REDUCTION OF PURINE CONTENT IN COMMONLY CONSUMED MEAT PRODUCTS THROUGH RINSING AND COOKING

by

Anna Ellington

(Under the direction of Yen-Con Hung)

Abstract

The commonly consumed meat products ground beef, ground turkey, and bacon were analyzed for purine content before and after a rinsing treatment. The rinsing treatment involved rinsing the meat samples using a wrist shaker in 5:1 ratio water: sample for 2 or 5 minutes then draining or centrifuging to remove water. The total purine content of 25% fat ground beef significantly decreased ($p<0.05$) from 8.58 mg/g protein to a range of 5.17-7.26 mg/g protein after rinsing treatments. After rinsing and cooking an even greater decrease was seen ranging from 4.59-6.32 mg/g protein. The total purine content of 7% fat ground beef significantly decreased from 7.80 mg/g protein to a range of 5.07-5.59 mg/g protein after rinsing treatments. A greater reduction was seen after rinsing and cooking in the range of 4.38-5.52 mg/g protein. Ground turkey samples showed no significant changes after rinsing, but significant decreases were seen after rinsing and cooking. Bacon samples showed significant decreases from 6.06 mg/g protein to 4.72 and 4.49 after 2 and 5 minute rinsing and to 4.53 and 4.68 mg/g protein after 2 and 5 minute rinsing and cooking. Overall, this study showed that rinsing foods in water effectively reduces total purine content and subsequent cooking after rinsing results in an even greater reduction of total purine content.

INDEX WORDS: Purine content, purine bases, water rinse treatment, cooking, gout, ground beef, ground turkey, bacon

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"I can do all things through Christ who strengthens me." Philippians 4:13

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CHAPTER 1

INTRODUCTION

According to the American College of Rheumatology, gout affects more than five million Americans and is considered the most common form of inflammatory arthritis in adult men (Lawrence 1998; Rheumatology 2005). Gout is a condition associated with the buildup of uric acid crystals in the joints, particularly in the lower extremities. The buildup is caused by either an overproduction or under excretion of uric acid, which is the end product of purine catabolism. The main sources of purines are dietary purines, those ingested in the foods we eat. Hence, gout attacks can be controlled by limiting the amount of purines consumed. Purines are mainly associated with high protein foods, and are found at highest concentrations in meat and fish products.

Previous studies have been done to measure the amount of purines present in foods before and after processing, including storage, washing, and cooking. Lou and his group have done several studies on purine content of fish products, and concluded that rinsing and cooking foods in water effectively reduces the amount of purines present (Lou 1998b; Lou and others 1998; Lou and others 2005a). The goal of this research was to reduce the amounts of purines present in commonly consumed meat products which would make these foods safer to consume for people suffering from gout. The objectives for this study were to compare the purine levels of meat products before and after a rinse treatment, as well as after cooking, and to determine if these treatments have any impact on purine content.

CHAPTER 2

LITERATURE REVIEW

PURINES

Deoxyribonucleic acid (DNA) functions as the storage area for all genetic information needed by cells and ribonucleic acid (RNA) functions to convey the information. DNA and RNA consist equally of nitrogenous bases, five carbon sugars (in DNA, 2-deoxyribose; in RNA, ribose), and phosphoric acid. The term nucleosides defines the compound formed when a base is linked to a sugar, and the term nucleotides defines the compound formed when the phosphoric acid links to the nucleoside. Nucleic acids are many nucleotides linked together by phosphodiester bridges, which forms the backbone structure for DNA and RNA.

Purines and pyrimidines are heterocyclic ring structures that function as the nitrogenous bases in DNA and RNA. The purine bases are adenine and guanine, and the pyrimidine bases are cytosine, uracil, and thymine (Garrett 2005). Hypoxanthine and xanthine are two naturally occurring purine derivatives that are rarely found as bases in DNA and RNA, but often act as important intermediates in the processes of making and breaking nucleotides (Angstadt 1997; Garrett 2005). The purine bases will be the main focus for this research. Adenine deaminates to form the purine base hypoxanthine and guanine deaminates to from the purine base xanthine. The purine adenine serves an important function in its role as adenosine triphosphate (ATP), which moves chemical energy between reactions. Free purine bases are not very water soluble, but when they are in the form of nucleosides they are much more water soluble due to the hydrophilicity of the sugar. Purine nucleosides are easily hydrolyzed in acid, resulting in the free

purine base and the sugar. Purines contain –OH and -NH_2 substituents which cause them to undergo keto-enol tautomeric shifts (Michelson 1963; Hurst 1980; Garrett 2005).

To form the double helix structure of DNA and RNA the nitrogenous bases join by means of two or three hydrogen bonds. In DNA, the adenine binds to the pyrimidine thymine, and in RNA adenine binds to the pyrimidine uracil. Guanine binds to the pyrimidine cytosine in DNA and RNA. Table 2.1 lists some characteristics about the four purines. The pKa values for purines range from 4.15 to 12.0 and all but guanine are soluble in water.

PURINE CATABOLISM AND GOUT

All foods are comprised of three basic components, proteins, carbohydrates, and fats. Proteins are in general made up of a combination of twenty different amino acids linked together by peptide bonds; carbohydrates are made up of carbon atoms, along with hydrogen and oxygen bonded together to form mono-to-polysaccharides; and fats are made up of fatty acids, which are basically chains of hydrocarbons terminating in a carboxylic acid group, esterified to glycerol molecules forming triacylglycerides (Fennema 1996). Nucleic acids, which contain purines, are important in the body because they are part of nucleoside di- and triphosphates which act as sources of energy, are used in different types of protein synthesis, and act as structural components in some coenzymes. Since purines can be synthesized de novo and salvaged from the body and reused, there is normally very little need for dietary purines. Dietary purines that are absorbed by the body but not needed are catabolized and the end product is urate or uric acid (Angstadt 1997). After food is ingested, the low pH of the stomach denatures the DNA and RNA into nucleic acids. Nucleases and phosphodiesterases from the pancreas hydrolytically remove the phosphate groups from the nucleic acids, converting them to mononucleotides that are eventually degraded into purines and pyrimidines (Champe and Harvey 1994).

Table 2.1. Properties of Purine Bases (Adapted from Bendich 1955; Budavari 1996)

Purine Base	**Molecular Formula**	**pKa Values**	**Molecular Weight**	**Melting Point (ºC)**	**Solubility in H_20**
Adenine	$C_5H_5N_5$	4.15, 9.80	135.13	360-365	Soluble
Guanine	$C_5H_5N_5O$	9.92	151.13	>360	Insoluble
Hypoxanthine	$C_5H_4N_4O$	8.8, 12.0	136.11	>300	Soluble
Xanthine	$C_5H_4N_4O_2$	7.5, 11.6	152.11	>300	Soluble

The purine bases are then converted by the intestinal mucosal cells to uric acid and excreted in the urine, and the pyrimidine bases are metabolized to urea and ammonia (Henderson 1973; Champe and Harvey 1994). At a pH of 7, uric acid usually exists as monosodium urate, a salt that is relatively insoluble in water. The low solubility of uric acid is not usually a problem and is still excreted unless the urine is very acidic or has high calcium concentration (Kelley and Schumacher 1993; Angstadt 1997). Because of an enzyme deletion in humans, we are the only mammals that don't produce an enzyme called uricase. Uricase degrades uric acid into allantoin, a much more soluble end product, which is more easily excreted (Seegmiller 1966). In healthy individuals, all of these byproducts of purine metabolism would be excreted as detailed previously (Henderson 1973). However, in humans with a condition known as gout, the uric acid that the body forms is not excreted efficiently, or excess uric acid is produced. It is converted to monosodium urate crystals and builds up in the body, in particular the joints (Seegmiller 1961). Gout is defined as "acute inflammatory monarthritis…often in middle-aged man" (Rott and Agudelo 2003) and is considered to be the most prevalent form of inflammatory arthritis in adult men with recent reports indicating that the incidences of gout in the U.S. are increasing (Lawrence 1998; Arromdee and others 2002)

Gout normally is exhibited in the body as monoarthric, meaning it affects only one joint, but polyarthric attacks can occur. In more than 75 percent of gout attacks, the joints in the lower extremity are affected, with over half of those being in the first metatarsophalangeal joint, or big toe (Lawry and others 1988). Gout is an extremely painful condition, and repeated gout attacks can lead to destruction of tissues and severe deformities in the joints (Angstadt 1997). Gout can occur due to a genetic disorder or decreased renal function, resulting in either an overproduction or under excretion of the uric acid, with 90% of cases caused by under excretion (Kelley and

Schumacher 1993; Wortman 1998). Gout is triggered most often by a diet high in purines, which are associated with high protein foods, and therefore are common in a wide variety of foods (Curto 1998; Lyu 2003). Some causes of overproduction of uric acid include severe psoriasis, exercise, alcohol, diet and obesity. Some causes of under excretion of uric acid include diabetes insipidus, renal insufficiency, hypertension, acidosis, and toxemia of pregnancy (Wortman 1998; Pittman and Bross 1999).

One risk factor for gout is a condition called hyperuricemia, which is diagnosed if the serum uric acid concentration in the body is above 7 mg per dL, (or 420 μmol per L) (deciliter $=10^{-1}$ L). When the uric acid concentration reaches 8 mg per dL or greater, monosodium urate begins to precipitate in the body's tissues, causing the typical symptoms of gout (Pittman and Bross 1999). However, there have been cases of gout without elevated serum uric acid levels as well as cases of hyperuricemia that never develop into gout, which leads to the conclusion that hyperuricemia is not the cause of gout (McCarty 1994).

Patients diagnosed with gout are usually encouraged to lose weight, avoid alcohol consumption, and decrease consumption of foods with high purine content (Pittman and Bross 1999). Obesity is a serious problem in the United States and people with a higher Body Mass Index (BMI) have been shown to have a significantly increased risk for developing gout (Choi and others 2005a). Studies show that people who are overweight have elevated uric acid levels in the body due to both an increased production as well as decreased renal excretion of uric acid (Dessein and others 2000). Alcohol consumption is discouraged because studies have shown that alcohol leads to increased uric acid production due to accelerated purine breakdown (Faller and Fox 1982). Certain alcoholic drinks contain high amounts of purines and this was believed to have been the cause of the increase in gout attacks (Gibson and others 1984). However, more

recent studies have shown that regardless of the type of alcohol consumed, there is still an increase in the risk of recurrent gout attacks. It is theorized that it is actually the total amount of ethanol that causes increased occurrence of attacks, not the components of the various types of alcoholic drinks, therefore it is suggested that people suffering from gout should avoid drinking alcoholic beverages completely (Zhang and others 2006).

When lifestyle changes have no impact on reducing the incidence of gout attacks, patients are treated with medication. The three most common medications used to relieve pain and symptoms after a gout attack are nonsteroidal anti-inflammatory drugs, colchicine, and coritcosteroids. Medication is also used to prevent recurrent gout attacks, the most common being uricosuric drugs, which increase renal excretion of uric acid, and allopurinol, which impairs the conversion of purines to uric acid. The down side of treating gout with medications is that many of the medications have unpleasant side effects such as gastrointestinal problems, skin rash, and even in some cases toxic effects (Pittman and Bross 1999). Long term treatment with allopurinol has even been shown to lead to liver problems (Wortman 1993).

As mentioned previously, purines are ingested into the body in the diet, therefore decreasing the consumption of purines from foods will lead to reduced risk of elevated uric acid levels and gout attacks. The dietary purines adenine and hypoxanthine in particular have been shown to cause a significant increase in blood uric acid levels in humans (Clifford 1976) and have been termed the uricogenic bases for this reason (Brule and others 1988). In a study conducted by Clifford (1976) after oral administration of the adenine and hypoxanthine, a marked increase in uric acid levels were seen in both normal and gouty individuals. However, administration of guanine and xanthine showed no effects on uric acid levels. It has been suggested that this method may be an inaccurate means of determining effect of purines because

of "artificial loading of purified purine" as opposed to the consumption of actual foods containing purines (Choi and others 2005b). A later study conducted using soy beans, which have high levels of guanine (99 mg/100 g), established that guanine did in fact have an impact on elevating uric acid levels in the body (Van Waeg and others 1986).

A study conducted by Brule and others (1992) showed that the consumption of the hypoxanthine-rich food haddock resulted in a higher increase in uric acid levels than did the adenine-rich foods liver and soybean (both 120 minutes after ingestion). This indicates that hypoxanthine has a greater impact on increases in uric acid than does adenine. However, this study also showed that after ingesting adenine rich foods, the uric acid levels did not decline as quickly as the uric acid levels did after consuming hypoxanthine-rich foods, meaning adenine is broken down by enzymes more slowly than hypoxanthine. Other studies also indicate that adenine and hypoxanthine may have a different impact on uric acid levels than the other purine bases because they are metabolized differently. For example, adenine may be phosphorylated to its nucleotide (AMP) before being converted to uric acid (Clifford 1976; Salati and others 1984; Brule and others 1992).

In the future, diets that restrict high purine content foods should begin to take into consideration the foods containing higher adenine and hypoxanthine contents, instead of just focusing on the total purine content of the food.

PURINE CONTENT IN FOODS

Since gout can be controlled by limiting the amount of dietary purines consumed, it is important to have an understanding of which foods have higher purine contents. According to the American Dietetic Association, foods are considered to be high purine content foods if they have a total purine content of 150-1000 mg/100g and it is recommended that people suffering

from gout avoid these foods. A study done by Ho (Ho 1986) suggests that foods containing purine contents of 0-25mg/100g be termed low purine content foods, food containing 25-100mg/100g be termed medium purine content foods, and foods containing more than 100mg/100g be considered high purine content foods.

Table 2.2 lists the total purine contents of some commonly consumed food products. As seen in the table, the foods with some of the highest purine content are meat and fish products, with the organ meats having particularly high levels of purines. According to the USDA, American consumption of total meat products for 2007 is projected to be 220 lbs per person. This demonstrates how difficult it is for people suffering from gout to follow a low purine diet A long term study recently completed by Dr. Hyon Choi and others (Choi and others 2004; 2005b) showed that higher consumption of meats (beef, pork, and lamb) and seafood (fish, shrimp, lobster, and scallops) resulted in an increased risk of gout but the consumption of purine rich vegetables (peas, beans, lentils, mushrooms, spinach, cauliflower, and oatmeal) did not result in an increased risk of gout. Their studies also showed that there was not a relationship between higher total protein consumption (from animal and vegetable sources) and increased risk of gout, indicating that protein content of food should not be used as an indication of purine content of a food. They also studied dairy products and their effect on uric acid levels, and concluded that consuming dairy products lowered uric acid levels in the body, and can actually lower the risk of gout (Choi and others 2005b). Another study on this concluded that the milk proteins casein and lactalbumin function to exert a urate lowering effect. This same study also revealed that people that consumed no dairy products in their diet suffered from significantly increased levels of uric acid (Ghadirian and others 1995).

As mentioned previously, adenine and hypoxanthine have been shown to have a greater

Table 2.2: Total purine content of commonly consumed foods (Adapted from Souci and others 2006)

Food Product	Purine Content mg/100g w.b
Yeast, brewer's	1810
Yeast, baker's	680
Beef liver	460
Fish, Trout	297
Fish, Tuna in oil	290
Fish, Tuna	257
Chicken liver	243
Lamb (muscle meat)	182
Fish, Halibut	178
Chicken breast (with skin)	175
Fish, Salmon	170
Pork (muscle meat)	166
Turkey (with skin)	150
Shrimp (brown)	147
Scallop	136
Ground beef (muscle meat)	133
Ham (cooked)	131
Lentil, seed	127
Beef, chuck	120
Lobster	118
Mussel	112
Beef, sirloin	110
Peas, chick (garbanzo)	110
Fish, cod	109
Oats, whole grain	94
Oyster	90
Frankfurter	89
Pea, green	84
Brocolli	81
Peanuts	79
Crawfish	60
Mushroom	58
Banana	57
Corn	52
Asparagus	23
Potato	16

impact on uric acid levels than guanine and xanthine. Thus, while knowing the total purine content of foods is very beneficial, it is also helpful to know the levels of individual purine bases in foods so those with higher adenine and hypoxanthine can be avoided as much as possible. Fish products contain moderate to high levels of purines, with hypoxanthine being the most abundant purine base in fish, crustacean, and mollusca, and adenine the most abundant base in shellfish. Since fish products are considered high purine content foods, studies have been conducted measuring the purine content of these products (Shinoda and others 1981; Lou and others 1996). There was substantial variation in levels of individual purine bases in some common fish and mollusk species. For example, the miiuy croaker fish, red bream, Spanish mackerel, and swordfish had total purine contents of 275.8, 152.1, 119.9, and 111.3 mg/100g (wet wt.), respectively, while the shark, tuna, and cod had total purine contents of 90.1, 81.9, and 55.4 mg/100g (wet wt.) respectively. For the mollusk species, the purine contents for the hard clam, grass shrimp, and sand shrimp were 200.3, 178.1, and 128.3 mg/100g (wet wt.) respectively, while the purine contents for the oyster, octopus, squid, and Pelagic crab were 108.9, 70.3, 62.2, and 50.9 mg/100g (wet wt.) respectively. Another interesting find was the variation of total purine content seen among the different sea fish species compared to the cultured fish species, where only minimum difference was seen in the purine contents. For example, cultured fish species such as the common carp, sea perch, and tilapia had purine contents of 116.0, 112.1, and 106.0 mg/100g (wet wt.) respectively.

Another study conducted by Clifford and Story (Clifford and Story 1976) analyzed various common food products for the individual levels of purine content, including meat, fish, and dried legumes. The highest levels of purines were seen in the meat and fish products, with levels of individual purine bases varying greatly. For example, chicken liver and beef liver had

purine contents of 243 and 197 mg/100 g (wet wt.) respectively, with guanine being the most abundant purine base present. Pork liver had a total purine content of 289 mg/100 g (wet wt.), and xanthine was the most abundant purine base present. For the seafood products tested, the fresh anchovies and clams had total purine contents of 411 and 136 mg/100 g (wet wt.) respectively, with xanthine being the most abundant purine base present. While the canned anchovies and clams had total purine contents of 321 and 62 mg/100g (wet wt.), respectively, with xanthine being the most abundant purine base present for the anchovies, but adenine being the most abundant purine base present for the clams. In some cases the purine contents of dried legumes were as high as some of the meat and fish products. For example, the blackeye pea, pinto bean, and garbanza bean had total purine contents of 230, 171, and 56 mg/100g (wet wt.) respectively, with guanine being the most abundant purine base present for the blackeye pea, adenine the most abundant for the pinto bean, and hypoxanthine being the most abundant purine base present for the garbanza bean.

Brule and others (Brule and others 1988) have also studied the purine contents of commonly consumed food products. They also analyzed the products for moisture, fat, and protein. All of their samples represent the purine content of the cooked product. The products were pan fried, boiled, roasted or broiled until medium well done. The meat products contained the highest levels of purines, from 12 to 287mg/100g. The bread, cereal and dairy products contained the lowest levels, from 1 to 16mg/100g. The veal, white fish and canned fish contained the highest amounts of hypoxanthine, and the organ meats contained the highest levels of adenine. Even though the meat products differed substantially in total purine content, the adenine and hypoxanthine contents for all the meat products were similar.

ANALYSIS OF PURINE CONTENT IN FOOD PRODUCTS

Because of the aromaticity of their ring structures, purines and pyrimidines exhibit a strong absorbance of ultraviolet (UV) light which is very useful in quantitative and qualitative analysis of nucleic acids (Garrett 2005). Reverse-phase high-pressure liquid chromatography (HPLC) has proven to be a very efficient for analyzing nucleic acids (Titkova and others 1983), and has been commonly used to separate and quantify the purine bases. The method was first introduced by Hartwick and Brown (Harwick and Brown 1976). Before this method was commonly recognized, HPLC with cation exchange column was used for the analysis of purines in foods (Clifford and Story 1976; Young 1983). HPLC works by using pressure to push the sample through narrow columns that are packed with particles, usually not any larger than 50 um. The separation is based on the differences of the speed of migration of the sample through the column. The samples are represented as peaks, with the first peak being the sample that elutes off the column fastest, and the last peak being the sample that takes the longest time to elute off the column. To quantify the amount of purine present, the area of the sample peak is compared to that of the area of the peak of the standard solution, where the amount used is already known, and the amount of purine present in the sample can be quantified. Different levels of standard solution are injected, for example, 20 um, 30 um, and 40 um may be injected, and a standard curve is generated based on the exact amount of standard used. The areas of the sample peaks are then inserted into the standard curve equation and the quantity of the samples are obtained (Engelhardt 1979).

The reverse-phase HPLC method requires the hydrolysis of nucleic acids into nucleotides and free bases by a strong acid. Brule and Sarwar (Brule and others 1988) modified a method developed by Marshak and Vogel (Marshak and Vogel 1951) making it easier and faster. 11.6 N

perchloric acid is used to hydrolyze the samples for 1 hour at 100ºC. The high heat in these acidic conditions results in the effective degradation of nucleic acids to bases, sugars, and phosphates. The samples are then cooled, the pH adjusted to 4.0 using NH_4OH, distilled water added to adjust the volume to 50 mL, and samples are filtered for analysis by HPLC. The bases were separated isocratically by reverse phase HPLC using a C-18 column and a mobile phase of 0.1 M potassium phosphate buffer adjusted to 4.0 pH with phosphoric acid. This method allows the elution of all four purine bases in 15 minutes. The efficiency of the method at recovering purine bases was determined to be 99-102%.

Brule and Sarwar (1989) also modified this method to determine free purine bases. A mild hydrolysis is performed on samples using 1.16 N perchloric acid for 1 hour at room temperature. This method can not break down the nucleic acid, leaving only the free bases available for measurement. After the hour, the pH of the mixture is adjusted to 4.0 using ammonium hydroxide, distilled water is added to a volume of 50 mL, and samples are filtered for analysis on HPLC, using the same conditions as the previous method. Recovery of the bases was reported at 99-102%.

Another method for analyzing purine content in food products is a method by Lou and others (Lou and Chen 1997). This method uses acid hydrolysis to release bound and free purines in foods to determine the total purine content. The method involves freeze drying the product, which effectively removes all moisture causing little or no change in the physical and chemical properties of the product. The samples are ground to powdered form making them a more uniform mixture and 5 mL of a 5:5:1 mixture of triflouroacetic acid, formic acid, and water is added to 100 mg of sample in a scrubbed glass tube. The mixture is heated at 100ºC for 35 minutes to effectively hydrolyze the sample. The sample is then rotary evaporated to dryness

and 5 mL of water is added twice, drying the sample each time, to remove all of the acid. 10 mL potassium phosphate (pH adjusted to 3.2 using phosphoric acid) is then added to the dried residue to dissolve the purine bases. After filtering, the sample is analyzed using reverse phase HPLC through a C18 column with a 0.02 M potassium phosphate buffer (adjusted to pH 3.2). All purine bases are eluted off the column in 8 minutes. The efficiency of the method at recovering the four purine bases was determined to be 87-100%.

PREVIOUSLY REPORTED RESULTS ON CHANGES OF PURINE CONTENT IN FOODS

Since gout is a relatively common disease and it has been proven that dietary purines have a significant impact on the occurrence of gout attacks, several food scientists have focused their efforts on researching the effects of different processing steps on the purine content of some foods, particularly meat and fish products.

Purine contents of mechanically separated meat products have been studied (Arasu and others 1981; Savaiano and others 1983; Sarwar and others 1985; Scarborough and others 1993). Mechanically separated meat (MS) is also referred to as mechanically deboned meat (MDM) and mechanically recovered meat (MRM). It is produced by mechanically removing all remaining attached skeletal muscle tissue from the bones of livestock carcasses and parts and is used for products like sausages and deli meats. During the removal process, small amounts of bone and bone marrow are added to the meat, and this has been shown to elevate the purine content of the meat (Arasu and others 1981). A study comparing the purine content of mechanically separated (MS) compared to hand separated (HS) poultry meats have been conducted which contrasted the previous conclusions (Sarwar and others 1985). Results for this study showed no significant difference in the two methods. A further study conducted by Scarborough (Scarborough and

others 1993) tested the hypothesis that elevated levels of free purine bases were present in MRM's due to ruptured muscle cells, which did not occur in carcass meat. The study concluded that a particular increase was seen in the purine base xanthine in poultry MRM as compared to hand separated meat, with xanthine from MS meat at a level of 100ug/g compared to HS meat at levels of 57ug/g, 33ug/g, and 14ug/g for neck, leg, and breast respectively (Scarborough and others 1993).

Studies have also been conducted on MS beef and veal. The results show that although the adenine and guanine levels in MS beef and veal are higher, the hypoxanthine levels are lower, resulting in the total purine content of MS and HS beef and veal being not significantly different (Savaiano and others 1983). The conclusion of the study was that MS beef and veal should have no greater effect on uric acid levels than HS beef and veal, because the total purine levels are not significantly different.

One study analyzed the effect of stewing on purine content in chicken meat (Young 1983). The researcher initially hypothesized that the hot water may work to alter the purine content in the chicken meat. Fresh chicken meat (never before frozen) was placed in boiling water until it reached an internal temperature of 80ºC in the thickest part of the meat. The breast, thigh, and skin were separated and purine content was measured for each individual section. Results showed slight increases in adenine and guanine contents after cooking, with significant differences ($p<0.05$) in increases seen in adenine in the breast sample and guanine in the thigh sample. Hypoxanthine content decreases in the breast and thigh, but not at a significant level. Significant increases in all purines were seen in skin samples after cooking, probably due to the decrease in collagen, which is found in large amounts in skin tissues and is soluble in hot water. Furthermore, since the skin only represents 20% of the weight of the cooked parts, this increase

is not as important overall. The study concluded that slight increases in purine content were seen in the cooked chicken meat due to the reduction of other components, such as fat and moisture (Young 1983).

Another study conducted by Brule and Sarwar (Brule and others 1989) evaluated the influence of two different cooking methods, dry heat (broiling) and moist heat (boiling in water) on the free and total purine bases in beef steak, beef livers, and haddock fillets. The beef was freshly cut and the haddock fillets were frozen. For the dry heat method, samples were broiled in an oven until the internal temperature reached 75ºC for the beef products, and 85ºC for the haddock. For the moist heat method, all samples were boiled in water until the internal temperature in the thickest part of the sample reached 80ºC. Samples were analyzed for total and free purine bases. Adenine and guanine levels increased in the beef steak and beef liver for both cooking methods. All purine bases decreased in the boiled haddock, but increased in the broiled haddock. It was concluded the increases were likely due to decreases in moisture and increases in protein. In the raw products, only small amounts of free adenine and guanine were detected, but large levels of free hypoxanthine and xanthine were present, constituting a major portion of the total purine content for those bases. This suggests that in the raw product adenine and guanine exist mostly as nucleotides and nucleosides, and hypoxanthine and xanthine exist mostly as free bases. The cooking juices were also analyzed for purine content. The results show that the bound and free purine bases were released into the cooking medium with a large percentage (38% to 97%) of the free bases released into the cooking medium. The results of this study also showed that free purine bases are more readily released into the cooking medium (Brule and others 1989).

Studies have also been done by Colling and Wolfram (1987, 1989) in Germany on the effects of cooking on purine content. Their studies also show that the purine content in foods changes after cooking and concluded that the purines are released into the cooking medium of foods cooked in water. For cooked samples, they saw increases in purine content from 188 to 209 mg/100g forcalf meat, and from 384 to 451 mg/100g, 319 to 400 mg/100g, and from 182 to 201 mg/100g for swine spleen, liver, and meat respectively (Colling and Wolfram 1987). Another study by this same group showed decreases in purine content after cooking in ham and soy meats. For ham products results show decreases from 168 to 131 mg/100g and from 127 to 108 mg/100g, and for soy products from 355 mg/100 g in the dried product to 50 mg/100 g in the cooked product (Colling and Wolfram 1989).

Lou has done extensive work on analyzing the changes in purine content of fish products during various phases of processing (Lou and others 1996; Lou and others 1997; Lou 1998b; Lou 1998; Lou and others 1998a; Lou and others 2005a; Lou and others 2005b). Grass shrimp are a popular commodity in Asian countries and contain a higher level of total purines than any other commercial seafood product (Ho 1986; Lou and others 1996). In a study conducted by Lou (Lou and others 1998a) the effects of various cooking times on purine content of grass shrimp were analyzed. Grass shrimp were cooked in water at 100ºC and purine content was analyzed after 0, 5, 10, 15, and 20 minutes of cooking. A decrease in the total purine content was seen, with levels reducing in greater quantities with each additional 5 minutes of cooking. The reduction in total purine content at each cooking duration was significantly different ($p<0.05$). The cooking juice was also analyzed for purine content and it was concluded that the purines were released into the cooking medium. Increases in purine content in the cooking medium were seen as the cooking times increased, which correlates with the reduction seen in the shrimp at each cooking

time. It should also be pointed out that for the individual purine bases, increases were seen at certain cooking times. After 5 minutes of cooking, guanine content increases from 7.88 to 8.57 umol/g and at 10 minutes and 20 minutes, hypoxanthine and xanthine increase from the previous measurements at 5 and 15 minutes (for hypoxanthine, from 2.0 to 2.7 umol/g, and 1.4 to 1.65 umol/g, and for xanthine, 0.20 to 0.28 umol/g and 0.15 to 0.20 umol/g, respectively). It should also be noted that the purine base adenine was seen in the greatest concentrations, constituting 82% of the total purine content (Lou and others 1998a).

A study similar to the previous study was also conducted by Lou (Lou and others 1997) in which the purine content was analyzed in various fish species after cooking (in water). The results were similar to those in the previous study. The total purine content was reduced by cooking in all instances, and a large decrease was seen particularly in hypoxanthine. The total purine content levels varied depending on the particular fish species. The Cinnamon flounder species showed the greatest reduction in purine content, losing 60% of the purines after cooking based on the content of the original sample. It was concluded that the decrease is due mainly to extraction by the hot water (Lou and others 1997).

Another study conducted by Lou (Lou and others 2001) analyzed the changes in purine content of the commonly consumed fish tilapia during storage, heating, and drying. The storage conditions for the tilapia were temperatures of 20ºC, 5ºC, and -20ºC at times of 16 hours, 96 hours, and 10 weeks, respectively. There were no significant changes in adenine, guanine, and xanthine in the fish stored at 5ºC, 20ºC, and -20ºC for the respective time frames, but there was a slight decrease in hypoxanthine content in all cases. In particular the fish stored at -20ºC for 10 weeks, the hypoxanthine decreased from 4.44 mg/g to 2.66 mg/g (dry weight). Hence, long term frozen storage does have a slight impact on purine content. The heating conditions for the tilapia

included boiling, steaming, and microwaving. Samples were analyzed after boiling and steaming at 10, 20, 30, and 40 minutes, and after microwaving at 4,5,6, and 7 minutes. A steady decrease in total purine content was seen in the boiled tilapia, with the most decrease seen after 40 minutes, with the purine hypoxanthine having the greatest decrease. The steam heating treatment resulted in a lower total purine content after 30 and 40 minutes of steaming, but an increase in total purine content was seen after 20 minutes of steaming due to increases in adenine and guanine. Xanthine content was found after 30 and 40 minutes of steaming, after not being previously detected. It is hypothesized that this may have been due to the degrading of hypoxanthine related compounds due to the heat, or could have been due to drip loss of nucleotides during the steaming process. There were no significant changes in total purine content in the microwave heating method. However, adenine and hypoxanthine showed a slight decrease of 16.6% after 7 minutes of cooking. There were significant decreases in the percentage moisture for all heating methods.

For the drying method, tilapia was dried at 70ºC and 80ºC and measured after 1, 2, and 3 hours. Significant decrease in total purine content was seen at both temperatures after 3 hours based on mg/g dry weight. A greater decrease in purine content was seen during the drying at 70ºC than at 80ºC, which could be due to differences in lipid content, which were not measured in this study. These results are reported on a dry weight basis, and when converted to a weight basis, fresh tilapia has total purine content of 126g/100g and after 3 hours of drying at 70ºC and 80ºC, the total purine content increases to 218 and 334 mg/100g respectively. It was concluded that moist heat methods have a greater impact on the reduction of purine content than dry heat methods. Boiling has the greatest impact on the reduction, most likely due to extraction by hot

water and heating degradation. Hypoxanthine decreased more than any other purine in all of the processing steps, however it is also the most abundant purine in tilapia (Lou and others 2001).

A more recent study done by Lou analyzed the effects of different processing steps on the changes in purine content in tilapia surimi products (Lou and others 2005a). The processing steps for surimi products include mincing, washing, grinding, and cooking. After mincing, the sample underwent a washing step, which involved washing the surimi product in a 5:1 water/mince ratio (water temperature was kept below 5ºC) at 10, 20, and 30 minute durations, and analyzed after each duration. After the washing steps, the samples were centrifuged to remove the wash water, and moisture content was adjusted back to 80%.

Analysis showed that during the first 10 minute interval of washing the greatest reduction of purine content occurred. The mince was also washed 3 consecutive times (at 10 minute duration), which is sometimes done in surimi processing to achieve a better quality product (gel). Analysis showed that after the first washing the greatest purine reduction occurred, with hypoxanthine continually decreasing with consecutive washes. No changes in guanine occurred during either of the washing steps. It was concluded that the more washes that were performed, the greater the loss in total purine from the tilapia.

Following the washing step were the grinding and cooking steps. The grinding of the surimi involved grinding the mince in an ice bath for 5 minutes, adding 2.5% NaCl, then grinding for 2 more minutes. For the heating step, the surimi paste was stuffed into a polyvinylidene casing and heated at 90ºC for 30 minutes, then quickly cooled with running water and stored at 4ºC. After grinding and cooking, the adenine and hypoxanthine content decreased slightly. Again, there were no changes in guanine content. This may be due to the low initial levels of guanine, as well as the low solubility of guanine, which was discussed previously. In

conclusion, the washing step of tilapia surimi processing resulted in the greatest loss of purine content. The reduction was so significant that Lou concluded that this previously considered high purine content food could be classified as a middle purine content food after processing (Lou and others 2005a).

A study very similar to the previous study was also conducted by Lou (Lou and others 2005b) and the purine content of the milkfish surimi was analyzed, which is popular in Taiwan. The same results were seen as in the previous study, with the washing step causing the greatest reduction in purine content, and subsequent washes having a greater impact each time. This study also concluded that the previously classified high purine content food milkfish surimi could after processing be classified as a middle purine content food (Lou and others 2005b).

PHYSICAL PROPERTIES OF GROUND BEEF, GROUND TURKEY, BACON

According to the USDA, the projected total consumption of red meats and poultry for the year 2007 is 220 pounds per person. Red meat accounts for 53% of this total, and chicken meat accounts for 47%. This is a big change compared to 1970, when total meat consumption per person was 165 pounds per year, with the red meat consumption representing 79% and poultry meat consumption representing 21% of total meat consumption (Haley 2000; USDA 2007).

Ground beef, ground turkey, and bacon are three commonly consumed meat products in the U.S.. Table 2.3 lists the proximate composition of these samples. All samples show an increase in protein content and decreases in moisture content after cooking. The 25% fat ground beef sample and the bacon sample show decreases in fat after cooking, while the 7% fat ground beef and the ground turkey show increases in fat content after cooking (USDA 2006). Reported values for purine content of ground beef is 133 mg/100g; turkey is 150 mg/100g; and bacon is 166 mg/100g (Souci and others 2006).

Table 2.3: Proximate composition of samples (USDA 2006)

Constituents	**Content (per 100g)**		
	Raw	**Cooked**	
Ground Beef, 25% Fat		**Crumbled**	**Broiled Patty**
Water (g)	58.16	54.49	55.5
Food Energy (kcal)	293	276	278
Protein (g)	15.76	26.28	25.56
Total lipids (g)	25	18.2	18.72
Total carbohydrates (g)	0	0	0
Ash (g)	0.77	1.1	0.98
	Raw	**Cooked**	
Ground Beef, 7% Fat		**Crumbled**	**Broiled Patty**
Water (g)	71.77	60.82	64
Food Energy (kcal)	152	209	191
Protein (g)	20.85	28.88	26.22
Total lipids (g)	7	9.51	8.79
Total carbohydrates (g)	0	0	0
Ash (g)	1.02	1.32	1.05
	Raw	**Cooked**	
Ground Turkey			
Water (g)	71.97	59.42	
Food Energy (kcal)	149	235	
Protein (g)	17.46	27.36	
Total lipids (g)	8.26	13.15	
Total carbohydrates (g)	0	0	
Ash (g)	0.87	1.19	
	Raw	**Cooked**	
Bacon			
Water (g)	40.2	12.32	
Food Energy (kcal)	458	541	
Protein (g)	11.6	37.04	
Total lipids (g)	45.04	41.78	
Total carbohydrates (g)	0.66	1.43	
Ash (g)	2.51	7.43	

THE POTENTIAL USE OF ELECTROLYZED WATER FOR REDUCING PURINE CONTENT

Electrolyzed water has been used in Japan for the last thirty years as a medical product, as well as for drinking and cooking (Koseki and others 2005). Over the last decade, researchers worldwide have proven its effectiveness at reducing food borne pathogens on food products as well as on processing equipment (Venkitanarayanan and others 1999; Kim and others 2000). Electrolyzed water is easily generated by subjecting water containing a very small amount of NaCl (usually around 0.1%) to an electrical current, which results in two forms of electrolyzed water, acidic and alkaline. The acidic form normally has a pH around 2.8 and functions to effectively eliminate pathogens. The alkaline form has a pH around 11 and is commonly used as a cleaning agent to reduce organic matter, as well as for drinking and cooking (Loi-Braden and others 2005). It is hypothesized that rinsing foods in alkaline EO water may potentially reduce the purine content more than rinsing in deionized water. Since EO water has strong oxidation-reduction potential and its strong reducing potential can dissolve and detach fats and proteins, it is possible that these properties may have an effect on breaking the ring structures of purines, leading to degradation, and a decrease in the purine content in foods.

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CHAPTER 3

REDUCTION OF PURINE CONTENT IN COMMONLY CONSUMED MEAT PRODUCTS THROUGH RINSING AND COOKING[1]

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ABSTRACT

The commonly consumed meat products ground beef, ground turkey, and bacon were analyzed for purine content before and after a rinsing treatment. The rinsing treatment involved rinsing the meat samples using a wrist shaker in a 5:1 ratio, 5 parts water to 1 part sample for 2 or 5 minutes then draining or centrifuging to remove water. The total purine content of 25% fat ground beef significantly decreased ($p<0.05$) from 8.58 mg/g protein to a range of 5.17-7.26 mg/g protein after rinsing treatments. After rinsing and cooking an even greater decrease was seen ranging from 4.59-6.32 mg/g protein. The total purine content of 7% fat ground beef significantly decreased from 7.80 mg/g protein to a range of 5.07-5.59 mg/g protein after rinsing treatments. A greater reduction was seen after rinsing and cooking in the range of 4.38-5.52 mg/g protein. Ground turkey samples showed no significant changes after rinsing, but significant decreases were seen after rinsing and cooking. Bacon samples showed significant decreases from 6.06 mg/g protein to 4.72 and 4.49 after 2 and 5 minute rinsing and to 4.53 and 4.68 mg/g protein after 2 and 5 minute rinsing and cooking. Overall, this study showed that rinsing foods in water effectively reduces total purine content and subsequent cooking after rinsing results in an even greater reduction of total purine content.

INDEX WORDS: Purine content, purine bases, water rinse treatment, cooking, gout, ground beef, ground turkey, bacon

INTRODUCTION

According to the American College of Rheumatology, gout affects more than five million Americans and is considered the most common form of inflammatory arthritis in adult men (Lawrence 1998; Rheumatology 2005). Gout is a condition associated with the buildup of uric acid crystals in the joints, particularly in the lower extremities. The buildup is caused by either an overproduction or under excretion of uric acid, which is the end product of purine catabolism (Kelley and Schumacher 1993). The main sources of purines are dietary purines, those ingested in the foods we eat; therefore, gout attacks can be controlled by limiting the amount of purines consumed.

Purines are mainly associated with high protein foods and are found at highest concentrations in meat and fish products (Choi and others 2004). Studies have shown that incidences of gout are rising in the U.S.(Arromdee and others 2002; Wallace and others 2004), and according to the USDA, the projected total consumption of red meats and poultry for the year 2007 is 220 pounds per person. Red meat accounts for 53% of this total, and chicken meat accounts for 47%. Today's meat consumption is a big increase compared to that of 1970, when each person consumed about 165 pounds per year. Red meat consumption represented 79% and poultry meat consumption represented 21% of total meat consumption in 1970 (Haley 2000; USDA 2007).

The average purine content of meat products usually exceeds 100mg/100g (Brule and others 1988). It is recommended that persons suffering from gout completely avoid foods such as liver, sardines, mussels, bacon, scallops, haddock, veal, venison, turkey, and alcoholic beverages. They should only occasionally consume foods such as beef, chicken, crab, ham, kidney beans, lima beans, mushrooms, oysters, pork and shrimp (Harris and others 1999).

According to the American Dietetic Association, foods containing more than 150mg/100g of total purines should be avoided by people suffering from gout (ADA 2003).

The four purine bases are adenine, guanine, hypoxanthine, and xanthine. Adenine deaminates to form the purine base hypoxanthine, and guanine deaminates to form the purine base xanthine (Garrett 2005). Studies have shown that the individual purines have different uricogenic effects. Adenine and hypoxanthine have been shown to have a greater impact on uric acid levels than guanine and xanthine (Clifford 1976), and it has been suggested that the restriction of some foods should be based on the content of uricogenic bases rather than on the total purine content (Brule and others 1989).

Previous studies have been done to measure the amount of purines present in foods before and after processing, including storage, washing, and cooking (Young 1983; Brule and others 1989; Lou and others 1997; Lou 1998; Lou and others 2001a; Lou and others 2005). Lou has done studies (Lou and others 2001b; Lou and others 2005) on how processing steps effect the levels of purines in fish products, and his studies indicate that rinsing and cooking foods in water reduces the total amount of purines present. A study conducted by Brule and Sarwar (1989) analyzed the effects of two different cooking methods, boiling and broiling, on purine content of beef steak, beef liver, and haddock fillets. Results showed increases in total purine content for beef steak and liver for both cooking methods. Total purine content decreased in the boiled haddock but increased in the broiled haddock. This study concluded that the increases in purine content after cooking were likely due to increases in dry matter as water and fat were lost, including the proportional increase in protein content. Another study conducted by Young (1983) studied the effect of stewing on chicken meat. Slight increases in adenine and guanine

content were reported, but it was concluded these increases were due to losses of other components, such as moisture and fat.

This study was designed to reduce the purine content of ground beef, ground turkey, and bacon. These products were chosen because they are considered high purine content foods, and they are commonly consumed products in the U.S.. It is hypothesized that by rinsing these products in water at a low rate of agitation for a predetermined amount of time, the total purine content of the product will decrease. This study also determined the effect of cooking on purine content of the samples before and after the rinse treatment.

MATERIALS AND METHODS

Materials

All samples were purchased from a local grocery store, stored at 4ºC, and used within 48 hours of purchase. The 25 % fat ground beef samples were labeled 100% beef, 75% lean, 25% fat. The 7% fat ground beef samples were labeled 100% beef, 93% lean, 7% fat. Ground turkey samples were labeled Premium Quality Honeysuckle White Ground Turkey. Bacon samples were labeled Oscar Mayer Naturally Hardwood Smoked America's Favorite Bacon.

Standards of purine compounds, adenine, guanine, hypoxanthine, and xanthine, were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Triflouroacetic acid was purchased from Pierce Biotechnology (Rockford, IL), formic acid was purchased from EMD Chemicals Inc.(Darmstadt, Germany), and potassium phosphate was purchased from Mallinckrodt Baker, Inc. (Phillipsburg, NJ).

Preparation of samples

Samples were weighed out into 100 gram portions. Each 100 gram portion was placed in 250 mL deionized water (chilled to 4ºC) and rinsed using a wrist action shaker (Burrell Corporation, Model 75, Pittsburgh, PA. U.S.A). Ground beef samples underwent a 2 minute rinse and drain, a 5 minute rinse and drain and a 5 minute rinse, followed by centrifugation (Figure 3.1). Ground turkey samples underwent a 2 minute rinse and drain and a 5 minute rinse and centrifugation. Bacon samples underwent a 2 minute or 5 minute rinse and drain. The draining step involved draining the sample on cheese cloth for approximately five minutes to remove the excess water. Centrifuged samples were obtained by placing sample into a centrifuge bottle after the rinsing step and centrifuging at 14,000 rpm for 10 minutes (Beckman Model J2-21M Induction Drive Centrifuge, Palo Alto, CA). Samples were then either frozen immediately or set aside for the cooking study.

Ground beef and ground turkey samples were cooked using two different methods. One method involved sautéing the meat in scrambled form on the stovetop (Amana Self Clean Oven, Maytag Corp., Benton Harbor, MI) for approximately 3 ½ minutes at medium heat (Figure 3.2). All drippings were collected with the sample. The other method involved grilling the meat in patty form on an electric, two sided griddle (George Foreman Grilling Machine, Model GR38SIL, Lake Forest, IL) for approximately five minutes on medium heat (Figure 3.3). Drippings drained from the meat were not collected. Samples were allowed to cool for approximately 5 minutes, then they were immediately placed in a freezer bag and stored in a -20ºC freezer. Bacon samples were pan fried on the stovetop on medium-low heat for four minutes on each side. The samples were then removed from the heat and allowed to drain on paper towels for approximately 5 minutes, then they were placed a freezer bag and stored in a -20ºC freezer.

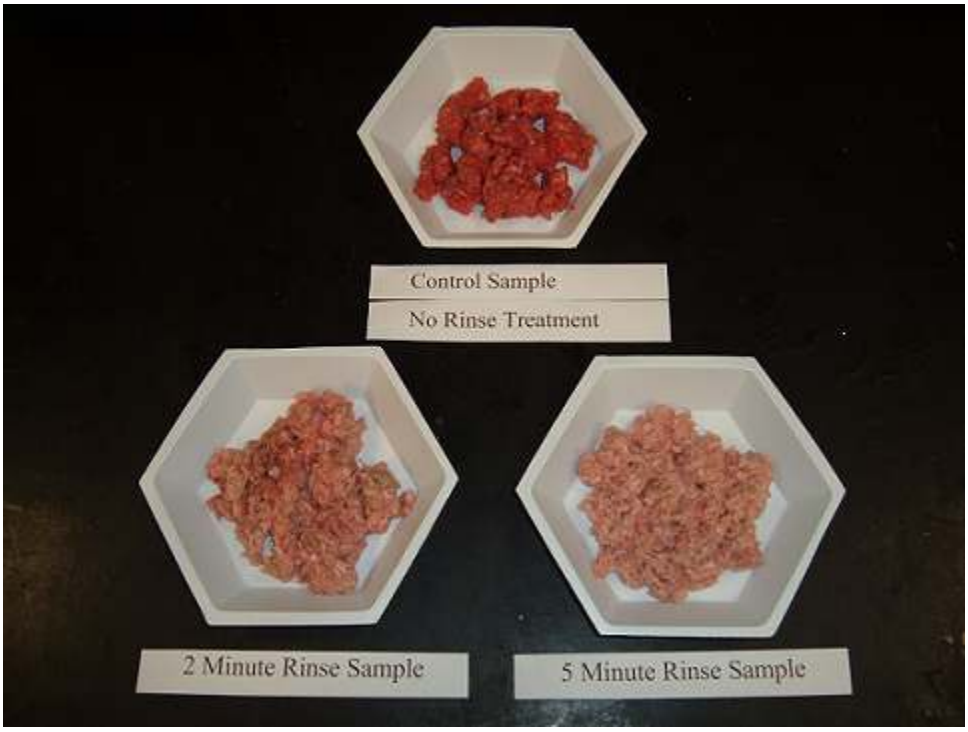


Figure 3.1: Comparison of control ground beef sample to rinsed ground beef samples

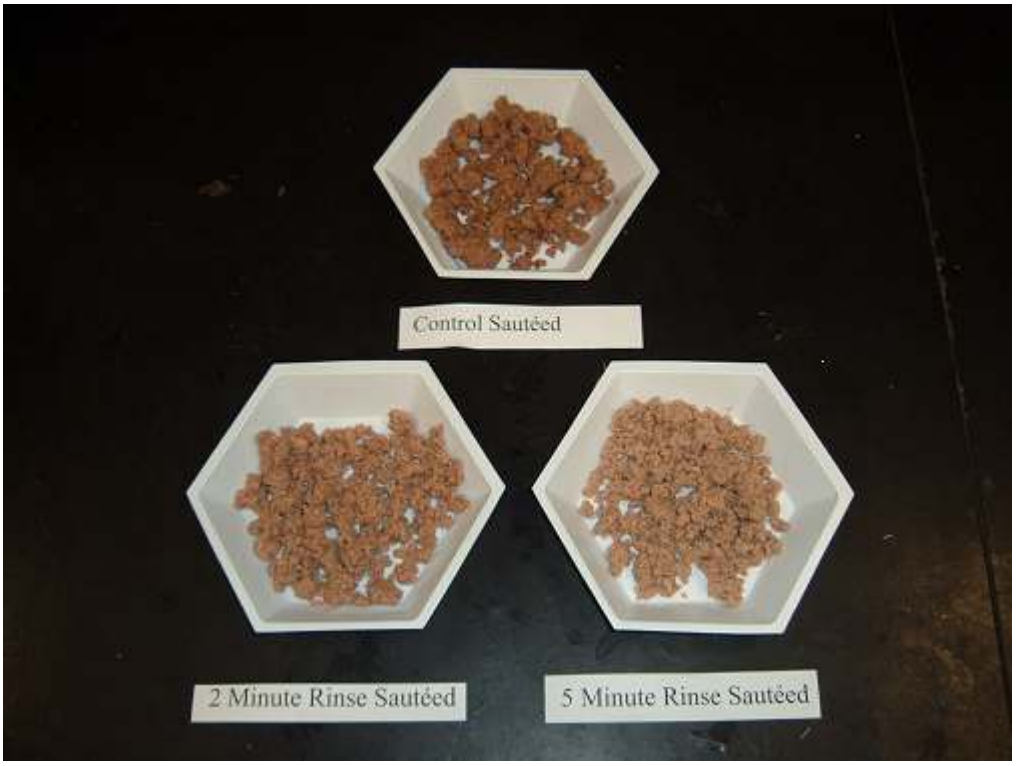


Figure 3.2: Comparison of control sautéed sample to rinsed and sautéed samples

Figure 3.3: Comparison of grilled control sample to rinsed and grilled samples

After all samples were completely frozen, they were placed in a freeze drier (Virtis Genesis 25ES, Gardiner, NY) for 24 hours at ~30 millitorr at a starting temperature of -20ºC and ending at ~25ºC to remove all of the moisture. The dried samples were then ground in an Osterizer blender for 30 seconds. 100 mg portions of dry samples were weighed and placed in glass test-tubes for further analyses.

Proximate composition determination

The moisture content of the samples was determined by vacuum drying at 70ºC for 24 hours (AOAC, 1951, Method 925.09). Total nitrogen was determined by a nitrogen combustion method (Leco, Model FP-2000, Warrendale, PA) (AOAC, 1998, Method 990.03) on moisture free samples. Protein was calculated by using a nitrogen-to-protein conversion factor of 6.25. Fat content of the samples was analyzed on moisture free samples using a Goldfisch fat extraction apparatus (Labconco, Kansas City, MO.) with petroleum ether for 24 hours (AOAC, 1999, Method 948.22a). The ash content was determined by placing the moisture free samples in a muffle furnace at 525ºC for 15 hours (Modified from AOAC, 1998, Method Ba 5-49). The carbohydrate content was calculated by subtracting the sum of the fat, protein, and ash contents from 100%.

Determination of purine contents

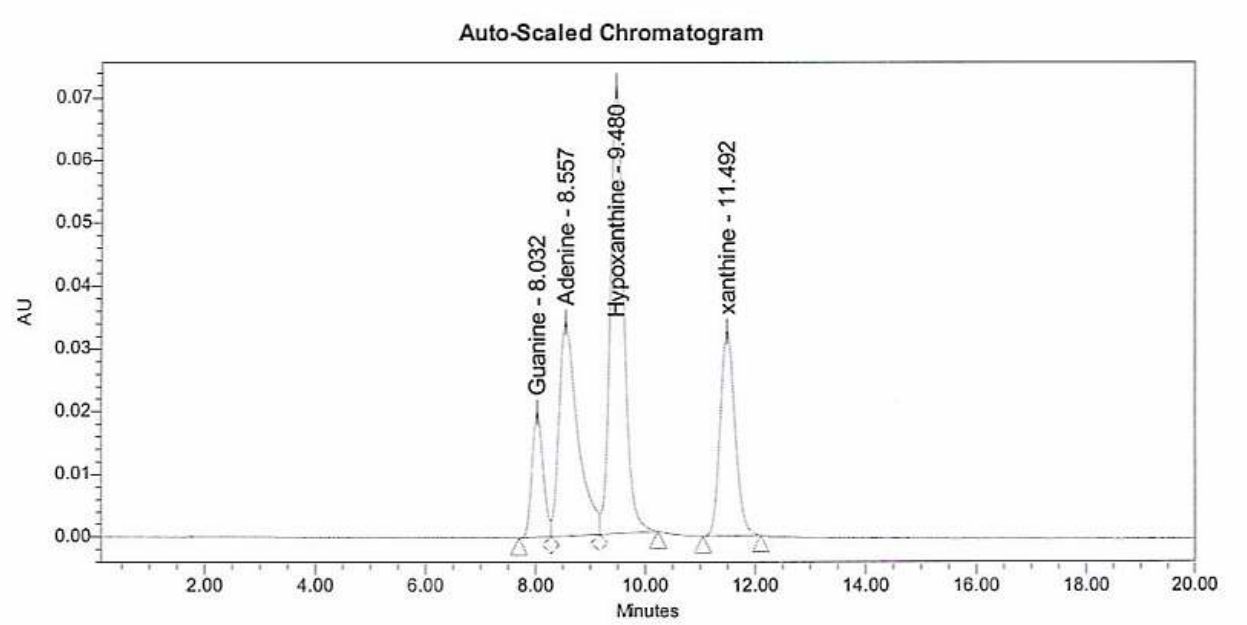
The method used by Lou and others (2005) was modified and used to determine the purine content of the samples. Five mL of a 5:5:1 mixture of CF_3COOH (Trifloroacetic acid)/HCOOH (Formic acid)/H_2O was added to 100 mg of powdered, freeze-dried sample in a capped glass test-tube. The mixture was placed in a 100ºC (Cober Electronics Microwave/Convection oven, Model LBM 1.2A/9276, Stamford, CT) oven for 35 minutes, then it was allowed to cool to room temperature. The resultant hydrolysates were placed in a 100 mL

flask and dried using a rotary evaporator (Buchi Labortechnik AG, Rotavapor R-215, Model CH-9230, Flawil, Switzerland) at 60ºC. The evaporation was repeated twice, each time with the addition of 5 mL of distilled water to the dried residue in the flask, ensuring that all acid was removed from the sample. Ten mL of KH_2PO_4 was then added to the dried residue, and the flask was placed in an ultrasonic water bath for 5 minutes to dissolve the residue. The solution was then transferred to a test tube and centrifuged for 5 minutes at 2000 rpm (International Equipment Comp., IEC Centra, Model CL-2, Needham Hts, MA). Then it was filtered through a 0.2µ nylon filter (Whatman syringe filter, Cat. No. 6870-2502, Florham Park, NJ). The purine bases were separated isocratically by high performance liquid chromatography (Waters Corporation, Model code SHC, 2695 Separations Module, Milford, MA) using a reversed phase column (Beckman Coulter ultrasphere C_{18}, 5µ, 4.6 mm * 25 cm) with an injection volume of 20 µl. The compounds were eluted off the column using a 0.02 M KH_2PO_4 buffer with pH adjusted to 3.4 using phosphoric acid (in order to achieve optimum separation). The flow rate was 1.0 mL/minute, and the elute passed through the ultraviolet detector at 254 nm. The concentration of bases was computed by calculating the area under each peak after comparison to the standard, which was in a 0.1mM solution. Identification of peaks was based on order of elution using known standard compounds (Figure 3.4). Data was analyzed using Millenium 32 software and Microsoft Excel.

Statistical analysis

Data was analyzed using analysis of variance, ANOVA, at a significance level of $\alpha > 0.05$, using SAS-JMP software. Mean comparisons were performed using Student's t Test (SAS 2004).

(a)



(b)

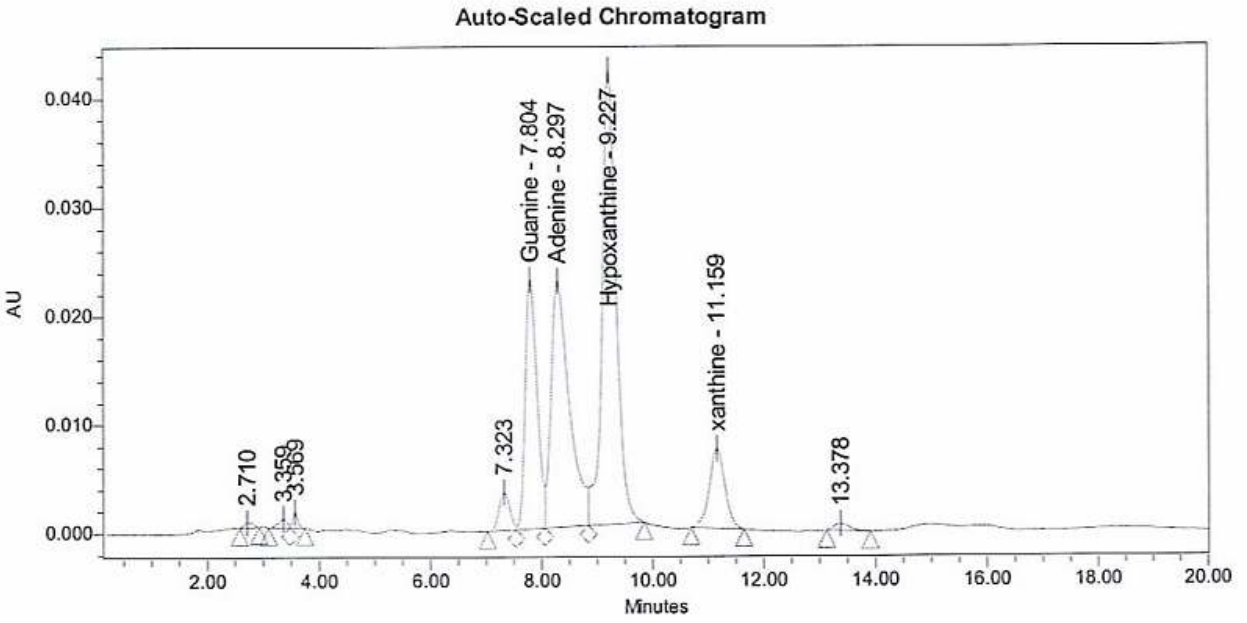


Figure 3.4: HPLC generated chromatogram of the four purine bases. (a) Standard purine solution. (b) Sample of ground beef

RESULTS AND DISCUSSION

Proximate composition and purine content of raw ground beef, ground turkey, and bacon are shown in Table 3.1. The 7% fat ground beef sample had the highest protein content and the bacon sample had the highest fat content of all the samples. The acid hydrolysis method used to determine the purine content of the samples releases the purine bases from nucleosides, nucleotides, and nucleic acid, meaning the total purine content of the samples are accurately represented (Lou and others 2005). Xanthine was the most abundant purine base present in the 25% fat ground beef and 7% fat ground beef constituting 44% and 35% of the total purine present, respectively. Guanine was the most abundant base present in the ground turkey, constituting 41% of the total purine content, and hypoxanthine was the most abundant purine base in the bacon, constituting 54% of the total purine content. Adenine was the least present base in the ground beef samples, and xanthine was the least present base in the ground turkey and bacon samples. The 7% ground beef samples had the highest purine content of all the samples tested at 5.57 mg/g dry basis (equal 157.85 mg/100 g wet basis), followed in decreasing order by 25% fat ground beef, bacon, and turkey samples.

The changes in purine content after rinsing and cooking are shown in Table 3.2 and Table 3.3. For the 25% fat ground beef, the total purine content significantly decreased ($p<0.05$) from 3.58 mg/g in the original sample to 3.07, 2.79, and 2.29 mg/g for the 2 minute rinse, 5 minute rinse, and 5 minute centrifuge, respectively. Hypoxanthine and xanthine also showed significant decreases during the rinsing steps. There was an increase in total purine content from 3.58 mg/g to 5.11 and 4.65 mg/g after sautéing and grilling, however purine content decreased when the samples were rinsed and cooked. For the sautéed samples, purine content decreased from 5.11 mg/g in the sautéed control to 3.06 and 2.52 mg/g in the rinsed and sautéed samples. For the

Table 3.1: Proximate composition and purine contents of ground beef, ground turkey, and bacon

	25% Fat Ground Beef	7% Fat Ground Beef	Ground Turkey	Bacon
Moisture (%)	60.81	71.66	69.18	30.61
Fat (%)	56.61	24.3	42.09	59.38
Ash (%)	1.73	3.3	2.85	5.36
Protein (%)	41.08	72.25	54.91	31.88
Carbohydrate (%)	0.58	0.15	0.15	3.4
Adenine (mg/g)	0.34	0.59	0.96	0.34
Guanine (mg/g)	0.97	1.58	1.58	0.54
Hypoxanthine (mg/g)	0.68	1.45	1.22	1.05
Xanthine (mg/g)	1.58	1.96	0.11	N.D.
Total purine (mg/g) dry wt.	3.58	5.57	3.88	1.93
Total purine (mg/100g) wet wt.	140.69	157.85	119.58	133.92

Fat, protein, and ash content are expressed on a moisture free basis; carbohydrate content was determined as 100% - (ash + fat + protein).

Table 3.2: Changes in purine content of 7% and 25% ground beef after rinsing and cooking[1]

Ground Beef	Protein (%)	Fat (%)	Ade*	Gua*	Hypo*	Xan*	Total Purine[2] (mg/g dry wt.)	Total Purine (mg/g protein)
25% Fat Control	41.08	56.61	0.34^{d}	0.97^{e}	0.68^{c}	1.58^{b}	3.58^{c}	8.58^{a}
Control, Sauteed	59.89	39.69	0.55^{a}	1.48^{c}	1.30^{a}	1.79^{a}	5.11^{a}	8.54^{b}
Control, Grilled	64.91	31.89	0.58^{a}	1.71^{a}	1.16^{b}	1.21^{c}	4.65^{b}	7.17^{cd}
2 M R and D[3]	42.49	55.8	0.33^{d}	0.94^{a}	0.59^{d}	1.21^{c}	3.07^{de}	7.26^{b}
5 M R and D[3]	43.81	56	0.36^{d}	0.99^{de}	0.48^{e}	0.95^{d}	2.79^{ef}	6.41^{bc}
5 M R and C[3]	44.43	55.5	0.32^{d}	1.01^{de}	0.32^{fg}	0.64^{e}	2.29^{g}	5.17^{ef}
2 M R and D , Sauteed	46.85	52.78	0.43^{c}	1.14^{d}	0.41^{ef}	1.09^{cd}	3.06^{de}	6.32^{bcd}
2 M R and D, Grilled	58.13	39.75	0.50^{b}	1.48^{bc}	0.37^{f}	0.99^{d}	3.33^{cd}	5.74^{cde}
5 M R and C, Sauteed	46.22	54.61	0.41^{c}	1.14^{d}	0.26^{g}	0.70^{e}	2.52^{fg}	5.48^{de}
5 M R and C, Grilled	62.01	42.44	0.49^{b}	1.63^{ab}	0.22^{g}	0.55^{e}	2.90^{e}	4.59^{f}
7% Fat Control	72.25	24.3	0.59^{ab}	1.58^{c}	1.45^{a}	1.96^{a}	5.57^{a}	7.80^{a}
Control, Sauteed	73.71	22.42	0.50^{d}	1.75^{abc}	1.25^{b}	1.99^{a}	5.49^{a}	7.46^{ab}
Control, Grilled	71.5	24.14	0.51^{e}	1.83^{ab}	1.15^{b}	1.78^{a}	5.27^{a}	7.36^{b}
2 M R and D[3]	73.08	26	0.61^{ab}	1.85^{a}	0.65^{de}	0.94^{bc}	4.05^{bc}	5.59^{c}
5 M R and D[3]	71	26.9	0.65^{a}	1.89^{a}	0.53^{f}	0.77^{cd}	3.83^{bcd}	5.35^{cd}
5 M R and C[3]	73.48	25.3	0.65^{a}	1.79^{ab}	0.52^{f}	0.78^{cd}	3.74^{cd}	5.07^{de}
2 M R and D, Sauteed	74.94	23.21	0.51^{d}	1.75^{ab}	0.84^{c}	1.03^{b}	4.13^{b}	5.52^{cd}
2 M R and D, Grilled	73.35	22.02	0.55^{cd}	1.65^{bc}	0.64^{de}	0.96^{bc}	3.81^{cd}	5.19^{de}
5 M R and C, Sauteed	76.13	21.58	0.62^{ab}	1.66^{bc}	0.68^{d}	0.61^{de}	3.58^{de}	4.75^{ef}
5 M R and C, Grilled	76.63	22.98	0.58^{abcd}	1.74^{abc}	0.55^{ef}	0.49^{de}	3.36^{e}	4.38^{f}

1. Mean values (n=3) in a column not followed by the same letter were significantly different ($p<0.05$).
2. Total purine = Ade+Gua+Hyp+Xan (due to rounding, the last digit for the total purine content may not be the same as the sum of the individual purines)
3. 2 minute rinse and drain, 5 minute rinse and drain, 5 minute rinse and centrifuge
* Ade= Adenine, Gua= Guanine, Hypo=Hypoxanthine, Xan= Xanthine, all reported in mg/g dry wt.

Table 3.3: Changes in purine content of ground turkey and bacon after rinsing and cooking[1]

Ground Turkey	Protein (%)	Fat (%)	Ade*	Gua*	Hypo*	Xan*	Total Purine[2] (mg/g dry wt.)	Total Purine (mg/g protein)
Control	55%	42.09	0.96^{c}	1.58^{e}	1.22^{a}	0.11^{ab}	3.88^{bcd}	7.07^{abc}
Control, Sauteed	56.85	37.98	1.07^{bc}	1.75^{de}	1.27^{a}	0.12^{a}	4.21^{abcd}	7.46^{a}
Control, Grilled	63.17	34.23	1.18^{b}	2.02^{bc}	1.23^{a}	0.11^{b}	4.54^{a}	7.19^{ab}
2 M R and D[4]	55.91	42.45	1.03^{bc}	1.66^{de}	1.01^{bc}	0.10^{c}	3.80^{cd}	6.70^{abc}
5 M R and C[4]	56.66	41.78	1.12^{b}	1.79^{cde}	0.86^{c}	N.D.[3]	3.77^{d}	6.64^{abc}
2 M R and D, Sauteed	59.3	39.16	1.12^{b}	1.95^{bcd}	1.07^{b}	0.11^{ab}	4.25^{abcd}	7.16^{abc}
2 M R and D, Grilled	62.38	37.63	1.11^{bc}	1.80^{cde}	0.84^{d}	0.10^{c}	3.86^{cd}	6.18^{c}
5 M R and C., Sauteed	63.35	35.87	1.33^{a}	2.20^{ab}	0.74^{de}	0.09^{d}	4.34^{abc}	6.88^{abc}
5 M R and C., Grilled	69.58	30.47	1.40^{a}	2.34^{a}	0.62^{e}	N.D.[3]	4.37^{ab}	6.28^{bc}
Bacon	**Protein (%)**	**Fat (%)**	**Ade***	**Gua***	**Hypo***	**Xan***	**Total Purine[2] (mg/g dry wt.)**	**Total Purine (mg/g protein)**
Control	31.88	59.38	0.34^{c}	0.54^{bc}	1.05^{a}	N.D.[3]	1.93^{b}	6.06^{a}
Fried	42.07	41.44	0.41^{b}	0.65^{b}	1.14^{a}	N.D.	2.21^{ab}	5.23^{b}
2 M R and D	29.98	69.02	0.34^{c}	0.43^{c}	0.64^{b}	N.D.	1.40^{c}	4.72^{c}
5 M R and D	30.64	66.32	0.32^{c}	0.46^{c}	0.59^{b}	N.D.	1.37^{c}	4.49^{c}
2 M R and D, Fried	53.94	37.43	0.48^{a}	0.87^{a}	1.09^{a}	N.D.	2.44^{a}	4.53^{c}
5 M R and D, Fried	55.18	37.53	0.52^{a}	0.93^{a}	1.14^{a}	N.D.	2.59^{a}	4.68^{c}

1. Mean values (n=3) in a column not followed by the same letter were significantly different ($p<0.05$).
2. Total purine = Ade+Gua+Hyp+Xan (due to rounding, the last digit for the total purine content may not be the same as the sum of the individual purines)
3. N.D.= not detected
4. 2 minute rinse and drain, 5 minute rinse and centrifuge

* Ade=Adenine, Gua=Guanine, Hypo=Hypoxanthine, Xan=Xanthine, all reported in mg/g dry wt.

grilled sample, purine content decreased from 4.65 mg/g in the control grilled to 3.33 and 2.90 mg/g in the rinsed and grilled samples. All individual purine bases, with the exception of guanine, showed significant decreases from the content of the control sample after the rinsing and cooking steps.

For the 7% fat ground beef sample, there was significant decrease in total purine from 5.57 mg/g in the original sample to 4.05, 3.83, and 3.74 mg/g for the 2 minute rinse, 5 minute rinse, and 5 minute centrifuge, respectively. For the individual purine bases, significant decreases after rinsing were seen in the hypoxanthine and xanthine, while increases were seen in the adenine and guanine. There was no significant change in total purine content or change in any of the individual purine bases after grilling and sautéing; however, significant decrease was seen in total purine content from 5.49 mg/g in the sautéed sample with no treatment to 4.13 and 3.58 mg/g in the rinsed and sautéed samples. A purine decrease was also seen from 5.27 mg/g in the grilled sample with no treatment to 3.81 and 3.36 mg/g for the rinsed and grilled samples. For the individual purine bases, hypoxanthine and xanthine, significant decreases were observed from the cooked control samples when compared to the rinsed and cooked samples.

Ground turkey had a total purine content of 3.88 mg/g sample and after rinsing there was insignificant decrease to 3.80 mg/g for the 2 minute rinse and 3.77 mg/g for the 5 minute rinse and centrifuge. Hypoxanthine and xanthine showed significant decreases in purine content after rinsing, while adenine showed significant increase for the 5 minute rinse and insignificant increase for the 2 minute rinse. Guanine showed insignificant increases for both rinses. There were significant increases from the control to the sautéed and cooked samples, from 3.88 mg/g to 4.21 and 4.54 mg/g. There was only one significant decrease from the cooked control samples to the rinsed and grilled samples. The significant decrease was for the 2 minute rinse, drained, and

grilled sample, from 4.54 mg/g to 3.86 mg/g. For the individual purine bases, adenine showed an increase from the cooked control sample compared to the rinsed and cooked in all cases and hypoxanthine showed significant decreases from the cooked control sample when compared to the rinsed and cooked samples in all cases.

Bacon had a total purine content of 1.93 mg/g dry wt.. After rinsing, there was significant decrease to 1.40 and 1.37 mg/g for the 2 minute rinse and drain sample and the 5 minute rinse and drain sample. Hypoxanthine was the only individual purine base that showed significant decrease from the control compared to the rinsed sample. The cooked bacon resulted in an increase in purine content from 1.93 mg/g to 2.21 mg/g, and all individual proportions of purine bases increased after cooking. Subsequent cooking of the rinsed samples caused an increase in total purine content from the cooked control sample, from 2.21 mg/g to 2.44 and 2.59 mg/g. The individual purine bases adenine and guanine showed significant increases from the cooked control samples to the rinsed and cooked samples.

As mentioned previously, other studies have reported increases in purine content after cooking and suggest that this is likely due to changes in other components, such as fat and moisture (Young 1983; Brule and others 1989). For this reason, all samples were also calculated in mg purine/g protein to account for decreases of moisture and fat and proportional increases of protein during the rinsing and cooking steps. This form more accurately represents the actual purine content of the samples. For the 25% fat ground beef samples there was significant decrease from the control sample compared to the rinsed samples, from 8.58 mg/g protein to 7.26, 6.41, and 5.17 mg/g protein. There was also significant decrease in total purine content from the cooked control at 8.54 mg/g protein to 6.32, 5.74, 5.48, and 4.59 mg/g protein in the rinsed and cooked samples. For the 7% fat ground beef samples, similar results were seen with

significant decreases from the control at 7.80 mg/g protein to the rinsed samples at 5.59, 5.35, and 5.07 mg/g protein. Significant decreases were also observed from the control cooked samples at 7.46 mg/g protein to the rinsed and cooked samples at 5.52, 5.19, 4.75, and 4.38 mg/g protein. The ground turkey samples showed no significant differences between the control samples and the rinsed samples. Significant decreases were seen from the cooked control sample at 7.46 mg/g protein for the sautéed sample; 7.19 mg/g protein for the grilled sample; and the 2 and 5 minute rinsed and grilled samples at 6.18 and 6.28 mg/g protein. For the bacon samples, significant differences were seen from the control at 6.06 mg/g protein to the rinsed samples at 4.72 and 4.49 mg/g protein and from the control to the cooked control sample at 5.23 mg/g protein. Significant differences also resulted from rinsing and cooking samples compared to the control, from 5.23 mg/g protein to 4.53 and 4.68 mg/g protein.

In summary, the rinsing step resulted in significant decreases ($p<0.05$) in the 25% and 7% ground beef samples and in the bacon samples. For both ground beef and bacon samples the rinsing procedure that resulted in the greatest decrease in purine content was the 5 minute centrifuge sample and all samples showed a decrease from the raw control sample. All samples that were rinsed then cooked showed a decrease in purine from the control cooked sample. The results indicate that rinsing has an impact on reducing the purine content of ground beef and bacon, with even greater decreases seen after rinsing and subsequent cooking. For the ground turkey samples, the results show that rinsing and grilling has a significant impact on reducing the purine content. The 25% fat ground beef, 7% fat ground beef, ground turkey and bacon had initial purine content of 3.58, 5.57, 3.88, and 1.93 mg/g dry wt., respectively. On this basis the 7% fat ground beef has higher total purine content than the 25% fat ground beef samples, as well as the ground turkey and bacon samples. After converting these values to mg/g protein basis,

total purine contents of the raw samples were 8.58, 7.80, 7.07, and 6.06 mg/g protein, respectively. The results show that the 25% fat ground beef sample contains the highest purine content based on protein content. Also, ground turkey and bacon samples had higher combined levels of adenine and hypoxanthine than the ground beef samples. Since these purines have been shown to result in greater increases in uric acid in the body (Clifford, 1976), ground turkey and bacon may have more of an impact on causing gout attacks than ground beef.

Previous studies have shown decreases in purine content after cooking (Young 1983; Lou and others 1997; Lou and others 1998; Lou and others 2001a; Lou and others 2005) and after washing (Lou and others 2005). Studies analyzing the effect of washing on purine content have only studied these effects on seafood products. Tilapia surimi products undergo a washing step as part of processing to improve the gel quality of the surimi. One study analyzed the effect of washing on the purine content of the tilapia surimi (Lou and others 2005). The washing step involved washing the surimi minse using a stirrer set at 100 rpm in cold water at a ratio of 5:1 water/sample and then centrifuging the mince for 2 minutes to remove the water. This procedure resulted in a decrease in total purine content by over 30%, concluding that the bases were released into the rinse water and that this previously high purine content food could was actually a medium purine content food (Lou and others 2005). Similar results were seen in the current study, which resulted in the average rinsing steps decreasing the total purine content by 27% for 25% ground beef, 32% for 7% ground beef, and 24% for bacon.

The effects of cooking have also proven to have an impact on decreasing the purine contents of other food products, particularly moist heat methods, such as boiling and steaming (Lou and others 2001a). A study analyzing the effect of boiling on purine content of various fish species showed a decrease in purine content of the Cinnamon flounder fish species by 60%, from

91.2 to 46.6 mg/100g on a dry wt. basis (Lou and others 1997). Decreases have also been shown during boiling of grass shrimp (Lou 1998). In this experiment, purine content was measured after every 5 minutes of cooking, and a decrease was seen each time. The greatest decrease was seen after 20 minutes of cooking, from 58.18 μmol/g fresh shrimp to 44.14 μmol/g after cooking (on a dry wt. basis). Other studies have reported increases in purine contents after cooking, but these studies concluded that this result was likely due to the decreases in other constituents, such as fat and moisture (Young 1983; Brule and others 1989). Brule reported purine increases in beef steak and beef liver after boiling and broiling, but he also analyzed the cooking juices and discovered that the purines were indeed being released into the cooking juices. The purine content of the raw beef steak was 105.9 mg/100 g and the purine content of the cooking juices was 58.88 mg/100 g for the beef steak. The purine content of the raw beef liver was 202.2 mg/100g, and the content in the cooking juices was 49.17 mg/100 g (on a wet wt. basis) (Brule and others 1989).

Electrolyzed water has recently gained recognition as an effective method of reducing food borne pathogens and has been used in Japan for the past thirty years as a medical product, as well as for drinking and cooking (Kim, 2000, Koseki, 2005). Electrolyzed water is generated using an electrolyzed water generator that operates by subjecting deionized water containing a very small amount of NaCl (usually around 0.1%) to an electrical current, which results in two forms of electrolyzed water, acidic and alkaline. The acidic form normally has a pH around 2.8 and functions to effectively eliminate pathogens. The alkaline form has a pH around 11 and is commonly used as a cleaning agent to reduce organic matter, as well as for drinking and cooking (Loi-Braden, 2005). Over the last decade, researchers worldwide have proven the effectiveness of electrolyzed water at reducing food borne pathogens on food products as well as on

processing equipment (Venkitanarayanan 1999; Kim 2000). In general, the purine bases have higher pKa values which represent weak acids. It is theorized that when food products containing high levels of purines are rinsed in alkaline EO water, a greater transfer of purines into the rinse water will occur. Preliminary analysis was conducted using alkaline EO water as the rinsing medium compared to deionized water on 25% fat ground beef and chicken meat samples. These samples were mixed in a 5:1 ratio and 10:1 ratio of EO water to sample, and mixing involved using a Hobart mixer set on low speed for 5 minutes. They were then drained for 5 minutes and subsequently frozen. The EO water that was used had a pH of 11.37 and an ORP of -837 mV. Results show a slightly greater decrease using the EO water as opposed to the regular tap water for the chicken samples, but there wasn't much difference for the ground beef samples (Table 3.4).

CONCLUSIONS

The process of rinsing does have a significant impact on reducing the purine content of high purine foods such as ground beef and bacon. It is hypothesized that the purines are being released into the rinse water. An even greater reduction in purine content occurs after rinsing and subsequent cooking.

OUTLOOK

Further research should be conducted on rinsing procedures to determine if altering this procedure could result in an even greater reduction in purine content. Rinsing studies should also be performed on other high purine content foods, and the rinse water should be analyzed for purine content. The effects of rinsing with alkaline electrolyzed water should also be further investigated.

Table 3.4: Comparison of purine content in chicken meat and ground beef rinsed in alkaline electrolyzed water vs. deionized water.

Chicken Meat, mg/g dry wt. basis					
	Adenine	Guanine	Hypo	Xanthine	Total Purine
Control	0.81	4.67	1.37	0.32	7.18
5:1 EO	0.69	3.44	0.44	0.12	4.69
10:1 EO	0.68	3.46	0.30	0.09	4.53
5:1 DI	0.78	4.02	0.45	0.11	5.36
10:1 DI	0.81	3.94	0.32	0.12	5.19
Ground Beef, mg/g dry wt. basis					
	Adenine	Guanine	Hypo	Xanthine	Total Purine
Control	0.33	0.78	0.63	1.99	3.74
5:1 EO	0.37	1.08	0.24	0.56	2.25
10:1 EO	0.33	0.92	0.23	0.55	2.03
5:1 DI	0.32	0.88	0.27	0.64	2.11
10:1 DI	0.36	0.98	0.26	0.66	2.27

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CHAPTER 4

SUMMARY AND CONCLUSIONS

This thesis described the effect of rinsing and cooking on the individual and total purine contents in foods. The rinse treatment involved rinsing 25% fat ground beef, 7% fat ground beef, ground turkey, and bacon samples in a 5:1 ratio (water: sample) for 2 or 5 minutes. Water was then removed by draining or centrifugation. The cooking methods for the ground beef and ground turkey samples were sautéing and grilling. The cooking method for the bacon samples was pan frying.

The total purine content of 25% fat ground beef significantly decreased ($p<0.05$) from 8.58 mg/g protein to a range of 5.17 – 7.26 mg/g protein after rinsing treatments. After rinsing and cooking, an even greater decrease was seen, with total purine ranging from 4.59 – 6.32 mg/g protein. The most abundant individual purine base in the raw sample was xanthine, and the most abundant individual purine base after rinsing and cooking was guanine. The total purine content of 7% fat ground beef significantly decreased from 7.80 mg/g protein to a range of 5.07 – 5.59 mg/g protein after rinsing treatments. A greater reduction was seen after rinsing and cooking, resulting in a purine range of 4.38 – 5.52 mg/g protein. The most abundant purine base present in this raw sample was xanthine, and the most abundant purine base present after rinsing and cooking was guanine. Ground turkey samples showed no significant changes after rinsing, but significant decreases were seen after rinsing and cooking. The ground turkey sample had higher levels of guanine than the other samples, with guanine being the most abundant individual purine base found in the raw sample. Guanine was also the most abundant base present after rinsing

and cooking. Since guanine is the only purine base that is insoluble in water, its elevated levels in ground turkey may be one reason very little significant reduction was observed in the ground turkey samples after the rinsing treatments. Bacon samples showed significant decreases in purine content from 6.06 mg/g protein to 4.72 and 4.49. After 2 and 5 minute rinsing and cooking, purine was further reduced to 4.53 and 4.68 mg/g protein, respectively.

For 25% fat ground beef and 7% fat ground beef, the percent reductions in purine comparing the cooked control sample to the average of the rinsed and sautéed samples was 31% and 28%, respectively, and the percent reduction for the average of the rinsed and grilled samples was 31% and 35%, respectively. For the ground turkey samples, the percent reduction from the cooked control to the average rinsed and sautéed samples was 6%, which is insignificant. Only one rinsed and grilled sample showed significant purine reduction from the cooked control ground turkey; the other rinsed and grilled sample showed insignificant reduction. The average reduction in purine content for these two samples was 13%. For the bacon samples, the percent reduction from the control cooked sample to the rinsed and cooked sample was 12%.

Overall, this study shows that rinsing is an effective method of reducing the purine content in certain high purine food products. Reducing the purine content of foods high in purines may result in a decreased risk of gout attacks in susceptible individuals. Further research should be conducted on other high purine foods to determine the potential reducing effect that rinsing may produce.