meet the metabolic needs for the constituent amino acids. Human protein requirements are defined by the FAO as the lowest level of dietary protein intake needed to balance the loss of nitrogen from the body, in turn maintaining the body's protein mass (42). A protein with an amino acid pattern that matches human amino acid needs is generally considered to be a higher quality protein. However, amino acid composition is only one component of quality. Consideration must also be given to the extent to which the protein is digested, and the constituent amino acids released, absorbed and made available to contribute to the protein needs of the consumer. Bioavailability is comprised of three food properties, which may alter the amount of an amino acid that can be ultimately used by the host. The three properties include: 1) digestibility, or the net amount of amino acid absorbed; 2) chemical integrity, or the portion absorbed that can be utilized; and 3) freedom from metabolic interference, given that within the food there are no substances that can limit the body's ability to utilize the amino acid (43). The digestibility of proteins can be determined by the fecal digestibility, which is the difference between nitrogen in (diet) versus nitrogen out (feces). It is suspected that colonic bacteria metabolize a portion of the unabsorbed amino acids and short peptides, which may create overestimations of the digestibility. As a result, ileal digestibility is considered by the FAO to be more accurate for the determination of amino acid digestibility and availability for human nutrition and forms the basis of the DIAAS method (42).

As a means of informing consumers about the protein value of a particular food, different jurisdictions allow the use of protein content claims, but the evidence required for their substantiation differs by region. In Canada, protein claims allow for foods to be deemed as a

"source of protein" when it presents with a protein rating of 20 or more, in which the rating is calculated as the reasonable intake multiplied by the protein efficiency rating (40,44). In the United States, the Food and Drug Administration will allow nutrient content claims on foods, such as an excellent source of protein, when the food contains a minimum of 20% or more of daily value (DV) per reference amount customarily consumed (RACC). For a good source claim, this value is 10-19% daily value per RACC (45). The FDA states that the daily recommended value for protein is 50 grams for adults and children older than 4 years old.

2.2.4 Methods of Determining Protein Quality

Determining protein quality takes into account the EAA composition, digestibility and bioavailability of the EAA (46). There are multiple methods to evaluate protein. These include the protein rating scale which includes protein efficiency ratio, biological value, net protein utilization and the PDCAAS.

The PER measures the growth of animals to determine the quality of protein. The method calls for the use of rats that are then fed the test protein in question (diet with 10% protein from the test article), and then measuring the weight gain of the rats in grams relative to the number of grams of protein consumed (46). The standard value of comparison is 2.5 as that is the standard value of casein, any value equal or greater than 2.5 is seen as an excellent source of protein.

The biological value method calculates the nitrogen used in tissue formation, divided by the nitrogen absorbed from the food (41). Higher values reflect a higher supply of EAA being utilized by the body. Similar to biological value, net protein utilization measures how a body retains nitrogen. The difference between the two methods is that net utilization is calculated from nitrogen ingested, not absorbed.

Table 9. Methods of Assessing Protein Quality

Method	Calculation	Advantages	Disadvantages
Net Protein Utilization (NPU)	$\frac{((0.16 \text{ x } (24 \text{ I})) - ((24 \text{ hour U}) + 2) - (0.1 \text{ x } (\text{ideal body weight kg})))}{(0.16 \text{ x}(24 \text{ hr protein intake})}$	Measured when protein content is diet below requirements	Not appropriate with adequate diet
Biological Value (BV)	$\frac{I-(F-F_o)-(U-U_o)}{I-(F-F_o)} x100$[b]	[c]Good measure of usability of proteins Detection of metabolic disease	Very strict diet under unnatural conditions Not to evaluated everyday use of proteins Dependant of age, weight, height, gender sex
Protein Digestibility-Corrected Amino Acid Score (PDCAAS)	$\left(\frac{\frac{\text{mg LAA}}{\text{gram test protein}}}{\frac{\text{mg LAA}}{\text{gram reference protein}}}\right) \text{x (\%TPD) x (100)}$	Simple Extensive AA data base	[d]No impact of anti-nutritive factors May need corrections for bioavailability of individual AA Scoring pattern does not include conditional LAA Rat bioassay – non reflective of human AA needs
Digestible Indispensable Amino Acid Score (DIAAS)	$\left(\frac{\frac{\text{mg Digestible dietary indispensibe AA}}{\text{gram test protein}})}{\frac{\text{mg Digestible dietary indispensibe AA}}{\text{gram reference protein}}}\right) \text{x } 100$	[e]True ileal digestibility Comprehensive representation of protein available in foods	Expensive Multiple analyses required for 1 value Comprehensive data lacking

[b](47)

[b]I=Nitrogen Intake of test protein

[b]F=Fecal Nitrogen; F_o= Fecal Nitrogen on Nitrogen Free diet; U=Urinary Nitrogen; U_o=Urinary Nitrogen on Nitrogen-Free Diet

[c](48)

[d](43)

[e](49)

Currently the Protein Digestibility-Correct Amino Acid Score (PDCAAS) is the United States' official method for determining protein quality for protein content claims on foods, and is currently positioned as the international standard by the FAO and World Health Organization WHO (41). This method uses the amino acid score, which reflects a ratio of the limiting amino acid in relation to the requirements of the human body. The Food and Agriculture Organization/ World Health Organization states that the essential amino acid requirement for humans for 2-5-year-old school children (**Table 1**) be used as the reference pattern. New reference patterns have been released over the years however the 1991 amino acid reference pattern is still used for determining PDCAAS values in the United States. The PDCAAS method can be analysed *in vitro* as well as *in vivo*. It should be noted that the *in vivo* method is the official method for the US Food and Drug Administration and the WHO. Health Canada accepts the PDCAAS method with alterations to reflect PER: PDCAAS x 2.5 = estimated PER (50). In 2013, a proposed method was introduced, the Digestible Indispensable Amino Acid Score (DIAAS), and it is currently under review by the FAO for becoming the recommended method as it incorporates ileal digestibility of amino acids (42). Upon investigation in 2011 by an expert committee of the FAO, it was determined that ileal digestibility was superior to fecal digestibility. However, due to a lack of published data for ileal digestibility coefficients on foods, the official method of protein quality determination was not altered from PDCAAS to DIAAS (43). DIAAS analysis within humans is difficult as ileal access is not direct, therefore invasive measures are required. In order for DIAAS to be considered as the ideal protein digestibility measurement, steps need to be taken in regards to gathering more data on foods and diets globally (43). Due to the invasive nature of assessing ileal digestibility in humans, animal models are required, with pigs being considered the best model to replicate adult human amino acid digestibility, followed by growing rats (43). The protocol requirements differ between pigs and rats, as has been addressed by the FAO (43).

2.3.5 Protein Digestibility-Corrected Amino Acid Score

The Protein Digestibility-Corrected Amino Acid Score (PDCAAS) method is the current recommended method for determining protein quality by the FAO and WHO. The PDCAAS method uses an amino acid scoring system (AAS) to determine the quality of protein. The amino acid scoring system uses the 1991 FAO/WHO reference pattern for each EAA and compares to the amount of EAA found in the sample. The 1991 reference pattern is the amount of AA

required for optima growth of 2-5-year-old school children. An AAS of 1.0 or higher indicates that the sample protein is equivalent to the reference pattern. If the AAS for each EAA in the sample protein is 1.0 or higher than the sample protein is a complete protein. The calculations include:

$$\text{AAS} = \frac{\text{mg of amino acid pre gram of protein (sample protein)}}{\text{mg of amino acid per gram of protein (reference protein)}}$$

$$\text{PDCAAS} = \text{AAS} \times \textit{ in vivo } \text{digestibility}$$

$$\textit{In vitro } \text{PDCAAS} = \text{AAS} \times \textit{ in vitro } \text{digestibility}$$

In vivo PDCAAS (IVPDCAAS) is determined using the feces of animal models, specifically the FDA-required rat bioassay (41). The amount of protein consumed is meticulously measured and compared to the amount found in the feces to determine how much remains in the system of the animal. This difference is what is used in the digestibility section of the equation to determine PDCAAS value.

With respect to PDCAAS, several limitations have been positioned, as discussed previously (43). The use of fecal nitrogen can lead to an overestimation of the digestibility coefficient, due the modifying effects from gut microflora. Additionally, the microflora differs between species, which may make data derived from rats less relevant for human digestibility measures. This overestimation is especially common in processed foods.

Although the PDCAAS method can be measured using *in vitro* methods, *in vivo* methods are the accepted approaches. This ultimately means that animal models are required for official methods of protein quality to be determined. This can be time consuming, expensive and represent ethical issues for certain stakeholders, including those companies who can't or won't use animal testing for regulatory purposes. Many plant-based food companies pride themselves on being a vegetarian/vegan option to the traditional animal-based proteins. The challenge of receiving protein quality claims at the expensive of using animal models is something some companies would rather not do. The ethical reasons behind companies not completing *in vivo* testing are in

line with a companies' morals. These ethical issues thus lead to products not qualifying for protein claims as all steps have not been completed.

Table 10. Protein digestibility-corrected amino acid scores for specific foods

Protein	Digestibility (%)	AAS (%)	PDCAAS
Casein	99	119	118[a]
Egg	98	121	118[a]
Cow's milk	95	127	121[a]
Beef	98	94	92
Soy protein isolate	95	96	91
Whole Wheat	91	44	40
Pinto Beans (canned)	73	78	57
Black Beans (autoclaved)	72	74	53
Pea protein isolate	92	79	73
Wheat Gluten	96	26	25
Sunflower protein isolate	94	39	37

AAS = Amino Acid Score; PDCAAS = Protein Digestibility-Corrected Amino Acid Score
Data from Protein quality evaluation by FAO/WHO (41).
[a] When data presented the maximum PDCAAS is 100, to correct for over estimations seen here.

2.3.6 *In vitro* Protein Digestibility Methods to Determine Protein Quality

Methods for the measurement of *in vitro* protein digestibility (IVPD) can be classified according to: 1) number of compartments (mono or bi-compartments); and 2) the nature of the progression through digestive processes (static or dynamic) (37). IVPD methods use incubation periods with the addition of enzymes including: pepsin, peptidase, pancreatin, trypsin, and chymotrypsin (36). Overestimation using IVPD models has been suspected for some proteins, as simulated digestion is often long in duration and a low constant pH is used in static IVPD methodology (36,37). However, evaluating PDCAAS using IVPD methods may not over predict protein digestibility given that TPD is also commonly over predicted, potentially mitigating this bias.

An example of a simple mono-compartment system is the pH drop method (37,51). This method allows for a good correlation between net change in pH in a 10-minute period and the probable fecal nitrogen digestibility in both plant and animal proteins (R^2= 0.81) (51). The pH drop method measures the pH change of a protein solution with an initial pH of 8.0, caused by enzymatic hydrolysis (37). The change in pH is due to the enzymatic reaction and the release of acidic amino acids, which allows for protein structure changes and digestibility to be measured (37,51).

Buffering by some food proteins, such as animal based proteins, and their constituent food matrices can lead to poor predictions of protein digestibility by the pH drop method. Another simple mono-compartment method is the pH stat method, created by Pedersen and Eggum, which is recommended by the FAO/WHO for predicting fecal protein digestibility (41,52). The pH of the protein solution is adjusted and held at 8.0 for a 10-minute incubation period by titration with 0.1M NaOH. Regression equations are predetermined for plant, animal or a combination of protein to predict true fecal protein digestibility (R^2= 0.9-0.96) (37). The pH drop method may be more effective than the pH stat method in predicting the fecal protein digestibility of plant-based proteins, due to the lower risk of buffering of pH changes. These two methods have been criticized for being over-simplified in their ability to predict true fecal protein digestibility in humans (35). This criticism stems from the facts that these methods are mono-compartmental, which results in the inability to reflect gastric and intestinal phases of digestion.

More complex methods for measuring IVPD include two-step digestion methods, which simulate the gastric and intestinal conditions of the human GIT. In short, these methods use HCl-pepsin at a low pH to mimic the conditions of the gastric phase, then the solutions are neutralized and

pancreatin at higher pH is added to mimic the intestinal phase of protein digestion. These methods lack data for *in vivo* protein digestibility comparisons, yet some two-step methods have been used to predict ileal protein digestibility (R^2=0.92) in pigs (36,37).

In both Canada and the United States, animal trials (*in vivo*) are required prior to making claims about protein quality in products. The latter includes the substantiation of protein content claims, such as “good source” or “excellent source” of protein, on labels. The use of animal trials are expensive, time consuming and regarded as unethical by the public (42). New and innovative plant protein products being introduced into the marketplace therefore may face a barrier due to lack of *in vivo* data and the resultant requirement by regulatory bodies for the use of *in vivo* methods to substantiate a protein claim. By using *in vitro* methods to predict the PDCAAS value, all the above barriers may be reduced. In order to select the appropriate method to determine protein digestibility, there is a need to further substantiate the validity of these models against *in vivo* data (36).

Table 11. Measures of *in vitro* Protein Digestibility, %True Fecal Protein Digestibility, and Protein Digestibility-Corrected Amino Acid Scores of plant-based protein foods

	AAS	%TPD	%IVPD	PDCAAS	IVPDCAAS
Extruded Red Lentil Flour[a]	0.68	92.38	88.01	63.01	60.03
Cooked Red Lentil Flour[a]	0.63	90.95	84.67	57.40	53.43
Extruded Green Lentil Flour[a]	0.66	86.02	84.30	57.09	55.95
Cooked Green Lentil Flour[a]	0.61	86.42	84.03	52.92	51.46
Butte Almonds[b]	0.530	86.2	78.3	45.7	41.5
Monterey Almonds[b]	0.493	89.9	80.6	44.3	39.7
Nonpareil Almonds[b]	0.557	85.7	78.6	47.8	43.8
Baked Black Beans[c]	0.91	63.55	74.26	57.52	67.22
Cooked Black Beans[c]	0.83	81.66	75.34	67.54	62.31
Baked Faba Beans[c]	0.75	88.63	76.79	66.36	57.49
Cooked Faba Beans[c]	0.61	88.49	81.41	54.14	49.81
Baked Navy Beans[c]	0.78	69.08	78.51	53.62	60.95
Cooked Navy Beans[c]	0.71	86.02	77.06	61.23	54.86

AAS = Amino Acid Score; %TPD = % true fecal protein digestibility; %IVPD = % *in vitro* protein digestibility; PDCAAS = protein digestibility-corrected amino acid score; IVPDCAAS = *in vitro* protein digestibility-corrected amino acid score

[a]Data provided by Nosworthy et al. 2018 (53)

[b]Data provided by House et al. 2019 (54)

[c]Data provided by Nosworthy et al. 2018 (55)

2.3.7 Dynamic Systems for Measuring *In vitro* Protein Digestibility

The TNO Gastro-Intestinal Model (TIM-1) is an *in vitro* dynamic system which closely follows *in vivo* physiological processes (56). The TIM-1 system consists of 4 compartments which mimic digestion in the stomach, and 3 discrete sections of the small intestine (duodenum, jejunum, and ileum). As the meal moves from one section to the next, it is exposed to digestive enzymes and fluids that closely mimic the human body with respect to pH levels throughout the gastrointestinal tract, enzyme secretion and activity, bile salt concentration, gastric emptying and transit time and removal of digested products and solubilisation/dissolution as nutrients are absorbed. The idea behind the TIM-1 is that, by simulating the dynamic digestive processes within the digestive tract, one can achieve greater control over processes and thus ensure reduced variability and increased reproducibility (57). Control is accomplished via a predesigned computer program that controls the physiological parameters of the system. There parameters include mixing, transit time of the meal, pH for specific time and place, secretion and formulation of digestive fluids as well as the removal of digestive compounds and water (57). In order to study digestion of protein in the TIM-1 model, bio-accessibility is expressed as the amount of protein nitrogen dialyzed as a percentage of the amount of protein in the food (34). Protein digestibility determined by Minekus in the TIM-1 system investigated the kinetics of nitrogen from the diet as a whole and not individual amino acids (34). In order for protein quality to be determined completely, an animal study must accompany the *in vitro* data for validation. There are ethical pushbacks for using rats and other animals and causing intentional decreased growth via amino acid deficiencies in diets. By using the TIM-1 system to determine the digestibility of the proteins, in conjunction with established methods for measuring the amino acid score, alternative methods can be positioned for determining PDCAAS without the use of animals (57). Previous research indicates that values derived from TIM-1 and ileal digestibility in canines were similar, indicating that the TIM-1 is a suitable substitute for *in vivo* canine tests (58). The results from the TIM-1 system and *in vivo* data from both calves and pigs yielded similar data for nitrogen delivery (34). Calf milk replacer digestibility using the TIM-1 resulted in digestibility coefficients that were replicated in ileal-cannulated calves (34). The skim milk protein digestibility coefficient using the TIM-1 was 97.9 ±1.1%, while the value determined *in vivo* was 99.5±1.1% (34).

The above research positions the potential for the use of *in vitro* digestibility procedures as cost and time effective methods to establish reliable and comparable data to those derived *in vivo* (37). The food sector is rapidly changing methods of extracting proteins from novel sources and creating new plant-based products. The ability to determine protein quality in a time and cost-effective way is essential to facilitate innovation in the protein food sector. Using *in vitro* methods allows for fast and comparable data to determine what proteins and/or processing technologies to utilize and further investigate. Research on how cooking methods impact protein quality is established for many common plant-based proteins such as soy, pea and rice. This data is currently lacking for many plant-based protein sources, including potatoes.

2.3 Factors Influencing Protein Quality

2.3.1 Amino Acid Composition

Amino acids are the organic compounds that form proteins required for life in every species. When considered from a nutritional standpoint, there are three groups of amino acids: Essential (indispensable), nonessential (dispensable) and conditionally essential (indispensable). There are nine amino acids generally considered be essential for human requirements. These amino acids cannot be made by the human body, thus must be consumed in the diet in appropriate amounts to meet requirements. The amount that each EAA is available within a given protein source, in comparison to the requirement pattern of 2-5 year old school children (FAO, 1991) is a determining factor in regard to protein quality (41). For proteins to be of high quality, the limiting EAA must be at a level close or higher than that of the reference pattern. Protein digestibility aids in determining the level at which these EAA are digested and used by the body; the higher the digestibility and corresponding AA composition determines the overall protein quality.

Table 12. Food and Agriculture Organization (1991) Essential amino acid requirement and content, mg/g protein

Essential Amino Acid	2-5 Year Child	Laboratory Rat	Casein	70% Rat Requirements	Ratio of 70% Rat Req. to Human Req.[1]
Arginine		503	37	35	
Histidine	19	25	32	18	0.92
Isoleucine	28	42	54	29	1.05
Leucine	66	62	95	43	0.65
Lysine	58	58	85	41	0.70
Methionine & Cystine	25	50	35	35	1.40
Phenylalanine& Tyrosine	63	66	111	46	0.73
Threonine	34	42	42	29	0.86
Tryptophan	11	12.5	14	10	0.89
Valine	35	50	63	44	1.26

[1]Rat to human requirements for age 2–5-year child

2.3.2 Factors influencing protein availability

The crude protein content of foods or food ingredients is generally determined by measuring the total nitrogen content of the material and multiplying this by an appropriate nitrogen conversion factor. The nitrogen content is generally determined by official methods including the Kjeldahl method or the Dumas method (59). The standard nitrogen conversion factor is 6.25, reflecting the fact that mixed proteins contain, on average, 16% nitrogen by weight. However not all nitrogen within foods or food ingredients are intact proteins, as some is found in the form of non-protein nitrogen (NPN). If this nitrogen cannot contribute to amino acid metabolic and protein synthesis, the crude protein value may overestimate the protein value of the food (60,61). Non-protein nitrogen in human milk make up 20-25% of total nitrogen; making up a complex array of compounds, from urea to oligosaccharides (62). Additionally, the use of 6.25 as a conversion factor may also overestimate the protein value of most plant foods, as these factors typically range from 4.71-5.65 for varying plant-based proteins and 5.53-6.15 for animal based proteins, with an average of 5.6 (60,61). With a conversion of 5.6 and not 6.25, a 10% reduction in protein values would be seen.

Anti-nutritional factors (ANFS) reduce the bioavailability of nutrients, and this group includes protease inhibitors, tannins and phytic acid (39). ANFS are naturally occurring in plants and seeds and can also be formed during storage or processing. There are 3 mechanisms in which ANFS can reduce the dietary protein quality, including by inhibiting digestive enzymes in the gut, chelating nutrients thus preventing digestion, and/or damaging the digestive tract (39). Heat processing as well as alkaline treatments can result in additional ANFS, including Maillard reaction products, oxidized sulphur amino acids, and D-amino acids (39).

Maillard reactions take place between amino acids and reducing sugars. Free asparagine is a major amino acid found in certain foods, including wheat and potatoes, which when heated in the presence of sugars yields acrylamide. Acrylamide is classified as a potential carcinogen towards humans (63). Raw potatoes do not contain any acrylamide however, they do contain the precursor asparagine and reducing sugars. Thus, when potatoes are fried, the end result is a food with a higher concentration of acrylamide (63). It was noted that temperatures above 100ºC is optimal for the production of acrylamide (64). Maillard reactions are also the main cause of the change of flavour and colour during baking and frying. The end-products of Maillard reactions are known to have strong antioxidant properties (63). Previous research investigating the amount

of acrylamide in fried potatoes found that the amount of reducing sugar within potatoes is a limiting factor, thus resulting in a limited amount of acrylamide formed that is not directly correlated with the amount of asparagine found in raw potatoes (63). This same study noted that total antioxidant concentration and acrylamide have a high correlation, and even more so that higher levels of total antioxidant concentrations are caused by Maillard reactions (63). Maillard reactions are also known as Strecker degradation products of amino acids, where an amino acid is decarboxylated and deaminated, ultimately forming an aldehyde (64).

2.3.3 Effect of Processing on Protein Composition

Processing conditions can lead to chemical modifications in food proteins. The manufacturing processes for protein isolates and concentrates requires the removal of macro and micronutrients, via both wet and dry milling processes. These modifications lead to alterations in the concentration of EAA. This is evident with potato protein isolates, as the isolation process causes an alteration in the amino acid score. The DIAAS score for potato protein isolates has been reported to be 100 (65). As protein isolation steps lead to the removal of key constituents, including fibre, carbohydrates and other plant constituents, this processing can modify the ultimate amino acid pattern of the isolate. The process of creating potato protein isolates effectively increases the protein to a range 77-83%, and also removes non-protein nitrogen compounds (free amino acids), thus allowing the EAA pattern (and AAS) of the isolate to increase, with the EAA of potato protein isolate being 37% of total AA, higher than both casein (34%) and egg (32%) (7).

The methods whereby proteins are processed yield differing results in the bioavailability of proteins. Previous research has documented that the method of pork processing results in changes in AA bioavailability, with *in vitro* bioavailability values being statistically lower for stewed pork than for either cooked pork or emulsion sausages (66). Research with soybean shows that irradiation and autoclaving increases IVPD in comparison to raw soybeans (67), as these processes reduce the content of ANFS. In terms of trypsin, chymotrypsin, and α-amylase inhibitors, extrusion techniques have been shown to reduce their content in faba and kidney bean flours, without compromising protein content (68). Radiation has shown to significantly reduce the levels of phytic acid, tannins and available lysine (69). The process of dehulling also significantly increases protein content in faba and kidney beans (68). All these method have an

effect on IVPD, for the majority of processing techniques cause ANFS to decrease and IVPD to increase (68,69).

Current literature shows the significant difference within cooking and/or processing methods of differing protein sources. Whole raw plant protein sources have a significantly lower protein digestibility than that of cooked plant proteins. There is no one single method that allows higher protein digestibility over another, as evident in the case of cooked black beans, which had a higher PER than those subjected to extrusion. The reverse was found with faba beans, however (55). Chickpeas also differ significantly in PER with differing processing methods as baked chickpeas have a lower PER than extruded (70).

2.3.4 Effect of Environmental, Genetic and Agronomic Factors

Extensive agricultural research has documented that geographical location of crop production can drastically alter the protein content of the crops (71,72). Differing geographical location brings differing climates, elevation, precipitation, growing season duration and agricultural practices. The genotype of plant species often produce significantly different data for mineral composition depending on the geographic location of crop growth (73). Protein content has been shown to differ significantly, up to ten fold, by potato cultivar, ranging from 1.8g/100g to 17.2g/100g DW (74). Documenting that not all potato genotypes produce the same content of macro and micronutrients is an area of active research and translation potential for consumers. Research on the content and digestibility of protein is rapidly emerging from both industrial- and academic-sourced research. Research has found that pulses, specifically beans and peas, have different protein digestibility values, as well as differences in their respective EAA contents and resulting AAS. (75).

Geographic location can also dramatically alter the composition of crops. Many crops are unable to be grown in the Canadian growing season as it is too short, making it unsuitable to certain higher protein foods including certain treenuts (e.g., almonds). Locally sourced products are becoming a growing consumer trend as many consumers are wanting to support locally sourced foods (76). Potatoes represent a crop well suited for storage to ensure year-round availability, as they can be stored and consumed for up to 11 months post-harvest with proper temperature, and treatment to prevent sprouting (77).

2.5 Factors Influencing the Protein Quality of Potatoes

Potatoes contain all nine of the amino acids essential for humans, however the relative balance of these amino acids need to match human requirements in order for a food source to be considered a "complete protein" (12). Animal proteins tend to have a balance of amino acids that better match human needs, as compared to most plant-based proteins, as the former are not typically limiting in any essential amino acids (limiting amino acid; LAA) (78). While individual plant-based proteins may have specific LAA, by mixing with other plant-based proteins, these limitations can be alleviated. An example of this is seen when mixing pulses, such as pinto bean, with other grains, including buckwheat. The LAA in pinto beans are typically the sulphur amino acids cysteine and methionine, yet the latter deficiencies can be overcome by blending with buckwheat, whose LAA is lysine. In fact, the LAA of buckwheat, lysine, is addressed by the relative excess of this amino acid in pinto beans, thus creating a high quality protein that better meets essential amino acid needs (79). The investigation of plant-based protein options is on the rise as animal proteins have been linked to the increase of greenhouse gas emissions, particularly methane (80). The health benefits of plant-based proteins has been shown to increase fibre consumption and reduce saturated fats in the diet, both are beneficial to heart health; as shown in the 2019 Canada Food Guide which suggests to consumer plant-based protein more often (81). The addition of more protein in place of carbohydrates has been linked to specific health improvements including glycemic control, and body weight regulation particularly in type 2 diabetics (82).

Within potatoes, about 50% of the total nitrogen is derived from intact protein, with the remaining 50% of nitrogen comprised of free amino acids, amide nitrogen associated with asparagine and glutamine and non-protein nitrogen (83). Some of this non-protein nitrogen, particularly the nitrogen found in free amino acids, will be available to the consumer as a component of dietary protein intake. In human feeding trials, potato protein isolate appears to be high quality when compared to other vegetable protein isolates, and it is thought that the high utilization by humans is due to the high free amino acid content of potatoes (83). Isolates are a concentrated protein (>90%), in which the majority of other macro nutrients are removed during the processing. The processing also removes non-protein nitrogen, thus increasing the amount of EAA within protein, as protein content is calculated using the Dumas method. Literature on the

non-protein nitrogen in potatoes is limited and what is available suggest that boiled potatoes have 1.8% nitrogen and of that percentage 37% is protein nitrogen (84).

When a variety of potatoes were evaluated for their amino acid composition, the results showed that the difference in species does not create a large difference in amino acid values. Russet Burbank potatoes have been shown to have a total essential amino acid value of 2219 mg amino acid/gram of nitrogen; while the same method to calculate amino acids scored whole eggs with a total essential amino acid value of 3215 (85). These results were based upon raw freeze-dried potatoes. **Table 13** below shows the amino acid values of boiled (without skin), baked (with skin) and microwaved (with skin) Russet potatoes (86)

There is a lack of current research on the effects of cooking methods on potato protein. The majority of data was conducted by Weaver et al who looked at the changes in nutritional composition of Russet potatoes. These changes included total nitrogen, protein and free amino acids, as well as reducing sugars and other carbohydrates within potatoes. They found that baked and fried potatoes had 75-80% of the raw potato values for total and protein nitrogen (87). The increase in protein and total nitrogen as potatoes were baked and fried may be the result of non-nitrogen protein. Boiled potatoes made into a mash contained 46% of the free amino acids of the raw material. Interestingly baked potatoes contained nearly double the amount of free amino acids compared to the raw material. This change in composition could predict that baking potatoes may increase the bioavailability of protein. The researchers also looked at flakes, granules and chips, and all 3 methods resulted in a loss of 30-40% loss of total nitrogen. Chips (33g serving) had 66% of total and protein nitrogen, and free-amino acids were twice that of a 100 g serving of boiled potatoes. Chips had half the total and protein nitrogen as a 100 g serving of baked potatoes and French-fries.

Toma et al looked at different varieties of potatoes and their changes in nutritional composition during home preparation (88). These researchers found that Russet Burbank potatoes had differing changes in protein depending on cooking method used, however each cooking method resulted in an increase of total protein available. The values of protein differed in the 2 growing seasons that the potatoes were chosen from indicating that a larger sample size is required.

Protein analysis conducted by Yilma et al resulted in 3 years of biochemical analysis from 4 different Russet varieties from varying parts in the western United States. The results show that on a dry weight basic the protein content of russet potatoes varies from 4.8g/100g in Russet

Burbank's to 5.8g/100g in Sage Russets (89). With respect to other key nutrients, it should be noted that microwaved potatoes can exhibit altered vitamin C levels with increases of 50% of those observed in baked potatoes. Furthermore, microwaving can reduce iron by 70% (16). Previous work also looked at the change in the contents of four vitamin after different cooking methods. The results for boiling and frying indicated a slight loss of riboflavin, niacin, ascorbic acid and thiamine relative to raw controls, while results for baking were less conclusive (87). Potassium (K) is used by the human body as an exchangeable cation within intracellular fluid, regulating fluid balance, muscle contractions, nerve signals, blood pressure regulation, and water retention. Potassium deficiency is a global issue, and within the US, K consumption is declining, leading to an increase in hypokalemia (K serum level <3.6mmol/L). There are current trends in agriculture that have been shown to reduce potassium within the food matrix (90). Potassium is removed from top-soil during harvest and added fertilizers containing nitrogen, phosphate and potash do not balance the amount removed, as data suggests 28% more K is removed than added (90). Of the fruit and vegetables examined by Sun and Weaver, potatoes were one of the few items that saw a potassium ratio for 2015/1999 over 1.0; others included cabbage, carrots and peppers (90). The structural properties of potassium make it one of the most leachable nutrients, as it is readily soluble in water.

The limited data on the bioavailability of potassium in potatoes indicates that 94% of K is available for digestion (91). Increasing consumption to 3300mg/d with potassium from potatoes (baked, boiled, pan fried, French fries) or from a potassium gluconate supplement did not show any significant difference in blood pressure compared to a control diet of 2300mg/day (92). As dietary consumption of K declines hypokalemia can ensue. This leads to the need for increases in global consumption of K as only 3% of the population currently met the adequate intake (4700mg/d). There also needs to be agricultural policies put in place to counter the soil loss of K across the US, and presumably in other countries, including Canada.

A point brought up in many peer reviewed articles relates to the glycemic response to different cooking methods for potatoes. When potatoes are fried or deep fried, the structure of the potato changes. Fried potatoes produce compacted structures on the surface which in turn limit the ability for enzymes to access the granules. This results in a slower release rate of total sugars, leading to lower glycemic index that don't spike as quickly as boiled potatoes (93). When baking and microwave cooking methods were compared both pith and cortex were analyzed. The results

of the observed changes in AA composition due to cooking methods is presented in **Table 14** (94).

Table 13. The amino acid composition (g/100 g serving) of potatoes, as impacted by cooking method.

Amino Acid	Potatoes, flesh and skin, raw 100g	Potatoes, Russet, flesh and skin, baked 100g	Potatoes, boiled, cooked without skin 100g	Potatoes, microwaved, cooked in skin 100g	Potatoes, French fried, salt added in processing 100g
Histidine	0.035	0.044	0.038	0.046	0.048
Serine	0.074	0.095	0.075	0.091	0.102
Arginine	0.101	0.13	0.079	0.097	0.135
Glycine	0.057	0.073	0.051	0.062	0.077
Aspartic acid	0.48	0.615	0.419	0.514	0.519
Glutamic acid	0.351	0.45	0.287	0.352	0.418
Threonine	0.067	0.086	0.062	0.076	0.081
Alanine	0.063	0.08	0.053	0.065	0.095
Proline	0.063	0.081	0.062	0.076	0.086
Cystine	0.024	0.031	0.022	0.027	0.037
Lysine	0.107	0.137	0.104	0.128	0.138
Tyrosine	0.048	0.062	0.064	0.078	0.08
Methionine	0.032	0.041	0.027	0.033	0.037
Valine	0.103	0.131	0.096	0.118	0.129
Isoleucine	0.066	0.085	0.07	0.085	0.082
Leucine	0.098	0.125	0.103	0.126	0.137
Phenylalanine	0.081	0.104	0.076	0.093	0.1
Tryptophan	0.021	0.027	0.027	0.033	0.021

USDA Branded Food Product Database (86)

Table 14. Effect of Conventional baking and microwave cooking methods for potatoes following a 70%

	Cortex			Pith		
Amino Acid	**Control**	**Conventional**	**Microwave**	**Control**	**Conventional**	**Microwave**
Aspartic acid	15.911	-0.688	-0.55	19.78	2.308	-0.67
Threonine	2.546	-0.168	-0.05	2.347	0.242	0.102
Serine	3.046	-0.058	0.054	3.07	0.314	0.006
Glutamic acid	11.532	-0.403	0.133	14.61	1.917	-1.024
Proline	2.511	0.225	0.662	2.16	0.55	0.57
Glycine	2.252	0.054	0.047	2.104	-0.028	-0.025
Alanine	2.413	0.123	-0.109	2.243	0.179	0.011
Valine	4.117	-0.62	-0.047	4.515	-0.068	-0.805
Methionine	1.525	-0.621	-0.027	1.688	-0.226	-0.538
Isoleucine	2.571	-0.037	0.033	2.573	0.17	-0.148
Leucine	4.127	0.05	-0.003	3.926	0.172	-0.008
Tyrosine	1.946	0.487	0.055	2.289	0.793	0.206
Phenylalanine	2.752	0.093	1.194	2.952	-0.079	-0.149
Lysine	4.305	-0.324	0.189	4.33	0.135	-0.035
Histidine	1.364	0.016	0.002	1.469	0.025	-0.161
Arginine	3.326	0.364	2.25	4.602	0.147	0.094
Aminobutyric acid	2.067	-0.611	-0.775	2.696	0.433	-0.182
Total % Change		-3.10%	2.30%		9.00%	-3.60%

Ethanol extraction for determining free amino acid content. All units are in mg/g of dry weight. Data obtained from Klein, L.B and Mondy, N.I. (94).

There is limited research in the field of potato PDCAAS. Previous research has found that potatoes have a biological value (BV) of 90-100, where compared to eggs that have a biological value of 100. For comparison to other plant-based proteins, dried beans have a BV of 73 and soybeans have a BV of 84 (28). In research investigating the protein content of commercially available plant-based protein sources, potato protein isolate was found to meet and exceed the FOA/WHO essential amino acid values (7). It was found that potato protein isolate contained 37% EAA as a component of the total protein (7). The composition of non-protein nitrogen has led to the assumption that non-protein nitrogen can not contribute to the protein needs of the consumer (95). In fact, 75% of non-protein nitrogen is made up of free amino acids(95) which are metabolically available. Glutamic acid, aspartic acid and valine were the three major amino acids in the total free amino acid fraction. There were also other amino compounds found in the tubers, including beta-alanine, gamma-aminobutyric acid, ornithine and homoserine (96). In both of the prior mentioned research studies, there was no free tryptophan or cystine found in the tubers, likely reflecting the fact that the researchers did not utilize methods needed to measure these amino acids. The most common amino acids present in potatoes were amides, glutamine and asparagine, which made up 20-40% and 20-50% of the total free amines (96). Lysine, tyrosine, tryptophan and glycine are suspected to produce the highest Maillard browning reaction due to producing L-amino acids, compared to other proteins (97). It was noted that the difference in the composition of the protein among the tubers from different harvest locations resulted in different colour intensity among the cooked chips (96).

All nine of the amino acids essential to humans can be found in potatoes, thus potatoes are a complete protein in respect to the amino acids available; however it is critical to be sure that the amino acids within potatoes are available in the correct amount for it to be considered a complete protein (12). Within potatoes, about 50% of the total nitrogen is derived from intact protein, thus leaving the remaining 50% of the nitrogen to be comprised of free amino acids, amide nitrogen associated with asparagine and glutamine and other non-protein nitrogen forms (83). In human feeding trials, potato protein appears to be high quality when compared to other vegetable protein, and it is thought that the high utilization by humans is due to the high free amino acid content of potatoes (83). Literature on the non-protein nitrogen in potatoes is limited and what is available suggest that boiled potatoes have 1.8% nitrogen and of that percentage 37% is protein nitrogen (84).

When different varieties of potatoes were evaluated for their amino acid composition, the results showed that the difference in varietal does not create a large difference in amino acid values. Russet Burbank potatoes have been shown to have a total essential amino acid value of 2219 mg amino acid/gram of nitrogen; while the same method to calculate amino acids scored whole eggs with a total essential amino acid value of 3215 (85). These results were based upon raw freeze-dried potatoes.

2.6 Gaps in the Research

Much of the data available has concluded that crude nitrogen is altered by different cooking methods. However, the difference between crude nitrogen levels and protein composition do not necessarily correlate, as LAA are essential in determining the quality of protein in a food product. There is a lack of knowledge on the effects of cooking upon the digestibility and AA composition of potatoes. As the current trend of plant proteins continues to increase, so does the need for new sustainable sources of protein. In the current literature, potato protein has been looked at with regards towards changes during cooking, but not with respect to the effect cooking methods have on the quality of the protein within Russet potatoes. Potassium digestibility literature is also lacking. Potatoes are a high in potassium, and are consumed globally. Therefore, the need to look at both protein quality and potassium digestibility is evident due to a current lack of literature.

Chapter 3: Hypothesis and Objectives

3.1 Hypotheses

H_{a1}: *In vitro* PDCAAS values of potato protein will be significantly increased by cooking method compared to raw potato protein.

- H_{01}: *In vitro* PDCAAS value of potato protein will not be significantly altered by cooking method compared to raw potato protein.

H_{a2}: *In vivo* PDCAAS value of potato protein will significantly be increased by cooking method compared to raw.

- H_{02}: *In vivo* PDCAAS value of potato protein will not be significantly altered by cooking method compared to raw potato protein.

H_{a3}: *In vitro* Potassium Digestibility will not be significantly altered by cooking method compared to raw.

- H_{03}: *In vitro* Potassium Digestibility will be significantly altered by cooking method compared to raw.

3.2 Objectives

The objectives of this research program include:

1. The determination of the impact of cooking method (RAW, BAKE, BOIL MICRO, FRIED) on the protein quality, determined via *in vivo* and *in vitro* PDCAAS methods, of Russet potatoes.
2. The determination of the relative availability of both nitrogen and potassium from cooked potatoes, using the efflux derived during the dynamic simulated multi-compartment digestion (TIM-1) system.

Chapter 4: Methods & Material

4.1 Chemicals

All chemicals and reagents were purchased from Sigma (Oakville, ON, Canada).

4.2 Materials

4.2.1 Sample Collections

Idaho Russet Potatoes were used for the current research. The potatoes were secured from a local farmer (location NW15-7-5) from an October 1, 2018 harvest, as facilitated by Kroecker Farms in Winkler, Manitoba, Canada. A total of 168kg of potatoes were transferred (May 2019) to the University of Manitoba's Food Science Pilot Plant and stored at 4ºC until utilized.

4.2.2 Cooking Preparation

The total composite sample of potatoes was divided into 7 primary treatment allocations of 24kg each, reflecting the designed cooking treatments: 1) RAW (control); 2) MICRO; 3) BAKE; 4) BOIL; 5) FRIED3M; 6) FRIED6M; and 7) FRIED9M.

Three independent replicates were conducted for each cooking treatment. No standardized methods could be found for baked, boiled, fried or microwaved, so trial runs were performed to establish methods that allowed for potatoes to be fully cooked, in a manner that mimics common home cooking methods. Once all cooking methods were performed, potatoes were mixed in an industrial Hobart mixer at speed 2 to ensure that individual cooking methods were homogenized. Specifics for the individual cooking treatments are found below.

***RAW* (Control):** Whole potatoes were peeled and placed into cold water. Once all potatoes were peeled, they were processed using a commercial food mill (Bird, mini-22 mini mixer grinder, 20kg capacity, L= 35, W= 25¼", H= 52") in order to have a consistent size for all samples.

***MICRO*:** Potatoes were left unpeeled and pierced with a fork. Potatoes were then cooked in a commercial microwave (0.7 cubic feet; 700 watts), individually, for 5 minutes on the highest setting. Once cooked, potatoes were removed and allowed to cool to room temperature. Once cool, the entire pith was removed and frozen. All weights pre and post cooking were recorded to determine the fresh weight composition of microwaved potatoes.

***BAKE*:** Potatoes were left unpeeled and poked with a fork. The entire batch (4kg per batch x 3) were then placed in the middle of a convection oven (Moffat ECP-3) at a temperature of 400°F for 55 minutes to ensure thorough cooking of the entire batch. Once cooked potatoes were cut

lengthwise and allowed to cool. Once potatoes had cooled, the interior portion (pith) of the potato was removed and frozen. All weights pre- and post-cooking were recorded to determine the fresh weight composition of baked potatoes.

***BOIL*:** Whole potatoes were peeled and cut into 2cm x 2cm x 2cm cubes then placed into a steam kettle (Groen, D-10, 10 U.S. Gallon) of boiling water, sufficient to cover potatoes and an additional 3 inches for 12 minutes. Following cooking, potatoes were removed, cooled to room temperature, and then frozen at-18°C until future processing.

***FRIED*:** Potatoes were first peeled and cut into 1cm x 1cm x 5cm with the use of French fry cutter. After cutting, potato strips were blotted dry and cooked in batches at a ratio of 1:7 of potato: oil (100% high-oleic canola oil), at a temperature of 375°F. Potatoes were cooked for three different time durations: 3, 6 or 9 minutes (Garland 80-03, oil capacity 40L). These times allowed for different cooked colours and textures to be obtained.

4.2.3 Frozen Processing

Each batch consisted of 4kg which were combined in Hobart mixer ensuring homogeneous batches. All samples were stored in 6" diameter disposable aluminum pie dishes, each containing between 200-500g of product, depending on the cooking method, to ensure even freezing and covered with plastic lids. Holes were punched into the plastic lids to allow for ventilation and reduce the amount of ice buildup from freezing. Once products were placed into metal pie plates with plastic lids which had holes punctured in them, they were placed in the walk-in freezer (-18°C) in the Ellis Building at the University of Manitoba. Frozen samples were then subjected to freeze drying using a SP VirTis Genesis 25Lfreeze drier (-70°C) until they were completely dry (approx. 3 days per batch).

4.2.4 Preparation for Analysis

Once the product was dried, the total amount of cooked potato sample from each replicate and cooking method was milled using a Retsch Ultra Centrifugal Mill ZM 200, with a 0.75mm sieve, and the total content of each replicate stored in airtight freezer bags at -18°C until analyzed.

4.3 Analytical Procedures

Prior to amino acid analysis, the samples derived from the fried treatments were subjected to defatting using a giant Soxhlet system (Electrothermal CMV12C). Defatting took place over a 16-hour period, using hexane as the solvent, in which 200g per batch of full fat fries were placed into cloth mesh bags with 50 grams each. All dried and defatted (FRIED) treatment replicates

were sub-sampled and the contents of crude protein (N x 6.25), crude fat, ash, crude fibre, dry matter, and potassium were determined by Central Testing Laboratory in Winnipeg, Manitoba, an ISO accredited lab by Standard Council of Canada. Data were expressed on an "as consumed" and "dry matter" basis.

For the measurement of total amino acid profile, AOAC Official Method 982.30 acid hydrolysis was utilized to determine the amino acid content of each sample, using 6N hydrochloric acid hydrolysis over a 24 hour period (98). Methionine and cysteine were assessed by acid oxidized hydrolysis following AOAC official method 985.28, with proteins initially oxidized with performic acid prior to acid hydrolysis. (98). Tryptophan was determined using alkaline hydrolysis as per the method of the International Organization of Standardization (ISO) 13904 (99). These methods are further explained below. All AA sets were derived and separated (AccQ-Tag Ultra C18, 1.7µm column) using AccQ-Tag Ultra chemistry system (Waters, Ltd., Mississauga, ON) on a Shimadzu UPLC system, with SIL-20AC autosampler.

4.3.1 In vitro Protein Digestibility

The *in vitro* protein digestibility values were determined using both static and dynamic methods. The static methods included the pH-drop protocol, pH-stat method and a two-step method. The dynamic method used the TIM-1 model.

4.3.2 Static Enzymatic pH Drop Method

The first method utilized was the pH-drop (PHD) protocol, which followed Hsu, Vavak, Setterlee and Miller's method, along with modifications (51,100). This static system followed the pH change of the protein digestate over a 10 minute time period, and has been shown to have a high correlation (R^2=0.90) with the *in vivo* true fecal protein digestibility in rats (51). In brief, 62.5 mg of protein equivalents from each treatment replicate were incubated, in triplicate, with an enzyme cocktail containing 1.6mg/mL trypsin (porcine pancreas 13,000-20,000 BAEE units/mg protein), 3.1mg/mL chymotrypsin (bovine pancreas ≥40 units/mg protein), and 1.3mg/mL protease (Streptomyces griseus ≥15 units/mg solid) which was prepared in 10mL of Milli-Q water and heated (Corning PC-420D hot plate) at 37°C. Combined sample protein and the enzyme cocktail (5mL of enzymes total) were brought to a pH of 8±0.5 with 1M Sodium hydroxide (NaOH) or HCl, after pH has stabilized following an hour of solubilization. The PHD was initialized with the addition of 1mL of the enzymatic cocktail to the protein solution. The initial pH was recorded before the introduction of the cocktail and at 30 second intervals, for a

total of 10 minutes. The PHD *in vitro* protein digestibility (PHDIVPD%) was calculated using the formula below:

$$PHDIVPD\% = 65.55 + 18.10 \times \Delta pH_{10\ min}$$

4.3.3 Static Enzymatic pH Stat Method

The second method used was the pH-static (PHS) method, which followed the protocol set out by Pedersen and Eggum (52). Similar to the pH-drop method, the pH-static (PHS) method involved the incubation of 62.5 mg of protein derived from the treatment replicates, in triplicate, with an enzymatic cocktail containing 1.61mg/mL trypsin (porcine pancreas 13,000-20,000 BAEE units/mg protein), 3.96 mg/mL chymotrypsin (bovine pancreas ≥40 units/mg protein), and 2.36mg/mL protease (Streptomyces griseus ≥15 units/mg solid), prepared in 10mL of Milli-Q water and heated at 37ºC. Both the sample protein and the enzyme cocktail were brought to a pH of 8±0.5 with 1M NaOH or HCl, following a 60 min pH stabilization process. Following the addition of enzymes, pH was held at 7.98 using 0.1N NaOH, and the volume of 0.1N NaOH used to hold the pH recorded. The PHS *in vitro* protein digestibility (PHSIVPD) was calculated using the formula:

$$PHSIVPD\% = 76.14 + 47.77 \times (\sum 0.1N\ NaOH)$$

4.3.4 *In vitro* Two- Step Protein Digestibility

A static, two compartment *in vitro* model was also used to determine protein digestibility, using the method of Boisen & Fernandez (36), with further modifications by A. Franczyk (37). The specific phases of the assay are positioned below:

Phase 1 – Gastric Digestion

The gastric digestion phase involved the digestion of 150 mg of protein from the treatment replicates, in triplicate, in a 50mL flask with 17.75mL of 0.1M pH 6.0 potassium phosphate. Following solubilization, 1mL of 1mg of amylase in 15mL of 0.1M pH 6.0 potassium phosphate was added, and the sample mixed in the shaking water bath at 150RPM at 40 ºC for 5 minutes. After that, 7.5mL of 0.2M HCl was added, and the pH adjusted to 2.0±0.05. Subsequently, 375µL of 8.33% chloramphenicol solution and 750µL of pepsin solution containing 10mg of pepsin were added to the flasks, and the flasks placed in a shaking water bath, within shaking for 6 hours at 150 revolutions per minutes (RPM) at 39ºC.

Phase 2- Intestinal Digestion

The samples from Phase1 were removed from the water bath, and 7.5mL of 0.2M 6.8 pH potassium phosphate and 3.75mL of 0.6M NaOH were added to the flasks, in order to neutralize the acidic pH. The pH of the flask contents was balanced to a pH of 7 using 1M NaOH with stirring at 300RPM at 40ºC. After the pH was balanced, 750µL of a pancreatin solution containing 50mg pancreatin (porcine pancreas, P1750; 4 × USP specifications) was added. Flasks were once again placed in a shaking water bath, with shaking for 18 hours at 150 RPM at 39ºC.

Phase 3- Sample Preparation

The flask contents were stirred at 300 RPM and 1.4mL from each flask was transferred to centrifuge tubes containing 200µL of 60% Trichloroacetic Acid (TCA) solution (7.5% w/v, final concentration) in order to stop enzymatic activity and precipitate undigested materials, including proteins. Samples were then vortexed for 6 seconds. All samples were centrifuged at 17,000g for 60 minutes. Following centrifugation, 1mL of the resulting supernatant was transferred to a 2mL cryogenic vial and stored at -20ºC.

Phase 4 – Amino Acid Hydrolysis

Following a slightly modified version of the AOAC official method (98), all samples were thawed and 200µL from each sample placed in hydrolysis tubes with 3.8mL of 6.3N HCl and ~20µL of 2-octanol. Samples were then incubated at 110°C for 24 hours. After 24 hours, 4mL of 25% NaOH was added to all samples. Samples were then transferred to 50mL beakers, the hydrolysis tubes were rinsed 3 times with 4mL of 0.1 sodium tetraborate buffer, and the rinsing's pooled with the hydrolysate. An aliquot was then filtered through a 0.22-micron syringe filter and analyzed, as per the methods below, within 2 hours.

4.3.4 Static Two-Step Digestion o-Phthaldialdehyde Assay Preparation

Total amino nitrogen content of the hydrolysates were measured according to the method of Church et al. (101). An o-Phthaldialdehyde (OPA) reagent was made fresh daily and contained 25mL of 0.1M sodium tetraborate, 2.5mL 5% SDS, 0.1mL ß-mercaptoethanol, 1.0mL OPA (prepared in methanol) and 21.4mL Milli-Q water in an amber flask (37). This solution was mixed at room temperature for an hour prior to usage.

4.3.5 Static Two-Step Digestion o-Phthaldialdehyde Spectrophotometric Analysis

Within 2 hours of completion of the acid hydrolysis step, samples were held at room temperature and 1.0mL of OPA reagent was added to 0.2mL of sample in a 1.5mL methacrylate cuvette, and cuvettes placed within an Agilent G1117AA spectrophotometer, and colour development was measured at 340nm at 2 minutes following the addition of OPA. A standard curve using 1mM L-leucine as the substrate, was subjected to the same incubation conditions to permit the calculation of total amino nitrogen (**Appendix C)**. The two-step *in vitro* protein digestibility (TSIVPD) was calculated as the quotient of the initial digest nitrogen concentration to the final digest nitrogen concentration, accounting for dilutions from all steps (37), according to the following equation:

$$\text{TSIVPD\%} = \frac{\text{Initial Digest Nitrogen}}{\text{Final Digest Nitrogen}} \times 100$$

4.3.6 TIM-1 Digestion

The dynamic, simulated TNO Gastro- Intestinal Model (TIM-1) contained four compartments that mimic the stomach, duodenum, jejunum, and ileum (56). In brief, each section was composed of flexible tubing surrounded by a glass fitting. The system was kept at body temperature (37°C) for the duration of the run, using water pumped between the glass jacket and the walls of the flexible tubing. Alternating compression allowed for peristaltic mixing. Prior to the addition of "food" to the TIM-1, pancreatin, bile, gastric enzyme solution, 1M hydrochloric acid, sodium bicarbonate, small intestine electrolyte solution, and stomach water (0.4% Hydroxypropyl Methylcellulose (HMPC) and 0.04% bile) were placed in appropriate locations to allow for uptake into the TIM-1. Starting residue gastric enzyme solution and stomach water was added to the stomach and duodenum compartments, and small intestine electrolyte solution was added to the jejunal and ileal compartments.

The gastric compartment contained 10g of gastric start residue (pH 1.7, 5g hydroxypropylmethylcelluose (0.4%)) and 5g gastric enzyme solution (2800 U/g pepsin; 47U/g α amylase, 20U/g lipase), the duodenum contained 55g duodenal start residues (15g pancreatin solution, 30g bile solution, 2mg trypsin and 15g small intestinal electrolyte solution), and both the jejunum and ileum contained 115g intestinal electrolyte solution (pH 7.4).

All samples contained 3 grams of protein, and the amount of all cooking method samples were adjusted accordingly, with roughly 30 grams of potato used for each run. In addition to the potato sample, 15 mg of pancreatic α amylase (1500units/mg) and 240 mg of blue dextrin were added. Freeze dried potato samples were mixed with warm water to bring the total amount of "fed product" to 200g and mixed for 10 minutes using a magnetic stir bar at 800 RPM. The pH of the food was adjusted to 5.2±0.2 using 1M HCl or 1M NaOH. Blue dextrin was only used for 50% of the samples due to issues with delivery of product. Blue dextrin was added in order to clearly visualize the duration of sample in each section of the TIM-1. An extra 100 mL of water was reserved to rinse the food beaker to ensure that the entire sample entered the TIM-1. Once all food (300g) was added and stomach temperature was brought up to and held at 37ºC, program Fed Water ECB V2 was selected, with the conditions established to allow a run for a total of 6 hours. Every 60 minutes, the dialysates from the jejunum and ileal were collected, and 12 mL of the excretion was subsampled for future glucose or amino acid testing. After the 6-hour run time, the total contents of efflux beaker (**Figure 1**), was recorded and material was freeze dried for analysis. A clean beaker replaced the efflux beaker which collected the residue. The entire system was then flushed with water to remove the residue, and this was recorded, frozen at -40°C, then freeze fried for analysis. The amount of freeze-dried residue collected ranged from 8.41g for the FRIED6MIN to 12.46g for the RAW. The amount of freeze-dried efflux collected ranged from 11.66g or FRIED6MIN to 26.72g for the RAW treatment.

4.3.7 TIM-1 Sample Analyses

Following dynamic digestion, pooled samples of the efflux and residual materials were frozen in Ziplock freezer bags with 300-500g per bag at-30°C until freeze-dried (Animal Science Building, University of Manitoba). Once dried, samples were then ground in a handheld commercial grinder (Black and Decker coffee grinder), and retained for subsequent analysis of dry matter, crude protein and potassium (as described above).

Table 15. TIM-1 Digestion Parameters

	Gastric compartment	**Duodenal compartment**	**Jejunal compartment**	**Ileal compartment**
pH	5.7 (T_0) 1.7 (T_{360})	~6.2	~7.4	~7.4
Volume	300 mL	55 mL	115 mL	115 mL
Secretion	520 U/min pepsin 2 U/min lipase 5 U/min amylase	20 mg/min Bile (T_0-T_{30}) 10 mg/min Bile (T_{30}-T_{360}) 80 mg/min pancreatic juice As required Sodium bicarbonate solution	As required Sodium bicarbonate solution	As required Sodium bicarbonate solution

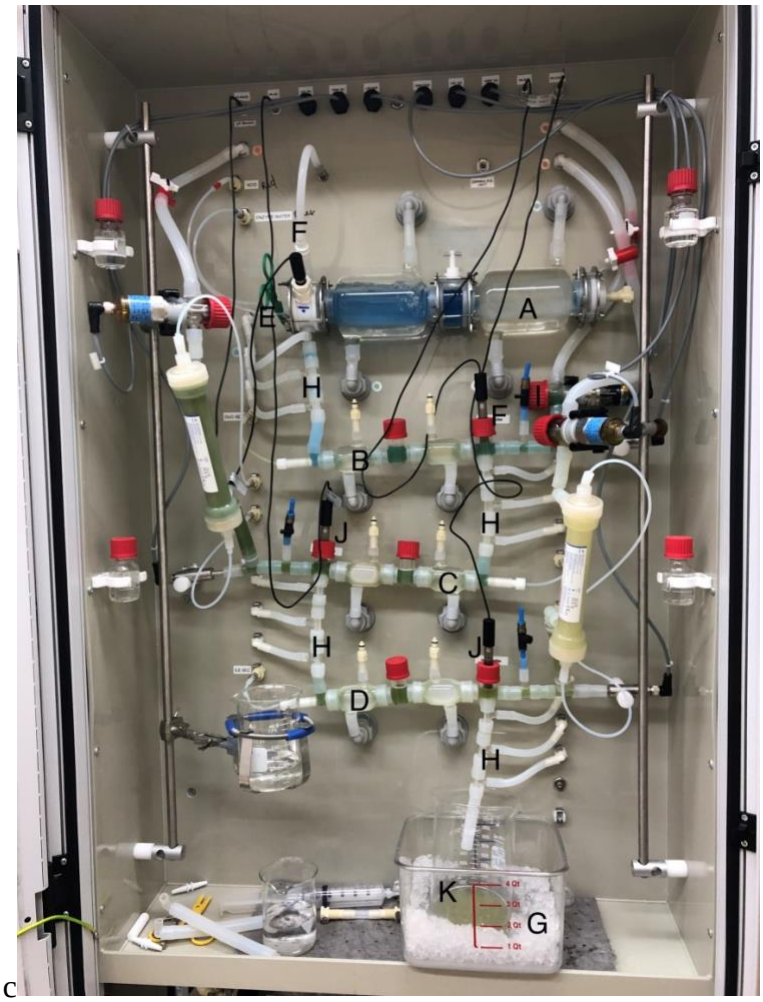
c 

Figure 1. Labelled photo of the TNO gastro-intestinal model (TIM-1)
A: gastric compartment B: duodenal compartment C: jejunal compartment D: ileal compartment E: gastric secretion pumps F: pH electrode G: water bath H: peristatic valve pump I: duodenal secretion pump J: pH electrode K: collecting vessel for ileal delivery

For the calculation of the TIM-1 *in vitro* protein digestibility (TIMIVPD%), input protein was calculated as the initial mass of protein minus the protein in the residue and protein found from other non-food sources within the residue. The output final protein was derived from the final protein content in the efflux subtracted by the non-food course proteins in the efflux. The TIMIVPD% was calculated as follows:

$$\text{TIMIVPD\%} = \frac{(\text{input protein} - \text{output final protein})}{\text{input protein}} \times 100$$

4.3.8 Calculation of *in vitro* Protein Digestibility-Corrected Amino Acid Score

The amino acid ratios from each sample were calculated by the abundance of each essential amino acid in the samples, with the values expressed in milligrams of amino acid per gram of sample protein. The amino acid score (AAS) was determined via the lowest ratio of amino acid in the sample divided by the corresponding reference requirement value in the FAO/ WHO 1991 reference pattern. The 1991 pattern is still used as reference for the United States PDCAAS calculations. From the AAS, the PDCAAS was calculated as the product of the AAS and the specific measure of digestibility, either *in vitro* or *in vivo* (see below), according to the following equations:

$$\text{AAS} = \frac{\text{mg of amino acid pre gram of protein (sample protein)}}{\text{mg of amino acid per gram of protein (reference protein)}}$$

$$\text{PDCAAS} = \text{AAS} \times \textit{in vitro}\ \text{digestibility}$$

The *in vitro* protein digestibility of the pH-drop, pH-stat, two-step were calculated from the product of the digestibility and the AAS to produce the corresponding *in vitro* protein quality.

$$\text{PHDIVPQ} = \text{PHDIVPD\%} \times \text{AAS} \qquad \text{PHSIVPQ} = \text{PHSIVPD\%} \times \text{AAS}$$

$$\text{TSIVPQ} = \text{TSIVPD\%} \times \text{AAS} \qquad \text{TIMIVPQ} = \text{TIMIVPD\%} \times \text{AAS}$$

4.3.9 *In vivo* Protein Digestibility-Correct Amino Acid Score

For the official determination of the PDCAAS, the rat bioassay was used to determine the impact of specific cooking methods on potato protein quality (41). Amino acid scores were determined as mentioned in section 4.9, in accordance with the FAO/WHO guidelines. True fecal protein digestibility was determined in accordance to the AOAC Official Method 991.29 (102). Briefly, test diets were formulated to have a 10% inclusion rate of protein derived from the individual potato treatments, with additional energy coming from corn oil and corn starch. A vitamin and mineral premix was added (AIN-93 formulations; Harlan Teklad, Madison, WI), to

meet the micronutrient requirements for the rodents (103). Male weaning laboratory rats (n=10 per treatments), which were housed individually in suspended wire-bottomed cages. Over an acclimation period of four days, rats were fed 15 g/day. Following this acclimation period, a five-day balance period occurred. During this time, feed intake and fecal output was weighed, and total fecal samples were retained, freeze dried, and analyzed for total nitrogen and dry matter determinations. True fecal protein digestibility was calculated using the following formula:

$$\text{TPD\%} = \left(\frac{\text{nitrogen intake} - (\text{fecal nitrogen loss} - \text{metabolic nitrogen loss})}{\text{nitrogen intake}}\right) \times 100$$

Metabolic nitrogen loss was determined as the fecal nitrogen produced per gram of diet consumed by the rats consuming a protein-free diet (104). The PDCAAS was calculated by

$$\text{PDCAAS (\%)} = \text{TPD\%} \times \text{AAS}$$

Rat weights were recorded during both the acclimation and balance periods for additional protein quality measurements.

4.4 Statistical Analysis

Data for composite samples are presented as means of triplicate analyses. Data for PHDIVPD%, PHSIVPD%, TSIVPD%, and TIMIVPD% were subjected to one-way ANOVA and Dunnett's test with RAW potatoes set as the control with p-values <0.05 indicating significant difference. Protein digestibility methods (PHDIVPD, PHSIVPD, TSIVPD, and TIMIVPD) were analyzed within the individual IVPD method using a one-way ANOVA and Dunnett's test and values from the RAW potato set as control with p-values <0.05 indicating significant difference. Values for protein quality (PDCAAS) were subjected to one-way model ANOVA and Dunnett's test with RAW set as a control with p-values <0.05 indicating significant difference. Statistical analysis was determined using Prism 9 (GraphPad).

Chapter 5: Results

5.1 Proximate Analysis

5.1.1 Crude Protein

Results for total nitrogen of processed samples were obtained from Central Testing Laboratory (CTL) in Winnipeg, Manitoba for all freeze-dried products. Fried samples for all three durations were analyzed, defatted then sent back for retesting. All initial results on an "as consumed basis" can be found in **Table 16**. Crude protein (CP) values increased as frying time increased from FRIED3M (3.07±0.07 g/100g) to FRIED9M (4.77±0.08 g/100g). Potassium values for all frying durations (555±19, 720±12, 877±21mg/100g); FRIED3M, FRIE6M FRIED9M respectively were significantly higher than the control of RAW (367±1 mg/100g). Potassium levels in BOIL (259±1 mg/100g) were significantly lower than that of the RAW control. Based upon the results of a one-way ANOVA (**Table 16)** was ran to indicate that there is a significant difference amount crude protein values using different consumer-based cooking methods.

When processed results were investigated on a dry-matter basis (**Table 17**), it is shown that CP was significantly increased when MICRO (10.19±0.09) and BAKE (9.89±0.11) cooking methods were applied and significantly decreased across all FRIED methods 3M, 6M and 9M (7.35±0.24, 7.48±0.02, and 7.47±0.16 respectively) compared to RAW control (8.66±0.27). Non-fibre carbohydrates were also significantly decreased among all cooking methods with the exception of BOIL. Fat content was significantly increase in all FRIED methods 3M, 6M and 9M (19.05±0.13, 19.28±0.14, and 19.09±0.32 respectively) compared to RAW control (0.243±0.08). Potassium values increased significantly across MICRO and BAKE, and showed a significant decrease in BOIL, FRIED3M, FRIE6M and FRIED9M.

Table 16. Nutrient composition of Russet potatoes subjected to different consumer-based cooking methods. Data are presented on "as consumed" basis (fresh weight)[1].

	Units	RAW	MICRO	BAKE	BOIL	FRIED3M	FRIED6M	FRIED9M	RAW (CNF)[2,3]
Dry Matter	g	21.39 (0.23)	29.38¤ (0.31)	24.77# (0.08)	19.15* (0.18)	41.8¤ (0.71)	51.79¤ (0.37)	63.93¤ (0.39)	20.66 (0.37)
Crude Protein	g	1.85 (0.04)	2.99¤ (0.06)	2.44¤ (0.03)	1.67 (0.04)	3.07¤ (0.07)	3.87¤ (0.03)	4.77¤ (0.08)	2.02 (0.03)
Non-Fibre Carbohydrate	g	18.10 (0.27)	24.51¤ (0.23)	20.57# (0.05)	16.36* (0.12)	28.25¤ (0.54)	35.10¤ (0.31)	43.56¤ (0.59)	17.47 (NA)
Crude Fibre	g	0.34 (0.01)	0.35 (0.01)	0.33 (0.01)	0.30 (0.02)	0.62# (0.04)	0.69¤ (0.05)	0.78¤ (0.03)	1.5 (0.0)
Fat	g	0.05 (0.02)	0.06 (0.02)	0.09 (0.01)	0.07 (0.01)	7.96¤ (0.11)	9.99¤ (0.08)	12.21¤ (0.24)	0.09 (0.01)
Ash	g	0.84 (0.01)	1.19¤ (0.03)	1.10# (0.02)	0.61# (0.02)	1.23¤ (0.06)	1.63¤ (0.03)	1.99¤ (0.03)	1.08 (0.1)
Calcium	mg	5.69 (0.00)	6.84¤ (0.00)	4.95¤ (0.00)	5.74¤ (0.00)	8.36¤ (0.00)	10.36¤ (0.00)	12.79¤ (0.00)	12 (1)
Phosphorus	mg	42.78 (0.15)	60.70¤ (0.07)	52.02# (0.17)	33.20# (0.10)	64.16¤ (0.25)	81.16¤ (0.01)	100.13¤ (0.15)	57 (1)
Magnesium	mg	19.25 (0.3)	26.44 (0.3)	23.12 (0.1)	14.05 (0.01	29.27 (0.1)	36.25 (0.1)	44.75 (0.1)	23 (0)
Potassium	mg	367 (1)	533 (4)	494 (8)	259 (1)	555 (19)	720* (12)	877# (21)	421 (12)
Sodium	mg	4.28 (0.00)	2.94¤ (0.00)	2.48¤ (0.00)	3.83 (0.00)	4.18# (0.00)	5.18¤ (0.00)	6.39¤ (0.00)	6 (1)
Copper	mg	0.04 (0.02)	0.06* (0.02)	0.04 (0.02)	0.04 (0.04)	0.07# (0.13)	0.12¤ (0.04)	0.14¤ (0.06)	0.108 (0.016)
Iron	mg	0.30 (0.02)	0.46 (0.02)	0.35 (0.01)	0.27 (0.02)	0.51 (0.03)	0.66 (0.03)	0.78 (0.06)	0.78 (0.04)
Manganese	mg	0.15 (0.02)	0.20 (0.07)	0.17 (0.01)	0.11 (0.00)	0.25# (0.03)	0.27# (0.01)	0.33¤ (0.01)	0.153 (0.026)
Zinc	mg	0.25 (0.00)	0.37 (0.01)	0.30 (0.01)	0.20 (0.00)	0.32 (0.09)	0.50# (0.02)	0.63¤ (0.02)	0.29 (0.00)

RAW= raw, peeled; MICRO = microwaved in skin (analyzed flesh only); BAKE = baked skin on (analyzed flesh only); BOIL = peeled boiled in water; FRIED3M = peeled, 3-minute frying in 100% high-oleic canola oil; FRIED6M = peeled, 6-minute frying in 100% high-oleic canola oil; FRIED9M = peeled potato, 9-minute frying in 100% high-oleic canola oil

[1]As consumed representing 100grams of potato flesh. Data are presented as Means, with Standard Error presented in brackets (n=3). The Potato, flesh, raw with a 100g serving size data is represtened in the table.

Using Raw as a control in the Dunnet's statistical test for comparison.

[2]Canadian Nutrient Files data based on raw potatoes, with no specification on the variety of potatoes analyzed. Data is presented as the Means with Standard Error presented in brackets (CNF n=11, sodium n=73, fibre n=20) in lab tests (n=3).

[3]CNF fibre reported as dietary fibre, in lab reported as crude fibre.

***** $p < 0.05$, # $p < 0.01$, ¤ $p < 0.0001$

Table 17. Nutrient composition of Russet potatoes subjected to different consumer-based cooking methods. Data are presented on dry weight basis[1] for 100g of potato.

	Units	RAW	MICRO	BAKE	BOIL	FRIED3M	FRIED6M	FRIED9M
Crude Protein	g	8.66 (0.27)	10.19¤ (0.09)	9.86# (0.11)	8.69 (0.13)	7.35¤ (0.24)	7.48# (0.02)	7.47# (0.16)
Non-Fibre Carbohydrates	g	84.61 (0.38)	83.41* (0.12)	83.05* (0.12)	85.44 (0.20)	68.04¤ (0.18)	67.78¤ (0.20)	68.14# (0.37)
Crude Fibre	g	1.6 (0.068)	1.20# (0.02)	1.34 (0.03)	1.58 (0.11)	1.48 (0.10)	1.34 (0.09)	1.22* (0.04)
Fat	g	0.243 (0.08)	0.20 (0.06)	0.36 (0.03)	0.36 (0.02)	19.05¤ (0.13)	19.28¤ (0.14)	19.09¤ (0.32)
Ash	g	3.91 (0.06)	4.04 (0.09)	4.42¤ (0.06)	3.16¤ (0.05)	2.94¤ (0.09)	3.15¤ (0.03)	3.11¤ (0.06)
Calcium	mg	27 (0)	23 (0)	20* (0)	30 (0)	20* (0)	20* (0)	20* (0)
Phosphorus	mg	200 (10)	210 (0)	210 (10)	170# (0)	150¤ (0)	160¤ (0)	160¤ (0)
Magnesium	mg	90 (0)	90 (0)	93 (3)	73# (3)	70¤ (0)	70¤ (0)	70¤ (0)
Potassium	mg	1720 (30)	1810* (10)	1990¤ (20)	1350¤ (30)	1330¤ (20)	1390¤ (20)	1360¤ (30)
Sodium	mg	20 (0)	10¤ (0)	10¤ (0)	20 (0)	10¤ (0)	10¤ (0)	10¤ (0)
Copper	mg	0.16 (0.01)	0.20 (0.01)	0.18 (0.01)	0.23* (0.02)	0.17 (0.03)	0.22* (0.01)	0.22* (0.01)
Iron	mg	1.42 (0.09)	1.57 (0.09)	1.43 (0.03)	1.40 (0.09)	1.24 (0.06)	1.27 (0.05)	1.22 (0.10)
Manganese	mg	0.72 (0.08)	0.68 (0.02)	0.71 (0.03)	0.59 (0.01)	0.59 (0.06)	0.51# (0.02)	0.52# (0.02)
Zinc	mg	1.16 (0.01)	1.25 (0.04)	1.21 (0.03)	1.03 (0.01)	0.76 (0.21)	0.97# (0.02)	0.99 (0.04)

RAW= raw, peeled; MICRO = microwaved in skin (analyzed flesh only); BAKE = baked skin on (analyzed flesh only); BOIL = peeled boiled in water; FRIED3M = peeled, 3-minute frying in 100% high-oleic canola oil; FRIED6M = peeled, 6-minute frying in 100% high-oleic canola oil; FRIED9M = peeled potato, 9-minute frying in 100% high-oleic canola oil

[1]Data are presented as Means, with Standard Error presented in brackets (n=3).

One-way ANOVA statistical model with Dunnett's test for comparison with Raw as the control.

***** $p < 0.05$, **#** $p < 0.01$, **¤** $p < 0.0001$

5.1.2 Amino Acid Composition

Results for amino acid composition on an as-consumed basis can be found in **Table 19.** The results indicated that cooking method altered the amino acid composition significantly, with FREID6M and FREID9M resulting in a significant increase ($p<0.05$) from the RAW. All AA in FRIED3M increased significantly except for HIS, ALA, CYS, and TRP. Review of the amino acid composition of BAKE found that ASP, GLU, LYS, TYR, VAL, and PHE saw significant increases in AA compared to raw ($p<0.05$). BOIL resulted in the fewest differences of AA composition with ASP, GLU, ALA, and PRO all resulting in a significant decrease ($p<0.05$). MICRO saw the following AA increase significantly in composition compared to RAW: AMM, SER, ARG, GLY, ASP, GLU, THR, LYS, TYR, VAL, ILE, LEU, PHE, and TRP. Total AA composition of each cooking method also resulted in significant differences compared to RAW. MICRO (2.425±0.039), BAKE (2.146±0.03), and all three frying methods: FREID3M (2.6970.037), FRIED6M (3.288±0.044) and FRIED9M (3.700±0.057) these methods all saw significant increases to total AA composition ($p<0.0001$). BOIL (1.446±0.023) saw a significant decrease to total AA composition compared to RAW (1.672±0.031).

Table 18. Amino acid composition[1] of Russet potatoes g/100g as received on a Dry Matter basis

	RAW	MICRO	BAKE	BOIL	FRIED3M	FRIED6M	FRIED9M
AMM	0.184	0.199	0.211	0.158	0.159	0.152	0.124#
HIS	0.095	0.097	0.102	0.097	0.075	0.069	0.054*
SER	0.239	0.242	0.250	0.246	0.192*	0.194*	0.194*
ARG	0.342	0.341	0.369	0.328	0.279#	0.279#	0.246¤
GLY	0.158	0.161	0.165	0.161	0.121	0.122	0.120
ASP	1.955	2.078¤	2.239¤	1.773¤	1.546¤	1.499¤	1.343¤
GLU	1.116	1.314¤	1.387¤	1.129	1.082	1.065#	0.981¤
THR	0.210	0.220	0.222	0.217	0.165*	0.163*	0.159#
ALA	0.180	0.128#	0.151	0.105¤	0.113#	0.103¤	0.094¤
PRO	0.190	0.197	0.198	0.198	0.155	0.151	0.146*
CYS	0.091	0.092	0.091	0.092	0.069	0.071	0.059
LYS	0.382	0.393	0.387	0.387	0.301¤	0.292¤	0.259¤
TYR	0.228	0.247	0.274*	0.228	0.205	0.205	0.181*
MET	0.140	0.141	0.147	0.133	0.116	0.117	0.093*
VAL	0.342	0.360	0.384*	0.338	0.284#	0.280#	0.259¤
ILE	0.224	0.229	0.229	0.226	0.178*	0.182*	0.168#
LEU	0.343	0.349	0.339	0.361	0.262¤	0.266¤	0.259¤
PHE	0.281	0.297	0.301	0.289	0.235*	0.238*	0.224#
TRP	0.089	0.088	0.080	0.091	0.076	0.074	0.065
Total Amino Acid	6.788	7.171¤	7.529¤	6.559¤	5.613¤	5.522¤	5.030¤
Crude Protein	8.66	10.19	9.86	8.69	7.35	7.48	7.47

AMM = Ammonia; HIS = Histidine; SER = Serine; ARG = Arginine; GLY = Glycine; ASP = Asparagine; GLU = Glutamine; THR = Threonine; ALA = Alanine; PRO = Proline; LYS = Lysine; TYR = Tyrosine; CYS = Cysteine; MET =Methionine; VAL = Valine; ILE = Isoleucine; LEU = Leucine; PHE = Phenylalanine; TRP = Tryptophan; RAW= raw, peeled; MICRO = microwaved in skin (analyzed flesh only); BAKE = baked skin on (analyzed flesh only); BOIL = peeled boiled in water; FRIED3M = peeled, 3 minute frying in 100% high-oleic canola oil; FRIED6M = peeled, 6 minute frying in 100% high-oleic canola oil; FRIED9M = peeled potato, 9 minute frying in 100% high-oleic canola oil

[1]Data presented as Means (n=3). Two-way ANOVA statistical model with Dunnett's test for comparison with Raw as the control.

* $p < 0.05$, # $p < 0.01$, ¤ $p < 0.0001$

Table 19. Amino acid composition[1] of Russet potatoes g/100g as received on an as consumed basis

	RAW	MICRO	BAKE	BOIL	FRIED3M	FRIED6M	FRIED9M
AMM	0.039	0.058#	0.052	0.030	0.066¤	0.079¤	0.079¤
HIS	0.023	0.032	0.029	0.021	0.036	0.041#	0.039*
SER	0.062	0.086¤	0.075	0.057	0.097¤	0.121¤	0.150¤
ARG	0.082	0.112¤	0.102	0.070	0.130¤	0.161¤	0.176¤
GLY	0.045	0.062*	0.054	0.041	0.067*	0.083¤	0.101¤
ASP	0.483	0.706¤	0.641¤	0.393¤	0.748¤	0.898¤	0.993¤
GLU	0.272	0.440¤	0.391¤	0.246¤	0.515¤	0.628¤	0.714¤
THR	0.053	0.076*	0.065	0.049	0.081¤	0.100¤	0.120¤
ALA	0.048	0.047	0.047	0.025#	0.059	0.067*	0.075¤
PRO	0.048	0.069	0.058	0.045*	0.077¤	0.093¤	0.111¤
CYS	0.023	0.032	0.027	0.021	0.034	0.043	0.045
LYS	0.093	0.132¤	0.109*	0.085	0.143¤	0.173¤	0.189¤
TYR	0.054	0.080¤	0.075#	0.049	0.095¤	0.118¤	0.129¤
MET	0.034	0.047	0.041	0.029	0.055#	0.069¤	0.068¤
VAL	0.086	0.125#	0.112¤	0.077	0.140¤	0.171¤	0.195¤
ILE	0.055	0.078#	0.066	0.050	0.086¤	0.109¤	0.124¤
LEU	0.085	0.119¤	0.097	0.080	0.127¤	0.160¤	0.192¤
PHE	0.067	0.098¤	0.084*	0.062	0.110¤	0.138¤	0.161¤
TRP	0.019	0.026	0.020	0.017	0.031	0.037#	0.041#
Total Amino Acid	1.672	2.425¤	2.146¤	1.446¤	2.697¤	3.288¤	3.700¤
Total Crude Protein	1.851	2.993	2.442	1.665	3.071	3.874	4.774

AMM = Ammonia; HIS = Histidine; SER = Serine; ARG = Arginine; GLY = Glycine; ASP = Asparagine; GLU = Glutamine; THR = Threonine; ALA = Alanine; PRO = Proline; LYS = Lysine; TYR = Tyrosine; CYS = Cysteine; MET =Methionine; VAL = Valine; ILE = Isoleucine; LEU = Leucine; PHE = Phenylalanine; TRP = Tryptophan; RAW= raw, peeled; MICRO = microwaved in skin (analyzed flesh only); BAKE = baked skin on (analyzed flesh only); BOIL = peeled boiled in water; FRIED3M = peeled, 3 minute frying in 100% high-oleic canola oil; FRIED6M = peeled, 6 minute frying in 100% high-oleic canola oil; FRIED9M = peeled potato, 9 minute frying in 100% high-oleic canola oil

[1]Data presented as Means (n=3). Two-way ANOVA statistical model with Dunnett's test for comparison with Raw as the control.

* $p < 0.05$, # $p < 0.01$, ¤ $p < 0.0001$

Table 20. Amino Acid score[1] of Russet potatoes subjected to different consumer-based cooking methods using the 1991 FAO reference pattern

	RAW	MICRO	BAKE	BOIL	FRIED3M	FRIED6M	FRIED9M
Histidine	0.667 (0.017)	0.589 (0.008)	0.636 (0.020)	0.675 (0.004)	0.476 (0.011)	0.444 (0.012)	0.360 (0.015)
Isoleucine	1.094 (0.014)	0.963 (0.017)	0.993 (0.015)	1.100 (0.021)	0.782 (0.007)	0.809 (0.005)	0.777 (0.014)
Leucine	0.712 (0.017)	0.623 (0.006)	0.623 (0.014)	0.744 (0.021)	0.489 (0.001)	0.502 (0.002)	0.509 (0.010)
Valine	1.364 (0.022)	1.233 (0.009)	1.356 (0.036)	1.340 (0.025)	1.020 (0.005)	1.014 (0.016)	0.976 (0.019)
Lysine	0.888 (0.022)	0.784 (0.012)	0.797 (0.018)	0.893 (0.011)	0.629 (0.005)	0.618 (0.006)	0.569 (0.012)
Threonine	0.862 (0.011)	0.774 (0.008)	0.806 (0.016)	0.883 (0.017)	0.608 (0.010)	0.607 (0.003)	0.615 (0.010)
Tryptophan	0.953 (0.025)	0.810 (0.101)	0.761 (0.142)	0.973 (0.26)	0.734 (0.015)	0.718 (0.012)	0.663 (0.054)
Sulphur Amino Acids	1.261 (0.023)	1.087 (0.031)	1.148 (0.018)	1.219 (0.009)	0.909 (0.012)	0.931 (0.016)	0.787 (0.017)
Aromatic Amino Acids	1.067 (0.009)	0.979 (0.008)	1.065 (0.017)	1.076 (0.017)	0.831 (0.026)	0.843 (0.008)	0.803 (0.031)
Amino Acid Score	0.667	0.589	0.623	0.675*	0.476¤	0.444¤	0.360¤

RAW= raw, peeled; MICRO = microwaved in skin (analyzed flesh only); BAKE = baked skin on (analyzed flesh only); BOIL = peeled boiled in water; FRIED3M = peeled, 3-minute frying in 100% high-oleic canola oil; FRIED6M = peeled, 6-minute frying in 100% high-oleic canola oil; FRIED9M = peeled potato, 9-minute frying in 100% high-oleic canola oil

[1]Data are presented as means with Standard Error presented in brackets (n=3). Amino acid scores calculated from the value of the limiting amino acid (highlighted in grey). A score of 1.0 or greater indicates that the amino acid meets or exceeds the established requirement pattern. Data were subjected to a one-way ANOVA using the Dunnett's test with Raw as a control for comparison.

* $p < 0.05$, # $p < 0.01$, ¤ $p < 0.0001$

5.2 Digestibility Values

5.2.1 *In vitro* Protein Digestibility Methods

Results of the IVPD testing (**Table 21)** indicated that the PHD method had the highest RAW IVPD (78.7), although differences were observed between different consumer-based cooking methods. The PHD resulted in lower IVPD values for all other cooking methods, and this pattern is not seen in the other IVPD methods. The PHS, TS and TIM-1 IVPD saw increases across all cooking methods compared to RAW. Not all methods resulted in the same cooking method consistently being higher among the others: PHD (RAW), PHD (BAKE), TS (FRIED6M) and TIM-1 (FRIED3M). TIM-1 resulted in the lowest IVPD for RAW, whereas the TS saw the highest overall IVPD for all other cooking methods, with the exception FRIED3M being higher in the TIM-1. The overall p-values of the F test from the one-way ANOVA indicated that there was a significant difference for the interaction of cooking method and IVPD.

Table 21. *In vitro* Protein Digestibility[1] of Russet potatoes subjected to different consumer-based cooking methods[2].

Method	RAW	MICRO	BAKE	BOIL	FRIED 3M	FRIED 6M	FRIED 9M	Casein[3]	p-value overall F-test
pH-Drop	78.7 (0.25)	76.9# (0.19)	76.5¤ (0.21)	76.5¤ (0.17)	77.3* (0.13)	78.3 (0.57)	76.2¤ (0.38)	89.42 (0.50)	<0.0001
pH-Stat	74.1 (0.56)	78.1# (1.23)	79.2# (0.87)	74.3 (0.43)	78.4# (0.86)	78.0* (0.69)	76.6 (1.00)	113.80 (1.00)	=0.0001
Two-Step	73.1 (0.33)	86.3¤ (1.41)	86.0¤ (1.3)	87.8¤ (1.11)	84.9¤ (3.39)	91.1¤ (1.29)	85.8¤ (1.45)	103.60 (1.30)	<0.0001
TIM-1[4]	71.8 (3.11)	85.3* (4.15)	81.7 (1.58)	83.0* (2.56)	86.5# (1.82)	84.8* (1.90)	82.5 (0.24)	89.8 (4.53)	<0.05

RAW= raw, peeled; MICRO = microwaved in skin (analyzed flesh only); BAKE = baked skin on (analyzed flesh only); BOIL = peeled boiled in water; FRIED3M = peeled, 3 minute frying in 100% high-oleic canola oil; FRIED6M = peeled, 6 minute frying in 100% high-oleic canola oil; FRIED9M = peeled potato, 9 minute frying in 100% high-oleic canola oil; PHD = Static Enzymatic pH-Drop Method, PHD = Static Enzymatic pH Stat Method; TS = Two-Step protein digestive method; TIM-1 = TNO gastro-intestinal model

[1]Protein Digestibility values are presented after being corrected for casein, assuming a casein values of 100% digestibility.

[2]Data are presented as Means, with Stand Error of the Mean presented in brackets (n=9), (TIM n=3)(pH-drop casein n=12)(pH-stat casein n=12)(Two-step casein n=32)(TIM-1 casein n=2). Each was subjected to a one-way ANOVA using the Dunnett's test with Raw as a control for comparison.

[3]Casein data shown without correction

[4]TIM-1 data presented without casein correction

***** $p < 0.05$, **#** $p < 0.01$, ¤ $p < 0.0001$

5.2.2 Potassium Digestibility

With respect to Potassium digestibility there were no significant differences among cooking methods. Potassium digestibility observed for the BOIL potatoes had the highest potassium digestibility (88.0%) and FRIED3M the lowest at (81.6) (**Table 22**).

Table 22. *In vitro* Potassium Digestibility of Russet potatoes subjected to different consumer-based cooking methods.

	RAW	**MICRO**	**BAKE**	**BOIL**	**FRIED3M**	**FRIED6M**	**FRIED9M**
TIM-1	81.7 (1.6)	85.9 (2.7)	83.8 (1.8)	88.0 (6.3)	81.6 (2.3)	86.5 (0.8)	85.0 (1.6)

RAW= raw, peeled; MICRO = microwaved in skin (analyzed flesh only); BAKE = baked skin on (analyzed flesh only); BOIL = peeled boiled in water; FRIED3M = peeled, 3-minute frying in 100% high-oleic canola oil; FRIED6M = peeled, 6-minute frying in 100% high-oleic canola oil; FRIED9M = peeled potato, 9-minute frying in 100% high-oleic canola oil; TIM-1 = TNO gastro-intestinal model

Each was subjected to a one-way ANOVA using the Dunnett's test with Raw as a control for comparison.

Data is presented as Means, with Stand Error presented in brackets (n=3).

***** $p < 0.05$, **#** $p < 0.01$, **¤** $p < 0.0001$

5.3 Protein Quality Values

5.3.1 *In vitro* Protein Quality

Potatoes contain high levels of asparagine and glutamine across all cooking methods, the two AA make up over 44% of total AA within each cooking method (**Table 23**). Within potatoes histidine is the common limiting amino acid except for BAKE where leucine was the limiting amino acid. AA values of VAL exceed the FAO 1991 reference pattern, with the exception of FRIED9M (0.976). Sulphur AA exceeded the FAO 1991 reference pattern for all cooking methods except for frying regardless of duration. Aromatic AA exceeded the reference pattern for RAW, BOIL and BAKE (1.067,10.076, 1.065 respectively). The AAS of BOIL Russet potatoes was the highest (0.675) and FRIED9M the lowest (0.360). All frying methods of FRIED3M, FRIED6M, FRIED9M (0.476, 0.444, 0.360 respectively) resulted in significantly decreased AAS compared to those observed with RAW (0.667), while BOIL saw a significant increase in AAS compared to raw.

5.3.2 *In vitro* Protein Quality Summary

For IVPDCAAS values derived from the PHD method demonstrated that all cooking methods, with the exception of BOIL, were significantly lower than the value observed for the RAW control ($p<0.0001$; **Table 22**). For the PHS method, only the frying treatments led to significantly reduced IVPDCAAS values, relative to the RAW control. The final static method, the TS IVPDCAAS method, data indicated that BOIL potatoes (0.602±0.013) had significantly higher ($p<0.05$) values than RAW (0.499±0.006). The results for the other treatments were not different from the RAW control.

For the dynamic digestibility methods, the TIM-1 resulted in the lowest IVPDCAAS overall value for RAW at 0.474±0.028. When the processed potatoes were compared to the RAW, for IVPDCAAS determined by TIM-1, the BOIL potatoes exhibited a significantly higher value than the raw controls, and the FRIED9M treatments presented with IVPDCAAS values of 0.297±0.012, which was significantly lower than RAW.

Table 23. *In vitro* Protein Digestibility Corrected Amino Acid Score[1] of Russet potatoes subjected to different consumer-based cooking methods[2].

	RAW	MICRO	BAKE	BOIL	FRIED 3M	FRIED 6M	FRIED 9M	P-values overall F test
PHD	0.519 (0.010)	0.453* (0.008)	0.435# (0.048)	0.516 (0.005)	0.367¤ (0.010)	0.348¤ (0.017)	0.275¤ (0.018)	<0.0001
PHS	0.489 (0.004)	0.460 (0.019)	0.486 (0.019)	0.502 (0.004)	0.372# (0.008)	0.347# (0.017)	0.277¤ (0.025)	<0.0001
TS	0.499 (0.006)	0.527 (0.018)	0.523 (0.044)	0.602* (0.013)	0.416 (0.016)	0.423 (0.023)	0.320# (0.018)	<0.0001
TIM-1[3]	0.474 (0.028)	0.503 (0.025)	0.464 (0.034)	0.560* (0.019)	0.411 (0.014)	0.377 (0.015)	0.297# (0.012)	<0.0001

RAW= raw, peeled; MICRO = microwaved in skin (analyzed flesh only); BAKE = baked skin on (analyzed flesh only); BOIL = peeled boiled in water; FRIED3M = peeled, 3-minute frying in 100% high-oleic canola oil; FRIED6M = peeled, 6-minute frying in 100% high-oleic canola oil; FRIED9M = peeled potato, 9-minute frying in 100% high-oleic canola oil; PHD = Static Enzymatic pH-Drop Method, PHD = Static Enzymatic pH Stat Method; TS = Two-Step protein digestive method; TIM-1 = TNO gastro-intestinal model

[1]*In vitro* Protein Digestibility Corrected Amino Acid Score values are shown after being corrected for casein, assuming a casein values of 100% digestibility.

[2]Data are presented as Means, with Standard Error of the Mean presented in brackets (n=9) (TIM n=3). Each was subjected to a one-way ANOVA using the Dunnett's test with Raw as a control for comparison.

[3]TIM-1 data presented without casein correction

***** $p < 0.05$, **#** $p < 0.01$, **¤** $p < 0.0001$.

5.3.3 *In vivo* Protein Digestibility and Quality

The data for the TPD and PDCAAS are presented in **Table 24** and **Table 25** respectively. The TPD value for the raw potato samples was 40.49±3.92 %. All methods of cooking resulted in significant improvements in the digestibility coefficients ($p<0.0001$), when compared to the raw control, with values reaching a maximum of 84.54±0.49% for the BAKED samples. The product of the TPD and the AAS yields the PDCAAS value, and the *in vivo* PDCAAS values aligned with the TPD, with the RAW control having the lowest value. The FRIED6M sample was also low and not significantly different from the RAW treatment (**Table 25**).

Table 24. *In vivo* True Fecal Protein Digestibility[1]

	RAW	MICRO	BAKE	BOIL	FRIED6M
True Fecal Protein Digestibility (TPD)	40.49 (3.92)	82.85¤ (0.59)	84.54¤ (0.49)	82.96¤ (0.65)	80.06¤ (1.59)

RAW= raw, peeled; MICRO = microwaved in skin (analyzed flesh only); BAKE = baked skin on (analyzed flesh only); BOIL = peeled boiled in water; FRIED6M = peeled potato, 6-minute frying in 100% high-oleic canola oil.

[1]Data are presented as Means, with Standard Error presented in brackets (TPD=10). Each was subjected to a two-way ANOVA using the Tukey's test with TPD as a control for comparison. ***** $p < 0.05$, **#** $p < 0.01$, ¤ $p < 0.0001$

Table 25. *In vivo* Protein Digestibility Corrected Amino Acid Score[1] of Russet potatoes subjected to different consumer-based cooking methods.

	RAW	MICRO	BAKE	BOIL	FRIED6M
TPD	0.270 (0.026)	0.488¤ (0.003)	0.527¤ (0.003)	0.560¤ (0.004)	0.289 (0.006)

RAW= raw, peeled; MICRO = microwaved in skin (analyzed flesh only); BAKE = baked skin on (analyzed flesh only); BOIL = peeled boiled in water; FRIED6M = peeled, 6-minute frying in 100% high-oleic canola oil; TPD = True Protein Digestibility

[1]Data are presented as Means, with Standard Error presented in brackets (TPD=10). Each was subjected to a two-way ANOVA using the Tukeys test with TPD as a control for comparison.
***** $p < 0.05$, # $p < 0.01$, ¤ $p < 0.0001$

Chapter 6: Discussion and Conclusion

6.1 Discussion

6.1.1 Nutritional Composition of cooked Russet potatoes

The data showed that, on an as-consumed basis, the cooking method used does lead to significant differences among key nutrients in Russet potatoes. For the purposes of this study, we compared all cooking methods against a RAW control. With respect to crude protein on an as-consumed basis, values were significantly higher for all of the cooking methods tested, relative to RAW, with the exception of the BOIL samples. Macronutrients increased significantly across all fried methods compared to RAW due to the removal of water from the potato. The latter resulted in an increase in the dry matter content and thus an increased nutrient density. The one exception to this increased nutrient density was the BOIL samples, where water content increased, thus decreasing the density of protein and non-fiber carbohydrates, in addition to several other nutrients including minerals. Although not all minerals were significantly higher than the RAW. Frying saw that the longer the duration, the greater the moisture loss and resultant increases in content. Iron was the one exception to this trend, as its content did not change. Work investigating mineral status of French fries found that iron decreased, where copper and zinc increased (105). This is on trend for what was found in this research. The majority of minerals were higher in all cooking methods although calcium saw a significant decrease in BAKE and BOIL. BOIL also saw a significant decrease in phosphorus. Boiling potatoes has been shown to have to have a negative effect on mineral content. Previous research discovered that boiling reduced potassium by up to 31% in various white potatoes, including Russet Norkotah (106). Levels of other minerals was shown to be significantly reduced as well including: phosphorus, manganese, sulfur, zinc, magnesium and iron (106). The current research does not follow this trend for all minerals, as only phosphorus and magnesium in the BOIL samples resulted in a significant decrease compared to RAW.

The data obtained from CNF did not indicate the variety of potato, therefore we are not able to directly compare CNF data to our data; it is more so used a guide. With respect to specific nutrients, there were large differences observed for calcium and copper, although it is unknown if the CNF data included values for the skin of the varietals reported. Previously reported data on the overall effects of cooking on Russet potato composition found that crude protein levels

were reduced when boiled (peeled/without skin), and increased when baked and fried (87). This is likely explained by the changes in moisture content of the potato as a function of the cooking method consistent with the results from the current study. All cooking methods except for BOIL saw an increase in protein on an as-consumed basis.

However, for a better understanding of the specific processing effects on the nutritional value of potato, it is best to also present data on a dry-matter basis. The current research shows that all macro nutrients, and minerals decrease during frying regardless of duration, as presented in **Table 17**, with the exception of fat; however, not all decreases are significant ($p<0.05$), as presented. Previous work showed that during frying crude protein content significantly increases; due to moisture loss increasing dry matter, of the 6 varietals tested two saw a decrease in crude protein content, attributed to leaching during bleaching (105). Significant increases in crude protein were shown in MICRO and BAKE samples. BOIL samples in dried samples again showed that phosphorus, magnesium and potassium decreased significantly from RAW control, following similar patterns to previous research (106). As expected, fat levels increased across all frying durations, as fat from the oil displaced the water during cooking. It has been proven that oil is absorbed at over 2000% when potatoes are fried (105). Potassium values increased in MICRO and BAKE at a significant level, current literature is lacking in respect to microwave and baking on mineral dispersion within potatoes regardless of varietal. Much of the known data were gathered from the CNF and USDA, and that data is commonly presented on an "as consumed" basis. On a dry weight basis potassium does equate to more than 1.3grams of potatoes nutritional composition, with the highest levels shown in MICRO and BAKE, both close to 2gram.

The use of cooking methods that do not involve water or oil produces the highest crude protein values on a dry-matter basis, as water is removed when cooked, the concentrations of macro and micronutrients increase across majority of those investigated. The use of water in BOIL samples follows previous trends of leaching of minerals. Whereas cooking in high temperature oil indicates, that as fat is absorbed, carbohydrates, protein and the majority of minerals decrease.

6.1.2 Amino Acid Composition

Regarding EAA, histidine was the limiting amino acid for all methods except BAKE (leucine). Data obtained from **Table 13** indicated that the AA values of Russet potatoes followed patterns established within existing USDA data. The USDA reported values, however, are not readily

comparable to the current data due to lack of information related to the specific cooking methods employed, as well as variety of potato, with only baked with skin and flesh indicating data is from Russet potatoes. In the current study, the contribution of the skin to the nutritional composition was not included, principally to provide a consistent comparison between the potato treatments. However, the data does suggest that the Russet potatoes utilized in this study have similar AA values of those used by the USDA used as a reference. In one study investigating microwaving versus baking, the cortex had an overall decrease in AA when microwaved and an increase during baking (94). When the pith was investigated, the opposite effect was seen. This study did not look at specific locations within the flesh of potatoes, as all flesh was investigated as a composite sample. Had the previous study investigated the flesh as a composite, it is likely that they would have observed that baking would have yielded an increase in AA, compared to an untreated control, while microwaving would have yielded the opposite effect. The present study does not follow this trend as overall AA values increase for microwaving and baking compared to RAW. Presenting data on an as-consumed basis is relevant for nutritional purposes, as it reflects the status in which the potato is consumed.

An amino acid score of 1.0 or greater indicates that the amino acid in question is equal to that of the FAO reference pattern for 2-5-year-old school children; this is not the case for Russet potatoes. Isoleucine matched or was close to matching the reference for cooking methods except frying, whereas valine the reference pattern for all cooking methods except FRIED9M. Sulphur amino acids (MET and CYS) exceed the reference pattern for all except for frying. The latter point has important implications for protein nutrition, which will be discuss below. The aromatic amino acids (PHE and TRP) were also matching or very close to the reference pattern. The same Amino Acid Score is used across all protein quality methods with only the digestibility changing between methods. It was found that AAS ranged from 0.360-0.675 with FRIED9M at the low end and BOIL at the high end, with histidine being the limiting AA for all except BAKE (LEU).

6.1.3 Potassium Digestibility

Potassium is a vital mineral required by the human body for the function of nerves and muscles. The recommended daily intake of potassium for healthy individuals over the age of 4 is 4700mg (107). The data suggests that an 85g serving of potatoes provides 420mg of potassium (9% Recommended Daily Intake), not including the skin. Thus, one small potato averaging a size of 170g provides 840mg of potassium equaling 18% of the Recommended Daily Intake for

potassium, and over 80% is digestible regardless of cooking method. Bananas are typically considered to be a high source of potassium as they contain 358mg/100g (108), with one large banana (150g) containing 537mg of potassium. As such, the current data highlight that potatoes contain more potassium per 100g than bananas.

The use of the TIM-1 to calculate *in vitro* digestibility allowed for potassium digestibility to be calculated also. The results of the digestibility can be seen in

Table *22*. Potassium is highly digestible in all cooking methods of Russet potatoes, with no significant differences due to any cooking method when compared to RAW. Prior i*n vitro* research on sweet potato potassium release found that, when stage of digestion was monitored, K released at a range of 40-100g/100g K in solids dependent of the stage of digestion (gastric or pancreatic) (109). Previous research indicated that various inclusions of potassium in the human diet ranging from 20-60 milliequivalents K from nonfried and fried potatoes saw no difference between cooking method with both resulting in >94%±12% absorption efficiency (91). The current research resulted in lower *in vitro* potassium digestibility values (81.6%±4.9%) than previous *in vivo* research, however the values are still all above 80%.

6.1.4 Monocompartment *In vitro* Protein Digestibility

When examined within in a treatment, the PHD and PHS values were very similar, except for RAW, where the PHD method yielded values higher than those observed for PHS. This was possibly due to high starch nature of potatoes thus, the PHS method resulted in lower raw IVPD values. It was suspected that the quantity of NaOH required to reduce/hold pH levels during the PHD and PHS were being buffered by the starch. Research conducted on processed beans found that PHDIVPD was in fact lower compared to TPD (55).

The addition of α amylase as an initial step of the TS was added in order to overcome the buffering of starch during IVPD. This additional step allowed for cooking methods to have increased values compared to PHD and PHS. For the TSIVPD method, RAW was still the lowest IVPD value, which is to be expected as raw potatoes are not as easily digested as cooked. Raw potato starch is made up of crystalline structure, which is resistant to human digestive enzymes, gelatinization of these starches when cooked increased digestibility (29). Work investigating potato flour digestibility found that cooking improves digestibility of proteins and starches (110). Protein digestibility of cooked products containing 40-60% potato increased from 12-17% when products were baked or roasted (110). Previous research on variety of proteins such as pulses and grains, concluded that pH drop *in vitro* protein digestibility under-predicted the digestibility values and Two-step *in vitro* protein digestibility may over-predict protein digestibility when compared to true fecal protein digestibility (37,42,51). The two-step saw significant increases across all cooking methods compared to RAW. This data shows that the pH-drop does indeed under-estimate the IVPD when compared to more advanced IVPD methods, such as the TS and TIM-1.

6.1.5 Multi-Compartment *In vitro* Protein Digestibility

When examining data derived during the TIM-1 digestibility method, the same significant increase across cooking methods was observed as those derived from the TS method. Using RAW as the control for comparison with a TIMIVPD, each cooking method yielded significantly higher digestibility coefficients. There is no current or previous literature investigating the use of the TIM-1 to investigate the IVPD of potatoes, however what is available indicates that the TIM-1 does have the potential to be used as an alternative to determine PDCAAS. Use of the TIM-1 on calf milk replacer yielded digestibility coefficients that replicated true ileal digestibility coefficients (34). In a study conducted by R. Havenaar et al., the authors found that DIAAS values, where the ileal digestibility coefficients were determined using the TIM-1, for immature herring egg protein digestibility was highly correlated to human ileal digestibility values (r^2=0.96), with digestibility values ranging from 71-92% (111). The potential for this technique is not fully utilized in literature for plant-based protein digestibility, as significant research is currently focussing on the use of this methodological approach for determining pharmaceutical bio accessibility. The present research found that the TIM-1 levels of digestibility were similar to those measured via the TS method. The values for TIMIVPD for the RAW treatment had the lowest level of digestibility, similar to that of the TS and PHS, with all other cooking methods yielding significantly higher values.

6.1.6 *In vivo* Protein Digestibility

In vivo protein digestibility results from this study indicated that the RAW treatment had the lowest percent true fecal protein digestibility (<50%). Untreated plant-based flours assessed with *in vivo* studies found that baking decreased protein digestibility in buckwheat (79). Data on protein digestibility determined via rodent bioassay is limited, therefore it is difficult to form a conclusive reason as to why RAW saw the highest SEM within cooking methods. Previous data on potato digestion, mostly in the form of protein isolates found that potatoes have a high bioavailability at 90% (12,29). However, research is lacking for raw potato consumption/digestibility data derived via *in vivo* methods, with only a few studies researching starch digestion. Research investigating raw starch consumption via rats found increased consumption of cooked starch; however fecal weight increased 3-fold in the raw starch group (112). As raw potatoes are over 80% starch, with a majority of those being resistant starches (resistant to intestinal digestion but available for microbial synthesis), this may have led to

increase microbial protein synthesis and excretion, which would lead to a reduction in protein digestibility values. While the TPD method accounts for endogenous nitrogen losses, the method is sensitive to food matrix effects that can influence microbial nitrogen synthesis. The studies that are currently available state the resistant starch as the cause of low digestion however do not provide reasoning for under consumption compared to other diets (112,113). With respect to cooking methods, clearly RAW potatoes presented with lower *in vivo* (and *in vitro*) digestibility coefficients, and the explanation for this is likely related to the interactions of the carbohydrate and protein fractions, and their ultimate effect on nitrogen availability.

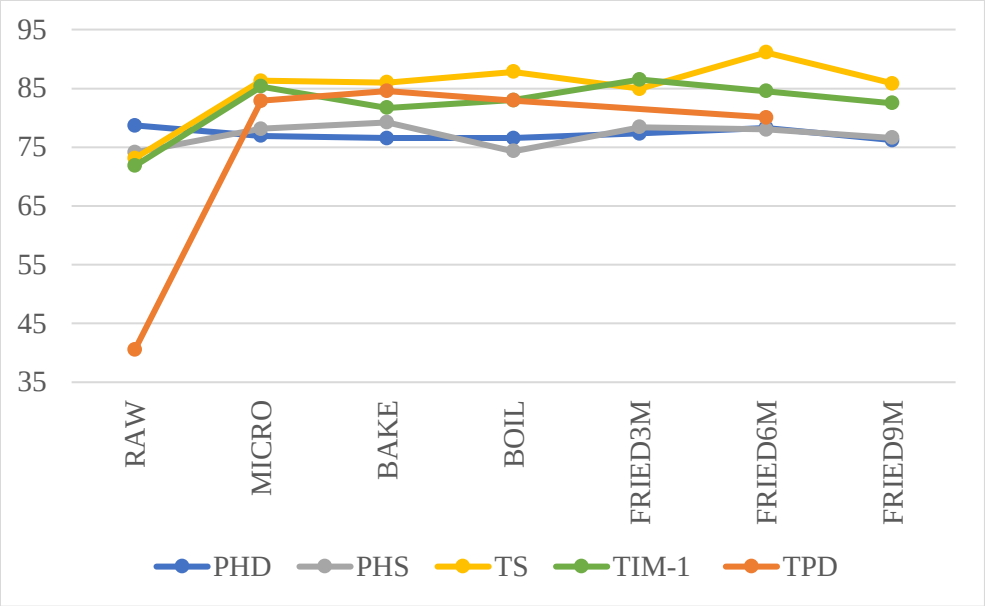


Figure 2. Protein digestibility of different cooking methods of Russet potatoes across different *in vitro* and *in vivo* methods
RAW= raw, peeled; MICRO=microwaved in skin (analyzed flesh only); BAKE=baked skin on (analyzed flesh only); BOIL= peeled boiled in water; FRIED3M= peeled, 3 minute frying in 100% high-oleic canola oil; FRIED6M= peeled, 6 minute frying in 100% high-oleic canola oil; FRIED9M= peeled potato, 9 minute frying in 100% high-oleic canola oil; PHD = Static Enzymatic pH-Drop Method, PHD = Static Enzymatic pH Stat Method; TS = Two-Step protein digestive method; TIM-1 = TNO gastro-intestinal model; TPD = True Protein Digestibility
In vitro Protein Digestibility values are shown after being corrected for casein, assuming a casine valuss of 100% digestibility.

6.1.7 *In vitro* Protein Quality

When IVPD and AAS data were combined to yield the IVPDCAAS values, the resultant data signified that cooking method does affect the quality of potato protein: MICRO and BAKE were shown to have significantly lower values than RAW using the pH-drop method. This was possibly due to the rapidly digesting starches in potatoes, making the change in pH difficult as these starches breakdown quickly and hinder protein digestion (112).

The two-step method IVPDCAAS indicated that BOIL was significantly higher than RAW, and FRIED9M was significantly lower, whereas all other cooking methods did not result in any significant changes relative to the RAW control. For application in this study, the two-step method was altered to include the use of alpha amylase, in order to aid in the breakdown of starch and better mimic *in vivo* digestibility conditions. Boiled potatoes have been shown to have increased RS once cooked and chilled, decreasing the content rapidly digested starches (30,93,112).

In the current study, the expense of running the TIM-1 led to the use of one composite sample being run for each cooking batch, resulting in a total of three samples per cooking method. The other IVPD methods allowed for greater replication, as each was run in triplicate for each of the 3 cooking batches. As such, the standard error of the mean for the TIM-1 *in vitro* protein digestibility data is likely to be higher, thus limiting the power of detecting significant differences via the Dunnett test.

With respect to the specific cooking methods, the frying of potatoes was clearly detrimental to the overall protein quality of the final product. While the IVPD values for all frying methods was not negatively impacted, the AAS values rapidly decreased as frying times increased, resulting in low IVPDCAAS. Effects of frying on protein quality has not been a topic of previous research, limiting further conclusions. However, looking at the data from this research it was found that longer duration of cooking resulted in lower quality. As all IVPD levels were high 71.8-91.1% for Russet potatoes across all cooking methods and IVPD methods, the quality of protein or IVPDCAAS relies on the AAS, which saw BOIL at the high end and FRIED9M at the low end, indicating that frying, regardless of duration, places a constraint on protein quality of potatoes.

6.1.8 *In vivo* Protein Quality

A PDCAAS score of 1.0 or greater indicates a complete protein that meets all EAA needs for an individual (using the FAO 1991 reference amino acid pattern). Casein and other animal proteins are complete proteins. Due to constraints on the animal testing with COVID-19 restriction, only one frying duration (FRIED6M) was analyzed. RAW Russet potatoes had a large standard deviation, possibly due to the rats not consuming the diet as it is not digested well, as noted with a TPD of 40.5%, whereas baked potatoes had a TPD of 84.5%.

Error! Reference source not found. Previous research has documented that complex multicompartment IVPD methods provide a stronger relationship towards TPD, when compared against static, monocompartment models. This research strengthens these observations and provides evidence that the TIM-1 method can be considered an alternative to animal studies for the prediction of PDCAAS. There is a lack of current research directly comparing the two methods against another, particularly in the realm of plant-based proteins. Data that is currently available investigates protein concentrates and meals as a whole, with a few exceptions.

Previous research found that frying decreased, while baking increased the total nitrogen content of Katahdin, Chipbelle and Rosa potatoes (94). When the potatoes were fried, there was a positive correlation of frying time to protein amount, with the longer the duration of frying yielding higher crude protein content on an "as consumed" basis; this is not the case on a "dry-matter" basis. The increase in total dry matter on an "as consumed" basis increased total crude protein, however when protein was analyzed for quality it was found that there was a significant decrease in FRIED6M compared to other cooking methods as the same AAS were used for PDCAAS as used for IVPDCAAS. Thus, RAW and FRIED6M saw the lowest PDCAAS values with BOIL, BAKE and MICRO having the highest respectively.

All methods, be they *in vitro* or *in vivo,* follow similar patterns in terms of protein quality (**Figure 3)**. *In vivo* RAW PDCAAS value is shown to be drastically reduced compared to all *in vitro* methods. MICRO, BAKE, BOIL and FRIED6M follow very similar trajectories between protein digestibility methods. Thus, indicating that *in vitro* methods provide a good reference point for cooked potato digestibility.

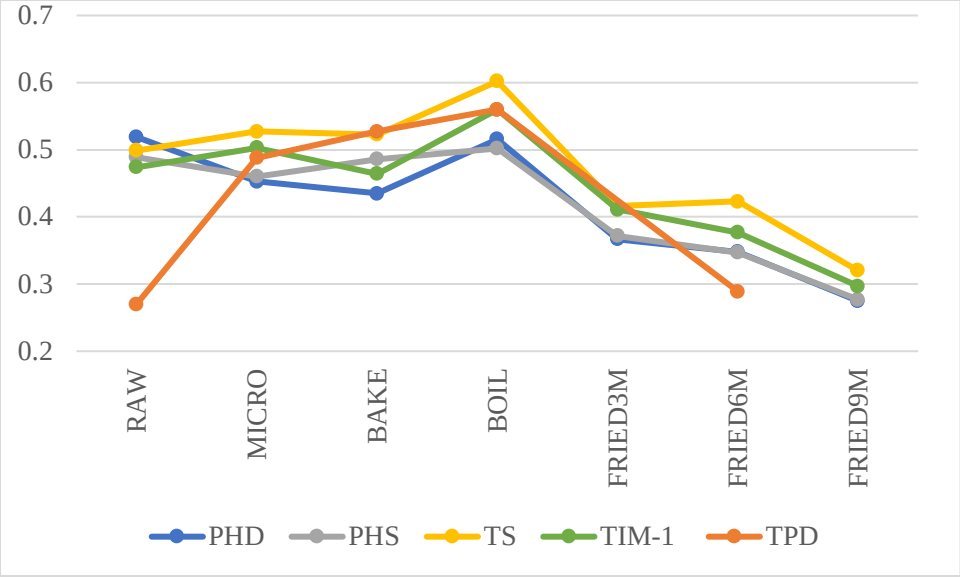


Figure 3. Protein Digestibility-Corrected Amino Acid Scores of Russet Potatoes across different *in vitro* and *in vivo* methods
RAW= raw, peeled; MICRO = microwaved in skin (analyzed flesh only); BAKE = baked skin on (analyzed flesh only); BOIL = peeled boiled in water; FRIED3M = peeled, 3 minute frying in 100% high-oleic canola oil; FRIED6M = peeled, 6 minute frying in 100% high-oleic canola oil; FRIED9M = peeled potato, 9 minute frying in 100% high-oleic canola oil; PHD = Static Enzymatic pH-Drop Method, PHD = Static Enzymatic pH Stat Method; TS = Two-Step protein digestive method; TIM-1 = TNO gastro-intestinal model; TPD = True Protein Digestibility

6.1.9 Russet Potatoes' Contribution to the North American Diet

As mentioned, potatoes contribute upwards of 4% of daily protein intake in the American diet, as one medium sized baked potato (1 cup, 160grams) contains 3.9 grams of crude protein. The amount of protein within a food product cannot alone contribute to the quality of protein, thus the protein quality was considered, using both *in vivo* and *in vitro* methods.

The recommended dietary allowance which is the average daily intake nutrient intake to meet 98% of the healthy population (107). Males and females aged 14-70 years require protein at a rate of 0.80 g/kg/day; representing a 70kg person 56 grams of protein are required daily. With this a medium sized baked potato with 3.9 grams of protein results in 6.9% of ones total protein as the RDA is based on crude protein content of food and not the complete protein values within those foods. Total fibre is required at 38g/day for males 14-50 years and 25 grams for females aged 19-50 years. Potatoes are considered a high source of fibre at an average of 2.4 grams in baked potatoes without peel (108). The addition of the peel increased that to 3.5 grams in a medium sized baked potato (108).

The ability to be grown in a wide variety of climates and soils hold the potential for potatoes to become a staple food in developing countries lacking the ability to consume complete proteins such as animal products due to the cost of these food sources. Potatoes are an excellent source of vitamin C, as well as being one of the most concentrated sources of potassium (12). Potatoes are a relatively cost effective and high yielding food that can be cooked in a variety of ways and provide substantial vitamins, minerals, carbohydrates, and fibre. MICRO, BAKE and BOIL have PDCAAS values over 0.4 qualifying as quality protein for foods intended for infants, however they do not meet the requirements of 10.00-19.99% of daily reference protein value of 50g of protein to qualify as a "good source of protein" (39). Even though potatoes do not constitute as a complete protein or a "good source" of protein potatoes do have the ability to contribute a substantial amount of protein to one's diet and therefore should be looked at as more than a source of carbohydrates.

The current research found that when total crude protein values were accounted for via the PDCAAS values, corrected protein values in an 85g serving were reduced drastically **(Table 26)**. FRIED6M having the highest crude protein in an 85g serving at 3.29g was then reduced to 0.95g/85g corrected protein serving. BOIL total crude protein (1.42g/85g serving) and a PDCAAS score of 0.56 causing the corrected protein to be reduced to 0.79g/85g serving. Higher

PDCAAS values found in BOIL, BAKE, and MICRO (>0.48) saw a reduction, however not as significant as RAW and FRIED6M with both having PDCAAS values <0.3. Hence the importance of finding complementary food sources to increase the total PDCAAS corrected protein.

Table 26. *In vivo* Protein Quality of Russet potatoes

Sample	% Crude Protein	% True Fecal Protein Digestibility		Amino Acid Score	Limiting Amino Acid	PDCAAS	Total Crude Protein (85g Serving)	PDCAAS-Corrected Protein (85g Serving)
		Mean	**SD**					
Casein	85.06	93.57	1.43	1.129	TRP	1.0		
RAW	1.85	40.49	11.74	0.668	HIS	**0.27**	1.57	**0.43**
MICRO	2.99	82.85	1.78	0.589	HIS	**0.488**	2.54	**1.24**
BAKE	2.44	84.54	1.45	0.623	LEU	**0.527**	2.07	**1.09**
BOIL	1.67	82.96	1.94	0.675	HIS	**0.56**	1.42	**0.79**
FRIED6M	3.87	80.06	4.77	0.361	HIS	**0.289**	3.29	**0.95**

SD = Standard Deviation (n=10); PDCAAS = Protein digestibility-corrected amino acid score; RAW= raw, peeled; MICRO=microwaved in skin (analyzed flesh only); BAKE=baked skin on (analyzed flesh only); BOIL= peeled boiled in water; FRIED6M= peeled, 6-minute frying in 100% high-oleic canola oil.

Amino acid score determined as the lowest amino acid score derived from **Table 20**

Values above 1.00 truncated to 1.00. Determined as the product of Amino Acid Score and % True Fecal Protein Digestibility

6.1.10 Complementary Food Sources

The entirety of one's diet needs to be examined as humans do not eat only one food source to acquire all dietary requirements. Finding complementary foods that create a balanced diet is important. In order to find complementary foods other factors besides the protein composition need to be assessed such as nutrient bioavailability. With potatoes having high levels of sulphur amino acids (cysteine and methionine) combining potatoes with foods low in sulphur amino acids will help to create a complementary protein in ones' diet. Foods that are complementary to potatoes include quinoa, split peas and other legumes. As potatoes are not deemed as a "source of protein" on their own, they do have the potential to create a balanced protein source when paired with other plant-based proteins.

Previous work investigating the digestibility of Canadian pulses found that for the majority of pulses the limiting essential amino acids are the sulphur containing Methionine and Cysteine. High TPD (>70%) in pulses and in potatoes would create a balanced AA profile for all essential amino acids. A serving size (90g = ½ cooked cup)of the corresponding pulses provide an "Good Source of Protein" in both the United States (75). Consuming vegan or vegetarian options can in turn provide difficulty in consuming foods with adequate nutrition, specifically protein with adequate essential amino acids. **Table 27** shows the combined PDCAAS of different home cooking methods with a variety of pulses, although the PDCAAS is not complete at 1.0, BAKE with split green peas has the highest combination at 0.73. The mixtures were calculated with the limiting AA alternating between sulphur AA and TRP. The sulphur AA are still limiting in a majority of the mixtures due to the low levels of protein found in potatoes; the high level of sulphur AA in a serving of potatoes is not enough to balance out the sulphur AA in lentil, however it does aid in increasing PDCAAS levels. All mixtures have a PDCAAS value greater than 0.5, concluding that the addition of pulses to meals that included whole potato cooked in a variety of ways aids in increasing the PDCAAS values, qualifying quality protein. Therefore, increasing the amount of complete protein found in a single meal.

Table 27. Examples of mixtures for protein quality of complementary blends of Russet potatoes and pulses

	Analytical Data										
	Weight (g)	**Protein (g/100g)**	**THR**	**VAL**	**M+C**	**ILE**	**LEU**	**P+T**	**HIS**	**LYS**	**TRP**
			mg/g protein								
RAW	85	1.85	29.31	47.75	31.52	30.63	46.97	67.21	12.67	51.52	10.48
MICRO	85	2.99	26.31	43.17	27.16	26.97	41.12	61.67	11.20	45.49	8.91
BOIL	85	1.67	30.03	46.91	30.48	30.79	49.12	67.80	12.83	51.82	10.71
BAKE	85	2.44	27.42	47.46	28.70	27.80	41.14	67.10	12.09	46.22	8.37
FRIED6M	85	4.98	20.65	35.48	23.27	22.65	33.13	53.08	8.44	35.85	7.90
Navy Bean	90	8.76	44.88	46.55	22.00	38.36	79.20	85.68	26.79	69.60	9.13
Split Green Pea	90	7.39	38.42	39.55	14.75	33.32	74.58	76.23	24.89	70.76	9.79
Black Bean	90	8.39	52.36	49.00	19.00	41.72	88.44	91.98	30.59	75.40	10.45
Red Kidney Bean	90	8.27	28.22	40.25	17.50	32.76	75.24	78.75	27.74	67.28	9.13
Chickpeas	90	7.57	40.46	48.65	27.00	45.64	84.48	94.50	29.26	73.66	6.71
			Qualities in Mixture								
	Digestibility	**Protein (g per serving)**	**THR**	**VAL**	**M+C**	**ILE**	**LEU**	**P+T**	**HIS**	**LYS**	**TRP**
			mg								
RAW	0.40	1.57	46.11	75.12	49.59	48.19	73.90	105.74	19.93	81.06	16.50
MICRO	0.83	2.54	66.94	109.83	69.10	68.60	104.61	156.88	28.48	115.72	22.67
BOIL	0.83	1.42	42.50	66.39	43.14	43.57	69.52	95.95	18.15	73.34	15.15
BAKE	0.85	2.08	56.91	98.51	59.58	57.70	85.40	139.28	25.10	95.94	17.38
FRIED6M	0.80	4.23	87.39	150.15	98.50	95.87	140.22	224.65	35.71	151.73	33.42
Navy Bean	0.77	7.88	353.83	367.00	173.45	302.43	624.41	675.50	211.21	548.73	71.98
Split Green Pea	0.85	6.65	255.53	263.05	98.10	221.61	496.03	507.01	165.54	470.62	65.11
Black Bean	0.70	7.55	395.37	370.00	143.47	315.03	667.81	694.54	230.99	569.35	78.91
Red Kidney Bean	0.79	7.44	210.04	299.58	130.25	243.83	560.01	586.14	206.47	500.77	67.95
Chickpeas	0.85	6.81	275.65	331.45	183.95	310.95	575.56	643.83	199.35	501.85	45.72

Table 27 (cont)

TOTALS										
BOIL + Red Kidney Bean	9.99	252.55	365.97	173.39	287.40	629.53	682.09	224.62	574.10	83.11
MICRO + Chickpea	9.36	342.60	441.28	253.06	379.55	680.18	800.71	227.83	617.57	68.39
BAKE + Split Green Pea	8.73	312.44	361.56	157.69	279.31	581.43	646.28	190.64	566.57	82.49
Fried6M + Black Bean	11.78	482.76	520.15	241.97	410.90	808.03	919.19	266.70	721.08	112.32
BOIL + Navy Bean	9.30	396.34	433.39	216.59	346.00	693.93	771.45	229.37	622.07	87.13
MICRO + Split Green Pea	9.20	322.47	372.88	167.21	290.21	600.64	663.89	194.03	586.35	87.78
BOIL + Split Green Pea	8.07	298.04	329.44	141.24	265.18	565.55	602.96	183.70	543.96	80.27
Amino Acid mg/g protein (total for each AA/total protein)										
	THR	**VAL**	**M+C**	**ILE**	**LEU**	**P+T**	**HIS**	**LYS**	**TRP**	
Reference Scoring Pattern	34	35	25	28	66	63	19	58	11	
BOIL + Red Kidney Bean	25	37	17	29	63	68	22	57	8	
MICRO + Chickpea	37	47	27	41	73	86	24	66	7	
BAKE + Split Green Pea	36	41	18	32	67	74	22	65	9	
Fried6M + Black Bean	41	44	21	35	69	78	23	61	10	
BOIL + Navy Bean	43	47	23	37	75	83	25	67	9	
MICRO + Split Green Pea	35	41	18	32	65	72	21	64	10	
BOIL + Split Green Pea	37	41	18	33	70	75	23	67	10	
Amino Acid Score for Mixture										
	THR	**VAL**	**M+C**	**ILE**	**LEU**	**P+T**	**HIS**	**LYS**	**TRP**	
BOIL + Red Kidney Bean	0.74	1.05	0.69	1.03	0.96	1.08	1.18	0.99	0.76	
MICRO + Chickpea	1.08	1.35	1.08	1.45	1.10	1.36	1.28	1.14	0.66	
BAKE + Split Green Pea	1.05	1.18	0.72	1.14	1.01	1.18	1.15	1.12	0.86	
Fried6M + Black Bean	1.21	1.26	0.82	1.25	1.04	1.24	1.19	1.06	0.87	
BOIL + Navy Bean	1.25	1.33	0.93	1.33	1.13	1.32	1.30	1.15	0.85	
MICRO + Split Green Pea	1.03	1.16	0.73	1.13	0.99	1.15	1.11	1.10	0.87	
BOIL + Split Green Pea	1.09	1.17	0.70	1.17	1.06	1.19	1.20	1.16	0.90	

Table 27 (cont)

Weighted Average Protein Digestibility sum of [(protein X Digestibility factor) / protein total)	
BOIL + Red Kidney Bean	0.70
MICRO + Chickpea	0.84
BAKE + Split Green Pea	0.85
Fried6M + Black Bean	0.74
BOIL + Navy Bean	0.78
MICRO Split Green Pea	0.85
BOIL Split Green Pea	0.85
Score Adjusted for Digestibility	
BOIL + Red Kidney Bean	0.52
MICRO + Chickpea	0.56
BAKE + Split Green Pea	0.73
Fried6M + Black Bean	0.60
BOIL + Navy Bean	0.66
MICRO + Split Green Pea	0.61
BOIL + Split Green Pea	0.59

Corrected Protein Content (PDCAAS)	**85g serving potato**	**90g serving lentil**	**Combined with serving size**	**Corrected protein**
BOIL + Red Kidney Bean	1.42	7.44	8.86	4.63
MICRO + Chickpea	2.54	6.81	9.36	5.25
BAKE + Split Green Pea	2.08	6.65	8.73	6.37
Fried6M + Black Bean	4.23	7.55	11.78	7.12
BOIL + Navy Bean	1.42	7.88	9.30	6.17
MICRO + Split Green Pea	2.54	6.65	9.20	5.65
BOIL + Split Green Pea	1.42	6.65	8.07	4.79

THR=Threonine; VAL=Valine; M+C= Methionine+Cysteine; ILE=Isoleucine; LEU=Leucine; P+T=Phenylalanine+Tyrosine; HIS = Histidine; LEU = Leucine; TRP = Tryptophan; RAW = raw, peeled; MICRO = microwaved in skin (analyzed flesh only); BAKE = baked skin on (analyzed flesh only); BOIL= peeled boiled in water; FRIED6M= peeled potato, 6-minute frying in 100% high-oleic canola oil

6.2 Summary of Findings

Overall, the data from this research can be summarized as follows:

- The use of cooking methods that do not involve water or oil produces the highest crude protein values on a dry-matter basis, as water is removed during cooking, leading to the concentrations of macro and micronutrients increasing across the majority of those investigated
- The AAS ranged from 0.360-0.675 with FRIED9M at the low end and BOIL at the high end, with histidine being the limiting AA for all except BAKE (LEU)
- *In vitro* Potassium digestibility values were found to be lower (81.6%±4.9%) than previous *in vivo* research; although still very high with no difference among cooking method
- The pH-drop method under-estimated the IVPD for Russet potatoes when compared to more advanced IVPD methods such as the TS and TIM-1
- The values for TIMIVPD found that RAW had the lowest level of digestibility, similar to that of the TS and PHS and all other cooking methods were significantly higher
- Russet potatoes have a high TPD, with the exception of the RAW treatment, which had the lowest TPD, with the highest observed at 84% for BAKE
- All IVPD levels were high 71.8-91.1% for Russet potatoes across all cooking and IVPD methods. The quality of protein relies on the AAS, and BOIL yielded the highest values, with FRIED9M at the low end, indicating that frying, regardless of duration, places a constraint on the protein quality of potatoes
- RAW and FRIED6M saw the lowest PDCAAS values with BOIL, BAKE and MICRO having the highest, respectively
- Potatoes are a relativity cost effective and high yielding plant that can be cooked in a variety of ways and provide substantial vitamins, minerals, carbohydrates, fibre
- Higher PDCAAS values found in BOIL, BAKE, and MICRO (>0.48) saw a reduction in PDCAAS corrected protein, however RAW and FRIED6M with both having PDCAAS values <0.3 saw a greater reduction. Hence the importance of finding complementary food sources to increase the total PDCAAS corrected protein.
- Sulphur AA are limiting in a majority of the mixtures containing potatoes and lentils due to the low levels of protein found in potatoes. The high levels of sulphur AA in a serving

of potatoes is not enough to balance out the sulphur AA in lentil, however it does aid increasing PDCAAS levels, thus contributing to a greater effective protein intake within a given meal.

6.3 Future Direction

6.3.1 *In vitro* and *In vivo* Protein Digestibility

The current research was the first to investigate *in vitro* and *in vivo* protein digestibility of potato protein as a whole food, not in isolated form. This research investigated multiple *in vitro* methods for protein digestibility. However, it was shown that not all *in vitro* methods predict similar digestibility, nor do all correlate to *in vivo* protein digestibility.

The ability to use four different *in vitro* methods allow for comparisons to be drawn between each method and determine which method was best suited for potatoes and possibly other high starch vegetables. Calculating PDCAAS via *in vivo* methods allowed for comparisons to be made in terms of which method of *in vitro* PDCAAS is best for potatoes. The ability for *in vitro* methods to predict *in vivo* protein digestibility in published work is evident, however it is often noted that over or under estimation is common. Finding a method that is cost- and time-effective while producing similar results to *in vivo* data is a major strength in this research. Investigating one method of *in vivo* is a limitation, as there are other methods that have been studied shown to provide more conclusive data such as PER and DIAAS. Future studies could allow for PER and DIAAS to be studied. As the TIM-1 protein digestibility is measured as ileal digestibility, the future inclusion of DIAAS could allow for further verification that the TIM-1 system predicts protein digestibility. This future study could create stronger evidence that *in vivo* methods are not required to establish protein claims. This justification of *in vitro* methods could allow for new novel proteins to enter the marketplace without being withheld due to ethical concerns around animal trials.

6.3.2 Primary Varietal

Russet potatoes are one of the most widely consumed potato varietals in North America, therefore investigating their digestibility in whole form has provided essential knowledge as to their contribution of protein to the human diet. A limitation of only investigating Russet potatoes in this research was that crops were provided from a single farm and field. Thus, the difference in geographical location was not able to be applied. Research has shown that factors such as geographical location play a large role in determining protein quantity of plant products. In order to combat this limitation, future studies with a larger geographical selection would be suggested. Another limitation of selecting only Russet potatoes is that there are other perhaps more commonly used potatoes for in home cooking, such as reds, purple, or baby potatoes. The current

research may not necessarily be applied to other varietals of potatoes, given differences in their carbohydrate and protein content. Future studies could include a wider range of varietals in order to create a better understanding of how each varietal differs in terms of protein quantity and quality.

6.3.3 Protein Concentrates and Whole Food

The impact of food in its whole source is of vital importance as the food matrix is often what needs to be considered regarding diet implications such as glycemic index. Much of the emerging research on novel proteins, such as those derived from potatoes, is studied in its concentrated or isolated form. Comparing concentrates to whole food is not always appropriate, as the processing method to create theses concentrates alters the AA composition and the factors that can influence both nitrogen and AA digestibility. For example, the process of protein isolation removes nonprotein nitrogen from the potato protein. Investigating the impact of the changes in these NPN fractions could aid in understanding the ultimate factors influencing the protein quality of Russet potatoes. These changes can provide differing protein digestibility coefficients, however current research investigating the impact of protein concentration on protein digestibility, in comparison to the whole potato, is limited. Investigating concentrated versus whole forms of potatoes with respect to amino acid content and protein digestibility could further advance our understanding of the importance of potato protein quality for the human diet.

6.3.4 Glucose Response

Potatoes have been classified as a medium to high glycemic index food, depending on cooking method (served cool vs. hot) (33). The importance of having knowledge on the glycemic index in relation to different cooking methods, as well as the GI of different varietals would be vasty important for consumer knowledge.

Glucose response testing would allow for consumers to have a better understanding on the impact cooking has on glycemic index. Using the TIM-1 model to determine GI would be recommended as sample were collected and are being held at the Richardson Centre of Functional Foods and Nutraceuticals. However, due to COVID-19 closures these sample were not able to be ran, and therefore glucose response was removed from this research. Analyzing the current samples would all for a comparison across cooking methods to determine how each method effects GI.

Adding other varietals to be tested would also be beneficial for consumer understanding of which variety of potato is best of those with monitoring GI of foods consumed.

6.4 Conclusion

Overall, Russet potato protein is of high bioavailability when cooked, and the proteins are highly digestible, as determined by both *in vivo* and *in vitro* methods. Through this research it was found that cooking method significantly alters the PDCAAS, as determined with both *in vivo* and *in vitro* protein digestibility methods. Each *in vitro* method provided differing data: When the TIM-1 and TPD were compared, only the RAW treatment yielded protein digestibility values that were significantly higher using the TIM-1. The level and quality of the protein in Russet potatoes does not offer reach a point where a serving of any of the cooked potatoes could be labelled as a "good source" of protein. However, when consumed in a meal with complementary protein sources, potatoes will provide value protein to the diet via complementation, particularly with other plant-based protein sources, including pulses. When protein content is corrected for PDCAAS, an 85 gram serving of baked Russet potato offers 1.09g of protein. In accordance with the Dietary Guidelines for Americans (2020-2025) potatoes are categorized as starchy vegetables; to which 4-8 cups per week should be consumed, depending on caloric intake (114). Combining a serving of potatoes with a serving of peas, beans or lentils (1/2 cup) would provide the essential amino acids required to make a complete protein, as these foods are limiting in sulphur amino acids, and the latter are found in potatoes in excess quantities. Thus, combining these sources will help to provide more quality-corrected protein for the human diet.

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Appendix

Appendix A

Processing of Russet Potatoes

Raw

1. Potatoes were peeled in potato peeler in Elis Pilot Plant using 8L of cold tap water ran over for 2 minutes.
2. Excess peel was removed by hand with the use of a peering knife.
3. Potatoes were cut into 2cm x 2cm cubes.
4. Samples were drained.
5. Drained samples were processed through a meat grinder to achieve uniform sizing.
6. Ground samples placed into trays containing 450-600g of samples.
7. Samples were then frozen and freeze dried.
8. After freeze drying samples were milled using a Retsch Ultra Centrifugal Mill SM 200 with a 0.75mm sieve

Microwave

1. Whole, unpeeled potatoes were poked with a fork.
2. Potatoes placed individually in a microwave (0.7 cubic feet; 700 watts)
3. Cooked for 5 minutes on power level 10.
4. Removed from microwaved.
5. Cooled to room temperature (22°C)
6. Cooled samples cut in half with pith removed.
7. Pith was then mixed in Hobart mixer on speed 1 for 90 seconds to allow for uniform sampling.
8. Samples were then placed into tin foil containers with holes in the lid and froze.
9. After samples were fully frozen, they were then freeze dried.
10. After freeze drying samples were milled using a Retsch Ultra Centrifugal Mill SM 200 with a 0.75mm sieve

Baking

1. Whole, unpeeled potatoes were poked with a fork
2. Potatoes were placed in an oven set to 400°F for 1 hour.
3. Once removed from oven potatoes were allowed to cool for 30 minutes.

4. Once cooled potatoes were cut in half and pith was removed.
5. Pith was then mixed in Hobart mixer on speed 1 for 90 seconds to allow for uniform sampling.
6. Samples were then placed into tin foil containers with holes in the lid and froze.
7. After samples were fully frozen, they were then freeze dried.
8. After freeze drying samples were milled using a Retsch Ultra Centrifugal Mill SM 200 with a 0.75mm sieve

Boiling

1. Whole potatoes were peeled using the potato peeler in the Elis food lab with cool water for 2 minutes.
2. Potatoes were cut into 2cm x 2cm cubes and placed in to boiling water in a 10L steam kettle.
3. Potatoes were boiled for 12 minutes (average time to cook fully, easy to poke with a fork)
4. When done water was drained
5. Potatoes cooled for 20 minutes.
6. Once cooled potatoes were blended in the Hobart mixer at speed 1 for 90 seconds to allow for uniform sampling.
7. Samples were then placed into tin foil containers with holes in the lid and frozen.
8. After samples were fully frozen, they were then freeze dried.
9. After freeze drying samples were milled using a Retsch Ultra Centrifugal Mill SM 200 with a 0.75mm sieve

Frying

1. Whole potatoes were peeled using the potato peeler found in the Elis food lab, with cool water for 2 minutes.
2. Once peeled potatoes were sliced using the fry cutter with the 1cm x 1cm cutter and placed in cool water.
3. Potato slices were removed from water and placed on paper towel and blotted dried for 2 minutes.
4. Once majority of water was removed potatoes were placed in 100% high-oleic canola oil at 375°F.

5. Potatoes were fried for 3 different times; 3, 6 and 9 minutes.
6. Once cooking time was concluded cooked potatoes were removed from oil and excess oil was removed by shaking basket for 30 seconds.
7. Potatoes were placed on paper towel for 2 minutes to allow further removal of oil.
8. Potatoes were cooled for 20 minutes.
9. Once potatoes were cooled, they were mixed in a Hobart mixer at speed 1 for 150 seconds.
10. Samples were then placed into tin foil containers with holes in the lid and froze.
11. After samples were fully frozen, they were then freeze dried.
12. After freeze drying samples were milled using a Retsch Ultra Centrifugal Mill SM 200 with a 0.75mm sieve

Freeze Drying

1. Frozen samples were removed from freezer and placed in freeze dryer for 3-4 days.
    a. Freeze dryer could only hold 20 containers at once, with that this process took 4 weeks to complete.
2. This process was done in The Elis Building at the University of Manitoba with the Genesis Pilot Lyophilizer.
    a. Shelf Temperature Control

Defatting

1. Only done to fried samples.
2. Fat content needed to >10% for amino acid analysis.
3. The was done in the Animal Science Laboratory at the University of Manitoba, using a Soxhlet.

Milling

All samples underwent milling to reduce the particle size. The mill used was a Retsch Ultra Centrifugal Mill ZM 200, 0.75mm sieve.

Appendix B

Methods in detail

Amino Acid Score Calculation using FAO reference pattern from 2007 and 2013.

Amino Acid score[1] of Russet potatoes subjected to different consumer-based cooking methods using the 2007 FOA reference pattern.

	Raw	Microwaved	Boil	Bake	Fried 3 mins	Fried 6 mins	Fried 9 mins
Histidine	0.507	0.448	0.513	0.484	0.362	0.338	0.264
Isoleucine	1.134	0.999	1.140	1.030	0.811	0.839	0.792
Leucine	0.870	0.761	0.910	0.762	0.598	0.614	0.617
Valine	1.326	1.199	1.303	1.318	0.992	0.986	0.933
Lysine	1.171	1.034	1.178	1.051	0.830	0.815	0.734
Threonine	1.221	1.096	1.251	1.142	0.861	0.860	0.867
Tryptophan	1.747	1.485	1.785	1.395	1.346	1.316	1.170
Sulphur Amino Acids	1.433	1.235	1.385	1.305	1.032	1.058	0.898
Aromatic Amino Acids	1.680	1.542	1.695	1.677	1.309	1.327	1.227
Amino Acid Score	0.507	0.448	0.513	0.484	0.362	0.338	0.264

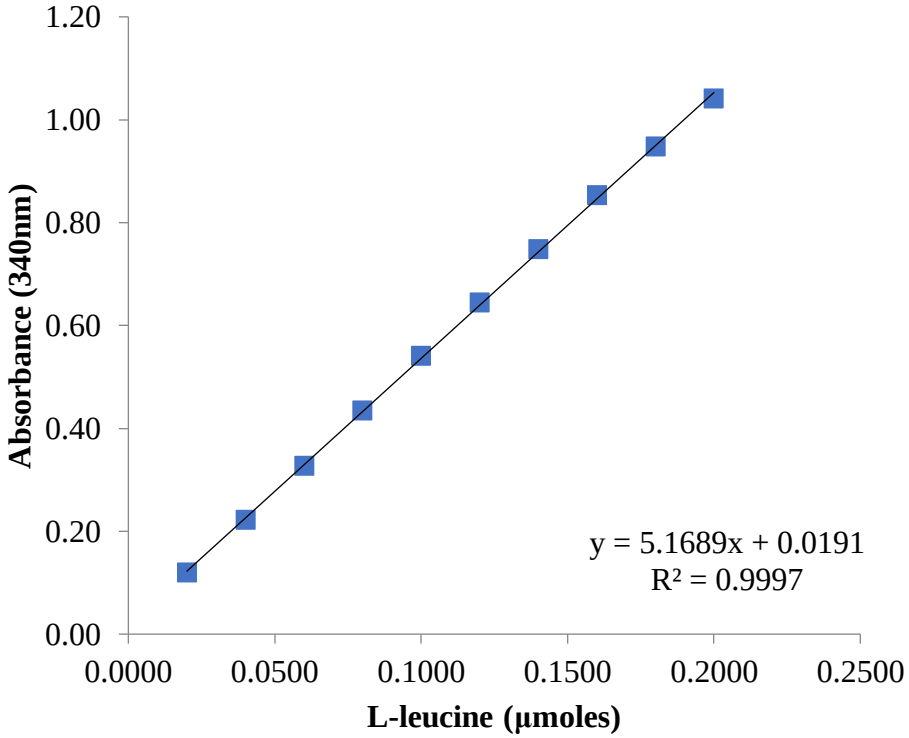
[1]Data are presented as Means, with Standard Errors, when available, presented in brackets (n=3). Amino acid score calculated from the value of the limiting amino acid (highlighted in grey). A score of 1.0 or greater indicates that the amino acid meets or exceeds the established requirement pattern.

Amino Acid score[1] of Russet potatoes subjected to different consumer-based cooking methods using the 2013 FAO reference pattern.

	Raw	Microwaved	Boil	Bake	Fried 3 mins	Fried 6 mins	Fried 9 mins
Histidine	0.704	0.622	0.713	0.672	0.503	0.469	0.380
Isoleucine	0.988	0.870	0.993	0.897	0.706	0.731	0.702
Leucine	0.746	0.653	0.780	0.653	0.513	0.526	0.533
Valine	1.137	1.028	1.117	1.130	0.850	0.845	0.813
Lysine	0.991	0.875	0.997	0.889	0.702	0.689	0.635
Threonine	1.085	0.975	1.112	1.015	0.765	0.765	0.775
Tryptophan	1.417	1.204	1.447	1.131	1.092	1.067	0.986
Sulphur Amino Acids	1.212	1.045	1.172	1.104	0.874	0.895	0.756
Aromatic Amino Acids	1.461	1.341	1.474	1.459	1.139	1.154	1.100
Amino Acid Score	0.704	0.622	0.713	0.653	0.503	0.469	0.380

[1]Data are presented as Means, with Standard Errors, when available, presented in brackets (n=3). Amino acid score calculated from the value of the limiting amino acid (highlighted in grey). A score of 1.0 or greater indicates that the amino acid meets or exceeds the established requirement pattern.

Appendix C



Sample standard curve (n=1) generated for ten dilutions of L-leucine measured for absorbance spectrophotometrically at 340nm after two-minute reaction with OPA reagent

Appendix D

Two-step digestion calculation

Protein target (mg) = 150	**Selection criteria**
Crude Protein (CP) (%) = 85.06	Previously measured in lab
Actual sample weight (mg) = 176.35	Measured
Max protein digested (mg) = 150.00	Actual sample weight x (CP%/100)
Max nitrogen digested = 24	Max protein digested / 6.25
Nitrogen content (µmol N) = 1714.29	Max nitrogen digested / 14 (MW of N) x 1000
Gastric HCl balance (mL) –=0.200	Measured
Intestinal NaOH balance (mL) = 0.700	Measured
Final digest volume (mL) = 40.275	39.375 mL (TSD methods) + additions above
Digest concentration (µmol N / mL) = 42.56	Nitrogen content / final digest volume
[1]Dilution 1 (D1; µmol N / mL) = 36.89	(digestion concentration x 1.3) / 1.5
[2]Dilution 1 (D1; µmol N / mL) = 1.757	(D1 /5) / 4.2
[3]Dilution 1 (D1; µmol N / mL) = 0.342	D2 / (20.5/4)
[4]Dilution 1 (D1; µmol N / mL) = 0.069	D3 / 5
Spec Measurement 1 = 0.31767	Measured
Spec Measurement 2 = 0.30228	Measured
Spec Average = 0.30823	Spec measurement1 + Spec measurement2) / 2
Regression x (Abs340) = 0.308	Spec Average
Regression m = 5.26199	Given/measured; leucine regression
Regression b = 0.00135	Given/measured; leucine regression
Regression y [µmol N] = 0.0588	x + b / m
Initial digest nitrogen (µmol N]) = 0.069	D4
Final digest nitrogen (µmol N]) = 0.0588	Y value from regression
***In vitro* digestibility = 85.22**	(final digest nitrogen / initial digest) x 100

[1]D1 = 200uL TCA in 1.3mL aliquot of final digest volume / 1.5 mL (final volume).

[2]D2 = 200uL of D1 (thus 1/5) in 4.2 mL (final volume; 4mL 6N HCl, hydrolysis step).

[3]D3 = 4.2 mL (above) in addition to 16.3mL (4mL 25% NaOH; 3x 4.1mL MQ water; end of hydrolysis).

[4]D4 = 200uL D3 (thus 1/5) added to cuvette (spectrophotometric analysis).

Appendix E

Absorbance (340nm) values for leucine dilution across all spectrophotometric analysis (n=5)

LEU (µmol)	Min	Max	Mean	STD	CV%
0.2000	1.0179	1.0511	1.0363	0.0154	1.49
0.1800	0.9144	0.9688	0.9401	0.0200	2.13
0.1600	0.8247	0.8693	0.8458	0.0181	2.14
0.1400	0.7438	0.7718	0.7536	0.0108	1.44
0.1200	0.6360	0.6507	0.6438	0.0061	0.95
0.1000	0.4913	0.5448	0.5240	0.0236	4.51
0.0800	0.4014	0.4425	0.4262	0.0162	3.81
0.0600	0.2916	0.3389	0.3206	0.0178	5.56
0.0400	0.1901	0.2330	0.2090	0.0187	8.94
0.0200	0.1068	0.1195	0.1126	0.0054	4.81

LEU= leucine; Min = minimum; Max = maximum; STD = standard deviation; CV% = coefficient of the variation

Appendix F

Detailed Two-Step Digestion Method

- Weigh out 150mg (±5) of protein for the test sample in a 50mL flask with screw cap.
- Add 2.5cm magnetic stirring rod to flask.
- Leave in freezer at -20°C for next day digestion.

Phase 1 – Gastric Digestion

1. Warm up water bath to 39°C.
2. Add 17.75mL of 0.1M pH 6.0 potassium phosphate buffer to the sample flask.
3. Mix briefly at 300 RPM at 40°C (to hydrate sample and reduce clumping).
4. After mixing add 1mL of α-amylase solution containing 1mg of amylase.
5. Add 7.5mL of 0.2M HCl.
6. While actively stirring at 300 RPM and 40°C, balance pH to 2.0 (±0.05) using 1M HCl
7. Add 375µL of 0.5% chloramphenicol solution to each digestion flask.
8. Add 750µL of pepsin solution containing 10mg pepsin.
9. Add flasks to water bath and shake for 6 hours at 150 RPM and 39°C.

Phase 2 – Intestinal Digestion

1. Stop water bath and remove samples (do not turn off).
2. Add 7.5mL of 0.2M pH 6.8 potassium phosphate buffer and 3.75mL of 0.6M NaOH to the sample flask.
3. While actively stirring at 300 RPM and 40°C, balance pH to 7.0 (±0.05) using 1M NaOH.
4. Add 750µL of pancreatin solution containing 50mg pancreatin.
5. Add flasks to water bath and shake for 18 hours at 150 RPM and 39°C.

Phase 3 (next day) – Sample Preparation

1. Prior to removal of samples, load 2mL centrifuge tubes with 200uL of ~56.25% TCA solution.
2. While actively stirring at 300 RPM and 40°C remove 1.3mL from the flask and transfer to the centrifuge tube (final [TCA]: ~7.5%).
3. When all sample aliquots have been transferred to centrifuge tubes, vortex each for approximately 5 seconds.

4. Centrifuge the sample at 17000g for 60 minutes 5. Transfer 750µL of the duplicate aliquots to a single labeled 2mL cryogenic vial and store at -20°C.

Enzymes

Prepared fresh

- Pepsin (13.3mg/mL): 150mg in 11.25mL 0.1M HCl; mixed at 300RPM and 40°C o Pepsin from porcine gastric mucosa (≥250 units/mg solid).
- Pancreatin (66.7mg/mL): 750mg in 11.25mL 0.2M pH 6.8 phosphate buffer; mixed at 300-700RPM and 40°C (Pancreatin from porcine pancreas (4 × USP specifications)).

Solutions

Potassium phosphate monobasic 1M

- Stock solution stored at room temperature o 68g of potassium phosphate monobasic in 432mL MQ water.
- Potassium phosphate dibasic 1M o Stock solution stored at room temperature.
- 87g of potassium phosphate dibasic in 413mL MQ water.

Potassium phosphate buffer 0.1M pH 6.0

- Stock solution stored at room temperature.
- 13.2mL of potassium phosphate dibasic 1M mixed with 86.8mL of potassium phosphate monobasic 1M and 900mL MQ water.

Potassium phosphate buffer 0.2M pH 6.8

- Stock solution stored at room temperature.
- 99.4mL of potassium phosphate dibasic 1M mixed with 100.6mL of potassium phosphate monobasic 1M and 800mL MQ water.

Chloramphenicol 0.5%

- Stock solution stored at room temperature.
- 500mg chloramphenicol solid in 100mL ethanol.

Trichloroacetic acid (TCA) 56.25%

- Made fresh o 5.625g TCA mixed with 4.375mL of MQ water at room temperature.
- Trichloroacetic acid (ACS reagent, ≥99.0%)

0.2M HCl

- Stock solution stored at room temperature.
- 40mL 6M HCl added to 1.16L of MQ water.

0.6M NaOH

- Stock solution stored at room temperature.
- 12g of NaOH solid in 488mL MQ water

Amylase

- 1mL transfer to each flask
- 1mg = 1500 units/mg protein
- 1mg in 15mL 0.1M pH 6.0 potassium phosphate buffer; mixed at 300RPM and 40°C.
- A6380-1G - α-Amylase from Bacillus sp. Type II-A, lyophilized powder, ≥1,500 units/mg protein
-

Regular Amino Acid Hydrolysis

Phase 1

1. Begin warming heating block to 126°C.
2. Thaw (approximately 1 hour) and transfer 200µL of test sample to a hydrolysis tube
3. Add 4mL of 6N HCl and ~20µL of octanol to the tube.
4. Moisten the hydrolysis tube caps in MQ water and briefly remove excess water by tapping on a paper towel.
5. Tie on the cap ¾ of the way, prior to sealing the tube.
6. Vacuum out air via the T cannula for approximately 45 seconds and seal the cap prior to the removal of the vacuum hose.
7. Place samples on heating block at 126°C for 24 hours

Phase 2

1. Add 4mL of 25% NaOH and a 1.5cm magnetic stirring rod to a 50mL beaker.
2. 2. Pour out contents of hydrolysis tube to the 50mL beaker
3. Rinse hydrolysis tube 3 times with 4.1mL of MQ water and transfer to the 50mL beaker
4. Stir samples briefly at 300 RPM.
5. Remove approximately 2-3mL of solution using a 5mL syringe from the beaker.
6. Apply a 0.22µm filter and transfer approximately 1.8mL of the syringe contents to a labeled cryogenic vial.
7. Samples are to be measured via OPA analysis prior to being stored at -20°C.

OPA Analysis (Church et al. 1983)

OPA Reagent

1. Add 25mL of 0.1M sodium tetraborate, 2.5mL of 20% SDS and 21.4mL of MQ water to a 150mL amber flask and stir (2.5cm stirring rod; 150-200 RPM) at room temperature.
2. Add 100µL of β-mercaptoethanol under a fume hood and allow solution to stir again at room temperature with non-translucent stopper.
3. Add 1mL of OPA solution containing 40mg phthaldialdehyde in methanol to flask with stopper.
4. Allow at least 1 hour for solution to stir prior to use.

L-Leucine Standard

1. Add 65.8mg of L-leucine to 50mL of MQ water and mix at room temperature (10mM solution)
2. Add 1mL of the 10mM L-Leucine solution to 9mL of MQ water and mix at room temperature (1mM solution)
3. Label 9 disposable 16x100mm test tubes from 2-10 (1 represents the 1mM L-Leucine solution)
4. From 10-2, pipette increasing volumes of 1mM L-Leucine solution being at 100µL and ending with 900µL (increments of 100µL)
5. From 10-2, pipette increasing volumes of MQ water beginning at 900µL and ending with 100µL (increments of 100µL), with the total volume of each test tube brought to 1mL.

Spectrophotometric Measurements (Agilent G1117AA)

1. Open UV-VIS Chem program and set the following parameters: Fixed wavelength to 340nm in a single box (allow program to turn on appropriate lamps, i.e., UV) and sampling to multicell (8-cell)
2. Load the first cell with a single 1.5mL methacrylate cuvette and pipette 200µL of MQ water, 1mL of OPA solution and mix via pipette 5-10 times.
3. After 2 minutes (from mixing) read the solution by clicking the ‘blank’ button
4. Load each cell with a 1.5mL methacrylate cuvette.

5. From cell 1-8 pipette 200µL of L-leucine standard from least to most concentrated (i.e., From 10-1)
6. Add 1mL of OPA solution to each cell, then follow-up with mixing each cell 5-10 times with the same pipette tip from least to most concentrated.
7. Begin 2-minute timer after mixing the first sample.
8. Click the 'sample' button to begin measuring the first sample after two minutes.
9. To measure next sample, click the cuvette image and click 'sample.'
10. Repeat for remaining L-leucine standards (10-1).
11. Repeat process for measuring samples using 200µL (change pipette tips for mixing different samples).

Solutions

Sodium Tetraborate 0.1M

- Stock solution stored at room temperature.
- 19g sodium tetraborate decahydrate in 481mL of MQ water stirred at 220RPM at 50°C.

Sodium dodecyl sulfate (SDS) 20%

- Stock solution stored at room temperature.
- 10g SDS in 40mL MQ water

Phthaldialdehyde (OPA)

- Made fresh in brown plastic vial.
- 60mg phthaldialdehyde in 1.25mL methanol

Appendix G

Amino acid Analysis Methods

Acid hydrolysis Procedure

50 mg of sample will be mixed with 2 drops 2-octanol and 4mL of 6N phenolic HCl in hydrolysis tubes under vacuum the sample will be degassed, sealed and incubated in an oven for 24hrs at 110□C. After 24hrs samples are to be neutralized with 4 mL of 25% (m/v) NaOH, then transferred to a 50 mL volumetric flask; then brought to volume with Milli-Q water. From there an aliquot was syringe filtered with a 0.22-micron filter and stored at -20°C prior to Ultra-Performance Liquid Chromatography.

Oxidized Hydrolysis Procedure

This method for specifically for methionine and cysteine, the method is as follows. 50 mg of sample is mixed with 2 drops of 2-octanol and 2 mL of fresh performic acid (9:1 mixture of phenolic formic acid (88%) and hydrogen peroxide (35%)). Samples are incubated at 4°C for 18 hrs. Once oxidation is complete, 0.35mg of sodium metabisulfite will be added and mixed repeatedly for 2 hours. After this time 2 mL of concentrated HCl is added, from there tubes are incubated for 16 hours at 110°C. After 16hrs samples are to be neutralized with 4 mL of 25% (m/v) NaOH, then transferred to a 50 mL volumetric flask; then brought to volume with deionized water. From there an aliquot will be syringe filtered with a 0.22-micron filter and stored at -20°C prior to Ultra-Performance Liquid Chromatography.

Alkaline Hydrolysis

The tryptophan contents of the samples are to be determined via HPLC with fluorescence detection, using ISO 13904 protocol (99). 50 mg of sample is to be placed into 125 mL polypropylene flasks and mixed with 8.4 mg of barium hydroxide octahydrate and 14 mL of water. The flasks will be autoclaved for 20 hours at 110°C. Following autoclaving, 30 mL of water and 1 mL of a concentrated internal standard (alpha methyltryptophan), 5 mL of orthophosphoric acid is added and the samples brought to a pH of 3-3.2 with 6M HCI. Lastly, 20 mL of methanol is added, and the sample will be brought to volume in a 100 ml volumetric flask. An aliquot was syringe filtered with a 0.22-micron filter and stored at -20°C prior to High-Performance Liquid Chromatography.

Ultra-Performance Liquid Chromatography of Regular and Oxidized Amino Acids

Following AOAC protocol Ultra-Performance Liquid Chromatography was utilized to determine individual amino acids in each sample (98). In polypropylene 300µL limited volume vials, 70 µL of AccQ-Tag borate buffer, followed by 10µL of each sample and finally 20µL of AccQ-Tag reagent. After vortexing samples are placed on derivatization block for 10 minutes, vials are derivatized 4 at a time. Once all samples are derivatized they were placed in the autosampler in installed on the Ultra-Performance Liquid Chromatography (UPLC). Sufficient amounts of Buffer A (dilution of AccQ-Tag Ultra Eluent A 20 times) and Buffer B (2% formic acid in acetonitrile) both are filtered through 22µm filters. Samples are loaded onto the UPLC with a flow rate of 0.7mL/minutes for regular and 0.4L/min for oxidized and 0.1%B, oven temperature was 51°C for regular, 40°C for Cysteine assay, and 60°C for methionine assay. The detection by UV at 260nm and a run time of 17 minutes for regular amino acids. Detection by fluorescence with excitation at 266 nm, and emission at 473 nm and a run time of 30 min for oxidized amino acids. Results were calculated as amino acid g/100g.

High-Performance Liquid Chromatography for Alkaline Amino Acid

For Alkaline amino acids the buffer contained 2.86mL glacial acetic acid and 50mL 1.0% 1,1,1-trichloro-2-methyl-2-propanol in methanol with 900mL of deionized water, pH to 5.0 with ethanolamine, volume brought to 1000mL in a graduated cylinder, it was then filtered through a 0.22µm filter. The High-Performance Liquid Chromatography (HPLC) was prepared with a fluorescence detector with a flow rate of 1.0mL/minute, excitation at 280nm, emission at 356nm at a temperature of 28°C with a run time of 34 minutes.

Appendix H

pH-Stat Method Prepared by Adam J. Franczyk

Sample Solution

1. Weigh 1 mg±0.05mg of nitrogen for each mL of solution.
2. Add 1 mL of milliQ water for each mg N and a magnetic stir bar.
3. Eliminate direct heat from hotplate on digest solution.
4. Heat to 37°C±1°C and stir at low to moderate RPM (100-300)
5. Allow test sample to solubilize; approximately 1 hour.
6. Bring to pH 8.00±0.05 using 0.1-1N NaOH or HCL.
    - Record the initial pH and the amount used to pH to 8.0; can be useful when determining sources of error, preparing duplicates/triplicates and/or similar samples.

Enzyme Solution

1. Freshly weigh 1.61mg trypsin, 3.96mg chymotrypsin and 2.36mg peptidase4 for each mL of solution
    a. Separately, on weigh paper, combine to single beaker.
2. Add 1 mL of milliQ water for a 1:1 ratio of the above enzymes and a magnetic stir bar.
    a. E.g., 16.1 mg trypsin, 39.6mg chymotrypsin and 23.6mg peptidase in 10mL milliQ water
3. Eliminate direct heat from hotplate on enzyme solution.
4. Heat to 37°C±1°C and stir at low to moderate RPM (100-300)
5. Allow solution to solubilize; approximately 10 minutes.
6. Bring to pH 8.00±0.05 using 0.1-1N NaOH or HCl.
    a. Record the initial pH and the amount used to pH to 8.0; can be useful when determining sources of error.
    b. Only balance solution pH once
7. Transfer solution to an ice bath, keep solution between 0-4°C.

Reaction Solution

1. Record sample solution pH (should be 8.00±0.05)

2. Immediately add 1mL of enzyme solution to sample solution (now the reaction solution)
3. Add and record the amount of 0.1N NaOH (μL) to maintain the pH at 7.98 for 10 minutes.
    - This is the "Σ 0.1N NaOH" (i.e., not the initial 0.1 – 1M NaOH used to bring the solution to 8.00±0.05)

Calculation

Amount of sample to weigh (mg) = 62.5 / (sample protein % / 100) in 10mL

All proteins = 76.14 + 47.77 x (Σ 0.1N NaOH)

Appendix I

In vivo True Fecal Protein Digestibility

Protein Digestibility	Mean	STD	CV%
Casein	93.57	1.43	1.52
RAW	40.49	11.74	29.01
BOIL	82.96	1.94	2.33
MICRO	82.85	1.78	2.15
BAKE	84.54	1.45	1.72
FRIED6M	80.06	4.77	5.96

Protein digestibility (n=10)

Appendix J

In Vivo True Fecal Protein Digestibility of Russet Potatoes

Protein Digestibility	Mean	SEM	N
RAW	40.49	3.92	10
BOIL	82.85	0.59	10
MICRO	84.54	0.49	10
BAKE	82.96	0.65	10
FRIED6M	80.06	1.59	10

Appendix K

In vivo True Fecal Amino Acid Score of Russet potatoes

	THR	VAL	M+C	ILE	LEU	P+T	HIS	LYS	TRP	AAS	PDCAAS
Casein	1.164	1.743	1.131	1.729	1.319	1.533	1.344	1.232	1.030	1.030	**0.964**
RAW	0.731	1.154	1.094	0.943	0.613	0.956	0.590	0.778	0.869	0.590	**0.239**
BOIL	0.749	1.133	1.057	0.948	0.641	0.963	0.597	0.783	0.887	0.597	**0.495**
MICRO	0.657	1.044	0.944	0.831	0.538	0.877	0.521	0.688	0.740	0.521	**0.432**
BAKE	0.684	1.147	0.997	0.857	0.538	0.954	0.562	0.699	0.697	0.538	**0.455**
FRIED6M	0.419	0.698	0.658	0.568	0.352	0.614	0.319	0.441	0.532	0.319	**0.256**

Shaded cells represent limiting amino acid

THR = Threonine VAL = Valine M+C = Methionine & Cystine ILE = Isoleucine LEU = Leucine P+T = Phenylalanine & Tyrosine HIS = Histidine LYS = Lysine TRP =Tryptophan AAS = Amino Acid Score PDCAAS = Protein Digestibility-Corrected Amino Acid Score