Meat Powder: A Value-Added Food Ingredient

by

JADE MICHELLE LONG

(Under the Direction of Anand Mohan)

ABSTRACT

Food waste accounts for $161 billion in loss in the US. Meat animal waste and meat products are considered a major contributor of food loss. Utilizing animal by-products in further processing would reduce meat waste and generate economic benefits. A recent study determined the biochemical composition of beef tongue, but no research has evaluated the potential of beef tongue as a value-added, multi-purpose food ingredient. In this study, beef tongue was skinned, chopped, micronized, filtered and spray-dried. The meat powder properties were characterized, and proximate composition, lipid composition, and proteomic profile were evaluated. Results indicated that beef tongue powder retains native protein structure, an abundance of unsaturated fatty acids, and other nutritional elements that show potential for it to be a high-quality functional food ingredient. Future research should include the application and evaluation of beef tongue powder for improvement of quality, sensory, and nutritional aspects of various food products.

INDEX WORDS: spray-drying, beef tongue, animal by-products, meat waste, product development

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DEDICATION

I would like to dedicate my thesis to my parents. Mom and Dad, you have supported me through all my endeavors and have taught me how to trust in the Lord through all circumstances. Mom, you showed me how to never give up, no matter what, and to remember the little things. Dad, you challenged me to be my best and to learn as much as I could in every situation. I’m so blessed to have you both in my life. Thank you for all you have done for me.

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CHAPTER 1

INTRODUCTION

Food waste and food loss are a growing economic, environmental, and social concern. Food loss is defined by the Food and Agriculture Organization as any food that is disposed of or discarded during harvesting and processing, and throughout the supply chain, while food waste is any food that is disposed of or discarded by foodservice, consumers, or retailers (FAO, 2019a). In 2019, about 14 % of globally produced food was lost prior to entering retail markets and 31 % of retail food became food waste (FAO, 2019b; Gustafson, 2019). This totals 1.3 billion tons of food and $161 billion per year according to the 2010 market (FDA, 2020). One of the contributors to food loss and food waste is meat. Meat and animal products account for approximately 13 % of global food waste and loss (FAO, 2019b) and continue to be a growing global issue. In developing countries, meat waste accrues due to poor processing and storage conditions, while in developed countries, meat waste accrues due to consumers discarding what is still fit for human consumption (Vilariño, Franco, & Quarrington, 2017). Meat waste has the highest environmental impact with greenhouse gas emissions representing 38 % of all food waste and food loss (Porter, Reay, Higgins, & Bomberg, 2016). Additionally, meat waste holds the highest value, 41 %, in economic loss (Buzby & Hyman, 2012), thus significantly impacting world hunger and food security (Kummu et al., 2016).

Commonly, meat by-products such as organs, blood, and bone are unused or discarded due to the lack of consumer interest/ palate preference. The majority of organs or rather variety meats are exported by the United States to markets in the Middle East, Egypt, Peru, and Mexico

(US Meat Export Federation Report, 2020), while animal blood and bone are repurposed as nutrient sources in animal feed and pet food (Javeed & Khan, 2016; Toldrá, Mora, & Reig, 2016). Variety meats, including heart, liver, kidney, tongue, and brain, are known as high-quality protein and a good source of vitamins and minerals (Biel, Czerniawska-Piatkowska, & Kowalczyk, 2019). Because of the increase in the global population, as well as, the need for affordable and nutritive protein sources, meat by-products are likely to increase in value.

To assist in the reduction of food loss and food waste, industry and academia are developing value-added products and ingredients. The conversion of food waste into a value-added product is a process in which the economic value of the food waste is evaluated and exploited for creating an ingredient or a product that has further utilization by the consumers or industry. Value addition includes the increase in nutritional macro and micronutrients, vitamins and minerals, as well as quality improvement. Recent reviews by Gullón, Astray, Gullón, Tomasevic, and Lorenzo (2020) and Munekata et al. (2020) discuss bioactive compounds with antioxidant ability, found in pomegranate peels and plant extracts, respectively, that have the ability to stabilize lipid oxidation and retain the quality of meat products. These polyphenols and essential oils enable a natural addition of an antioxidant and antimicrobial with health benefiting properties while utilizing food waste.

The stability of a food product is vital to its safety and quality. Food powders are a historically shelf-stable product and are convenient for transportation and storage (Intipunya & Bhandari, 2010). Low water activity (< 0.4) and moisture content, make food powders unsuitable for microbial growth and a relatively stable product with a long shelf-life. Although food powder is easy to handle and using meat by-products as an ingredient would reduce food waste, muscle powders are yet to be explored. Beef tongue is a widely available, underutilized meat by-product that may have potential use as a muscle-based food powder. Beef tongue is determined to possess not only protein, but fat consisting of mostly mono- and poly-unsaturated fatty acids that would improve the nutrient content of a food product (Warren, Bowker, & Mohan, 2020). This study hypothesized that a spray-dried meat powder would retain valuable nutrients and healthy fats found in raw beef tongue as well as potential non-denatured proteins that may possess functional properties. The objective of this study was to develop and characterize beef tongue powder for value-added applications. Specific objectives included evaluating beef tongue for the absence of tissue defects through meat color, NADH concentration, pH, and metmyoglobin reducing ability (experiment 1), and determining the characteristics and properties of beef tongue powder through proximate composition, lipid composition, and proteomic profiling (experiment 2). Little research has been done on the development of meat powder, the opportunity to grow the application of meat by-products, specifically beef tongue, into a value-added ingredient will increase the economic value of these by-products in the meat industry.

CHAPTER 2

LITERATURE REVIEW

2.1. Animal by-products and variety meats

2.1.1. Commercial impact

For the past 10 years, over 20,000 metric tons of variety meats are exported from the U.S. each month. This reaches a value of almost $1 million annually in product value (US Meat Export Federation Report, 2020). Variety meats and animal by-products continue to play a major role in the meat export business, due to the palate preferences of the American consumer. For Americans, beef is expected to be a burger or steak and nothing more, therefore, the organs and other by-products must be internationally purchased in order to be profitable. Specifically, over 90% of beef tongues are exported yearly, mostly to Mexico and the Middle East, at a price of $5 per pound (USDA-AMS, 2020). In 2015, the U.S. Meat Export Federation reported 82% of beef livers were exported to Egypt, and that Peru has an increasing interest in beef hearts. The beef variety meat market is expanding, therefore, growing the industry's involvement through value-added products would be of economic interest.

2.1.2. Regulation and classification

With the consideration of using variety meats and animal by-products as an ingredient for food applications, regulations and classification must be utilized for the safety of consumers. The USDA has methods for evaluating the safety of meat, poultry and eggs to ensure the health and benefit of consumers. The method used to regulate and classify animal by-products is the hazard

Specific Risk Material (SRM) standards. These standards classify the meat products into three categories. Animal by-product Category 1 is deemed the most severe as it includes the cuts with possible bovine spongiform encephalopathy (BSE) and what can only be co-incinerated and/or rendered. Category 2 is for the cuts derived from an animal who died on the farm or had too high of a drug concentration (i.e. antibiotic) and the digestive tract cuts. Category 3 was meat intended for human consumption but were retail and catering wastes (Henchion & McCarthy, 2019). Aside from these categories, the edible by-products and offal are inspected, frozen, and often exported (US Meat Export Federation Report, 2020).

2.1.3. Other uses

Animal by-products are often considered “unfit for human consumption”, meaning they are either considered contaminated with bovine spongiform encephalopathy (BSE), antibiotics, or harmful microorganisms. In order to make some profit, by-products are redirected to industries outside of human consumption. For example, due to the high protein composition of organs, including brain, heart, kidney, liver, lung, spleen, and tongue, bioactive compounds can be extracted and sold as a value-added product. Furthermore, co-products such as bovine skin and tendon tissues are used in biomedicine for tissue engineering. They are considered an ideal raw material because of their abundance in extracellular matrix proteins (collagen) as well as their ability to be shaped or reconstructed (Mullen et al., 2017). One of the most economical uses involves the pet food and animal feed industry. As mentioned before, category 3 meat (retail and catering waste) does not meet the requirements for human consumption but it still contains desired nutritional properties, and it is more digestible compared to plant-based ingredients thus making it the preferable ingredient. Additional animal by-products can include meat and bone meal ashes that are a good source of minerals and trace elements (Javeed & Khan, 2016; Toldrá et al., 2016).

2.1.4. Chemical composition

Animal by-products are known as processing coproducts to meat scientists. Specifically, liver, heart, kidneys, tongue and sweetbreads are defined as "edible offal" (Henchion & McCarthy, 2019). These meat cuts (edible offal) contain all essential amino acids as well as digestible phosphorus and does not include anti-nutritional factors or allergenic proteins, thus making these an excellent feed ingredient (Mora, Toldrá-Reig, Reig, & Toldrá, 2019). A study completed by Biel et al. (2019) quantified the chemical composition of beef by-products/offal. The experiment enabled the comparison of the liver, heart, kidney, tongue, and brain in protein, fat, and ash as a percentage of wet tissue, as well as the concentration of minerals in the ash (Figure 1.). Another study by Warren et al. (2020) determined the physicochemical properties of beef tongue and the proximate composition values are shown in Figure 1. Furthermore, Figure 1. indicates that the liver has the highest concentration of protein, followed closely by the kidney, heart and tongue, while the brain has the lowest. The tongue and the brain have the highest percentage of fat, and the percentage of ash is indistinguishable among all by-products. The characterization of the fat found in the beef tongue was included in the experiment by Warren et al. (2020) and concluded that oleic acid (33.63%) and palmitic acid (23.87%) were the predominant fatty acids. By understanding the basic composition of each variety meat, it allows for further consideration of their nutritional and functional value.

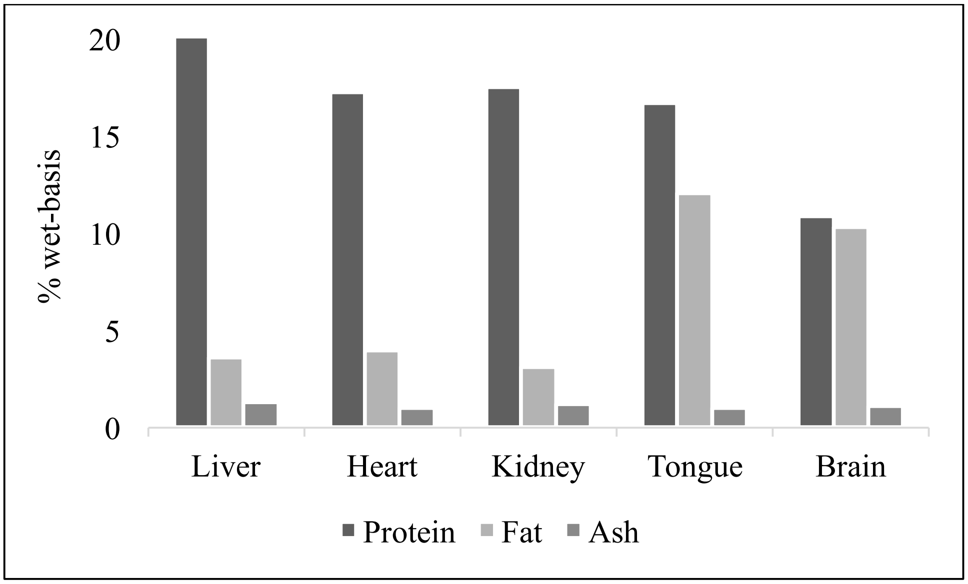

Figure 1. Percent composition of protein, fat, and ash in beef by-products (liver, heart, kidney, tongue and brain) in wet tissue (Biel et al., 2019).

2.2. Beef tongue

Beef tongue is a skeletal muscle that consists of primarily Type I (slow-twitch) oxidative fibers, and contains an abundance of mitochondria and enzymes, including structural proteins, such as actin and myosin. (Li et al., 2015; Warren et al., 2020). Aside from some recent studies that investigated the physicochemical properties, chemical composition, and the development of a cured beef tongue product, there is limited research involving further application of beef tongue as a meat product/ source of meat protein (Wioletta, Ewa, & Alicja, 2019; Zhu et al., 2018). The majority of beef tongue research focuses on the muscle in its raw state. One study published in 2008 investigated the risk of exposure to BSE through consumption of the lymphoid tissue in beef tongue (European Food Safety Authority, 2008). The conclusions of this data stated that the risk of exposure to BSE is low in beef tongue even with the current harvesting methods. Another study by Gonulalan, Kose, and Yetim (2004) compared quality characteristics when smoking beef tongues with liquid smoke versus vaporous smoke, and determined liquid smoke as an acceptable alternative. The implementation of the liquid smoking method would provide an acceptable product while transitioning this sector of the meat industry to a simple method that is more sanitary and sustainable (Schneck, 1981). Before these studies were conducted, the only published research involved beef tongue jerky and lactic acid treatments for quality retention of beef tongue; therefore, further investigation into the biochemical properties and functional proteins of beef tongue will expand the knowledge and application of this bovine muscle (Carr et al., 1997; Visser, Koolmees, & Bijker, 1988).

2.3. Beef heart

There is a plethora of research involving beef heart, with the majority of the studies focusing on the mitochondria. Many of these studies concentrated on the function, enzymatic processes, and response characterization of mitochondria in beef heart (Fernández-Morán, Oda, Blair, & Green, 1964; Hall & Crane, 1971; Kohutiar et al., 2016). One article by Kohutiar, Eckhardt, Mikšík, Šantorová, and Wilhelm (2018) investigated protein nitration in beef heart mitochondria and determined that proteins, including serum albumin, cytochrome C, myosin and NADH dehydrogenase, are endangered by the presence of peroxynitrite. Due to the oxidative role of nitric oxide, maintaining the balance of pH and nitration is vital when evaluating mitochondrial proteins and their functions (Schiffer, Lundberg, Weitzberg, & Carlström, 2020). Beef heart is the central focus of these research studies because it is known to contain large amounts of mitochondria (Smith, 1967) due to its energy demanding functions (Knutsen, 2019), and the methods for extraction and isolation of mitochondria were determined and simplified (Truman, 1964). There are some studies on beef heart that are not focused on mitochondria, such as, using beef heart as an animal feed component to improve the nutritional value, and the quantification of the retention and digestibility of coenzyme Q10 in beef heart when cooking or digesting. (Ercan & El, 2011; Wen, Chen, Qu, & Gao, 2018). From a meat industry standpoint, beef heart is considered edible offal that is a less expensive protein option for the middle class in foreign countries. In fact, countries such as Indonesia have adjusted their trade policies to include more offal in order to stabilize beef prices, thus creating a larger market for beef heart (Tenrisanna, Rahman, & Khanam, 2016).

2.4. Muscle-based products with value-added applications

Value-added products are a rapidly growing sector in the food industry. As more attention is drawn to food waste and the discovery of valuable compounds within food, the industry has begun developing new products. A few studies, such as the ones conducted by Chan, Omana, and Betti (2011), Li, Kang, Zhao, Xu, and Zhou (2014) and Zhao et al. (2016) prove that myofibrillar protein (MP) gelation properties of pale, soft, exudative (PSE)-like chicken can be improved when processed under pressure (50 and 100 MPa), using high-intensity ultrasound, or isoelectric solubilization/precipitation (ISP). Furthermore, a review by Chen et al. (2018) discussed how the improvement of MP gelation properties on inferior muscle, PSE-like chicken, can be used in industry to create products with lower sodium chloride and phosphate concentrations that have similar textures and water holding capacity (WHC) to those using normal chicken meat. Additional meat products such as, pork frankfurters, were evaluated under high pressure processing and the research showed that the pressure gradient highly influences protein solubility and gelatinization, which allows for a lower concentration of sodium chloride with an improvement in WHC (Tintchev et al., 2013). Both the findings in the studies conducted on PSE-like chicken and pork frankfurters indicate an ability to create a value-added product. Lower sodium chloride would decrease the total sodium level in the product which improves the nutritional value of the product. With the creation of value-added products, food waste is reduced, products' health attributes are influenced, and the functionality of ingredients is expanded (Laufenberg, Kunz, & Nystroem, 2003; Rasane, Jha, Sabikhi, Kumar, & Unnikrishnan, 2015).

2.5. Food Powders

Food powders are well integrated into the industry as spices but it was infant formula that truly sparked the exploration of method development for encapsulating fat, improving shelf-life, and creating uniform particle size (Mohammed, Tan, Manap, Muhialdin, & Hussin, 2020; Moskop & Nasea, 2007). Powder is now the most common format of food and is considered the "technical solution for food production" (Bhandari, 2013; Cuq et al., 2013). This title is attributed to the characteristics that powder can bring: low bulk weight, diverse application, relatively high stability, and convenience in storage and transport (Intipunya & Bhandari, 2010).

The food industry uses three common methods for food powder production: vacuum-drying, freeze-drying, and spray-drying. Of these three, freeze-drying is considered potentially the best method for removing water and producing a high quality product, since the low temperature and absence of liquid water prevent microbial and other deterioration reactions (Ratti, 2012). Freeze-drying uses a two-step drying process preceded by freezing. By inducing the phase change from solid to liquid, decreasing the vapor pressure below equilibrium, and then heating causing sublimation, the water is removed from the product. The secondary drying stage occurs with desorption (Ratti, 2012; Stapley, 2008). Although freeze-drying is highly effective in the production of food powders, it is also the most expensive. The amount of energy necessary to freeze, vacuum, condense, and reheat a product while avoiding temperatures that would deteriorate the product, poses an economical hurdle. A study was conducted in 2014 on the intensification of freeze-drying (Pisano, Fissore, & Barresi, 2014). The process of vacuum drying is effective in removing water however, it is time consuming. For example, 7 hours at 50 °C were required to dry a disk of egg yolk 4-mm thick (Xiting, Jiaqi, Yu, Wenxue, & Jinling, 2020). In order to correct this, a new method was developed using microwaves that allowed a decrease in drying times, however, the resulting food product still presented inferior food quality due to the prolonged heat exposure (Shu et al., 2020). Both methods, vacuum-drying and freeze-drying, produce a product that has low water activity and is easier to handle and ship, but they still require post processing of the product, such as grinding, in order to create a food powder. Unlike these two methods, spray-drying simultaneously dries and creates a powder.

2.6. Spray-drying

2.6.1. Purpose and potential benefits of spray-drying

Spray-drying is an inexpensive and flexible process that is continuously being improved for optimization and quality (Dyvelkov & Sloth, 2014; Mohammed et al., 2020). It possesses a multi-scale operating capacity with high throughputs at a relatively low cost (Dyvelkov & Sloth, 2014; Zuidam, 2012). The purpose of spray-drying is defined as: (i) dehydration for preservation, ease of transport, storage and handling, (ii) the encapsulation of bioactive compounds, and (iii) the inducement of phase transformation (Ho, Truong, & Bhandari, 2017). Although spray-drying presents a low temperature drying process, spray-drying does reach a time-temperature ratio that poses an issue when working with probiotics. The positive aspect is the time-temperature component which is proven to reduce microbial loads and eliminate select viruses (Polo et al., 2005). Dehydration decreased the moisture content and thermal processing reduced the survival of bacteria and pathogens (Champagne & Fustier, 2007; Ghandi, Adhikari, & Powell, 2013). Spray-drying is not a proven kill step for pathogen and/or spoilage bacteria, however, a microbial reduction does occur because of the process design. With these characteristics and abilities, spray-drying has made its way to being a top contender for the production of powders (Ghandi et al., 2013).

2.6.2. Spray-drying processing method

Spray-drying is a single-step process of converting a liquid feed solution or slurry into a dried powder. The method for this conversion is described as the atomization of the feed liquid into solid microparticles through flash drying with a hot drying gas (Gaspar, 2014). The microparticles are separated from the gas by means of a cyclone and are collected as either a powder or granules. A diagram of spray-drying equipment is shown in Figure 2. The feed liquid is pumped into the nozzle/atomizer concurrently with the hot gas and is sprayed into a drying chamber. The particles are drawn and collected into a cyclone after the particles are separated from the gas. Residual feed liquids (not fully dried) are collected at the base of the drying chamber and the excess gas is expelled as exhaust.

In order for spray-drying to be effective, the settings, inlet temperature (temperature of drying gas), pump rate, Q-flow (pressure of trying gas), vacuum, and aspirator, must be selected and defined (Dobry et al., 2009). A study by Kurozawa, Morassi, Vanzo, Park, and Hubinger (2009) evaluated the effect of processing parameters on product properties. They determined that the inlet temperature is directly related to the temperature of the particles (outlet temperature) which is known to affect the moisture content and color of the product; a high Q-flow coupled with high moisture in the slurry decreases the density of the product, and high inlet temperature combined with high Q-flow and a thin slurry creates a more uniform particle size. In order to make sure uniform atomization is achieved, the slurry must have a consistent temperature, homogeneity, density, and moisture content throughout (Kurozawa et al., 2009; Masters, 1972).

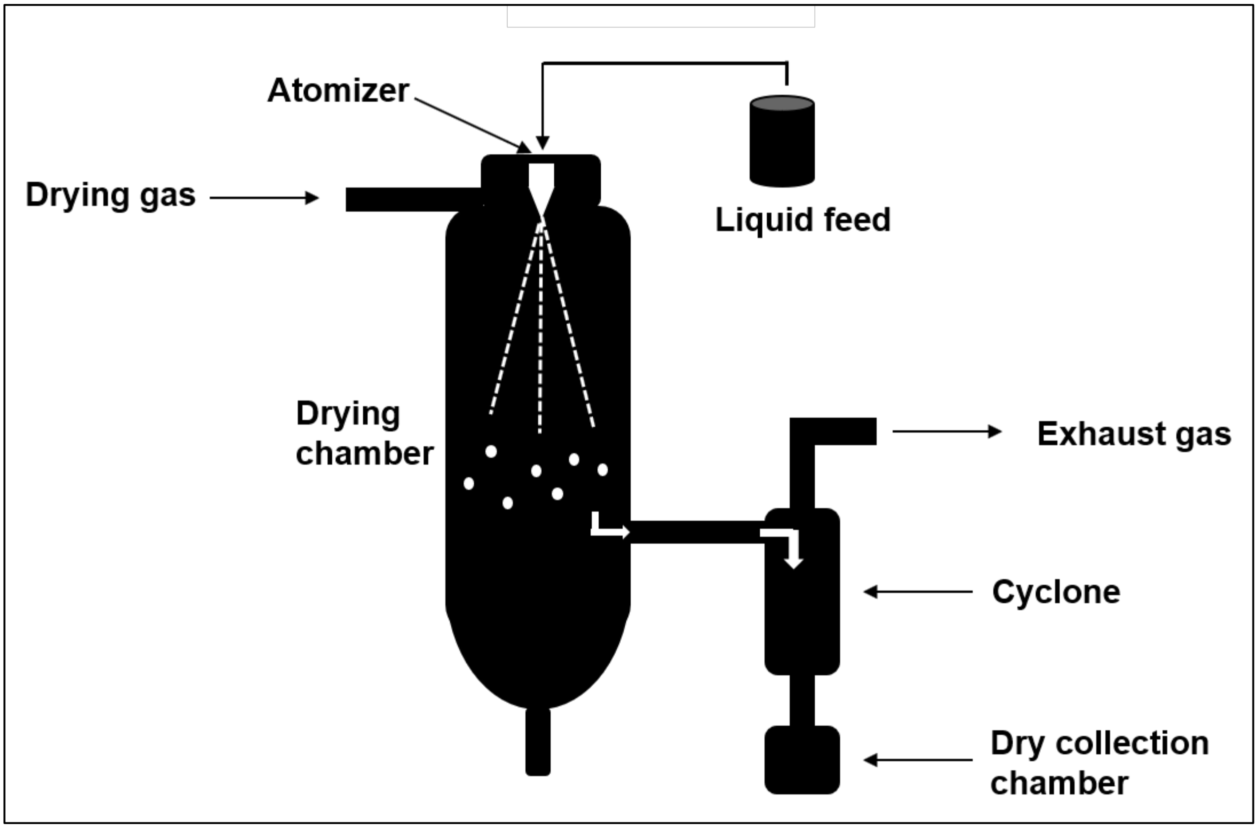

Figure 2. Diagrammatic representation of spray drying equipment and its process flow.

Processing limitations such as the clogging of the pump and/or nozzle and improper drying affect the efficiency of the process as well as the quality of the powder. The feed liquid must contain homogenate particles that are small enough to fit the diameter of the pump and/or nozzle in order to avoid clogging and variation in particle sizes of the powder. Spray-drying with a high pump rate can cause improper drying and agglomeration of the particles, which can lead to high moisture content and variation in particle size (Santos et al., 2017). Through proper preparation of the feed liquid, these processing limitations can be avoided.

2.6.3. Spray-dried animal derived foods

Spray-drying of skeletal muscle tissue has yet to be explored in detail, therefore, concerns regarding the validity of this process may arise. The concerns likely include, the preservation of native protein throughout thermal processing, effects of the muscle composition, and whether there is successful methodology for spray-drying meat. Because the functionality of the protein is desired to remain intact, the temperature selected for processing must be low enough to keep the proteins from fully denaturing. The spray-drying method ensures the best outcome for native proteins due to the outlet temperature (temperature of the sample/powder) being much lower than the temperature of the gas that is drying the sample (H. Wang et al., 2020). Skeletal muscle contains a substantial amount of fat, which can pose an issue while spray-drying, therefore, carrier agents such as maltodextrin (MD) are used. Carrier agents assist in spray-drying through their properties of low viscosity and high solubility (Varastegani et al., 2017). A study by Haluk, Yeliz, and Orhan (2018), compared whey powder isolate (WPI), MD and a combination of the two as carrier agents for spray-dried bone broth powder. MD was determined as the most suitable regarding encapsulation, shelf-life studies, and solubility. A study conducted by Ran, Zhang, Wang, and Liu (2019) outlined effective methods for spray-drying chicken products. Chicken breast meat and skeletons were trimmed of fat, cooked, homogenized, and incorporated with a protease and flavoenzymes to shorten peptide lengths for improved flavor. The slurry was then diluted and spray-dried. This is a proven method for spray-drying meat. Products such as, chicken powder, dehydrated chicken powder, and chicken broth powder, are all examples of industry available spray-dried meat that follow this processing methodology (Ran et al., 2019).

2.7. Functionality of skeletal muscle protein

2.7.1. Protein functions in food systems

Proteins play an important role in food systems. Due to their abilities to form gels and protein networks, aerate, bind ions, participate in mineral transport etc., proteins have physical and biological functionality (Bhat, Kumar, & Bhat, 2015). Proteins can decrease the drip loss by improving the water binding capacity (WBC) of food product (Li et al., 2015); they set a matrix for stability, i.e. gelation; they act as an adhesive material through cohesion and adhesion; and they create elasticity with the formation of disulfide bridges (Weder & Belitz, 2003). Additionally, they interact with fats for stabilization with emulsification and adsorption. The intensity of protein's functionality is determined by the characteristics of molar mass, amino acid profile, and number of intramolecular disulfide bonds. It is desired for them to have a low molar mass, a balanced amino acid profile in terms of charges, a high number of intramolecular disulfide bonds, and good solubility in water.

2.7.2. Water holding capacity

WHC is defined as the ability of a food to hold its own water or added water during the application of force, pressure, centrifugation, or heating. It is important to evaluate its Water Holding Capacity (WHC), solubility, isoelectric point (pI), and protein composition when considering a protein's functionality. Each of these properties plays a role in the effectiveness of the protein's ability to exhibit functional properties in a food system and in turn impact macro traits such as meat quality. In the meat industry, the focus lies primarily on WHC since millions of dollars rest in the success of water retention in muscle tissue (Huff-Lonergan & Lonergan, 2005). In meat or meat products, WHC determines the visual acceptability, cook yield, weight loss, and sensory traits (Warner, 2017). Furthermore, WHC affects the protein composition.

When meat purges it loses a significant amount of protein (Huff-Lonergan & Lonergan, 2005). One study demonstrated approximately 112 mg of protein is lost per mL of fluid (Savage, Warriss, & Jolley, 1990). During the post-mortem conversion of muscle to meat, the WHC of the meat can be influenced by protein oxidation, the rate and extent of pH decline, and proteolysis (Huff-Lonergan & Lonergan, 2005).

2.7.3. Location of water

In order to understand WHC, it is imperative to know the locations of water in the muscle. Water is found in both the intra- and extramyofibrillar spaces. Specifically, the water is held within myofibrils, between myofibrils, between myofibrils and the cell membrane, between muscle cells, and between muscle bundles. The water is classified as either *bound water*, *entrapped/ immobilized water*, or *free water* depending on the location. Bound water exists around non-aqueous constituents and has extremely low mobility. The polar water is attracted to charged molecules such as proteins. Bound water makes up a very small portion of total water in the muscle and is resistant to freezing, and loss when heated. Entrapped water is prone to freezing but still has a slight resistance to movement due to the attraction to bound water and due to steric effects. This type of water is known to leach out when the cell structure changes, which most often occurs because of a decrease in pH. Free water is the source for the majority of water loss. This is because the water molecules are bound only by weak surface forces that are easily broken by heating, freezing, compression…etc. (Huff-Lonergan & Lonergan, 2005).

2.7.4. Post-mortem muscle pH and rigor mortis

The stiffening of muscle post mortem is known as rigor mortis. During rigor mortis, skeletal muscle fibers contract and lose flexibility (Cadavez, Xavier, & Gonzales-Barron, 2019) which influences WHC. Post-mortem muscle pH declines because of the buildup of lactic acid.

As the pI is reached for major proteins, such as myosin (pI= 5.3), the positive and negative charges, of the proteins begin to neutralize resulting in the entrapped/immobilized water becoming free and easily lost (Huff-Lonergan & Lonergan, 2005; Vega-Warner & Smith, 2001). Furthermore, the repulsion of the proteins decrease as the net charge approaches zero allowing the myofibrils to pack tighter, thus reducing the space available for water. These changes cause the entrapped/immobilized water to move to the extramyofibrillar spaces. In conjunction with the effects of pH, rigor mortis includes permanent cross-bridging between myosin molecules that constitute the thick myofilaments and actin molecules that constitute the thin myofilaments. This also affects the movement of the entrapped/immobilized water and the water already present in the extramyofibrillar space. Both the free and entrapped/immobilized water is forced out of the muscle, and the water that is leached is known as purge (Huff-Lonergan & Lonergan, 2005). A recent study using Nuclear Magnetic Resonance (NMR) supports the theory that water located in a myofibrillar protein matrix that includes actin and myosin filament structures can be detected and assigned to a specific marker, and it proved that WHC is mainly determined by the amount of entrapped/immobilized water in the extramyofibrillar space (Bertram, Purslow, & Andersen, 2002). This study demonstrated that NMR is a successful tool in understanding WHC and drip loss by the movement and dispersion of water as well as the structure of the meat.

2.7.5. Amino acid composition

In addition to determining total protein content in a protein source, it is also important to identify what amino acids are present, especially the presence of essential amino acids. The nine essential amino acids are: histidine, lysine, tryptophan, isoleucine, leucine, valine, methionine, threonine, and phenylalanine (Wax, 2019). The Essential Amino Acid Index (EAAI) is defined as the ratios of essential amino acids in a protein relative to their respective amounts in the entire protein. There is a flaw with this measurement since it recognizes protein when fully digested and therefore over-estimates the biological value. This can be corrected with the inclusion of the digestibility factor specific to the food type. This measurement is referred to as the Modified Essential Amino Acid Index (MEAAI). In a study that obtained the MEAAI, biological value, and Chemical Score Index (CSI) of various food types in comparison to whole egg protein, lean beef has a MEAAI of 62.3, biological value or 74.3 as reported by the FAO, and a CSI of 61.4. This study also revealed that methionine and cystine were the limiting amino acids (Ihekoronye, 1988).

2.7.6. Amino acid analysis

As mentioned in section 2.7.2, protein composition also has a role in protein functional properties. Amino acid analysis (AAA), involves the determination of the overall protein content as well as identifying specific proteins. Protein content is measured by nitrogen concentration as determined by the Kjeldahl method, which is a standard accepted by the Association of Analytical Communities. The Food and Agriculture Organization (FAO) recommends a 6.25 factor for meat when determining protein content by nitrogen concentration. The FAO also recommends identifying proteins with ion exchange and gas-liquid or High Performance Liquid Chromatography (HPLC) followed by mass spectrometry (FAO, 2003). By determining the specific proteins present as well as their concentrations, the functional properties of meat can be further understood.

2.7.7. Amino acid score, protein digestibility, and cooking effects

A standard used in order to determine if a protein is complete, meaning it contains all essential amino acids, is the Protein Digestibility Corrected Amino Acid Score (PDCAAS). This score is out of 1.0, and beef/red meat possesses the score of 0.9. Red meat has 94% digestibility compared to beans with 78% and whole wheat with 86% (Williams, 2007). Protein digestibility is often evaluated for nutritional claims but is also an indicator of the integrity of protein properties. The cooking process affects the physical, chemical, and structural properties of meat proteins, specifically, the temperature duration and the method of cooking. It is proven that cooking at 100 °C decreases protein digestibility, and protein denaturation has been shown to occur at 70 °C. Protein digestibility is evaluated using a Digestible Indispensable Amino Acid Score (DIAAS) per FAO recommendation. The DIAAS is a test for true ileal amino acids and evaluates what is actually absorbed/digested. A study conducted in 2018 studied the effects of various cooking methods on the DIAAS. For beef, the DIAAS were not significantly different from the raw values when boiled and pan fried. The beef was boiled at 80 °C for approximately 15 minutes and pan-fried at 186 °C for approximately 12 minutes. The only notable effect the cooking process had on the beef was the ileal digestibility of histidine. This study showed that DIAAS of beef is affected by the cooking method (Hodgkinson, Montoya, Scholten, Rutherfurd, & Moughan, 2018).

2.7.8. Radicals and oxidation

Meat oxidation affects lipids, proteins, pigments and vitamins which can lead to nutritional loss and sensory degradation (Domínguez et al., 2019). Therefore, further AAA involves evaluating protein radicals and protein oxidation. Prior to slaughter, animal muscle contains an antioxidant system that allows for the radicals (compounds with unpaired electrons), specifically tyrosine and histidine, to be formed and captured as participants in electron transfer (Morozova, 2020). As proteins are oxidized, the antioxidant system is lost since the proteins can no longer bind to the radicals. Reactive species, such as molecular oxygen, hydrogen peroxide, hypochlorous acid…etc. are responsible for the oxidation of proteins. One protein that undergoes this process is perferrylmyoglobin, which derives from the reaction of ferryl hemoglobin and hydrogen peroxide. When perferrylmyoglobin is oxidized it can induce intra- and intermolecular protein cross-linking via tyrosine and it can generate protein radicals. With the presence of unregulated radicals, issues with the nutritional value of meat and toxic oxidized amino acid formation can arise (Papuc, 2016). In order to observe the radicals, NMR can detect hyperpolarized signals of the diamagnetic radical products (Morozova, 2020).

2.8. Myoglobin

Myoglobin (Mb) is a globular protein molecule consisting of two parts: heme, the non-protein portion, and globin, the peptide portion. It is a low molecular weight protein (16 kD) that is exclusively found in the heart and skeletal muscle (Simsa et al., 2019), specifically, the sarcoplasmic fraction (Wilson & Reeder, 2006). The abundance of this protein is dependent on the muscle tissue type, as the more oxidative the muscle fiber, the higher the Mb concentration (Feher, 2017). This correlation is based on the oxygen-binding properties of heme or rather the presence of Fe^{2+} located within the heme group (Harvey, 2008). Aside from the primary role, to facilitate oxygen transportation in muscle cells, Mb also stores oxygen for later use by mitochondria in oxidative processes such as respiration (Suman & Joseph, 2013). Additionally, Mb participates in the regulation of NO, the transportation of fatty acids, and provides antioxidant protection in tissues (Postnikova & Shekhovtsova, 2018).

In comparison to hemoglobin (Hb), Mb is a monomer consisting of one globin with an affiliated porphyrin ring, while Hb is a large molecule with four porphyrin groups and four polypeptide chains (Powell, 2015; Richards, 2010). Although Hb contains four iron molecules that enable four oxygen molecules to be carried simultaneously, Mb has a higher affinity for oxygen and therefore accepts oxygen from Hb at the muscle cell interface and releases the bound oxygen under low oxygen pressures (Meuse, Hill, Nguyen, Frignoca, & Wells, 2019). Hb is the predominant iron containing protein in meat tissue prior to slaughter, but due to Hb being located in the blood, a post slaughter, properly bled animal carcass will contain little Hb and mostly Mb (Suman & Joseph, 2013). In fact, Mb accounts for roughly 95% of the iron in the muscle tissue of a slaughtered animal, thus making it the primary protein influence in meat color and therefore, meat quality (Sammel, Hunt, & Kropf, 2001).

2.8.1. Heme

Heme has a planar structure centralized by an iron atom which is surrounded by four nitrogen containing heterocyclic pyrrole rings connected by methine bridges (Layer, 2020) (Figure 1). This system is known as protoporphyrin IX and is the protein active site of Mb (Ohkouchi & Takano, 2014). Iron has six outer-shell electrons that can participate in bonding. Four of these are used to bind with nitrogen in the pyrroles, leaving the other two to bind with ligands that are capable of approaching an octahedral environment. The apoprotein, fifth ligand, is often a histidine residue but can also be methionine, cysteine or tyrosine residues (Everse, 2004). The sixth ligand determines the energy level of the molecule and the reactivity of the iron center (Sahu & Goldberg, 2016). Heme iron can bind to high energy ligands such as O_2, NO, CO, CN^-, and N_3, create a powerful electrostatic field that lengthens the gap between the outermost orbitals in iron (Sammel et al., 2001). Electron movement induced by the molecule bound at the sixth coordination accounts for the colors of Mb and the colors seen in meat.

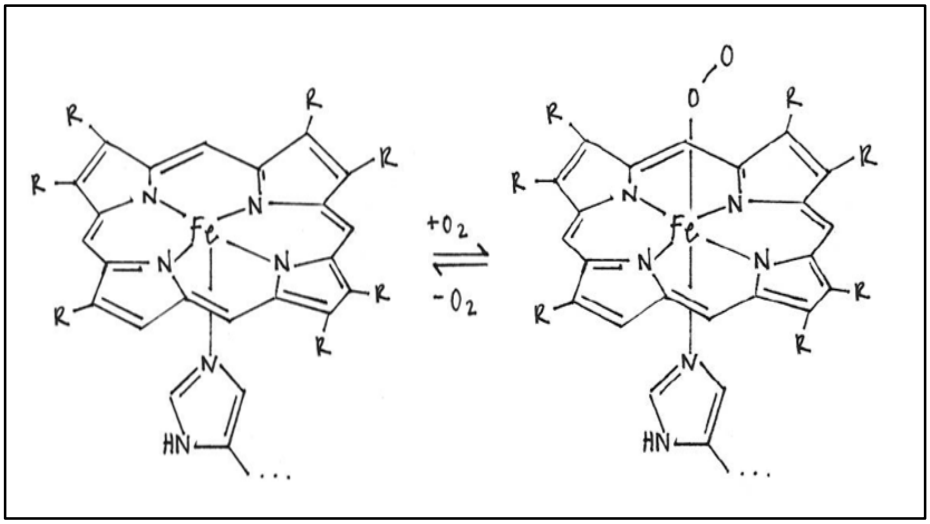

Figure 3. Diagrammatic representation of heme structure and reaction of heme with a dioxygen molecule.

2.8.2. Globin

Globin consists of approximately 150 amino acids (Hankeln & Burmester, 2008). These peptide chains form eight alpha helical ribbons that fold and surround the hydrophobic heme protoporphyrin IX (Wilson & Reeder, 2006). Although the hydrophobic core favors binding to heme, the actual connection is bound through the histidine, located on helix F in position 8, and the heme cofactor, using iron's fifth coordination (Freitas, Saito, Wan, Hou, & Alam, 2008). Globin is found in several oxygen regulating molecules, whether as a monomer such as Mb, or as a polymer such as Hb (de Vaal, 2017). This plays a role in the function of globin when oxygen is bound to the molecule. An effective way to observe the interaction is through oxygen saturation of Mb and Hb as it relates to the partial pressure of oxygen. This is known as the hyperbolic dissociation curve. Due to the cooperativity of globins in Hb, the oxygen affinity decreases more rapidly than Mb (Williamson, Carrell, & Lehmann, 1994). This property explains the storage and transport functions of Mb and Hb, respectively. Additionally, the configuration of these molecules ensures stabilization of the electrons gained/loss during oxygenation/deoxygenation. In relation to meat, factors such as high temperatures and acidic pH are known to cause negative effects on the structure of globin. Under these conditions, the molecule will change the structure and expose the iron core for potential oxidation (Sammel et al., 2001).

2.8.3. Color stability

Color stability is the retention of meat color during its shelf-life. This parameter is measured separately from microbial shelf-life due to the fluctuation of color throughout the shelf-life. Myoglobin exists in various forms depending on the ligand in the sixth position of the heme porphyrin ring. Each one of these forms influence the color of meat. Myoglobin in its native state (deoxymyoglobin) has a ferrous iron center and displays a purplish-red color. It is in the presence of a dioxygen molecule that the color will change to a bright cherry red which indicates the formation of oxymyoglobin (American Meat Science Association, 2012). Furthermore, in the presence of nitric oxide, the meat will also turn bright red. When myoglobin is oxidized the ferrous iron becomes ferric, thus creating metmyoglobin, and the meat will change from purple to brown. Often times other deriviatives form throughout the redox reaction of myoglobin. For example, oxidized porphyrins will create a faded green color which can still remain after heat is applied. Figure 4 shows the reversible and irreversible reactions of Mb in the presence of nitric oxide, oxygen, and heat.

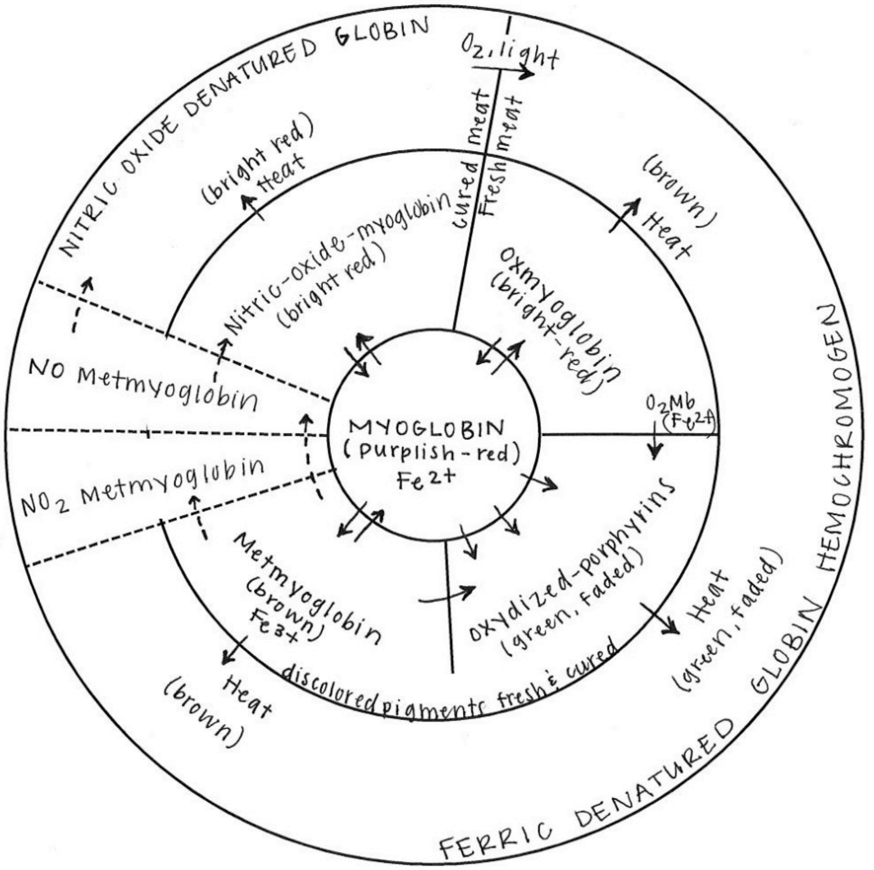

Figure 4. Myoglobin isoforms and derivatives in meat.

Due to the emphasis placed on meat color by the consumer (Ramanathan, Hunt, et al., 2020; Tomasević et al., 2019), many studies have evaluated the need to quantify acceptable and unacceptable color. The American Meat Science Association (AMSA) released a color guide in 2012 that outlines specific methods for measuring meat color. These methods have become a standard but the efficiency of these methods are constantly being improved. One study by Hernández, Sáenz, Alberdi, and Diñeiro (2016) evaluated the Krzywicki (1979) and KS methods using total pigment content and CIELAB to determine an accurate description of the appearance of fresh meat. The quantification of the content of oxymyoglobin, deoxymyoglobin and metmyglobin during storage enables the determination of the relationship between storage time and consumer acceptable meat. The results proved that the KS method was more accurate, metmyoglobin was a strong indicator for storage time, and using total pigment content fully explains the L* values (lightness).

An excellent review published in 2020 provides a collection of material about the color stability as it pertains to a specific muscle. Skeletal muscle such as *Psoas major* and beef tongue are conducive to continuous contraction and relaxation of the muscle. The color of the tissue correlates to the fiber type (Type I or Type II) (Ramanathan, Hunt, et al., 2020). Type I fibers are known as red fibers and have a higher content of mitochondria and myoglobin, while Type II fibers are known as white fibers and have a lower concentration of mitochondria and myoglobin (Warren et al., 2020). The characterization and classification of color stability in 19 different bovine muscles was completed by McKenna et al. (2005). The results created a benchmark for determining the color stability through the proportion of reducing activity and oxygen consumption rates. Although this study was extremely valuable, animal-to-animal variation in color stability needs to be considered. King, Shackelford, and Wheeler (2011) determined that the combination of initial oxygen consumption capacity and the initial and final metmyoglobin reducing activities is a good indicator of the variation. Additional factors and conditions such as packaging conditions and postmortem metabolic mechanisms also influence color stability (Yu et al., 2017).

2.8.4. Effect of pH and temperature

The pH of muscle tissue is considered an intrinsic factor that affects color stability and quality of meat (Yu et al., 2017). Muscle tissue begins at a pH of 7.0-7.2 with a decline to a pH of 5.5-5.7 over roughly 24 hours (Boles & Pegg, 2010) post slaughter due to the build up of lactic acid and ATP hydrolysis (Ramanathan, Kiyimba, Gonzalez, Mafi, & DeSilva, 2020).

Muscle that has an ultimate pH of > 6.0 is commonly referred to as dark, firm, and dry (DFD). The higher pH (> 6.0) is caused by lower glycogen levels prior to death (Ramanathan, Hunt, et al., 2020). DFD influences the quality and sensory perception of the meat. Due to the higher pH, the water holding capacity is increased which immobilizes the internal water flow and creates a dense, compact tissue (Lawrie & Ledward, 2006). Additionally, the mitochondria and the myoglobin are competing for the residual oxygen available causing a higher concentration of metmyoglobin and a darker meat color (Neethling, Sigge, Hoffman, Suman, & Hunt, 2017).

Temperature, or rather cold-chain management, of post-mortem skeletal muscle is considered an extrinsic factor that affects the color of the meat (Yu et al., 2017). When the temperature of a post-harvest carcass is higher than normal the pH of the muscle tends to decline quicker (Neethling et al., 2017). The combination of high temperature and a rapidly dropping pH causes the denaturing of myofibrillar and sarcoplasmic proteins (Adzitey & Huda, 2012). With the denaturing of protein, the functionality of the protein is diminished, therefore, the water holding capacity is decreased and the extrafibrillar water shifts along with the Mb (Adzitey & Nurul, 2011). This results in a muscle that is pale and soft, better known as PSE. Neither DFD or PSE are acceptable to consumers, therefore, extensive studies were conducted and continue to be conducted on how to reduce this waste (Adzitey & Nurul, 2011; Holdstock et al., 2014; M. Miller, 2007; R. K. Miller, 2017).

2.9. Mitochondria

Mitochondria is a double-membrane organelle (Figure 3) responsible for energy production in both eukaryotic and prokaryotic cells using oxidative phosphorylation (Sierra & Oliván, 2013). Oxidative phosphorylation is the process by which ATP synthase and the respiratory chain convert ADP to ATP (du Plessis, Agarwal, Mohanty, & van der Linde, 2015).

England et al. (2018) notes that even though the role of mitochondria is thought to cease post mortem, studies show that mitochondria may play a part in the post mortem energy metabolism and thus influences other factors such as pH decline and meat quality (Matarneh et al., 2017; Ramanathan & Mancini, 2018). In order for mitochondria to function, oxygen must be present. Oxygen is the last electron acceptor in the electron transport chain and therefore necessary to complete the mechanism. Post harvest, small amounts of oxygen remain in the muscle tissue (Ramanathan & Mancini, 2018). The residual oxygen can support limited mitochondrial function, and even with low function, a single mitochondrion can oxidize one pyruvate molecule into 15 molecules of ATP (England et al., 2018). This suggests that mitochondria may play a larger role in meat quality than originally determined.

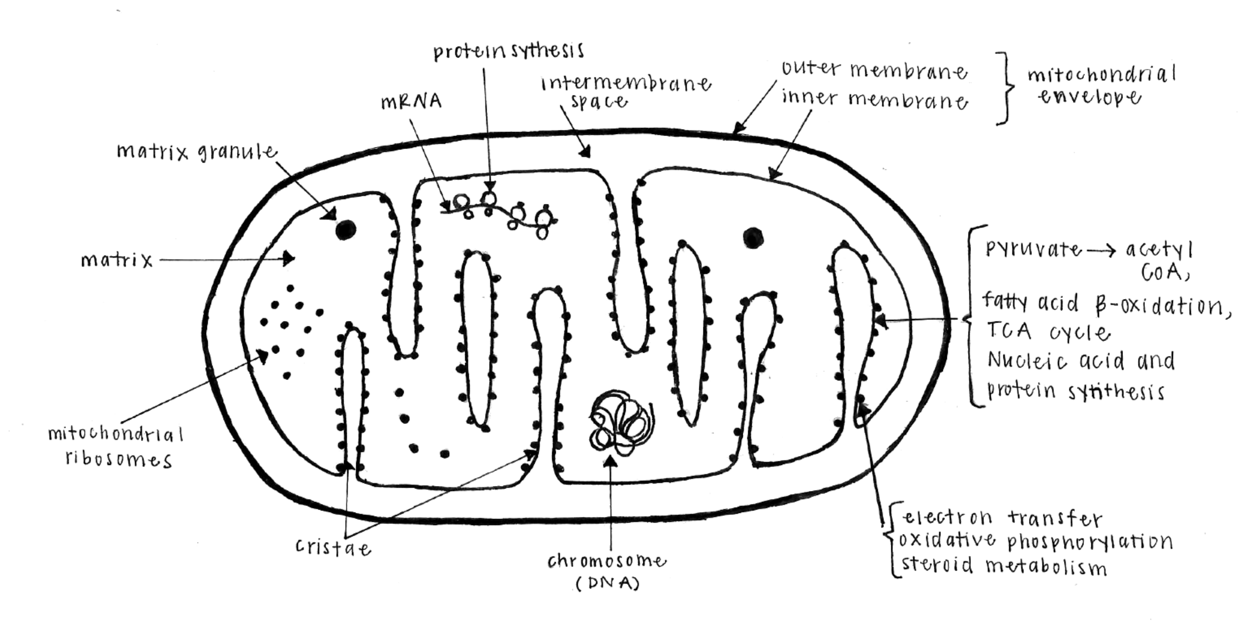

Figure 5. Diagrammatic representation of mitochondrion structure (redrawn from Kumar (2015).

2.9.1. Sarcoplasm

The sarcoplasm is the protein containing fluid surrounding myofibrils (Kahraman & Gurbuz, 2016). Sarcoplasmic proteins are water-soluble and account for 20 to 40% of the entire protein content in muscle (Lopez-Enriquez, Ocano-Higuera, Torres-Arreola, Ezquerra-Brauer, & Marquez-Rios, 2015) with myoglobin, glycolytic enzymes (Przybylski et al., 2016), and creatine kinase (Bowker, Eastridge, & Solomon, 2014) being the majority of this fraction. Many studies have determined sarcoplasmic proteins to be a biomarker for meat quality attributes such as color stability, water holding capacity, texture and other sensory traits (Gao, Wu, Ma, Li, & Dai, 2016; Nair et al., 2016; Przybylski et al., 2016). In order to determine the content of the sarcoplasmic fraction, methods of sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) coupled with mass spectrometry were employed. Results reported by Przybylski et al. (2016) include a positive correlation between hardness, chewiness, and elasticity of meat when there is a high content of sarcoplasmic protein. Specifically, certain enzymes such as pyruvate kinase, lactate dehydrogenase, phosphorylase b kinase, phosphorylase b and a few others that participate in select post mortem glycolysis pathways were reported to influence texture (Bowker et al., 2014; Grabež et al., 2015; Holman, Collins, Kilgannon, & Hopkins, 2020). Furthermore, Nair et al. (2016) discusses the critical role and correlation of antioxidant proteins, found in the sarcoplasmic proteome, in regard to meat color, surface redness, and metmyoglobin reducing activity (MRA). Since the composition of sarcoplasmic protein fraction varies from animal-to-animal and muscle-to-muscle (Nair, Li, Beach, Rentfrow, & Suman, 2017), it is essential to analyze and understand this property of muscle tissue and its effects on meat quality.

2.9.2. NAD^+/NADH

The oxidized nicotinamide adenine dinucleotide (NAD^+) and its reduced form, NADH, participate in a continuous transfer of electrons (Figure 4) that is essential to oxidation reactions in cell metabolism (Bogan & Brenner, 2013). NAD^+/NADH are coenzymes found in the mitochondrial matrix (Blanco & Blanco, 2017) and are involved in the Krebs cycle, glycolysis and mitochondrial electron transport (Casem, 2016). These coenzymes work in conjunction with dehydrogenases within the mitochondrial matrix to dehydrogenate substrates such as pyruvate, malate, glutamate, 3-OH-acy-coenzyme A, and isocitrate (Blanco & Blanco, 2017). Additionally, the reduced form (NADH) is the first acceptor of the electron transport chain and becomes oxidized through the donation of an electron (Yoshida & Imai, 2018). The role and derivation of NADH in the mitochondria is defined expertly by Davila et al. (2018). The NADH is produced from nicotinamide mononucleotide (NMN) using a specified transferase. Berger, Lau, Dahlmann, and Ziegler (2005) determined that these transferases are located in three locations- nucleus, Golgi apparatus and mitochondria. The specific transferases located in the mitochondria are known as nicotinamide mononucleotide adenylyltransferase 2 and 3 (NMNAT2 and NMNAT3), and they provide evidence that mitochondria can maintain a NADH pool (Davila et al., 2018). This explains the ability of mitochondria to continue limited aerobic metabolism in post-mortem tissue (Belskie, Van Buiten, Ramanathan, & Mancini, 2015).

The depletion of NADH in mitochondria induces an irreversible color change in meat. In 2017, Nerimetla et al. (2017) stated that the electrons generated from NAD^+/NADH and mitochondrial-interposed electron transfer participate in metmyoglobin reduction. Metmyoglobin reducing ability (MRA) is the ability of a post-mortem muscle to decrease the formation of metmyoglobin by reducing it to deoxymyoglobin. MRA is also a method used to measure the color stability in meat (Kim, Keeton, Smith, Berghman, & Savell, 2009). If the presence of NAD^+/NADH were removed, the aerobic production of adenosine triphosphate (ATP) would cease because the electron transport chain would lack a first acceptor (Yoshida & Imai, 2018). Therefore, regeneration of NADH in the mitochondria can affect the stability of meat color in beef (Belskie et al., 2015).

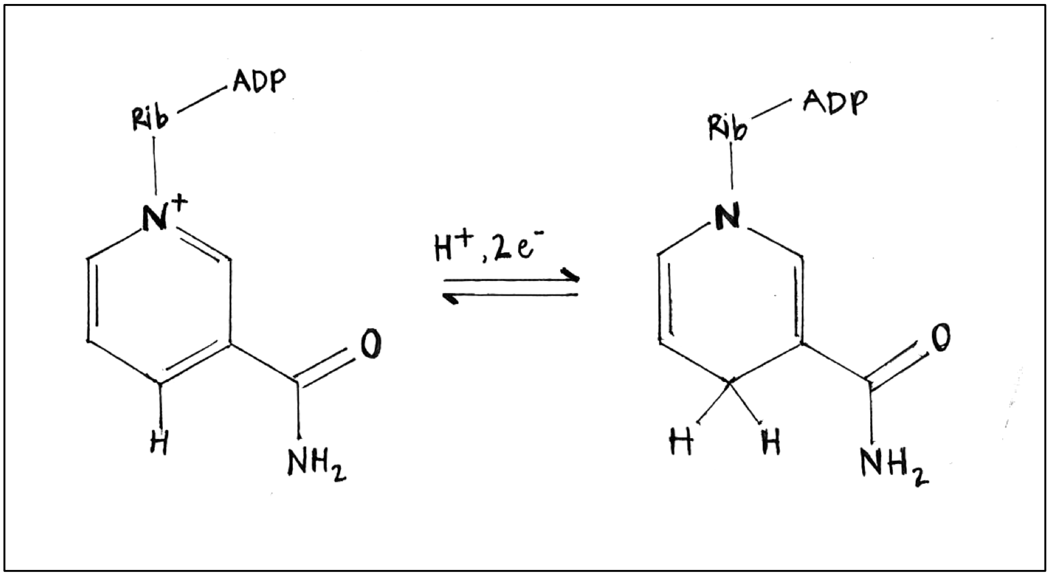

Figure 6. Diagrammatic representation of the redox reaction of NAD^+/NADH.

CHAPTER 3

MATERIALS AND METHODS

3.1. Raw materials and chemicals

Two experiments were conducted to develop and characterize beef tongue powder. In experiment 1, the physiochemical properties of beef tongue were determined and compared with beef heart and *Psoas major*. In experiment 2, a method for spray-drying beef tongue was developed, and the physical characteristics, composition, and powder properties were evaluated.

Three different bovine muscles, tongue, *Psoas major* (IMPS # 189) and heart, (experiment 1, n=6; experiment 2, n=3), from grass-fed cattle slaughtered at ≥ 30 months age were obtained from a local meat purveyor. All three muscles were shipped to the University of Georgia Food Science Department in vacuum sealed packaging and stored frozen (-20 °C) until further analysis. Beef tongue was skinned prior to analysis and was sampled from all three interior regions, posterior, medial, and anterior (Figure 1). Beef heart and *Psoas major* were sample from the interior portion of the muscle tissue. All chemicals and reagents were of analytical grade.

3.2. Experiment 1: Comparison of proximate composition and the determination of NAD$^+$/NADH concentration, and metmyoglobin reducing ability in beef tongue, beef heart and *Psoas major*.

3.2.1. ***pH and proximate composition***. For determining pH, the method by Kim et al. (2009) was followed with some modifications. A sample from each bovine muscle tissue was frozen (-20

°C) and pulverized in a (Cuisinart® MINI-PREP® Plus, Stamford, CT, USA). A 5 g sample of muscle tissue was mixed with 20 mL of deionized water and homogenized (VDI 25 S41; VWR International, Radnor, PA, 13.5 rpm). pH measurements were performed in triplicate using a pH benchtop meter (Versa Star Pro; ThermoFisher Scientific, Waltham, MA, USA) and were averaged for statistical analysis.

For the proximate composition analysis, a 15 g sample was cut from the interior portion of the muscle tissue and trimmed for visible fat and connective tissue. A muffle furnace at 600 °C for two hours was used to analyze total ash (AOAC Method 942.05). Moisture content was determined by oven drying at 105 °C for three hours (AOAC Method 930.15). Protein was analyzed by the Kjeldahl method (AOAC Methods 4500-Norg C and 4500-NH3 C) and the fat content was determined using an ANKOM^{XT15} Extractor (PVM 1:2003 and AOCS Procedure Am 5-04). Three samples of each muscle tissue were analyzed and replicated for a minimum of three times.

3.2.2. ***NAD$^+$/NADH Concentration.*** NADH and NAD$^+$ concentrations were determined by using an NAD$^+$/NADH quantification kit (K337, BioVision Research Products, Mountain View, CA, USA). Briefly, 20 mg of muscle tissue was mixed with 400 μL of NADH alkaline extraction buffer, homogenized, and centrifuged for 5 minutes at 14000 × *g*. A 50 μL extracted sample was transferred to the 96-well plate in triplicate to detect NADt (NAD$^+$ and NADH). A 200 μL remaining sample was heated to 60°C for 30 minutes in a water bath in order to extract NADH. The sample was cooled on ice and 50 μL were transferred to the 96-well plate in triplicate. The color developer was added and the absorbance was recorded at 450nm. The concentrations of NADt and NADH (ng/mg) were calculated from the standard curve equation, and the NAD$^+$

concentration (ng/mg) was calculated using the difference of NADt and NADH. Three replications were conducted for each muscle tissue.

3.2.3. ***Metmyoglobin reducing ability (MRA)***. Concentration of myoglobin redox forms and MRA were determined following the methods outlined by Sammel (2000) with slight modifications. A 3 × 2 × 1.3 cm^3 sample of muscle tissue was cut and trimmed free of visible fat and connective tissue, placed on a Styrofoam tray, and covered with overwrap film (18 in clear, 40 gauge). The overwrap film was removed and the sample was immersed in 0.3% sodium nitrite for 20 minutes with intermittent stirring. The muscle tissue was removed from the solution, blotted dry, and sealed in a vacuum bag (VacPak-It 30.5 cm × 71 cm × 3 mm chamber bags, 80/20 polyethylene/nylon, VAC PAK packaging solutions, Clifton NJ, USA). Reflectance measurements were recorded immediately through the vacuum bag. The muscle tissue was incubated at 30°C and spectrophotometric measurements were collected in 30-minute increments for 2 hours. Surface color (CIE *L*a*b**) was evaluated, and reflectance from 400-700 nm in 10 nm increments were obtained using a HunterLab MiniScan™ EZ Spectrophotometer (Model 45/0 LAV, Illuminant A, 10° standard observer; Hunter Associates Laboratory Inc.; Reston, VA) in order to calculate the relative percentage of surface oxymyoglobin (OMb), metmyoglobin (MMb), and deoxymyoglobin (DMb). A minimum of three measurements from each muscle tissue were recorded and averaged for statistical analysis.

3.3. Experiment 2: Processing parameters and determination of composition and physical properties of spray-dried beef tongue powder.

3.3.1. ***Development and optimization of the spray-drying process.*** The final method for spray-drying beef tongue is displayed in Figure 2. The final method for spray-drying was determined and optimized after several adjustments and trials. Tables 1 and 2 show the various experiments that were conducted to determine the final method. Raw beef tongue was evaluated for the best method to clean, peel, reduce particle size and form a slurry, and filter (Table 1). The spray-drying process was evaluated for producing a powder that was dry, slightly pink, and had a high yield (Table 2).

3.3.2. ***Preparation of feed liquid.*** Three independent batches (n=3) of feed liquids were made. The beef tongues were thawed at 4 °C, scrubbed with 3.0% lactic acid solution for at least 30 seconds on all sides and blotted dry. Their outer skin was removed and the tongues were sliced (Globe S13, Globe Food Equipment Co., Dayton, OH, USA) to a thickness of 2 mm. The slices were bowl chopped (Stephan UM 5 universal, Vertical-Cutter/ Mixer, Stephan Machinery Corporation, Columbus, OH, USA) with 1 % salt and 10 % ice and emulsified (VDI 25 S41; VWR International, Radnor, PA, 24,000 rpm**)** in three passes, each 30 seconds, with the addition of 1-2 liters of isotonic solution (0.85 % w/v salt). The homogenate was vacuum filtered using a metal strainer and gravity filtered using a nylon screen. Maltodextrin (STAR-DRI® 100 Maltodextrin, Tate & Lyle, Decatur, IL, USA) was dissolved in the filtrate to increase the solids content by 10% (amount determined as ~8.5 % of slurry weight). The filtrate with the maltodextrin was used for spray-drying. All experimental steps were carried out at 4 °C.

3.3.3. ***Spray-drying method.*** The beef tongue feed liquid was spray-dried using a Mini Spray Dryer B-290 (Büchi, Switzerland) with an inlet temperature of 155 °C, an outlet of (60-75 °C) and pump rate of 6 mL/min (25 % pump). Additional settings used were: Q-flow= 50, aspirator= 100%, vacuum= -60mbar, nozzle diameter= 0.7mm. Three replications of spray-drying were completed and yield was calculated using the mass of the starting feed liquid and the mass of the final powder (Eq. 1).

$$solids\ yield\ (\%) = \frac{m_p \times p_p}{m_s \times p_s} \tag{1}$$

In this equation, m_p is the final weight (g) of the powder; p_p is the percent solids of the powder; m_s is the weight (g) of the feed liquid, and p_s is the percent solids in the feed liquid.

The percent solids content was determined as the percentage that was calculated (Eq. 2).

$$solids\ content\ (\%) = sliced\ tissue\ (g) \times 0.3016 \tag{2}$$

In this equation, the constant, 0.3016, is the combined average percentage of protein, fat, and ash of tongue muscle tissue as determined in proximate composition by percent wet-basis.

3.3.4. *Proximate composition.* Proximate composition of the spray-dried powder was determined following the same methods listed in section 3.2.1 with slight modification. A 15 g of powder was used instead of muscle tissue. Three samples of powder (n=3) were analyzed and replicated for a minimum of three times.

3.3.5. *Bulk density.* Bulk density was determined following the method outlined by (Erickson & Kerr, 2018). A 2 g sample of powder was added to a 10 mL graduated cylinder and compressed with a glass rod until completely packed. Bulk density was calculated as the lowest volume after packing per 2 g. Samples were analyzed in triplicate and averaged for statistical analysis.

3.3.6. *Color parameters.* The color parameters (CIE L^*, a^*, b^*) of the beef tongue powder were obtained using a HunterLab MiniScan™ EZ Spectrophotometer (Model 45/0 LAV, Illuminant A, 10° standard observer; Hunter Associates Laboratory Inc.; Reston, VA). For each measurement, 2.0 g of powder was packed evenly on black paper and the surface was smoothed using aluminum foil. Measurements were performed in triplicate for three independent samples of powder.

3.3.7. ***Water activity.*** Powder (1 ± 0.3 g) was placed in a sample dish (Novasina 11 0601) and the water activity (a_w) was determined using a LabSwift-a_w water activity meter (Model Novasina AG, CH-8853, Lachen, Switzerland). Measurements were collected for each powder (n=3) in duplicate and averaged for statistical analysis.

3.3.8. ***Differential scanning calorimetric analysis (DSC).*** Tongue muscle (6 ± 2 g) and *Psoas major* muscle (200 ± 5 g) was seared, covered, and cooked in a pan on a hot plate to an internal temperature of 60 °C, 67.5 °C, and 75 °C as was monitored and measured by a thermocouple placed in the center of the meat. Samples were removed from the center of each muscle portion for analysis. Thermal denaturation of proteins was measured using a 204F1 Phoenix DSC (NETZSCH, Geraetebau GmbH Wittelsbacherstrasse 42, Germany) according to the methods of Srinivasan, Xiong, Blanchard, and Tidwell (1997) with minor modifications. Samples were sealed in 30 μL aluminum pans (Perkin-Elmer, Waltham, MA, USA) for DSC analysis and ran in duplicate. Beef tongue powder was placed directly into the pan for analysis and was also ran in duplicate. Experimental samples were thermally scanned from 20 to 100 °C at a heating rate of 10 °C/min. Using Proteus thermal analysis software (Ver 6.0.0., NETZSCH, Geraetebau GmbH Wittelsbacherstrasse 42, Germany) the maximum heat flow for each peak was determined.

3.3.9. ***Free and bound water.*** The ratio of free and bound water was determined using Nuclear Magnetic Resonance (NMR) as similar to Popineau, Rondeau-Mouro, Sulpice-Gaillet, and

Shanahan (2005). Beef tongue powder was placed into standard 5 mm NMR tubes and lightly packed to a minimum of 25 mm. A 1D proton spectra were collected at 25 °C on a Varian Inova 550 MHz spectrometer (Varian Medical Systems Co. Palo Alto, CA, USA) using a standard single pulse program (20-degree pulse, acquisition time = 100 milliseconds, relaxation delay = 1 second, spectral width = 500000 Hz). MNova analysis software (V.14.2, MestreLab, Inc., Compostela, Spain) was used to deconvolute the narrow and broad components of the signal using the line fitting tool. A minimum of three samples were analyzed and each sample was processed using line fitting for a minimum of three times and averaged. Results were reported as a ratio of bound to free water.

3.3.10. ***Scanning electron microscopy (SEM).*** In order to evaluate the particle structure of beef tongue powder, scanning electron microscopy (SEM) was conducted. The powder was vapor fixed with 4% osmium tetroxide for one hour and then was placed on an aluminum SEM stub with a carbon adhesive. The sample was vacuum dried at 4 x 10^{-2} mBar for 3 hours and plated with 16 nm gold using a sputter coater (Structure Probe, Inc., West Chester, PA, USA). Imaging was performed on a Teneo field-emission SEM (Thermo Fisher Scientific, Hillsboro, OR, USA) at magnifications of 2500x, 6500x, 15000x, and 20000x.

3.3.11. ***SDS-PAGE and protein identification.*** Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was used to separate the protein fractions according to the methods by Warren et al. (2020) with slight modification. Raw beef tongue, *Psoas major* and beef heart samples (2.5 g) were homogenized with 25 mL of DI water in three passes using Tissue Tearor ™ at 30,000 rpm (Biospec Product, Inc., Model 398, 120 V, ~1.15A). Samples of beef tongue powder (~2 mg), filtered raw beef tongue homogenate, *Psoas major* homogenate and beef heart homogenate were combined with 1 mL SDS sample loading buffer (Tris-HCl, glycerol, SDS, bromophenol blue, dithiothreitol; Fisher Scientific, Suwanee, GA, USA), vortexed and placed in a boiling water bath for 7 minutes. Samples were cooled and stored frozen (-40 °C) until analysis. The first lane of the gel was loaded with 10 μL of Precision Plus Protein™ duel color standards (Bio-Rad Laboratories, Hercules, CA, USA) and 10 μL of each denatured sample was loaded for each additional lane. The gel was ran using a Tris-Glycine-SDS running buffer (Fisher Scientific, Suwanee, GA, USA) at ambient temperature. The gel was ran at 100 V for 20 minutes and then at 200 V for a total run time of 1.5- 2 hours. The gel was stained in Coomassie brilliant blue R-250 (Bio-Rad Laboratories, Hercules, CA, USA) for 1 hour and destained using Bio-Rad destain (Hercules, CA, USA).

Protein identification was determined using liquid chromatography-mass spectrometry (LC-MS) analysis. A 20 mg sample of powder was mixed with 2 mL of isolation buffer (220mM mannitol, 80mM sucrose, 0.02mM EDTA, 20mM Tris-HCl, and 5mM Pi) centrifuged at 4 °C at 12500 ×g for 10 minutes. The pellet was discarded and the supernatant was mixed with ethanol (9:1) and cooled to -40 °C for 2 hours. The sample was thawed and centrifuged (4 °C at 12500 ×g for 10 minutes), and the protein pellet was saved for analysis. LC-MS analysis was conducted according to Warren et al. (2020) with slight modification. The isolated protein sample was diluted with ammonium bicarbonate, denatured by heat block, reacted with dithioreitol (~20 °C), and alkylated with iodoacetic acid. The sample was then digested at 37 °C overnight, dried with a vacufuge, and resuspended in 2 % acetonitrile/ 0.1% formic acid.

Mass spectrometry analysis of the sample was conducted with an LTQ Orbitrap Elite Mass Spectrometer (Proteomics and Mass Spectrometry Facility, University of Georgia) coupled with a Proxeon Easy NanoLC system (Thermo Fisher Scientific, Massachusetts, USA). A self-packed reversed-phase column (200 Å 5 μM Bruker MagicAQ C18 resin) with a two-buffer gradient elution was utilized for peptide separation. MS data was collected using data-dependent acquisition (DDA), collision-induced dissociation (CID), higher energy collisional dissociation (HCD), and tandem mass spectrometry (MS/MS). Peak identification required Xcalibur software (v.2.2, Thermo Fisher Scientific, Massachusetts, USA), and modification characterization and protein identification used the Uniprot database. Three samples, one from each batch of beef tongue powder, were ran a minimum of three times and protein abundance was determined and reported using averaged peak area.

3.3.12. *Fatty acid profile.* The fatty acid profile of the beef tongue powder was determined using methods outlined by Nagachinta and Akoh (2013) and Warren et al. (2020) with minor modifications. Lipids were extracted, derivatized for fatty acid methyl esters (FAMEs) and analyzed using gas chromatography-flame ionization detector (GC-FID). A 0.5 g sample of powder was vortexed with 2 mL of distilled water, combined with hexane/isopropanol (3:1, v/v) and centrifuged at 3000 × *g* for 30 minutes. Lipids were further extracted from the aqueous phase, filtered using a sodium sulfate column, and separated by evaporation. Extracted lipids were combined with an internal standard, heptadecanoic acid, and a transmethylation reagent and placed in a Reacti-Block™ B-1 aluminum block (Reacti-Therm III™ Heating/Stirring Model; Thermo Fisher Scientific, Massachusetts, USA) for 18 hours. Derivatized FAMEs were washed twice before the hexanes were removed with nitrogen steam. FAMEs were dissolved in hexane and sealed with an electronic crimper and analyzed with an Agilent 6890 N GC-FID system with a highly polar biscyanopropyl column. A split ratio 50:1 with ultra-high purity (UHP) helium was used (60 psi) with an oven temperature of 140 °C for 5 minutes. The temperature was increased by 4 °C/min until 240 °C and then held for 15 minutes. FAMEs were identified by retention time, and were compared to a FAMEs standard reference mixture. Quantification of each FAME was calculated by response factor. Six samples were analyzed in duplicate and averaged for statistical analysis.

3.4. Statistical analysis. Statistical analyses were preformed using JMP Pro Version 15 software. One-way ANOVA was conducted for statistical difference ($p < 0.05$) for proximate composition, color parameters, pH, NAD$^+$/NADH concentrations, and the percent composition of myoglobin forms. Tukey's HSD test was performed to separate the means and determine statistical difference ($p < 0.05$) of each analysis.

CHAPTER 4

RESULTS

4.1. Experiment 1: Comparison of proximate composition and the determination of NAD$^+$/NADH concentration, and metmyoglobin reducing ability in beef tongue, beef heart and *Psoas major*.

4.1.1. Proximate composition

Proximate composition analysis of beef tongue, beef heart, and *Psoas major* was performed to determine the absence of tissue defects in the raw muscle tissue. Proximate composition showed similarities ($p>0.05$) between tongue, heart, and *Psoas major* muscles for protein, fat, ash, and moisture content (Table 3). Beef tongue, as a whole muscle, contained 17.7 % protein and 11.5 % fat, while beef heart and *Psoas major* contained only 2.2 % and 6.2 % fat, respectively. Beef tongue, beef heart, and *Psoas major* showed similar trends for all data in both wet-basis and dry-basis analyses ($p< 0.05$).

4.1.2. NAD$^+$/NADH concentration and pH

NAD$^+$/NADH concentration and pH of postmortem muscle revealed differences among beef tongue, heart, and *Psoas major* (Table 4). Beef tongue had the highest pH (6.0; $p<0.05$) and *Psoas major* had the lowest (5.8). Beef heart and beef tongue had statistically similar ($p>0.05$) pH values as did beef heart and *Psoas major*. All three muscle tissues contained relatively similar NAD$^+$ concentrations ($p>0.05$) with a range of 1.9 pmol/mg. *Psoas major* had the highest NADH concentration (52.5 pmol/mg) and was statistically different ($p<0.05$) from both beef tongue and beef heart ($p<0.05$). Beef tongue and beef heart contained similar concentrations of NADH ($p>0.05$).

4.1.3. Metmyoglobin reducing ability

The MRA assay was conducted to assure the absence of tissue defect in the muscle by measuring the reducing ability of metmyoglobin. MRA displayed color similarities between beef tongue, beef heart, and *Psoas major* (Figure 3). *L**-values of the three muscles shown in Figure 3A increased after 30 minutes and maintained the brightness for the remainder of the treatment. Tongue had a higher *L**-values from 60 to 150 minutes as compared with heart and *Psoas major* but was not statistically different. All three muscles had similar redness initially (*a**-values of 20.7-21.5) and followed a similar pattern of decrease after 30 minutes (11.6-13.4) and a continuous increase from 60 to 150 minutes (Figure 3B). While the *a**-value of heart increased to 20.1 at 60 minutes and then began to plateau between 120 and 150 minutes. The *a**-values of tongue and *Psoas major* continued to increase, however, this trend was not statistically different between the three muscles. *Psoas major* had the reddest (highest *a** value) surface color by the end of the treatment, followed by tongue and then heart with the lowest (23.3), but the two were not statistically different. Chroma values shown in part F of Figure 1 followed a similar trend as *a** for each respective bovine muscle. Overall, the three muscles performed similarity at each time as indicated by the error bars.

The MRA assay also showed the differences in percentages of metmyoglobin (MMb), deoxymyoglobin (DMb) and oxymyoglobin (OMb) between beef tongue, beef heart, and *Psoas major* before and after nitrite treatment and vacuum-sealed incubation (Figure 4). Percent OMb (Figure 4A) shows statistically similar abundance between all three bovine muscles except at 60 and 90 minutes, in which tongue, 13.7 % and 26.2 % respectively, was lower than heart (36.5 %

and 46.1 %) and *Psoas major* (48.3 % and 58.6 %). OMb continued to increase from 60 to 150 minutes for each muscle and resulted with statistically similar percentages at 150 minutes. Tongue showed a statistically higher amount of MMb compared with heart and *Psoas major* at 60 and 90 minutes (Figure 4B), which correlates with the difference found in OMb abundance. At the end of the treatment (150 minutes) all three muscles had statistically similar abundance of OMb and MMb, but were statistically different in DMb abundance.

4.2. Experiment 2: Processing parameters and determination of composition and physical properties of spray-dried beef tongue powder.

4.2.1. Development and optimization of the spray-drying process

The processing trials were performed and evaluated to find the best option for each processing step in spray-drying beef tongue. In order to clean the tongue, a simple detergent wash was determined as a less effective method than lactic acid. The scrubbing process was selected as opposed to dipping in order to remove both large and small debris. Using a knife and keeping the tongue whole proved to be the most effective way for removing the skin since the peeler was determined to be too shallow for removing the thick portions of the skin. Particle size reduction and slurry formation was the most successful when the meat was homogenized with 1 L of saline after slicing and bowl chopping. Filtration through cheesecloth was difficult and did not remove all the connective tissue, but using vacuum and gravity filtration with metal and nylon strainers was successful. Spray-drying settings 1-4 produced powder that was low in yield due to the fat adhering to the drying chamber, while settings 5 and 6 used maltodextrin and resulted in an acceptable yield that reduced fat loss. An inlet temperature of 150 °C was slightly low for the slurry that contained maltodextrin (setting 5) and caused improper drying, but the increase to 155 °C improved the dryness of the powder. Ultimately, setting 6 was determined as the optimal settings for spray-drying raw beef tongue.

4.2.1. Proximate composition and powder characterization

Proximate composition and powder characteristics are shown in Table 5. Spray-dried beef tongue powder contained protein (25.5 %), fat (14.0 %), and ash (13.5 %), with the remainder likely representing the maltodextrin content. Moisture content, bulk density, water activity, and solids yield were determined to be 3.9 %, 0.8 g/mL, 0.2, and 0.5 %, respectively. The ratio of tightly bound and freely bound water was 4:1. The color parameters are also found in Table 3. The powder has a brightness (L^*) of 78.6, a redness (a^*) of 10.5, and a yellowness (b^*) of 16.7.

4.2.2. Differential scanning calorimetric analysis

Differential scanning calorimetric (DSC) analysis was performed to determine the presence of non-denatured proteins. DSC produced thermograms indicating the denaturation of proteins in beef tongue, beef tongue powder (Figure 5.1), and *Psoas major* (Figure 5.2). Figure 5.1A shows three transition peaks for beef tongue powder located at 34.0 °C (I), 52.6 °C (II), and 72.5 °C (III). The four muscle samples (Figure 5.1B-1E) had broad peaks around similar temperatures (~53 °C and 73 °C, respectively) as peak II and III. Raw beef tongue (Figure 5E) showed the most similar II peak to thermogram A at 54.9 °C, and 75 °C beef tongue (Figure 5B) showed the most similar III peak to thermogram A at 74.6 °C. Peak I in thermogram A was not found in the thermograms of the beef tongue muscle samples (B-E). In Figure 5.2, thermogram D showed three distinct peaks at 50.8 °C (I), 68.3 °C (II), and 77.2 °C (III). *Psoas major* cooked to 60 °C displays two peaks, one at 58.3 °C and another at 74.6 °C (Figure 4C). Thermogram B in

Figure 5 shows one broad peak with a maximum of 56.3 °C, and thermogram A in Figure 5 displays no distinct peaks.

4.2.3. Free and bound water

Nuclear magnetic resonance (NMR) analysis was performed to determine the ratio of free and bound water of a 4% moisture beef tongue powder at 25 °C (Table 5). The line shape was deconvoluted into two curves that sum to the total line shape as shown in Figure 6. The best fit simple line shape for both the broad (5.5 kHz, 77.1% total peak intensity) and narrow (1.8 kHz, 22.9% total peak intensity) proton components was Lorentzian.

4.2.4. Scanning electron microscopy

Particle size of spray-dried beef tongue powder containing 10% maltodextrin was evaluated at various magnifications using scanning electron microscopy (SEM) (Figure 7). Imaging was performed using a Teneo field-emission SEM. Magnification of the powder was increased per micrograph A, D, B, C, respectively (Figure 7). Micrographs B, C and D show the formation of spherical particle structure, and micrograph B displays surface texture. All micrographs show various particle sizes and shapes (irregular, spherical and shrunk) with bridging between particles/ agglomeration. Particles in A and C appear smooth, while C shows cracking, and B and D show a rough, “raisin-like” surface.

4.2.5. SDS-PAGE and protein identification

SDS-PAGE separations of the protein profile of raw beef tongue (T), *Psoas major* (P), beef heart (H), and beef tongue powder (S) are shown in Figure 8. The band patterns developed from the separation distinguished beef tongue powder from beef heart and *Psoas major,* and revealed the similarity to beef tongue. The proteins most prominent in all samples are myosin, actin and myoglobin. These three proteins are identified with arrows in Figure 8. Myosin, actin and myoglobin show similar band patterns among *Psoas major,* beef tongue, and beef tongue powder, however, the bands for beef heart are more abundant for all three major proteins. Beef heart showed more numerous and prominent bands between 20 kDa and 70 kDa as compared with *Psoas major,* beef tongue and beef tongue powder. Beef tongue shows similar high molecular weight proteins as *Psoas major* which are indicated by the bands greater than ~60 kDa. Beef tongue powder bands are nearly identical to the band pattern of beef tongue; however, the bands are slightly more numerous and prominent in the powder sample. Both beef tongue samples, powder and raw, favor the band pattern of *Psoas major* more than beef heart, with beef tongue resembling *Psoas major* in band prominence.

Protein identification analysis was conducted with LC-MS/MS for the total protein fraction of beef tongue powder. This analysis identified 235 proteins and determined their abundance in each fraction by peak area. All proteins with a peak area of 5.0×10^7 or greater were selected and summarized in Table 6. The analysis showed that serum albumin, mitochondrial creatine kinase, and myoglobin were the three most abundant proteins with peak areas of 2.7×10^9, 2.1×10^9, and 1.1×10^9, respectively. In regard to the proteins involved in glycolysis, triosephosphate isomerase, phosphoglycerate mutase, fructose-bisphosphate aldolase, and glyceraldehyde-3-phosphate-dehydrogenase were the most abundant. Pyruvate kinase, phosphoglycerate kinase, and L-lactate dehydrogenase were also identified and had peak areas of 7.2×10^7, 5.5×10^7, and 5.1×10^7, respectively. Malate dehydrogenase, a TCA cycle protein, had an abundance of 1.7×10^8 for its mitochondrial isoform and 6.9×10^7 for the cytoplasmic form. Additional mitochondrial proteins, including ATPase inhibitor and cytochrome *c* were also detected.

4.2.6. Fatty acid profile

Differences in the content of polyunsaturated fatty acids (PUFAs), monounsaturated fatty acids (MUFAs) and saturated fatty acids (SFAs) of beef tongue powder were determined by fatty acid analyses and are displayed in Table 7. Beef tongue powder has nearly equal amounts of MUFAs (46.6%) and SFAs (45.9%) with a very low PUFAs content (6.8%). The averaged percent composition of individual fatty acids found in each sample of beef tongue powder are displayed in Table 7. Oleic acid (C18:1n9), palmitic acid (C16:0), and stearic acid (C18:0) composed the top three most prominent fatty acids in the samples, with averages of 38.0 ± 0.3%, 27.5 ± 0.1 %, and 13.8 ± 1.0 %, respectively.

CHAPTER 5

DISCUSSION

The main objective of this study was to develop and characterize beef tongue powder for value-added application. The processing methods developed for spray-drying raw beef tongue resulted in a dry (moisture = 3.9 %, a_w= 0.2) powder with an acceptable color (a^*-value of 10.5 and L^*-value of 78.6), a high protein content (25.5 %) and uniform particle size (Figure 7). The particle micro-appearance agrees with a study by Maidannyk et al. (2020) that determined that a spray-dried milk powder with a 40 % protein content displayed a "shriveled" surface texture that "filled-out" as the protein content was increased. The use of maltodextrin to increase yield and "carry" the lipids through atomization was found in agreement with several previous studies that spray-dried high fat feed liquids (Premi & Sharma, 2017; Y. Wang et al., 2017). Bulk density of typical spray-dried powder ranges from 0.30 g/mL to 0.55 g/mL and refers to the solids content found in the particles thus providing an estimate of the composition per gram of powder (Huntington, 2004).

Results of proximate composition analysis, pH, and NAD^+/NADH concentration of raw beef tongue revealed that the muscle behaves similarly to beef heart and *Psoas major,* and that it was free of tissue defects that could alter the powder composition and characteristics, i.e. protein content, abundance of native proteins, color…etc. *Psoas major* and beef heart were selected as a comparison to beef tongue for potential defects due to their abundance of myoglobin, NADH, and red fibers (Ramanathan & Mancini, 2018; Yu, Tian, Shao, Li, & Dai, 2019), as beef tongue is known to have an abundance of red fibers (Warren et al., 2020). Warren et al. (2020) also determined that the proximate composition of beef tongue varies based on the location within the tongue. They found that the protein content ranged from 13.8 % to 19.2 %, fatty acid content ranged from 1.6 % to 22.9 % and moisture content ranged from 61.2 % to 77.6 % (by wet-basis). The values obtained within this current study fall in the ranges of these findings (Table 3). Biel et al. (2019) determined beef heart to have a proximate composition of 77.0 % moisture, 17.3 % protein, 4.0 % fat, and 1.0 % ash (by wet-basis). These values are also similar to the proximate analysis of beef heart conducted in this study (Table 3). The proximate composition of *Psoas major* is comparable to the results found by Canto et al. (2016), which determined a 75.9 % moisture content, 20.2 % protein content, 2.5 % fat content, and 1.1 % ash content. MRA analysis and the percentage of myoglobin redox forms further confirmed the expectation that beef tongue was relatively similar to beef heart and *Psoas major* in myoglobin and color chemistry, as further indicated by the *a**-values of the three muscles (Figure 3). By understanding the composition of beef tongue and recognizing the chemical mechanisms (i.e. metmyoglobin reduction) that affect overall characteristics of beef tongue, the hypotheses for the composition and characteristics (i.e. pink color of the powder, potential for powder color stability…etc.) of the meat powder were determined.

The beef skeletal muscle proteins including myofibrillar, sarcoplasmic and collagen constitute major structural framework of meat products. Modifications in bovine skeletal muscle proteins and denaturation during thermal processing could potentially alter the textural and functional properties of the meat. Application of thermal energy during spray-drying may influence the functional properties of structural proteins in beef tongue powder. DSC was used to analyze the denaturation of beef tongue proteins. Cook temperatures of 60 °C, 67.5 °C, and 75

°C were selected as because they are the maximum, median, and minimum temperatures of the outlet range. These cook temperatures mimic the powder temperature throughout spray-drying process and were an appropriate comparative for the potential protein denaturation of the meat powder. Results obtained from DSC (Figure 5.1) analysis demonstrated that the use of thermal processing in spray-drying of beef tongue did not denature all the structural proteins found in raw beef tongue. Peaks observed in the beef tongue powder DSC thermogram (Figure 5.1, thermogram A), correspond with the denaturing temperatures of myosin and sarcoplasmic proteins (II) and actin (III) and indicate that these proteins retained native structure in the sample. A study by Srinivasan et al. (1997) evaluated the non-denatured proteins in prawns using DSC and found peaks at similar temperatures that represent actin, myosin, and sarcoplasmic proteins. The results of DSC indicate suitability of beef tongue for enduring the spray-drying process and retain potential protein functional properties. A study by H. Wang et al. (2020) determined that spray drying was an effective method for the retention of the functional properties of soybean hydrolysates. Native proteins are known to possess functional properties, such as high WHC, that are valuable to the quality of food products (Warner, 2017), and more specifically meat products (Li et al., 2015). Retention of the native structure of the proteins in beef tongue powder may have potentials of exhibiting functional properties and capabilities such as gel formation, moisture retention, and foaming ability.

NMR analysis of beef tongue powder determined the ratio of bound (tight and loose) to free water, and the water activity (a_w) of beef tongue powder (0.2) confirmed and validated water availability. This analysis indicated that the powder's moisture content (3.9 %) primarily refers to bound moisture, which is important in understanding the microbial safety and quality retention of the powder. With an abundance of free water, the oxidation of proteins and lipids is more likely to occur and in turn would reduce the shelf-life and shelf-stability of the meat powder. Having a food product with a low water activity and primarily bound water can limit the production of free radicals and/ or reactive oxygen which contribute to the deterioration of the quality of a product through off-odors and flavors (Barden, 2014). Low water activity (< 0.7) food is known to have a long shelf-life with potential to be shelf-stable (Tapia, Alzamora, & Chirife, 2020). Additionally, low water activity acts a hurdle for microbial growth, and therefore attributes to the safety and quality of the food. A study by Lloyd, Hess, and Drake (2009) determined through sensory testing that a whole milk powder with a 30 % fat content, 3 % moisture content, and 0.2 water activity has a shelf-life of a year when stored with in an oxygen impermeable package that has been flushed with nitrogen gas. Products such as these are considered shelf-stable as well as an ingredient for further processing (Olaimat et al., 2020). Beef tongue powder has the potential to have a long shelf-life and could be a shelf-stable ingredient. The quantification and determination of shelf-life and shelf-stability should be included in future research in order to confirm this hypothesis.

Results from SDS-PAGE protein separation showed that beef tongue powder contained protein bands in a nearly identical pattern as raw beef tongue. The similarity of protein bands in raw beef tongue and beef tongue powder suggest that the conversion of raw beef tongue to powder may not have altered the protein profile. The retention of the protein composition profile in beef tongue powder would have potential for the same functional and nutritional value as raw beef tongue. The data from LC-MS revealed that beef tongue powder has several cytoplasmic and mitochondrial proteins such as myoglobin, malate dehydrogenase, glyeceraldehyde-3-phosphate dehydrogenase, and cytochrome *c*. This result showed that these proteins in beef tongue powder may have thermal stability during spray-drying (Wuhan Huamei Biotech Co.;

Yin & Konermann, 2020). Warren et al. (2020) reported a similar sarcoplasmic and mitochondrial protein composition, but also found that raw beef tongue contains an abundance of pyruvate dehydrogenase, NAD(P)H- hydrate epimerase, and succinate dehydrogenase that were absent in beef tongue powder. The loss of some proteins during spray drying was expected due to processing requirements such as the solvent containing both water and salt, which could bind some proteins and be lost during the evaporation of the solvent. The absence of NADH hydrogenase and other proteins that participate in the reduction of MMb suggests potential retention and stability of the color and therefore the quality of beef tongue powder (Ijaz et al., 2020).

Proximate composition of beef tongue powder revealed a high fat content (14.0 ± 0.3 %) that was identified by GC-FID analysis as containing mostly oleic acid (38.0 %). Warren et al. (2020) reported that raw beef tongue also contained primarily oleic acid (33.7 %). Oleic acid is a monounsaturated fatty acid that is known to have positive health effects. A study by Adams, Walzem, Smith, Tseng, and Smith (2010) determined that the consumption of beef high in MUFAs, specifically oleic acid, have a negative correlation to risk of cardiovascular heart disease (CVD). The results of beef tongue powder composition and fatty acid identification suggest that this meat powder could be a source of dietary fatty acids.

The data collected in this study provided characteristics and composition of meat powder that was developed from the animal by-product, beef tongue. This beef tongue powder has potential to be a shelf-stable, functional food ingredient that is a good source of healthy fat. Utilizing beef tongue powder as a value-added food ingredient would have a positive economic impact on the meat industry by generating revenue from meat waste. Future research should include the determination of functional properties, shelf-stability, shelf-life, and color stability of beef tongue powder.

CHAPTER 6

SUMMARY

Our findings indicate that beef tongue powder can be developed by spray-drying and that the beef tongue powder retains both protein and fat content from the raw tongue. Additionally, the findings indicate the presence of native proteins and the retention of unsaturated fatty acids and oleic acid in the beef tongue powder. These conclusions provide reason that beef tongue powder has properties that could classify it as a functional ingredient with health benefits in a value-added product. This study also determined that beef tongue powder has a low water activity and does not include major proteins involved in the reduction of metmyoglobin. These conclusions provide reason that spray-drying beef tongue acts as a method that potentially creates shelf-stability and increased shelf-life, as well as, a stabilization of the meat powder color. The beef tongue powder that was developed in this study provides a sustainable solution for a meat byproduct to be converted into a value-added food ingredient with potential nutritional and functional application.

During the processing of raw beef tongue into a meat powder, precautions were taken to eliminate and reduce microorganisms, such as lactic acid treatments of the tongue's skin and thermal processing by spray-drying. However, future studies should include the determination of the initial microbial load on the raw beef tongue and in the meat slurry, as well as the effectiveness of spray-drying as a potential kill step. Additionally, a shelf-life study should be conducted in order to determine the safety and quality of the beef tongue powder. Through the potential conclusion of beef tongue powder being an ingredient that is safe to consume, a wider application of this product could be considered.

Two processing difficulties, skinning the beef tongue and removing the connective tissue, were presented during the development of beef tongue powder. The removal of the beef tongue skin, while necessary, was a timely and difficult task. Investigation of a method to remove the skin in a more efficient manner would decrease processing time and would be more economically feasible for the meat industry. Connective tissue consists of strong fibers that are difficult to sheer and therefore must be removed prior to spray-drying. A vacuum filtration method was used but also was timely. Further research should include the determination of an efficient and large-scale separation method for the connective tissue and the homogenized beef tongue slurry.

This study determined that a raw, whole beef tongue muscle can be converted to a meat powder that retains a similar structural protein composition and nearly identical fatty acid composition as raw beef tongue, and has potential meat industry applications. Next steps should include the evaluation of the nutritional aspect by studying the bioavailability of the protein found in beef tongue powder, as well as determining the nutritional increase when used as a value-added food ingredient in food products such as sausage and beef stew. Additionally, future work should include identifying additional traits such as amino acid profiles, mineral composition, sodium content, carbohydrates…etc., and functional properties of the meat powder such as gelation, solubility, WHC, ability to form stable emulsions, foamability…etc. Furthermore, the development of a value-added product using beef tongue powder would enable the evaluation of the sensory properties of beef tongue powder which can be determined through sensory panels and degree of difference testing.

Beef tongue is a meat byproduct that is often considered meat waste due to the disinterest of American consumers regarding variety meats. The development of a meat powder through sustainable processing would generate revenue for the meat industry by converting meat waste into a value-added food ingredient that consumers would likely find more palatable or even consider it a preferred natural ingredient to other additives. Additional research should be conducted to develop and characterize meat powders made from other meat byproducts such as beef heart, liver, and kidney.

CHAPTER 7

CONCLUSION

This study was conducted to provide the meat industry with a sustainable approach of adding value to meat waste. The raw beef tongue was micronized, spray dried and evaluated for powder properties, proximate composition, lipid composition, and proteomic profile. NMR results showed that beef tongue powder retained tightly bound water and had a very low water activity (a_w, 0.2). Fatty acid and DSC analyses showed that beef tongue powder has an abundance of nutritional fat and non-denatured proteins. More specifically, beef tongue powder retained MUFAs (i.e. oleic acid, palmitoleic acid…etc.) and non-denatured structural protein such as actin. LC-MS analysis showed that spray-dried raw beef tongue retained sarcoplasmic and mitochondrial proteins such as myoglobin, malate dehydrogenase, glyeceraldehyde-3-phosphate dehydrogenase, and cytochrome *c*. Further application of beef tongue powder could include its addition to products such as sausage, ground meat or stew. Furthermore, the nutritional aspect should be evaluated by studying the potential increase in bioavailability of the protein found in beef tongue powder. Future studies should focus on exploring the use of other meat animal waste for value-added application.

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Table 1. Trial methods for cleaning, peeling, reducing particle size and slurry formation, and filtering for raw beef tongue.

Processing Step[*]	**Major Steps**[†]	**Minor Steps**[‡]
Cleaning	Water + detergent	Dipping Scrubbing
	Water + lactic acid	Dipping Scrubbing
Peeling	Knife	Whole Butterfly
	Peeler	Whole Butterfly
Particle size reduction and slurry formation	Slicing + Bowl chopping	Megatron + 2 L saline Homogenize + 1 L saline
Filter	Cheesecloth	-
	Strainer	Vacuum Gravity

[*] Processing steps progress sequentially from top to bottom.

[†] Major steps are independent from one another but are subjective to the processing step.

[‡] Minor steps are independent from one another and follow the major steps.

Table 2. Trial setting methods for spray-drying raw beef tongue slurry with and without maltodextrin.

Setting	IT[*] (°C)	%, Pump[†]	Q-flow[‡]	%, Aspirator[§]	Vacuum[¶]	%, MD[#]
1	150	25	40	100	60	-
2	150	20	40	100	60	-
3	165	25	40	100	60	-
4	150	25	50	100	60	-
5	150	25	50	100	60	10
6	155	25	50	100	60	10

[*] IT = inlet temperature of drying gas

[†] rate of feed liquid pump

[‡] flow rate of drying gas into the nozzle

[§] flow rate of drying gas into spray-drier

[¶] vacuum pressure in the spray-drier (mbar)

[#] MD = maltodextrin, carrier agent

Table 3. Proximate composition of beef tongue, beef heart, and *Psoas major* (n=3).

Muscle Tissue	Moisture	Protein	Fat	Ash
		% (Wet-Basis)		
Tongue[†]	68.1	17.7	11.5	1.0
Heart	77.3	17.7	2.2	1.4
Psoas major	71.7	19.7	6.2	1.4
**SE*	*2.7*	*1.0*	*3.2*	0.2
		% (Dry-Basis)		
Tongue[†]	-	58.9	32.2	3.5
Heart	-	78.1	9.4	6.0
Psoas major	-	69.6	21.7	4.9
*SE	-	*7.8*	*8.1*	*0.6*

*standard error

[†] represents average of anterior, medial, and posterior portions of beef tongue

Each column was analyzed for statistical difference ($p < 0.05$).

Table 4. pH and NAD^+/NADH concentrations of beef tongue, heart and *Psoas major* (n=3).

	Tongue	**Heart**	***Psoas major***
pH	6.0 ± 0.1^{a}	5.9 ± 0.0^{ab}	5.8 ± 0.2^{b}
NAD^+ (pmol/mg)	7.8 ± 0.8	9.7 ± 0.5	9.6 ± 0.4
NADH (pmol/mg)	15.0 ± 0.1^{b}	12.4 ± 0.2^{b}	52.5 ± 8.0^{a}

Value are mean ± SD

Numbers in a row with different letters (*a-b*) are statistically different ($p<0.05$).

Table 5. Beef tongue powder proximate composition and physical characteristics.

Protein (%)	25.5 ± 4.2
Fat (%)	14.0 ± 0.3
Ash (%)	13.5 ± 2.1
Moisture (%)	3.9 ± 1.1
Bulk density (g/mL)	0.8 ± 0.0
Water activity (a_w)	0.2 ± 0.1
Solids yield (%)	0.5 ± 0.1
Bound: free water ratio*	4:1
Color	
L^*	78.6 ± 3.5
a^*	10.5 ± 1.3
b^*	16.7 ± 1.5

*Bound water = loosely and tightly bound water
Free water = freely bound water

Table 6. Proteomic profile of beef tongue powder by LC-MS.

Protein		***Averaged Peak Area**
Serum albumin		2.7×10^9
Creatine kinase		
	*M-type**	2.1×10^9
	*S-type**	1.1×10^8
Myoglobin		1.1×10^9
Phosphatidylethanolamine-binding protein 1		7.7×10^8
Triosephosphate isomerase		5.3×10^8
Serotransferrin		3.9×10^8
Phosphoglycerate mutase 2*		3.6×10^8
Fructose-bisphosphate aldolase A		3.5×10^8
Glyeceraldehyde-3-phosphate dehydrogenase		3.1×10^8
α-1-acid glycoprotein		2.7×10^8
Actin		
	aortic smooth muscle	1.9×10^8
	γ-enteric smooth muscle	1.3×10^8
	cytoplasmic 1	9.3×10^7
Tropomyosin		
	*α-3 chain**	1.8×10^8
	*β chain**	1.3×10^8
	*α-1 chain**	1.3×10^8
	*α-4 chain**	8.8×10^7
Malate dehydrogenase		
	*Mitochondrial**	1.7×10^8
	*Cytoplasmic**	6.9×10^7
Cytochrome c		1.6×10^8
Hemoglobin		
	α subunit	1.5×10^8
	β subunit	9.1×10^7
Superoxide dismutase [Cu-Zn]		1.3×10^8
β-enolase		1.3×10^8
Myosin		
	*light chain 1/3**	9.5×10^7
	*light chain 3**	8.8×10^7
Pyruvate kinase PKM*		7.2×10^7
ATP*ase inhibitor, mitochondrial		7.2×10^7

Aspartate aminotransferase, cytoplasmic	7.1×10^7
Hemopexin	6.4×10^7
Fatty acid-binding protein	6.2×10^7
Pancreatic trypsin inhibitor	5.8×10^7
Peroxiredoxin-1	5.7×10^7
Phosphoglycerate kinase 1	5.5×10^7
Acetyl-CoA acetyltransferase, mitochondrial	5.5×10^7
Carbonic anhydrase 3	5.5×10^7
Musculoskeletal embryonic nuclear protein 1	5.5×10^7
Peptidyl-prolyl cis-trans isomerase FKBP1A*	5.4×10^7
L-lactate dehydrogenase B chain*	5.1×10^7

Proteins identified with LC-MS/MS.

*Averaged peak area calculated using isolated protein samples from 3 batches of beef tongue powder. RMSE = 5.3×10^8.

Creatine kinase M-type = cytoplasmic isoenzyme, S-type = mitochondrial isoenzyme.

Phosphoglycerate mutase 2 = muscle-specific form.

Myosin light chain 1/3 and light chain 3 = skeletal muscle isoforms.

Tropomyosin α-3, β, α-1 and α-4 chains = subunits of the protein.

Mitochondrial and cytoplasmic malate dehydrogenase = enzymes main isoforms.

Pyruvate kinase PKM = skeletal muscle isoform.

ATP = adenosine triphosphate.

FKBP1A of peptidyl-prolyl cis-trans isomerase = enzyme isoform.

B chain of L-lactate dehydrogenase = enzyme subunit.

Table 7. Fatty acid composition of beef tongue powder.

Fatty Acid	Average % Composition*
C4:0	ND
C6:0	ND
C8:0	0.5
C10:0	0.3
C12:0	0.1
C13:0	0.1
C14:0	3.5
C14:1n-5	0.7
C15:0	0.5
C15:1	0.2
C16:0	27.5
C16:1n-7	4.6
C17:1	0.1
C18:0	13.8
C18:1 Trans	1.3
C18:1n-9	38.0
C18:1n-7	1.0
C18:2	0.3
C18:2n-6	4.6
C20:0	0.1
C18:3n-6 (GLA)	0.0
C20:1n-9	0.7
C18:3n-3 (ALA)	0.7
C21:0	0.1
C20:2n-6	0.0
C22:0	0.1
C18:3n-6 (DGLA)	0.2
C20:3n-3 (ETE)	0.1
C20:4n-6 (AA)	0.6
C22:2n-6	0.1
C24:0	0.0
C20:5n-3 (EPA)	0.1
C22:5n-3	0.2
C22:6n-3 (DHA)	0.0
Saturated	45.9
Monounsaturated	46.6
Polyunsaturated	6.8

*Standard error = ± 0.2.

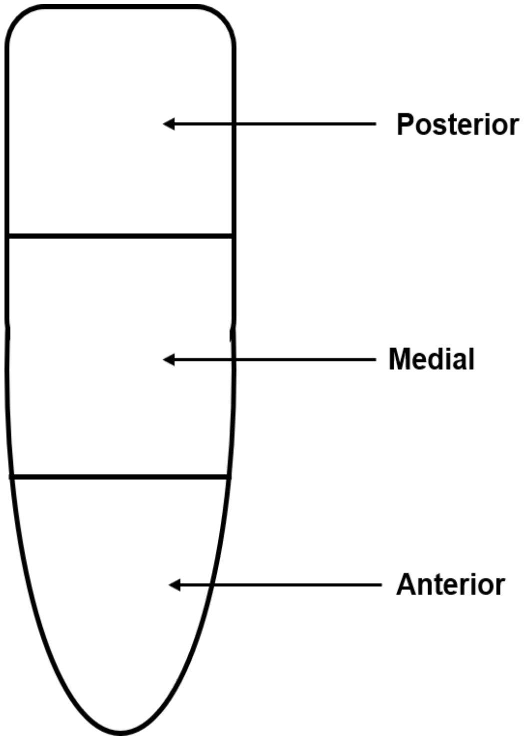

Figure 1. Diagrammatic representation of beef tongue sampling regions.

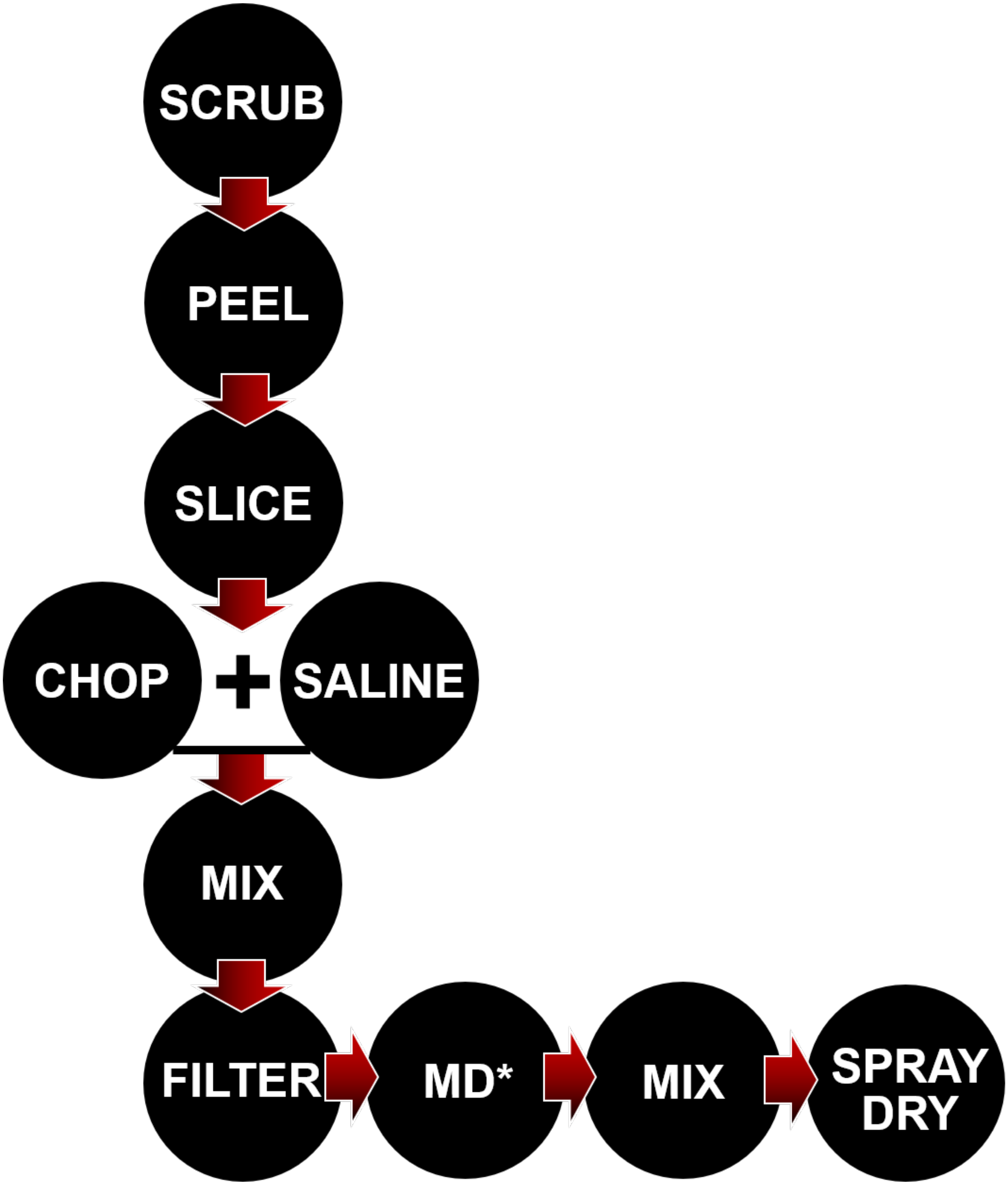

Figure 2. Flow diagram for the method of spray-drying raw beef tongue. MD* indicates the addition of maltodextrin.

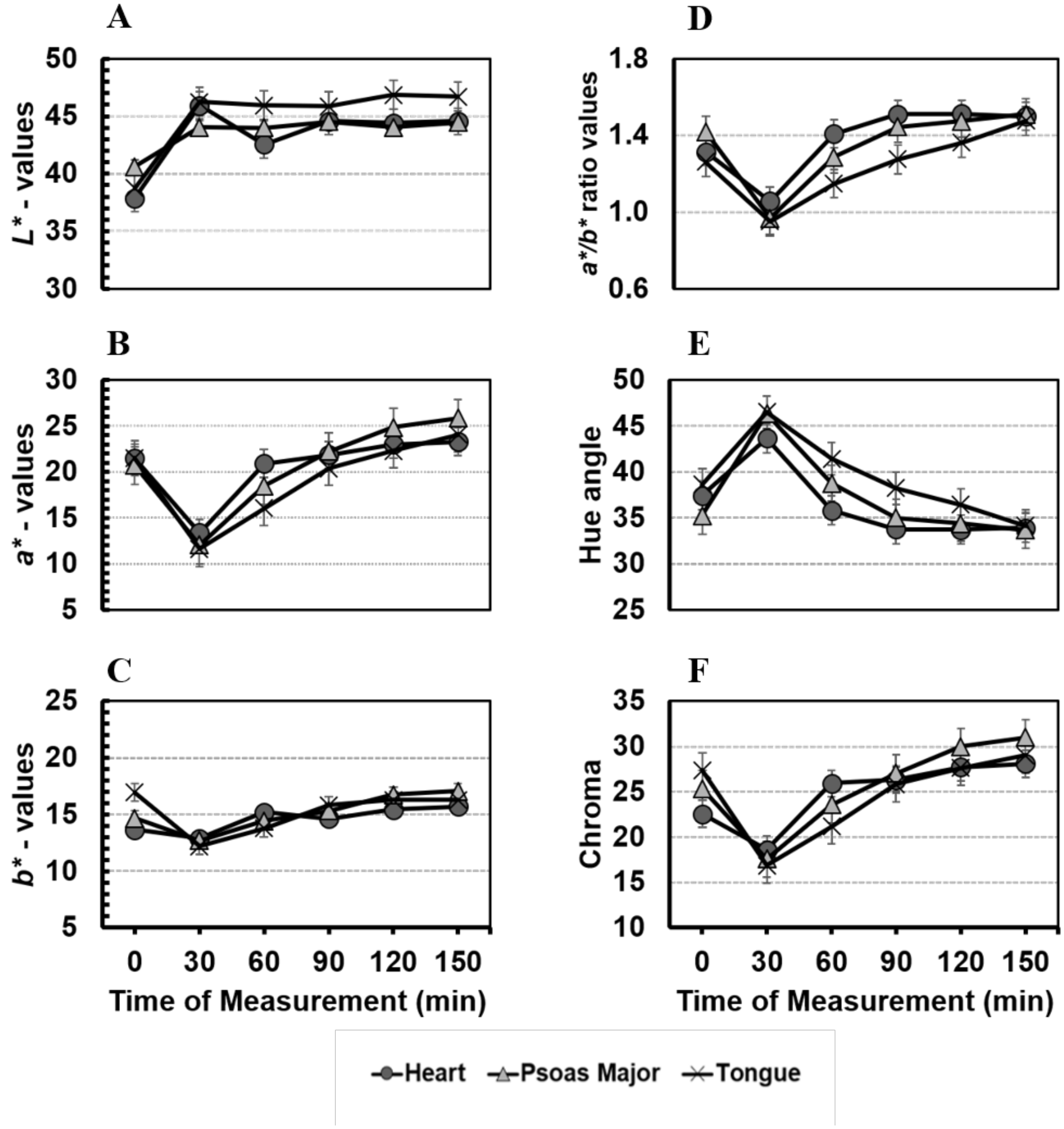

Figure 3. Reflecto-spectrophotometer readings displaying the effects of muscle tissue type on *L*, a*, b**, hue angle, and chroma of beef tongue, beef heart, and *Psoas major* before and after nitrite treatment and vacuum-sealed incubation. Bars represent standard error per muscle type per time.

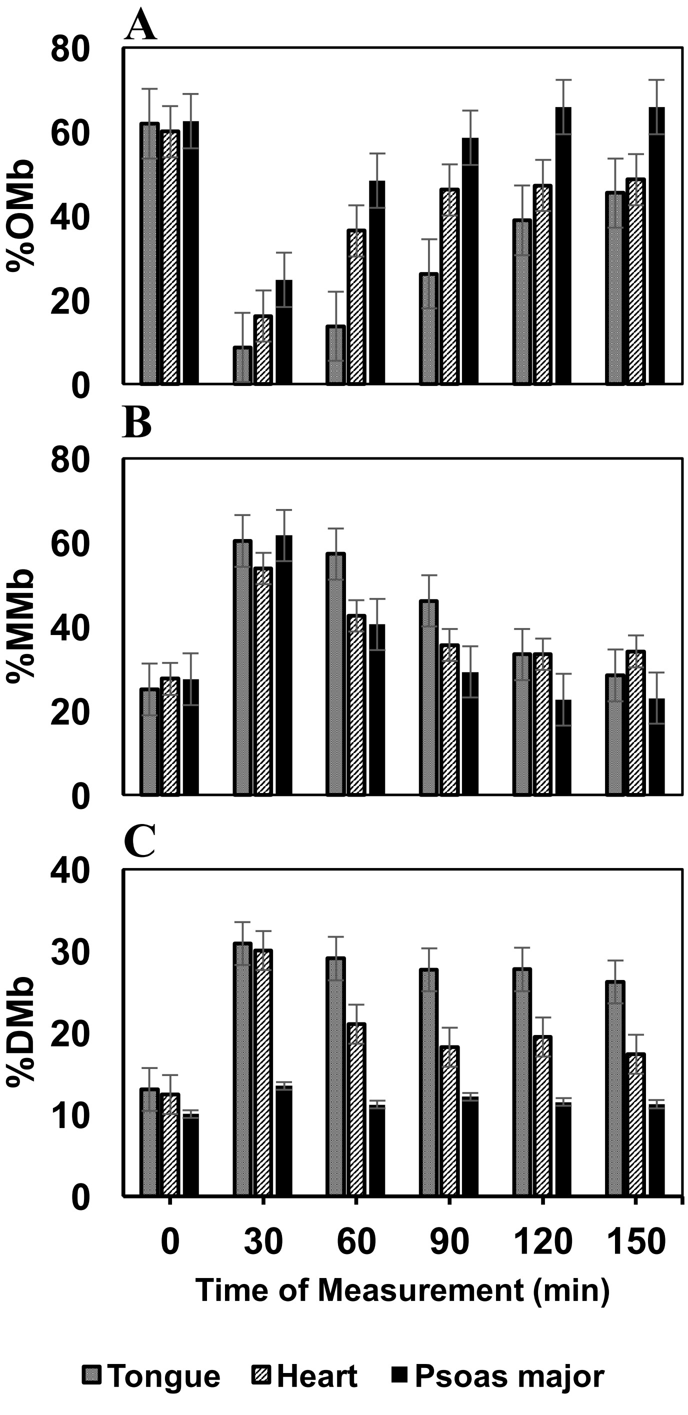

Figure 4. Percentage of metmyoglobin (MMb), deoxymyoglobin (DMb) and oxymyoglobin (OMb) in three types of bovine muscle by reflecto-spectrophotometer. Percent abundance determined using % surface MMb before and after nitrite treatment and vacuum-sealed incubation. (A) oxymyoglobin; (B) metmyoglobin; (C) deoxymyoglobin. Bars represent standard error per time.

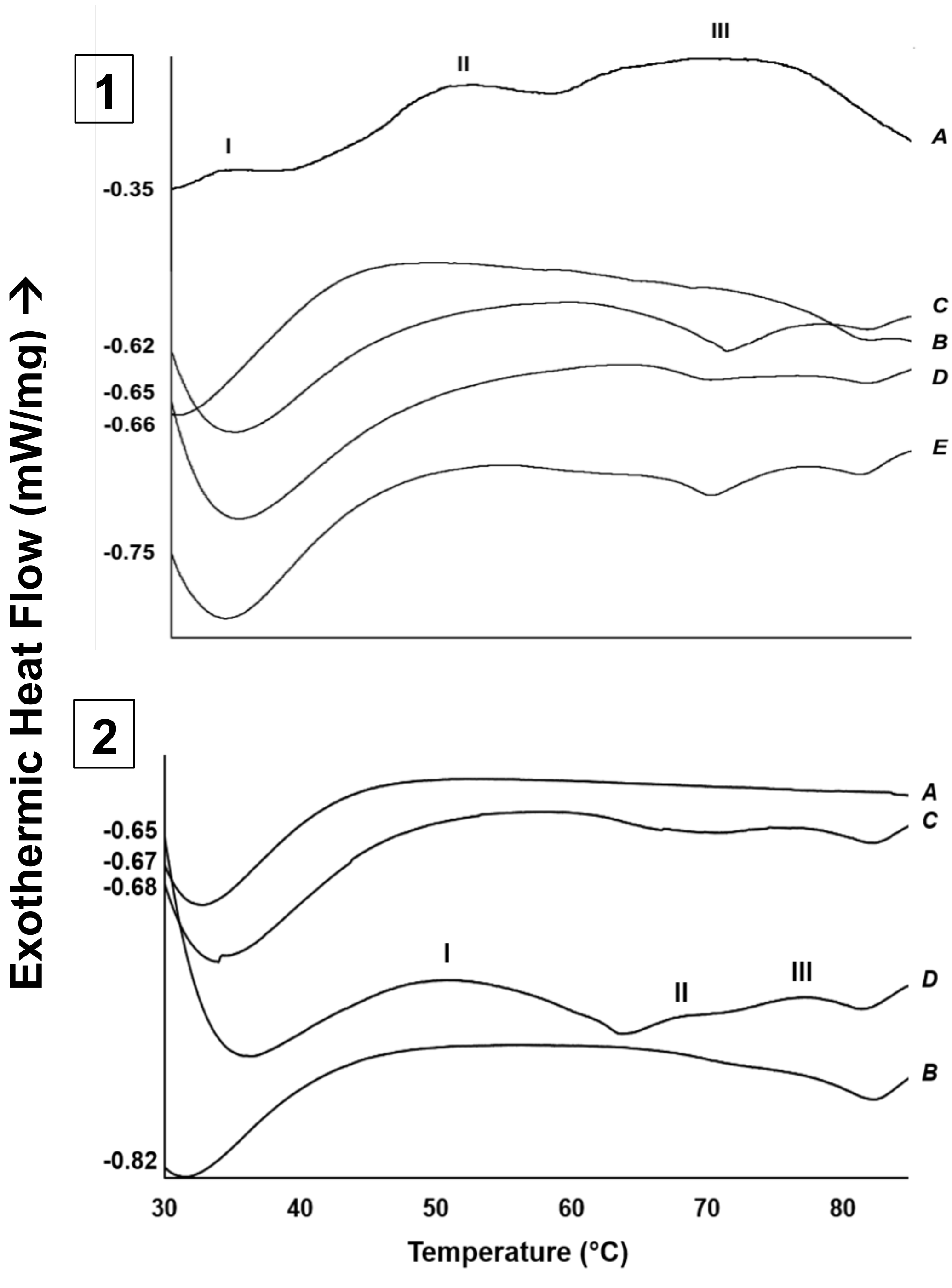

Figure 5. Differential scanning calorimetric (DSC) thermograms of beef tongue and *Psoas major* subjected to thermal treatments. (1A) spray-dried powder; (1B-1D) tongue muscle cooked to internal temperature of 75 °C, 67.5 °C; and 60 °C, respectively; (1E) raw tongue muscle. (2A-2C) *Psoas major* muscle cooked to internal temperature of 75 °C, 67.5 °C; and 60 °C, respectively; (2D) *Psoas major* raw muscle. Roman numerals (I-III) indicate peaks associated with specific protein denaturation.

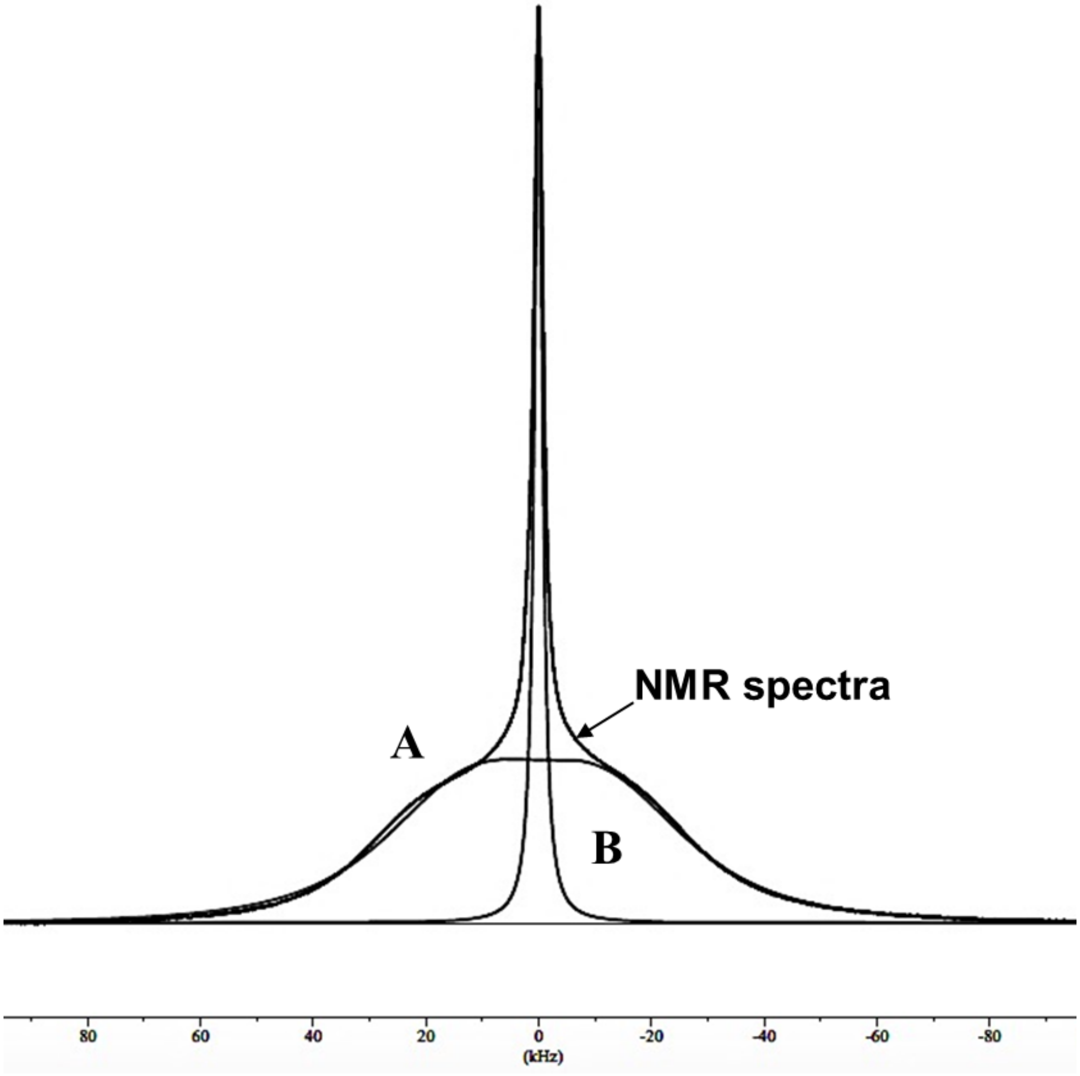

Figure 6. Wide-line ^{1}H NMR spectrum for spray-dried beef tongue powder of 4 % moisture. Line shape was deconvoluted into two Lorentzian curves in which the summation represents the total line shape. The broad curve (A) represents percent loosely and tightly bound water, and the narrow curve (B) represents percent freely bound water.

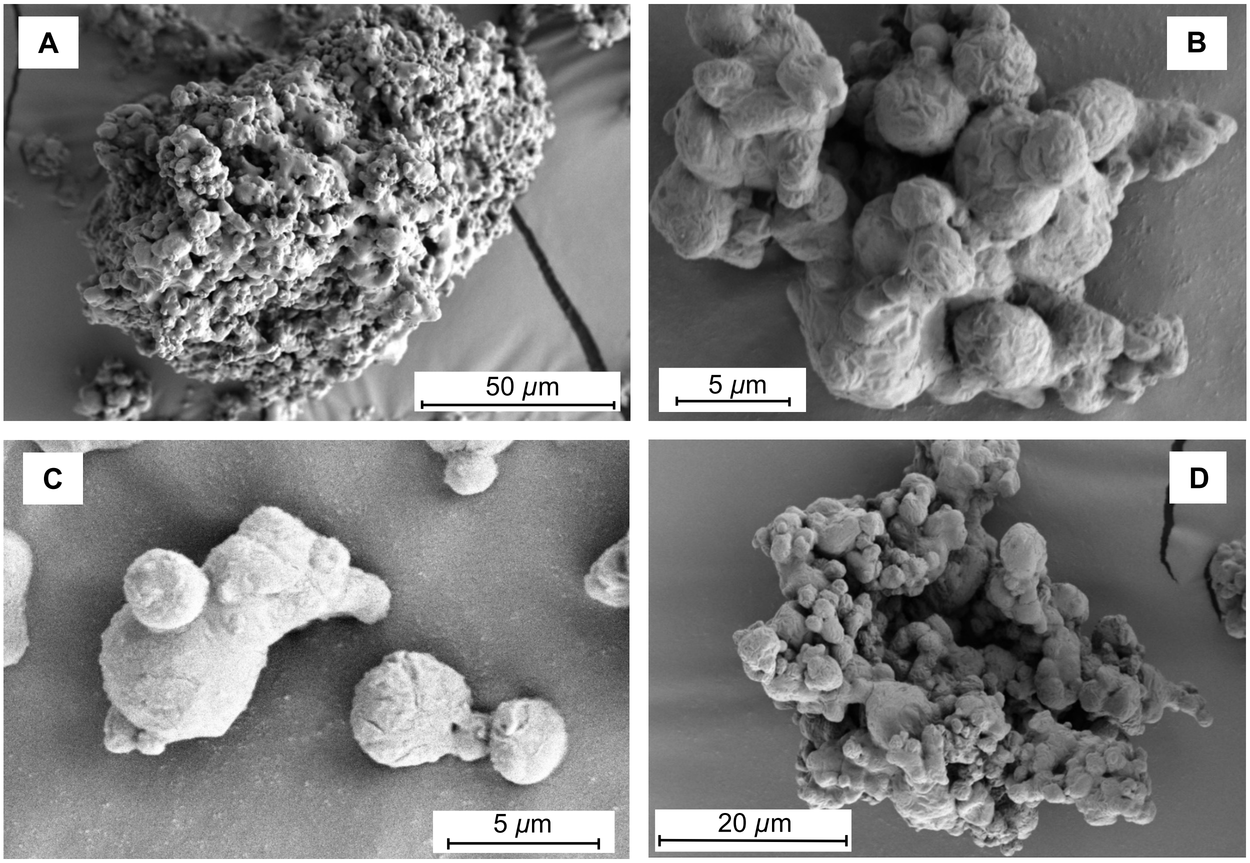

Figure 7. Micrographs of spray-dried beef tongue powder formulated with 10% maltodextrin. Images with magnification of (A) 2500×; (B) 15000×; (C) 20000×; and (D) 6500×.

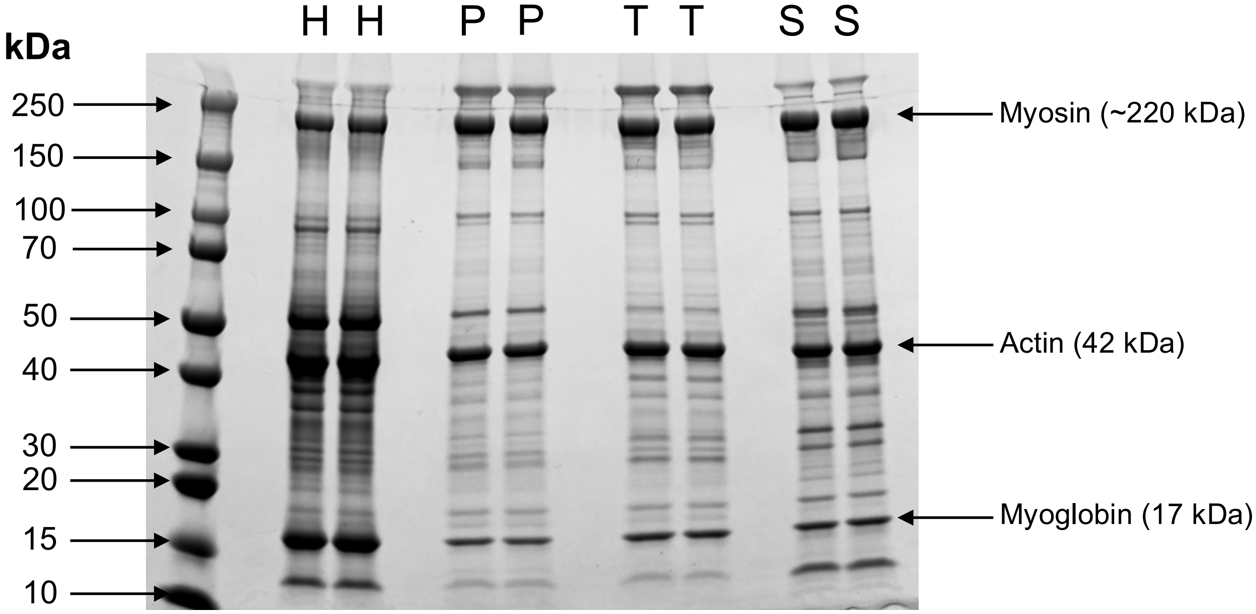

Figure 8. SDS-PAGE gel of isolated protein fractions from beef tongue powder (S), raw beef heart homogenate (H), raw *Psoas major* homogenate (P), and raw beef tongue homogenate (T) at *10 μ*L stained with Coomassie brilliant blue.

APPENDIX

RELATIVE ABUNDANCE OF BEEF TONGUE POWDER PROTEINS

***Batch**	**Protein**	**Peak Area**
B1	Serum albumin OS=Bos taurus GN=ALB PE=1 SV=4 - [ALBU_BOVIN]	4.0×10^9
B1	Creatine kinase M-type OS=Bos taurus OX=9913 GN=CKM PE=1 SV=2 - [KCRM_BOVIN]	2.7×10^9
B1	Serotransferrin OS=Bos taurus GN=TF PE=2 SV=1 - [TRFE_BOVIN]	6.4×10^8
B1	Triosephosphate isomerase OS=Bos taurus GN=TPI1 PE=2 SV=3 - [TPIS_BOVIN]	8.4×10^8
B1	Myoglobin OS=Bison bison OX=9901 GN=MB PE=1 SV=1 - [MYG_BISBI]	2.2×10^9
B1	Glyceraldehyde-3-phosphate dehydrogenase OS=Bos taurus GN=GAPDH PE=1 SV=4 - [G3P_BOVIN]	4.6×10^8
B1	Tropomyosin beta chain OS=Bos taurus GN=TPM2 PE=2 SV=1 - [TPM2_BOVIN]	1.8×10^8
B1	Malate dehydrogenase, mitochondrial OS=Bos taurus GN=MDH2 PE=1 SV=1 - [MDHM_BOVIN]	2.5×10^8
B1	Phosphoglycerate mutase 2 OS=Bos taurus GN=PGAM2 PE=2 SV=1 - [PGAM2_BOVIN]	6.3×10^8
B1	Phosphatidylethanolamine-binding protein 1 OS=Bos taurus OX=9913 GN=PEBP1 PE=1 SV=2 - [PEBP1_BOVIN]	1.1×10^9
B1	Tropomyosin alpha-1 chain OS=Homo sapiens GN=TPM1 PE=1 SV=2 - [TPM1_HUMAN]	1.8×10^8
B1	Fructose-bisphosphate aldolase A OS=Rattus norvegicus GN=Aldoa PE=1 SV=2 - [ALDOA_RAT]	3.5×10^8
B1	Tropomyosin alpha-3 chain OS=Bos taurus GN=TPM3 PE=2 SV=1 - [TPM3_BOVIN]	1.8×10^8
B1	Alpha-1-acid glycoprotein OS=Bos taurus GN=ORM1 PE=2 SV=1 - [A1AG_BOVIN]	4.7×10^8
B1	Creatine kinase S-type, mitochondrial OS=Bos taurus GN=CKMT2 PE=2 SV=1 - [KCRS_BOVIN]	1.1×10^8
B1	Cytochrome c OS=Bos taurus GN=CYCS PE=1 SV=2 - [CYC_BOVIN]	2.1×10^8

B1	Beta-enolase OS=Bos taurus GN=ENO3 PE=2 SV=1 - [ENOB_BOVIN]	1.3×10^8
B1	Phosphoglycerate kinase 1 OS=Bos taurus GN=PGK1 PE=2 SV=3 - [PGK1_BOVIN]	5.5×10^7
B1	Hemoglobin subunit alpha OS=Bos taurus OX=9913 GN=HBA PE=1 SV=2 - [HBA_BOVIN]	6.5×10^8
B1	Hemoglobin subunit beta OS=Bos taurus GN=HBB PE=1 SV=1 - [HBB_BOVIN]	9.1×10^7
B1	Malate dehydrogenase, cytoplasmic OS=Bos taurus GN=MDH1 PE=2 SV=3 - [MDHC_BOVIN]	6.9×10^7
B1	Tropomyosin alpha-3 chain OS=Rattus norvegicus GN=Tpm3 PE=1 SV=2 - [TPM3_RAT]	1.8×10^8
B1	Acetyl-CoA acetyltransferase, mitochondrial OS=Bos taurus OX=9913 GN=ACAT1 PE=2 SV=1 - [THIL_BOVIN]	5.5×10^7
B1	Creatine kinase S-type, mitochondrial OS=Oryctolagus cuniculus GN=CKMT2 PE=2 SV=1 - [KCRS_RABIT]	9.1×10^7
B1	Aconitate hydratase, mitochondrial OS=Bos taurus GN=ACO2 PE=1 SV=4 - [ACON_BOVIN]	2.5×10^7
B1	Myosin light chain 1/3, skeletal muscle isoform OS=Bos taurus OX=9913 GN=MYL1 PE=2 SV=1 - [MYL1_BOVIN]	9.5×10^7
B1	Aspartate aminotransferase, cytoplasmic OS=Bos taurus GN=GOT1 PE=1 SV=3 - [AATC_BOVIN]	7.1×10^7
B1	Myosin light chain 3 OS=Bos taurus OX=9913 GN=MYL3 PE=1 SV=1 - [MYL3_BOVIN]	8.8×10^7
B1	Actin, aortic smooth muscle OS=Bos taurus GN=ACTA2 PE=1 SV=1 - [ACTA_BOVIN]	1.9×10^8
B1	Alpha-1B-glycoprotein OS=Bos taurus GN=A1BG PE=1 SV=1 - [A1BG_BOVIN]	4.2×10^7
B1	Myosin-7 OS=Mesocricetus auratus GN=MYH7 PE=2 SV=2 - [MYH7_MESAU]	1.9×10^7
B1	Heat shock 70 kDa protein 1B OS=Bos taurus GN=HSPA1B PE=2 SV=1 - [HS71B_BOVIN]	1.5×10^7
B1	Pyruvate kinase PKM OS=Felis catus GN=PKM PE=1 SV=2 - [KPYM_FELCA]	7.2×10^7
B1	Protein/nucleic acid deglycase DJ-1 OS=Bos taurus OX=9913 GN=PARK7 PE=2 SV=1 - [PARK7_BOVIN]	2.8×10^7
B1	Pancreatic trypsin inhibitor OS=Bos taurus PE=1 SV=2 - [BPT1_BOVIN]	5.7×10^7
B1	Superoxide dismutase [Cu-Zn] OS=Bos taurus OX=9913 GN=SOD1 PE=1 SV=2 - [SODC_BOVIN]	1.7×10^8

B1	Adenylate kinase isoenzyme 1 OS=Bos taurus GN=AK1 PE=1 SV=2 - [KAD1_BOVIN]	2.6×10^{7}
B1	Heat shock cognate 71 kDa protein OS=Equus caballus GN=HSPA8 PE=2 SV=1 - [HSP7C_HORSE]	1.5×10^{7}
B1	Troponin C, skeletal muscle OS=Sus scrofa OX=9823 GN=TNNC2 PE=1 SV=2 - [TNNC2_PIG]	2.1×10^{7}
B1	Carbonic anhydrase 3 OS=Bos taurus OX=9913 GN=CA3 PE=2 SV=3 - [CAH3_BOVIN]	5.5×10^{7}
B1	Musculoskeletal embryonic nuclear protein 1 OS=Bos taurus OX=9913 GN=MUSTN1 PE=3 SV=1 - [MSTN1_BOVIN]	5.0×10^{7}
B1	Hemopexin OS=Bos taurus GN=HPX PE=2 SV=1 - [HEMO_BOVIN]	6.4×10^{7}
B1	L-lactate dehydrogenase B chain OS=Bos taurus GN=LDHB PE=2 SV=4 - [LDHB_BOVIN]	5.1×10^{7}
B1	Vitamin D-binding protein OS=Bos taurus GN=GC PE=2 SV=1 - [VTDB_BOVIN]	2.9×10^{7}
B1	Peroxiredoxin-6 OS=Bos taurus OX=9913 GN=PRDX6 PE=1 SV=3 - [PRDX6_BOVIN]	2.3×10^{7}
B1	Complement C3 OS=Bos taurus GN=C3 PE=1 SV=2 - [CO3_BOVIN]	1.1×10^{7}
B1	ATP synthase subunit alpha, mitochondrial OS=Bos taurus GN=ATP5A1 PE=1 SV=1 - [ATPA_BOVIN]	3.0×10^{6}
B1	Peptidyl-prolyl cis-trans isomerase FKBP3 OS=Bos taurus GN=FKBP3 PE=1 SV=2 - [FKBP3_BOVIN]	2.6×10^{7}
B1	Peroxiredoxin-1 OS=Bos taurus GN=PRDX1 PE=2 SV=1 - [PRDX1_BOVIN]	5.7×10^{7}
B1	Thioredoxin OS=Bos taurus GN=TXN PE=3 SV=3 - [THIO_BOVIN]	9.5×10^{6}
B1	Cofilin-2 OS=Bos taurus GN=CFL2 PE=2 SV=1 - [COF2_BOVIN]	1.6×10^{7}
B1	Cadherin-13 OS=Homo sapiens OX=9606 GN=CDH13 PE=1 SV=1 - [CAD13_HUMAN]	5.2×10^{6}
B1	14 kDa phosphohistidine phosphatase OS=Bos taurus OX=9913 GN=PHPT1 PE=2 SV=1 - [PHP14_BOVIN]	2.4×10^{7}
B1	Troponin T, fast skeletal muscle OS=Bos taurus OX=9913 GN=Tnnt3 PE=2 SV=1 - [TNNT3_BOVIN]	7.7×10^{6}
B1	Alpha-1-antiproteinase OS=Bos taurus GN=SERPINA1 PE=1 SV=1 - [A1AT_BOVIN]	7.7×10^{6}
B1	ATP synthase-coupling factor 6, mitochondrial OS=Sus scrofa OX=9823 GN=ATP5PF PE=1 SV=1 - [ATP5J_PIG]	1.1×10^{7}

B1	Fatty acid-binding protein 5 OS=Bos taurus OX=9913 GN=FABP5 PE=1 SV=4 - [FABP5_BOVIN]	1.1×10^{7}
B1	Prothymosin alpha OS=Bos taurus GN=PTMA PE=1 SV=2 - [PTMA_BOVIN]	5.6×10^{6}
B1	Myosin-2 OS=Equus caballus GN=MYH2 PE=2 SV=1 - [MYH2_HORSE]	8.4×10^{6}
B1	Peptidyl-prolyl cis-trans isomerase A OS=Bos taurus GN=PPIA PE=1 SV=2 - [PPIA_BOVIN]	8.7×10^{6}
B1	Fatty acid-binding protein, heart OS=Sus scrofa OX=9823 GN=FABP3 PE=2 SV=3 - [FABPH_PIG]	6.2×10^{7}
B1	Myc box-dependent-interacting protein 1 OS=Mus musculus OX=10090 GN=Bin1 PE=1 SV=1 - [BIN1_MOUSE]	3.1×10^{7}
B1	Dihydrolipoyl dehydrogenase, mitochondrial OS=Homo sapiens OX=9606 GN=DLD PE=1 SV=2 - [DLDH_HUMAN]	2.8×10^{7}
B1	Ceruloplasmin OS=Ovis aries OX=9940 GN=CP PE=2 SV=1 - [CERU_SHEEP]	4.4×10^{6}
B1	Catalase OS=Bos taurus GN=CAT PE=1 SV=3 - [CATA_BOVIN]	7.2×10^{6}
B1	Troponin C, slow skeletal and cardiac muscles OS=Bos taurus OX=9913 GN=TNNC1 PE=1 SV=1 - [TNNC1_BOVIN]	3.8×10^{6}
B1	Radixin OS=Mus musculus GN=Rdx PE=1 SV=3 - [RADI_MOUSE]	1.1×10^{7}
B1	Small muscular protein OS=Bos taurus OX=9913 GN=SMPX PE=3 SV=1 - [SMPX_BOVIN]	3.0×10^{7}
B1	Stress-70 protein, mitochondrial (Fragments) OS=Mesocricetus auratus GN=HSPA9 PE=1 SV=1 - [GRP75_MESAU]	5.8×10^{6}
B1	Peptidyl-prolyl cis-trans isomerase FKBP1A OS=Bos taurus OX=9913 GN=FKBP1A PE=1 SV=2 - [FKB1A_BOVIN]	5.4×10^{7}
B1	Myosin regulatory light chain 2, ventricular/cardiac muscle isoform OS=Oryctolagus cuniculus OX=9986 GN=MYL2 PE=1 SV=2 - [MLRV_RABIT]	2.0×10^{7}
B1	Protein HP-20 homolog OS=Bos taurus OX=9913 PE=2 SV=1 - [HP20_BOVIN]	2.0×10^{7}
B1	Lumican OS=Bos taurus OX=9913 GN=LUM PE=1 SV=1 - [LUM_BOVIN]	1.9×10^{7}
B1	Four and a half LIM domains protein 1 OS=Rattus norvegicus OX=10116 GN=Fhl1 PE=2 SV=1 - [FHL1_RAT]	8.0×10^{6}
B1	10 kDa heat shock protein, mitochondrial OS=Rattus norvegicus GN=Hspe1 PE=1 SV=3 - [CH10_RAT]	1.3×10^{7}
B1	Non-histone chromosomal protein HMG-14 OS=Bos taurus OX=9913 GN=HMGN1 PE=1 SV=2 - [HMGN1_BOVIN]	1.1×10^{6}

B1	Alpha-aminoadipic semialdehyde dehydrogenase OS=Mus musculus OX=10090 GN=Aldh7a1 PE=1 SV=4 - [AL7A1_MOUSE]	4.3×10^6
B1	Protein HP-25 homolog 1 OS=Bos taurus OX=9913 PE=1 SV=1 - [HP251_BOVIN]	1.0×10^7
B1	Non-muscle caldesmon OS=Rattus norvegicus OX=10116 GN=Cald1 PE=1 SV=1 - [CALD1_RAT]	1.1×10^6
B1	Troponin I, slow skeletal muscle OS=Rattus norvegicus GN=Tnni1 PE=1 SV=2 - [TNNI1_RAT]	3.3×10^6
B1	Prothrombin OS=Bos taurus OX=9913 GN=F2 PE=1 SV=2 - [THRB_BOVIN]	7.0×10^6
B1	Myoglobin OS=Zalophus californianus OX=9704 GN=MB PE=1 SV=2 - [MYG_ZALCA]	2.9×10^8
B1	Zinc-alpha-2-glycoprotein OS=Bos taurus OX=9913 GN=AZGP1 PE=2 SV=1 - [ZA2G_BOVIN]	1.2×10^7
B1	Histidine triad nucleotide-binding protein 1 OS=Bos taurus GN=HINT1 PE=1 SV=2 - [HINT1_BOVIN]	1.1×10^7
B1	Glutathione S-transferase P OS=Bos taurus GN=GSTP1 PE=1 SV=2 - [GSTP1_BOVIN]	1.5×10^7
B1	ATPase inhibitor, mitochondrial OS=Bos taurus OX=9913 GN=ATP5IF1 PE=1 SV=2 - [ATIF1_BOVIN]	7.2×10^7
B1	Alpha-2-HS-glycoprotein OS=Bos taurus GN=AHSG PE=1 SV=2 - [FETUA_BOVIN]	1.1×10^7
B1	Glycogen phosphorylase, muscle form OS=Ovis aries OX=9940 GN=PYGM PE=2 SV=3 - [PYGM_SHEEP]	4.2×10^7
B1	Myosin regulatory light chain 2, skeletal muscle isoform OS=Rattus norvegicus OX=10116 GN=Mylpf PE=1 SV=2 - [MLRS_RAT]	1.9×10^7
B1	Macrophage migration inhibitory factor OS=Bos taurus OX=9913 GN=MIF PE=1 SV=6 - [MIF_BOVIN]	1.0×10^7
B1	CapZ-interacting protein OS=Bos taurus OX=9913 GN=RCSD1 PE=2 SV=1 - [CPZIP_BOVIN]	2.4×10^6
B1	Vimentin (Fragment) OS=Cricetulus griseus GN=VIM PE=2 SV=1 - [VIME_CRIGR]	4.4×10^7
B1	Kininogen-2 OS=Bos taurus GN=KNG2 PE=1 SV=1 - [KNG2_BOVIN]	4.3×10^6
B1	Protein HP-25 homolog 2 OS=Bos taurus OX=9913 PE=2 SV=1 - [HP252_BOVIN]	8.0×10^6
B1	Hepatoma-derived growth factor OS=Bos taurus OX=9913 GN=HDGF PE=2 SV=1 - [HDGF_BOVIN]	1.4×10^7

B1	28 kDa heat- and acid-stable phosphoprotein OS=Homo sapiens GN=PDAP1 PE=1 SV=1 - [HAP28_HUMAN]	1.5×10^6
B1	Filamin-C OS=Homo sapiens GN=FLNC PE=1 SV=3 - [FLNC_HUMAN]	1.2×10^7
B1	Transthyretin OS=Bos taurus GN=TTR PE=1 SV=1 - [TTHY_BOVIN]	1.1×10^7
B1	NADH dehydrogenase [ubiquinone] iron-sulfur protein 6, mitochondrial OS=Bos taurus OX=9913 GN=NDUFS6 PE=1 SV=2 - [NDUS6_BOVIN]	2.9×10^6
B1	Proteasome subunit beta type-6 OS=Bos taurus OX=9913 GN=PSMB6 PE=1 SV=1 - [PSB6_BOVIN]	3.7×10^6
B1	ATP synthase subunit beta, mitochondrial OS=Bos taurus GN=ATP5B PE=1 SV=2 - [ATPB_BOVIN]	1.3×10^7
B1	ADP-ribose glycohydrolase MACROD1 OS=Bos taurus OX=9913 GN=MACROD1 PE=2 SV=1 - [MACD1_BOVIN]	3.7×10^6
B1	Plasminogen activator inhibitor 1 RNA-binding protein OS=Rattus norvegicus GN=Serbp1 PE=1 SV=2 - [PAIRB_RAT]	1.1×10^6
B1	Stress-induced-phosphoprotein 1 OS=Bos taurus GN=STIP1 PE=2 SV=1 - [STIP1_BOVIN]	4.4×10^6
B1	Myristoylated alanine-rich C-kinase substrate OS=Bos taurus OX=9913 GN=MARCKS PE=1 SV=6 - [MARCS_BOVIN]	2.9×10^6
B2	Serum albumin OS=Bos taurus GN=ALB PE=1 SV=4 - [ALBU_BOVIN]	1.4×10^9
B2	Creatine kinase M-type OS=Bos taurus OX=9913 GN=CKM PE=1 SV=2 - [KCRM_BOVIN]	1.5×10^9
B2	Triosephosphate isomerase OS=Bos taurus GN=TPI1 PE=2 SV=3 - [TPIS_BOVIN]	4.6×10^8
B2	Tropomyosin beta chain OS=Bos taurus GN=TPM2 PE=2 SV=1 - [TPM2_BOVIN]	8.8×10^7
B2	Myoglobin OS=Bison bison OX=9901 GN=MB PE=1 SV=1 - [MYG_BISBI]	6.6×10^8
B2	Serotransferrin OS=Bos taurus GN=TF PE=2 SV=1 - [TRFE_BOVIN]	1.4×10^8
B2	Malate dehydrogenase, mitochondrial OS=Bos taurus GN=MDH2 PE=1 SV=1 - [MDHM_BOVIN]	8.1×10^7
B2	Phosphatidylethanolamine-binding protein 1 OS=Bos taurus OX=9913 GN=PEBP1 PE=1 SV=2 - [PEBP1_BOVIN]	7.6×10^8
B2	Tropomyosin alpha-1 chain OS=Homo sapiens GN=TPM1 PE=1 SV=2 - [TPM1_HUMAN]	8.8×10^7

B2	Phosphoglycerate mutase 2 OS=Bos taurus GN=PGAM2 PE=2 SV=1 - [PGAM2_BOVIN]	3.1×10^{8}
B2	Tropomyosin alpha-3 chain OS=Bos taurus GN=TPM3 PE=2 SV=1 - [TPM3_BOVIN]	8.8×10^{7}
B2	Alpha-1-acid glycoprotein OS=Bos taurus GN=ORM1 PE=2 SV=1 - [A1AG_BOVIN]	2.3×10^{8}
B2	Tropomyosin alpha-4 chain OS=Equus caballus GN=TPM4 PE=1 SV=2 - [TPM4_HORSE]	8.8×10^{7}
B2	Glyceraldehyde-3-phosphate dehydrogenase OS=Sus scrofa GN=GAPDH PE=1 SV=4 - [G3P_PIG]	1.5×10^{8}
B2	Acetyl-CoA acetyltransferase, mitochondrial OS=Bos taurus OX=9913 GN=ACAT1 PE=2 SV=1 - [THIL_BOVIN]	3.1×10^{7}
B2	Hemoglobin subunit alpha OS=Bos taurus OX=9913 GN=HBA PE=1 SV=2 - [HBA_BOVIN]	2.1×10^{8}
B2	Fructose-bisphosphate aldolase A OS=Rattus norvegicus GN=Aldoa PE=1 SV=2 - [ALDOA_RAT]	3.5×10^{7}
B2	Beta-enolase OS=Rattus norvegicus GN=Eno3 PE=1 SV=3 - [ENOB_RAT]	4.8×10^{7}
B2	Myosin light chain 3 OS=Bos taurus OX=9913 GN=MYL3 PE=1 SV=1 - [MYL3_BOVIN]	4.4×10^{7}
B2	Superoxide dismutase [Cu-Zn] OS=Bos taurus OX=9913 GN=SOD1 PE=1 SV=2 - [SODC_BOVIN]	1.3×10^{8}
B2	Alpha-1B-glycoprotein OS=Bos taurus GN=A1BG PE=1 SV=1 - [A1BG_BOVIN]	2.3×10^{7}
B2	Hemoglobin subunit beta OS=Bos taurus GN=HBB PE=1 SV=1 - [HBB_BOVIN]	3.5×10^{7}
B2	Actin, cytoplasmic 1 OS=Cercopithecus pygerythrus GN=ACTB PE=1 SV=1 - [ACTB_CERPY]	9.3×10^{7}
B2	Cytochrome c OS=Bos taurus GN=CYCS PE=1 SV=2 - [CYC_BOVIN]	1.0×10^{8}
B2	Myosin light chain 1/3, skeletal muscle isoform OS=Mus musculus OX=10090 GN=Myl1 PE=1 SV=2 - [MYL1_MOUSE]	3.1×10^{7}
B2	Phosphoglycerate kinase 1 OS=Equus caballus GN=PGK1 PE=1 SV=2 - [PGK1_HORSE]	1.2×10^{7}
B2	Pancreatic trypsin inhibitor OS=Bos taurus PE=1 SV=2 - [BPT1_BOVIN]	6.0×10^{7}
B2	Creatine kinase S-type, mitochondrial OS=Bos taurus GN=CKMT2 PE=2 SV=1 - [KCRS_BOVIN]	2.7×10^{7}
B2	ATP synthase-coupling factor 6, mitochondrial OS=Bos taurus OX=9913 GN=ATP5PF PE=1 SV=2 - [ATP5J_BOVIN]	9.2×10^{6}

B2	Protein/nucleic acid deglycase DJ-1 OS=Bos taurus OX=9913 GN=PARK7 PE=2 SV=1 - [PARK7_BOVIN]	2.0×10^7
B2	Musculoskeletal embryonic nuclear protein 1 OS=Bos taurus OX=9913 GN=MUSTN1 PE=3 SV=1 - [MSTN1_BOVIN]	3.6×10^7
B2	Myosin-7 OS=Mesocricetus auratus GN=MYH7 PE=2 SV=2 - [MYH7_MESAU]	1.1×10^7
B2	Peroxiredoxin-6 OS=Bos taurus OX=9913 GN=PRDX6 PE=1 SV=3 - [PRDX6_BOVIN]	1.8×10^7
B2	Small muscular protein OS=Bos taurus OX=9913 GN=SMPX PE=3 SV=1 - [SMPX_BOVIN]	3.8×10^7
B2	Peptidyl-prolyl cis-trans isomerase A OS=Bos taurus GN=PPIA PE=1 SV=2 - [PPIA_BOVIN]	8.2×10^6
B2	Transthyretin OS=Bos taurus GN=TTR PE=1 SV=1 - [TTHY_BOVIN]	1.0×10^7
B2	Aspartate aminotransferase, cytoplasmic OS=Bos taurus GN=GOT1 PE=1 SV=3 - [AATC_BOVIN]	1.5×10^7
B2	Myosin regulatory light chain 2, ventricular/cardiac muscle isoform OS=Oryctolagus cuniculus OX=9986 GN=MYL2 PE=1 SV=2 - [MLRV_RABIT]	1.7×10^7
B2	Carbonic anhydrase 3 OS=Bos taurus OX=9913 GN=CA3 PE=2 SV=3 - [CAH3_BOVIN]	2.1×10^7
B2	10 kDa heat shock protein, mitochondrial OS=Rattus norvegicus GN=Hspe1 PE=1 SV=3 - [CH10_RAT]	8.3×10^6
B2	28 kDa heat- and acid-stable phosphoprotein OS=Homo sapiens GN=PDAP1 PE=1 SV=1 - [HAP28_HUMAN]	1.6×10^6
B2	Cysteine and glycine-rich protein 3 OS=Bos taurus OX=9913 GN=CSRP3 PE=2 SV=1 - [CSRP3_BOVIN]	9.2×10^6
B2	Myc box-dependent-interacting protein 1 OS=Mus musculus OX=10090 GN=Bin1 PE=1 SV=1 - [BIN1_MOUSE]	1.6×10^7
B2	Tubulin polymerization-promoting protein family member 3 OS=Bos taurus OX=9913 GN=TPPP3 PE=1 SV=1 - [TPPP3_BOVIN]	2.0×10^6
B2	Nuclease-sensitive element-binding protein 1 OS=Mus musculus GN=Ybx1 PE=1 SV=3 - [YBOX1_MOUSE]	2.9×10^6
B2	Aconitate hydratase, mitochondrial OS=Bos taurus GN=ACO2 PE=1 SV=4 - [ACON_BOVIN]	1.9×10^6
B2	Parathymosin OS=Bos taurus GN=PTMS PE=1 SV=2 - [PTMS_BOVIN]	1.3×10^7
B2	Metallothionein-1B OS=Equus caballus OX=9796 PE=1 SV=2 - [MT1B_HORSE]	3.7×10^6

B2	14 kDa phosphohistidine phosphatase OS=Bos taurus OX=9913 GN=PHPT1 PE=2 SV=1 - [PHP14_BOVIN]	1.9×10^7
B2	Malate dehydrogenase, cytoplasmic OS=Bos taurus GN=MDH1 PE=2 SV=3 - [MDHC_BOVIN]	1.2×10^7
B2	Troponin C, skeletal muscle OS=Sus scrofa OX=9823 GN=TNNC2 PE=1 SV=2 - [TNNC2_PIG]	5.9×10^6
B2	Adenylate kinase isoenzyme 1 OS=Bos taurus GN=AK1 PE=1 SV=2 - [KAD1_BOVIN]	6.6×10^6
B2	L-lactate dehydrogenase B chain OS=Bos taurus GN=LDHB PE=2 SV=4 - [LDHB_BOVIN]	1.2×10^7
B2	Cytochrome c oxidase subunit 6B1 OS=Bos taurus OX=9913 GN=COX6B1 PE=1 SV=2 - [CX6B1_BOVIN]	2.0×10^7
B2	Catalase OS=Bos taurus GN=CAT PE=1 SV=3 - [CATA_BOVIN]	4.1×10^6
B2	Heat shock 70 kDa protein 1 OS=Chlorocebus aethiops GN=HSPA1 PE=2 SV=1 - [HSP71_CHLAE]	1.5×10^6
B2	Radixin OS=Mus musculus GN=Rdx PE=1 SV=3 - [RADI_MOUSE]	6.1×10^6
B2	Peptidyl-prolyl cis-trans isomerase FKBP1A OS=Bos taurus OX=9913 GN=FKBP1A PE=1 SV=2 - [FKB1A_BOVIN]	2.3×10^7
B2	Zinc-alpha-2-glycoprotein OS=Bos taurus OX=9913 GN=AZGP1 PE=2 SV=1 - [ZA2G_BOVIN]	1.1×10^7
B2	Protein HP-25 homolog 1 OS=Bos taurus OX=9913 PE=1 SV=1 - [HP251_BOVIN]	4.4×10^6
B2	Troponin I, cardiac muscle OS=Bos taurus OX=9913 GN=TNNI3 PE=1 SV=2 - [TNNI3_BOVIN]	2.3×10^7
B2	Protein HP-25 homolog 2 OS=Bos taurus OX=9913 PE=2 SV=1 - [HP252_BOVIN]	5.1×10^6
B2	Protein CutA OS=Bos taurus OX=9913 GN=CUTA PE=1 SV=1 - [CUTA_BOVIN]	8.1×10^6
B2	Hemopexin OS=Bos taurus GN=HPX PE=2 SV=1 - [HEMO_BOVIN]	3.2×10^7
B2	Cytochrome c oxidase assembly factor 6 homolog OS=Bos taurus OX=9913 GN=COA6 PE=3 SV=1 - [COA6_BOVIN]	1.4×10^6
B2	ATPase inhibitor, mitochondrial OS=Bos taurus OX=9913 GN=ATP5IF1 PE=1 SV=2 - [ATIF1_BOVIN]	4.6×10^7
B2	Macrophage migration inhibitory factor OS=Bos taurus OX=9913 GN=MIF PE=1 SV=6 - [MIF_BOVIN]	1.0×10^7
B2	Peroxiredoxin-1 OS=Bos taurus GN=PRDX1 PE=2 SV=1 - [PRDX1_BOVIN]	1.5×10^7

B2	Galectin-1 OS=Bos taurus OX=9913 GN=LGALS1 PE=1 SV=2 - [LEG1_BOVIN]	3.2×10^{6}
B2	DNA-(apurinic or apyrimidinic site) lyase OS=Bos taurus GN=APEX1 PE=1 SV=2 - [APEX1_BOVIN]	1.7×10^{6}
B2	Protein phosphatase inhibitor 2 OS=Bos taurus OX=9913 GN=PPP1R2 PE=2 SV=1 - [IPP2_BOVIN]	1.4×10^{6}
B2	Craniofacial development protein 2 OS=Bos taurus GN=CFDP2 PE=1 SV=2 - [CFDP2_BOVIN]	6.3×10^{5}
B2	NADH dehydrogenase [ubiquinone] iron-sulfur protein 6, mitochondrial OS=Bos taurus OX=9913 GN=NDUFS6 PE=1 SV=2 - [NDUS6_BOVIN]	4.4×10^{6}
B3	Serum albumin OS=Bos taurus GN=ALB PE=1 SV=4 - [ALBU_BOVIN]	2.5×10^{8}
B3	Triosephosphate isomerase OS=Bos taurus GN=TPI1 PE=2 SV=3 - [TPIS_BOVIN]	2.8×10^{8}
B3	Creatine kinase M-type OS=Bos taurus OX=9913 GN=CKM PE=1 SV=2 - [KCRM_BOVIN]	5.6×10^{8}
B3	Serotransferrin OS=Bos taurus GN=TF PE=2 SV=1 - [TRFE_BOVIN]	9.6×10^{7}
B3	Myoglobin OS=Bison bison OX=9901 GN=MB PE=1 SV=1 - [MYG_BISBI]	3.6×10^{8}
B3	Tropomyosin beta chain OS=Bos taurus GN=TPM2 PE=2 SV=1 - [TPM2_BOVIN]	4.0×10^{7}
B3	Phosphatidylethanolamine-binding protein 1 OS=Bos taurus OX=9913 GN=PEBP1 PE=1 SV=2 - [PEBP1_BOVIN]	4.5×10^{8}
B3	Pancreatic trypsin inhibitor OS=Bos taurus PE=1 SV=2 - [BPT1_BOVIN]	5.8×10^{7}
B3	Hemoglobin subunit alpha-1 OS=Bos mutus grunniens OX=30521 PE=1 SV=1 - [HBA1_BOSMU]	1.8×10^{8}
B3	Malate dehydrogenase, mitochondrial OS=Bos taurus GN=MDH2 PE=1 SV=1 - [MDHM_BOVIN]	2.0×10^{7}
B3	Tropomyosin alpha-1 chain OS=Homo sapiens GN=TPM1 PE=1 SV=2 - [TPM1_HUMAN]	4.0×10^{7}
B3	Alpha-1-acid glycoprotein OS=Bos taurus GN=ORM1 PE=2 SV=1 - [A1AG_BOVIN]	1.1×10^{8}
B3	Phosphoglycerate mutase 2 OS=Bos taurus GN=PGAM2 PE=2 SV=1 - [PGAM2_BOVIN]	1.3×10^{8}
B3	Glyceraldehyde-3-phosphate dehydrogenase OS=Mustela putorius furo GN=GAPDH PE=2 SV=1 - [G3P_MUSPF]	4.2×10^{7}
B3	Beta-enolase OS=Rattus norvegicus GN=Eno3 PE=1 SV=3 - [ENOB_RAT]	2.7×10^{7}

B3	Tropomyosin alpha-3 chain OS=Bos taurus GN=TPM3 PE=2 SV=1 - [TPM3_BOVIN]	4.0×10^7
B3	Musculoskeletal embryonic nuclear protein 1 OS=Bos taurus OX=9913 GN=MUSTN1 PE=3 SV=1 - [MSTN1_BOVIN]	6.0×10^7
B3	Actin, gamma-enteric smooth muscle OS=Bos taurus GN=ACTG2 PE=2 SV=1 - [ACTH_BOVIN]	1.3×10^8
B3	Fructose-bisphosphate aldolase A OS=Rattus norvegicus GN=Aldoa PE=1 SV=2 - [ALDOA_RAT]	2.7×10^7
B3	Superoxide dismutase [Cu-Zn] OS=Bos taurus OX=9913 GN=SOD1 PE=1 SV=2 - [SODC_BOVIN]	7.0×10^7
B3	Cytochrome c OS=Bos taurus GN=CYCS PE=1 SV=2 - [CYC_BOVIN]	7.3×10^7
B3	Myosin light chain 3 OS=Bos taurus OX=9913 GN=MYL3 PE=1 SV=1 - [MYL3_BOVIN]	2.2×10^7
B3	Hemoglobin subunit beta OS=Bos taurus GN=HBB PE=1 SV=1 - [HBB_BOVIN]	1.4×10^7
B3	Phosphoglycerate mutase 1 OS=Homo sapiens GN=PGAM1 PE=1 SV=2 - [PGAM1_HUMAN]	2.0×10^7
B3	Alpha-1B-glycoprotein OS=Bos taurus GN=A1BG PE=1 SV=1 - [A1BG_BOVIN]	9.5×10^6
B3	Myosin light chain 1/3, skeletal muscle isoform OS=Mus musculus OX=10090 GN=Myl1 PE=1 SV=2 - [MYL1_MOUSE]	1.4×10^7
B3	Myosin-7 OS=Mesocricetus auratus GN=MYH7 PE=2 SV=2 - [MYH7_MESAU]	2.2×10^7
B3	Peptidyl-prolyl cis-trans isomerase FKBP1A OS=Bos taurus OX=9913 GN=FKBP1A PE=1 SV=2 - [FKB1A_BOVIN]	2.2×10^7
B3	Small muscular protein OS=Bos taurus OX=9913 GN=SMPX PE=3 SV=1 - [SMPX_BOVIN]	3.8×10^7
B3	Nebulin OS=Homo sapiens GN=NEB PE=1 SV=5 - [NEBU_HUMAN]	4.7×10^6
B3	Craniofacial development protein 2 OS=Bos taurus GN=CFDP2 PE=1 SV=2 - [CFDP2_BOVIN]	1.1×10^6
B3	10 kDa heat shock protein, mitochondrial OS=Rattus norvegicus GN=Hspe1 PE=1 SV=3 - [CH10_RAT]	4.1×10^6
B3	Peroxiredoxin-6 OS=Bos taurus OX=9913 GN=PRDX6 PE=1 SV=3 - [PRDX6_BOVIN]	6.3×10^6
B3	Creatine kinase S-type, mitochondrial OS=Bos taurus GN=CKMT2 PE=2 SV=1 - [KCRS_BOVIN]	2.5×10^7

B3	Myosin regulatory light chain 2, skeletal muscle isoform OS=Homo sapiens OX=9606 GN=MYLPF PE=2 SV=1 - [MLRS_HUMAN]	4.2×10^6
B3	Cysteine and glycine-rich protein 3 OS=Rattus norvegicus OX=10116 GN=Csrp3 PE=1 SV=1 - [CSRP3_RAT]	6.7×10^6
B3	Adenylate kinase isoenzyme 1 OS=Bos taurus GN=AK1 PE=1 SV=2 - [KAD1_BOVIN]	1.1×10^7
B3	Microtubule-associated protein 4 OS=Bos taurus OX=9913 GN=MAP4 PE=1 SV=1 - [MAP4_BOVIN]	1.0×10^7
B3	Myc box-dependent-interacting protein 1 OS=Mus musculus OX=10090 GN=Bin1 PE=1 SV=1 - [BIN1_MOUSE]	2.0×10^7
B3	Vimentin OS=Homo sapiens GN=VIM PE=1 SV=4 - [VIME_HUMAN]	1.8×10^7
B3	Y-box-binding protein 3 OS=Rattus norvegicus OX=10116 GN=Ybx3 PE=1 SV=1 - [YBOX3_RAT]	4.6×10^6
B3	Acetyl-CoA acetyltransferase, mitochondrial OS=Bos taurus OX=9913 GN=ACAT1 PE=2 SV=1 - [THIL_BOVIN]	7.4×10^6
B3	ATPase inhibitor, mitochondrial OS=Bos taurus OX=9913 GN=ATP5IF1 PE=1 SV=2 - [ATIF1_BOVIN]	3.5×10^7
B3	Hemopexin OS=Bos taurus GN=HPX PE=2 SV=1 - [HEMO_BOVIN]	1.1×10^7
B3	Troponin T, fast skeletal muscle OS=Bos taurus OX=9913 GN=Tnnt3 PE=2 SV=1 - [TNNT3_BOVIN]	2.2×10^7
B3	Peptidyl-prolyl cis-trans isomerase A OS=Bos taurus GN=PPIA PE=1 SV=2 - [PPIA_BOVIN]	5.9×10^6
B3	Metallothionein-1B OS=Equus caballus OX=9796 PE=1 SV=2 - [MT1B_HORSE]	3.9×10^6
B3	Protein/nucleic acid deglycase DJ-1 OS=Bos taurus OX=9913 GN=PARK7 PE=2 SV=1 - [PARK7_BOVIN]	1.0×10^7
B3	Myosin regulatory light chain 2, ventricular/cardiac muscle isoform OS=Oryctolagus cuniculus OX=9986 GN=MYL2 PE=1 SV=2 - [MLRV_RABIT]	7.4×10^6
B3	Cytochrome c oxidase assembly factor 6 homolog OS=Bos taurus OX=9913 GN=COA6 PE=3 SV=1 - [COA6_BOVIN]	1.3×10^6
B3	Aspartate aminotransferase, cytoplasmic OS=Bos taurus GN=GOT1 PE=1 SV=3 - [AATC_BOVIN]	2.0×10^6
B3	Hepatoma-derived growth factor OS=Bos taurus OX=9913 GN=HDGF PE=2 SV=1 - [HDGF_BOVIN]	8.5×10^6
B3	14 kDa phosphohistidine phosphatase OS=Bos taurus OX=9913 GN=PHPT1 PE=2 SV=1 - [PHP14_BOVIN]	9.2×10^6

B3	L-lactate dehydrogenase B chain OS=Bos taurus GN=LDHB PE=2 SV=4 - [LDHB_BOVIN]	4.4×10^6
B3	Synaptopodin 2-like protein OS=Homo sapiens OX=9606 GN=SYNPO2L PE=2 SV=3 - [SYP2L_HUMAN]	1.6×10^6
B3	Radixin OS=Mus musculus GN=Rdx PE=1 SV=3 - [RADI_MOUSE]	2.4×10^6
B3	Macrophage migration inhibitory factor OS=Bos taurus OX=9913 GN=MIF PE=1 SV=6 - [MIF_BOVIN]	4.6×10^6

*batch 1, 2 and 3 of spray-dried beef tongue powder