THESIS

NUTRITIONAL COMPOSITION AND FOOD SAFETY INTERVENTIONS OF PLANT AND ANIMAL-SOURCED FOODS

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ABSTRACT

NUTRITIONAL COMPOSITION AND FOOD SAFETY INTERVENTIONS OF PLANT AND ANIMAL-SOURCED FOODS

Nutritional composition of plant- and animal-sourced food is important for human growth and development, and yet even nutritious food-groups can be detrimental to human health if contaminated with harmful pathogens upon consumption. Therefore, two studies were performed to assess the nutritive quality of plant- and animal-sourced proteins; as well as, the antimicrobial efficacy of novel sanitizers against a foodborne pathogen attributed to illness from plant- and animal-sourced food consumption. In the first study, nutrient profiles of animal-derived meat products, which are traditionally an important source of nutrients in the human diet, were compared to novel plant-based meat alternatives, which have been growing in popularity among modern consumers. Nutritional composition of two different formulations of the Beyond Meat Burger (BMB1 and BMB2), Impossible Food Burger (IFB1 and IFB2), 80/20 ground pork (GP), and 80/20 ground beef (GB) were analyzed for proximate, mineral, vitamin, fatty acid, and amino acid profiles. Crude protein and crude fat content did not differ ($P > 0.05$) for each product in cooked states. Plant-based meat alternatives were either numerically greater than or did not differ statistically ($P < 0.05$) from animal-derived meat products in every mineral tested. Fat soluble vitamin A, D2, D3, and K1 were below detection limits (< 0.3 mcg/g for vitamin A; < 0.001 mcg/g for vitamin A, D2, D3, and K1) in all raw and cooked samples. Vitamin E content in raw and cooked plant-based meat alternatives was substantially greater ($P < 0.05$) than in raw and cooked animal-derived meat products. Raw and cooked GP and GB were substantially greater ($P < 0.05$)

than IFB1 and IFB2 in pantothenic acid (B5) but otherwise were numerically similar to or statistically less ($P < 0.05$) than IFB1 and IFB2 in most B vitamins tested. Total saturated and monounsaturated fatty acids did not differ ($P > 0.05$) for BMB2, IFB2, GP, and GB. IFB1 and IFB2 were greater ($P < 0.05$) than GP and GB in oleic acid (C18:1) content. Fatty acid profiles of raw and cooked BMB2 and IFB2 did not differ ($P > 0.05$) from one another. Essential amino acid composition of raw and cooked plant-based meat alternatives and animal-derived meat products were numerically comparable. Raw BMB2 did not differ ($P < 0.05$) from raw GP in histidine, lysine, and threonine content and was otherwise greater ($P < 0.05$) than raw GP in tyrosine, isoleucine, leucine, and valine. Raw GP was only numerically greater ($P > 0.05$) than raw BMB2 in methionine and tryptophan. In conclusion, plant-based meat alternatives assessed in this study were comparable to animal-derived GP and GB in most nutrient profiles assessed, providing high values of minerals, vitamins, fatty acids, and amino acids. Nonetheless, the high concentrations of certain nutrients as well as the integration of these nutrients into a food matrix may have implications for bioavailability and must be further investigated.

In the second study, efficacy of novel antimicrobial sanitizers was assessed in relation to reducing *Listeria monocytogenes* contamination on a plant-based food. Both plant and animal-sourced foods have proven to be vectors of *L. monocytogenes* contamination, but a largescale, multistate listeriosis outbreak was attributed to whole cantaloupes raising concerns for the potential contamination of other fresh produce not previously associated with *L. monocytogenes* contamination. This study assessed efficacy of chlorine as well as different concentrations of novel sanitizer and sulfuric-acid based surfactant blends, peroxyacetic acid (PAA) and ProduceShield Plus (PSP), against inoculated *L. monocytogenes* populations on whole cantaloupe melons (*Cucumis melo* L. var. *reticulatus*). Cantaloupe melons (n = 6) were inoculated with a five strain

mixture of *L. monocytogenes* (7 - 8 log CFU/cantaloupe) and immersed in water, chlorine (40 ppm), PSP (pH 1.81), PAA (40, 80, 250 ppm), or PAA+PSP (40, 80, 250 ppm and PSP blend) sanitizer solutions, under slight agitation for 0.5, 1, and 5 min exposure times. Recovery of surviving *L. monocytogenes* populations after immersion treatment, was accomplished by vigorously shaking whole cantaloupes in D/E neutralizing broth and plating the rinsates on PALCAM agar. The *L. monocytogenes* inoculation level achieved on whole cantaloupes was 7.9 ± 0.4 log CFU/cantaloupe. Immersion of inoculated whole cantaloupes in water or PSP achieved pathogen reductions that ranged between 0.3 to 0.5 log CFU/cantaloupe, and 0.9 to 1.8 log CFU/cantaloupe, respectively, across the three different exposure times (0.5, 1, 5 min). Reductions of *L. monocytogenes* populations on inoculated cantaloupes treated with 40 ppm chlorine achieved less than or equal to 3.3 log CFU/cantaloupe reductions across the different exposure times; while different concentrations of PAA (40, 80, 250 ppm) all achieved greater than or equal to 3.1 log CFU/cantaloupe reductions across the three exposure times. Different concentrations of PAA (40, 80, 250 ppm) blended with PSP resulted in pathogen reductions of between 3. 2 and > 4.9 log CFU/cantaloupe across the different exposure times. Decontamination efficacy of each PAA concentration level, within each treatment and exposure time, was similar ($P > 0.05$) to that of its corresponding PAA+PSP blend for most cases, although the PAA+PSP blends had numerically greater reductions than each corresponding PAA treatment and contained several samples which were below the detection limit of (2.7 log CFU/cantaloupe). In summary, PAA and the PAA+PSP blends demonstrated the greatest antimicrobial efficacy against *L. monocytogenes* populations on inoculated whole cantaloupes. More research should be conducted to elucidate a possible synergistic effect between PAA and sulfuric acid-based surfactants, such as PSP, on plant and animal-sourced foods susceptible to *L. monocytogenes* contamination.

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1. CHAPTER 1: REVIEW OF LITERATURE

Consumption of plant- and animal-sourced food is fundamental to the survival, development, and prosperity of humankind (Kremer, 1993; Mann, 2018). Humans are characteristically and habitually omnivorous by nature, and therefore require nutrients derived from both plant and animal sources. In the present review, we briefly outline the nutritional characteristics of plant and animal sourced foods, to better understand how animal-derived meat products and plant-based meat alternatives may differ in nutrient profiles.

1.1. NUTRITION OVERVIEW

Plants synthesize nutrients from water, carbon dioxide, and elements from the environment through the process of photosynthesis, whereby prokaryotic and eukaryotic organisms are able to derive and store energy (Mann and Truswell, 2017). Omnivorous and herbivorous species, such as porcine and bovine animals, are well-suited to convert plant-based foods into energy-dense animal-derived macronutrients and micronutrients for human consumption.

Macronutrients (carbohydrates, lipids, and proteins) must be consumed in the largest quantities and comprise the majority of energy intake in humans. Carbohydrates are synthesized in plants from water and carbon dioxide through the process of photosynthesis, and comprise 40 to 80% of total energy intake in human diets (FAO, 1998; Mann and Truswell, 2017). Lipids are a group of compounds synthesized in plant and animal organisms from acetyl CoA obtained from the catabolism of carbohydrates and comprise 30 to 40% of total energy intake in human diets (Bjorntorp, 1991; Cooper, 2000; Smuts and Wolmarans, 2013). Proteins are synthesized in plants and animals from nitrogen and other components in the soil, or from amino acids and nutrients

directly obtained from food and is the second most abundant component in animals, following water (Mann and Truswell, 2017).

Micronutrients must be consumed in lower concentrations and generally refer to minerals and vitamins, which must be obtained directly from the diet (Fairfield and Fletcher, 2002; Gupta and Gupta, 2014; Mann and Truswell, 2017). Minerals are chemical elements sequestered by plants and animals from the environment and are often involved as structural components of proteins or as cofactors for enzymes (Gharibzahedi and Jafari, 2017). Dietary minerals are subdivided into macro-minerals and trace-minerals based on the metabolic demand of the element. Vitamins are a complex group of organic molecules synthesized in plants and some animals for use in cellular metabolism (McDowell, 2008). Vitamins are characterized by their solubility as either being fat or water soluble (Anderson and Young, 2003).

1.2. ANIMAL-DERIVED MEAT NUTRITION

Animal-derived meats are regarded as a nutrient-dense group of foods required for optimal human growth and development, and have become a significant component of the human diet (Higgs, 2000; Mann, 2007). The United States Department of Agriculture (USDA) identifies meat obtained from mammals, such as beef, pork, and lamb, as red meats, while meat obtained from poultry and fish as white meat (Boler and Woerner, 2017). Increased consumption of animal-derived meat has been associated with higher GDP and longer life expectancies compared to low meat consuming countries, but has also been associated with increased risk of all-cause mortality, cardiovascular heart disease, cancer and other malignancies, within high meat consuming countries (FAO, 2001; Song et al., 2016; Kim et al., 2018; Kim Hyunju et al., 2019).

1.2.1. Nutrient profiles

Animal-derived meats are an excellent source of essential amino acids, long chain saturated and unsaturated fatty acids, B vitamins, and trace minerals (Pereira and Vicente, 2013; De Smet and Vossen, 2016; Bohrer, 2017). The amino acid composition of animal-derived meat closely resembles the amino acid composition of the human body and confers high anabolic capacity due to high leucine, lysine, and methionine content (Xiong and Yada, 2004; Gorissen and Witard, 2018). Animal-derived meats, particularly from ruminants, are relatively high in saturated fatty acids due to the biohydrogenation of unsaturated fatty acids during fermentation (Smet et al., 2004). Animal-derived meat products are also relatively high in monounsaturated oleic acid (C18:1) and polyunsaturated linoleic (C18:2) and linolenic (C18:3) acids. Lean meats are poor sources of fat soluble vitamin A and D, but are an excellent source of vitamin B12, as well as thiamin (B1), riboflavin (B2), niacin (B3), pantothenic acid (B5), and pyridoxine (B6) (Purchas et al., 2007; Williams, 2007; McAfee et al., 2010). Finally, red meats are excellent sources of bioavailable iron, zinc, phosphorus, selenium, and copper (Williams, 2007).

1.2.2. Health impacts of animal-derived meat

Although saturated fatty acid and cholesterol content of red meats has been traditionally and conventionally considered a risk factor for the development of heart disease, more recent observational studies have not found evidence for a Signiant association of dietary cholesterol and cardiovascular heart disease (Lipid Research Clinics Program, 1984; Higgs, 2000; Carson Jo Ann S. et al., 2020). Nonetheless, the cooking method and temperature of animal-derived meats may contribute to the formation of heterocyclic amines (HCAs) or polycyclic aromatic hydrocarbons (PAHs) which may be a risk factor for the development of colon and other cancers (Jägerstad et

al., 1991; Cross and Sinha, 2004; McAfee et al., 2010). Alternatively, the presence of nitrates or nitrites used in meat curing and exposed to high thermal and acidic treatments may form nitrosamines which may cause disease in humans (Hill et al., 1973; Issenberg, 1976).

Many largescale epidemiological studies suggest a positive association between red meat and human disease (Cross and Sinha, 2004; Larsson and Wolk, 2006; Lippi et al., 2016). Nonetheless, convincing and adequately-powered research that draw conclusive results are lacking (McAfee et al., 2010). As a result, the effect of red meat or other meat types, independent of the carcinogenicity of food additives, cooking temperatures, and other genetic or behavioral risk factors has yet to be determined (Larsson and Wolk, 2006). Certainly, human pathogenesis is a complex study of genetic, environmental, physical, biological, behavioral, and dietary risk factors, making it difficult to attribute pathogenicity to one food group (Lippi et al., 2016).

1.3. ANIMAL-DERIVED MEAT MARKET

The United States remains the highest consumer of total meat per capita, at more than three times the global average (Speedy, 2003; Daniel et al., 2011). The global average of daily meat consumption in 2005, was 110 g per person, but had a 10-fold variation between high and low consuming populations and varies within different socioeconomic or cultural strata (FAO, 2009; De Smet, 2012). The global annual per capita average of meat consumption is 37.97 kg, with low consuming countries ranging between 3 and 5 kg per capita per year, and high consuming countries exceeding 100 kg per capita per year (Speedy, 2003). Some estimates predict that one-third of the world's population consumes less than 10 kg of animal-derived meat every year and that cereal grains supply more than half of the human requirements for energy and protein (Bender, 1992; Speedy, 2003).

Due to changes in population, urbanization, and industrialization, as well as improved economics for developing countries, the global demand for animal-derived meat is predicted to double by year 2050 (Steinfeld et al., 2006; Fiala, 2008; Bruinsma, 2009). While economic factors have been proposed as a primary driver for increased meat consumption in developing countries, reduction in meat prices and trade liberalization have also been characterized as factors that affect this increase in consumption as well (Delgado, 2003; Wood, 2011; Henchion et al., 2014). Nonetheless, alternative protein sources other than animal-derived meats are a growing market and potentially important alternative protein source for the human population.

1.4. PLANT-BASED MEAT ALTERNATIVE BACKGROUND AND NUTRITION

Meat alternatives are a group of foods that do not contain meat but target similar taste, appearance, and texture as meat, poultry, fish, or shellfish (Shurtleff and Aoyagi, 2014). The American Meat Science Association, National Cattlemen's Beef Association, and United States Cattlemen's Association do not consider plant-based meat alternatives as meat, but the Plant Based Foods Association argues that meat alternatives should be considered meat products (Boler and Woerner, 2017; Hermesauto, 2019; PBFA, 2019).

1.4.1. Nutrient profiles

Plant-based proteins such as legumes, oilseeds, and cereals are primarily used in plant-based meat alternative formulations, with legumes such as soybeans, peas, and black beans being the primary protein sources in most plant-based meat alternatives. Soy, wheat, pea, mung bean, and potato proteins meet the joint World Health Organization/Food and Agriculture Organization (WHO/FAO) regulations for essential amino acid intakes (FAO and WHO, 1991; Joint WHO/FAO/UNU Expert Consultation, 2007; Yi-Shen et al., 2018). Black bean and rice proteins

meet WHO/FAO requirements for most essential amino acids except sulfur-containing amino acids and lysine, respectively (Joint WHO/FAO/UNU Expert Consultation, 2007).

Important plant-based lipids used in meat alternative formulations are often a combination of solid fats such as coconut oil and cocoa butter and liquid oils such as sunflower oil and canola oil (Moskin, 2019; Sha and Xiong, 2020a). Coconut oil and cocoa butter contain high levels of saturated fat, primarily from lauric acid (C12:0) for coconut oil and palmitic (C16:0) and stearic (C18:0) acid for cocoa butter (Lipp and Anklam, 1998; Shankar et al., 2013; Freeman et al., 2017). Sunflower oil is relatively high in unsaturated long chain fatty acid content (91.5 %), with the predominant fatty acids in sunflower oil being oleic acid (C18:1) and linoleic acid (C18:2) (Chowdhury et al., 2007). Canola oil contains the lowest amount of saturated fatty acids (6.98 %), compared to most vegetables oils (Zambiazi et al., 2007). While plant-based oils do not contain cholesterol, plant-based meat alternatives are often higher than many minimally processed plant proteins in saturated fat content (Hu et al., 2019).

Plant-based foods, especially vegetables, are good sources of vitamin C, D, and E, but are relatively poor sources of A and B vitamins (Booth et al., 1992; García-Closas et al., 2004; Moore et al., 2004). The formulations of many plant-based meat alternatives are often comprised of purified plant proteins and fats which may lack certain nutrients and phytochemicals naturally present in vegetables (Hu et al., 2019). Therefore, many plant-based meat alternative formulations supplement their products with vitamins, especially thiamin (B1), riboflavin (B2), niacinamide (B3), pyridoxine hydrochloride (B6), folic acid (B9), and cobalamin (B12) (Sha and Xiong, 2020b).

Minerals obtained from animal sources, were originally consumed from plant and environmental sources (Gupta and Gupta, 2014). Consequently, plants are often good sources of minerals, but the bioavailability of plant-sourced minerals is variable compared to that of animal-

sourced minerals (Grusak, 2002). Many plant-based meat alternatives are supplemented with the following minerals: sodium chloride, potassium chloride, calcium chloride, calcium acetate, ferrous sulfate (iron), calcium phosphate, sodium phosphate, magnesium carbonate and other minerals (Sha and Xiong, 2020b).

1.4.2. Antinutritional factors and health impacts of plant-based meat alternatives

Plant-based food products are generally marketed as healthier alternatives to animal meat or processed foods (Slade, 2018). However, compounds such as tannins, phytates, oxalates, saponins, lectins, and protein inhibitors are commonly found in plant foods, which may inhibit the absorption of nutrients in human intestines (Gemede and Ratta, 2014). Tannins are polyphenolic compounds found in plant tissues that can bind to proteins or inhibit digestive enzymes (Chung et al., 1998). Phytates and oxalates are widely distributed in plant tissues and have a propensity to bind to minerals, such as zinc, iron, calcium, magnesium, manganese, copper, and potassium (Noonan, 1999; Bohn et al., 2008). Saponins, lectins, protease inhibitors, and other antinutritional factors are also present in plant tissues and may impact the digestibility of carbohydrates, proteins, minerals and other nutrients, although the concentration of antinutrient factors may be variable in purified protein isolates used in meat alternative formulations (Fernández-Quintela et al., 1997; Soetan and Oyewole, 2009). Additionally, plant-based meat alternatives are characterized as ultra-processed foods, and usually consumed in a “fast-food” setting, alongside other processed foods which may be high in refined sugars, sodium, or saturated fat (Hu et al., 2019; Kyriakopoulou et al., 2019). Therefore, the presence of antinutrient components present in plant-sourced foods, as well as the format in which plant-based meat alternatives are consumed, may have implications on human health.

1.5. PLANT-BASED MEAT ALTERNATIVE MARKET

Vegetarian diets have been on the rise in recent years, but vegetarians account for less than 5% of the American population (Richardson et al., 1994; Segovia-Siapco and Sabaté, 2019). An increase in flexitarian and vegan diets has also been observed, alongside general meat reduction or avoidance from traditionally omnivorous consumers (Beardsworth and Keil, 1991; Nezlek and Forestell, 2020). The motivations for these plant-based diets are usually oriented on human health, animal welfare, or environmental sustainability (Kessler et al., 2016). Issues regarding the environmental impact and sustainability of animal production have been gaining interest, influencing consumer choices (Kumar et al., 2017; Sanchez-Sabate and Sabaté, 2019). These environmental concerns, in conjunction with other factors, have influenced meat eating consumers to reduce meat intake, and incorporate plant-based meat alternatives into their diet. As a result, vegetarian, vegan, and other niche markets which have traditionally been the target for plant-based meat alternative consumption, has now shifted to include habitual "meat eaters" who are affable to plant-based meat alternative consumption (Ruby and Heine, 2012; Nezlek and Forestell, 2020).

The plant-based meat alternative market is expected to reach $ 30 billion in 2026 and $ 85 billion by 2030, having grown from $ 4.8 billion in 2018 (Watson, 2019; Sha and Xiong, 2020a). The plant-based meat alternative market is expanding at more than three times the rate of other animal-derived meat products markets, although this market still only accounts for 1% of the total retail meat sold in the US (U.S. Plant-Based Market Overview, 2018).

Beyond Meat and Impossible Foods are popular brands with novel meat alternative products, although Tyson, Smithfield, Perdue Farms, Hormel Foods, and Maple Leaf have also developed meat alternative products (Sha and Xiong, 2020a). Beyond Meat products are being

sold at many popular US-based supermarket chains as well as some restaurant chains, such as Dunkin' Donuts. Impossible Foods products have gained popularity and are being sold in Burger King fast food chains, as well as featured in many local restaurants throughout the United States.

While these products are gaining popularity among modern consumers, little is yet known about the nutrient composition of modern plant-based meat alternatives and possible health implications. Researchers at Colorado State University (Fort Collins, CO) have previously determined the nutrient profiles of original formulations of the Beyond Meat Burger (BMB1), Impossible Food Burger (IFB1), and 80/20 ground pork (GP) (Thompson, 2019). Nonetheless, new ingredients are being used in the current formulations of the Beyond Meat Burger (BMB2) and Impossible Food Burger (IFB2). Therefore, the objective of the current study was to determine the nutrient profiles of current formulations of BMB2 and IFB2, in comparison to BMB1, IFB1, GP, and 80/20 ground beef.

2. CHAPTER 2: NUTRITIONAL COMPOSITION OF PLANT- AND ANIMAL-SOURCED FOODS

2.1. INTRODUCTION

Plant- and animal-sourced foods provide essential nutrients for human growth and development (Mann and Truswell, 2017). Traditionally, animal-derived foods, such as milk, eggs, and meat have been regarded as a nutrient dense group of foods that are optimal for human growth and development (Higgs, 2000). In recent years, however, plant-based lifestyles, such as veganism, vegetarianism, or flexitarianism have been on the increase, which has coincided with the development of novel plant-based meat alternatives that simulate the taste, appearance, or texture of animal-derived meat products (Richardson et al., 1994; Shurtleff and Aoyagi, 2014; Segovia-Siapco and Sabaté, 2019). These products have become very popular among modern consumers, with the plant-based meat alternative market currently growing at three times the rate of other animal-derived meat products (Watson, 2019; Sha and Xiong, 2020a).

Considering the current public health circumstances surrounding food security and nutrition in both the developing and developed world, as well as the growing consumption of both animal-derived meat products and plant-based meat alternatives, it is important to understand the nutrient profiles of these food groups and their implications on human health. Researchers at Colorado State University (Fort Collins, CO) have previously determined the nutrient profiles of original formulations of the Beyond Meat Burger-original (BMB1), Impossible Food Burger-original (IFB1), and 80/20 ground pork (GP) (Thompson, 2019). In 2019, new formulations of these plant-based meat alternatives were developed, for which it has become the objective of the

present work to determine nutrient profiles for the current formulations of the Beyond Meat Burger-current (BMB2) and Impossible Food Burger-current (IFB2). Additionally, in an effort to draw accurate comparisons between plant-based meat alternatives and animal-derived meat products, nutrient profiles for 80/20 ground beef (GB) were retrieved from the United States Department of Agriculture (USDA) nutrient database (USDA, 2020).

2.2. MATERIALS AND METHODS

2.2.1. Sample collection and preparation

To adhere to USDA nutrient database guidelines, original and current formulations of the Beyond Meat Burger (BMB1, BMB2) and Impossible Food Burger (IFB1, IFB2), along with the 80/20 (i.e., 80% lean, 20% fat) ground pork (GP) were purchased at food service companies and supermarkets from six randomly selected cities (Seattle, WA; Peyton, CO; Memphis, TN; Newburgh, IN; Houston, TX; and Brooklyn, NY) throughout the United States. Approximately 5 lbs of frozen product, with the same lot number, were purchased and transported under refrigeration (4°C) to Colorado State University (Fort Collins, CO), where they were frozen (-20˚C) until further analysis. Six replicates (n = 6) of each product, designated as raw or cooked, were analyzed for nutrient content separately. Nutrient profiles for raw and cooked 80/20 ground beef (GB) were retrieved form the USDA nutrient database and utilized for comparisons in the present work (USDA, 2020).

Samples (BMB1, BMB2, IFB1, IFB2, and GP) were formed into 4 oz patties and cooked on non-stick anodized aluminum skillet to an internal temperature of 71°C. Internal temperature was determined with a digital thermocouple thermometer. After cooking, the product was placed on a stainless-steel rack to cool for 10 min. Both uncooked and cooked patties were chilled,

uncovered, at refrigerated temperatures (0 to 4°C) for 12 to 24 h prior to homogenization. Samples were frozen by immersion into liquid nitrogen and immediately homogenized for 10 s on a low speed (1500 rpm) and 30 s on a high speed (3500 rpm) with a Robot Coupe BLITZER 6V (Robot Coupe USA Inc., Ridgeland, MS) blender, until a fine homogenized powder was obtained. Homogenized samples of raw and cooked products were stored at -80˚C for further analysis.

2.2.2. Proximate analysis

All proximate data in the present work are reported on an as fed basis, as opposed to a dry matter basis. Moisture analysis was performed using the AOAC oven drying method 950.46 (AOAC International, 1995) at Colorado State University (Fort Collins, CO). Approximately 1 g of samples were weighed 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] × 100.

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

Total lipid content was extracted using the method described by Folch and Stanley (1957) method along with a processes described in AOAC official method 983.23 (AOAC International, 2006) at Colorado State University. Approximately 1 g of sample was homogenized in a 2:1 ratio of chloroform and methanol solution respectively. Homogenized samples were placed onto an orbital shaker at room temperature for 20 min, followed by filtering through ashless filter paper.

Four mL of 0.9% NaCl was added to the filtered sample, and the sample was placed in a refrigerator (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 N_2 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) × 100.

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

Acid detergent fiber (ADF) and neutral detergent fiber (NDF) was evaluated according to the method proposed by Van Soest et al. (1991) at Colorado State University. Samples were digested in an Ancom 200 Fiber analyzer (Ankom Technology Corp.) with 10 liters of NDF solution, 4 mL of heat stable alpha amylase, and 20 g of sodium sulfate. Following agitation and heating for 70 min, the liquid was drained and alpha amylase was re-applied twice for 5 min durations each, to completely hydrolize starches. Samples were placed in beaker immersed in acetone, left in an oven at 60ºC to dry overnight, and later weighed to determine NDF percentage. ADF analysis was performed by adding 2 liters of ADF solution to the sample, and agitating under constant heat for 60 min. Following the initial reaction, liquid was drained from the sample and alpha amylase was re-applied to the sample twice for 5 min, afterwhich the sample was immersed in acetone and placed in an oven at 60ºC to dry overnight.

2.2.3. Fatty Acid analysis

Fatty acid analysis was conducted at Colorado State University. Total lipid was extracted from 1.0 g of homogenized sample using the method described by Folch and Stanley (1957) and modified by Bligh and Dyer (1959). Saponification and methylation of lipids was accomplished using the method described by Parks and Goins (1994). Individual lipids were separated via gas chromatography using a Hewlett Packard (Avondale, PA) Model 6890 series II gas chromatograph fixed with a series 7683 injector and flame ionization detector and fitted with a 100 m × 0.25 mm (id) fused silica capillary column (SP-2560 Supelco Inc. Bellefonte, PA) as described by Phillips et al. (2010).

2.2.4. Cholesterol analysis

Cholesterol content was analyzed at Eurofins Laboratories (Madison, WI). Samples were saponified using ethanolic potassium hydroxide. The unsaponifiable fraction that contained cholesterol and other sterols was extracted with toluene. Toluene was evaporated, and the residue was dissolved into dimethylformamide (DMF). Samples were derivatized to form trimethylsilyl ethers and content was quantitatively determined by gas chromatography using 5 alpha-cholestenol as an internal standard.

2.2.5. Inductively Coupled Plasma Mass Spectrometry analysis

Minerals (Ca, Mg, K, Na, Fe, Zn, Cu, Mn, P) were analyzed by Eurofins Laboratories using the USDA wet ashing procedure and AOAC official methods 985.35, 984.27, 985.01 (AOAC International, 2006) and AOAC official method 2011.14 (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 mass spectrometer by comparing the emission of the unknown sample against emissions from standard solutions.

2.2.6. Fat-soluble vitamins analysis

Vitamin A content was measured by Eurofins Laboratories using HPLC methods described by Njeru et al. (1992) and Alosilla et al. (2007). Vitamin D and 25-hydroxy-Vitamin D analyses was performed by Eurofins Laboratories using HPCL with UV detection. Vitamin E content was measured by Craft Technologies (Wilson, NC) using HPLC with a normal phase column, and UV detection with external calibration, and internal standard recovery post analysis. Vitamin K1 analysis was performed by Eurofins Laboratories using AOAC official method 999.15 including HPLC and fluorescence detection (AOAC International, 2006).

2.2.7. B-vitamin analysis

B-vitamins were analyzed by Eurofins Laboratories . The AOAC official methods utilized in the analysis of each vitamin was as follows: vitamin B-12 AOAC 952.20 and 960.46; niacin AOAC 944.13 and 960.46; vitamin B-6 AOAC 961.15; riboflavin AOAC 960.46 and 940.33; thiamin AOAC 942.23, 953.17, and 957.17; pantothenic acid AOAC 945.74, 960.46, and 992.07 (AOAC International, 2006).

2.2.8. Amino acid profile

Amino acid profile was determined by Eurofins Laboratories . Samples were hydrolyzed in 6 N hydrochloic 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 hydrolyzed from proteins by heating at approximately 110°C in 4.2 N sodium hydroxide. Samples were analyzed by HPLC after pre-injection derivatization. Primary amino acids were derivatized with o-phthalaldehyde (OPA) and secondary amino acids were derivatized with fluorenylmethyl chloroformate (FMOC) before injection.

2.2.9. Statistical analysis

Analyses were performed using R software (v.3.6.1), whereby the simple means and standard deviations for each nutrient component were obtained. The Anova type III function from the Car package (Fox and Weisberg, 2019) was used to determine statistical differences. The emmeans function with a CLD display, from the emmeans package (Lenth 2019) was utilized to identify respective statistically significant differences. Tukey adjusted pairwise comparisons were used for each test. The alpha level for this study was 0.05 to determine statistically significant differences. Results for nutrient profiles of BMB1, BMB2, IFB1, IFB2, and GP are reported as least square means (n = 6) with standard deviation, and a letter superscript designating statistical difference. Results for GB are reported as means with no standard deviation and no statistical superscript, as data was directly retrieved from USDA nutrient database as a mean, with no standard deviations. Finally, nutrient intakes per serving size were determined by multiplying the

nutrient component mean by 113 g/serving as obtained from the nutrient label of the plant-based meat alternatives.

2.3. RESULTS

2.3.1. Proximates

Results of proximate analysis of raw and cooked samples are reported in Tables 2.1 and 2.2, respectively. Moisture content ranged between 57 - 63 % in raw products and 50 - 55% in cooked products. Raw and cooked animal-derived meat products contained greater ($P < 0.05$; Tables 2.1 and 2.2) moisture content than the plant-based meat alternatives. BMB2 and GP lost 12.9 and 12.2% moisture from raw to cooked states, respectively. IFB2 and GB had 9.3 and 9.7 % moisture loss from raw to cooked states, respectively.

Crude protein content of raw GP was greater ($P < 0.05$; Table 2.1) than the other products. Cooked BMB2 contained greater ($P < 0.05$; Tables 2.1 and 2.2) crude protein (as fed) than cooked IFB2, although GB was numerically greater (Table 2.2) in crude protein content, than the other products. Crude fat content was numerically greatest (Tables 2.1 and 2.2) in raw and cooked GB compared to the other products. Crude fat content did not differ ($P > 0.05$; Table 2.2) between BMB2 and IFB2 and GP after cooking.

Dry matter (as fed) content was greatest ($P < 0.05$; Tables 2.1 and 2.2) in raw and cooked plant-based meat alternatives than animal-derived meat products. BMB2 and IFB2 did not differ ($P > 0.05$; Table 2.2) in dry matter (as fed) content before cooking but BMB2 was greater ($P < 0.05$; Table 2.2) than IFB2 in dry matter content after cooking. Acid detergent fiber (as fed) content

did not differ ($P < 0.05$; Tables 2.1 and 2.2) between BMB2 and IFB2 in raw and cooked states. Neutral detergent fiber did not differ ($P > 0.05$; Tables 2.1 and 2.2) either in BMB2 and IFB2.

2.3.2. Minerals

Results from mineral analysis of raw and cooked samples are reported in Tables 2.3 and 2.4, respectively. Raw and cooked IFB2 was greater ($P < 0.05$; Tables 2.3 and 2.4) than raw and cooked BMB2 and GP in each macromineral tested (calcium, magnesium, potassium, sodium), except phosphorus, for which raw and cooked BMB2 was greater ($P < 0.05$; Tables 2.3 and 2.4). Raw and cooked IFB2 contained more than twice the amount of most macrominerals (calcium, magnesium, potassium) found in raw and cooked BMB2, and more than 2 to 4 times the amount of magnesium, potassium and sodium found in raw and cooked animal-derived meat products. Furthermore, raw and cooked IFB2 contained approximately 17 and 15 times the amount of calcium found in raw and cooked GP, respectively. Raw and cooked GP and GB were consistently less ($P < 0.05$; Tables 2.3 and 2.4) than raw and cooked BMB2 and IFB2 in each macro-mineral (calcium, magnesium, phosphorus, sodium), except for potassium, for which raw and cooked animal-derived meat products were greater ($P < 0.05$; Tables 2.3 and 2.4) than BMB2, but still less ($P < 0.05$; Tables 2.3 and 2.4) than IFB2.

Raw IFB2 was greater ($P < 0.05$; Table 2.3) than raw BMB2 in most trace elements tested (copper, manganese, and zinc). Iron content did not differ ($P > 0.05$; Table 2.3) between raw BMB2 and IFB2. Additionally, copper content did not differ ($P > 0.05$; Table 2.4) between BMB2 and IFB2 after cooking. Raw and cooked GP and GB were consistently less ($P < 0.05$; Tables 2.3 and 2.4) than raw and cooked BMB2 and IFB2 in copper, iron, and manganese content. Raw and cooked GP did not differ ($P > 0.05$; Tables 2.3 and 2.4) from BMB2 in zinc content. Cooked GB

was numerically greater (Table 2.4) than the other cooked products in zinc content. The magnitude of difference for trace minerals (copper, iron, manganese, and zinc) between raw and cooked IFB2 and BMB2 ranged between 1 and 2 times that of the respective trace mineral. Raw and cooked IFB2 was approximately 2 to 4.5 times greater than raw and cooked GP and GB in copper, iron, and zinc content. Raw and cooked BMB2 and IFB2 ranged between 38 and 108 times the manganese content found in raw and cooked GP and GB. Iodine, cobalt, fluoride, and selenium trace minerals were not tested in this study.

2.3.3. Vitamins

Results from vitamin analysis of raw and cooked samples are reported in Tables 2.5 and 2.6. Fat soluble vitamin A, D2, D3, and K1 were below the detection limit in all raw and cooked samples, except for trace amounts of vitamin D3 in raw and cooked GP and vitamin K1 in raw and cooked BMB2. Vitamin E content in raw and cooked plant-based meat alternatives was substantially greater ($P < 0.05$; Tables 2.5 and 2.6) than in raw and cooked animal-derived meat products. Raw and cooked IFB2 contained approximately 4 and 14 times the amount of vitamin E found in raw and cooked BMB2 and GP, respectively.

B vitamin content of raw and cooked IFB2 was greater ($P < 0.05$; Tables 2.5 and 2.6) than raw and cooked BMB2 in each B vitamin, except pantothenic acid (B5), for which the raw and cooked states of BMB2 and IFB2 did not differ ($P > 0.05$; Tables 2.5 and 2.6). Raw IFB2 was only greater ($P < 0.05$; Table 2.5) than raw GP in thiamin (B1), pyridoxine free base (B6), and biotin (B7) content. Otherwise, raw IFB2 and GP did not differ ($P > 0.05$; Table 2.5) in riboflavin (B2) and niacin (B3) content. Raw and cooked GB was less (Tables 2.5 and 2.6) than raw and cooked

GP in each B vitamin. Raw and cooked GB was greater (Tables 2.5 and2.6) than raw and cooked BMB2 and IFB2 in pantothenic acid (B5).

2.3.4. Fatty acids

Results of the fatty acid analysis of raw and cooked samples is reported in Tables 2.7 and 2.8, respectively. Fatty acid profiles of raw and cooked BMB2 and IFB2 did not differ ($P > 0.05$; Tables 2.7 and2.8) from one another. Raw and cooked BMB2 and IFB2 were greater ($P < 0.05$; Tables 2.7 and2.8) than raw and cooked GP and GB in myristic acid (C14:0) and arachidonic acid (C20:0); otherwise, raw and cooked GP and GB were greater ($P < 0.05$; Tables 2.7 and2.8) than BMB2 and IFB2 in most of the saturated fatty acids. Raw and cooked GP was similar to (Tables 2.7 and 2.8) raw and cooked GB in most saturated fatty acids, except myristic acid (C14:0).

Regarding monounsaturated fatty acids, plant-based meat alternatives only contained oleic acid (C18:1), for which the raw and cooked plant-based meat alternatives were greater ($P < 0.05$; Tables 2.7 and2.8) than raw and cooked animal-derived meat products. Raw and cooked GP and GB were comparable in palmitoleic acid (C16:1), vaccenic acid (C18:1), and eicosenoic acid (C20:1). Regarding polyunsaturated fatty acids, raw and cooked plant-based meat alternatives only contained linoleic acid (C18:2) for which BMB2 and IFB2 did not differ ($P > 0.05$; Tables 2.7 and2.8) in their raw or cooked states. Raw and cooked GB were similar (Tables 2.7 and& 2.8) to the plant-based meat alternatives in linoleic acid (C18:2) content, while raw and cooked GP were numerically greater (Tables 2.7 and2.8) than the other products. Raw and cooked GP were numerically greater (Tables 2.7 and2.8) in a-linoleic acid (C18:3), arachidonic acid (C20:4) than GB. GP contained trace amounts of DPA C22:5) and DHA (C22:6). Finally, cholesterol content

was greatest ($P < 0.05$; Tables 2.7 and2.8) in the animal-derived meat products and was below detection limit (< 0.01 mg/g) in raw and cooked BMB2 and IFB2 products.

2.3.5. Amino acids

Results from amino acid analysis of raw and cooked samples are reported in Tables 2.9 and 2.10, respectively. The essential amino acid composition of raw and cooked plant-based meat alternatives and animal-derived meat products were comparable. Essential amino acids were greater in raw and cooked BMB2 ($P < 0.05$; Tables 2.9 and2.10), compared to raw and cooked IFB2, except tryptophan, which was greater ($P > 0.05$; Tables 2.9 and2.10) in raw and cooked IFB2. Raw BMB2 did not differ ($P < 0.05$; Table 2.9) from raw GP in histidine, lysine, and threonine content and was otherwise greater than raw GP in tyrosine, isoleucine, leucine, and valine. Raw GP was only numerically greater (Table 2.9) than raw BMB2 in methionine and tryptophan. The essential amino acid profile of raw and cooked GB was similar to that of GP.

Regarding non-essential amino acids, raw and cooked BMB2 was greater ($P < 0.05$; Tables 2.9 and2.10) than IFB2 in arginine and tyrosine. Raw and cooked IFB2 was greater ($P < 0.05$; Tables 2.9 and 2.10) than other plant-based meat alternatives and animal-derived meat products in cystine and glutamic acid. Raw and cooked GP and GB were substantially greater ($P < 0.05$; Tables 2.9 and 2.10) in glycine than the plant-based meat alternatives. Proline content did not differ ($P > 0.05$; Tables 2.9 and 2.10) between plant- and animal-sourced products. Raw BMB2 was greater ($P < 0.05$; Table 2.9) than raw IFB2 in each obligatory non-essential amino acid (alanine, aspartic acid, and serine). Alanine content did not differ ($P > 0.05$; Table 2.9) between raw BMB2 and GP. Raw and cooked GP and GB were less ($P < 0.05$; Tables 2.9 and 2.10) than BMB2 and IFB2 in aspartic acid and serine content.

2.4. DISCUSSION

2.4.1. Proximates

Results from proximate analysis of raw and cooked samples are reported in Tables 2.11 and 2.12, respectively. As fed crude protein and crude fat content did not differ ($P > 0.05$; Tables 2.11 and 2.12) for each product in raw and cooked states, although GB contained the greatest (Tables 2.11 and 2.12) numerical crude protein and crude fat content before and after cooking. Only new formulations of BMB2 and IFB2 were analyzed for acid detergent fiber and found to contain relatively high percentages of plant cell and fibrous materials.

2.4.2. Minerals

Results from mineral analysis for previous and current formulations of raw and cooked samples are reported in Tables 2.13 and 2.14, respectively. Calcium, and sodium were considerably greater ($P < 0.05$; Tables 2.13 and 2.14) in plant-based meat alternatives (BMB1, BMB2, IFB1, and IFB2) than in GP and GB. One serving of cooked plant-based meat alternative could supply between 3.0 - 24.4% of the adult (19 - 30) calcium Recommended Dietary Allowance (RDA) for males and females, and meet approximately 27.8 and 42.7% the adult sodium RDA for males and females, respectively (National Academies of Sciences et al., 2019). Iron and zinc content was substantially greater ($P < 0.05$; Tables 2.13 and2.14) in plant-based meat alternatives than animal-derived meat products. One serving of cooked plant-based meat alternative may supply between 28.8 – 83.7 % and 15.3 – 44.7 % of the adult iron RDA for males and females, respectively (National Academies of Sciences et al., 2019). Nonetheless, iron found in plant-sources is exclusively non-heme iron, which is substantially less bioavailable than heme iron found in red meats (Hurrell et al., 1992; Bohn et al., 2008).

While these minerals may be nutritionally important, the presence of phytates, fibrous plant materials, mineral interactions present in the digestive system and the incorporation of minerals in the food matrix can inhibit absorption (Philipp Schuchardt and Hahn, 2017; Reinhold et al., 1976; Wapnir, 1998; Brink, 1992). High calcium and magnesium levels have been known to contribute to iron and potassium inhibition, while high iron levels have been known to contribute to manganese inhibition (Slatopolsky et al., 1986; Kies, 1987; Lynch, 2000). Nonetheless, Troesch et al., (2009) demonstrated that calcium doses (200 mg), similar to those present in IFB1, did not significantly reduce iron absorption, but calcium has been shown to inhibit iron absorption in single-meal studies, where nutrients were not obtained from different food sources (Lynch, 2000). Additionally, high sodium and potassium levels may contribute to increased urinary mineral losses (Matkovic et al., 1995; Whiting et al., 1997).

2.4.3. Vitamins

Results from vitamin analysis for previous and current formulations of raw and cooked samples are reported in Tables 2.15 and 2.16, respectively. Fat soluble vitamin A, D2, and D3 were found to be below the detection limit in all raw and cooked products, with vitamin K1 being only found in BMB1 and BMB2 samples at levels slightly above the detection limit. Vitamin E content in raw and cooked plant-based meat alternatives was substantially greater ($P < 0.05$; Tables 2.15 and 2.16) than in raw and cooked GP and GB. One serving of cooked plant-based meat alternative may supply between 14.7 – 60.1 % of the RDA for adult (19 – 30 y) males and females (National Academies of Sciences et al., 2019). Typically, vitamin E supplementation in foods contributes a significant portion to the American diet, but absorption in human intestines is highly variable and impacted by the amount consumed, fat content of food, food matrix, and the presence of other fat-soluble nutrients (Radimer et al., 2004; Borel et al., 2013).

IFB1 and IFB2 were numerically comparable to or statistically greater ($P < 0.05$) than GP and GB in each B-vitamin assessed, except niacin (B3) and pantothenic acid (B5), for which GP were greater ($P < 0.05$; Tables 2.15 and 2.16). One serving of IFB1 or IFB2 would surpass the adult RDA for males and females of 1.2 and 1.1 mg/d, respectively. One serving of plant-based meat alternatives would supply between 41.2 – 43.8% of the adult niacin (B3) RDA, while one serving of cooked GP or GB would supply approximately 36.2 – 56.3% adult niacin RDA (National Academies of Sciences et al., 2019). One serving of cooked GP or GB would supply approximately 14.9 – 20.0 % adult RDA for pantothenic acid, while plant-based meat alternatives would supply between 4.8 – 9.1% of the adult pantothenic acid RDA (National Academies of Sciences et al., 2019). Finally, cooked BMB2 and IFB2 would supply approximately 11.6 - 32.1% of adult folate RDA, while cooked GB would only supply approximately 2.8% of adult pantothenic acid RDA (National Academies of Sciences et al., 2019).

While B-vitamins have demonstrated poor thermostability, photostability, and evaporation loss during storage and cooking, the fluorometric and microbial analysis performed in this study did not demonstrate B-vitamin loss from evaporation, high temperature, or light exposure (Farrer, 1955; Hilker and Somogyi, 1982; Woodcock et al., 1982; Saidi and Warthesen, 1983; Furuya et al., 1984; Baker, 1995). Niacin (B3) is usually chemically bound when found in plant materials, and thiamin (B1) and pyridoxine (B6) can undergo Maillard reactions which may affect bioavailability (Ghosh et al., 1963; Wall and Carpenter, 1988; Hoppner and Lampi, 1993). The crystalline nature of thiamin (B1) supplements and the presence of other minerals may impact bioavailability (Gadient, 1986). Considering the high concentrations of thiamin (B1) and pyridoxine (B6) in IFB1 and IFB2 products, there may be a chance of reduced availability through Maillard reaction products formed during cooking (Reynolds, 1988). High thiamin (B1)

concentrations may inhibit riboflavin (B2) and pyridoxine (B6) absorption, but riboflavin (B2), pyridoxine (B6), and B12 conversely may inhibit thiamin (B1) absorption (Rindi and Laforenza, 2000; Sriram et al., 2012). Copper, calcium, iron, and other minerals, in high concentrations of plant-based meat alternatives can act as antagonists of B-vitamin absorption, although their effect within plant-based meat alternatives may be variable.

2.4.4. Fatty acids

Results from fatty acid analysis for previous and current formulations of raw and cooked samples are reported in Tables 2.17 and 2.18, respectively. Raw and cooked plant products were below detection limit (< 0.01 mg/g) for cholesterol levels, while cooked animal products (GP and GB) contained approximately 0.86 mg/g. Palmitic (C16:0) and stearic (C18:0) acids were greater ($P < 0.05$; Tables 2.17 and 2.18) in GP and GB than in the plant-based meat alternatives. BMB2 and IFB2 contained greater ($P < 0.05$; Tables 2.17 and 2.18) oleic acid (C18:1) content than GP and GB. Substantial increases ($P < 0.05$; Tables 2.17 and 2.18) in oleic (C18:1) and linoleic acid (C18:2) content from IFB1 to IFB2 may be a contribution of sunflower oil which was used in the IFB2 formulation (Bhatnagar et al., 2009). BMB2 substituted cocoa butter for sunflower oil, which may explain the increase ($P < 0.05$; Tables 2.17 and 2.18) in oleic acid (C18:1) content from BMB1 and decrease ($P < 0.05$; Tables 2.17 and 2.18) in linoleic acid (C18:2) content from BMB1 (Lipp et al., 2001; Bhatnagar et al., 2009).

Fatty acids in mammals are synthesized from acetyl CoA obtained from the catabolism of carbohydrates (Cooper, 2000). Linoleic and a-linolenic acids, however, cannot be synthesized *de novo* and are essential for human growth and development. BMB1 proved to be a potentially excellent source of these essential fatty acids, compared to the other plant-based meat alternatives,

GP, and GB. GP was an excellent source of linoleic acid (C18:2) compared to GB, BMB2, and IFB2. GP was relatively high in arachidonic acid (C20:4), but trace amounts of the fatty acid were found in BMB1 and IFB1. Fatty acids are readily absorbed in the human body, but the presence of fibrous materials and various thickeners and binders incorporated into meat alternative formulations may inhibit fatty acid absorption (Fuse et al., 1989; Roediger, 1994). Physical characteristics of the food products, such as water activity, storage temperature, packaging characteristics, the presence of some minerals or proteins, and the cooking temperature, may contribute to the oxidation of unsaturated fatty acids (Shahidi and Zhong, 2010). While GP and GB have been criticized for being high in saturated fatty acid content, BMB2 and IFB2 resembled the saturated fatty acid profile of GB and GP.

2.4.5. Amino acids

Results from amino acid analysis for previous and current formulations of raw and cooked samples are reported in Tables 2.19 and 2.20, respectively. The major differences between cooked animal products (GP and GB) and plant-based meat alternatives were in histidine and methionine, for which GP and GB were greater ($P < 0.05$; Table 2.20) than plant-based meat alternatives. Cooked BMB2 was greater ($P < 0.05$; Table 2.20) than GP and GB in isoleucine and phenylalanine content, probably as pea and mung bean protein isolates are excellent sources of these essential amino acids (Pownall et al., 2010; Du et al., 2018). BMB2 substantially increased ($P < 0.05$; Tables 2.19 and 2.20) methionine content from BMB1, probably from the use of rice protein isolate which is high in methionine, although GP and GB were still greater ($P < 0.05$; Tables 2.19 and 2.20) than BMB2 in methionine content (Shih and Daigle, 2000). IFB2 was either numerically less than or statistically less than ($P < 0.05$; Tables 2.19 and 2.20) GP and GB in each essential amino acid. IFB2 substantially decreased ($P < 0.05$; Tables 2.19 and 2.20) isoleucine, leucine, methionine,

phenylalanine, and valine content from IFB1, probably as wheat protein used in IFB1 is generally a good source of leucine, lysine, phenylalanine, and valine compared to soy protein isolate (Gorissen and Witard, 2018). IFB2 was less than ($P < 0.05$; Tables 2.19 and 2.20) BMB2 in each essential amino acid assessed, except threonine and tryptophan.

Plant-based meat alternatives were either numerically comparable to or statistically greater than animal-derived meat products in most non-essential amino acid profiles. BMB2 was greater than ($P < 0.05$; Tables 2.19 and 2.20) GP and GB in arginine, aspartic acid, glutamic acid, serine, and tyrosine content. Substantial increases ($P < 0.05$; Table 2.19) in glutamic acid, glycine, serine, and proline were observed from BMB1 to BMB2 formulation, probably because rice and mung bean protein isolates are relatively high in the aforementioned amino acids (Shih and Daigle, 2000; Du et al., 2018). IFB2 was either numerically comparable to or statistically ($P < 0.05$) less than GP and GB in most non-essential amino acids assessed. Substantial decreases ($P < 0.05$; Tables 2.19 and 2.20) in nearly each non-essential amino acid were observed from IFB1 to IFB2, as wheat protein isolates are particularly high in glutamic acid, proline, and potato protein is high in many of the other non-essential amino acids (Gorissen et al., 2018; Gorissen and Witard, 2018).

The presence of fibrous material and antinutrient components present in some plant-products may inhibit protein digestibility of plant-based meat alternatives (Popova and Mihaylova, 2019). The protein digestibility-corrected amino acid score (PDCAAS) is a simple evaluation of protein quality, as it relates to the amount of the first liming amino acid in a test protein to the human metabolic requirement of that corresponding amino acid. The PDCAAS of soy, wheat, pea, mung bean, and bean proteins are 95, 96, 88, 76, and 78%, respectively (Mubarak, 2005; Anwar et al., 2007; Joint WHO/FAO/UNU Expert Consultation, 2007). However, Sarwar (1997) suggested that PDCAAS digestibility scores substantially over-estimate the digestibility of many

plant proteins, as they fail to account for antinutrient factors present in plant materials. Antinutrient components of these plant-based meat alternatives were not assessed in this study. However, plant-based protein isolates have been assessed to contain lower bioavailability compared to animal-derived meat proteins (Mariotti et al., 1999).

2.5. CONCLUSIONS

Plant-based meat alternatives were comparable to GP and GB in crude protein and crude fat content. Some plant-based meat alternatives were high in several minerals, which would compete for absorption, thus potentially reducing nutritional values. IFB2 was high in thiamin (B1), niacin (B3), pyridoxine (B6), biotin (B7), and folates (B9), which may potentially be a good source of some B vitamins. However, high thiamin (B1) concentrations may inhibit absorption of riboflavin (B2) and pyridoxine (B6). Additionally, the accessibility of these nutrients in the presence of antagonists, antinutrients, fibers, and food matrix would have to be further investigated.

The crude fat content and many of the saturated fats in the plant-based meat alternatives were comparable to GP. Plant-based meat alternatives were generally comparable to GP and GB in most essential amino acid profiles. However, BMB2 demonstrated the highest levels of most essential amino acids among the plant-based meat alternatives. GP and GB were still excellent sources of histidine and methionine, although BMB2 proved to be a good source of isoleucine, phenylalanine, and tryptophan. Overall, plant-based meat alternatives were generally comparable to animal-derived meat products in many of the nutrient profiles assessed. However, it is important to investigate further the digestibility, and the effects of fibrous materials, food matrix, and antinutrient compounds on the bioavailability of these nutrients.

3. CHAPTER 3: REVIEW OF LITERATURE

The integrity and safety of plant and animal-sourced food is necessary to support human health after consumption of nutrients. Plant- and animal-sourced foods, however, are often produced in natural environments which support microbiological growth and contamination. As a result, humans culturally and habitually administer various combinations of temperature, salts, organic acids, or other preservatives to plant- and animal-sourced foods as a means to decrease the prevalence of microbiological species on raw foods and/or to inhibit their growth during distribution or storage (Mintz and Du Bois, 2002). Plant-sourced foods however, are often consumed in a raw state in our modern diet, which is conducive for humans to potentially contract foodborne illnesses (Dao and Yen, 2006). In the present review we briefly outline the pathogenesis of an important foodborne microorganism associated with illness in plant- and animal-sourced foods.

3.1. FOODBORNE ILLNESS

More than 250 human illnesses have been determined to result from the consumption of contaminated food (Miliotis and Bier, 2003). Foodborne illness is responsible for 30% of deaths in children under 5 years of age, following malnutrition and malaria (WHO, 2015). The World Health Organization (WHO) estimates foodborne disease to be annually responsible for 600 million illnesses and 420,000 deaths globally; while epidemiological surveillance within the United States estimates foodborne bacterial pathogens to be responsible for 9.4 million illnesses and 1,351 deaths every year (Scallan et al., 2011; Havelaar et al., 2015). Among these, *L. monocytogenes* is annually responsible for approximately 1,600 illnesses and 260 deaths, becoming the third leading cause of death from foodborne illness within the United States (Scallan

et al., 2011). The economic impact of *L. monocytogenes* illness within the US approximates to $ 2.7 billion every year, while the total economic toll of foodborne illness from 14 pathogens in the United States accounts for $ 14.1 billion every year (Batz et al., 2011).

3.2. LISTERIA MONOCYTOGENES CHARACTERISTICS

L. monocytogenes is a gram-positive, non-spore forming bacterium, initially described by Murray et al. (1926), and is characterized as able to grow at low temperatures (> 1°C) , high salinity (10%), within a broad pH range (5.3 – 9.6), and in the presence of low concentrations of oxygen (Ryser and Marth, 2007). The genus *Listeria* contains 17 recognized species, of which only *L. monocytogenes* is known to cause disease in humans (Chen and Knabel, 2007).

3.2.1. Pathogenesis

The disease caused by *L. monocytogenes* infections is recognized as listeriosis, which is usually contracted by the ingestion of contaminated food or water and can be manifested in humans, other mammals, birds, crustaceans, and fish (Dhama et al., 2015). Listeriosis is characterized by severe sepsis, meningitis, encephalitis, gastroenteritis, spontaneous birth, or abortion, or intrauterine or cervical infections in humans (Janakiraman, 2008; Arslan et al., 2015). *L. monocytogenes* can survive in the digestive tract and can enter the bloodstream via the intestinal lumen, where it proceeds to invade other cells or organs in the body (Kathariou, 2002; Coelho et al., 2019). *L. monocytogenes* evades immune responses by secreting certain virulence factors, such as listeriolysin O (LLO) and other compounds, or through cytoplasmic propulsion, mediated by actin-assembly-inducing proteins (ActA) (Bielecki et al., 1990; Domann et al., 1992; Kocks et al., 1992). People who are immunosuppressed, such as extremely young children, the elderly, pregnant

women, or people with underlying medical conditions have a higher risk of contracting listeriosis after exposure,(Donnelly, 2001).

3.2.2. Attachment to surfaces and biofilm formation

Bacteria are capable of undergoing reversible and irreversible attachment to inert and organic material surfaces (Hinsa et al., 2003). Reversible attachment involves the interactions of chemical and physical forces between the bacterial cell and material surface, such as van der Waals forces, electrostatic forces and hydrophobic interactions (Loosdrecht et al., 1987). The cell surface of *L. monocytogenes* is negatively charged and extremely hydrophobic, which makes *L. monocytogenes* well-suited for reversible cell attachment on both hydrophobic and hydrophilic surfaces (Ukuku and Fett, 2002; Hsu et al., 2013; Huang and Nitin, 2017).

Irreversible attachment involves the interaction of stronger forces between the cell and substrate, such as covalent bonds, hydrogen bonds, hydrophobic interactions, and extracellular surface structures (Jones and Isaacson, 1982; Van Oss et al., 1988). Provided the right conditions, *L. monocytogenes* attaches to material surfaces by secreting a long polysaccharide fibril (Herald and Zottola, 1988; Mafu et al., 1990). Contact times of 20 min at low temperatures (4°C) was sufficient for bacterial attachment of *L. monocytogenes* on various surfaces (Mafu et al., 1990). Irreversible attachment requires stronger physical or chemical interactions to detach bacteria from a substrate surface.

L. monocytogenes is well recognized to form biofilms on material surfaces (Borucki et al., 2003; Pan et al., 2006). Biofilms are structured matrices containing microorganisms embedded in extracellular polymeric substances (Carpentier and Cerf, 1993). Biofilms are formed when a freely moving planktonic cell, adheres to a surface, forming a thin monolayer of cells that begin to colonize and synthesize an extracellular matrix (Flemming et al., 2007). Mature biofilms are able

to release cells from the biofilm structure, after a certain duration, to colonize new surfaces (Colagiorgi et al., 2017).

Bacterial biofilms are more resistant to bactericides than free-moving, planktonic cells (Pan et al., 2006). *L. monocytogenes* biofilms formed in food processing facilities have been observed to grow despite regular sanitization and at temperatures as low as 4°C (Pan et al., 2006; Bonaventura et al., 2008). Indeed, the physical adaptation of *L. monocytogenes* provided through surface attachment, biofilm formation, growth rates, and quiescence in the presence of high saline conditions, broad temperature ranges, and low acidity are thought to be responsible for the persistence of *L. monocytogenes* populations in food processing facilities despite regular sanitization (Holah et al., 2002).

3.2.3. L. monocytogenes *contamination in environment*

L. monocytogenes is not often detected in natural soil environments that are not farms but is a characteristic microorganism of spoiling plant tissues (MacGowan et al., 1994; Carlin et al., 1995). It has been hypothesized by Fenlon et al. (1996) that *L. monocytogenes* may survive at very low concentrations in the soil and root interface of the grass stem, where a thin layer of decaying plant tissue may support growth and act as an inoculum following harvest. Certainly, low quality silage, administered to animals as feed, has been well documented as a common reservoir of *L. monocytogenes* contamination in animals (Gray, 1960; Fensterbank et al., 1984; Fenlon, 1986). Fecal material from animals infected with *L. monocytogenes* may directly contaminate soil and water supplies on animal farms; as well as, contaminated fecal material administered to agricultural crops as a fertilizer may be a source of pre-harvest contamination of produce (Dowe et al., 1997; Lyautey et al., 2007). Likewise, *L. monocytogenes* from contaminated environmental sources can be easily carried into food processing facilities, where they are able to contaminate

food products post-harvest (Muhterem-Uyar et al., 2015). Among the many *L. monocytogenes* serotypes distributed throughout the environment, three serotypes (1/2a, 1/2b, 4b) account for the majority (>90%) of infections in humans (Tappero et al., 1995).

3.3. *L. MONOCYTOGENES* OUTBREAKS IN PRODUCE

The first largescale *L. monocytogenes* outbreak in humans associated with vegetables was linked to contaminated coleslaw which utilized cabbage obtained from a farm fertilized with untreated sheep manure (Schlech et al., 1983). Sheep manure was obtained from a farm which had a history of ovine listeriosis. Although conclusive evidence linking the *L. monocytogenes* outbreak to infected sheep on the farm was not obtained, this outbreak was one of the few suspected pre-harvest contaminations of *L. monocytogenes*.

The majority of *L. monocytogenes* outbreaks involving produce have been attributed to post-harvest contamination of produce. A largescale *L. monocytogenes* outbreak in 1999 at two primary schools and a university in Italy was attributed to contaminated canned corn product, for which there were 2,930 reported infections and no deaths (Aureli et al., 2000). The investigation of this outbreak revealed that utensils and drains within the processing facility tested positive for *L. monocytogenes*, which likely served as the source of contamination.

In 2010, an *L. monocytogenes* outbreak in a series of hospitals in Texas was attributed to a chopped celery ingredient used in chicken salad, infecting 10 elderly people, for which there were 5 deaths in the months that followed (Gaul et al., 2013). In 2011, a multistate listeriosis outbreak was attributed to romaine lettuce, for which there were 84 recorded infections and 15 deaths (Shrivastava, 2011; Zhu et al., 2017). In 2014, a listeriosis outbreak linked to caramel apples contaminated with *L. monocytogenes* at the processing facility resulted in 35 infections, 7 deaths, and 1 fetal loss, among the 11 infections reported in pregnant women (CDC, 2015). Again, in 2016

another multistate listeriosis outbreak was attributed to packaged salads contaminated with *L. monocytogenes* for which there were 19 infections and 1 death (CDC, 2018).

In 2011, a large multistate listeriosis outbreak was associated with whole cantaloupes that were contaminated with *L. monocytogenes* for which there were 147 cases, 143 hospitalizations, 33 deaths, and one fetal loss (McCollum et al., 2013; CDC, 2018). This particular cantaloupe outbreak is the largest listeriosis outbreak on record in the United States and one of the largest foodborne illness outbreaks in recent times (McCollum et al., 2013). A new piece of equipment previously used in potato harvesting may not have been adequately sanitized and the construction of the processing facility did not support adequate cleaning and sanitization, which likely contributed to the contamination of whole cantaloupes with *L. monocytogenes* (McCollum et al., 2013).

3.4. POST-HARVEST CHEMICAL DECONTAMINATION INTERVENTIONS

Considering the impact of post-harvest contamination of *L. monocytogenes* on produce, it is important to implement post-harvest chemical and physical interventions to decrease the risk of contracting listeriosis from raw produce. Chlorine, chlorine dioxide, trisodium phosphate, quaternary ammonium compounds, hydrogen peroxide, ozone, and various acids have been assessed for chemical decontamination of produce (WHO-FSU, 1998; Fatemi and Frank, 1999; Bastos et al., 2005; Dell'Erba et al., 2007; Ryser and Marth, 2007; Gerba, 2015; Scott et al., 2015). Halogenation, oxidation, emulsification, and cell lysis are common antimicrobial mechanisms used in sanitizers utilized for chemical interventions of produce (Mohan and Pohlman, 2016).

3.4.1. Halogen-based sanitizers

Halogens commonly used for disinfection are iodine, chlorine, and fluorine. These compounds interact with important cellular components on the cell surface and within the cytoplasm of the cell, forming halogen-containing compounds (Ryser and Marth, 2007). This oxidation destabilizes important biological components of the cell, resulting in cellular rupture or death.

Elemental chlorine or several hypochlorites are commonly utilized as disinfectants in wash, spray, and fume chemical interventions on raw produce (Eckert and Ogawa, 1988). Sodium hypochlorite (pH 6.5), specifically, is the most common sanitizer used in the produce industry (Shen et al., 2012). Chlorine is commonly utilized at concentrations of 50 - 200 ppm, with a contact time of between 1 - 2 min, and pH between 6.0 - 7.5 (to avoid metal corrosion) (WHO-FSU, 1998). Chlorine dioxide (ClO_2) is becoming more popular for use in produce safety due to relatively high efficacy in wide pH ranges and in the presence of organic matter compared to liquid chlorine and hypochlorites. Additionally, chlorine dioxide has 2.5 times the oxidation capacity of chlorine (Beuchat et al., 2004).

Some limitations to the use of hypochlorite and chlorine dioxide in produce, however, is that chlorine may react with ammonia residues on plants, forming organochlorines, which may be carcinogenic (Beuchat et al., 2004). Additionally, the concentrations of free available chlorine may be variable, depending on the pH and temperature of the solution, or the exposure of chlorine to organic materials, air, light, or metals, which may inactivate chlorine disinfectants (Brackett, 1987; Han et al., 2000). Chlorine dioxide has low chemical stability, breaking down when exposed to

light or high temperatures (> 30°C) and has the risk to be explosive during mixing (WHO-FSU, 1998).

3.4.2. Oxidation-based sanitizers

Oxidizing agents rely on the release of oxygen species to disrupt the osmotic function of lipoproteins in the cytoplasmic membrane of microorganisms (Luukkonen and Pehkonen, 2017; Kitis, 2004). This action causes rupture of the cell wall, resulting in cell death. Ozone has a long history of use in water treatment for the elimination of pathogens in produce (WHO-FSU, 1998; WHO, 2008). Ozone, however, has relatively low chemical stability and may cause corrosion of facility or equipment surfaces (Khadre et al., 2001). Peroxyacetic acid (PAA), however, is an oxidizing agent widely used in the meat production industry and is gaining popularity in produce chemical intervention systems.

PAA is synthesized by a mixture of acetic acid and hydrogen peroxide in equilibrium, whereby a strong oxidation potential is generated (Dell'Erba et al., 2007; Carrasco and Urrestarazu, 2010; Hua et al., 2011). PAA disrupts the cell permeability of bacteria through oxidation and alters protein synthesis (Oyarzabal, 2005). Peroxyacetic acid has been approved as a food-grade sanitizer in the United States since 1986 and is approved for use in produce at concentrations not exceeding 80 ppm (CFR, 2020).

PAA is not affected by changes in temperature and has little reactivity with organic matter, unlike chlorine (Banach et al., 2015). PAA treatment demonstrates similar efficacy against pathogens within a wide pH range (2.5 – 6.3), and varying water hardness (20 – 460 ppm) (Artés et al., 2007; Shen et al., 2019). PAA does not form toxic byproducts with organic matter and decomposes to acetic acid and oxygen (Monarca et al., 2002). PAA is commonly used as an

antimicrobial intervention during meat and poultry processing but has also been used for decontamination of fresh produce such as iceberg lettuce, mung bean sprouts, cantaloupe, etc (Shrivastava, 2011; Shen et al., 2019).

3.4.3. Sulfuric acid-based surfactant sanitizers

Organic and inorganic acids are commonly used to decontaminate produce and other foods (Maris, 1995; Gilbert and Moore, 2005; Huang and Nitin, 2017). Sulfuric acid is a strong inorganic acid, which dissociates protons into the cell cytoplasm, which causes the cell to efflux protons to stabilize intracellular pH, consuming energy sources, resulting in cell death (Maris, 1995; Coelho et al., 2019). Conversely, surfactants are able to interact with the hydrophobic regions of the cytoplasmic membrane of bacteria, whereby they are able to penetrate the cell wall, disrupting membrane organization, leaking intracellular material, and resulting in cell wall lysis caused by autolytic enzymes (Moore et al., 2000; McDonnell, 2007).

Non-ionic surfactants, such as with sulfuric acid-based surfactants, do not carry a charge on the hydrophilic head of the molecule, which allows these surfactants to easily emulsify fats and oils (Zhang and Farber, 1996). Some newly developed non-ionic surfactants have the capability of disrupting outer cell membranes through hydrophobic and acid interactions. Such is the case with ProduceShield Plus (PSP), a non-ionic surfactant that contains sulfuric acid as the active ingredient and an amphiphilic component (D-glucopyranose oligomer blend) as a surfactant component; which has the potential of executing a synergistic effect against foodborne pathogens (Kang et al., 2020).

Considering the intrinsic ability of *L. monocytogenes* to survive in hostile natural, man-made, and biological environments, as well as cause severe illness in humans and animals alike, it

is important to maximize the efficacy of chemical post-harvest interventions. Though chlorine has been widely used as a sanitizer in the produce industry, PAA and novel non-ionic surfactant sanitizers may have potential for maximizing the antimicrobial activity against foodborne pathogens associated with raw produce. As a result, the objective of the present work was to evaluate efficacy of chlorine as well different concentrations of peroxyacetic acid (PAA) and ProduceShield Plus (PSP), against *L. monocytogenes* populations on inoculated whole cantaloupe melons

4. CHAPTER 4: USE OF NOVEL SANITIZER BLENDS TO REDUCE *LISTERIA MONCYTOGENES* CONTAMINATION ON WHOLE CANTALOUPES

4.1. INTRODUCTION

The World Health Organization (WHO) identified *Listeria monocytogenes* to be one of the five main causes of foodborne illness (Anon, 2000). Listeriosis is the third leading cause of death from foodborne illnesses in the United States, owing to the high fatality rates of the disease, exceeding that of *Salmonella* spp. and *Clostridium botulinum* (Scallan et al., 2011). This pathogen is only responsible for 1% of food-borne illnesses but up to 28% of foodborne related deaths in the United States (Mead et al., 1999; Scallan et al., 2011)

In recent years, largescale *L. monocytogenes* outbreaks have been attributed to contaminated produce, and have had devastating consequences (Zhu et al., 2017). One of the largest listeriosis outbreaks, with the highest death rate, was attributed to whole cantaloupes that were contaminated with *L. monocytogenes* during post-harvest processing, suggesting current food safety measures to reduce pathogen contamination on fresh produce must be continually addressed (McCollum et al., 2013; CDC, 2018).

Various chemical solutions utilizing chlorine as the active ingredient are the most popular sanitizers currently used in the produce industry, although these products have the ability to form carcinogenic compounds when exposed to organic material and have variable chemical stability in different environments (Han et al., 2000; Wu and Kim, 2007). Peroxyacetic acid (PAA) has demonstrated good efficacy in the fresh meat and produce industries for eliminating the presence of pathogens on food surfaces (Shen et al., 2019). However, the hydrophobic nature of fresh

produce, as well as the ability for *L. monocytogenes* to attach and form biofilms may implicate the use of surfactants, such as novel ProduceShield Plus (PSP) in produce sanitization (Kang et al., 2020). Therefore, the objective of the present study was to assess the efficacy of chlorine, PAA, and PAA+PSP surfactant blends on reducing *L. monocytogenes* populations on inoculated whole cantaloupes.

4.2. MATERIALS AND METHODS

4.2.1. L. monocytogenes *strains and inoculum preparation*

The inoculum was comprised of a mixture of five *L. monocytogenes* strains, including four strains associated with the 2011 Jensen Farms cantaloupe outbreak (ATCC-BAA 2657, ATCC-BAA 2658, ATCC-BAA 2659, ATCC-BAA 2660; Lomonaco et al., 2013; CDC, 2018) and one human clinical isolate (Scott A) . Working cultures of the strains were maintained at 4°C on plates of PALCAM agar (Difco, Becton, Dickinson and Company [BD], Sparks, MD). The strains were separately activated three days before each of the two trials of the study by transferring a single colony from the PALCAM agar plate into 10 mL of tryptic soy broth (Difco, BD) supplemented with 0.6% yeast extract (Acumedia-Neogen, Lansing, MI) (TSBYE). The inoculated broths were incubated at 35°C for 22 h and subsequently subcultured by transferring a 0.5 mL aliquot of the initial TSBYE culture into 50 mL of TSBYE. After incubation (35°C, 22 h), equal rations of broth cultures of each strain was combined and cells were harvested by centrifugation (6000 × *g*, 15 min, 4°C; Sorvall Legend X1R centrifuge, Thermo Scientific, Waltham, MA). Harvested cells were washed twice with 10 mL aliquots of phosphate-buffered saline (pH 7.4; PBS; Sigma-Aldrich, St. Louis, MO) and after the second wash, were resuspended in 250 mL of PBS. Two-mL volumes of this 5-strain *L. monocytogenes* suspension were distributed into 50 mL conical centrifuge tubes

and used to inoculate the cantaloupes. The approximate concentration of the inoculum suspension was 9 log CFU/mL.

4.2.2. Cantaloupe inoculation

Whole cantaloupes (*Cucumis melo* L. var. *reticulatus*; 9 count per case) were procured from a local distribution center (Del Monte Fresh Produce, Aurora, CO) one day prior to each trial. The average weight and circumference of the cantaloupes was 1.78 ± 0.17 kg and 15.03 ± 0.52 cm, respectively. Preliminary work conducted before the start of the study indicated the presence of high levels of non-*Listeria* bacterial populations on the cantaloupe surface that were able to grow on PALCAM agar (the *Listeria* spp. recovery medium used in the study). In order to lower the levels of these microflora so as not to interfere with the recovery of inoculated *L. monocytogenes* populations from treated and untreated product, cantaloupes were subjected to an ethanol spray treatment followed by rinsing with water. More specifically, after the cantaloupes were weighed, measured, and inspected for abrasions or lacerations, they were placed on sterile wire racks inside of sterile open plastic laboratory totes. The upper portion of each cantaloupe was then liberally sprayed with 70% ethanol (approximately 20 mL per cantaloupe) and allowed to sit for 5 min. Cantaloupes were then rotated so that the portion of the fruit that was previously on the bottom was now on top. The fruit was again sprayed with 70% ethanol and after 5 min, the cantaloupes were thoroughly rinsed (20 s) under running room-temperature tap water to remove ethanol residues and any remaining dirt or debris. The washed cantaloupes were placed on absorbent pads and were left to air dry at room temperature (16 to 28 h) prior to inoculation and treatment the next day.

Two trials (repetitions) of the study were performed on two separate days with different lots of cantaloupe and separately prepared inoculum. For each trial, three cantaloupes were

randomly assigned to each treatment to be evaluated (Table 4.1). Cantaloupes were inoculated, under a biosafety cabinet, to a target *L. monocytogenes* level of 7 to 8 log CFU/cantaloupe. For inoculation, individual cantaloupes were placed on trays, and were held in place by standing the fruit on one of its ends on an autoclave-sterilized foil ring. A separate hog bristle paintbrush (0.75-in; U.S. Art Supply, TCP Global Corporation, San Diego, CA) was used to inoculate each cantaloupe surface with a 2-mL volume of the inoculum suspension. The entire surface, except for 2 cm around the stem scar end and anterior end of the fruit was inoculated. Inoculated cantaloupes were left under the biosafety cabinet for 1 h, to allow for bacterial cell attachment and for the cantaloupe surface to air-dry, before sanitizer treatment application or microbial analysis of untreated cantaloupes.

4.2.3. Sanitizer treatment of whole cantaloupes

Inoculated cantaloupes either received a water or sanitizer treatment for 0.5, 1 or 5 min, or were left untreated and analyzed to determine the *L. monocytogenes* inoculation level. As shown in Table 4.1, the sanitizer treatments evaluated included 40 ppm free chlorine (Clorox, Oakland, CA) that was adjusted to pH 6.5 with citric acid (Fisher Scientific, Fair Lawn, NJ), 40, 80 and 250 ppm PAA (OxypHresh 15, CMS Technology, Bridgewater, NJ), ProduceShield Plus (PSP; CMS Technology) at a pH of 1.8, and three blends of PAA and PSP (PAA+PSP). In all cases, tap water was used to prepare the sanitizer solutions from their respective concentrates. The concentrations of free chlorine and PAA were verified with a Kemio Disinfection unit (Palintest, Gateshead, England, United Kingdom), and the pH of all solutions was measured with an Orion (Thermo Scientific, Beverly, MA) pH meter and pH electrode.

Treatments were applied by placing individual cantaloupes into Whirl-Pak bags (184-oz; Nasco, Fort Atkinson, WI) and pouring 2 L of the test solution into the bag. A separate Whirl-Pak

bag and fresh, unused solution was used to treat each cantaloupe. Cantaloupes were completely immersed in the test solution and gently agitated for 0.5, 1 or 5 min. Following treatment, cantaloupes were transferred to sterile plastic colanders to drain for 5 min before microbiological analysis for surviving pathogen populations. Additionally, for selected treatments, the treatment solution in which each of the cantaloupes was immersed was also analyzed for any surviving *L. monocytogenes* populations. For this analysis, a 1-mL volume of the treatment solution was taken immediately after the cantaloupe was removed from the solution and was transferred into a test tube containing 9 mL of double-strength Dey/Engley (D/E) neutralizing broth (Difco, BD).

4.2.4. Microbiological analysis

Untreated (control) and treated whole cantaloupes were placed in individual Whirl-Pak bags (184-oz) containing 500 mL of D/E neutralizing broth (single-strength) and were vigorously shaken by hand 60 times to recover cells. A 20-mL aliquot of the rinsate was transferred to a 50-mL conical centrifuge tube for microbial analysis. Cantaloupe rinsates were serially diluted tenfold in maximum recovery diluent (Acumedia-Neogen) and appropriate dilutions were plated, in duplicate, onto PALCAM agar (Difco, BD) for enumeration of *L. monocytogenes* populations. Similarly, the treatment solution aliquot in 9 mL of double-strength D/E neutralizing broth was diluted and plated on PALCAM agar. Plates were incubated at 35°C and colonies counted after 48 ± 2 h of incubation. Detection limits of the microbial analysis of the cantaloupes and treatment solutions were 2.7 log CFU/cantaloupe and 1 log CFU/mL, respectively.

In addition to the above analyses, on each of the trial days, three washed (i.e., subjected to the 70% ethanol spray treatment followed by rinsing with water and air drying), uninoculated and untreated cantaloupes were analyzed for natural microbial population levels (on tryptic soy agar

[Acumedia-Neogen] supplemented with 0.6% yeast extract) and for any naturally present *Listeria* spp. populations (on PALCAM agar).

4.2.5. Statistical analysis

The study was a randomized complete block design with an augmented or enhanced factorial arrangement of treatment (nine sanitizer treatments), treatment exposure time (0.5, 1, and 5 min), and one untreated control treatment. Two trials (repetitions) of the study were performed on two separate days. For each trial, three cantaloupes were analyzed for a total of $n = 6$ experimental units per treatment and exposure time. *L. monocytogenes* counts of cantaloupes were transformed to values expressed as log CFU/cantaloupe. For the purpose of statistical analysis, cantaloupes with no detectable *L. monocytogenes* survivors were assigned a value equal to the microbial analysis detection limit (i.e., 2.7 log CFU/cantaloupe). Pathogen reductions for each individual treated cantaloupe were determined by subtracting the log CFU/cantaloupe value of each treated cantaloupe from the mean initial inoculated *L. monocytogenes* level (log CFU/cantaloupe) determined from the six untreated cantaloupes. Mean reductions were determined by averaging the reductions of the six cantaloupes within each treatment and treatment exposure time combination.

A linear model was fit to the data, containing the mean log CFU/cantaloupe reductions as the response variable. The linear model contained a blocking predictor variable for trial day and an interaction predictor term for treatment and exposure time. An ANOVA type 3 test was used to determine the effect of interaction and blocking variables. Tukey adjusted pairwise comparisons were used to determine statistical difference between factorial arrangements. Data were analyzed

with the CRAN-R package (Lenth, 2020) in R (version 3.5.1). All differences are reported using a significance level of $\alpha = 0.05$.

4.1. RESULTS AND DISCUSSION

4.1.1. L. monocytogenes *populations of untreated cantaloupes*

Surviving pathogen populations recovered from inoculated cantaloupes subjected to the various immersion decontamination treatments are summarized in Table 4.1, and corresponding pathogen reductions are presented in Table 4.2. The *L. monocytogenes* inoculation level on whole cantaloupes following the inoculation procedure, as determined by microbial analysis of inoculated untreated samples, was 7.9 ± 0.4 log CFU/cantaloupe (Table 4.1).

Naturally-occurring *Listeria* spp. populations were not detected (2.7 log CFU/cantaloupe detection limit) on any of the washed, uninoculated cantaloupes analyzed. As such, *L. monocytogenes* counts recovered with the PALCAM agar from inoculated untreated (control) and treated cantaloupes were those of the *L. monocytogenes* inoculum used in this study. Total aerobic microbial population counts of 5.4 ± 0.3 log CFU/cantaloupe were recovered from the washed, uninoculated cantaloupes. Aerobic plate counts obtained from sampling whole cantaloupes during different stages of transportation, processing, and packaging on different farms ranged between 6.76 and 7.15 log CFU/mL (Deann Akins et al., 2008).

4.1.2. L. monocytogenes *populations of water-treated whole cantaloupes*

Water immersion, at room temperature, has been shown to remove cells from vegetable surfaces and was included in this study to determine the rinsing effect of the 0.5, 1, and 5 min immersion treatments in a liquid solution (Han et al., 2000). Irrespective of exposure time, the

water treatment reduced initial *L. monocytogenes* levels by 0.3 to 0.5 log CFU/cantaloupe (Table 4.2). The relatively low pathogen reductions from water immersion across exposure times (0.5, 1, and 5 min) affirms good *L. monocytogenes* cell attachment to the cantaloupe surface and accounts for reduction effects as a consequence of agitation, solubility, reversible cell attachment, and other physical and chemical factors (Loosdrecht et al., 1987; Walter et al., 2009). This confirms previous reports of the effects of water washes on *L. monocytogenes* counts. For example, Rodgers et al. (2004) Rodgers et al. (2004) reported that cantaloupe inoculated with *L. monocytogenes* and immersed in water for 5 min resulted in 1 log CFU/g reductions of *pathogen* populations. In another study, cantaloupe inoculated with *L. monocytogenes* (8.8 log CFU/cantaloupe) and immersed in deionized water for 5 min resulted in 0.2 log CFU/cantaloupe reductions of inoculated populations (Singh et al., 2018).

4.1.3. Effect of chlorine immersion treatment

Treatment of cantaloupes with 40 ppm chlorine for 0.5, 1 or 5 min reduced L. monocytogenes populations by 2.1, 2.7, and 3.3 log CFU/cantaloupe, respectively (Table 4.2). The 1.2 log CFU/cantaloupe difference between the 0.5 min and 5 min exposure times was significant ($P < 0.05$; Table 4.2). Svoboda et al. (2016) reported that whole cantaloupes inoculated with L. monocytogenes (9.1 log CFU/mL) and immersed in 65 ppm chlorine solution for 5 min at 4°C resulted in 1.9 log CFU/mL reductions in L. monocytogenes populations. Similarly, Singh et al. (2018) reported 1.9 log CFU/cantaloupe reductions of L. monocytogenes populations following immersion of inoculated whole cantaloupes (8.8 log CFU/cantaloupe) in 15 L of 100 ppm chlorine for 5 min. effect of PSP immersion treatments

In this study, immersion of cantaloupes in PSP resulted in substantially lower ($P < 0.05$; Table 4.2) reductions of *L. monocytogenes* populations than were observed with the chlorine, PAA, or PAA+PSP treatments. PSP is an antimicrobial consisting of sulfuric acid combined with a surfactant. Pathogen reductions obtained with PSP ranged between 0.9 and 1.8 log CFU/cantaloupe across the different exposure times. Antimicrobial effects of surfactants when used individually, are variable and highly concentration dependent, among other factors (Gerba, 2015). Tomatoes inoculated with *Salmonella*, mechanically diced, and treated for 60 s in a flume tank resulted in less than 1.0 log CFU/g reductions of *Salmonella* populations for both the water (control) and PSP (pH 1.8) (Kang et al., 2020). A sulfuric acid and sodium sulfate blend (SSS) is an antimicrobial approved for use on meat and poultry products and has similar sulfuric acid components to PSP but does not contain a surfactant component. Chicken wings inoculated with *Salmonella* and immersed in a SSS (pH 1.1) solution for 10 and 20 s resulted in approximately 0.8 to 1.2 log CFU/mL reductions in *Salmonella* populations (Scott et al., 2015). Additionally, *Salmonella* reductions of 1.0 to 1.5 log CFU/cm^2 were observed in beef cheek meat inoculated with *Salmonella* (4.1 log CFU/cm^2) immersed in 0.05% SSS for 1, 2.5, and 5 min (Schmidt et al., 2014).

4.1.4. Effect of PAA immersion treatment

L. monocytogenes reductions for PAA-treated cantaloupes, regardless of concentration, ranged from 3.0 to 4.1 log CFU/cantaloupe (0.5 and 1 min exposure) and >3.6 to >4.6 log CFU/cantaloupe (5 min exposure) (Table 4.2). Singh et al. (2018) reported that whole cantaloupes inoculated with *L. monocytogenes* (8.8 log CFU/cantaloupe) and immersed in 15 L of 85 ppm PAA for 5 min resulted in 3.0 log CFU/cantaloupe reductions of *L. monocytogenes* cells. Fan et al. (2009) reported that whole cantaloupes inoculated with *Salmonella* Poona (3.5 log CFU/cm2)

and immersed in 10 L of 80 ppm PAA only resulted in a 0.5 log CFU/cm^2 reduction in *Salmonella* cells, which did not differ from the control.

In most instances, reductions from PAA immersion were greater ($P < 0.05$; Table 4.2) than those obtained for 40 ppm chlorine treatment. A noticeable exception to this finding was 40 ppm PAA, which had similar ($P \geq 0.05$) efficacy to that of 40 ppm chlorine, irrespective of exposure time. Greater reductions of *L. monocytogenes* populations on inoculated whole cantaloupe immersed in 85 ppm PAA than in 100 ppm chlorine were reported by Singh et al. (2018). Furthermore, PAA treatment has resulted in greater reductions of *L. monocytogenes* populations on inoculated produce, than chlorine is multiple studies (Fatemi and Frank, 1999; Rodgers et al., 2004; Walter et al., 2009; Belessi et al., 2011; Shen et al., 2019). The hydrophobic nature of waxy plant cuticles and the *Listeria* cell membrane make it difficult for extremely polar antimicrobials, like chlorine, to exhibit bactericidal effects (Ukuku and Fett, 2002; Gil et al., 2009; Hsu et al., 2013; Yaron and Römling, 2014; Huang and Nitin, 2017). Nonetheless, PAA has demonstrated hydrophobic properties, which may facilitate the penetration of PAA into the hydrophobic cuticle of plant epidermis (Fatemi and Frank, 1999).

4.1.5. Effect of PAA + PSP immersion treatments

Across all treatment exposure times, PAA+PSP blends, at all tested concentrations, effectively ($P < 0.05$; Table 4.2) reduced pathogen levels by 3.2 to > 4.9 log CFU/cantaloupe. Increasing sanitizer treatment duration did not ($P \geq 0.05$) enhance the antimicrobial efficacy of each of the PAA+PSP blends. The decontamination efficacy of each PAA concentration level, within each treatment exposure time, was, in general, similar ($P \geq 0.05$) to that of its corresponding PAA+PSP blend; however, the blended PAA (80 ppm and 250 ppm) + PSP solutions were able to

achieve numerically greater ($P \geq 0.05$) reductions of *L. monocytogenes* populations on inoculated cantaloupes for each exposure time compared to when PAA and PSP were used individually. This may be an effect of the different modes of action from each antimicrobial, such as oxidative stress supplied by PAA, and the effect of low pH from sulfuric acid and hydrophobic interactions from the surfactant agent in PSP (Huang and Nitin, 2017; Singh et al., 2018).

Kang et al. (2020) observed numerically greater ($P > 0.05$) reductions of *Salmonella* populations on inoculated tomatoes for each exposure time (20, 40, and 60 s) when PAA and PSP were used in combination than when PAA and PSP were used individually. Similarly, PAA (200 ppm) used in combination with a non-ionic surfactant (ethoxylated glycerol; 5000 ppm) was able to significantly ($P < 0.05$) reduce the presence of *Escherichia coli* O157:H7 on inoculated beef trimmings, than when PAA was used alone (Mohan and Pohlman, 2016). The use of a surfactant (Tween 80) when used in addition to PAA (60 ppm) was able to cause numerically greater reductions of mesophilic aerobes on inoculated cantaloupes, than when PAA was used without a surfactant; although this relationship was also not significant ($P > 0.05$) (Bastos et al., 2005).

It is logical that the use of multiple sanitizers, with different modes of action, could result in numerically greater reductions in pathogenic bacteria (Mohan and Pohlman, 2016; Shen et al., 2019). Huang and Nitin (2017) previously demonstrated that surfactants (Tween-20, sodium dodecyl sulfate, and lauric arginate) were able to decrease the surface tension between plant cuticles and the sanitizer solution, which allows sanitizers to more effectively interact with pathogens attached to vegetable surfaces. Additionally, PAA has demonstrated hydrophobic properties, which may provide a bactericidal advantage compared to chlorine, when interacting with highly hydrophobic pathogens, such as *L. monocytogenes*, which are attached to the hydrophobic cuticle of cantaloupe (Ukuku and Fett, 2002; Ukuku, 2006). The active component

of PAA is capable of disrupting proteins and permeability of cell membranes, which can result in cell death (Maris, 1995; Shen et al., 2019). The sulfuric acid component of PSP, is actively lowering solution and cytoplasmic pH, which can enhance the disruption of cell membranes, protein denaturation, and cell lysis (Hua et al., 2011; Scott et al., 2015). Considering the hydrophobic nature of plant cuticles, the ability for *L. monocytogenes* to form biofilms and evade constant sanitization in food processing facilities, it is possible that different modes of action from PAA+PSP sanitizer blends may be important for decreasing *L. monocytogenes* viability. (Fatemi and Frank, 1999; Gilbert and Moore, 2005; Huang and Nitin, 2017; Kang et al., 2020).

Certainly, there are limitations associated with chlorine sanitization of produce, such as sensitivity to solution pH, water hardness, temperature, and presence of organic material (Lawrence and Block, 1968; Brackett, 1987). PAA has demonstrated good bactericidal effects against *L. monocytogenes* in the present study as well as other literature (Fatemi and Frank, 1999; Rodgers et al., 2004; Walter et al., 2009; Belessi et al., 2011; Shen et al., 2019). The use of sulfuric acid, as well as surfactants has also been demonstrated to numerically increase the reductions of pathogenic populations on produce exposed to immersion and spray treatments (Bastos et al., 2005; Mohan and Pohlman, 2016; Kang et al., 2020). Considering the antimicrobial effect of PAA and PSP observed in this study and the characteristics associated with *L. monocytogenes* and cantaloupe melons discussed in the paper, more research should be conducted to elucidate whether a synergistic relationship exists between PAA and PSP.

4.1.1. Surviving L. monocytogenes *cells in treatment solution*

The presence of surviving *L. monocytogenes* cells within some treatment solutions (water, 40 ppm chlorine, PSP, 40 ppm PAA, and 40 ppm PAA+PSP) was determined immediately

following immersion treatment of inoculated cantaloupes, and these results are presented in Table 4.3. *L. monocytogenes* populations were recovered from the water and PSP treatment solutions, but not from the chlorine, PAA, and PAA+PSP solutions. Pathogen cells in treatments solutions were between 2.6 to 3.8 log CFU/mL for the water treatment solution and <1.0 to <1.5 log CFU/mL for the PSP treatment solution. It is interesting to note the lower *L. monocytogenes* populations in the PSP treatment solution than in the water solution, indicating the bactericidal effects of the PSP treatment. Singh et al. (2018) reported surviving *L. monocytogenes* populations in treatment solutions of water and chlorine (100 ppm) but not PAA (45, 85, 100 ppm) after immersion treatment (5 min) of inoculated cantaloupe.

4.2. CONCLUSIONS

All evaluated concentrations of PAA and the PAA+PSP blends effectively ($P < 0.05$) reduced *L. monocytogenes* contamination on the surface of whole cantaloupes. Immersion of inoculated cantaloupe in PAA solutions, at different concentrations, resulted in numerically greater reductions of *L. monocytogenes* populations than were obtained by immersion in 40 ppm chlorine solutions. In general, pathogen reductions were similar ($P > 0.05$) between each PAA concentration level and its corresponding PAA+PSP blend. Reductions ranging from 4.1 to > 4.9 log CFU/cantaloupe were achieved with the 5 min, 80 ppm PAA treatment, and all tested exposure times (0.5, 1 or 5 min) of 250 ppm PAA, 80 ppm PAA+PSP, and 250 ppm PAA+PSP. Blended PAA and PSP solutions resulted in numerically greater *L. monocytogenes* reductions on inoculated cantaloupe than was obtained when PAA and PSP were utilized individually. The results of this study offer alternatives to using chlorine for reducing *L. monocytogenes* contamination on the surface of cantaloupes and may elucidate a potential application for PSP and PAA blends in produce decontamination systems.

TABLES

Table 2.1: As fed proximate analysis (percent ± standard deviation) of raw Beyond Meats Burger-New (BMB2), Impossible Foods Burger-New (IFB2), 80/20 ground pork (GP), and 80/20 ground beef (GB) (n = 6)

Component	**BMB2**	**IFB2**	**GP**	**GB***
Dry Matter	42.93 ± 0.79^{a}	42.28 ± 0.4^{a}	37.09 ± 1.29^{b}	38.0
Moisture	57.07 ± 0.79^{b}	57.72 ± 0.4^{b}	62.91 ± 1.29^{a}	62.0
Ash	1.72 ± 0.2^{b}	2.51 ± 0.22^{a}	1.76 ± 0.36^{b}	0.84
Crude Fat	13.08 ± 2.2^{a}	11.67 ± 1.85^{b}	10.15 ± 4.68^{a}	20.0
Crude Protein	18.59 ± 0.87^{b}	17.18 ± 0.82^{b}	22.62 ± 3.24^{a}	17.0
Acid Detergent Fiber	9.92 ± 1.95^{a}	9.39 ± 1.81^{a}	NA	NA
Neutral Detergent Fiber	18.75 ± 4.09^{a}	18.63 ± 3.27^{a}	NA	NA

*Data collected from USDA database(USDA, 2020)

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

NA: Not Applicable

Table 2.2: As fed proximate analysis (percent ± standard deviation) of cooked Beyond Meats Burger-New (BMB2), Impossible Foods Burger-New (IFB2), 80/20 ground pork (GP), and 80/20 ground beef (GB) (n = 6)

Component	**BMB2**	**IFB2**	**GP**	**GB***
Dry Matter	50.3 ± 0.93^{a}	47.67 ± 0.99^{b}	44.77 ± 2.18^{c}	44.0
Moisture	49.7 ± 0.93^{c}	52.33 ± 0.99^{b}	55.23 ± 2.18^{a}	56.0
Ash	2.08 ± 0.16^{b}	3 ± 0.16^{a}	1.27 ± 0.41^{c}	1.0
Crude Fat	11.6 ± 3.91^{a}	11.22 ± 1.68^{a}	11.09 ± 5.87^{a}	18.0
Crude Protein	23.77 ± 1.54^{a}	20.22 ± 0.48^{b}	21.48 ± 3.04^{ab}	26.0
Acid Detergent Fiber	10.84 ± 0.8^{a}	12.92 ± 2.34^{a}	NA	NA
Neutral Detergent Fiber	20.67 ± 4.19^{a}	23.85 ± 4.86^{a}	NA	NA

*Data collected from USDA database(USDA, 2020)

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

NA: Not Applicable

Table 2.3: Mineral composition (ppm ± standard deviation) of raw Beyond Meats Burger-New (BMB2), Impossible Foods Burger-New (IFB2), 80/20 ground pork (GP), and 80/20 ground beef (GB) (n = 6)

Component	**BMB2**	**IFB2**	**GP**	**GB***
Calcium	819.83 ± 73.96^{b}	1860 ± 46.48^{a}	105.8 ± 61.89^{c}	180
Magnesium	350 ± 30.09^{b}	714 ± 19.33^{a}	178.5 ± 16.32^{c}	170
Phosphorus	2423.33 ± 160.71^{a}	1840 ± 54.77^{b}	1596.67 ± 126.6^{c}	1580
Potassium	2431.67 ± 247.9^{c}	5760 ± 197.89^{a}	3176.67 ± 702.67^{b}	2700
Sodium	3230 ± 275.32^{a}	3608.33 ± 203.9^{a}	995.5 ± 1281.44^{b}	660
Copper	2.1 ± 0.25^{b}	2.67 ± 0.45^{a}	0.71 ± 0.09^{c}	0.61
Iron	36.6 ± 2.59^{a}	36.33 ± 3.55^{a}	7.91 ± 2.32^{b}	19.4
Manganese	6.94 ± 0.77^{b}	10.32 ± 0.73^{a}	0.18 ± 0^{c}	0.1
Selenium	NT	NT	NT	0.15
Zinc	23.33 ± 2.68^{b}	48.33 ± 2.53^{a}	20.77 ± 5.03^{b}	41.8

*Data collected from USDA database(USDA, 2020)

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

NT: Not Tested

Table 2.4: Mineral composition (ppm ± standard deviation) of cooked Beyond Meats Burger-New (BMB2), Impossible Foods Burger-New (IFB2), 80/20 ground pork (GP), and 80/20 ground beef (GB) (n = 6)

Component	**BMB2**	**IFB2**	**GP**	**GB***
Calcium	1068.17 ± 89.29^{b}	2165 ± 69.5^{a}	139.13 ± 87.32^{c}	240
Magnesium	446.67 ± 31.7^{b}	827 ± 23.07^{a}	239.17 ± 17.67^{c}	200
Phosphorus	3120 ± 182.54^{a}	2143.33 ± 60.22^{b}	2146.67 ± 121.76^{b}	1940
Potassium	3056.67 ± 287.31^{c}	6753.33 ± 326.17^{a}	4220 ± 834.94^{b}	3040
Sodium	4186.67 ± 261.97^{a}	4240 ± 219.27^{a}	1277.83 ± 1586.4^{b}	750
Copper	2.8 ± 0.37^{a}	3.07 ± 0.43^{a}	1.4 ± 0.71^{b}	0.8
Iron	47.42 ± 2.98^{a}	42.63 ± 3.23^{b}	10.83 ± 2.58^{c}	24.8
Manganese	8.97 ± 0.83^{b}	11.97 ± 0.78^{a}	0.18 ± 0^{c}	0.11
Selenium	NT	NT	NT	0.22
Zinc	30.27 ± 2.75^{b}	56.77 ± 3.37^{a}	28.15 ± 5.53^{b}	62.5

*Data collected from USDA database(USDA, 2020)

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

NT: Not Tested

Table 2.5: Vitamin composition (mcg/g ± standard deviation) of raw Beyond Meats Burger-New (BMB2), Impossible Foods Burger-New (IFB2), 80/20 ground pork (GP), and 80/20 ground beef (GB) (n = 6)

Component	**BMB2**	**IFB2**	**GP**	**GB***
Vitamin A	< 0.3	< 0.3	< 0.3	0.04
Vitamin D2	< 0.001	< 0.001	< 0.001	0.07
Vitamin D3	< 0.001	< 0.001	< 0.001	0.00
Vitamin E	17.08 ± 3.44[b]	71 ± 4.88[a]	5.08 ± 0.2[c]	1.7
Vitamin K1	0.13 ± 0.03[a]	0.04 ± 0[b]	0.04 ± 0[b]	0.02
Betaine	NT	NT	NT	82
Choline	NT	NT	NT	564
Vitamin C	NT	NT	NT	0.00
Thiamin (B1)	0.53 ± 0.05[b]	190.5 ± 19.53[a]	3.33 ± 1.04[b]	0.43
Riboflavin (B2)	1.13 ± 0.15[b]	2.87 ± 0.21[a]	2.53 ± 0.46[a]	1.51
Niacin (B3)	5.55 ± 0.44[b]	51.72 ± 4.91[a]	55.98 ± 13.65[a]	42.27
Pantothenic Acid (B5)	1.75 ± 0.2[b]	1.97 ± 0.23[b]	8.13 ± 2.02[a]	4.98
Pyridoxine Free Base (B6)	0.52 ± 0.14[c]	4.92 ± 0.2[a]	3.64 ± 1.15[b]	3.23
Biotin (B7)	0.05 ± 0[b]	0.14 ± 0.02[a]	0.04 ± 0.01[b]	NT
Folates (B9)	0.33 ± 0.04[b]	1.08 ± 0.13[a]	0.05**	0.07
Vitamin_B12	NT	NT	NT	0.02

*Data collected from USDA database(USDA, 2020)

**Data collected from USDA database([a]FoodData Central Search Results: https://fdc.nal.usda.gov/fdc-app.html#/food-details/167902/nutrients; accessed on 05/11/2020)

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

NT: Not Tested

Table 2.6: Vitamin composition (mcg/g ± standard deviation) of cooked Beyond Meats Burger-New (BMB2), Impossible Foods Burger-New (IFB2), 80/20 ground pork (GP), and 80/20 ground beef (GB) (n = 6)

Component	**BMB2**	**IFB2**	**GP**	**GB***
Vitamin A	< 0.3	< 0.3	< 0.3	0.03
Vitamin D2	< 0.001	< 0.001	< 0.001	0.05
Vitamin D3	< 0.001	< 0.001	< 0.001	0.00
Vitamin E	18.4 ± 4.34^{b}	80.25 ± 4.8^{a}	5.74 ± 1.82^{c}	1.2
Vitamin K1	0.15 ± 0.03^{a}	0.04 ± 0^{b}	0.04 ± 0^{b}	0.02
Betaine	NT	NT	NT	90
Choline	NT	NT	NT	808
Vitamin C	NT	NT	NT	0.00
Thiamin (B1)	0.65 ± 0.08^{b}	206.5 ± 13.29^{a}	3.97 ± 1.3^{b}	0.47
Riboflavin (B2)	1.5 ± 0.06^{b}	3.23 ± 0.19^{a}	3.08 ± 0.43^{a}	1.76
Niacin (B3)	6.02 ± 0.54^{c}	58.23 ± 2.87^{b}	80.03 ± 6.86^{a}	50.98
Pantothenic Acid (B5)	3 ± 0.39^{b}	2.12 ± 0.22^{b}	8.85 ± 1.28^{a}	6.58
Pyridoxine Free Base (B6)	0.48 ± 0.06^{c}	5.61 ± 0.45^{a}	2.98 ± 0.51^{b}	3.66
Biotin (B7)	0.07 ± 0^{b}	0.16 ± 0.01^{a}	0.05 ± 0.01^{c}	NT
Folates (B9)	0.41 ± 0.09^{b}	1.14 ± 0.12^{a}	0.06**	0.1
Vitamin_B12	NT	NT	NT	0.03

*Data collected from USDA database(USDA, 2020)

**Data collected from USDA database([b]FoodData Central Search Results: https://fdc.nal.usda.gov/fdc-app.html#/food-details/167903/nutrients; accessed on 05/11/2020)

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

NT: Not Tested

Table 2.7: Cholesterol content (mg/g ± standard deviation) of raw Beyond Meats Burger-New (BMB2), Impossible Foods Burger-New (IFB2), 80/20 ground pork (GP), and 80/20 ground beef (GB) (n = 6)

Component	**BMB2**	**IFB2**	**GP**	**GB***
Cholesterol	0.01 ± 0^{b}	0.01 ± 0^{b}	0.7 ± 0.05^{a}	0.71
C8:0 (Caprylic acid)	ND	ND	0.21 ± 0.51	ND
C10:0 (Decanoic acid)	ND	ND	0.05 ± 0.01	ND
C12:0 (Lauric acid)	ND	ND	0.14 ± 0.02	0.08
C14:0 (Myristic acid)	5.2 ± 0.92^{a}	5.86 ± 0.81^{a}	1.35 ± 0.07^{b}	3.26
C15:0 (Pentadecylic acid)	ND	ND	ND	0.53
C16:0 (Palmitic acid)	11.76 ± 0.52^{b}	11.65 ± 0.97^{b}	23.9 ± 0.73^{a}	23.68
C17:0 (Margaric acid)	ND	ND	0.42 ± 0.03	1.24
C18:0 (Stearic acid)	8.38 ± 0.48^{b}	7.89 ± 0.97^{b}	12.75 ± 1.27^{a}	13.07
C20:0 (Arachidic acid)	18.35 ± 0.54^{a}	18.66 ± 1.6^{a}	0.1 ± 0.12^{b}	0.09
C24:0 (Lignoceric acid)	ND	ND	ND	ND
C14:1	ND	ND	ND	0.09
C16:1 (Palmitoleic acid)	ND	ND	2.52 ± 0.23	4.01
C18:1 (Oleic acid)	53.84 ± 1.2^{a}	53.56 ± 1.45^{a}	33.44 ± 2.89^{b}	42.46
C18:1 n7 (vaccenic acid)	ND	ND	4.55 ± 0.36	6.53
C20:1 n9 (Eicosenoic acid)	ND	ND	0.56 ± 0.04	0.37
C18:2 (linoleic acid)	2.48 ± 0.09^{b}	2.37 ± 0.29^{b}	14.35 ± 1.4^{a}	2.32
C18:2t10c12	ND	ND	0.01 ± 0.01	ND
C18:3 (a-linolenic acid)	ND	ND	1.75 ± 0.59	0.36
C20:2 (Eicosadienoic acid)	ND	ND	0.41 ± 0.09	ND
C20:4 (Arachidonic acid)	ND	ND	3.64 ± 2.52	0.19
C22:5 (DPA)	ND	ND	0.04 ± 0.02	ND
C22:6 (DHA)	ND	ND	0.02 ± 0.01	ND

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

*Data collected from USDA database(USDA, 2020)

ND: Not Detected

Table 2.8: Cholesterol content (mg/g ± standard deviation) of cooked Beyond Meats Burger-New (BMB2), Impossible Foods Burger-New (IFB2), 80/20 ground pork (GP), and 80/20 ground beef (GB) (n = 6)

Component	**BMB2**	**IFB2**	**GP**	**GB***
Cholesterol	0.01 ± 0[b]	0.01 ± 0[b]	0.86 ± 0.06[a]	0.88
C8:0 (Caprylic acid)	ND	ND	ND	ND
C10:0 (Decanoic acid)	ND	ND	0.05 ± 0.01	ND
C12:0 (Lauric acid)	ND	ND	0.13 ± 0.02	0.08
C14:0 (Myristic acid)	5.21 ± 1.2[a]	5.46 ± 0.7[a]	1.26 ± 0.08[b]	3.21
C15:0 (Pentadecylic acid)	ND	ND	ND	0.52
C16:0 (Palmitic acid)	11.95 ± 1.12[b]	11.88 ± 0.7[b]	24.18 ± 0.85[a]	24.04
C17:0 (Margaric acid)	ND	ND	0.43 ± 0.03	1.21
C18:0 (Stearic acid)	8.19 ± 0.86[b]	8.9 ± 0.63[b]	13.04 ± 1.34[a]	13.36
C20:0 (Arachidic acid)	17.63 ± 3.17[a]	16.19 ± 2.84[a]	0.1 ± 0.12[b]	0.08
C24:0 (Lignoceric acid)	ND	ND	ND	ND
C14:1	ND	ND	ND	0.90
C16:1 (Palmitoleic acid)	ND	ND	2.39 ± 0.3	4.10
C17:1 (Heptadecanoic acid)	ND	ND	ND	1.02
C18:1 (Oleic acid)	54.46 ± 1.39[a]	54.98 ± 2.44[a]	33.25 ± 2.55[b]	43.0
C18:1 n7 (vaccenic acid)	ND	ND	4.57 ± 0.42	4.90
C20:1 n9 (Eicosenoic acid)	ND	ND	0.56 ± 0.03	0.36
C18:2 (linoleic acid)	2.56 ± 0.1[b]	2.59 ± 0.26[b]	14.13 ± 1.73[a]	2.53
C18:2t10c12	ND	ND	0.01 ± 0.01	ND
C18:3 (a-linolenic acid)	ND	ND	1.77 ± 0.61	0.36
C20:2 (Eicosadienoic acid)	ND	ND	0.4 ± 0.09	ND
C20:4 (Arachidonic acid)	ND	ND	3.68 ± 2.52	0.30
C22:5 (DPA)	ND	ND	0.04 ± 0.02	ND
C22:6 (DHA)	ND	ND	0.02 ± 0.01	ND

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

*Data collected from USDA database(USDA, 2020)

ND: Not Detected

Table 2.9: Amino Acid composition (mg/g ± standard deviation) of raw Beyond Meats Burger-New (BMB2), Impossible Foods Burger-New (IFB2), 80/20 ground pork (GP), and 80/20 ground beef (GB) (n = 6)

Component	**BMB2**	**IFB2**	**GP**	**GB***
Arginine	16.5 ± 1.89^{a}	11.4 ± 0.66^{b}	11.08 ± 0.72^{b}	11.18
Cystine	2.55 ± 0.27^{b}	4.42 ± 0.25^{a}	1.83 ± 0.24^{c}	1.77
Glutamic Acid	33.17 ± 3.57^{b}	37.87 ± 2.2^{a}	23.72 ± 2.24^{c}	25.75
Glycine	8.06 ± 0.92^{b}	7.09 ± 0.37^{b}	9.75 ± 0.66^{a}	11.66
Proline	8.89 ± 1.03^{a}	8.34 ± 0.51^{a}	7.93 ± 0.53^{a}	8.75
Tyrosine	8.25 ± 0.97^{a}	6.49 ± 0.36^{b}	5.51 ± 0.5^{b}	5.28
Histidine	4.86 ± 0.54^{a}	3.85 ± 0.2^{b}	5.46 ± 0.79^{a}	5.58
Isoleucine	9.41 ± 1.04^{a}	7.82 ± 0.46^{b}	7.28 ± 0.78^{b}	7.59
Leucine	16.52 ± 1.73^{a}	13 ± 0.72^{b}	12.7 ± 1.2^{b}	13.39
Lysine	12.82 ± 1.58^{a}	10.29 ± 0.81^{b}	13.28 ± 1^{a}	14.23
Methionine	2.53 ± 0.38^{b}	2.01 ± 0.15^{b}	4.3 ± 0.45^{a}	4.42
Phenylalanine	11.04 ± 1.09^{a}	8.65 ± 0.51^{b}	6.3 ± 0.56^{c}	6.7
Threonine	7.3 ± 0.86^{a}	6.57 ± 0.37^{a}	7.1 ± 0.7^{a}	6.65
Tryptophan	1.75 ± 0.07^{c}	2.26 ± 0.08^{a}	1.9 ± 0.12^{b}	0.87
Valine	10.4 ± 1.11^{a}	8.17 ± 0.48^{b}	7.81 ± 0.74^{b}	8.44
Alanine	9.12 ± 1.08^{a}	7.7 ± 0.47^{b}	10.14 ± 0.9^{a}	10.76
Aspartic Acid	22.17 ± 2.4^{a}	18.63 ± 1.05^{b}	14.68 ± 1.41^{c}	15.47
Serine	10.16 ± 1.18^{a}	8.39 ± 0.47^{b}	6.38 ± 0.52^{c}	6.88

$^{a\text{-}e}$ Means within a row with different subscripts differ statistically ($P < 0.05$)
*Data collected from USDA database(USDA, 2020)

Table 2.10: Amino Acid composition (mg/g ± standard deviation) of cooked Beyond Meats Burger-New (BMB2), Impossible Foods Burger-New (IFB2), 80/20 ground pork (GP), and 80/20 ground beef (GB) (n = 6)

Component	**BMB2**	**IFB2**	**GP**	**GB***
Arginine	20.17 ± 0.87^{a}	13.82 ± 0.6^{c}	14.95 ± 1.11^{b}	16.75
Cystine	3.11 ± 0.14^{b}	4.93 ± 0.23^{a}	2.5 ± 0.19^{c}	2.65
Glutamic Acid	40.75 ± 1.45^{b}	45.17 ± 1.23^{a}	32.05 ± 3.04^{c}	38.58
Glycine	9.86 ± 0.45^{b}	8.6 ± 0.23^{b}	12.97 ± 2.23^{a}	17.47
Proline	10.97 ± 0.37^{a}	10.07 ± 0.36^{a}	11.14 ± 1.2^{a}	13.11
Tyrosine	10.13 ± 0.46^{a}	7.83 ± 0.21^{b}	7.44 ± 0.51^{b}	7.92
Histidine	5.87 ± 0.27^{b}	4.65 ± 0.17^{c}	7.57 ± 0.62^{a}	8.36
Isoleucine	11.67 ± 0.48^{a}	9.45 ± 0.24^{b}	9.97 ± 0.73^{b}	11.38
Leucine	20.27 ± 0.83^{a}	15.77 ± 0.44^{c}	17.47 ± 1.34^{b}	20.07
Lysine	15.62 ± 0.55^{b}	12.18 ± 0.57^{c}	18.22 ± 1.13^{a}	21.31
Methionine	3 ± 0.14^{b}	2.41 ± 0.13^{c}	5.91 ± 0.51^{a}	6.62
Phenylalanine	13.63 ± 0.5^{a}	10.52 ± 0.27^{b}	8.6 ± 0.62^{c}	10.04
Threonine	8.92 ± 0.42^{a}	7.93 ± 0.24^{b}	9.58 ± 0.7^{a}	9.96
Tryptophan	2.36 ± 0.12^{b}	2.6 ± 0.08^{a}	2.69 ± 0.16^{a}	1.31
Valine	12.83 ± 0.53^{a}	9.93 ± 0.23^{b}	10.69 ± 0.86^{b}	12.64
Alanine	11.08 ± 0.48^{b}	9.24 ± 0.26^{c}	14.02 ± 1.41^{a}	16.12
Aspartic Acid	27.23 ± 0.97^{a}	22.43 ± 0.62^{b}	20.25 ± 1.61^{c}	23.17
Serine	12.3 ± 0.57^{a}	10.02 ± 0.35^{b}	8.62 ± 0.7^{c}	10.3

$^{a\text{-}e}$ Means within a row with different subscripts differ statistically ($P < 0.05$)
*Data collected from USDA database(USDA, 2020)

Table 2.11: As fed proximate analysis (percent ± standard deviation) of raw Beyond Meats Burger-Old (BMB1), Beyond Meats Burger-New (BMB2), Impossible Foods Burger-Old (IFB1), Impossible Foods Burger-New (IFB2), 80/20 ground pork (GP), and 80/20 ground beef (GP) (n = 6)

Component	**BMB1**	**BMB2**	**IFB1**	**IFB2**	**GP**	**GB***
Dry Matter	32.13 ± 0.83^{c}	42.93 ± 0.79^{a}	37.19 ± 1.96^{b}	42.28 ± 0.4^{a}	37.09 ± 1.29^{b}	38.0
Moisture	67.87 ± 0.83^{a}	57.07 ± 0.79^{c}	62.81 ± 1.96^{b}	57.72 ± 0.4^{c}	62.91 ± 1.29^{b}	62.0
Ash	1.54 ± 0.7^{b}	1.72 ± 0.2^{b}	1.54 ± 0.46^{b}	2.51 ± 0.22^{a}	1.76 ± 0.36^{b}	0.84
Crude Fat	10.77 ± 3.81^{a}	13.08 ± 2.2^{a}	11.98 ± 4.99^{a}	11.67 ± 1.85^{a}	10.15 ± 4.68^{a}	20.0
Crude Protein	20 ± 3.35^{ab}	18.59 ± 0.87^{ab}	22.03 ± 4.14^{a}	17.18 ± 0.82^{b}	22.62 ± 3.24^{a}	17.
Acid Detergent Fiber	NA	9.92 ± 1.95^{a}	NA	9.39 ± 1.81^{a}	NA	NA
Neutral Detergent Fiber	NA	18.75 ± 4.09^{a}	NA	18.63 ± 3.27^{a}	NA	NA

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

*Data collected from USDA database(USDA, 2020)

NA: Not Applicable

Table 2.12: As fed proximate analysis (percent ± standard deviation) of cooked Beyond Meats Burger-Old (BMB1), Beyond Meats Burger-New (BMB2), Impossible Foods Burger-Old (IFB1), Impossible Foods Burger-New (IFB2), 80/20 ground pork (GP), and 80/20 ground beef (GP) (n = 6)

Component	**BMB1**	**BMB2**	**IFB1**	**IFB2**	**GP**	**GB***
Dry Matter	36.74 ± 3.46^{c}	50.3 ± 0.93^{a}	44.98 ± 0.72^{b}	47.67 ± 0.99^{ab}	44.77 ± 2.18^{b}	44.0
Moisture	63.26 ± 3.46^{a}	49.7 ± 0.93^{c}	55.02 ± 0.72^{b}	52.33 ± 0.99^{bc}	55.23 ± 2.18^{b}	56.0
Ash	1.79 ± 0.62^{bc}	2.08 ± 0.16^{b}	1.56 ± 0.19^{bc}	3 ± 0.16^{a}	1.27 ± 0.41^{c}	1
Crude Fat	11.93 ± 5.19^{a}	11.6 ± 3.91^{a}	9.24 ± 3.08^{a}	11.22 ± 1.68^{a}	11.09 ± 5.87^{a}	18.0
Crude Protein	23.29 ± 4.39^{a}	23.77 ± 1.54^{a}	20.29 ± 3.62^{a}	20.22 ± 0.48^{a}	21.48 ± 3.04^{a}	26.0
Acid Detergent Fiber	NA	10.84 ± 0.8^{a}	NA	12.92 ± 2.34^{a}	NA	NA
Neutral Detergent Fiber	NA	20.67 ± 4.19^{a}	NA	23.85 ± 4.86^{a}	NA	NA

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

*Data collected from USDA database(USDA, 2020)

NA: Not Applicable

Table 2.13: Mineral composition (ppm ± standard deviation) of raw Beyond Meats Burger-Old (BMB1), Beyond Meats Burger-New (BMB2), Impossible Foods Burger-Old (IFB1), Impossible Foods Burger-New (IFB2), 80/20 ground pork (GP), and 80/20 ground beef (GP) (n = 6)

Component	BMB1	BMB2	IFB1	IFB2	GP	GB*
Calcium	213.83 ± 11.6^{c}	819.83 ± 73.96^{b}	257.5 ± 6.66^{c}	1860 ± 46.48^{a}	105.8 ± 61.89^{d}	180
Magnesium	190.83 ± 7.88^{c}	350 ± 30.09^{b}	120.67 ± 7.2^{d}	714 ± 19.33^{a}	178.5 ± 16.32^{c}	170
Phosphorus	1888.33 ± 74.41^{b}	2423.33 ± 160.71^{a}	1296.67 ± 28.75^{d}	1840 ± 54.77^{b}	1596.67 ± 126.6^{c}	1580
Potassium	2828.33 ± 188.09^{bc}	2431.67 ± 247.9^{c}	3096.67 ± 89.37^{b}	5760 ± 197.89^{a}	3176.67 ± 702.67^{b}	2700
Sodium	3328.33 ± 205.47^{b}	3230 ± 275.32^{b}	4935 ± 166.94^{a}	3608.33 ± 203.9^{b}	995.5 ± 1281.44^{c}	660
Copper	3.38 ± 0.42^{a}	2.1 ± 0.25^{b}	3.82 ± 0.38^{a}	2.67 ± 0.45^{b}	0.71 ± 0.09^{c}	0.61
Iron	43.43 ± 2.9^{a}	36.6 ± 2.59^{b}	22.28 ± 0.87^{c}	36.33 ± 3.55^{b}	7.91 ± 2.32^{d}	19.4
Manganese	2.46 ± 0.46^{d}	6.94 ± 0.77^{b}	4.36 ± 0.31^{c}	10.32 ± 0.73^{a}	0.18 ± 0^{e}	0.1
Selenium	NT	NT	NT	NT	NT	0.15
Zinc	20.9 ± 1.29^{c}	23.33 ± 2.68^{c}	29.72 ± 1.44^{b}	48.33 ± 2.53^{a}	20.77 ± 5.03^{c}	41.8

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

*Data collected from USDA database(USDA, 2020)

NT: Not Tested

Table 2.14: Mineral composition (ppm ± standard deviation) of cooked Beyond Meats Burger-Old (BMB1), Beyond Meats Burger-New (BMB2), Impossible Foods Burger-Old (IFB1), Impossible Foods Burger-New (IFB2), 80/20 ground pork (GP), and 80/20 ground beef (GP) (n = 6)

Component	BMB1	BMB2	IFB1	IFB2	GP	GB*
Calcium	267.33 ± 15.19^{c}	1068.17 ± 89.29^{b}	297 ± 15.19^{c}	2165 ± 69.5^{a}	139.13 ± 87.32^{d}	240
Magnesium	235.33 ± 6.31^{c}	446.67 ± 31.7^{b}	140.83 ± 6.49^{d}	827 ± 23.07^{a}	239.17 ± 17.67^{c}	200
Phosphorus	2315 ± 79.44^{b}	3120 ± 182.54^{a}	1513.33 ± 28.75^{c}	2143.33 ± 60.22^{b}	2146.67 ± 121.76^{b}	1940
Potassium	3378.33 ± 367.12^{c}	3056.67 ± 287.31^{c}	3590 ± 60.99^{bc}	6753.33 ± 326.17^{a}	4220 ± 834.94^{b}	3040
Sodium	4135 ± 355.79^{b}	4186.67 ± 261.97^{b}	5666.67 ± 212.95^{a}	4240 ± 219.27^{b}	1277.83 ± 1586.4^{c}	750
Copper	4.88 ± 0.52^{a}	2.8 ± 0.37^{b}	4.45 ± 0.59^{a}	3.07 ± 0.43^{b}	1.4 ± 0.71^{c}	0.8
Iron	60.02 ± 5.51^{a}	47.42 ± 2.98^{b}	26.98 ± 1.37^{c}	42.63 ± 3.23^{b}	10.83 ± 2.58^{d}	24.8
Manganese	3.03 ± 0.29^{d}	8.97 ± 0.83^{b}	5.04 ± 0.39^{c}	11.97 ± 0.78^{a}	0.18 ± 0^{e}	0.11
Selenium	NT	NT	NT	NT	NT	0.22
Zinc	25.55 ± 1.97^{c}	30.27 ± 2.75^{bc}	34.17 ± 1.76^{b}	56.77 ± 3.37^{a}	28.15 ± 5.53^{c}	62.5

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

*Data collected from USDA database(USDA, 2020)

NT: Not Tested

Table 2.15: Vitamin composition (mcg/g ± standard deviation) of raw Beyond Meats Burger-Old (BMB1), Beyond Meats Burger-New (BMB2), Impossible Foods Burger-Old (IFB1), Impossible Foods Burger-New (IFB2), 80/20 ground pork (GP), and 80/20 ground beef (GB) (n = 6)

Component	BMB1	BMB2	IFB1	IFB2	GP	GB*
Vitamin A	< 0.3	< 0.3	< 0.3	< 0.3	< 0.3	0.04
Vitamin D2	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.07
Vitamin D3	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.00
Vitamin E	21.65 ± 4.49^{c}	17.08 ± 3.44^{c}	33.93 ± 5.88^{b}	71 ± 4.88^{a}	5.08 ± 0.2^{d}	1.7
Vitamin K1	0.22 ± 0.02^{a}	0.13 ± 0.03^{b}	0.04 ± 0^{c}	0.04 ± 0^{c}	0.04 ± 0^{c}	0.02
Betaine	NT	NT	NT	NT	NT	82
Choline	NT	NT	NT	NT	NT	564
Vitamin C	NT	NT	NT	NT	NT	0.00
Thiamin (B1)	0.6 ± 0.46^{b}	0.53 ± 0.05^{b}	182.33 ± 7.5^{a}	190.5 ± 19.53^{a}	3.33 ± 1.04^{b}	0.43
Riboflavin (B2)	1.17 ± 0.1^{c}	1.13 ± 0.15^{c}	3.83 ± 0.34^{a}	2.87 ± 0.21^{b}	2.53 ± 0.46^{b}	1.51
Niacin (B3)	3.47 ± 0.31^{b}	5.55 ± 0.44^{b}	52.58 ± 4.85^{a}	51.72 ± 4.91^{a}	55.98 ± 13.65^{a}	42.27
Pantothenic Acid (B5)	3.62 ± 0.29^{b}	1.75 ± 0.2^{d}	3.43 ± 0.26^{bc}	1.97 ± 0.23^{cd}	8.13 ± 2.02^{a}	4.98
Pyridoxine Free Base (B6)	0.41 ± 0.09^{c}	0.52 ± 0.14^{c}	2.86 ± 0.16^{b}	4.92 ± 0.2^{a}	3.64 ± 1.15^{b}	3.23
Biotin (B7)	0.06 ± 0.01^{b}	0.05 ± 0^{bc}	0.04 ± 0.01^{c}	0.14 ± 0.02^{a}	0.04 ± 0.01^{c}	NT
Folates (B9)	ND	0.33 ± 0.04^{b}	ND	1.08 ± 0.13^{a}	NT	0.07
Vitamin B12	NT	NT	NT	NT	NT	0.02

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

*Data collected from USDA database(USDA, 2020)

NT: Not Tested

Table 2.16: Vitamin composition (mcg/g ± standard deviation) of cooked Beyond Meats Burger-Old (BMB1), Beyond Meats Burger-New (BMB2), Impossible Foods Burger-Old (IFB1), Impossible Foods Burger-New (IFB2), 80/20 ground pork (GP), and 80/20 ground beef (GB) (n = 6)

Component	BMB1	BMB2	IFB1	IFB2	GP	GB*
Vitamin A	< 0.3	< 0.3	< 0.3	< 0.3	< 0.3	0.03
Vitamin D2	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.05
Vitamin D3	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.00
Vitamin E	26.58 ± 6.73^{c}	18.4 ± 4.34^{d}	38.75 ± 2.13^{b}	80.25 ± 4.8^{a}	5.74 ± 1.82^{e}	1.2
Vitamin K1	0.25 ± 0.03^{a}	0.15 ± 0.03^{b}	0.04 ± 0^{c}	0.04 ± 0^{c}	0.04 ± 0^{c}	0.02
Betaine	NT	NT	NT	NT	NT	90
Choline	NT	NT	NT	NT	NT	808
Vitamin C	NT	NT	NT	NT	NT	0.00
Thiamin (B1)	0.32 ± 0.12^{b}	0.65 ± 0.08^{b}	197.33 ± 9.65^{a}	206.5 ± 13.29^{a}	3.97 ± 1.3^{b}	0.47
Riboflavin (B2)	1.55 ± 0.15^{c}	1.5 ± 0.06^{c}	4.37 ± 0.15^{a}	3.23 ± 0.19^{b}	3.08 ± 0.43^{b}	1.76
Niacin (B3)	4.28 ± 0.34^{c}	6.02 ± 0.54^{c}	62.2 ± 2.97^{b}	58.23 ± 2.87^{b}	80.03 ± 6.86^{a}	50.98
Pantothenic Acid (B5)	4.03 ± 0.16^{b}	3 ± 0.39^{bc}	3.52 ± 0.21^{b}	2.12 ± 0.22^{c}	8.85 ± 1.28^{a}	6.58
Pyridoxine Free Base (B6)	0.47 ± 0.06^{c}	0.48 ± 0.06^{c}	3.14 ± 0.23^{b}	5.61 ± 0.45^{a}	2.98 ± 0.51^{b}	3.66
Biotin (B7)	0.07 ± 0.01^{b}	0.07 ± 0^{b}	0.05 ± 0.01^{c}	0.16 ± 0.01^{a}	0.05 ± 0.01^{c}	NT
Folates (B9)	ND	0.41 ± 0.09^{b}	ND	1.14 ± 0.12^{a}	ND	0.1
Vitamin B12	NT	NT	NT	NT	NT	0.03

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

*Data collected from USDA database(USDA, 2020)

NT: Not Tested

Table 2.17: Cholesterol content (mg/g ± standard deviation) of raw Beyond Meats Burger-Old (BMB1), Beyond Meats Burger-New (BMB2), Impossible Foods Burger-Old (IFB1), Impossible Foods Burger-New (IFB2), 80/20 ground pork (GP), and 80/20 ground beef (GB) (n = 6)

Component	**BMB1**	**BMB2**	**IFB1**	**IFB2**	**GP**	**GB***
Cholesterol	< 0.01	< 0.01	< 0.01	< 0.01	0.7 ± 0.05	0.71
C8:0 (Caprylic acid)	1.22 ± 0.17^{b}	ND	8.36 ± 0.27^{a}	ND	0.21 ± 0.51^{c}	ND
C10:0 (Decanoic acid)	ND	ND	6.98 ± 0.8^{a}	ND	0.05 ± 0.01^{b}	ND
C12:0 (Lauric acid)	3.48 ± 0.81^{b}	NA	46.8 ± 1.08^{a}	ND	0.14 ± 0.02^{c}	0.08
C14:0 (Myristic acid)	ND	5.2 ± 0.92^{b}	22.76 ± 1.09^{a}	5.86 ± 0.81^{b}	1.35 ± 0.07^{c}	3.26
C15:0 (Pentadecylic acid)	ND	ND	ND	ND	ND	0.53
C16:0 (Palmitic acid)	7.44 ± 0.57^{c}	11.76 ± 0.52^{b}	2.03 ± 0.28^{d}	11.65 ± 0.97^{b}	23.9 ± 0.73^{a}	23.68
C17:0 (Margaric acid)	ND	ND	ND	ND	0.42 ± 0.03^{a}	1.24
C18:0 (Stearic acid)	5.26 ± 0.38^{c}	8.38 ± 0.48^{b}	2.28 ± 0.2^{d}	7.89 ± 0.97^{b}	12.75 ± 1.27^{a}	13.07
C20:0 (Arachidic acid)	1.08 ± 0.06^{b}	18.35 ± 0.54^{a}	0.2 ± 0.06^{b}	18.66 ± 1.6^{a}	0.1 ± 0.12^{b}	0.09
C24:0 (Lignoceric acid)	1.1 ± 0.69^{a}	NA	0.48 ± 1.18^{a}	ND	ND	ND
C14:1	ND	ND	ND	ND	ND	0.89
C16:1 (Palmitoleic acid)	ND	ND	ND	ND	2.52 ± 0.23	4.01
C17:1 (Heptadecanoic acid)	ND	ND	ND	ND	ND	0.9
C18:1 (Oleic)	31.88 ± 2.19^{b}	53.84 ± 1.2^{a}	6.98 ± 0.31^{c}	53.56 ± 1.45^{a}	33.44 ± 2.89^{b}	42.46
C18:1 n7(Vaccenic acid)	ND	ND	ND	ND	4.55 ± 0.36^{a}	6.53
C20:1 n9 (Eicosenoic acid)	1.07 ± 0.06^{a}	ND	0.18 ± 0.04^{c}	ND	0.56 ± 0.04^{b}	0.37
C18:2 (Linoleic acid)	34.59 ± 3.39^{a}	2.48 ± 0.09^{c}	2.05 ± 1.03^{c}	2.37 ± 0.29^{c}	14.35 ± 1.4^{b}	2.32
C18:2t10c12	ND	ND	ND	ND	0.01 ± 0.01^{a}	ND
C18:3 (a-linolenic acid)	12.46 ± 0.5^{a}	ND	0.73 ± 0.13^{c}	ND	1.75 ± 0.59^{b}	0.36
C20:2 (Eicosadienoic acid)	ND	ND	ND	ND	0.41 ± 0.09^{a}	ND
C20:4 (Arachidonic acid)	0.41 ± 0.03^{b}	ND	0.13 ± 0.06^{b}	ND	3.64 ± 2.52^{a}	0.19

C22:5 (DPA)	ND	ND	ND	ND	0.04 ± 0.02^{a}	ND
C22:6 (DHA)	ND	ND	0.02 ± 0.04^{a}	ND	0.02 ± 0.01^{a}	ND

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

*Data collected from USDA database(USDA, 2020)

ND: Not Detected

Table 2.18: Cholesterol content (mg/g ± standard deviation) of cooked Beyond Meats Burger-Old (BMB1), Beyond Meats Burger-New (BMB2), Impossible Foods Burger-Old (IFB1), Impossible Foods Burger-New (IFB2), 80/20 ground pork (GP), and 80/20 ground beef (GB) (n = 6)

Component	**BMB1**	**BMB2**	**IFB1**	**IFB2**	**GP**	**GB***
Cholesterol	< 0.01	< 0.01	< 0.01	< 0.01	0.86 ± 0.06	0.88
C8:0 (Caprylic acid)	1.12 ± 0.13^{b}	ND	6.67 ± 3.33^{a}	ND	ND	ND
C10:0 (Decanoic acid)	NA	ND	5.78 ± 2.82^{a}	ND	0.05 ± 0.01^{b}	ND
C12:0 (Lauric acid)	2.79 ± 0.41^{b}	ND	38.95 ± 19.1^{a}	ND	0.13 ± 0.02^{b}	0.08
C14:0 (Myristic acid)	ND	5.21 ± 1.2^{b}	19.2 ± 7.93^{a}	5.46 ± 0.7^{b}	1.26 ± 0.08^{b}	3.21
C15:0 (Pentadecylic acid)	ND	ND	ND	ND	ND	0.52
C16:0 (Palmitic acid)	7.5 ± 0.74^{c}	11.95 ± 1.12^{b}	1.76 ± 0.9^{d}	11.88 ± 0.7^{b}	24.18 ± 0.85^{a}	24.04
C17:0 (Margaric acid)	ND	ND	ND	ND	0.43 ± 0.03	1.21
C18:0 (Stearic acid)	5.41 ± 0.32^{c}	8.19 ± 0.86^{b}	2.04 ± 1.02^{d}	8.9 ± 0.63^{b}	13.04 ± 1.34^{a}	13.36
C20:0 (Arachidic acid)	1.11 ± 0.07^{b}	17.63 ± 3.17^{a}	0.18 ± 0.1^{b}	16.19 ± 2.84^{a}	0.1 ± 0.12^{b}	0.08
C24:0 (Lignoceric acid)	1.14 ± 0.74^{a}	ND	ND	ND	ND	ND
C14:1	ND	ND	ND	ND	ND	0.90
C16:1 (Palmitoleic acid)	ND	ND	ND	ND	2.39 ± 0.3	4.10
C17:1 (Heptadecanoic acid)	ND	ND	ND	ND	ND	1.02
C18:1 (Oleic)	32.15 ± 1.78^{b}	54.46 ± 1.39^{a}	6.19 ± 3.05^{c}	54.98 ± 2.44^{a}	33.25 ± 2.55^{b}	43.0
C18:1 n7(Vaccenic acid)	ND	ND	ND	ND	4.57 ± 0.42^{a}	4.90
C20:1 n9 (Eicosenoic acid)	1.1 ± 0.07^{a}	ND	0.17 ± 0.09^{c}	NA	0.56 ± 0.03^{b}	0.36
C22:1 (Erucic acid)	ND	ND	ND	ND	ND	ND
C18:2 (Linoleic acid)	35.42 ± 2.12^{a}	2.56 ± 0.1^{c}	2.13 ± 1.08^{c}	2.59 ± 0.26^{c}	14.13 ± 1.73^{b}	2.53
C18:2t10c12	ND	ND	ND	ND	0.01 ± 0.01^{a}	ND
C18:3 (a-linolenic acid)	11.84 ± 0.94^{a}	ND	0.68 ± 0.35^{c}	ND	1.77 ± 0.61^{b}	0.36
C20:2 (Eicosadienoic acid)	ND	ND	ND	ND	0.4 ± 0.09^{a}	ND

C20:4 (Arachidonic acid)	0.42 ± 0.03^{b}	ND	0.09 ± 0.06^{b}	ND	3.68 ± 2.52^{a}	0.30
C22:5 (DPA)	ND	ND	ND	ND	0.04 ± 0.02^{a}	ND
C22:6 (DHA)	ND	ND	0.02 ± 0.04^{a}	ND	0.02 ± 0.01^{a}	ND

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

*Data collected from USDA database(USDA, 2020)

ND: Not Detected

Table 2.19: Amino Acid composition (mg/g ± standard deviation) of raw Beyond Meats Burger-Old (BMB1), Beyond Meats Burger-New (BMB2), Impossible Foods Burger-Old (IFB1), Impossible Foods Burger-New (IFB2), 80/20 ground pork (GP), and 80/20 ground beef (GP) (n = 6)

Component	BMB1	BMB2	IFB1	IFB2	GP	GB*
Arginine	15.32 ± 0.55^{a}	16.5 ± 1.89^{a}	9.17 ± 0.79^{c}	11.4 ± 0.66^{b}	11.08 ± 0.72^{b}	11.18
Cystine	2.45 ± 0.17^{c}	2.55 ± 0.27^{c}	6.22 ± 0.61^{a}	4.42 ± 0.25^{b}	1.83 ± 0.24^{d}	1.77
Glutamic Acid	30.1 ± 0.93^{c}	33.17 ± 3.57^{c}	69.57 ± 3.39^{a}	37.87 ± 2.2^{b}	23.72 ± 2.24^{d}	25.75
Glycine	7.36 ± 0.13^{cd}	8.06 ± 0.92^{bc}	8.85 ± 0.3^{ab}	7.09 ± 0.37^{d}	9.75 ± 0.66^{a}	11.66
Proline	7.96 ± 0.27^{b}	8.89 ± 1.03^{b}	21.97 ± 1.01^{a}	8.34 ± 0.51^{b}	7.93 ± 0.53^{b}	8.75
Tyrosine	7.05 ± 0.14^{b}	8.25 ± 0.97^{a}	8.84 ± 0.25^{a}	6.49 ± 0.36^{b}	5.51 ± 0.5^{c}	5.28
Histidine	4.24 ± 0.08^{bc}	4.86 ± 0.54^{ab}	4.08 ± 0.17^{c}	3.85 ± 0.2^{c}	5.46 ± 0.79^{a}	5.58
Isoleucine	8.74 ± 0.14^{ab}	9.41 ± 1.04^{a}	9.19 ± 0.27^{a}	7.82 ± 0.46^{bc}	7.28 ± 0.78^{c}	7.59
Leucine	15.3 ± 0.24^{a}	16.52 ± 1.73^{a}	16.68 ± 0.53^{a}	13 ± 0.72^{b}	12.7 ± 1.2^{b}	13.39
Lysine	13.28 ± 0.36^{a}	12.82 ± 1.58^{a}	7.63 ± 0.33^{c}	10.29 ± 0.81^{b}	13.28 ± 1^{a}	14.23
Methionine	1.61 ± 0.13^{d}	2.53 ± 0.38^{c}	3.28 ± 0.13^{b}	2.01 ± 0.15^{d}	4.3 ± 0.45^{a}	4.42
Phenylalanine	9.87 ± 0.18^{b}	11.04 ± 1.09^{a}	11.82 ± 0.35^{a}	8.65 ± 0.51^{c}	6.3 ± 0.56^{d}	6.7
Threonine	6.67 ± 0.13^{a}	7.3 ± 0.86^{a}	6.98 ± 0.2^{a}	6.57 ± 0.37^{a}	7.1 ± 0.7^{a}	6.65
Tryptophan	1.67 ± 0.06^{c}	1.75 ± 0.07^{bc}	2.29 ± 0.1^{a}	2.26 ± 0.08^{a}	1.9 ± 0.12^{b}	0.87
Valine	9.12 ± 0.19^{b}	10.4 ± 1.11^{a}	10.52 ± 0.21^{a}	8.17 ± 0.48^{bc}	7.81 ± 0.74^{c}	8.44
Alanine	7.93 ± 0.19^{b}	9.12 ± 1.08^{a}	6.66 ± 0.22^{c}	7.7 ± 0.47^{bc}	10.14 ± 0.9^{a}	10.76
Aspartic Acid	21.17 ± 0.49^{a}	22.17 ± 2.4^{a}	13.27 ± 0.4^{c}	18.63 ± 1.05^{b}	14.68 ± 1.41^{c}	15.47
Serine	9.34 ± 0.23^{bc}	10.16 ± 1.18^{ab}	10.75 ± 0.42^{a}	8.39 ± 0.47^{c}	6.38 ± 0.52^{d}	6.88

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

*Data collected from USDA database(USDA, 2020)

Table 2.20: Amino Acid composition (mg/g ± standard deviation) of cooked Beyond Meats Burger-Old (BMB1), Beyond Meats Burger-New (BMB2), Impossible Foods Burger-Old (IFB1), Impossible Foods Burger-New (IFB2), 80/20 ground pork (GP), and 80/20 ground beef (GP) (n = 6)

Component	BMB1	BMB2	IFB1	IFB2	GP	GB*
Arginine	16.82 ± 1.09^{b}	20.17 ± 0.87^{a}	9.73 ± 0.56^{d}	13.82 ± 0.6^{c}	14.95 ± 1.11^{c}	16.75
Cystine	2.67 ± 0.22^{cd}	3.11 ± 0.14^{c}	7.05 ± 0.62^{a}	4.93 ± 0.23^{b}	2.5 ± 0.19^{d}	2.65
Glutamic Acid	33.6 ± 1.73^{c}	40.75 ± 1.45^{b}	80.93 ± 5.03^{a}	45.17 ± 1.23^{b}	32.05 ± 3.04^{c}	38.58
Glycine	8.17 ± 0.36^{c}	9.86 ± 0.45^{bc}	10.18 ± 0.48^{b}	8.6 ± 0.23^{bc}	12.97 ± 2.23^{a}	17.47
Proline	8.98 ± 0.46^{c}	10.97 ± 0.37^{b}	25.27 ± 1.48^{a}	10.07 ± 0.36^{bc}	11.14 ± 1.2^{b}	13.11
Tyrosine	7.73 ± 0.35^{b}	10.13 ± 0.46^{a}	10.04 ± 0.49^{a}	7.83 ± 0.21^{b}	7.44 ± 0.51^{b}	7.92
Histidine	4.75 ± 0.23^{c}	5.87 ± 0.27^{b}	4.78 ± 0.31^{c}	4.65 ± 0.17^{c}	7.57 ± 0.62^{a}	8.36
Isoleucine	9.78 ± 0.5^{bc}	11.67 ± 0.48^{a}	10.65 ± 0.59^{b}	9.45 ± 0.24^{c}	9.97 ± 0.73^{bc}	11.38
Leucine	17.22 ± 0.76^{bc}	20.27 ± 0.83^{a}	19.45 ± 1^{a}	15.77 ± 0.44^{c}	17.47 ± 1.34^{b}	20.07
Lysine	14.52 ± 0.69^{b}	15.62 ± 0.55^{b}	8.48 ± 0.54^{d}	12.18 ± 0.57^{c}	18.22 ± 1.13^{a}	21.31
Methionine	1.81 ± 0.29^{e}	3 ± 0.14^{c}	3.83 ± 0.2^{b}	2.41 ± 0.13^{d}	5.91 ± 0.51^{a}	6.62
Phenylalanine	10.85 ± 0.55^{b}	13.63 ± 0.5^{a}	13.57 ± 0.6^{a}	10.52 ± 0.27^{b}	8.6 ± 0.62^{c}	10.04
Threonine	7.38 ± 0.37^{b}	8.92 ± 0.42^{a}	7.97 ± 0.37^{b}	7.93 ± 0.24^{b}	9.58 ± 0.7^{a}	9.96
Tryptophan	1.95 ± 0.22^{c}	2.36 ± 0.12^{b}	2.63 ± 0.11^{a}	2.6 ± 0.08^{ab}	2.69 ± 0.16^{a}	1.31
Valine	9.97 ± 0.45^{c}	12.83 ± 0.53^{a}	11.85 ± 0.54^{b}	9.93 ± 0.23^{c}	10.69 ± 0.86^{c}	12.64
Alanine	8.98 ± 0.35^{c}	11.08 ± 0.48^{b}	7.76 ± 0.36^{d}	9.24 ± 0.26^{c}	14.02 ± 1.41^{a}	16.12
Aspartic Acid	23.87 ± 1.29^{b}	27.23 ± 0.97^{a}	15.57 ± 0.99^{d}	22.43 ± 0.62^{b}	20.25 ± 1.61^{c}	23.17
Serine	10.29 ± 0.47^{b}	12.3 ± 0.57^{a}	12.37 ± 0.71^{a}	10.02 ± 0.35^{b}	8.62 ± 0.7^{c}	10.3

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

*Data collected from USDA database(USDA, 2020)

Table 4.1: Mean (n = 6) surviving *Listeria monocytogenes* populations (log CFU/cantaloupe ± SD) following immersion treatment of inoculated (five-strain mixture; 7 to 8 log CFU/cantaloupe) whole cantaloupes in water or various sanitizer solutions for 0.5, 1 or 5 min.

Treatment	Mean surviving populations (log CFU/cantaloupe ± SD) for indicated exposure time (min)			
	0	0.5	1	5
Control (untreated)	7.9 ± 0.4			
Water		7.6 ± 0.3	7.7 ± 0.2	7.4 ± 0.2
40 ppm chlorine		5.8 ± 0.4	5.3 ± 0.3	4.7 ± 0.5
PSP		7.0 ± 0.3	7.0 ± 0.2	6.1 ± 0.3
40 ppm PAA		4.8 ± 0.4	4.6 ± 0.7	<4.3 ± 0.9*
80 ppm PAA		4.4 ± 0.8	4.9 ± 0.6	3.7 ± 0.2
250 ppm PAA		3.8 ± 0.9	3.9 ± 0.3	<3.4 ± 0.6†
40 ppm PAA+PSP blend		4.7 ± 0.7	4.2 ± 1.0	4.2 ± 0.7
80 ppm PAA+PSP blend		<3.4 ± 0.5*	3.5 ± 0.7	3.2 ± 0.5
250 ppm PAA+PSP blend		<3.0 ± 0.4†	<3.0 ± 0.5‡	<3.4 ± 0.8†

SD: standard deviation; PSP: ProduceShield Plus (pH 1.8); PAA: peroxyacetic acid

* *L. monocytogenes* was not detected (<2.7 log CFU/cantaloupe) in one of the six cantaloupes analyzed

† *L. monocytogenes* was not detected (<2.7 log CFU/cantaloupe) in two of the six cantaloupes analyzed

‡ *L. monocytogenes* was not detected (<2.7 log CFU/cantaloupe) in four of the six cantaloupes analyzed

Table 4.2: Mean (n = 6) *Listeria monocytogenes* reductions (log CFU/cantaloupe ± SD) following immersion treatment of whole cantaloupes in water or various sanitizer solutions for 0.5, 1 or 5 min.

Treatment	Solution pH ± SD	Mean reduction (log CFU/cantaloupe ± SD) for indicated exposure time (min)1		
		0.5	1	5
Water	Not Measured	0.3 ± 0.3 $^{E\text{-}z}$	0.3 ± 0.2 $^{E\text{-}z}$	0.5 ± 0.2 $^{E\text{-}z}$
40 ppm chlorine	6.52 ± 0.02	2.1 ± 0.4 $^{D\text{-}y}$	2.7 ± 0.3 $^{D\text{-}yz}$	3.3 ± 0.5 $^{C\text{-}z}$
PSP	1.82 ± 0.01	0.9 ± 0.3 $^{E\text{-}y}$	0.9 ± 0.2 $^{E\text{-}y}$	1.8 ± 0.3 $^{D\text{-}z}$
40 ppm PAA	5.50 ± 0.26	3.1 ± 0.4 $^{CD\text{-}z}$	3.4 ± 0.7 $^{CD\text{-}z}$	>3.6 ± 0.9 $^{BC\text{-}z,*}$
80 ppm PAA	4.43 ± 0.08	3.5 ± 0.8 $^{BC\text{-}yz}$	3.0 ± 0.6 $^{CD\text{-}y}$	4.2 ± 0.2 $^{ABC\text{-}z}$
250 ppm PAA	3.77 ± 0.06	4.1 ± 0.9 $^{ABC\text{-}z}$	4.1 ± 0.3 $^{ABC\text{-}z}$	>4.6 ± 0.6 $^{AB\text{-}z,\dagger}$
40 ppm PAA+PSP blend	1.81 ± 0.02	3.2 ± 0.7 $^{C\text{-}z}$	3.7 ± 1.0 $^{BC\text{-}z}$	3.7 ± 0.7 $^{BC\text{-}z}$
80 ppm PAA+PSP blend	1.81 ± 0.01	>4.5 ± 0.5 $^{AB\text{-}z,*}$	4.4 ± 0.7 $^{AB\text{-}z}$	4.8 ± 0.5 $^{A\text{-}z}$
250 ppm PAA+PSP blend	1.81 ± 0.02	>4.9 ± 0.4 $^{A\text{-}z,\dagger}$	>4.9 ± 0.5 $^{A\text{-}z,\ddagger}$	>4.6 ± 0.8 $^{AB\text{-}z,\dagger}$

SD: standard deviation; PSP: ProduceShield Plus (pH 1.8); PAA: peroxyacetic acid

[1] Cantaloupes had an average initial *L. monocytogenes* level of 7.9 ± 0.4 log CFU/cantaloupe

[*] *L. monocytogenes* was not detected (<2.7 log CFU/cantaloupe) in one of the six cantaloupes analyzed

[†] *L. monocytogenes* was not detected (<2.7 log CFU/cantaloupe) in two of the six cantaloupes analyzed

[‡] *L. monocytogenes* was not detected (<2.7 log CFU/cantaloupe) in four of the six cantaloupes analyzed

[A-E] Means within a column with different superscript letters differ ($P < 0.05$)

[x-z] Means within a row with different superscript letters differ ($P < 0.05$)

Table 4.3: Mean (n = 6) surviving *Listeria monocytogenes* populations (log CFU/mL ± SD) in treatment solutions immediately following immersion treatment (0.5, 1 or 5 min) of inoculated (five-strain mixture; 7 to 8 log CFU/cantaloupe) whole cantaloupes.

Treatment solution	Mean surviving populations (log CFU/mL ± SD) for indicated exposure time (min)		
	0.5	1	5
Water	2.6 ± 0.6	3.3 ± 0.4	3.8 ± 0.2
40 ppm chlorine	ND	ND	ND
PSP	<1.1 ± 0.2*	<1.5 ± 0.5†	<1.0 ± 0.0‡
40 ppm PAA	ND	ND	ND
40 ppm PAA+PSP blend	ND	ND	ND

SD: standard deviation; PSP: ProduceShield Plus (pH 1.8); PAA: peroxyacetic acid; ND: not detected (<1.0 log CFU/mL) in all six samples analyzed

* *L. monocytogenes* was not detected (<1.0 log CFU/mL) in two of the six samples analyzed

† *L. monocytogenes* was not detected (<1.0 log CFU/mL) in one of the six samples analyzed

‡ *L. monocytogenes* was not detected (<1.0 log CFU/mL) in five of the six samples analyzed

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