THESIS

QUALITY AND NUTRITIONAL ASPECTS OF CONVENTIONAL AND NOVEL FOOD PROTEINS

Submitted by

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ABSTRACT

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Cattle weights have increased during the last couple of decades and have not always been accompanied by improvements in facility capabilities and management. Alongside quality issues of color, tenderness, and water holding capacity, issues such as sour muscles and bone taints are now appearing with high frequency in the meat industry. Development of off-flavor/sourness in deep muscles such as knuckles (*vastus femoris, vastus lateralis, vastus medialis,* and *rectus femoris*) has been a long-standing issue in the beef industry, however, has not been well characterized. Therefore, the objective of this study was to investigate the potential cause, and to characterize the sour odor associated with beef knuckles using microbial, odor panel, and gas chromatography-mass spectrometric (GC-MS) analyses. Knuckles (n = 10) identified as having no sour odor (control), slight sour odor (SLI-SO), or severe sour odor (SVR-SO) were collected from the fabrication line of a commercial beef processing plant. Upon collection of knuckles, synovial fluid and the femur surface were swabbed to determine psychrotrophic anaerobic sporeformer presence. The collected knuckles were transported on ice to the laboratory where they were aseptically separated into two halves, with one half destined for microbial, odor, and GC-MS analyses on the day of collection (day 0) and the other half for the same analyses (excluding GC-MS) after 35 days of vacuum packaged storage at 0 - 2°C (day 35). For microbial analysis, 15 g of tissue was excised from the muscle surface and was analyzed for aerobic plate counts (Petrifilm Aerobic Count plates) and lactic acid bacteria counts (Lactobacilli MRS agar). Samples (5 g) for

GC-MS were held at -80°C until analysis. The remainder of the sample was diced and used for trained odor panels. Odor panelists identified differences ($P < 0.05$) for all tested attributes (off odor, oxidation, putrid, and sour notes) between control and sour knuckles (SLI-SO and SVR-SO) on day 0. Similarly, on day 35, differences ($P < 0.05$) were observed between control, SLI-SO, and SVR-SO knuckles for all attributes, with SVR-SO samples receiving the highest score for all categories. However, the microbiological analysis found no differences between aerobic plate counts and lactic acid bacteria counts of control, SLI-SO, and SVR-SO knuckles on day 0 or day 35. In addition, GC-MS analysis did not indicate a difference ($P > 0.05$) in the abundance of volatiles between the treatments (probably due to high variations within treatment groups). Overall, compounds such as acetic, acetoin, propionic, butyric, and isobutyric acid were trending towards having greater abundance in sour samples.

Although animal proteins have been the primary source of protein in the human diet, plant-based proteins have gained popularity in recent years. While some studies have indicated lesser environmental impacts, the nutritional composition of plant-proteins has not been readily investigated. Therefore, the objectives were to evaluate the nutritional composition of Morning Star Farms spicy black bean burger (VB), Beyond Meat's Beyond Burger (BB), Impossible Food's Impossible Burger (IB), a boneless top loin pork chop (PC), and 80% lean 20% fat ground pork (GP). Six different cities were selected for product collection to give a representative view of the products (Seattle, WA; Peyton, CO; Memphis, TN; Newburgh, IN; Houston, TX; and Brooklyn, NY). Following collection, products were brought back to Colorado State University. Half of the products sampled from each city were cooked, and the remaining half were left in their raw state. All ground products were cooked to an internal temperature of 71°C while the PC was cooked to

63°C. Samples (both raw and cooked) were then homogenized individually and stored under vacuum-packaged conditions at -80°C until further analysis. Methodologies for proximate analysis, amino acids, fatty acids, minerals, vitamins, organic acids, and allergens were conducted following the Association of Official Analytical Chemist (AOAC) guidelines. Overall, the product state (raw or cooked) had little effect on nutritional composition. Analysis indicated that the PC contained the highest ($P < 0.05$) amounts of protein, essential amino acids, and B-vitamins. Cholesterol was found highest ($P < 0.05$) in the pork products (PC and GP) with no cholesterol being identified in the plant-based products (VB, BB, and IB). However, when evaluating mineral make-up, the plant-based products contained the highest ($P < 0.05$) amounts, especially in sodium and iron levels. Sodium levels were about ten times higher, along with iron levels being 3 to 4 times higher in plant-based products. Overall, the pork products were found to contain the greatest amounts of amino acids, and B-vitamins needed in a diet. While the plant-based products were generally lower in nutrients, the IB was found at nutritional levels close to the GP and PC.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

REVIEW OF LITERATURE

Safety and quality of meat is critical in maintaining consumer acceptance and has been extensively researched. Even with all these advances in the knowledge of meat processing systems, there remains several unknown quality defects. Incidences of these quality defects can affect consumer acceptance adversely and could affect repurchasing decisions. To develop an understanding of these quality defects, it is imperative to have a clear understanding of the process of muscle to meat conversion.

1.1. Rigor Mortis

During the process of meat harvesting, cattle are stunned to render them unconscious, and then exsanguinated. Upon exsanguination, a biological countdown starts as rigor mortis begins to occur in muscle tissues. The animal's biological systems can no longer utilize blood to dispose of waste and supply oxygen to the cells; to counteract this, the metabolism transitions from aerobic to anaerobic. Under the anaerobic conditions, cells begin to convert glucose and glycogen stores into lactate, protons (H+), and ATP. While the total amount of ATP is decreased, cells begin to accumulate lactate and hydrogen protons that result in a drop of pH (Honikel et al., 1983; Bendall 1973).

1.2. Muscle pH

In live animals, muscle is found at a pH of 7.0 - 7.2 and gradually declines to a final pH of 5.4 - 5.8 upon the completion of rigor mortis (Bendall 1973). However, factors such as antemortem

and environmental stressors, and cooling can result in changes that alter this natural pH decline. When carcasses are observed to have a lagged drop in pH that results in a final muscle tissue pH of 7.0, the lean tissue exhibits a dark, firm, and dry (DFD) appearance. In contrast, a rapid pH drop resulting in a muscle pH of 5.5 within the first two hours of rigor mortis, causes proteins to denature due to the acidic environment that results in lean tissue with a pale, soft, and exudative (PSE) appearance.

1.2.1. Dark, Firm, and Dry Meat

Dark, firm, and dry (DFD) meat is most commonly observed in beef production systems. When livestock are exposed to long-term stress, the animal's sympathetic nervous system is upregulated, resulting in a depletion of glycogen stores within muscle fibers as the animal enters a fight or flight state. By removing stressors from the animal's environment along with a resting period, muscles glycogen stores will naturally replenish. However, without proper glycogen stores in muscle fibers upon slaughter, livestock species are predisposed to express DFD attributes in muscle tissue. Due to the darker appearance of DFD meat, consumers are often less accepting of products exhibiting these defects. Additionally, DFD products have a reduced shelf life due to the high-water activity and lower pH that favor microbial growth.

1.2.2. Pale, Soft, and Exudative Meat

Carcasses expressing the PSE condition are associated with undesirable color, lower processing yields, increased cooking losses, and reduced juiciness of the meat product. While PSE is most often due to a genetic predisposition of the halothane and/or Rendement Napole genes in swine, this condition has been additionally identified in beef carcasses. While these conditions are similar in appearance, locations and origins differ between the species. While in pork the PSE

condition is apparent in the longissimus dorsi, PSE in beef has been identified to occur in slower chilling tenderloins, and large muscle groups of the round (Aalhus et al., 1998).

1.2.2.1. Pale, Soft, and Exudative-Like Condition in Beef

Unacceptable changes in color, water holding capacity, tenderness, and decrease in postmortem enzyme functionality are observed during the occurrence of the PSE-like condition in beef (Kim et al., 2014; Warner et al., 2014; Aalhus et al., 1998). Hunt and Hendrick (1977) reported a similar condition in the semimembranosus muscle of the round and separated the muscle into an inside (ISM) and outside (OSM) region based on appearance and color. The ISM had a larger amount of glycolytic muscle fibers and lower color stability compared to OSM which contained more oxidative muscle fibers and had higher color stability (Sammel et al., 2002; Nair et al., 2016). When evaluating the origins of the PSE-like condition in ISM, Nair et al. (2016) reported that the greater abundance of glycolytic enzymes along with the higher postmortem temperature of ISM compared to OSM could result in the decreased meat color stability. Moreover, research by Sammel et al. (2002) reported that the removal of the semimembranosus muscle from the femur pre-chilling resulted in similar color stability, pH levels, and temperature decline for ISM and OSM. All these studies indicate that proper chilling is a crucial step to prevent PSE-like conditions in deep muscles such as those present in the round.

1.3. Increasing Carcass Sizes

The proper chilling of carcasses allows for the proper onset of rigor mortis to occur while simultaneously limiting the potential for bacterial spoilage. As live cattle are sold to meat processors on a poundage basis, producers are often utilizing practices that allow for maximum performance and weight gain of cattle. With increases in technology and livestock management,

the average carcass weights are reported to be increasing at a rate of 1.8 kg per year (Boykin et al., 2017). Additionally, when evaluating carcasses from 1991 to 2016, a 13% increase in carcass weight along with a larger amount of cattle were identified as belonging to the heavyweight classification (McKenna et al., 2002). Due to a lack of processing facility accommodations, larger carcasses require the necks and fore shanks to be tied up to prevent product from dragging on production facility floors. Additionally, some facilities will reject loads of livestock due to the inability of processors to accommodate these large framed cattle. Furthermore, problems within the production floor are amplified in carcass coolers as cooling systems must dissipate more considerable amounts of heat from larger framed and heavier weighing carcasses.

1.4. Cold Shortening

By minimizing animal stress pre-slaughter, PSE and DFD conditions can be negated in meat production. However, other quality conditions can occur regardless of antemortem factors. This class of quality issues is based on the production facilities' ability to ensure proper dissipation of heat from carcasses. When proper cooling techniques are applied, carcasses can complete the process of rigor successfully while lowering temperatures quickly enough to stave off bacterial growth and protein denaturation.

When chilling is impeded, a faster decline in muscle pH occurs that results in the denaturation of proteins and, ultimately, an undesirable pale meat color (Kim et al., 2014). When muscle pH is above 6.0, and the temperature drops to below 12°C, rigor mortis process is halted (Bendall 1973). Due to the drastic drop in temperature, the sarcoplasmic reticulum is restricted in function and unable to release calcium into the muscle fibers (Savell et al., 2005). With the surplus of ATP left in muscle, the fibers contract resulting in the filaments crossing over each other that results in the elimination of the I–band of the sarcomere (Savell et al., 2005). With the elimination

of the I-band, a decrease in tenderness has been reported (Herring et al., 1965). In addition to cold shortening, when a muscle is frozen prerigor, a similar condition, known as thaw rigor, can occur when the product is thawed. Upon thawing, muscle cells are flooded with previously withheld calcium that was stored in the sarcoplasmic reticulum that rushes into the muscle cells causing violent contractions that physically shorten the muscle to half of the original size (Savell et al., 2005). Due to the compression of sarcomeres within the shortened product, a reduction in tenderness is observed. To prevent the development of these issues it is imperative that production facilities properly chill carcasses at an appropriate rate.

1.5. Heat Distribution

With the increase of carcass weights and sizes, as discussed in sections before, cooling carcasses has become more problematic as greater amounts of product require proper cooling (Boykin et al., 2017; McKenna et al., 2002). Along with higher carcass weights, an increasing number of carcasses are being classified as United States Department of Agriculture (USDA) 4 and 5 yield grades (Boykin et al., 2017). USDA yield grades are indications of the cutability of closely trimmed retail cuts that can be acquired from a carcass and are determined on a 1 – 5 scale. A USDA 1 would express the highest amount of cutability from a carcass, while a USDA 5 would have the lowest cutability. These yield grades are determined by the (1) external fat, (2) kidney, pelvic, and heart fat (3) ribeye area, and (4) hot carcass weight. With USDA yield grade 4 and 5 carcasses, a greater amount of external fat is observed, which has been found to impede the rate at which heat can dissipate from a carcass (Boykin et al., 2017; Aalhus et al., 2001). These hypotheses are further supported as Klauer et al. (2018) found that higher-yielding carcasses (USDA yield grade 1 and 2) were able to more appropriately dissipate heat at a proper rate when compared to lower-yielding carcasses (USDA yield grade 4 and 5). Along with these factors identified by

Aalhus et al. (2001) and Boykin et al. (2017), heat distribution of a carcass is a unique challenge due to the varying thickness and non-uniform shape of carcasses. When observing primals, rounds and chucks are typically thicker and as a result, exhibit more difficulty cooling. At the same time, middle meats such as the loin and sirloin can dissipate heat at an appropriate rate. When further evaluating the heat transfer of larger muscles such as those found in the round, the surface of these muscles was able to reach adequately low temperatures when chilled (Kuffi et al., 2016). However, as the muscle gets further away from the surface, a gradient consisting of a slower drop in temperature along with drops in glucose concentration and pH is identified (Kuffi et al., 2016). Furthermore, this gradient found in deeper muscle tissues may be amplified by lower-yielding cattle and increased muscle size (Aalhus et al., 2001; Klauer et al., 2018). When observing light, medium, and heavyweight classifications of cattle, after 28 h of chilling, carcasses with a light frame averaged 2.88°C lower than those of heavy frame carcasses (Klauer et al., 2018). When evaluating individual primals, the chuck, due to its large surface area, lacked any significant difference in overall temperature between weight classifications (Klauer et al., 2018). However, when evaluating deep tissues of the round, a significant difference between weight classes was apparent, with the highest temperatures being recorded in the heavyweight classification (Klauer et al., 2018). Similarly, Djimsa et al. (2019) reported that lightweight cattle carcasses dropped below 7°C, while heavyweight carcasses exhibited temperatures above 10°C after a 24-h cooling period. To ensure that carcasses of different size classifications are allowed to cool properly, it has been suggested to sort carcasses before cooling and adjust cooler settings appropriately to match the respectively framed cattle (Klauer et al., 2018). While this process could ensure proper cooling, no current production facility utilizes such practices.

1.6. Souring Condition

With the identified temperature gradient of large round muscles, it is apparent that cooling methods are unable to cool large-framed carcasses consistently. Rounds from beef carcasses will randomly and sporadically exhibit a sour-like smell upon opening the patella joint during fabrication of the round into wholesale cuts. Producers and research studies have identified this condition in the round as the "sour knuckle." The sour knuckle condition is associated with the *vastus femoris, vastus lateralis, vastus medialis,* and *rectus femoris* muscles that make up the knuckle (Nottingham 1960). The souring condition is unique in that souring is identifiable at varying intensities, and occurrence is not predictable within rounds. Slightly soured knuckles are found to express aromas that are associated with dairy product souring, while those intensely soured knuckles have pungent aromatics similar to that of raw sewage. Due to these offensive odors, the knuckles are typically discarded or rejected by consumers upon delivery from a supplier. When determining the potential cause of this condition, researchers have suggested the cause to originate from the growth of microorganisms or complications of the postmortem metabolism (De Lacy et al., 1998; Shank et al., 1962; Ingram 1952).

1.6.1. Bone Souring

Bone souring (also known as bone taint) is a condition similar to sour knuckles that commonly occurs in hams. Research has indicated that bone souring in hams is due to the growth of bacterial organisms within the product. These bacteria are gram-positive sporeformers that can grow in a low pH setting, accompanied by mechanisms of heat resistance (Ingram 1952). In the muscles near the bone of the ham, where heat transfer would be limited, the majority of the culturable bacteria identified were clostridia and streptococci (Ingram 1952). To impede the

formation of the bone souring condition, researchers determined that producers must maintain a low temperature throughout the ham in production facilities (Boyer 1926; Ingram 1952).

1.6.2. Potential Causes of Sour Knuckles

Although the sour knuckle condition has been around the beef industry for several decades, the exact cause of this condition has not been identified, which makes prevention of souring even more challenging. Among the factors discussed as potential mechanisms, the major ones are of microbial or enzymatic origin and are further discussed below.

1.7. Microbial Origin

Due to the similarities of the bone and knuckle souring conditions, the majority of research to determine the potential cause of the souring condition in beef knuckles has focused on identifying a microorganism(s) responsible for this condition (Nottingham 1960; De Lacy et al., 1998). When observing bone souring in hams, often, *Bacillaceae* bacteria were recovered from samples. Upon sampling the ischiatic lymph nodes (a lymph node found in the deep tissues of the round) of cattle, Nottingham (1960) was able to identify and culture a similar species of *Bacillaceae* bacteria identified to cause the bone souring condition in hams. On the other hand, due to the range of sourness observed among rounds, it has been hypothesized that one microorganism could not be directly responsible (Nottingham 1960). Instead, a combination of *Bacillaceae* and clostridia, accompanied by additional aerobic and anaerobic organisms, could be necessary to create this range of souring intensities (Nottingham 1960). Furthermore, the growth of clostridia bacteria, propionate along with other short-chain fatty acids are produced by the organism's metabolomic processes, which could potentially be the source of the offensive odors (De Lacy et al., 1998). Similar clostridia organisms are also observed in the occurrence of blown

packaged products (Hungaro et al., 2016). In vacuum packaged products, the low oxygen environment promotes the growth of *Clostridium estertheticum* (the primary bacterium associated with blown pack products), which results in a putrid odor and production of large volumes of gas resulting in mis-shaped packaging (Hungaro et al., 2016). While a putrid odor is associated with the sour knuckle condition, gas production has not been observed in studies examining sour knuckles (Nottingham 1960; De Lacy et al., 1998). When rounds were inoculated with a mixture of 14 individual clostridia strains isolated from sour knuckles and blown package defects, the souring condition was successfully induced (De Lacy etal., 1998). In conclusion, this study strengthened the hypothesis that a mixture of clostridia species is necessary for the occurrence of the sour knuckle condition (De Lacy et al., 1998). However, with the De Lacy et al. (1998) study, rounds were inoculated with levels of bacteria that have not been observed in rounds expressing the condition naturally as past investigations have only identified 10 - 100 colonies upon isolation (Shank et al., 1962; Nottingham, 1960).

1.8. Enzymatic Origin

A primary difference when comparing the souring conditions of hams and beef knuckles is the location of the defect. While in the knuckle, the bone marrow is never soured, the defect is solely located in the muscle tissue, which is contrary to the bone souring condition expressed in hams (Lepovetsky et al., 1953). While the De Lacy et al. (1998) study was able to recreate the souring condition in rounds through inoculation, such levels of bacterial contamination would not be observed naturally in sour rounds as indicated by previous studies that evaluated microbial concentrations (Shank et al., 1962; Nottingham 1960). Ultimately, further studies would be necessary to determine if *Clostridium* spp. at naturally occurring levels would be able to induce the souring condition (De Lacy et al., 1998).

Due to the rounds naturally containing varying levels of clostridia and *Bacillaceae* bacteria regardless of the souring defect, some research has indicated that the condition may originate from the postmortem metabolism (Shank et al., 1962). Contrary to the prior studies, Shank et al. (1962) was unable to find a difference between culturable bacterial populations recovered from soured and non-soured rounds. However, propionic acid was found abundantly in rounds that expressed the souring condition. At the same time, bacterial populations recovered were at levels hypothesized as being far too low to generate such pungent flavors (Shank et al., 1962).

A previous study (Shank et al., 1962) investigated potential factors that could induce the souring condition with a lamb leg model. These factors included: (i) exercise prior to slaughter, (ii) induced propionate or lactate concentrations in the blood, and (iii) delayed cooling (Shank et al., 1962). Following six days of storage, all of the legs that were held at 37°C for 18 h before chilling expressed some degree of sourness regardless of the other treatments evaluated within the study. These results are interesting because regardless of the state of exercise or propionate/acetate blood levels, the souring condition was observed, suggesting that improper cooling might be a primary cause of the condition. In addition, of the lamb legs that were subjected to delayed cooling, those injected with a propionate or lactate solution expressed a higher degree of sourness (Shank et al., 1962). From these results, Shank et al. (1962) hypothesized that at least two of the three following factors must be present for the souring condition to occur: (i) the animals must be in an excited state, (ii) blood levels of propionate must be increased, and (iii) the round must not be adequately chilled.

CHAPTER 2

INVESTINGATING THE ETIOLOGY OF INCREASED INCIDENCE OF SOUR KNUCKLES IN COMMERCIAL BEEF PROCESSING FACILITIES

2.1 Introduction

The 2017 National Beef Quality Audit has reported that the average carcass weight is increasing at a rate of 1.8 kg per year since 1991 (Boykin et al., 2017). The average carcass weight in 1991 was 345.0 kg, whereas the average carcass weight in 2016 was 390.3 kg (Boykin et al., 2017). This increase in carcass weight poses several challenges for beef processors. With the increased weight, carcass coolers must dissipate considerably higher amounts of heat from larger framed and heavier weighing carcasses (Boykin et al., 2017; McKenna et al., 2002). Moreover, with considerably more of the product requiring cooling, quality issues of color, tenderness, and water holding capacity could occur (Savell et al., 2005). Along with these, issues such as bone sours and sour knuckles are appearing with greater frequency in processing plants.

Sour knuckle is a quality defect seen in the round of beef carcasses and is associated with a sour/pungent odor upon opening of the patella joint. When this condition is identified during beef fabrication, the meat is removed from the production line and is often rendered, resulting in product loss. If, however, the product enters the market because it was not detected during fabrication, negative consumer perception could develop due to the intense off odors. Although this condition has been associated with beef carcasses for a long time, its origin and etiology are not clearly understood.

The sour knuckle condition is associated with *vastus femoris*, *vastus lateralis*, *vastus medialis*, and *rectus femoris* muscles expressing a sour/pungent odor that varies in intensity and was first reported in 1941 (Haines 1941). It is considered similar to bone souring in hams due to similar anatomical location, and similar clostridial species have been isolated from both product types (Ingram 1952; Nottingham 1960; Cosnett et al., 1956). On the other hand, some studies have noted that unlike bone souring in hams, the femur marrow is never soured in sour knuckles, with the defect being solely located in the muscle tissues alongside the femur (Lepovestky et al., 1953).

With the range of souring present in beef knuckles, it has been hypothesized that a mixture of several microorganism types might be responsible for the condition rather than a single microorganism (Nottingham 1960). However, when trying to evaluate possible bacterial populations for their ability to induce the condition, results were inconclusive (De Lacy et al., 1998; Shank et al., 1962). By utilizing a mixture of clostridial species associated with meat spoilage, De Lacy et al. (1998) was able to induce the souring condition. However, inoculation concentrations utilized were not representative of naturally occurring clostridial levels in beef rounds, which have been reported to be in the range of 0 - 2.0 log CFU/mL (Shank et al., 1962; Nottingham 1960; De Lacy et al., 1998). In addition, upon examining naturally sour and non-sour rounds, Shank et al. (1962) reported that the only samples to contain contaminated synovial fluid, which was hypothesized to be the origin of the condition, were from non-sour rounds. Furthermore, investigations are inconclusive about the role of clostridial species in the occurrence of the souring condition of beef rounds.

Due to these inconclusive microbiological results, another theory was hypothesized and that was that the condition could originate from complications in the postmortem metabolism (Shank et al., 1962, De Lacy et al., 1998). In support, volatile acid analysis indicated higher

amounts of propionic, acetic, and butyric acid in sour rounds compared to non-sour rounds (Shank et al., 1962). Further, Shank et al. (1962) were able to induce the souring condition in lamb legs with improper cooling (37°C overnight, followed by 4°C for five days). In addition, a gradient of souring intensities could be recreated by either incorporating propionate into the bloodstream or extraneous exercise of the ewes prior to slaughter (Shank et al., 1962). Even then, the cause of the sour knuckle condition remains uncertain, with most of the studies being done several decades ago. Since then, many changes have happened in the beef processing system, and the sour knuckle is starting to appear with greater frequency in beef processing plants. Therefore, the objective of the current study was to evaluate the microbial populations and volatile acids associated with the sour knuckle condition in beef carcasses.

2.2 Materials and Methods

2.2.1. Beef Knuckle Collection

Beef knuckles (*vastus femoris*, *vastus lateralis*, *vastus medialis*, and *rectus femoris* muscles) were collected during fabrication, over a two-day period, from a commercial fed-beef processing facility in the western region of the United States. During the fabrication process, the souring condition was identified by the employees working in the fabrication line. Upon identification, the knuckles were collected and separated into two categories based on the intensity of the souring condition, namely (i) slightly sour (SLI-SO; knuckles that had a lesser degree of souring expressing diary sour notes), and (ii) severely sour (SVR-SO; knuckles that had a greater degree of souring expressing raw swedge like notes). Additionally, knuckles that did not express any odors indicating the souring condition were collected randomly during the sample collection procedure and were designated as control samples. At the end of each of the two collection days, 15 knuckles (5 knuckles/treatment) were transported on ice to the Center of Meat Safety & Quality

at Colorado State University (N=30). Upon arrival, knuckles were aseptically halved, and halves were randomly assigned to be processed within 12 h (day 0) of collection or after 35 days of storage at 0 ± 2°C under vacuum packaged conditions (day 35).

2.2.2. Psychrotrophic Anaerobic Sporeformers

Upon collection of the knuckles at the beef processing facility, synovial fluid and the femur surface were sampled using separate sterile polyurethane sponges hydrated with 10 mL of HiCap neutralizing broth (Whirl-Pak, Nasco; Fort Atkinson, WI). The sponges were placed in a cooler, together with ice packs, and shipped overnight to a commercial testing laboratory (Food Safety Net Services [FSNS], San Antonio, TX) for psychrotrophic anaerobic sporeformer count analysis. Upon arrival at the testing laboratory, 15 mL of peptone water diluent (prepared in house by FSNS) was added to each sponge, followed by mechanical pummeling for 60 s. An aliquot (10 mL) of sample was then removed and added to a sterile flask containing 90 mL of tempered (approximately 45 ± 2°C) molten tryptone glucose extract agar (Neogen, Lansing, MI). Flasks were gently agitated and immediately placed in a circulating water bath set at 80 ± 1°C. They were held at this temperature for 30 min with occasional agitation. Following incubation, flasks were cooled in tepid (45 ± 2°C) water for ≤ 10 min before being poured into five petri dishes (approximately 20 mL per plate). Upon solidification of the agar, plates were incubated anaerobically at 25 ± 2°C for 10 days. After the incubation period, colonies exhibiting typical morphology were counted and the number of colony forming units (CFU) per sponge determined. The detection limit of the analysis was 0.4 log CFU/sponge.

2.2.3. Microbiological Analysis of Knuckle Muscle Tissue

Muscle tissue that was previously in direct contact with the femur was analyzed for aerobic plate counts (APC) and lactic acid bacteria counts (LABC) on day-0 and day-35 of storage. For each sample analyzed, 15 g was aseptically excised and placed into a 24 oz filter bag (Whirl-Pak) together with 30 mL of maximum recovery diluent (MRD; Acumedia-Neogen, Lansing, MI). Samples were then mechanically pummeled (Masticator, IUL Instruments, Barcelona, Spain) for 2 min and diluted in 0.1% buffered peptone water (BPW; Difco, Becton Dickinson and Company [BD], Sparks, MD). Appropriate dilutions were then plated, in duplicate, onto Petrifilm Aerobic Count Plates (3M, Maplewood, MN) for determination of APC. To obtain LABC for the samples, 1 mL of appropriate dilutions was transferred, in duplicate, to empty petri dishes. Then, 10 mL of molten (45-50°C) Lactobacilli MRS agar (Difco, BD) was added to each petri dish, swirled and allowed to set at room temperature. After the agar had set, a 10 mL overlay of molten Lactobacilli MRS agar was added to each plate. Colonies were counted after incubation of plates at 25 ± 2°C for 72 h (Petrifilm Aerobic Count Plate) or 5 days (Lactobacilli MRS agar). The detection limit was 0.5 log CFU/g for both analyses (i.e., APC and LABC).

2.2.4. Odor Panels

A seven-member trained odor panel evaluated raw beef knuckles for overall off odor, oxidation, putrid, and sour notes using a 10-point scale (10 = extreme off odor, extreme oxidation, extreme putridity, and extreme sourness; 1 = no off odor, no oxidation, no putrid, and no sour notes). Oxidized and unoxidized oil were used to train the oxidized notes, whereas the sour attribute was trained using fresh and old sour cream. Putrid notes were trained using putrefied and fresh meat whereas overall off odor was trained using a severely sour and a non-sour knuckle (Table 1). Meat samples from knuckles were collected from lean muscle tissue with immediate

contact of the femur surface. Upon collection, samples were diced and stored in capped glass test tubes at 0 ± 2°C. Prior to panels, samples were held at room temperature for 20 min prior to panelist evaluation. Sensory panels were conducted on day 0 and 35, with 15 samples (5 samples per treatment) presented per session. Samples were randomized prior to being assigned random 3-digit codes to mask sample identity. Panelist were presented samples in individual booths with instructions to shake the samples for 5 seconds before evaluation and to record results on the ballot provided (Figure 1).

2.2.5. Gas Chromatography-Mass Spectrometry

Gas chromatography-mass spectrometry (GS-MS) analysis was performed only on the day 0 control and SVR-SO (severely sour) samples to examine the two extremes. A portion (5 g) of the meat surface in contact with the femur was collected during the sample processing. Samples were then diced into small cubes and frozen with liquid nitrogen before being transferred into a 20 mL headspace vial and stored at -80°C until analysis.

For the GC-MS, the samples were incubated at 40°C for 30 min followed by extraction using a Carboxen/PDMS (SPME) fiber (85 µm, Stableflex, Sigma-Aldrich, St. Louis, MO) as described by Perez et al. (2008). Following extraction, compounds were injected into a DB-WAXUI column (30 m x 0.25 µm; Agilent Technologies, Santa Clara, CA) in a TRACE 1310 GC (Thermo, Waltham, MA) coupled to an ISQ-LT MS (Thermo). The SPME fiber was desorbed at the injection port (250°C) for 3 min, and additionally at the fiber conditioning port (270°C) for 3 min with the GC inlet operating under splitless mode. The oven program started at 35°C for 5 min, with the first ramp to 100°C at a rate of 8°C/min, the second ramp to 240°C at a rate of 12°C/min, and a final hold at 240°C for 5 min. These data were collected under the electron impact mode with a full scan of 35 -350 amu at a scan rate of 10 scans/second.

2.2.6. Statistical Analysis

Psychrotrophic anaerobic sporeformer counts recovered from sponge samples of the femur surface and synovial fluid were expressed as least squares means for log CFU/sponge, and, bacterial populations (APC and LABC) recovered from muscle tissue samples were expressed as least squares means for log CFU/g. The analysis was conducted as a paired comparison design. All variables were analyzed using R Studio (v. 3.5.1.), with treatment (control, SLI-SO, or SVR-SO) as the factor of the study design with significance at an alpha level of 0.05. The ANOVA function was utilized from R Studio (v. 3.5.1) to determine significant differences. Upon identification of a significant difference ($P < 0.05$), the lsmeans function was used to determine the statistical difference between treatment groups.

2.3. Results and Discussion

2.3.1. Psychrotrophic Anaerobic Sporeformers in Synovial Fluid and Femur Surface

Mean psychrotrophic anaerobic sporeformer counts for synovial fluid samples ranged from < 0.8 (SLI-SO and SVR-SO) to < 1.0 (control) log CFU/sponge, with no ($P > 0.05$) differences identified between the three treatment groups (Table 2). It should be noted that counts of 50% of the control and SLI-SO samples, and 20% of the SVR-SO samples, were below the analysis detection limit (0.4 log CFU/sponge). Similarly, psychrotrophic anaerobic sporeformer counts recovered from sponge samples of the femur surface were low, ranging from < 0.8 (SVR-SO) to 1.2 (control) log CFU/sponge (Table 2). Additionally, non-detectable counts (< 0.4 log CFU/sponge) were obtained for 40% of the SLI-SO samples and 10% of the SVR-SO samples (Table 2). A significant difference ($P < 0.05$) was identified between the counts of the control and sour samples; however, the log-unit difference between these treatments was small (Table 2).

While a few studies have suggested that mesophilic anaerobic sporeformers such as clostridia could be responsible for the off odors (Ingram 1956; De Lacy et al., 1998), results from the current study could not differentiate ($P > 0.05$) sour and non-sour knuckles based on psychrotrophic anaerobic sporeformer contamination. Although, De Lacy et al. (1998) was able to induce the souring condition by inoculating the synovial fluid of beef knuckles with several *Clostridium* spp. associated with meat spoilage. However, the authors indicated that the high concentration of *Clostridium* spp. used in the inoculation was not representative of populations identified in other studies (Haines & Scott 1940; Nottingham 1960).

2.3.2. Microbiological Counts of Knuckle Muscle Tissue

Muscle tissue from control, SLI-SO and SVR-SO knuckles was analyzed for APC and LABC in a further attempt to determine if there are microbial population differences between sour and non-sour knuckles. Results showed similar APC and LABC for control, SLI-SO, and SVR-SO samples, irrespective of sampling day (day 0 and day 35) (Table 3). Nottingham (1960) found similar results and concluded that due to low populations of aerobic bacteria in sour knuckles, such organisms are most likely not responsible for the condition. Findings reported by Shank et al. (1962) further supported this idea as sour and non-sour knuckles could not be differentiated based on aerobic counts of the bone marrow, muscle tissue, and popliteal lymph nodes sampled. In addition, Shank et al. (1962) aseptically transferred plugs of meat from soured knuckles into knuckles lacking the condition, but was not able to induce the souring odor, further suggesting that the condition might not be of bacterial origin. In the current study, APC and LABC of muscle tissue samples reached approximately 7 log CFU/g after 35 days of vacuum-packaged storage at 0 ± 2°C (Table 2). The increase in bacterial counts during storage is expected as the low initial numbers of microbial populations recovered on day 0 of storage would be expected to replicate

and increase in number through 35 days of refrigerated storage. Recovery of approximately 7 log CFU/g of lactic acid bacteria was also not unexpected since the product was stored vacuum-packaged, and lactic acid bacteria are known to predominate under such packaging conditions (Egan 1983).

2.3.3. Odor Panel

Odor panels performed on knuckles on day 0 indicated significant differences ($P < 0.05$) for off odor, oxidation, putrid, and sour notes between control and sour knuckles (both SLI-SO and SVR-SO) (Figure 2A). However, the panelists were not able ($P > 0.05$) to differentiate between SLI-SO and SVR-SO knuckles on day 0 (Figure 2A). After 35 days of storage, a significant difference ($P < 0.05$) was observed between control, SLI-SO, and SVR-SO knuckles for off odor, oxidation, putrid, and sour notes (Figure 2B). With current productions systems, it is unlikely that a soured knuckle entering the retail market would be similar to the sour knuckles in the day 0 panel results. Taking an average of 20.5 days for a beef product to reach the retail market, products exhibiting the souring condition in the retail setting would be better speculated to resemble results from day 35 panels with the consumer being able to differentiate between a slight and severely sour knuckle (Guelker et al., 2013). With this, producers risk inflicting negative perceptions of their products due to the foul odors present and harming consumer trust. While the souring odors have been identified to range from sweet to sewer-like, further research is needed to determine when consumers will reject a product exhibiting sour odors (Nottingham 1960, Shank et al., 1962).

2.3.4. Gas Chromatography-Mass Spectrometry (GC-MS)

Although the odor panelists were able to identify the difference in sourness between treatments, GC-MS results indicated no significant differences ($P > 0.05$) between SVR-SO and control day 0 samples. These results are interesting as the GC-MS analysis was performed only on control and the severely sour (SVR-SO) treatment groups to compare the two extremes. When evaluating acetoin, along with acetic, butanoic, and propionic acids, a wide variation was observed between the two treatment groups (Figure 3). However, when evaluating individual samples, SVR-SO knuckles were found to express greater amounts of acetoin, along with acetic, butanoic, and propionic acids (Figure 4). These results suggest that the extent of sourness was not consistent between samples within the treatment group and could have possibly led to the lack of statistical differences ($P > 0.05$). In contrast, when evaluating sour and non-sour knuckles, Shank et al. (1962) observed greater amounts of propionic, butyric, and acetic acids in sour knuckles.

Overall, the sensory analysis identified differences ($P > 0.05$) between sour and non-sour beef knuckles on day 0 and between all three categories after 35 days of refrigerated vacuum-packaged storage. However, the culturable microorganisms tested for were not different between the treatments. This suggests the possibility that the causative microorganism, if the sour condition is in fact of a microbial nature, may be non-culturable. It is estimated that fewer than 1% of the prokaryotes in most environments can be cultivated in isolation, and possibly the microorganism(s) responsible for producing the sourness was unable to be detected through this investigation's methodology (Schloss & Handelsman 2005). This can be due to the inability for microbiologists to recreate the complex set of environmental conditions required to grow many bacterial species. Culture-independent DNA sequencing techniques provide a powerful way to rapidly and inexpensively characterize the unculturable majority. Specifically, 16S ribosomal RNA (rRNA)

sequencing analysis could be utilized to determine if microbial communities of control (not sour) and sour knuckles differ. 16S rRNA sequencing is ideal when working with host-associated samples with high amounts of host DNA (e.g., any meat samples) because a bacterial-specific, taxonomically informative gene is amplified using the polymerase chain reaction (PCR) and sequenced. Unlike shotgun metagenomics, in which all DNA in a sample is sequenced, 16S rRNA sequencing limits DNA sequencing specifically to microbial DNA.

2.4. Conclusions

Upon examination of sour and non-sour knuckles, a clear odor difference between control and sour (SLI-SO and SVR-SO) knuckles was identified by the panelists. Furthermore, following 35 days of refrigerated vacuum-packaged storage conditions, the panelists identified differences between all three treatment groups. However, the microbiological evaluations indicated no differences among the treatment groups with regards to APC and LABC of muscle tissue, and psychrotrophic anaerobic sporeformers of femur surface and synovial fluid sponge samples. It is possible that the sour condition could be due to the presence of non-culturable microorganisms (organisms that cannot be cultured with traditional methods). The best approach to investigate these would be to conduct a 16S rRNA analysis. In contrast, gas chromatography-mass spectrometry results showed that compounds such as acetic, acetoin, propionic, butyric, and isobutyric acid tended to be different between severely sour and non-sour (control) samples. Due to the limited sample size and the spectrum of sourness, there was no statistical difference in the volatiles analyzed. However, a definite pattern emerged in the volatile analysis differentiating sour and control knuckles. These data could be used as the base for further research to develop rapid detection techniques for sour knuckles. Improper chilling and animal stress could also be potential causes for this condition; however, further research is necessary to investigate the possibility.

CHAPTER 3

REVIEW OF LITERATURE

For millennia, humans have relied on animals as the primary source of protein in the diet. As early humans hunted and gathered food, high fiber, and low energy foods were not often worth the energy gained during collecting when contrasted to the net energy gained from the hunting of animals for dietary energy (Mann 2007). With the agricultural revolution, selective breeding of plants allowed for the selection of lower fiber and energy-dense varieties.

Additionally, the domestication of livestock allowed for a continuous source of protein that ultimately replaced the hunting of wild game. Livestock animals are known for their ability to convert low quality forages into energy-dense meat and milk products (Augustin et al., 2016). With the introduction of the feedlot systems, producers started formulating rations that allow for maximum animal performance. However, when feeding these animals in feedlot systems, sources such as grain and corn must be utilized to meet dietary requirements. Due to the ruminant's ability to convert low quality food into high-quality protein, often more of the feedstuffs is required to meet nutritional needs. Improvements in nutrition, health, and management of livestock in feedlot production systems have resulted in increased individual animal performance (Capper, 2011). For example, when evaluating beef production systems of 1977 and 2007, operations in 2007 were able to generate 1 billion kg of beef with 30.1% fewer cattle (Capper, 2011). Even with these vast improvements, various studies have identified between 6 to 20 kg of grain is required to produce a single kg of beef (Eshel et al., 2014). Also, it is estimated that 30-40% of the plants used as animal feedstuffs could be fed directly to humans as a food source (Erb et al., 2012). Due to these

findings, a popular viewpoint is to take the grain and corn that would be fed to livestock and use it for human consumption.

This feed or food dilemma is apparent in monogastric and ruminant animals and is speculated to increase as populations rise (Makkar, 2016). While ruminants can utilize feedstuffs that do not compete with human feed sources, monogastric animals require higher quality feed that directly competes with human food sources (Mottet et al., 2017). Even with more efficient feed-to-gain ratios identified in monogastric animals, the direct competition for human food sources is a problem for growing populations. Currently, to help reduce the amount of human-edible plants in rations, producers are incorporating byproducts that would be otherwise wasted. While these byproducts may not entirely replace corn and grain within rations, they can provide a portion of the protein, energy, and fiber to meet livestock requirements while reducing the amount of human-edible foods in rations (Salami et al., 2019). With a predicted population of 9.6 - 12.3 billion people by 2100, food systems must become even more effective at generating more food from fewer resources due to the growing population. It is speculated that by 2020, to meet worldwide demand, 303 million metric tons of meat will be required, which is close to twice the amount that was needed in 1983 (Delgado et al., 2001). Furthermore, a growing demand for protein-rich diets is increasing in both developed and developing countries due to rapid growth, urbanization, and increasing socioeconomic status around the world (Steinfeld et al., 2006; Slingo et al., 2005). Even with the improvements in production systems, the task will be daunting for upcoming generations of future agriculturalists.

3. Meat Alternatives in the Marketplace

For the consumption of meat to occur, animals must be slaughtered and processed. Due to the cost of other sentient beings to generate meat, many consumers feel conflicted. Due to the high

sensory and nutritional attributes of meat, people enjoy meat consumption, but simultaneously dislike hurting animals (Loughnan et al., 2010). This condition, as described by Loughnan et al. (2010), has been identified as the "meat paradox." In recent years, there has been a growing availability of alternative proteins that offer consumers options that are free of the meat paradox. Rabobank (2017) speculates that the current market size for alternative protein products is 130,000 tons for the European Union and 120,000 tons for the United States and Canada per year and predicts a 6 to 8 percent growth in demand for alternative protein sources in the next five years. Thus, alternative protein sources have the potential to play a significant role in meeting the demand in protein over the next decade.

To compete in this market, meat companies such as Tyson, Smithfield, JBS and Cargill have begun developing plant-based product lines to meet this growing consumer demand. Alongside these companies, billionaires such as Bill Gates and Richard Branson, have invested in alternative protein sources either through direct ownership or minority investments. While there are many alternative protein sources available, they can be generally categorized as (i) meat substitutes (plant protein-based products), (ii) emerging alternatives (insect protein), and (iii) laboratory-grown meat. Of the alternatives identified in this review, the plant-based products have the greatest retail market presence. While plant-based products have been in retail markets for some time, a new era of these products has emerged that is focused on simulating nutritional and sensory aspects of animal protein in a plant-based matrix. While not prevalent in western culture, insect proteins have been consumed worldwide throughout history (de Castro et al., 2018). Containing large amounts of amino acids along with low environmental impacts, insect proteins will be discussed in greater detail in subsequent sections (Oonincx & de Bore, 2012; de Castro et al., 2018). Currently unavailable in the protein marketplace, cell cultured meat has gained large

amounts of media attention and consumer interest. This product is unique because it generates animal muscle tissues for consumption without harming livestock species. Additionally, cell cultured meat has been reported to reduce environmental impacts when compared to conventional livestock production (Tuosmisto & de Mattos 2011).

Recent studies have indicated that animal proteins cannot be replaced entirely, because, for populations living in developing countries, livestock is more than food and often serve as a livelihood for rural populations (Springman et al., 2019). In recent years, the modernized livestock systems have received criticism regarding animal welfare, environmental impact, sustainability, and human health concerns with consumption of red and processed meats (Hoquette et al., 2016). Reports such as EAT from the Lancet journal suggest a healthy and environmentally sustainable diet consists of consuming none or little amounts of red and processed meat (Willett et al., 2019). In the meantime, consumers' interest in non-animal or alternative protein sources have gained popularity and appear poised to increase in market share within the protein marketplace. The overall longevity of these products in the marketplace could be determined by the product's environmental impact, consumer acceptance, and nutritional aspects.

3.1. Environmental Impacts

3.1.1. Life Cycle Assessments

To evaluate the effects of livestock and food production practices on the environment, researchers utilize a modeling tool known as a life cycle assessment (LCA). LCA's can determine inputs required in a production system and present findings on a cradle to grave basis. When building LCA's, the researcher gathers information from databases and past reports (such as feed efficiency and water usage reports) from years before and generate estimated numbers to predict future trends. Due to several LCA's reporting a high environmental impact associated with

livestock production, several researchers have evaluated the replacement of animal protein with alternative sources (Goldstein et al., 2017).

3.1.2. Animal Proteins

Worldwide livestock production was estimated to generate 14.5% of greenhouse gas emissions (Gerber et al., 2013). Although livestock production in the United States is amongst the most efficient globally, there are significant environmental impacts (Matthews 2013; Goldstein et al., 2017). As livestock are commonly reared in intensive settings, land required for grazing has been replaced by cropland that is necessary to supply feedstuffs to animals in commercial feedlots (Naylor et al., 2005). However, when livestock are finished on pasture, some environmental impact is reduced, but contributions are mitigated by poor land management and overgrazing (Heller & Keoleian 2018).

When evaluating the environmental impact of livestock, it is dependent on the species under evaluation (Mattick et al., 2015; Goldstein et al., 2017). Typically, of all the livestock animals, cattle are identified to contribute to the highest environmental impact per kg of edible meat produced. However, feed to gain ratios found in poultry or swine are speculated to be equal to or less than in environmental impact than alternative protein sources (Mattick et al., 2015).

While not as prevalent in the United States, the demand for animals' feedstuffs has led to a loss of forest and grazing lands worldwide. The most prominent example of this is the large-scale soybean production systems present in Brazil. With a higher demand in soybean feedstuffs production, an increase in deforestation and reduction in biodiversity has occurred over the years as forest is converted into arable cropland throughout Brazil (Fearnside 2000).

3.1.3. Insect Proteins

When evaluating insect protein, previous studies have identified a reduction in overall environmental impact when contrasted to livestock production systems (Oonincx & de Boer 2012; Smetana et al., 2015). While there are few LCA's conducted on insect protein, complications have been identified within recent studies. While insect protein production occurs throughout the world, the LCA's reported in literature focus primarily on European style production systems (Halloran et al., 2016). Additionally, most research conducted focuses on a small selection of insect species and lacks processing and storing methods due to a lack of standardization within the industry (Oonincx & de Boer, 2012; Halloran et al., 2016). In short, a few LCA's published show an overall reduction in environmental impact, but to truly estimate the effects, more information on production and processing procedures will be required (Halloran et al., 2016; Oonincx & de Boer, 2012).

3.1.4. Plant-Based Proteins

The LCA's of plant-based proteins have identified a reduction in environmental impact when compared to beef production systems (Goldstein et al., 2017; Heller & Keoleian 2018). When comparing beef production to the Beyond burger (a pea protein-based plant product), reductions in water, energy, and global warming potential were reported (Heller & Keoleian 2018). However, when compared to other livestock species, the plant-based protein was found similar to swine production in environmental impact, along with poultry and insect proteins (Goldstein et al., 2017). Furthermore, replacing beef with chicken might result in a lower environmental impact than that of a plant-based protein, due to the animals' efficiency at converting feedstuffs to edible lean (Goldstein et al., 2017).

3.1.5. Cell Cultured Protein

When utilizing LCA's to determine the impacts of cultured proteins, results are often contradictory. Due to the deficit in knowledge of how the production and processing happens exactly, researchers have to speculate on the application of the technology in a large-scale setting, which makes it impossible to set the LCA parameters accurately. Tuosmisto and de Mattos (2011) reported lower greenhouse gas emissions (GHG), along with land and water use to produce cell cultured meat compared to meat produced from livestock. In this study, due to the lack of information on production, procedures were based on cell culturing protocols conducted at the University of Amsterdam. In contrast, Mattick et al. (2015) reported that the cell cultured production required the most industrial energy, along with a global warming potential similar to pork and poultry production. The conclusions from the Mattick et al. (2015) study were based on the use of protocols for the cell cultivation of hamster ovary cells. While there is no currently accepted production procedure, these papers exhibit how changing production procedures can change the total environmental impacts of the production system. Regardless of the protocols, both studies identified that the majority of energy requirements originated from the basal media production used for growing the muscle cells (Tuosmisto & de Mattos 2011; Mattick et al., 2015). Until large-scale processing methods and materials are standardized, researchers will only be able to roughly estimate the environmental impacts of cultured protein production. Without this knowledge, researchers must estimate the industrial energy required to produce the product in a large-scale setting.

3.2. Consumer Acceptance

3.2.1. Animal Proteins

Animal proteins have played a significant role in society. From their origins as valuable nutrient sources to early hunters and gatherers, throughout humanity's timeline, meat has ingrained itself into social and cultural aspects of human life (Leroy & Praet 2015). When evaluating the United States and other western societies, diets based on animal protein are the norm among consumers (Sanchez & Sabate 2019). Upon cooking, the mallard reaction creates a myriad of desirable flavor attributes such as roasted, browned, meaty, juicy, and tenderness that consumers desire (Kerth & Miller 2015). Due to these attributes, consumers who eat any amount of animal protein are typically unwilling for a complete replacement with an alternative in their diet. When evaluating consumer opinions on reducing meat consumption, consistently, a group of consumers was identified that are defiant to give up animal protein (Lemken et al., 2019; Elzerman et al., 2013; Graça et al., 2015; Hoek et al., 2011). When evaluating American consumers, 25.3% identified themselves as unwilling to give up animal proteins within their diet (Bryant et al., 2018).

With animal proteins, both heavy and moderate meat-eating consumer groups have been identified. Studies have shown that the heavy meat consumer group typically consists of older males that express the greatest reluctance to try any alternative protein sources (Hoek et al., 2011; Graça et al., 2015). This is further supported by a study that identified that heavy animal protein consumers valued familiarity, and found this familiarity in animal proteins, while the plant-based alternative was viewed as taboo (Hoek et al., 2011). To overcome this rejection, alternative protein sources must replicate the sensory and textural attributes of animal protein (Schouteten et al., 2016; Hoek et al., 2011). Overall, those who consume meat are unwilling to give up their consumption and see no reason to replace their preferred protein source. When evaluating the reasoning of heavy

meat consumers, the aspects of hedonism, affinity, entitlement, and dependence were identified (Graça et al., 2015). When observing the opposite consumer (such as vegetarian or vegan), the most crucial motive for replacement was animal welfare aspects followed by dietary health concerns (Graça et al., 2015). This ideology changes, however, when evaluating consumers who are willing to replace or reduce, but not eliminate, animal protein in the diet. While those who do not consume meat rank animal welfare as the most significant concern, those who are willing to reduce the amount of meat in their diet are motivated by environmental impacts (Graça et al., 2015).

3.2.2. Plant-Based Proteins

Upon first encounter, consumers whose diet consists of animal protein commonly express neophobia (the fear of foods) to plant-based alternatives (Tuorila et al., 2001). However, while the initial acceptance of plant proteins may be low, a long-term study determined that consumers showed no preference between plant, animal, and tofu protein sources (Hoek et al., 2013). Even though no preference is apparent between animal and plant proteins, following long-term incorporation into the diet, the likelihood of initial incorporation is low due to the presence of food neophobia (Hoek et al., 2013; Tuorila et al., 2001). When purchasing plant-based protein, consumers expect a sensory experience similar to that of animal protein (Sexton 2016; Hoek et al., 2011, 2013). In early plant-based protein alternatives, comprised of soy-based products, consumers commonly identified an undesirable uniform taste and negative soy flavor (Elzerman et al., 2013). Additionally, negative mouthfeel attributes associated with compactness, dryness, and softness of the soy-based alternatives were also identified (Elzerman et al., 2013). While some products currently in the marketplace (such as the Beyond Burger and Impossible Burger) claim to replicate

the textural and mouthful components of animal proteins, no current research has investigated the sensory attributes of these newer products.

3.2.2.1. The Flexitarian Consumer

While many consumers are unwilling to give up or reduce the intake of animal proteins in their diet, a fraction of consumers are. This fraction of consumers that is willing to reduce but not entirely replace animal protein in their diet have been identified as the "flexitarian" consumer by several studies (Sanchez-Sabete & Sabate 2019; Vanhonacker et al., 2013; Graça et al., 2015). A study by de Boer et al. (2014) identified the "flexitarian" consumer group with results showing that 81% of 253 people surveyed in the study were intentionally replacing meat with an alternative protein source at least once every week. These purchasing and consumption decisions may contribute to the reason for a reduction of animal protein in the diet compared to a complete replacement.

When motivations for reduction were studied it was determined that environmental concerns are the driving factor in flexitarians, while those consuming a vegan and vegetarian diet rated animal welfare as the driving factor (Haverstock & Forgays 2012; Sanchez-Sabete & Sabate 2019; Fox et al., 2008; Hussar et al., 2010; Jabs et al., 1998).

3.2.3. Cell Cultured Meat

Cell cultured meat is currently unavailable to consumers in the marketplace. While many consumers are willing to try the product, the majority of consumers already express a negative view of the product due to the perceived unnaturalness (Wilks & Phillips 2017; Slade 2018). While the majority of consumers are not accepting of the product, consumers do speculate that cell cultured meat would have lesser environmental impacts when compared to conventional

agriculture (Wilks & Phillips 2017). However, when sensory attributes were stated to be similar, a majority of consumers preferred the animal-based product, with only 13% of consumers preferring a cell cultured meat product (Slade 2018). Additionally, when the price was incorporated, if given a choice between a cheaper beef burger a plant-based protein or a cell cultured protein that are a dollar higher in cost than the beef product, approximately two-thirds of consumers would purchase the beef product (Slade 2018). When heavy meat consumers were asked to replace meat in their diet, the cell cultured product received higher acceptance when compared to a plant-based protein (Slade 2018; Bryant & Barnett 2018). To further support these findings, when asked to replace meat in their diets, 19.3% of consumers reported a greater acceptance of cell cultured meat over a plant based product along with 28.4% reporting a slightly greater acceptance of cell cultured meat in the diet over a plant-based product (Wilks & Philips 2017).

3.2.4. Insect Proteins

Many western consumers astoundingly reject insect proteins as these products are seen as a protein source only consumed in dire situations (Verkerk et al., 2007). Due to this bias, insect proteins have a minimal share of the western protein market, regardless of the nutritional density of the products (Verkerk et al., 2007). However, while consumers reject a completely insect-based product, when consumers sampled a blend of insect and animal protein, greater acceptance was identified (Caparros Megido et al., 2016). These findings indicated that while a pure insect product may not be accepted, integration would be possible if insect protein was first introduced in a product that is mixed with a more consumer-accepted protein (Caparros Megido et al., 2016).

3.3. Nutrition

3.3.1. Animal Proteins

Animal proteins are regarded highly for their nutritional and caloric densities. When evaluating nutritional aspects, Vitamin B12 is found most commonly in animal proteins. Vitamin B12 is vital to the diet and when a deficiency occurs, the body is unable to produce enough healthy red blood cells to deliver oxygen adequately. Along with vitamin B 12, vitamin A is found in the most bioavailable state in animal protein sources (Biesalski 2005). While vitamin A can be supplemented with a provitamin, these supplements must contain higher amounts of provitamin A due to a reduced conversion rate (Biesalski 2005). When evaluating those who practice vegetarian and vegan diets, deficiencies of dietary iron, vitamin B12, and selenium were identified as potential problems (Sanders 1999). While these nutrients could be supplemented, animal protein is considered a highly bioavailable source of selenium, iron, and folic acids that can be immediately utilized by the body. While there has been a negative connotation of red meat in the diet, studies focused on lean red meat (meat products with < 10% total fat) have positive effects on health (Li et al., 2005). Furthermore, a review by Li et al. (2005) found that diets with lean red meat do not raise blood cholesterol and low-density lipoprotein levels in the body.

3.3.2. Plant-Based Proteins

In recent years, several manufacturers have developed products with much improved sensory properties. Products such as the Impossible Food's Impossible Burger and Beyond Meat's Beyond Burger have grown in popularity. For the Impossible Burger, the primary protein ingredients are textured wheat protein, potato protein, and soy protein isolate. With the deficiency of several vital nutrients, this product is supplemented with vitamin B12, vitamins E and C,

thiamin, zinc, niacin, riboflavin, and vitamin B6 to give the product nutritional density. The Beyond Burger only utilizes pea protein isolate as the primary source of protein. This product is then mainly composed of oils, including canola, coconut, and sunflower oils, along with other ingredients such as beet juice extract that simulates a red color similar to meat.

While there is no current research (other than the nutritional label) on the Beyond Burger or Impossible Burger, several studies have evaluated nutritional densities of the individual ingredients that may help to approximate the nutrient density of these new food products. When evaluating overall protein makeup, wheat (81% protein), potato (80% protein), and pea (80% protein) were identified as the most protein-dense of plant-based isolates (Gorissen et al., 2018). This study further evaluated these foods for essential amino acid makeup and identified the potato protein as having the highest amount (at 37%), followed by pea (30%) and soy protein isolates (27%) (Gorissen et al., 2018). Additionally, this study evaluated lysine levels of the three protein isolates and found that both potato and pea had significantly higher amounts of lysine than soy protein (Gorissen et al., 2018). While the exact makeup of the two plant-based protein sources, one could assume that the amino acid makeup of the Impossible Burger could be closer to meeting dietary requirements.

3.3.3. Contrasting Plant- and Animal-Based Protein Diets

Currently, more than 1 billion people over the age of 20 are considered to be overweight worldwide (Finucane et al., 2011). When evaluating plant and animal protein-based diets, reductions in body mass index (BMI) scores have been identified in plant-based diets (McEvoy et al., 2012; Turner-McGrievy et al., 2017; Barnard et al., 2015; Tonstad et al., 2013). In addition to decreased BMI scores, lower risk for coronary heart disease (CHD) have been identified (Marsh et al., 2012; McEvoy et al., 2012). However, with the uptake of a plant-based diet, there is a high

possibility that iron, vitamin D, vitamin B12, and n-3 fatty acids will be restricted in the diet (McEvoy et al., 2012). While these nutrients are identified in plant-based products, the bioavailability is uncertain. With the presence of phytate in plant proteins, the absorption of minerals such as iron, zinc, and calcium can be restricted (Gibson et al., 2010). With dephytinization methods, cereals and legumes can eliminate phytate levels resulting in higher amounts of bioavailable nutrients (Gibson et al., 2010).However, some cereal and legume sources are naturally low in essential nutrients and must receive further enrichment to meet nutritional requirements, regardless of phytate content (McEvoy et al., 2012; Gibson et al., 2010).

In addition to vitamin and mineral differences between plant- and animal-based products, the protein composition and digestibility also vary (van Vilet et al., 2015; Gorissen et al., 2018). When evaluating amino acids, leucine has been identified to have the most significant impact on postprandial stimulation of muscle protein synthesis (MPS) of all amino acids (van Loon 2012). Due to lower amounts of leucine in soy protein (6.0 – 8.0% of amino acid makeup) when compared to animal proteins (8.5 – 9.0% of amino acid makeup), a lower amount of MPS was observed when investigated by van Vliet et al. (2015). While these issues can be overcome with supplementation and proper diet formulations, more research is needed to determine the protein makeup of other plant sources (van Vliet et al., 2015).

CHAPTER 4

NUTRITIONAL ANLAYSIS OF PLANT AND ANIMAL PROTEINS

4.1. Introduction

For centuries, humans have relied heavily on animals as the primary source of high-quality protein in the diet. The high nutritional value of meat, which provides proteins in quantity and quality, along with many micronutrients such as heme iron, has been a significant driver behind the popularity of meat. However, in recent years the use of livestock for protein production has received negative media publicity regarding animal welfare, environmental impacts, and health concerns (Hoquette et al., 2016). Due to the high sensory and nutritional attributes of meat, people enjoy meat consumption, but simultaneously dislike hurting animals (Loughnan et al., 2010). To combat this quandary, a growing amount of plant-based proteins are entering the market with the claim of having a product that replicates the sensory attributes of meat without harming livestock.

In addition to addressing animal welfare concerns, another major marketing point of plant-based proteins is environmental sustainability. A recent study by Heller and Keoleian (2018) reported reductions in water, energy, and global warming potential when substituting a beef burger with a plant-based alternative. Additionally, when evaluating all livestock species, 14.5% of total greenhouse gas emissions worldwide are estimated to originate from livestock production (Gerber et al., 2013). However, when evaluating individual livestock species, such as swine, environmental impacts have been identified to be similar to the production of plant-based proteins (Mattick et al., 2015; Goldstein et al., 2017). Furthermore, when evaluating the quality of protein present in plant protein isolates, typically, lower amounts of essential amino acids are identified compared to

animal proteins (Gorissen et al., 2018). In addition to amino acids, vitamins and minerals such as vitamin B-12 and iron, along with many other macro and micronutrients are provided in animal proteins (Biegalski 2005; Sanders 1999). While some of these nutrients can be incorporated into product formulations for enrichment, no current research has investigated the nutritional components of these complex plant-based products. Therefore, the objective of this study was to evaluate the nutritional densities of three major plant-based protein products (Morning Star Farms Spicy Black Bean Burgers, Beyond Food's Beyond Burger, and Impossible Food's Impossible Burger) compared to two pork products (center cut boneless pork chops, and 80% lean 20% fat ground pork).

4.2. Materials and Methods

4.2.1. Sample Collection

Six cities (Seattle, WA; Peyton, CO; Memphis, TN; Newburgh, IN; Houston, TX; and Brooklyn, NY) were randomly selected from a list provided by the USDA for product collection. From each city, 800 g of 80% lean 20% fat ground pork (GP), boneless center cut pork chops (PC), Morning Star Farms Spicy Black Bean Burger (VB), Beyond Meats Beyond Burger (BB), and Impossible Foods Impossible Burger (IB) were collected from grocery stores or restaurants. Upon collection, products were kept on ice and transported Colorado State University's Meat Laboratory (Fort Collins, CO) within 24 h of collection before they were frozen and kept at -20°C until further processing. Replicates (n = 6) of each product were analyzed in both raw and cooked states for nutrient density.

4.2.2. Sample Cooking

Half of the total amount of products collected from the cities (i.e., 400 g per product) was subjected to cooking with product temperature being monitored with a digital thermocouple thermometer. Ground products (GP and all plant-based products) were cooked by pan-grilling on a non-stick anodized aluminum skillet to an internal temperature of 71°C. The PC was grilled utilizing a Salton two-sided grill (Spectrum Brands, Middleton, WI). Upon cooking, the PC was flipped at an internal temperature of 20°C, and then cooked to a final temperature of 63°C. Post cooking, cooked products were placed onto a stainless-steel wire rack and cooled at room temperature (approximately 22 ± 2°C) for 10 min. After cooling, cooked products on wire racks were refrigerated (0 to 4°C) for 12 - 24 h before sample homogenization.

4.2.3. Sample Homogenization

Due to sensitivity of B-vitamins and nutrients, and to prevent sample cross contamination, standardized protocols were used for sample homogenization. Samples were diced and then frozen using liquid nitrogen prior to homogenization. A stainless-steel spoon was used to transfer frozen samples into a 7 – quart (6.62-L) Robot Coupe BLIZER 6V (Robot Coupe USA Inc., Ridgeland, MS) and blended until samples appeared as a homogenized fine powder. Each sample was blended for 10 s on a low speed (1,500 rpm) and 30 s on a higher speed (3,500 rpm) and then stored under vacuum-packed conditions at -80°C for further analysis. Following, sample homogenization samples were sent to the Colorado State University Nutrition Lab for proximate and fatty acid analysis. While amino acid, mineral, vitamin, and allergen content of the samples was determined by an outside lab (Eurofins).

4.2.4. Proximate Analysis

Moisture analysis was performed using the Association of Official Agricultural Chemist (AOAC) oven drying method 950.46 (AOAC International, 1995). Approximately 1 g samples were weighed out into aluminum tins and allowed to dry for 24 h at 100°C in a forced air-drying oven. Percent moisture (%MC) was calculated using the following formula: %MC = [(wet weight – dry weight) / wet weight] x 100.

Percent ash was determined using the ashing method described by 923.03 of the AOAC International official methods (AOAC International, 1995). Approximately 1 g samples were placed into a pre-weighed crucible. Samples were then placed into a Thermolyne box furnace at 600°C for 18 h. Percent ash was then calculated utilizing the following formula:
%Ash = (ash weight / original wet sample weight) x 100.

Total lipid content was extracted using the Folch et al. (1957) method along with processes dictated in the AOAC official method 983.23 (AOAC International, 2006). One gram of the samples was homogenized in a 2:1 ratio of chloroform and methanol solution, respectively. The homogenized sample was placed onto an orbital shaker at room temperature for 20 min. The homogenate was then filtered through ashless filter paper. Following filtration, 4 mL of 0.9% NaCl was added to each of the filtered samples. Once all samples had received the solution, they were refrigerated (3 ± 2°C) for 24 h. When the filtrate separated into two phases, the lower phase was aspirated and placed into a pre-weighed scintillation vial. The vial was then dried under nitrogen gas. Following drying, the vial was allowed to air dry under a fume hood for 2 h and then placed into a forced air-drying oven to dry for 12 h at 100°C. Percent fat was then calculated using the following formula: %Fat = (fat weight / original wet sample weight) x 100.

Crude protein was determined by the AOAC method number 992.15 utilizing a TruSpec CN Carbon/Nitrogen Analyzer (Leco Corporation, St. Joseph, MI) (AOAC International, 2006). Percent protein was then calculated by multiplying the total percentage of nitrogen by a factor of 6.25.

4.2.5. Fatty Acid Analysis

Prior to analysis, total lipid content was extracted from 1.0 g of homogenized samples using methodology developed by Folch et al. (1957) as modified by Bligh and Dyer (1959). Methodology for saponification and methylation of lipids was based off processes conducted by Parks and Goins (1994). Individual lipids were separated via gas chromatograph fixed with a series 7683 injector and flame ionization detector fitted with a 100-m×0.25-mm (id) fused silica capillary column (SP-2560 Supelco Inc. Bellefonte, PA).

Cholesterol was. quantified following the AOAC Official Method 994.10 (AOAC International, 2006). The samples were saponified using ethanolic potassium hydroxide. The unsaponifiable fraction that contained cholesterol and other sterols was extracted with toluene. The toluene was evaporated, and the residue was dissolved into dimethylformamide where samples derivatized to form trimethylsilyl ethers. The derivatized cholesterol was quantitatively determined by gas chromatography using 5 alpha-cholestenol as an internal standard.

4.2.6. Inductively Coupled Plasma Mass Spectrometry Analysis

Analysis of elements (Ca, Mg, K, Na, Fe, Zn, Cu, Mn, and P) was conducted by inductively coupled plasma (ICP) emission spectrometry using the 984.27, 985.01, and 2011.14 methods according to AOAC International protocol (AOAC International, 2011). Samples were either dry-ashed, wet-ashed, or read directly. If dry-ashed, samples were placed in a muffle furnace set to

500°C until the sample was completely ashed. The resulting ash was treated with concentrated hydrochloric acid, dried and re-dissolved in a hydrochloric acid solution. If wet-ashed, samples were digested in a microwave or on a hot plate with nitric acid, hydrochloric acid, and/or hydrogen peroxide. The amount of each element was determined with an ICP spectrometer by comparing the emission of the unknown sample against emissions from standard solutions.

4.2.7. Vitamins

Vitamin A, as retinol, was quantified by following AOAC methods 992.04, 992.06, and 2001.13(AOAC International, 2006). Samples were saponified to break down fat and release vitamins within the sample matrix. The digest was then extracted with an organic solvent. Vitamin A was then quantitated along with all-trans retinol and 13-cis retinol by ultra or high-performance liquid chromatography.

Vitamin D was quantified by liquid chromatography – mass spectrometry (LCMS) based on the protocol from Huang et al. (2009). Upon saponification, vitamin D was released from the matrix. Following saponification, vitamin D was then extracted via liquid partitioning, dried down, reconstituted, and analyzed by liquid chromatography-tandem mass spectrometry.

Vitamin E was measured according to the methods of Speek et al. (1985), Cort et al. (1983), and McMurray et al. (1980). Vitamin E is typically saponified to break down the fat and release the B-vitamins, with the digest being extracted with an organic solvent. Tocopherol was then quantitated by ultra or high-performance liquid chromatography (UHPLC or HPLC) with fluorescence detection.

Vitamin K levels were determined from AOAC methods 992.27 and 999.15 (AOAC International, 2009). Samples were extracted with organic solvents and injected on a reverse-phase

high-performance liquid chromatography (HPLC) system with post column reduction and fluorescence detection.

Thiamin content was determined by fluorometric methods dictated by AOAC methodology 942.23, 953.17, and 957.17 (AOAC International, 2006). Samples were autoclaved under weak acidic conditions to free thiamin forms bound to protein. The resulting solution was incubated with a buffered enzyme solution to complete the release of any bound thiamine with samples purified on a cation-exchange column. An aliquot was taken and reacted with potassium ferricyanide to convert thiamin to thiochromone. The thiochromone was extracted and read on a fluorometer with samples being quantified using an external standard.

Riboflavin content was determined by following AOAC 940.33 and 960.46 protocols (AOAC International, 2006). Samples were hydrolyzed with diluted hydrochloric acid and pH was adjusted to remove interferences. The amount of riboflavin was determined by comparing the growth response of the sample using the bacterium, *Lactobacillus rhamnosus*, with the growth response of a riboflavin standard, with growth responses being measured turbidimetrically.

Biotin was determined with procedures established by Scheiner et al. (1975), Wright et al. (1944), and Scheiner (1996). Unbound biotin within samples was extracted with water, while bound biotin was extracted with diluted sodium hydroxide, sulfuric acid, or disodium ethylenediaminetetraacetic acid solution. The amount of biotin was determined by comparing the growth response of the sample to a standard, using the bacterium, *Lactobacillus plantarum*, with the growth response measured turbidimetrically.

Niacin was determined following AOAC 944.13 and 960.46 methodology(AOAC International, 2006). Samples were hydrolyzed with diluted sulfuric acid and pH was adjusted to remove any interference. The amount of vitamin B3 was determined by comparing the growth

response of the sample, using the bacterium, *Lactobacillus plantarum*, compared to the growth response of a Vitamin B3 standard. Responses from testing were measured turbidimetrically.

Pantothenic acid was determined following AOAC methods 945.74, 992.07 and 960.46 (AOAC International, 2006). Samples were treated with an enzyme mixture to liberate any bound pantothenic acid to assay for total pantothenic acid, with pH adjusted to remove interferences within samples. The presence of pantothenic acid was determined by comparing the growth response of the sample using the bacterium, *Lactobacillus plantarum*, with the growth response of a calcium pantothenate standard with growth responses measured turbidimetrically.

Pyridoxine Hydrochloride was determined following AOAC method 961.15 protocol (AOAC, 2005). Samples were hydrolyzed with diluted sulfuric acid in an autoclave, with pH adjusted to further liberate vitamin B6 and remove interferences. The amount of vitamin B6 was determined by comparing the growth response of the sample using the yeast, *Saccharomyces cerevisiae*, to a vitamin B6 standard with responses measured turbidimetrically.

4.2.8. Amino Acids

Cystine, cysteine, and tryptophan were determined following methodology dictated by previous studies (Schuster et al., 1998; Henderson et al., 1989; Henderson et al., 2010). This process consisted of hydrolyzation in 4 N hydrochloric acid for 24 h at approximately 110°C. Phenol was added to the 6 N hydrochloric acid to prevent halogenation of tyrosine. Cystine and cysteine were converted to S-2-carboxyethylthiocysteine by the addition of dithiodipropionic acid. Tryptophan was then hydrolyzed from proteins by heating at approximately 110°C in 4.2 M sodium hydroxide. Samples were then subjected to analysis via HPLC after pre-injection deracemization. The primary amino acids were derivatized with o-phthalaldehyde (OPA), while the secondary amino acids were derivatized with fluorenyl methyl chloroformate before injection.

4.2.9. Organic Acids

Sorbate and benzoate levels were obtained using procedures described by Bui and Cooper (1987). Samples were extracted with water and methanol. The organic acids were then separated using reverse HPLC and measured with UV detection.

Succinate, formate, tartrate, and fumarate were quantified by following AOAC International standards Method 986.13 (AOAC International, 2005). Samples were extracted with 0.016 M sulfuric acid; then the extract was analyzed on an HPLC system equipped with a UV detector.

Acetate, citrate, malate, lactate, pyruvate, quinate, and oxalate levels were obtained following AOAC official method 986.13 procedures(AOAC International, 2006). Samples were treated with an acidic solution to begin the extraction of organic acids. The samples were then filtered, centrifuged, and re-filtered again before organic acids were separated utilizing reverse-phase HPLC and UV detection.

Propionate levels were determined by following the procedure in method 986.13 as described in the AOAC (AOAC International, 2006). This process consists of treating samples with an acidic solution to extract organic acids. Samples were filtered, centrifuged, and then additionally filtered to remove any contaminants before samples were subjected to separation via HPLC and UV detection.

4.2.10. Allergens

Almond, egg, gluten, hazelnut, milk, soy and walnut allergens were evaluated using ELISA kits. Each kit is designed to detect the appropriate allergen protein 30 min post extraction. Following the test procedure, color changes in sample wells were compared to standards in control

wells using a microwell reader. Quantified allergen protein concentrations were then computed using comparisons.

4.2.11. Statistical Analysis

The analysis was conducted as a paired comparison design. Variables were analyzed using R studio (v.3.5.1.), with product (VB, BB, IB, PC, GP) and cooked state (raw or cooked) as the factors of the study design with significance set at an alpha level of 0.05. The ANOVA function was utilized from R Studio to determine significant differences. Upon identification of a significant difference between products, the CLD function in the emmeans package was used to assign statistical groupings with a Tukey adjustment being applied to all P-values.

4.3. Results and Discussion

4.3.1. Proximate Analysis

The results of the proximate analysis for raw and cooked samples are presented in Tables 4 and 5, respectively. When evaluating dry matter, the IB and GP contained similar ($P > 0.05$) percentages but were greater ($P < 0.05$) than all other samples examined regardless of product state (raw or cooked). The ash content of samples was numerically similar among products, with levels less than or equal to 2.00%. Product moisture directly relates to juiciness, which is an essential sensory attribute to consumers (Hughes et al., 2014). Of the products tested, the IB and GP had the lowest moisture as raw products and had the greatest ($P < 0.05$) moisture loss during cooking. In contrast, the VB contained the highest ($P < 0.05$) percentage of moisture post-cooking when compared to all products tested. The lack of moisture reduction of the VB could be attributed to prior thermal processing during product production. Protein concentrations of the products were numerically similar before cooking; however, the IB and PC products contained slightly greater

($P < 0.05$) amounts of protein when compared to the BB product. However, post-cooking the PC contained the highest ($P < 0.05$) percentage of protein. To no surprise, the crude fat content of the GP was highest ($P < 0.05$) regardless of the product state (raw or cooked). Furthermore, post cooking the IB contained a greater ($P < 0.05$) percentage of fat than the VB, BB, and PC samples. The numerical differences of the high-fat (GP and IB) compared to the low fat (VB, BB, and PC) products showed that the high fat products had close to double the percentage of crude fat present.

4.3.2. Cholesterol and Fatty Acids

Dietary cholesterol is commonly associated with greater risk for the development of heart disease (Blesso & Luz Fernandez 2018). In this study, cholesterol levels were found to be higher ($P < 0.05$) in animal-based proteins than plant-based products. All plant-based products had less than or equal to 0.02 mg/g of cholesterol, with BB and IB products containing less than 0.01 mg/g of cholesterol (Table 6 and Table 7). In contrast, the cooked PC had 0.57 mg/g and cooked GP had 0.7 mg/g of cholesterol. The higher amount of cholesterol in GP compared to PC is expected as it is typically considered a leaner product. Even though cholesterol was present in the PC, it has been found that lean meat cuts may not negatively contribute to a rise in blood cholesterol (Duo Li et al., 2005).

Fatty acid profiles were similar regardless of the product state (Table 6 and Table 7). The IB product was found to contain the highest ($P < 0.05$) amounts of short and medium-chain fatty acids. Of the fatty acids present in the IB product, the medium-chain C12:0 and C14:0 saturated fatty acids were the most abundant ($P < 0.05$). This appearance of the short and medium-chain fatty acids within the IB could be due to the inclusion of coconut oil in the product formulation as coconut oil is abundant in C12:0 and C14:0 fatty acids (Caballero et al., 2003). Furthermore, when evaluating long-chain fatty acids, the GP and PC contained the highest ($P < 0.05$) amounts when

compared to the plant-based proteins (VB, BB, and IB). Of the long-chain fatty acids identified in the PC, C16:0 and C18:1 n-9 were found in the greatest amounts. These findings support previous studies that identified the prevalence of C16 and C18 fatty acids in pork products (Enser et al., 1996). Interestingly, of the long-chain fatty acids identified, C18:2 n-6 was highest ($P < 0.05$) in VB and BB products. The presence of C18:2 n-6 could be due to the canola oil used in the formulation of the BB product as canola oil naturally contains these fatty acids (Ghazani et al., 2016). Overall, the IB was found to have the most significant ($P < 0.05$) amounts of saturated fat when compared to all other products.

4.3.3. Minerals

The results for mineral analysis are presented in Tables 8 and 9. Calcium levels were highest ($P < 0.05$) in VB and followed the order: VB > BB = IB > PC = GP ($P < 0.05$), irrespective of product state (raw or cooked). The calcium in the VB product was almost 4 times higher than calcium levels of both BB and IB products. This significant ($P < 0.05$) difference is most likely due to the incorporation of calcium caseinate in the VB product formulation. Interestingly, regardless of product state, BB contained the highest ($P < 0.05$) amounts of iron along with quantities of copper similar to the IB product. Additionally, the iron and copper identified in the BB was 3 to 4 times numerically greater than both of the pork products. However, when evaluating diets that replace animal protein with plant protein, iron deficiencies are commonly observed (Sanders, 1999). Even though results showed that the plant products contain considerably higher amounts of iron, the bioavailability is uncertain. With the natural presence of phytic acid (commonly found in legumes and cereals), the absorption of iron, zinc, calcium, and manganese are negatively affected (Hurrell, 2019). Due to the lack of bioavailability of the plant-based iron, it is reasonable to speculate that iron absorption would be reduced for the plant-based products.

The VB had the greatest amounts ($P < 0.05$) of manganese and magnesium regardless of product state. However, the IB product contained levels of manganese similar to VB following cooking. Manganese in the pork products (GP and PC) could not be detected or was observed below test limitations of 0.18 ppm. In contrast, when evaluating potassium, the PC and GP samples contained the highest ($P < 0.05$) amounts regardless of the product state. When evaluating sodium levels in the current study, the IB was highest ($P < 0.05$) with the VB and BB products containing similar levels that were greater ($P < 0.05$) than the amounts of sodium found in the pork products (PC and GP).The numerical differences observed between the plant and pork products is vast. With these differences between the plant-based and pork products, it is essential to note that the plant products do not require any additional seasoning before consumption. While the pork products contained the lowest amounts of sodium, higher amounts would be expected as consumers would typically season these products prior to consumption in the home. The amounts of additional sodium being added to the products would be based on consumer preferences; therefore, the evaluation of additional sodium was not explored in the current study. Furthermore, the IB product contained approximately ten times greater amounts of sodium when compared to the PC. The 2015-2020 dietary guidelines recommend Americans to consume less than 2,300 mg of sodium each day (Dietary Guidelines, 2010). The sodium content observed in the IB product could contribute to 25% of the daily sodium allowance, while the BB and VB products contained approximately 20% of the recommended daily allowance of sodium.

4.3.4. Vitamins

While some differences were found between fat soluble vitamin content of the products, these differences were often numerically miniscule between raw (Table 10) and cooked (Table 11) samples. Among the differences identified, the IB had the greatest ($P < 0.05$) vitamin E

concentration while the BB contained the highest ($P < 0.05$) amount of vitamin K. When evaluating water-soluble vitamins, the IB product contained the highest ($P < 0.05$) amounts of thiamin and riboflavin regardless of product state. This abundance could be due to the product formulation supplementing thiamin and riboflavin to increase nutritional density. Additionally, due to supplementation, the IB product contained pyridoxine levels similar ($P > 0.05$) to the PC and GP products. The pork products (PC and GP) were found to contain the highest ($P > 0.05$) amount of niacin, pantothenic acid, and pyridoxine. Overall, the PC, GP, and IB contained greater amounts of B-vitamins when compared to the BB and VB products.

4.3.5. Amino Acids

The amino acid profiles of raw and cooked products are presented in Tables 12 and 13. During the analysis, asparagine was converted to aspartic, and glutamine was converted to glutamic during the hydrolysis step. Hence, asparagine and glutamine concentration, along with aspartic acid and glutamic acid concentration, are indicated as aspartic and glutamic values in Tables 12 and 13. In general, amino acid compositions between the products were comparable, although PC tended to have the highest values for most amino acids, most probably due to a high protein concentration. In the raw form, PC and GP had a greater ($P < 0.05$) amount of alanine than the plant-based products (VB, BB, and IB), whereas arginine was greatest ($P < 0.05$) in BB. Interestingly, the IB had almost double the amount of cysteine and glutamic acid compared to other products. The levels of histidine followed the order of the products as listed: PC > GP > IB = BB > VB ($P < 0.05$). Upon cooking products, similar results were observed for the amino acid profiles (Table 13). Alanine levels were different ($P < 0.05$) between the products, with PC having the highest ($P < 0.05$) concentration, followed by GP, BB, and then VB, with IB being similar ($P > 0.05$) to the VB and BB products. Identical to the raw products, cysteine, and glutamic acid

levels were highest ($P < 0.05$) in IB compared to other products. When evaluating the VB, BB, and IB nutrient profiles, it is critical to consider the ingredients in the formulation. With the incorporation of wheat, soy, and potato protein in the IB, a greater amount of protein sources are utilized to cover deficiencies that would otherwise be present due to the utilization of a singular protein source (Gorissen et al., 2018). These findings are further supported by the fact that BB product was lacking in some amino acids, possibly due to only utilizing pea protein isolate as the primary and only protein source (Gorissen et al., 2018). While the body can generate some amino acids required for metabolomic functions, there are nine essential amino acids (EAAs) that the body cannot produce and must be supplied by the diet. When evaluating the nine EAAs of plant and animal-based proteins, Goriseen et al. (2018) found that plant-based proteins typically contain lower amounts of EAAs when compared to animal proteins. When isoleucine (an EAA) content of the raw products were evaluated, the following order was observed: PC > IB > BB > GP > VB ($P < 0.05$). Furthermore, the raw PC contained the greatest amount of lysine, methionine, threonine, tryptophan, and valine ($P < 0.05$) when compared to other products. Upon cooking, histidine, leucine, isoleucine, and lysine levels were highest ($P < 0.05$) in the PC, whereas IB had the highest amount ($P < 0.05$) of phenylalanine, proline, and serine. Of the EAAs, leucine, lysine, and methionine are critical to muscle protein synthesis (MPS; van Vilet et al., 2015). When evaluating the cooked products (as consumers would eat), greater amounts of these amino acids necessary for appropriate MPS were identified in the PC and GP products. Furthermore, legumes and soybeans have been identified to have limited amounts of sulfur-containing amino acids, such as methionine that are important for MPS (Berrazaga et al., 2019; van Vilet et al., 2015). In addition, even though proteins are present in the plant-based products, the bioavailability and digestibility may restrict proper utilization of proteins by the body. When observing plant-based

proteins, a greater amount of proteolysis resistant proteins in the beta-sheet formation have been identified, which can result in lower digestibility (Carbonaro et al., 2012; Nguyen et al., 2015). In addition, with protease inhibitors being present in raw legumes, cereal grains, and potatoes, true protein digestion can only be speculated without further investigation (Gilani et al., 2005).

4.3.6. Organic Acids

For both raw and cooked products, data could not be gathered for organic acids except for sorbic, citric, oxalic, and pyruvic acids, most likely due to low amounts or matrix interference (Table 14 and Table 15). In order to overcome these testing limitations, new mass spectrometry-based methodologies would need to be developed for each of these products. Among the quantifiable organic acids, acetic acid was found in the highest ($P < 0.05$) amount in VB and BB products at levels much greater than the other products. IB was found to have the greatest ($P < 0.05$) amount of sorbic acid when compared to the other products. When comparing citric acid, the plant-based products had higher ($P < 0.05$) amounts when compared to pork products. In general, the test results are suggestive of lacking concentrations of organic acids in these products.

4.3.7. Allergens

The results for the allergen testing indicated no difference ($P > 0.05$) between the products for almond, hazelnut, peanut, and walnut proteins either in cooked or raw form (Table 16 and Table 17). Milk proteins were identified in the highest ($P < 0.05$) concentration in the VB in raw form and then in similar amounts ($P > 0.05$) with the other two plant-based proteins when cooked. Egg proteins were detected at similar levels ($P > 0.05$; regardless of product state) in the BB, IB, and GP. When evaluating gluten levels in products, the black bean had the highest ($P < 0.05$) amount before cooking. However, upon cooking, no difference ($P > 0.05$) was observed in gluten

levels, most likely due to protein denaturation. When evaluating the presence of soy allergens, the IB and VP products had the highest ($P < 0.05$) amounts pre- and post-cooking. Due to varying allergen sensitivity among individuals, there is no set amount for a particular allergen that dictates whether an allergic reaction will occur (Bindslev-Jensen et al., 2002). Testing protocols can detect allergens greater than 2.5 ppm or lower than 25 ppm. If the products contain proteins outside of this range, they were indicated as >25 or <2.5 ppm (Table 16 and Table 17). The exact level of milk, egg, soy, and gluten allergens in the products were inconsistent. With products being handled and cooked in the same facility, even with precautions taken, cross-contamination might have occurred. Looking at the food allergens listed on product labels, the VB had egg, milk, soy, and wheat allergens and had the most significant amount of these allergens during our testing (Table 16). The only other product to have allergens listed was IB, with soy used in the product formulation. As expected, IB and VB products had the highest ($P < 0.05$) amount of soy allergens (Table 16). From the information provided on the labels and high standard errors reported, the VB and IB products could be the likely source of the allergen contamination for BB, PC, and GP samples.

4.4. Conclusion

Overall, the product state (raw or cooked) did not have a great influence on the nutritional composition of these products. The nutritional analysis indicated that PC could be considered the most nutritionally dense among the products evaluated in the current study with highest amounts of protein and essential amino acids. When evaluating plant-based proteins, the IB was found to be the most nutritionally dense with similar protein content to PC and GP. Cholesterol was found in the highest concentrations among the pork products, while it was not detected in the plant-based proteins. When evaluating the mineral make-up of the products, the plant-based products typically

contained higher amounts, especially when evaluating iron and sodium content. Sodium levels were about ten times higher in plant-based proteins, along with iron content being about 3 to 4 times greater when compared to the pork products. Vitamin E content was the highest in plant-based products, whereas the pork products had higher amounts of B-vitamins. Overall, the current research provided an overview of the nutritional compositional differences of animal and plant-based proteins. However, to understand the ability of the human body to utilize these nutrients, further digestibility studies needs to be undertaken.

Table 1. Definitions and references for sensory notes evaluated.

Attribute	Definition	Reference
Oxidation	The aromatics commonly associated with oxidized fat and oils. These aromatics may include cardboard, painty, varnish and fishy.*	Wesson vegetable oil = 1.0 Microwaved Wesson vegetable oil (3 min at high) = 8.0
Putridity	The aromatics associated with spoiled meat.	Fresh beef NY strip steak = 0.0 Beef NY strip steak held at 22 ± 2°C for 24 h = 6.0
Sour	Sour, fermented aromatics associated with dairy products such as buttermilk and sour cream.*	Fresh sour cream = 2.0 Sour creamed held at 22 ± 2°C for 24 h = 5.0
Overall Offodor	The combination of sour, putrid, and oxidative notes	Non-sour kuckle – 2.0 Putrid meat – 7.0

* For further reference consult Adhikari et al. (2010)

Table 2. Mean (n = 10) psychrotrophic anaerobic sporeformer counts (log CFU/sponge) for synovial fluid sponge samples and femur surface sponge samples collected from knuckles categorized (by plant personnel) as having a slight sour odor (SLI-SO), severe sour odor (SVR-SO) or no sour odor (control).

Sample Type Analyzed	**Treatment (extent of sourness)**	**Mean ± Standard Deviation (log CFU/sponge)**	**%BDL***
Synovial fluid	Control	$<1.0 \pm 0.9^{a}$	50%
	SLI-SO	$<0.8 \pm 0.6^{a}$	50%
	SVR-SO	$<0.8 \pm 0.4^{a}$	20%
Femur surface	Control	1.2 ± 0.7^{a}	0%
	SLI-SO	$<1.2 \pm 0.8^{b}$	40%
	SVR-SO	$<0.8 \pm 0.5^{b}$	10%

[a-b]Means with a different superscript letter within each sample type analyzed (synovial fluid or femur surface) differ statistically ($P < 0.05$). Means with a less than symbol (<) indicate that at least one sample within the treatment had a count that was below the analysis detection limit (0.4 log CFU/sponge)

***** %BDL indicates the percent of samples, of the 10 samples analyzed, with bacterial counts that were below the analysis detection limit (0.4 log CFU/sponge)

Table 3. Mean (n = 10) bacterial counts (log CFU/g) for muscle tissue surface samples from knuckles categorized (by plant personnel) as having a slight sour odor (SLI-SO), severe sour odor (SVR-SO) or no sour odor (control). Samples were analyzed on the day of collection at the plant (day 0) and after 35 days of refrigerated (0 - 2°C) vacuum-packaged storage.

Days of Storage	Bacterial Count Type	Treatment (extent of sourness)	Mean ± Standard Deviation (log CFU/g)	%BDL*
Day 0	Aerobic plate count	Control	$<1.4 \pm 0.6^{b}$	20%
		SLI-SO	1.6 ± 0.7^{a}	0%
		SVR-SO	1.7 ± 0.3^{a}	0%
	Lactic acid bacteria count	Control	$<1.2 \pm 0.5^{b}$	10%
		SLI-SO	1.3 ± 0.4^{a}	0%
		SVR-SO	$<1.3 \pm 0.5^{b}$	10%
Day 35				
	Aerobic plate count	Control	7.0 ± 0.8^{a}	0%
		SLI-SO	6.9 ± 0.6^{a}	0%
		SVR-SO	6.7 ± 1.0^{a}	0%
	Lactic acid bacteria count	Control	7.1 ± 0.7^{a}	0%
		SLI-SO	7.3 ± 0.9^{a}	0%
		SVR-SO	6.7 ± 0.6^{a}	0%

[a-b] Means with a different superscript letter within each storage day (day 0 or day 35) and bacterial count type (aerobic plate count or lactic acid bacteria count) differ statistically ($P < 0.05$). Means with a less than symbol (<) indicate that at least one sample within the treatment had a count that was below the analysis detection limit (0.5 log CFU/g)

***** %BDL indicates the percent of samples, of the 10 samples analyzed, with bacterial counts that were below the analysis detection limit (0.5 log CFU/g)

Table 4. Proximate analysis (percent ± standard deviation) for raw Spicy Black Bean Burgers, Beyond Burgers, Impossible Burgers, Pork chops, and Ground pork (n=6).

Proximate Analysis (%)	Black Bean Burger (VB)	Beyond Burger (BB)	Impossible Burger (IB)	Pork Chop (PC)	Ground Pork (GP)
Dry Matter	32.48 ± 2.68^{b}	32.13 ± 0.83^{b}	37.19 ± 1.96^{a}	30.82 ± 2.27^{b}	37.09 ± 1.29^{a}
Crude Fat	7.44 ± 1.56^{cd}	10.05 ± 2.10^{bc}	12.53 ± 1.58^{ab}	5.26 ± 2.25^{d}	15.21 ± 1.23^{a}
Ash	1.69 ± 0.21^{ab}	1.29 ± 0.18^{bc}	1.79 ± 0.27^{a}	1.14 ± 0.22^{c}	1.11 ± 0.46^{c}
Protein	19.46 ± 2.54^{ab}	18.28 ± 1.78^{b}	22.18 ± 1.82^{a}	22.33 ± 1.80^{a}	19.37 ± 1.32^{ab}
Moisture	67.52 ± 2.68^{a}	67.87 ± 0.83^{a}	62.81 ± 1.96^{b}	69.18 ± 2.27^{a}	62.91 ± 1.29^{b}

$^{a\text{-}d}$ Means within a row with different superscripts differ statistically ($P < 0.05$)

Table 5. Proximate analysis (percent ± standard deviation) for cooked Spicy Black Bean Burgers, Beyond Burgers, Impossible Burgers, Pork chops, and Ground pork (n=6).

Proximate Analysis	Black Bean Burger (VB)	Beyond Burger (BB)	Impossible Burger (IB)	Pork Chop (PC)	Ground Pork (GP)
Dry Matter	30.73 ± 2.58^{c}	36.74 ± 3.46^{b}	44.98 ± 0.72^{a}	38.64 ± 3.30^{b}	44.77 ± 2.18^{a}
Crude Fat	8.42 ± 2.22^{c}	7.71 ± 3.29^{c}	14.33 ± 1.70^{b}	7.76 ± 1.51^{c}	18.48 ± 1.56^{a}
Ash	1.77 ± 0.38^{ab}	1.91 ± 0.36^{a}	2.00 ± 0.14^{a}	1.11 ± 0.15^{c}	1.33 ± 0.49^{bc}
Protein	16.65 ± 1.90^{c}	23.56 ± 1.34^{b}	25.92 ± 0.79^{ab}	28.71 ± 3.16^{a}	23.51 ± 1.13^{b}
Moisture	69.27 ± 2.58^{a}	63.26 ± 3.46^{b}	55.02 ± 0.72^{c}	61.36 ± 3.30^{b}	55.23 ± 2.18^{c}

$^{a\text{-}c}$ Means with in a row with different superscripts differ statistically ($P < 0.05$)

Table 6. Fatty acid (percentage ± standard deviation) and cholesterol (mg/g ± standard deviation) content for raw Spicy Black Bean Burgers, Beyond Burgers, Impossible Burgers, Pork chops, and Ground pork (n=6).

Fatty Acid	Black Bean Burger (VB)	Beyond Burger (BB)	Impossible Burger (IB)	Pork Chop (PC)	Ground Pork (GP)
Cholesterol	0.01 ± 0.002^{c}	$< 0.01^{c}$	$< 0.01^{c}$	0.72 ± 0.04^{b}	0.86 ± 0.06^{a}
C 8:0	N/A	1.05 ± 0.17^{b}	7.16 ± 0.27^{a}	N/A	N/A
C 10:0	N/A	N/A	5.99 ± 0.80^{a}	0.04 ± 0.01^{b}	0.05 ± 0.01^{b}
C 12:0	N/A	2.99 ± 0.81^{b}	40.12 ± 1.08^{a}	0.10 ± 0.01^{c}	0.12 ± 0.02^{c}
C 14:0	N/A	N/A	19.51 ± 1.09^{a}	1.14 ± 1.14^{b}	1.15 ± 0.07^{b}
C 16:0	7.29 ± 0.09^{b}	6.38 ± 0.57^{b}	1.74 ± 0.28^{c}	21.17 ± 1.09^{a}	20.49 ± 0.73^{a}
C 16:1 n-7	N/A	N/A	N/A	2.10 ± 0.19^{a}	2.16 ± 0.23^{a}
C 17:0	N/A	N/A	N/A	0.35 ± 0.01^{a}	0.35 ± 0.03^{a}
C 18:0	1.68 ± 0.02^{c}	4.51 ± 0.38^{b}	1.95 ± 0.20^{c}	11.36 ± 0.87^{a}	10.93 ± 1.27^{a}
C 18:1 n-9	8.55 ± 0.37^{b}	27.33 ± 2.19^{a}	5.98 ± 0.31^{b}	28.52 ± 1.72^{a}	28.66 ± 2.89^{a}
C 18:1 n-7	1.67 ± 0.02^{b}	N/A	0.03 ± 0.07^{c}	3.86 ± 0.47^{a}	3.90 ± 0.36^{a}
C 18:2 n-6	29.30 ± 0.64^{a}	29.65 ± 3.39^{a}	1.76 ± 1.03^{c}	12.10 ± 0.87b	12.30 ± 1.40^{b}
C 20:0	N/A	0.92 ± 0.06^{a}	0.17 ± 0.06^{b}	0.08 ± 0.11^{b}	0.09 ± 0.12^{b}
C 20:1 n-9	N/A	0.91 ± 0.06^{a}	0.15 ± 0.04^{c}	0.36 ± 0.22^{b}	0.48 ± 0.04^{b}
C 20:2	N/A	N/A	N/A	0.38 ± 0.04^{a}	0.35 ± 0.09^{a}
C 20:4 n-6	N/A	0.35 ± 0.03^{b}	0.11 ± 0.06^{b}	2.56 ± 2.05^{a}	3.12 ± 2.52^{a}
C 22:5 n-3	N/A	N/A	N/A	0.03 ± 0.03^{a}	0.03 ± 0.02^{a}
C 24:0	N/A	0.95 ± 0.69	0.41 ± 1.18	N/A	N/A
C 22:6 n-3	N/A	N/A	0.01 ± 0.04	0.02 ± 0.01	0.02 ± 0.01

$^{a-b}$ Means with in a row with different superscripts differ statistically ($P < 0.05$)

N/A Fatty acids were not clearly identified within sample matrices due to lack of prevalence

Table 7. Fatty acid (percentage ± standard deviation) and cholesterol (mg/g ± standard deviation) content for cooked Spicy Black Bean Burgers, Beyond Burgers, Impossible Burgers, Pork chops, and Ground pork (n=6).

Fatty Acid	Black Bean Burger (VB)	Beyond Burger (BB)	Impossible Burger (IB)	Pork Chop (PC)	Ground Pork (GP)
Cholesterol	0.02 ± 0.002[c]	< 0.01[c]	< 0.01[c]	0.57 ± .05[b]	0.7 ± 0.046[a]
C 8:0	N/A	0.96 ± 0.13[b]	5.72 ± 3.33[a]	N/A	N/A
C 10:0	N/A	N/A	4.95 ± 2.82[a]	0.04 ± 0.01[b]	0.04 ± 0.01[b]
C 12:0	N/A	2.39 ± 0.41[b]	33.39 ± 19.10[a]	0.10 ± 0.01[b]	0.11 ± 0.02[b]
C 14:0	N/A	N/A	16.45 ± 7.93[a]	1.06 ± 0.04[b]	1.08 ± 0.08[b]
C 16:0	6.51 ± 1.17[b]	6.43 ± 0.74[b]	1.51 ± 0.90[c]	21.34 ± 1.12[a]	20.72 ± 0.85[a]
C 16:1 n-7	0.36 ± 0.01[b]	N/A	N/A	2.11 ± 0.22[a]	2.05 ± 0.30[a]
C 17:0	N/A	N/A	N/A	0.35 ± 0.01[a]	0.37 ± 0.03[a]
C 18:0	1.73 ± 0.03[a]	4.64 ± 0.32[b]	1.75 ± 1.02[a]	11.45 ± 0.82[a]	11.18 ± 1.34[a]
C 18:1 n-9	8.66 ± 0.46[b]	27.56 ± 1.78[a]	5.31 ± 3.05[c]	28.61 ± 2.00[a]	28.5 ± 2.55[a]
C 18:1 n-7	1.72 ± 0.06	N/A	0.03 ± 0.08[b]	3.89 ± 0.49[a]	3.92 ± 0.42[a]
C 18:2 n-6	30.18 ± 0.87[a]	30.36 ± 2.12[a]	1.82 ± 1.08[c]	11.92 ± 1.02[b]	12.11 ± 1.73[b]
C 20:0	0.37 ± 0.01[b]	0.95 ± 0.07[a]	0.15 ± 0.10[c]	0.08 ± 0.11[c]	0.09 ± 0.12[c]
C 20:1 n-9	0.18 ± 0.01[c]	0.94 ± 0.07[a]	0.14 ± 0.09[c]	0.36 ± 0.22[b]	0.48 ± 0.03[b]
C 20:2	N/A	N/A	N/A	0.38 ± 0.04[a]	0.35 ± 0.09[a]
C 20:4 n-6	0.33 ± 0.01[b]	0.36 ± 0.03[b]	0.08 ± 0.06[b]	2.58 ± 2.05[a]	3.15 ± 2.52[a]
C 22:5 n-3	N/A	N/A	N/A	0.03 ± 0.03[a]	0.03 ± 0.02[a]
C 24:0	N/A	0.98 ± 0.74[a]	N/A	N/A	N/A
C 22:6 n-3	N/A	N/A	0.01 ± 0.04	0.02 ± 0.01	0.02 ± 0.01

[a-b] Means with in a row with different superscripts differ statistically ($P < 0.05$)

N/A Fatty acids were not clearly identified within sample matrices due to lack of prevalence

Table 8. Mineral composition (ppm ± standard deviation) of raw Spicy Black Bean Burgers, Beyond Burgers, Impossible Burgers, Pork chops, and Ground pork (n=6)

Mineral Tested	Black Bean Burger (VB)	Beyond Burger (BB)	Impossible Burger (IB)	Pork Chop (PC)	Ground Pork (GP)
Calcium	881.83 ± 40.45^{a}	213.83 ± 11.60^{b}	257.50 ± 6.66^{b}	71.57 ± 36.78^{c}	105.80 ± 61.89^{c}
Copper	2.42 ± 0.16^{b}	3.38 ± 0.42^{a}	3.82 ± 0.38^{a}	0.51 ± 0.14^{c}	0.71 ± 0.09^{c}
Iron	17.62 ± 0.69^{c}	43.43 ± 2.90^{a}	22.28 ± 0.87^{b}	3.98 ± 0.69^{e}	7.91 ± 2.32^{d}
Magnesium	367.33 ± 8.96^{a}	190.83 ± 7.88^{c}	120.67 ± 7.20^{d}	246 ± 13.49^{b}	178.50 ± 16.32^{c}
Manganese	4.92 ± 0.28^{a}	2.46 ± 0.46^{c}	4.36 ± 0.31^{b}	< 0.18^{c}	< 0.18^{c}
Phosphorus	1263.33 ± 48.44^{d}	1888.33 ± 74.41^{b}	1296.67 ± 28.75^{d}	2028.33 ± 90.42^{a}	1596.67 ± 126.60^{c}
Potassium	2930 ± 129.61^{b}	2828.33 ± 188.09^{b}	3096.67 ± 89.37^{b}	3690 ± 207.46^{a}	3176.67 ± 702.67^{ab}
Sodium	3273.33 ± 190.96^{b}	3328.33 ± 205.47^{b}	4935 ± 166.94^{a}	426.67 ± 38.29^{c}	995.50 ± 60.17^{c}
Zinc	8.73 ± 0.75^{d}	20.90 ± 1.29^{b}	29.72 ± 1.44^{a}	14.13 ± 0.71^{c}	20.77 ± 5.03^{b}

$^{a\text{-}e}$ Means with in a row with different superscripts differ statistically ($P < 0.05$)

Table 9. Mineral composition (ppm ± standard deviation) of cooked Spicy Black Bean Burgers, Beyond Burgers, Impossible Burgers, Pork chops, and Ground pork (n=6)

Mineral Tested	Black Bean Burger (VB)	Beyond Burger (BB)	Impossible Burger (IB)	Pork Chop (PC)	Ground Pork (GP)
Calcium	987.67 ± 68.27[a]	267.33 ± 15.19[b]	297 ± 15.19[b]	79.18 ± 40.06[c]	139.13 ± 87.32[c]
Copper	2.85 ± 0.27[b]	4.88 ± 0.52[a]	4.45 ± 0.59[a]	1.06 ± 0.76[c]	1.40 ± 0.71[c]
Iron	20.25 ± 1.67[c]	60.02 ± 5.51[a]	26.98 ± 1.37[b]	6.64 ± 2.58[d]	10.83 ± 2.58[d]
Magnesium	404.83 ± 18.73[a]	235.33 ± 6.31[c]	140.83 ± 6.49[d]	281.50 ± 9.69[b]	239.17 ± 17.67[c]
Manganese	5.39 ± 0.41[a]	3.03 ± 0.29[b]	5.04 ± 0.39[a]	< 0.18 ± .002[c]	< 0.18 ± .002[c]
Phosphorus	1411.67 ± 75.48[c]	2315 ± 79.44[a]	1513.33 ± 28.75[c]	2331.67 ± 74.14[a]	2146.67 ± 121.76[b]
Potassium	3236.67 ± 190.86[b]	3378.33 ± 367.12[b]	3590 ± 60.99[ab]	4156.67 ± 180.19[a]	4220 ± 834.94[a]
Sodium	3618.33 ± 284.77[b]	4135 ± 355.79[b]	5666.67 ± 212.95[a]	462.5 ± 51.45[c]	1277.83 ± 1586.40[c]
Zinc	9.80 ± 0.97[d]	25.55 ± 1.97[b]	34.17 ± 1.76[a]	17.23 ± 1.99[c]	28.15 ± 5.53[b]

[a-e] Means with in a row with different superscripts differ statistically ($P < 0.05$)

Table 10. Vitamin profile (mg/g ± standard deviation) of raw Spicy Black Bean Burgers, Beyond Burgers, Impossible Burgers, Pork chops, and Ground pork (n=6).

Vitamin Tested	Black Bean Burger (VB)	Beyond Burger (BB)	Impossible Burger (IB)	Pork Chop (PC)	Ground Pork (GP)
A	< 0.30[a]	< 0.30[a]	< 0.30[a]	< 0.30[a]	< 0.30[a]
D2	< 0.001[a]	< 0.001[a]	< 0.001[a]	< 0.001[a]	< 0.001[a]
D3	< 0.001[b]	< 0.001[b]	< 0.001[b]	0.0021 ± 0.0016[b]	0.0099 ± 0.003[a]
E	15.92 ± 1.31[c]	21.65 ± 4.49[b]	33.93 ± 5.88[a]	< 5.00[d]	5.08 ± 0.20[d]
K1	0.06 ± .013[b]	0.22 ± .023[a]	.04 ± .001[b]	.04 ± .001[b]	.04 ± .001[b]
Thiamin (B1)	0.11 ± 0.11[b]	0.06 ± 0.05[b]	18.23 ± 0.48[a]	0.48 ± 0.33[b]	0.33 ± 0.10[b]
Riboflavin (B2)	2.53 ± 0.33[b]	1.17 ± 0.10[d]	3.83 ± 0.34[a]	1.95 ± 0.19[c]	2.53 ± 0.46[b]
Niacin (B3)	8.40 ± 0.44[c]	3.47 ± 0.31[c]	52.58 ± 4.85[b]	81.30 ± 14.14[a]	55.98 ± 13.65[b]
Pantothenic (B5)	3.65 ± 0.25[c]	3.62 ± 0.29[c]	3.43 ± 0.26[c]	6.33 ± 0.52[b]	8.13 ± 2.02[a]
Pyridoxine (B6)	1.03 ± 0.11[c]	0.41 ± 0.09[c]	2.86 ± 0.16[b]	4.16 ± 0.97[a]	3.64 ± 1.15[ab]
Biotin (B7)	0.05 ± 0.01[ab]	0.06 ± 0.01[a]	0.04 ± 0.01[bc]	0.02 ± 0.01[d]	0.04 ± 0.01[c]

[a-d] Means with in a row with different superscripts differ statistically ($P < 0.05$)

Table 11. Vitamin profile (mg/g ± standard deviation) of cooked Spicy Black Bean Burgers, Beyond Burgers, Impossible Burgers, Pork chops, and Ground pork (n=6).

Vitamin Tested	Black Bean Burger (VB)	Beyond Burger (BB)	Impossible Burger (IB)	Pork Chop (PC)	Ground Pork (GP)
A	$< 0.03^{a}$	$< 0.03^{a}$	$< 0.03^{a}$	$< 0.03^{a}$	$< 0.03^{a}$
D2	$< 0.01^{a}$	$< 0.01^{a}$	$< 0.01^{a}$	$< 0.01^{a}$	$< 0.01^{a}$
D3	$< 0.001^{a}$	$< 0.001^{a}$	$< 0.001^{a}$	0.0019 ± 0.0015^{a}	0.0059 ± 0.0064^{a}
E	19.33 ± 1.69^{c}	26.58 ± 6.73^{b}	38.75 ± 2.13^{a}	$< 5.00^{d}$	5.75 ± 1.82^{d}
K1	0.06 ± 0.009^{b}	0.25 ± 0.03^{a}	0.04 ± 0.001^{b}	0.039 ± 0.0004^{b}	0.039 ± 0.0004^{b}
Thiamin (B1)	0.07 ± 0.01^{b}	0.03 ± 0.01^{b}	19.73 ± 0.96^{a}	0.46 ± 0.20^{b}	0.40 ± 0.13^{b}
Riboflavin (B2)	2.83 ± 0.42^{b}	1.55 ± 0.15^{d}	4.37 ± 0.15^{a}	2.28 ± 0.20^{c}	3.08 ± 0.43^{b}
Niacin (B3)	9.90 ± 1.07^{c}	4.28 ± 0.34^{c}	62.20 ± 2.97^{b}	84.15 ± 14.84^{a}	80.03 ± 6.86^{a}
Pantothenic (B5)	3.65 ± 0.25^{c}	3.62 ± 0.29^{c}	3.43 ± 0.26^{c}	6.33 ± 0.52^{b}	8.13 ± 2.02^{a}
Pyridoxine (B6)	1.17 ± 0.15^{b}	0.47 ± 0.06^{b}	3.14 ± 0.23^{a}	3.64 ± 1.22^{a}	2.98 ± 0.51^{a}
Biotin (B7)	0.07 ± 0.01^{a}	0.08 ± 0.01^{a}	0.05 ± 0.01^{b}	$0.03 \pm .010^{c}$	0.05 ± 0.01^{b}

$^{a\text{-}d}$ Means with in a row with different superscripts differ statistically ($P < 0.05$)

Table 12. Amino acid composition (mg/g ± standard deviation) of raw Spicy Black Bean Burgers, Beyond Burgers, Impossible Burgers, Pork chops, and Ground pork (n=6).

Amino Acid	Black Bean Burger (VB)	Beyond Burger (BB)	Impossible Burger (IB)	Pork Chop (PC)	Ground Pork (GP)
Alanine	5.82 ± 0.28^{d}	7.94 ± 0.19^{c}	6.66 ± 0.22^{d}	13.12 ± 0.57^{a}	10.14 ± 0.90^{b}
Arginine	8.16 ± 0.30^{e}	15.32 ± 0.55^{a}	9.17 ± 0.79^{d}	14.28 ± .43^{b}	11.08 ± 0.72^{c}
Aspartic	13.40 ± 0.5^{bc}	21.17 ± 0.49^{a}	13.27 ± 0.40^{c}	19.90 ± 0.91^{a}	14.68 ± 1.41^{b}
Cysteine	2.58 ± 0.30^{b}	2.45 ± 0.17^{b}	6.22 ± 0.61^{a}	2.38 ± 0.20^{bc}	1.83 ± 0.24^{c}
Glutamic	29.87 ± 1.66^{b}	30.10 ± 0.93^{b}	69.57 ± 3.39^{a}	32.18 ± 1.54^{b}	23.72 ± 2.24^{c}
Glycine	4.71 ± 0.18^{e}	7.36 ± 0.13^{d}	8.85 ± 0.30^{c}	10.65 ± 0.77^{a}	9.75 ± 0.66^{b}
Histidine	3.06 ± 0.06^{d}	4.24 ± 0.08^{c}	4.08 ± 0.17^{c}	8.24 ± 0.54^{a}	5.46 ± 0.79^{b}
Isoleucine	6.08 ± 0.25^{d}	8.74 ± 0.14^{b}	9.19 ± 0.27^{b}	10.16 ± 0.50^{a}	7.28 ± 0.78^{c}
Leucine	10.75 ± 0.41^{d}	15.3 ± 0.24^{b}	16.68 ± 0.53^{a}	17.20 ± 0.78^{a}	12.70 ± 1.20^{c}
Lysine	6.82 ± 0.12^{c}	13.28 ± 0.36^{b}	7.63 ± 0.33^{c}	18.85 ± 1.07^{a}	13.28 ± 1.00^{b}
Methionine	2.79 ± 0.13^{d}	1.61 ± 0.13^{e}	3.28 ± 0.13^{c}	6.06 ± 0.32^{a}	4.30 ± 0.45^{b}
Phenylalanine	7.15 ± 0.2^{d}	9.87 ± 0.18^{b}	11.82 ± 0.35^{a}	8.47 ± 0.35^{c}	6.30 ± 0.56^{e}
Proline	9.81 ± 0.38^{b}	7.96 ± 0.27^{c}	21.97 ± 1.01^{a}	9.46 ± 0.92^{b}	7.93 ± 0.53^{c}
Serine	7.27 ± 0.29^{d}	9.34 ± 0.23^{b}	10.75 ± 0.42^{a}	8.44 ± 0.40^{c}	6.38 ± 0.52^{e}
Threonine	5.03 ± 0.18^{c}	6.67 ± 0.13^{b}	6.99 ± 0.20^{b}	9.68 ± 0.48^{a}	7.10 ± 0.70^{b}
Tryptophan	1.7 ± 0.08^{d}	1.67 ± 0.06^{d}	2.29 ± 0.10^{b}	2.67 ± 0.15^{a}	1.90 ± 0.12^{c}
Tyrosine	5.31 ± 0.20^{d}	7.05 ± 0.14^{c}	8.84 ± 0.25^{b}	7.47 ± 0.28^{a}	5.51 ± 0.50^{d}
Valine	6.86 ± 0.14^{d}	9.12 ± 0.19^{b}	10.52 ± 0.21^{a}	10.49 ± 0.45^{a}	7.81 ± 0.74^{c}

$^{a\text{-}e}$ Means with in a row with different superscripts differ statistically ($P < 0.05$)

Table 13. Amino acid composition (mg/g ± standard deviation) of cooked Spicy Black Bean Burgers, Beyond Burgers, Impossible Burgers, Pork chops, and Ground pork (n=6)

Amino Acid	Black Bean Burger (VB)	Beyond Burger (BB)	Impossible Burger (IB)	Pork Chop (PC)	Ground Pork (GP)
Alanine	6.49 ± 0.49^{d}	8.98 ± 0.35^{c}	7.76 ± 0.36^{cd}	17.03 ± 1.06^{a}	14.02 ±1.41^{b}
Arginine	8.59 ± 0.52^{c}	16.82 ± 1.09^{a}	9.73 ± 0.56^{c}	18.18 ± 1.14^{a}	14.95 ± 1.11^{b}
Aspartic	14.98 ± 1.12^{c}	23.87 ± 1.29^{a}	15.57 ± 0.99^{c}	26.00 ± 2.25^{a}	20.25 ±1.61^{b}
Cysteine	2.95 ± 0.53^{b}	2.67 ± 0.22^{b}	7.05 ± 0.62^{a}	2.94 ± 0.29^{b}	2.51 ± 0.19^{b}
Glutamic	33.5 ± 5.61^{c}	33.6 ± 1.73^{c}	80.93 ± 5.03^{a}	41.27 ± 3.36^{b}	32.05 ± 3.04^{c}
Glycine	5.18 ± 0.47^{d}	8.17 ± 0.36^{c}	10.18 ± 0.48^{b}	13.77 ± 1.05^{a}	12.97 ± 2.23^{a}
Histidine	3.44 ± 0.33^{d}	4.75 ± 0.23^{c}	4.78 ± 0.31^{c}	10.28 ± 1.11^{a}	7.57 ± 0.62^{b}
Isoleucine	6.73 ± 0.62^{c}	9.78 ± 0.50^{b}	10.65 ± 0.59^{b}	13.05 ± 1.16^{a}	9.97 ± 0.73^{b}
Leucine	12.05 ± 1.19^{d}	17.22 ± 0.76^{c}	19.45 ± 1.00^{b}	22.33 ± 1.96^{a}	17.47 ± 1.34^{bc}
Lysine	7.44 ± 0.55^{d}	14.52 ± 0.69^{c}	8.48 ± 0.54^{d}	23.48 ± 2.24^{a}	18.22 ±1.13^{b}
Methionine	3.22 ± 0.28^{c}	1.82 ± 0.29^{d}	3.83 ± 0.20^{c}	7.79 ± 0.90^{b}	5.91 ± 0.51^{a}
Phenylalanine	7.91 ± 0.76^{c}	10.85 ± 0.55^{b}	13.57 ± 0.60^{a}	10.88 ± 0.87^{b}	8.60 ± 0.62^{c}
Proline	11.10 ± 1.97^{bc}	8.98 ± 0.46^{c}	25.27 ± 1.48^{a}	12.28 ± 0.74^{b}	11.14 ± 1.20^{bc}
Serine	8.10 ± 0.75^{c}	10.29 ± 0.47^{b}	12.37 ± 0.71^{a}	10.74 ± 0.79^{b}	8.62 ± 0.70^{c}
Threonine	5.57 ± 0.45^{d}	7.38 ± 0.37^{c}	7.98 ± 0.37^{c}	12.25 ± 0.95^{a}	9.58 ± 0.70^{b}
Tryptophan	5.87 ± 0.58^{c}	7.73 ± 0.35^{b}	10.05 ± 0.49^{a}	9.55 ± 0.84^{a}	7.45 ± 0.51^{b}
Tyrosine	7.56 ± 0.68^{d}	9.97 ± 0.45^{c}	11.85 ± 0.54^{b}	13.47 ± 1.06^{a}	10.69 ± 0.86^{bc}
Valine	1.80 ± 0.18^{c}	1.95 ± 0.22^{c}	2.63 ± 0.11^{b}	3.76 ± 0.22^{a}	2.69 ± 0.16^{b}

$^{a\text{-}e}$ Means with in a row with different superscripts differ statistically ($P < 0.05$)

Table 14. Organic acids concentrations (ppm ± standard deviations) for raw Spicy Black Bean Burgers, Beyond Burgers, Impossible Burgers, Pork chops, and Ground pork (n=6).

Organic Acid Tested	Black Bean Burger (VB)	Beyond Burger (BB)	Impossible Burger (IB)	Pork Chop (PC)	Ground Pork (GP)
Sorbic	$< 4.00^{b}$	$< 4.00^{b}$	171.67 ± 20.62^{a}	$< 4.00^{b}$	$< 4.00^{b}$
Acetic	621.00 ± 31.34^{b}	1187.83 ± 193.02^{a}	$<400.00^{c}$	$< 400.00^{c}$	462.50 ± 145.40^{bc}
Citric	4328.33 ± 179.16^{a}	960 ± 58.98^{b}	481.83 ± 200.45^{c}	$< 400.00^{c}$	$< 400.00^{c}$
Oxalic	501.25 ± 71.56^{a}	$< 400.00^{b}$	$< 400.00^{b}$	$< 400.00^{b}$	$< 400.00^{b}$
Pyruvic	$< 400.00^{a}$	$< 400.00^{a}$	$< 400.00^{a}$	621.8 ± 116.25^{a}	623.83 ± 464.43^{a}
Benzoic	N/A	N/A	N/A	N/A	N/A
Lactic	N/A	N/A	N/A	N/A	N/A
Malic	N/A	N/A	N/A	N/A	N/A
Quinic	N/A	N/A	N/A	N/A	N/A
Formic	N/A	N/A	N/A	N/A	N/A
Fumaric	N/A	N/A	N/A	N/A	N/A
Succinic	N/A	N/A	N/A	N/A	N/A
Tartaric	N/A	N/A	N/A	N/A	N/A
Propionic	N/A	N/A	N/A	N/A	N/A

$^{a\text{-}c}$ Means with in a row with different superscripts differ statistically ($P < 0.05$)

N/A – Sample matrix error occurred and, as such, data could not be gathered

Table 15. Organic acids concentrations (ppm ± standard deviations) for cooked Spicy Black Bean Burgers, Beyond Burgers, Impossible Burgers, Pork chops, and Ground pork (n=6).

Organic Acid Tested	Black Bean Burger (VB)	Beyond Burger (BB)	Impossible Burger (IB)	Pork Chop (PC)	Ground Pork (GP)
Sorbic	$< 4.00^{b}$	$< 4.00^{b}$	176.67 ± 22.58^{a}	$< 4.00^{b}$	$< 4.00^{b}$
Acetic	690.00 ± 66.30^{b}	1334.17 ± 269.15^{a}	$< 400.00^{b}$	$< 400.00^{b}$	$< 400.00^{b}$
Citric	4863.33 ± 264.85^{a}	1088.17 ± 93.98^{b}	$< 400.00^{c}$	$< 400.00^{c}$	$< 400.00^{c}$
Oxalic	582.17 ± 37.66^{a}	$< 400.00^{b}$	$< 400.00^{b}$	$< 400.00^{b}$	$< 400.00^{b}$
Pyruvic	$< 400.00^{b}$	$< 400.00^{b}$	$< 400.00^{b}$	592.25 ± 133.81^{a}	445.8 ± 69.24^{b}
Benzoic	N/A	N/A	N/A	N/A	N/A
Lactic	N/A	N/A	N/A	N/A	N/A
Malic	N/A	N/A	N/A	N/A	N/A
Quinic	N/A	N/A	N/A	N/A	N/A
Formic	N/A	N/A	N/A	N/A	N/A
Fumaric	N/A	N/A	N/A	N/A	N/A
Succinic	N/A	N/A	N/A	N/A	N/A
Tartaric	N/A	N/A	N/A	N/A	N/A
Propionic	N/A	N/A	N/A	N/A	N/A

$^{a\text{-}c}$ Means with in a row with different superscripts differ statistically ($P < 0.05$)
N/A – Sample matrix error occurred and, as such, data could not be gathered

Table 16. Allergens (ppm ± standard deviation) for raw Spicy Black Bean Burgers, Beyond Burgers, Impossible Burgers, Pork chops, and Ground pork (n=6).

Allergen	Black Bean Burger (VB)	Beyond Burger (BB)	Impossible Burger (IB)	Pork Chop (PC)	Ground Pork (GP)
Milk	> 25.00[a]	3.18 ± .84[b]	3.95 ± 1.75[b]	< 2.50[b]	< 2.50[b]
Egg	18.00 ± 9.04[a]	4.60 ± 1.96[b]	8.23 ± 8.95[ab]	3.15 ± 1.59[b]	7.63 ± 9.13[ab]
Gluten	76.55 ± 8.45[a]	37.12 ± 35.34[b]	38.37 ± 17.70[b]	10.75 ± 9.32[b]	15.17 ± 15.84[b]
Soy	21030 ± 2950[a]	6250 ± 9190[b]	21250 ± 9190[a]	< 2500[b]	< 2500[b]
Almond	< 2.50[a]	< 2.50[a]	< 2.50[a]	< 2.50[a]	< 2.50[a]
Hazelnut	< 2.50[a]	< 2.50[a]	< 2.50[a]	< 2.50[a]	< 2.50[a]
Peanut	< 2.50[a]	< 2.50[a]	< 2.50[a]	< 2.50[a]	< 2.50[a]
Walnut	< 2.40[a]	< 2.40[a]	< 2.40[a]	< 2.40[a]	< 2.40[a]

[a-b] Means with in a row with different superscripts differ statistically ($P < 0.05$)

Table 17. Allergens (ppm ± standard deviations) for cooked Spicy Black Bean Burgers, Beyond Burgers, Impossible Burgers, Pork chops, and Ground pork (n=6).

Allergen	Black Bean Burger (VB)	Beyond Burger (BB)	Impossible Burger (IB)	Pork Chop (PC)	Ground Pork (GP)
Milk	21.25 ± 9.19^{a}	14.37 ± 7.39^{ab}	9.65 ± 4.08^{ab}	3.15 ± 1.18^{b}	6.93 ± 8.90^{b}
Egg	21.95 ± 7.47^{b}	15.35 ± 10.27^{ab}	13.87 ± 10.74^{ab}	3.07 ± 1.01^{b}	12.68 ± 11.40^{ab}
Gluten	63.45 ± 12.32^{a}	26.63 ± 23.62^{a}	44.17 ± 4.01^{a}	41.78 ± 33.59^{a}	32.57 ± 36.99^{a}
Soy	18750 ± 6690^{a}	$< 2500^{b}$	$> 25000^{a}$	$< 2500^{b}$	6250 ± 9190^{b}
Almond	$< 2.50^{a}$	$< 2.50^{a}$	$< 2.50^{a}$	$< 2.50^{a}$	$< 2.50^{a}$
Hazelnut	$< 2.50^{a}$	$< 2.50^{a}$	$< 2.50^{a}$	$< 2.50^{a}$	$< 2.50^{a}$
Peanut	$< 2.50^{a}$	$< 2.50^{a}$	$< 2.50^{a}$	$< 2.50^{a}$	$< 2.50^{a}$
Walnut	$< 2.40^{a}$	$< 2.40^{a}$	$< 2.40^{a}$	$< 2.40^{a}$	$< 2.40^{a}$

$^{a\text{-}b}$ Means with in a row with different superscripts differ statistically ($P < 0.05$)

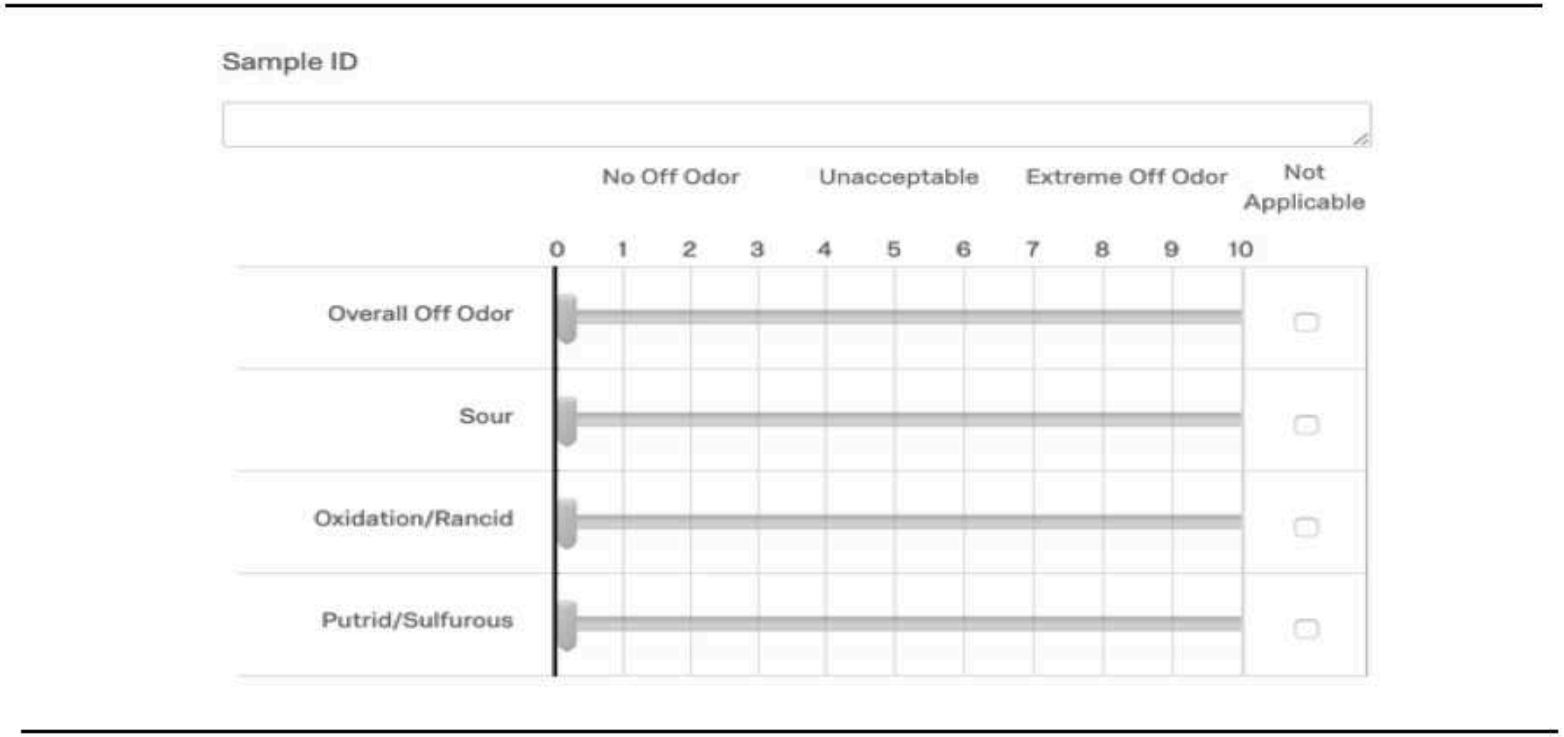

Figure 1. Ballot presented to panelist for evaluating beef knuckles.

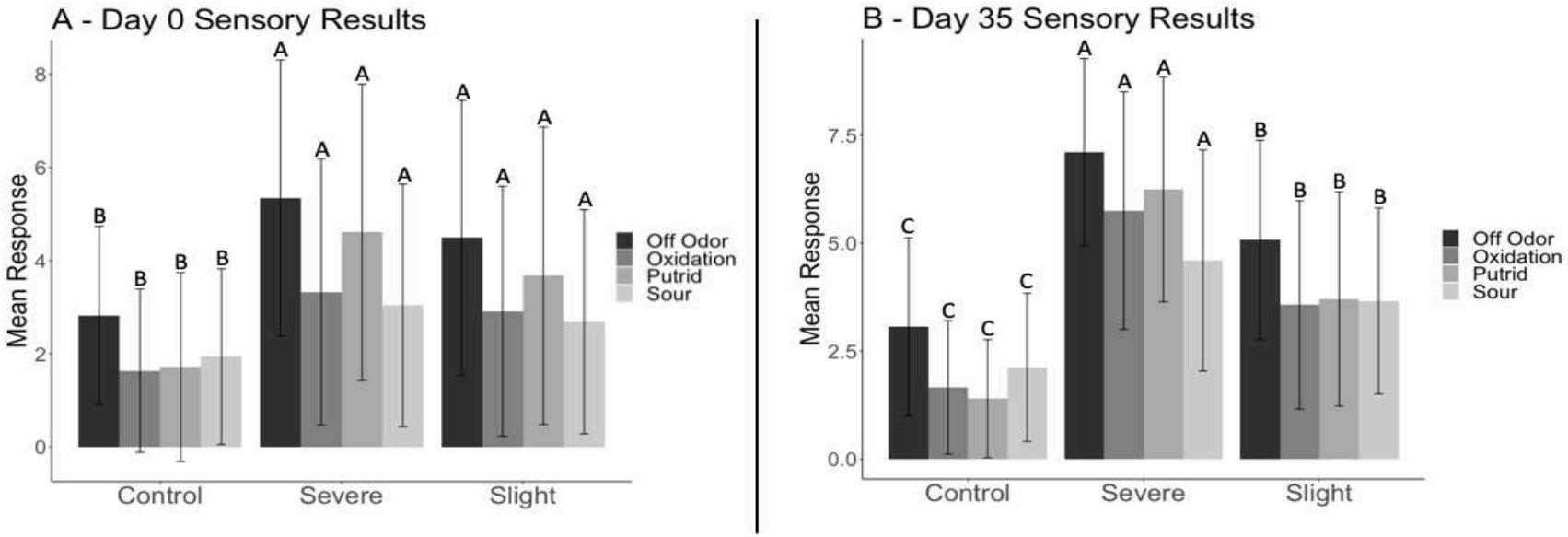

Figure 2. Odor panel results from day 0 (A) and day 35 (B) for control, slighlty sour, and severely sour knuckles.

A-C Bars within the same graph with different letter assignments differ statistically ($P < 0.05$)

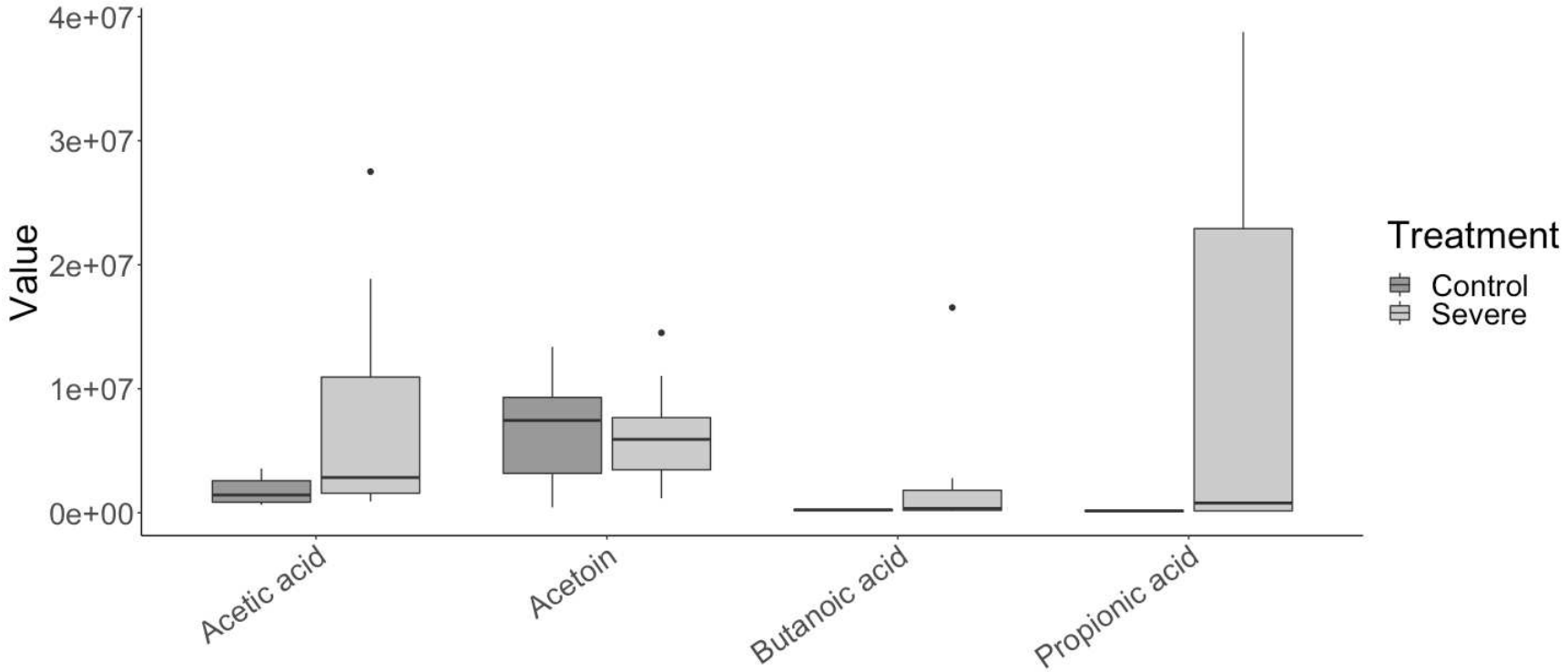

Figure 3. Compounds of interest identified from day 0 beef knuckles lacking and severely expressing the souring condition.

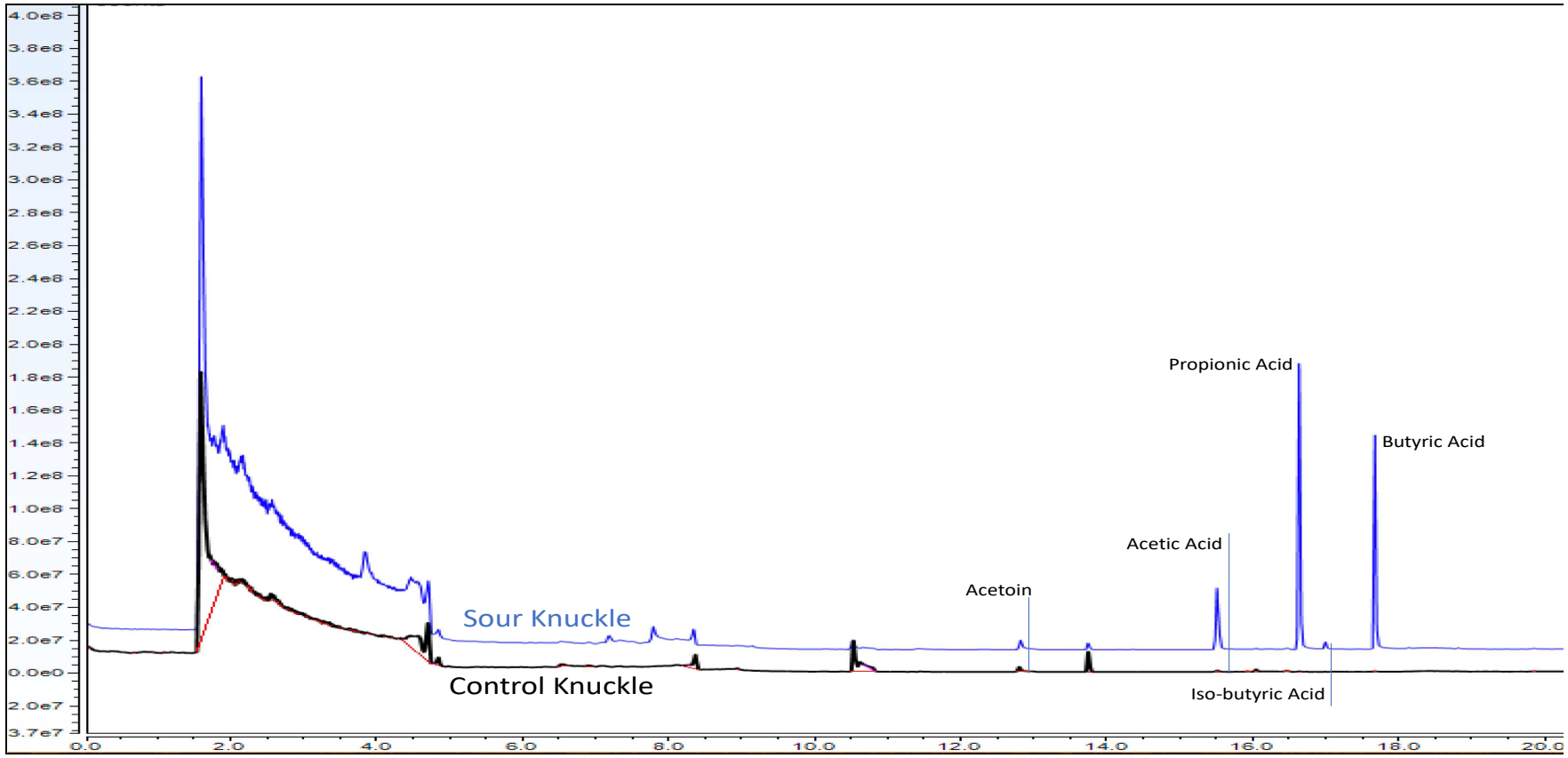

Figure 4. Representative GC-MS spectra from a severely sour and non-sour day 0 beef knuckle.

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