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A Study of Cooking and Varietal Effects on Potato in Vitro Bile Acid Binding Capacity

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A STUDY OF COOKING AND VARIETAL EFFECTS ON POTATO IN VITRO BILE
ACID BINDING CAPACITY

By

Emily A. Hinkle

A Thesis Submitted in Partial Fulfillment
of the Requirements for a Degree with Honors
(Food Science and Human Nutrition)

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Abstract

Potatoes have received negative press about being unhealthy due to having high starch content, but these vegetables contain many healthful components. Many compounds in food, such as soluble dietary fiber, help reduce serum cholesterol levels by binding to bile acids in the digestive tract and causing the body to draw from serum cholesterol to create new bile acids. Potatoes were prepared three different ways (raw, steamed, steamed then cooled) and different varieties of potatoes were used, each with different chemical compositions (King Harry, Elba, Yukon Gold and All-Blue). The potatoes were subjected to an *in vitro* digestion to simulate the human body's natural digestion. After digestion a bile acid binding procedure was applied to the potatoes and the absorbance of each sample was read with a spectrophotometer. Results were expressed as amount of bile acids bound and percent bound relative to cholestyramine for each potato sample. It was observed that cooling potatoes after steaming significantly increased bile acid binding ability of the potato. The amount bound for All-Blue and Elba potatoes was significantly higher than Yukon Gold and King Harry potatoes. These findings can help UMaine secure additional research funding and aid the Maine potato industry in promoting potatoes as a healthful food.

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Manuscript

Introduction

Potatoes (*Solanum tuberosum* L) have gained a negative reputation due to being considered a high starch food¹, but there are numerous health improving components within potatoes². Potatoes have been associated with being high in calories, but due to their low fat content they have a lower energy density comparable to legumes². According to a review by Camire et al. (2009), potatoes, including the skin, contain many vitamins and minerals such as vitamin C, calcium, potassium, iron and B vitamins². Potatoes also contain numerous phytochemicals, which are nutrients that protect against diseases, such as cancer and heart disease³. Another important class of health-promoting compounds in potatoes is dietary fiber, including resistant starch². Both have numerous health benefits that can decrease one's risk for heart disease. The main focus of this study is to assess the ability of potatoes to potentially lower serum cholesterol risks via bile acid binding.

Potatoes receive a lot of negative press since they are a high glycemic index food. A study done by Halton et al. (2006) stated that potatoes are associated with increased risk of type 2 diabetes¹. Unfortunately, the data provided by this research was only from mailed-in questionnaires. These questionnaires asked women how often they ate potatoes and French fries. Based on the report of the study, there were no questions asked regarding butter or margarine consumed with the potato, and no questions on the type of

fries consumed, such as homemade vs. store bought. Healthful food preparation techniques for cooking potatoes were not probed. While the study reported a statistical significant relationship between potatoes and French fries with type 2 diabetes risk, there was no direct evidence proving the potatoes were the cause of the disease. Studies, like the one by Halton and co-workers, give potatoes a negative reputation despite the health benefits they provide. Thus, it is important to continue studies promoting the health benefits of potatoes.

Background Research

Dietary Fiber:

As defined by the Institute of Medicine dietary fiber (DF) is defined as “carbohydrates and lignin that are intrinsic and intact in plants and that are not digested and absorbed in the small intestines”⁴. The AACC defines dietary fiber as “the edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestines”⁵.

Dietary fiber can be soluble, which can generally be fermented in the colon by bacteria, or insoluble, which has a bulking effect but may not ferment in the colon. Dietary fiber has numerous health benefits making it a key component in plant foods, and an important part of the human diet. Increased levels of dietary fiber have been linked with lower incidences of chronic heart disease, stroke and peripheral vascular disease⁴. In addition to cardiovascular benefits, foods high in dietary fiber can provide satiety better than plant foods without fiber⁶. Improved satiety can decrease consumption of

calories and prevent weight gain. Dietary fiber also benefits the gastrointestinal tract by improving fecal bulking and promoting regularity⁶. It is hypothesized that DF can help prevent the polyps in the colon that lead to colon cancer. DF might prevent polyps by reducing the amount of time carcinogens and procarcinogens are in the colon⁶. Dietary fiber also binds to bile acids in the intestinal tract, which will be discussed later.

Resistant Starch:

Resistant starch (RS) is considered a type of fiber as it is detected during the AOAC International total dietary fiber (TDF) analysis⁷. RS is nutritionally and physiologically similar to other kinds of fiber. Resistant starch is a starch or a fraction of starch that is not digested by amylolytic enzymes in the digestive tract. RS can be resistant to digestion due to being physically inaccessible, retrograded, or chemically modified. There are multiple types of RS: RS1, RS2, RS3 and RS4. RS1 is tightly bound starch molecules that are physically inaccessible to the digestive enzymes due to a fiber shell around it⁷. An example of a RS1 is the intact native starch granules of raw grains. RS1 can only be digested if properly milled prior to ingestion. RS2 is an ungelatinized starch granule that is inaccessible to digestive enzymes due to its compact structure⁷. RS2 can be completely digested if cooked or processed. RS3 is retrograded or recrystallized starch that develops when cooked foods are cooled⁷. RS4 is chemically modified to resist digestion by esterification or by cross-linking⁷. Similar to other types of dietary fiber, resistant starch prevents colon cancer, increases fecal bulking and lowers one's risk for heart disease⁷. Also, like dietary fiber, resistant starch binds to bile acids and prevents their re-absorption, thus removing them from the body⁷.

Bile Acid Binding:

In this study, the focus is the role of resistant starch, dietary fiber and other plant compounds in bile acid binding. Figure 1 displays the normal circulation of bile acids in the body. During a meal the bile acids are expelled from the gall bladder into the small intestines. Some bile acids are reabsorbed in the duodenum, but 95% are reabsorbed in the ileum⁸. Between 0.2 and 0.6 grams of bile acids are lost each day in the feces; the liver replenishes these losses⁸. The binding of bile acids in the intestinal tract hinders the formation of micelles. By preventing the formation of micelles, the bile acids are excreted from the intestinal tract and the bile acid stores are diminished⁶. After the consumption of high bile acid binding food components like resistant starch and dietary fiber, the amount of bile acids lost in the feces increases. Approximately 50% of bile acids are excreted causing the bile acid pool to decrease⁸. The decrease in the bile acid pool in the gall bladder signals the liver to increase bile acid synthesis from serum low-density lipoprotein (LDL) cholesterol. The LDL cholesterol is removed from the blood stream and used to create new bile acids, causing serum cholesterol levels to drop. Thus, the excretion of bile acids leads to the lowering of serum LDL cholesterol⁶.

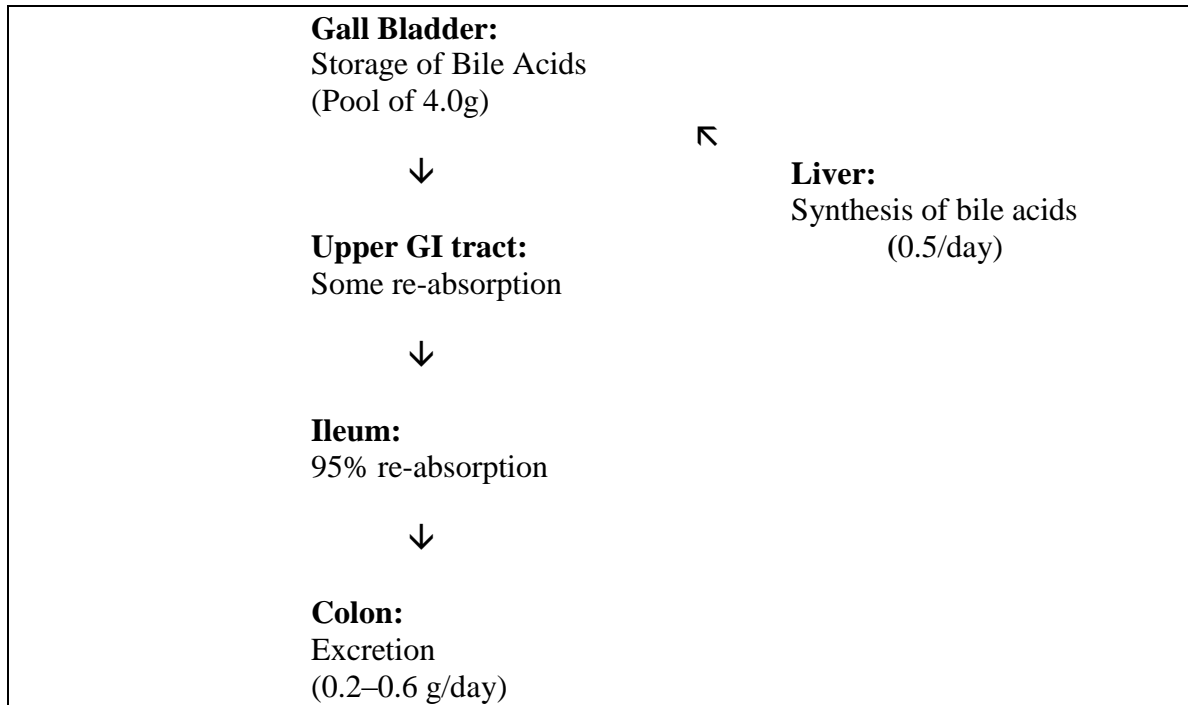


Figure 1: Diagram of the normal circulation of bile acids in the digestive system⁷.

A high serum level of low-density lipoprotein cholesterol is a risk factor for coronary heart disease. Coronary heart disease is a major cause of death in the United States⁹. Hypercholesteremia, also known as high cholesterol, is a risk factor for arteriosclerosis, commonly referred to as hardening of arteries¹⁰. By lowering serum cholesterol, the bile acid binding by resistant starch and dietary fiber helps reduce the risk of coronary heart disease. Currently the drug cholestyramine is used to lower serum LDL cholesterol. Like dietary fiber and resistant starch, cholestyramine binds to bile acids and removes them from the body. Cholestyramine is a positively-charged anion exchange resin and bile acids are negatively-charged anions. The opposing charges cause the bile acids to bind to cholestyramine¹¹. Cholestyramine causes bile acids to be excreted, decreasing the bile acid pool and increasing bile acid synthesis.

Several studies have confirmed the bile acid binding ability of potatoes and the cholesterol lowering benefits. A study by Hashimoto et al. (2006) found that the amount of resistant starch in potato pulp is enough to impact serum cholesterol levels¹⁰. Potato pulp, a by-product of purifying potato compounds, was given to rats in their diet. Cornstarch was given to the control group¹⁰. Serum cholesterol levels were significantly lower for the rats consuming the potato pulp. A study by Camire et al. (1993) provided evidence that potato peels contain enough dietary fiber and resistant starch to result in bile acid binding¹². A study by Ebihara et al. (1998) concluded that rats fed potato starch excreted significantly more bile acids in their feces than did the control rats¹³. A study by Robert et al. (2008) reported that rats fed potatoes had significantly lower cholesterol compared with the control rats that were fed sucrose¹⁴. The rats that consumed potatoes had 37% less cholesterol than the test rats. A study by Kanazawa et al. (2008) reported increased fecal bile acid excretion in rats fed potato starch¹⁵. These studies suggest that the resistant starch and dietary fiber content in potatoes is enough to cause bile acid binding and lower serum cholesterol.

Effects of Potato Preparation:

Various preparation methods can alter the composition of resistant starch in potatoes. When cooking potatoes the resistant starch will change from little digestibility to totally digestible. Raw potato starch granules (RS2) are in a crystalline structure composed largely of amylose, and they have a large surface area preventing digestion by digestive enzymes, such as amylase¹⁶. Raw potato starch is tightly packed in a radial pattern that limits accessibility to digestive enzymes. After heat processing, raw potato

starch becomes more available for digestion, but it still has a slow rate of digestion resulting in only a partial digestion. Cooked and then cooled potatoes are considered RS3 potatoes. RS3 is retrograded amylose that recrystallizes during cooling of gelatinized potato starch. Potatoes are commonly consumed steamed, or steamed and then cooled for dishes, such as potato salad. Evidence from a study by Yadav et al. (2009) showed how heating and cooling cycles impact resistant starch concentration in potatoes¹⁶. Tubers did not differ significantly in resistant starch content between cycles of repeated heating and cooling. However, compared to the control raw tubers the first cooling cycle greatly increased the resistant starch content¹⁷. Similar findings were found in a study by Gormley and Walshe (1999) that looked into the effects of cooling on the levels of enzyme-resistant potato starch¹⁷. Gormley and Walshe found that potatoes that were heated then cooled had more resistant starch than did raw potatoes. According to a review by Sharma et al. (2008), thermal processes have been found to increase resistant starch in potatoes⁷. The thermal effect on increasing resistant starch is due to the effects on gelatinization and retrogradation of the resistant starch⁷. While it is concluded that both steaming and steaming then cooling potatoes increases resistant starch, few studies compare the two methods¹⁶.

Fiber composition in potatoes changes with different processing. During wet thermal treatment the cell membrane is broken down and contents are exposed to the processing water. Higher temperatures may break weak polysaccharide chains as well as glycosidic linkages in dietary fiber polysaccharides increasing the solubility of fiber¹⁸. During thermal reactions, plant cell walls are altered making fiber, a cell wall component,

more soluble. The solubility of the fiber increases after the thermal reaction due to the increased ability of water molecules to reach the dietary fiber¹⁹. Little is concluded about the solubility of dietary fiber in cooled foods after being heated.

Phytochemicals and Potatoes:

Phytochemicals are secondary products of plant metabolism used to protect the plant against physical damage, such as bruising, and protection from various pests, such as insects³. Some phytochemicals have antioxidant properties, which can decrease risk of chronic diseases and cancers¹⁵. Antioxidants interfere with oxidation by breaking the chain reaction or scavenging for highly reactive free radicals²⁰. Common phytochemicals in potatoes are phenolics acids, flavonoids, carotenoids and folates³.

Phenolic acids are the most abundant antioxidant class and neutralize free radicals²⁰. There are currently over 8,000 phenolics identified. The phenolics content of a potato ranges from 530 to 1770 µg/g, and phenolics are in both the skin and the flesh of potatoes, but in lower concentrations in the flesh³. A study by Lewis et al. (1998), reported that purple-skinned potatoes contain twice the concentration of phenolics than do white-skinned potatoes²¹. The most common phenolics in potatoes are chlorogenic acid, protocatechic acid, vanillic acid, and p-coumaric acid³. Chlorogenic acid is the most abundant phenolic acid in potatoes³. Potatoes have been considered the third most important source of phenolics in all food, after apples and oranges³.

Flavonoids, another class of phytochemicals, in potatoes ranges from 200 to 300

$\mu\text{g}/\text{g}$ ³. The common flavonoids in potatoes are anthocyanins, catechin, epicatechin, erodictyol, kaempferol and naringenin³. Flavonoids are found more predominantly in purple potatoes compared to white. There is a substantial amount of anthocyanins (a subgroup of flavonoids with antioxidant properties) in potatoes, ranging from 5.5 - 35mg/100 g³. Anthocyanins provides red, blue and purple pigmentation to foods. Anthocyanins have been linked with lower LDL-cholesterol in a human study by Qin et al. (2009)²². Purple potatoes containing anthocyanins may help lower cholesterol, but it is uncertain if this is due to increased bile acid binding.

Carotenoids are phytochemicals that act as antioxidants²³. The major carotenoids in potatoes are lutein, zeaxanthin, violaxanthin and neoxanthin³. β -carotene, a carotenoid, is present in potatoes but only in small amounts. The yellow color in yellow potatoes is mostly due to the zeaxanthin and lutein. Thus, potatoes with yellow flesh contain a higher concentration of carotenoids. The carotenoid content ranges from 50–350 $\mu\text{g}/100\text{ g}$ and 800–2000 $\mu\text{g}/100\text{ g}$ depending on the variety of potato³. In table 1, there is a comparison of the potato flesh color and amount of various phytochemicals (modified from a table in “Beneficial phytochemicals in potato” by Ezekiel, R., Singh, N., Sharma, S., and Kaur, A.)³.

Potato flesh color	Total Phenolics ($\mu\text{g/g}$)^a	Anthocyanin Content ($\text{mg}/100\text{ g FW}^c$)	Chlorogenic Acid ($\text{mg}/100\text{ g DM}^d$)	Total Carotenoids ($\text{mg}/100\text{ g FW}$)
Purple Flesh	-	368	3.8-32.7	-
White Flesh	369.1-527.2	-	2.8-22.8	50-100
Yellow Flesh	237.7-407.0	-	12.6-63.9	100-350

^aThe - represents unreported results

^b Modified from a table in “Beneficial phytochemicals in potato” by Ezekiel, R., Singh, N., Sharma, S., and Kaur, A.

^cFW= Formula Weight

^dDM= Dry Matter

Potatoes do not contain a substantial amount of folate. The folate content of a potato is 12 to 37 $\mu\text{g}/100\text{ g}^3$. Yellow potatoes have been reported to have a larger amount of folates compared to other varieties³. Folate deficiency is one of the leading causes of birth defects. Folate deficiency is also associated with some cancers, cardiovascular diseases and anemia²⁴. Despite not having a dense amount of folate, compared to other phytochemicals, potatoes are still an important source.

Potato preparation can impact the phytochemical content of a potato³. In a study by Mattila and Hellsrom (2007), there was a higher content of phenolics in unpeeled and cooked potatoes (92% soluble phenolic acid) compared to peeled and cooked potatoes (71% soluble phenolics)²⁵. This is due to the migration of phenolics from the peel into the internal tissues of the potato. Anthocyanin, carotenoid and folate content were not significantly changed in potatoes after cooking³. Potato skin has a higher content of chlorogenic acid than the flesh; so peeling decreases the amount of chlorogenic acid in

the potato²¹. Overall, when looking to retain phytochemical content in a cooked potato it is important to cook potatoes with the skin on³.

Potatoes and Maine:

Potatoes have been associated with Maine longer than they have been with Idaho or any other state²⁶. The cool, damp climate of Maine is ideal for growing potatoes. In 2012, potatoes brought in more cash receipts than any other agricultural product in Maine²⁴. As of 2012, 68% of potatoes were sold for processing, such as potato chips or French fries, 20% of potatoes were sold as seeds, and 12% were sold for tablestock, such as in homes or restaurants²⁶. Maine potatoes are distributed to over 20 other states. However, Maine potato production has dropped from around 70 thousand acres in 1998 to around 55 thousand acres in 2012²⁶. Research demonstrating that potatoes could decrease cholesterol might aid farmers who could use the results to promote Maine potatoes.

Potato Variety Components:

Hundreds of potato cultivars exist around the world. The potato's texture description is determined by two factors, starch ratio and solids content. The potatoes with more amylopectin hold firmly together when cooked, while potatoes with more amylose tend to become mealy when cooked. A study by Hallstrom et al. (2011) concluded that wheat with a higher content of amylose increases resistant starch formation in bread²⁷. This relationship of higher amylose to increased resistant starch could be a factor in the bile acid binding of potatoes. The solids content is the potato's

density compared to the density of water. Potatoes with fewer solids tend to be moist, while potatoes with higher solids tend to be dry²⁷. There are no current studies relating potato solid content to bile acid binding ability.

Conclusion of Introduction:

In conclusion, this study will assess the bile acid binding of potatoes to show their potential health benefits. Identifying which cooking method of potatoes produces the most bile acid binding could help Mainers determine which method of cooking promotes the most lowering of cholesterol. Four different varieties of potatoes will be tested to determine the impact of potato variety composition on bile acid binding. There are two research hypotheses for this paper: (1) the type of potato variety will have an effect on the bile acid binding of the potato and (2) the cooking method used will affect the bile acid binding of the potato.

Materials and Methods

Potatoes:

King Harry (white flesh), Elba (white flesh), Yukon Gold (yellow flesh) and All Blue (purple skin and flesh) potatoes from Wood Prairie Farm (Woodville, ME) were used as samples for this study. Table 2 provides the texture description and best preparation method for each potato²⁸.

<i>Potatoes</i>	<i>All-Blue</i>	<i>Elba King Harry</i>	<i>Yukon Gold</i>
<i>Texture Description</i>	Soft moist	Waxy mid-dry	Firm dry
<i>Starch Ratio</i>	Medium amylose-amylopectin	Medium amylose-amylopectin	High amylopectin
<i>Solids Content</i>	Low solids	Medium solids	High solids
<i>Best preparation method</i>	Steamed Au Gratin Sautéed	All purpose Baked Boiled Salads	Baked Boiled Fried

Potato Preparation:

Approximately 20-25 pounds of each type of potato were purchased from Wood Prairie Farm. Nine potatoes from each variety were selected and washed thoroughly with water to remove any debris. Three potatoes of each variety were placed in a Magic Bullet (Model MB1001C, China) blender until well blended, approximately 10 seconds. These potatoes were blended uncooked and were referred to as the “Raw” potato samples. The potatoes were blended with their skins on, because the skins hold most of the phytochemicals within the potatoes. Six potatoes of each variety were steamed whole in a Cleveland Steam Cub Steamer (Model 1SCE, Cleveland, OH). By steaming the potatoes whole, they held the heat longer and lost less phytochemicals through leaching. The potatoes were steamed for 45 minutes at 200°C at the same time. The Cleveland steamer instructions suggestion steaming potatoes from 30-35 minutes, however after testing the potatoes for that time, the temperature had not reached 200°C. After further temperature tests, it was concluded that 45 minutes was the appropriate steaming time for whole potatoes. Once steamed, three potatoes of each variety were placed into a TRUE refrigerator (St. Louis, MO) to be cooled for 24 hours and were referred to as the “cool”

or “cooled” potato sample. The last three potatoes of each variety were quickly (within 10 minutes) blended and underwent the digestion phase while still hot. These potatoes were referred at the “steam” or “steamed” potato samples. Each variety of potato was blended separately and immediately weighed prior to blending. Figure 2 displays a flow chart of the potato preparation steps.

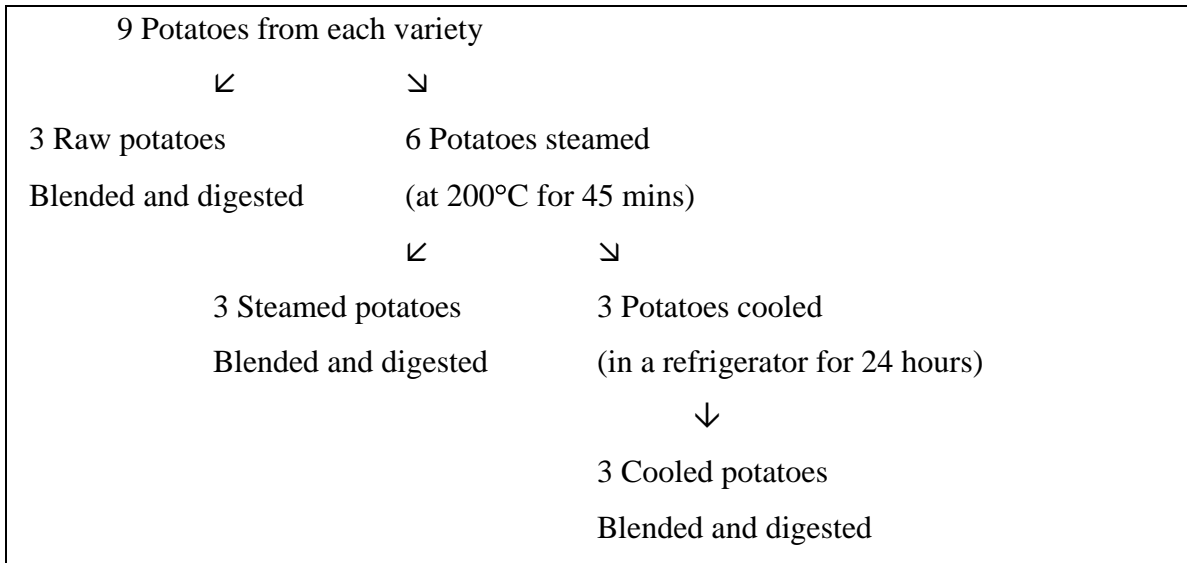


Figure 2: Flow chart of the potato preparation

The potatoes were measured to approximately (± 0.1 mg) 400 mg using a Sartorius GMBH scale (Gottingen, Germany). The protocol suggested 100 mg dry matter and due to the moisture of the potatoes, 400 mg (100 mg DM @ 23% solids content \approx 400mg) was necessary to achieve the appropriate amount. The moisture was calculated by collecting samples into aluminum dishes and drying them in a Fisher Isotemp forced air oven (model 350, Fair Lawn, NJ). Samples were weighed before and after drying, and percent moisture was calculated. The moisture content of each variety of potato can be found in Table 3. The approximately 400mg samples were placed into 50mL centrifuge

tubes. Each variety had three tubes containing samples that will receive bile acids and one tube with a sample as the negative blank, which would receive buffer instead of bile acids.

Table 3: Calculations for Percent Moisture and Dry Matter of Potato Samples

<i>Sample ID</i>	<i>Sample Wt (mg)</i>	<i>Tare + Sample (mg)</i>	<i>Dry wt (mg)</i>	<i>% Moisture</i>	<i>% Dry Matter</i>
Blue Cool	11.37	11.96	0.60	78.99	21.01
Blue Cool	7.50	8.08	0.58	78.29	21.71
Blue Raw	7.84	8.40	0.57	78.53	21.47
Blue Raw	7.22	7.77	0.55	79.05	20.95
Blue Steam	10.11	10.66	0.55	78.07	21.93
Blue Steam	9.83	10.37	0.54	78.24	21.76
Elba Cool	10.11	10.62	0.51	80.71	19.29
Elba Cool	10.37	10.93	0.56	80.15	19.85
Elba Raw	10.88	11.47	0.59	78.84	21.16
Elba Raw	11.31	11.84	0.53	78.91	21.09
Elba Steam	11.14	11.62	0.49	81.09	18.91
Elba Steam	7.43	7.94	0.51	81.16	18.84
Gold Cool	10.69	11.31	0.62	75.92	24.08
Gold Cool	7.56	8.19	0.63	75.45	24.55
Gold Raw	9.92	10.53	0.61	78.00	22.00
Gold Raw	9.52	10.04	0.53	78.41	21.59
Gold Steam	10.13	10.75	0.63	74.12	25.88
Gold Steam	10.89	11.57	0.68	73.90	26.10
Harry Cool	7.59	8.23	0.64	75.88	24.12
Harry Cool	7.47	8.06	0.59	76.02	23.98
Harry Raw	9.46	10.09	0.64	73.09	26.91
Harry Raw	10.39	11.12	0.72	73.91	26.09
Harry Steam	9.55	10.30	0.75	75.21	24.79
Harry Steam	10.50	11.12	0.63	74.96	25.04
Average				77.37	22.63

In addition to the tubes with samples, two 50 mL centrifuge tubes were labeled as reagent blanks but contained no samples. Another 50 mL centrifuge tube contained the reagent positive blank, with 1 mL of 2.88 μ M/L bile acid mixture. This bile acid mixture

was made by diluting the 36 mM/L solution 1:12.5 with pH 6.3 phosphate buffer. Into four centrifuge tubes, two samples of cholestyramine and cellulose were included in the process. Cholestyramine (MP Biomedicals, LLC, Cholestyramine Resin 5mg, Solon, OH) was used as the positive bile acid binding control, while cellulose (SIGMA α -cellulose, C-8002, St. Louis, MO) served as a negative bile acid binding control because it is a poor binding agent as indicated by the protocol used by Camire et al. (2003)²⁹. The potato samples were found as a percent bound relative of cholestyramine, with cholestyramine's amount bound set as 100%. Cellulose is a negative control and should bind little bile acid. The potatoes' bile acid binding was also calculated as a percent of cholestyramine's bile acid binding, with cholestyramine's binding set as 100%. Two 50 mL centrifuge tubes each had 25 mg of cholestyramine and two 50 mL centrifuge tubes each had 26 mg of cellulose²⁹.

Digestion:

To every centrifuge tube, 1 mL 0.01 N HCl was added. Each tube was vortexed with a Fisher Scientific Touch Mixer (Model 232) and placed into a 37°C Julabo LABORTECHNIK (Model GMBH, Seelbach, Germany) shaking water bath for 1 hour²⁹. The addition of the hydrochloric acid and the incubation at body temperature mimics the stomach phase of digestion. Following the stomach phase is the intestinal phase. All tubes were removed from the water bath and 0.1 mL of 0.1 N NaOH was added to neutralize the pH to 6.3²⁹. After neutralizing the pH, 4 mL of bile acid solution was added to three of the four test tubes of each potato sample (for each variety and cooking method): one of the reagent blank tubes, one of the cholestyramine tubes, and one of the

cellulose tubes. The bile acid solution was formulated from multiple bile acids used by Kahlon and co-workers (2008)³⁰. Nine mM/mL of each bile acid (SIGMA-ALDRICH, CBA-1KT, St. Louis, MO) was combined to create the 36 mM/L mixture. Due to the varying formula weights, a different amount of each bile acid was added to 5.8 mL of buffer and combined to form the 36 mM/L mixture (Table 4). The 36 mM/L mixture was diluted 1:50 with 6.3 phosphate buffer to create a 0.72 μ M/mL bile mixture, which was added to the appropriate test tube.

Table 4: Bile acids and amounts in 36mM/L bile acid mixture.

<i>Bile Acid CBA-1KT</i>	<i>Sigma</i>	<i>Formula Weight (mg)</i>	<i>mM/mL</i>	<i>Amount added to 5.8mL of buffer</i>
Sodium glycocholate	G7132	487.60	9	25.5 mg
Sodium glycochenodeoxycholate	G0759	471.61	9	24.6 mg
Sodium glycodeoxycholate	G9910 G2878	471.60	9	24.6 mg
Sodium taurocholate	T4009	537.68	3	9.4 mg
Sodium taurochendeoxycholate	T6260	521.69	3	9.1 mg
Sodium taurodeoxycholate	T0875	521.69	3	9.1 mg

The test tubes that did not receive the bile acids instead received 4 mL of phosphate buffer. The phosphate buffer was made from 14.2g sodium phosphate monobasic, monohydrate (SIGMA, S-9638, St Louis, MO) in 1L reverse osmosis water and 13.8 g sodium phosphate dibasic, anhydrous (SIGMA, S-9763, St. Louis, MO) in 1L reverse osmosis water. The phosphate buffer was composed of 775 mL sodium phosphate monobasic and 225 mL sodium phosphate dibasic with the pH adjusted to 6.3. The phosphate buffer was composed of 775 mL sodium phosphate monobasic and 225 mL sodium phosphate dibasic with the pH adjusted to 6.3. All test tubes received 5 mL

of porcine pancreatic solution. Since pigs have a similar digestive system to that of humans, the porcine pancreatic extract was assumed to function like a human's. The porcine pancreatic solution contained 10 mg of porcine pancreatic extract (ICN Biomedicals, Inc, Aurora, Ohio) per 1 mL of 0.1M phosphate buffer at a pH of 6.3. The test tubes were then incubated in a 37°C shaking water bath for 1 hour²⁷. A summary of the content of each test tube can be seen in Table 5.

TABLE 5: Samples and Contents in the Digestion Phase.

<i>Sample</i>	<i>Sample Amount</i>	<i>Bile Acid Solution 0.72µM/mL</i>	<i>0.1M 6.3 pH Phosphate buffer.</i>	<i>Porcine Pancreatin</i>
Reagent Blank with BA solution		4 mL		5 mL
Reagent Blank with Buffer			4 mL	5 mL
Reagent Positive Blank	1 mL 2.88µM/mL bile acid mixture		4 mL	5 mL
Cholestyramine	25 mg	4 mL		5 mL
Cholestyramine with buffer	25 mg		4 mL	5 mL
Cellulose	26 mg	4 mL		5 mL
Cellulose with buffer	26 mg		4 mL	5 mL
Sample 1 rep 1	100 mg DM ^a (150 mg wet)	4 mL		5 mL
Sample 1 rep 2	100 mg DM (150 mg wet)	4 mL		5 mL
Sample 1 rep 3	100 mg DM (150 mg wet)	4 mL		5 mL
Sample 1 with buffer	100 mg DM (150 mg wet)		4 mL	5 mL
Continued for all samples 1-4				

^aDM= Dry Matter

After the shaking water bath, each tube was centrifuged with a Beckman centrifuge (Model TJ9, Palo Alto, CA) for 15 minutes at 26,890 XG²⁹. Then supernatant

was decanted and transferred into a second set of labeled tubes. Each pellet was rinsed with 5 mL phosphate buffer, vortexed and centrifuged again. Then the supernatant was decanted and added to the first supernatant. Then the samples were stored at -20°C until bile acid binding analysis. The entire process was duplicated with a replicate set of potatoes, and the order of the variety of potatoes varied with each duplicate.

Bile Acid Binding:

To determine the bile acid binding value for each sample a Trinity Biotech Inc Kit 450-A (Wicklow, Ireland) was used^{29,30}. The bile acid analysis followed the procedure included in the Trinity Biotech Kit. The kit supplied a Reagent A and a Reagent B to be used to create the test and blank reagent. Reagent A was composed of 2.5 mmol/L NAD, 0.61 mmol/L NBT, 625 U/L diaphorase, pH 7.0±0.1 buffer and nonreactive stabilizers. Reagent B was composed of 1250 U/L 3 α HSD and nonreactive stabilizers and fillers. To each bottle of Reagent A 10 mL of deionized water was added, and the bottle was inverted until dissolved. To each bottle of Reagent B 5 mL of deionized water was added, and the bottle was inverted until dissolved. To make the test reagent, 4 mL of Reagent A and 1 mL of Reagent B were combined in a separate vial labeled “test”. To make the blank reagent, 4 mL of Reagent A and 1 mL of deionized water were combined in a separate vial labeled “blank”. The test and blank reagents were warmed to 37°C prior to use in a Fisher Isotemp drybath (Model 145).

The Trinity bile acid calibrator set 450-11 (Wicklow, Ireland) was used as standards. The standards used were 5 μ mol/L, 25 μ mol/L, 50 μ mol/L, 100 μ mol/L and

200 $\mu\text{mol/L}$. Disposable 12 x 75mm borosilicate glass test tubes (Fisher Scientific, USA) were labeled for each sample, blank reagent and standards as “test” and “blank”. Two hundred μl of each sample, blank reagent and standard was added to both the test and blank tube. Then 0.5 mL of test reagent was added to each “test” tube and 0.5 mL of blank reagent was added to each “blank” tube with an Eppendorf repeater pipetter (Model 4780, Hamburg, Germany). The repeater pipetter made the pipetting consistent and accurate. The tubes were incubated at 37°C for 5 minutes. After the incubation, 0.1 mL of stop reagent (1.33M phosphoric acid) was added to each tube to stop the color reaction. The stop reagent (Trinity Biotech, Wicklow, Ireland) was added to the tube in the same order as the test and blank reagents were added to the tube. A repeater pipetter was used to dispense the stop reagent into each tube. Once the stop reagent was added the color was stable for 1 hour. One at a time, the contents of the test tube were poured into a disposable 3 ½ mL, acrylic plastic cuvette (Fisher Scientific, China) and read with a Spectronic Instruments spectrophotometer (Spectronic 20D+, Rochester, NY). A flow chart of the entire protocol can be seen in Figure 3²⁹.

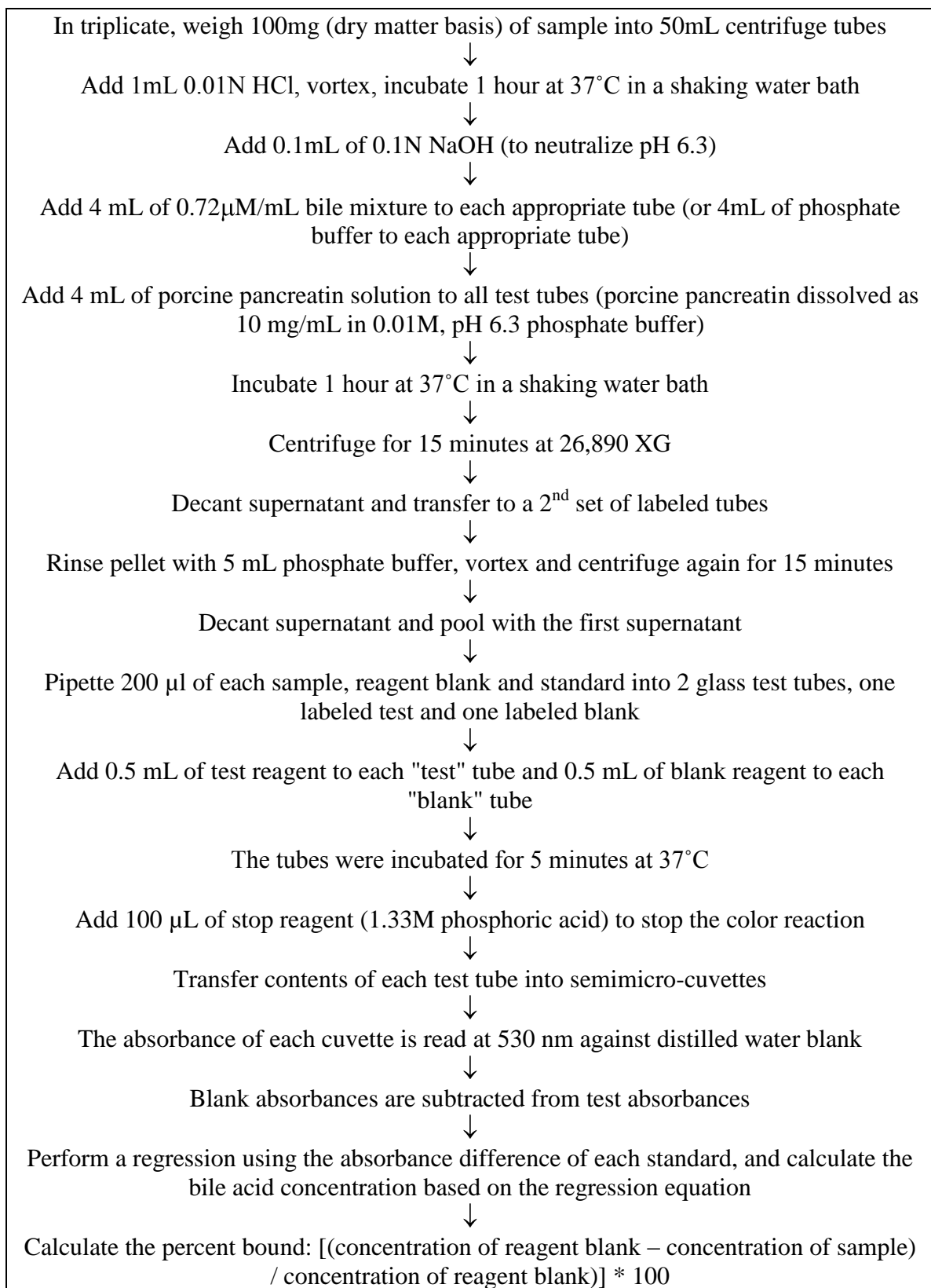


Figure 3: Flow chart of the digestion and bile acid binding protocol

Calculations:

The absorbance values versus the concentrations of each standard were plotted and fitted to a linear regression equation, and the bile acid concentration was calculated using the regression equation. In order to calculate the percent bound, the following equation was used for each sample²⁹: [(concentration of reagent blank – concentration of sample) / concentration of reagent blank] * 100. Using the mg of dry matter weight calculated previously, the amount bound uM/100mg DM was calculated using the following equation: {[wt mg DM/100]*2.88] / %Bound}*100. The amount bound uM/100mg DM of each potato sample was then divided by the amount bound uM/100mg DM for cholestyramine in order to calculate the % relative to cholestyramine²⁹.

Statistical Analysis

The statistical analysis of the data was done using SYSTAT, and after a two-way analysis of variance with cultivar and processing as factors, a Tukey's honest significant difference test was run²⁹. With Tukey's significant difference it is harder to find differences within data, but it was chosen to be certain differences existed. Differences using the Tukey's significant difference had a probability value of ≤ 0.05 .

Results

Processing, cultivar and their interaction significantly affected bile acid binding ($p=0.00$). The potato samples will be referred to as the type of potato variety followed by treatment, for example an All-Blue potato that was digested steamed and then cooled would be considered “All-Blue Cool”. Table 6 displays the results of the amount bound and % bound relative to cholestyramine³⁰. Elba Cool bound the most bile acids and Yukon Gold Steamed bound the least.

Table 6: *In vitro* bile-acid binding by potato cultivars as raw, steamed or steamed then cooled. ^a

<i>Treatment</i>	<i>Amount Bound</i> (uM/100mg DM)	<i>Percent Bound Relative to</i> <i>Cholestyramine (%)</i>
Blue		
Raw	2.25±0.39 abc	31.42±4.75 abc
Steam	1.75±0.24 cde	24.42±2.40 ef
Cool	2.22± 0.018 abc	31.10±2.66 abcd
Elba		
Raw	2.05±0.22 bcd	28.54±1.38 bcde
Steam	1.80±0.20 cde	25.10±3.32 def
Cool	2.62±0.18 a	36.50±4.60 a
Yukon Gold		
Raw	1.87±0.36 bcd	25.95±3.70 cdef
Steam	1.50±0.28 e	20.55±3.00 f
Cool	2.26±0.20 abc	31.61±2.36 abc
King Harry		
Raw	1.67±0.39 de	23.16±3.97 f
Steam	1.63±0.19 de	22.17±2.65 f
Cool	2.35±0.11 ab	32.87±2.01 ab
Cholestyramine	7.20±0.69	100±0.00
Cellulose	0.00±0.05	0±0.50

^aMeans within columns followed by different letters are significantly different ($p\leq 0.05$, Tukey’s HSD test)

Elba Cool, King Harry Cool, Yukon Gold Cool, All-Blue Raw, All-Blue Cool and Elba Raw were all statistically similar in results for the amount bound (Figure 4). Elba Steam, All-Blue Steam, King Harry Raw, King Harry Steam and Yukon Gold Steam were all statistically similar. Percent bound relative to cholestyramine followed the same trends (Figure 5). The only difference was Elba Raw which was also statistically similar to Elba Steam, All-Blue Steam, King Harry Raw, King Harry Steam and Yukon Gold Steam.

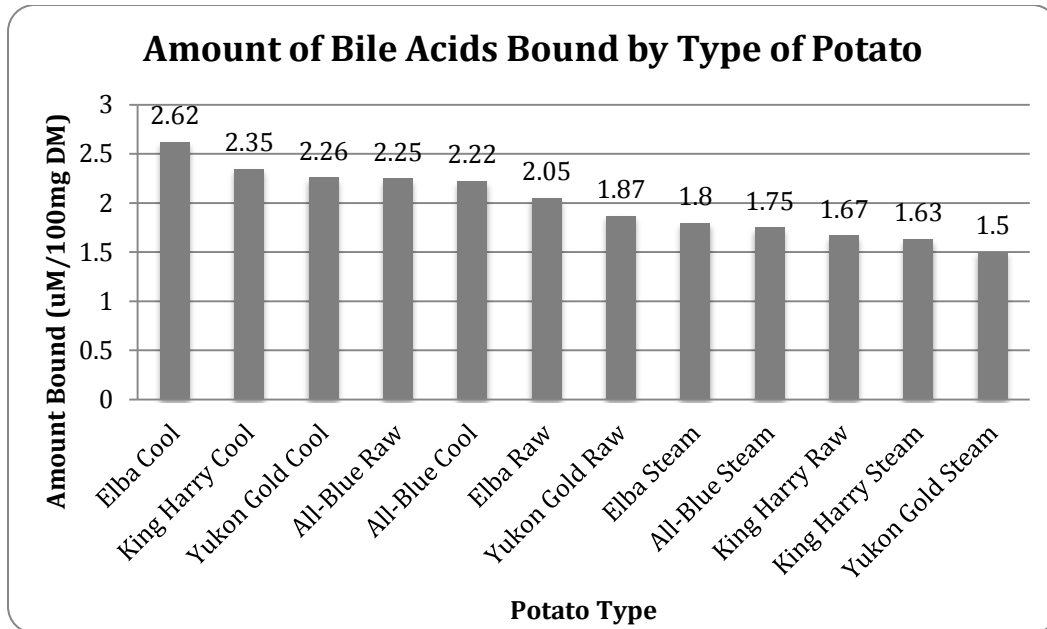


Figure 4: Amount of bile acids bound for each potato cultivar and type of processing.

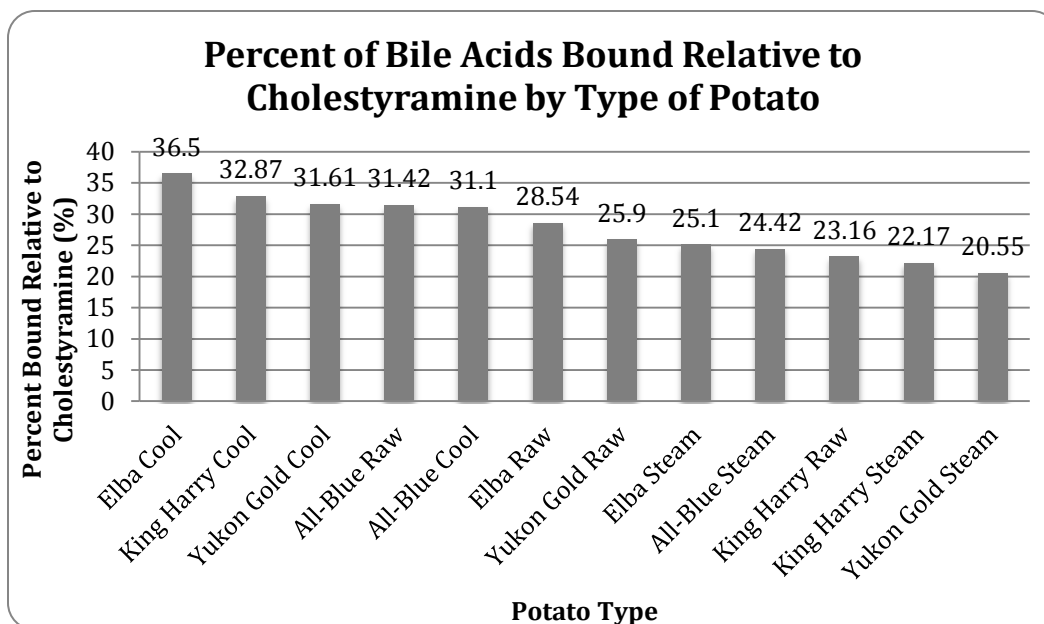


Figure 5: Percentage of bile acid binding relative to 100% cholestyramine for each potato cultivar and type of processing.

Based on the results of the analysis of variance, the potatoes that were cooled after steaming bound significantly more bile acids than did the raw ($p= 0.00$) and the steamed ($p= 0.00$). Also, the steamed potatoes bound significantly less bile acids than did the raw ($p= 0.00$). The average amount bound by steamed then cooled potatoes was $2.36 \mu\text{mol}/100\text{mg DM}$ while the amount bound by just steamed potatoes was $1.67 \mu\text{mol}/100\text{mg DM}$. As seen in Figure 6, the average percent bound of cooled potatoes was higher than the percent bound for just steamed potatoes. Similar results were found for the percent relative to cholestyramine for each process. The average percent of cholestyramine of the cooled potatoes, 33.02% , was significantly higher ($p= 0.00$) than the average percent of cholestyramine for steamed potatoes, 23.06% (Figure 7).

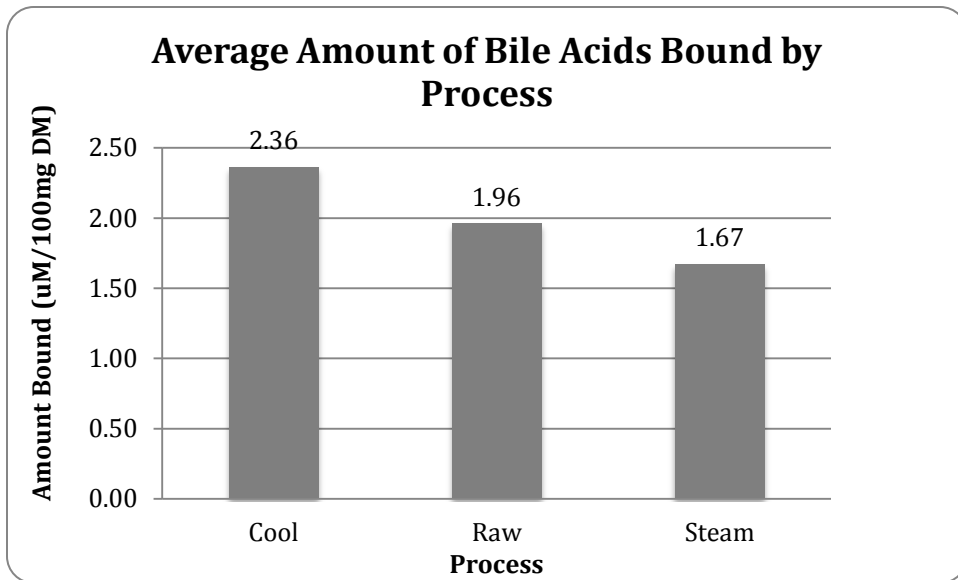


Figure 6: Comparison of the average amount of bile acids bound for each type of processing.

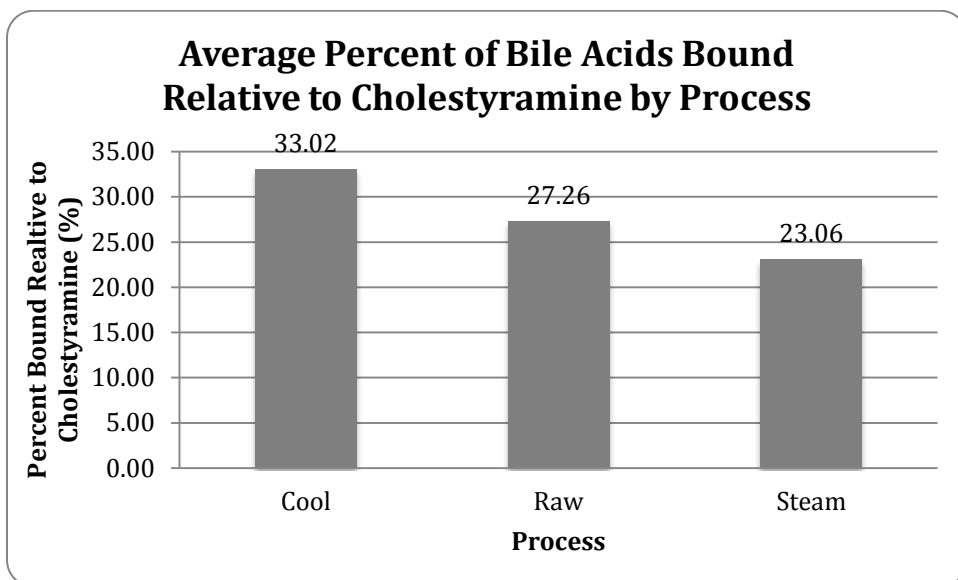


Figure 7: Comparison of the average percentage of bile acid binding relative to 100% cholestyramine for each type of processing.

Based on the analysis of variance, Elba and All-Blue potatoes bound significantly more bile acids than did the other cultivars. The average amount bound was significantly higher for Elba, 2.16 $\mu\text{mol}/100\text{mg DM}$, and All-Blue, 2.07 $\mu\text{mol}/100\text{mg DM}$, than King Harry, 1.88 $\mu\text{mol}/100\text{mg DM}$ and Yukon Gold, 1.88 $\mu\text{mol}/100\text{mg DM}$ (Figure 8). Elba

and All-Blue did not differ significantly ($p \geq 0.05$) and King Harry and Yukon Gold did not differ significantly ($p \geq 0.05$). The average percentage bound relative to cholestyramine was significantly higher for Elba, 30.05%, and All-Blue, 28.98%, than King Harry, 26.07%, and Yukon Gold, 26.02% (Figure 9). Elba and All-Blue did not differ significantly ($p \geq 0.05$) and King Harry and Yukon Gold did not differ significantly ($p \geq 0.05$).

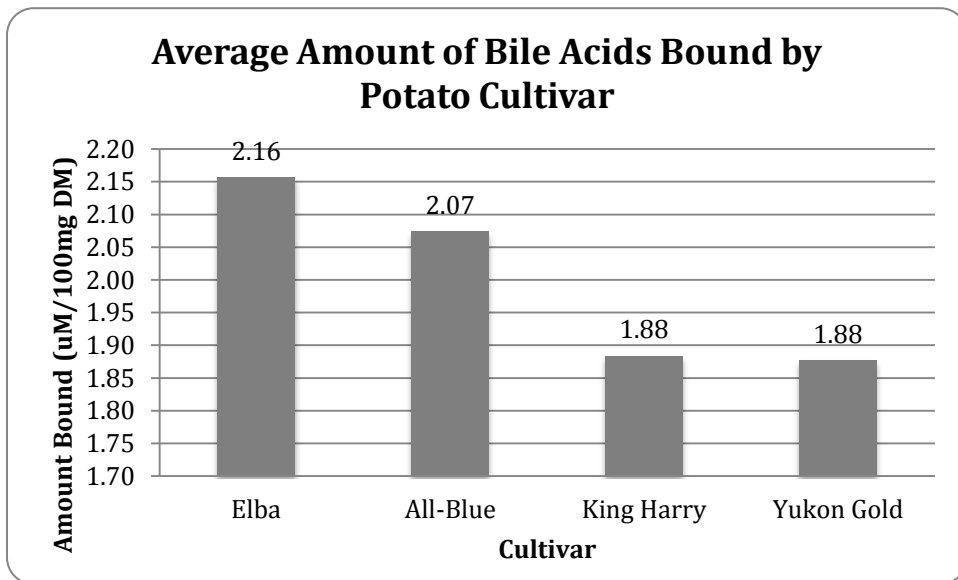


Figure 8: Comparison of the average amount of bile acids bound by each potato cultivar.

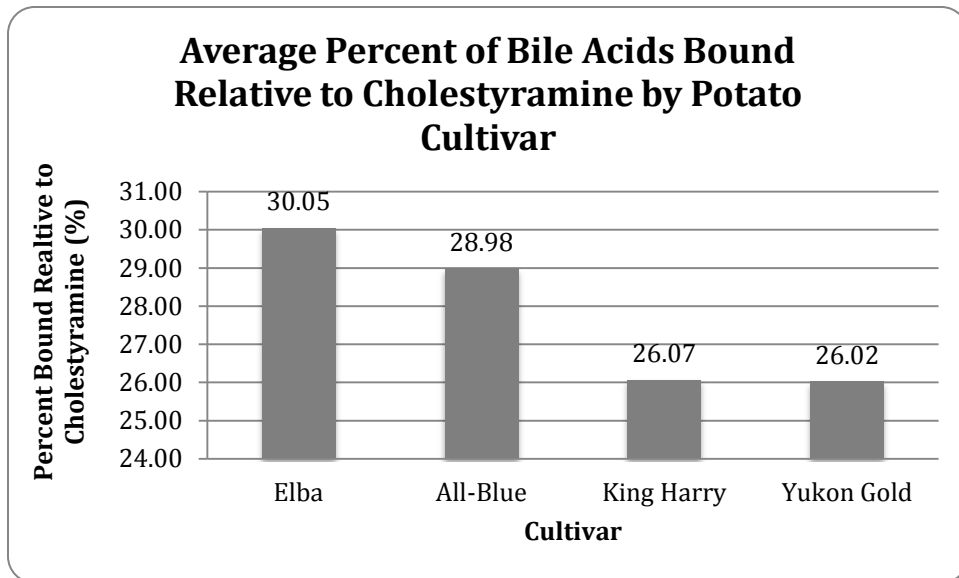


Figure 9: Comparison of the average percentage of bile acid binding relative to 100% cholestyramine by each potato cultivar.

Discussion

Processing:

Steamed and then cooled potatoes bound significantly more bile acids than the raw or steamed potatoes. This could be due to the changes in resistant starch during the cooling process. RS2 is converted to RS3 during the cooling process, creating more resistant starch. The increased resistant starch could have increased the amount of bile acid binding interactions between the resistant starch and bile acids. Additionally, cooking increases dietary fiber solubility. This change in fiber solubility could make more fiber available for bile acid binding. Thus, the change from RS2 to RS3 and the increase in fiber solubility could have been the factors that caused the increased bile acid binding in potatoes that were steamed then cooled. Further analysis of potato

composition when raw, steamed, and steamed then cooled is recommended in order to learn which components of the cooled potatoes increased bile acid binding.

Thermal processing of resistant starch and dietary fiber should increase the potatoes ability to bind bile acids when compared to the raw. While raw potato starch can bind bile acids, consumption of raw potato starch is not recommended.

Cultivar:

Elba and All-Blue potatoes bound significantly more bile acids than the Yukon Gold and the King Harry potatoes. The increased bile acid binding of All-Blue potatoes could have been due to medium ratio of amylose and amylopectin, since amylose is suggested to increase resistant starch content²⁵. All-Blue potatoes have a large amount of anthocyanin, giving it its purple color, which have been associated with lowering cholesterol²⁰. The anthocyanin content of All-Blue potatoes could have been a factor in bile acid binding ability. The low solids or starch and fiber composition of All-Blue potatoes could have been factors in the increased bile acid binding. Since the All-Blue potatoes differed from the other varieties in multiple ways, it cannot be concluded which factors caused the increased bile acids binding. The increased bile acid binding of Elba potatoes could have been due to the medium ratio of amylose and amylopectin, medium solids content or starch and fiber composition. However, since the King Harry potatoes have a similar color and texture to the Elba potatoes, it cannot be concluded what factors caused in the increased bile acid binding. In order to conclude what factors of the variety

of potato caused the increase in bile acid binding, further research in the composition of each potato is needed.

Comparison to Cholestyramine:

The suggested dosage of cholestyramine from the Physician's Desk Reference (PDR) is initially 4 g orally once a day and maintenance is 8 g orally twice a day³³. In this study 25 mg of cholestyramine was used as positive bile acid binding control; this amount is 160 times less than the initial daily dose. The potato samples contained approximately 100 mg dry weight and bound around an average of 30% relative to cholestyramine. Thus it would take approximately 48 g of dry weight potato sample to have the same bile acid binding potential of an initial dose of cholestyramine. According to the USDA, 1 medium potato, 202 grams (approximately 46 g dry weight), is a typical serving size for potatoes; therefore potatoes may contribute significantly to serum cholesterol reduction as a part of lower cholesterol regimen.

Comparison to Other Foods:

The amount of bile-acid-binding of the potatoes in this study was less than the amount bound by potato peels recorded by Camire et al. (1993) (Table 7). The potato peel typically contains more of certain phytochemicals than does the flesh of the potato¹². Potato peels also contain dietary fiber, and peeled potatoes have lower dietary fiber content³. The difference could be due to a higher concentration of fiber and phytochemicals in the peels versus the peels and flesh. The bile acid binding of wheat in a study by Kahlon et al (2009), was slightly lower than the results for the potatoes in this

study, which is expected since wheat is high in insoluble fiber. In another study by Kahlon et al (2007), the bile acid binding of blueberries, apples, plums and cranberries was less than the results of the amount bound in the potatoes of this study. However, there are numerous factors that could cause a variation in results between studies. Overall, potatoes may be a food to include in a diet to reduce serum cholesterol.

Sample	Bile-Acid-Binding (uM/100mg DM)
Potato peels extruded 104°C, 36% ¹²	7.23± 0.58
Potato peels extruded 143°C, 36% ¹²	8.14±0.73
Potato peels not extruded ¹²	3.80±0.52
Whole grain wheat, hard red winter ³¹	0.80± 0.01
Pearled grain wheat, hard red winter ³¹	0.62±0.01
Blueberries ³²	0.73±0.02
Apples ³²	0.12±0.01
Plums ³²	0.60±0.01
Cranberries ³²	0.12±0.04

¹²Results from “*In vitro* binding of bile acids by extruded potato peels” by Camire, M.E., Zhao, J., & Violette, D.

³¹Results from “*In vitro* bile-acid binding of whole vs. pearled wheat grain.” by Kahlon, T.S., Chiu, M.M., & Chapman, M.H.

³²Results from “*In vitro* binding of bile acids by blueberries (*Vaccinium* spp.), plums (*Prunus* spp.), prunes (*Prunus* spp.), strawberries (*Fragaria X ananassa*), cherries (*Malpighia punicifolia*), cranberries (*Vaccinium macrocarpon*) and apples (*Malus sylvestris*)” by Kahlon, T.S., & Smith, G.E.

Conclusions

In conclusion, this study suggested that when potatoes are cooled after steaming they are capable of more bile acid binding. Potatoes of the Elba and All-Blue variety performed more bile acid binding than did King Harry or Yukon Gold potatoes. In addition to additional quantitation of potato composition, other recommended research

for the future would be studying the effects of bile acid binding with other forms of food preparation. This study examined only prepared steamed potatoes, but people prepare potatoes in a variety of ways, such as baking, roasting, frying and boiling. Identifying the bile acid binding associated with other thermal processing methods could help identify which processing has the greatest bile acid binding ability. Additional varieties of potatoes should be tested in the future. Measuring bile acid binding ability of red-skinned potatoes, red-fleshed potatoes and more frequently consumed varieties of potatoes would help give consumers more insight on the cholesterol lowering ability of more potato varieties. Overall, future research would provide evidence to promote health benefits of potatoes.

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Author's Biography

Emily Adrienne Hinkle was born in Kittery, Maine on January 5, 1990. She was raised in Kittery and graduated from R.W. Traip Academy in 2008. Emily entered the University of Maine already awarded the George Mitchell Scholarship and the Distinguished Scholars Scholarship. Emily double majored in Food Science and Human Nutrition and Biology. She was a member of the UMaine's Nutrition Club, where she held a volunteer coordinator position. She was also a member of Umaine's improvisational acting group, Improv In Sanity, and was the president from May 2011 to May 2012. Emily was awarded the Center for Undergraduate Research (CUGR) Fellowship and awarded second place at the CUGR showcase for her research presentation. Emily plans to graduate in May 2013.

Upon graduation, Emily plans to attend Cornell University's Dietetic Internship. Emily plans to apply to master's programs after completion of the internship.