A Nutritional Analysis of *Phaseolus vulgaris* Grown in an Aquaponics System vs. Grown Commercially vs. Grown Organically

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Bachelor of Sciences

Submitted in partial fulfilment of the requirements for

College Honors

Departmental Distinction in Biology

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Abstract

The purpose of this project was to perform several biochemical analyses on common green bean (*Phaseolus vulgaris*), samples grown in a variety of environments. Seven samples of *P. vulgaris* from an aquaponics system as well as various modern commercial agricultural and organic sources were obtained, lyophilized, and prepared. Bomb calorimetry, loss on ignition (percent organic content), CHN elemental analysis, BCA protein, and anthrone carbohydrate assays were performed on the sample tissues. There were detectable differences in the nutritional quality of the beans dependent on growing conditions. Aquaponics reared beans had higher protein concentration and may be more nutritious than beans from other growing techniques. These findings support that more research needs to be completed on the nutritional quality of plants grown in aquaponics systems and other alternative sustainable agricultural methods.

Introduction

According to the World Health Organization's recommendations, a healthy diet includes a great deal of variation in foodstuffs in order to both ensure proper macro- and micronutrient intake. Macronutrients, such as fat, protein, and carbohydrates, are responsible for storing and delivering energy throughout the body as well as providing the building blocks for other crucial structural roles. Micronutrients, consisting of vitamins, essential fatty acids, and various minerals, are also necessary to support bodily functions and play vital roles in physical and cognitive development. They cannot be generated by the body, and it is therefore balanced diets are crucial to supply the necessary micronutrients. (Hans and Jana 2018). To properly supply the necessary nutrients and promote lifelong health, the World Health Organization (WHO) recommends a diet that minimizes the intake of salt, sugars, and fats while maximizing intake of vegetables, fruits, and legumes. Specifically, WHO recommends that adults consume about 500 grams of fruits and vegetables, less than 50 grams of sugar, and less than 5 grams of salt per day

(World Health Organization 2015). Consistent fruit and vegetable consumption has been shown to be a protective factor against several chronic diseases, including diabetes mellitus II and a plethora of cardiovascular diseases (Miller *et al.* 2017; Bazzano 2005). Green beans or *Phaseolus vulgaris* (*P. vulgaris*), belonging to the vegetable family *Leguminosae* or legumes, can be a crucial component of a balanced diet, as they are one of the most important sources of plant protein for humans. They are also a good source of carbohydrates, fats, antioxidants, minerals, vitamins E and K, folic acid (crucial for pregnant women), β-carotene (precursor to vitamin A), and several other water-soluble B vitamins—all of which are necessary for the body to complete its baseline functions and maintain health as discussed previously (Bailey 1921; Beaulac *et al.* 2009; Castro-Guerrero *et al*. 2016; Dos Santos 2016; Fabbri and Crosby 2016; Smit *et al*. 2001).

The environment and method of growth— primarily divided into conventional or organic methodologies—determines much of the nutritional benefit and quality of various produce products within a single species. The United States Department of Agriculture (USDA) has strict guidelines that define the differences between organic and conventional agriculture in the United States. The major difference between the two is that organic agriculture is typically considered to be more sustainable and more environmentally friendly, as it does not use synthetic compounds to control undesirable weeds or pests, or fertilizers to promote crop growth. Additionally, organically-raised livestock are protected from conventional handling methods, such as prophylactic antibiotic or hormone administration, and overcrowded living conditions. These practices are considered to be beneficial for the environment, as they do not generate equal waste to conventional methods, and the waste that is generated is considered to be less harmful for the environment as well (National Organic Standards Board 2010; United States Department of Agriculture 2016). Some studies also suggest that organic agriculture produces produce with a higher macro- and micronutrient density than conventional agriculture. The advantages to conventional agricultural methods are higher production rate per acre and lower costs leading to greater accessibility of produce. On average, organic farming demonstrates a lower average crop yield than conventional methods at dramatically increased prices; this is problematic for people shopping on a limited budget or with limited access to farmers utilizing organic methodology. This is also problematic in a world with an ever-growing population that requires consistent access to a variety of crops (Gomiero 2017; Maffei *et al.* 2016; National Organic Standards

Board 2010; Schrama *et al*. 2018; Seufert *et al.* 2017; United States Department of Agriculture 2016).

A subset of organic agriculture, aquaponics, has been gaining popularity by small producers over the past few years as a way to meet the ever-increasing demand for two cash crops (vegetables and fish) in a more environmentally conscious way than traditional agriculture or aquaculture. These closed systems combine hydroponics (growing plants without soil) and aquaculture so that the waste created by the fish provides nutrients to the plants. The plants help to purify the water by recycling the carbon dioxide, other gases, and wastes that accumulate in the tank. Feeding the fish and ensuring the integrity of the system are the only tasks required of human caretakers (Fang *et al*. 2017; Kyaw and Ng 2017).

The three most common aquaponics system designs are floating disc, media-based bed, and nutrient film. The floating disc method involves a fish tank with the plants of interest being grown on a substrate floating in the water so that the roots directly interact with the fishes' environment. In the media-based system, the fish and the plants are kept physically separate, with the water being recycled through a series of pipes and powered by a pump. Rather than soil, however, a variety of rocks or other substances, which do not provide additional nutrients can be used to hold the plants. In the nutrient film method, the plants and fish are again kept separate; the waste water from the fish tank runs through both a pump and a filter before watering the roots of several rows of plants encased in PVC pipes, before returning to the fish tank (Fang *et al*. 2017; Graber and Junge 2009).

Aquaponics systems are heavily under-researched and their full potential has not been thoroughly explored. They are relatively easy to maintain, largely self-sustaining, and can be built in diverse environments (Edwards 2015; Fang *et al.* 2017; Forchino *et al.* 2017; Graber and Junge 2009; Love *et al.* 2015; Rakocy 2007; Rakocy *et al.* 2006). Because they can be so compact, there are potential commercial as well as household implications for aquaponics systems which grants the potential for food production in almost any location (Konig *et al.* 2018). Therefore, they have enormous potential to fulfill a public health niche with the ability to aid urban centers and poorer regions (also known as "food deserts") that may lack consistent access to fresh, healthy foods (Beaulac *et al.* 2009; Dos Santos 2016; Smit et al. 2001). They can also be used as an educational tool in such environments, helping to inform about nutrition and balanced diets. The need for such systems becomes more and more relevant in the present climate of increasing urbanization, reducing agricultural land, and elevated human populations whose demands for food ever-increase (Konig *et al*. 2018; Kyaw and Ng 2017). They could also help reduce the agricultural waste and carbon footprint of the traditional agricultural industry. Additionally, aquaponics systems require less resources such as water and minerals to be added to the system and reduce waste when compared with traditional agricultural methods. (Johnson *et al.* 2007; Reay et al. 2012).

As promising as these systems could be, since they are so under-researched. There remain unanswered questions, which has led to problems in large scale deployment (Love et al. 2014). The systems suffer primarily from high start-up costs, difficulty recycling gases within systems, as well as a limited knowledge of crops that can grow in these systems (Badiola *et al.* 2012; Bosma *et al.* 2017; Fang *et al*. 2017; Hu *et al.* 2015; Tyson et al. 2011).

P. vulgaris show promise for use in aquaculture. They host bacteria in their roots that fix atmospheric nitrogen into a biologically useful form. Thus, legumes could help facilitate the recycling of nitrogenous gases through an aquaponics system yet have not been well-documented in these systems. Testing the ability of the beans to recycle nitrogen more efficiently was beyond the scope of this project. However, we sought to demonstrate that the green beans could survive in an aquaponics system as few researchers grow them in favor of herbs, tomatoes, and leafy green vegetables (such as lettuce or bak choy) (Ako and Baker 2009). We also sought to determine the macronutritional quality of the beans. If the experimentally grown beans have equivalent nutritional content to the commercially or organically grown beans, then this will hopefully encourage more research to be done on making these systems more efficient. Even though this project does not immediately solve all of the existing problems concerning aquaponic systems, it will set the groundwork to explore new solutions to existing problems barring these systems from functioning as well as they could. The nutritional quality of the *P. vulgaris* grown in the aquaponics system will be equivalent to the organic samples and equal, if not greater, than the traditionally grown samples.

Sample Procurement, Materials, and Methods

Aquaponics Growth Experiments

To grow *P. vulgaris*, the floating disc aquaponics method was used with tilapia for the fish component and Burpee Garden Bean Bush Blue Lake 274 bean seeds for the plant component. Six holes in the floating disc were marked to grow the beans in – each about a half a meter apart with two seeds planted in coconut coir. The seeds were left to germinate and grow in the Albright College greenhouse for five months, then the beans were harvested. The tank was cleaned and the fish were fed every 2-3 days. Fresh beans were bought from a Giant food store and others were harvested from the Albright College permaculture garden. Additionally, two commercially frozen samples, one fresh organic sample from western Pennsylvania, and one commercial organic frozen sample were obtained.

Biochemical Analysis

Samples were collected, lyophilized, and weighed. Then, about 5 grams of wet beans were placed in a drying oven until their weights stabilized and were weighed. The dried beans were then placed in a muffler oven for 48 hours to ash them, and the ash masses were taken. The weights were used to calculate loss on ignition (LOI) and percent organic content. Lyophilized samples were then run through a Wiley mill to ground them into a fine powder.

About one gram of each lyophilized sample was measured in triplicate for bomb calorimetry runs. The samples were made into pellets using deionized water to help the pellet retain its shape. While the bomb calorimeter was running, about two grams of lyophilized samples were weighed out into small aluminum weigh boats to be run through the 2400 Perkin Elmer Series II CHN Elemental Analyzer. This instrument was used to determine the C:N ratios and percent carbon, hydrogen, and nitrogen content for each sample. Four aluminum packets containing each sample were weighed and folded, as well as several blanks and k-standards. Lyophilized samples were stored in the freezer to be used for later assays.

All seven lyophilized samples were prepared to conduct carbohydrate and protein determination assays as well. The anthrone determination of total carbohydrate concentration first required sample extractions to be prepared. This was done as outlined by Brylawski and Manigat using a Fisher Scientific/Brandon Sonifier Model 150 Cell Disruptor ultrasonic dismembranator (2018). Two separate methods of anthrone determination were attempted and adapted to be read using a ThermoFisher Multiskan FC Microplate Photometer reader. The first protocol was followed according to Brylawski (2014). The second protocol was followed as described by Brylawski and Manigat (2018). The Pierce BCA protein assay was prepared according to the Thermo Fisher Scientific, Inc. protocol (2014). Standard curves were created using the blank-corrected standards (plotting the absorbance vs. known concentration), and the unknown carbohydrate or protein concentrations were then determined from the curve. A single factor ANOVA was run on the results, and standard error was calculated in Microsoft Excel for graphing.

Results

Overall, there was a great amount of variability among and between sample types. The beans grown in the Albright permaculture garden and in the aquaponics system had the greatest average protein concentration, while the commercially-grown organic frozen samples had the least (Figure 1). The commercially frozen and fresh samples had the greatest average carbohydrate concentration per gram dry mass, while the Albright aquaponics samples had the least (Figure 2). The greatest organic carbon content was seen in the Albright aquaponics samples, and the least in the fresh organic samples. (Figures 3 and 4). The greatest energy yield was seen with the commercially-grown organic frozen beans, while the least was seen with the Albright College permaculture garden beans (Figure 5). The frozen, commercially-grown samples had the highest carbon-nitrogen ratios, while the fresh samples had the lowest (Figure 6). The commercially-grown samples had a higher overall percentage of carbon than the organically grown samples. The percentages of hydrogen and nitrogen were similar across all samples, except the WFF sample, which had lower percentages than the other samples (Figure 7).

Table 1. Sample IDs, their origin, and whether the samples were obtained fresh or frozen.

Sample ID	Sample Origin	Fresh or Frozen
AAR	Albright College aquaponics system	Fresh
APR	Albright College permaculture garden	Fresh
BFF	Bird's Eye commercially-grown	Frozen
BOF	Bird's Eye commercially-grown organic	Frozen
CFR	Giant Food Stores commercially-grown	Fresh
WFF	Weis Markets commercially-grown	Frozen
WPR	Western Pennsylvania organic	Fresh

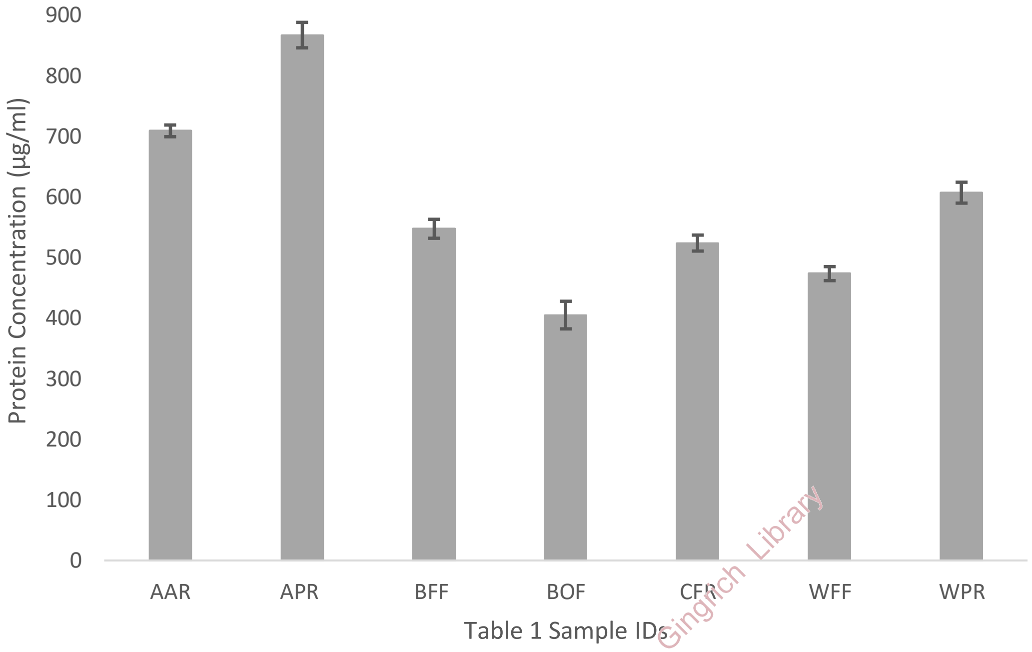

Figure 1. Mean ± SE protein concentration of the seven *P. vulgaris* samples (ANOVA: single factor, df 6,14, $p < 2.43^{-10}$, error bars represent the standard error values).

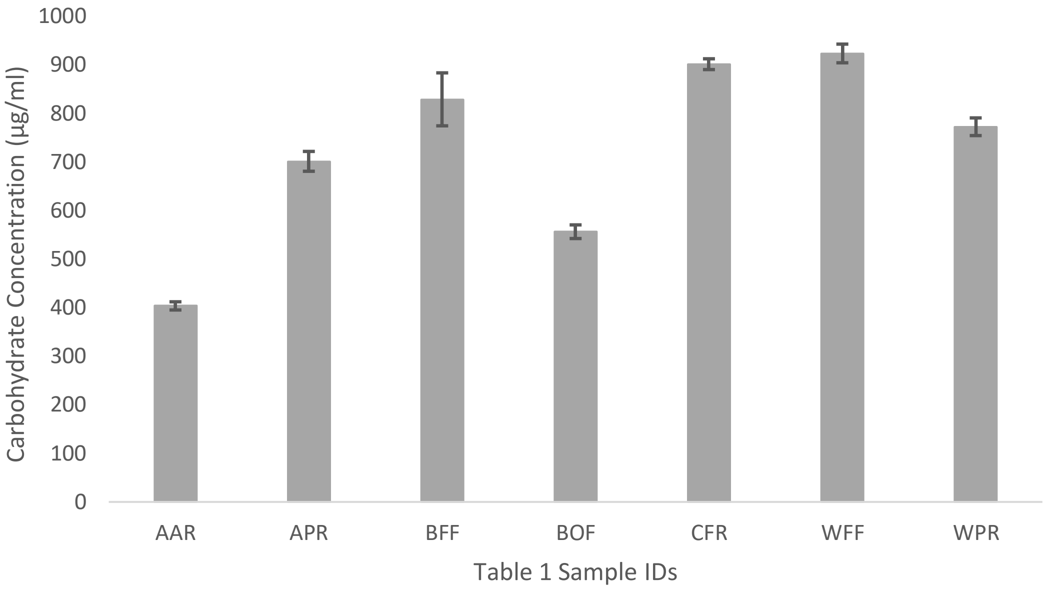

Figure 2. Mean + SE carbohydrate concentration of the seven *P. vulgaris* samples (ANOVA: single factor, df 6,14, $p < 5.38^{-9}$, error bars represent the standard error values).

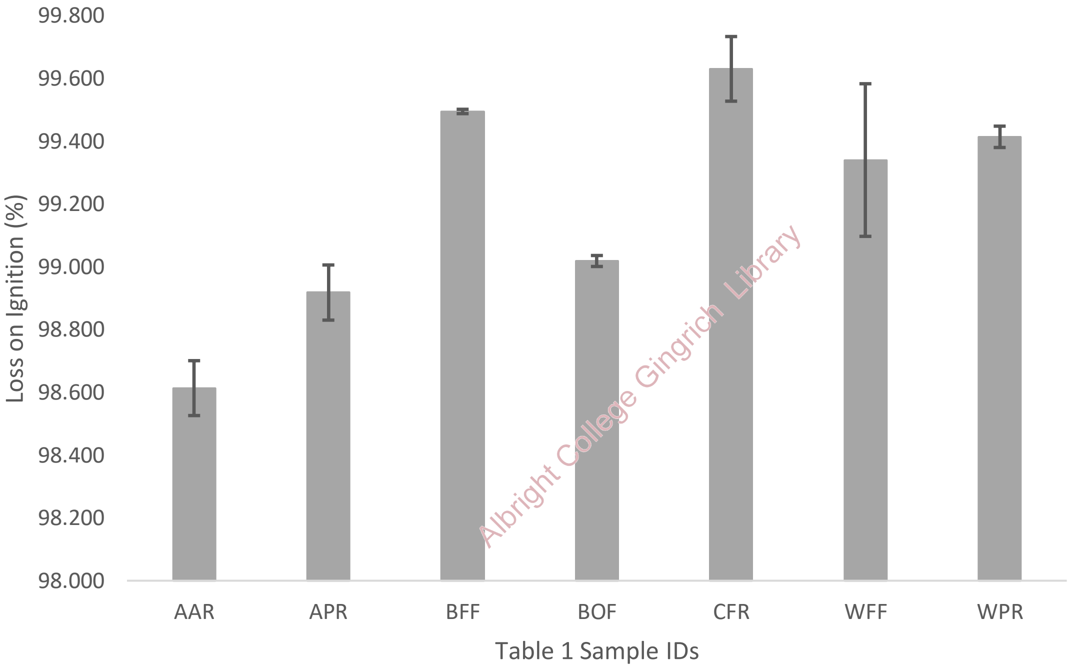

Figure 3. Mean + SE loss on ignition (LOI), calculated by taking (wet mass - ash mass)/ wet mass. (ANOVA: single factor, df 6,14, $p > 0.05$, error bars represent the standard error values).

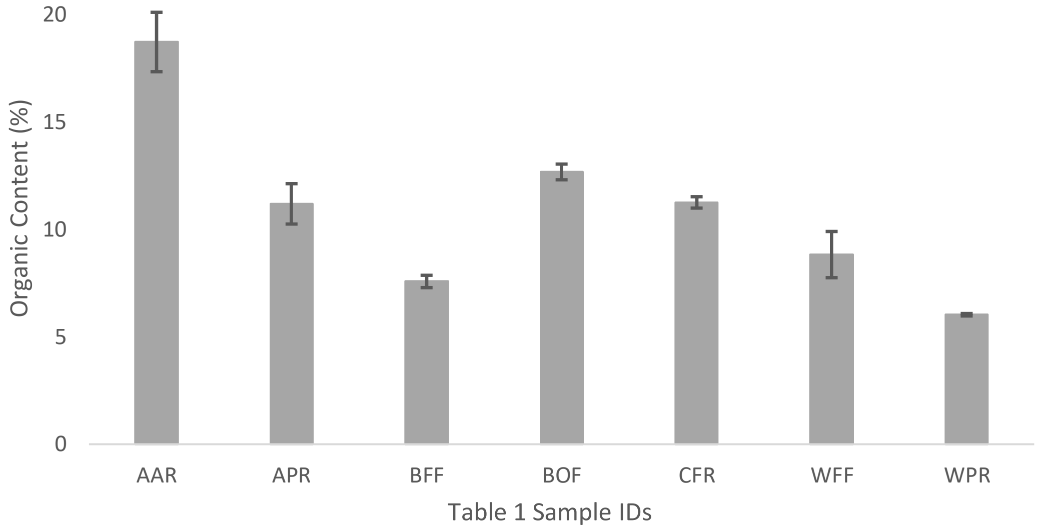

Figure 4. Mean + SE percent organic content determined by first calculating the percent moisture and then subtracting that value from 100. (ANOVA: single factor, df 6,20, $p < 6.56^{-7}$, error bars represent the standard error values).

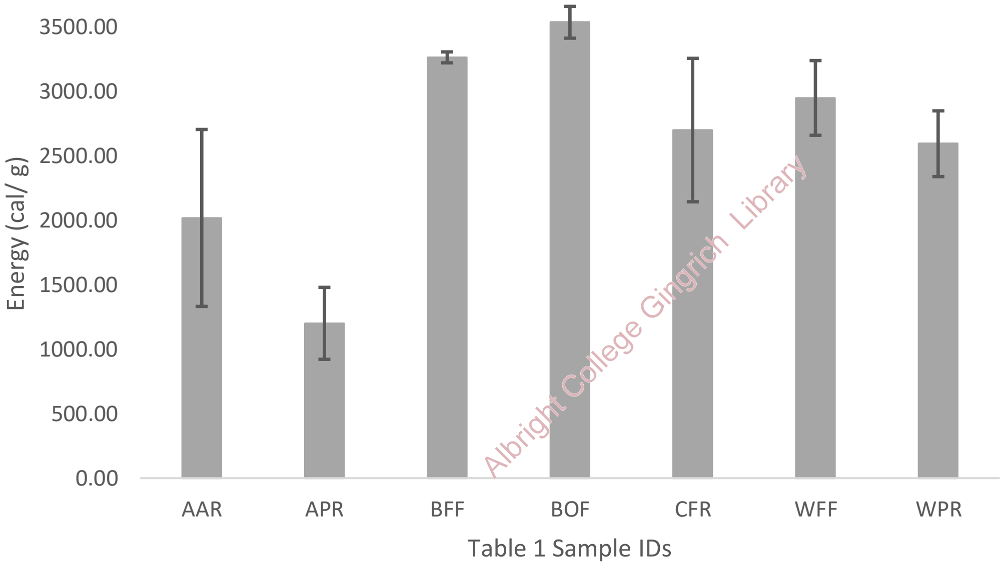

Figure 5. Mean + SE energy yield in calories/ gram for samples run through the bomb calorimeter. (ANOVA: single factor, df 6,14, $p > 0.05$, error bars represent the standard error values).

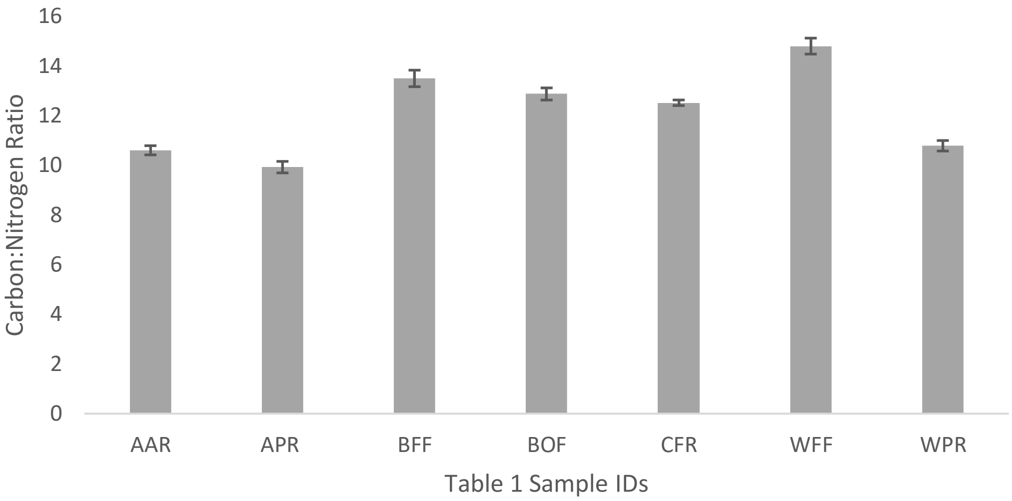

Figure 6. Mean + SE carbon and nitrogen ratio for samples run through the elemental analyzer. (ANOVA: single factor, df, 6,21, $p < 1.60^{-11}$, error bars represent the standard error values).

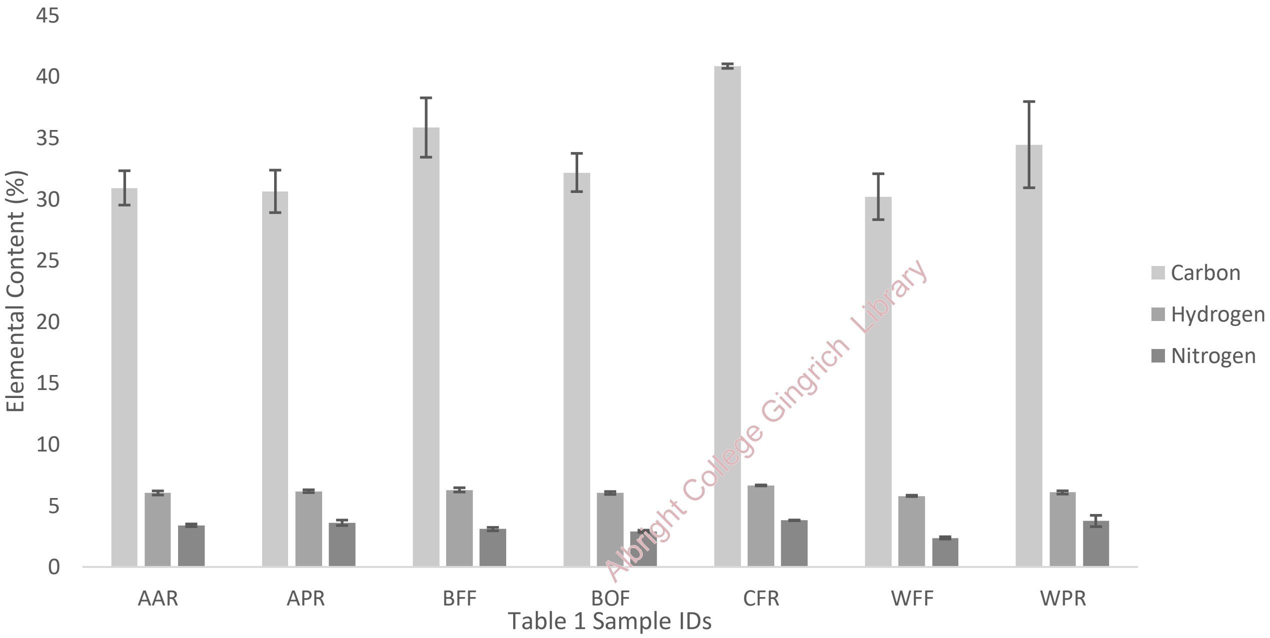

Figure 7. Mean + SE percent content of carbon, hydrogen, and nitrogen for samples (about 2g each) run through the elemental analyzer. (Carbon ANOVA: single factor, df 6,21, $p < 0.014$; Hydrogen ANOVA: single factor, df 6,21, $p < 0.0037$; Nitrogen ANOVA: single factor, df 6,21, $p < 0.009$, error bars represent the standard error values).

Discussion

The variability between and among sample types is most likely due to the precise location and method of bean growth, as the environment determines the bioavailability of certain compounds and therefore the content of the beans. The higher protein concentration and lower carbohydrate concentration observed with the aquaponics and Western Pennsylvania organic samples is due to the availability of nitrogen in the system and soil respectively. This would allow the beans to generate and utilize proteins more than carbohydrates for energy storage due to the abundance of nitrogen, which would promote the synthesis of amino acids over monosaccharides. The opposite is true for the other conventionally and organically grown samples, which were most likely grown in soil with less bioavailable forms of nitrogen; therefore the beans used other available compounds in the soil to generate carbohydrates as their primary energy storage molecules. This is supported by the carbon: nitrogen ratios as well as the percentage of nitrogen and carbon in the samples; which highlights the C/N balance theory that states in the presence of more nitrogen, a plant will preferentially produce proteins as opposed to carbohydrates. This is also supported by the difference in physical locations, and therefore soil content and quality, in which the samples were grown. Additionally, the samples were grown and harvested at different times, which would also change the bioavailable compounds during bean growth. The organically and commercially grown samples with the greater concentrations of carbohydrates also showed higher energy yields than the aquaponics and permaculture samples. This is because carbohydrates generate more energy than proteins do when metabolized (Elmadfa and Meyer 2010; Herencia *et al.* 2011; Passioura 2002). The loss on ignition represents the percent of the beans that are lost as volatile compounds, such as water or carbon dioxide. The remaining content is considered to be the organic matter. Again, this is most likely due to the environment in which the individual bean samples were grown, and which compounds were available during germination and growth (Hoogsteen *et al.* 2015; Salehi *et al.* 2011).

In the future, there needs to be more extensive research dedicated to the nutritional quality of produce grown in a variety of environments, a more complete analysis of the difference between frozen and fresh produce, as well as an analysis of the micronutrient content of the various samples. Additionally, it would be helpful for future researchers to gather soil samples from the location where each crop was grown, as an analysis of the differences in soil might help to more concretely explain the differences among and between sample types' nutritional qualities. It would also help to expand this nutritional analysis to look for specific micronutrients and lipids in addition to the protein and carbohydrate macronutrients. Ultimately, this experiment supported that *P. vulgaris* grown in aquaponics systems can be equally nutritious to those grown via organic methods.

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