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Determination of Cane Berry Pomaces Benefits Through In Vitro Model for Human Colonic Fermentation

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DETERMINATION OF CANE BERRY POMACES BENEFITS THROUGH IN VITRO MODEL FOR HUMAN COLONIC FERMENTATION

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ABSTRACT

Cane berry pomaces have traditionally been considered waste products with little or no value. Yet these pomaces' high levels of phenolic compounds such as anthocyanins, is a good source of dietary fiber. Because the pomaces are low in sugar and rich in fiber and dietary antioxidants, they have potential as food ingredients for the health food market. The pomace delivers health benefits associated with high fiber and the antioxidant polyphenolic compounds associated with whole fruits and juices without the high sugar content.

Some dietary fibers are substrates for anaerobic fermentation by the microbiome in the lower gastrointestinal (GI) tract. The anaerobic fermentation of dietary fiber results in a lowering of the pH in the biomass and production of short chain fatty acids. In this study, a model system mimicking colonic fermentation in the digestive tract was developed and used to assess fermentation of cane berry pomace by colonic bacteria. Pomace samples from blueberry and black raspberry, and Hi-Maize© resistant starch (a high amylose cornstarch used as the positive control) were treated with pepsin followed by pancreatin digestion to simulate the digestive changes in the upper gastrointestinal (GI) tract. After the digestion, the remaining undigested material was washed and air dried. The fiber was then fermented anaerobically with an inoculum of colonic bacteria prepared from fecal donations of healthy volunteers.

The fermentation of the blueberry and black raspberry pomaces using the inoculum from 5 individuals resulted in a wide range of SCFA production. Fermentation with Hi-maize© resistant starch resulted in production of higher concentrations of SCFAs compared to the cane berry pomace substrates. An inoculum, prepared by mixing individual stool samples from five individuals, provided a microbiota that

represented a broader population, thus resulting in a more generalized result. The production of acetate, propionate and butyrate was significantly higher in the pooled sample compared to results from individual donors.

A different group of 5 individuals consumed a diet rich in resistant starch (RS) for a month and at the end of that period their stool samples were collected and used to prepare a pooled inoculum. The pooled inoculum from subjects with RS-fortified diet produced higher level of SCFAs than the pooled inoculum with non-fortified diet expect the Hi-Maize© RS which did not show difference at 0.05 significance level.

INTRODUCTION

SIGNIFICANCE OF RESEARCH

Overweight and obesity are defined as an abnormal or excessive gain in body fat and are associated with many chronic diseases including diabetes, cardiovascular disease and some cancers. The body mass index (BMI=mass/height^2) is an approximate measure of human body shape and obesity conditions based on the individual mass and height (Flegal et al., 2001, McOrist et al., 2011, Meyer et al., 2011). A BMI equal or greater than 25 is considered as overweight and a BMI over 30 is considered as obese (Cook et al., 2005, Flegal et al., 2001, McOrist et al., 2011, Meyer et al., 2011).

The worldwide occurrence of obesity has doubled since 1980. In the US, overweight and obesity conditions occur in more than two thirds (68.8%) of the adult population and in about a third (~31.8%) of children and adolescents (Ogden et al., 2012). In 1995 obesity alone was about 15.9% of the population and that number increased to 35.5% by 2010 (Finkelstein et al., 2009, Flegal et al., 2012). In addition the impact on individual's quality of life, medical cost of obesity and related conditions such as diabetes, coronary heart diseases and some cancers is estimated to be $147 billion per year in the U.S.(Finkelstein et al., 2009).

Although there are many diverse causes for obesity, most cases are the result of an energy imbalance. When the energy from food intake is greater than the energy expended through physical activity, the excess energy is stored in the body as fat (Swinburn et al., 2009). Additional, such as stress and genetic make-up, can further complicate energy balance and promote an even greater weight gain (Apovian, 2010). Typically health professionals recommend more physical activity and a balanced diet

which matches caloric expenditure to prevent obesity or promote weight loss. This is difficult for many people to accomplish with a more sedentary lifestyle and since the typical American diet includes excessive consumption of energy dense food. Over the last decades, a significant increase of the portion size and caloric contents of food products has been observed and this increase leads to more consumption and is a contributing factor to obesity and related conditions (Schwartz and Byrd-Bredbenner, 2006).

In recent years the food industry has introduced many products such as low fat, low sugar, and reduced portions to help meet the consumer needs, but one of the most beneficial approaches is to decrease the energy density of foods by increasing the level of dietary fiber. Modern food processing such as flour milling or juice production, however, removes much of the dietary fiber in plant products leaving highly digestible sugars and starches in the food. Dietary fiber includes both non-fermentable cellulose fiber and fermentable fiber such as pentosans, gums and fructooligiosaccharides. Plant fiber increases food volume without increasing energy density, thus providing satiety (Gropper and Smith, 2013). In addition to diluting the dietary energy, fibers can aid in digestion, slow the absorption of glucose, and improve the absorption of nutrients, particularly minerals (King et al., 2012, Lanza et al., 1987, Miyazato et al., 2010). When reaching the lower GI tract, fermentable fiber can be fermented by gut microflora to balance intestinal pH and produce short chain fatty acids. (Asa Henningsson, 2001, Parrett et al., 1997).

The typical American diet is below the recommended daily intake for total fiber. Adults should consume 25-38 grams per day (Institute of Medicine . Standing Committee on the Scientific Evaluation of Dietary Reference and Institute of Medicine .

Panel on, 2005). According to the American Heart Association; the average American consumes an average of 15 grams of fiber per day (Marlett et al., 2002). Some consumers prefer taking fiber supplements (from refined sources) rather than consume whole grain and vegetable products that are naturally rich in fiber, but often considered less desirable because of flavor and texture. An alternative to using supplements or adding refined fiber to foods is to produce and incorporate a low cost fiber into highly palatable food products. Adding a low cost fiber to food would increase the fiber content of food products, thus increasing satiety and lowering energy density.

In Juice processing, most of the fiber is removed, thus removing one of the major benefits of fruit consumption. The pomace, including skin, seeds, and pulp from fruit and vegetables after juice pressing, is produced from cane berry fruits such as grape, raspberry, cranberry and blueberry. Currently pomace is an underutilized by-product and is considered a waste product with little value; it is typically used as animal feed or field dressings. However, many of the health promoting compounds, such as anthocyanins and other phenolics, and 70 to 80% of the dietary fiber, remain in the pomace. Novel reprocessing methods have made the pomace a value added product that can be used in foods to increase the phytonutrient value and fiber content. We hypothesize that fiber rich co-products removed during processing can be reintroduced to the diet without affecting the taste and texture of the food.

The fiber in pomace not only decreases the energy density of foods, but has a functional value since it is fermented by microflora in the lower gut. Each source of pomace produces a unique composition of substrate that can provide an advantage to certain microflora. The symbiotic combination of prebiotics (substrate used as food for the gut microflora) and probiotics (specific microflora species unique to the substrate)

results in the production of specific end products including enzymes, peptides and short chain fatty acids (SCFA). The SCFAs are utilized by the epithelial cells in the colon and some portions are absorbed and enter the circulatory system. SCFAs, primarily acetate, propionate and butyrate are produced when dietary fiber is fermented in the colon. Acetate and propionate are readily absorbed and enter the peripheral circulation to be metabolized by the peripheral tissues while the butyrate is used by the colonocytes as primary source of energy (McOrist et al., 2008). SCFAs are important to colonic and systemic health and can reduce the risk of developing gastrointestinal disorders, cancer, cardiovascular disease, obesity and obesity related disorders. Therefore, inclusion of dietary fiber which is readily fermented to SCFAs could provide health benefits beyond simple energy dilution.

OBJECTIVE

The distribution of microflora in the individual biome is influenced by a number of factors including “inoculation” in early life, genetics and environmental factors including diets. These factors help account for the large diversity in the microflora found in the large intestines of individuals across many populaces. Diets play a very important role in the species and the number of bacteria that flourish at any given time in the gut. The purpose of this study is to determine the differences in fermentation end products resulting from different dietary berry pomace fibers. This project was designed to assess the influence of anaerobic fermentation on dietary fibers from berry pomaces that are resistant to digestion in a model system. The pomace will be predigested with pepsin followed by pancreatin pre-digestion which will concentrate the non-digestible oligosaccharides and polysaccharides from berry pomaces. The pomace will then be fermented with human fecal inoculum and the short chain fatty acid production will be

measured. The study also assessed the differences in fermentation of pomace fiber by individuals consuming different dietary levels of fiber.

HYPOTHESIS

Increasing fermentable dietary fiber will alter the microbiota of many individuals and will result in increased production of short chain fatty acids. Our hypothesis is that when provided a fermentable substrate as pre-treatment of gut microbiota, it will become more responsive to other fiber substrates such as resistant starch or cane berries pomace, therefore will produce more butyrate which is an indication of a shift toward butyrate producing bacteria.

LITERATURE REVIEW

DIETARY FIBERS

Dietary fiber is defined as components of a diet that resist digestion and absorption in the upper gastrointestinal tract and reach the illium and large intestine. Some types of fiber stay unaltered until excretion. Others undergo a complete or partial fermentation by microbiota in the large intestine (Du et al., 2010, Eastwood and Morris, 1992, Wijnands et al., 1999). There are a wide variety of fiber sources, mainly from plant cells walls and can be found in cereals, fruits and vegetables. They can be classified depending on their physical and chemical structures, and properties such as volume, viscosity, water holding capacity, solubility and fermentability (Burton-Freeman, 2000, Howarth et al., 2001). Dietary fiber has historically been defined by many scientist as complex non-starch carbohydrates formed from cellulose, hemicellulose, β-glucans, pectins, mucilages, gums and non-polysaccharide lignins (polymers of phenylpropane) (Burton-Freeman, 2000, Eastwood and Morris, 1992). More recently, residues such as lactulose, resistant starch, condensed tannins and some non-digestible protein can be included in this category since they are also fermented by bacteria in the lower gut (Saura-Calixto et al., 1991).

Properties of dietary fibers

Dietary fibers have three primary modes of action; bulking, viscosity and fermentation. The physical and chemical properties of the various dietary fibers determine the functional benefits provided to the host. Chemical properties include type or mixture of monosaccharides; how they are joined, including branching; and the molecular weight of the polymers. The physical organization of the fiber and cell wall material, including the complexion with non-digestible or other fermentable components

such as lignins, can also influence the ability of enzymes or microbes to access the specific fiber component (Eastwood and Morris, 1992).

All fiber is defined as resistant to digestion in the small intestine; however, it can be further classified as fermentable and non-fermentable. Dietary fiber is also divided according to the physical properties and defined by viscosity and solubility. Other factors such as the physicochemical nature (lipid, protein, carbohydrate) of the meal and the microflora present in the lower gut can influence fermentability (Bird et al., 2007). The degree of fermentability varies with the type of fiber, and the fiber contributes varying amounts of energy from fermentation products such as SFAs which are absorbed by the body.

Viscosity and solubility of dietary fibers

Soluble fiber just means it disperses in water. Most soluble fiber is viscous, allowing it to absorb and retain water, forming a gel. Plant foods contain both soluble and insoluble types of fiber in varying degrees. The solubility plays an important role in its functional properties and resulting health benefits. The water soluble fibers increase the viscosity in the intestine to slow gastric emptying time. Because of this, it has a tendency to hinder macronutrient (glucose and sterol) absorption to stabilize blood glucose and reduce blood cholesterol in the upper GI tract. And soluble fiber is mostly fermentable by bacteria in the lower GI tract which contributes to the production of short chain fatty acids and absorption of nutrients in the lower digestive tract (Isken et al., 2010, Mallillin et al., 2008). Types of soluble dietary fibers can include a variety of complex carbohydrates (oligosaccharides or polysaccharides) such as gums, polydextrose and pectins (Theuwissen and Mensink, 2008). Insoluble fibers, composed of lignins, cellulose and hemicellulose can bind water and add bulk to stool but are not

fermented in the human colon. Both soluble and insoluble fibers are believed to reduce the exposure of colonocytes to potential carcinogens by diluting the fecal toxins contents and are attributed to moderate weight loss due to low energy density and increased satiety (Chen et al., 2010, Isken et al., 2010, Mallillin et al., 2008, Theuwissen and Mensink, 2008).

DIETARY FIBER SOURCES

Fiber is defined to be the components of plants that resist human digestive enzymes that include lignin, polysaccharides, resistant starches, inulin and other oligosaccharides (Anderson et al., 2009, Burton-Freeman, 2000). Some edible portions of plants such as the skin, pulp, seeds, stems, leaves, and roots, contain both insoluble and soluble fiber components. The three main categories are dietary, functional and total fiber. Total fiber is the combination of both dietary and functional fiber. Dietary fiber includes non-digestible carbohydrates and lignin that are part of the plant cell walls and act as bulking agents. Functional fiber includes carbohydrates that have beneficial physiological effects in humans and can also be classified as fermentable fiber. Fermentable fibers are non-digestible carbohydrates that are metabolized by the gut microflora and include Resistant starches (RS), some non-starch polysaccharides (NSP), and oligosaccharides. This type fiber will increase the beneficial bacterial loads in the large intestine and are also known as prebiotics (Cani et al., 2007b, Topping and Clifton, 2001, Wang et al., 2004).

Lignin, considered part of the dietary fiber complex, is insoluble and can be found in plants filling the spaces in the cell wall between cellulose, hemicellulose, and pectin components (Burton-Freeman, 2000). Lignin is covalently linked to hemicellulose, thus crosslinking different plant polysaccharides and conferring mechanical strength to the

cell wall and, by extension, to the plant as a whole. The polysaccharide components of plant cell walls are highly hydrophilic and thus permeable to water, whereas lignin is more hydrophobic. Lignin is indigestible by animal enzymes, but some fungi and bacteria are able to secrete lignases that can biodegrade the polymer (Chabannes et al., 2001).

Oligosaccharides are polymers of simple sugars with 2 to 20 monomer units and are often found as a component of glycoproteins or glycolipids (Macfarlane et al., 2008). These compounds can be only partially digested by humans and are fermented by gut microflora. Galactooligosaccharides (GOS), which also occur naturally, consist of short chains of galactose molecules. GOS is naturally found in soybeans and can be synthesized from lactose (milk sugar). Fructooligosaccharides (FOS), which are found in many vegetables, consist of short chains of fructose molecules. FOS products derived from chicory root contain significant quantities of inulin (a β 2,1-linked fructosyl residue ending with a glucose). Inulin has a higher degree of polymerization than FOS and belongs to a group of naturally occurring polysaccharides known as fructans. It is highly available to the gut bacterial flora making it similar to resistant starches and other fermentable carbohydrates. FOS and inulin are found naturally in Jerusalem artichoke, burdock, chicory, leeks, onions, and asparagus (Meyer et al., 2011)

Non-Starch Polysaccharides

Fiber in the human diet is principally non-starch polysaccharides (NSP) and is subdivided into soluble and insoluble NSP. The classification is based on their solubility in aqueous solutions, but not necessarily under physiological conditions (Topping and Clifton, 2001). They consist of long chains of repeating monosaccharide units joined by bonds called beta-acetal linkages that cannot be split by the enzymes in the digestive

tract. Non-starch polysaccharides include celluloses, hemicelluloses, gums, pectins, xylans, mannans, glucans and mucilages. The effects of slowly fermentable NSP may be due to increased fecal bulk or reduced transit time that dilutes toxins and their contact time with the colonic epithelium. Increased SCFA production, in particular butyrate, is another proposed mechanism for the protective effect of insoluble NSP (Le Leu et al., 2002).

Resistant Starch

RS is considered as a type of dietary fiber and is found in unprocessed foods, in high amylose grain foods or as a refined food additive. Physiologically, resistant starch (RS) behaves like soluble fiber, but it also has some characteristics of an insoluble fiber (Cummings et al., 2001). RS is defined as starches or products of starch degradation not absorbed in the small intestine (Brouns et al., 2007, Goni et al., 1996). It is not hydrolyzed in by digestive enzymes, but it is fermented in the colon by the gut microflora (Zheng et al., 2010). RS can be found in many food products (for examples bananas, corn, potatoes, yams, whole grain bread, beans, oatmeal, and brown rice). The resistance to digestion is dependent on factors such as amylose-amylopectin ratio, physical form, and processing (Asp, 1992).

RS can be categorized into 5 types: RS1, found usually in seeds, legumes or unprocessed whole grains are physically inaccessible by digestive enzymes; RS2 found in uncooked potatoes, green bananas and high amylose corn usually occurs in natural granular form and is inaccessible by digestive enzymes due to the physical structure of the starch (d-glucose units linked by α-1,4/α-1,6 glucosidic bonds) or the starch backbone; RS3 is found after retrogradation (recrystallization) of starchy food that have been cooked and cooled such as bread, pasta, rice, potatoes, and cornflakes; RS4, not

found in nature, are chemically modified starches, for example cross-linked starches, starch ethers, starch esters, that are water-insoluble semi-crystalline structures preventing enzymes from digesting them; and RS5, recently introduced and not commercially available, occurs as starch-lipid or amylose-lipid complex that forms when starchy foods are heated or cooked in the presence of fats or lipids and offers resistance to enzymatic digestion based on steric hindrance by the complex (Aparicio-Saguilán et al., 2008, Brouns et al., 2007, Ratnayake and Jackson, 2008, Reed, 2012)

Berry pomaces

Small berries such as grapes, blueberries, blackberries, raspberries, and other cane berries are recognized for their health promoting materials. These fruits are rich in phenolic acids, anthocyanins, proanthocyanindins and flavonoids (Zadernowski et al., 2005). Most berries are harvested from cultivators for the purpose of fresh market or production of juices. They are often considered as “super fruits” because of the high content of anthocyanins, the water-soluble flavonoids responsible for the red, blue and purple color in the fruits (Su and Chien, 2007, Zadernowski et al., 2005). The content of phenolics in berries is influenced by the variety of the fruit, the degree of maturity at harvest, and harvest conditions (Zadernowski et al., 2005). Processing the fruits into juice increases the commercial life of the product and provides convenient consumer access to the healthy products (Brambilla et al., 2008). The phenolic compounds in berries have been reported to have antioxidant, anticancer, anti-inflammatory, and anti-neurodegenerative biological properties (Seeram, 2008). Although many of the beneficial components in fruit are retained in the juice, much of the bioactive components remain in the pomace that is discarded after juice production.

Pomace is the by-product residue consisting of skins, pulps, seeds and stems remaining after fruits and vegetables have been pressed for juice, wine or other products. Millions of pounds of pomace are produced each year with most of it disposed of in landfills. The primary use currently is in animal feeds and field dressings, have an additional value in the food industry, potential applications include production of flavors and value added components such as anthocyanins, citric acid, and seed oils (Su and Silva, 2006). Pomace constitutes approximately 20% of the initial fruit weight and can contain 25-50% of the polyphenolics, thus containing many of the potential beneficial health components found in fresh fruit, including the anthocyanins, other phenolics and dietary fiber (Khanal et al., 2009). The bioactive compounds, such as anthocyanins in fresh fruits are poorly absorbed in the upper gastrointestinal tract, however, when the pomaces are used as a dietary fiber additive in foods, the anthocyanins are fermented by the microbiome in the lower gastrointestinal tract resulting in improved bioavailability.

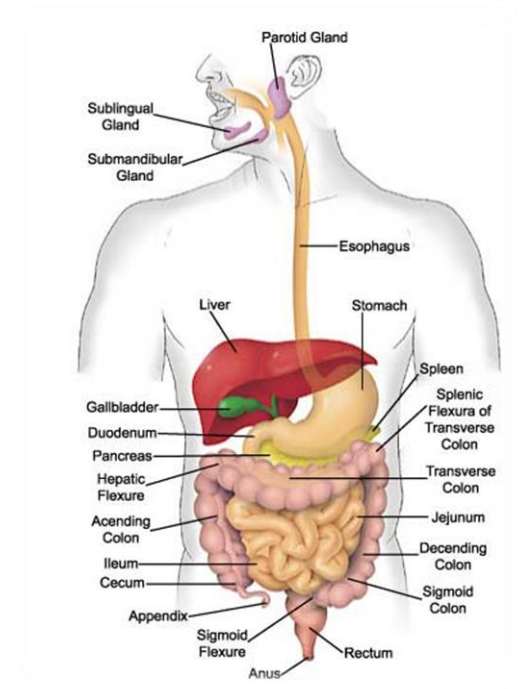
HUMAN COLONIC FERMENTATION

Physiologically, humans do not have the enzymes capable of metabolizing dietary fibers in the GI tract. When these non-digestible carbohydrates reach the large intestine, they are met by an entire anaerobic metabolic system comprised of bacteria which possess enzymes capable of doing the process. (Hernot et al., 2009, Louis and Flint, 2009). The human large intestine serves as a vessel for fermentation and the microbiota generated by-products can be utilized by the host (Cummings et al., 2001). These by-products play an essential role in the host's health with the production of nutrients and energy and the protection against diseases (Backhed et al., 2005).

The GI tract has different type of bacterial populations according to the location and environmental conditions. Each bacteria affects digestion and absorption of

intestinal contents during passage throughout the GI Tract (Table 1). The mouth has the second largest population of bacteria including Firmicutes, Bacteroidetes, Proteobacteria, Fusobacteria and Actinobacteria (Tlaskalova-Hogenova et al., 2004)(Figure 1). The stomach has less bacterial populations, due to high acidity and digestive enzymes, which are mostly gram positive and gram negative (Helicobacter pylori) aerobic bacteria (Tlaskalova-Hogenova et al., 2004). The small intestine has a relatively higher concentration of bacteria that are mainly Firmicutes such as Lactobacilli, Bacilli, gram positive Coci and some Bacteroidetes, Proteobacteria, Fusobacteria and Actinobacteria. The large intestine contains an average of the largest, mostly anaerobic bacterial populations. The colonic microbiota changes due to a nutritional shifts, aging, stress or variation of food intake (Topping and Clifton, 2001).

Table 1. Dimensions of the Human GI Tract, passage time of intestinal contents, and corresponding microbial density (Rajilic-Stojanovic et al., 2007)



Gastrointestinal segment	Length (cm)	Passage time (hours)	Density of microbiota cells/ml (g)
Stomach	12	2--6	10^0-10^4
Duodenum	25	3--5	10^4-10^5
Jejunum	160		10^5-10^7
Ileum	215		10^7-10^5
Caecum	6	41567.00	10^{10}-10^{11}
Ascending colon	15		
Transverse colon	50		
Descending colon	25		
Signoid colon	40		
Rectum	18	1.00	10^{10}-10^{11}

1 Mouth/Esophagus
Firmicutes, Bacteroidetes, Proteobacteria, Fusobacteria and Actinobacteria
3 Stomach
Helicobacter pylori
5-10 Small intestines
Bacteroides, Lactobacillus, Enterococcus Clostridium, Enterobacterium, Gram positive Coci
11 Colon
Bacteroides, Bifidobacterium, Eubacterium, Lactobacillus, Fusobacterium, Enterococcus Clostridium, Escherichia coli, Staphylococcus, Streptococcus

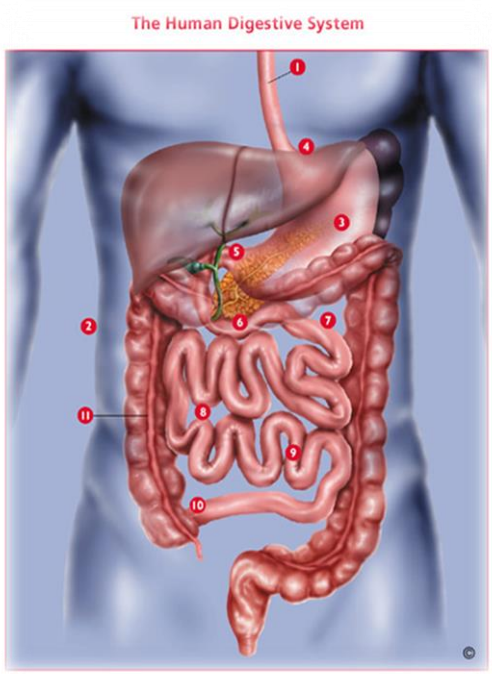


Figure 1. Human GI Tract Microbiota (Rajilic-Stojanovic et al., 2007)

Prebiotics and Probiotics

The term prebiotic, which overlaps the definition of dietary fiber, was defined as "a non-digestible food ingredient that is beneficial to the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon" (Gibson et al., 2004, Schrezenmeir and de Vrese, 2001).Dietary fibers that increase the beneficial bacterial load in the large intestine are known as prebiotics. The term probiotic was defined as "A live microbial feed supplement which beneficially affects the host by improving its intestinal microbial balance" (Fuller, 1986, 1989, Parker, 1974). Probiotics have n vast range of health benefits including inhibiting the attachment and growth of pathogenic microbes in the epithelium of the intestinal tract (Macfarlane and Cummings, 1999). Probiotics can improve the immune response, increased the ability to digest food, and alleviate many common digestive disorders such as constipation, diarrhea and Irritable bowel syndrome IBS. In-vitro and in-vivo studies showed that when pH-controlled co-culture of Bifidobacterium infantis was inoculated together with

Escherichia coli, and Clostridium perfringens, with the presence of oligo-fructose the Bifidobacteria has expressed an inhibitory effect on the growth of the other two species (Cummings et al., 2001, Senevirathne, 2010). Prebiotic fiber supplements do not always need a probiotic supplementation since the normal microbial populations in the human body exceeds 100 trillion cells with close to 1000 different species of bacteria in the adult GI tract (Cani et al., 2007b, Kurokawa et al., 2007)Even though the bacterial community in the GI tract is extremely diverse; the majority of species belongs to a few bacterial divisions or pyla, namely Firmicutes, Bacteroidetes Actinobacteria and Proteobacteria (Backhed et al., 2005).

Digestion and Microflora

The digestive system is a complex and complicated biological system. Digestion of food starts in the mouth. During the oral phase of digestion food starts with mechanical breakdown, then salivary α-amylase hydrolysis and finally bolus formation prior to swallowing (Woolnough et al., 2008). In the stomach hydrolysis of protein, carbohydrate and fat occurs. Protein digestion is initiated by pepsin and hydrochloric acid resulting in to peptide formation; Carbohydrate digestion is initiated by salivary amylase and lipid digestion occurs with gastric lipase. Further hydrolyzation occurs in the small intestine. Protein and peptide passing from the stomach are exposed to the pancreatic converted into free amino acids and smaller peptides. Carbohydrates are further hydrolyzed by pancreatic amylase to maltose and to dextrin, then, after exposure to maltase, lactase, sucrose, and isomaltase are converted to monosaccharides. Lipid is also exposed to enzymes and co-enzymes such as pancreatic lipase, carboxylic ester hydrolase, phospholipase and colipase. Pancreatic lipase breaks down triacylglycerols into monoacylglycerols and fatty acids, the carboxylic ester hydrolase breaks down

carboxylic esters, and phospholipase breaks down fatty acids into the 2-position of glycerophospholipid. The presence of bile salts enhances the activity of lipase and the absorption of long-chain fatty acids and monoacylglycerols. The amino acids, peptides simple sugar molecules and digested fatty acid are absorbed in small intestine (Boisen and Eggum, 1991).

The remaining undigested fat, proteins and carbohydrates pass into the large intestine (Saunders and Sillery, 1988). From 10-20 % of nitrogen from protein is recovered by microflora in the large intestine. Protein (mucin) and carbohydrates (Resistant starch (RS), oligo saccharides, Inulin) are metabolized by bacterial enzymes. The 2% of undigested fatty acids reaching the large intestines cannot be fermented by bacterial enzymes, so lipids are only slightly affected during passage through the large intestine. The major by-product of dietary fiber and protein fermentation are the production of short chain fatty acids (SCFA), which can be absorbed and act as an energy source in humans (Boisen and Eggum, 1991).

Fermentation and short chain fatty acids

When food products are consumed, normal digestive enzymes convert complex food components into a range of absorbable compounds for use throughout the body. Many dietary fibers, because of their structure do not get metabolized in the upper digestive tract, but are fermented in the lower intestine by the resident microflora. (Valeur and Berstad, 2010). The metabolism of these carbohydrates is dependent on the specific bacteria present, the non-digested substrate available, the enzymes produced and most importantly the anaerobic conditions (Duncan et al., 2002b, Schröder et al., 1994, Yasuda et al., 2007). SCFAs and gases (carbon dioxide, hydrogen, and methane) are produced in the large intestines when non-digested

carbohydrates go through this anaerobic fermentation process (Cherbut, 2002, Hernot et al., 2009, Macfarlane and Englyst, 1986). Three (3) major SCFAs of interest, acetate, propionate and butyrate, can be metabolized and produced through various pathways (Asa Henningsson, 2001).

Acetate the most abundant of the three SCFAs and can be produced through several different pathways. In glycolysis, the pyruvate can be converted to Acetyl CoA and later into acetate. It also can be synthesized by the Wood–Ljungdahl pathway, also called the reductive Acetyl-CoA pathway. This pathway occurs when acetogenic and methanogenic bacteria are present with the help of two enzymes, carbon monoxide dehydrogenase and acetyl-CoA synthase, and acetyl CoA is produced from the synthesis of CO_2, CO and hydrogen used as the electron donor (de Graaf and Venema, 2007, Westermann et al., 1989).

Propionate is formed through the succinate pathway by decarboxylation of succinate in presence of CO_2. It can also be produced by some bacteria in the Acrylate pathway, where pyruvate, through a series of reactions is transformed into propionyl-CoA, which is later converted to propionate (Hosseini et al., 2011, Miller and Wolin, 1996). Propionate, mainly metabolized in the liver, has been shown to inhibit gluconeogenesis and increase glycolysis in rat hepatocytes (Asa Henningsson, 2001). It has also been shown to lower cholesterol synthesis by inhibiting the 3-hydroxy-3-methylglutaryl-CoA reductase activity (Mallillin et al., 2008).

Butyrate is probably the most studied of all the SCFAs because of its utilization as an energy source by the epithelial cells in the colon. Acetate produced during fermentation is utilized by some bacteria to make butyrate (Duncan et al., 2004). Also, depending on the species, enzymes such as butyryl CoA and acetate CoA transferase

or phosphotransbutyrylase/butyrate kinase are used to produce butyrate (Pryde et al., 2002).

In vitro studies about gut anaerobic fermentation of various sources of fiber have been conducted, and the produced SCFAs vary greatly with the substrates and also the fermentation. Table 2 is an example of variability between produced SCFAs from numerous sources of fibers in raw and processed forms (Hernot et al., 2008).

Table 2: pH Change and Acetate, Propionate, Butyrate, And Total Short-Chain Fatty Acid (tSCFA) Production following 12 h of in Vitro Fermentation of Native and Extruded Cereal Grains and Components (Hernot et al., 2008).

Substrates	pH change	Mg/g of DMG						
		acetate*		propionate		butyrate		tSCFA
barley								
native WG	-0.16	3.8	(6.5)	14.7	(24.9)	40.5	(68.6)	59.0
processed WG	-0.14	57.2	(55.0)	20.4	(19.6)	26.3	(25.3)	104.0
Corn								
Native WG	0.06	0.0		1.2	(2.6)	45.5	(45.5)	46.7
Processed WG	-0.03	0.0		13.0	(40.3)	19.3	(59.7)	32.2
bran	0	0.0		10.1	(62.7)	6.0	(37.3)	16.0
Oat								
native WG	0.08	5.4	(8.6)	21.3	(34.3)	35.4	(57.1)	62.0
processed WG	0	31.4	(32.1)	27.0	(27.6)	39.4	(40.3)	97.8
bran	-0.07	11.2	(14.2)	28.0	(35.8)	39.4	(50.2)	78.6
Rice								
native WG	0.03	9.4	(27.7)	4.3	(12.8)	20.1	(59.5)	33.8
processed WG	-0.02	0.0		8.1	(62.8)	4.8	(37.2)	12.9
Wheat								
native WG	0.17	58.1	(46.7)	12.1	(9.7)	54.2	(43.6)	124.4
processed WG	0.09	8.2	(18.4)	12.6	(28.3)	23.8	(53.3)	44.5
bran	-0.13	52.3	(46.4)	20.2	(17.9)	40.1	(35.6)	112.6
germ	-0.16	53.5	(37.7)	33.4	(23.6)	54.9	(38.7)	141.8
Standards								
Solka Floc	-0.12	0.8	(100.0)	0.0		0.0		0.8
inulin Ultra-FOS	-1.78	212.6	(44.5)	94.4	(19.7)	171.2	(35.8)	478.2
Pectin HM Rapid	-1.44	308.3	(67.6)	70.6	(15.5)	77.3	(16.9)	456.1
SEM	0.02	4.25		1		1.8		5.8

*Values in parentheses are individual fractions expressed as a percentage of total SCFA.

SCFA Production

The gut microflora is comprised of a variety of bacteria species which play a very important role in human health. They coexist with the host, and mutually benefit both of them by providing nutrients, energy and protection while feeding on the indigestible carbohydrates (Backhed et al., 2005, Belenguer et al., 2006, Xu and Gordon, 2003). The human colon host bacteria species mostly belonging to the Bacteroidetes and Firmicutes phyla (Turroni et al., 2008, Xu and Gordon, 2003). A new born is sterile at birth but acquires the first set of bacteria from contact with the mother and the environment (Morelli, 2008). Gut microflora become dominated by bifidobacteria and lactobacilli through breast milk, but later in life other bacteria are found in the population including Enterobacteriaceae, enterococci, Bacteroides, and clostridia (Fooks and Gibson, 2002, Morelli, 2008). The population and types of some of the most dominating species, including bifidobacteria and lactobacilli, differ individually because of the provided substrate (Hernot et al., 2009, Parrett et al., 1997). The species and the substrates play a very important role in SCFA production. Several strains of Roseburia sp., Faecalibacterium prausnitzii, and Coprococcus sp in the colon produce a significant amount of butyric acid (Duncan et al., 2002b).

Bifidobacterium spp is classified under phylum Actinobacteria; it is a Gram-positive strictly anaerobic branched rod that produces lactic and acetic acid without generation of CO_2. The genus Bifidobacterium is the third most numerous bacterial populations in the human intestine after the genera Bacteroides and Eubacterium. Bifidobacterium is about 6% of total fecal bacteria (Matsuki et al., 2004). Bifidobacterium spp can be used as a probiotic because it improves digestion, absorption and helps to decrease the side-effects of antibiotic therapy. This organism has a symbiotic

relationship in the metabolism of resistant starches, fructo-oligosaccharides and inulin, transgalactosylated oligosaccharides and soybean oligosaccharides (Cani et al., 2004, Falony et al., 2006, Gibson G R, 1995, Gibson et al., 1995, Gibson et al., 2004, Ito et al., 1993, Topping and Clifton, 2001, Wang et al., 2004). Bifidobacterium spp can change the position of starch molecules and produces acetate and lactate as end products, which helps to promote butyrate.

Lactobacilli are Gram-positive non-spore-forming rods that belong to the general category of lactic acid bacteria under the phylum Firmicutes. Lactobacillus spp. produces lactic acid as the major acid during fermentation of glucose with acetic, succinic and formic acids in minor quantities.

Bacteroides spp. is strictly anaerobic Gram-negative, dominant bacillus bacterial group in human gastrointestinal tract. Bacteroides spp. can break down a wide variety of indigestible dietary carbohydrate by producing acetate and succinate as the major metabolic end products. This organism increased rapidly soon after the introduction of prebiotics such as Resistant Starch. This bacterium attaches to starch molecules and starts the fermentation process (Bird et al., 2000, Brown et al., 1997, Wang and Gibson, 1993). During young age higher population of Bacteroides spp. are seen in the intestinal tract compared to the elderly age.

Clostridium clusters categorization has done based on16S rRNA sequence analysis, According to Phylogenetic analysis of 16S rRNA genes shows that the group is very diverse, it includes non-clostridial species in deeply branching clusters. The main two butyrate producing Clostridium clusters groups are normally present in healthy humans (Senevirathne, 2010).

HEALTH BENEFITS OF DIETARY FIBER

Benefits of Microbiota

Microbes in the digestive system have a symbiotic relationship with the host providing a stable environment with nutrients for the microbes (Leser and Molbak, 2009). Gut microbiota help the host by providing defense against pathogens, synthesis of vitamins, fermentation of dietary fibers and dietary proteins; priming the immune system early in life; and stimulating the gut motility (Cani et al., 2007a, Cani and Delzenne, 2007, Leser and Molbak, 2009, Topping and Clifton, 2001). The gut microbiota helps to increase thickness of the villi in the intestinal wall and exhibits fast epithelial cell turnover (Leser & Molbak, 2009).The microbiota in the gut contributes to the development of healthy conditions within the intestinal tract by suppressing colonization of pathogenic organisms (Itoh et al., 1995, Spinler et al., 2008). Production of Short chain fatty acids (SCFA) is one of the most useful benefits of gut microbes.

Nutrient and Mineral Absorption

Fermentation of dietary fibers by the gut microflora results in SCFAs that are absorbed through the intestinal lumen providing direct and indirect health benefits. SCFAs, especially butyrate, are the primary energy source required by the colonocytes resulting in growth of intestinal epithelial cells producing a thicker mucosa and increasing the number of villia in the intestinal lining (Campbell et al., 1997). The healthier epithelial cells allow for more effective absorption of nutrients, minerals, peptides, and SCFAs which are transported to the liver and metabolized for circulation to various other tissues in the body.

Benefits of SCFA

The basic fermentative reaction in the human colon is similar to that of obligate herbivores (Topping and Clifton, 2001). Intestinal microbial communities hydrolyze non-digestible plant polysaccharides such as lignin, hemicelluloses, pectin, cellulose and RS into SCFA. The acetate, propionate, and butyrate are found in greater concentration in the feces of those who consume a diet containing dietary fibers (Morita et al., 1999). The typical ratios of SCFA in feces are proportion of 3:1:1 in acetate: propionate: butyrate (Duncan et al., 2002a). These SCFA help regulate the colonic physiological processes and maintain normal bowel function. Reduction of pH values from SCFA help to reduce the growth pathogenic organisms and reduce absorption of toxic compounds with carcinogenic potential in the gut (Bird et al., 2000).

Butyrate is a major energy source for epithelial cells of colonic mucosa which stimulates cell proliferation (Sato et al., 2008, Schrezenmeir and de Vrese, 2001). Butyrate is the major SCFA that in providing protection against cancer and ulcerative colitis by reducing cell proliferation, blocking the absorption of cancer-causing substances and making the colon less vulnerable to DNA damage (Pitcher and Cummings, 1996). It also helps to boost the absorption of calcium to maintain a healthy epithelium (Cummings and Macfarlane, 1991, Gibson and McCartney, 1998, Hagopian et al., 1977). Dietary resistant starch was associated with increased gene and hormone expression for peptide YY (PYY) and glucagon-like peptide-1(GLP-1), which are also associated with increased butyrate in the cecum in rats (Keenan et al., 2006, Zhou et al., 2006).

Propionate is the primary precursor for gluconeogenesis and may inhibit liponeogenesis and protein synthesis (Louis et al., 2007, Schwiertz et al., 2010).

Propionate is believed to inhibit the synthesis of fatty acids in the liver and also involved in the control of hepatic cholesterol synthesis. It helps to lowers plasma cholesterol concentrations by inhibiting hepatic cholesterogenesis through colonic fermentation (Cheng and Lai, 2000). The propionate concentration and proportion of total SCFA increases significantly in lean subjects and could be an important factor that contributes to weight gain in obese subjects (Schwiertz et al., 2010).

Acetate is the major SCFA produced by the colonic microflora. It is around 60–75% of the total SFCA detected in feces and is formed by many of the colonic microflora with one-third coming from reductive acetogenesis which is produced by anaerobic bacteria (Louis et al., 2007, Miller and Wolin, 1996). Acetate is not metabolized in the colon because It is quickly absorbed after production and transported to the liver. Remaining acetate is further utilized by colonic microbiota and covert into butyrate. Acetate is essential for cholesterol synthesis in the body (Hijova and Chmelarova, 2007). It acts as an energy substrate for muscles and has been shown to suppress harmful bacteria (Araya-Kojima et al., 1995)

Hunger, Satiety, Energy control and Gut Hormones

One of the most beneficial aspects of dietary fibers is the capability to control (delay or inhibit) hunger after being consumed because of the bulking and viscous capabilities. This results in stomach expansion, an longer time and effort for chewing, and a prolonged intestinal phase for nutrient digestion and absorption (Slavin and Green, 2007). Dietary fibers have zero or low energy value and fiber rich foods play an important role in energy balance because their bulking properties dilute the energy of the food. Energy density is defined as the number of kilojoules per unit weight of food, and most high fiber foods have a reduced energy density (Burton-Freeman, 2000).

Some fibers have water retention properties and are not metabolized in the GI tract, but contribute to the stool size. Furthermore the consumption of dietary fiber also affects the concentration of Peptide Tyrosine-Tyrosine (PYY) and Glucagon-like peptide-1 (GLP) secreted in the blood (Bosch et al., 2009). GLP-1 and PYY are satiety hormones secreted by the gut in response to nutrient intake. GLP-1, secreted from the L-cells of the intestinal mucosa, and PYY inhibits esophageal muscle contraction and gastric acid secretion and delays gastric emptying (Bohorquez et al., 2011, Näslund et al., 1999). Both hormones decrease the food intake and body weight in animal and human models (Buddington and Weiher, 1999, Näslund et al., 1999).

Peptide YY (PYY) and glucagon-like peptide 1 (GLP-1), are two main hindgut hormones that are produced in greater amounts in response to large amounts of food passing through the small intestine in to the large intestine (Cani et al., 2005, Keenan et al., 2006, Shen et al., 2009, Zhou et al., 2006). The gut hormone PYY is produced by L endocrine cells mainly located in the ileum, large intestine and rectum (McGowan & Bloom, 2004). GLP-1 is also produced by L endocrine cells that are located in the ileum and large intestine (Kreymann et al., 1987, Kreymann et al., 1988). GLP-1 acts through binding to the GLP-1 receptors that are found on many cell types including, beta cells of the pancreas, neuronal cells in the brain, adrenal, pituitary, kidney, and throughout the gastrointestinal tract (Gotthardt et al., 2006). GLP-1 enhances both early and late phase of insulin secretion stimulated by glucose and is important in the treatment of diabetes mellitus (Wicki et al., 2007). GLP-1 controls feeding behavior in the brain that affects energy intake and energy expenditure making it a potential treatment for weight control (Perez-Tilve et al., 2006). Peptide YY (PYY) is also plays a role in energy balance and adiposity since it helps to inhibit both food intake and gut motility and fat

oxidation (Adams et al., 2006, Adams et al., 2004)PYY is also important in the control of insulin sensitivity and controlling obesity (Boey et al., 2006a, Boey et al., 2006b, Boey et al., 2007).

The increase of butyrate in the intestinal tract by fermentation of prebiotics may help to increase PYY and GLP-1 (Keenan et al., 2006, Zhou et al., 2006). PYY and GLP-1 are associated with reduced body fat and decreasing the blood glucose level, reducing body weight and improving insulin sensitivity in mammalians (Young et al., 1999). Lower respiratory exchange ratio is prominent in mice fed RS, indicating a partitioning of fat to oxidation rather than storage (Zhou et al., 2009).

Disease Prevention and Treatment

The metabolic processing in the colon by microflora yields SCFA end-products, primarily butyrate, that controls the proliferation and differentiation of intestinal epithelial cells, thus altering intestinal growth by changing the expression of the cell surface. The healthier epithelial cells prevent injury to the gut mucosa from occurring with significant, beneficial health effects such as reduction of infectious inflammatory bowel disorders and colonic tumors. Dietary fiber reduces the exposure time of colonocytes to potential carcinogens by diluting fecal toxins. It also prevents the reabsorption of bile acid in the liver that can aid in lowering circulating triglycerides (Chen et al., 2010, Mallillin et al., 2008). Dietary fiber improves carbohydrate metabolism and glucose tolerance. The possible mechanisms include a relaxed glycemic response to a meal that reduces the highs or lows that stimulate appetite as well as the increased release of gut satiety peptides, GLP-1 and PYY. This can also prevent or reduce obesity and related disorders by decreasing blood glucose levels for storage as fat, increasing insulin

sensitivity resulting in less insulin to push lipogenesis and decreased fatty acid synthase activity.

Beneficial bacteria reinforces the intestinal walls by crowding out pathogenic organisms that compete for available nutrients, receptors, and growth factors and increases antimicrobial and antibacterial compounds such as bacteriocins, cytokines and butyrate. Improvement in mineral absorption and balance can prevent osteoporosis and improve bone density. Also, the increased absorption through the intestinal epithelium causes lymphoid tissue near the gut to grow to stimulate immunomodulatory cells to boost the immune system for an overall health effect.

MATERIALS AND METHODS

MONOSACCHARIDE ANALYSIS OF BLUEBERRY AND BLACK RASPBERRY POMACES

The carbohydrate analysis was conducted by the LSU Ag Center Audubon Sugar Institute Research Station using the following method: 0.5 g of freeze dried pomace was mixed with 3 ml of DI water. A duplicate sample was run with L-arabinose pre-mixed and dried on the sample. Samples were mixed for 40 minutes with a wrist action shaker, and then sonicated with regular shaking for 20 minutes. The samples were centrifuged at 10,000 RPM for 10min and filtered (0.45m nylon) before being analyzed via HPLC. The operational conditions are described in table 1.

Table 3: HPLC operational parameters

Pump	Agilent 1200, G1310A Isocrate Pump
Eluent	18.3MΩ deionized water
Flow	1.0mL/min
Detector	G1362A Agilent Differential Refractive Index (DRI) Detector, 45°C
Injection	20μL
Column	BioRad Aminex HPX-87P(P), Lead form, 300mm x 4mm (ID), 9 μm particles, 80°C
Back Pressure	18kg/cm^2 (255psi) at 85°C and mL/min
Plates (N)	>7000

ACID DETERGENT FIBER (ADF) AND NEUTRAL DETERGENT FIBERS (NDF) PROFILES

The analysis was conducted by the LSU AgCenter Forage Quality Laboratory. The Association of Analytical Communities (AOAC) method Number 930.15 was followed to determine the dry matter for the substrates. Black raspberry and blueberry) pomace was dried for 2 hours at 135°C in a conventional drying oven. Acid Detergent Fiber (ADF) and Neutral Detergent Fiber (NDF) were determined following the Goering

and Van Soest method (McOrist et al., 2008). The neutral-detergent procedure for cell-wall constituents is a rapid method for analyzing the total fiber while the acid-detergent fiber procedure provides a rapid method for lignocellulose determination in feedstuffs.

The NDF was determined as described below:

Into a beaker with reflux, the following was added in the specific order listed, with a calibrated scoop: 0.5-1.0g of dried sample (ground), 100 ml of a neutral-detergent solution (at room temperature), 2 ml of decahydronaphthalene, and 0.5 g of sodium sulfite. The mixture was heated to boiling in 5 to 10 minutes while avoiding foaming. Temperature was adjusted as needed for an even boiling and reflux level for 60 minutes, timed from onset of boiling.

After suspending the solids, the solution was transferred into Gooch crucibles, previously tared on filter manifold. No vacuum was admitted until the crucible was filled. A low vacuum was initially applied, then increased as more force was needed. The sample was rinsed into the crucible with minimum amount of hot (90°-100° C.) water.

The vacuum was removed and the crucible was filled with hot water. The liquid was filtered and the washing steps were repeated, and then washed twice with acetone in the same manner and suck dry. The crucibles were then dried at 100° C for 8 hours and weighed. The percent of cell-wall constituents was reported as yield of recovered NDF. Estimate cell soluble material determined by subtracting this value from 100.

The residue was ashed in the crucible for 3 hours at 500° to 550° C, weighed and reported as ash insoluble in neutral-detergent.

The ADF procedure was done as described below:

Into a beaker with reflux, the following was added, in the specific order listed: 1.0g of dried sample (ground), 100 ml of cold (room temperature) acid detergent solution and 2

ml. decahydronaphthalene. The mixture was heated to boiling for 5 to 10 minutes and the heat was reduced as needed to avoid foaming and reflux for 60 minutes, timed from onset of boiling.

The mixture was filtered on a previously tared crucible using a filter manifold with a light suction. After breaking it up with a rod, the filter mat was washed twice with hot water (90°-100° C). The sides of the crucibles were washed in the same manner. The wash procedure was repeated with acetone until all the colors were removed. The ADF was dried at 100° C. for 8 hours and weighed.

The ADF was calculated using the equation below:

$$\frac{(\text{weight of oven} - \text{dry crucible including fiber} - \text{tared weight of oven} - \text{dry crucible})}{\text{oven} - \text{dry sample weight}}$$

FECAL COLLECTION AND PREPARATION OF BACTERIAL INOCULUM MIXTURES

The in-vitro bacteria inoculum was prepared using feces that were taken from three different groups and were prepared as followed. Group 1, where the fecal samples were freshly collected from Sprague Dawley (SD) rats that were on a hi-maize controlled diet from 8 weeks prior collection. The samples were then stored at -80°C until usage. The fecal samples for the next two groups were collected from human volunteers (male and female of varied ages). The donors were healthy and free of antibiotics for at least three weeks prior to the collection. The non-fortified group (group 2) that was free feeding without diet restriction or dietary fiber additives and the RS-fortified group (group3) had yogurt with RS added to their daily diet. The amount of RS started at 25 g per day and increased by 25 g every week until reaching 75 g per day. The feces samples were frozen (-4°C) immediately after collection, then transferred to an ultra-low freezer (-80°C) until usage. For the non-fortified group, each individual fecal

sample was mixed with distilled water at a 1 to 1 weight by weight ratio, and stored in aliquots of 50 g. The pooled fecal samples were prepared by mixing equal amount of the individual mixtures, and divided in 50 g aliquots. The pooled fecal samples for the RS-fortified group was prepared following the same process and also divided in 50 g aliquots. Individual mixtures were not made or this group. All the aliquots (individual, unfortified pooled and RS-fortified pooled mixture) were stored at -80°C until usage.

ENZYMATIC DIGESTION OF FOOD PRODUCTS

A method mimicking the human digestion and absorption process before entering the large intestine was applied to the berry pomace substrates in order to prepare the products for fermentation. The enzymatic digestion using pepsin followed by pancreatin, was conducted to remove the digestible, non-fibrous contents of the substrate. Pepsin is an enzyme used to degrade protein into peptides and the pancreatin is a mixture of amylase, lipase and protease produced by pancreatic exocrine cells. The protease works to hydrolyze proteins into oligopeptides; amylase hydrolyzes starches into oligosaccharides and the disaccharide maltose; and lipase hydrolyzes triglycerides into fatty acids and glycerols. The digestion was done as followed:

Sixty (60) g of dried pomace substrate was added to 100 mL distilled water with Hydrochloric acid (HCl) to bring the solution to a pH of 2. The mixture was incubated for ten (10) min while shaking to bring the temperature to 37°C. Then 0.5 g (3500 U/mg) of pepsin was added to the mixture and incubated while shaking for another 3 hours. Next, the pH was brought to 7.5 with a sodium hydroxide (NaOH) or sodium bicarbonate ($NaHCO_3$) solution, and incubated for 10 min to bring the temperature back to 37°C before adding 4.0 g of pancreatin (SIGMA-ALDRICH) and 1g of bile salts (Mixture of

sodium cholate and sodium deoxycholate from SIGMA-ALDRICH). The solution was mixed thoroughly for 10 min then incubated at 37°C for another 2 hours. The mixture was microwaved for 3-5 min to denature the enzymes and then filtered to obtain the solids. Those remaining solids of the mixture were then washed with ethanol and filtered again before being dried overnight. The dried substrate was later made into a powder and kept frozen until utilized for the fermentation step. All substrates except for the ones used with the rats fecal samples went through the pre-digestion process.

FERMENTATION PROCESS

The anaerobic buffer solution was prepared according to the following steps and was used throughout the fermentation process. The anaerobic solution was a mixture of two solutions, A and B, at the ratio 9:1 and prepared as follows:

- Solution A (per liter of distilled water) contained 11.76 g of sodium bicarbonate ($NaHCO_3$), 11.1 ml of Hemin (0.78 mmol/L of water), 1.1ml of menadione (0.36 mmol/L of water) and 1.1 ml of resazurin (3.98 mmol/L of water) as a redox indicator. The solution was autoclaved at 121°C for 15min after preparation.
- Solution B (per liter of autoclaved distilled water) contained 0.48 mol NaCl, 0.02 mmol (Dipotassium phosphate) K_2HPO_4, and 0.63 mmol L-Cysteine-HCl.

The in-vitro bacteria inoculum was prepared using 50 g of the feces that was mixed with 200 mL of the buffer then filtered with either cheese cloth or a filter whirlpak bag. The solution is incubated at 37°C under anaerobic conditions for one hour.

One (1) liter glass bottles used as vessels were autoclaved at 121°C for 15 min prior to usage. To each vessel was added: 350mL of the anaerobic solution (solution A+B) and 10 g of the fermentation substrate. Then an aliquot of 50mL of the incubated solution was added to each vessel and flushed with an anaerobic gas mixture (10% CO_2, 80%

N_2 and 10% H_2) for 10 min. The samples were then incubated at 37°C, while shaking or stirring. Samples were collected at times 0, 12, and 24 hours, stored in 15 mL falcon tubes and frozen until usage. The process was done in triplicate for each individual mixture, the unfortified pooled and RS-fortified pooled mixture.

PH AND SCFA ANALYSIS

For each of the samples, the pH was measured as an indicator of SCFA fermentation using a with Mettler Toledo seven easy pH meter. One milliliter (1mL) of the fermentation samples was thoroughly mixed with 1mL of distilled water. To it, 1ml of an acid solution (metaphosphoric acid (3.72M) plus 2-ethylbutyrate (3.72μM)) was added and vortexed. The mixture was then centrifuged for 10 min at 3000rpm. An aliquot of the supernatant was collected in auto sampling vials and analyzed using gas chromatography (GC) for short chain fatty acids (butyrate, propionate and acetate).

Table 4. GC operational conditions

GC	HP 6890 SERIES GC SYSTEM			
AUTOSAMPLER	Agilent Technologies 7683 Series			
	°C/min	**Next °C**	**Hold Min**	**Run time**
Initial			0.1	0.10
Ramp 1	10	150C	0.1	3.70
Ramp 2	11	170C	2.0	7.52

DATA ANALYSIS

The obtained results were used to calculate the actual amount of SCFAs produced from the original amount of substrate. Data was analyzed using SAS 9.3. ANOVA with two factors used as a test comparison of the means (expressed as means ± Standard deviation), and the results were presented at a significance $p<0.05$.

RESULTS

CARBOHYDRATE PROFILE OF BLUEBERRY AND BLACK RASPBERRY POMACE

The carbohydrate analysis of both dried pomaces showed the presence of simple sugars such as sucrose, D-glucose, L-Arabinose, D-fructose. Blueberry had smaller values compared to the black raspberry. As showed in table 5, both pomaces show levels ranging from 0 to 16%. The sucrose level was lower than other sugars for black raspberry and was not at a detectable level in the blueberry pomace with fructose levels highest in the profile for each pomace. As shown in table 6, the blueberry pomace was much higher in fiber and lower in digestible nutrients.

Table 5. Carbohydrate profile of blueberry and black raspberry pomace.

Samples	Analytes	µg/g of sample	Percentage (%)
Blueberry pomace	Sucrose	0	0.00
	D-Glucose	52573	5.26
	L-Arabinose	2580	0.26
	D-Fructose	57881	5.79
Black Raspberry pomace	Sucrose	2932	0.29
	D-Glucose	160265	16.03
	L-Arabinose	3530	0.35
	D-Fructose	206375	20.64

Table 6. Fiber profile of berry pomaces

	Blueberry	Black Raspberry
Dry Matter %	100.00	100.00
Acid Detergent Fiber (ADF) %	26.14	5.61
Neutral detergent fiber (NDF) %	43.28	7.43
Total Digestible Nutrients (TDN) %	70.72	92.65

PH PROFILES DURING THE ANAEROBIC FERMENTATION

Changes in pH with rat fecal samples

The fermentation using the rat fecal samples to prepare inoculum was able to decrease the pH for the Hi-Maize RS for after 12 hours and stayed constant. The black

raspberry and blueberry pomaces as shown in figure 2 did not have significant changes in the pHs during the fermentation process.

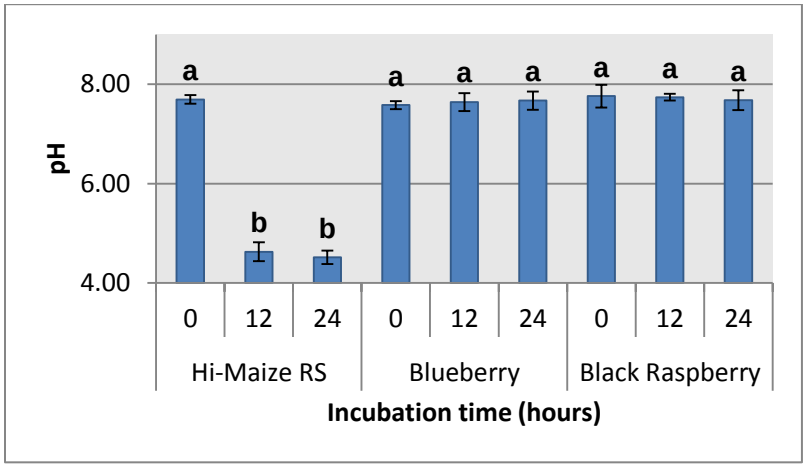
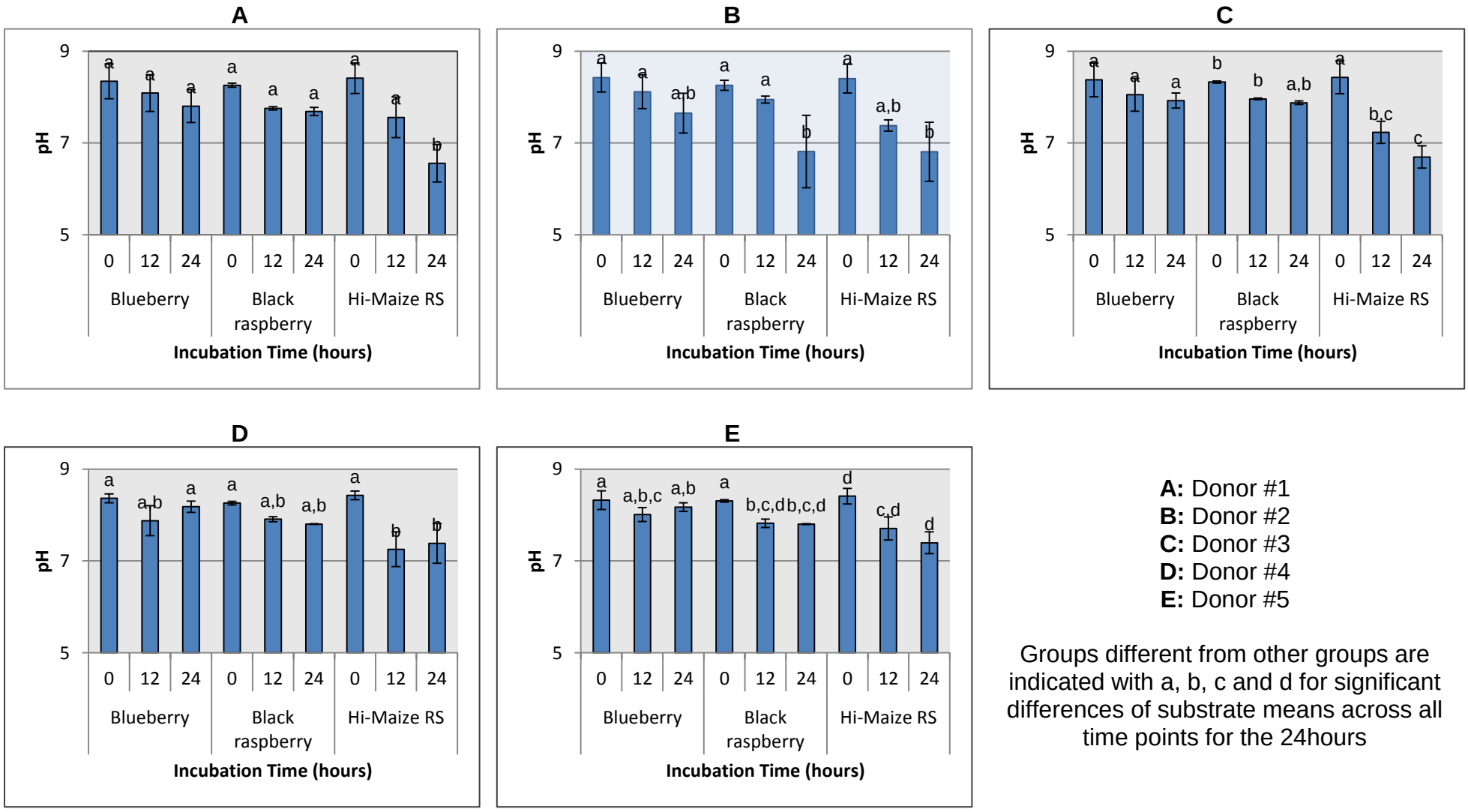


Figure 2: pH of fermentation products using rat fecal inoculum
Groups different from other groups are indicated with a and b for significant differences of substrate means across all time points for the 24hours

Changes in pH for individual donors

Figures 2, A through E represent the measurement of the pH which indicated differences between individual donors and the three substrates. The changes in pH from the 5 donors using the substrates from Hi-Maize RS, blueberry pomace and black raspberry pomace were fermented over a 24 hour period. For each individual donor there was distinctive fermentation pattern. Donors 1 and 3 showed a significant difference in pH for the hi-maize RS. The significant differences could also be noticed in other fermentation substrates. Blueberry pomace for donor 1 through 3 showed a continuous decrease of pH until the 24 hours pull time whereas for the same substrate the pH increased after the 12 hours incubation time for donors 4 and 5. All 5 donors showed similarities in the decrease rate for the pH except for donor 2 who had significantly lower pH for the 24 hours incubation period.



A: Donor #1
B: Donor #2
C: Donor #3
D: Donor #4
E: Donor #5

Groups different from other groups are indicated with a, b, c and d for significant differences of substrate means across all time points for the 24hours

Figure 3: Comparison in pH for individual stools samples

Comparison of mixed pooled samples

The pooling of donated samples allowed the mixture to contain a wide variety of microorganisms. The pH changes of the three substrates (Hi-Maize RS, blueberry pomace and black raspberry pomace) respectively in pooled non-fortified and pooled RS-fortified donors.

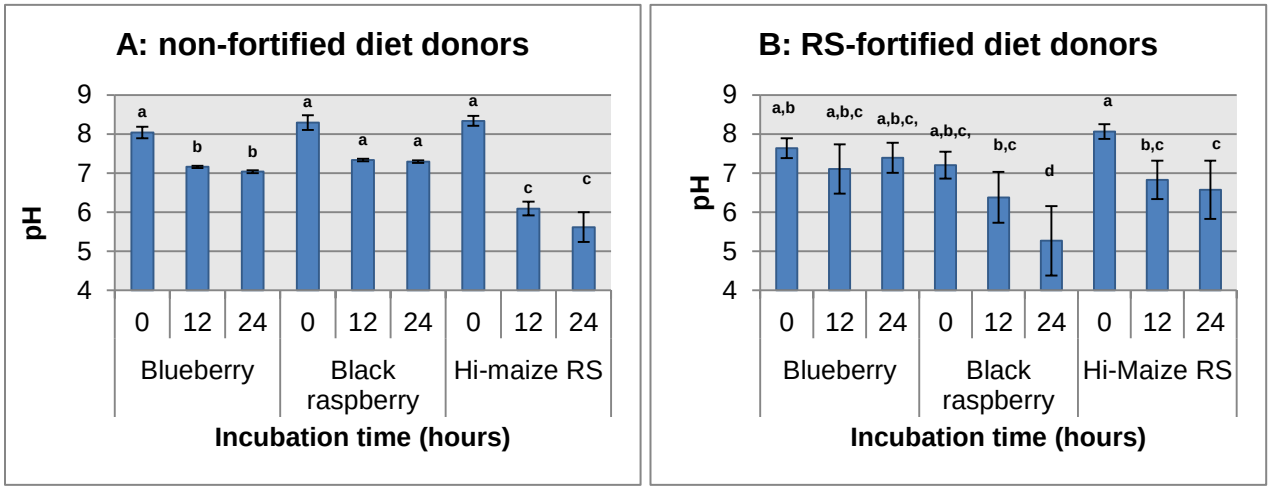


Figure 4: Comparison in pH for pooled mixed stools samples Groups different from other groups are indicated with a, b, c and d for significant differences of substrate means across all time points for the 24hours

None of the substrates showed significant changes after 12 hours of fermentation in the non-fortified diet. For the RS fortified diets, the blueberry pomace did not show significant differences in pH while hi-maize RS and black raspberry showed a pH decrease by the 12 hour measurement and was significantly lower at the 24 hours pull time suggesting that the microflora were adjusting to the substrate.

SHORT CHAIN FATTY ACID PROFILES

Rat fecal samples

The fermentation products showed an increasing concentration of SCFAs over the 24 hours period time. As inlustrated on table 7, the measured SCFAs indicated variations with all the utilized substrates. Hi-Maize RS as expected produced highest

the highest acetate after 12 hours (567.76 ±96.30 μg/g) and stayed unchanged at the 24 hour pull time. The blueberry and black raspberry pomaces on the other hand had a slow increase over the 24 hours periods. The pomaces produced higher amounts of propionate and butyrate in comparison to the control (hi-maize RS).

Table 7 : Short Chain Fatty Acid Profile for pooled samples - rats

Substrates	Time (hours)	short chain fatty acids (μg/g of fermented substrate)		
		acetate	Propionate	butyrate
blueberry	0	54.67 ± 9.67	0.89 ± 0.89	2.92 ± 1.19
	12	159.79 ± 31.36	24.61 ± 4.68	54.42 ± 27.97
	24	203.99 ± 29.93	42.85 ± 3.61	76.39 ± 33.74
Raspberry	0	57.53 ± 8.86	0.90 ± 0.90	3.02 ± 1.15
	12	166.50 ± 27.62	25.49 ± 3.64	50.75 ± 19.57
	24	219.05 ± 20.86	43.82 ± 5.24	63.84 ± 17.67
Hi maize RS	0	86.02 ± 27.45	0.94 ± 0.66	3.25 ± 1.03
	12	567.76 ± 96.30	6.05 ± 2.35	18.86 ± 17.84
	24	564.67 ± 73.96	5.58 ± 1.82	17.08 ± 15.55

Individual donors

The measurements of the SCFAs for each individual donor showed differences in the fatty acid profiles as shown in table 8. For Donor #1 greater production of acetate was observed with the RS substrate later in the fermentation time suggesting adaptive growth of advantageous organisms. The production of acetate with black raspberry pomace and blueberry pomace increased significantly after the 12 hours and remained stable for the remaining time. This may suggest that the beneficial organisms grew up in 12 hours and ran out of substrate after 12 hours. With the other donors, the responsiveness for the production of acetate differed from individual to individual, and substrate to substrate. The production of acetate of blueberry was greater than the RS for donors #2 and #4. Donors #3 and #5 did not show significant difference between the black raspberry and the blueberry pomaces after 12 hours. Although the production of

propionate was negligible compared to the acetate, significant variations in the types of substrates used, incubation times and donors were observed. As expected, the produced butyrate was much lower than acetate. The control (hi-maize resistant starch) for donor #1 had the highest butyrate production. The next observed measurements were the same for donors #4 and #5. After 24 hours, the measurements for the other substrates shows detection, but much lower than the amount produced from the fermentation Hi-Maize RS.

Table 8: Acetate µg/g of fermented substrate from individual donors

Substrates	Time (hours)	Donors				
		# 1	# 2	# 3	# 4	# 5
Blueberry	0	8.58 ± 2.95	7.85 ± 2.96	6.78 ± 1.92	9.58 ± 2.60	8.85 ± 2.63
	12	42.37 ± 9.24	42.47 ± 12.30	30.96 ± 28.20	47.32 ± 4.56	37.24 ± 9.24
	24	51.56 ± 7.43	52.31 ± 10.04	41.93 ± 11.56	58.77 ± 8.37	49.04 ± 11.02
Black raspberry	0	4.00 ± 0.40	3.07 ± 0.40	2.60 ± 0.24	3.92 ± 1.13	3.10 ± 0.49
	12	45.67 ± 10.14	25.01 ± 0.62	31.96 ± 3.06	29.69 ± 2.23	40.90 ± 2.52
	24	41.62 ± 1.35	29.48 ± 1.30	31.25 ± 1.69	36.71 ± 1.26	44.78 ± 5.85
Hi-maize RS	0	6.65 ± 9.67	1.61 ± 0.36	0.47 ± 0.57	2.08 ± 0.45	0.00 ± 0.00
	12	34.06 ± 8.80	18.49 ± 6.42	22.09 ± 7.67	23.18 ± 7.58	25.59 ± 3.82
	24	83.86 ± 32.49	37.77 ± 2.33	31.04 ± 24.20	40.59 ± 9.65	52.40 ± 14.13

Table 9: Propionate µg/g of fermented substrate from individual donors

Substrates	Time (hours)	Donors				
		# 1	# 2	# 3	# 4	# 5
Blueberry	0	0.34 ± 0.29	0.00 ± 0.00	0.00 ± 0.00	0.63 ± 0.01	0.00 ± 0.00
	12	0.54 ± 0.47	0.54 ± 0.54	0.96 ± 0.24	0.80 ± 0.18	0.17 ± 0.30
	24	2.47 ± 0.97	3.06 ± 1.31	4.36 ± 0.91	1.27 ± 0.39	3.58 ± 0.40
Black raspberry	0	0.42 ± 0.07	0.00 ± 0.00	0.00 ± 0.00	0.54 ± 0.02	0.00 ± 0.00
	12	0.59 ± 0.14	0.84 ± 0.03	0.74 ± 0.07	0.14 ± 0.25	0.84 ± 0.09
	24	1.53 ± 0.05	1.72 ± 0.05	1.92 ± 0.07	1.07 ± 0.09	2.73 ± 0.31
Hi-maize RS	0	0.35 ± 0.24	0.00 ± 0.00	0.00 ± 0.00	0.66 ± 0.07	0.00 ± 0.00
	12	0.13 ± 0.26	0.27 ± 0.31	0.29 ± 0.35	0.36 ± 0.33	0.11 ± 0.22
	24	1.15 ± 0.78	2.18 ± 0.67	3.28 ± 2.11	0.36 ± 0.32	1.26 ± 0.65

Table 10: Butyrate µg/g of fermented substrate from individual donors

Substrates	Time (hours)	Donors				
		# 1	# 2	# 3	# 4	# 5
Blueberry	0	0.86 ± 0.14	0.00 ± 0.00	0.00 ± 0.00	0.60 ± 0.05	0.00 ± 0.00
	12	0.96 ± 0.32	0.00 ± 0.00	0.00 ± 0.00	0.59 ± 0.06	0.00 ± 0.00
	24	1.21 ± 0.33	0.67 ± 0.55	0.00 ± 0.00	0.91 ± 0.15	0.22 ± 0.38
Black raspberry	0	0.75 ± 0.03	0.00 ± 0.00	0.00 ± 0.00	0.56 ± 0.01	0.00 ± 0.00
	12	1.07 ± 0.22	0.00 ± 0.00	0.60 ± 0.04	0.00 ± 0.00	0.94 ± 0.07
	24	1.09 ± 0.04	0.87 ± 0.09	0.00 ± 0.00	0.61 ± 0.02	0.00 ± 0.00
Hi-maize RS	0	0.95 ± 0.08	0.00 ± 0.00	0.00 ± 0.00	0.61 ± 0.04	0.00 ± 0.00
	12	21.33 ± 5.37	0.00 ± 0.00	0.00 ± 0.00	6.76 ± 2.09	3.98 ± 2.54
	24	43.60 ± 7.72	2.16 ± 1.19	2.58 ± 2.43	19.00 ± 19.45	11.84 ± 8.02

Pooled samples

The inoculum prepared from mixed pooled samples resulted in a significantly different SCFA profile during fermentation than observed for individual inocula. As shown in table 11, for pooled non-fortified diet, there is increase in all the SCFAs during the fermentation period. The highest concentration of short chain fatty acids (acetate and butyrate) was recorded for the blueberry and hi-maize RS, but the black raspberry had the smallest for all the fermentation times. While being significantly lower than acetate, there is still we still increased propionate over the 24 hours fermentation times. With blueberry and black raspberry pomace; butyrate increased consistently over time, but less total butyrate was produced over time. It is likely that this is because the berry pomaces were lower in fermentable carbohydrate than the RS. For the RS fortified diet, on table 12, the SCFA profile appeared significantly different from the one in the non-controlled diet. The substrate hi-Maize RS as expected, had good production of SCFAs, but the gut microbiota became more responsive in the fermentation of other substrate and which produced more acetate and butyrate compared to the non-fortified diet. The

production of acetate for blueberry and black raspberry pomaces were comparable or even higher than the hi-maize RS ones.

All 3 SCFAs profiles (rats, non-fortified and RS-fortified diets) show significant differences with the rat fecal inoculum that produced a higher amount of SCFAs.

Table 11 : Short Chain Fatty Acid Profile for pooled samples - Non-fortified diet

Substrates	Time (hours)	short chain fatty acids (µg/g of fermented substrate)		
		Acetate	Propionate	Butyrate
Blueberry	0	5.10 ± 0.98	0.00 ± 0.00	0.00 ± 0.00
	12	43.04 ± 6.63	0.65 ± 1.10	0.11 ± 0.33
	24	59.15 ± 4.89	11.63 ± 6.69	1.19 ± 1.06
Black raspberry	0	6.88 ± 0.89	0.00 ± 0.00	0.63 ± 0.92
	12	31.95 ± 6.02	0.57 ± 0.44	0.14 ± 0.21
	24	33.52 ± 2.99	1.71 ± 0.65	0.34 ± 0.32
Hi-maize RS	0	0.00 ± 0.00	0.00 ± 0.00	0.63 ± 0.73
	12	38.70 ± 11.73	1.17 ± 1.04	12.65 ± 8.18
	24	68.22 ± 21.70	3.50 ± 2.07	24.71 ± 18.54

Table 12 : Short Chain Fatty Acid Profile for pooled samples - RS Fortified diet

Substrates	Time (hours)	short chain fatty acids (µg/g of fermented substrate)		
		Acetate	Propionate	Butyrate
Blueberry	0	0.00 ± 0.00	0.00 ± 0.00	1.76 ± 2.73
	12	116.10 ± 100.86	1.80 ± 2.80	0.00 ± 0.00
	24	157.02 ± 105.93	8.21 ± 9.07	1.44 ± 2.23
Black raspberry	0	15.17 ± 21.03	0.00 ± 0.00	16.75 ± 5.56
	12	227.44 ± 182.95	0.00 ± 0.00	21.46 ± 4.59
	24	285.70 ± 183.43	0.00 ± 0.00	4.98 ± 1.87
Hi-maize RS	0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	12	77.16 ± 60.14	0.00 ± 0.00	11.80 ± 13.84
	24	115.72 ± 77.78	2.42 ± 3.34	20.55 ± 16.57

DISCUSSION

The objective of the study was the utilization of a developed in-vitro model capable of mimicking the gut fermentation of undigested carbohydrates and also the assessment of the fermentability of dietary fibers present in the black raspberry and blueberry pomaces through the model. Our expectation for the study was to develop and perfect a system capable of mimicking the digestion of food products and the in vitro colonic fermentation of undigested dietary fiber. The system will also allow us to assess the fermentability of dietary fibers in food products and difference between the individual microbiota.

CARBOHYDRATE ANALYSIS

The analysis of the dried pomaces from the blueberry and black raspberry determined a composition of 70-90% dietary fiber and included low levels of phenolic compounds, proteins and others carbohydrates. The concentration of nutrients and carbohydrates remaining in the pomace can vary significantly between different of the species of cane berry fruits, but also varies due to processing and juice pressing methods (Goni et al., 2007, Silva et al., 2005). Food components such as sugars, proteins and lipids, even though present in pomaces in very small amounts, may influence the fermentation process, however, those nutrients were removed through the pre-digestion process. The chemical composition of the fiber and the presence of other compounds are a factor in the fermentation process, causing the fibers to react differently from one source to another. The fermentability of dietary fibers in the gut may be dependent on the linkages (α-1,4 and β-1,4 glycosidic bonds, and β-glucan bonds) which vary in different type of dietary fiber.

MICROBIAL FERMENTATION

In a previous study (unpublished data) the effects of RS on the gut microbiota was assessed using an animal model and the results showed that the microbial population was favorable in butyrate formation. Lactobacillus spp Bifidobacterium spp, Clostridial clusters IV and XIVa & b and Bacteroides in the cecum of the rats fed an RS diet were significantly higher than those from the cecum of rats fed an energy control diet. Another animal study conducted by Keenan et al (2006), where rats were fed an RS diet, had a lower pH and significantly higher SCFA concentration compared to rats fed the control diet (Keenan et al., 2006). A study done with pigs fed hi-amylose starches showed that the animal fed 85% amylose starch had the fecal pH drop by one unit compared to the fecal pH of those fed 50% amylose starch (Bird et al., 2007). These results are comparable to the data presented in this study where the Hi-maize RS control substrate had a lower pH and higher SCFA levels in all individual and pooled samples when compared to the berry pomaces.

The second factor to consider during fermentation is the diversity of microflora population found in individuals. The human colon is host to more than 500 species of bacteria and each bacterial population differs from one individual to another (Turroni et al., 2008, Xu and Gordon, 2003). The difference between each individual can be identified by the resulting fermentation by-products.

PH DETERMINATION

The pH is an important measurement in the fermentation because it is an indicator of microflora growth. In many species, the rate of fermentation can be controlled by the pH. For example, an increase in the rate of fermentation corresponded to the lowering of the gut pH while inhibiting metabolism of lactate (McOrist et al., 2011).

Research conducted by Walker et al, showed that during anaerobic fermentation, a low pH of 5.5 produced a higher level of butyrate compared to samples with a pH of 6.0 (Walker et al., 2005). Likewise, a study on the effects of the pH on anaerobic fermentation showed that, in a pH environment below 5, methanogenic activities are inhibited while yielding butyrate as the main by-product (Flegal et al., 2001).

As expected in this study, in all individual or pooled samples, the HI-maize controls showed that lower the pH gets, the higher the amount of SCFAs produced. Hi-maize RS substrates had a lower reduction in the pH for each pull time. When compared to the berry pomaces, the pH did not significantly change after 12 hours. There was a reduction in pH with both the pre-digested blueberry and black raspberry pomaces, but they did not reach the pH of 6.0 that was measured with the Hi-Maize RS.

SCFAS ANALYSIS

Bacterial fermentation is dependent on the conditions that can generate several pathways to yield different compounds as end products such as SCFAs (acetate, propionate, butyrate), lactate, formate, hydrogen and carbon dioxide (Cole et al., 2005, Louis and Flint, 2009). The production of SCFAs with the rat fecal inoculum did not correlate with the pH with the black raspberry and blueberry pomaces. In comparison to the human fecal inoculum, the rat's showed higher SCFAs. The presence of other food components beside the fibers in the pomaces which did not go through the enzymatic pre-digestion may have played an important role in the higher amount of SCFAs. When monosaccharides are readily available, they are used by anaerobic bacteria before processing of the oligo and polysaccharides. With the individual inoculum used, there was a higher production of acetate with the resistant starch as the substrate. The berry pomace substrates produced different SCFAs and fermentation patterns. Overall, the

Hi-maize RS produced more acetate, propionate and butyrate than the berry pomaces for the individuals.

The resident microflora present in each individual was shown by the varied SCFA profile from each donor. The presence and the growth of bacteria such as Bacteroides, Bifidobacteria, Ruminococci, Eubacteria, or Clostridia and their anaerobic fermentation of dietary fibers can produce different pH levels. A pH reduction can selectively facilitate the growth of butyrigenic producing bacteria (Macfarlane and Macfarlane, 1993, Palframan et al., 2002).

There were significant differences in SCFAs during the fermentation between the rats, non-fortified diet and the RS-fortified diets. In the non-fortified diets, the ratio of produced acetate came up significantly higher than butyrate and propionate whereas, in the RS-fortified diets, the produced butyrate was comparable to the acetate. Similar observations have been seen in a study conducted by Belenguer et al., where some bacteria produced butyrate in the gut ecosystem by the consumption of the acetate and lactate end products of fermentation in addition cross-feeding and partial breakdown of other end products (Belenguer et al., 2006). The two pathways, “pyruvate to butyrate” and “acetate to butyrate” through the help of butyrate CoA: acetate CoA transferase could have caused of the higher amount of the butyrate in this case (Duncan et al., 2004). It has been shown by Duncan et al. that strains of butyrate-producing bacteria such as F. prausnitzii, which constitute about 15% of the human fecal microflora, require an acetate rich environment for growth, and about 50% of the butyrate producing strains proved to have net acetate consumption (Duncan et al., 2002b). A diet such as RS can be a way to induce an increase and a switch of the microbial population of butyrigenic bacteria (McOrist et al., 2011).

SUMMARY AND CONCLUSION

Dietary fibers are defined as carbohydrates or complexes that do not get broken down by the enzymes present in the digestive tract and do not get absorbed in the small intestine. The gut ecosystem is comprised of several types of bacteria which help metabolize these food components that escape digestion. These non-digestive components are mainly grouped as prebiotics and serve as nutrient sources for the bacteria which metabolize them through the anaerobic fermentation taking place in the large intestine. The short chain fatty acids (SCFAs) such as butyrate propionate and acetate are the major by-products of this fermentation process. SCFAs are beneficial to human health.

The anaerobic fermentation process used in our experiment was designed to completely mimic the in vivo digestion in the large intestine with in vitro fermentation of non-digestible carbohydrates in anaerobic conditions.

Hi-maize RS, blueberry pomace and black raspberry pomace were used to assess the individuality and the fermentability of the microflora of each donor. This individuality was indicated by the differences in the pH and the amount of SCFAs produced from each substrate for each donor. Hi-Maize RS showed a better response in the production of acetate and butyrate with higher production of propionate for the blueberry pomace and black raspberry pomace.

The prepared pooled inoculates produced lower pHs during the fermentation process. The production of SCFAs was much higher in pooled samples compared to the individual samples. After mixing the RS fortified diets, the microflora became more proficient of fermentation. The berry pomaces using the RS-fecal inoculum fermented significantly better compared to the non-fortified diet. Even though, the berry pomaces

substrates' fermentability was higher, the hi-maize RS did not show any significant difference between the two diets for the 24 hours period. The SD rats, being on a high RS diet allowed them to development a microbiota capable of fermentation of dietary fibers and better production of SCFAs. Using their fecal samples to make the bacteria inoculum in the in vitro model led to a greater SCFAs production, even for himaize RS.

We could conclude that dietary fibers, when put through the system will ferment and produce SCFAs. The fermentability of a fiber depends on its chemical structure and on the microflora available for the breakdown of that substrate. Every individual has a specific native microflora and will ferment differently from one another. The consumption of RS for a prolonged period causes the gut microbiota to be able to produce more SCFAs and can aid in the fermentability of other sources of fiber.

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APPENDICES

A. POLYSACCHARIDES KNOWN TO BE DEGRADED BY CERTAIN HUMAN INTESTINAL BACTERIA*

Bacteria	Polysaccharides
Bacteroides	Arabinogalactan, pectin, cellulose, xylan, guar gum, mucins, heparin. Chondroitin sulphate, starch gum arabic, xylan
Bifidobacteria	Arabinogalactan, pectin, starch, mucin,
Ruminococci	Mucins, guar gum
Eubacteria	Starch, pectin
Clostridia	Starch, pectin

*Types of bacteria and main polysaccharides they degrades data are from (Macfarlane and Macfarlane, 1993).

B. FDA RECOMMENDED DAILY INTAKE/VALUE OF FOOD COMPONENT

Food Component	Daily Value
Total Fat	65 grams (g)
Saturated Fat	20 g
Cholesterol	300 milligrams (mg)
Sodium	2,400 mg
Potassium	3,500 mg
Total Carbohydrate	300 g
Dietary Fiber	25 g
Protein	50 g
Vitamin A	5,000 International Units (IU)
Vitamin C	60 mg
Calcium	1,000 mg
Iron	18 mg
Vitamin D	400 IU
Vitamin E	30 IU
Vitamin K	80 micrograms µg
Thiamin	1.5 mg
Riboflavin	1.7 mg
Niacin	20 mg
Vitamin B6	2 mg
Folate	400 µg
Vitamin B12	6 µg
Biotin	300 µg
Pantothenic acid	10 mg
Phosphorus	1,000 mg
Iodine	150 µg
Magnesium	400 mg
Zinc	15 mg
Selenium	70 µg
Copper	2 mg
Manganese	2 mg
Chromium	120 µg
Molybdenum	75 µg
Chloride	3,400 mg

C – SOLUTIONS FOR FIBER ANALYSIS

Neutral Detergent Solution

Reagents	
Distilled water (liters)	1.00
Sodium lauryl sulfate, USP (grams)	30.00
Disodium ethylene diamine tetra acetate (EDTA),dihydrate crystal, reagent grade (grams)	18.61
Sodium borate decahydrate, reagent grade (grams)	6.81
Disodium hydrogen phosphate, anhydrous, reagent grade (grams)	4.56
2-ethoxyethanol (ethylene glycol monoethyl ether), purified grade (milliliters)	10.00
Preparation	
Put EDTA and Na2B4O7 • 10H2O together in a large beaker, add some of the distilled water, and heat until dissolved; then add to solution containing sodium lauryl sulfate and 2-ethoxyethanol (ethylene glycol monoethyl ether). Put Na2HP04 in beaker, add some of the distilled water, and heat until dissolved; and then add to solution containing other ingredients. Check pH to range 6.9 to 7.1. If solution is properly made, pH adjustment will rarely be required.	

Acid Detergent Solution

Reagents	
Sulfuric acid, reagent grade standardized to 1N (100=percent assay) (grams)	16.04
Cetyl trimethylammonium bromide (CTAB), technical grade (grams)	20.00
Preparation	
Weigh sulfuric acid and make up to volume with distilled water at 20° C. Check normality by titration before addition of detergent. Then add CTAB and stir.	

VITA

M'famara Goita was born in 1985 in Baton Rouge, Louisiana USA. He received his primary and secondary educations in Jean Paul II catholic school in Bamako, Mali W. Africa. He went to high school at "La Chaine Grise Monseigneur Didier de Montclos de Bamako", where he received his Bachalaureat in 2002. Two years later, he started his undergraduate studies at Louisiana State University. He participated in couple of Research Experience for Undergraduates (REU) programs, then presented his work at the SERMACS at Memphis, TN in 2008. In 2008, he received his Bachelor of Science degree in Chemsitry, then he started working as a researcher associate for the department of food science at LSU.

He started his graduate studies under at Louisiana State University. During his graduate career, Mfamara worked as a graduate research assistant. He worked in the development of couple of products included a healthy breakfast bar named "Cocoa Bleuet", a Protein ball and Vitamin Candies. He also presented his work at the Experimental Biology 2012, San Diego, CA.

Mfamara plans to graduate with his Master's in Food Science with a concentration in Food Chemistry. Eventually, Mfamara would like to obtain a Doctorate degree and pursue his professional career in a food manufacturing company.