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Development of a Soy-blueberry Burger and the Changes in Anthocyanins and Phenolics During Storage and Broiling

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**DEVELOPMENT OF A SOY-BLUEBERRY BURGER AND THE CHANGES IN
ANTHOCYANINS AND PHENOLICS DURING STORAGE AND BROILING**

By

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A THESIS

Submitted in Partial Fulfillment of the

Requirements for the Degree of

Doctor of Philosophy

(in Food and Nutrition Sciences)

The Graduate School

The University of Maine

August, 2007

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By Pamela Beth Small

Thesis Advisor: Dr. Alfred Bushway

An Abstract of the Thesis Presented
in Partial Fulfillment of the Requirements for the
Degree of Doctor of Philosophy
(in Food and Nutrition Sciences)
August, 2007

Blueberries are high in phenolic acids and flavonoids, which make them one of the leading sources of antioxidants. When added to foods, antioxidants can increase shelf life, maintain nutritional quality, and retard production of heterocyclic amines in meats during cooking thus having the potential to be of great significance to the food industry.

Soy and soy-based foods have been gaining in popularity as a functional food for specific health conditions. Increased media coverage touting the health benefits of soy has generated a rise in consumer awareness.

Two soy-blueberry burgers were prepared using 10 and 15% blueberry puree. A soy burger with no blueberry puree was formulated as a control. Once

the final recipe was determined, the soy-blueberry burgers were made in bulk, blast frozen and placed in frozen storage.

Total phenolics were determined in triplicate from six samples. SAS and Tukey's ($\alpha=0.05$) was utilized to determine the differences in total phenolics between cooked and uncooked samples of the burgers and the total phenolic change in the samples over time (uncooked). The differences in total phenolics were statistically different from each other ($P \leq 0.05$). The total phenolic changes in samples over time: 0, 3 and 6 months were statistically different from each other.

Three sensory tests were conducted. A quantitative affective test was utilized to determine the overall liking for the soy-blueberry burger in regards to appearance, texture, and flavor. Overall appearance and texture were favored in the 10% burger in two of the three tests. All tests showed the same trends in flavor, overall acceptability, and preference with the 15% burger being favored.

A lower sodium burger was utilized in the third sensory test. All burgers had a 30% reduction in sodium. These burgers had the highest overall acceptance in the study.

To date, there are no vegetable burgers on the commercial market that combine the possible health benefits of soy and blueberries. The objectives of this research were 1) to develop a soy-blueberry burger that would promote a healthy diet and utilize Maine blueberries and 2) to determine the changes in anthocyanins and phenolics during storage and broiling.

DEDICATION

I dedicate this manuscript to my family without whose love and support I would not be where I am or who I am today. Life is not about finding yourself it is about creating yourself. 40 and 40+

Where would one be without friends? I have many who have and continue to make my life a most enjoyable journey. I am truly blessed.

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It may seem silly to thank my menagerie of four legged companions but I would be remiss if I did not. I strive to be as wonderful of a person as they think I am.

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Chapter 1

INTRODUCTION

Blueberries

Blueberries are produced commercially in 16 countries worldwide on about 120,000 acres. They are a crop native to North America with production concentrated in the United States and Canada. Presently, the United States supplies more than half of the world's production of 525 million pounds (Figure 1).

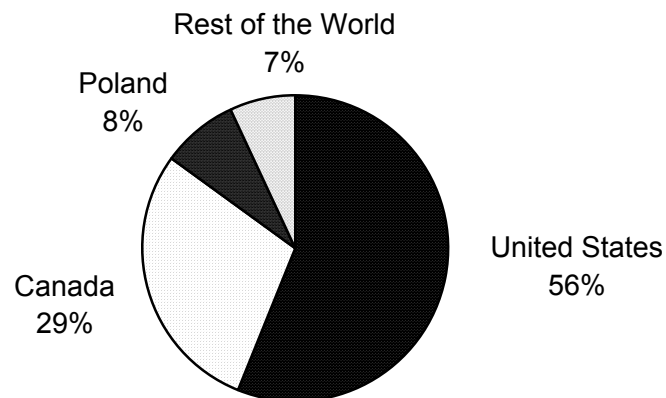


Figure 1. Top Four World's Producers of Blueberries

Source: USDA 2006 FAO

The North American blueberry industry produces 350 million pounds of wild and cultivated blueberries annually (Figure 2). Wild blueberries make up approximately half of this 350 million pound crop. Maine averages nearly 75 million pounds annually with another 30,000 acres of blueberry fields in the non-

fruit bearing stage every year. Currently, 99 percent of the crop is frozen, but five to ten percent of those berries are canned after the harvest is complete. Less than one percent of the wild blueberry crop is sold fresh (Yarborough 2004).

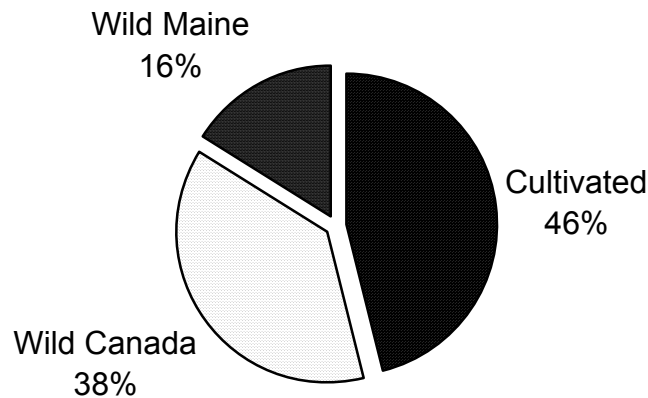


Figure 2. North American Blueberry Production

Source: USDA 2006 FAO

Blueberries and Health

Health benefits obtained from the consumption of blueberries has increased commercial interest by the industry (Camire 2002). Howell and others (1998) have shown that blueberries contain proanthocyanidins that reduce the ability of *Escherichia coli*, a bacterium responsible for urinary tract infections, to adhere to the epithelial cells that line the urinary tract. A study done by Schmidt in 2004 used extracts from wild blueberry (*Vaccinium angustifolium Ait.*). The extracts were separated into proanthocyanidin-rich fractions. The fraction composition was correlated with bioactivity using antiproliferation and

antiadhesion in vitro assays. Specifically, the assays showed inhibited adhesion of *Escherichia coli* responsible for urinary tract infections. As well, this study suggests both antiadhesion and antiproliferation activity are associated with high molecular weight proanthocyanidin oligomers found in wild blueberry fruits.

Among fruit and vegetables, blueberries are one of the richest sources of antioxidants, which may provide protection against coronary heart disease and stroke. Glycosaminoglycans (GAG) are functionally and structurally important carbohydrate components of the aorta; they interact with various compounds e.g. lipoproteins and are affected in the development of atherosclerosis. In 2006, a study done by Kalea and others, the effects of a diet rich in blueberries on the content and structure of aortic GAG were investigated in Sprague-Dawley rats. The rats were fed on control or a blueberry-rich feeds for 13 wk. At the end of the feeding period, rats were anaesthetized and their thoracic aortas were removed. GAG populations were lower in the aortas of blueberry fed rats than in control fed rats. Results indicate that diets rich in blueberries produce structural alterations in rat aortic tissue GAG. Other investigations have indicated that the moderate consumption of anthocyanins through the intake of products such as bilberry extract (Xue and others 2001) or red wine (Renaud and de Logeril 1992) is associated with a lower risk of coronary heart disease.

Bickford and others (2000) reported that rat diets supplemented with either spinach, strawberries or blueberries reversed age-induced declines in beta-adrenergic receptor function. In addition the spinach diet improved learning on a runway motor task, previously shown to be modulated by cerebellar

norepinephrine. The researchers state that motor learning is important for adaptation to changes in the environment and is thus critical for rehabilitation following stroke, spinal cord injury, and the onset of some neurodegenerative diseases. These data indicates that age-related deficits in motor learning and memory can be reversed with nutritional interventions.

Joseph and others (1999) theorized increasing antioxidant levels in the diet could reverse brain aging and other age-related diseases that are related to oxidative stress. Bickford and others in 1999 investigated the effects of aging on cerebellar noradrenergic function and motor learning. Aging is associated with a decline in motor coordination and the ability to learn new motor learning skills. This loss of function is correlated with a decline in cerebellar beta-adrenergic receptor function. Foods such as blueberries and spinach can prevent and/or reverse age related declines in cerebellar noradrenergic receptor function.

Zhao and others (2004) conducted a study on HT-29 colon cancer cell lines and found that anthocyanins in blueberry extracts inhibited the growth of those cells. As well, Schmidt and others (2004) found that wild blueberry extracts of proanthocyanidins prevented the growth of human prostate cancer cells and mice cancer cell lines. Roy and others (2002) conducted a study using edible berry extracts. In the study, the researchers state that edible berries may have chemopreventive properties and that anti-angiogenic approaches to prevent and treat cancer represent a priority area in investigative tumor biology. Angiogenesis is a term used to describe formation of new blood vessels and is unwanted in situations including varicose veins and tumor formation. Vascular

endothelial growth factor (VEGF) plays a crucial role for the vascularization of tumors. While the vasculature in adult skin remains normally quiet, the skin retains the capacity for brisk initiation of angiogenesis during inflammatory skin diseases such as psoriasis and skin cancers. The study investigated six berry extracts (wild blueberry, bilberry, cranberry, elderberry, raspberry seed, and strawberry) and a grape seed proanthocyanidin extract (GSPE) and found that the antioxidant capacity of the extracts inhibited VEGF.

Blueberries and Antioxidants

Formation of free radicals: bonds don't typically split in a way that leaves a molecule with an odd, unpaired electron. But when weak bonds split, free radicals are formed. Free radicals are very unstable and react quickly with other compounds, trying to capture the needed electron to gain stability. Generally, free radicals attack the nearest stable molecule, "stealing" its electron. When the "attacked" molecule loses its electron, it becomes a free radical itself, beginning a chain reaction. Once the process is started, it can cascade, finally resulting in the disruption of a living cell. Normally, the body can handle free radicals, but if antioxidants are unavailable, or if the free-radical production becomes excessive, damage can occur. Of particular importance is that free radical damage accumulates with age.

Antioxidants neutralize free radicals by donating one of their own electrons. The antioxidant nutrients themselves don't become free radicals by donating an electron because they are stable in either form. They act as scavengers,

helping to prevent cell and tissue damage. Interest in finding and utilizing naturally occurring antioxidants (Fukumoto and Mazza, 2000) is growing. The protection provided against disease by vegetables and fruits have been attributed to the various antioxidants found in these foods (Ames and others 1993). The antioxidants, which neutralize free radicals, include catechins, flavones, isoflavones, phenolics and anthocyanins (Cao and others 1996, Wang and others 1996). Antioxidants offer protection against the oxidative stress that has been associated with many chronic and degenerative diseases (Wu and others 2004).

Lipid oxidation occurs when oxygen reacts with lipids in a series of free radical chain reactions that lead to complex chemical changes. Oxidation of lipids in foods causes quality losses. In vivo, lipid oxidation may play a role in coronary heart disease, atherosclerosis, cancer, and the aging process (Jadhav and others 1996). Antioxidants are compounds that can delay or inhibit lipid oxidation. Blueberries vary in their antioxidant capacities (Prior and others 1998). The anthocyanin content, a major contributor to the antioxidant capacity, may be affected by differences in growing season, the location of growth, rainfall, species, and maturity of the fruit. In general, blueberries are high in phenolic acids and flavonoids (particularly anthocyanins), which make them one of the leading sources of antioxidants (Kalt and others 1999; Wu and others 2004; Prior and others 1998; Cao and others 1997). Total phenolic content and antioxidant capacity have been shown to have a strong linear relationship in fruit (Kim and others 2003). Where blueberries are one of the richest sources of

antioxidants (Kalt and others 2001; Wu and others 2004; Wang and others 1996; Prior and others 1998), they have the potential to be of great significance to the food industry. Recently, interest has been growing in finding naturally occurring antioxidants for use in foods to replace synthetic antioxidants and for possible in vivo use. Blueberries may relieve eyestrain from staring at a computer screen (Kalt and Dufour 1997). These are tied to increased blood circulation to the eye due to vasorelaxation, and reduced damage suffered by the eye due to exposure to free radicals. Similarly, antioxidants keep eye lens protein radicals from cross-linking potentially leading to cataracts (Kalt and Dufour 1997). When added to foods, antioxidants minimize rancidity, retard the formation of toxic oxidation products, maintain nutritional quality, increase shelf life (Jadhav and others 1996) and retard production of heterocyclic amines (HAs) in meats during cooking (Wang and others 1982; Pearson and others 1992).

Heterocyclic Amines

Heterocyclic amines are mutagenic compounds formed from single amino acids and/or proteins that are naturally present in meat and other proteinaceous foods. To date, more than 20 HA's have been identified in heat-treated foods. Not only does the content of mutagen precursors vary depending on the details of cooking but human dietary habits differ as well. Some studies suggest an association between meat consumption and mutagenic tumors (DeStefani and others 1997; Sinha and others 2001; Thorogood and others 1994). Other

studies find no correlation between ingested HA's and cancer (Augustsson and others 1999; DeStefani and others 2001; Gertig and others 1999).

Several studies have investigated the reduction of mutagenic activity by the addition of antioxidants to meat: (Wang 1982; Pearson 1992; Persson and others 2003; Shon and others 2004; Murkovic and others 1998; Oguri and others 1998),

The formation of HAs is not limited to muscle food. Utilizing the Ames/*Salmonella* test, the Lawrence Livermore National Laboratory (LLNL) has detected mutagenic activity in cooked non-meat protein-containing foods, such as those high in wheat gluten. As well, Hotchkiss and Parker (1990) suggest that nearly all proteinaceous foods will form HAs. The consumption of meat is a controversial dietary topic.

The LLNL has recognized 2-amino-1-methyl-6-phenylimazo[4,5-b]pyridine (PhIP) as the most abundant heterocyclic amine in proteinaceous foods and although it is produced during the cooking of soy (Thizbaud and others 1995), the levels are negligible in comparison to muscle foods.

Soy Production and Use

In the United States, soybeans were planted on 75.5 million acres (30.6 million hectares) in 2006, producing a record 3.188 billion bushels (86.77 million metric tons) of soybeans (Figure 3). The average price paid to farmers was \$6.20 per bushel (\$228 per metric ton). The total 2006 crop value exceeded \$19.7 billion (USDA 2006).



Alabama	160	Maryland	470	Oklahoma	310
Arkansas	3,110	Michigan	2,000	Pennsylvania	430
Delaware	180	Minnesota	7,350	S.Carolina	400
Florida	7	Mississippi	1,670	S.Dakota	3,950
Georgia	155	Missouri	5,150	Tennessee	1,160
Illinois	10,100	Nebraska	5050	Texas	225
Indiana	5,700	New Jersey	88	Virginia	520
Iowa	10,150	New York	200	W.Virginia	17
Kansas	3,150	N.Carolina	1,370	Wisconsin	1,650
Kentucky	1,380	N.Dakota	3,900		
Louisiana	870	Ohio	4,650		

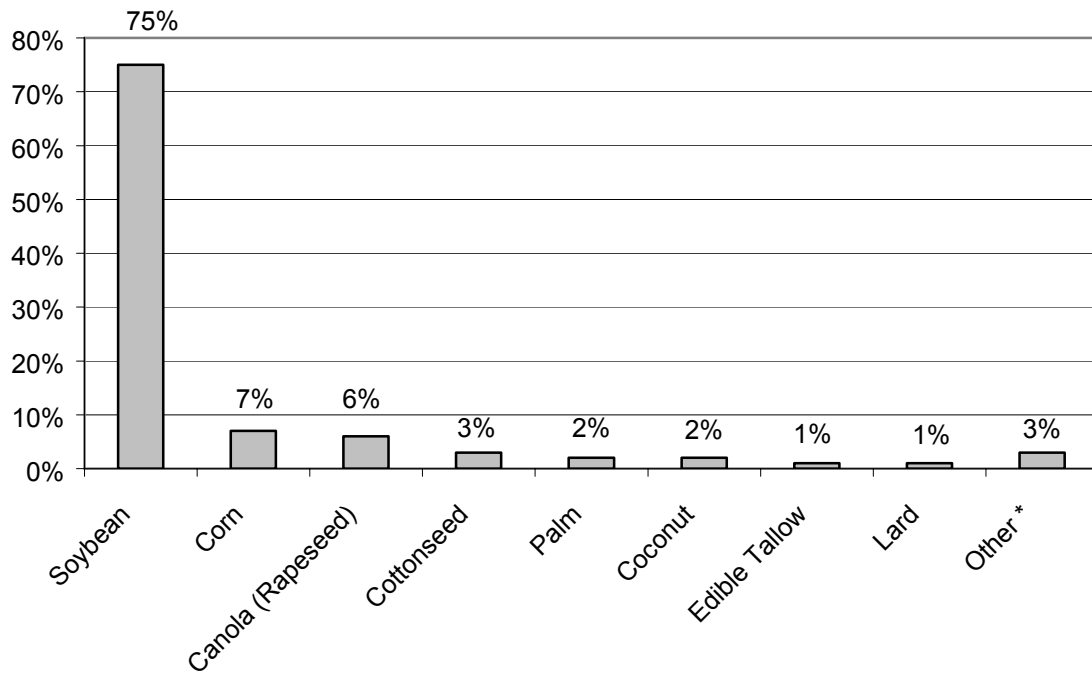
Figure 3. Soybean Area per Thousand Acres Planted by State 2006

The map also shows thousand hectares in italic font.

Source: USDA 2006

In 2006, soybeans represented 57 percent of world oilseed production, and 38 percent of those soybeans were produced in the United States. The United States exported a record 1.1 billion bushels (29.9 million metric tons) of soybeans, which accounted for 42 percent of the world's soybean trade. U.S. soybean and product exports were \$8.9 billion in 2006. China was the largest customer for U.S. soybeans with purchases totaling \$2.5 billion. Mexico was the second largest market for U.S. soybeans with purchases of \$906 million. Other significant buyers included Japan with purchases of \$863, and the European Union with purchases of \$720 million. Mexico was the largest customer for U.S. soybean meal at \$377 million, Canada was second with purchases of \$283 million, and The Philippines was third with purchases of \$123 million. Mexico was the largest customer for U.S. soybean oil with purchases of \$60 million, and China was second with purchases of \$59 million (USDA 2006).

Domestically, soybeans provided 75 percent of the edible consumption of fats and oils in the United States (Figure 4).



Soybean	17,045 million pounds
Corn	1,483
Canola (Rapeseed)	730
Palm	471
Coconut	360
Edible Tallow	214
Lard	176
Other*	751
Total	22,871

Figure 4. U.S. Fats & Oils Percent Edible Consumption 2006

Source: U.S. Census Bureau, 2006

*Other includes sunflower, safflower, palm, palm kernel, and peanut.

Soy protein products have a long history of usage by the meat industry. This growth in utilization can be attributed to a number of factors. 1) The protein products have been improving in taste and functionality. 2) The processor learned the proper use of soy protein products discovering that if little was good, more was not necessarily better unless major adjustments were made. 3) Economic benefits were realized without a loss in quality. 4) Consumer interest in nutrition helped focus attention on the soy protein products as excellent supplemental ingredients (Rakosky 1974).

Fat is an important constituent in meat. Its purpose is to improve texture and to add flavor. Without fat, meat tends to be tough and lacks the richness of flavor expected in meats. The meat processor attempts to duplicate nature in this respect when he combines lean meat with fat. This is accomplished more efficiently if the meat ingredients are comminuted, which is usually achieved by chopping, flaking, grinding, or similar processes.

Soy proteins are considered a natural addition to processed meats for several reasons. 1) Soy proteins are functional; many have emulsification and binding properties. Soy proteins have an affinity for the meat juices. This not only helps reduce cooking losses, but the resulting product is more juicy and flavorful. 2) Soy protein products are high in nutritious protein that will complement the meat protein. 3) Soy proteins provide functional properties at a reasonable cost. On the basis of protein they are among the lowest cost products available (Rakosky 1974).

Soybean processing yields a number of products. There are industrial applications as well as food and feed applications. Although they can be eaten whole after being boiled or roasted, most soybeans are transformed into a variety of foods. In the main form of processing, or "crushing", the soybeans are cleaned, cracked, de-hulled and rolled into flakes. The crushing process ruptures the oil cells for extraction. The oil is removed with solvents or screw presses, and after further processing; the refined soybean oil goes into such products as margarine, salad dressings and cooking oils. After the oil is extracted, the flakes are toasted and ground to produce soybean meal, most of which is used as a high-protein component of animal feeds. However, some of it is also processed into products for human consumption. These products include soy flour and grits, soy protein isolate and soy protein concentrate.

Soy Flour and Soy Grits

Soy flour and soy grit products are obtained by grinding defatted flakes. The protein content and other characteristics of these flakes are the same. Soy flours are 100 mesh or finer products and have been used in cooked sausage and nonspecific loaves for several years. Its primary purpose has been to extend meat, and it was used because it was an inexpensive product high in nutritious protein. It was recognized early that soy flour has the advantage of holding both the meat juices and the fat. Its main disadvantage has been its taste and mouthfeel. These factors tended to limit its use.

Soy grits are obtained by grinding flakes to particle sizes larger than 100 mesh. Like soy flour the toasted product is preferred in meat applications. Soy grits also are used in sausage products but to a lesser degree than soy flour. Soy grits have greater utility in coarse ground meat products such as hamburger-type products. In the U.S. additives such as soy grits are not permitted in hamburgers. The disadvantage of mouthfeel in products containing soy flour is not noticed in similar products containing soy grits. This may be due to expectancy on the part of the taster, i.e. if it can be seen it is expected (Rakosky 1974).

Isolated Soy Protein

Isolated soy protein (ISP) is produced by extracting a white flake with water or mild alkali. The protein-containing liquor is separated from the flake residue and the protein in the liquor is precipitated with food-grade acid. The resulting curd is washed and spray dried in the isoelectric form, or the curd is neutralized before spray drying to produce a water-dispersible sodium proteinate (Rakosky 1974). In both cases the protein content is greater than 90% on a dry weight basis. The sodium proteinate is the form used most widely by the meat industry. Isolated soy protein (ISP) is available in either the isoelectric form or as a proteinate. Although it can be made as the salt of various cations, its usual form is the sodium proteinate. ISP is a globulin-like fraction selectively extracted from defatted flakes. It is both an emulsifier and a binder.

ISP also is used advantageously in canned meat items because the high processing temperatures do not affect it adversely. In this application, there appears to be a protective action for the meat protein against the effects of heat. ISP also functions to an advantage in sausage products where large percentages of poor water binding capacity meats are used.

Soy Protein Concentrates

The development of soybean protein concentrates stemmed principally from two considerations: to improve flavor and increase protein concentration. Beany flavor is one of the major objectionable characteristics, which limits the use of soy flours. It is difficult to avoid the occurrence of the beany flavor of soybeans in untoasted full-fat or defatted soy flour. Further processing of flours into concentrates extracts the components lipoxidase, urease and antitrypsin, which are responsible for the beany taste and bitterness (Campbell 1985). Concentrates have a protein content on a moisture free basis of 70%. Depending on the application, soy protein concentrates can be classified as a flour-like product or granular.

Textured Soy Protein (TSP)

Textured soy protein (TSP) is made wholly from either defatted soy meal flakes or soy protein concentrate. This soy product, also called granulated soy protein concentrate (GSPC) is available as either a mince/crumble or chunk form. The former is similar in texture to ground beef, while the chunks resemble

cubed meat used in stews. It can be hydrated to a greater degree and used at higher levels. The advantages over soy grits are that it is blander and has a higher protein content. The advantage in using GSPC/TSP in patties is that shrink is reduced 10% (Rakosky 1974). Good dimensional stability, as well as a better tasting, juicier product, is an added benefit. For best results from the use of GSPC/TSP, it is recommended that the product be presoaked with water for a short time before it is added to the meat (Soya Bluebook 1989). The product is kosher and pareve. The functional properties of TSP are estimated to remain excellent for one year. The nutritional quality of the product is excellent for several years. Flavor changes can occur when TSP is stored under adverse conditions for periods longer than one year. Storage below 75° F and 60% relative humidity will promote longer shelf life. Based on reports from manufacturers, TSP has been successfully used worldwide for more than 30 years in developing countries. Uses of soy protein in food include but are not limited to meat food products such as: emulsified meats, coarsely-chopped meats, canned meats, whole muscle meats, poultry products, seafood products, analogs, pet foods; dairy-type products: beverage powders, cheeses, coffee whiteners, frozen desserts, whipped toppings, infant formulas, milk replacers for young animals, bakery products, cereals, pasta, and miscellaneous foods such as: soups, gravies, sauces, candies, confections and oriental foods (U.S. Soybean Export Council 2006).

Considerations

Salt has a masking effect on soy, as does lemon oil (Rakosky 1974). Gardze and others (1979) also found that salt caused decreased cereal-like aroma and flavor of soy and generally increased desirability scores. Salt also decreased the oily mouth coating. The flatus factor found in soy flour and grits can cause problems to those individuals who have sensitive intestinal tracts. In such cases, both soy protein concentrates and ISP are recommended since the sugars causing these problems (stachyose and raffinose) are absent.

Soy and Health

Dietary and lifestyle habits differ thus posing varying cancer risks (Augustsson and others 1997; Williams, 1985; Doll and Peto, 1981; Commoner and others 1978). The consumption of meat is a controversial dietary topic. Some studies suggest an association between meat consumption and mutagenic tumors (DeStefani and others 1997; Sinha and others 2001; Thorogood and others 1994). Soy and soy-based foods provide many health benefits (Anderson and Garner, 1997; Slavin, 1991) and they have been gaining in popularity as a functional food for specific health conditions (IFICF 2003). Increased media coverage touting the health benefits of soy has generated a rise in consumer awareness (United Soybean Board 2001).

Soy research today includes but is not limited to cancer, cardiovascular health, osteoporosis, and menopausal relief (Messina and Messina, 2003; Anderson and others 1999). Messina (2003) and Adlercreutz (2003) proposed

that the isoflavone genistein plays a role in breast and prostate cancer prevention. Current research has demonstrated no clear evidence that soy prevents (or causes) breast cancer. The guideline for soy consumption by women with or without breast cancer is moderate (Messina and Loprinzi, 2001). Women with ER+ tumors are not to increase their soy intake (Duffy and Cyr, 2003) and women who are at risk for breast cancer should avoid soy isoflavone supplements (Kurzer, 2003). In 1999, the US Food and Drug Administration passed a soy protein health claim stating that 25g of soy protein in a low-fat, low-cholesterol diet can help reduce the risk of heart disease. A study by Anderson and others (1995) evaluated 38 clinical studies on the relationship between soy protein and total cholesterol, low-density lipoprotein cholesterol, and triglyceride levels with all three values decreasing significantly with consumption of soy protein. Sirtori and others in 1995 clinically explored the effects of soy on lowering cholesterol. Anderson and others in 1999 investigated the positive cardiovascular and renal benefits of dry bean and soybean intake. In contrast, a meta-analysis of 68 studies of soy protein and cholesterol recently found only an average 3% reduction in LDL and 8% reduction in triglycerides. The report was compiled by the Agency for Healthcare Research and Quality (AHRQ)'s Tufts-New England Medical Center Evidence-Based Practice Center. Also, a 2004 Dutch study published in the Journal of the American Medical Association tested the effect of soy protein intake on a daily basis. Researchers found no significant difference in cholesterol or other plasma lipids between subjects taking soy protein and

those on a placebo. A 2005 University of Massachusetts study, published in the *Journal of the American College of Nutrition*, had similarly disappointing results (Tufts University Health & Nutrition Letter 2005).

Osteoporosis is a worldwide problem and the most prevalent metabolic bone disease in developed countries, including the United States (Wasnich 1996). There is considerable interest in the skeletal benefits of soy foods. In part, this is because Asian epidemiologic studies generally show that soy intake is positively associated with higher bone mineral density (Messina 2004). Both the hormonal and non-hormonal properties of isoflavones may contribute to possible skeletal benefits of soy foods (Branca 2003). The clinical data suggest that isoflavones reduce bone loss, but the inconsistent results and relatively small size and short duration of most of the trials prevent definitive conclusions from being made. Despite this, the data are encouraging enough to recommend soy foods consumption for postmenopausal women who are concerned about bone health. Setchell and Lydeking-Olsen looked at dietary soy phytoestrogens and their sparing effect on bone (2003). In controversy, the 2004 Dutch study reported in *JAMA* also looked for bone-health benefits and found little difference between soy protein and a placebo (Tufts University Health & Nutrition Letter 2005).

Soy isoflavones are also purported to lower hot flash occurrence by 30 – 50% in menopausal women (Kurzer, 2003). However, in two separate studies in 2001, Margo N. Woods, DSc, and Barry R. Goldin, PhD, both of Tufts' School of Medicine, found no difference in the number or intensity of hot flashes between

the periods her subjects took extra isoflavones and the time the same women took a placebo instead (Tufts University Health & Nutrition Letter 2005).

Some evidence suggests that soy foods may be valuable in maintaining control of glucose and insulin levels. Ho and Chen (2005) found that among 173 postmenopausal women from Hong Kong, habitual soy protein intake was inversely related to fasting serum glucose levels in women with baseline fasting glucose levels above the median. As well, Yang and others (2004) found that, among Chinese postmenopausal women with a body mass index of $<25 \text{ kg/m}^2$, the risk of glycosuria was reduced by about two-thirds in high-versus low-soy consumers. A few human intervention studies suggest that soy protein or isoflavones increase insulin sensitivity, but many other studies do not (Jayagopal and others 2002; Duncan and others 1999; Ham and others 1993; Lang and others 1999). At this time, no firm conclusions can be made (Messina 2005) but it is important to note that soy foods have a low glycemic index (Foster-Powell and others 2002). The glycemic index refers to the relative blood glucose response to carbohydrate-containing foods. Some evidence indicates that foods with a high glycemic index increase risk for a variety of chronic diseases, including diabetes and obesity (Hodge and others 2004). Thus, the low glycemic index of soy foods suggests that they have a role to play in helping control diabetes and obesity. Increased rates of diabetes have resulted in a dramatic rise in the incidence of kidney, or renal, disease (Hostetter 2001; Sims and others 2003), which is often a complication of diabetes. Studies have shown that the type and amount of protein can play an important role in renal

function. One of the first human studies to suggest that soy protein might favorably affect renal function was published by Kontessis and others in 1990.

Soy protein is one of the eight major food allergens, along with proteins from milk, eggs, peanuts, tree nuts, fish, shellfish and wheat. Although a person can be allergic to any food, these eight account for 90 percent of all food-allergic reactions. It is important to note that among adults, soy allergy is relatively uncommon and is much less prevalent than the common food allergies mentioned above (Cordle 2004).

Soy Trends

From 1992 to 2004, soyfood sales in the United States increased from \$300 million to \$4.1 billion over 12 years. This increase can be attributed to new soyfood categories being introduced, soyfoods being repositioned in the market place, and new customers selecting soy for health and philosophical reasons. New growth for soy will come with more consumers making a commitment to following healthier diets and more research linking soy with disease prevention. The wide variety of soyfoods will help consumers meet the 2005 Federal Dietary Guidelines that call for eating foods like soy that are high in fiber, omega 3 fatty acids, key vitamins and minerals, and lower in saturated fat, cholesterol and calories. From 2000 to 2005, food manufacturers in the United States introduced over 2,100 new foods with soy as an ingredient, averaging about 350 new products per year. According to the Mintel's Global New Products Database, the 1999 FDA-approved health claim for soy and heart

health brought many new introductions, leading to 406 new products in 2001, 278 in 2002, 336 in 2003 and 448 in 2004. (Soyfoods Association of North America; U.S. Soybean Export Council 2006).

Consumer Attitude

According to Hymowitz (1990) soy foods have been available for human consumption within the United States for several decades. However, there are several barriers to soy consumption (Schyver and Smith, 2005), the greatest being its unfavorable image. Taste, texture, and visual appearance are often described in unfavorable terms. Soy is also viewed by many as a substitute food; food for “vegetarians, hippies, Asians and/or for those with food allergies.” As well, those who do not consume soy products do not know how to prepare them much less find them or know their cost. Then there are the soy consumers that won’t eat soy because of excessive processing and packaging; those who are concerned about genetically modified soy in soy foods, and those that suffer gastrointestinal discomfort.

In 2006, 82 percent of consumers perceived soy products as healthy. In addition, 31 percent of consumers, for health reasons, specifically purchased products that contained soy. In comparison, only 26 percent intentionally bought soy foods in 2005. According to the 2006 Consumer Attitudes Report, 30 percent of Americans consume soy foods or soy beverages once a month or more. The report also states that over half of consumers have tried soy foods or beverages in restaurants, and over one-third said they would order soy

products in restaurants if they could find soy on the menu. As a versatile source of food, the soybean is hard to beat. It is the highest natural source of dietary fiber. Nine essential amino acids, which are necessary for human nutrition and are not produced naturally in the body, are found in soybeans (Source: American Soybean Association.). As consumers become more health conscious (Sloan, 2004) the development of a non-muscle burger, with acceptable organoleptic properties, may provide encouragement for the general population to consume a healthier diet. Soy can be a good source of protein, and replacing a beef hamburger with a soy "burger" can have positive health effects because you are reducing saturated-fat and cholesterol intake.

Vegetable Burgers

Currently there are several brands of vegetable burgers available to consumers. Each brand has numerous "flavors" or "styles" in the freezer case, so this study focused on the "flavor/style" labeled as "original". The name brands in the area are packaged similarly: frozen, individually wrapped, four to a box, each burger weighing 2.5 ounces with the serving size being one burger. The burgers are approximately the same size at 9 x 8 cm and 1cm thick. The prices are comparable ranging from \$2.99 a box to \$4.99 a box. The prices varied by grocery.

	Brand 1	Brand 2	Brand 3
Calories	70	100	100
Calories from Fat	5	30	22
Total Fat	0.5g	3.5g	2.5g
Saturated Fat	0	1g	0.5g
Cholesterol	0	5mg	0
Sodium	280mg	420mg	350mg
Total Carbohydrates	6g	14g	9g
Dietary Fiber	4g	5g	4g
Sugar	1g	1g	1g
Protein	13g	5g	10g
Vitamin A	0	4%	4%
Vitamin C	0	2%	0
Calcium	6%	2%	0
Iron	4%	4%	4%

Table 1. Comparison of Three Brand Name Vegetable Burger Nutrition Labels
Source: Brand name product labels

To date, there are no vegetable burgers on the commercial market that incorporate the possible health benefits that could be obtained from the addition of blueberry puree. Therefore, the objectives of this research were 1) to develop a soy-blueberry burger that would promote a healthy diet and utilize Maine blueberries thus taking advantage of the health benefits of soy and blueberries and 2) to determine the changes in anthocyanins and phenolics during storage and broiling.

Chapter 2

DEVELOPMENT OF A SOY-BLUEBERRY BURGER

Materials, Production, and Storage

Textured soy protein was purchased from the Natural Living Center in bulk (lot #165-11825 Doug Jeffords Company, Franklin, TN). The following ingredients were purchased at Sam's Club (Bangor, ME) in bulk: soy sauce (Kikkoman Foods Inc, Walworth, WI), canola oil (ConAgra, Omaha, NE), sesame oil (Kikkoman Foods Inc, Walworth, WI), chopped garlic (Spice World Inc, Orlando, FL), and dehydrated minced onion (Tone Brother's Inc, Ankeny, IA). The guar gum was a gift from Danisco (Vernon, TX). The blueberry puree, also a gift, was from Maine Wild Blueberry Company (Machias, ME).

The researcher read the ingredient labels on the brands of available vegetable burgers – original flavor. A list of common ingredients from the three brands was compiled as a base recipe for the soy-blueberry burgers. The base ingredients were (in no particular order): soy protein, water, canola oil, garlic, dehydrated onion, soy sauce and guar gum. To this base recipe, sesame oil was added for flavor and blueberry puree was added at 10% and 15% by weight.

A prototype was developed in a home kitchen via several trial and error recipes. Samples were originally mixed by tablespoon, teaspoon and less amounts. Without knowledge of the ingredients or amounts, two average

consumer volunteers tasted the samples. Per comments as to flavor and mouth - feel, ingredients such as soy sauce, garlic, onion, and oil were increased or decreased accordingly. The volunteers eventually decided on the sample they liked best.

The selected sample recipes were brought to the commercial kitchen (University of Maine Orono), measurement conversions were made and the measurements adjusted so that small batches of the prototype burgers could be made (four - $\frac{1}{4}$ lb burgers for each sample). The soy-blueberry burgers were made with three concentrations of blueberry puree: none (control), 10%, and 15% by weight. Each burger was 113.4 grams ($\frac{1}{4}$ pound). The amount of blueberry puree added was a percent by weight basis (10% or 15% of a 113.4 g burger). All other ingredients were in exactly the same amounts with the exception of TSP. The amount of TSP decreased by weight as blueberry puree was added. The control burger had no blueberry puree (Table 2).

	Control	10%	15%
Ingredient	Amount (g)	Amount (g)	Amount (g)
Textured soy protein	165.84	165.84	165.84
Tap water	168.16	125.62	104.36
Soy sauce	28.35	28.35	28.35
Vegetable oil	21.26	21.26	21.26
Chopped garlic	24.81	24.81	24.81
Dehydrated minced onion	24.81	24.81	24.81
Guar gum	15.96	15.96	15.96
Sesame oil	4.42	4.42	4.42
Blueberry puree	0.0	42.53	63.79

Table 2. Soy-Blueberry Burger Ingredients List and Amounts in Grams

Each recipe makes 4 – ¼ lb burgers.

The amounts in grams are shown for each the control burger, 10% and 15% blueberry puree by ¼ lb precooked burger weight.

The researcher calculated how many burgers of each sample were needed for 150 sensory test participants (two sensory tests). The ingredient amounts for each burger were increased proportionally so that the researcher could easily mix the samples in bulk amounts. Each combination of the three soy-blueberry burgers were mixed, minus the guar gum, placed in individual re-closeable

storage bags (Hannaford Bros Co, Scarborough, ME), placed in a commercial refrigerator at 10°C and allowed to hydrate overnight. The following day, the prescribed amount of guar gum was thoroughly mixed into each sample. The mixtures were weighed out by 113.4 grams ($\frac{1}{4}$ lb = 4 oz) and pressed into a burger using a burger press (Univex Salem, NH). The burgers were packaged by the dozen with two pieces of waxed paper (Reynolds, Richmond, VA) between each burger and each dozen was wrapped in freezer paper (Reynolds, Richmond, VA), tape sealed, and waterproof sharpie marked with the date and sample type. The packages of samples were placed on metal trays in a blast freezer (Southeast Cooler Lithia Springs, GA) at -30°C for one half hour. Once frozen, the packaged samples were transferred to -20°C walk-in freezer until analyzed.

Chapter 3

DETECTION AND QUANTIFICATION OF SOY-BLUEBERRY BURGER TOTAL PHENOLICS

Method and Materials

Total phenolic content of the three different soy-blueberry burgers (control, 10% blueberry puree by weight, and 15% blueberry puree by weight) was determined on day one, and following three and six months of frozen storage at -20°C using Folin-Ciocalteu reagent (Sigma; St. Louis, MO) according to the method of Velioglu and others (1998). Samples from each treatment were analyzed in triplicate in an uncooked and in a cooked (broiled) state. The standard regression curve was prepared using a stock solution of gallic acid (500 $\mu\text{g/ml}$) (Sigma; St. Louis, MO) and 85% MeOH (Fisher Scientific; Lawn Fair, NJ). The stock was diluted $\mu\text{g/ml}$: 450, 400, 350, 300, 250, and 200.

Absorbance was read at 725nm against distilled water using a Spectronic 20D+ (Spectronic Instruments, Rochester, NY). The regression equation of the gallic acid standard and the absorbance values of each sample were used to calculate gallic acid equivalents with results reported as gallic acid concentration ($\mu\text{g/g}$ dry weight) (Figure 5).

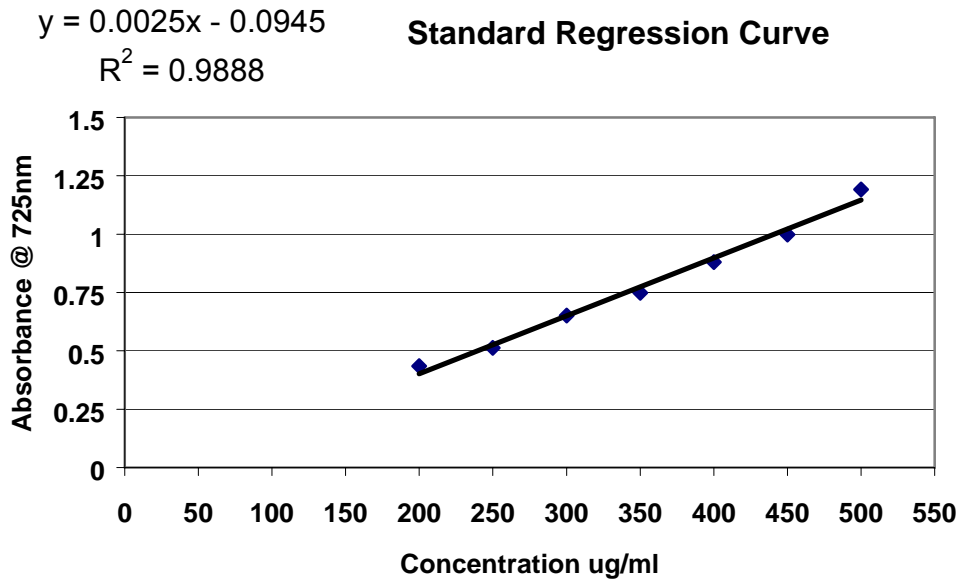


Figure 5. Standard Regression Curve

Prepared using a stock solution of gallic acid (500 µg/ml) and 85% MeOH (X axis concentration µg/ml). Absorbance was read at 725nm against distilled water

Total phenolic concentration calculations:

$$\mu\text{g/ml} = (\text{Absorbance}_{725\text{nm}} - \text{intercept})/\text{slope}$$

$$\mu\text{g extracted} = \mu\text{g/ml} \times \text{extraction volume}$$

$$\text{mg extracted} = \mu\text{g extracted}/100$$

$$\text{mg}/100\text{g} = (\text{mg extracted}/\text{sample weight}) \times 100$$

Statistical Analysis

SAS and Tukey's Studentized Range (HSD) Test ($\alpha = 0.05$) was utilized to determine the statistical differences in total phenolics between cooked and uncooked samples and the total phenolic change in the samples over time (uncooked only).

Results and Discussion

Total phenolics were determined in 6 samples (3 burgers in triplicate): Control cooked & uncooked, 10% (blueberry puree by $\frac{1}{4}$ burger weight) cooked & uncooked, and 15% (blueberry puree by $\frac{1}{4}$ burger weight) cooked & uncooked. All of the burgers: control, 10% and 15%, cooked and uncooked were statistically different from each other (Table 3).

Tukey Grouping	Mean $\mu\text{g/g}$	N	Treatment
A	310.4	9	15% cooked
B	308.0	9	15% uncooked
C	304.5	9	10% cooked
D	301.3	9	10% uncooked
E	284.4	9	control cooked
F	283.0	9	control uncooked

Table 3. Differences in Total Phenolics in Cooked & Uncooked Soy-Blueberry Burgers.

Means expressed in $\mu\text{g/g}$ on a dry weight basis. Means with the same letter are not significantly different. ($\alpha = 0.05$).

The total phenolic changes in the samples over time (0, 3 and 6 months frozen storage) were also significantly different from each other (Tables 4, 5, & 6).

Tukey Grouping	Mean $\mu\text{g/g}$	N	Time (months)
A	270.1	9	0
B	268.2	9	3
C	266.1	9	6

Table 4. Total Phenolic Changes in Control Soy-Blueberry Burgers Over Time (uncooked only)

Means expressed in $\mu\text{g/g}$ on a dry weight basis

Means with the same letter are not significantly different. ($\alpha = 0.05$)

Tukey Grouping	Mean $\mu\text{g/g}$	N	Time (months)
A	306.2	9	0
B	303.8	9	3
C	293.9	9	6

Table 5. Total Phenolic Changes in 10% Soy-Blueberry Burgers Over Time (uncooked only)

Means expressed in $\mu\text{g/g}$ on a dry weight basis

Means with the same letter are not significantly different. ($\alpha = 0.05$)

Tukey Grouping	Mean µg/g	N	Time (months)
A	314.1	9	0
B	310.1	9	3
C	299.8	9	6

Table 6. Total Phenolic Changes in 15% Soy-Blueberry Burgers Over Time (uncooked only)

Means expressed in µg/g on a dry weight basis

Means with the same letter are not significantly different. ($\alpha = 0.05$)

Although significant, the difference between the cooked and uncooked soy-blueberry burger samples was only 1% for the 10% and 15%. The difference in phenolics of the cooked and uncooked control burger, that has no blueberry puree, was merely 0.45% but was still considered significant. The degradation of total phenolics over time within each sample burger was found to be significant via Tukeys post hoc. The control burger, which has no blueberry puree, suffered the least loss of total phenolics. Degradation of the phenolics were nearly the same from day zero to 3 months frozen storage and from 3 months frozen storage to 6 months frozen storage, each 0.75%. Phenolic loss over time was also 0.75% for the 10% soy-blueberry burger from day zero to 3 months frozen storage. It was, however greater for the 10% soy-blueberry burgers from 3 months frozen storage to 6 months frozen storage: 3.2%. The

15% soy-blueberry burgers had the most dramatic decrease in total phenolics from day zero to 3 months frozen storage (1.25% loss) and from 3 months frozen storage to 6 months frozen storage (3.3% loss). These findings are not surprising. It is reasonable to surmise that there would be a larger percent of phenolic degradation as the amount of blueberry puree (more phenolics) increases. Although the food (type of fruit/vegetable) and numbers differ, the trend of decreasing phenolics during storage over time is seen in other research (Kalt and others 2000, Kujala and others 2000, Chaudry and others 1998, Skrede and others 2000).

The above mentioned differences may be due to the fact that phenolics are susceptible to degradation during various processing operations (Skrede and others, 2000) including pH, oxygen, heat, and storage temperatures as well, phenolics and anthocyanins are readily oxidized because of their antioxidant properties thus, susceptible to degradative reactions during processing and storage. Blueberry puree experienced losses ($P < 0.001$) in total phenolics, anthocyanins, and antioxidant capacity during processing; phenolics from 351 mg gallic acid eq/100g to 213 and anthocyanins from 79.6 mg malvidin 3-glucoside/100g to 31.3 (Kalt and others 2000). A study by Kujala and others (2000) investigated the effect of cold storage on the content of total phenolics and three individual compounds in red beetroot. The researchers saw significant differences ($P < 0.0001$) in the contents of total phenolics and individual compounds at 5 °C from 0 to 196 days of storage. The amount of total phenolics ranged from 15.5 ± 0.1 to 13.1 ± 0.3 mg GAE/g; it decreased steadily

until 63 days of storage and the changes after that were minor. Chaudry and others (1998) studied the phenolic compounds of solar-cabinet-dried persimmon during storage. Their research indicated that phenolic compounds and total phenols showed a decreasing trend during storage.

Chapter 4

DETECTION AND QUANTIFICATION OF SOY-BLUEBERRY BURGER ANTHOCYANINS

Materials and Methods

The anthocyanin extraction method of Rodriguex-Saono and Wrolstad (2001) was utilized. Each soy-blueberry burger was sampled and analyzed in triplicate in an uncooked and in a cooked (broiled) state at three time periods: one day of frozen storage, three months of frozen storage, and six months of frozen storage at -20°C. All beakers were appropriately covered with aluminum foil to prevent degradation of the anthocyanins by light. Fifteen grams of each burger were individually mixed with 35 ml acetone and vigorously stirred via magnetic plates and bars (Fisher Scientific, Lawn Fair, NJ) for 20 minutes. The anthocyanin extract (filtrate) was separated from the insoluble material by Buchner funnel, Whatman #1 filter paper and vacuum and stored in an aluminum foil covered 150ml centrifuge bottles (Nalgene Labware, Rochester, NY). The sample was extracted and separated in the same manner twice more with the anthocyanin filtrate being combined and the final volume recorded. The total volumes of filtrate were individually transferred into separatory funnels and two volumes of chloroform were added to each, inverted gently to mix, and allowed to separate until a clear partition between phases was obtained (approximately ½ hour). The lower, colorless layer of acetone/chloroform was appropriately discarded while the top phase containing the anthocyanins was

drained into individual 150ml centrifuge tubes (Nalgene Labware, Rochester, NY). The samples were centrifuged in a Sorvall RC-5B Refrigerated Superspeed Centrifuge (DuPont Company, Wilmington, DE) with a GSA Head at 8,000 rpm for ten minutes. The supernatant was placed in a boiling flask. The residual acetone/chloroform was removed in a rotary evaporator at 40°C under vacuum. The volume was recorded for later calculations.

The total monomeric anthocyanin content was calculated according to the pH differential procedure by Giusti and Wrolsted (2001). The samples were measured at 510nm, the maximum absorbance wavelength for anthocyanins and 700nm to correct for haze in the samples with a Spectronic 20D+ (Spectronic Instrument, Rochester, NY). Results were expressed as mg/100g fresh weight of cyanin-3-glucoside. The molecular weight of cyanin-3-glucoside was 449.2 g/mole and the extinction coefficient (ϵ) was 26,900 L cm⁻¹ mg⁻¹.

The following calculation was used to determine the absorbencies of the samples:

$$\text{Absorbance of sample} = (\text{Absorbance}_{510} - \text{Absorbance}_{700}) \text{ at pH } 1.0 - (\text{Absorbance}_{510} - \text{Absorbance}_{700}) \text{ at pH } 4.5$$

The following calculation was used to determine the monomeric anthocyanin concentration in each sample: monomeric anthocyanin concentration mg/L =
absorbance of sample x molecular weight of predominant anthocyanin x dilution factor x 1000

Results and Discussion

The samples were analyzed in triplicate and final results were averaged. The results were reported as mg/100g of fresh wt. of cyanin-3-glucoside (Table 7).

Sample	Time	Mean Anthocyanin Content (mg/100g) Fresh Wt of Cyanin-3-glucoside
Control uncooked	1 day frozen storage	0.09 mg/100g
	3 months frozen storage	0.06
	6 months frozen storage	0.02
Control cooked	1 day frozen storage	0.12
	3 months frozen storage	0.09
	6 months frozen storage	0.04
10% uncooked	1 day frozen storage	0.79
	3 months frozen storage	0.60
	6 months frozen storage	0.49
10% cooked	1 day frozen storage	0.96
	3 months frozen storage	0.73
	6 months frozen storage	0.52
15% uncooked	1 day frozen storage	1.21
	3 months frozen storage	1.17
	6 months frozen storage	0.99
15% cooked	1 day frozen storage	1.48
	3 months frozen storage	1.34
	6 months frozen storage	1.21

Table 7. Total Anthocyanin Content of Soy-Blueberry Burgers

Each soy-blueberry burger was sampled and analyzed in triplicate in an uncooked and in a cooked (broiled) state at three time periods: one day of frozen storage, three months of frozen storage, and six months of frozen storage at -20°C. Anthocyanin content results were expressed as mg/100g fresh weight of cyanin-3-glucoside.

Total anthocyanins were determined in 6 samples (3 burgers in triplicate): Control cooked & uncooked, 10% (blueberry puree by ¼ burger weight) cooked & uncooked, and 15% (blueberry puree by ¼ burger weight) cooked & uncooked. The control burgers, cooked and uncooked, were not significantly different from each other. The 10% and 15% burgers, cooked and uncooked, were statistically different from each other (Table 8).

Tukey Grouping	Mean µg/g	N	Treatment
A	1.21	9	15% cooked
B	1.09	9	15% uncooked
C	0.86	9	10%cooked
D	0.79	9	10% uncooked
E	0.09	9	Control cooked
E	0.08	9	Control uncooked

Table 8. Differences in Total Anthocyanins in Cooked & Uncooked Samples
Means with the same letter are not significantly different. $\alpha = 0.05$

The total anthocyanin changes in the samples over time (0, 3 and 6 months frozen storage) were significantly different (Tables 9, 10, & 11).

Tukey Grouping	Mean $\mu\text{g/g}$	N	Time (months)
A	0.09	9	0
B	0.08	9	3
C	0.07	9	6

Table 9. Total Anthocyanin Changes in Control Soy-Blueberry Burgers Over Time (uncooked only)

Means expressed in $\mu\text{g/g}$ on a dry weight basis

Means with the same letter are not significantly different. ($\alpha = 0.05$)

Tukey Grouping	Mean $\mu\text{g/g}$	N	Time (months)
A	0.79	9	0
B	0.60	9	3
C	0.49	9	6

Table 10. Total Anthocyanin Changes in 10% Soy-Blueberry Burgers Over Time (uncooked only)

Means expressed in $\mu\text{g/g}$ on a dry weight basis

Means with the same letter are not significantly different. ($\alpha = 0.05$)

Tukey Grouping	Mean µg/g	N	Time (months)
A	1.21	9	0
B	1.17	9	3
C	0.99	9	6

Table 11. Total Anthocyanin Changes in 15% Soy-Blueberry Burgers Over Time (uncooked only)

Means expressed in µg/g on a dry weight basis

Means with the same letter are not significantly different. ($\alpha = 0.05$)

The total anthocyanin content of soy-blueberry burgers was proportionate to the amount of blueberry puree in the sample. The control had little anthocyanin content while the 15% soy-blueberry burger had the highest amount. The same trend seen with the phenolics was observed with the anthocyanins. There was a decrease in anthocyanins in storage and over time. These findings, as with the phenolics, vary in the type of fruit and/or vegetable tested. As well, the numbers are different. However, the trend remains the same. These differences may be due to the fact that anthocyanins are susceptible to degradation during various processing operations (Skrede and others, 2000) including pH, oxygen, heat, and storage temperatures (Kalt and others 2000). Anthocyanins as well as other polyphenolics are readily oxidized because of their antioxidant properties thus, susceptible to degradative reactions during various processing unit

operations (Kalt and others 2000). A study done by Kalt and others in 2000, indicated that blueberry puree anthocyanin content declined by more than half during 20°C storage, and the total phenolic content decreased by 30%. Ochoa and others (1999) investigated the physical and chemical characteristics of raspberry pulp. Their data indicated that raspberry anthocyanins decreased significantly during storage. Morais and others (2002) state that the anthocyanin concentration decreases with increasing length of storage and the decomposition rate is higher at elevated temperatures and although degradation may be slower at frozen temperatures, under long-term storage degradation can still be significant.

Chapter 5

SENSORY TESTING OF THE SOY-BLUEBERRY BURGERS

Materials and Methods

Consumer acceptance testing was conducted on the three soy-blueberry burgers. The use of human subjects approval was obtained from the College of Natural Sciences, Forestry and Agriculture Human Subjects Protection Committee. Sensory testing was performed, under the direction of Dr. Mary Ellen Camire and with incomparable help of Mike Dougherty, in the Consumer Testing Center in the Department of Food Science & Human Nutrition at the University of Maine. Volunteers for the sensory test were recruited through the University of Maine; Orono FirstClass email services where a notice was posted to several folders and via printed flyers posted about campus. All subjects were required to be at least 18 years of age and not have any allergies to soy, blueberries, garlic, onion or soy sauce. A total of 75 subjects were recruited for each sensory test. Volunteers were required to sign an informed consent form prior to the tasting session. The evaluation rooms were climate controlled with positive-pressure air flow to prevent odors from the preparation area to bias judgments. A combination of incandescent and fluorescent lighting was used. Subjects were seated in booths separated by partitions.

Participants were first requested to answer demographic questions: age, gender, how often they consume muscle foods, how often they consume soy, and how important it is to them to eat healthy foods. A nine point hedonic scale

was used in the questionnaire (9 = like extremely, 1 = dislike extremely) (Peryam and Pilgrim 1957). A quantitative affective test was utilized to determine the overall liking for the soy-blueberry burger in regards to appearance, texture, flavor, and overall acceptance (opinion).

Affective tests, when done properly: 1) allow different treatments to be judged to find the optimum accepted product, 2) break the masses of consumers down into smaller groups to allow an understanding of who will buy the product and how to market it to them, and 3) assess the market share potential for the new product. This information is obtained by asking specific questions about a persons age, sex, geographic location, nationality, religion, education and employment along with their preferences on the product being tested. Simply stated, it stereotypes user groups based on these variables and learns the preferences of particular groups' eating habits. This is not done because of prejudicial motivation, but simply because consumer preferences tend to be very grouped based on sensory characteristics.

Participants in this sensory test were also asked to rank the soy-blueberry burgers. The burger that one liked the best was ranked as 1. The second choice was ranked second (2), and the burger least preferred was ranked third (3). The burger with the LOWEST rank value was the burger that received the most # 1's and is the MOST LIKED burger. The burger with the HIGHEST rank value received the most # 3's and is the LEAST LIKED burger.

On the day of testing, the soy-blueberry burgers (control, 10%, and 15% blueberry puree) were taken from frozen storage (-20°C) broiled on an

EmberGlo E24 electric charbroiled (EmberGlo, Chicago, IL) at 200°C until the internal temperature reached 71.1°C as measured with a thermocouple (SensorTec, Fort Wayne, IN). Each of the three soy-blueberry burgers was assigned a random, 3-digit code. The participants were served the samples, each 1/4 of a patty, in a randomized order determined by the SIMS software.

The sensory test (sensory test two) was replicated six months later to determine if frozen storage had an effect on the acceptance of the soy-blueberry burgers.

The SIMS Software 2000 program for Windows (version 3.3, Sensory Computer Systems, Morristown, N.J., U.S.A.) (includes SAS statistical software) was used to generate the questionnaire, collect data, and analyze the sensory results for both sensory tests. Differences between burgers were analyzed using SYSTAT Version 9 software and Tukey's post hoc test.

Chapter 6

SENSORY TESTING OF A LOWER SODIUM SOY-BLUEBERRY BURGER

Materials and Methods

Because several of the comments from the first and second sensory tests stated that the soy-blueberry burger tasted “salty” and because of health concerns over high sodium foods, a low sodium soy sauce (Kikkoman Foods Inc, Walworth, WI) was used to produce a “lower sodium soy-blueberry burger”. Through an amendment made to the use of human subjects approval that was obtained from the College of Natural Sciences, Forestry and Agriculture Human Subjects Protection Committee, a third sensory test was conducted at the Natural Living Center (NLC) in Bangor, Maine. Patrons of the NLC were recruited for the sensory test by printed flyers posted in the shop. Volunteers were required to sign an informed consent form prior to the tasting session.

A paper form of the quantitative affective test was utilized to determine the overall liking for the soy-blueberry burger in regards to appearance, texture, flavor and overall acceptance (opinion). A nine point hedonic scale was used in the questionnaire. Participants in this sensory test were also asked to rank the soy-blueberry burgers. The burger that one liked the best was ranked as 1. The second choice was ranked second (2), and the burger least preferred was ranked third (3). The burger with the LOWEST rank value was the burger that received the most # 1's and is the MOST LIKED burger. The burger with the HIGHEST rank value received the most # 3's and is the LEAST LIKED burger.

Low sodium soy-blueberry burgers were made, blast frozen and stored according to the method described previously. The one alteration was the use of low sodium soy sauce in the recipe in lieu of regular soy sauce.

On the day of testing, the three concentrations (Control, 10%, and 15% blueberry puree) of soy-blueberry burgers were transported from frozen storage via an ice packed cooler and placed in the NLC's walk-in cooler. As needed, burgers were grilled at 200°C until the internal temperature reached 71.1°C as measured with a thermocouple (SensorTec, Fort Wayne, IN). Three treatments, each a 1/4 of a patty, were presented by random code to panelists. Seventy-five (75) volunteers completed the sensory test.

The researcher keyed the paper questionnaire responses into the SIMS Software 2000 program and utilized the SIMS program, as before, to analyze the sensory results. Differences between burgers were analyzed using SYSTAT Version 9 software and Tukey's post hoc test.

Results and Discussion all Three Sensory Tests

All three sensory tests had 75 participants each. Demographic questions were asked to determine age (Figure 6), gender (Figure 7), the frequency of soy consumption (Figure 8), and the importance of a healthy diet (Figure 9). These questions break the masses of consumers down into smaller groups to allow an understanding of who will buy the product and how to market it to them and assess the market share potential for a new product.

The first two sensory tests (day 1 frozen storage and 3 months frozen storage) were conducted at the Consumer Testing Center, University of Maine, Orono. The third sensory test (day 1 frozen storage – low sodium) was conducted at the Natural Living Center, Bangor, ME.

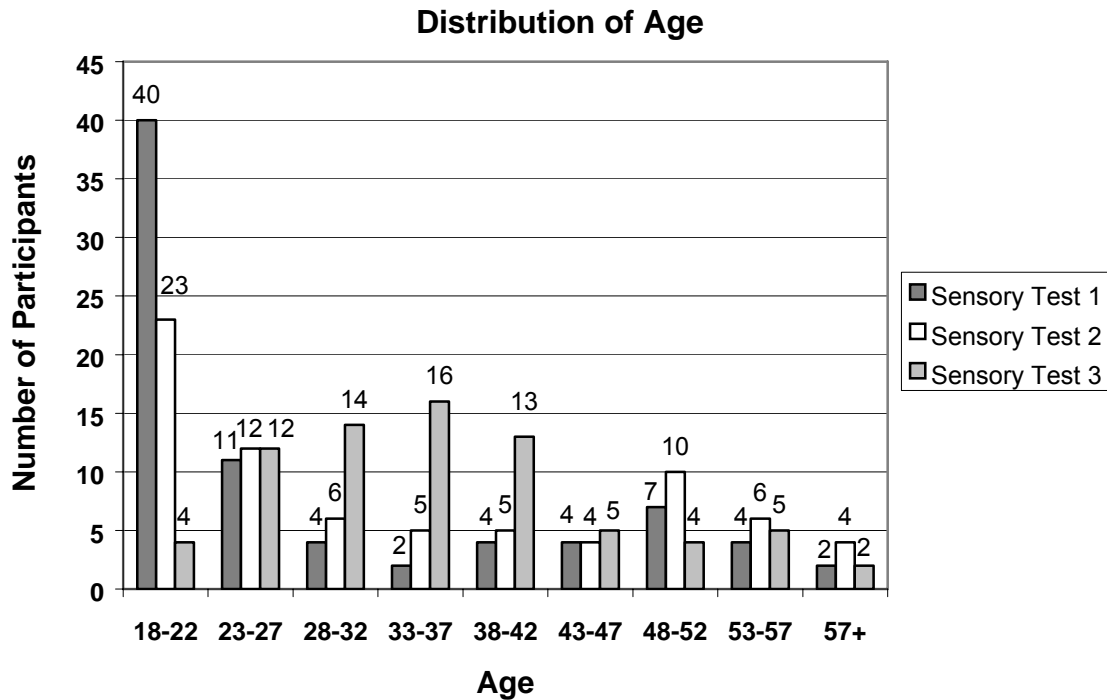


Figure 6. Distribution of Age

All three sensory tests. Each test had 75 participants.

Sensory test 1 was conducted at the University of Maine, Orono. Therefore, it is not surprising to see that the majority of participants were between the ages of 18 and 22 years. Sensory test 2 was also conducted at the University of Maine, Orono. However, the researcher offered extra credit points to the

students enrolled in a class she was teaching. The 18 – 22 years of age range is still the highest in number but the other age ranges are more evenly distributed. The third sensory test was conducted at the Natural Living Center (NLC) in Bangor, ME. NLC is essentially a “healthful” grocery market. The majority of consumers that participated were between the ages of 23 and 42 with the peak at 33 – 37 years of age. Because of their age and the fact that they shop at the NLC indicates that this group of people would be the most likely to be interested in a product such as the soy-blueberry burger.

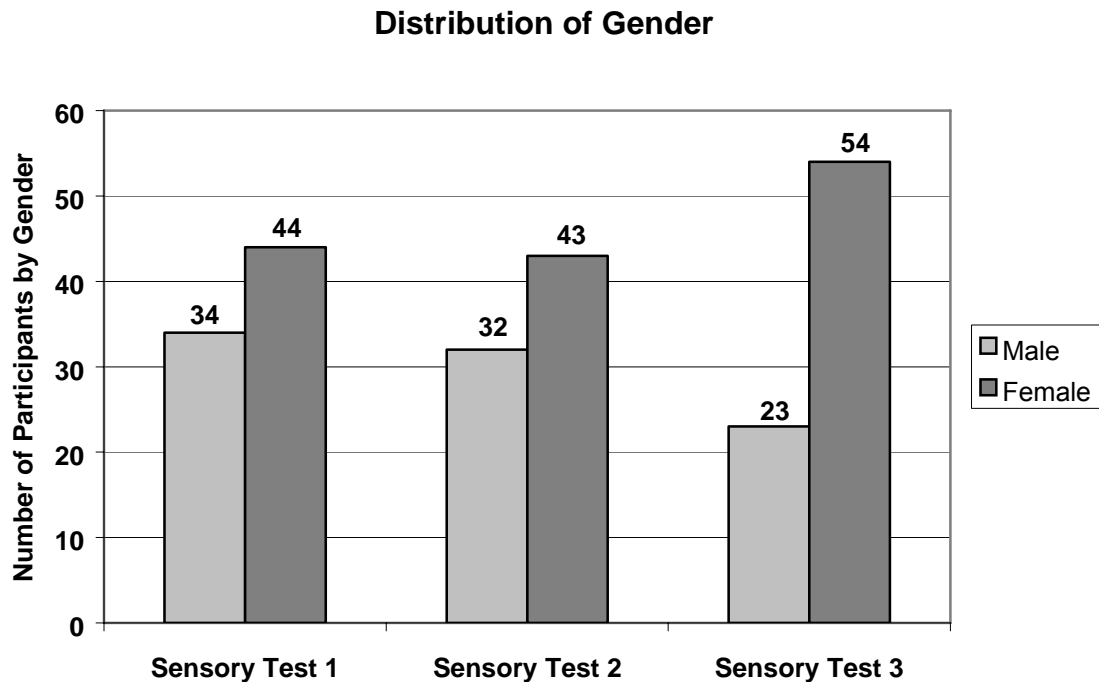


Figure 7. Distribution of Gender

All three sensory tests. Each test had 75 participants.

Sensory tests one and two are similar in the numbers of male vs. female with the females predominating. Both were conducted at a University during the school year. As well, the females outnumber the males in sensory test three but in greater numbers. Several comments read that the male was participating in the test because their female counterpart was. It seems as if this product appealed to women more than men.

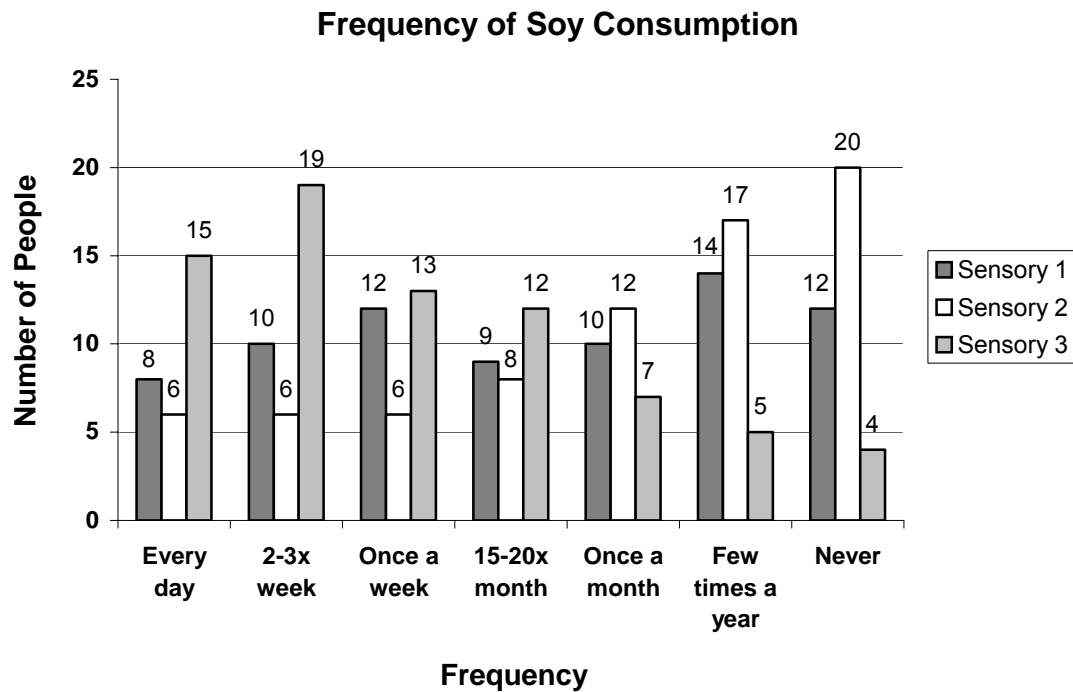


Figure 8. Frequency of Soy Consumption

All three sensory tests. 75 participants per each test

There were no stipulations on whether or not a participant regularly consumed soy or if they even consumed soy at all. Frankly, the researcher was afraid there wouldn't be enough participants. The panelists in the first test were spread the most evenly across the gamut of soy consumption choices. They are, however skewed towards the "once a month" and "few times a year". At least half of the 75 participants in sensory test two were bribed with extra credit points towards their final exam. They were not soy consumers as seen by Figure 8. Sensory test three is a more true representation of the consumer that would be interested in this product as these people consume soy on a regular basis.

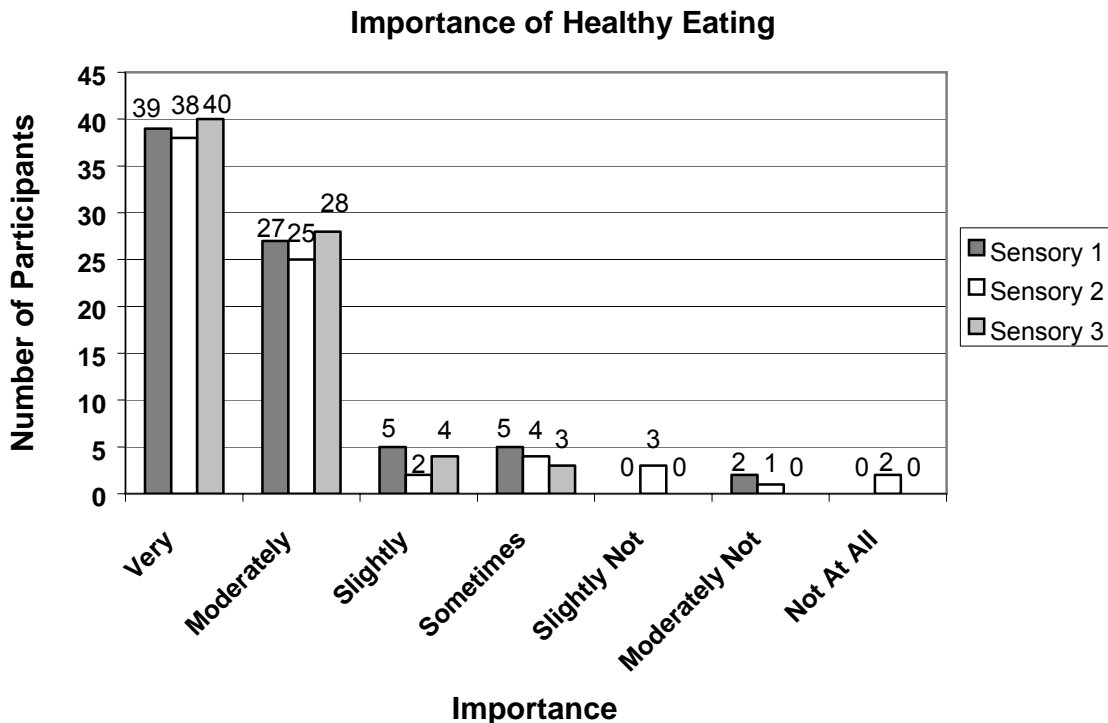


Figure 9. Importance of Healthy Diet

All three sensory tests. 75 participants per each test

Of course all participants think it is important to eat a healthy diet. The NLC patrons from sensory test three had the highest numbers and are probably the people that do as they say as far as eating healthy foods.

A quantitative affective test was utilized to determine the overall liking for the soy-blueberry burger in regards to appearance, texture, flavor, overall acceptance, soy flavor, and blueberry flavor (Tables 12, 13, & 14).

Soy-Blueberry Burger Acceptance - Sensory Test One					
<u>Attribute</u>	<u>Control</u>	<u>10%</u>	<u>15%</u>	<u>P-value</u>	<u>Significance</u>
Overall Appearance	6.32 ^b	7.08 ^a	6.37 ^b	0.0024	**
Texture	5.88 ^b	6.63 ^a	6.31 ^{ab}	0.0043	**
Flavor	5.92 ^b	6.43 ^{ab}	6.55 ^a	0.0134	*
Overall Acceptance	5.71 ^b	6.45 ^a	6.48 ^a	0.0003	***
Soy Flavor	3.15	3.03	3.05	0.6267	not significant
Blueberry Flavor	4.58 ^a	4.42 ^a	4.14 ^b	0.0009	***

Table 12. Soy-Blueberry Burger Acceptance - Day 1

* = 0.05 significant ** = 0.01 highly significant *** = 0.001 very highly significant. Numbers with the same letter are not significantly different. Sensory test one with 75 participants.

Soy-Blueberry Burger Acceptance - Sensory Test Two					
<u>Attribute</u>	<u>Control</u>	<u>10%</u>	<u>15%</u>	<u>P-value</u>	<u>Significance</u>
Overall Appearance	6.27	6.72	6.28	0.0752	not significant
Texture	6.06	6.32	5.97	0.2288	not significant
Flavor	6.00 ^b	6.35 ^{ab}	6.69 ^a	0.0111	*
Overall Acceptance	5.95 ^b	6.42 ^a	6.59 ^a	0.0035	**
Soy Flavor	3.08	2.87	3.03	0.3003	not significant
Blueberry Flavor	4.60 ^a	4.57 ^a	4.27 ^b	0.0141	*

Table 13. Soy-Blueberry Burger Acceptance - 3 Months Frozen Storage

* = 0.05 significant ** = 0.01 highly significant *** = 0.001 very highly significant. Numbers with the same letter are not significantly different. Sensory test two with 75 participants.

Soy-Blueberry Burger Acceptance - Sensory Test Three – Low Sodium					
<u>Attribute</u>	<u>Control</u>	<u>10%</u>	<u>15%</u>	<u>P-value</u>	<u>Significance</u>
Overall Appearance	6.88 ^b	6.65 ^b	7.39 ^a	0.0004	***
Texture	6.37 ^b	6.87 ^a	7.01 ^a	0.0001	***
Flavor	7.09 ^b	7.53 ^a	7.65 ^a	0.0001	***
Overall Acceptance	6.75 ^b	7.08 ^a	7.25 ^a	0.0001	***
Soy Flavor	2.92 ^a	2.97 ^a	2.60 ^b	0.3003	**
Blueberry Flavor	4.56 ^a	3.59 ^a	2.93 ^c	0.0001	***

Table 14. Soy-Blueberry Burger Acceptance - Natural Living Center - Day 1 – Low Sodium

* = 0.05 significant ** = 0.01 highly significant *** = 0.001 very highly significant. Numbers with the same letter are not significantly different. Sensory test three with 75 participants.

Regular soy soy-blueberry burgers were utilized in the first two sensory tests: Day 1 and 3 months frozen storage. A low sodium soy sauce was utilized to make the soy-blueberry burgers for sensory test three, conducted at the Natural Living Center, Bangor, ME.

The 10% soy-blueberry burger ranked the highest for overall appearance and texture. The differences were not considered significant in the first test (Table 12) but were highly significant ($P \leq 0.01$) in the second test (Table 13). The 15% soy-blueberry burger ranked second in the category but in neither test

was the difference significant. The control burger was the least liked in appearance. In the flavor and overall acceptance categories all three tests were in concurrence – participants scored the 15% burger the highest, the 10% burger second, and the control burger the lowest. In tests one and two (Tables 12 & 13), the 15% blueberry puree soy burger was not significantly scored higher than the 10% burger in these categories ($P = 0.05$). Both burgers were significantly different ($P = 0.05$) from the control burger for overall acceptance. Both the 10% and 15% burgers scored higher than 6 on the 9 point hedonic scale for overall acceptance (range for three sensory tests: 6.42 – 7.25). The highest scores for overall acceptance came from the patrons of the Natural Living Center. These overall acceptance scores of 6 – 7 for the soy-blueberry burgers are sufficient to successfully introduce this product into the marketplace. The control burger scored 5.95, 5.71, and 6.75 respectively. In flavor the 10% was not significantly different ($P = 0.05$) from the control burger nor was it significantly different from the 15% burger. In test three (Table 14), the 10% burger was significantly different from the control in flavor. For texture, test one (Table 12) saw no significant differences between the three-burger formulations. In test two (Table 13), the 10% burger was significantly different from the control but not the 15% burger. Overall appearance and texture were favored in the 10% soy-blueberry burger in the first two tests. In the third test (Table 14) of lower sodium burgers the 10% was third to the control burger in overall appearance. Panelists stated that it was “too gray”. The 10% was second to the 15% burger in texture with several comments stating that it was

“slimy” compared to the 15% burger. In all three tests, all three of the burgers received texture remarks such as: “mushy”, and “crumbly” as well as “delicious”, “as good as any soy burger”. The 15% burger again was rated the best in flavor and overall acceptance in the third test with the 10% burger close behind. In test three (Table 14), the texture of the 15% and 10% burgers were significantly different ($P = 0.05$) from the control. The participants of test three preferred the texture of the 15% burger more than the 10% burger.

The control burger was considered, in tests one and two (Tables 12 & 13), to have the most soy flavor but none of the burgers were significantly different in this category for either test. In test three it was the 10% burger that participants said had the most soy flavor with the control not being significantly different. The control scored the lowest for blueberry flavor in both tests. The flavor was not significantly different from the 10% burger but both the control and the 10% burger were significantly different than the 15% blueberry puree soy burger. Some panelists in the test commented that the 15% burger was “sweet”. The 10% and the control burger were not different in this category but both were highly significantly different ($P = 0.01$) than the 15% burger. It was commented that the “sweetness of the blueberries masked the soy flavor”. In all three of the tests, the highest mark for soy flavor was 3.15 on a scale of 1 - 5 with all the other marks being at 3 or below. The third test followed the same trend as the two previous tests in blueberry flavor. However, the participants of the third test reported very highly significant differences ($P = 0.001$) in the blueberry flavor

between all three samples with the 15% having the most blueberry flavor and the control having the least

Participants in this sensory test were asked to rank the soy-blueberry burgers. The burger that one liked the best was ranked as 1. The second choice was ranked second (2), and the burger least preferred was ranked third (3). The burger with the LOWEST rank value was the burger that received the most # 1's and is the MOST LIKED burger. The burger with the HIGHEST rank value received the most # 3's and is the LEAST LIKED burger (Tables 15,16, & 17).

Soy-Blueberry Burger Preference - Sensory Test One					
<u>Attribute</u>	<u>Control</u>	<u>10%</u>	<u>15%</u>	<u>P-value</u>	<u>Significance</u>
Preference	2.37 ^b	1.91 ^a	1.72 ^a	0 .0002	***

Table 15. Soy-Blueberry Burger Preference - Day 1

The soy-blueberry burger with the LOWEST score is the MOST preferred. Numbers with the same letter are not significantly different.
 * = 0.05 significant ** = 0.01 highly significant *** = 0.001 very highly significant. Sensory test one with 75 participants.

Soy-Blueberry Burger Preference – Sensory Test Two					
<u>Attribute</u>	<u>Control</u>	<u>10%</u>	<u>15%</u>	<u>P-value</u>	<u>Significance</u>
Preference	2.27 ^b	1.96 ^{ab}	1.77 ^a	0 .0070	**

Table 16. Soy-Blueberry Burger Preference - 3 Months Frozen Storage

The soy-blueberry burger with the LOWEST score is the MOST preferred. Numbers with the same letter are not significantly different.

* = 0.05 significant ** = 0.01 highly significant *** = 0.001 very highly significant. Sensory test two with 75 participants.

Soy-Blueberry Burger Preference – Sensory Test Three – Low Sodium					
<u>Attribute</u>	<u>Control</u>	<u>10%</u>	<u>15%</u>	<u>P-value</u>	<u>Significance</u>
Preference	2.47 ^c	1.95 ^b	1.59 ^a	0 .0000	***

Table 17. Soy-Blueberry Burger Preference - Natural Living Center - Day 1

The soy-blueberry burger with the LOWEST score is the MOST preferred. Numbers with the same letter are not significantly different.

* = 0.05 significant ** = 0.01 highly significant *** = 0.001 very highly significant. Sensory test three with 75 participants.

Regular soy-blueberry burgers were utilized in the first two sensory tests (Tables 15 & 16): Day 1 and 3 months frozen storage. A low sodium soy sauce was utilized to make the soy-blueberry burgers for sensory test three (Table

17). In all three sensory tests the 15% soy-blueberry burger was preferred, the 10% was second choice and the control (no blueberry puree) was the least preferred. In tests one and two (Tables 15 &16), the 15% and the 10% burgers were not significantly different ($P = 0.05$) from each other but were significantly different from the control. In sensory test three (Table 17) the three-burger formulations were all significantly different from each other.

Participants were asked to rate the soy flavor and the blueberry flavor in each of the three formulations of soy-blueberry burger: control (no blueberry puree), 10%, and 15% blueberry puree. The scale is as follows: 1 = no soy or no blueberry flavor, 3 = just right, and 5 = strong soy or strong blueberry flavor. In sensory test one, more than half of the 75 participants scored the soy flavor of the control, 10%, and 15% soy-blueberry burgers as just-right (Table 18). The blueberry flavor for the control and 10% burgers were scored 73.3% and 72%, respectively for no blueberry flavor, which is not necessarily detrimental. The 15% burger scored a 57.3% for no blueberry flavor (Table 18).

Just-Right-Score	Soy-flavor			Blueberry flavor		
	Control	10%	15%	Control	10%	15%
1	12.0	13.6	12.7	73.3	72.0	57.3
2	18.7	12.7	14.3	14.7	16.0	21.3
3	42.7	48.7	48.0	10.7	10.7	14.7
4	18.6	15.7	17.0	1.30	0.0	4.0
5	8.0	9.3	8.0	0.0	1.3	2.7

Table 18. Percent Distribution of Just-Right-Scores - Day 1

Sensory test one. 75 observations for soy flavor. 75 observations for blueberry flavor. 1 = no soy flavor or no blueberry flavor 3 = just right 5 = strong soy flavor or strong blueberry flavor

In sensory test two, again more than half of the 75 participants scored the soy flavor of the control, 10%, and 15% soy-blueberry burgers as just-right (Table 19). The blueberry flavor for the control, 10%, and 15% burgers were scored 63.3%, 61.1%, and 60.4%, respectively for no blueberry flavor. This is lower than the results for just right scores in test one. About half of the participants were offered extra credit to taste test the product and perhaps they “thought” they tasted blueberry just because they were informed that test product was a soy-blueberry burger.

Just-Right-Score	Soy-flavor			Blueberry flavor		
	Control	10%	15%	Control	10%	15%
1	9.0	9.7	8.6	63.3	61.1	60.4
2	18.8	19.6	19.3	18.9	19.7	17.2
3	50.0	48.7	48.0	14.6	15.4	15.7
4	15.4	14.7	16.2	3.2	2.8	4.0
5	6.8	7.3	7.9	0.0	1.0	2.7

Table 19. Percent Distribution of Just-Right-Scores - 3 Months Frozen Storage

Sensory test two. 75 observations for soy flavor. 75 observations for blueberry flavor. 1: no soy flavor or no blueberry flavor 3: just right 5: strong soy flavor or strong blueberry flavor

Table 20 shows the percent distribution for just right scores as chosen by 75 patrons of the Natural Living Center. Again, more than half of the 75 participants scored the soy flavor just right: control at 51.4% distribution, 10% at 48.8%, and 15% at 48.0%. This group of people seemed to be a little more discerning when it came scoring the blueberry flavor. They surmised correctly that the control burger shouldn't taste like blueberry, as there was no blueberry in it. The scores were distributed well below just right: 40.5% and 37.8%. However, there was the 20.7% distribution of just right. Per the percent distribution, the 10% and 15% burgers were scored just right by more than half of the 75 participants (Table20).

Just-Right-Score	Soy-flavor			Blueberry flavor		
	Control	10%	15%	Control	10%	15%
1	8.0	16.6	17.7	40.5	12.0	15.5
2	21.9	18.6	20.0	37.8	28.0	23.7
3	51.4	48.8	48.0	20.7	48.7	47.0
4	12.4	10.7	9.3	2.0	10.0	12.0
5	6.3	5.3	5.0	0.0	1.3	1.8

Table 20. Percent Distribution for Just-Right-Scores - Natural Living Center – Day 1.

75 observations for soy flavor. 75 observations for blueberry flavor.

1: no soy flavor or no blueberry flavor 3: just right 5: strong soy flavor or strong blueberry flavor

These sensory tests, particularly the overall acceptance scores of 6 – 7 and the percent distribution of just right scores, indicate that a soy-blueberry burger containing 10 –15% blueberry puree by weight was acceptable by panelists. This development of a non-muscle burger, with acceptable organoleptic properties, may provide encouragement for the general population to consume a healthier diet.

Chapter 7

MINERAL ANALYSIS OF THE SOY-BLUEBERRY BURGER AND THE LOWER SODIUM SOY-BLUEBERRY BURGER

Method and Materials

Mineral analysis was performed on day 1 frozen storage uncooked control, 10% blueberry by weight and 15% blueberry by weight regular soy-blueberry burgers and on day 1 frozen storage uncooked control, 10% blueberry by weight and 15% blueberry by weight low sodium soy-blueberry burgers utilizing the method described by Shearer (1984). All samples were analyzed in triplicate and the final data were averaged.

Day 1: Nine regular soy-blueberry (three of each formulation) and nine low sodium soy-blueberry burgers (3 of each formulation) were allowed to come to room temperature. Eighteen (18) 15ml glass scintillation vials (Wheaton Science Products, Millville, NJ) were labeled appropriately with a diamond pen. The vials were weighed (Sartorius, Brinkman Instruments, Westbury, NY) and the weights recorded. One gram samples were weighed into the vials. The samples were placed in 100°C Fisher Isotemp 350 drying oven (Fisher Scientific, Lawnfair, NJ) overnight so that the sample reached a constant weight. After drying, the samples/vials were re-weighed. The average moisture content of each burger was calculated per the AOAC method 24.035 (1970).

Calculation for % moisture:

$$\frac{(\text{vial weight} + \text{sample weight}) - (\text{vial} + \text{dry sample weight})}{\text{sample weight}} \times 100$$

	Soy-Blueberry Burger	Low-Sodium Soy-Blueberry Burger
Control (no blueberry puree)	58%	58%
10% blueberry puree	51%	51%
15% blueberry puree	50%	50%

Table 21. Percent Moisture Content of Soy-Blueberry Burgers & Low-Sodium Soy-Blueberry Burgers

The moisture content of each formulation of the soy-blueberry burgers was calculated on day 1 frozen storage, in triplicate, and averaged per AOAC method 24.035, 1970.

The vials were placed on a hotplate (Fisher Scientific, Lawnfair, NJ) to pre-ash samples. After cooling, the samples/vials were placed in the muffle oven (Thermolyne, Dubuque, IA) for six hours at 550°C for ashing. After cooling the samples/vials were again re-weighed and the weights recorded.

Calculation for % ash:

$$\frac{(\text{vial weight} + \text{ash weight}) - (\text{vial weight})}{\text{sample weight}} \times 100$$

Calculation for % ash on a dry weight basis:

$$\frac{(\text{vial weight} + \text{ash weight}) - (\text{vial weight})}{(\text{sample weight})[1 - (\% \text{ moisture}/100)]} \times 100$$

In the hood, one ml of concentrated HCL 12.1 N (Fisher Scientific, Lawnfair, NJ) and one ml concentrated nitric acid –15.8 N (Fisher Scientific, Lawnfair, NJ) were pipetted into each vial. The ash was allowed to dissolve for a minimum of 45 minutes. Ten ml of distilled water were added to each followed by gentle vortexing. The contents of each vial were then transferred into 100 ml volumetric flasks and the volume diluted to 100 ml with distilled water. The contents were allowed to settle overnight.

The next day, 15 ml of each sample were transferred into new, labeled, glass vials and taken to the Analytical Laboratory in Deering Hall at the University of Maine for Inductively Coupled Plasma – Optical Emission Spectroscopy (ICP-OES) mineral analysis.

Calculating mineral concentration:

Receive results from IPC and convert into ppm

$\text{ppm} \times \text{original dilution factor} / \text{original sample weight} = \text{ppm } (\mu\text{g/g}) \text{ in wet sample}$

The GLM procedure was performed via the SAS System for Windows. Tukey's Studentized Range (HSD) Test for each mineral was run with α set at 0.05

Results and Discussion

Because several of the comments from the first two sensory tests stated that the soy-blueberry burger tasted "salty" and because of health concerns over high sodium foods, a low sodium soy sauce was used to produce a "lower sodium soy-blueberry burger". For each mineral, differences between treatments and differences between the regular soy sauce burgers and the low sodium burgers were determined.

For all the minerals (Table 22) there were no significant differences between the control, 10% and 15% burgers. For all the minerals except Na, there were no significant differences between the regular sodium soy-blueberry burgers and the low sodium soy-blueberry burgers.

Control									
Ca	K	Mg	Na	P	B	Cu	Fe	Mn	Zn
3.30	16.4	2.53	14.7	6.76	0.32	0.02	0.06	0.03	0.04
<i>3.00</i>	<i>16.4</i>	<i>2.51</i>	<i>10.2</i>	<i>6.77</i>	<i>0.34</i>	<i>0.01</i>	<i>0.06</i>	<i>0.03</i>	<i>0.04</i>
10% blueberry puree by weight									
Ca	K	Mg	Na	P	B	Cu	Fe	Mn	Zn
2.00	14.8	1.92	16.1	5.18	0.25	0.01	0.04	0.03	0.04
<i>2.00</i>	<i>14.8</i>	<i>1.92</i>	<i>11.2</i>	<i>5.18</i>	<i>0.24</i>	<i>0.01</i>	<i>0.04</i>	<i>0.02</i>	<i>0.04</i>
15% blueberry puree by weight									
Ca	K	Mg	Na	P	B	Cu	Fe	Mn	Zn
2.20	16.5	2.15	17.4	5.92	0.24	0.01	0.04	0.03	0.04
<i>2.20</i>	<i>16.5</i>	<i>2.16</i>	<i>12.1</i>	<i>5.92</i>	<i>0.24</i>	<i>0.01</i>	<i>0.05</i>	<i>0.03</i>	<i>0.04</i>

Table 22. Mineral Concentration on a Dry Weight Basis

Mineral amounts are expressed on a dry weight basis as mg/100g
Regular soy sauce in regular font. *Low sodium soy sauce in italic font.*
Samples analyzed in triplicate, calculated individually, final numbers averaged.

Per Tukey groupings, sodium was significantly different between all of the regular soy sauce burgers and all of the low sodium soy sauce burgers ($P \leq 0.05$). Percent reduction of sodium was calculated. The sodium concentrations

expressed on a dry weight basis as mg/100g were reduced in all three burgers. The control burger had a 31% reduction, the 10% soy-blueberry burger, a 30% reduction and the 15% soy-blueberry burger, also a 30% reduction. One of the controversies in preventive medicine is, whether a general reduction in sodium intake can decrease the blood pressure of a population and thereby reduce cardiovascular mortality and morbidity. Many people with high blood pressure find that cutting down on sodium lowers their blood pressure (Mayo Foundation for Medical Education and Research 2007). However, Jürgens and Graudal (2003) determined that the level of the effect in Caucasians with normal blood pressure does not warrant a general recommendation to reduce sodium intake. They did find that reduced sodium intake in Caucasians with elevated blood pressure had a useful effect to reduce blood pressure in the short-term. Their results suggest that the effect of low versus high sodium intake on blood pressure was greater in Black and Asian patients than in Caucasians.

Chapter 8

SOY-BLUEBERRY BURGER NUTRITION LABELS

The nutrition labels were generated using Nutritionist Pro 2007 nutrient analysis software program (version 2.4.1, Stafford, TX).

Nutrition Facts			
Serving Size 4.00 ounce(s) (113g)			
Amount Per Serving			
Calories	239	Calories from Fat	76
% Daily Value			
Total Fat	8.4g		13 %
Saturated Fat	1.0g		5 %
Trans Fat	0.0g		
Cholesterol	0.0mg		0 %
Sodium	836.5mg		35 %
Total Carbohydrate	20.2g		7 %
Dietary Fiber	13.8g		55 %
Sugars	2.5g		
Protein	28.7g		
Vitamin A	0 %	Calcium	18 %
Vitamin C	11 %	Iron	29 %

Figure 10. Nutrition Label for Soy-Blueberry Burger Control

Nutrition Facts			
Serving Size 4.00 ounce(s) (113g)			
Amount Per Serving			
Calories	236	Calories from Fat	76
		% Daily Value	
Total Fat	8.5g		13 %
Saturated Fat	1.0g		5 %
Trans Fat	0.0g		
Cholesterol	0.0mg		0 %
Sodium	809.1mg		34 %
Total Carbohydrate	20.8g		7 %
Dietary Fiber	13.4g		54 %
Sugars	3.4g		
Protein	26.9g		
Vitamin A	1 %	Calcium	17 %
Vitamin C	12 %	Iron	27 %

Figure 11. Nutrition Label for Soy-Blueberry Burger 10%

Nutrition Facts			
Serving Size 4.00 ounce(s) (113g)			
Amount Per Serving			
Calories	236	Calories from Fat	76
		% Daily Value	
Total Fat	8.5g		13 %
Saturated Fat	1.0g		5 %
Trans Fat	0.0g		
Cholesterol	0.0mg		0 %
Sodium	809.1mg		34 %
Total Carbohydrate	20.8g		7 %
Dietary Fiber	13.4g		54 %
Sugars	3.4g		
Protein	26.9g		
Vitamin A	1 %	Calcium	17 %
Vitamin C	12 %	Iron	27 %

Figure 12. Nutrition Label for Soy-Blueberry Burger 15%

Comparing the nutrition labels from three brand name veggie burgers to the three samples formulated for this research, one can see that in order for the samples to be comparable some adjustments must be made. Using less oil and/ or perhaps a more healthy oil can reduce calories, fat, and saturated fat. There is no cholesterol! The salt content was decreased by 30% with the use of low sodium soy sauce. With the 30% decrease the sodium values are: control - 585.9mg, 10% - 566mg, and 15% - 566mg. Using less soy sauce will decrease the salt content but may affect the flavor of the soy-blueberry burger. Also, salt masks the beany taste of soy. Dieters will want the carbohydrates decreased although 20.8g is not much carbohydrate for the average person especially if he or she is not dieting. Healthy children will utilize the carbohydrates. Well, who cares about the calories and the carbs when one has three times as much fiber?! Blueberries are part of a balanced healthy diet – the added sugar may be negligible. The textured soy protein used to make the research burgers add 2 to 5 times as much protein to the veggie burger. There is a 3% decrease in vitamin A but the vitamin C is 11 times, 10 times, and 12 times greater in the research burgers than in the brand name burgers. The calcium levels in the experimental burgers are 12% to 17% higher than in the brand name burgers. Iron is 20% greater in the soy-blueberry burgers.

With manipulation to decrease the calories and especially the sodium, combined with the high overall acceptance scores, the soy-blueberry burger has the potential to be a healthy and tasty addition to ones diet.

	Brand 1	Brand 2	Brand 3	Control		10%		15%	
				R	<i>LS</i>	R	<i>LS</i>	R	<i>LS</i>
Calories	70	100	100	239	<i>239</i>	236	<i>236</i>	236	<i>236</i>
Calories from Fat	5	30	22	76	<i>76</i>	76	<i>76</i>	76	<i>76</i>
Total Fat	0.5g	3.5g	2.5g	8.4g	<i>8.4g</i>	8.5g	<i>8.5g</i>	8.5g	<i>8.5g</i>
Saturated Fat	0	1g	0.5g	1g	<i>1g</i>	1g	<i>1g</i>	1g	<i>1g</i>
Cholesterol	0	5mg	0	0	<i>0</i>	0	<i>0</i>	0	<i>0</i>
Sodium	280mg	420mg	350mg	837 mg	<i>586</i> <i>mg</i>	809 mg	<i>566</i> <i>mg</i>	809 mg	<i>566</i> <i>mg</i>
Total Carbohydrates	6g	14g	9g	20.8g	<i>20.8g</i>	20.8g	<i>20.8g</i>	20.2g	<i>20.8g</i>
Dietary Fiber	4g	5g	4g	13.4g	<i>13.4g</i>	13.4g	<i>13.4g</i>	13.8g	<i>13.8g</i>
Sugar	1g	1g	1g	3.4g	<i>3.4g</i>	3.4g	<i>3.4g</i>	2.5g	<i>2.5g</i>
Protein	13g	5g	10g	28.7g	<i>27.8g</i>	26.9g	<i>26.9g</i>	26.9g	<i>26.9g</i>
Vitamin A	0	4%	4%	0	<i>0</i>	1%	<i>1%</i>	1%	<i>1%</i>
Vitamin C	0	2%	0	11%	<i>11%</i>	12%	<i>12%</i>	12%	<i>12%</i>
Calcium	6%	2%	0	18%	<i>18%</i>	17%	<i>17%</i>	17%	<i>17%</i>
Iron	4%	4%	4%	29%	<i>29%</i>	24%	<i>24%</i>	24%	<i>24%</i>

Table 23. Comparison of the Nutrition Labels for Commercial and Experimental Veggie Burgers

Regular soy sauce burger values are in regular (R) font and the low – sodium (*LS*) soy sauce burger value are in *italic font*. Source: Brand name product nutrition labels. Regular and low-sodium soy sauce soy-blueberry burgers: control, 10%, and 15% burger values were generated using Nutritionist Pro 2007 nutrient analysis software program.

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APPENDICES

Appendix A

Recruitment Flyer

Volunteers needed to taste

Soy-Blueberry Burgers

Wednesday, September 13th

11:00pm – 3:00pm

Consumer Testing Center, 158 Hitchner Hall

The healthful benefits of soy and blueberries together!

Persons interested in participating must:

Be at least 18 years of age

Be interested in evaluating the soy-blueberry product

Not have allergies to soy, blueberry, sesame, onion, and/or garlic

Participants will be offered the choice of a 60-minute phone card or
2 Consumer Testing Center points that can be accrued towards gift certificates.

If you have any questions or to sign up for a testing appointment, please

contact Pamela Small at 581-2773 or via FirstClass

(pamela.small@umit.maine.edu)

Appendix B

Informed Consent

Product being tested: Textured soy protein burgers with blueberry puree

If you have any known allergies to the following products, you may not participate in this study: Soy, soy sauce, blueberry, sesame, onion, and/or garlic

The evaluation of the soy-blueberry burger will take approximately 20 minutes. You will be asked to complete a questionnaire pertaining to each sample. I understand that I have the right to refuse to participate in, or withdraw from this study at any time. I understand that the study may involve the following risks/discomforts: No more than those encountered in the course of everyday eating.

Confidentiality

All data will be kept in the password-protected, locked computer server. All data will be destroyed one year after the study has been completed.

Further questions, concerns, or comments about this study or the informed consent process, may be directed to:

Pamela Small, Principle Investigator at pamela.small@umit.maine.edu or 581-2773,

Dr. Alfred Bushway, Co-investigator, at 581-1629

Dr. Mary Ellen Camire, Co-investigator and Sensory Testing Center Coordinator, at 581-1733 or email Mary.Camire@umit.maine.edu

If you have any questions about your rights as a research participant, please contact Ms. Gayle Anderson, Office of Research and Sponsored Programs, 581-1498. Your participation in the study indicates that you have read and understand the above and agree to participate in the study.

Appendix C

Soy-Blueberry Burger Acceptance Questionnaire

Please answer the following questions.

When finished, please click on the hand at the bottom of the screen to begin testing samples.

Please mark the box that best describes your age in years.

18-22 23-27 28-32 33-37 38-42 43-47 48-52 53-57 57+

Please mark the box that best describes your gender. female male

How often do you consume muscle food (beef, pork, poultry, seafood, other)?

Every day 2-3 times a week Once a week 15-20 times a month
 Once a month A few times a year Never

How often do you consume soy (textured soy protein, tofu, other)?

Every day 2-3 times a week Once a week 15-20 times a month
 Once a month A few times a year Never

How important is it to you to eat healthful foods?

Very important
 Moderately important
 Slightly important
 Sometimes important
 Slightly not important
 Moderately not important
 Not important at all

(click on hand at the bottom of the screen now)

Please take a drink of water before tasting the sample. Make sure the sample code on the plate matches the code on the screen. Please mark your appropriate response.

Please look at the burger before tasting it.

1. How do you like the appearance of this soy-blueberry burger?

- Like extremely
- Like very much
- Like moderately
- Like slightly
- Neither like nor dislike
- Dislike slightly
- Dislike moderately
- Dislike very much
- Dislike extremely

2. How do you like the texture of this burger?

- Like extremely
- Like very much
- Like moderately
- Like slightly
- Neither like nor dislike
- Dislike slightly
- Dislike moderately
- Dislike very much
- Dislike extremely

3. How do you like the overall flavor of this burger?

- Like extremely
- Like very much
- Like moderately
- Like slightly
- Neither like nor dislike
- Dislike slightly
- Dislike moderately
- Dislike very much
- Dislike extremely

4. What is your overall opinion of this burger?

- Like extremely
- Like very much
- Like moderately
- Like slightly
- Neither like nor dislike
- Dislike slightly
- Dislike moderately
- Dislike very much
- Dislike extremely

5. Please rate the soy flavor in this soy-blueberry burger

- | | | | | |
|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|
| <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| No soy flavor | | Just right | | Strong soy flavor |

6. Please rate the blueberry flavor in this soy-blueberry burger

No blueberry flavor Just right Strong blueberry flavor

5. Would you buy this soy-blueberry burger?

- Definitely would buy
- Probably would buy
- Maybe/maybe not buy
- Probably would not buy
- Definitely would not buy

Comments on this sample:

Now that you have tasted the three (3) samples, please rank them in order of your preference with your most favorite being number one (1).

Sample 294:

Sample 517:

Sample 783:

Your opinions are very important to this study. Thank you.

Appendix D

Soy-Blueberry Burger Acceptance Rating – Day 1

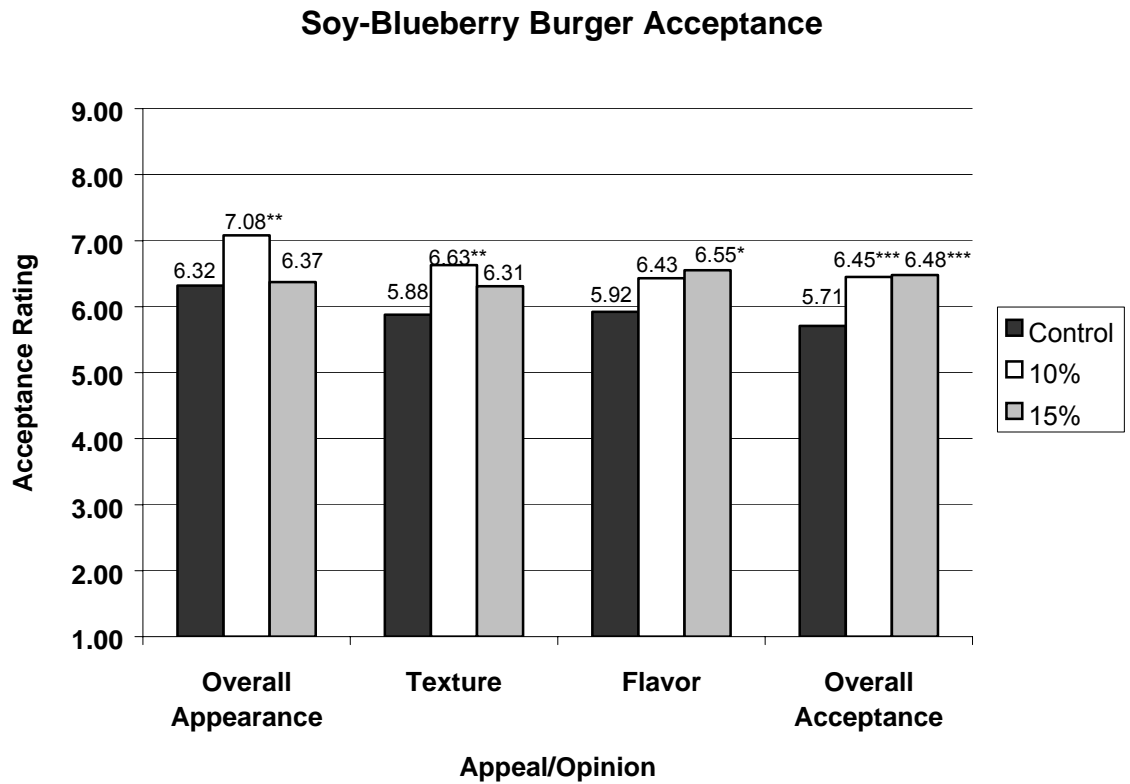


Figure D.1. Soy-Blueberry Acceptance Rating – Day 1

* = 0.05 significant ** = 0.01 highly significant *** = 0.001 very highly significant. Nine point hedonic scale: 9 = like extremely, 1 = dislike extremely

Appendix E

Preference Ranking of Soy-Blueberry Burgers - Day 1

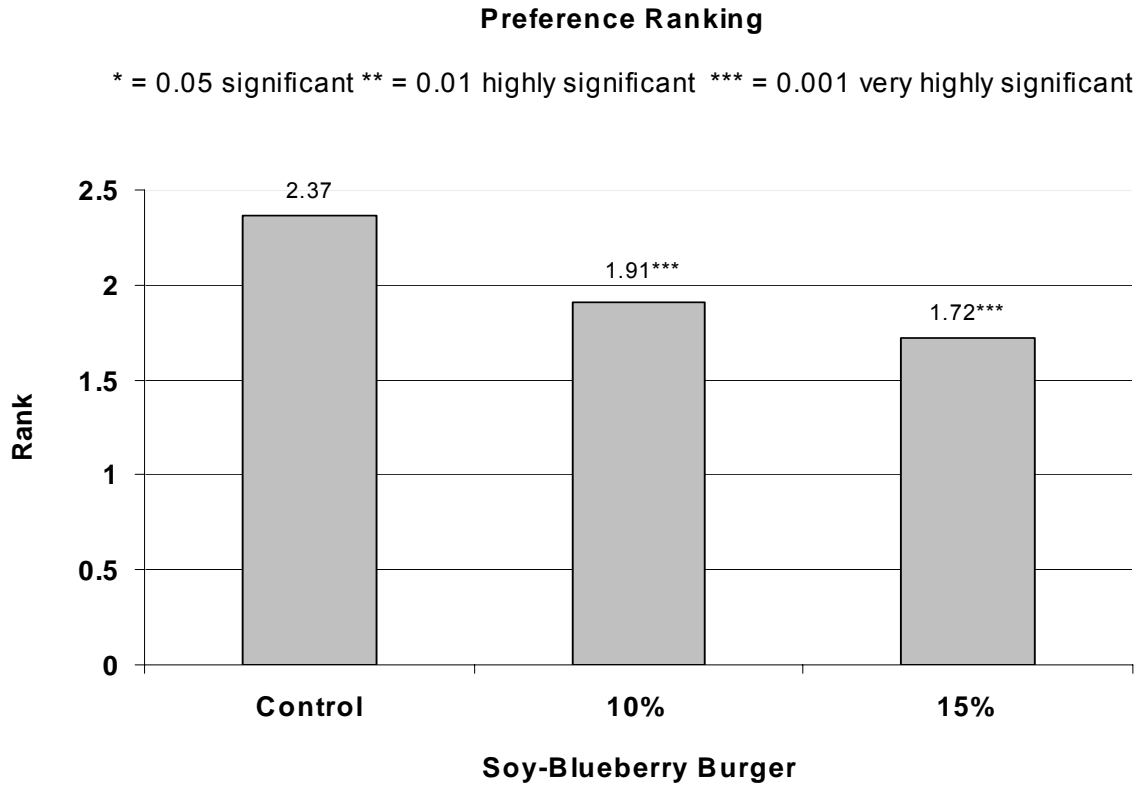


Figure E.1. Preference Ranking of Soy-Blueberry Burgers - Day 1

The burger with the LOWEST number was the MOST preferred.

Appendix F

Soy-Blueberry Burger Acceptance Rating - 3 Months Frozen Storage

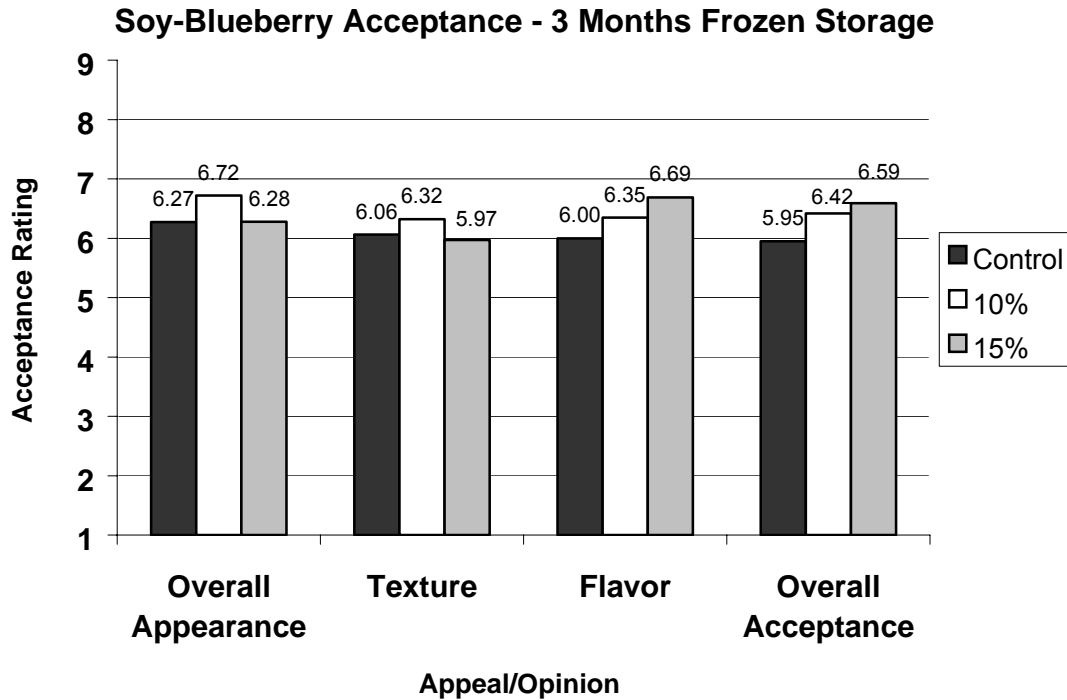


Figure F.1. Soy-Blueberry Burger Acceptance Rating - 3 Months Frozen Storage

* = 0.05 significant ** = 0.01 highly significant *** = 0.001 very highly significant. Nine point hedonic scale: 9 = like extremely, 1 = dislike extremely

Appendix G

Preference Ranking of Soy-Blueberry Burgers – 3 Months Frozen Storage

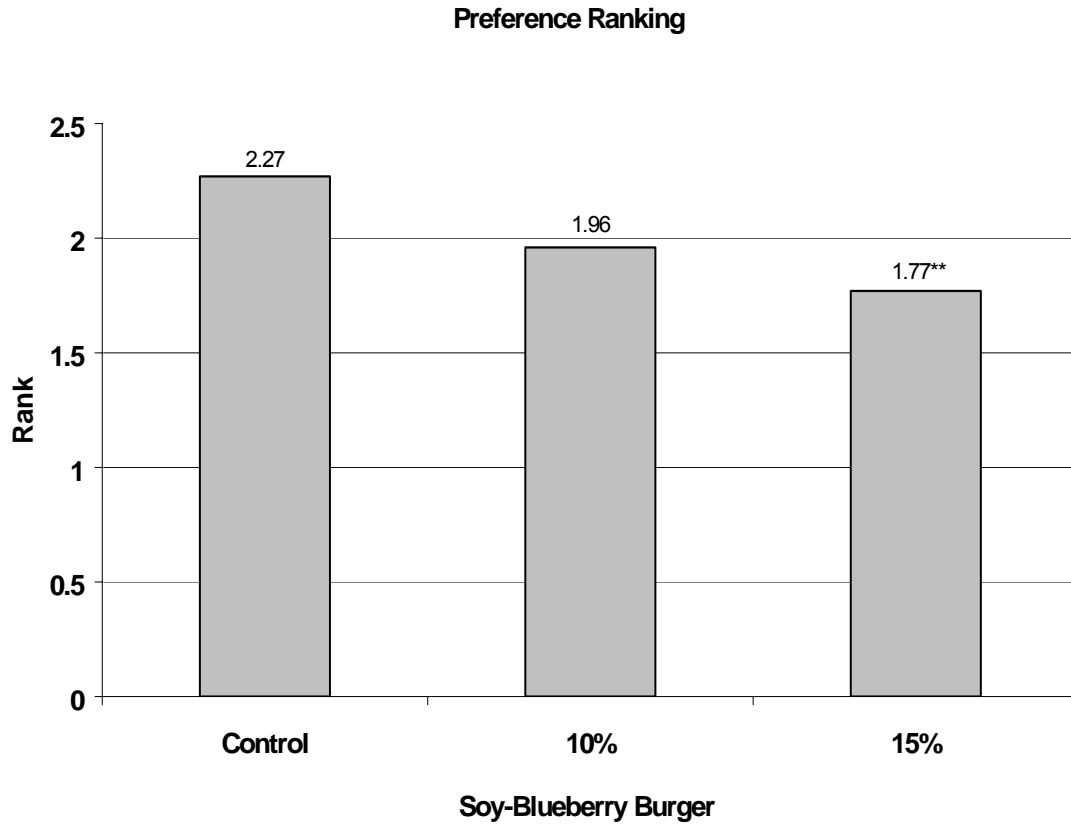


Figure G.1. Preference Ranking of Soy-Blueberry Burgers - 3 Months Frozen Storage

* = 0.05 significant ** = 0.01 highly significant *** = 0.001 very highly significant
Soy-blueberry burger with the LOWEST number is the MOST preferred.

Appendix H

Soy-Blueberry Burger Acceptance Rating - Natural Living Center - Day 1 – Low Sodium

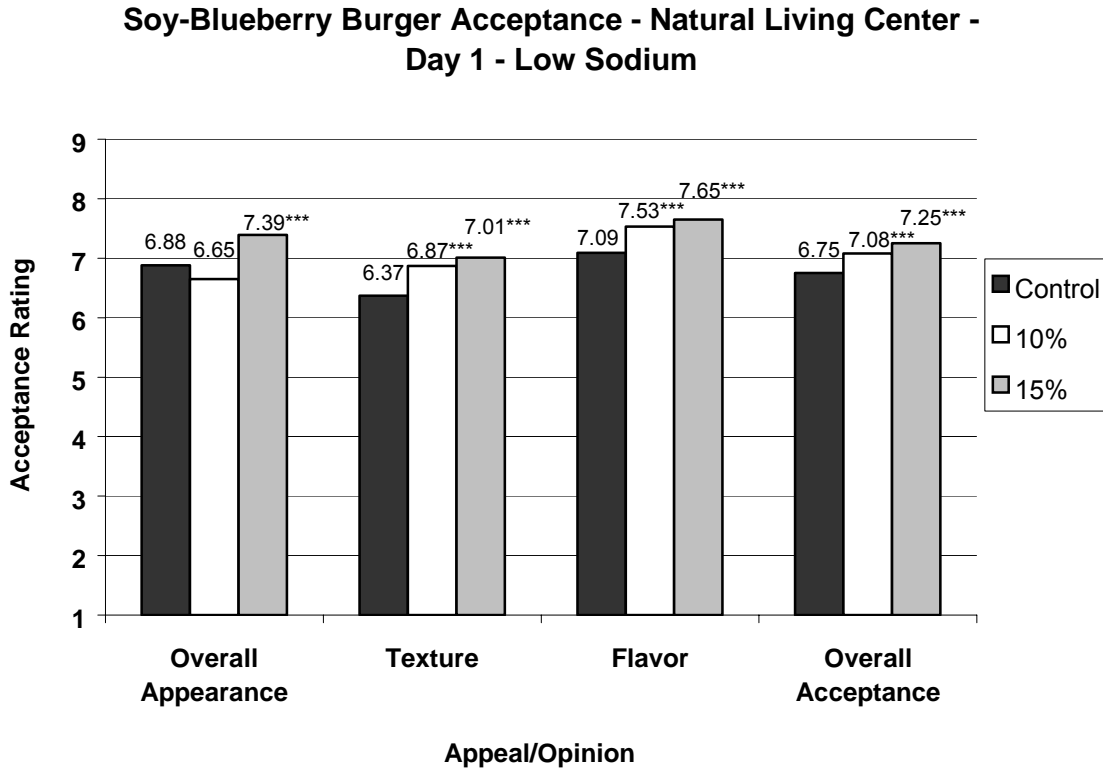


Figure H.1. Soy-Blueberry Burger Acceptance Rating - Natural Living Center -
Day 1 – Low Sodium

* = 0.05 significant ** = 0.01 highly significant *** = 0.001 very highly significant. Nine point hedonic scale: 9 = like extremely, 1 = dislike extremely

Appendix I

Preference Ranking of Soy-Blueberry Burgers – Natural Living Center - Day 1 – Low Sodium

Preference Ranking - Low Sodium Soy-Blueberry Burger

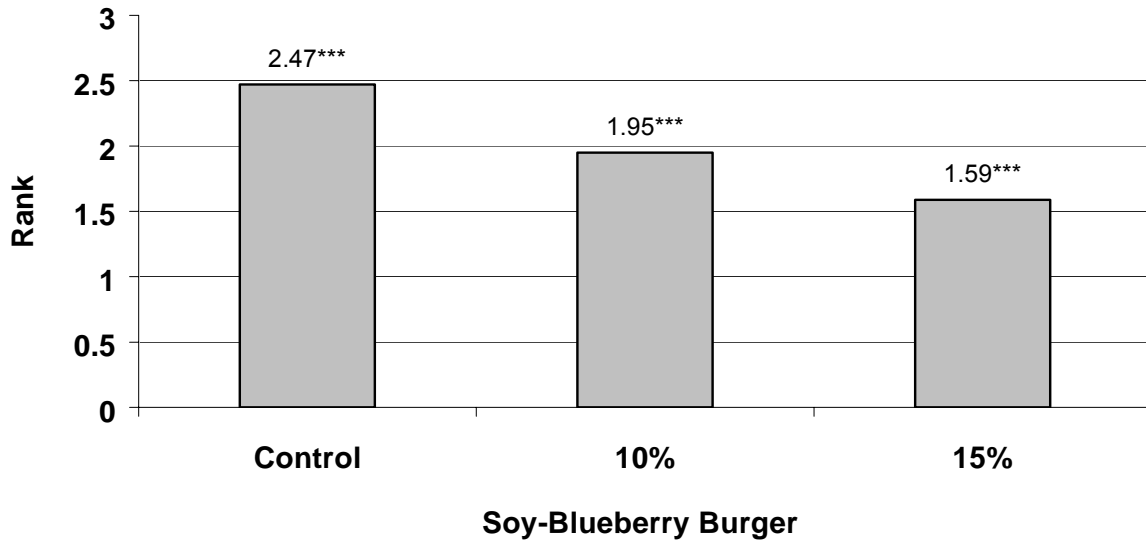


Figure I.1. Preference Ranking of Soy-Blueberry Burgers – Natural Living Center Day 1 - Low Sodium

* = 0.05 significant ** = 0.01 highly significant *** = 0.001 very highly significant. Soy-Blueberry burger with the LOWEST number is the MOST preferred

Appendix J

Effectiveness of Feeding Blueberry Derived Proanthocyanidins in Preventing Colonization of *Salmonella enteritidis* In Experimentally Challenged Chicks

Pamela Small, Dr. Alfred Bushway, Dr. H. Michael Opitz, Dawna Beane, Brenda Kennedy-Wade, and Kathy Davis –Dentici

ABSTRACT: Fifty commercial 6-week-old SPAFAS SPF (specific pathogen free) chickens were divided into five sets of 10 chicks each. Feed was mixed for each cage with the following amount of blueberry powder: Cage A: 150g, Cage B: 300g, Cage C: 600g; Cages D and E received feed with no blueberry treatment. Each chicken in groups E1, E2, and E3 received daily two doses of blueberry powder mixed in warmed water via crop gavage: E1: 3.5ml, E2: 8.5ml, E3: 17ml. Half of the chicks in cages A, B, C, and D and two chicks in group E4 were challenged with 10^8 colony forming units (CFUs) of a nalidixic acid resistant *Salmonella enteritidis* (SE-NA) culture on day 3. The remaining chicks were exposed by contact with cage mates throughout the experiment. On day 4 p. i., cecal colonization was evaluated. On day 7 cecal, gut, and organ colonization was evaluated for each chick. The experiment was duplicated. Per Systat Tukey's HSD multiple comparisons ($P = 0.05$), there were no statistical differences found between any of the chicken groups in the anti-adhesion effectiveness of blueberry proanthocyanidins in reducing the gut and organ

colonization of *Salmonella* in poultry. It did however provide valuable information that will be useful in further studies.

Keywords: blueberry, chicken, proanthocyanidins, PACs, Salmonella

Introduction

Salmonella infections of domestic poultry are costly to both the poultry industry and to society. It is acquired vertically bird to bird causing significant growth depression and mortality among young chicks (Snoeyenbos and others 1969; Gast and Beard 1990; Gast and Beard 1990; Gordon and Tucker 1965; Nakamura and others 1994; Zecha and others 1977). Biosecurity measures can increase production costs and negative publicity ultimately affects the profitability of producers. *Salmonella* is a major cause of human foodborne illness associated with poultry and eggs (Tauxe 1991). Infections of humans in the United States are estimated at over more than 3.5 billion dollars. The adherence of *Salmonella* to intestinal epithelial cells is the first step in the sequence of events that produces disease. Adherence of *Salmonella* has been associated with type 1 fimbriae (Aslanzadeh and Paulissen 1990; Ernst and others 1990; Linqvist and others 1987).

It is known that cranberry proanthocyanidins (PACs) have anti-adhesive properties that prevent the type 1 fimbriae of *Escherichia coli* from attaching to epithelial cells (Zafriri and others 1989; Howell and others 1998). The characteristic was also noted with blueberries (Howell and others 1998; Ofek

1991). The objective of this research was to evaluate the anti-adhesion effectiveness of blueberry PACs in reducing the gut and organ colonization of *Salmonella* in poultry.

Material and Methods

Experimental animals

All chickens were wing-banded upon arrival. Fifty commercial 6-week-old SPAFAS SPF (specific pathogen free) chickens were used for each replicate group. The chicks were housed in isolator 5 at the University of Maine poultry facility on campus, divided into five groups of 10 chicks. Each group was placed in pullet grower cages equipped with an external feeder and drinker trough. An environmental temperature of 75-85°F was maintained throughout the trial. Regular daylight was provided. Commercial grower mash and water was provided ad lib until the chickens were 6 weeks old. At the age of six weeks, all feed was replaced with the same commercial grower mash with blueberry powder mixed in. Ten pounds of feed was mixed for each cage (A, B, and C) with the following amount of blueberry powder: Cage A: 150g, Cage B: 300g, Cage C: 600g; Cages D and E also received 10 lbs of feed with no blueberry treatment. The chicks were cared for daily at least twice. This experiment was repeated once in isolator 6.

Biosecurity

The isolators were ventilated with positive pressure coarse filtered air. All personnel entering the isolator wore clean coveralls, protective footwear, dust mask, bouffant cap, and disposable gloves for all operations for all activity inside the isolator throughout the experiment. A rubber mat, filled with disinfectant, was placed at the entrance of the isolator. Every person entering and leaving the isolator was required to step into the disinfectant. All protective gear used during activities inside the isolator was taken off before leaving the isolator.

All disposable items used during the experiment as well as manure were placed into garbage bags. These bags remained in the isolator until the end of the experiment. All items sent to the testing labs were placed onto tray, which was inserted into a plastic bag for transport to the lab.

Treatments

Treatment consisted of administration of blueberry powder containing 9mg of PACs per gram powder. Each chicken in groups E1, E2 and E3 received daily two doses of blueberry powder mixed in warmed water (at a rate of 3 grams blueberry powder in 15ml warmed water): E1: 3.5ml, E2: 8.5ml, E3: 17ml. The blueberry powder was administered either in the feed or by crop gavage as follows (Table 1):

Cage	# of Chickens	PAC Route	Dose (mg) PAC/Day /Chicken	Dose (g) Blueberry Powder Day/Chicken	Conc. of PAC in Feed (ppm)	Conc. of Blueberry Powder In Feed (%)
A	10	Feed	13.5	1.5	288	3.2
B	10	Feed	27	3	588	6.2
C	10	Feed	54	6	1051	11.7
D	10	None	None	None	0	0
E1	2	Crop Gavage	13.5	1.5	NA	NA
E2	2	Crop Gavage	27	3	NA	NA
E3	2	Crop Gavage	54	6	NA	NA
E4	2	None	None	None	0	0
E5	2	None	None	None	0	0

Table J.1. Treatments

Each chicken in groups E1, E2 and E3 received daily two doses of blueberry powder mixed in warmed water (at a rate of 3 grams blueberry powder in 15ml warmed water): E1: 3.5ml, E2: 8.5ml, E3: 17ml. The blueberry powder was administered either in the feed or by crop gavage.

Salmonella challenge

Half of the chicks in cages A, B, C, and D and two chicks in group E4 were challenged with 10^8 colony forming units (CFUs) of a nalidixic acid resistant *Salmonella enteritidis* (SE-NA) culture on day 3 (Table 2). The remaining chicks were exposed by contact with cage mates throughout the experiment.

Direct challenge was by gavage into the crop of each chick in groups 1, 3, and 5 with a suspension of washed SE-NA cells in PBS from a 24-hour culture. The appropriate dose of salmonellae was suspended in 1.0 ml PBS. A licensed poultry veterinarian performed all procedures.

Cage	Group	SE Challenge	
		Direct	Contact
A	1	Yes	No
	2	No	Yes
B	1	Yes	No
	2	No	Yes
C	1	Yes	No
	2	No	Yes
D	1	Yes	No
	2	No	Yes
E	1	Yes	No
	2	Yes	No
	3	Yes	No
	4	Yes	No
	5	No	Yes

Table J.2. Salmonella Challenge

Half of the chicks in cages A, B, C, and D and two chicks in group E4 were challenged with 10^8 colony forming units (CFUs) of a nalidixic acid resistant *Salmonella enteritidis* (SE-NA) culture on day 3.

Evaluation of colonization

A drag swab was taken from the manure under each cage one-day prior to challenge to verify the Salmonella free status of each group of chickens. Colonization with SE-NA was evaluated on day 4 and 7 post challenge. On day

4, only cecal colonization was evaluated and on day 7 cecal, gut, and organ colonization was evaluated for each chick. On day 4 p.i. each chick was placed into a mouse cage and was inoculated intraperitoneally with 0.25ml of 5% Pilocarpin. Approximately 1g ejected cecal content was collected (approx. 1-3 minutes later) and placed into 100ml PBS (pre-weighed). Quantitation of CFUs was by spiral counter, in duplicate, on BG-NA agar. On day 7 p.i., every chick was euthanized. Cecal content, a 1cm long piece of the cecum, and a pool of equal sized pieces of the spleen and liver was collected from each chick. A licensed poultry veterinarian and the resident pathology lab technician performed all procedures.

Results

The cecal content was quantitated on days 4 and 7 p. i., while the cecum and organ samples were noted only as either positive or negative for Salmonella growth on day 7 p.i.. Observations made by the veterinarian and pathology lab technician were noted. Per Systat Tukey's HSD multiple comparisons ($P = 0.05$) no statistical differences between any of the groups were found.

Day 4 p. i. cecal results show that 26 of 28 chicks (Trial 1) that were challenged were positive for Salmonella growth. In the repeat experiment (Trial 2), 27 of 28 challenged chicks were positive. On Day 7 p. i., the cecal results were Trial 1: 27 out of 28 birds were positive and Trial 2: 27 of 28 were positive. Out of 100 chicks total, only one unchallenged chick gave a positive and it was a low colony count. From these results one can conclude that the challenged

chicks harbored a Salmonella infection and the unchallenged chicks did not. This was regardless of blueberry dose or method of intake.

Trial 1, Day 7 p. i. 24 h cecum sample XLT 4 and BGN-AL plates: 15 of 28 challenged chicks harbored Salmonella in their cecums. After 72 h the number of positives increased to 22. All of the positives were seen in challenged chicks. All unchallenged chicks remained negative. This was regardless of blueberry dose of method of intake. In Trial 2, 20 of 28 challenged chicks were positive after 24 h. There was no increase in this number after 72 h incubation. Regardless of blueberry dose or method of intake, all unchallenged chicks remained negative for Salmonella.

Trial 1, Day 7 p. i. 24 h organ pool sample XLT 4 and BGN-AL plates: 15 of 28 challenged chicks harbored Salmonella in their organs (spleen and liver pool). After 72 h the number of positives increased to 22. All unchallenged chicks remained negative for Salmonella. This was regardless of blueberry dose of method of intake. In Trial 2, 20 of 28 challenged chicks were positive after 24 h. There was no increase in this number after 72 h incubation. Regardless of blueberry dose or method of intake, all unchallenged chicks remained negative for Salmonella. The organ colonization mimicked that of cecum harborization.

Conclusions

There were several factors that may have contributed to the inconclusiveness of this study. The commercial feed purchased contained pro-

biotics. What effect this may have had on the outcome of this trial is unknown at this time. Environmental and management factors influence the susceptibility of poultry to Salmonella. Stressful conditions have been shown to facilitate infections and horizontal transmission (Holt and Porter 1992; Thaxton and others 1975). Although all challenged chicks in group E were cecal positive, isolator 5 had lower plate counts. Isolator 5 and isolator 6 are also in different buildings with different heating/cooling setups, which may have been an environmental stressor.

Newly hatched birds are most susceptible to salmonellae but this decreases rapidly with age by the day (Fagerberg and others 1976; Smith and others 1980). Because of facilities, cost, and mortality rates this study utilized 6-week-old specific pathogen free chicks.

Although no significant differences were found between blueberry dose amounts and/or method of intake, there were several observations made by the Ph.D. student, the veterinarian, and the pathology lab technician. It was observed that the chickens receiving the blueberry supplement, either in their feed or via direct placement into the crop, consumed 10 to 20% more feed based on dosage. There was also an increase in fecal output. Upon necropsy, per veterinary observation, there were no signs of nutritional deficits or excesses. It was observed that the positive cecum sample plates and positive organ sample plates had a fewer CFUs than previously seen with other samples in the lab. The greatest observation was the lack of horizontal transmission.

This feeding trial was an inconclusive evaluation to the effectiveness of the anti-adhesion effectiveness of blueberry proanthocyanidins in reducing the gut and organ colonization of *Salmonella* in poultry. It did, however, provide several helpful observations that may be useful in further studies.

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BIOGRAPHY OF THE AUTHOR

Pam grew up in Colchester, Connecticut. After attending college on a sports scholarship, she decided to experience the world.

She spent ten years traveling the world and professionally training/driving harness horses. Pam was the leading female trainer/driver at several racetracks on the East Coast and set several track records. Later, she returned to school part-time, worked full time, and continued to train horses as a hobby. Pam graduated from Parkland College, Champaign, IL with an A.A. in 1996. She received several "Outstanding Service" awards for mentoring at Parkland College as well as Champaign-Urbana community service awards. From IL, Pam traveled the Caribbean.

In 1998, while working full time, Pam enrolled at the University of Maine. In 1999 she graduated with a Bachelor's degree in Animal and Veterinary Sciences. She has taught several courses at the University and at Husson College. Pam and her various non-human companions spend much of their free time sharing themselves and educating others in several public venues including: grade schools, rehabilitation services, veterans' homes, and working with hearing impaired children.

From Olympic trials, world travel, horse training, various work experiences, obtaining a higher education, teaching, being an aunt and numerous other experiences, Pam's life continues to be a cornucopia of adventure. She is a candidate for the Doctor of Philosophy degree in Food and Nutrition Sciences from The University of Maine in August 2007.