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Chemistry and Application of Pulses as Value-Added Ingredients in Processed Foods

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CHEMISTRY AND APPLICATION OF PULSES AS VALUE-ADDED INGREDIENTS IN PROCESSED FOODS

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This work is dedicated to the many people that will go nameless, for all you did, I thank you. I thank God and the Spirits that Be for the many ways I have been blessed and the opportunities provided to me. I cannot give enough thanks to my caring, and forever patient wife April McKeel Holliday for supporting me and being my rock and drill sergeant when I needed it. Thanks to my mom, Claire M. Brown, for making sure I was raised in an environment that instilled a hard work ethic, desire for knowledge, and opportunity for success. And thank you to all the teachers and friends in my life who have guided me through my different stages. Specifically, Bill Koren, George Charlet Jr., Gavin Estes, Paul Cook, Joyce Ann Stewart, Dr. Christopher Loss, Dr. Michael Cheng, Matthew Cael, Dr. John Marcy and the faculties of the Food Science Department of Louisiana State University and John Folse Culinary Institute at Nicholls State University.

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ABSTRACT

Chemical characterization of pulses allows for varying rates of water absorption, least gelation capacity, and retrogradation depending on species. Nutritionally, pulses are good sources of protein while being low in fat. Pulses deliver a readily bioavailable food form of several key minerals. Additionally, they deliver fiber. The insoluble fiber components are both natural and formed resistant starch in addition to the oligosaccharide content. Therefore, pulses can serve both a nutritional and functional role when used as a value-added ingredient. Meat patties were produced from beef and 23 different pulses at 35%, 42.5%, and 50% ratios. Each patty was tested for weight loss, diameter loss, color, and texture. The 50:50 ratio samples had the least amount of cook loss but the greatest visible bean fraction. All fractions improved nutritional profile. Navy, Light Red Kidney (LRB), and Small Red Beans were found to be most beneficial/acceptable as partial meat substitutes. The 42.5% patties were tested using two consumer focus groups. The recommendations from the focus group was used in a consumer study for both liking and difference. Panelists found significant differences for overall liking; however, panelists failed to determine difference Therefore, LRB modified meat patty (MMP) could be implemented at the USDA National School Lunch Program. The health impact of the MMP verses a control diet (CD) was tested using Syrian hamsters. The hamsters were fed for four weeks with weekly measurements of weight gain. After necropsy, organ weights and blood lipid levels were measured. All non-CD diet hamsters resulted in higher finished body weights. Hamsters on LRB or MMP diets had reduced LDL and VLDL averages of 22.7 and 8.1 mg/dL respectively compared to the CD. Additionally, average HDL:LDL ratios for the MMP and LRB diets increased from 1.47:1 for the CD to 1.9:1 and 2.2:1 respectively. Hamsters on CD and LRB

diets had lower liver weights and reduced epididymal adipose weight compared to diets containing MMP or GB. The results suggest partial substitution of LRB in GB can have significant impact on cholesterol levels and visceral fat deposition due to synergism between sat fat and cholesterol in the diet.

CHAPTER 1. LITERATURE REVIEW

1.1 Introduction

One of the biggest health concerns in the United States of late has been the rise in obesity among both adults and children over the last few decades. Focused media coverage has raised public awareness of obesity as a global epidemic and major public health crisis that carries severe health implications. The risk factors for diseases associated with obesity, notably heart disease, cancer, and especially type-2 diabetes, are determined to a great extent by behaviors learned in childhood and continued into adulthood. As more and more obese children become obese adults, healthcare costs associated with obesity are rising as well and will continue to rise to astronomical levels. Two questions arise from this information: how did obesity get out of control, and more importantly, how do we stop it?

Obesity is a problem for all groups and genders; it is particularly severe among certain ethnic groups. Additionally, obesity is more prevalent in low- and middle-income populations, particularly in urban settings. The dietary habits and patterns leading to the obesity epidemic are a result of what foods are being served, what foods are available, taste preferences, and cultural practices. Cultural views, in particular, can affect popular opinion on what is perceived as healthy or obese. Additionally, as many populations migrate to developed countries (or as their home country becomes more developed), their diets often evolve to include more energy-dense foods high in fat (particularly saturated fat, sugar, and salt), while decreasing in micronutirents, dietary fiber, and important bioactive phtyochemicals. In combination with lifestyle changes, their new diets are often accompanied by a corresponding increase in diet-

related chronic non-communicable diseases (NCDs). Furthermore, obesity related NCDs co-exist with problems related to undernutrition in many countries[1]. Obesity is not just a nutritional and medical concern but also impacts social and environmental issues as well. Understanding the complexity of these issues allows researchers to develop better solutions for reducing the impact of obesity in all critical areas.

1.2 Obesity and Health-Related Problems

1.2.1 Introduction

Obesity rates in the United States are on the rise. In 1980, no state had an obesity rate over 15% and in 1991, no state was over 20%. However, in 2007-2008, forty-nine of the fifty states had obesity exceeding 20% of the population and 25% of the population in thirty-one states. Additionally, in 2008, sixteen states saw an increase in obesity rates for the second year in a row and eleven states saw a rise for the third year in a row. Furthermore, eight of the ten states with the highest rates of obesity were in the south. Unfortunately, not a single state saw a decrease despite the national effort to decrease obesity. In the 2013 report from the Robert Wood Johnson Foundation, the obesity epidemic is shown to have gotten worse. Every state now has an adult obesity rate over 20%, while forty-one states have rates of at least 25% and thirteen states now have rates over 30%[2].

According to the 2009 Center for Disease Control (CDC) report on the adult population[3], African Americans had the highest rates of obesity, followed by Hispanics, and then whites. The prevalence of obesity per state for black Americans ranged from 23.0% to 45.1%. Forty states

showed rates of ≥30% in black Americans (Louisiana had 35.9%), and five states (Alabama, Maine, Mississippi, Ohio, and Oregon) showed rates of ≥40%. Among Hispanics, the prevalence of obesity per state ranged from 21.0% to 36.7%, with eleven states showing rates of ≥30%. Among whites, the prevalence of obesity per state ranged from 9.0% to 30.2%, with only West Virginia showing rates of ≥30%[3]. Figure 1 below represents the average rate of obesity for race and gender.

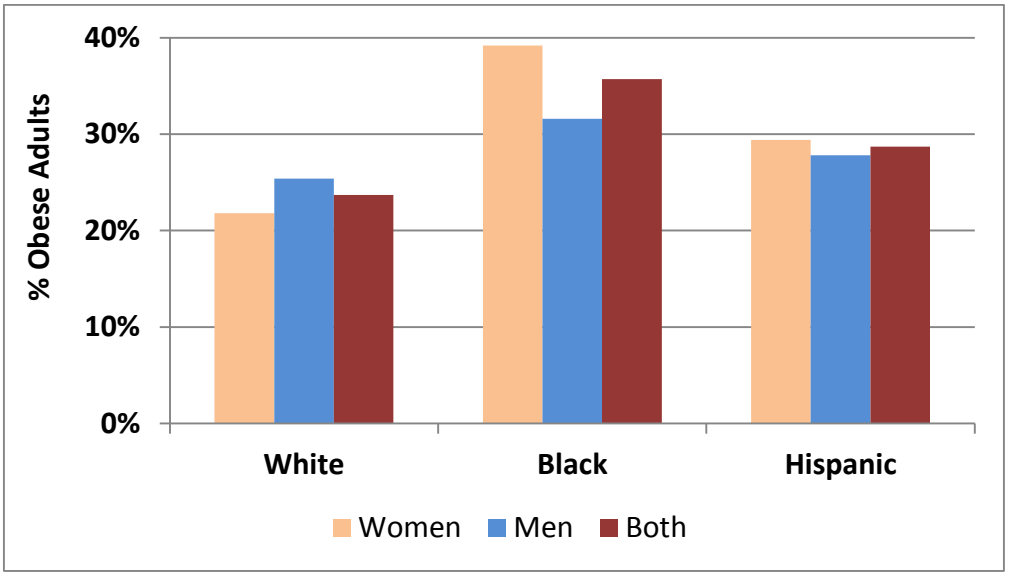

Figure 1: Obesity Among Adults, by Race & Gender in the United States: CDC 2006-2008 Data[3]

The terms “overweight” and “obese” are standard labels for ranges of weight that are greater than what is generally considered healthy for a given height. These terms also identify ranges of weight that have been shown to increase the likelihood of certain diseases and other health problems. For adults, overweight and obesity ranges are determined by using an individual’s weight and height to calculate a "body mass index" (BMI).

$$BMI = \frac{\text{Weight (lbs)} * 703}{\text{Height}^2\ (\text{in}^2)} \quad \text{or} \quad \frac{\text{Weight (kgs)}}{\text{Height}^2\ (\text{m}^2)}$$

An adult with a BMI between 25 and 29.9 is considered overweight while a BMI of 30 or higher is considered obese. BMI is used as a correlate of body fat for an average person and although BMI is indicative of a standard range of body fat, BMI does not directly measure body fat. As a result, BMI calculations have limitations and can lead to the misclassification of certain individuals such as athletes with increased muscle mass or the elderly.

Waist circumference may be a better indicator of health risk than BMI, ideally they should be used in combination. Waist circumference is particularly useful for individuals with a BMI of 25-34. For individuals with a BMI of more than 35, waist circumference adds little predictive power on the disease risk classification of BMI. Measuring waist circumference is a simple check to tell how much body fat an individual has and where it is placed around the body. Where the fat is located can be an important sign of the individual's risk of developing an ongoing health problem. If the majority of fat is around the waist rather than at the hips, there is a greater risk for heart disease and type-2 diabetes[4]. Besides the direct impact on health, one concern with higher BMI levels in adults is the tendency for their children to become overweight or obese.

1.2.2 Childhood Obesity

One of the major concerns with childhood obesity is the increased likelihood of remaining obese as an adolescent and an adult[3]. It takes little imagine to project that as more and more

obese children become obese adults, the diseases associated with obesity will surge. Increasing rates of childhood obesity in American children (as illustrated in Figure 2 below) is a growing health concern in the United States.

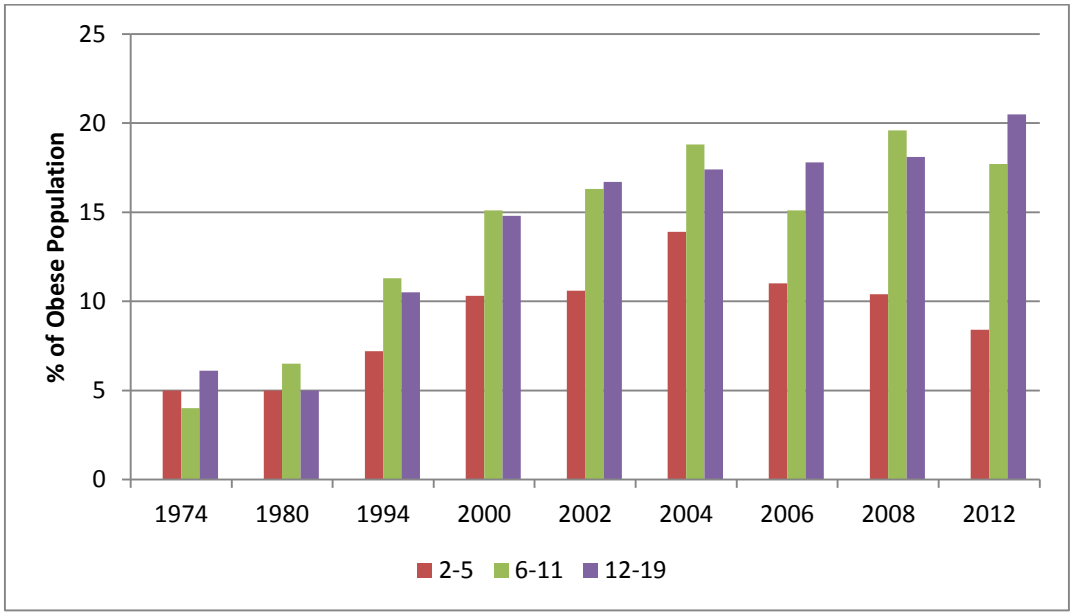

Figure 2: Percentage of Overweight Children & Adolescents (Ages 2-19): 2012 NHANES Data[5]

In 2003-2004, 13.9% of children (two to five years old) were overweight, with an additional 26.2% at risk for becoming overweight. In the six to eleven age group, 18.8% were overweight and a staggering 37.2% were at risk for becoming overweight. The study showed 17.4% of adolescents (twelve to nineteen years old) were overweight and 34.3% were at risk for becoming overweight[5]. Among US children two to seven years of age, an estimated energy imbalance of only 110-165 kcal/day was sufficient to account for the excess weight gain[6].

Interestingly, a study in 2012 showed a reduction in obesity in both the two to five and six to eleven age groups. The reduction in obesity of the two to five age group began between 2004 and 2006 and has continued to decline ever since. However, the twelve to nineteen age group is continuing to climb and in 2012, topped the 20% threshold value.

Minority children are at particularly high risk for childhood obesity. Among non-Hispanic black children aged twelve to nineteen, the 2006 female population had a total obese population of 27.7%. Meanwhile, the male population had a total obese population of 18.5%. In Hispanic children aged twelve to nineteen, however, the male population was at greater risk with a total obese population of 22.1% compared to females having a total obese population rate of 19.9%[4]. These trends can be seen in Figure 3 below.

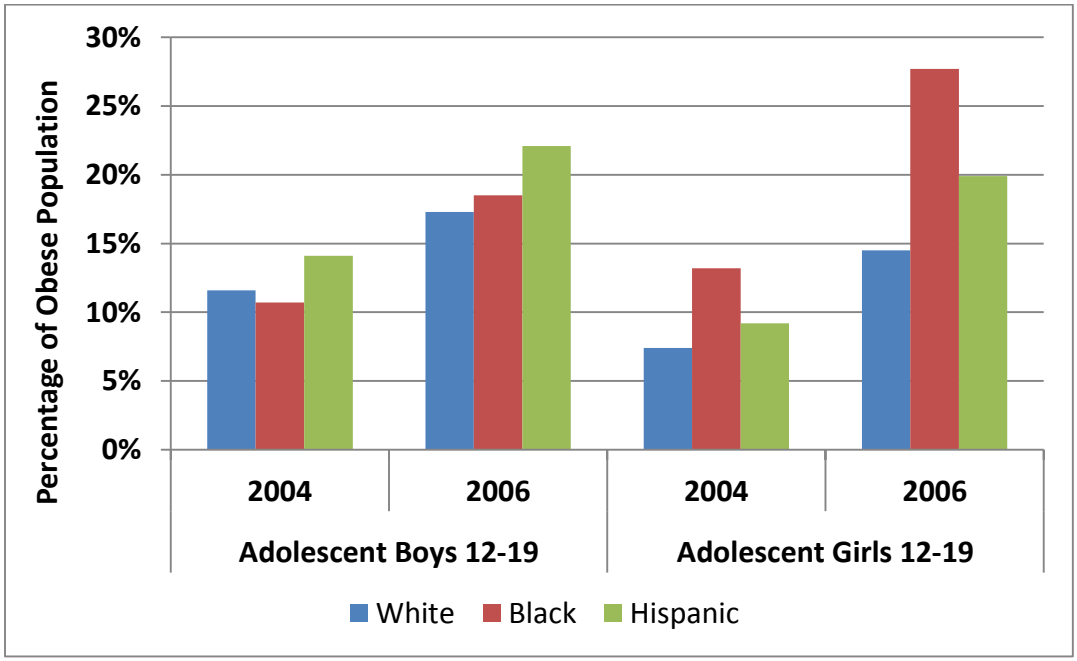

Figure 3: Childhood Obesity by Race: 2008 NHANES Data[5]

Studies show that many Latino mothers believe their obese child to be healthy and are unconcerned about their child's weight. However, these same parents believe that obese individuals need assistance from nutritionists or physicians to help with weight reduction[7]. Among African American parents, there is greater awareness of acute health conditions than obesity[7]. Specifically, obese African American girls and their female caregivers were unaware of the potential health consequences associated with increased body size[8].

BMI is also used to determine overweight and obesity in children. Although it is calculated using a child's weight and height, a child's weight status is determined using an age- and sex-specific percentile for BMI rather than the BMI categories used for adults as seen in Figure 4.

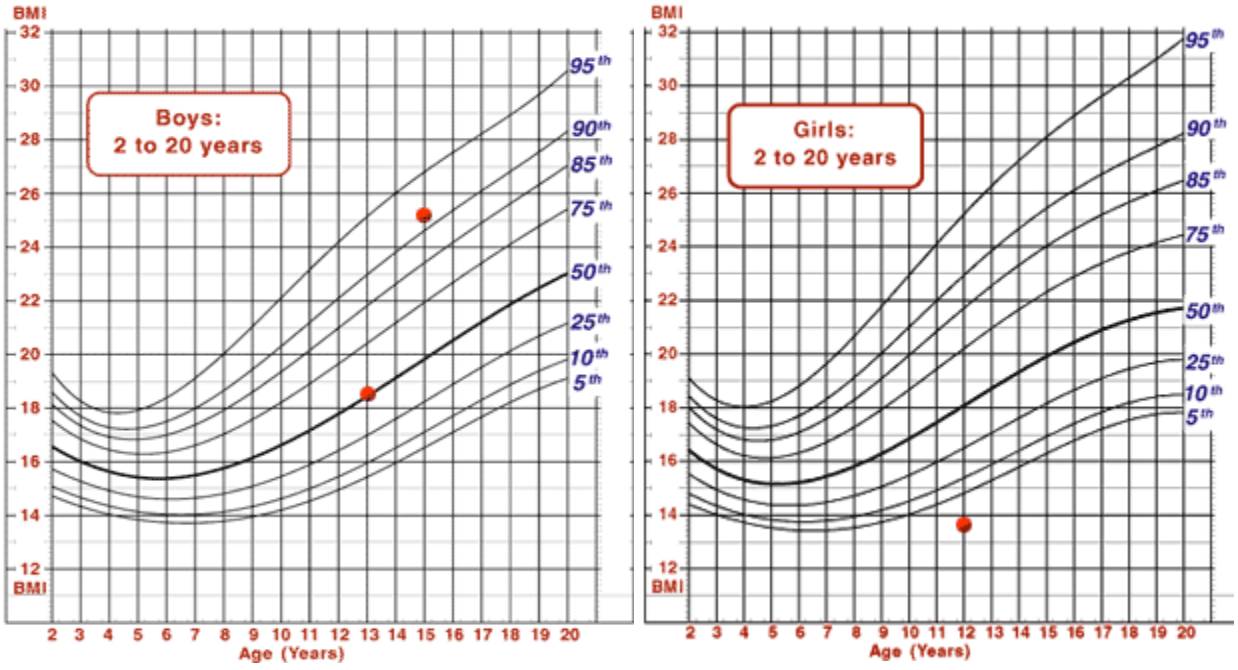

Figure 4: Childhood BMI Growth Charts[5]

Children's body composition varies as they age and varies between boys and girls. Since BMI does not measure body fat directly, this variation is a reasonable indicator of excess weight for most children and teens. For children and adolescents (aged 2-19 years), being overweight is defined as having a BMI between the 85th and 94th percentile for children of the same age and sex, whereas, childhood obesity is defined as a BMI of ≥95th percentile for children of the same age and sex.

In the United States, twenty million children and teens are overweight or obese. This means that we are looking at the first modern generation of young people that may not live longer than their parents since childhood obesity can have numerous harmful effects on the body. Moderate obesity, which is now common, reduces life expectancy by about three years. Severe obesity, which is still uncommon in children, can shorten a person's life by ten years. This ten-year loss is equal to the effects of lifelong smoking. This makes obesity the second leading preventable cause of death in the United States, second only to smoking[9]. A recent study showed that 70% of children had at least one cardiovascular disease risk factor and that 39% had two or more. Additionally, obese children are more likely to have impaired glucose tolerance, insulin resistance, type-2 diabetes, breathing problems, musculoskeletal discomfort, and fatty liver disease. Furthermore, if children are overweight or obese, they are more likely to become overweight or obese adults and to a more severe degree. A 2010 study showed that obese adolescents were sixteen times more likely to become severely obese adults compared to adolescents of normal weight or those who were classified as overweight[6,9].

1.2.3 Heart Disease/Cardiovascular Disease

According to the 2009 report *F as in Fat: How Obesity Policies are Failing in America*[10], "the obesity epidemic is a big contributor to the skyrocketing health care costs in the United States." Risa Lavizzo-Mourey, president and chief executive officer of the Robert Wood John Foundation.

In 2010, cardiovascular disease (CVD) in the United States accounted for 31 .9% of all deaths. These numbers indicate that over 2,150 Americans die of CVD each day, or an average of one death every forty seconds. In 2010, 34% of deaths attributable to CVD occurred before the age of 75 years, a reduction in the current average life expectancy of 78 .7 years[6]. This disease is not just costing lives; it is putting a burden on the US economy as well. The total direct and indirect costs of CVD and stroke in the United States for 2010 are estimated to have been $315.4 billion. By comparison, the total estimated costs for cancer in 2008 were a mere $201 .5 billion.

There is strong evidence that childhood obesity has led to a significant increase in the development of precursors for CVD, such as type-2 diabetes, hypertension, dyslipidemia, metabolic syndrome, and plaque deposits in the arteries. The arterial walls of overweight children are looking more like those of an average forty-five year old (see Figure 5 & Figure 6 below) according to a study presented at the American Heart Association's 2008 convention[,11,12,13]. The results of a poor diet and sedentary lifestyle traditionally seen in middle-aged and older adults are now seen in adolescents and in more pronounced stages in adults in their early twenties.

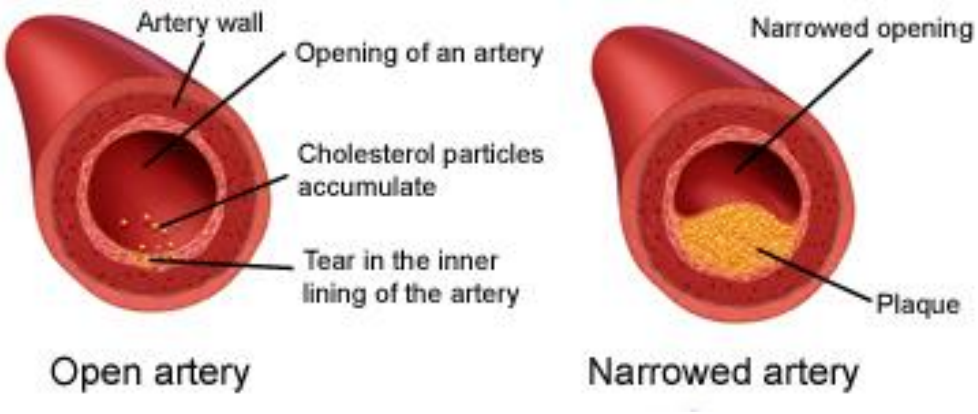

Figure 5: Plaque Deposits in Artery

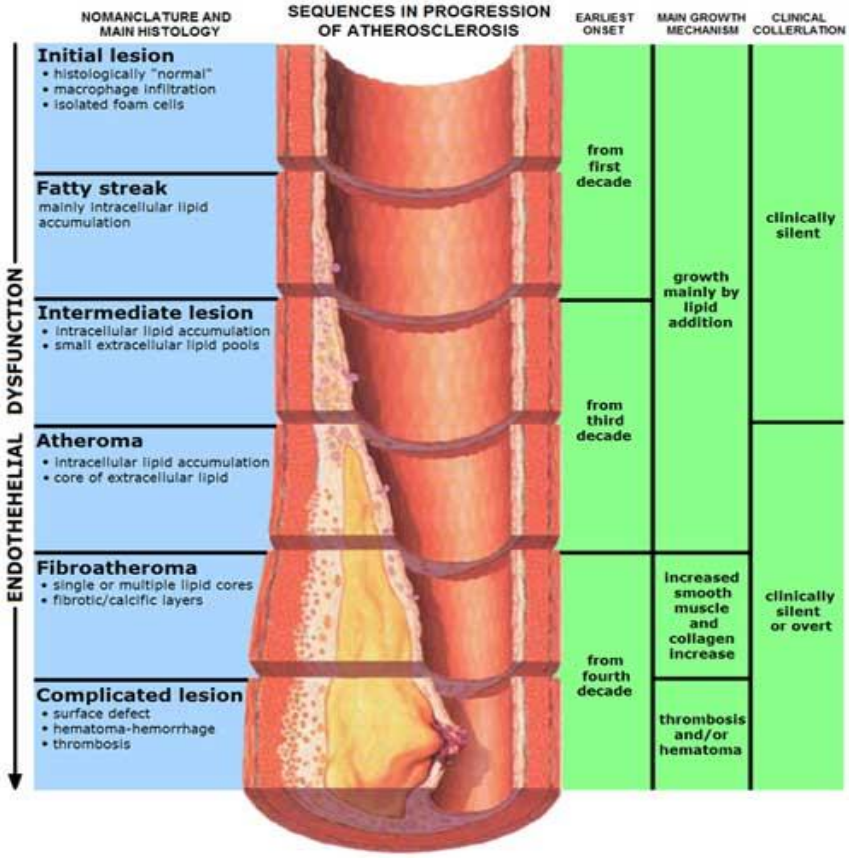

Figure 6: Progression of Atherosclerosis
http://en.wikipedia.org/wiki/Atherosclerosis[14]

Fortunately, the build-up of plaque in arterial walls can be slowed and even reversed if necessary changes are made. A reduction in refined carbohydrates, excessive fat consumption, and an increased consumption of lean protein and fiber has been shown to reverse many of the negative symptoms[13,15].

1.2.4 Diabetes

Until the 1990's, the majority of cases of diabetes mellitus in children and adolescents were immune-mediated type-1 diabetes. The rise of childhood obesity over the last two decades has led to a dramatic increase in the incidence of type-2 diabetes in children and adolescents. In fact, type-2 diabetes is now the dominant form of diabetes in children and adolescents in some populations. Obesity is strongly correlated with insulin resistance, which, when coupled with relative insulin deficiency, progresses from metabolic syndrome to type-2 diabetes. Children and adolescents with type-2 diabetes often experience the microvascular and macrovascular complications of this disease at younger ages than individuals who develop diabetes in adulthood, including elevated blood pressure, dyslipidemia, and a higher prevalence of factors associated with atherosclerotic cardiovascular disease, stroke, myocardial infarction, and sudden death. Children from racial minority groups suffer disproportionately in the development of early onset type-2 diabetes. Helping children achieve or maintain a healthy weight requires accurate identification by health care professionals and promotion of lifestyle modifications. It will also require significant societal change to create a healthier environment for children[16,17].

1.2.5 Health Care Costs

The increase in obesity and overweight is growing faster in adults than in children, and in women faster than in men. If these trends continue, by 2030, 86.3% adults will be overweight or obese. Black women (96.9%) and Mexican-American men (91.1%) would be the most affected. In children, the prevalence of overweight and obesity (BMI ≥ 85th percentile) will nearly double by 2030[6,18]. The economic impact of these increases will impact health-care costs most severly. A recent study estimated that medical expenditures attributed to diseases associated with overweight and obesity accounted for 9.1% of total US medical expenditures in 1998 and reached $78.5 billion but have increased drastically in the following years. The breakdown of the 2010 costs can be found in Figure 7 below with data provided by the National Heart, Lung, and Blood Institute[6,18].

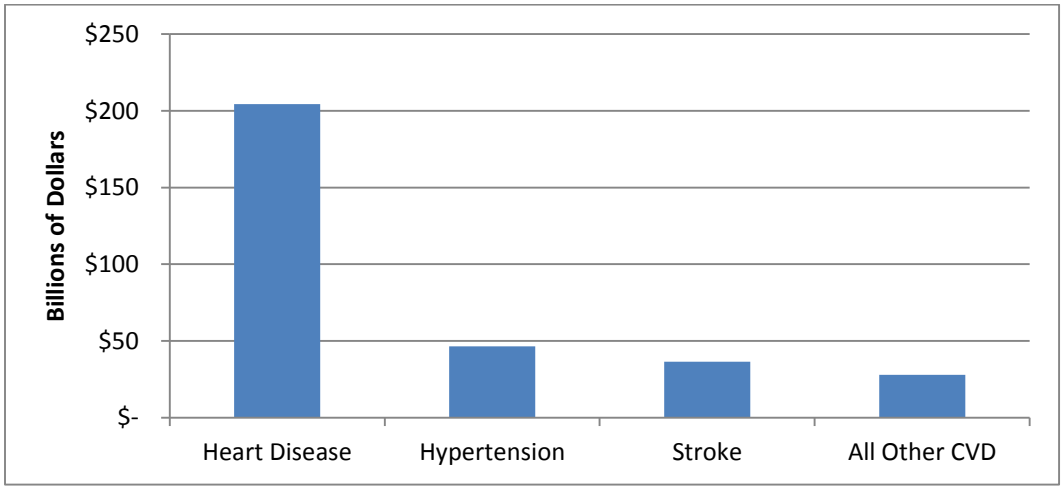

Figure 7: Costs of Cardiovascular Disease and Stroke in 2010

Of great economic concern is the fact that obesity-related health care expenditures will continue to rise unless solutions for the obesity epidemic are implemented. If trends remain the same, it is anticipated that by 2030, 43.9% of the American population will have some form of cardiovascular disease or a related illness. Additionally, between 2012 and 2030, total direct healthcare costs associated with cardiovascular disease are projected to rise from $396 billion to $918 billion. The estimation also implies that one of every six dollars spent on health care will be spent treating diseases associated with overweight and obesity. Unfortunately, the same trend will be seen in health care expenditures related to childhood and adolescent obesity. Studies show that medical costs related to overweight and obese children have already tripled in the last 20 years[6,18].

1.3 Dietary Patterns

1.3.1 What is Being Served

Food consumption is variably affected by a wide variety of factors including food availability, food accessibility, and food choice, which in turn are further influenced by geography, demography, disposable income, socio-economic status, urbanization, globalization, marketing, religion, culture, and consumer attitudes[19]. While studies have shown that fruit and vegetable consumption has remained constant over the past 25 years, new evidence is suggesting a different story emerging across the US and Europe. While organizations on both continents spend millions of dollars on marketing, subsidies, purchase, and distribution of fruits and vegetables in an effort to increase consumption, both US and European averages have begun to decline while obesity levels have increased[20]. The most recent School Dietary Assessment Study

conducted by the USDA found that 90% of school lunch menus offer entrees such as pizza and cheeseburgers despite the fact that Americans would like to see improved nutrition in school lunches[21]. Recent changes instigated by First Lady Michele Obama's "Chefs Move to Schools" campaign, as well as federal legislation for the USDA National School lunch program, promise increased servings of fruit and vegetables. However, there is no evidence to date that the children are actually consuming the increased servings.

Nutrient-dense foods that are associated with better health outcomes tend to cost more per kilocalorie than do refined grains, sweets, and fats. In fact, research has shown that the price disparity between healthful and less healthful foods appears to be growing[22]. Conversely, other research attests to the ability to eat healthier without increased spending. Using 2008 Nielsen Homescan data, price and calorie per portion of 20 fruits and vegetables were compared with 20 common snack foods such as cookies, chips, pastries, and crackers. The averages per portion for fruits and vegetables were 31 cents and 57 calories. The averages per portion for snack foods were 33 cents and 183 calories per portion for snack foods. According to the study, a person would save 11 cents and reduce caloric intake by 194 calories by replacing a 2.6-ounce danish with a 5.2-ounce apple[23]. The comparison study demonstrates that it is, in fact, possible to eat/serve healthier foods for the same or lower costs.

Data has shown that children's eating habits are influenced by food available in their immediate environments. Therefore, the quantity and quality of healthy choices in a child's diet are highly affected by the National School Lunch Program. Studies show that 47% of a school-aged

panelist's daily calories are obtained at school (including breakfast, lunch, and snacks). The 2010 dietary consumption by US children and teenagers of selected foods and nutrients related to cardiometabolic health revealed that the average consumption of saturated fat was about 11% of calories with approximately 30-40% youth consuming over 10% of their caloric energy from saturated fat. The average consumption of dietary cholesterol ranged from 225 to 250 mg/day while over 75% consumed roughly 300 mg of dietary cholesterol per day. Meanwhile, the average consumption of dietary fiber ranged from 14 to 15 g/day with less than 2% of children in all age and sex subgroups consuming the recommended 28 g/day[24].

Food "deserts" are defined by the CDC as areas that lack access to fruits, vegetables, and other foods that make up a full and healthy diet. Many Americans living in rural, minority, or low-income areas are subjected to food deserts[12]. The relative cost of fruits and vegetables has increased dramatically, making it even more difficult for lower income families to purchase these types of products even if they were so inclined[11]. In addition to it becoming more expensive to purchase fresh fruits and vegetables, it is anticipated that the cost of meat may double in the next few years[25,26].

1.3.2 Taste preferences

Despite the rise in consumer demand of gourmet foods in both restaurants and supermarkets, the hamburger remains a staple of the American diet with billions of burgers being consumed in the United States each year. However, the demand for reduced-fat ground beef products has been increasing steadily presumably based on consumers' concerns for their health. Consumers

expect reduced-fat ground beef products to have similar tenderness, juiciness, and flavor compared to the full-fat versions. But when these products do not meet their expectations, consumers often reject the new products, as seen in the McDonald's Corporation's national launch of the McLean hamburger. Past research has demonstrated that reducing fat from 20% to 10% in ground beef patties results in a reduction in tenderness, juiciness, and flavor[27]. Additionally, further research has shown that consumers quickly begin to rate low calorie alternatives as less tasty with repeated exposure compared to full calorie versions[28].

1.3.3 Cultural Practices

It would appear that food, in general, has become less expensive over time in the United States. As a share of personal disposable income, the average total spent on food expenditures by families and individuals has decreased steadily as seen in Figure 8 below.

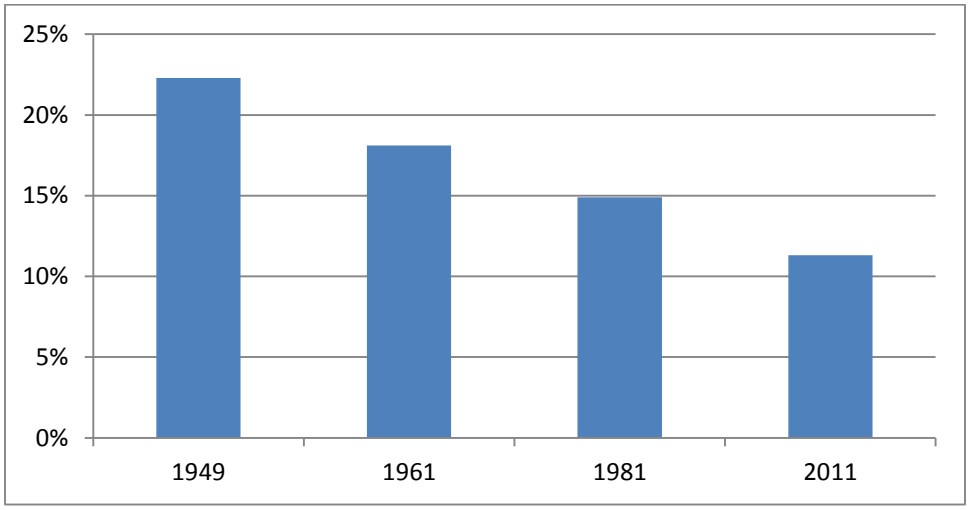

Figure 8: Food Expenditure as a Percentage of Disposable Income[6]

While people in different socioeconomic groups are spending similar percentages of their income on food, they are purchasing differently. The consumption of whole grains was associated with higher socioeconomic status, whereas the consumption of refined cereals, breads, pasta, and rice was associated with lower income levels. Additionally, the more affluent were more likely to consume a greater variety and higher quantity of fresh fruits and vegetables[29].

Every day in the United States, children and adults are faced with thousands of food choices. People with a sedentary lifestyle are at an even greater risk of being exposed to poor food choices through marketing. Food choices advertised on television tend to promote unbalanced diets compared with recommendations set forth in the USDA nutritional guidelines. These advertised foods tend to oversupply nutrients associated with chronic illness (sugar, starch, saturated fat, cholesterol, and sodium) while undersupplying nutrients that help protect against illness (fiber, fat soluble vitamins, and key minerals such as calcium and potassium)[30]. In a meta-analysis of prospective cohort studies, each daily serving of fruits or vegetables was associated with a 4% lower risk of chronic heart disease and a 5% lower risk of stroke[6].

1.4 Agricultural Impacts

Interactions among food, energy, and water are currently, and historically have always been, complex and inseparable[31]. Agriculture today faces an important challenge – to produce more food for a growing population with a smaller labor force[1]. With the supply of open land for agricultural use shrinking and a limited supply of fresh water in certain parts of the world, the

relationship of food, energy, and water is one of the most complex, yet, critical issues facing modern society[1,31]. Additionally, maximizing the edible food product per acre is necessary to improve food security as a social issue. Furthermore, by increasing the number of plant based crops which thereby improves food security factors can also have an impact on lowering obesity rates[1].

1.4.1 Water Usage

Agriculture is one of the largest consumers of water. In 2000, 82 billion gallons of water per day were used in irrigation and farming. This number increased dramatically in just a few short years, to over 129 billion gallons per day in 2005, and is continually on the rise. Surprisingly, the water demand for animal protein foods is much higher than for plant based foods. One head of cattle requires 4000 cubic meters of water or about 1.06 million gallons in its lifetime compared to one cubic meter of water or about 265 gallons needed for one kilogram of pulses[31]. The Food and Agricultural Organization (FAO) of the United Nations projects that to feed the nine billion people in 2050, global food production will need to rise by 70% and double in the developing world[1]. The projected increases in food production will be challenged by the rising energy prices, depletion of aquifers available for water withdrawal, and the continuing loss of farmland to urbanization[31].

1.4.2 Land Usage

Not only is water usage a concern, but the total land being used for agricultural purposes is decreasing. On top of that, food production is inherently inefficient, as photosynthesis converts

less than 2% of incoming solar radiation into the plant's biomass. The conversion of this biomass into animal protein compounds the inefficiency, with only 5-15% of feed converted into edible protein[31]. Edible portion or edible protein per unit of land is used as a measure of agricultural productivity and often used to compare agricultural commodities[31]. For example, the edible protein for beef is roughly 20 pounds per acre compared to poultry which averages close to 60 pounds per acre. Meanwhile, plant based proteins such as legumes average 192 pounds per acre. Other plant based foods offer edible portions of 138 pounds per acre for wheat, 211 pounds per acre for corn/maize, and 260 pounds per acre for rice[1,32].

The FAO has reported that in 2050, the world will be far from solving the problem of economic deprivation and malnutrition in significant parts of the world's population. Its current projections are that 350 million people will be undernourished despite current increases in agricultural technology[1]. Feeding the world's population adequately means producing the types of foods to ensure food security and reducing current food waste [1,31,33]. The involvement in sustainability initiatives to improve the environment can be positive for businesses because customers are increasingly aware of the links between the energy it takes to produce, package, transport, and dispose of food and its waste and the impact on global warming[33]. However, most consumers are unaware of the total amount of food wasted. As Figure 9 below demonstrates, a large portion of our food supply is lost to waste[31]. This waste creates higher food prices, decreases the amount of resources available for reducing worldwide food insecurity, and further highlights the inefficiency of our current food system[31, 33].

FOOD GROUP	PERCENT ULTIMATELY WASTED
Grains	32%
Vegetables	25%
Fruit	23%
Tree Nuts, Peanuts	16%
Dairy	33%
Meat (Beef, Pork, Poultry, & Seafood)	16%
Eggs	31%
Average	25%

Figure 9: Food Wasted in the United States[34]

1.4.3 Cost

Higher food cost is a known deterrent to higher nutritional food products[22]. Because of the projected price increases on various foods and ingredients, school programs, such as the National Head Start Association and the United States Department of Agriculture's (USDA) National School Lunch Programs, will be financially constrained and providing high quality nutritious meals will become even more difficult. However, as previously stated, it is possible to consume more nutritious food products in spite of rising food costs[23]. The purchase and consumption of plant based proteins, especially in the form of pulses, are an ideal option for consumers, as well as school programs, looking to follow and/or provide a more nutritious diet while minimizing costs. Pulses are inexpensive and are favored for their culinary versatility, as well as for their nutritional benefits[35]. The recent prices and expected increases of pulses can be seen in Figure 10 below.

	2010	2012	2014	2016	2018	2020	2022
Farm Production (Billion Pounds)	5.5	4.9	4.8	5	5.2	5.4	5.6
Farm Value (Billion $US Dollars)	$1.2	$2.1	$1.4	$1.6	$1.7	$1.8	$1.9
Average $US Dollar/Pound	$ 0.22	$ 0.43	$ 0.29	$ 0.32	$ 0.33	$ 0.33	$ 0.34

Figure 10: Production and Crop Value For Pulses, 2010-2022
*Source: USDA, National Agricultural Statistics Services
*Projections: USDA, Economic Research Services

This means that while other proteins may double in cost, adding multiple dollars per pound to their price, pulse prices are only estimated to increase at a rate of less than $0.01 per year. In the recent past, the highest prices for pulses were still only about $0.20 higher per pound than recent lowest market price. This means that the current "cost in use" would rise from around $0.12 per pound to about $0.18 per pound[36].

As demonstrated, plant based food items have historically been less expensive then animal proteins. Therefore, it comes as no surprise that plant based proteins can be used to off-set food costs associated with meats. Pulses represent one of the least expensive and versatile sources of food proteins. Additionally, pulses fall into both the "meat and bean" group and the legumes (dry beans) subgroup under vegetables as labeled by the USDA in the MyPyramid Dietary Guidelines for Americans 2005[37]. In addition to the nutritional and financial benefits of pulses, the various compositions offer a variety of physical chemistry features that can be useful during processing when pulses are used as value-added ingredients in food manufacturing.

Using pulses as value-added ingredients will also provide a substantial cost reduction over the 100% animal protein-based products since the cost of animal protein is expected to increase disproportionately for an indeterminable length of time due to the rise of feed prices and fluctuating fuel prices[25]. As feed prices increase, the feed to muscle ratio further exacerbates the rising meat prices. It takes upwards of eleven pounds of plant protein to produce one pound of animal protein in some cases. The replacement of 100% animal protein with either a legume-based protein source or a combination of plant and animal protein would constitute a substantial cost savings to consumers and particularly to local and national Head Start and school lunch programs.

1.5 Obesity and Food Security

Not only would a switch from 100% animal protein options to partial or full substitution of pulse-based products result in lower economic costs to food service operations, but it would help to reduce the excessive caloric intake currently found in most American diets, thereby becoming an important player in the fight against obesity. A greater understanding of plant-based proteins, specifically pulses, as well as their chemical and functional role as a processed/manufactured food ingredient, could effectively lead to creating ways to reduce obesity, environmental impact (water, energy, and land requirements), and food insecurity. From a global perspective, food security will exist "when all people at all times have physical, social, and economic access to sufficient, safe, nutritious food to meet dietary needs and food preferences"[31]. A reduction of animal protein would open the agricultural land for additional crops which thereby could be used to reduce food insecurity by having a greater total quantity

of “sufficient, safe, nutritious food”. Additionally, commercially-available blended proteins could deliver the flavors, colors, and textures associated with 100% animal proteins while delivering the lower costs and nutritional qualities of plant proteins.

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CHAPTER 2. CHEMICAL CHARACTERIZATION AND FUNCTIONAL PROPERTIES OF PULSES FOR USE AS VALUE-ADDED INGREDIENTS IN PROCESSED FOODS

2.1 Abstract

Pulses offer unique functional and nutritional properties. Their chemical characterization allows for varying rates of water absorption, least gelation capacity, and retrogradation rates depending on species. Nutritionally, pulses are good sources of protein while being low in fat. Meanwhile, the mineral content of pluses can benefit certain populations that are normally micronutrient deficient with a readily bioavailable food form of iron, calcium, potassium, phosphorus, and zinc. Additionally, they deliver soluble and insoluble fiber. The insoluble fiber components are both natural and formed resistant starch in addition to the oligosaccharide content. Lastly, pulses demonstrate antioxidant activity that may play a role in human health but could impart antioxidant attributes as a food ingredient. Therefore, pulses can serve both a nutritional role and functional role when used as a value-added ingredient in processed foods.

2.2 Introduction

Many ingredients have special functions in the baking and cooking process with some being critical to the success of the finished product. However, some ingredients can be substituted with others to produce the same outcome. However, most substitutions will have at least a minor effect on the finished product. Therefore, it is essential to understand the chemical and functional characteristics of potential ingredient substitutions. This includes pulses when looking to use them as value-added ingredients in processed foods.

2.2.2 Protein

2.2.2.1 Total Protein

Pulses are consumed worldwide with consumption highest in areas where animal protein is scarce or expensive. Pulses are known to have high protein values that are about twice that of grains and several times that of root vegetables. In human nutrition, protein plays a role in tissue repair, enzyme and hormone synthesis, and energy supply[1]. While pulse protein quality is limited by the sulphur amino acids, tryptophan and threonine, this is compensated in most diets by combining with rice or other grains[2]. Apart from their nutritional qualities, proteins offer many functional attributes as well.

2.2.2.2 Gelation

One of the first physical chemistry benefits of pulse proteins is gelation. Protein sols (slurries) can be converted into high-viscosity progels though a heat-induced protein gelation involving dissociation and denaturation of the protein, which will set upon cooling as seen in Figure 11 below. This reaction is irreversible although some protein gels can be melted and reset with controlled temperature changes. Further heating then converts the progel into a metasol, a disruption of the gel by partially refolding the protein, which does not gel upon cooling[3,4].

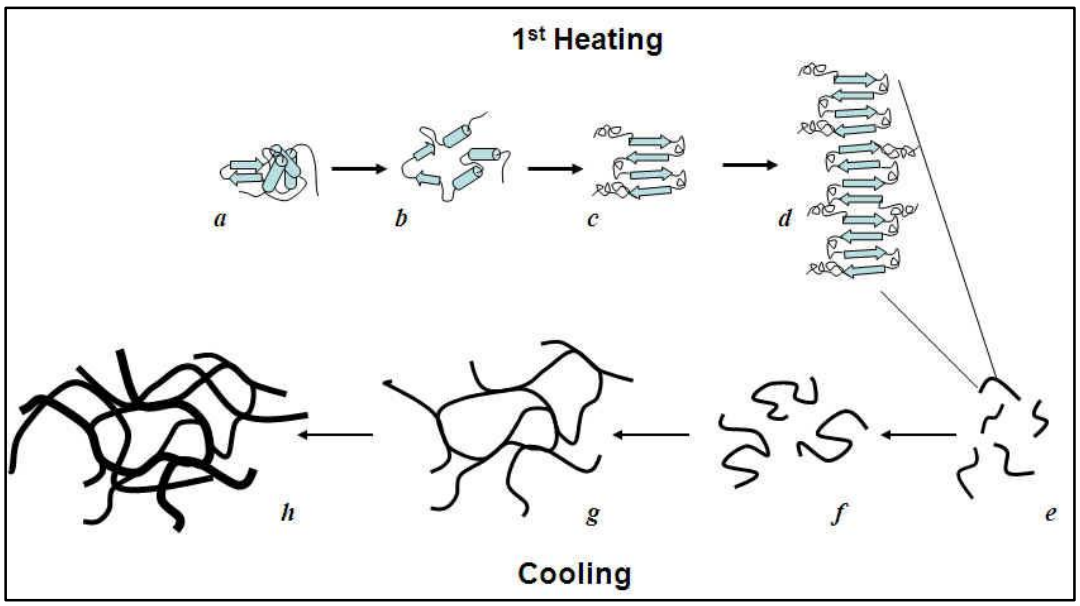

Figure 11: Protein Structural Changes During Gelation[5]

Pulse proteins are globular in nature and tend to form gels when minimally disrupted following heating above the unfolding temperature. Physical interactions are mostly hydrophobic and hydrogen bonding which are the key forces involved in the development of globular protein gels, although, disulfide bonds can further contribute to the gel structure. The ability of pulse protein molecules to interact and form three-dimensional network structures following thermally induced denaturation and molecular folding, is a key functional property of pulses. The tertiary and quaternary structure of these protein complexes influences the texture and potentially flavor characteristics of food products containing them[3,4].

Processing conditions can affect the physical properties of proteins and influences the texture and flavor characteristics of the finished food product[3,4]. These flavor interactions have been well documented in plant based protein sources and starts with the natural flavors of the plant

proteins. These flavors need to be muted in order for any further flavoring component to be added. Secondly, the concerns of flavor interactions with proteins are a result of chemical interactions from the wide range of chemical structures and mass transfer effects. As the proteins begin to unfold during processing, the issues of off-flavor absorption and desirable flavor binding can occur simultaneously. Therefore, understanding the processing properties of the plant protein source can lead to better finished goods[6,7,8].

2.2.2.3 Trypsin Inhibitor Levels

Plants produce a wide variety of proteins and while general nutrition focuses on the energy storage protein, many plant proteins also serve a role in protecting the plant. The existence of naturally occurring proteinase inhibitors in pulses is well established. In the preparation of pulses for human consumption, as pulses are cooked to an acceptable softness, a decrease in the levels of antinutrional components is seen. The inactivation of antinutritional factors such as protease inhibitors and lectins (hemagglutinins) is very important[4,9].

Trypsin is an enzyme secreted by the pancreas that breaks down protein in the small intestine, specifically in the duodenum. Trypsin catalyzes the hydrolysis of peptide bonds, hydrolyzing proteins into smaller peptides. These peptides are then further hydrolyzed into amino acids by other proteases, enzymes that break down proteins and peptides, where they can be absorbed into the blood stream. Trypsin digestion is a necessary step in protein absorption as the vast majority of proteins are too large to be absorbed through the lining of the small intestine[4,9].

2.2.2.4 Reducing Trypsin Inhibition

Previous research has shown that extended soaking (up to four days) reduces antinutritional components, especially trypsin inhibitors, but does not completely remove them. Cooking presoaked pulses at 90°C for 15 minutes was enough to destroy 80% of the trypsin inhibitor activity. However, cooking unsoaked pulses resulted in only a 4% reduction in trypsin inhibitor activity[10]. Therefore, a combination of soaking and cooking was utilized in this research.

2.2.3 Carbohydrates

Carbohydrates perform numerous roles in living organisms including energy storage, structural components and important component of coenzymes and genetic molecules. Additionally, saccharides are included in many other important biomolecules that play key functions in the immune system, fertilization, preventing pathogenesis, blood clotting, and development[11].

2.2.3.1 Fiber

However, humans cannot metabolize all types of carbohydrates to yield energy. Dietary fiber is defined as is the indigestible portion of food derived from plants and can be separated into two classifications: soluble and insoluble[12].

Soluble fiber consists of the gums, pectins and mucilage compounds located on the inside of plant cells. In the digestive system, these fibers absorb water and swell to form a thick, viscous mass that slows down the rate at which you digest food. Eating foods rich in soluble fiber may help prevent high serum cholesterol and diabetes[13,14].

Insoluble fiber is made up of cellulose, lignin, and pectin, which are resistant to the action of digestive enzymes and are one set of the many polysaccharides found in pulses. Each type plays important roles in human nutrition including lowering cholesterol, controlling blood sugar levels, and weight management. Figure 12 below was modified from Johnson[15] and outlines the basic types of fiber and their role in nutrition.

Types of Fiber	**Soluble or Insoluble**	**Natural Sources**	**Health Benefits**
Cellulose,some hemicellulose	Insoluble	Found in nuts, whole grains, seeds, brown rice, skins of produce.	Reduces constipation, lowers risk of diverticulitis, can aid weight loss.
Inulin oligofructose	Soluble	Extracted from onions or chicory root and byproducts of sugar production.	May increase beneficial bacteria in the gut and enhance immune function.
Lignin	Insoluble	Found in flax, rye, some vegetables.	Benefits heart health and possibly immune function.
Mucilage, beta-glucans	Soluble	Found in oats, beans, peas, barley, flaxseed, berries, soybeans, bananas, oranges, apples, carrots.	Helps lower LDL cholesterol, reduces risk of heart disease and type 2 diabetes.
Pectin and gums	Soluble (some insoluble)	Naturally found in fruits, berries, and seeds. Also extracted from skins of produce and other plants.	Slows the passage of food through the intestinal GI tract, helps lower blood cholesterol.
Polydextrose polyols	Soluble	None. Synthesized from dextrose (glucose), sorbitol and citric acid.	Adds bulk to stools, helps prevent constipation.
Resistant starch	Soluble	Starch in plant cell walls naturally found in unripe bananas, oatmeal, and legumes.	Helps weight management by increasing fullness.

Figure 12: Types of Fiber and Their Role in Nutrition[15]

Additionally, a fiber rich diet offers many benefits for human nutrition as seen in Figure 13 below from the FAO.org[16]. Dietary fiber increases the weight and size of your stool and softens

it. A bulky stool is easier to pass, decreasing the chance of constipation. Fiber also helps solidify watery, loose stool because it absorbs water and adds bulk to stool. A high-fiber diet can also lower the risk of digestive disorders like hemorrhoids, diverticular disease, duodenal ulcers and colon cancer[16,17,18]. Additionally, dietary fiber increases mastication rates which increases satiety which has been shown to decrease caloric intake and increase fat oxidation[16].

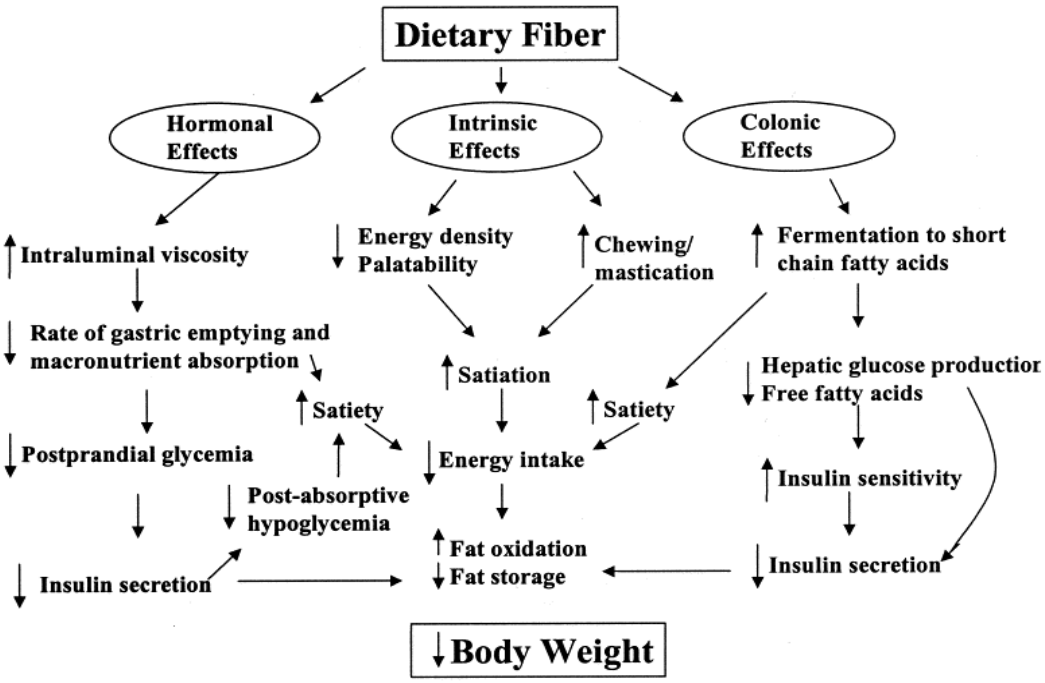

Figure 13: Benefits of Fiber[16]

Furthermore, some fiber is fermented in the colon and there is ongoing research at how this may play a role in preventing diseases. For example, the soluble fiber found in beans has been shown to lower total blood cholesterol levels by reducing low-density lipoprotein, or "bad," cholesterol. Studies also have shown that fiber may have other heart-health benefits, such as aiding blood pressure regulation and reducing inflammation. Furthermore, soluble fiber can

slow the absorption of sugar and help improve blood sugar levels, an important aspect for people with diabetes.

A diet rich in insoluble fiber may also reduce the risk of ever developing type 2 diabetes. This may be due to fiber rich food requiring more chewing time, allowing the stomach time to register it is full, reducing the likelihood to overeat. Also, a high-fiber diet is more filling and satiating in addition to generally being less "energy dense," meaning it has fewer calories for the same volume of food[19].

According to an Institute of Medicine formula based on getting 14 grams of dietary fiber for every 1,000 calories, women need 25 grams per day and men should get 38 grams per day, whereas, the FDA recommends 25g/day of dietary fiber based on a caloric intake of 2,000 calories, for all adults and children four or more years of age[20].

2.2.3.2 Oligosaccharides

Dietary carbohydrates can range in molecular size from simple sugars to complex polymers such as cellulose chains and galactomannins. The simple sugars consist of three basic subgroups: monosaccharides, disaccharides, and oligosaccharides. Monosaccharides are any sugar that cannot be reduced into simpler sugars by hydrolysis and are often referred to as *simple sugar* (i.e. glucose, fructose, and galactose). Disaccharides are any of a class of 38 sugars that yield two monosaccharide molecules upon hydrolysis (i.e. sucrose = fructose + glucose, maltose = glucose + glucose, and lactose = glucose +galactose). They are small easy to

absorb molecules often simply referred to as “sugars”. Oligosaccharides are any carbohydrate consisting of 3 to 9 monomeric sugar units although some sources have defined oligosaccharides as up to 20 monomeric units. Oligosaccharides are regular components of the human diet but have not received the same amount of attention simple sugars, starch or dietary fiber. Most of the naturally occurring oligosaccharides are found in plants.

Recently, interest in oligosaccharides has increased partly due to their functional properties that include sweetening ability and fat replacement in addition to past research showing their resistance to digestion in the upper gastrointestinal tract and fermentation in the large bowel. Frequently these oligomers are not well digested by humans and as a consequence reach the lower gastrointestinal tract where they are fermented by organisms in the microbiome. Thus, some oligosaccharides have been shown to offer nutritional effects similar to soluble dietary fiber by promoting fermentation that results in a healthy gastrointestinal tract, improving glucose control, and aiding in the metabolism of triglycerides[21]. These oligosaccharides are nondigestable in the stomach due to extended branching but can be metabolized and fermented by colonic bacteria. The predominate oligosaccharides in pulses are polymers of sucrose with extended branching of additional galactose units as seen in Figure 14 below. For purposes of this study, the total saccharide content is defined as the total combination of monosaccharides, disaccharides, and oligosaccharides.

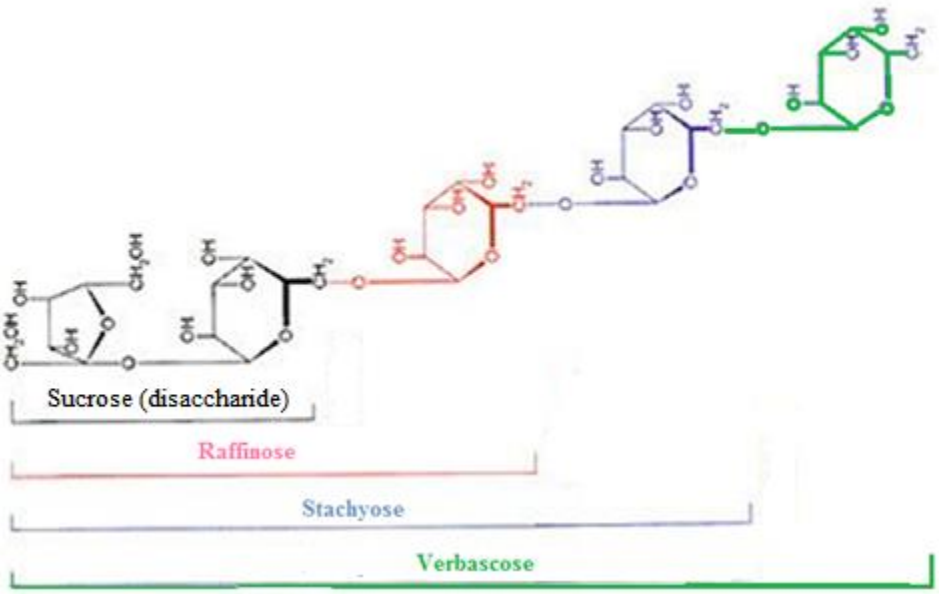

Figure 14: Building of Oligosaccharides

2.2.4 Antioxidants

Free radicals are highly unstable molecules that are formed during exercise and when the body converts food into energy. Additionally, free radicals are also produced by macrophage activity during inflammation and as side products of the body's ability to kill pathogens during infection. Additional exposure to free radicals can occur from a variety of environmental sources, such as smoke, pollution, and sunlight. Free radicals cause "oxidative stress," a process that triggers cell damage. Free radical oxidative damage/stress can play a role in a variety of diseases including cancer, cardiovascular diseases, diabetes, Alzheimer's disease, Parkinson's disease, and eye diseases such as cataracts and age-related macular degeneration[22].

Antioxidants are that delay or inhibit oxidative damage when present in small quantities compared to the oxidizable substrate[22]. Antioxidant molecules have been shown to counteract oxidative stress in laboratory experiments; however, research has not shown antioxidant supplements to be beneficial in preventing diseases. The ATTICA study has shown that dietary modification including higher consumption of antioxidants is associated with improved control of glycemic markers and lower incidence of diabetes in epidemiological studies[22].

Both DPPH and ORAC Values have been used to compare antioxidant activity. However, ORAC has been extensively applied for relative comparison of antioxidant values of foods. Pulses tend to show high degrees of antioxidant activity as determined by the ORAC assay. The ORAC method is designed to demonstrate antioxidant capacity *in vitro*. However the USDA has stated that no physiological proof *in vivo* existed in support of the free-radical theory since no correlation between test results and biological activity could be determined[23]. However, that does not mean ORAC is useless. While the value might not demonstrate a nutritive antioxidant, there is evidence demonstrating foods with high ORAC values as stabilizers in food systems[24].

2.3 Materials

Twenty-three types of pulses (three unique sets each) were donated by Archer Daniel Midland (ADM, Decatur, Il.) or purchased at a local produce market (LPM, Baton Rouge, LA). The dried pulses included Black Beans (ADM), Cranberry Beans (LPM), Pinto Beans (ADM), Pink Beans (ADM), Small Red Beans (ADM), Dark Red Beans (ADM), Light Red Beans (LPM), White Kidney Beans (ADM), Mayocoba Beans (ADM), Navy Beans (ADM), Great Northern Beans (ADM), Large

Lima Beans (LPM), Baby Lima Beans (LPM), Garbanzo Beans (LPM), Black Eyed Peas (ADM), Green Split Peas (LPM), Yellow Split Peas (LPM), Lentils (LPM), and Red Lentils (LPM). Fresh frozen pulses included Speckled Butter Beans (LPM), Purple Hull Peas (LPM), Butter Peas (LPM), and Crowder Peas (LPM). Each pulse was examined to remove stones and other debris.

2.4 Methods

2.4.1 Analytical Sample Preparation

Sample preparation for pulse chemical analysis started with grinding dry/dried samples to less than 0.75mm using a centrifugal mill (Retsch ZM 200; Haan Germany).

2.4.2 Moisture

Moisture content was determined by weighing 10 g samples of each pulse in triplicate into aluminum pans before placing in a 100°C forced draft oven for 24 hours. Samples were quickly transferred to a desiccator and weighed after reaching room temperature.

2.4.3 Hydration

Hydration of the dry pulses was determined in two ways: total weight gain and weight gain over time. Two methodologies were used because each offered unique insight into the different hydration properties of the different pulses.

For total weight gain, 50g of each pulse was soaked in tap water for 24 hrs at 4°C. The pluses were drained and then reweighed to determine total hydration based on water weight gain.

This methodology was used to ensure that full hydration was reached based on values from previous publications. Expectations were that the pulses would gain on average two times their starting weight in additional water weight when completely hydrated to the pulse core.

For hydration, as weight gain over time, 50g of each pulse was soaked in tap water at 4°C, similar to above. However, the pulses were soaked in 2 hour increments before draining, weighing, and restarting the soaking process. This process was carried out with 12 soakings for a total of 24 hours. This methodology was used to determine the minimum soaking time needed to reach hydration. The desire is that depending on the pulse used the soaking time could be reduced from 24 hours to aid industrial processing.

2.4.4 Ash

Ashing was performed in triplicate following AOAC Method 900.02[25]. Porcelain crucibles were washed with nitric acid, marked, and heated at 525°C, cooled to room temperature and weighed. For analysis, 5-10 g of dried pulses, taken from moisture determination, were added to each crucible then heated at 525°C for 12 hours. Ash content was determined by subtracting the final ash weight from the original dry sample weigh accounting for the crucible weight.

2.4.5 Fat

Crude fat content was analyzed in triplicate using AOAC 920.39C[26] Soxhlet gravimetric analysis with the Soxtec System HT6 and Soxtec Avanti 2050 instruments (Foss, Hillerod, Denmark).

Sample Testing: The pre-ground, pre-dried (around 8% moisture) samples (3 g) were weighed into the glass vials of the Soxtec System and boiled in 50 ml of petroleum ether (PE) for 30 minutes. The PE was drained and then the samples automatically rinsed using the same PE in a continuous rinsing process for 45 minutes. The PE was then evaporated out of the samples over the course of 15 minutes using the internal heating block. The samples in vials were allowed to cool at room temperature off the extractor for an additional 5 minutes before further drying in a 100°C forced air dryer for an additional 5 minutes to ensure all solvent was removed. Crude fat was determined as a percentage on the dry weight basis of the difference in starting sample weight verses post extraction and drying weight.

2.4.6 Protein Analysis

2.4.6.1 Crude/Total Protein

The crude protein content was analyzed in triplicate using the AOAC 981.10 Crude Protein in Meat by Block Digest Method[27] by the Louisiana State Agriculture and Forestry Laboratory.

2.4.6.1.1 Sample Preparation: Pulse flour samples (1-1.10 g) were weighed onto a tared Whatman No. 1 filter paper (90mm diameter) recording the sample weight. The paper with the sample was folded and placed in a 250 mL calibrated digestion tube. Sulfuric acid (20 mL) was added to the digestion tube and swirled lightly until both the filter paper and the sample were submerged in acid. The digestion tube was placed on a digestion rack and covered with foil. The samples then sat overnight.

2.4.6.1.2 Reagent Preperation:

- Salt sulfuric (NaCl/ H_2SO_4) solution 2000 mL: 200 g of salt (NaCl) was weighed into a 2000 mL beaker. Approximately 1500ml of deionized water was added followed by 15ml of sulfuric acid (H_2SO_4). The mixer was placed onto a stirrer under the solution was clear. Finally the solution was brought to volume (2000 mL) with deionized water before adding 2 mL of Brij® (Polyethylene glycol hexadecyl ether)**.**
- 6% Sulfuric solution (for rinse): Approximately 1000 mL of deionized water was added to a 2000 mL volumetric flask followed by 120 mL of sulfuric acid. The mixture was allowed to cool before bringing the solution to volume (2000 mL) with deionized water.
- Sodium salicylate/ Sodium nitroprusside: Sodium salicylate (75 g) was weighed into a 600 mL beaker before adding approximately 400 mL of deionized water. The solution was placed on a stirrer. Sodium nitroprusside (0.15 g) was weighed onto a watchglass and rinsed into the 600 mL beaker. Stirring was continued until all the solids had dissolved. The solution was brought to 500 mL volume followed by the addition of 0.5 mL of Brij. This solution was stored in an amber bottle and kept in a cabinet in dark.
- Buffer solution (1000ml): Potassium sodium tartrate (50 g) was weighed in a 2000 mL beaker. Approximately 500 mL of deionized water was placed on stirrer for 30 minutes before adding 14.2 g of anhydrous sodium phosphate to the 2000ml beaker on stirrer. The solution was mixed for an additional 30 minutes. Then, 54 g of sodium hydroxide was added and mixed for another 30 minutes. The final solution was brought to volume (1000 mL) with deionized water before adding 1 mL of Brij.

- Clorox solution: Clorox (6.7 mL) was brought to 200 mL volume with deionized water before adding 7 drops of Brij. This solution was made fresh for each run.

2.4.6.1.3 Sample Digestion: The digestion block was heated to 410^{o}C before setting the digestion rack with tubes on it. The rack was covered with the manifold and the water valve was set for optimum draw of acid fumes to avoid the sample from being drawn into the manifold. The samples were digested for 15 minutes before removing from the block and allowing them to cool for 10 minutes.

The catalyst, 20 P PRO PAC (a mixture of 10 g K_2SO_4, 0.3 g of $CuSO_4$ and 0.1 g of Pumice) was used to aid in the digestion of the samples. One 20P PRO PAC was added to each tube before rinsing with 3.75 mL of 30% hydrogen peroxide. The samples were transferred back to the digestion block. The manifold was set to digest the samples once again but with the addition of metal shields on the front and back of the rack to contain the heat for digestion. Digestion occurred for 1 hour and 45 minutes. At the end of the digestion period, the samples were removed and allowed to cool. The manifold was removed and the samples rested for another 10 minutes. The samples were a bright green color when they come off the block. But once cool, the color became a light blue. Once the sample had turned blue, approximately 200 mL of deionized water was added slowly to each sample while swirling lightly to prevent the sample from solidifying. The samples were then fully cooled to room temperature. The samples were brought to volume, stoppered, and shook by hand. The sample was poured into a 4 mL cup and ran on a Bran+Luebbe AutoAnalyzer 3 (SEAL Analytical Inc., Mequon, Wisconsin) with digital

colorimeter and AACE computer program. Excluding the salicylate line, all reagent lines were placed in their respective containers, the sample probe was connected to the sampler and the proportioning pump was started. After the reagents had been pumping for at least ten minutes, the salicylate line was placed in its respective container and the system was allowed to equilibrate. After stable baseline had been obtained, the sampler was started.

2.4.6.1.4 Standards Preparation: Ammonia sulfate (5 g) was weighed into a small beaker and placed in a 100^{o}C oven for 1 hour. After baking, the beaker was removed from the oven and allowed to cool. **T**he baked ammonium sulfate (2.3585 g) was weighed into a 1000ml beaker. Approximately 250 mL of the blank digest was added. The ammonium sulfate solution was poured into a 500ml volumetric flask, brought to volume with blank digest and mixed well. This created the stock standard at 1000 ppm. It was stored away from light and made fresh at least every 6 months. The blank digest was made by digesting 20 mL sulfuric acid and 12 g of 20P PRO PAC catalyst in a 250 mL tube not including the sample. When digesting the samples, 2-3 empty tubes of blank digest were digested for making fresh standards with each digestion and ran in order to keep the samples and standards in the same matrix.

2.4.6.1.5 Working Standards: Using the freshly made blank digest, each standard was filled to volume.

- 50ppm: 5 ml stock (1000ml) standard/ 100ml flask
- 100ppm: 10 ml stock (1000ml) standard/ 100ml flask
- 150ppm: 15 ml stock (1000ml) standard/ 100ml flask
- 200ppm: 20 ml stock (1000ml) standard/ 100ml flask

2.4.6.1.6 Running Sample Set-Up:

- Position: 1 Sample ID: Primer Concentration: 200 ppm
- Position: 2 Sample ID: Drift Concentration: 100 ppm
- Position: 3 Sample ID: Calibration Standard Concentration: 50 ppm
- Position: 4 Sample ID: Calibration Standard Concentration: 100 ppm
- Position: 5 Sample ID: Calibration Standard Concentration: 150 ppm
- Position: 6 Sample ID: Calibration Standard Concentration: 200 ppm
- Position: 7 Sample ID: High Concentration: 200 ppm
- Position: 8 Sample ID: Low Concentration: Blank Digest
- Position: 9 Sample ID: Low Concentration: Blank Digest

2.4.6.2 Least Gelation Capacity

The method of Coffman and Garcia[28] has been used extensively in different studies to determine the least gelation concentration/capacity (LGC) of proteins and was followed for this research. Briefly, sample suspensions (5, 10, 15, 20% w/v ground pulse sample in 10 mL of distilled water) were prepared. The test tubes, 150 mm tall x 16 mm with 14 mm inside diameter, containing the suspensions were heated for 30 minutes in a 90°C water bath followed by cooling in 15.5°C water for 15 minutes. The tubes were then further chilled at 4°C for 2 hours. LGC is defined as the concentration where the samples from an inverted test tube do not fall out or slip down from incomplete swelling and partial adhesion. The globulin fraction forms a gel at a concentration of around 20% with better gelling properties shown in lower LGC.

2.4.7 Carbohydrate Analyzes

2.4.7.1 Total Carbohydrates

Carbohydrates were calculated by difference from total solids using crude fat, crude protein, and ash. Further carbohydrate analyses were performed on the pulses in order to develop a greater understanding of their differences.

2.4.7.2 Fiber

The crude fiber content of the pulse samples was analyzed in triplicate using the filter bag technique with the ANKOM 2000 Fiber Analyzer (Macedon, NY) following the AOCS approved procedure Ba 6a-05. Filter bags encapsulate the sample which prevents error and allows filtration to occur passively. Beans were dried, ground, and portioned (0.8-1.2 g) before being heat sealed into filter bags. One empty filter bag was included for correction of calculations. The samples and the empty bag were extracted by soaking in 250 ml petroleum ether for 10 minutes to remove fat. The filter bags were then allowed to air dry for 5 minutes. The filter bags were then put onto the trays and placed in the extractor. The vessel was cooled to below 20°C with chilled running water before starting. The samples were digested with 0.255 N H_2SO_4, followed by 0.313 N NaOH, before rinsing with hot water. The samples were then gently pressed to remove any excess water before being submerged in 250 ml of acetone for 3 minutes. The filter bags were then removed and air dried on wire screens to ensure air circulation while drying at 100°C in a forced air oven for 2 hours before ashing.

2.4.7.3 Saccharide Content

The procedure used during analysis was a combination of AOAC2001.02[29] and AOAC 980.13[30] to ensure both the mono- and di-saccharides as well as the oligosaccharides were quantified. The saccharides (both simple and complex sugars) of the pulse samples were extracted with 80% ethanol (w/w) for 10 hours under slight agitation. The supernatant was carefully removed and filtered through a 0.22 µm polyvinylidene difluoride (PVDF) membranes (MFS, Adventec, Quebec, Canada) before HPLC separation. The analysis was performed using an HPLC (Waters Alliance 2690 Separation Module and Waters Pulse Ampherometric Detector) (Waters Corp. Milford, MA) with CarboPac PA10 (4x250mm) column and guard for separation of different sugars alongside a gold, quadruple waveform electrode with an ED40, pulsed Electrochemical detector. The conditions for running samples were from AOAC method 982.14[31] for determination of oligosaccharides. An initial column wash with the running buffer of 90 mM NaOH for 1 minute was completed before injecting 10 µL of sample solution. The samples were eluted using a flow rate of 1.5 mL/minute of the running buffer for 20 minutes and tested in triplicate. Results were calculated using a standard curve prepared from 0-1.0 mg/mL of a single saccharide in the 80% ethanol extraction solution. Total saccharides were determined as the combination of mono- and di- saccharides and oligosaccharides The specific oligosaccharides of interest were raffinose, stachyose, and verbascose. Cellobiose is not present in pulses and was therefore used as an internal standard at 0.25 mg/g of solution. Its consistent presence with little fluctuation served as a marker of consistency during sample testing.

2.4.7.4 Resistant Starch

The enzymatic-chemical method was performed according to the AOAC Method 2002.02[32] and AACC Method 32-40[31] using the Megazyme kit (Megazyme International Ireland Ltd., Co. Wicklow, Ireland) by the Louisiana State University Nutrition Laboratory. Pulse samples and resistant starch (RS) control (52.5% dry weight basis (dwb) resistant starch) provided in the kit were weighed at 100±5 mg into screw cap tubes, which were tapped to ensure no sample adhered to the sides of the tube. Then, 4.0 mL of pancreatic α-amylase (3 Ceralpha Units/mg, 10 mg/mL) containing amyloglucosidase (AMG) (3 U/mL) was added to each tube. The tube was tightly capped, dispersed thoroughly on a vortex mixer, and attached horizontally in a shaking water bath, aligned in the direction of motion. The tubes were incubated at 37ºC with continuous shaking (200 strokes/min). After shaking for 16 hours, the tubes were removed, uncapped, and 4.0 mL of ethanol (99%) was added to each tube before vigorous mixing on a vortex mixer. After this, the tube was centrifuged at 1,500 x g (approx. 3,000 rpm) for 10 min. The supernatant was decanted and the precipitate suspended in 2 mL of 50% ethanol and shaken. A further 6 mL of 50% ethanol was added to the tubes before being mixed and centrifuged again. The supernatant was decanted and the process repeated for a third extraction. After the final supernatant was decanted, the tubes were inverted on absorbent paper to drain any excess liquid. A magnetic stirrer bar (5 x 15 mm) was added to each tube, followed by 2 mL of 2 M KOH. The precipitate was resuspended (and any RS dissolved) by stirring in an ice/water bath over a magnetic stirrer for 20 minutes. Then, 8 mL of 1.2 M sodium acetate buffer (pH 3.8) was added to the tubes while stirring. Immediately, 0.1 mL of AMG (3300 U/mL) was added, the contents were mixed well, and the tubes were placed in a water

bath at 50ºC. The samples were incubated for 30 min with intermittent mixing before being centrifuged at 1,500 x g for 10 minutes one last time. The final volume in the tube was approximately 10.3 mL (+0.05 mL). For the RS control, the contents of the tube was transferred into a 100-mL volumetric flask and then diluted to volume with distilled water. From this, an aliquot was taken and transferred into a screw cap tube. This was centrifuged together with the samples. From each tube, 0.1 mL aliquot (in triplicate) of the supernatant was transferred into glass tubes, added with 3.0 mL of GOPOD reagent, and mixed well using a vortex mixer. A reagent blank was prepared by mixing 0.1 mL of 0.1 M sodium acetate buffer (pH 4.5) and 3.0 mL of GOPOD reagent. Glucose standards were prepared (in triplicate) by mixing 0.1 mL glucose (1 mg/ mL) and 3.0 mL GOPOD reagent. The samples, blank and standards were incubated for 20 min at 50°C, cooled to room temperature. The absorbance was measured at 510 nm against the reagent blank. The percentage of RS was calculated using the following formulas:

For samples:
RS (g/100 g sample) = A*F*(10.3/0.1)*(1/1000)*(100/W)*(162/180) = A*F/W*9.27

For Resistant Starch Control and samples over >10% RS:
RS (g/100 g sample) = A*F*(100/0.1)*(1/1000)*(100/W)*(162/180) = A*F/W*90

Where:
A= absorbance
F = conversion factor (100 (μg glucose / GOPOD absorbance for 100 g of glucose)
W = weight of test portion analyzed;

2.4.7.5 Pentosan Content

Pentosan content was determined following the method by Douglas[33]. A calibration curve was made using 0.1g D-(+) xylose per 100 ml distilled water (w/v). Aliquots of 0.5, 1.0, 1.5, and 2.0 ml of the D-(+) xylose solution were adjusted to 2 ml with distilled water.

Two levels of pulse flours were weighed (4.5 and 5.5 mg) into stoppered glass test tubes with 2 ml of distilled water and 10 ml of freshly made extracting solution composed of: 110 ml glacial acetic acid AR, 2 ml hydrochloric acid, AR, 5ml 20% phloroglucinol in ethanol (w/v), and 1 ml 1.75% glucose in distilled water (w/v).The tubes were placed into boiling water for 25 minutes. After allowing the tubes to briefly cool for 10 minutes under flowing water, the absorbance of the supernatant was immediately measured at 552 nm and 510 nm after adjusting for background using distilled water. Pentosan content was determined by subtracting the reading at 510nm from that at 522nm and comparing the value with the calibration curve.

2.4.7.6 Rapid Viscosity Analysis

Pasting characteristics of the pulse flours were evaluated with a RVA-4 machine (Newport Scientific Pty. Ltd., Warriewood NSW, Australia) using the AACC Method 61-02[34]. Prior to analysis, the volume of water and weight of starch sample were determined based on the following formula:

$S = 88 \times 3.00 / (100 - M)$
$W = 28.0 - S$

Where:
S=corrected sample mass (g)
M =moisture % of the sample
W=corrected water volume (mL)

Briefly, the distilled water, W, was measured into a new RVA canister. Then, the sample, S, was weighed into a pan and transferred into the RVA canister with water. The paddle was placed into the canister and the sample was thoroughly dispersed into the liquid by vigorously "jogging" or moving the blade up and down at least 10 times through the sample. The canister and paddle were inserted into the analyzer. Each sample was first held at 50°C with a spindle speed of 960 rpm. After 10 sec, the rotating speed was reduced to 160 rpm for the remainder of the test. Next, the temperature was increased at a rate of 12°C /min to 95ºC and held at the temperature for 2.5 min. The sample was then cooled to 50°C. Analysis was done in triplicate.

The pasting temperature (PT), peak viscosity (PV), minimum viscosity (MV), final viscosity (FV), and peak time (PTime) were measured by the RVA with the ThermoCline for Windows v.3 (TCW3) software. The peak viscosity is defined as the maximum viscosity that occurs prior to the initiation of sample cooling. The minimum viscosity is the lowest viscosity recorded after the peak viscosity. The final viscosity is the viscosity at the end of the test. Calculations for the Total setback (TSB) and Breakdown (BD) were determined based on the following formula:

TSB=FV-MV
BD=PV-MV

2.4.8 Antioxidant Capacity

2.4.8.1 2,2-diphenyl-1-picrylhydrazyl (DPPH) Antioxidant Assay

The DPPH assay provides evidence on the reactivity of compounds with a stable free radical. DPPH shows a strong absorption band at 517 nm in visible spectroscopy due to an odd number of electrons. As this unpaired electron attaches to a free radical scavenger, the absorption characteristic vanishes, and the resulting discoloration is proportional to the number of electrons taken up[35]. The pulse extract solutions for the DPPH test were prepared by adding 0.2 g of ground flour in 10 ml methanol. This mixture was stirred for 30 minutes and then the supernatant was removed. Two ml of a DPPH solution with a concentration of 0.025 g of DPPH in 1000 ml of methanol was mixed with 40, 80, 120 µL of the extract solution in a cuvette. After a 30-minute incubation at room temperature, the reaction solution was examined by spectrophotometer at 515 nm. The inhibition percentage of the absorbance of DPPH solution was calculated using the following equation:

$$\text{Inhibition } \% = [(Abst0 - Abst30min)/Abst0] \times 100$$

Where Abst0min was the absorbance reading of DPPH at time zero and Abst30min was the absorbance reading of DPPH after the 30 minutes of incubation with the extract.

The inhibition percentage determined from the absorbance of DPPH was compared between each concentration of the pulse extract solution added.

2.4.8.2 Oxygen Radical Absorbance Capacity (ORAC)

Ground pulse samples were extracted (5 g in 20 mL) with ethanol/acetone/water/acetic acid (40:40:20:0.1) in triplicate. Samples were placed in screw-cap vials to prevent solvent evaporation and heated for 60 minutes in a 60°C water bath. Samples were allowed to cool for 10 minutes then homogenized for 1 minute before filtering through MiraCloth (CalBiochem, LaJolla, CA) before freezing at -20°C until analysis.

Further completion of ORAC testing was performed by the USDA ARS Arkansas (Little Rock Arkansas) following the procedures established by Cao et al[36]. Prior to analyzing, the extracts were evaporated to dryness and then dissolved in 950 g 1^{-1} ethanol. A 40 µL portion of the diluted sample was added to a well in a 48-well microplate. A fluorescein solution was prepared fresh by dissolving 0.0225 g Fluorescein NA salt (Sigma Aldrich, Milwaukee, Wisconsin) in 50 mL of 0.075 M phosphate buffer (pH 7.0). A second dilution was prepared by adding 50 µL of solution #1 in 10 mL of phosphate buffer. A 320 µL portion of solution #2 was added to 20 mL of phosphate buffer to create the working fluorescein solution. Both fluorescein solution (400 µL) and 75 µL of 2,2-azobiz(2-amidino-propane) dihydrochoride (AAPH) (Waco Chemicals, Richmond, VA) were added to the assay mixture with reading initiated immediately.

2.4.9 Statistical Analyses

The statistical analysis of the chemical and physical analyses data was completed using Microsoft Excel 2010 (Microsoft, Redmond, WA). All data was analyzed for analysis of variance

(ANOVA) and standard deviation (STD) with an alpha of 0.05 to maintain a confidence interval of 95%. Fisher's least significant difference test was performed alongside ANOVA.

2.5 Results

2.5.1 Moisture

Pulse moisture content fell in to distinct groups as seen in Figure 15 below based on whether they were obtained as a dry product or frozen.

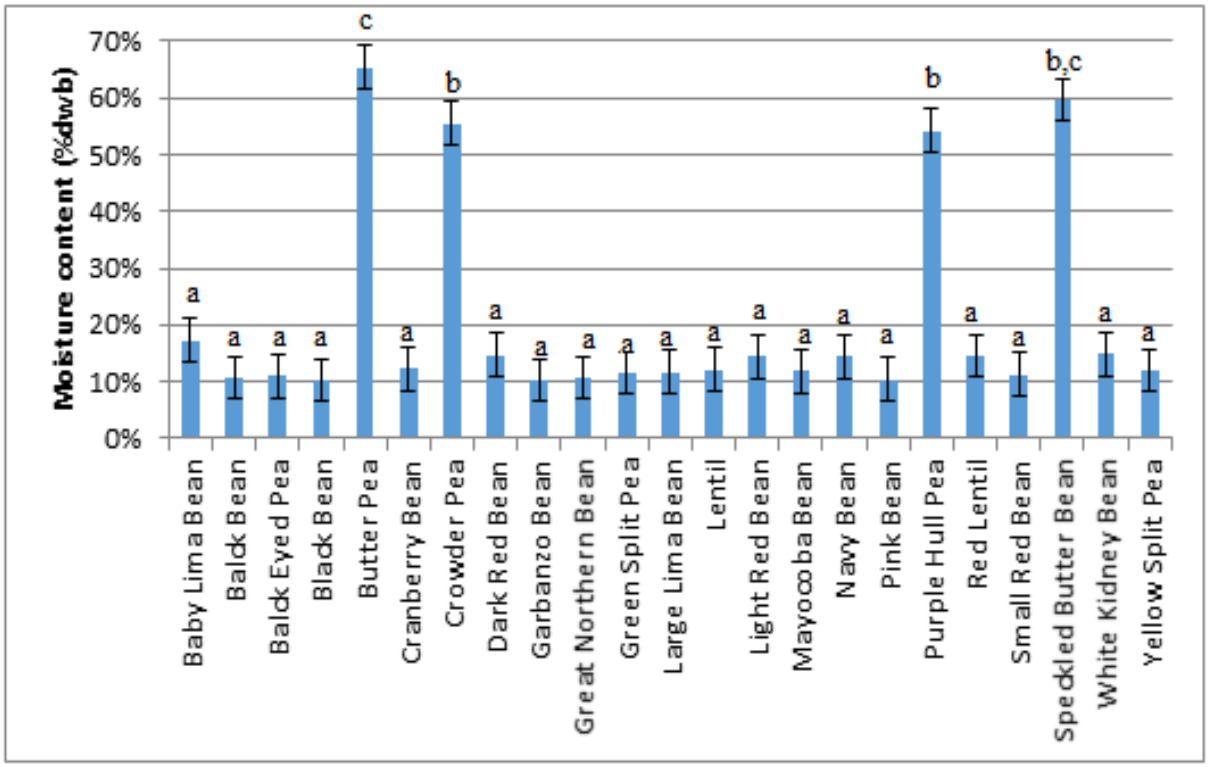

Figure 15: Moisture Content of Pulses
Bars with the same character are not significantly different (P>0.05).

For the dry samples, the average moisture content ranged from as high as 17.27% for the baby lima bean to as low as 10.27% for the black beans. However, the frozen pulses had higher moisture contents with a range from 54.16% for the purple hull pea to 65.34% for the butter pea. The variability among the dry pulses is just due to variety while differences between the frozen and dry pulses is due to moisture being absorbed during the blanching process before freezing. While the dry pulses have both national and international distribution, the frozen pulse varieties are mostly a southern regional variety with a more limited market.

2.5.2 Hydration

The dry pulses were allowed to soak and hydrate before cooking to reduce the "hard bean" effect. Hard bean has been referred to as the inability for a dry bean to soften during the traditional cooking process in the time that it takes the remaining batch to cook and soften[37]. However, there is little research published on the rate of hydration on dry pulses in water. By measuring the average weight increase every two hours, a hydration curve was determined as seen in Figure 16 below. Peas hydrated to 60% total hydration within 2 hours whereas it took the beans over 6 hours to reach the 60% total hydration. Also the peas, reached over 90% total hydration within 12 hours while the bean samples took almost 18 hours. Weights were stopped at 24hrs due to minimal further hydration. All pulses finished with an average water uptake of 3x the starting weight.

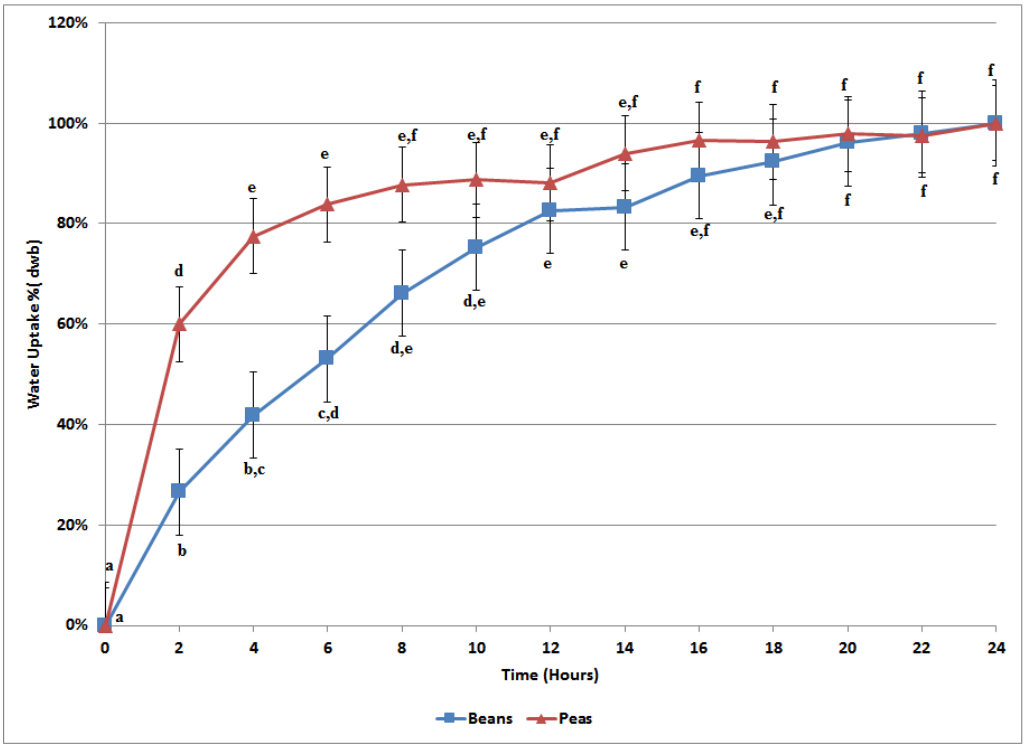

Figure 16: Percent Water Uptake Over Time
Bars with the same character are not significantly different (P>0.05).

2.5.3 Ash

The ash content is a measure of the total amount of minerals present within a food. During dry ashing, water and other volatile materials are vaporized and organic substances are burned in the presence of oxygen in air to CO_2, H_2O and N_2. Most minerals are converted to oxides, sulfates, phosphates, chlorides or silicates. Although most minerals have fairly low volatility at these high temperatures, some are volatile and may be partially lost, including iron, lead and mercury. Figure 17 shows the overall ash content of pulses demonstrates the levels of minerals in pulses. The lowest levels of minerals in the butter pea, crowder pea, purple hull pea, and speckled butter bean could be due to being grown in the southern United States as opposed to the other pulses coming from the northern central US.

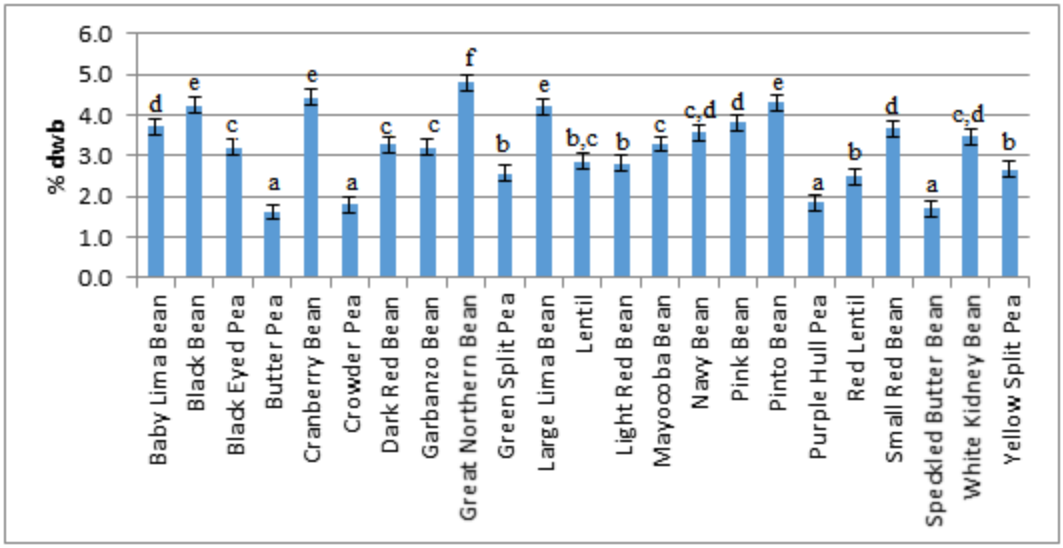

Figure 17: Ash/Total Mineral Content of Pulses
Bars with the same character are not significantly different (P>0.05).

The mineral content of most dry pulses is similar when comparing the same species, i.e one bean to another or one pea to another. This is especially true with the lentils tested. However, there are some major fluctuations even in similar species. Past research has shown that the greatest fluctuations occur in the calcium content of beans[38]. White beans (204 mg) have over twice the calcium of lima beans (81 mg), while pink beans (130 mg) are somewhere in between. Additionally, the purple hull pea (110 mg) has twice the calcium of the green and yellow split peas (55 mg)[39] as seen in Figure 18 below.

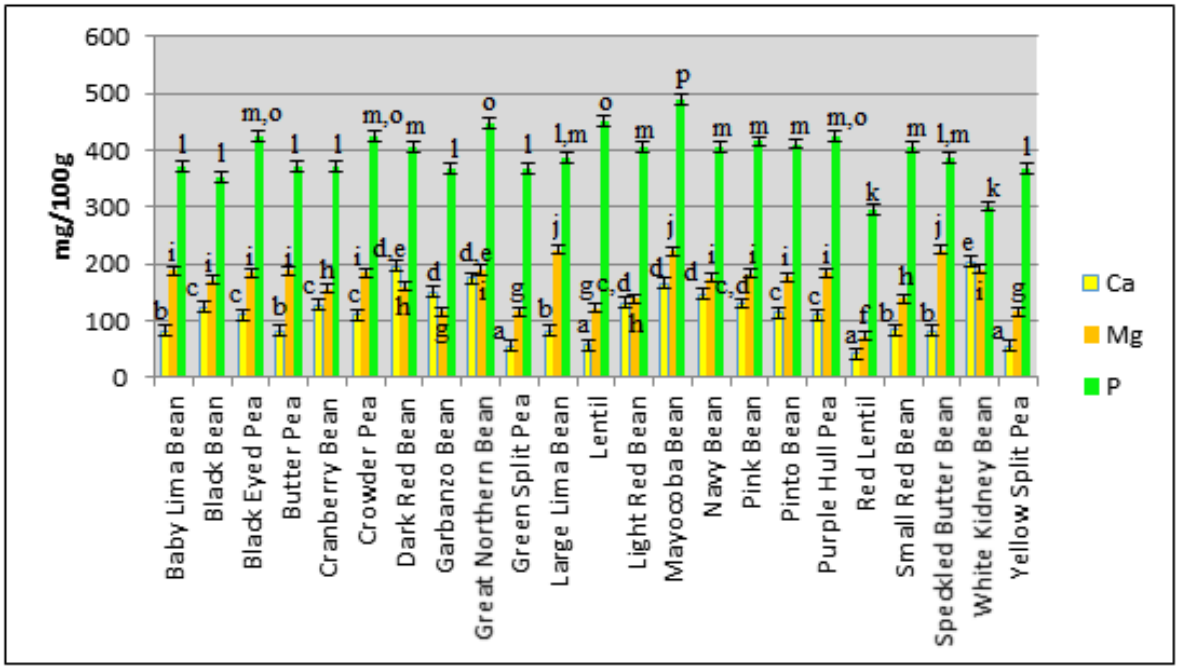

Figure 18: Ca, Mg, & P Content of Pulses: Data from USDA National Nutrient Database[39]
Bars with the same character are not significantly different (P>0.05).

When comparing species, research has shown beans have the highest calcium and potassium content (Figure 19) while lentils are higher in iron and zinc[39,40]. Pea varieties shared similarities with both.

Furthermore, iron contents can vary between varieties. The cranberry bean (5.00 mg) and black bean (5.02 mg) are near the bottom in terms of iron content while the small red bean (6.69 mg) and pink bean (6.77 mg) are more in the middle, with the white kidney bean (10.44 mg) having the highest iron content. The values for all 23 varieties can be seen in Figure 20 below[39,40].

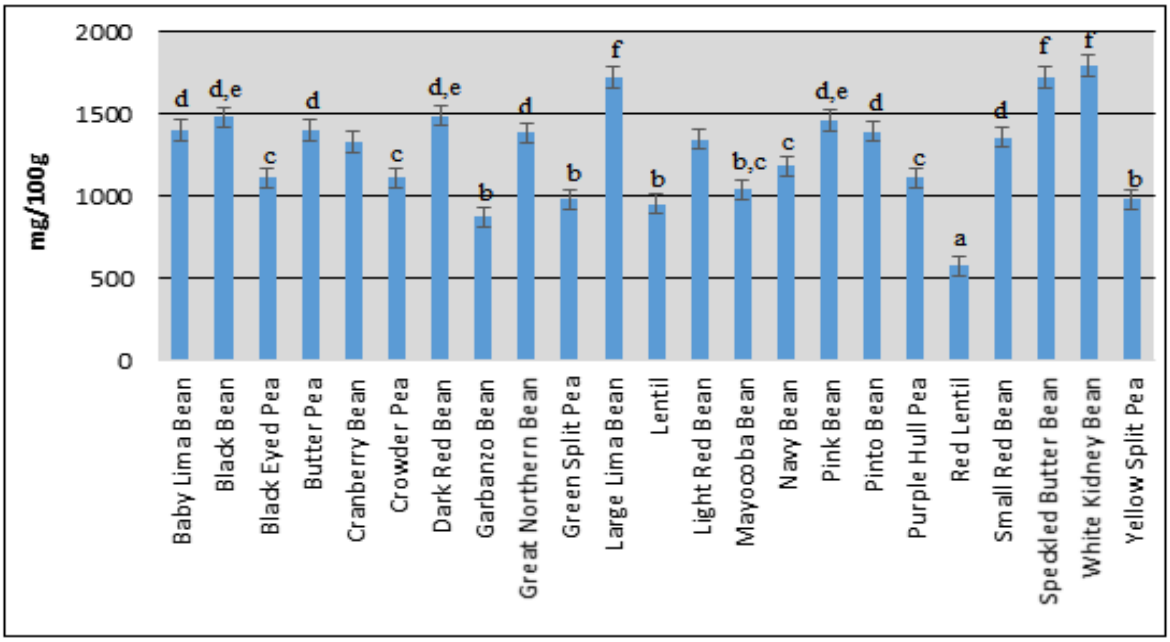

Figure 19: K Content of Pulses: Data from USDA National Nutrient Database[39]
Bars with the same character are not significantly different (P>0.05).

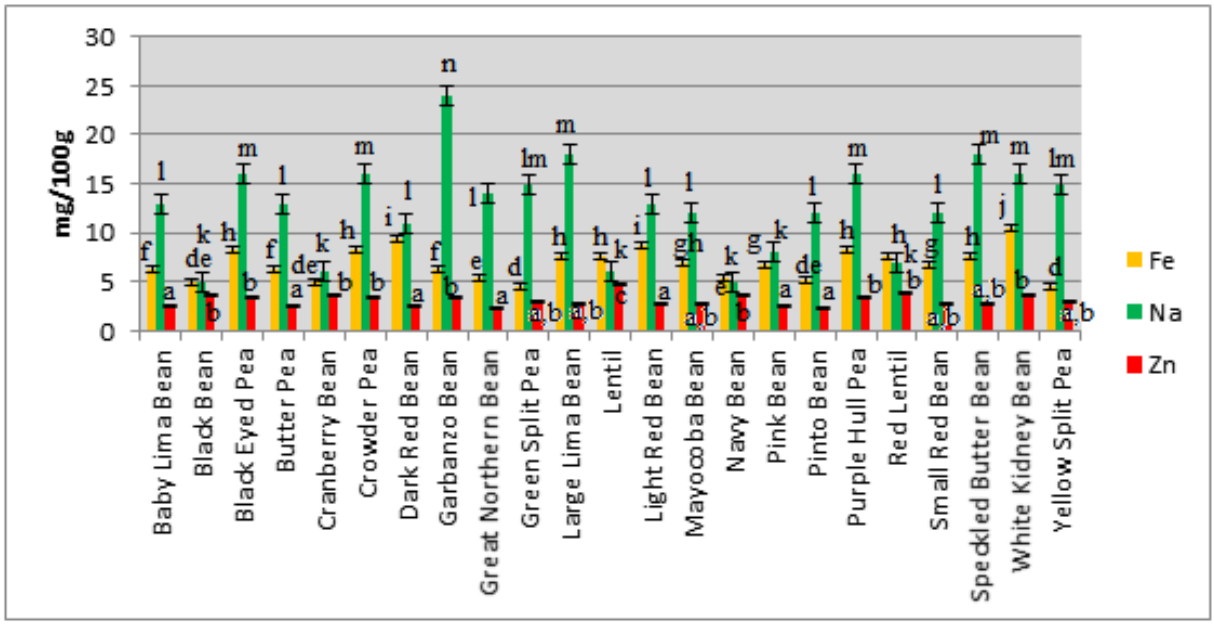

Figure 20: Fe, Na, & Zn Content of Pulses: Data from USDA National Nutrient Database[39]
Bars with the same character are not significantly different (P>0.05).

2.5.4 Fat

Pulses are known for being very low fat sources of protein. Figure 21 demonstrates the fat content of pulses. Even at the highest level, garbanzo beans are less than 5.4% fat. On average, pulses contain less than 1.5% percent fat which is in agreement with past published research[38,39,41].

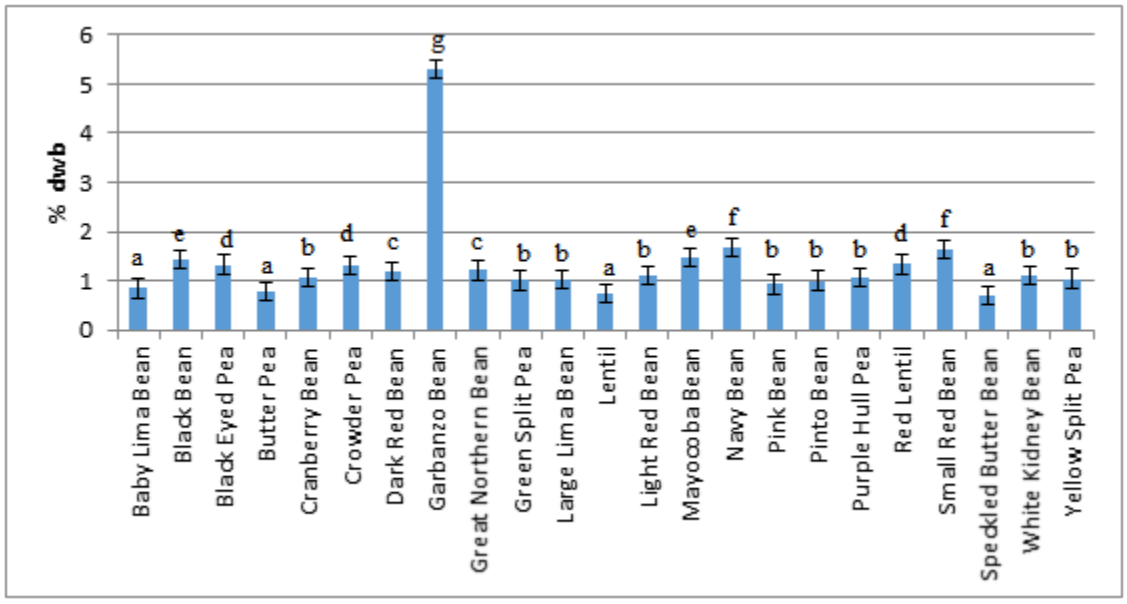

Figure 21: Fat Content of Pulses
Bars with the same character are not significantly different (P>0.05).

2.5.5 Protein

2.5.5.1 Total/Crude Protein

The classic assay for protein concentration in food is to measures crude protein by quantifying total nitrogen. The amount of nitrogen is multiplied by a factor of 6.25 for pulses is used for food labels and the total or crude protein can be determined.

The crude protein values (Figure 22) for the pulses ranged from 15.0% for the speckled butter bean to 26.1% for the red lentil with an average of 21.0%. The USDA published Nutrient Database for Standard Reference data for dry kidney beans list 23.6%. Our results are close with the dark red kidney bean at 22.1% and light red kidney bean at 21.46%. Additionally, our results are similar to published data on crude protein levels in pulses from other studies[38,39,42].

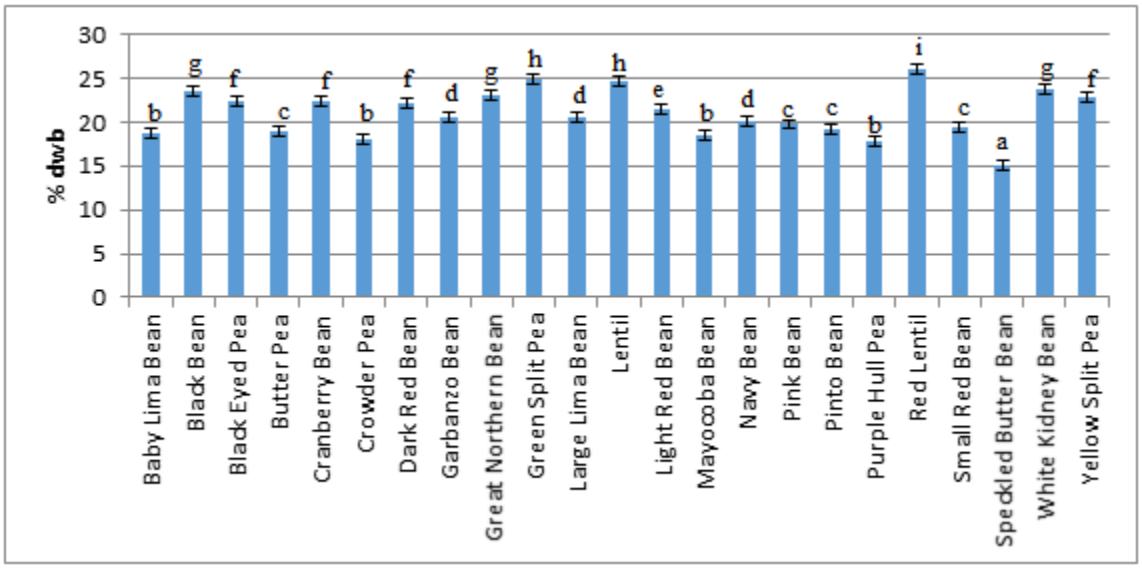

Figure 22: Crude Protein Content of Pulses
Bars with the same character are not significantly different (P>0.05).

2.5.5.2 Least Gelation

Least gelation capacity was reported as gel formation (single gel), partial gel formation (gel separated when inverted), and no gel formation (all liquid). The results for the pulse samples can be seen in Figure 23 below. No samples gelled at a 5% concentration although the beginning of partial gelation was evident in some samples. The small lima bean, crowder pea, garbanzo bean, green split pea, large lima bean, purple hull pea, speckled butter bean, and

yellow split pea samples did not gel at the 10% concentration. However, at the 10% concentration, the butter pea and red lentil samples had partial gel formation and all remaining samples had complete gel formation. All samples gelled at the 15% and 20% concentrations.

	Concentration of Sample			
PULSE SAMPLE	5%	10%	15%	20%
Baby Lima Bean	X	X	✓	✓
Black Bean	X	✓	✓	✓
Black Eyed Pea	X	✓	✓	✓
Butter Pea	X	P	✓	✓
Cranberry Bean	X	✓	✓	✓
Crowder Pea	X	X	✓	✓
Dark Red Bean	X	✓	✓	✓
Garbanzo Bean	X	X	✓	✓
Great Northern Bean	X	✓	✓	✓
Green Split Pea	X	X	✓	✓
Large Lima Bean	X	X	✓	✓
Lentil	X	✓	✓	✓
Light Red Bean	X	✓	✓	✓
Mayacoba Bean	X	✓	✓	✓
Navy Bean	X	✓	✓	✓
Pink Bean	X	✓	✓	✓
Pinto Bean	X	✓	✓	✓
Purple Hull Pea	X	X	✓	✓
Red Lentil	X	P	✓	✓
Small Red Bean	X	✓	✓	✓
Speckled Butter Bean	X	X	✓	✓
White Kidney Bean	X	✓	✓	✓
Yellow Split Pea	X	X	✓	✓
X=Did not gel				
P=Partial gel formation				
✓=Gel Formation				

Figure 23: Least Gelation Capacity of Pulse Flour

2.5.6 Carbohydrates

2.5.6.2 Fiber

Various publications have researched the specific types of fiber in various categories of pulses, but there is little literature available comparing total fiber levels across multiple pulse varieties and between members of each variety. Figure 24 below shows the total fiber content of the 23 pulses on a dry weight basis.

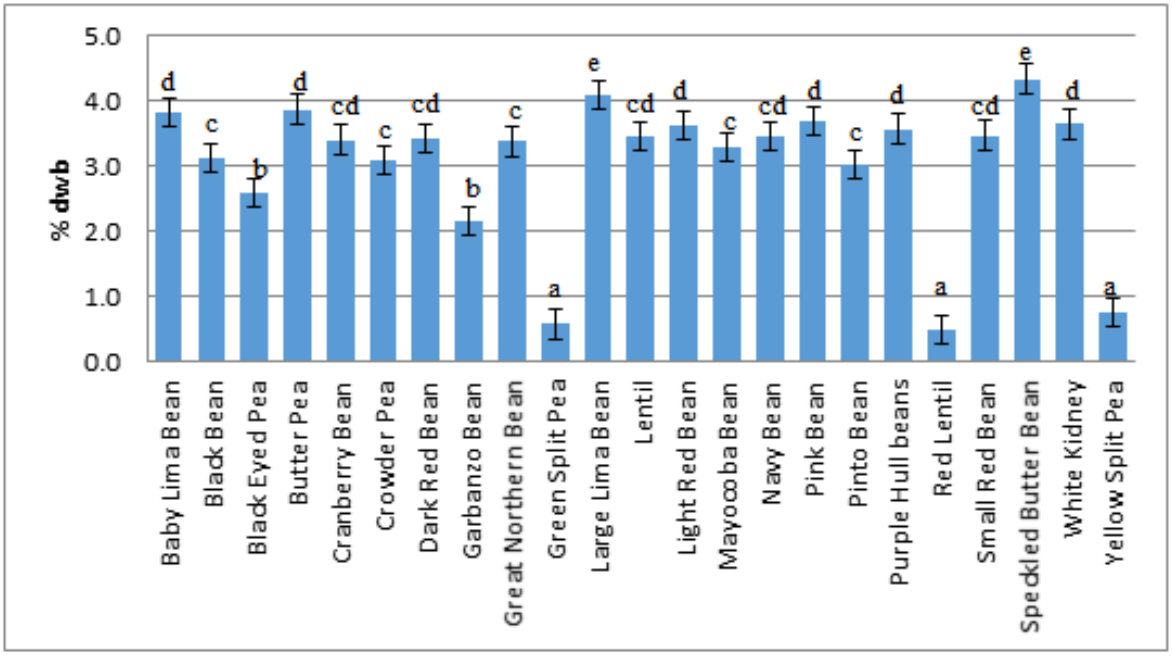

Figure 24: Average Fiber Content of Pulses
Bars with the same character are not significantly different (P>0.05).

The samples had an average fiber content of 3.06% with red lentil on the low end with 0.49% and speckled butter bean on the high end with 4.34% fiber on a dry weight basis. Pulses with thicker fibrous skins (speckled butter bean, baby lima bean, large lima bean, and butter pea) tended to have higher levels of fiber which is in agreement with past research[38,39,41].

2.5.6.3.1 Total Saccharide Content: In general, peas had the highest total saccharide content followed by beans and then lentils. The peas had an average total saccharide content of 59.4 mg/g as compared to beans and lentils with 54.5 mg/g and 37.5 mg/g respectively. Additionally, 3 out of the 6 varieties of peas had total saccharide contents over 60 mg/g compared to only 2 out of 15 varieties of beans. Neither of the lentils had total saccharide content greater than 40 mg/g. The total saccharide content for the pulse samples can be seen in Figure 25 below.

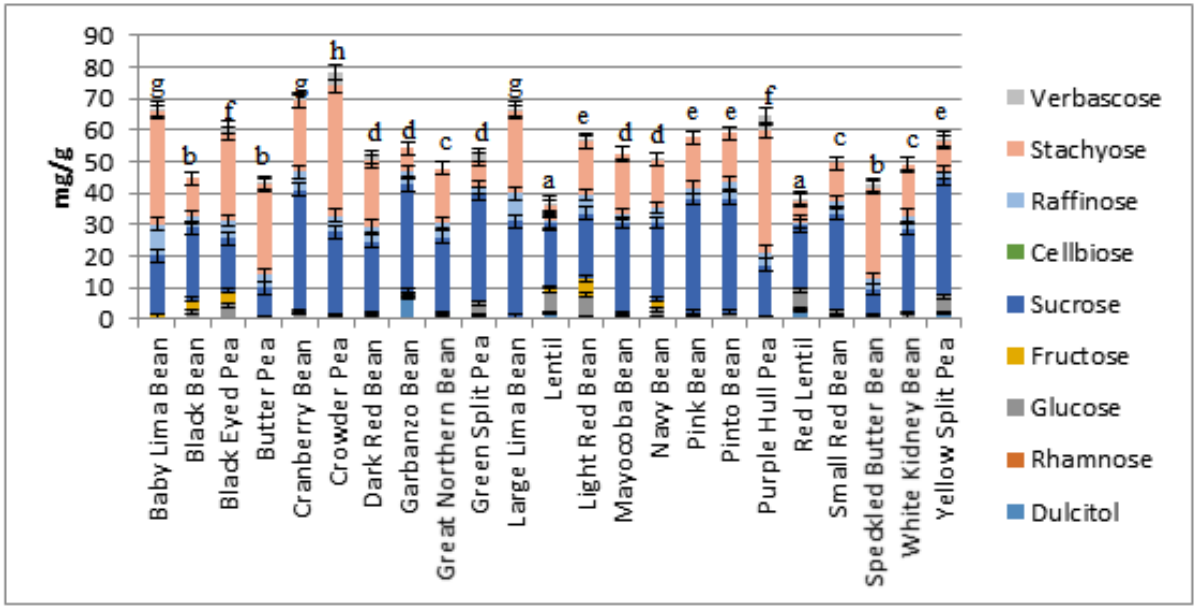

Figure 25: Total Saccharide Content of Dry Pulses
Bars with the same character are not significantly different (P>0.05).

2.5.6.3.2 Oligosaccharides: The oligosaccharides in pulses are of greater significance in human nutrition. The oligosaccharides (raffinose, stachiose and verbascose are not digestible in the

human gut but are instead fermented by colonic bacteria into butyric acid. Unfortunately, this fermentation also produces hydrogen and methane which can cause discomfort and flatulence.

As seen in Figure 26 below, stachyose was the most abundant oligosaccharide followed by raffinose and verbascose respectively. Peas had the highest levels of stachyose and also the greatest percentage of total saccharides as oligosaccharides. Lentils had the lowest total levels.

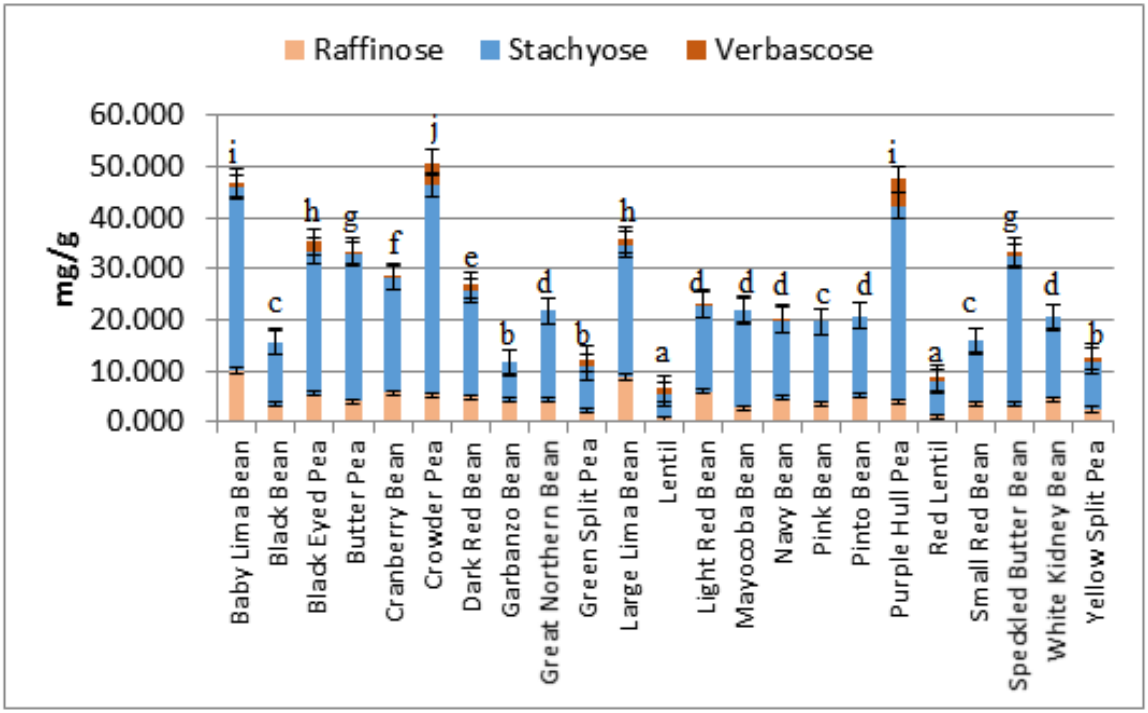

Figure 26: Oligosaccharide Content of Dry Pulses
Bars with the same character are not significantly different (P>0.05).

2.5.6.4 Resistant Starch

2.5.6.4.1 Pulse Flour: Pulses can have a significant resistant starch content and can contain up to almost 30% as seen in Figure 27 below.

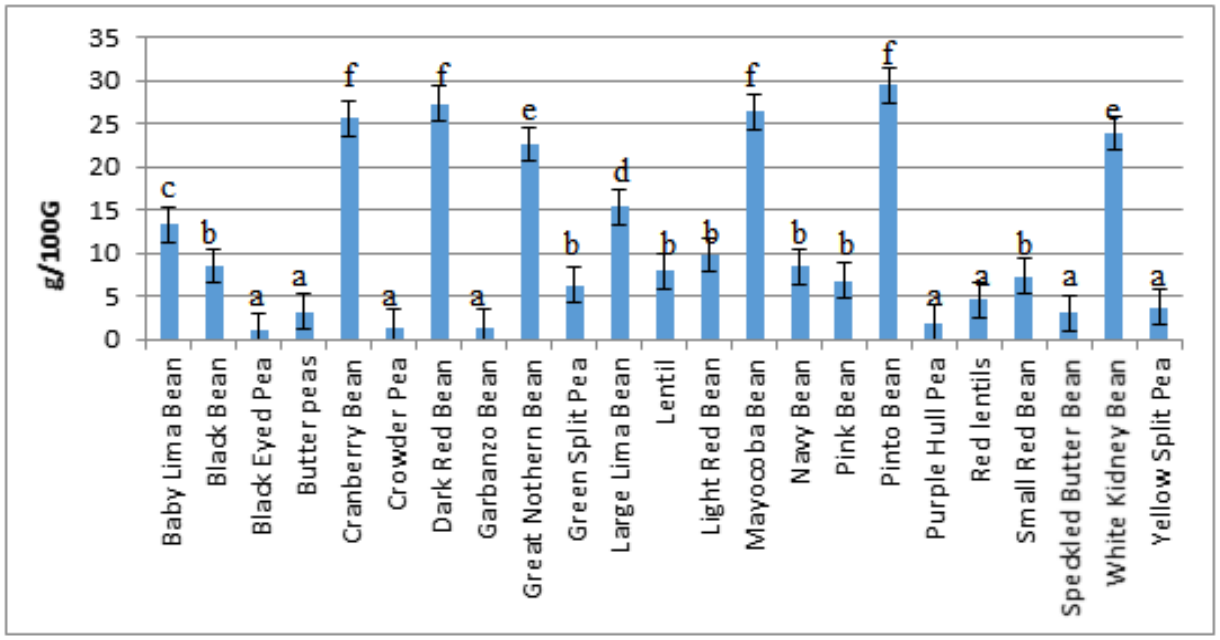

Figure 27: Resistant Starch Content of Raw Pulse Flour
Bars with the same character are not significantly different (P>0.05).

The resistant starch in the raw pulse flours would fall under Resistance Starch Type 1 and Type 2 (RS1 and RS2) classifications. RS1 is found in grains, seeds and legumes and resists digestion because it is bound within the fibrous cell walls and RS2 is found in some starchy foods, including raw potatoes and green (unripe) bananas.

2.5.6.4.1 Hydrated and Baked Pulse Fractions: However, humans do not eat dry pulse flour. When comparing the raw flour resistant starch content in the figure above to the resistant starch found in hydrated and bake pulse fractions (Figure 28), there is a significant reduction in resistant starch. However, an increase in the amount of resistant starch is seen in some samples

especially the samples with little resistant starch to begin with. Previous research has shown that this is due to starch retrogradation and classified as Resistance Starch Type 3 (RS3). RS3 is formed when certain starchy foods, including potatoes and rice, are cooked and then cooled turning some of the digestible starches into resistant starches via retrogradation.

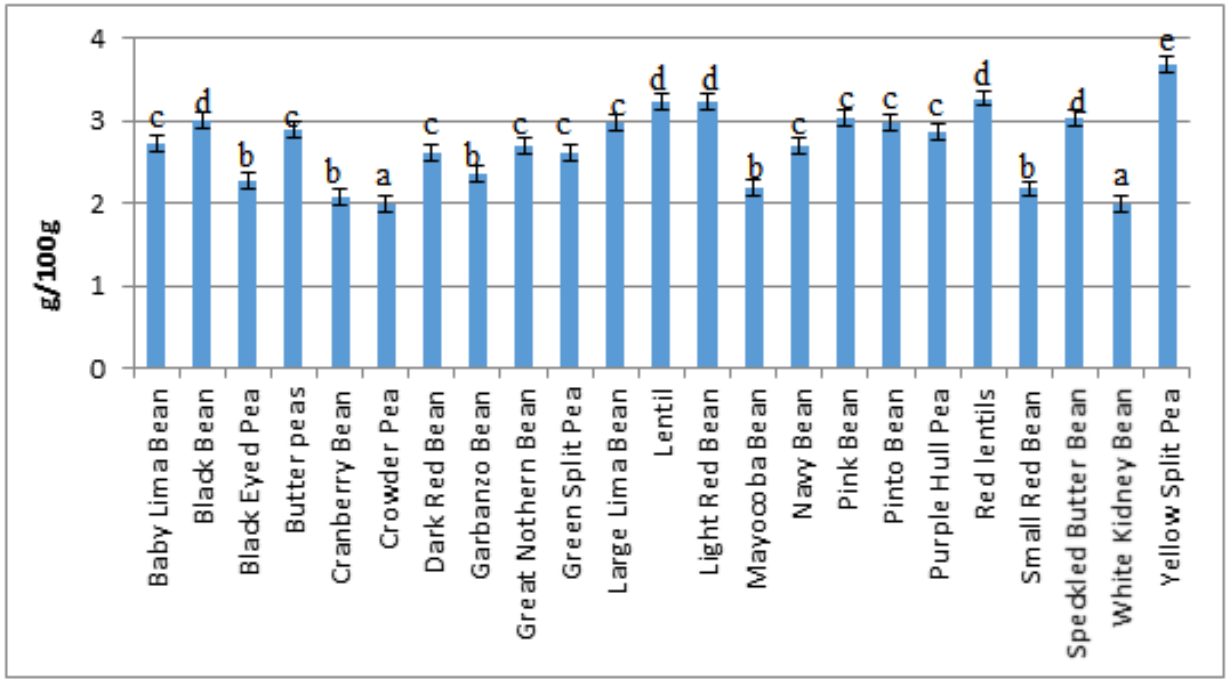

Figure 28: Resistant Starch Content of Hydrated & Baked Pulse Fractions
Bars with the same character are not significantly different (P>0.05).

2.5.6.5 Pentosans

The pulse content of pentosans is less than that found in grains where pentosan content plays an important role in starch analysis. However, with the intent to convert dry pulses into hydrated bean fractions, the pentosan content can play an important role as a food ingredient in absorbing and holding moisture in processed foods. The bean samples had the greatest

pentosan contents, specifically the large lima bean followed by the mayocoba bean, pinto bean, light red bean, pink bean, and navy bean respectively. All samples can be seen in Figure 29 below.

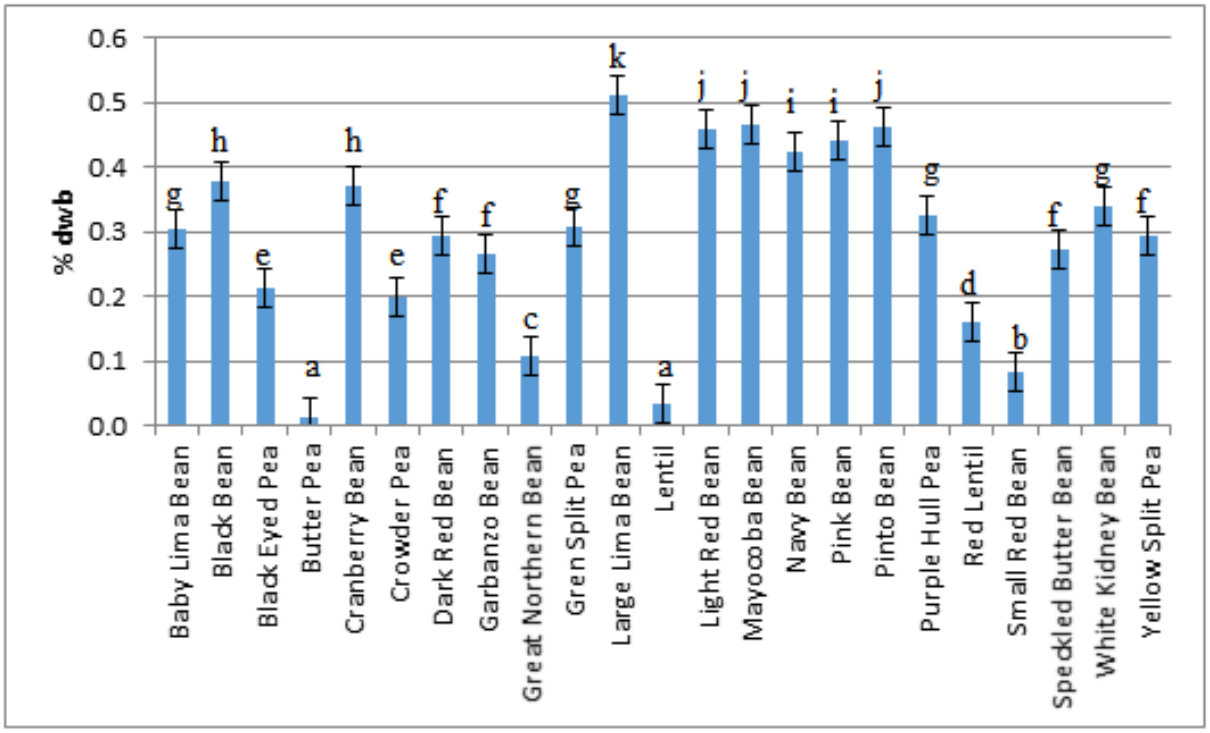

Figure 29: Pentosan Content in Pulse Flour
Bars with the same character are not significantly different (P>0.05).

2.5.6.6 Rapid Viscoanalysis

The raw pulse flours had lower peak viscosities but a higher starch pasting temperature than the wheat flour control, as seen in Figure 30 below. Additionally, reduced minimum viscosity, breakdown, final viscosity, and total setback were seen compared to the control. The cooked pulse flour samples were significantly lower that the raw pulse flour in every category except peak time which was only slightly lower and pasting temperature which could not be

determined due to the starches having already been activated by the previous cooking and drying processes.

Flour	Peak	Peak Time	Pasting Temperature	Minimum Viscosity	Breakdown	Final Viscosity	Total Setback
	(cP)	(min)	(˚C)	(cP)	(cP)	(cP)	(cP)
CONTROL:							
Wheat Flour	4281[a]	5.71[d]	65.40[f]	2601[h]	1687[k]	4315[o]	1714[r]
RAW:							
Bean Flours	1142[b]	6.91[e]	82.85[g]	1087[i]	54.5[l]	1940[p]	852.4[s]
Pea Flours	1432[b]	6.19[e]	81.68[g]	1341[i]	103.4[m]	2229[p]	900.5[s]
*Pulse Flours	1243[b]	6.66[e]	82.44[g]	1176[i]	71.5[l,m]	2040[p]	869.1[s]
PRECOOKED:							
Bean Flours	117.7[c]	6.28[e]	N/A	111.4[j]	5.60[n]	199.3[q]	87.9[t]
Pea Flours	114.7[c]	5.16[d]	N/A	95.2[j]	19.5[n]	123.8[q]	28.7[u]
*Pulse Flours	105.2[c]	5.96[d,e]	N/A	94.7[j]	9.92[n]	165.9[q]	71.2[t]

Figure 30: RVA Analysis Results
*Pulse Flours Data is the average of bean and pea flours
**Columns with the same character are not significantly different (P>0.05).

2.5.7 Antioxidants

2.5.7.1 DPPH

The ability of a compound to decolorize DPPH free radical signifies the radical scavenging activity of the tested compound. When reviewing data generated from a DPPH assay, it is essential to consider both the inhibition percent as well as the concentration. All extracts were prepared using the same dry weight basis of pulse with only the concentration volumes changing in the study.

The values for the lentil, cranberry bean, pinto bean, pink bean, and light red bean had the highest radical scavenging activity values compared to the butter pea, garbanzo bean, baby lima bean, red lentil, and large lima bean which all demonstrated the lowest radical scavenging activity values at the 40µL concentration as seen in Figure 31 below.

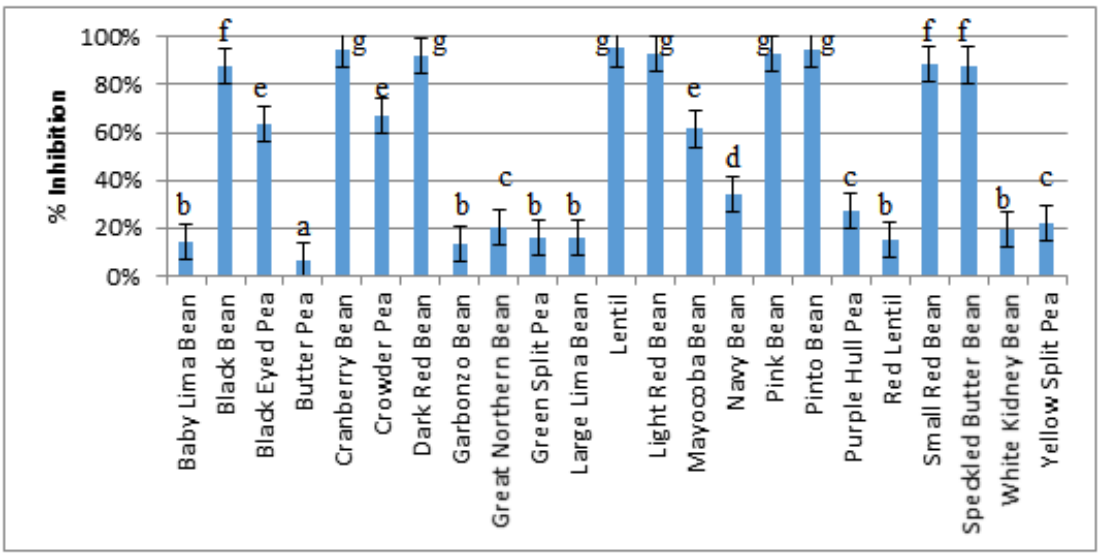

Figure 31: DPPH Inhibition Percent with 40µL of Pulse Extract
Bars with the same character are not significantly different (P>0.05).

However, as the pulse extract is increased to 80 µL, there were changes in which samples presented the highest and lowest radical scavenging activity values. Figure 32 shows that as the concentration of the pulse extract is increased, the highest antioxidant capacities are seen in the black bean, cranberry bean, speckled butter bean, pinto bean, and pink bean samples.

It has been demonstrated that an overabundance of antioxidants within a sample can act as pro-oxidants depending on the substrate and the radical source present[43,44]. Four of the

samples tested demonstrated reduced radical scavenging activity values after increasing the pulse extract percentage with the dark red bean, light red bean, and small red bean showing the greatest impact. This might be due to pro-oxidation in the system but further research would be necessary to determine if this is accurate or due to some other cause. Additionally, the same samples offered the lowest levels of inhibition at both concentration levels.

As the amount of pulse extract is increased again, the samples exhibiting the highest inhibition percent or radical scavenging activity values were the crowder pea, black bean, pink bean, pinto bean, lentil, cranberry bean, and speckled butter bean as seen in Figure 33.

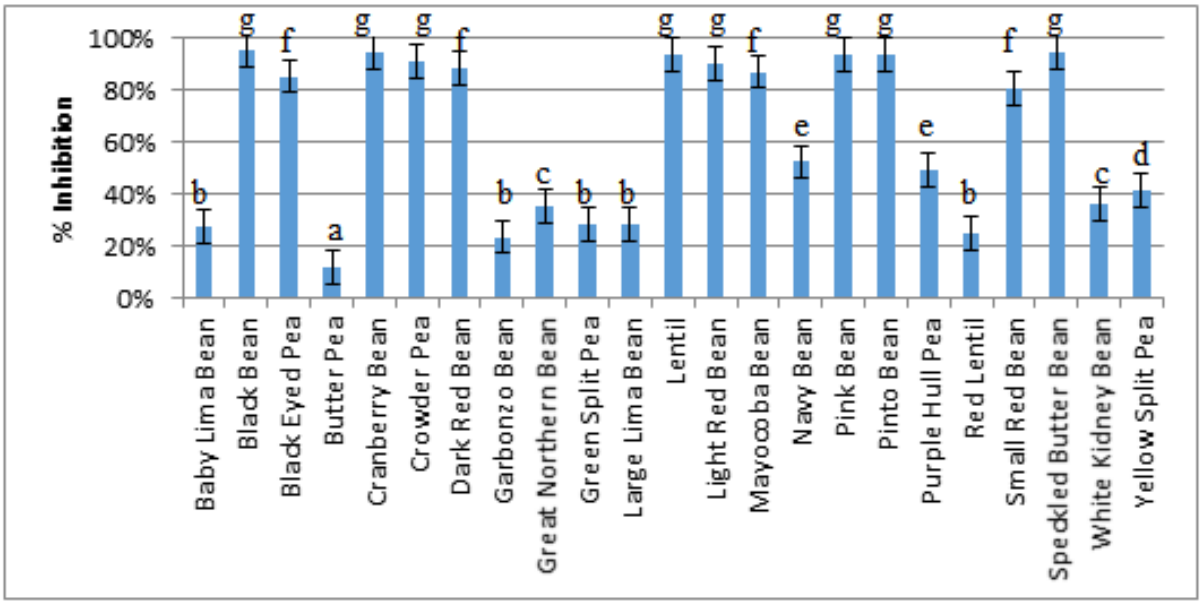

Figure 32: DPPH Inhibition Percent with 80µL of Pulse Extract
Bars with the same character are not significantly different (P>0.05).

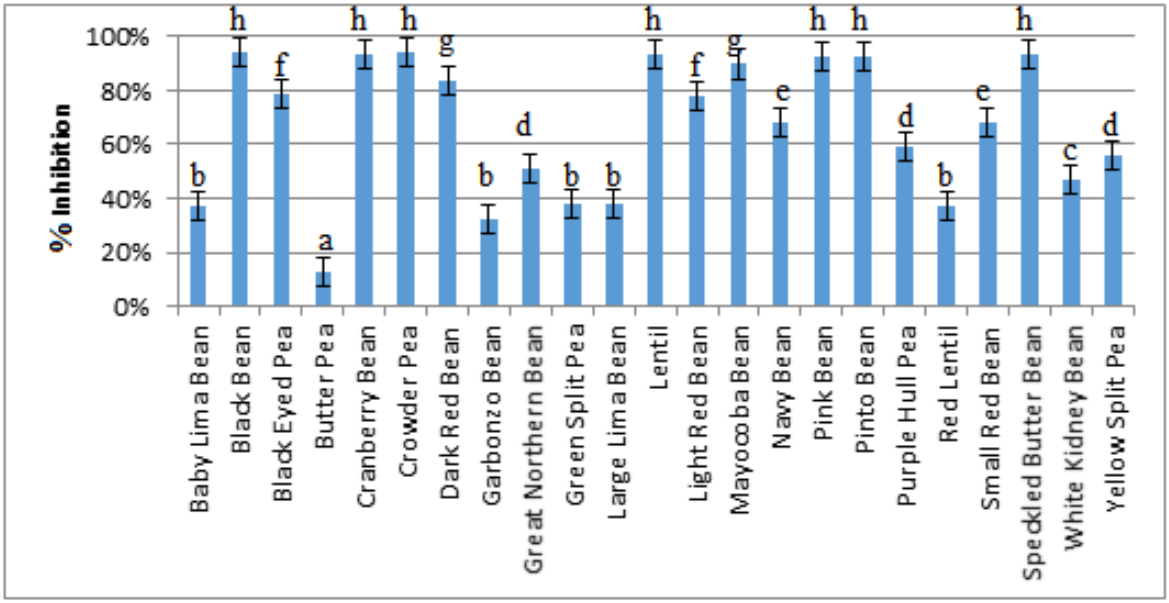

Figure 33: DPPH Inhibition Percent with 120µL of Pulse Extract
Bars with the same character are not significantly different (P>0.05).

If the three previous figures are superimposed upon each other, a rise in inhibition percent is seen over 50% of the time when the amount of pulse extract added is increased. However, the cranberry bean, dark red bean, light red bean, pink bean, and small red bean show the reverse of this trend with lowering of inhibition percent as higher amounts of pulse extract are added.

2.5.7.2 ORAC

As previously stated, ORAC values are more generally used to compare different foods for marketing purposes. Comparison of the ORAC values with the DPPH values for the pulse samples shows similar results (see Figure 34 below). The small red bean, lentil, pinto bean, dark red bean, and light red bean demonstrated the highest antioxidant capacities.

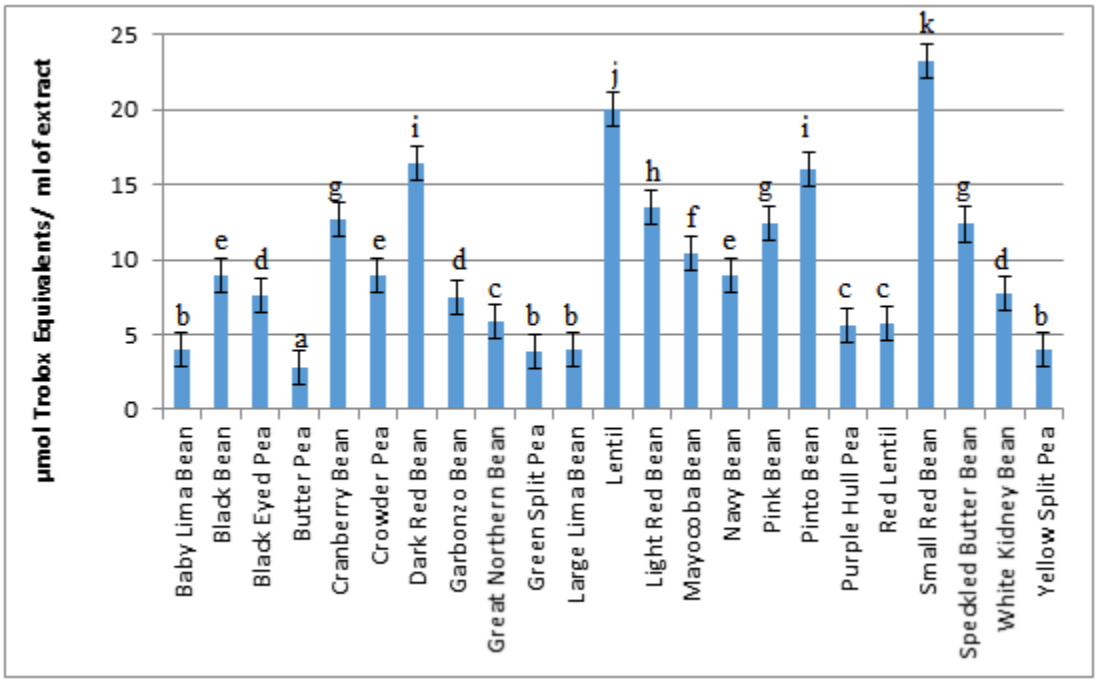

Figure 34: ORAC Values of Pulses
Bars with the same character are not significantly different (P>0.05).

2.6 Discussion

The results from the study have shown the nutritional value and some of physical attributes of dry pulses. But in order to utilize the pulse as a food ingredient, pulses need to be further treated in order to be edible and most nutritious for human consumption. The first method for this is the soaking or hydration process due to most pulses being a dry seed with minimal moisture content allowing for extended dry storage and stability. The hydration rate results suggest that dry whole pulses should be soaked on average for 16-18 hours at 4°C to achieve a minimum of 90% total hydration.

The hydration process has not been shown to have any impact on the ash and mineral content. When comparing pulses to other foods, they are one of the highest sources of several micronutrient minerals including iron, magnesium, potassium, and zinc. The data used from the USDA nutritional database has been validated and is in agreement with the compiled data from Campos-Vega et al[39,41]. for pulses grown outside of the US. All of the previous listed minerals are needed at proper levels in the diet to ensure optimum health. Iron deficiency is the most common nutritional deficiency and the leading cause of anemia in the United States. Several population groups are at risk especially young children, females after reaching puberty, and anyone who regularly takes antacids, especially the elderly[40]. Magnesium deficiency is suspected to be present in some form in over 80% of the US population although true symptomatic magnesium deficiency due to low dietary intake is uncommon. However, habitually low intakes or excessive losses due to certain health conditions, chronic alcoholism, and/or the use of certain medications can lead to magnesium deficiency. The populations of greatest risk include people with alcohol dependence, gastrointestinal disease, type II diabetes, and the elderly[45].Potassium deficiency, or hypokalemia, affects a smaller percentage of the population than the other two mineral deficiencies but has a greater impact on the lives of the individuals it does affect. The most common causes for hypokalemia include use of antibiotics, diarrhea and vomiting, kidney disease, eating disorders, sweating and low magnesium levels. While small drops in potassium levels may only have small effects on the overall health, serious health conditions can become a concern as levels decrease further. Hypokalemia is known to cause dysrhythmias, heart palpitations, fatigue, muscle damage, and at extremely low levels can cause the heart to stop beating completely[46,47]. In all three nutrient deficiencies, the major

population of concern is the elderly, but studies are also showing these deficiencies among people of low socio-economic households where food quality and variety may be limited[48,49]. With the quality and quantity of mineral nutrients available in pulses, an increase in pulse consumption could help alleviate the number of cases with mineral deficiencies.

The low level of fat is consistent with previously published information specifically with pulses averaging 1% total fat except for garbanzo beans which are at 5%[39,41]. The higher level of total fat seen in the garbanzo bean could be one reason it works well as a creaming agent in sauces and dips. Additionally, using this pulse in other traditionally higher fat items could maintain mouth feel while reducing the caloric load and improving the protein level which is normally deficient in high fat manufactured food items.

Protein, besides being a trendy buzz word for food marketing groups, is the nutrient needed for growth and rebuilding of cells in the human body. The roughly 21 g of protein per 1/2 cup of cooked dry pulses delivers over 40% of the RDI[39]. But besides nutrition, the protein in the various pulses demonstrated varying levels of gelation at similar concentrations. This information can allow for different pulses to serve different needs in food systems. For example, if a product developer was looking to increase gel strength then they would want to use one of the samples such as the navy bean which gelled at the 10% concentration rather than the butter pea which only had partial gelation.

The dietary consumption of pulses has long been praised not only for its protein value but also for its fiber content. The fiber results demonstrated that pulses have an average fiber content of 3 percent or about 15 grams per ½ cup of cooked pulses. These values represent 60% of the recommended daily intake per serving[39]. The fiber however is just one of the beneficial carbohydrates found in pulses. The high oligosaccharide content will allow for butyric fermentation in the gut by intestinal microflora and create flatulence. Additionally, the resistant starch, both the remaining naturally occurring, and any created through retrogradation from cooling the cooked pulses, will be fermented in the lower gastrointestinal system. This fermentation is a secondary benefit to health and can be used as a marketing strategy when using pulses as a value-added food ingredient.

But besides health, pulses can offer functional properties as well. Although there is very little published information on pentosan content in pulses. What could be found agreed with our findings of less than 0.5% on average[50,51,52]. There are some studies showing that the soybean legume has higher levels but not near the 4.5-6.0% pentosans found in wheat[27,52]. These minor components have been credited with many functional properties. However, their high water absorbing capacity is one of the most important characteristics. This functional attribute might reflect the ability of pentosans to swell and retain large amounts of water in their structure. This large increase in water holding capacity might also result in substantial redistribution of moisture among food systems[53]. The ability of a pulse to bind water can be a necessary feature when trying to reduce the cost of a finished product.

Additionally, the differences in the results of the pasting properties between the beans and peas as well as the differences seen in the raw versus precooked pulse flours offers various attributes to product developers looking for specific functional properties with controlled thickening and retrogradation by using various pulse types of a blend of the two. The lower peak viscosity of the pulse flours indicates a thinner slurry, possibly due to reduced amounts of amylose, despite having a higher gelation or pasting temperature. The total setback values and lower breakdown indicate a lower amount of retrogradation and a more consistent viscosity throughout the heating and cooling process, respectively[54].

Another overlooked marketing feature is the antioxidant capacity of pulses. DPPH is a cell-permeable, stable free radical that acts as a hydrogen radical scavenger and is a screening tool for detecting the free radical scavenging activity of antioxidants. As inhibition percent increases, the radical scavenging activity or antioxidant capacity is more abundant and more active[55]. This information demonstrates that different extracts showed different kinetics. It is not surprising to see differences emerge and these differences could play an important role in future research and food applications. Further research is needed to explain the trend of certain pulses for decreasing inhibition percentage as concentration increased but the specific anthocyanin unique to this subgroup could play a factor[23]. Overall, the data demonstrates the concentration dependent response referenced by Sharma and Bhat[56]. Additionally, at some point, the system was either overwhelmed or completely saturated with high phenolic containing pulses such as black bean, cranberry bean, lentil, pink, pinto, and speckled butter bean.

In comparison of the ORAC values for the pulse samples with the DPPH values for the pulse samples, there is some agreement in the trends. Each of the pulses that had the highest ORAC values and DPPH inhibition are red to brown tinted including: cranberry bean, crowder pea, dark red bean, lentil, light red bean, pink bean, pinto bean, small red bean, and speckled butter bean. While it is not known what specific phenolics or antioxidants are responsible, it can be assumed that the trend for these to demonstrate higher antioxidant capacity in both the ORAC and DDPH experiments is an indication of some antioxidant activity in the food matrix as well as possibly in the body. However, past research has shown that red beans contain a wide variety of flavonoids (including their flavonols, their glycosides, anthocyanins, proanthocyanidins and isoflavones) as well as some phenolic acids. Additionally, all beans contain the same hydroxycinnamic acid derivatives, but the flavonoid components are unique among groups[23].

2.7 Conclusion

The current food industry revolves around the transformation of raw materials into food products with various levels of production, processing, distribution, and preparation. Food companies are quickly having to become more focused on ingredient availability, and food access and utilization which are among the key factors in food security. The world's constantly expanding population has resulted in a greater pressure for novel foods while reducing the agricultural impact. This has resulted in a greater emphasis on the need for food ingredients with multiple functional properties. Functional properties can be defined as the physical and chemical properties influencing the behavior foods during processing, storage, cooking and nutritionally during consumption. Such functional properties can include hydration/water

holding capacity, gelation, retrogradation, antioxidant activity and many others. Pulses offer a multitude of nutritional benefits and their impact as a functional ingredient is becoming ever-more needed and apparent to fight food security, obesity, and rising animal protein costs.

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CHAPTER 3. CHARACTERISTICS OF MEAT OR SAUSAGE PATTIES USING PULSES AS EXTENDERS

3.1 Abstract

Meat patties were produced from either beef (20% fat) or pork (18% fat) and 23 different pulses. The pulses were blended with meat at 35%, 42.5%, and 50% ratios. The blends were formed into 113.4g (4 ounce) meat patties or 56.7g (2 ounce) pork sausage patties. Each patty was blast frozen, stored at -20ºF (-29ºC) in food-grade resealable freezer bags, and then baked in a 74ºC oven for 15 minutes before testing for weight loss, diameter loss, color, and texture. The 50:50 ratio samples had the least amount of cook loss but the greatest visible bean fraction. All fractions improved nutritional profile. Navy, Light Red Kidney, and Small Red Beans were found to be most beneficial/acceptable as partial meat substitutes.

3.2 Introduction

The Third National Health and Nutrition Examination Survey (NHANES III) reports that 66.3±1.1% of all adults over the age of 20 are overweight, 32.2±1.2% are obese, and in children 37.2±1.9% of 6-11year olds are overweight and 18.8±1.3% of 6-11year olds are obese[1]. NHANES III also reported the waist circumference of 1,803 children showed that 18.85% of participants were classified with central obesity[2]. From 1988-2004 the percent relative change in 6-11year olds in abdominal obesity was 42% in boys and 83.4% in girls. Abdominal obesity can be interpreted as abdominal subcutaneous and visceral fat combined[3]. In children and adults, healthy diet and regular exercise helps prevent excessive weight gain and promotes weight loss[4]. Pulses have been shown to play a role in healthy diets and reduce obesity by lowering body mass with increased consumption[5].

Pulses are the edible seeds of legume crops such as peas, lentils or beans. They are good sources of protein, thiamin, iron, magnesium and zinc and are high in dietary fiber and folate. Pulses also contain polyphenols which have antioxidant properties that may provide additional health benefits[6]. Consumption of pulses has been associated with lower rates of coronary heart disease, diabetes, and obesity[5,7,8]. In the United States, the average US adult consumption of pulses is 0.1-0.3 servings per day as compared to the recommended amount of 0.9 servings/d[9]. Therefore, an average US adult is only consuming one ½ cup serving of pulses every third day, whereas the recommendation is one ½ cup serving per day. In other parts of the world, pulses are an integral part of the diet. Pulses are inexpensive and are favored for their culinary, versatility, and nutritional benefits[10].

Many food professionals and dietitians agree that one approach to increase fruit and vegetable consumption is to disguise fruits and vegetables as pieces, purees, or powders in normally and frequently consumed foods to increase the nutritional value "unknowingly"[11,12]. Pulses are well suited for substitution in many food products because they are low in fat, high in good quality protein, and provide fiber, dietary resistant starch and a variety of phytochemicals with purported health benefits.

Currently the food industry adds either stabilized rice bran or textured soy protein to decrease cook loss in preformed meat and sausage patties. There is increasing concern about broad use of soy because of its allergenicity. Soy is one of the more common food allergies, especially

among babies and children. Additionally, with the inclusion of soy as one of the big eight allergens, many manufacturers are looking for soy alternatives and the use of rice bran is limited to no more than 3% of the meat product due to its low protein content[13,14]. Pulses, unlike soy, do not pose a significant allergen concern and are not limited by labeling requirements of the FDA because of their protein content[13,15]. The acceptability in a finished product has only briefly been studied.

The focus of this research was to establish practical ranges of substitution for pulses in ground meat products. Ground beef or pork sausage patties with up to fifty percent (50%) replacement of meat with pulses were tested to determine the properties of the resulting products.

3.3 Materials and Methods

3.3.1 Pulses

Twenty-three types of pulses were obtained from Archer Daniel Midland (ADM, Decatur, Il.) or a local produce market (LPM, Baton Rouge, LA). The dried pulses included Black Beans (ADM), Cranberry Beans (LPM), Pinto Beans (ADM), Pink Beans (ADM), Small Red Beans (ADM), Dark Red Kidney Beans (ADM), Light Red Kidney Beans (LPM), White Kidney Beans (ADM), Mayocoba Beans (ADM), Navy Beans (ADM), Great Northern Beans (ADM), Large Lima Beans (LPM), Baby Lima Beans (LPM), Chickpeas (LPM), Black-eyed Peas (ADM), Green Split Peas (LPM), Yellow Split Peas (LPM), Lentils (LPM), Red Lentils (LPM). Fresh frozen pulses included Speckled Butter Beans (LPM), Purple Hull Peas (LPM), Butter Peas (LPM), Crowder Peas (LPM). Each pulse was tested in both the beef and pork meat and bean analogs. Each pulse was carefully examined to

remove stones or other debris. The cleaned pulses were hydrated by adding 100ml of room temperature tap water to 50g of pulses and held overnight (about 18 hours) at 40˚F (4.5˚C). The hydrated pulses were drained and ground (3/16" plate, KitchenAid Food Grinder Stand Mixer Attachment, Professional 600 Stand Mixer, KitchenAid, St. Joseph, MI).

3.3.2 Patties

All patties were based on either 80/20 ground beef (GB) or an 18% fat fresh pork sausage (GP) purchased from a local grocery store and verified for fat content. Three control patty formulations were prepared for comparison against the GB/HPF and GP/HPF formulations. The 100% Meat Control (MC) patties were made from 100% GB or GP. The Fiber Controls (FC) were formulated with GB or GP and three percent (3%) stabilized rice bran (Nutracea, Phoenix, Arizona). Soy Controls (SC) were formulated with GB or GP with three percent (3%) textured soy protein (Nexsoy Non-GMO/Organic, Nexcel Natural Ingredients, Springfield, IL). The test patties were made using the ground pulses at 35%, 42.5%, or 50% replacement of the GB or GP by mixing. The patties were formed into 56.7 g sausage patties or 113.4g meat patties using a Hollymatic Model 200-U Patty Machine (Hollymatic, Countryside, IL). Patties were frozen in stacks of three patties interleaved with patty paper at -20˚F (-29˚C) in a blast freezer before packaging into sets of 12 and storing at -20˚F (-29˚C) to mimic the frozen patties used in the local school lunch program.

3.3.3 Cook Loss

Before cooking, all of the patties were weighed and the diameters measured twice at 90˚ angles and averaged. The patties were then baked on commercial half sheet pans (42cmX29cm) in a

Moffat Turbofan 32 Oven (Moffat, Christchurch, New Zealand) at 177°C for 15 minutes (the patties reached an internal temperature of 165˚F/74°C) mimicking the procedure used in most cafeterias[16]. The difference in frozen weight to cooked weight was recorded as percent cook loss and the difference in frozen diameter to cooked diameter was recorded as shrinkage. All patty formulations were tested in triplicate.

$=(W_f-W_c)/W_f*100$
W_f: Frozen weight
W_c: Cooked weight

or

$=(D_f-D_c)/D_f*100$
D_f: Frozen diameter average
D_c: Cooked diameter average

3.3.4 Color Testing

Each patty was tested for both raw and cooked color differences in terms of L*, a*, and b* using a Spectrophotometer Cm-508D (Minolta, Ramsey, New Jersey). Each variety was analyzed in triplicate with five measurements per patty. Measurements were taken directly on the surface of the patty at a 90º angle after a true white calibration.

3.3.5 Texture as an Indication of Tenderness

The texture of cooked test samples was measured with a TA.HDPlus Texture Analyzer (Texture Technologies Corp., Scarsdale, NY/Stable Micro Systems, Godalming, Surrey, UK) with the

Kramer Shear Attachment and 250kg load cell. Each patty was trimmed using a chef's knife to create a rectangle shape to fit into the 4cm by 3cm Kramer cell. Shearing rate was 1mm/second for a distance of 30.0mm with the results reported in kg of shear force.

3.3.6 Nutritional Profiles

The nutritional profiles of the samples were estimated using Genesis version 7.9.0. Database version: June 2006**. (**ESHA Research, Salem, Oregon).

3.3.7 Statistical Analysis

Statistical analysis was performed using MYSTAT (2008 Edition, Systat Software Inc., Chicago, IL) and SAS 9.2 (SAS Institute Inc. Cary, NC). All data was run in triplicate and was compared using the standard deviation of the group, co-efficient of variance, one-way ANOVA and Tukey's Studentized Range. All statistical differences were determined at $p<0.05$.

3.4 Results

3.4.1 Cook loss

The cook loss in the meat patty control ranged from 37.9% cook loss for the meat control (MC) to 29.9% cook loss by weight for the fiber control (FC) (Figure 35). The pulse replacement meat patties were all significantly different ($p<0.05$) from the control samples and ranged from an 8.0% average cook loss in the Yellow Split Pea patty samples to a 15.1% average cook loss in the Pink Bean patty samples. The average cook loss for all the bean samples was 10.6% (Figure 35).

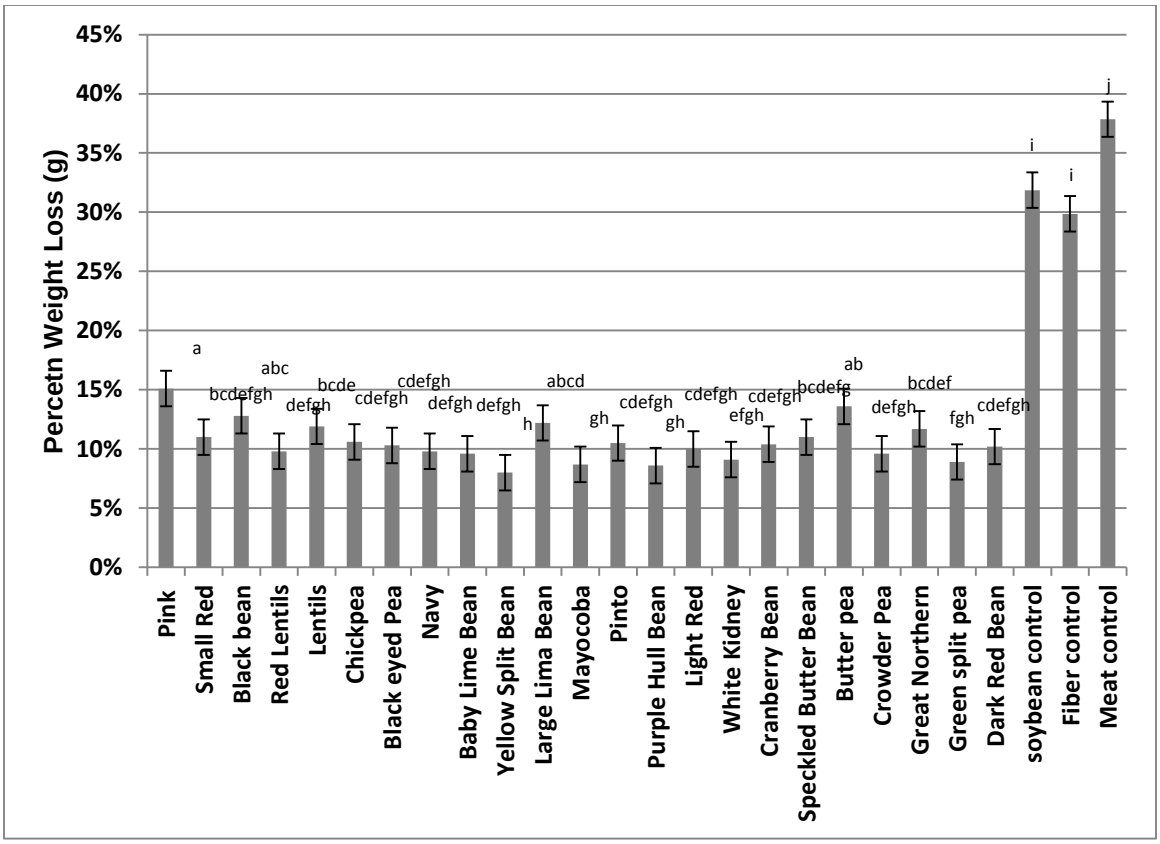

Figure 35: Average Percent Cook Loss of Meat Patties
Bars with the same character are not significantly different (P>0.05).
Coefficient of Variation=0.58

The sausage patties had cook losses of 19.3% by weight (g) in the fiber control, 16.0% in the soy control patties, and 22.3% in the GP control. The pulse replacement sausage patties were significantly different ($p<0.05$) from all three control samples. They ranged from a 5.6% average cook loss in the Small Red Bean patty samples to a 10.7% average cook loss in the Speckled Butter Bean patties with an average cook loss of 8.0% for all pulse samples (Figure 36).

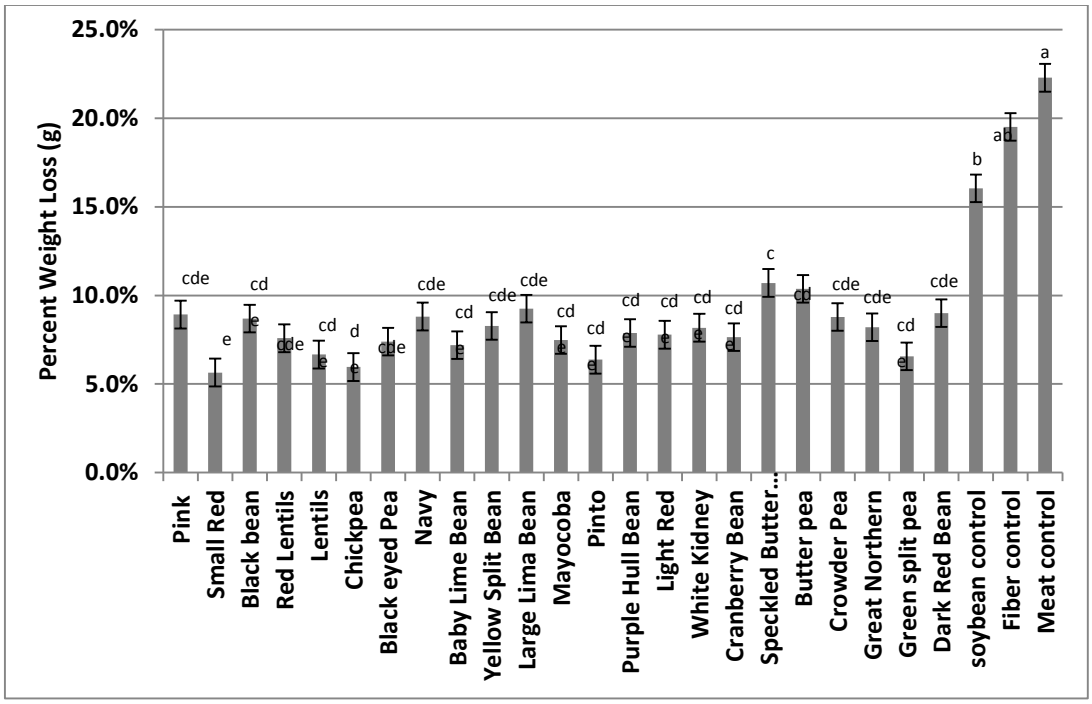

Figure 36: Average Percent Cook Loss of Sausage Patties
Bars the same character are not different (P>0.05).
Coefficient of Variation=0.43

3.4.2 Shrinkage

The three meat patty control samples had shrinkage of 25.7±0.1%. The pulse replacement meat patty samples ranged from an 8.6% average diameter cook loss in the Purple Hull Pea patties to a 17.5% average diameter cook loss in the Butter Pea samples. All experimental sample results were different ($p<0.05$) from the control samples and varied from the average diameter cook loss of 14.1±5.5% as seen in Figure 37.

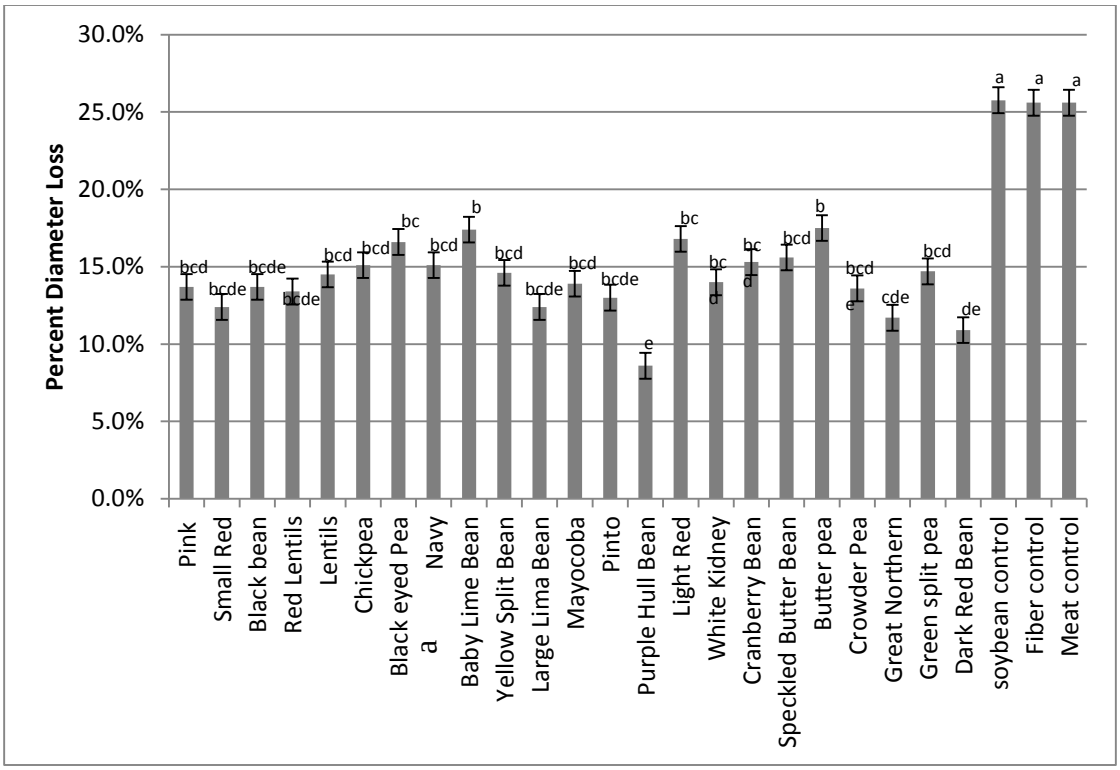

Figure 37: Average Shrinkage of Beef formulated Patties
Bars with the same letter are not different (P>0.05).
Coefficient of Variation=0.27

The three control sausage patties had an average shrinkage of 17.9%, the fiber control had 19.4% shrinkage and the meat control had 17.1% shrinkage. The pulse sausage patties ranged from 6.0% shrinkage in the Small Red Bean patties to 15.6% shrinkage in the Speckled Butter Bean samples. Figure 38 shows that eight experimental patties (Pink Beans, Black Beans, Red Lentils, Speckled Butter Beans, White Kidney Beans, Baby Lima Beans, Yellow Split Peas and Butter Peas) were not different ($p<0.05$) from the soy or meat control and all but the Speckled Butter Bean samples were different than the fiber control.

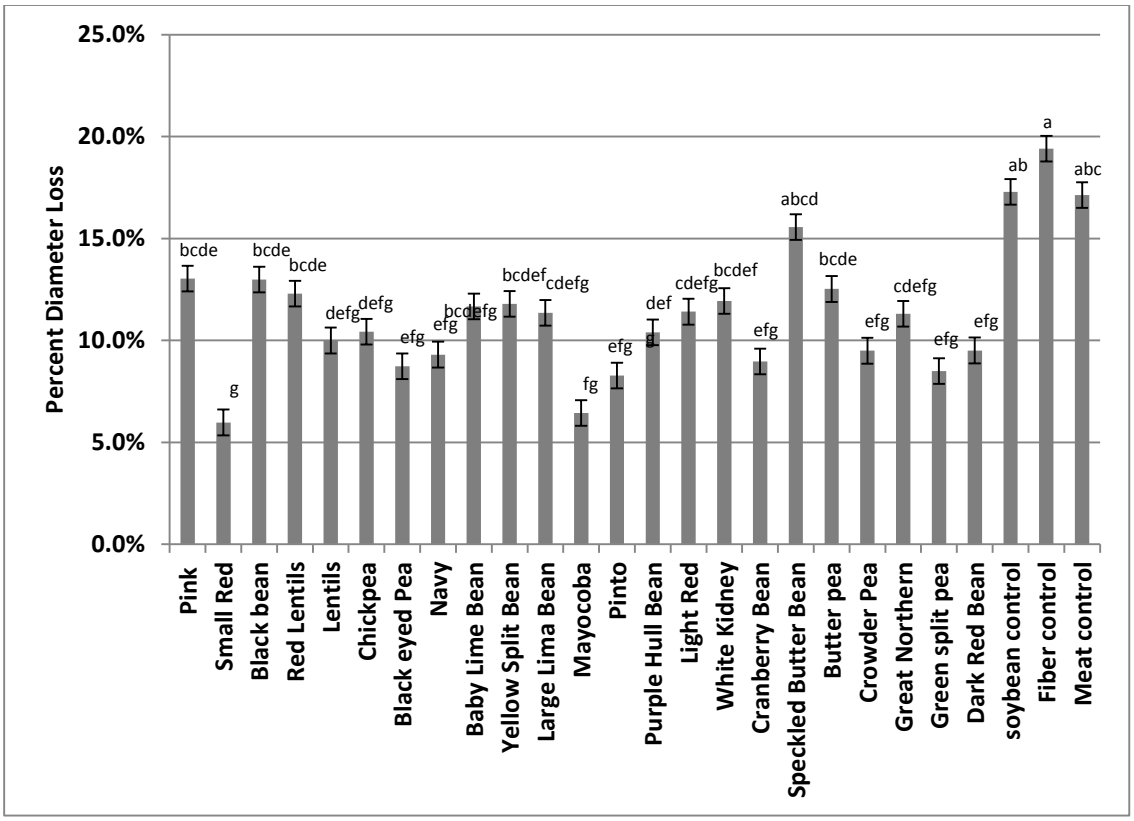

Figure 38: Average Shrinkage of Sausage Patties
Bars with the same letter are not different (P>0.05).
Coefficient of Variation=0.28

3.4.3 Color

Color values for meat patties made with the raw pulses were tested because the product is intended to be purchased frozen and uncooked. Since there was no statistical difference between the 35%, 42.5%, and 50% substituted raw samples, the results were averaged and compared to the control samples. None of the test patties had significantly different L* values (lightness to darkness of the product) from those of the three control meat patties. Only the Pink Bean, Red Lentil, Baby Lima Bean, Pinto Bean, Speckled Butter Bean, Butter Pea, Green Split Pea, and Dark Red Bean patties had significantly different a* values (green to magenta

hues) than the three controls. Additionally, the Cranberry Bean and Small Red Bean patties were significantly different from the Fiber and Meat Controls. The Black Bean and Dark Red Bean test patties were significantly different in b* values (yellow to blue hues) than the control samples as seen in Figure 39.

Patty Information	Color		
Bean	**L* Average**	**a* Average**	**b* Average**
Pink Beans	46.53 ^{ab}	8.67 ^{bcd}	14.75 ^{abc}
Small Red Beans	47.19 ^{ab}	8.21 ^{bcdef}	11.43 ^{cde}
Black Beans	42.13 ^{b}	4.12 ^{fgh}	8.20 ^{e}
Red Lentils	48.60 ^{ab}	10.93 ^{ab}	15.91 ^{abc}
Lentils	49.60 ^{ab}	5.00 ^{defgh}	19.15 ^{a}
Chickpeas	49.69 ^{ab}	8.28 ^{bcdef}	18.06 ^{a}
Black-eyed Peas	48.21 ^{ab}	6.60 ^{bcdefgh}	14.68 ^{abc}
Navy Beans	51.22 ^{ab}	6.35 ^{cdefgh}	16.56 ^{abc}
Baby Lima Beans	48.85 ^{ab}	13.14	16.86 ^{abc}
Yellow Split Peas	49.86 ^{ab}	7.40 ^{bcdefgh}	17.57 ^{a}
Large Lima Beans	48.02 ^{ab}	7.96 ^{bcdefg}	18.78 ^{a}
Mayocoba Beans	50.72 ^{ab}	7.06 ^{bcdefgh}	14.77 ^{abc}
Pinto Beans	46.24 ^{ab}	9.89 ^{abc}	11.90 ^{bcde}
Purple Hull Peas	53.31 ^{ab}	4.37 ^{defgh}	11.35 ^{cde}
Light Red Beans	49.73 ^{ab}	7.35 ^{bcdefgh}	13.92 ^{abcd}
White Kidney Beans	51.80 ^{ab}	6.72 ^{bcdefgh}	14.50 ^{abc}
Cranberry Beans	49.42 ^{ab}	8.53 ^{bcde}	13.82 ^{abcd}
Speckled Butter Beans	48.26 ^{ab}	9.99 ^{abc}	14.66 ^{abc}
Butter Peas	54.95 ^{a}	8.64 ^{bcd}	16.87 ^{abc}
Crowder Peas	52.99 ^{ab}	7.24 ^{bcdefgh}	13.71 ^{abcde}
Great Northern Beans	56.36 ^{a}	4.65 ^{defgh}	17.44 ^{ab}
Green Split Peas	49.76 ^{ab}	3.28 ^{h}	13.81 ^{abcd}
Dark Red Beans	42.22 ^{b}	10.34 ^{abc}	8.79 ^{de}
Soybean Control	47.69 ^{ab}	4.14 ^{efgh}	15.82 ^{abc}
Fiber Control	49.85 ^{ab}	3.76 ^{gh}	16.71 ^{abc}
Meat Control	50.13 ^{ab}	3.99 ^{fgh}	16.60 ^{abc}

Figure 39: Color Results for Raw Beef formulated Patties
Data for a variable with the same letter are not different (P>0.05).
Coefficient of Variation= 0.09(L*), 0.36 (a*), 0.22 (b*)

The uncooked sausage patties with partial pulse replacement were also compared to the control samples. None of the test patties showed a difference in L* values from the fiber control. There was a significant difference in a* values of the meat control from those of the Pink Bean, Small Red Bean, Black Bean, Lentil, and Pinto Bean patties. The soybean control had only a significant difference from the Crowder Pea patty. The a* values were significantly different between 15 of the pulse patties and the soybean control patties, while only the Crowder Pea and Green Split Pea patties were different from the fiber and meat control patties. The Lentil patty was different in b* from the soybean and meat control patties. Seven sample patties (Small Red Bean, Black Bean, Chickpea, Black-eyed Pea, Navy Bean, Purple Hull Pea, Light Red Bean) were significantly different in b* values from the fiber control and of those, only the Small Red Bean, Black Bean, and Navy Bean patties were not significantly different from the Meat Control as seen in Figure 40.

The L*, a*, and b* values for cooked patties produced with Pink Beans, Small Red Beans, Lentils, Black-eyed Peas, Navy Beans, Pinto Beans, Purple Hull Peas, Light Red beans, White Kidney Beans, Cranberry Beans, Speckled Butter Beans, and Crowder Peas, at 35%, 42.5% and 50% replacement, were not significantly different than the values of the three control meat patties (Figure 41).

Patty Information	Color		
Bean	**L* Average**	**a* Average**	**b* Average**
Pink Beans	46.53^{de}	8.67 ^{bcde}	14.75^{bcdefgh}
Small Red Beans	47.19^{cde}	8.21 ^{abcd}	11.43^{hi}
Black Beans	42.13^{cde}	4.12^{cde}	8.20^{i}
Red Lentils	48.60 ^{abcde}	10.93 ^{bcde}	15.91^{cdefgh}
Lentils	49.60^{e}	5.00 ^{bcde}	19.15^{a}
Chickpeas	49.69^{abc}	8.28 ^{bcde}	18.06^{fghi}
Black-eyed Peas	48.21 ^{abcde}	6.60 ^{bcde}	14.68^{fghi}
Navy Beans	51.22 ^{abcde}	6.35 ^{abcd}	16.56^{ghi}
Baby Lima Beans	48.85^{abc}	13.14 ^{abcd}	16.86^{cdefgh}
Yellow Split Peas	49.86 ^{abcde}	7.40^{abcd}	17.57^{bcdefg}
Large Lima Beans	48.02^{ab}	7.96 ^{bcde}	18.78^{defgh}
Mayocoba Beans	50.72 ^{abcde}	7.06 ^{bcd}	14.77^{bcd}
Pinto Beans	46.24^{e}	9.89 ^{abcd}	11.90^{abcd}
Purple Hull Peas	53.31^{abcd}	4.37 ^{bcde}	11.35^{efghi}
Light Red Beans	49.73^{abcde}	7.35 ^{bcde}	13.92^{defgh}
White Kidney Beans	51.80 ^{abcde}	6.72 ^{bcde}	14.50^{ab}
Cranberry Beans	49.42^{bcde}	8.53 ^{abcd}	13.82^{efghi}
Speckled Butter Beans	48.26 ^{abcde}	9.99^{ab}	14.66^{bcde}
Butter Peas	54.95 ^{abcde}	8.64 ^{bcde}	16.87^{abc}
Crowder Peas	52.99^{a}	7.24^{de}	13.71^{cdefgh}
Great Northern Beans	56.36 ^{abcde}	4.65 ^{abcd}	17.44^{bcdef}
Green Split Peas	49.76 ^{abcde}	3.28^{e}	13.81^{cdefgh}
Dark Red Beans	42.22 ^{abcde}	10.34 ^{bcde}	8.79^{fghi}
Soybean Control	47.69 ^{abcde}	4.14^{a}	15.82^{bcdef}
Fiber Control	49.85^{bcde}	3.76^{abc}	16.71^{abc}
Meat Control	50.13^{ab}	3.99 ^{bcde}	16.60^{hi}

Figure 40: Color Results for Raw Sausage Patties
Data for a variable with the same letter are not different (P>0.05).
Coefficient of Variation= 0.08(L*), 0.26 (a*), 0.44 (b*)

Patty Information		Color		
Bean	**% Replacement**	**L* Average**	**a* Average**	**b* Average**
Pink Beans	35	43.25^{a}	9.66^{cd}	13.62 ^{cdef}
Small Red Beans	35	30.49 ^{de}	9.12^{d}	10.11^{f}
Black Beans	35	33.17 ^{bcde}	9.88^{cd}	11.53^{ef}
Red Lentils	35	36.00 ^{abcde}	14.57^{abc}	16.92 ^{abcdef}
Lentils	35	31.53 ^{cde}	8.70^{d}	12.41 ^{cdef}
Chickpeas	35	37.76 ^{abcde}	10.09^{cd}	17.66 ^{abcde}
Black-eyed Peas	35	38.94 ^{abcd}	9.95^{cd}	14.13 ^{cdef}
Navy Beans	35	43.23^{a}	10.96 ^{abcd}	14.99 ^{cdef}
Baby Lima Beans	35	39.62 ^{abcd}	15.22^{ab}	19.39^{abcd}
Yellow Split Peas	35	41.15^{abc}	12.42 ^{abcd}	17.94^{abcde}
Large Lima Beans	35	41.12 ^{abc}	15.31^{ab}	20.08^{abc}
Mayocoba Beans	35	40.74 ^{abc}	12.92^{abcd}	15.57 ^{bcdef}
Pinto Beans	35	39.29 ^{abcd}	9.79^{cd}	13.31 ^{cdef}
Purple Hull Peas	35	40.60 ^{abc}	8.39^{d}	15.16 ^{cdef}
Light Red Beans	35	38.42 ^{abcd}	10.44 ^{bcd}	13.22 ^{cdef}
White Kidney Beans	35	42.13^{ab}	12.21 ^{abcd}	16.83 ^{abcdef}
Cranberry Beans	35	33.92 ^{abcde}	10.33 ^{bcd}	11.09^{ef}
Speckled Butter Beans	35	41.70^{ab}	11.26 ^{abcd}	15.12 ^{cdef}
Butter Peas	35	36.24 ^{abcde}	11.04 ^{abcd}	15.14 ^{cdef}
Crowder Peas	35	36.48 ^{abcde}	12.14 ^{abcd}	17.79 ^{abcde}
Great Northern Beans	35	33.99 ^{abcde}	9.66^{cd}	23.30^{a}
Green Split Peas	35	40.13 ^{abcd}	8.74^{d}	16.79 ^{abcdef}
Dark Red Beans	35	28.27^{e}	15.90^{a}	22.57^{ab}
Soybean Control		37.55 ^{abcde}	10.39 ^{bcd}	12.05^{ef}
Fiber Control		38.21 ^{abcd}	11.71 ^{abcd}	14.24 ^{cdef}
Meat Control		36.97 ^{abcde}	11.31 ^{abcd}	13.03 ^{cdef}
Pink Beans	42.5	43.70 ^{abcd}	10.47 ^{bcde}	14.01 ^{bcd}
Small Red Beans	42.5	33.92 ^{def}	10.27 ^{bcde}	11.51^{d}
Black Beans	42.5	31.22^{ef}	3.41^{f}	4.54^{e}
Red Lentils	42.5	40.40 ^{abcde}	16.26^{a}	17.80 ^{ab}
Lentils	42.5	35.14 ^{def}	8.93^{de}	16.10 ^{bcd}
Chickpeas	42.5	40.60 ^{abcde}	9.76 ^{cde}	17.62 ^{ab}
Black-eyed Peas	42.5	35.06 ^{def}	12.19 ^{abcd}	16.32 ^{bcd}
Navy Beans	42.5	39.70 ^{abcdef}	12.52 ^{abcd}	16.22 ^{bcd}

Figure 41: Color Results for Cooked Beef formulated Patties
Data for a variable with the same letter are not different (P>0.05).
Coefficient of Variation= 0.11(L*), 0.21 (a*), 0.20 (b*)

(Figure 41 continued)

Patty Information		Color		
Bean	**% Replacement**	**L* Average**	**a* Average**	**b* Average**
Baby Lima Beans	42.5	40.77 abcde	12.67abcd	16.40 bcd
Yellow Split Peas	42.5	43.57 abcd	13.37abc	18.12ab
Large Lima Beans	42.5	47.70ab	12.09 abcd	16.49 bcd
Mayocoba Beans	42.5	48.73a	11.68 bcd	16.65 abcd
Pinto Beans	42.5	39.04 abcdef	9.67 cde	13.67 bcd
Purple Hull Peas	42.5	42.38 abcd	9.86 cde	15.76 bcd
Light Red Beans	42.5	35.69 cdef	10.88 bcde	13.05 bcd
White Kidney Beans	42.5	45.52abc	10.49 bcde	16.63 abcd
Cranberry Beans	42.5	39.17 abcdef	11.32 bcd	13.57 bcd
Speckled Butter Beans	42.5	44.01abcd	11.42 bcd	17.08 abc
Butter Peas	42.5	39.63 abcdef	12.43 abcd	16.90 abcd
Crowder Peas	42.5	40.11 abcdef	9.56 cde	13.83 bcd
Great Northern Beans	42.5	34.03 def	9.63 cde	22.03a
Green Split Peas	42.5	38.64 abcdef	6.49ef	17.41 abc
Dark Red Beans	42.5	30.27f	14.67ab	21.97a
Soybean Control		37.55 cdef	10.39 bcde	12.05 cd
Fiber Control		38.21 bcdef	11.71 bcd	14.24 bcd
Meat Control		36.97 cdef	11.31 bcd	13.03 bcd
Pink Beans	50	37.94 abc	11.45 ab	13.87 cde
Small Red Beans	50	36.54 abc	10.65 ab	12.54 def
Black Beans	50	30.11c	5.69b	8.09f
Red Lentils	50	39.00 abc	14.05a	20.74ab
Lentils	50	37.13 abc	8.32 ab	16.39 abcde
Chickpeas	50	37.83 abc	9.67 ab	16.41 abcde
Black-eyed Peas	50	38.66 abc	9.23 ab	14.37 cde
Navy Beans	50	45.21a	10.07 ab	16.47 abcde
Baby Lima Beans	50	40.37 abc	9.73 ab	13.15 def
Yellow Split Peas	50	42.96 ab	10.03 ab	21.51a
Large Lima Beans	50	42.47 ab	11.63 ab	16.17 abcde
Mayocoba Beans	50	42.52 ab	11.47 ab	16.94 abcde
Pinto Beans	50	37.94 abc	10.19 ab	13.44 def
Purple Hull Peas	50	44.92a	9.28 ab	15.04cde
Light Red Beans	50	40.06 abc	10.94 ab	14.68 cde
White Kidney Beans	50	43.53 ab	10.95 ab	16.53 abcde
Cranberry Beans	50	39.08 abc	11.65 ab	16.35 abcde
Speckled Butter Beans	50	44.20ab	9.84 ab	15.43 bcde
Butter Peas	50	44.75a	11.03 ab	19.32abc

(Figure 41 continued)

Patty Information		Color		
Bean	**% Replacement**	**L* Average**	**a* Average**	**b* Average**
Crowder Peas	50	39.73 abc	9.00 ab	13.15 def
Great Northern Beans	50	35.94 abc	7.41 b	16.98 abcde
Green Split Peas	50	40.59 abc	5.84 b	17.80 abcd
Dark Red Beans	50	33.65 bc	10.35 ab	19.22 abc
Soybean Control		37.55 abc	10.39 ab	12.05 ef
Fiber Control		38.21 abc	11.71 ab	14.24 cde
Meat Control		36.97 abc	11.31 ab	13.03 def

Similarly, the L*, a*, and b* results of the cooked sausage patties with 35%, 42.5% and 50% replacement levels of the Pink Bean, Small Red Bean, Yellow Split Pea, Mayocoba Bean, Purple Hull Pea, Light Red Bean, and Dark Red Bean patties were not significantly different than the values of the three control sausage patties (Figure 42).

Patty Information		Color		
Beans	**% Replacement**	**L* Average**	**a* Average**	**b* Average**
Pink Beans	35	38.81 bcdef	8.05 bc	14.04 cd
Small Red Beans	35	36.29 def	9.93 ab	12.61 de
Black Beans	35	34.22 f	2.05 d	7.34 d
Red Lentils	35	40.69 bcdef	15.01 a	21.27 a
Lentils	35	36.33 def	5.32 bcd	15.51 abcd
Chickpeas	35	43.09 abcde	9.17 b	19.62 abc
Black-eyed Peas	35	42.56 abcde	8.62 bc	16.86 abcd
Navy Beans	35	40.35 bcdef	9.08 b	16.16 abcd
Baby Lima Beans	35	49.76 a	10.21 ab	20.43 ab
Yellow Split Peas	35	39.30 bcdef	6.27 bcd	18.23 abcd
Large Lima Beans	35	45.15 abc	6.88 bcd	17.36 abcd
Mayocoba Beans	35	39.88 bcdef	8.86 b	17.41 abcd

Figure 42: Color Results for Cooked Sausage Patties
Data for a variable with the same letter are not different (P>0.05).
Coefficient of Variation= 0.10(L*), 0.33 (a*), 0.20 (b*)

(Figure 42 Continued)

Patty Information		Color		
Beans	**% Replacement**	**L* Average**	**a* Average**	**b* Average**
Pinto Beans	35	44.49^{abcd}	8.48^{bc}	16.13^{abcd}
Purple Hull Peas	35	40.88^{bcdef}	7.06^{bcd}	13.97^{cd}
Light Red Beans	35	38.77^{bcdef}	8.45^{bc}	15.39^{bcd}
White Kidney Beans	35	41.94^{abcdef}	8.98^{b}	17.47^{abcd}
Cranberry Beans	35	44.46^{abcd}	5.72^{bcd}	15.31^{bcd}
Speckled Butter Beans	35	44.01^{abcd}	5.88^{bcd}	13.94^{cd}
Butter Peas	35	43.02^{abcde}	7.68^{bc}	17.92^{abcd}
Crowder Peas	35	39.87^{bcdef}	5.76^{bcd}	13.98^{cd}
Great Northern Beans	35	46.30^{ab}	6.32^{bcd}	15.96^{abcd}
Green Split Peas	35	37.28^{cdef}	3.11^{cd}	18.18^{abcd}
Dark Red Beans	35	38.85^{bcdef}	4.82^{bcd}	13.27^{d}
Soybean Control		38.80^{bcdef}	7.18^{bcd}	14.14^{cd}
Fiber Control		39.14^{bcdef}	6.98^{bcd}	13.75^{d}
Meat Control		34.82^{ef}	6.90^{bcd}	13.39^{d}
Pink Beans	42.5	40.71^{bcde}	7.65^{b}	15.44^{bcd}
Small Red Beans	42.5	41.37^{bcde}	9.22^{ab}	13.58^{bcde}
Black Beans	42.5	31.49^{f}	2.01^{c}	7.39^{e}
Red Lentils	42.5	37.67^{def}	13.59^{a}	23.41^{a}
Lentils	42.5	40.25^{bcde}	4.99^{bc}	19.94^{ab}
Chickpeas	42.5	43.09^{abcd}	6.20^{bc}	18.02^{abcd}
Black-eyed Peas	42.5	44.32^{abcd}	5.73^{bc}	15.86^{bcd}
Navy Beans	42.5	40.44^{bcde}	8.16^{b}	19.51^{abc}
Baby Lima Beans	42.5	42.88^{abcd}	8.12^{b}	17.66^{abcd}
Yellow Split Peas	42.5	39.24^{cde}	7.56^{b}	17.30^{abcd}
Large Lima Beans	42.5	43.13^{abcd}	5.21^{bc}	14.98^{bcd}
Mayocoba Beans	42.5	40.31^{bcde}	6.51^{bc}	16.97^{bcd}
Pinto Beans	42.5	44.43^{abcd}	8.56^{b}	16.65^{bcd}
Purple Hull Peas	42.5	41.76^{bcde}	6.40^{bc}	15.31^{bcd}
Light Red Beans	42.5	37.94^{def}	6.53^{bc}	14.06^{bcd}
White Kidney Beans	42.5	47.57^{ab}	7.31^{b}	17.31^{abcd}
Cranberry Beans	42.5	39.45^{cde}	5.39^{bc}	12.91^{de}
Speckled Butter Beans	42.5	39.56^{cde}	8.73^{ab}	18.40^{abcd}
Butter Peas	42.5	46.22^{abc}	5.04^{bc}	19.03^{abcd}
Crowder Peas	42.5	38.05^{def}	5.47^{bc}	12.98^{de}
Great Northern Beans	42.5	50.26^{a}	6.30^{bc}	17.52^{abcd}
Green Split Peas	42.5	38.32^{def}	4.47^{bc}	18.80^{abcd}
Dark Red Beans	42.5	39.26^{cde}	5.52^{bc}	14.50^{bcd}

(Figure 42 continued)

Patty Information		Color		
Beans	**Beans**	**L* Average**	**a* Average**	**b* Average**
Soybean Control		38.80 cdef	5.85 b	14.14 bcd
Fiber Control		39.14 cde	6.98 b	13.75 bcde
Meat Control		34.82 ef	6.90 bc	13.39cde
Pink Beans	50	41.27 abcdef	6.10 bc	14.49 bcd
Small Red Beans	50	41.91 abcdef	7.69 bc	14.94 bcd
Black Beans	50	31.31g	1.68d	3.64e
Red Lentils	50	38.08defg	12.91a	22.86a
Lentils	50	37.91 defg	5.65 bcd	17.51 abcd
Chickpeas	50	42.70 abcdef	7.14 bc	18.69 abcd
Black-eyed Peas	50	37.12 defg	8.65b	17.65 abcd
Navy Beans	50	47.69abc	7.61 bc	18.76 abcd
Baby Lima Beans	50	45.84abcde	8.30bc	18.54 abcd
Yellow Split Peas	50	38.71 cdefg	7.38 bc	18.88 abcd
Large Lima Beans	50	45.29 abcde	6.75 bc	17.27 abcd
Mayocoba Beans	50	43.97 abcdef	5.98 bc	17.33 abcd
Pinto Beans	50	43.96 abcdef	7.53 bc	16.44 bcd
Purple Hull Peas	50	42.99 abcdef	6.87 bc	15.41 bcd
Light Red Beans	50	36.61efg	5.78 bcd	13.57cd
White Kidney Beans	50	49.54ab	7.63 bc	19.38abc
Cranberry Beans	50	40.38 bcdefg	6.51 bc	13.52d
Speckled Butter Beans	50	39.60cdefg	7.26 bc	13.20d
Butter Peas	50	46.48abcd	5.09 bcd	19.82ab
Crowder Peas	50	44.40 abcde	4.21cd	14.57 bcd
Great Northern Beans	50	50.52a	6.30 bc	18.90abcd
Green Split Peas	50	41.68 abcdef	4.49 bcd	22.65a
Dark Red Beans	50	37.76 defg	5.87 bcd	14.06 bcd
Soybean Control		38.80 cdefg	5.85 bc	14.14 bcd
Fiber Control		39.14cdefg	6.98 bc	13.75cd
Meat Control		34.82fg	6.90 bc	13.39d

3.4.4 Tenderness

The tenderness of the meat patty samples prepared with Black-Eyed Pea, Baby Lima Bean, Purple Hull Pea, and Crowder Pea, at 35%, 42.5% and 50% replacement, were not significantly different than the control samples. The experimental pulse samples ranged from 17.319kg of

shear force for the Cranberry Bean meat patty samples indicating the soft texture of this patty, to 29.602kg for Black-Eyed Peas indicating the firmer texture of this patty (Figure 43).

Percentage Beans	35.0%	42.5%	50.0%	Overall Average
Small Red Beans	22.386 fghi	19.088 gh	21.063 defg	20.846
Pink Beans	21.868 efghi	17.501 h	17.978 fgh	19.116
Black Beans	28.324bcde	23.222 cdefgh	21.660 cdefg	24.402
Lentils	26.934bcdefg	22.900 cdefgh	22.708 cdefg	24.181
Red Lentils	26.057 cdefgh	22.678 cdefgh	20.689 defg	23.141
Black-eyed Peas	32.670ab	28.307 bcd	27.829 bcd	29.602
Chickpeas	25.156 efgh	24.600 bcdefgh	26.724 bcde	25.493
Navy Beans	24.058 efghi	24.413 bcdefgh	22.989 cdefg	23.820
Green Split Peas	25.300 defgh	26.655 bcdef	23.598 cdef	25.184
Mayocoba Beans	27.203bcdef	24.407 bcdefgh	23.097 cdefg	24.903
Large Lima Beans	25.288 defgh	24.492 bcdefgh	20.027 efgh	23.269
Baby Lima Beans	31.335abc	28.565bcd	25.656 bcde	28.519
Yellow Split Peas	24.576 efgh	22.924 cdefgh	25.181 bcdef	24.227
Butter Peas	20.348hi	19.572fgh	16.292 gh	18.737
White Kidney Beans	20.264hi	18.729 gh	19.530 efgh	19.508
Pinto Beans	18.447i	19.010 gh	21.823 cdefg	19.760
Purple Hull Peas	26.028 cdefgh	27.152 bcde	28.434 bc	27.205
Crowder Peas	26.191 cdefgh	29.345abc	27.361 bcdef	27.633
Light Red Beans	21.323 fghi	20.196 efgh	24.489 bcdef	22.003
Speckled Butter Beans	20.740hi	21.269 defgh	22.598 cdefg	21.536
Cranberry Beans	21.090ghi	17.579 h	13.288 h	17.319
Great Northern Beans	32.411ab	19.439 fgh	27.620 bcd	26.490
Dark Red Beans	25.246 defgh	17.976 gh	20.007 efgh	21.076
Fiber Control	25.257 defgh	25.257 bcdefg	25.257 bcdef	25.257
Soybean Control	36.488a	36.488a	36.488 a	36.488
Meat Control	31.210 abcd	31.210ab	31.210 ab	31.210

Figure 43: Kramer Results in KG Shear Force for Beef formulated Patties
Data within a replacement level with the same letter are not different (P>0.05).
Coefficient of Variation=0.16 (35%), 0.16 (42.5%), 0.17 (50%), 0.14 (Average)

Sausage patties produced with Black Bean, Lentil, Black-eyed Pea, Green Split Pea, and Baby Lima Bean samples were the only test patties with shear forces that were not statistically different from the control patties. The average kilograms of shear force for the samples ranged from 18.264kg for Great Northern Bean samples to 29.553g for Crowder Pea patties (Figure 44).

Percentage Beans	35.0%	42.5%	50.0%	Average
Small Red Beans	24.988 abc	20.733 efghi	17.172 jk	20.964
Pink Beans	23.852 abcd	22.610 bcdefghi	20.856 fghij	22.439
Black Beans	24.534 abcd	25.021 abcdefg	23.374 defgh	24.310
Lentils	22.928 abcd	25.703 abcdef	23.801 defgh	24.144
Red Lentils	20.222 cd	21.236 defghi	22.169 efghi	21.209
Black-eyed Peas	22.590 abcd	23.837 bcdefghi	23.050 defgh	23.159
Chickpeas	22.270 abcd	25.834 abcde	21.944 fghi	23.350
Navy Beans	20.345 cd	20.584 efghi	24.483 cdefg	21.804
Green Split Peas	24.576 abcd	21.319 defghi	22.309 efghi	22.735
Mayocoba Beans	22.423 abcd	22.255 bcdefghi	21.618 fghij	22.099
Large Lima Beans	22.623 abcd	21.307 defghi	19.935 hijk	21.288
Baby Lima Beans	27.401 a	27.007 abc	27.452 abcd	27.287
Yellow Split Peas	23.960 abcd	24.277 bcdefgh	28.424 abc	25.553
Butter Peas	20.381 cd	19.986 ghi	20.509 ghij	20.292
White Kidney Beans	25.742 ab	23.592 bcdefghi	20.733 fghij	23.356
Pinto Beans	19.821 d	19.137 hi	17.906 ijk	18.954
Purple Hull Peas	23.508 abcd	30.359 a	27.413 abcd	27.093
Crowder Peas	26.872 a	30.206 a	31.581 a	29.553
Light Red Beans	24.692 abcd	27.192 ab	30.371 ab	27.418
Speckled Butter Beans	21.155 bcd	21.696 bcdefghi	23.161 defgh	22.004
Cranberry Beans	22.782 abcd	21.592 cdefghi	20.784 fghij	21.719
Great Northern Beans	20.521 cd	18.577 i	15.695 k	18.264
Dark Red Beans	21.068 bcd	20.153 fghi	20.416 ghij	20.546
Fiber Control	23.320 abcd	23.320 bcdefghi	23.320 defgh	23.320
Soybean Control	26.478 a	26.478 abcd	26.478 bcde	26.478
Meat Control	25.132 abc	25.132 abcdefg	25.132 cdef	25.132

Figure 44: Kramer Results in KG Shear Force for Sausage Patties
Data within a replacement level with the same letter are not different (P>0.05).
Coefficient of Variation=0.09 (35%), 0.14 (42.5%), 0.18 (50%), 0.12 (Average)

3.4.5 Nutritional Profiles

Based on physical property measurements, the Light Red Kidney Bean was used to calculate nutritional values since they offered ideal cook loss reduction, matched color, and resulted in ideal texture range. However, any pulse would offer similar nutritional profiles do to their being low fat and high in fiber. Light Red Bean meat patties were analyzed at 35%, 42.5% and 50% pulse substitution and the control patties were analyzed based on a 4oz patty as seen in Figure 45. As bean percentage increased there is a reduction in kcal, fat, saturated fat, and cholesterol than the control meat patties.

Nutritional Facts	Control Patties-Average	35% Light Red Bean Patty	42.5% Light Red Bean Patty	50% Light Red Bean Patty
Serving size	1 patty (112g)	1 patty (112g)	1 patty (112g)	1 patty (112g)
Calories	287	250	240	230
Calories from Fat	207	144	117	108
Total Fat	23g	16g	13g	12g
Saturated Fat	9g	7g	6g	4.5g
Cholesterol	77mg	50mg	45mg	40mg
Sodium	75mg	55mg	50mg	45mg
Total Carbohydrates	1g	11g	14g	16g
Dietary Fiber	1g	5g	6g	7g
Sugars	0g	1g	1g	2g
Protein	29g	17g	16g	15g

Figure 45: Nutritional Information for Beef formulated Patties

Similarly, 2 ounce control patties and Light Red Bean sausage patties at 35%, 42.5% and 50% pulse substitution were analyzed as seen in Figure 46 below. Again the bean substituted

sausage patties contained few calories, less fat, saturated fat, and cholesterol than the control sausage patties.

Nutritional Facts	Control Patties-Average	35% Light Red Bean Patty	42.5% Light Red Bean Patty	50% Light Red Bean Patty
Serving size	1 patty (57g)	1 patty (57g)	1 patty (57g)	1 patty (57g)
Calories	140	120	120	110
Calories from Fat	90	60	50	45
Total Fat	10g	7g	6g	5g
Saturated Fat	3g	2g	1.5g	1.5g
Cholesterol	40mg	25mg	25mg	20mg
Sodium	280mg	190mg	170mg	150mg
Total Carbohydrates	1g	6g	7g	8g
Dietary Fiber	0g	2g	3g	3g

Figure 46: Nutritional Information for Sausage Patties

3.5 DISCUSSION

The results from the cook loss experiment show the benefits of the fiber from the pulses in the meat patties[17,18]. As the fiber level of the patties increased (highest in the patties with the addition of pulses, followed by the textured soy protein, then the rice bran and lowest in the 100% GB patty), the cook loss was reduced in both the meat patties and sausage patties. Past research shows that as fat percentage increases, cook loss increases since the proportion of moisture lost is much less than the proportion of fat lost[19,20,21,22]. Additionally, research has shown that adding fiber to meat patties increases cooking yield and water holding capacity[20,23]. By substituting hydrated pulse fractions, we reduced the percentage of fat and increased fiber in the final patty; therefore we were able to reduce cook loss in the test patties significantly.

The spectrophotometer results for color differentiation of the meat patties show that the pulses affected the a* value the greatest for the raw meat patty and the b* for the cooked meat patties. The sausage patties had the greatest difference in L* values for both the raw and cooked sausage patties. The obvious difference in the results has to do with the two sets of patties, beef verse pork. In the raw set, we see the greatest differences in a* and L* values due to the hydrated pulse fractions having a greater color difference against the raw beef or pork since 15 to 17% fat is barely noticeable in ground meat[24]. However, when comparing the test and control cooked patties, the patties had the greatest differences in b* and L*. This is different from the results obtained from Mansour and Khalil. In their 1999 publication, they stated that fiber increased a* values and lowered b* values[23]. Our results show that the addition of pulses positively affected both values. The difference may arise from our use of pulses with the seed coat attached and not the addition of a pure fiber as seen in their publication.

The Kramer shear force values from this study showed that as fat decreased and fiber increased in the patties, there was a reduction in kilograms of shear force values, possibly due to the increase in water holding capacity of fiber over protein. This agrees with published literature which says that as fat is decreased (to a certain level) or fiber is increased, there is a decrease in shear force due to water holding capacity of the patties[19,20,21,23].

In the course of this research, it has been determined that by using specific pulses at the correct ratio to meat, a meat and analog hybrid can successfully be developed to meet the

needs of today's consumers (health, appearance, juiciness, and tenderness)[25]. By using pulses, the overall nutritional profile can be improved by reducing calories, total fat, saturated fat, cholesterol, and increasing fiber. Therefore, this information gives rise to the questions of consumer acceptability and clinical analysis to determine if the improved nutritional profile is in substantial enough quality to see an improvement in health.

3.6 Conclusion

Many consumers, as well as many food service operations such as the National School Lunch Program and National School Breakfast Program, survive on a very fixed budget. Pulses are commonly consumed around the United States and the world for many different reasons but predominately for their taste and low price. Therefore, the development of a successful meat and sausage patty made using a partial replacement with pulses offers the possibility to reduce cost and improve nutrition, through increased vegetable consumption. Additionally, the reduced cook loss and shear values demonstrate a juicier patty. This would allow for food service professionals to ensure that they can meet the quality expectations of their consumers without having to worry about exceeding price points. This research shows that the pulse extended patties exceed the quantitative quality parameters of the control patties but, a full scale consumer panel will be needed to compare the patties on taste.

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CHAPTER 4: SENSORY ANALYSIS OF A MODIFIED MEAT PATTY MADE USING HYDRATED LIGHT RED BEAN FRACTIONS

4.1 Abstract

Patties were formulated using either 100% ground beef or a combination of ground beef and hydrated light red bean pulse fractions. Both patties were prepared and microbiological tested for both total *Escherichia coli* and salmonella species counts in order to ensure safety. The patties were first tested against each other using two consumer focus groups composed of a total of 34 seventh grade children aged 11-13 years old. The patties were served in buns with ketchup, mayonnaise, and/or mustard. The recommendations from the focus group (addition of pickles and cheese) was used to test the two patties in a consumer study for both liking and difference. The 76 consumer panelists aged 5-13 years old (average age of 9 years old) rated the two patties using a 5-point hedonic scale for overall liking, tenderness, and juiciness. Panelists found significant differences between the two different patties for overall liking; however, panelists failed to determine difference of the two patties using a same-different test. Therefore, the two patties were deemed not different, and the cost reduced and nutritionally improved hydrated light red bean fraction patty could be implemented at the USDA National School Lunch Program.

4.2 Introduction

In the 1960s and 1970s only 5 to 7 percent of U.S. children were obese. Today, 17 percent of children are obese. Additionally, obesity is now the second leading cause of death in the United States[1]. Unless this epidemic is successfully corrected, life expectancy will begin to decline[2]. Not only do obese individuals have shorter life expectancies, but their quality of life is also

compromised as they are more likely to suffer from diabetes, kidney failure, stroke, breast and colorectal cancer, osteoarthritis and depression[3].

Obesity that begins in childhood is linked to a variety of psychological problems, asthma, diabetes and early onset cardiovascular risk factors. Because many obese children grow up to become obese adults, childhood obesity is strongly linked to an increased lower age mortality and morbidity[4]. Additionally, obesity disproportionately affects certain racial and ethnic minority groups in both child and adult populations. Therefore, it underlies many of the health disparities facing our nation.

The East Baton Rouge Parish Public School System (EBRPSS) is the largest school district in the state and among the top 75 nationally in student enrollment. Seventy-one percent (over 45,000) of all students enrolled in pre-kindergarten through grade 12 in the parish are enrolled in public schools. Of these 71%, African American/Non-Hispanic Blacks make up 79.5%, Caucasians/Non-Hispanic Whites are 16.1%, and all other ethnicities combined are 4.4%. Additionally, 69.4% of all public school students receive free lunches, with an additional 6.7% receiving reduced price lunch (no more than $0.40 per meal)[5].

Being a predominately low socio-economic student population, the risk of being overweight or obese is greatly increased. This is partially due to the increased consumption of refined grains and added fats in the diet of those with a lower socioeconomic status[6]. Additionally, food deserts and the increased prevalence of fast food restaurants has created diet patterns and

preferences for the less nutritious food items frequently served[6]. Therefore, the objective of this research was to test if consumers (aged 5-13 years old) accepted and could differentiate between 100% ground beef patties and a cost reduced nutritionally improved meat patty made using hydrated light red bean fractions that was previously developed by Holliday et al (2011)[7].

4.3 Materials and Methods

4.3.1 Pulses

Light red beans were donated by Archer Daniel Midland (ADM, Decatur, Il.) Beans were carefully examined to remove stones and other debris. The cleaned pulses were hydrated by adding 2:1 tap water to beans and held overnight (about 18 hours) at 40 °F (4.5 ˚C). The hydrated beans were drained and ground (3/16" plate, KitchenAid Food Grinder Stand Mixer Attachment, Professional 600 Stand Mixer, KitchenAid, St. Joseph, MI) to produce the hydrated light red bean fractions (HLRBF).

4.3.2 Patty Forming

Both patties were prepared using 80/20 ground beef (GB) purchased from a local grocery store (Baton Rouge, LA). The control sample was prepared using 100% GB while the test sample were prepared using 42.5% HLRBF and 57.5% GB. The samples were formed into 113.4 g patties using a Hollymatic Model 200-U Patty Machine (Hollymatic, Countryside, IL). Patties were frozen in stacks of three interleaved with patty paper at -150 ˚C in a cryogenic freezer until reaching an internal temperature of -25 °C. Temperature data probes were used to monitor the internal temperature of the patties every second during the freezing process.

4.3.3 Microbiological Analysis

4.3.3.1 *Escherichia coli* (E. coli) Testing

Both the test and control samples were tested for total E. coli by the Louisiana State University Food Microbiology Laboratory. The raw patties were diced and 25 g of the sample was added to 225 g of phosphate buffered saline (PBS). The mixture was homogenized and 1 ml of the liquid portion was diluted in 9 ml of PBS. Decimal dilutions were performed as needed before dispensing 1 ml of dilution onto the Petrifilm (3M Corporation, St. Paul, MN). Samples were tested in triplicate with duplicates of a homogenized sample of each patty. The samples were incubated 24-48 hours at 37 °C and counted. Colonies of E. coli produced gas and were blue to red-blue in color.

4.3.3.2 Salmonella Testing

Both samples were tested for total salmonella by the Louisiana State University Food Microbiology Laboratory. The raw patties were diced and 25 g of the sample was added to 225 g of phosphate buffered saline (PBS). The mixture was homogenized and 1 ml of the liquid portion was diluted in 9 ml of PBS. Decimal dilutions were performed as needed before dispensing 1 ml of dilution onto a *xylose lysine deoxycholate* (XLD) 3M Petrifilm plate. Samples were tested in triplicate with duplicates of a homogenized sample of each patty. The samples were incubated 24-48 hours at 37 °C and counted. Salmonella colonies were identified by a black center.

4.3.4. Sensory Analysis

Following the procedure of Holliday et al. (2011), the 42.5% hydrated light red bean fraction meat patty was chosen for testing because it closely mimicked the 100% GB control in color and sheer force while greatly improved the cook loss and shrinkage results. This choice was also based on previous research showing that appearance had more influence than taste for meat substitutes[8]. Additionally, the improved cost and nutritional profile showed promise for use in school lunch programs.

4.3.4.1 Focus Group

The study was conducted at McKinley Middle School, Baton Rouge, Louisiana with 34 seventh grade panelists to determine the attributes that they find important in a hamburger product. Institutional Review Board (IRB) permission was obtained through an application for exemption from the Louisiana State University Agricultural Center (Exemption number HHE0925).

Samples were prepared following the procedure listed earlier. The patties and buns were split in half and transported to the middle school in an insulated food transport carrier (Cambro USA, Huntington Beach, CA). White whole wheat hamburger buns were donated from Flowers Bakery (Flowers Foods, Thomasville, GA).

The panelists were provided a parental consent form (Appendix 1) two weeks prior to the study and all participants were required to obtain parental/guardian consent before participating. The consent form excluded any participation if a subject was allergic or opposed to eating to

wheat, beef or legumes. The consent form also asked if the study participants were also participants in the National School Lunch Program. On the day of the consumer study, the panelists were asked to complete a panelist research assent form (Appendix 2) which indicated their willingness to participate in the study. After all forms were collected, the panelists were given a four question survey form (Appendix 3) using a modified 5-point hedonic scale (1=very bad and 5=super good) following Chen and Resurreccion[9]. Once panelists had sufficient time to record their responses, the panelists were asked to verbally share their answer with the rest of the group; this was done one panelist at a time. After all panelists had responded to the first question, we moved onto the next question and so on. The response sheets were then collected.

The panelists were instructed to pick up two hamburger samples (½ meat patty and ½ bun assembled as ½ a hamburger). The panelists could then pick from any of 3 condiments to dress their hamburger as they normally would when consuming hamburgers (ketchup, mustard and/or mayonnaise). Panelists were then asked to taste their hamburger and provide their feedback (Appendix 4). The forms were then collected and study participants were rewarded with 12 oz bottles of PowerAde (Coca-Cola Company, Atlanta, GA). The process was repeated the following day in the same classroom with a different group of students. The first group had 15 participants and the second group had 19 participants.

4.3.4.2 Consumer Panels

The study was conducted at several East Baton Rouge Parish Park Summer Day Camps in Baton Rouge, Louisiana with a planned 150 third through fifth grade panelists over the course of 5

days to determine which patty they preferred in a hamburger product and if they could determine difference between the two. Institutional Review Board (IRB) permission was obtained through an application for exemption from the Louisiana State University Agricultural Center (Exemption number HHE0925).

Patties were prepared following the same procedure listed earlier. The patties and buns were split in half and transported to the consumer test site in an insulated food transport carrier (Cambro USA, Huntington Beach, CA). The hamburger buns were donated from Flowers Bakery (Flowers Foods, Thomasville, GA) and were either 100% white whole wheat for the control or 70% white whole wheat and 30% bean flour for the test sample. The panelists could then pick from any of 3 condiments to dress their hamburger as they normally would when consuming hamburgers (ketchup, mustard and/or mayonnaise) as well as cheese and pickles (based on feedback from the focus group).

The panelists were given a parental consent form (Appendix 5) two weeks prior to the study and required to get parental/guardian consent before participating. The consent form excluded any participation if a consumer was allergic or opposed to eating to wheat, beef or legumes. The consent form also asked if the study participants were also participants in the National School Lunch Program. On the day of the consumer study, the panelists were asked to complete a panelist research assent form (Appendix 6) which indicated their willingness to participate in the study. After completion of the both taste tests, the forms were then collected and participants were again rewarded with PowerAde.

4.3.4.2.1 Acceptance Testing: The plan for the testing was explained to all participants and they were introduced to members of the research team. They were encouraged to ask for help if they had any questions. Panelists were provided a record sheet and two randomly coded ½ hamburger set ups (1 test and 1 control) following a balanced block design. The panelists were asked to rate each of the two samples on three attributes: how much they liked the sample overall (overall liking), how much they liked the juiciness of the sample (juiciness), and how much they liked the tenderness of the sample (tenderness). A 5-point hedonic scale was used to rate the attributes (1=very bad and 5=super good).

4.3.4.2.2 Difference Testing: The panelists were then given a second a set of two randomly coded ½ hamburger set ups (either two like or two different samples) and asked to determine if the samples were same or different and mark their results on the same form.

4.3.5 Statistical Analyses

The statistical analysis of the data from the sensory analyses was completed using Statistical Analysis Systems statistical software package version 9.1.3 (SAS Institute, Cary, NC, USA). All data was analyzed for analysis of variance (ANOVA) and standard deviation (STD). An alpha of 0.05 was used to maintain a confidence interval of 95%.

4.4 Results

4.4.1 Patty Forming

By monitoring the temperature of the patties during the cryogenic freezing cycle, it was determined that the patties were fully frozen (0 °C) in 125 seconds or about 2 minutes as seen in Figure 47 below.

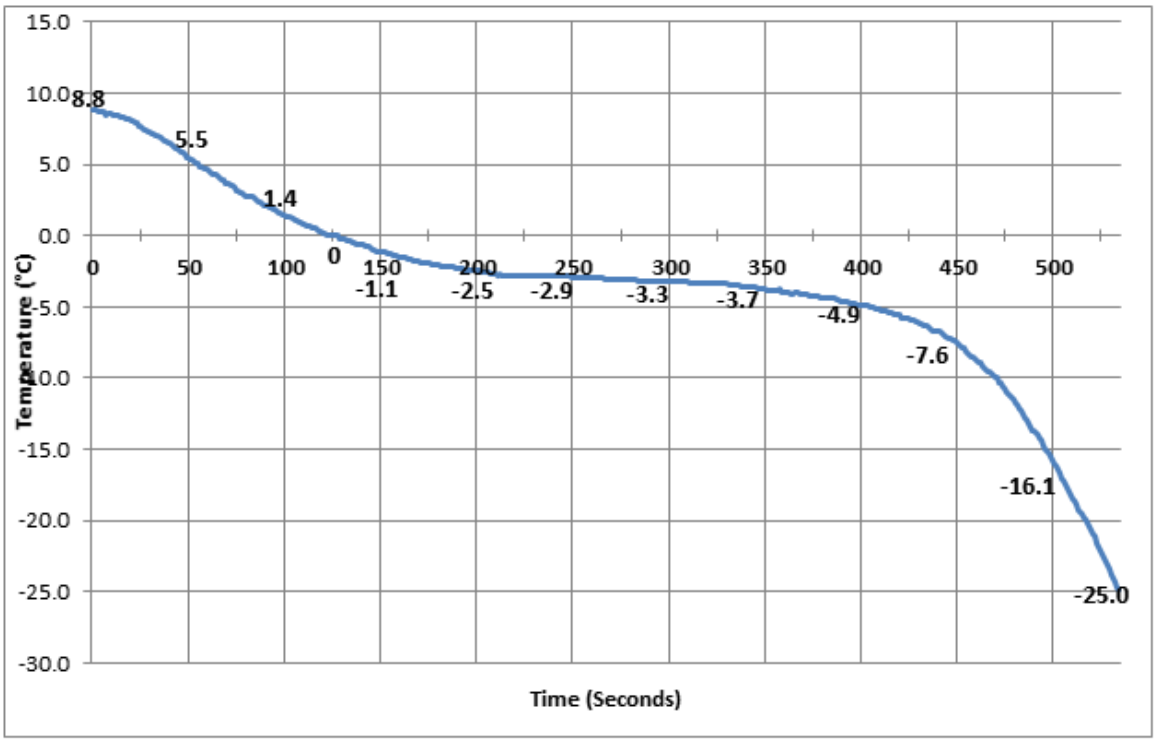

Figure 47: Average Temperature of Patties during Freezing

The patties spent a little under 8.5 minutes in the chamber freezer going from an initial temperature of 8.8 °C down to -25 °C.

4.4.2 Microbiological results

Microbial contaminations are a concern with all manufactured foods, but raw meat products carry particular concerns of coliforms, E. coli, and salmonella. Coliforms are present in meat and are almost impossible to completely eliminate, especially for ground meat. For coliforms, higher levels indicate greater concerns associated with processing. Total coliforms are related to fecal contamination but do not include all aerobic bacteria. The USDA regulations for boneless and ground meat are absence of *E. coli* and Salmonella and less than 1,000 CFUs/gram. As seen in Figure 48 below, the 100% beef patty sample is under 500 CFUs while the light red bean patty is slightly above, with no detectable Salmonella nor E. coli defining the products as acceptable.

100% Beef Control Patty:	42.5% Light Red Bean Patty:
Salmonella: No Detectable Levels	*Salmonella*: No Detectable Levels
E. coli: No Detectable Levels	*E. coli*: No Detectable Levels
Total coliforms: 440 CFU/g	*Total coliforms*: 560 CFU/g

Figure 48: Microbial Load of Patty Samples

4.4.3 Sensory

4.4.3.1 Focus Group

The 34 member focus group composed of 7th graders revealed the attributes that middle school students in Baton Rouge, Louisiana find important in a hamburger product. The students felt strongly about the additional inclusion of strong flavors from seasonings, cheese and condiments in a hamburger product as seen in Figure 49 below.

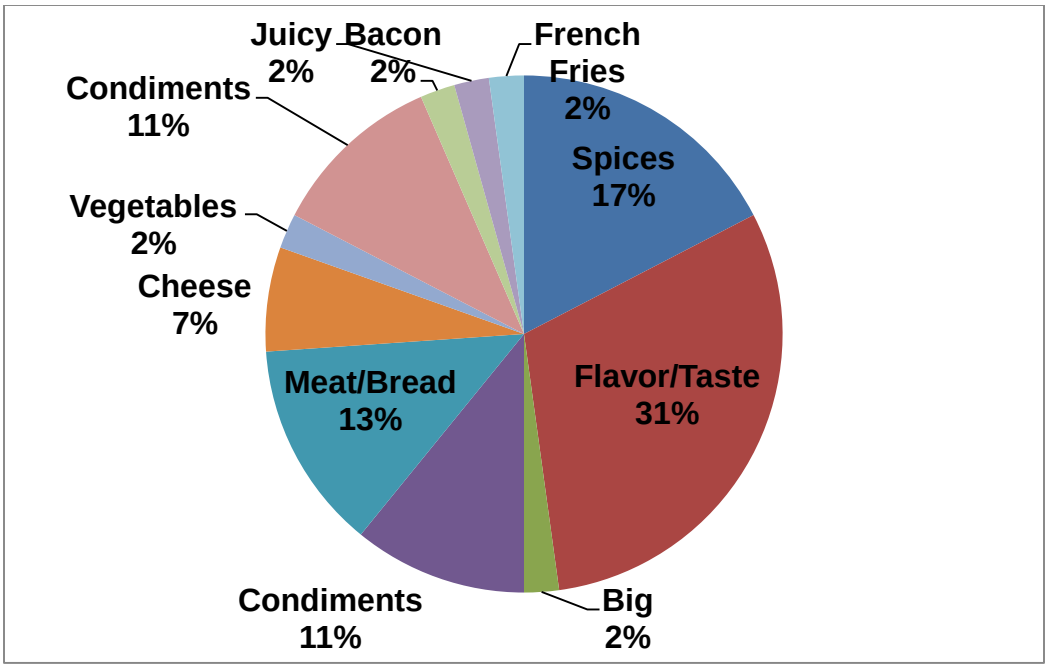

Figure 49: Results for "What do you like about hamburgers?"

After presenting the students with a meat and bean patty, students in both groups commented that both of the patties needed more seasoning and suggested using a local Creole seasoning made from mostly salt and cayenne pepper. Additionally, similar response levels for changing the flavor, juiciness, and seasoning profiles in both patties can be seen in Figure 50. However, it is important to note the "no change" percentages for the control and the test hamburger. There is a difference between the control and the test product (31% vs. 8%) and this can be attributed to a lack of flavor, juiciness or other attributes.

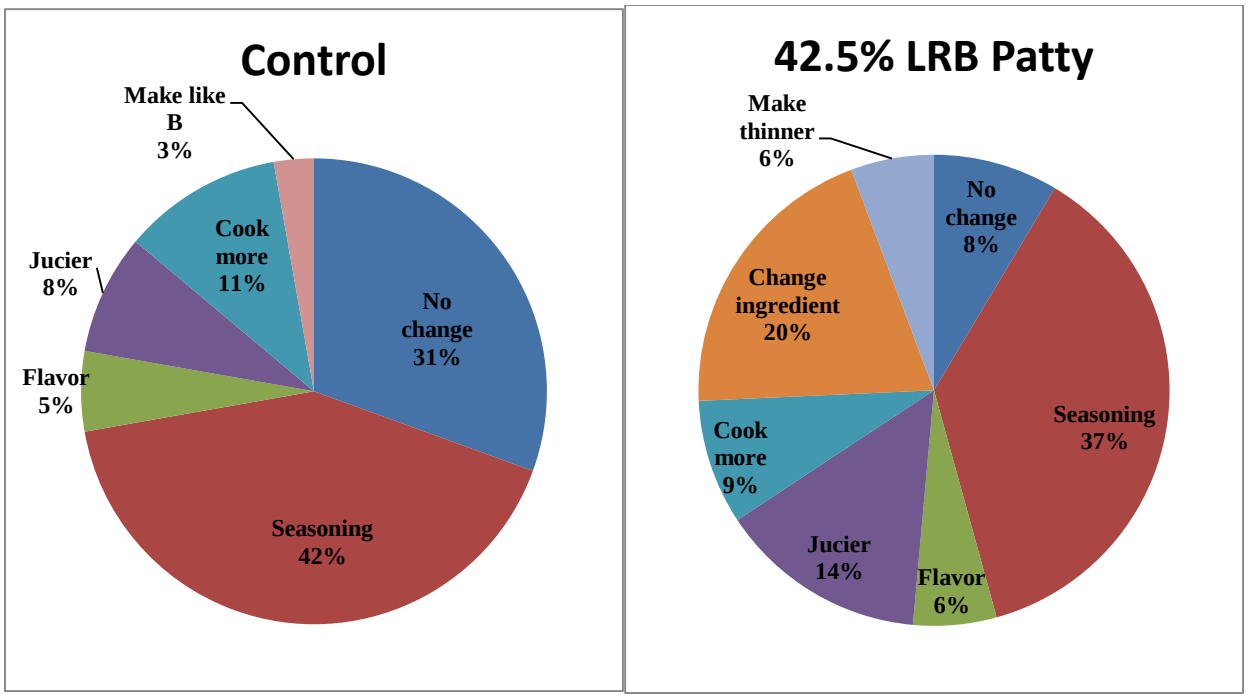

Figure 50: Results for "What should be changed?"

4.4.3.2 Consumer Tests

4.4.3.2.1 Preference Testing: The study had originally planned to use 150 elementary and middle school children as test panelists. However, after the first two days of testing, it was reported that some children (8 total) had some stomach discomfort the afternoon following testing. Therefore, the research was immediately discontinued. Therefore, the data from the 76 panelists who had completed the study was used. It was our belief that the discomfort was a result of gas production in the gut due to the increase of beans in the diet for the students. A population of panelists who eat oligosaccharide rich foods would be needed to prevent this from happening again.

The panelists were comprised of 56% male sand 46% females between the ages of 5-13 with the average age of 9 years old. As seen in Figure 51 below, the panelists ranked the control patty higher in overall liking but there was no significant differences in juiciness and tenderness.

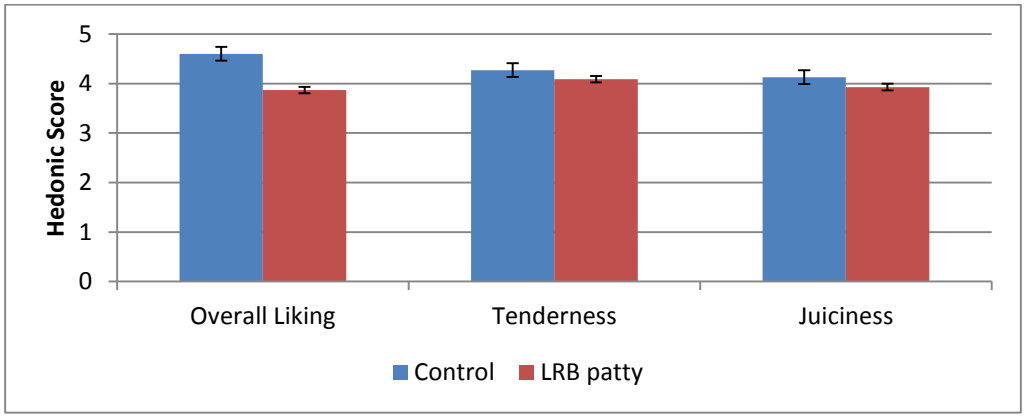

Figure 51: Hedonic Ranking of Patties

4.4.3.2.2 Difference Testing: While the panelists had a significantly higher overall liking score for the control compared to the LRB patty (4.6 vs. 3.87). This may not directly mean that panelists prefer one sample over the other since they could not distinguish between the two patties ($p=0.05$) when compared side by side. Of the 72 panelists, only 28 gave correct responses while 66% of the panelists had incorrect responses.

4.5 Discussion

By quick freezing, bacterial growth was controlled. While E. coli is a coliform, having coliforms does not mean you have E. coli in your product. It is a fecal contaminate and a sign of contamination during processing. Additionally, salmonella is mostly found in poultry and thus it

is not related to cattle farms where you are most likely to find coliforms and E. coli, so it is used specifically as a sign of contamination.

Berry stated in his 1993 publication that faster freezing increased fat retention during cooking and is necessary for ensuring tenderness in low-fat ground beef. Previous research has also shown that beef patties of 10% or lower fat content had lower juiciness ratings that those of 20% fat. However, another study demonstrated that juiciness scores can be misinterpreted since low fat patties result in greater initial juice release while traditional 20% fat patties had sustained juice release patties[10]. Following the recommendation to ensure tenderness and juiciness, the samples were frozen as quickly as possible and the sensory results showed no difference between the control and reduced fat test samples containing the HLRBF. This information helps to interpret the results of this study since no difference was determined between the samples by the panelists. Other studies testing the addition of soy okara in meat patties showed that the addition of up to 7.5% soy okara had better sensory results in all categories (appearance, flavor, juiciness, tenderness, and overall acceptability) than the lean 10% fat patty and only slightly reduced sensory scores to the 20% fat patty[11]. While there was not a boost in sensory rating, the addition of HLRBF into the meat was at a much higher level than the soy okara and no difference was determined for tenderness and juiciness scores. The previous research along with this research has shown promise to improving both quality and nutritional content in meat patties.

The focus group specifically asked for creole seasoning to improve the flavor but this may be a regional preference for salty and spicy food. The results from the panelist can serve as guidance for future research and market testing. However, the consumer panel ran into a specific problem/concern. With children getting stomach aches, the research team decided that further research would need to use a reduced amount of pulses in the beginning before slowly dialing up the percentage of the HLRBF. However, with the data generated there was a small difference between the overall liking scores but not the juiciness and tenderness. But when asked to differentiate between the samples, the panelists were not able to determine a difference. This is not uncommon trend and shows that the slight differences in overall liking can be muted in terms of comparison. The term for this relationship is equivalence testing and it recognizes that two products can be perceptually different and yet still be similar enough to each other to be used interchangeably or statistically not different[12,13]. This is further demonstrated when the two samples (control and test) were not significantly different in juiciness and tenderness liking scores.

4.6 Conclusion

Obesity is an important childhood concern to correct but most children have developed into picky eaters. Therefore, foods need to be developed that are improved nutritionally while meeting the satisfaction in terms of taste and flavor profiles and in an approachable form, such as meat patties. These results show promise for moving forward into a larger consumer test and possible market trial for a HLRBF modified patty as well as other food options containing hidden nutrition.

4.7 References

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CHAPTER 5: USING A C. ELEGAN AND SYRAIN HAMSTER MODEL TO DEMONSTRATE THE BENEFIT OF A MODIFIED MEAT PATTY MADE USING HYDRATED PULSE FRACTIONS

5.1 Abstract

Beef is the most widely consumed protein in the United States with nearly 5 billion pounds sold in 2009. Light Red Beans (LRB) represent a lower cost source of good quality protein. A 3oz. serving of ground beef (GB) delivers zero fiber, 3.5g of saturated fat and 50mg of cholesterol. LRB provide fiber, are very low in fat, and have been shown to significantly reduce serum cholesterol levels. The objective of this research was to determine the health impact of a modified meat patty (MMP) verses a control diet (CD) composed of 20% fat, 20% protein, 5% fiber, 4.8% supplement, and 50.2% starch using Syrian hamsters. The diets compared to the CD were 25% LRB, 25% GB, 50% LRB, 50% GB, 25% MMP (12.5% LRB + 12.5% GB), and 50% MMP (25% LRB + 25% GB). The LRB, GB, and MMP were baked in an oven at 305^{o}F for 20 minutes before freeze-drying. The diets were prepared in advance and kept refrigerated until feeding. The hamsters were fed for four weeks with weekly measurements of weight gain. After necropsy, organ weights and blood lipid levels were measured. All non-CD diet hamsters resulted in higher finished body weights. Hamsters on LRB or MMP diets had reduced LDL and VLDL averages of 22.7 and 8.1 mg/dL respectively compared to the CD. Additionally, average HDL:LDL ratios for the MMP and LRB diets increased from 1.47:1 for the CD to 1.9:1 and 2.2:1 respectively. Hamsters on CD and LRB diets had lower liver weights and reduced epididymal adipose weight compared to diets containing MMP or GB. The results suggest partial substitution of LRB in GB can have significant impact on cholesterol levels and visceral fat deposition due to synergism between sat fat and cholesterol in the diet.

5.2 Introduction

Animal studies are used as model systems to demonstrate the impact of diet changes for the purpose of better understanding the disease process without the added risk of harming actual human participants. The animal chosen should meet a determined taxonomic equivalency to humans, so as to react to disease or its treatment in a way that resembles human physiology. *Caenorhabditis elegans* (C. elegans) are microscopic nematodes (see Figure 52 below) that are widely used in biological sciences.

Figure 52: Caenorhabditis elegans (C. elegans):[1]

The organism is a useful model system because of its short life span (approximately 18-21 days), it is inexpensive for testing, and its entire genome has been sequenced. A comparison between the genome project for C. elegans and humans has shown similar conserved neurotransmitter receptors, and neurotransmitter synthesis and release pathways. Additionally, humans and C. elegans share 22 gene families[2]. Recently it has been used for metabolic and nutrigenomic studies since most of the pathways involved in energy homeostasis are similar to human pathways. Furthermore, C. elegans are useful in understanding the biochemistry of nutrient interactions and obesity because of their insulin-like pathway that

regulates glycogenesis, lipogenesis, and lipid homeostasis. A disruption in this pathway results in a disturbance in longevity, reproductive development and metabolism. Additioanlly, the release of serotonin in C. elegans controls fat deposition and feeding behavior[3].

A C. elegans model was jointly developed between Louisiana State University's (LSU) Food Science Department and LSU's Nutrition Department for assessing the biological effects of food ingredients by looking at travel distance, pumping rate, and internal fat deposits in C. elegans. Travel distance is defined as the mean distance traveled across the NGM agar plates. C. elegans have the ability to locate their food and determine if it is a high quality food source based on its nutritional value. They tend to "dwell" when there is a high concentration of food and tend to "roam" when food is scarce[3,4]. Pumping rate is defined as the oscillatory movement at the terminal bulb of each organism's pharynx. Pumping rate in the terminal bulb of the pharynx is a direct correlate of the feeding rate of the organism. This rate is highly correlated to aging and it is proven be higher at a younger age and lower at a more advanced age. An ideal pumping rate for *C. elegans* in the first five days of life may be as high as 250-300 pumps per minute. As they age, the ideal pumping rate decreases to around 150-240 pumps per minute. As the *C. elegans* reach the end of their lives an ideal pumping rate would be 100-150 pumps per minute. A higher pharyngeal pumping rate in these ranges may correlate to increased longevity.[3,4]. Previous research utilizing this method evaluated 9 different pulses for their effects on lifespan. The results from the study showed that travel distance was not significantly different between treatment and control groups. Therefore, C. elegans showed a similar preference for the E. coli diet and the E. coli bean blend. The study also showed that the great northern bean, cranberry

bean, lentil and dark red kidney bean had a significant increase in pumping rate throughout the study compared to the control E. coli diet. This suggests improved longevity as C. elegan activity is a measure of health. Therefore, the increased pumping rate found the in C. elegans with the beans fortified diet showed improved overall health and reduced markers of aging. In addition, a decrease in fat deposition was seen in the C. elegans fed cranberry bean, black bean, light red bean, navy bean and white kidney bean diets. This research furthers the understanding that fat deposition is not solely dependent on feeding rate or amount consumed and can include a vast number of hormonal triggers. Other studies have also noted the fermentation of oligosaccharides into short chain fatty acids and lower intestinal fat deposition. This agrees with the results of the C. elegen bean feeding study. The results suggest the benefits of sustained lifespan and decreased fat deposition in C. elegans when fed a bean based diet[4,5].

The golden hamster or Syrian hamster, *Mesocricetus auratus*, is a member of the subfamily *Cricetinae*. The Syrian hamster has a short stocky body 15 to 20 cm long, with a lifespan of two to three years. Adult hamsters weigh from 110 to 140 g, with females slightly larger than males. Hamsters are the fifth most commonly used animal in research based on several factors. First, sexually mature female hamsters are ready for breeding every four days and have the shortest gestation period in any known placental mammal of only 16 days. They can produce large litters of 20 or more young, although the average litter size is between eight and ten pups. Second, they have a strong relative freedom from naturally acquired disease. Third, they are susceptible to many pathogens, including human strains. Fourth, their unique anatomic and physiologic features, including their propensity for adipose fat deposition, blood lipids markers

analogous to humans, and temperament, allow for short feeding trials. Finally, their rapid development and short life cycle can be used to showcase lifestyle effects in only a few years[6].

Syrian hamsters serve a good model for visceral fat deposition, similar to the C. elegans, as well as serum cholesterol models. This is mostly due to the use of high saturated fat and cholesterol diets to promote atherosclerosis in these animals and the resulting atherosclerotic lesions are similar to those found in humans. Additionally, the hamsters, like humans, take up approximately 80% of LDL-C via the LDL receptor pathway which serves as a reference for diet triggered serum cholesterol. Therefore, Syrian hamsters can be used to further demonstrate the impact of light red beans when included as part of the high beef consuming western diet.

5.3 Materials and Methods

5.3.1 Light Red Beans

Light red beans were donated by Archer Daniel Midland (ADM, Decatur, Il.) The beans were carefully examined to debris. The cleaned pulses were hydrated by adding 2:1 tap water to beans and held overnight (about 18 hours) at 40˚F (4.5˚C). The hydrated beans were drained and ground (3/16" plate, KitchenAid Food Grinder Stand Mixer Attachment, Professional 600 Stand Mixer, KitchenAid, St. Joseph, MI) to produce hydrated light red bean fractions (HLRBF). The HLRBF were then baked on commercial half sheet pans (42cmX29cm) in a Moffat Turbofan 32 Oven (Moffat, Christchurch, New Zealand) at 177°C for 15 minutes to a temperature of 165˚F/74°C. The HLRBF were frozen, freeze dried, and ground into a flour.

5.3.2 Ground Beef

Meat patties were prepared using 80/20 ground beef (GB) purchased from a local grocery store (Baton Rouge, LA). The patties were formed into 113.4g patties using a Hollymatic Model 200-U Patty Machine (Hollymatic, Countryside, IL) before baking on commercial half sheet pans (42cmX29cm) in a Moffat Turbofan 32 Oven (Moffat, Christchurch, New Zealand) at 177°C for 15 minutes (the HLRBF reached an internal temperature of 165˚F/74°C). The baked patties along with the fat and juice in the baking pan were then frozen, freeze dried, and ground.

5.3.3 Syrian Hamsters

The hamster model was performed by the Processed Foods Lab of the USDA Western Regional Research Center in Albany, CA and was approved by the Animal Care and Use Committee of the Western Regional Research Center.

Male Golden Syrian hamsters (approximately 80g, LVG strain, Charles River, Wilmington, MA) were acclimated and given water and 5001 rodent diet (LabDiet, PMI International, Redwood, CA; protein 239 g/kg; fat 50g/kg; nonnitrogenous substances 487 g/kg; crude fiber 51g/kg; ash 70 g/kg; energy 17mJ/kg; and sufficient amounts of minerals and vitamins for healthy maintenance) ad libitum for one week prior to the initiation of the experimental diets.

5.3.4 Diets

Hamsters were divided into 7 groups of 8 hamsters each. Hamsters were fed diets varying the amount of HLRBF flour (Bn) and beef meal (Bf) as seen in Figure 53 below for 2 weeks with water available ad libitum.

Ingredient	**Control**	**Bn25**	**Bf25**	**BnBf25**	**Bn50**	**Bf50**	**BnBf50**
Fat 20%							
Butter	80.0	60.0	60.0	60.0	40.0	40.0	40.0
Corn Oil	100.0	75.0	75.0	75.0	50.0	50.0	50.0
Fish	20.0	15.0	15.0	15.0	10.0	10.0	10.0
Cholesterol*	1.0	0.8	0.8	0.8	0.5	0.5	0.5
Fiber, 5%							
Cellulose (.95 solids)	52.6	39.5	39.5	39.5	26.3	26.3	26.3
Protein 20%							
Casein	210.5	157.9	157.9	157.9	105.3	105.3	105.3
Diet Additive	0.0	284.1	284.1	284.1	568.2	568.2	568.2
Starch, Balance							
Corn Starch	547.3	410.4	410.4	410.4	273.6	273.6	273.6
Other:							
DL Methionine	3.0	2.3	2.3	2.3	1.5	1.5	1.5
Choline Bitartrate	3.0	2.3	2.3	2.3	1.5	1.5	1.5
Mineral Mix	35.0	26.3	26.3	26.3	17.5	17.5	17.5
Vitamin Mix	10.0	7.5	7.5	7.5	5.0	5.0	5.0
TOTAL WT	***1062.4***	***1080.9***	***1080.9***	***1080.9***	***1099.4***	***1099.4***	***1099.4***

Figure 53: Syrian Hamster Diets
Bn25/Bf25/BnBf25=25% replacement of casein with diet additive or equal combination
Bn50/Bf50/BnBf50=50% replacement of casein with diet additive or equal combination

5.3.5 Body Weight & Feed Intake

The body weights of each hamster were recorded weekly and food intake was measured twice a week. Weights were averaged for each group. After the last weighing, the hamsters were food deprived for 12 hours before being anesthetized with a mixture of isoflurane™ and oxygen. Immediately after the hamsters were euthanized, sample tissues were collected, weighed, and immediately frozen in liquid nitrogen for further analysis.

5.3.6 Cecal pH

Cecal pH was determined by clamping off the cecum and squeezing the contents into an Eppendorf tube. A small amount of DI water was added to make the contents more fluid and the pH was recorded with a pH meter.

5.3.7 Plasma Triglycerides

Blood was collected by cardiac puncture into EDTA rinsed syringes. The blood was transferred to 5 mL polypropylene tubes containing potassium EDTA, mixed on a rocker, then kept on ice until centrifugation at 2,000 x g for 30 minutes at 4ºC. The plasma was aliquoted into Eppendorf tubes and stored at -80ºC for analysis. Plasma lipoproteins were separated, and cholesterol was measured using HPLC with slight modification to the method described by German et al. and Yokoyama et al[7,8]. To prevent oxidation of the analytes, all solvents and reagents were kept iced while deoxygenated by purging with nitrogen before freezing. The 1.0-mL aliquots of plasma were thawed and an additional 100 μL phosphate buffered ascorbic acid (PBA, 200 g/L ascorbic acid, 0.4 mol/L NaH_2PO_4, pH 3.6) was added. The plasma was divided into 500-μL samples for replicates and 250μL of a 0.6 mol/L calcium chloride solution was added to each sample.

Taxifolin (2165 nmol/L in PBA) was added to all plasma samples as an internal standard at 82 nmol/L. The plasma was incubated at 37°C in a shaking water bath containing 100 U sulfatase and 2500 U β-glucuronidase dissolved in 120 μL water. After incubation, the plasma was extracted with 1 mL methylene chloride and 500 μL water, vortexed for 1 min and centrifuged at 4500 × *g* for 10 min at 4°C. The aqueous supernatant was removed, and the remaining portion was extracted again with 750 μL water. The aqueous extracts were mixed and extracted twice with ethyl acetate (first with 2.0 mL, then with 1.5 mL). The combined ethyl acetate extracts were passed through anhydrous sodium sulfate packed into Pasteur pipettes, dried under nitrogen and then redissolved in 20 μL pyridine and derivatized with 30 μL N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) at 65–75°C for 2 hours. Plasma triglycerides were determined by enzymatic colorimetric assays using a Roche Diagnostics/ Hitachi 914 Clinical Analyzer (Roche Diagnostics, Indianapolis, IN) with assay kits.

5.3.8 Fat Deposition

On the day of euthanasia, visceral fat mass was excised (mesenteric, epididymal and retroperitoneal white adipose tissues) and weighed for evaluation of central adiposity. Liver weights were taken to compare fat deposits.

5.3.9 Statistical Analysis

The statistical analysis of the Syrian Hamster feeding data was completed using Microsoft Excel 2010 using the Statistical Functions (Microsoft, Redmond, WA). All data was analyzed for analysis of variance (ANOVA) and standard deviation (STD). An alpha of 0.05 was used to

maintain a confidence interval of 95%. Fisher's least significant difference test was performed alongside ANOVA for determination of the differences among the means.

5.4 Results

5.4.1 Feed Intake

The hamsters gained more weight per kilocalorie of food consumed as the ratio of beef to bean increased. All bean containing diets helped control weight gain. These trends can be seen in the Figure 54 below along with the regression equation (Figure 55) for weight gain with beef.

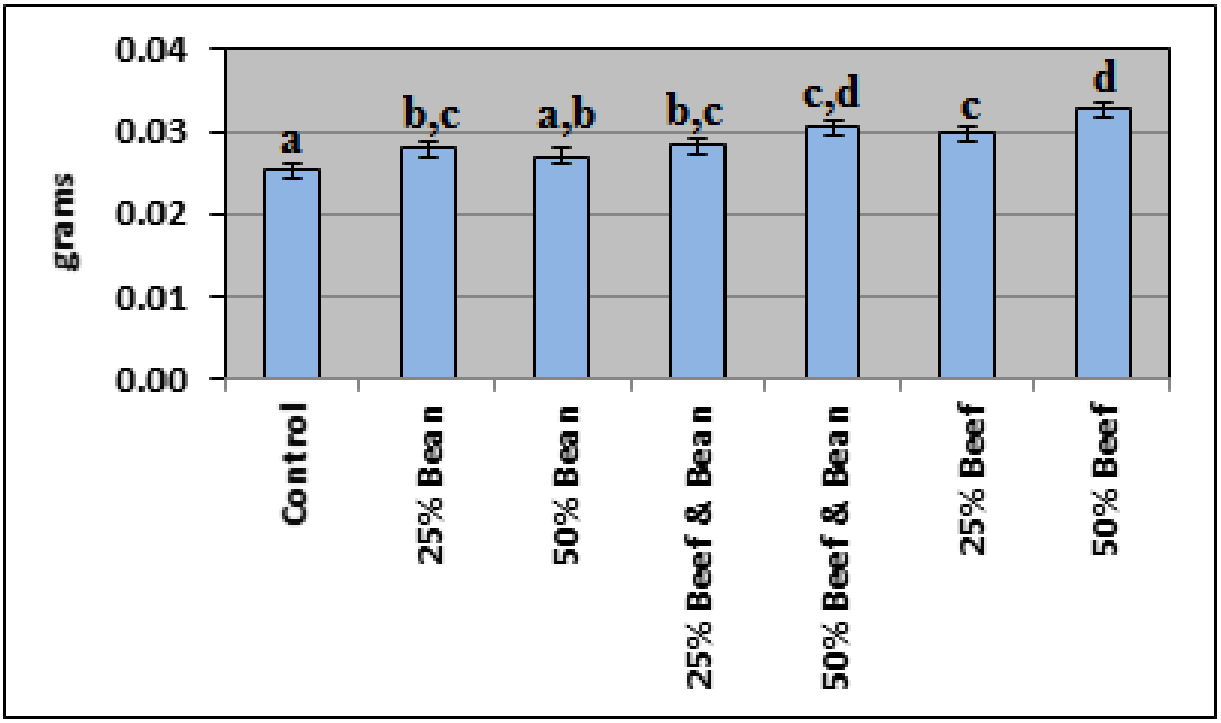

Figure 54: Weight Gain (g) per Kilocalories Consumed
Bars with the same character are not significantly different (P>0.05).

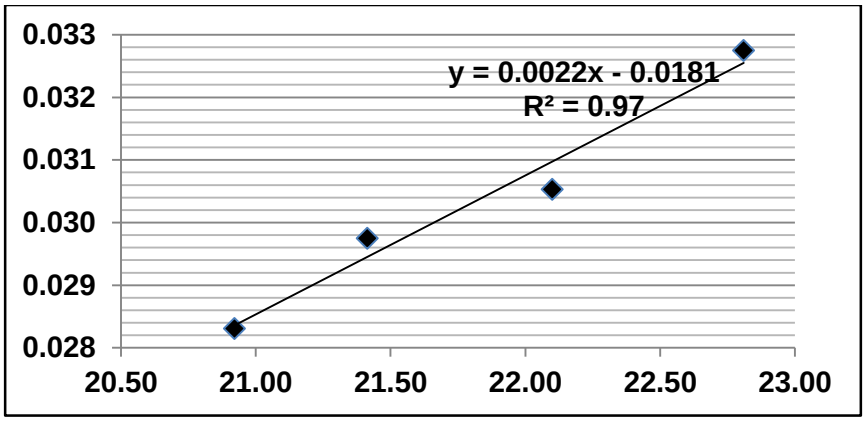

Figure 55: Regression for Weight Gain for Beef Containing Diets

This is further demonstrated by the following two figures (Figure 56 and Figure 57) showing total feed intake over the 18 day study as well as the total calories consumed.

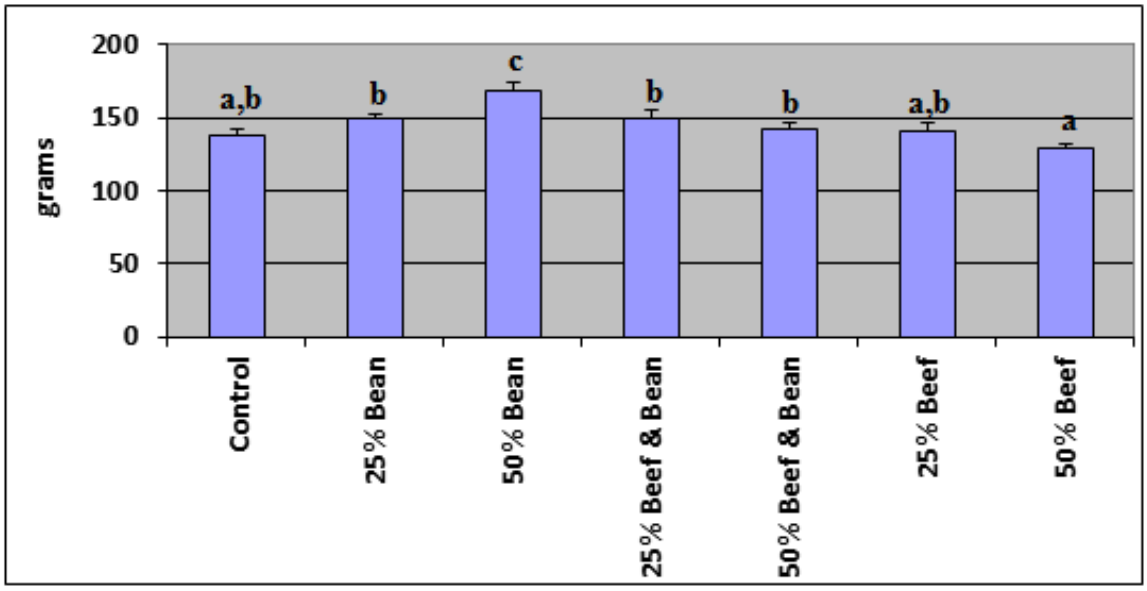

Figure 56: Feed Intake (g) over the 18 days
Bars with the same character are not significantly different (P>0.05).

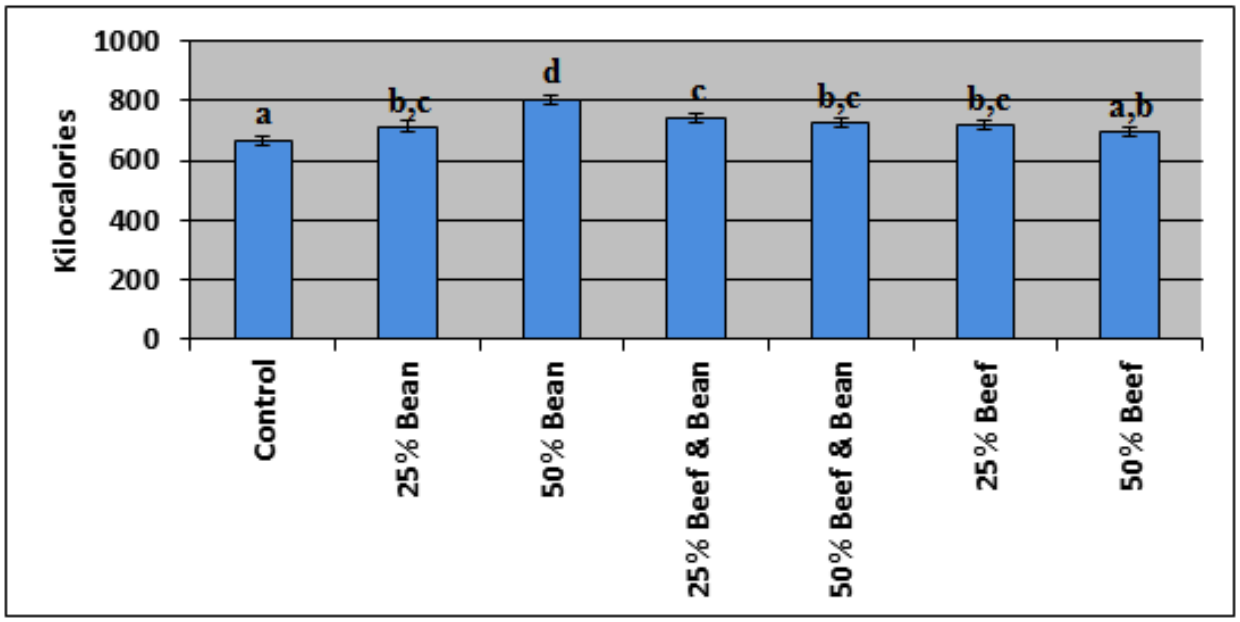

Figure 57: Total Calories Consumed
Bars with the same character are not significantly different (P>0.05).

5.4.2 Cecal pH

In this study, hamsters consuming the bean fortified diets had the lowest cecal pH levels. Additionally, beans reduced the cecal pH level in the blended diets verses the beef fortified diets as seen in Figure 58.

5.4.3 Plasma Triglycerides

When looking at the results in Figure 58 and Figure 59 below, the bean fortified diets lowered both the low density lipoprotein (LDL) and very low density lipoprotein (VLDL) levels. In the blended bean and meat diets, the beans reduced the adverse effects of the beef on the LDL and VLDL levels.

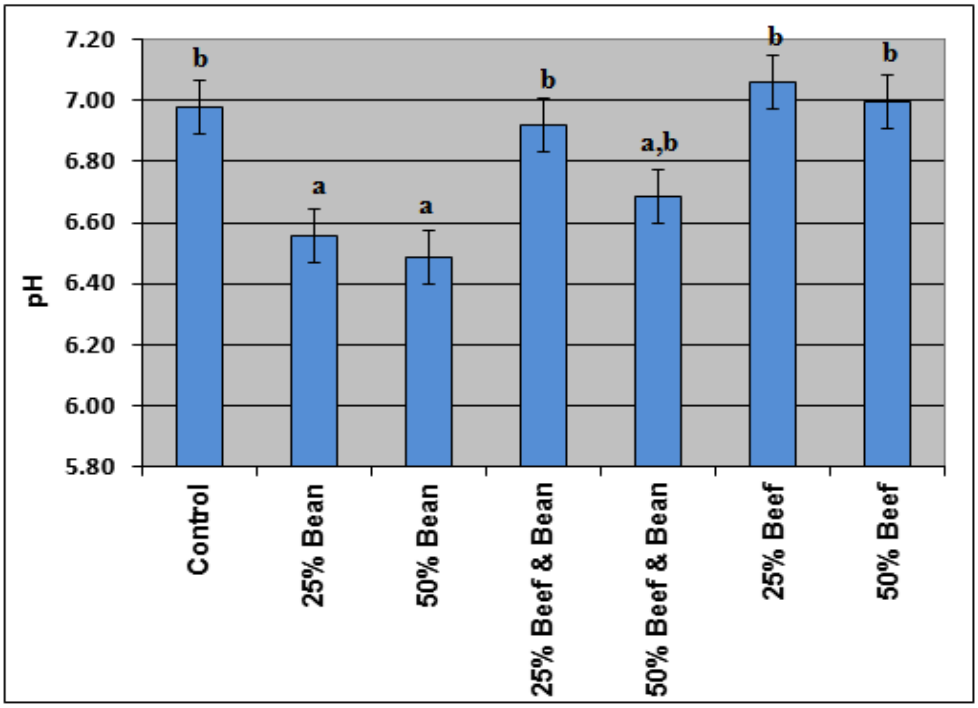

Figure 58: Cecal pH of Hamsters
Bars with the same character are not significantly different (P>0.05).

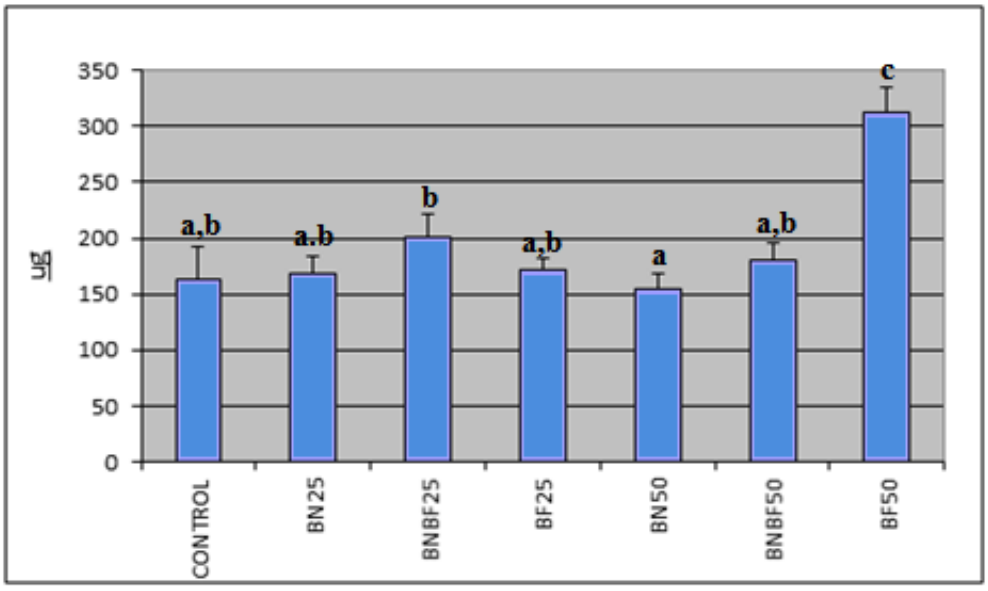

Figure 59:Total Plasma Triglyceride Levels (ug) After Feeding Study
Bars with the same character are not significantly different (P>0.05).

Figure 60 shows that the hamsters on the bean or beef and bean diets had reduced LDL and VLDL averages of 22.7 and 8.1 mg/dL respectively compared to the control diet. Additionally, the average HDL:LDL ratios for the beef and bean and bean diets increased from 1.47:1 for the control diet to 1.9:1 and 2.2:1 respectively. Plasma lipoprotein levels for LDLs and VLDLs demonstrated a dose dependent response for both LDL and VLDL levels with bean containing diets.

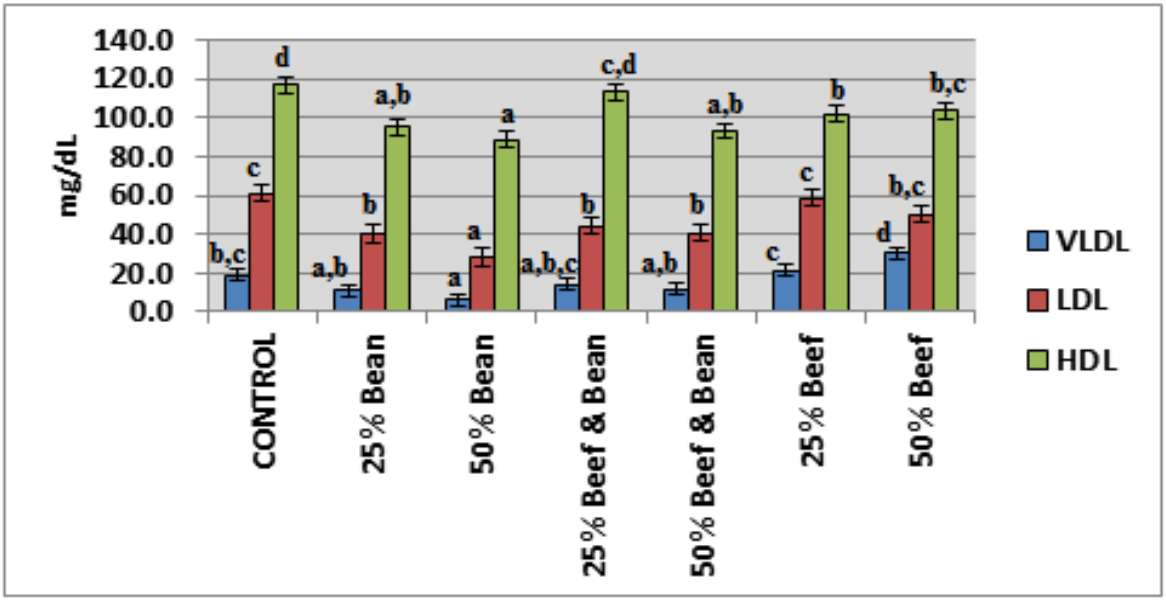

Figure 60: Plasma Lipoprotein Cholesterol Distribution
Bars with the same character are not significantly different (P>0.05).

5.4.4 Fat Deposition

Hamsters on the bean only diets and the 50% blended diet had lower liver weights than the control and all beef containing diets as seen in Figure 61 below.

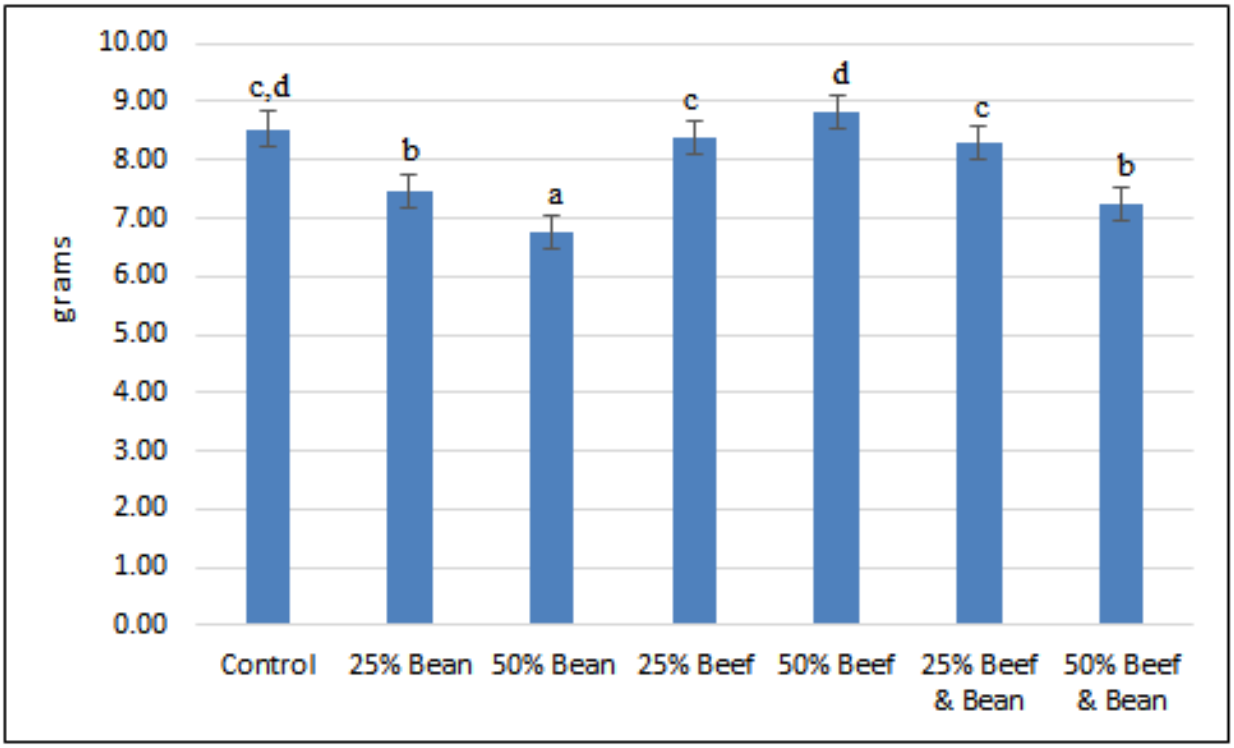

Figure 61: Liver Weights
Bars with the same character are not significantly different (P>0.05).

Reduced epididymal adipose weight was seen at the 50% addition rates when comparing the bean fortified to the beef fortified diet. The blended diet showed a reduction in epididymal and retroperitoneal adipose weight compared to the beef fortified diet but not as low as the bean fortified diet. All modified diets showed higher epididymal and retroperitoneal adipose weights than the control diet as seen in Figure 62.

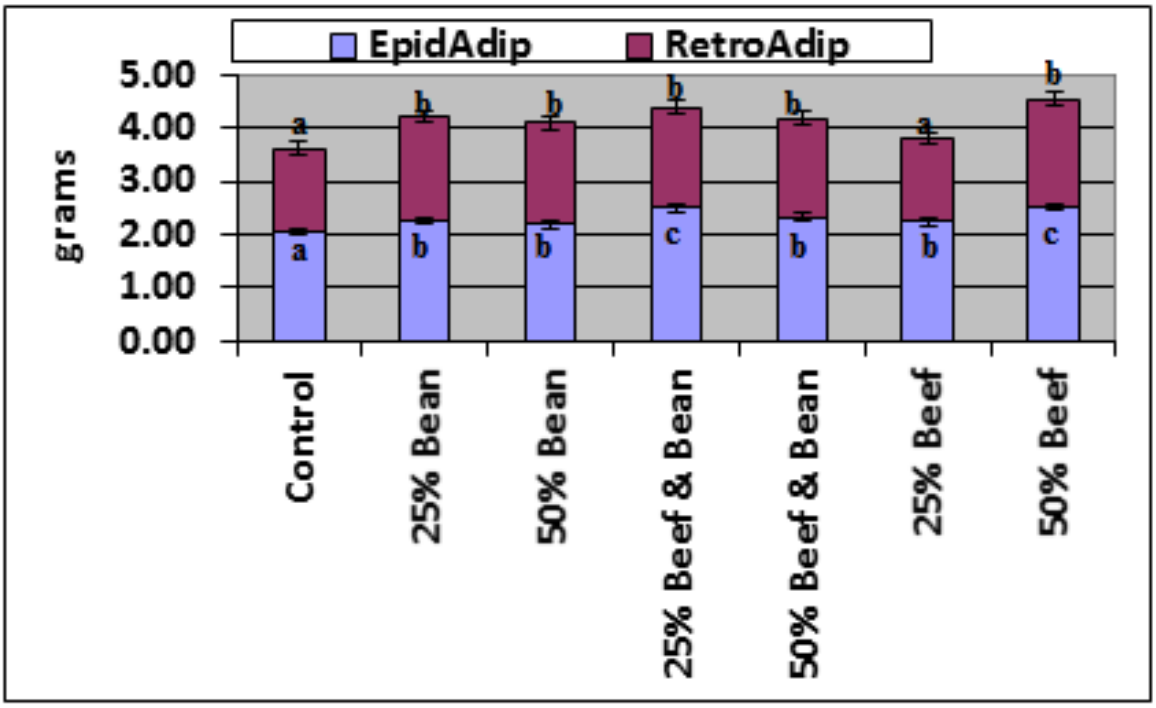

Figure 62: Epididymal & Retroperitoneal Adipose Weight
Bars with the same character are not significantly different (P>0.05).

5.5 Discussion

This research was able to demonstrate the improved nutritional quality of extended meat products in a hamster model. With the goal of reducing obesity, weight gain of the animals was the first target. The feed intake of the hamsters consuming beans was greater than in the meat diets though they gained less weight. However, weight is only one marker of health. It has been previously demonstrated that pulses are good sources of dietary fiber, some of which is fermentable in the lower gastrointestinal tract[9]. When beans were added to the hamster diets, the pH decreased suggesting more fermentation of fiber into short chain fatty acids. The specific short chain fatty acids resulting from fermentation have been shown to be beneficial for gastrointestinal health. Another benefit found in lowered cecal pH is improved triglyceride

levels[10]. The presence of resistant starch or other fermentable fiber in the diet has been shown to have a direct correlation with cecal pH through an increased number and concentration of short chain fatty acids. The microbial breakdown in the large intestine may promote fermentation, producing acetate, propionate, and butyrate. However, these products and their concentrations is dependent upon the type of starch and associated microflora[11]. Although, all have been shown to improve triglyceride levels in the blood.

VLDL contains the highest amount of triglyceride and thus, high VLDL levels may lead to an increased risk of coronary artery disease (CAD), which can further lead to a heart attack or stroke. While it is important to lower LDL and VLDL level to prevent CAD, it is also important to consider the overall ratio of the high density lipoprotein (HDL) compared to the total combination of the LDL and VLDL as well as the ratio of HDL to total cholesterol (TC) levels. A higher ratio of HDL to LDL and VLDL offers insight into a cholesterol protection buffer because the larger and less dense HDL particles are considered protective. Meanwhile, a lower ratio of HDL to TC is an indicator of a lower risk of heart disease[12].

Fatty liver, or fatty liver disease (FLD), is a condition where large pockets of triglycerides accumulate in liver cells. FLD is observed in up to 75% of obese people. The addition of the HLRBF into the hamster diets reduced liver weights, therefore, reducing the chance for FLD development.

As adipose weight increases, the prevalence of adipose fat increases. The indicators for obesity (BMI and waist circumference) are closely associated with measurements of adipose fat. However, the higher substitution level of bean in the diets reduced overall epididymal and retroperitoneal adipose weights compared to the beef containing diets.

5.6 Conclusion

The results suggest that partial substitution with light red kidney beans in a ground beef mixture can have significant impact on cholesterol levels and visceral fat deposition due to synergism between saturated fat and cholesterol in the diet.

5.7 References

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APPENDIX 1: PARENTAL RESEARCH CONSENT FORM

I, _____, agree to allow my child _____to
Please Print Please Print
participate in the research entitled "Foods for Health, Meat Patty with Added Legumes" which is being conducted by Dr. John Finley of the Department of Food Science at Louisiana State University, phone number (225)578-5207. For this particular research about 45 minutes of time will be required during their science period at school. I understand that my child's participation is entirely voluntary and whether or not they participate will not affect my child's status with their school or LSU. It will be a short focus group where my child will sample a meat patty with added beans and give his/her opinion.

The following will **EXCLUDE** my child:

1. A beef or dry bean (pinto, etc.) **ALLERGY**.
2. A religious or social preference against eating beef.
3. Child **DOESN'T** like **HAMBURGERS** or doesn't participate in the **SCHOOL LUNCH PROGRAM**.

The only **RISKS** foreseen in this study are complications due to beef or dry bean allergy.
The following **PRECAUTIONS WILL BE TAKEN** to protect your child:

- The meat patty will be cooked to an internal temperature of 160◦F, measured with a thermometer, just as in the school cafeteria.
- Excluding children from the study who are allergic to wheat, beef or dry beans.

Privacy:
The results of this study will not be released in any identifiable form without my prior consent unless required by law.

Questions:
The child's teacher has explained the project and the investigator will answer any further questions about the research, either now or during the course of the project. Carla Sandlin (225)578-5207. Email: csandl1@lsu.edu

The study has been discussed with me, and all of my questions have been answered. I understand that additional questions regarding the study should be directed to the investigators Carla Sandlin or Dr. John Finley. In addition, I understand the research at Louisiana State University AgCenter that involves human participation is carried out under the oversight of the Institutional Review Board. Questions or problems regarding these activities should be addressed to Dr. David Morrison, Assistant Vice Chancellor of LSU AgCenter at (225)578-4182.

I agree with the terms above.

_____ _____
Signature of Parent Date

APPENDIX 2: CHILD RESEARCH ASSENT FORM

I _____ am willing to participate in the focus group
Please Print
with prior permission from my parent/guardian. I have given this permission form to the investigator. The study has been discussed with me and all of my questions have been answered.

_____ _____
Student Signature Date

_____ _____
Investigator Signature Date

APPENDIX 3: STUDENT RREPSONSE SHEET FOR FOCUS GROUP: PART 1

Student Response Sheet for Focus Group

1. What is a Hamburger?

_____

_____

2. What is a hamburger made of?

_____

_____

3. What do you like about hamburgers?

_____

_____

4. How often do you eat hamburgers?

_____

_____

APPENDIX 4: STUDENT RESPONSE SHEET FOR FOCUS GROUP: PART 2

Student Response Sheet for Focus Group

1. Is this what you normally eat at school or a home?

_____

_____

2. How is it different or the same?

_____

_____

3. How should we change it?

_____

_____

4. How would you react if you knew it was healthier for you?

_____

_____

APPENDIX 5: PARENTAL RESERCH CONSENT FORM

I, _____, agree to allow my child _____to
Please Print Please Print

participate in the research entitled "Foods for Health, Meat Patty with Added Legumes" which is being conducted by Dr. John Finley of the Department of Food Science at Louisiana State University, phone number (225)578-5207. For this particular research about 30 minutes of time will be required. I understand that my child's participation is entirely voluntary and whether or not they participate will not affect my child's status with BREC, their school, or LSU. It will be a short study where my child will sample both a regular meat patty and a meat patty with added beans and give his/her opinions.

The following will **EXCLUDE** my child:

4. A beef or dry bean (pinto, etc.) **ALLERGY**.
5. A religious or social preference against eating beef.
6. Child **DOESN'T** like **HAMBURGERS** or doesn't participate in the **SCHOOL LUNCH PROGRAM**.

The only **RISKS** foreseen in this study are complications due to beef or dry bean allergy.
The following **PRECAUTIONS WILL BE TAKEN** to protect your child:

- The meat patty will be cooked to an internal temperature of 165°F, measured with a thermometer, just as in the school cafeteria.
- Excluding children from the study who are allergic to wheat, beef or dry beans.

Privacy:
The results of this study will not be released in any identifiable form without my prior consent unless required by law.

Questions:
The child's teacher has explained the project and the investigator will answer any further questions about the research, either now or during the course of the project. Darryl Holliday (225) 578-5207. Email: DHolliday@agcenter.lsu.edu

The study has been discussed with me, and all of my questions have been answered. I understand that additional questions regarding the study should be directed to the investigators Darryl Holliday or Dr. John Finley. In addition, I understand the research at Louisiana State University AgCenter that involves human participation is carried out under the oversight of the Institutional Review Board. Questions or problems regarding these activities should be addressed to Dr. David Morrison, Assistant Vice Chancellor of LSU AgCenter at (225)578-4182.

I agree with the terms above.

_____ _____
Signature of Parent Date

APPENDIX 6: CHILD RESEARCH ASSENT FORM

I _____ am willing to participate in the focus group
Please Print
with prior permission from my parent/guardian. I have given this permission form to the investigator. The study has been discussed with me and all of my questions have been answered.

_____ _____
Student Signature Date

_____ _____
Investigator Signature Date

VITA

Darryl L. Holliday was born in August of 1983, in Baton Rouge, Louisiana to Dwight Holliday and the now Claire Brown. He resided in Baton Rouge until after he graduated high school.

After completing his undergraduate education at The Chef John Folse Culinary Institute at Nicholls State University in Thibodaux, Louisiana, Chef Holliday continued his culinary training through internships with both an international multi-unit restaurant chain and international ingredient application team. He then furthered his education at Louisiana State University where he completed a Master's of Science in Food Science with a focus in food engineering/flavor chemistry with a minor in business marketing. His educational background as well as his product development experience with everything from start-up operations to multi-national companies allowed him to become a Certified Research Chef through the Research Chefs Association. His 15+ years in the food industry have included work in bakeries, fine dining establishments, multi-unit chain restaurants (both kitchen and management), specialty ingredient companies, and finished product manufacturers.

Chef Holliday is a senior level food and beverage professional with expertise in culinary and food science product development, ingredient functionality, cost reduction, and business development/market presence. Mr. Holliday is now a candidate for a PhD from the School of Nutrition and Food Science with an emphasis in food processing/product development with areas of focus in Culinology®, food chemistry, human nutrition, and organic chemistry at

Louisiana State University and Agricultural and Mechanical College, which is expected to be awarded in December 2014.