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Effects of Sous-Vide Processing and Acidic Marination on Physicochemical Quality, Shelf-Life, and Consumer Acceptability of Blue Mussel (Mytilus edulis) Meats

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EFFECTS OF SOUS-VIDE PROCESSING AND ACIDIC MARINATION ON PHYSICOCHEMICAL QUALITY, SHELF-LIFE, AND CONSUMER ACCEPTABILITY OF BLUE MUSSEL (*MYTILUS EDULIS*) MEATS

by

Sara M. Gundermann

B.S. University of Maine, 2022

A THESIS

Submitted in Partial Fulfillment of the

Requirements for the Degree of

Master of Science

(in Food Science and Human Nutrition)

The Graduate School

The University of Maine

December 2023

Advisory Committee:

Denise I. Skonberg, Professor of Food Science, Advisor

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EFFECTS OF SOUS-VIDE PROCESSING AND ACIDIC MARINATION ON PHYSICOCHEMICAL QUALITY, SHELF-LIFE, AND CONSUMER ACCEPTABILITY OF BLUE MUSSEL (*MYTILUS EDULIS*) MEATS

By Sara Marie Gundermann

Thesis advisor: Dr. Denise Skonberg

An Abstract of the Thesis Presented in
Partial Fulfillment of the Requirements for the
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December 2023

Sous-vide cooking is a thermal processing method in which a raw food is vacuum sealed in a pouch and then placed into water below 100°C for a controlled amount of time. It is particularly good for cooking meats because of its precise control over temperature and time, which results in an ideal food texture for consumers. This method also prevents the food from coming in contact with oxygen, which can lead to spoilage that reduces product quality and shelf life. Acidification is a food preservation method that utilizes acids to lower the pH of foods, making the environment less conducive to the growth of certain spoilage and/or pathogenic microorganisms. Mussels are economically important; however, they are commonly sold live in mesh bags, with little effort to increase their value in the market. The development of value-added mussel products would benefit a sustainable seafood economy and make it more convenient for consumers to increase their consumption of healthy seafood products.

The objectives of this research included: (1) evaluating the impacts of two sous-vide cooking temperatures (65°C or 75°C) and three lactic acid treatments (0%, 0.5%, or 1%) on physicochemical properties and microbial quality of mussel meats over 35 days refrigerated

storage and (2) determining the impacts of three potential home preparation methods (consuming immediately after sous-vide cooking, reheating in a bag submerged in boiling water, reheating in a saucepan) on the physicochemical and consumer acceptability of sous-vide mussel meats in an acidic marinade.

In the first study, shucked mussel meats were vacuum-packaged in bags with lactic acid solutions (0%, 0.5%, or 1%) and sous-vide cooked at 65°C or 75°C for 30 minutes. Sous-vide processing at 75°C, combined with the 1% lactic acid solution, maintained total volatile base nitrogen values, a seafood spoilage indicator, at a "good" quality level over the course of the 35 refrigerated storage days. Adding 1% lactic acid solution reduced the initial pH of the product, significantly reducing total plate counts, psychrotroph counts, and TVBN production compared to the control. Therefore, acidification coupled with sous-vide processing at 75°C was selected for subsequent evaluation.

In the second study, consumer acceptability and physicochemical analysis of the impact of thermal home preparation methods on acidified (marinated) sous-vide mussel meats were evaluated. Reheating did not significantly impact the sensory acceptability of the mussels compared to the sous-vide control. The lack of differences in consumer acceptability between home preparation methods suggests that consumers have a lot of flexibility in preparing the value-added product. Participants appeared to be receptive to the product concept, with over 80% stating they would be likely to purchase the product in the retail environment. The results of these studies have important implications for the mussel industry and value-added mussel products and suggest that there is room for further innovation of acidified sous-vide mussel products at the retail level.

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LITERATURE REVIEW

1.1 Mussels

1.1.1 Mussel Biology

Mussels are bivalve mollusks that are found in coastal waters all over the world (Veiga et al., 2020). They can also be found in freshwater and deep seas (De Paula et al., 2020). Mussels are part of the phylum Mollusca, one of the most diverse and largest taxa in the animal kingdom. It has been estimated that there are around 93,000 molluscan species (Bouchet et al., 2002). Mussel anatomy includes a bivalve shell, filtering gills, no differential head, and a lack of a radula (Murgarella et al., 2016). They also have an adductor muscle that allows them to keep their shells shut to protect their internal organs from predators (Figure 1.1.). Mussels are found in a variety of shell colors and sizes based on species and where they are harvested. Some examples of colors include silver, blue, green, black, and brown.

Filter feeding allows certain marine species a unique way of capturing food such as phytoplankton. Mussels are referred to as filter feeders meaning they obtain their food by filtering organic matter or organisms from the water. Bivalves act as a small living pump, and as water is pulled in, nutrients and oxygen are extracted before the water is released (Murgarella et al., 2016). The shells of mussels are long and asymmetrical compared to other mollusks like clams and oysters, which may be an evolutionary adaptation allowing the mussels to burrow and avoid predation (Wilson et al., 2012).

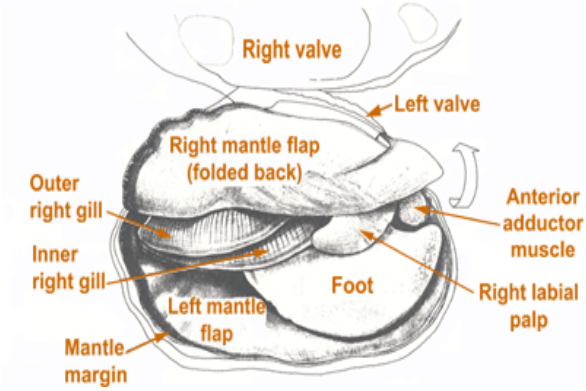

Figure 1.1. Anatomy of a mussel (Illinois State Museum)

In addition to the presence of mussels being an indicator of good water quality (Best, 2023), mussels are extremely unique on an environmental level. Their ability to filter feed allows these bivalves to play a role in cleaning up their habitat, in this case, the surrounding water. They can absorb heavy metals and filter out harmful toxins and algae that may be in the water (Krasota & Kostylev, 1988). Their empty shells can also act as habitats for a number of other marine organisms including fish, algae, and crustaceans. Eutrophication is when water becomes highly nutrient dense, most frequently due to run off from the land (US Department of Commerce, 2019). This is dangerous to the ecosystem because the waters become highly saturated with algae and other plant life leaving little to no oxygen available. However, mussel farming was found to control eutrophication in the Baltic Sea (Kotta et al., 2020). Kotta et al. (2020) also discovered that their effectiveness at cleaning the water was dependent on the method of mussel farming.

1.1.2 Mussel Industry

Mussels are farmed all over the world contributing to a value of 4.5 billion U.S. dollars in 2016, equivalent to production of around 2.1 million tons of mussels (FAO, 2018). The leading mussel-producing countries in 2021 included China (829,481 tons), Chile (425,849 tons), and Spain (203,226 tons) (FAO, 2021). Mussels are a highly perishable food making them difficult to transport internationally. They are currently the top imported bivalve by value in the United

States, totaling more than $102 million dollars in value (NOAA Fisheries, 2023). In 2022, there were close to 2 million pounds of mussels produced in the United States, totaling around 5.3 million dollars (NOAA, 2023). Currently, the United States produced 181,672 tons of mussels at a $4.7 billion dollar value while the United States imported $102 million dollars' worth (NOAA, 2023). The U.S. has many bodies of water between 32-55°F, ideal conditions for mussel farming of the majority of species (Arrieche et al., 2020).

Harvested mussels can be either wild caught or farm raised. Wild caught mussels are the most cost-efficient option but harvesters have no control over the amount or quality of the product when they are wild caught. Some examples of mussel species that are regularly farmed across the globe are the green mussel (*Perna viridis),* Mediterranean mussel *(Mytilus galloprovincialis),* and the blue mussel *(Mytilus edulis)* (Holmyard, 2020).

There are two primary ways to farm mussels, rope culture and bottom culture. Most commonly in rope culture juvenile mussels (spat) are allowed to adhere to a rope, and then are moved to a raft at an ocean grow-out site when they reach the correct size, which depends on the species (Alves et al., 2020). There is concern that the seabed-raised mussels are detrimental to the environment because they must be dredged from the ocean floor during harvest. This dredging can damage the bottom habitat for other marine life (Holmyard, 2020). For this reason about 90% of the mussel industry now solely uses rope culture to farm mussels (Holmyard, 2020). Neither method of mussel farming requires supplemental feeding since mussels are filter feeders and obtain their nutrients from the environment.

1.1.3 Mussel Nutrition and Sustainability

One of the reasons that researchers care about mussels is because they are a nutritious source of protein (Bongiorno et al., 2015). They contain essential vitamins and minerals as well

as amino acids that human bodies cannot produce naturally. Some of the nutrients that are found in mussels include Vitamins A and B12, as well as the minerals zinc and iron (Rodriguez-Hernandez et al., 2019). Mussels also contain omega-3 fatty acids, which are beneficial for preventing heart disease, among other medical issues. According to the USDA (FoodData Central, 2023), a 100 gram serving of cooked mussels contains about 24 grams of protein, 56 milligrams of cholesterol, 0.46 grams of omega-3 fatty acids, and 155 calories. Mussels are quite high in omega-3 fatty acids and low in calories compared to other land animal protein sources.

Animal-derived proteins are looked at as some of the highest quality proteins which can contribute to a healthy diet. Mussels have an amino acid score, which is a measure of protein quality, of 107 which is considerably high, similar to the score for whole egg, at 100 (Valverde et al., 2013). Based on the Australian Food Nutrient Database, it was shown that mussels outperformed other protein sources in many areas but especially for iron and vitamin B-12 content (Yaghubi et al., 2021). Salmon has a higher omega-3 fatty acids content than mussels but also a much higher greenhouse gas emission rate, making mussels a good alternative option, particularly with regard to sustainability (Hammer et al., 2022).

The beneficial environmental impact of mussels was already mentioned earlier in this review, but there are many layers to this topic. Mussels are low on the food chain compared to many other marine organisms that are commonly consumed by humans. This is a good thing because foods lower on the chain have a lower carbon and water footprint than other foods (Coluccia et al., 2022). Mussels in particular are filter feeders, so they do not require excess food and nutrients meaning that less environmental resources are expended to grow them (Ferreira et al., 2018*)*. Bridger et al. (2022) investigated how the introduction of mussel farm infrastructure could have a positive ecosystem effect and restorative properties. The authors deployed several

ropes in the pre-selected sites, and these sites were monitored over five years. It was found that the long-line mussel farm created in Lyme Bay was able to modify the benthic habitat for the better in just two years as well as the biodiversity of the environment. The size of the mussel clumps and the percentage of yield increased each year of the study as well as the biodiversity of the environment. This discovery is important because it shows the successful application of rope-grown mussel farming, particularly with regard to environmental and economic sustainability.

1.1.4 Availability and Product Forms

In 2016, the U.S. produced approximately 900,000 pounds of various species of mussels (NOAA Fisheries, 2023). In 2022, that number increased to 1,939,125 pounds. In 2016, the U.S. was the largest global importer of frozen mussels at 14,000 tons valued at over 15 million dollars (New Zealand Ministry for Primary Industries, 2017). However, according to a 2014 survey, 88% of U.S. consumers said that they never purchase/consume mussels compared to 48% of consumers in France (New Zealand Ministry for Primary Industries, 2017). Consumers that were most interested in consuming mussels were located primarily on the east and west coasts, in Michigan, or in Texas (New Zealand Ministry for Primary Industries, 2017). Additionally, mussels did not make the top 10 seafood list in the U.S. in 2021 (aboutseafood.com), meaning the average per capita consumption is less than 4oz per year. This low level of mussel consumption leaves opportunities in the U.S. for product innovation.

In the U.S., there is not a lot of diversity in retail mussel products. Mussels can commonly be purchased live from supermarkets, in 1 to 2 pound mesh bags. In general, there is a lack of value-added products when it comes to this protein source. The most common ways that mussels are available in the market, other than live in mesh bags, are frozen in-shell or smoked meats. The use of freezing can be seen in products such as shelled and pre-shucked frozen

mussels in plastic bags from Pana Pesca (panapesca.com). Pana Pesca is a premium seafood wholesaler located in Massachusetts. The majority of Pana Pesca's customers include chefs, large food service operations as well as restaurant chains, emphasizing the fact that there is little product diversity available at supermarket retailers (panapesca.com).

Many people enjoy eating hot smoked or cold smoked mussels which can be found in the refrigerated section of the store or shelf stable in a can. The smoking process typically involves minimal cooking of the mussels (usually a low temperature for a short amount of time for the cold smoked products) and provides characteristic flavor and aroma notes that consumers enjoy. These mussels are usually then preserved in some kind of oil; examples include rapeseed and olive oil. Some companies merchandising products in the U.S. in the smoked mussel space include Patagonia (patagoniaprovisions.com) even though the mussels are imported from Spain, and Cole's Seafood located in Maryland (colesseafood.com). Patagonia and other smoked mussel brands have focused on shelf stable canned products leaving a gap in the refrigerated ready-to-eat section of the market.

1.2 Sous-Vide Cooking

1.2.1 Introduction to Sous-Vide

Sous-vide cooking is a thermal processing method in which the raw food is placed in a pouch and vacuum sealed, removing the air. The bag is then placed into water or steam at a specific temperature for a controlled amount of time, and then cooled (Church and Parsons, 2000). Sous-vide equipment is good for cooking meats because of the precise control over temperature and time, which results in an ideal food texture for consumers. There is usually less toughening of the muscle, a problem that can occur frequently in foods cooked using more traditional methods (Bongiorno et al., 2018). It also prevents the food from coming in contact

with oxygen which can lead to oxidation, off-flavors and off-odors (Ghazala et al., 1995). Food scientists and culinologists have been studying sous-vide processing since the 1990's and it has been used in select upscale restaurants since the 1970's. However, there has been a surge in sous-vide cooking technology for retail applications since the 2000's (Roca and Brugue's, 2005). Products produced via sous-vide cooking range from fruit and vegetable dishes, to center of the plate proteins.

One of the most notable benefits associated with sous-vide cooking is the precise temperature control that one has over the process. Precise temperature control can allow food to retain a better texture but still be considered safe and can make product characteristics more reproducible. The vacuum package allows the meat to be submerged but not in direct contact with the heating medium, allowing for a less tough product (Ismail et al., 2022). This can be seen in a study (Sanchez et al. 2012) performed on pork cheeks sous-vide cooked at 60 and 80°C. The investigators found that hardness values for the controls, cooked in a saucepan, were higher than both sous-vide (60 and 80°C) treatment values. Water retention also plays a large role in the quality of muscle foods. Gómez et al. (2019) sous-vide cooked both beef and beef analog products at different temperature and time combinations. The samples were either cooked at 70 or 80°C; the beef product was cooked for 60, 90, or 120 min while the beef analog was cooked for 90, 120, or 150 min. They found that both cook loss and texture were affected by the differences in cooking parameters. At 80°C, the longer the product was cooked, the more moisture loss took place; however, this trend was not seen for the products that were cooked at 70°C. Regarding texture, beef samples cooked at 70°C showed less toughness than the samples cooked at 80°C. Even though lower temperature and shorter time sous-vide cooking has been shown to provide better texture, that is not the only draw.

People should always be aware of food safety, but it is particularly important in vacuum-packaged products. In order to ensure the safety of sous-vide foods, determining if something is "done" based on appearance is a practice that should be avoided (Ismail et al., 2022). Instead, a thermometer should be used to ensure that a product is cooked according to safety guidelines. One of the largest microbial concerns is with botulism, because of the vacuum packaging. The vacuum packaging inhibits the growth of aerobic bacteria and can promote the growth of anaerobic bacteria in the small amount of oxygen that is present (NSW, 2012). In order to prevent this concern sous-vide processed products are immediately chilled after cooking and kept below 3.3 °C to prevent *Clostridium botulinum* growth and toxin formation (Center for Food Safety and Applied Nutrition, n.d.). According to the FDA Fish and Fisheries Hazard Guide, the risks associated with sous-vide can be reduced if cooking temperatures below 54.5°C are held for 6 hours or longer (Center for Food Safety and Applied Nutrition, 2022). This can be somewhat unrealistic to produce in mass scale, so it is common to see sous-vide cooking temperatures ranging from 60-80°C.

There are many benefits to sous-vide processing that should not be overlooked. A key benefit of sous-vide processing is that it can preserve the nutritional value of foods. In all cooking processes, there is some degradation of nutrients, but sous-vide cooking may contribute to less nutrient loss. Antioxidants in food reduce the formation of oxidative products that can be harmful to the body (Lorenzo et al., 2018). In a study on seven different vegetables, Natella et al. (2010) reported that antioxidant activity was dependent on the type of vegetable as well as the cooking process, sous-vide being one of the methods evaluated. The researchers found that the sous-vide processed vegetables maintained higher antioxidant values than the other treatments, including microwaving and boiling. Rondanelli et al. (2017) determined ash and metal content in

ready-to-eat cereal grains before and after boiling and sous-vide cooking. They found that ash content increased in the legumes that were cooked with sous-vide. This was beneficial because it indicated an increase in minerals. Also, mineral salts seemed to dissipate in the cooking water during boiling but not during sous-vide cooking. In another example, vegetables appeared to lose fewer anthocyanins and other phenolic compounds when sous-vide cooking was used (Baardseth et al., 2010). While a lot of research has been done on nutrient content of vegetables, grains, and muscle foods, further investigation into impacts of sous-vide on seafood is warranted.

1.2.2 Impact of Sous-Vide Time/Temperature Conditions on Safety

The shelf life of sous-vide products typically depends on the temperature and length of cooking as well as the length and condition of storage (Mol et al., 2012). It is important that the correct temperatures and times are selected as those decisions are vital to the safety of sous-vide processing. Balancing the safety of the food and the quality of the food can be a challenge. Spoilage microorganisms are those that contribute to food deterioration by producing unwanted tastes, smells, and textures in the food. In contrast, pathogenic organisms (e.g., viruses, bacteria, parasites) are organisms capable of causing a foodborne illness event (Bintsis, 2017). The most common pathogens of concern related to sous-vide cooking are *Clostridium perfringens*, *Bacillus cereus*, *Listeria monocytogenes,* and *C. botulinum* (Carlin, 2014). These pathogens are of most interest because of the spores and/or toxins that they produce.

In order to prevent any kind of foodborne intoxication or illness the FDA has many guidelines and regulations that food producers must follow. The first pathogen of concern for seafood, specifically raw or vacuum packaged products is *Listeria monocytogenes.* This pathogen is extremely heat resistant and can survive the long-time low temperature cooking that occurs with some types of sous-vide processing (Farber and Peterkin, 1991). *L. monocytogenes*

can also cause Listeriosis, a serious foodborne illness with a 15% mortality rate that can cause spontaneous abortion (FDA, n.d.). Taking this into account, the FDA enforces that there must be a 6-log reduction of *L. monocytogenes* because it is such a heat resistant pathogen (FDA, n.d.). However, thermal processing may not control *L. monocytogenes* because of heat shocking, meaning that if the system does not reach a high enough temperature for enough time, the application of heat can cause the bacteria to become more resistant to extreme temperature (Vanderveen, 2007). Another pathogen that must receive a 6-log reduction for the food to be considered safe is non-proteolytic *Clostridium botulinum*. C. *botulinum* is classified into two different categories 1) proteolytic and 2) non-proteolytic. The difference is that non-proteolytic strains can grow at lower temperatures, but their spores have much lower heat resistance (Lynt et al., 1982). Botulism is a foodborne intoxication resulting from spores producing botulinum toxin. The illness can be deadly and can cause symptoms like vertigo and paralysis. Type A strains are all proteolytic and type E stains are all non-proteolytic while type B and F have both proteolytic and non-proteolytic strains. Type E is most common in marine species and would be of concern for sous-vide mussels. The concern about *C. botulinum* also applies strongly to sous-vide cooked seafood products, because non-proteolytic strains grow without oxygen and at the low chilled temperatures typical of refrigerated storage. Thermal processing will not control this hazard because spores are heat resistant, and the spores are what produce the botulinum toxin.

Taking into consideration how serious these food safety concerns are, hurdles are recommended and sometimes required in order to prevent foodborne illness events. Storing the product at a temperature below 3.3°C is a hurdle; however, if any temperature abuse occurs, the product is not considered to be safe. For this reason, additional hurdles are required for low acid foods or foods that have a pH higher than 4.6 to ensure that no harm comes to the consumer.

These additional hurdles can include acidifying the product to a low pH (< 4.6), making sure that the product has a low water activity, or adding a high concentration of NaCl to the product (Maier et al., 2018). The thermal profile of vacuum sealed reduced oxygen packaged products must be recorded from sourcing to processing and distribution. A method to record temperature/time and ensure safety is time-temperature indicators or TTI's. A TTI is a device that attaches to bulk packages (i.e. packages that go to restaurants and grocery stores) and shows in an irreversible way how long the product has been exposed to temperatures that are higher than safety allows (Center for Food Safety and Applied Nutrition, 2022). They show the retailers and consumers that proper temperature protocols were followed from the time the product was produced to the time that they received it. One of the key components is that the device must show an irreversible change of color if temperature abuse takes place (Center for Food Safety and Applied Nutrition. 2021). If temperature abuse took place not only would the TTI show it but there would also be a risk of C. botulinum because of temperature exposure over 3.3°C. Also, if temperature abuse took place to the point where the TTI was affected the product would not be able to sell due to safety concerns. While TTI's can be a useful tool they can also be a hindrance and not cost-effective for producers when producing high volumes of product.

1.2.3 Impact of Sous-Vide Time/Temperature Conditions on Seafood Quality Attributes

Seafood is a particularly interesting application of sous-vide cooking for multiple reasons. The first reason is that the texture of seafood is naturally very delicate compared to most other muscle foods. Another reason is that fish and shellfish have such a short shelf life. Sous-vide cooking has been shown to retain the texture of foods and to extend the shelf life so that quality does not diminish as quickly. For example, Gonzalez -Fandos et al. (2004) determined that the average shelf life of a sous-vide seafood product ranges from 15 days to 16 weeks

compared to fresh seafood lasting only a few days (Gonzalez-Fandos et al., 2004). The process of sous-vide cooking is convenient and could lead to an increase in seafood consumption because consumers may be more likely to consume their own seafood at home since the product is ready-to-eat. Making seafood proteins more accessible to home consumers would be beneficial since individuals with a higher intake of fish have been reported to have lower coronary heart disease mortality compared to those who eat less or none at all (He et al., 2004). Consumption, processing, and storage challenges of seafood can potentially be addressed by the increased application of sous-vide cooking.

Another reason why there is interest in this method is because of its ability to gently extend the shelf life of refrigerated foods (Popovici, 2018). Some common seafood quality analyses include water holding capacity, pH, total volatile base nitrogen (TVBN), thiobarbituric acid reactive substances (TBARS), cook loss, color, and texture. TVBN is often used as a measure of protein degradation. The TVBN assay measures the amount of volatile nitrogenous compounds (i.e., ammonia, dimethyl, trimethylamine) that contribute significantly to off odors (Brady, 2013). Since these are predominantly caused by microbial spoilage, the assay is an indicator of seafood quality and can be directly related to the microbial populations present in food. The levels of acceptability with regard to TVBN are as follows: 25 mg N/100g is considered to be high quality, 30 mg N/100g is good and anything above that is considered to be spoiled (Bongiorno et al., 2018). In a study by Larsen et al. (2010), the water holding capacity of trout fillets was measured after various cooking methods were performed. After different forms of wet cooking (i.e., poaching, streaming, sous-vide cooking), the product maintained a higher water holding capacity than when the fillets were fried or baked (Larsen et al, 2010). Lower

water holding capacity and moisture content both had negative impacts on the overall texture of the fish muscle.

Selectively choosing sous-vide cooking parameters has the ability to prevent overcooking of seafood products. The amount of heat applied to a product can affect the amount of force that is required to shear or slice the product. Typically, the larger the shear force value, the tougher the protein. In sous-vide Atlantic mackerel it was found that cooking at 65-75°C compared to boiling increased the tenderness of the sarcoplasmic proteins (Cropotova et al., 2019). The sous-vide cooking also reduced the solubility of myofibrillar proteins less than boiling did, meaning less of it was denatured and aggregated as a result of sous-vide cooking. Certain sous-vide cooking processes have also been shown to preserve the color of various foods. When protein denaturation occurs due to high cooking temperatures, color change can occur. Because seafood proteins are very sensitive to overcooking at high temperatures, it is common that they are sous-vide cooked below 90°C (Sampels et al., 2015; Kato et al., 2017). Sous-vide cooking below 90°C can preserve protein quality and promote the stability of pigments of fish. In Atlantic mackerel cooked both with and without sous-vide technology at different temperatures, the fish subjected to harsher thermal processing displayed significantly lighter color flesh than what most people prefer (Cropotova et al., 2019). In addition to the actual sous-vide cooking process, storage time also played a role in the color of the sample. Oxidation of the lipids in the mackerel caused the fish to turn yellow but not as quickly as fish that were not sous-vide processed.

Sous-vide processing has been reported to be effective at reducing lipid oxidation in foods. Lipid oxidation is a complex set of reactions in which unsaturated fatty acids react with free radicals and oxygen (Domínguez et al., 2019). This has a negative effect on food because it can cause an unpleasant appearance, taste, and smell. Lipid oxidation is more prone to happening

in foods that are high in unsaturated fatty acids, such as seafood. One way that lipid oxidation can be measured is by thiobarbituric acid reactive substances (TBARS) content. The TBARS assay works by measuring the quantity of the secondary product malondialdehyde (MDA), which is formed as a byproduct of lipid peroxidation (Brady, 2013). In one study, salmon fillets were vacuum sealed in pouches and cooked under sous-vide processing conditions or in an oven prior to refrigerated storage for 0, 5, or 10 weeks (Díaz et al., 2011). Lipid oxidation did not significantly increase in the sous-vide salmon fillets from day 0 (1.10mg MDA/kg) to week 10 (2.30mg MDA/kg), both values being lower than those of the salmon that was cooked in an oven and not vacuum packaged. This suggests that vacuum sealing and sous-vide cooking can extend the time before lipid oxidation, thereby improving the shelf life of lipid-containing foods. Gittleson et al. (1992) also investigated the oxidation rates of sous-vide salmon during refrigerated storage and reported that even after 12 weeks, rancidity as measured by TBARS was not detected. Sous-vide processed and raw sturgeon were also evaluated, followed by refrigerated storage for 9 days. With regard to TBARS, it was seen that the group that was cooked at the highest temperature (60°C) for the longest amount of time and the raw material had the highest TBARS values (Cai et al., 2021).

The amount of time foods are sous-vide processed, and the temperature at which they are processed can also impact their microbial populations. Singh et al. (2016) evaluated the effect of different sous-vide processing parameters on seerfish steaks during refrigerated storage. The steaks were cooked at 70, 80, and 90°C for 5, 10, and 15 minutes. In this study, some steaks were also brined with salt. TVBN values, which are strongly correlated with microbial growth, for all of the sous-vide cooked fish steaks, remained acceptable for 65 days of storage, with TVBN levels remaining below 25 mg N/100g, the acceptable limit for good quality seafood (Smaldone

et al., 2011). In another study (Olatunde and Benjakul, 2021) assessing the effects of sous-vide cooking temperatures on microbial quality, the researchers extracted, vacuum sealed, and sous-vide cooked lump crab meat. The crab was cooked for 1 or 2 hours at 75, 80, or 85°C with raw controls that were crab vacuum packed or crab packed in air. Olatunde and Benjakul (2021) found that all of the crab meat samples that were sous-vide cooked had improved microbial safety compared to both sets of controls and fewer negative impacts on chemical qualities. For example, TBARS values for the 85°C treatment were significantly higher than the lower temperature treatments, even at 1 hour of processing, meaning that cooking at 85°C led to more oxidation. Also, the treatment cooked at 75°C for 1 hour had higher aerobic plate counts than any other SV temperature and time combination.

Many different time and temperature combinations can be used when processing seafood by sous-vide cooking. Jeya et al. (2009) assessed the microbial populations of vacuum packed raw minced fish and sous-vide cooked fish cakes (*Lethrinus lethrinus*) over time during refrigerated storage (3°C). These fish cakes were either conventionally cooked by pasteurizing at (100°C for 20 minutes), or sous-vide cooked (70°C), and a control of bagged but not vacuum sealed fish was also used (Jeya et al., 2009). The samples were then stored at 3°C for 16 weeks. For total bacteria count, it is notable that the sous-vide cooked sample counts initially increased slightly but then remained at around 3 log CFU/g for all 16 weeks, while the other treatments reached 5 log CFU/g after only 6 weeks. The acceptable limit above which seafood is considered spoiled is usually around 6-7 log CFU/g, while the acceptable limit for good quality cooked products is 5 log CFU/g (Center for Food and Applied Nutrition, 2022). Sous-vide cooking appeared to extend the shelf life of the fish cakes an extra 10 weeks compared to steaming of the fish (100°C for 20 minutes) and the raw product. Also, after 16 weeks, the sous-vide fish cakes

became unacceptable due to their sensory attributes (flavor, odor, texture), but aerobic plate counts remained around 3 log CFU/g. Lactic acid bacteria are a type of spoilage organism that can grow in microaerophilic/anaerobic environments and may be associated with sous-vide seafood (Carlin et al., 1999). Lactic acid bacteria were not found on the sous-vide cooked fish cakes during the entire study, while the raw product and conventionally cooked product had ~2 log CFU/g after just 2 weeks of storage (Jeya et al., 2009).

Sous-vide cooking can help to preserve/improve the sensory characteristics of a product compared to other conventional cooking methods (Coşansu et al., 2022). It can be especially effective in preserving the texture of seafood because the protein is so delicate. Diaz et al. (2009) investigated sous-vide salmon (80°C for 43 minutes) and found that the sensory texture (hardness) values remained acceptable for up to 25 days of 3°C storage compared to pan roasted salmon, which had acceptable hardness values for 15 days. However, based on descriptive sensory analysis of odor and flavor, the shelf life of the sous-vide salmon was 18 days. Thus, although sous-vide cooking has been shown to be a beneficial method for producing high quality seafood, overcooking is still possible and a 'one-size fits all' processing approach is not appropriate for all species or products. Appropriate sous-vide processing parameters for mollusks, in particular, have not been thoroughly investigated.

1.2.4 Impact of Sous-Vide Time/Temperature Conditions on Mollusk Quality Attributes

Bivalve mollusks commonly consumed and subjected to sous-vide processing in various research studies include clams, oysters, scallops, and mussels. Zhan et al. (2022) investigated the impact of sous-vide cooking on physicochemical properties of scallops. The shucked scallops were split into three groups; scallops sous-vide cooked at 70 or 75°C, and a control that was boiled at 100°C to represent conventional cooking. The scallop samples were stored at 4°C and

were evaluated over the course of 30 days for various quality attributes. Over the first 10 days of storage, scallops sous-vide cooked at 70°C had significantly more lipid oxidation than those cooked at 75°C. That trend continued, and by day 30, the scallops that were processed at 75°C had significantly lower TBARS values (~1.8 mg/kg) compared to ~3 mg/kg for the 70°C treatment. However, the boiled scallops had approximately twice the TBARS values compared to both of the sous-vide treatments. There were no significant differences in texture (shear force, N) between either of the sous-vide treatments (~4.30 N) until day 20 and both sous-vide treatments were significantly more tender than the boiled scallop (10.04 N). The researchers reported that by day 30 of storage, the 70°C sous-vide treatment had the highest TVBN values, followed by the 75°C sous-vide treatment and then the boiling treatment.

Mussels have also been researched in relation to sous-vide processing conditions and subsequent quality. Bongiorno et al. (2018) investigated the physicochemical properties of sous-vide and conventionally cooked in-shell mussels. The treatments included steaming at 90°C for 10 minutes, sous-vide processing (85°C) with no brine, and sous-vide processing with a brine added to the vacuum sealed bag. TVBN values for both sous-vide treatments remained below the acceptable limit (35mg N/100g) for the entirety of the 50 days of storage. However, the other treatments surpassed the acceptable limit by around day 30. The investigators also found that sous-vide cooking coupled with brining at 85°C increased the moisture content and weight of the mussels compared to the steamed sample (90°C) sample. Both sous-vide treated mussel samples had higher overall sensory acceptability scores compared to the conventionally cooked mussels.

In another study, sous-vide cooking helped to reduce microbial growth and extended the shelf life of mussels at refrigerated temperatures. Samsudin and Karim (2021) sous-vide cooked (85°C) and boiled (100°C) green mussels, then evaluated their microbial counts every 5 days for

20 days. The mussels were stored in chilled conditions, and the authors measured the following microbial populations: total bacteria count, total coliform count, Enterobacteriaceae, *Pseudomonas* spp., and yeast/mold count. The total bacteria counts for the sous-vide treatment (4.01 log CFU/g) remained significantly lower than for the boiled treatment (7.82 log CFU/g) over the 20 days. These studies indicate that the time and temperature at which mollusks, and specifically mussels, are sous-vide processed can directly impact quality attributes and shelf life and that brining coupled with sous-vide processing may offer additional benefits.

1.3 Acidification

1.3.1 Introduction to Acidification

Acidification is a food preservation method that utilizes acids to lower the pH of foods, making the environment less conducive to the growth of certain spoilage and/or pathogenic microorganisms. These acids can be both naturally formed in the foods or added to the formulation. Acid can be applied on the outside of the food, injected into the food, or added as an ingredient in a marinade or sauce (Kolman et al., 2020). Some specific acids added to food products or produced naturally include acetic, lactic, malic, citric, and tartaric acid (Kolman et al., 2020). An acidified food is defined as a food that has either acid or acid ingredients added to it in order to achieve a final pH value of 4.6 or lower (Dogan et al., 2022). Some examples of acidified foods are pickles and salsa. The main benefit of food acidification is reducing microbial growth and extending the shelf life of products.

Adding acids or acid ingredients to foods is one of the ways to potentially extend their shelf life. However, food systems are complex, and just because an acid works well in one system does not necessarily mean that it will work well in another. This means that acids prevent the growth of different microbes to different extents. There are also safety concerns to be aware

of when using acidification as a method of preservation, especially when it comes to seafood. The first thing is that *C. botulinum* growth must be prevented. This is done in acidified foods by ensuring the pH is below 4.6, in some cases even 4.4 to be extra safe (Center for Food Safety and Applied Nutrition, 2021).

1.3.2 Impact of Acidification on Seafood

One of the main contributing factors to the short shelf life of seafood, along with its high-water content and rich nutrient profile, is that the proteins have a neutral pH (Cosansu et al., 2022). The neutral pH can make it easier for spoilage microorganisms to grow, thus reducing the shelf life even further. Acids can be used as ingredients in marinades and sauces to improve the appearance and taste of the seafood product for the consumer. Some examples of acidified seafood products with characteristic flavors and aromas enjoyed by some consumers are fish sauce, pickled herring, and fish/shrimp paste. Acidification is a hurdle that can be used to improve the safety and other characteristics of a product, but it is also a method that can be coupled with other technology, such as sous-vide or high-pressure processing, to create an even better product. Sampels et al. (2010) marinated herring fillets in various berry marinades that consisted of elderberry, cranberry, and black currant. They hypothesized that the acidic nature of the berries, along with their antioxidant properties, could reduce lipid oxidation in this high fat fish. The fillets were marinated for 24 hours, vacuum packaged, and then put into frozen storage. The pH results showed that the cranberry marinade (5.88) and black currant marinade (5.95) were significantly more effective at maintaining a lower pH of the herring fillets than the elderberry (6.18) and control (6.38). Lipid oxidation of the fish during frozen storage was measured by TBARS analysis. The researchers reported that the berry marinades, specifically cranberry (21.3 nM/g) and black currant (15.9 nM/g), produced lower TBARS values than the

other berry marinades and the control (25.4 nM/g). Marination using highly acidic ingredients can be beneficial to the quality of seafood.

Acidification can also be used to extend the shelf life of seafood other than fish. Cadun et al. (2008) investigated how a marinade including citric, sorbic, and benzoic acid would affect the refrigerated shelf life of deep-water pink shrimp. The shrimp were boiled in a water bath (100°C) for 10 minutes, then the marinade was applied, and the samples were stored for 75 days at 1°C. No significant differences were observed with regard to pH but the TVBN values of the non-marinated shrimp (7.0 mg/100 g) were higher than those of the acid marinated samples (5.6 mg/100 g) by day 75. The marinated shrimp (2.4 mg MDA/kg) also had significantly lower TBARS values by day 75 compared to the control shrimp (6.6 mg MDA/kg). Thus, the authors concluded that applying the acid marinades delayed oxidation and spoilage in shrimp compared to the non-marinated control. In another study, Stamatis and Vafidis (2009) marinated vacuum sealed sea urchins in either 3, 5, or 7% acetic acid solution and enumerated their microbial populations before, during, and after 75 days of storage (6°C). The concentration of the acetic acid solution added to the samples did not seem to have a significant effect on the microbial growth (TBC, LAB, yeast/mold) among acid treatments. However, all of the acetic acid treatments produced significantly lower total bacteria counts compared to the control, which was not marinated. Evidently, acidification has the potential to couple well with other preservation methods such as chilled or frozen storage.

1.3.3 Impact of Acidification on Sous-Vide Seafood

It can be beneficial to couple processing technologies that have been proven successful on their own. This can be seen in a study by Cosansu et al. (2013), where lemon juice marination was coupled with sous-vide cooking to extend the shelf life of whiting. One group of

fillets was vacuum packaged and sous-vide cooked without marination, while the other group was treated with lemon juice for 30 minutes before sous-vide processing. Lemon juice was shown to significantly decrease the pH of the experimental fillets compared to the control for the entire 42 days of the refrigerated storage study. The lemon juice had no significant effect on TBARS or TVBN levels of the sous-vide processed fish; however, both sous-vide treatments exhibited lower values than the raw fish control. Cosansu et al. (2013) also measured the microbial populations in the fillets with and without lemon juice application. The lemon juice marinated samples (5.37 log cfu/g) produced significantly lower psychrophilic aerobic bacteria counts than the fish that were not marinated (7.04 log cfu/g) through day 42. The shelf life of the acidified sous-vide fillets based on physicochemical, microbial, and sensory data was 35 days compared to 28 days for the fish that were not acidified.

There is a lack of research on the physicochemical quality of acidified sous-vide seafood. However, Dogruyol et al. (2020) conducted research on the thermal inactivation of *L. monocytogenes* in acidified sous-vide Atlantic salmon. Minced salmon was divided into four groups: 0.5% citric acid (w/v), 1% oregano essential oil (w/v), citric acid and oregano essential oil, and non-treated control. The cooking temperatures ranged from 55°C to 62.5°C. The authors found that the inactivation times (D-values) for the control group were significantly longer than for all of the marinated treatments. The combination of citric acid and oregano essential oil produced significantly shorter inactivation times than either treatment alone. Further research is needed to investigate how the marinades could affect the quality of the product during storage.

1.3.4 Impact of Acidification on Mussels

Utilizing acidification, sauces, or marinades can increase the consumer's liking of sensory attributes as well as extend the shelf life of a food product (Maxwell et al., 2018). The

goal of processors is to make their products attractive, nutritious, and good tasting. Green mussel meats were pretreated in 2% citric acid or 2% lactic acid, then cooked, and then put into vacuum sealed bags that each contained one of the acids. There was then a separate non-acidified control (Arcales and Nacional, 2018). The mussels were stored at 3˚C for 18 days and every 3rd day, acceptability and descriptive assessments were performed. Both acid pretreatments were found to have protected the odor of the sample compared to the control, which was rejected on the 9th day due to the smell of ammonia. The naturally sour flavors of lactic and citric acid produced lower flavor acceptability scores throughout the study than the control. The 2% concentration of the acids likely contributed to the low acceptability scores. However, no significant difference was found in overall acceptability between the two acids. The two treatments extended the overall acceptability of the mussels until day 15 compared to the control, which was rejected as unacceptable at day 6. Arcales and Nacional (2018) also found that the citric acid and lactic acid pretreated samples exhibited significantly lower psychrophilic bacteria counts than the control. Psychrophilic bacteria counts are important because these bacteria grow at refrigerator temperatures, and many sous-vide seafood products are retailed in chilled storage. The authors also found that from days 12 to 15, lactic acid treated green mussels had significantly lower TVBN values compared to the samples that were treated with citric acid.

In another study, the goal was to determine the shelf life of green mussels marinated with tamarind fish sauce enriched with iron and zinc (Tien et al., 2019). The mussels were packed in modified atmosphere packaging and stored at 4°C for 27 days. The samples consisted of a marinated green mussel, a marinated green mussel packaged in modified atmosphere packaging, and a non-marinated mussel. Panelists were asked hedonic questions about product appearance, aroma, texture, taste, and overall liking. All sensory attributes degraded over time for all of the

samples, but they all remained at an acceptable level. The control had the highest initial overall acceptability score (~7), while the two samples that were marinated were rated at ~5. The authors defined acceptable as higher than a hedonic score of 5. The actual components of the sauce, like the ingredients being too pungent and overpowering, as the authors mentioned that both fish sauce and zinc can have unpleasant aftertastes could have led to the fairly low overall acceptability scores. Treating seafood proteins with marinades can be beneficial for consumer acceptability, convenience, and extending shelf life. There is room for more research on coupling sous-vide technology with acidification to produce consumer acceptable and convenient seafood products.

1.4 Justification

Mussel farming has been shown to have a positive impact on the aquatic environment by reducing eutrophication that can lead to algal blooms, hypoxia, and damage to essential fish and shellfish habitats. In U.S. retail markets, mussels are mainly sold live in 1-2 pound mesh bags, and can quickly spoil in 7-10 days (Pemaquid Mussel Farms). There is room in the market for more convenient, refrigerated mussel meat products with a longer target shelf life of four weeks. Sous-vide cooking and acidification are both methods of shelf-life extension that can contribute to the development of value-added shellfish products. While some sous-vide research has been conducted on mussels, there is a lack of research on mussel meats that have already been shucked. Acidification has been shown to extend the shelf life of steamed green mussels by lowering the overall pH and decreasing microbial growth, with some off-odor/off-flavor development noted under the acid concentrations applied. However, there is a considerable knowledge gap when it comes to coupling sous-vide and acidification to produce a better quality mussel product. The overall goal of this research was to develop an acidified sous-vide mussel

product having an extended refrigerated shelf life that is tasty and convenient for U.S. consumers. Mussels are an underutilized seafood with many nutritional and environmental benefits, and increasing the retail availability of convenient, high quality, sous vide mussel products may contribute to increased consumption and enjoyment of this sustainable seafood.

1.5 Objectives

The specific objectives of the two studies were to: (1) evaluate the effect of sous-vide cooking temperatures (65°C and 75°C for 30 minutes) and acidification levels (0%, 0.5%, and 1% lactic acid) on physiochemical and microbial quality of mussel meats, and (2) determine the impact of different potential "at home" reheating methods (reheating sous vide pouch in boiling water, reheating mussels in a saucepan) on consumer acceptability and select physicochemical qualities of marinated (acidified) sous-vide mussel meats, in comparison to a non-reheated control.

CHAPTER 2

EFFECTS OF TEMPERATURE AND ACID PRETREATMENT ON SHELF-LIFE OF SOUS-VIDE PROCESSED MUSSEL MEATS DURING REFRIGERATED STORAGE

2.1. Introduction

Seafood consumers are looking for safe and convenient products with premium quality characteristics. Sous-vide and acidification are methods that can be coupled to accomplish these challenges (Cosansu et al., 2013). Both technologies have the promise to be used for retail and food service applications. Sous-vide cooking is a thermal processing method in which the raw food is placed in a pouch and vacuum sealed, removing the air. The bag is then placed into water or steam at a specific temperature for a controlled amount of time (Church and Parsons, 2000). The product must be immediately cooled to ≤3.3°C to ensure safety and reduce potential toxin production of *Clostridium botulinum* (Food and Drug Administration, 2022). Time-temperature indicators (TTI's) must also be used as a hurdle to ensure that the products have not been time-temperature abused (Center for Food Safety and Applied Nutrition. 2021). A key benefit of sous-vide cooking is its ability to reduce overcooking and help retain texture properties in muscle foods (Bongiorno et al., 2018). Sous-vide cooking also extends the peak quality and shelf life of raw food (Gonzalez-Fandos et al., 2004). Sous-vide ready-to-eat products are convenient for consumers to prepare at home because they are already fully cooked when purchased.

Acidification is another beneficial process that has not been thoroughly researched on seafood products, specifically mollusks. Acidification can be applied to food through injection, soaking, or as an ingredient in a sauce or marinade (Koleman et al, 2020). Many different acids can be used in food applications, including citric, acetic, lactic, and tartaric acids. Citric acid is one of the most common pairings for seafood because of its tangy and bright flavor (Cosansu et

al., 2013). However, Arcales & Nacional (2018) reported that adding citric acid to green mussels decreased the yield compared to lactic acid application. The main benefit of acidification is extending the shelf life of food products by reducing microbial growth. Additionally, acidification and sous-vide cooking of whiting allowed for the fish to retain its texture and other quality attributes (Cosansu et al., 2013). However, to the best of our knowledge, no previous studies have reported the impact of sous-vide cooking and acidification on mussel meats. The objective of this experiment was to evaluate the impacts of two sous-vide cooking temperatures (65°C or 75°C) and three lactic acid treatments (0%, 0.5%, or 1%) on physiochemical properties and microbial quality of mussel meats over 35 days refrigerated storage.

2.2 Methods

2.2.1. Experimental Design Overview

Approximately 100 pounds of live blue mussels (*Mytilus edulis*) were purchased from Pemaquid Mussel Farms (Bucksport, ME, USA) in January 2023. Mussels were shucked, and the meats were added to plastic bags in groups of eight (~60 grams). The bags were then vacuum sealed and sous-vide cooked for 30 minutes at two different temperatures (65°C or 75°C) and three different acidification levels (0%, 0.5%, or 1% lactic acid) for a 2x3 factorial design. Each of the six treatments was prepared in triplicate, and the bags were stored at 3°C for 35 days. Liquid loss, pH, thiobarbituric acid reactive substances (TBARS), total volatile base nitrogen (TVBN), and microbial counts were determined on days 1, 7, 14, 21, and 35. Instrumental texture and color (L*, a*, b*) analysis were conducted on days 1, 7, 14, and 35.

2.2.2. Preparation of Mussel Treatments

Live mussels were shucked by blanching. Before blanching, 150 mussel shell lengths were measured using a ruler. Live mussels were immersed in boiling water in a steam kettle for

30 seconds in 10-pound batches, followed by a 2-minute immersion in a ~0°C ice water slurry. Based on preliminary experimentation, this method caused the mussel shell to open by releasing the adductor muscle while not noticeably cooking the meat (Arcales and Nacional, 2018). The mussel meats were then placed in snack-size Ziploc bags on ice in coolers (Coleman, USA) and then stored in a walk-in cooler (Matthew Highlands Pilot Plant, Orono, ME) at 4°C overnight. The following day, large (8" x 10") plastic sous-vide bags (UltraSource, Kansas, MO, USA) were organized according to process replicate (A,B,C). Eight mussel meats were placed into each bag, and their total weight was recorded. Each bag contained either 25 mL of 0.5% or 1% lactic acid (Fisher Scientific, Lactic Acid, MA, USA) solution (w/v).

Small (4" x 3") sous-vide bags (UltraSource, Kansas, MO, USA) were used to validate the temperature and time of the sous-vide cooking processes by placing one mussel in each bag. Each small sous-vide bag had 3 mL of 0.5% or 1% lactic acid solution (w/v) added to it. The bags were then sealed under 97% vacuum (Model UV550, Wichita, KS, USA).

The 6 treatments were coded as 65-0, 65-0.5, 65-1, 75-0, 75-0.5, and 75-1. The 65 and 75 represent the temperature (˚C) at which the mussels were cooked. The 0.5 and 1 represent the concentration (%) of lactic acid solution (25 mL) added. The 0 represents that no liquid was added to the bag. Thirty-six large sous-vide bags per treatment were prepared (2 bags/replicate) x 3 replicates x 6 sample days. An additional 72 small sous-vide bags containing one mussel each were prepared and used for recording sample core temperature throughout the sous-vide cooking process. A small (0.5 inch x 0.5 inch) foam square (ThermoWorks, Salt Lake City, UT) was placed on the surface of the sous-vide bag after first using a needle to pierce the foam. The thermocouple (Omega, Stamford, CT) wire was threaded through the foam and into the bag in order to reach the center of the mussel meat.

2.2.3. Sous-vide Cooking

Sample bags containing mussel meats were cooked in polycarbonate bins (5-gal StorplusTM; Carlisle, OK) using immersion cookers (Sous-videTM Professional Creative, PolyScience, Niles,IL) with a control of ± 0.05 °C. The water baths were set to 65 or 75°C. The containers were filled with warm tap water up to the maximum fill line. Once the machine was switched on, circulation began, and the direction of the water flow was tested by the addition of food coloring. The temperature of the water was tested with a K-type thermocouple (RDXL4SD, Omega, Stamford, CT) to ensure that the reading on the machine was equivalent to the actual temperature. The thermocouple was placed in the water in three random places. This process was repeated three different times, confirming that the water temperature was within ± 0.05 °C of the target. Once the temperature and circulation were validated, randomly selected sous-vide bags (six large and two small) were clipped to a wire rack and fully submerged in the water. A tray was used to cover the top of the containers in order to prevent evaporation of the water.

In order to monitor the internal temperature of the samples, a K-type thermocouple was inserted into each of the individually vacuum-packed mussel meats. The probes were placed into the center of the thickest part of each mussel meat. The thermocouple probes were attached to a data logger thermometer (RDXL4SD, Omega, Stamford, CT), and temperatures were recorded every 30 seconds throughout cooking. Prior to cooking, the thermocouple probes were calibrated with both boiled water and an ice water slurry (2:1 w/v) ice: water, such that the probe did not touch the bottom or sides of the container. The temperature was within ± 0.05 °C of 100°C for the boiled water and 0°C for the ice water slurry. Each bag of mussels was cooked for 30 minutes at either 65°C or 75°C. Following cooking, all of the bags were immediately placed in an ice water slurry (2:1 w/v) to lower the temperature quickly. It is important to lower the temperature

to <3.3°C within 30 minutes in order to prevent microbial growth (Center for Food Safety and Applied Nutrition, n.d.). Cooked samples in bags were placed on metal trays and stored in the walk-in refrigerator (4°C) until analysis.

2.2.4. Liquid Loss Measurement

Liquid loss was defined as the liquid released from the mussel meats during cooking and storage. The calculation accounts for the lactic acid solution that was added to the bags before cooking. At each sampling period, one bag per treatment replicate containing eight mussel meats was drained using a colander for 10 seconds, and the liquid volume was weighed. The percentage of liquid loss from the eight mussels during the cooking process and storage was determined by the following equation:

$$\%\ \text{Liquid Loss} = \frac{\text{final liquid weight (mL)} - 25\ \text{(mL)}}{\text{initial mussel weight (g)}} * 100$$

2.2.5. pH

The pH meter (Orion Star A111 pH meter, Thermo Scientific, Waltham, MA, USA) and probe were calibrated (pH 4, pH 7, and pH 10 standards) based on the manufacturer's instructions. On each test day, eight mussels per treatment replicate were homogenized together using a Magic Bullet Blender (Nutribullet, CA, USA) for 30 seconds. A 1:9 ratio (w/v) of homogenized mussel meat to distilled water was prepared for the analysis by vortexing for 10 seconds. Individual pH values of each sample were determined singly per replicate, and replicate values were averaged to derive the mean pH value for each treatment.

2.2.6. Total Volatile Base Nitrogen

The TVBN content of the samples was determined once per treatment replicate according to the method published by Botta et al. (1986), with modifications. Mussel meat homogenate (15 g) was weighed out, and 7.5% trichloroacetic acid solution (25 mL) was added to a Waring

blender. The mixture was blended for 30 seconds and poured into P8 Whatman filter paper (Fisher Scientific, P8 Grade, MA, USA). The filtrates were then frozen at ~ -18°C until use.

The defrosted extract (15 mL) was added to a micro-Kjeldahl distillation unit (Rapid distillation unit, Labconco, Kansas City, MO), followed by 4 mL of 10% sodium hydroxide solution. Prior to adding the sample, 15 mL of 4% boric acid solution and 8 drops of indicator (0.2% methyl red and 0.2% methylene blue, 2:1 in ethanol) were added to an Erlenmeyer flask at the outlet. The sample was distilled until the contents of the Erlenmeyer flask reached an approximate volume of 45 mL. The distillate was then titrated with 0.05 N hydrochloric acid (HCl) until the mixture turned from a green to a constant blue color. The volume (mL) of titrant used was recorded, and the following equation was used to calculate TVBN:

TVBN= [(Volume (mL) HCl used for titrating the sample) * (Normality of HCl) * (Molecular weight of Nitrogen)] * $\frac{100\ (\text{mL})}{\text{undiluted sample volume (mL)}}$ * $\frac{(\text{total extract in (mL) also factoring in water})}{\text{undiluted sample volume (mL)}}$

TVBN values were expressed as mg N/100 g.

2.2.7. Thiobarbituric Acid Reactive Substances

The TBARS content of the samples was determined in duplicate for each treatment replicate using a modification of the Nielsen (1998) method. A standard curve was made using 1,1,3,3 tetraethoxypropane (TEP) as the base at 10^{-5} M. Aliquots of 0, 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 mL of the TEP standard solution were transferred into screw top test tubes. The test tube volumes were brought to 4 mL with buffer (50nM PO4, 0.1% EDTA, 0.1% PG): 30% TCA (trichloroacetic acid solution). Thiobarbituric acid solution (20 mM TBA) was added (4 mL) to each tube, and then they were capped and vortexed for 10 seconds.

The homogenized mussel meats (4 g) were weighed and added to a 50 mL falcon tube. Cold buffer (16 mL) was then added to the meats and homogenized using a polytron benchtop homogenizer (Weber Scientific) for 30 seconds. The 30% TCA solution (4 mL) was then added to each tube and vortexed for 15 seconds, followed by filtering the samples using p8 Whatman paper until a volume of 15 mL was reached. Four mL of filtrate were added to a screw-top test tube, followed by the addition of 20 mM TBA solution (4mL) and vortexing for 10 seconds.

The sample and standard curve test tubes were put in boiling water for 20 minutes and then were immediately cooled in water for 10 minutes. Sample absorbance were read using a DU 530 spectrophotometer (Beckman Coulter, Brea, CA, USA) set at a wavelength of 530 nanometers. TBARS values (mg MDA/kg meat) were determined by the following equation: TBARS value=

$$\frac{(\text{total extract in (mL)})\left(\text{concentration of MDA}\left(\frac{\text{nmol}}{\text{mL}}\right)\right) * \frac{1\text{ mol MDA}}{10^9\text{nmol MDA}} * \frac{72.0636\text{ g MDA}}{1\text{ mol MDA}} * \frac{10^6\ \mu\text{g MDA}}{1\text{ g MDA}}}{4\text{ grams (mussel mince)}}$$

2.2.8. Microbial Analysis

Mussel treatments were sampled on days 0, 1, 7, 14, 21, 35, and 38 for total bacteria count. On day 38, mussel samples were also evaluated for the presence of lactic acid bacteria. Two mussels were aseptically removed from each treatment replicate bag (four mussels per treatment replicate). Those four mussels were homogenized in a Waring blender with a 1:9 ratio of mussel meat to sterile 0.1% bacto peptone (BD Diagnostics, Sparks, MD, USA). An aliquot of 1 mL of that solution was transferred into a 2 mL microcentrifuge tube (10^{-1} dilution). This process was repeated to attain 10^{-2} and 10^{-3} dilutions by taking 100 µL from the 10^{-1} and 10^{-2} dilution tubes into the next highest dilution tube mixed with 900 μL of sterile 0.1% bacto peptone dilution tubes were vortexed (Weber Scientific, Hamilton Township, NJ, USA) for 10 seconds. On days 0 and 7, raw mussels were also analyzed for total bacteria counts.

All culture media for the study were prepared in 100 mm x 15 mm plastic Petri dishes (Weber Scientific, Hamilton Township, NJ, USA). Tryptic soy agar (TSA) (Alpha Sciences, Pharmacy Avenue, Toronto, Ontario, Canada) was prepared based on the manufacturer's instructions and used to evaluate total mesophilic bacterial count (TBC). To determine total bacteria counts, aliquots of 100 uL of the appropriate dilutions were spread-plated every day on (TSA), except day 38. Half of the (TSA) plates were incubated at 35°C for 48 hours to determine mesophilic count, and the other half were incubated at 7°C for 10 days to determine psychrotroph count. DeMann Rogosa Sharpe agar (MRS) (Alpha Biosciences, Baltimore, MD, USA) was prepared based on the manufacturer's instructions and used to evaluate lactic acid bacterial counts. On day 38, MRS was used to determine lactic acid bacteria counts (LAB), and samples were plated in duplicate for each treatment replicate. The day 38, plates on MRS agar were incubated at 30°C for 48 hours.

After incubation, bacterial colonies were enumerated, and the dilution factors were used to calculate microbial populations (CFU/g). All treatment replicates were plated in duplicate, and the counts were averaged. The counts were log-transformed and then averaged for statistical analysis. If no colonies were found, the results were reported as the detection limit of the plating method (< 2.0 log CFU/mL).

2.2.9. Color

A colorimeter (LabScan XE, Hunter Labs, Reston, VA, USA) was used to measure differences in color among the treatments. The external color of each individual mussel (n=8 mussels per treatment replicate) was measured in a plastic sample cup with a 2’ diameter (Fisherbrand, Waltham, MA). The colorimeter was standardized using white and black tiles. The area view was 1.00”, and the port size was 1 3/16”. The Hunter L*, a*, b* values of the mussel

meat were recorded as the average of three (initial and rotated 120° twice) readings per sample by the colorimeter software (Universal, version 4.10, 2001, Hunter Labs, Reston, VA).

2.2.10. Texture

After the color analyses, texture measurements were conducted on the mussels using a calibrated texture analyzer (TA-XTi2, Texture Technologies Inc., Scarsdale, NY, USA). A TA-44 craft blade was used for slicing the individual mussel meats (n=8 mussels per treatment replicate) (Figure 2.1). Each mussel was placed on the platform with the flatter side down, with the blade perpendicular to the length of the mussel. Each mussel was sheared twice with the cuts approximately 2 centimeters apart. The texture analyzer was configured to a 100% depth and a 2 mm/s test speed. The maximum peak positive force (N) required to shear the mussel meat and positive area (N/sec) were both recorded by the texture analysis software (Exponent 32, version 5,0,6,0 2010, Texture Technologies Inc., Scarsdale, NY).

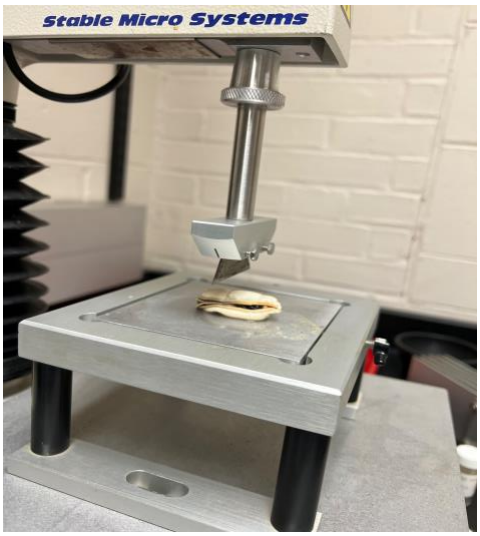

Figure 2.1. Shear force analysis

2.2.11. Statistical Analysis

The data were analyzed using IBM SPSS 28 (International Business Machines – Statistical Package for Social Sciences) at a significance level of $p \leq 0.05$. The Shapiro-Wilk test

was used to assess normality, and Levene's test was run to assess the homogeneity of variances. One-way ANOVA was performed to detect statistical differences for all one-level treatments. Multi-way ANOVA was run for all dependent variables to determine treatment and time effects. Tukey's honest significant differences (HSD) post-hoc was used to separate means.

2.3 Results and Discussion

2.3.1. Time-temperature profiles during sous-vide cooking

Before the mussels were shucked, the shell lengths (cm) of approximately 150 mussel were measured. The mean mussel shell length was 6.9 $\pm$ 0.4 cm. After shucking, the mean mussel meat weight was 7.4 $\pm$ 0.8 g. The internal temperature profiles of mussel meats during the 30-minute sous-vide cooking process are shown in Figure 2.2. These cooking processes were sufficient to control the target foodborne pathogen, *L. monocytogenes*. The minimum time required to cook each sample was determined by the equation $y= 5 \times 10^9 e^{-0.309x}$ (Humaid, 2020) which represents the relationship between temperature in degrees Celsius (x) and time in minutes (y). The calculated equivalent time values were 13 and 1 min for a core product temperature of 64°C and 74°C, respectively. The internal mussel temperatures reached the water bath temperatures close to the end of cooking, however they reached the 64°C and 75°C targets within 14 and 8 minutes, respectively. The 30-minute processing time was chosen as a conservative overestimate to ensure the safety of mussels processed at both temperatures. In future studies the cool down time would need to be monitored to ensure that all mussels cooled to <3.3°C within 30 minutes (Center for Food Safety and Applied Nutrition, n,d).

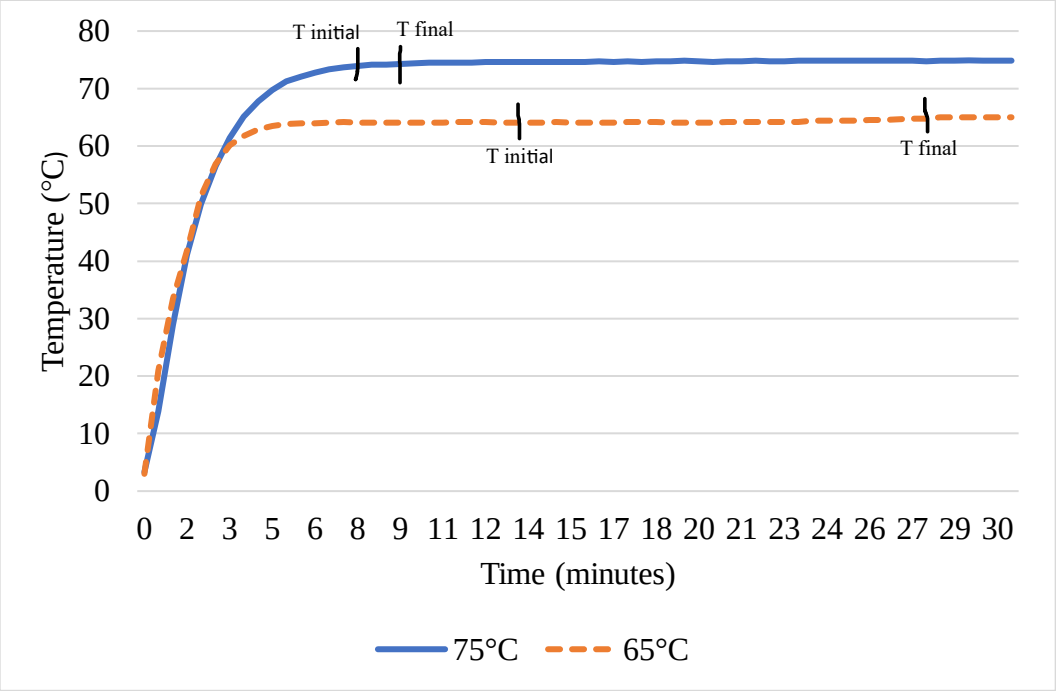

Figure 2.2. Representative core temperature profiles of mussel meats during 30 min cooking in a 65°C and 75°C water bath
T initial represents the time at which the mussels reached the target temperature of 64°C or 74°C. T final represents the end of the calculated required hold time.

2.3.2 pH

Temperature, lactic acid, and storage time all had significant effects on the pH of the mussel meats (Table 2.1), with individual treatment means ranging from 5.1 to 6.5 over the course of the study (Table 2.2). Overall, the pH values for the 65°C treatments were significantly ($p \leq 0.05$) lower than for the 75°C treatments. The magnitude of the difference between the two temperatures was minimal; at 65°C, the mean pH was 5.74 $\pm$ .02 and at 75°C, the mean pH was 5.83 $\pm$ .02. As expected, acidification with lactic acid significantly reduced the pH of the mussels. The lactic acid solutions were added in an approximately 1:3 (lactic acid to mussel meat) ratio which was enough to significantly acidify the mussel meats despite the buffering capacity of the proteins. The most concentrated acid (1%) made the mussel meats about ten times more acidic initially (pH 5.21) than the control samples (pH 6.36). At a mean pH of 5.79, the

0.5% model treatment was significantly different from the 0% and 1% acidification treatments. Overall mean pH values dropped over the course of 35 days of storage. Day 1 had the highest mean pH (5.87), and day 35 had the lowest mean pH (5.68), which was unexpected. However, there were no significant differences among overall pH values on days 7, 14 and 21. In contrast, in scallops sous-vide cooked at 70°C and 75°C, the pH values increased over the course of 30 days in chilled storage along with TVBN content, and the production of ammonia and trimethylamine by spoilage bacteria (Zhan et al., 2022). The mean pH for both the control and 75°C sous-vide treatment was initially around 6.8 and increased significantly to approximately 7.1 by day 30. The pH most likely increased due to the accumulation of alkaline substances in the muscle tissue caused by microbial degradation of proteins.

Table 2.1. Model effects (p-values) on dependent variables

Dependent Variables	Temperature	Lactic Acid Treatment	Storage Days
pH	***	***	***
Liquid Loss	***	**	-
TVBN (mg N/100 g)	***	***	***
TBARS (mg MDA/g)	***	***	**
APC (log CFU/g)	*	*	**
Psychrotrophs (log CFU/g)	***	***	***
L*	***	*	-
a*	-	**	***
b*	-	**	***
Peak Force (N)	-	*	***
Positive Area (N/sec)	***	***	***

* = ≲ 0.05
** = ≲ 0.01
*** = ≲ 0.001
- = NS (not significant)
Interaction terms shown in Appendix A.

Samples from the three lactic acid concentrations (0%, 0.5%, and 1%) remained significantly different from each other until day 35 (Figure 2.3). The pH of the 1% lactic acid treated samples increased slightly over the course of the 35 days, while the mean pH values of the controls dropped from 6.5 to 6.0 by day 35. Vacuum packaging can inhibit aerobic Gram-negative bacteria, which can allow for the growth of lactic acid bacteria (Francoise, 2010). The drop in pH for the unacidified control samples at day 21 may have been related to the growth of lactic acid bacteria, as they generate acidic products such as organic acids, including acetic acid, lactic acid, and citric acid, among others (Punia Bangar et al., 2022). On day 35, lactic acid bacteria counts for all treatments were ~3.00 log CFU/g (Figure 2.7).

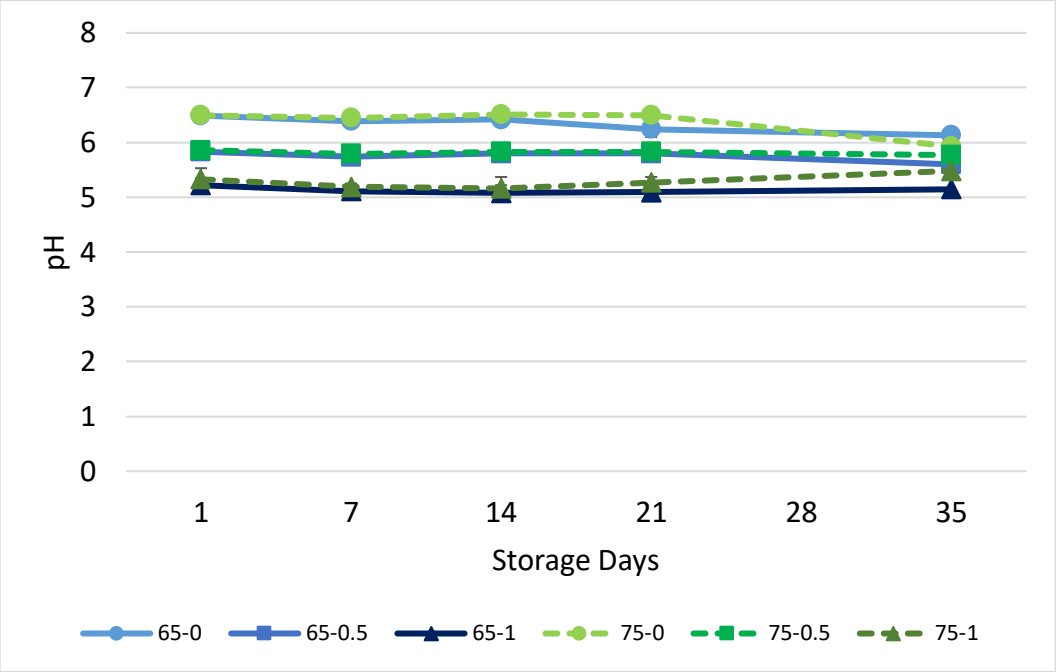

Figure 2.3. Effects of processing temperature, lactic acid treatment, and storage time on pH of mussel meats over 35 days
The error bars represent standard deviation. (n=3)

Table 2.2. Mean pH values of mussel meat treatments over 35 days

Treatment	Day 1	Day 7	Day 14	Day 21	Day 35
65-0	6.5 ± 0.0cB	6.4 ± 0.0cAB	6.4 ± 0.1cB	6.2±0.1cAB	6.1± 0.2dA
65-0.5	5.8 ± 0.1bA	5.7 ± 0.1 bA	5.8 ± 0.1 bA	5.8 ± 0.1bA	5.6 ± 0.0bcA
65-1	5.2 ± 0.1aA	5.1 ± 0.1aA	5.1 ± 0.1aA	5.1 ± 0.1aA	5.2 ± 0.1aA
75-0	6.5 ± 0.1cB	6.5 ± 0.0cB	6.5 ± 0.1cB	6.5 ± 0.1cB	5.9 ± 0.2cdA
75-0.5	5.9 ± 0.1bA	5.8 ± 0.0bA	5.8 ± 0.1bA	5.8 ± 0.1bA	5.8 ± 0.1bcA
75-1	5.3 ± 0.2aA	5.2 ± 0.1aA	5.2± 0.2aA	5.3 ± 0.1aA	5.5 ± 0.1abA

Data are expressed as mean ± standard deviations (n=3). Lowercase letters designate significant differences (in columns) among treatments at each time point (1-way ANOVA). Uppercase letters designate significant differences (in rows) within treatments over time (1-way ANOVA).

2.3.3 Liquid Loss

Temperature and lactic acid treatment had significant effects on the liquid loss of the mussel meats at the model level, while storage time did not (Table 2.1). Liquid loss in all

treatments over time ranged from 18.8 to 28.2% (Table 2.3). Based on multi-way ANOVA, the liquid loss values for the 75°C treatments were significantly ($p \leq 0.05$) higher than for the 65°C treatments. Similarly, when cod fillets were sous-vide cooked at 82°C and 55°C, the fillets that were cooked at 82°C had significantly higher cook loss values (Stormo & Skara, 2023). In addition, Cropotova et al. (2019) discovered that cook loss was significantly higher over time in mackerel fillets that were sous-vide cooked at 75°C and 90°C compared to 60°C. Thus, lower sous-vide cook temperature may increase juiciness and provide a softer texture to seafood products. In the current study, the extent of difference between the two cooking temperatures was moderate; 65°C treatments had a mean liquid loss value of 21.1% and the 75°C treatments had a mean value of 25.5%. However, it's possible that even a 4% difference in liquid loss levels may be associated with differences in juiciness and texture among muscle foods. Araujo et al. (2020) recorded when smoking catfish that an approximately 8% difference in liquid loss values (39.8% vs. 31.7%) resulted in significant differences in sensory texture scores, with the higher liquid loss value corresponding to a lower liking score (4.71) than the lower liquid loss treatment (6.69) on a 9-point hedonic scale.

Increasing the lactic acid concentration overall increased the liquid loss values. There was no significant effect of acid on mean liquid loss values between the control mussel meats (23.5%) and those treated with 0.5% lactic acid (22.1%) on a model level (Table 2.1). However, the 1% lactic acid treatment caused the mussel meats to have a significantly higher mean liquid loss value (24.3%) than both the control and 0.5% lactic acid treatment (Table 2.3). Santos & Regenstein (1990) reported that treating hake and mackerel fillets with 0.5% erythorbic acid resulted in significantly higher cook loss values than those not treated with acid. The authors also found that vacuum packaging increased the cook loss values as well. In hake specifically,

vacuum-packaging combined with acidification produced the highest cook loss values (~40%) compared to any other treatments. Reportedly, when introduced to meat, acid acts to unwind (denature) the long proteins in the muscle allowing water to escape the muscle matrix (Ke et al., 2009).

The impacts of processing temperature on liquid loss were acidification dependent (Figure 2.4). There were significant differences in liquid loss between temperatures (65°C and 75°C) in the 0% and 0.5% lactic acid samples. When 0% and 0.5% lactic acid were applied at 65°C, the liquid loss was approximately 20%, compared to approximately 25% at 75°C. Ofstad et al. (1996) reported that storage time was the largest contributing factor to the liquid loss of raw cod and salmon post-mortem, while the current study shows that acid and temperature were more significant. However, in the salmon and cod study the samples were not cooked, which causes a lot of liquid loss due to the shrinkage of the proteins. The mean liquid loss of the raw salmon was 9.5%, and the average liquid loss of sous-vide cooked cod at 90°C was 9.78% (Ofstad et al., 1996; Cropotova et al., 2019). Both values are about half as much liquid loss as compared to the sous-vide mussels in lactic acid solution. One experimental design difference between Cropotova et al. (2019) and the current study is that the length of storage was only 7 days compared to 35 days for the mussels. Also, mussels and mackerel have different water contents in that the moisture content for mussels can be up to 90% compared to 70-80% for fish. It is also possible that cooking and storing the mussels in a liquid altered the extent of liquid loss over time.

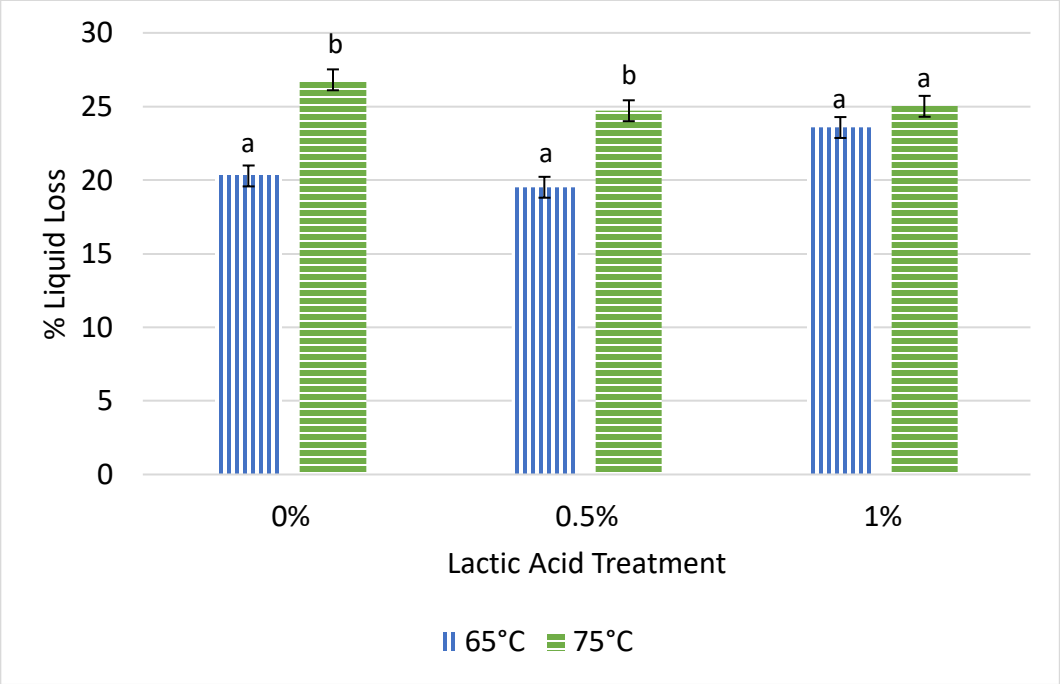

Figure 2.4. Effects of processing temperature and lactic acid treatment on the (%) liquid loss of mussel meats

Each column represents the mean plus/minus standard error (n=15) Columns within each lactic acid treatment group not sharing a letter are significantly different ($p \leq 0.05$).

Table 2.3 Mean liquid loss (%) values of mussel meat treatments over 35 days

Treatment	Day 1	Day 7	Day 14	Day 21	Day 35
65-0	20.2 ± 3.6 abA	18.7 ± 3.0 aA	20.7 ± 2.9 abA	19.8 ± 0.7abA	22.0 ± 0.5abA
65-0.5	20.1 ± 1.4 aA	22.0 ± 2.1 aA	18.8 ± 2.8 aA	17.8 ± 4.8 aA	18.9 ± 1.2aA
65-1	23.8 ± 0.8abAB	21.2 ± 2.6 aA	20.7 ± 1.2 abA	24.6 ± 3.9abAB	27.5 ± 1.8 cB
75-0	26.0 ± 4.5 abA	26.6 ± 1.9 aA	27.1 ± 1.9 bA	28.2 ± 2.7 bA	26.2 ± 1.9bcA
75-0.5	27.7 ± 1.4 bA	24.1 ± 4.6 aA	22.3 ± 1.9 abA	27.2 ± 4.9 abA	22.3± 3.3abA
75-1	25.2 ± 2.8 abA	26.4 ± 2.7 aA	23.2 ± 3.3 abA	24.8 ± 1.7 abA	25.4 ± 1.3bcA

Data are expressed as mean ± standard deviations (n=3). Lowercase letters designate significant differences (in columns) among treatments at each time point (1-way ANOVA). Uppercase letters designate significant differences (in rows) within treatments over time (1-way ANOVA).

2.3.4 Total Volatile Base Nitrogen (TVBN)

TVBN content is an important microbial spoilage indicator for seafood (Yang et al., 2016). Several authors have estimated the TVBN limit for the acceptability of shrimp as 30 mg/100 g (Smaldone et al., 2011; Altissimi et al., 2018). Altissimi et al. (2018) reported that

fresh, just-purchased seafood TVBN values ranged from 13 mg/100 g for defrosted cuttlefish to 34 mg/100 g for fresh and defrosted red shrimp. In the present study, processing temperature, lactic acid concentration, and storage time all had significant model level effects on the TVBN values of the mussel meats (Table 2.1). Overall, the 65°C processing treatment (19.3 mg N/100 g) produced significantly higher mean TVBN values than the 75°C processing treatment (14.1 mg N/100 g) over the course of the storage study. The 1% lactic acid treatment (12.4 mg N/100 g) had significantly lower overall TVBN values compared to the 0% (19.5 mg N/100 g) and 0.5% treatments (18.2 mg N/100 g), which were not significantly different from each other. Similarly, acidification reduced TVBN levels of blue-jack mackerel fish silage intended for use in animal feeds (Enes et al., 2007). The samples that were acidified with formic acid and propionic acid maintained TVBN values approximately three times lower after 21 days of storage than samples that were not treated. The acidified samples had a mean TVBN value of 18 mg/g N, while the other samples ranged from 53-73 mg/g N. In the current study, acidifying with 1% lactic acid or sous-vide cooking at 75°C kept the samples below the acceptable limit, 30 mg N/100 g, throughout 35 days of refrigerated storage (Figure 2.5).

TVBN values increased in all treatments over time. Day 1 had the lowest overall mean TVBN value at 9.3 mg N/100 g while day 35 had a mean value about four times higher, at 38.5 mg N/100 g. Compounds including dimethylamine, trimethylamine, and ammonia are produced over time in fish and shellfish throughout refrigerated and frozen storage (Singh et al., 2016; Bongiorno et al., 2018), primarily through microbial metabolism. It is also important to note that we are not sure exactly when the TVBN values spiked in the 65-0, 65-0.5, and 75-0.5 treatments (Table 2.4). Because TVBN values were so constant up through day 21, we decided to skip analysis on day 28 and extend the shelf-life study to 35 days. This left us with missing

information and being unable to determine exactly when the TVBN went over the level of acceptability for those treatments. However, based on having TBVN values under 25 mg/100g, both the 1% lactic acid treatments maintained “good quality” for the entire 35-day storage study.

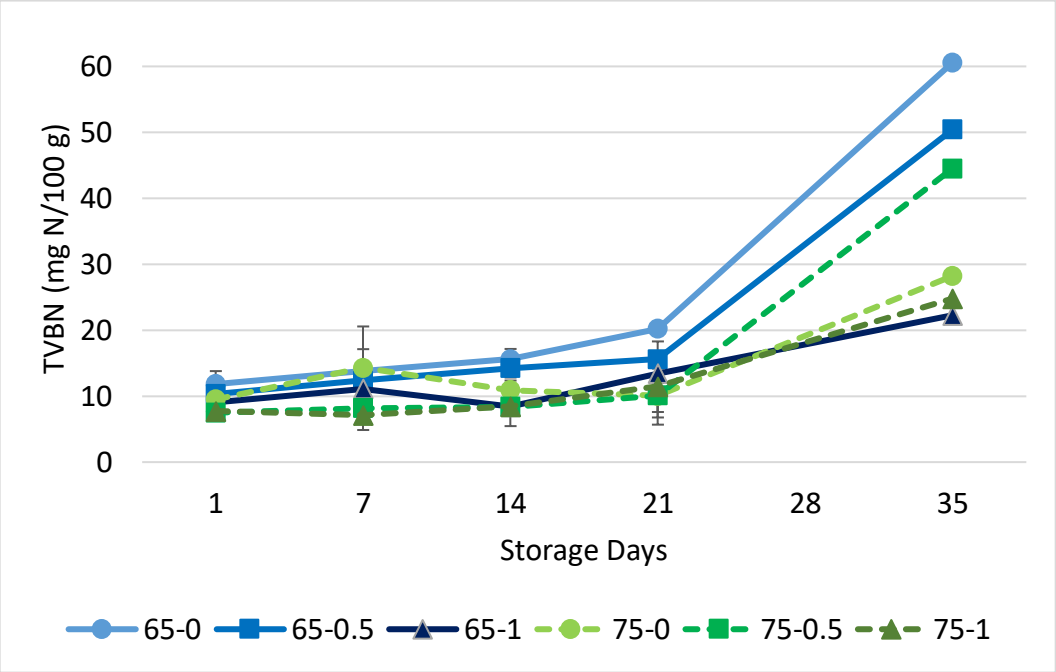

Figure 2.5. Effects of processing temperature, lactic acid treatment, and storage time on TVBN values (mg N/100 g) of mussel meats over 35 days
The error bars represent standard deviation. (n=3)

Table 2.4. Mean total volatile base nitrogen (mg N/100 g) content of mussel meat treatments over 35 days

Treatment	Day 1	Day 7	Day 14	Day 21	Day 35
65-0	11.8 ± 2.0 cA	13.8 ± 3.4 aA	15.6 ± 0.9bAB	20.2 ± 0.0 bB	60.9 ± 2.9cC
65-0.5	10.3 ± 0.4 bcA	12.3 ± 0.6 aA	14.3 ± 2.9 bA	15.6±2.7abA	50.4 ±4.6 bcB
65-1	9.1 ± 0.4 abA	11.1 ± 0.8aAB	8.4 ± 1.4 aA	13.4 ±1.4abB	22.3 ± 2.6 aC
75-0	9.5 ± 0.8 abcA	14.3 ± 6.3aAB	10.9 ± 1.4abA	10.1 ± 4.3 aA	28.2 ± 9.9 aB
75-0.5	7.5 ± 0.2 aA	8.2 ± 0.6 aA	8.4 ± 2.9 aA	10.1 ±2.5aAB	44.5 ± 3.2 bB
75-1	7.7 ± 1.1 abA	7.1 ± 2.3 aA	8.4 ± 1.4 aA	11.5 ± 4.7 aA	24.8 ± 7.4 aB

Data are expressed as mean ± standard deviations (n=3). Lowercase letters designate significant differences (in columns) among treatments at each time point (1-way ANOVA). Uppercase letters designate significant differences (in rows) within treatments over time (1-way ANOVA).

2.3.5 Thiobarbituric Acid Reactive Substances (TBARS)

TBARS measures secondary products that are produced due to lipid oxidation. Foods that are high in polyunsaturated fatty acids are more likely to have a faster rate of lipid oxidation because of the double bonds that they contain. Temperature, lactic acid concentration, and storage time all significantly affected the TBARS values of the mussel meats (Table 2.1). Mussels sous-vide cooked at 75°C had significantly higher values (1.58 mg MDA/kg) than the 65°C (1.26 mg MDA/kg) treatment samples. We would typically expect foods cooked at a higher temperature to have higher TBARS values (Broncano et al., 2009) since chemical reaction rates (e.g., oxidation) should increase as the temperature increases; a lower temperature lowers the reaction rate. However, vacuum packaging has the potential to lower oxidation rates because it removes oxygen, which is needed for the oxidation process and small differences in oxidation may be negligible (Roldan et al., 2014). The 75°C processing temperature resulted in higher initial TBARS values compared to the 65°C treatments (Figure 2.6). Similarly, Pongsetkul et al. (2022) found that in tilapia, higher sous-vide cooking temperatures contributed to larger levels of oxidation.

The lactic acid treatments also had a significant effect on overall TBARS values, with the most concentrated acid (1%) having significantly higher TBARS values (1.76 mg MDA/kg) than the control (1.13 mg MDA/kg) and 0.5% (1.37 mg MDA/kg) lactic acid treatments. These were not significantly different from each other. It's not clear why the lactic acid treatment apparently promoted oxidation of the mussel samples. In contrast, Cosansu et al. (2011) reported that when whiting was sous-vide cooked at 70°C and half of the samples were dipped in a 1:4 (w/v) solution of lemon juice for 30 minutes there was no significant difference in TBARS value over the course of 42 days. However, in the present study the mussels were cooked and stored in the lactic acid solution instead of dipping. Simply dipping may have led to the lack of significant differences if the fish did not have time to absorb the lemon juice. Differences among studies may also have been due to different acids (lactic versus citric) or to differences in lipid composition of mussels versus whiting. Raw blue mussels were recorded to have an average lipid content of 2.24% (FoodData Central, 2023).

Unexpectedly, TBARS values of the samples decreased significantly during storage (Figure 2.6, Table 2.5). Day 1 had the highest mean TBARS value (1.77 mg MDA/kg while day 35 (1.21 mg MDA/kg) had the lowest. We would expect TBARS values first to increase with storage time, then plateau and decrease after all the unsaturated fatty acids become oxidized and converted into tertiary reaction products. With less oxygen, this would still be expected to happen, but at a slower rate. An increase may have occurred between days 1 and 7, however samples were not analyzed during that period. Cetinkaya et al., (2017) recorded that in sous-vide cooked rainbow trout, each treatment, no matter if the fish were vacuum packaged or treated with rosemary extract, had an increase in TBARS values between day 1 and day 45. An increase in TBARS values was also seen in tilapia that was sous-vide cooked at 80°C with day 0 values at

around 0.2 mg MDA/kg and day 49 values being approximately 1.4 mg MDA/kg (Karki et al., 2023). In shrimp, TBARS values of 1-2 mg MDA/kg are related to unpleasant odor and taste (Farajzaedeh et al., 2016). Since the lipid content of blue mussels was reported to be ~2.25% (Khan et al., 2005), which is higher than the fat content of shrimp (~1.79%), the cut off value for acceptability of mussels could be even different, but that would have to be determined experimentally with a trained panel.

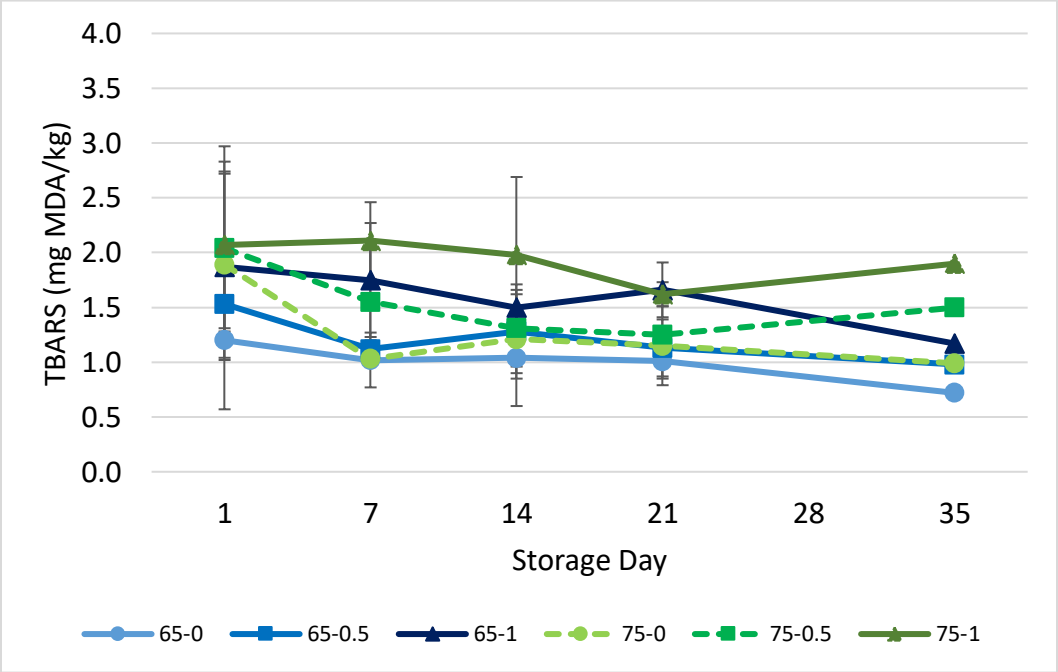

Figure 2.6. Effects of processing temperature, lactic acid treatment, and storage time on TBARS values (mg MDA/kg) of mussel meats over 35 days
The error bars represent standard deviation. (n=3)

Table 2.5. Mean thiobarbituric acid reactive substances (mg MDA/kg) content of mussel meat treatments over 35 days

Treatment	Day 1	Day 7	Day 14	Day 21	Day 35
65-0	1.2 ± 0.6aA	1.0 ± 0.3aA	1.0 ± 0.2aA	1.0 ± 0.2aA	0.7 ± 0.1aA
65-0.5	1.5 ± 0.3aA	1.1 ± 0.1aA	1.3 ± 0.4aA	1.1 ± 0.3abA	1.0 ± 0.2abA
65-1	1.9 ± 0.9aA	1.8 ± 0.5abA	1.5 ± 0.2aA	1.7 ± 0.2bA	1.2±0.3abcA
75-0	1.9 ± 0.8aA	1.0 ± 0.0aA	1.2 ± 0.3aA	1.2 ± 0.4abA	1.0 ± 0.0abA
75-0.5	2.0 ± 0.9aA	1.6 ± 0.5abA	1.3 ± 0.3aA	1.3 ± 0.1abA	1.5 ± 0.5bcA
75-1	2.1 ± 0.8aA	2.1 ± 0.3bA	2.0 ± 0.7aA	1.6 ± 0.1bA	1.9 ± 0.2cA

Data are expressed as mean ± standard deviations (n=3). Lowercase letters designate significant differences (in columns) among treatments at each time point (1-way ANOVA). Uppercase letters designate significant differences (in rows) within treatments over time (1-way ANOVA).

2.3.6 Microbial Analysis

Total plate count is a measure of the population of all of the aerobic bacteria present in a sample. It is particularly useful for shelf-life testing because it is somewhat of an all-encompassing measurement that can describe the general microbial quality of the product. The total bacteria count for cooked foods must be below 5 log CFU/g to be judged acceptable (Huss, 1995). Total plate counts for the treatments over the course of the study remained below 3 log CFU/g, which was not unexpected due to the fact that the product was both cooked and vacuum packaged. Lactic acid treatment and storage day both had a significant effect on total plate count at the model level (Table 2.1), whereas processing temperature did not. The mean overall total plate count of the control (2.61 log CFU/g) was significantly higher than that of the 1% acid treatments (2.47 log CFU/g), although the difference was minimal. At 2.59 log CFU/g, the mean total plate count for the 0.5% lactic acid treatment was not significantly different than the other treatments. In another study, when 2% and 3% lactic acid solutions were applied to raw catfish fillets, the 3% samples had significantly lower microbial counts compared to the control during refrigerated storage (Kim et al., 1995). Cosansu et al. (2011) reported that by day 21 of storage,

sous-vide fish fillets acidified with lemon juice had significantly less mesophilic aerobic bacteria (2.91 log CFU/g) compared to the fillets that were only sous-vide processed and not acidified (4.28 log CFU/g). Citric acid may be more effective than lactic acid at hindering microbial growth because it has a lower pKa, indicating that it is a stronger acid.

Storage time also had a significant effect on total plate counts at the model level. Day 1 (2.69 log CFU/g) of storage overall did not have significantly different total plate counts from day 21 (2.49 log CFU/g) or day 35 (2.60 log CFU/g). However, the in-between days (7 and 14) had significantly higher counts than the first day and the last day. This trend was unusual and was likely related to the environment in the sous-vide pouch and other competitive bacteria. In the bag, there were also aerotolerant anaerobes and facultative anaerobes, which prefer to grow when oxygen is present but can also grow when there is no oxygen. The low total plate counts on day 1 were due to the effects of cooking, and as storage continued and as time went on the aerotolerant anaerobes and facultative anaerobes that remained after cooking grew slowly. However, if large amounts of anaerobic bacteria grew later on, then the anaerobic bacteria may have outcompeted the aerobic bacteria. Unfortunately, anaerobic bacteria were not enumerated in this study, with the exception of lactic acid bacteria on day 35 of storage. Similarly, Hollingworth et al. (1991) reported that when imitation crabmeat was vacuum sealed and stored at 22°C, total plate counts increased up to day 13 (8.2 log CFU/g). Then, on day 19, the total plate counts decreased (7.9 log CFU/g) while the proteolytic counts increased (8.2 – 8.4 log CFU/g). In the present study, although storage day significantly impacted overall total plate counts, the magnitude of differences among days were small. There were no significant differences in total plate counts among individual treatments of mussel meats at any time point (Table 2.6).

Table 2.6. Mean total plate counts (log CFU/g) of mussel meat treatments over 35 days

Treatment	Day 1	Day 7	Day 14	Day 21	Day 35
65-0	2.8 ± 0.1aA	2.6 ± 0.2aA	2.5 ± 0.3 aA	2.5 ± 0.2 aA	2.6 ± 0.0 aA
65-0.5	2.7 ± 0.2 aA	2.5 ± 0.3aA	2.4 ± 0.2 aA	2.6 ± 0.3 aA	2.6 ± 0.2aA
65-1	2.6 ± 0.1aA	2.5 ± 0.3aA	2.2 ± 0.2 aA	2.3 ± 0.4 aA	2.5 ± 0.1aA
75-0	2.9 ± 0.1 aA	2.5 ± 0.2 aA	2.7 ± 0.4 aA	2.4 ± 0.1aA	2.8 ± 0.2 aA
75-0.5	2.7 ± 0.2 aA	2.5 ± 0.2 aA	2.7± 0.3 aA	2.6 ± 0.1 aA	2.6 ± 0.1aA
75-1	2.6 ± 0.2 aA	2.6 ± 0.2aA	2.4 ± 0.2 aA	2.5 ± 0.0 aA	2.6 ± 0.1 aA

Data are expressed as mean ± standard deviations (n=3). Lowercase letters designate significant differences (in columns) among treatments at each time point (1-way ANOVA). Uppercase letters designate significant differences (in rows) within treatments over time (1-way ANOVA).

Processing temperature, lactic acid treatment, and storage time all had a significant effect on psychrotrophic bacterial populations at the model level (Table 2.1). Psychrotrophic bacteria are responsible for the spoilage of seafood at chilled temperatures (Gram & Huss, 1996). This product was not aerobically stored, rather, it was held in reduced oxygen or anaerobic environment, meaning that bacteria that prefer oxygen but function in non-oxygen environments can still grow. Based on multi-way ANOVA, the overall psychrotrophic bacterial populations for the 75°C treatments (2.43 log CFU/g) were significantly ($p \leq 0.05$) higher than for the 65°C treatment (2.13 log CFU/g). This is the opposite of what was expected because higher thermal processing temperatures are typically associated with more inactivation of vegetative bacteria. The low thermal processing temperatures of sous-vide cooking may not kill all bacteria that are present or spores meaning even in an anaerobic environment bacteria will continue to grow. For example, when salmon was sous-vide cooked at either 40°C or 50°C, the 40°C processing temperature resulted in significantly higher bacteria counts (Abel et al., 2019). Typically, higher processing temperatures would be associated with fewer bacterial colonies. At 0.3 log CFU/g, the extent of difference between the two processing temperatures was statistically significant but minimal. On days 21 and 35, the 75-0 treatment had significantly higher psychrotrophic bacteria

populations, at 3.41 log CFU/g and 2.79 log CFU/g, respectively, than all the other treatments, which had values ranging from 2.00-2.50 log CFU/g (Table 2.7).

Both the 0.5% (2.21 log CFU/g) and 1% lactic acid treatments (2.12 log CFU/g) had significantly lower psychrotrophic bacterial counts than the 0% lactic acid treatment (2.53 log CFU/g). The addition of lemon juice to whiting significantly lowered the psychrophilic aerobic bacteria counts from day 28 on compared to samples that were only sous-vide cooked and not treated with lemon juice (Cosansu et al., 2011). Similarly, Arcales & Nacional (2018) reported that green mussels treated with either 2% citric or lactic acid produced significantly fewer psychrophilic bacteria colonies than the control samples. Dropping the pH typically reduces microbial growth because lower pH values can adversely impact the structure and function of bacterial cells. Typically, it would be expected that combining a higher temperature and acidification would decrease microbial counts however, in this specific study there was no interaction seen between the two.

Length of refrigerated storage also had a significant effect on psychrotrophic bacterial populations at the model level in that the mean count across treatments at day 35 (2.54 log CFU/g) was significantly higher than any other storage day. The mean count at day 1 was 2.15 log CFU/g, and there were no significant differences in overall psychrotrophic bacterial populations until day 35. Thermal processing at either temperature combined with the vacuum packaging was sufficient to maintain psychrotophic and total plate count at fairly low levels. Similarly, when fish cakes were sous-vide processed, the sous-vide psychrotrophic counts stayed consistent (~3.00 log CFU/g) for the entire 16 weeks of storage, while the control treatment, which was conventionally cooked had a steep spike at week 3 (~5.50 log CFU/g) (Jeya et al.,

2009). Similarly, all of the sous-vide treatments in our study had psychrotrophic counts that stayed under 3.50 log CFU/g (Table 2.7).

Table 2.7. Mean total psychrotrophic populations (log CFU/g) of mussel meat treatments over 35 days

Treatment	Day 1	Day 7	Day 14	Day 21	Day 35
65-0	2.1 ± 0.1aA	2.1 ± 0.1 aA	2.0 ± 0.0 aA	2.4 ± 0.3 aA	2.4 ± 0.2 aA
65-0.5	2.1 ± 0.1 aA	2.3 ± 0.2 aA	2.0 ± 0.0 aA	2.2 ± 0.2 aA	2.1 ± 0.2 aA
65-1	2.1 ± 0.1 aA	2.4 ± 0.2 aB	2.0 ± 0.0 aA	2.0 ± 0.0 aA	2.0 ± 0.0 aA
75-0	2.2 ± 0.3aA	2.3 ± 0.1 aA	2.5 ± 0.4 aA	3.4 ± 0.1 bB	2.8 ± 0.2 aB
75-0.5	2.3 ± 0.1 aA	2.2 ± 0.1 aA	2.3 ± 0.3 aA	2.1 ± 0.1 aA	2.5 ± 0.7 aA
75-1	2.2 ± 0.2 aA	2.4 ± 0.1 aA	2.0 ± 0.0 aA	2.0 ± 0.0 aA	2.2 ± 0.3 aA

Data are expressed as mean ± standard deviations (n=3). Lowercase letters designate significant differences (in columns) among treatments at each time point (1-way ANOVA). Uppercase letters designate significant differences (in rows) within treatments over time (1-way ANOVA).

Lactic acid bacteria counts were evaluated only on Day 35 due to suspicion that they could be contributing to the off odors and softening of the mussels. It was reported in the current study that on day 35 both 1% lactic acid treatments had neutral and briny aromas while all other samples had notes of pungency, fishy, and musky. Lactic acid bacteria found in sous-vide products are frequently associated with off-odor and swelling of the packs (Gonzales-Fandos, 2004). Treatment did not have a significant effect on lactic acid bacteria population counts (Figure 2.7), with the average counts for all treatments being ~3.00 log CFU/g. This was somewhat unexpected because the rationale for measuring lactic acid bacteria on the final day of storage was that there might be large populations that would have contributed to the spoilage of the product. Lyhs et al. (1998) reported that sous-vide trout fillets with lactic acid bacteria counts ranging from 10^4-10^6 CFU/g were considered spoiled. Jeya Shakila et al. (2009) reported that minced cooked fish that had been sous-vide processed had higher lactic acid bacteria counts (4.12 log CFU/g) compared to raw fish (3.81 log CFU/g) and fish cakes that were conventionally cooked (not detected). This suggests that the sous-vide environment from the current experiment

has the potential to be associated with lactic acid bacteria growth due to the lack of oxygen and the acidic environment that can also facilitate growth. Most lactic acid bacteria prefer a pH of 6-7 but many are acidophilic, meaning that they can tolerate lower pH values (Saraoui et al., 2016).

It would be beneficial to enumerate lactic acid bacteria populations (log CFU/g) during the entire shelf life of the product and to test for the presence of *C. botulinum.* Lactic acid bacteria have been reported to be the main spoilage organism for sous-vide products in several other studies, and it would be useful to more clearly understand the microbial population dynamics inside the sous-vide pouches during storage (Jeya Shakila et al., 2009; Lyhs et al., 1998). In the present study, total plate counts and psychotropic counts were enumerated, but they only measured aerobic bacteria, so anaerobic bacteria were not accounted for. The sous-vide bag environment was anaerobic, with conditions suitable for *C. botulinum*. In order to prevent growth and toxin production by this pathogen, the product must be stored at <3.3°C and have a pH of less than 4.6 (Center for Food Safety and Applied Nutrition, 2023). The lactic acid concentrations applied in the present study were selected based on their potential to reduce growth of spoilage bacteria without negatively impacting aroma and flavor of the mussels, not their ability to reduce pH to below 4.6. Acidification with 1% lactic acid reduced initial mussel pH from ~6.5 to ~5.2; an environment conducive to the growth of *C. botulinum* More importantly, storage temperature was not controlled to <3.3°C, hovering around 4°C for the entirety of the experiment. Although slightly lower than most domestic refrigerator temperatures (Evans & Redmond, 2016), this lack of adequate temperature control for a vacuum packaged product emphasizes the need for TTI's on retail packaging, or the addition of another hurdle. It would have been useful to look for the presence of *C. botulinum* or possibly other clostridia

species, which can also be associated with off-odor and gas expansion of food packaging similar to lactic acid bacteria (Center for Food Safety and Applied Nutrition, n.d.).

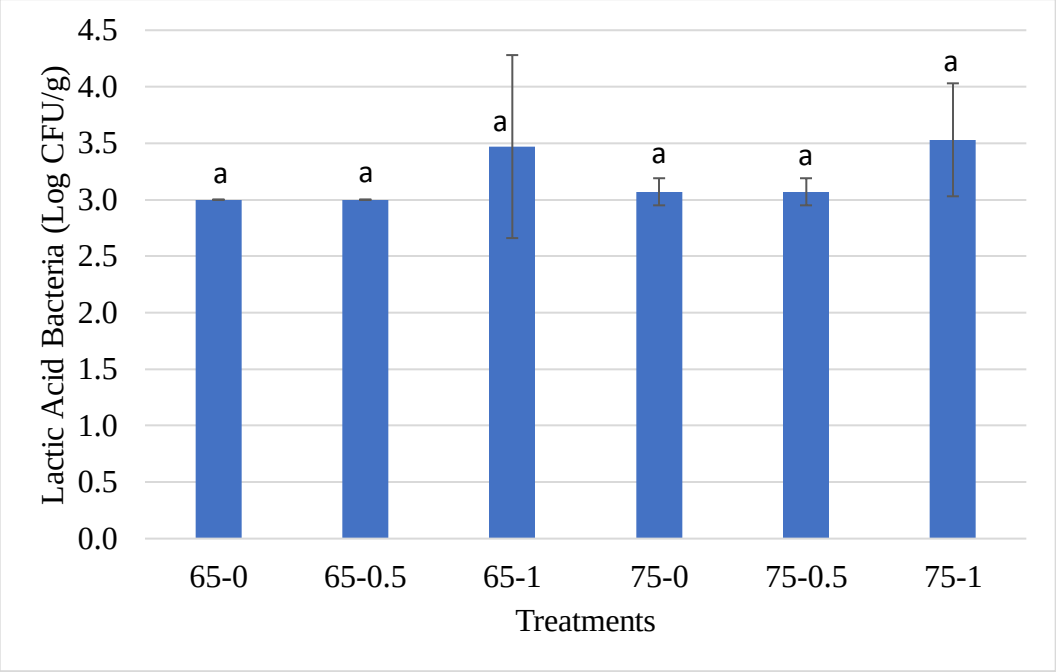

Figure 2.7. Mean total lactic acid bacteria populations (log CFU/g) of mussel meat treatments on day 35
Each bar represents the mean values ± standard deviation (n=3). Treatments not sharing an uppercase letter are significantly different ($p \leq 0.05$) based on one-way ANOVA followed by Tukey's HSD post hoc test.

2.3.7 Color

Processing temperature and lactic acid treatment had a significant effect on L* value at the model level (Table 2.1), while storage day did not. Overall, the 65°C processing treatment (52.31) had significantly higher mean L* values than the 75°C processing treatment (50.44), although the magnitude of difference was minimal. Cropotova et al. (2019) reported that increasing the temperature of sous-vide processing increased the lightness of the mackerel. This was likely due to higher aggregation and denaturation of the proteins, increasing light scattering, as seen by Christensen et al. (2011) in pork. Cooking time can also have a bleaching effect on the

L* values of mussels. Palamae et al. (2023) noted that sous-vide mussels that were cooked for 2 minutes (48.56) had significantly higher L* values than those that were only cooked for 1 minute (44.61) meaning that the longer cooking time produced lighter colored mussels. The current study appeared to have the opposite outcome in that the higher sous-vide processing temperature decreased overall lightness. Russo et al. (2023) noted that when Mediterranean mussels were sous-vide cooked at different temperatures (72°C, 80°C, 90°C, and 100°C), there were no significant differences in L* value between 72°C (87.4) and 80°C (91.3) sous-vide processing temperatures. However, these L* values were significantly higher, almost double, the values measured in the present study. One reason for the darker mussel color in the present study could be that the mussels were fresh while the other study used frozen product. Sun et al. (2023) reported that there were significant differences in L* values between fresh and frozen shrimp with the frozen shrimp having significantly higher L* values. Ice crystals form during the freezing process that can damage the structure of the muscle tissue, increasing lightness. Also, the L* values from the study done by Palamae et al. (2023) using fresh mussels were more in line with the present values.

Acid treatment also had a significant effect on L* value at the model level. There was no significant difference between the 0.5% (51.80) lactic acid treatment and the 1% (52.01) treatment, but both produced a slight bleaching effect and significantly greater L* values than the 0% control (50.32). In contrast, Kamireddy et al. (2008) reported that the addition of citric acid and acidified sodium chlorite solution to trout fillets had no effect on initial L* values. This could be due to the fact that the trout fillets were only dipped into the acid solutions and then removed, whereas the mussels were soaked in the lactic acid solutions for the duration of chilled storage. There were no significant differences in L* values among the lactic acid treatments

within the 65°C or 75°C processing temperature (Figure 2.8). In addition, one-way ANOVA showed that there were no significant differences among any individual treatments storage days (Table 2.8).

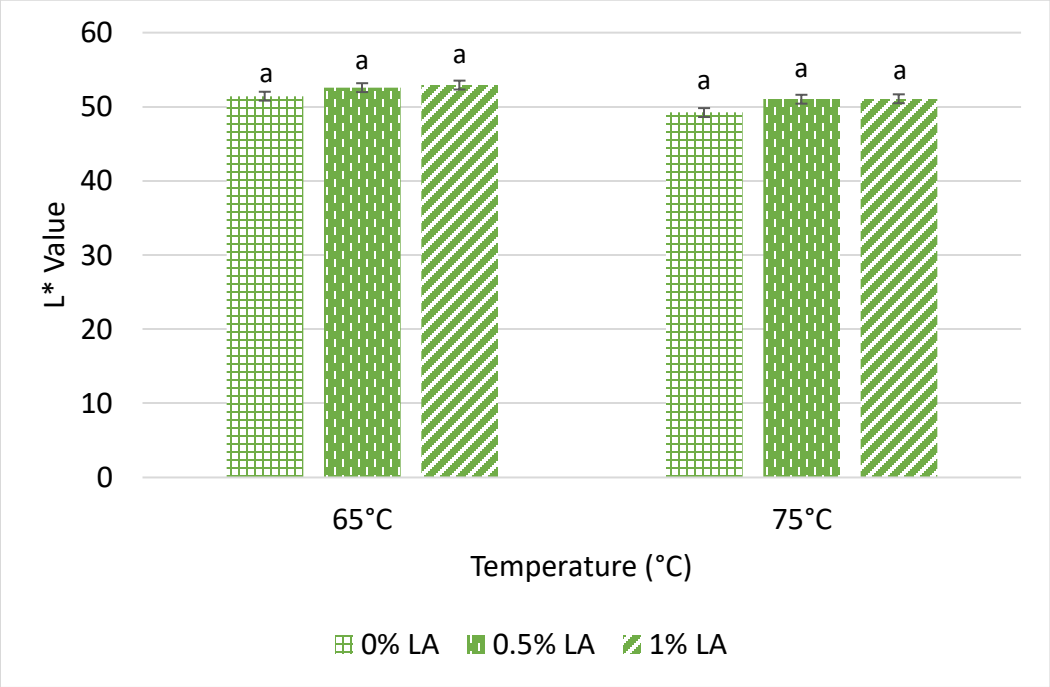

Figure 2.8. Effects of processing temperature and lactic acid treatment on L* value of mussel meats

Each represents the mean plus/minus standard error (n=12) Columns not sharing a letter within each processing temperature are significantly different ($p \leq 0.05$) based on multi-way ANOVA followed by Tukey's HSD post hoc test.

Table 2.8. Mean L* values of mussel meat treatments over 35 days

Treatment	Day 1	Day 7	Day 14	Day 35
65-0	52.44 ± 1.63 aA	51.14 ± 1.46 aA	52.18 ± 1.03 aA	49.96 ± 0.95 aA
65-0.5	52.44 ± 1.95 aA	51.84 ± 1.68 aA	52.97 ± 1.89 aA	53.05 ± 3.41 aA
65-1	53.82 ± 1.47 aA	53.46 ± 2.10 aA	51.42 ± 1.98 aA	53.03 ± 0.64 aA
75-0	50.11 ± 0.87 aA	50.28 ± 1.29 aA	49.21 ± 2.47 aA	47.26 ± 4.10 aA
75-0.5	51.55 ± 3.84 aA	49.36 ± 2.50 aA	52.44 ± 2.46 aA	50.72 ± 2.15 aA
75-1	52.74 ± 0.22 aA	50.84 ± 0.89 aA	49.37 ± 2.65 aA	51.37 ± 0.84 aA

Data are expressed as mean ± standard deviations (n=3). Lowercase letters designate significant differences (in columns) among treatments at each time point (1-way ANOVA). Uppercase letters designate significant differences (in rows) within treatments over time (1-way ANOVA).

The a* value indicates the redness or greenness of the sample, with negative values being green and positive values being red. The red color in seafood is often due to carotenoid pigments which are particularly prevalent in products like salmon and lobster. The a* values for this study ranged between 4.12 and 8.16 (Table 2.9). Russo et al. (2023) reported that the a* values for mussels at 72°C and 80°C processing temperatures ranged from -2.0 and -1.5, indicating more of green color than red. One factor could be that different species of mussels were used. The present study investigated blue mussels while Russo et al. (2023) used Mediterranean mussels. The color of mussel meats is partly due to their diet, and as filter feeders their environments can allow access to different diets. More information is needed on the instrumental color of blue mussels specifically.

Acid treatment and storage day had a significant effect on a* value at the model level (Table 2.1) while processing temperature did not. There was no significant difference in overall mean a* values between the 0% lactic acid treatment (5.58) and the 0.5% lactic acid treatment (5.60), however the 1% lactic acid treatment (4.91) lowered the overall a* values significantly compared to the 0% and 0.5% treatments. In contrast, Kamireddy et al. (2008) stated that the addition of citric acid and acidified sodium chlorite to trout fillets produced no significant differences in a* values over time, even when two different concentrations of acidified solution were applied. It is possible that lactic acid could have a different effect on a* value than citric acid; further research on instrumental color analysis is warranted. It is important to note that mussels obtain pigments from filter feeding and farmed trout does not contain the same pigments. By day 35, the mean overall a* values (6.08) were significantly higher than for any other storage day meaning samples displayed increased redness over time, driven primarily by the extremely high a* value (~7.10) for the day 35 control samples (Figure 2.9). There were no

significant differences seen among storage days 1 (5.01), 7 (5.07), and 14 (5.28) and no trend observed regarding the impact of storage time on a* values. As stated by Cropotova et al. (2019), some species of seafood, like mackerel, have so much natural variation in a* values that it is not possible to conclude any trends.

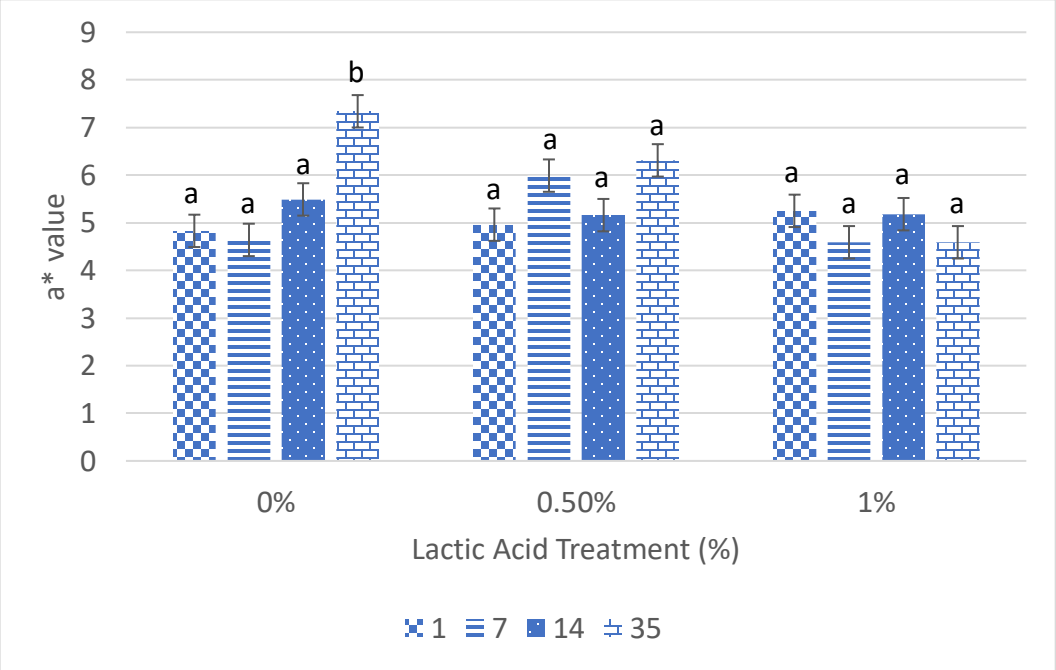

Figure 2.9. Effects of lactic acid treatment and storage time on a* values of mussel meats

Each column represents the mean ± standard error (n=6). Columns not sharing a letter within the same lactic acid treatment are significantly different ($p \leq 0.05$)

Table 2.9. Mean a* values of mussel meat treatments over 35 days

Treatment	Day 1	Day 7	Day 14	Day 35
65-0	4.58 ± 0.32 aA	4.81 ± 0.28 abA	4.83 ± 0.73 aA	8.16 ± 1.00 bB
65-0.5	4.63 ± 0.64 aA	6.36 ± 1.45 bA	4.92 ± 0.64 aA	6.46 ± 0.33 abA
65-1	5.35 ± 0.85 aA	5.06 ± 0.65 abA	4.32 ± 0.52 aA	4.73 ± 0.83 aA
75-0	5.08 ± 0.81 aA	4.47 ± 0.63 abA	6.15 ± 1.00 aA	6.52 ± 1.13 abA
75-0.5	5.28 ± 0.52 aA	5.62 ± 0.55 abA	5.40 ± 1.44 aA	6.15 ± 1.62 abA
75-1	5.10 ± 0.54 aAB	4.12 ± 0.46 aA	6.05 ± 0.53 aB	4.45 ± 0.65 aA

Data are expressed as mean ± standard deviations (n=3). Lowercase letters designate significant differences (in columns) among treatments at each time point (1-way ANOVA). Uppercase letters designate significant differences (in rows) within treatments over time (1-way ANOVA).

2.10. Pearson correlation between dependent variables of mussel meats

	pH	Liquid Loss	TVBN	TBARS	Meso-philes	Psychro-trophs	L*	a*	b*	Shear Force
pH	1									
Liquid Loss	-.027	1								
TVBN	.093	-.175	1							
TBARS	-.407**	.207*	-.339**	1						
Meso-philes	.268*	-.085	.030	-.038	1					
Psychro-trophs	.257*	.225*	.161	-.123	.174	1				
L*	-.043	-.273*	.130	-.123	-.062	-.025	1			
a*	.154	-.060	.032	-.056	.085	.090	-.217	1		
b*	-.017	-.069	-.026	-.016	.047	.092	-.163	.882**	1	
Shear Force	.142	.032	.106	-.031	.003	-.070	.033	-.260*	-.401**	1
Positive Area	.038	.297*	-.103	.084	-.040	.034	-.114	-.286*	-.459**	.780**

*Correlation is significant ($p < 0.05$). **Correlation is highly significant ($p < 0.01$).

The b* value indicates the blueness or yellowness of the sample, with negative values indicating blue and positive values associated with red coloration (Palamae et al., 2023). The b* values for this study ranged from 15.8 to 23.6 (Table 2.11). Likewise, Russo et al. (2023)

reported that b* values for mussels sous-vide cooked at 72°C and 80°C ranged from 14.7 to 21.4. Acid treatment and storage day had an overall significant effect on b* value (Table 2.1), while processing temperature did not. There was no significant difference in mean b* values between the 0% lactic acid treatment (18.89) and the 0.5% lactic acid treatment (18.56). Storage day also had a significant effect on b* value, with a mean day 35 value (20.37) significantly higher than for day 1 (16.81). Similarly, Kamireddy et al. (2008) found that over the length of 15 day storage, cooked trout fillets that were not acidified had a significant increase in b* value, almost doubling between day 0 (4.19) and day 8 (9.10). It was theorized that the yellowing, a negative attribute, over the course of storage could be due to light exposure from a walk-in cooler which can trigger lipid oxidation reactions and color change. However, the initial b* values of the mussel samples were much higher than those of the trout samples, and the magnitude of increase was not as large. When trout fillets were treated with 1000 ppm of a citric acid sodium chlorite solution the fillets had lower b* values compared to the non-acidified samples over time, but not significantly so (Kamireddy et al., 2008). In contrast, mussel meats treated with the 1% lactic acid solution did not exhibit an increase in b* values over time (Figure 2.10), indicating that acidification was able to retard yellowing in cooked mussels associated with storage. Correlations showed that there was a highly significant positive correlation between a* values and b* values ($r=.882$, $p \leq .001$) (Table 2.10), meaning that both increased together throughout chilled storage. Very notably, both a* values and b* values in the control sample increased over time. Clearly, more research is needed to understand how and why the application of organic acids impacts the instrumental color of bivalves. Typically, color change is associated with consumer acceptability in that yellowing and darker colors are considered negative attributes

regarding seafood, but mussels have a lot of variety in color due to unrelated factors (Palamae et al., 2023).

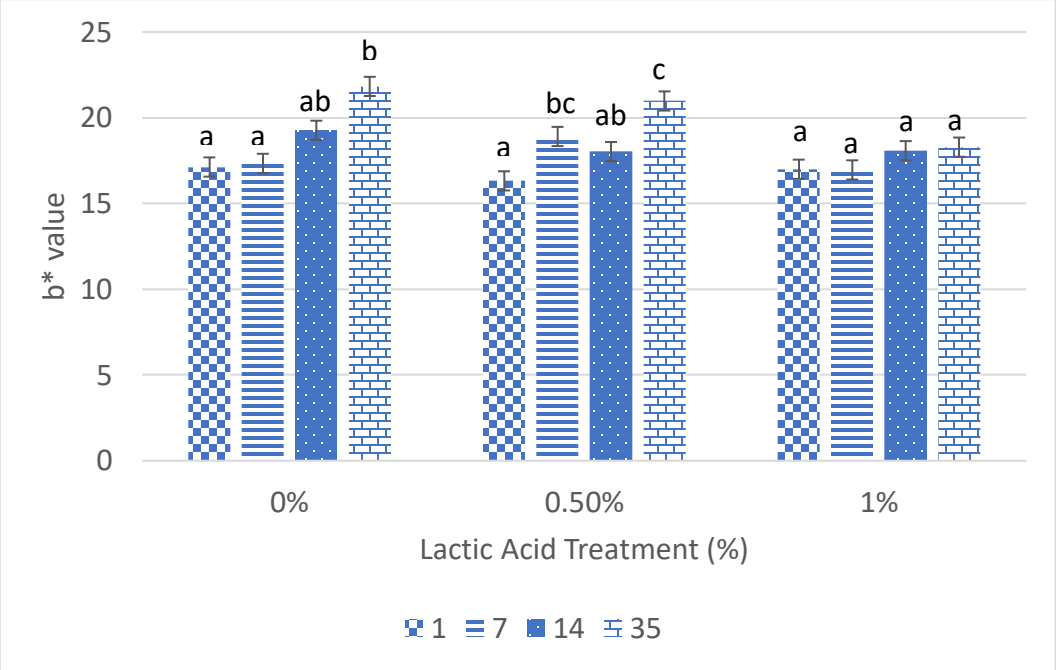

Figure 2.10. Effects of lactic acid treatment and storage time on b* values of mussel meats

Each column represents the mean ± standard error. Columns not sharing a letter in the same treatment group are significantly different ($p \leq 0.05$. The error bars represent standard error. (n=6)

Table 2.11. Mean b* values of mussel meat treatments over 35 days

Treatment	Day 1	Day 7	Day 14	Day 35
65-0	16.3 ± 0.8 aA	17.9 ± 1.7 abA	17.9 ± 1.1 aA	23.6 ± 1.0 bB
65-0.5	15.8 ± 1.3 aA	19.7 ± 2.1 bAB	18.4 ± 1.4 aAB	21.4 ± 1.3 abB
65-1	16.9 ± 1.3 aA	17.9 ± 1.0 abA	17.1 ± 1.0 aA	19.0 ± 0.4 aA
75-0	17.9 ± 2.0 aA	16.8 ± 1.1 abA	20.6 ± 2.1 aA	20.1 ± 2.6 abA
75-0.5	16.8 ± 0.5 aA	18.1 ± 0.8 abAB	17.6 ± 1.7 aAB	20.6 ± 2.1 abB
75-1	17.0 ± 1.2 aA	15.9 ± 0.7 aA	19.1 ± 0.1 aB	17.6 ± 0.6 aAB

Data are expressed as mean ± standard deviations (n=3). Lowercase letters designate significant differences (in columns) among treatments at each time point (1-way ANOVA). Uppercase letters designate significant differences (in rows) within treatments over time (1-way ANOVA).

2.3.8 Texture

Texture analysis is important because it can be an indicator of quality. Sous-vide specifically is a technology that has been proven to help seafood retain positive textural properties. This study had peak shear force values ranging between 13.15 N and 18.86 N. Russo et al. (2023) reported shear force (N) values ranging from 4.5 N to 5 N for mussels that were cooked at 72°C and 80°C. The lower values are most likely because the product was frozen and went through many freeze thaw cycles which can cause damage, softening the muscle. Acid treatment and storage day had a significant effect on peak force values at the model level (Table 2.1), however, processing temperature did not. There were no significant differences on day 35 in peak shear force values between the 0.5% (15.78 N) lactic acid treatment when compared to the 0% (15.19 N) or 1% (16.34 N) acid treatments, however, there was a significant difference though minimal in magnitude, between the 0% and 1% lactic acid treated samples. There is limited research on how acidification affects the instrumental texture (shear force) of bivalves. Tien et al. (2019) investigated how an acidified tamarind fish sauce enriched with iron and zinc affected the sensory texture of mussels. There were no significant differences in how panelists rated their liking of texture between mussels treated with the marinade and mussels not treated (Tien et al., 2019), meaning acidification did not have an effect on texture of the mussels. Kamireddy et al. (2008) also recorded that for trout fillets the amount of shear force required to slice the 1,000 ppm sodium chlorite solution acidified fish (290.33N) compared to the control (266.43 N) was significantly greater. In the current study, the slightly higher force required to shear the 1% lactic acid samples was likely due to the higher concentration lowering the pH even further and denaturing the protein networks of the mussels.

The general impact of storage time on texture was that values slightly increased on day 7 and then decreased until day 35 of storage. In contrast, Kamireddy et al. (2008) reported that shear force values of trout decreased at day 8 and then increased by day 15. These fillets were not acidified, and acidification could be one of reasons for an increase in shear force of the mussels, due to denaturation of the surface proteins. Day 1 (15.82 N) samples had peak force values that were significantly lower than day 7 (17.20 N) values. From day 7, peak positive force values significantly decreased for days 14 (15.83 N) and 35 (14.23 N).

Russo et al. (2023) sous-vide cooked mussels at four different temperatures (72°C, 80°C, 90°C, and 100°C), but similar to the results in the current study, the temperature did not have a significant effect on instrumental texture results. However, it was found that over the course of 21 days, all of the shear force values of the mussels significantly dropped due to the softening of the muscle. The texture of muscle foods softens during refrigerated storage due to water that is lost and structural collapse of the protein. Ge et al. (2016) noted that muscle softening during refrigerated storage is also related to endogenous enzyme (e.g., proteases) activities as well as microbial growth. In the current study, the peak force for the 0% and 0.5% lactic acid treatments dropped significantly during refrigerated storage, while it remained constant for the 1% acid treatment, possibly suggesting that the 1% acid treatment inhibited softening by causing denaturation of the structural proteins (or proteases), making the mussels tougher and firmer (Figure 2.11). Based on one-way ANOVA, there were no significant differences among any of the treatments within each individual storage day (Table 2.12).

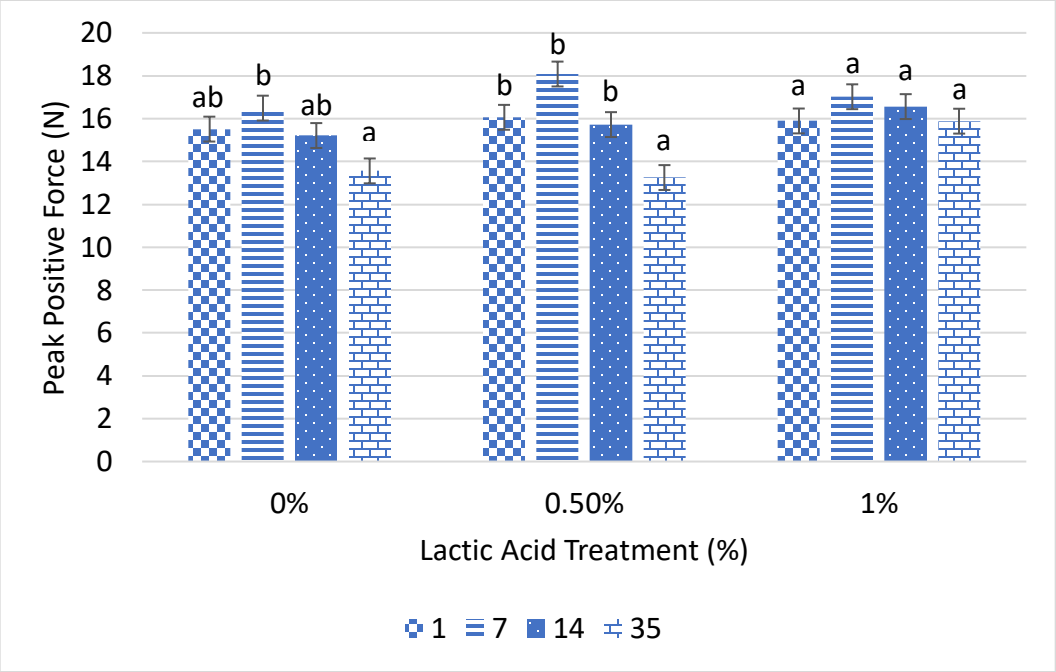

Figure 2.11. Effects of lactic acid treatment and storage time on peak force values (N) of mussel meats

Each column represents the mean ± standard error (n=6). Columns not sharing a letter in the same lactic acid treatment group are significantly different ($p \leq 0.05$).

Table 2.12. Mean peak force values (N) of mussel meat treatments over 35 days

Treatment	Day 1	Day 7	Day 14	Day 35
65-0	16.38 ± 1.23 aA	16.80 ± 1.34 aA	15.41 ± 1.27 aA	13.97 ± 1.13 aA
65-0.5	15.88 ± 0.89 aAB	17.30 ± 1.62 aB	14.89 ± 0.79 aAB	12.74 ± 1.39 aA
65-1	15.49 ± 1.16 aA	18.16 ± 1.78 aA	15.12 ± 0.94 aA	15.21 ± 1.63 aA
75-0	14.64 ± 1.06 aA	16.17 ± 1.14 aA	15.01 ± 2.12 aA	13.15 ± 1.84 aA
75-0.5	16.24 ± 1.77 aAB	18.86 ± 1.42 aB	16.54 ± 0.60 aAB	13.77 ± 2.53 aA
75-1	16.30 ± 0.40 aA	15.88 ± 1.35 aA	17.99 ± 0.74 aA	16.56 ± 1.87 aA

Data are expressed as mean ± standard deviations (n=3). Lowercase letters designate significant differences (in columns) among treatments at each time point (1-way ANOVA). Uppercase letters designate significant differences (in rows) within treatments over time (1-way ANOVA).

Processing temperature, acid treatment, and storage day all had a significant effect on the area under the shear force curve (N/sec) at the model level (Table 2.1). Based on multi-way ANOVA, the area under the curve values for the 75°C treatments (17.00 N/sec) were significantly ($p \leq 0.05$) higher than for the 65°C treatments (13.70 N/sec). A larger value typically

indicates a firmer or tougher muscle sample as seen with the firmness of uncooked and cooked shrimp investigated by Martinez-Alvarez et al. (2009). The extent of difference between the two temperatures was significant, with the 65°C treatment values about 3 N/sec lower. It would be expected that muscle protein cooked at a higher temperature would require more force to shear. The main reason for this is the muscle fibers denature and aggregate in response to heat, causing water loss and shrinkage of the product, thereby toughening the muscle (Shen et al., 2022). It's difficult to compare these results with other research since few texture analysis studies on seafood have reported area under the shear force curve. Area under the curve could be measured instead of peak force if overall firmness of the product and not just the initial shear, or "bite," were being investigated. The 0.5% (15.47 N/sec) and 1% lactic acid treatments (16.48 N/sec) were not significantly different from each other. However, the 0% lactic acid treatment (14.09 N/sec) samples exhibited significantly lower area under the curve compared to the other two lactic acid treatments, similar to the results observed for peak force values.

There were no significant differences in area under the curve values among days 1, 7, or 14 of storage, however, day 35 had significantly lower values compared to all other storage days (Figure 2.12). The area under the curve values for all treatments except for 65-1, regardless of temperature or lactic acid treatment, decreased from day 14 to day 35 (Table 2.13). This indicates that muscle softening occurred during that 3 week window. The visual and tactile deterioration of mussel texture became readily apparent to the laboratory analysts upon extended weekly sampling. There was a significant positive correlation between peak force values and positive area values ($r=.780$, $p \leq .001$) (Table 2.10). This was expected because positive area under the curve is dependent on peak force values, although it also includes a component of time in the measurement. Peak shear force is usually associated with the amount of force required for a

consumer to initially bite through a sample, while the amount of total effort required to chew the sample would be better compared to the area under the curve.

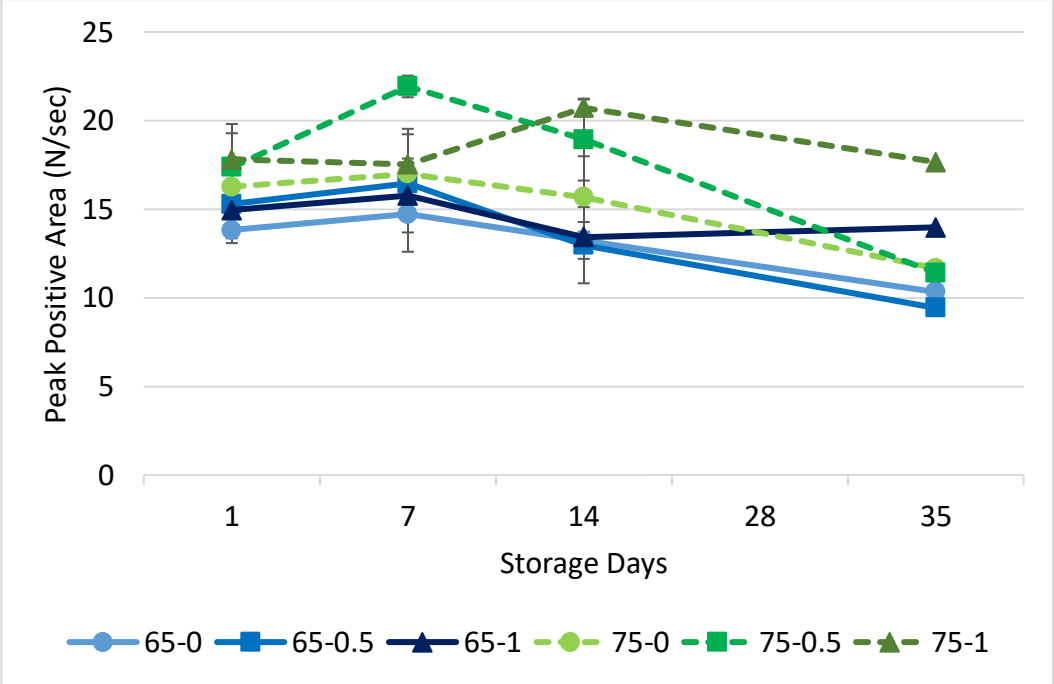

Figure 2.12. Effects of processing temperature, lactic acid treatment, and storage time on positive area values (N/sec) of mussel meats over 35 days
The error bars represent standard deviation. (n=3)

Table 2.13. Mean positive area (N/sec) of mussel meat treatments over 35 days

Treatment	Day 1	Day 7	Day 14	Day 35
65-0	13.8 ± 0.7 aAB	14.7 ± 2.1 aB	13.2 ± 1.0 aAB	10.3 ± 1.4 aA
65-0.5	15.3 ± 1.3 aB	16.5 ± 2.8 aB	13.0 ± 2.2 aAB	9.4 ± 1.2 aA
65-1	14.9 ± 1.6 aA	15.8 ± 0.9 aA	13.4 ± 0.3 aA	14.0 ± 1.5 abA
75-0	16.3 ± 0.7 aA	16.9 ± 0.9 aA	15.7 ± 2.3 abA	11.7 ± 3.6abA
75-0.5	17.4 ± 1.9 aAB	18.9 ± 1.4 aB	18.9 ± 2.3 bcB	11.4 ± 3.6 abA
75-1	17.8 ± 2.0 aA	17.5 ± 2.0 aA	20.7 ± 0.5 cA	17.7 ± 1.9 bA

Data are expressed as mean ± standard deviations (n=3). Lowercase letters designate significant differences (in columns) among treatments at each time point (1-way ANOVA). Uppercase letters designate significant differences (in rows) within treatments over time (1-way ANOVA).

2.4. Conclusions

The objective of this experiment was to evaluate the impacts of two sous-vide cooking temperatures (65°C or 75°C) and three lactic acid treatments (0%, 0.5%, or 1%) on physiochemical properties and microbial quality of mussel meats over 35 days refrigerated storage. Both sous-vide processing and acidification have the potential to retain texture and sensory properties as well as extend the shelf-life of foods. Sous-vide processing at 75°C, combined with adding 1% lactic acid solution to the bag, maintained TVBN values, a seafood spoilage indicator, at a "good" quality level over the course of the 35 refrigerated storage days. Having a cooked and vacuum-packaged sous-vide product allowed total plate counts and psychrotrophic bacteria counts to remain below the acceptable limit of 5 log CFU/g over the course of the study. Adding 1% lactic acid solution reduced the initial pH of the product from 6.5 to ~5.2, significantly reducing total plate counts, psychrotroph counts, and TVBN production compared to the control.

The target shelf life was 28 days (4 weeks), which would exceed the typical shelf-life of live mussels by 2 weeks and allow time for distribution. As measured by TVBN, the acceptable quality shelf life of both 1% lactic acid solution treatments was 35 days. The 1% lactic acid treatment also maintained a better texture for 35 days compared to the control as determined by peak shear force and area under the curve. This was very beneficial because by day 35 the control samples were notably degraded and mushy. A lower pH could potentially increase the shelf life past 35 days of storage. An acid other than lactic could potentially lower pH values if an acid with a lower pKa were chosen, however, its impact on other physicochemical quality indicators would need to be assessed. The 75°C and 1% lactic acid solution did increase liquid loss and TBARS values significantly, but the differences were minimal. The higher sous-vide

cooking temperature also imparted a lower L* value (darker color) to the mussel meats, while the 1% lactic acid solution resulted in lower a* and b* values by day 35 of refrigerated storage; however, it's unclear whether these changes in color would impact consumer acceptability of the product.

The application of sous-vide processing and acidification provides an opportunity to develop convenient and innovative seafood products with extended refrigerated shelf life. However, sensory acceptability testing should be performed to see how the acidic pH and sous-vide cooking at 75°C affect the overall liking of the product. It would also be necessary to further investigate the microbial population dynamics, specifically with regard to *C. botulinum* and anaerobic bacteria. Time-temperature indicators would have to be used because the product is ready-to-eat, and the overall pH was not low enough to ensure safety. The use of TTIs on retail packaging may be cost prohibitive on an industrial scale, so investigating the use of an acid solution that can provide a lower pH to the mussel product may be warranted.

CHAPTER 3

CONSUMER ACCEPTABILITY AND PHYSICOCHEMICAL ANALYSIS OF A MARINATED SOUS-VIDE MUSSEL MEAT PRODUCT

3.1. Introduction

Convenience is an important characteristic when it comes to what consumers value in a product (Buckley et al., 2007). Sous-vide cooking provides many benefits while also allowing consumers to purchase ready-to-eat products from retail locations. Acidification is a technique that contributes not only to the safety and shelf life of food but also to consumer enjoyment and acceptability. Acids can be applied to food through injection, soaking, or as an ingredient in a sauce or marinade (Fadiloglu & Serdaroglu, 2018). Arcales & Nacional (2018) observed that when marinating mussels directly in acid, the consumer acceptability scores were extremely low because the acid was not incorporated into the other marinade ingredients or diluted with other flavors. However, acidification reduced the microbial growth and extended the shelf life of the samples. Since sous-vide processing has also been shown to limit microbial growth, extend shelf life, and improve consumer acceptability of foods, there is potential for positive results when combining the technologies (Cosansu et al., 2013).

Fresh mussels perish quickly, which can pose a challenge when trying to make seafood more accessible for everyone, no matter their location. Russo et al. (2023) noted that treating mussels with citric acid and sous-vide cooking provided the best outcome for shelf life but not necessarily sensory acceptability compared to no acidification and conventional cooking. When choosing preservatives and other food additives, it is important to consider how best to incorporate them to extend shelf life and reduce microbial growth; however, they also need to be acceptable to consumers. Richard & Pivarnik (2020) reported that 77% of Rhode Island citizens

considered the convenience of seafood home preparation to be very important. However, there has been limited research on how home preparation, specifically reheating methods, influences the quality of the products that consumers purchase. The objective of this experiment was to evaluate the impacts of three potential home preparation methods (consuming immediately after sous-vide cooking, reheating in a bag submerged in boiling water, reheating in a saucepan) on the physiochemical and consumer acceptability of sous-vide mussel meats in an acidic marinade. It was expected that the reheated treatments would have a lower moisture content, higher shear force values, and lower protein solubility values in comparison to the sous-vide control.

3.2. Methods

3.2.1. Experimental Design Overview

This study was designed to evaluate the impacts of reheating sous-vide mussels in the bag and reheating sous-vide mussels in a pan, compared to sous-vide mussels not reheated. Approximately 30 pounds of live blue mussels (*Mytilus edulis*) were purchased from Pemaquid Mussel Farms (Bucksport, ME, USA) in June 2023. Mussels were shucked via blanching and added to sous-vide bags along with a marinade. The bags were then vacuum sealed and sous-vide cooked for 30 minutes at 75°C. After sous-vide processing the bags of mussels were immediately packed in ice and stored in a walk-in refrigerator overnight until consumer testing the following day. All mussel treatments were also evaluated for pH, moisture content, protein solubility, and shear force. The pH and titratable acidity of the marinade were measured and compared to the lactic acid solution applied in the previous study (Chapter 2).

3.2.2. Preparation of Mussels

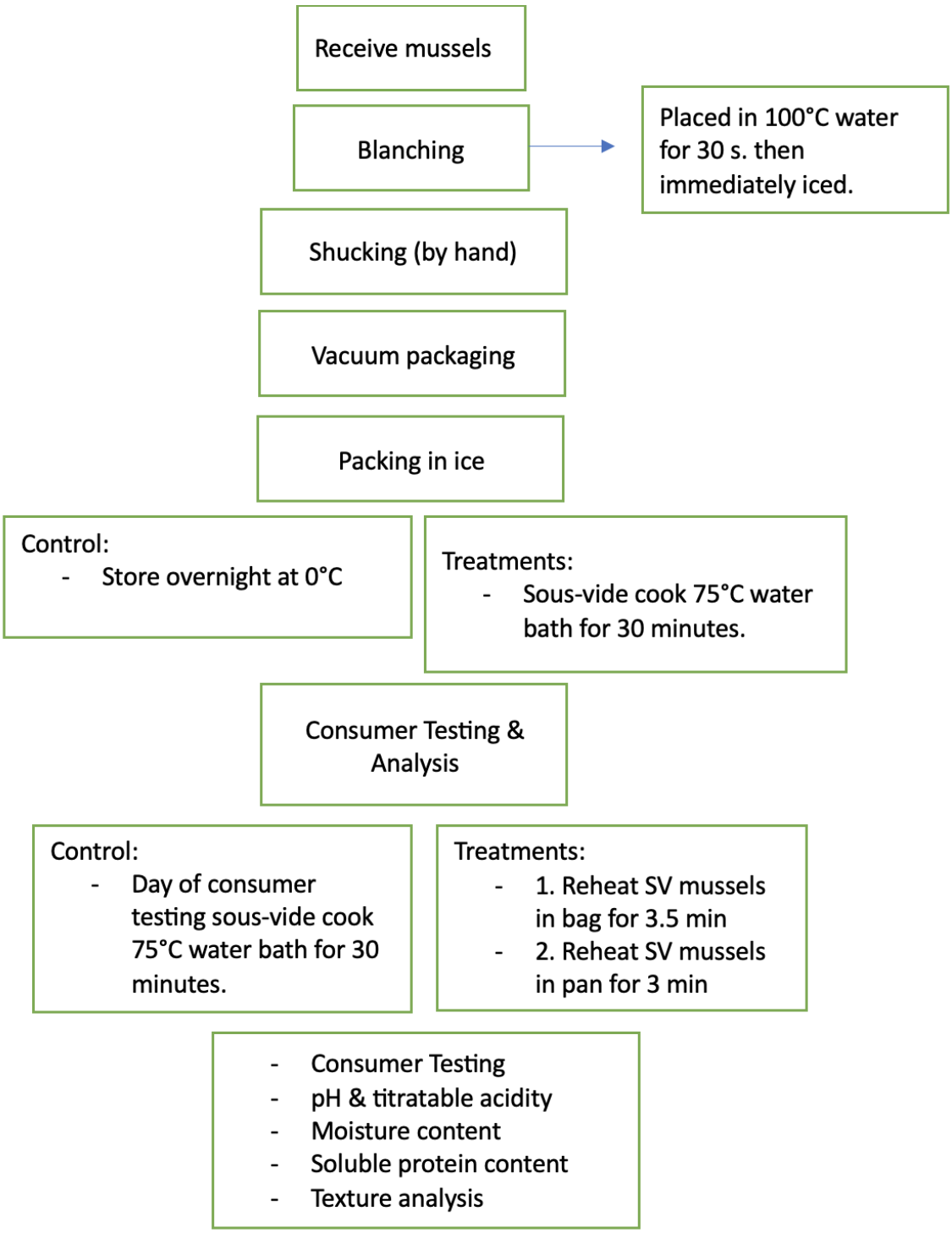

Figure 3.1. Process Flowchart

Before boiling, shell lengths of 50 live mussels were measured (mm, +/- 0.05) using a digital micrometer (Marathon, USA)**.** Live mussels were washed using a scrub brush and tap water before being immersed in boiling water using a steam kettle for 30 seconds. Four, ten pound batches were blanched one at a time followed by 2 minutes in a ~0°C ice water slurry (Figure 3.1). Based on experimentation, this method allowed the mollusk to open by releasing the adductor muscle while not noticeably cooking the meat (Arcales and Nacional, 2018). The mussels were then shucked by hand and placed in snack size Ziploc bags on ice in coolers (Coleman, USA) stored in a walk-in cooler (Matthew Highlands Pilot Plant, Orono, ME) at 3°C overnight. The following day, large sous-vide bags (8" x 10") (UltraSource, Kansas, MO, USA) were organized by treatments and type of analysis, and eight mussels were placed in each bag. Each large sous- vide bag had marinade added to it in a 1:1 (w/v) mussel meat to marinade ratio. The bags were sealed under 97% vacuum (Model UV550, Wichita, KS, USA) and labeled with the following codes: C, B, P, and R. The letters represent the control treatment (C) which was not reheated, the treatment reheated by boiling (B) in the bag, and the treatment reheated in a pan (P). The R represents raw material. There were 15 bags per treatment for sensory evaluation and 3 bags per treatment for laboratory analysis. Laboratory analysis for each of the three treatments was done in triplicate. All sous-vide bags were packed in ice and in coolers (Coleman, USA) and stored in a walk in-cooler (Matthew Highlands Pilot Plant, Orono, ME) at 3°C overnight.

3.2.3. Marinade Production

The marinade recipe was loosely based on a Spanish mussel dish (Spain on a Fork, 2019). The marinade included the following ingredients: white wine, water, garlic powder, onion powder, paprika, salt, pepper, cornstarch, and honey. All ingredients (Table 3.1) were measured (g) using a digital balance. The spices were ground using an automatic coffee and spice grinder

(Krups, USA). A medium-sized aluminum saucepan was put on medium heat on a gas stove and left for 2 minutes. Water and Pinot Grigio white wine (Clos du Bois, USA) were poured into the pan and stirred for 2-3 minutes. The ground garlic powder (McCormick), onion powder (McCormick), paprika (McCormick), salt (Morton), and pepper (McCormick) were added to the saucepan and cooked for an additional 3 minutes. The cornstarch (Argo) slurry was added with the honey (Great Value) while stirring. The marinade was then cooked until it could glaze the back of a spoon (approximately 3-4 minutes). About 6 liters of marinade was made for the experiment.

Table 3.1. Marinade Recipe

Ingredient	Weight percent (g)
White wine	36.32
Water	36.32
Garlic powder	2.32
Onion powder	2.16
Salt	2.52
Pepper	0.24
Paprika	1.68
Cornstarch	2.92
Water (for slurry)	8.76
Honey	6.76

3.2.4. Sous-Vide Cooking

Sample bags containing mussel meats were randomly cooked in polycarbonate bins (5-gal StorplusTM; Carlisle, OK) using immersion cookers (Sous-videTM Professional Creative, PolyScience, Niles,IL) with a temperature control of ± 0.05 °C. The containers were filled with warm tap water up to the maximum fill line and the water baths were set to 75°C. Once the machine was switched on, circulation began, and the direction was tested by the addition of food coloring. The temperature of the water was tested with a K-type thermocouple (RDXL4SD,

Omega, Stamford, CT) to ensure that the reading on the machine was equivalent to the actual temperature. The thermocouple was placed in the water in three random places. This process was repeated three different times, confirming that the temperature was within ± 0.05 °C of the target temperature. Once the temperature and circulation were validated, randomly selected (six large sous-vide bags) were clipped to a wire rack and fully submerged into the container. A tray was used to cover the top of the containers in order to prevent evaporation of the water.

Each bag of mussels was cooked for 30 minutes in 75°C water, ensuring that the internal temperature of the mussel meats reached 75°C for at least 10 minutes. The starting temperatures of the bags were ~3°C. Each bag contained 8 mussel meats and each water bath contained 6 bags. These conditions were validated by monitoring the internal temperature of a mussel in the center of the bag and verifying that an internal temperature of 75°C was reached for at least 10 minutes. This process was repeated three times before the study commenced to complete the validation process. The thermal processing was important to prevent the growth of *L. monocytogenes*. Following cooking, all of the bags were immediately placed in an ice water slurry (2:1 w/v) to quickly lower the temperature. It is important to lower the temperature to <3.3°C within 30 minutes in order to prevent *C. botulinum* (Center for Food Safety and Applied Nutrition, n.d.). Cooked samples were sorted, packed in ice in coolers and stored in the walk-in refrigerator (3°C) until analysis.

3.2.5. Sensory Evaluation

The goal of this study was to investigate the effects of different home preparation methods on the quality and liking of a sous vide mussel meat product. Eighty-two participants (18 and older) were recruited by email, posters (Appendix B), and word of mouth for the sensory testing to assess the acceptability of sous-vide cooked marinated mussel meats and to provide

feedback on the new product concept. People who are allergic to mussels, white wine, garlic powder, onion powder, salt, pepper, cornstarch, or honey or do not enjoy consuming mussels were requested to not participate in this study. Participants were asked to refrain from eating, drinking (except water), or smoking for a minimum of 30 minutes prior to the test. Mussels were served at 50°C with a coating of marinade. The three different sous-vide mussel treatments (not reheated, reheated by boiling the bag, and reheated in a pan) were presented to the panelists, who used a 9-point hedonic scale to assess their liking of sensory attributes, including appearance, aroma, flavor, texture, and overall liking (Pilgrim et al., 1955). Prior to tasting the product, panelists were asked demographic questions and questions about their mussel consumption habits. There were also questions after the sensory evaluation that assessed the panelists' opinions on the product concept (Figure 3.2). The questionnaire (Appendix B) was distributed, collected, and analyzed using SIMS 2000 Sensory software (Berkeley Heights, NJ).

This new product is a pouch of convenient, ready-to-eat mussel meats in a marinade that would be available in the refrigerated section of your supermarket. The mussels are already sous-vide processed (cooked under vacuum in the heat-resistant plastic pouch) to help retain their flavor and ensure meat tenderness. These shucked and cooked mussels can be consumed as is, or briefly re-heated prior to serving.

Figure 3.2. Product Concept Statement

Samples were sous-vide cooked as described in 3.2.4. All bags of mussels were stored and packed in ice in coolers until the product was ready to be prepared for consumer acceptability testing. Three mussel preparation treatments were evaluated: (1) mussels sous-vide processed the previous day, reheated for 3.5 minutes to 50°C in the bag, (2) mussels sous-vide processed the previous day, reheated for 3 minutes to 50°C in a saucepan, (3) control mussels sous-vide processed immediately prior to sensory evaluation, served at 50°C. The treatment (1)

mussels were reheated by filling a medium sized aluminum saucepan with water, turning to high heat, and boiling the water. The bag of mussels and marinade was then placed in the boiling water for 3.5 minutes. The treatment (2) mussels were reheated by pouring the contents of the bag into a medium sized aluminum pan on low heat and heating for 3 minutes. The treatment (3) mussels were sous-vide cooked at 75°C for 30 minutes directly before evaluation. All treatments were then maintained at 50°C by putting reheated sous-vide bags and pans containing reheated mussels in 50°C sous-vide baths. All treatments were held in those sous-vide baths, which were kept at 50°C using immersion circulators, for approximately 15 minutes between reheating and serving. The portions were large enough to evaluate appearance, aroma, texture, flavor, and overall liking.

Samples, which consisted of one mussel meat (~9 g) of each of the three treatments with a tablespoon of sauce, were placed in 2-oz ceramic ramekins (Figure 3.3). The samples were labeled with random codes, and there was a randomized order of presentation. Participants were seated individually in booths with fluorescent lighting at the Sensory Evaluation Center at the University of Maine. A tray containing the three different sous-vide mussel product samples labeled with 3-digit random codes was served along with spring water for palate cleansing between samples, a napkin, and a spoon. The questionnaire first asked about demographics (age, gender, ethnicity) and mussel consumption habits, followed by hedonic questions and a comment section. Each participant was instructed to evaluate the samples from left to right in the order shown on the tablet screen, to take a sip of spring water before testing each sample and rate the specific sensory attributes of the samples. A 9-point hedonic scale (from 1=“Dislike Extremely" to 9=“Like Extremely,” with 5=“Neither Like Nor Dislike”) was used to assess the acceptability of appearance, aroma, flavor, texture, and overall liking of samples. The panelists then read a

concept statement and answered questions about the overall product concept. Participants were pre-scheduled to show up at thirty-minute intervals from 11:00 am to 1:30 pm or 4:00 pm to 5:30 pm. There were 12 participants per testing interval. Participants were requested to read an informed consent form (Appendix C) before taking the test. Responses were collected anonymously. At the end of the test, participants were compensated with $5 cash for their participation. The consumer acceptability study was approved (application number, 2023-06-01; approval date, June 14th, 2023) by the University of Maine Institutional Review Board (IRB) for the Protection of Human Subjects.

Figure 3.3. Sous-vide mussel meats in marinade ready for consumer acceptance test.

3.2.6. pH

The pH meter (Orion Star A111 pH meter, Thermo Scientific, Waltham, MA, USA) and probe were calibrated (pH 4, pH 7, and pH 10 standards) based on the manufacturer's instructions. Eight mussels per treatment replicate were homogenized together using a Magic Bullet Blender (Nutribullet, CA, USA) for 30 seconds. A 1:9 ratio of homogenized mussel meat to distilled water was prepared by vortexing for 10 seconds. To evaluate the marinade a 1:9 ratio of marinade to distilled water was prepared for the analysis by vortexing for 10 seconds. Individual pH values of each sample (both mince and marinade) were determined singly per

replicate. The replicates were represented by three different sous-vide bags that were cooked at the same time. The replicate values were averaged to derive the mean pH value for each treatment.

3.2.7. Moisture Content

Eight mussels per treatment replicate were homogenized using a Magic Bullet Blender (Nutribullet, CA, USA) for 30 seconds. Moisture content values (%) were determined by drying samples (5 g) of minced mussel meat overnight in a 105 °C oven (Fisher Isotemp, Barrington, IL) (AOAC, 2005). Once samples cooled, each pan was weighed. Moisture content was determined by using the following equation:

$$\%\ \text{Moisture Content (wwb)} = \frac{\text{initital sample weight (g)} - \text{dry sample weight (g)}}{\text{initial sample weight (g)}} * 100$$

3.2.8. Texture

Texture measurements were conducted on the mussels using a calibrated texture analyzer (TA-XTi2, Texture Technologies Inc., Scarsdale, NY, USA). The thickness of each mussel (mm, +/- 0.05) was measured using a digital micrometer (Marathon, USA). The TA-44 craft blade was used for slicing the mussel meats (n=8 mussels per treatment replicate). Each mussel was placed on the platform with the flatter side down, with the blade perpendicular to the length of the mussel, and then sheared once in the middle. The texture analyzer was configured to a 100% depth and a 2 mm/s test speed. The maximum peak force (N) required to shear the mussel meat and positive area (N/sec) under the curve were both recorded by the texture analysis software (Exponent 32, version 5,0,6,0 2010, Texture Technologies Inc., Scarsdale, NY).

3.2.9. Titratable Acidity

A modified titratable acidity (TA) procedure (UC Davis, 2018) was used to estimate the titratable acidity of the marinade and to allow comparison to the lactic acid solutions prepared in

the previous study. Marinade (10 g) or lactic acid solution was placed into a beaker with 100 mL of distilled water and a magnetic stir bar. Then, sodium hydroxide (0.1 N NaOH) (Fisher Scientific, Waltham, ME) was slowly added until a pH of 8.2 was reached. The total volume (mL) of titrant added was recorded, and used to calculate titratable acidity (%) based on lactic acid equivalents:

$$\%\,TA = \frac{Normality\ of\ NaOH\ \left(\frac{mol}{L}\right) * Volume\ of\ NaOH\ (mL) * Equivalent\ weight\ of\ lactic\ acid\ \left(\frac{g}{mol}\right)}{Sample\ mass\ (g)} * 100$$

3.2.10. Protein Solubility

To analyze sarcoplasmic and total soluble protein of the mussel mince, a modified method of Li et al. (2013) was utilized. Each treatment replicate was homogenized separately using a Magic Bullet Mini Blender (Nutribullet, CA, USA) for 30 seconds. To determine sarcoplasmic protein the sample (2g) of minced mussel was homogenized using a polytron benchtop homogenizer (Weber Scientific) for 30 seconds in 20 mL ice cold 0.025 mol/L potassium phosphate buffer solution (pH~7.2). Samples were placed on a rotary shaker at 210 rpm for 12 hours at 4°C. Samples were then centrifuged for 20 minutes at 1500 x *g* at 2 °C in a bench top centrifuge (model 5430, Eppendorf, Hamburg, Germany). To determine total soluble protein the sample (1g) was homogenized in 20 mL of ice cold 1.1 mol/L potassium iodide in 0.1 mol/L phosphate buffer (pH~7.2). The same homogenization, shaking, and centrifuge processes were followed as above.

The collected supernatants were used to determine soluble protein content following the method of Lowry et al, (1951). Solution A (2% Na2CO3 anhydrous in 0.4% NaOH), Solution B (1% cupric sulfate * 5 H2O), Solution C (2.7% sodium potassium tartrate in H2O), Solution D (100 mL solution A + 1 mL solution B + 1 mL solution C), Solution E (diluted 1:1 v/v Folin-Ciocalteu phenol reagent), and bovine serum albumin (BSA) were used. A standard curve

consisting of 0, 20, 40, 80, 100, 200, and 300 µL of BSA (1 mg/mL) was constructed. Total soluble and sarcoplasmic protein concentrations were calculated based on absorbance at 700 nm using a UV spectrophotometer (DU 530, Beckman Coulter, Fullerton, CA) in comparison to the BSA standard curve. Soluble myofibrillar protein was calculated by subtracting sarcoplasmic from total soluble protein. Values were expressed as mg soluble protein/ g of mussel meat.

3.2.11. Statistical Analysis

Sensory evaluation data were extracted and analyzed using SIMS 2000. Physiochemical data input and statistical analysis were performed using SPSS 28 (International Business Machines – Statistical Package for Social Sciences) at a significance level of $p \leq 0.05$. The Shapiro-Wilk test was used to assess normality and Levene's test was run to assess homogeneity of variances. One-way ANOVA was performed to detect statistical differences for all one-level treatments. Tukey's honest significant difference (HSD) was used as the post-hoc test to separate means. Pearson's correlation was performed to evaluate correlations among variables.

3.3. Results and Discussion

3.3.1. Sensory analysis

The consumer sensory evaluation was conducted with 82 panelists: 58% were female, and 41% were male. There was a good range of ages, with 30% of participants being between the ages of 18-25, 42% being 26 -55, and 28% over 56 years of age (Table 3.2). Out of the participants, 83% described themselves as Caucasian. Out of all 82 participants, 63% said that they consume/purchase mussels once or twice a year or less. Approximately 26% of people consume mussels about 6 times per year, while 7% do not consume or purchase mussels at all. In contrast, almost 80% of the panelists said that they purchase/consume mussels from the store

once or twice a year or never. This implies that many people who consume or purchase mussels are doing so from somewhere other than the store, e.g., they are ordering them at a restaurant. Also, 7% of participants said that they do not eat mussels at all whereas 30% noted that they do not purchase mussels from the store. It is possible that people are not fully aware of mussels at the grocery store or how to cook them at home. Consumers may find a 3 pound bag of live mussels intimidating, limiting the amount of mussel purchasing that occurs from stores. Richard & Pivarnik (2020) reported that within Rhode Island, those that lived on or near the coast were significantly more likely to purchase seafood twice a week or more, most likely due to convenience and familiarity. Also, in Rhode Island, participants rated that they were a 3 out of 5 when it came to knowledge about preparing seafood but considered easy preparation to be a 4 out of 5 for importance, with 77% considering easy home preparation to be very important. In the present study, convenience with regard to home food preparation was rated as at least "important" to 66% of participants, and only 2% of participants stated that convenience is "not important" to them at all (Table 3.3). The data communicates just how important easy preparation is in getting consumers to purchase more seafood at the store level.

Table 3.2. Demographics of sensory participants

Question	Response	Percent (%)
What is your current gender identity?	Female (Cis or trans)	58
	Male (Cis or trans)	41
	Non-binary, genderqueer, or genderfluid	0
	Prefer not to reply	1
Please indicate your age.	18-25	30
	26-35	10
	36-45	15
	46-55	17
	56-65	21
	66 years or older	7
	Prefer not to answer	0
How do you describe yourself? (Please select all that apply)	Black or African American	1
	American Indian/Alaska Native	1
	Asian	10
	Caucasian (White)	83
	Native Hawaiian/Other Pacific Islander	0
	Prefer not to answer	5

n=82 participant

Table 3.3. Mussel consumption frequency and opinions of sensory participants

Question	Response	Percent (%)
Approximately how often do you purchase/consume mussels?	Every week	0
	Every two weeks	1
	Once a month	10
	Every other month	26
	Once or twice a year	56
	I do not purchase/consume mussels	7
Approximately how often do you purchase/consume mussels <u>from the store</u>?	Every week	0
	Every two weeks	0
	Once a month	7
	Every other month	15
	Once or twice a year	48
	I do not purchase/consume mussels from the store	30
How important is convenience to you as a consumer with regard to home food preparation?	Extremely important	20
	Important	46
	Somewhat important	32
	Not important	2

n=82 participants

There were no significant differences ($p < 0.05$) in liking scores among the three treatments for any of the individual sensory attributes or for overall acceptability (Table 3.4). This suggests that the method of preparation or reheating of the sous-vide mussel meat product did not affect consumer liking. It is important to note the high standard deviations for hedonic testing, meaning that there was a large variation between scores for the same treatment. Similarly to our results, reheating chicken patties via the microwave, roasting, boiling, or grilling had a negligible effect on their hedonic sensory panel scores (Ferreira et al., 2016). Li et al. (2023) reheated braised beef by microwave, steaming, boiling, and open flame, and there were no significant differences found in overall acceptability between samples reheated by open flame and by boiling. Our study was different in that the control was only cooked once and the other two samples were reheated. It was expected that reheating would have a negative effect on hedonic scores, especially texture, since reheating can dry out a product, so it was surprising when there were no significant differences among any of the treatments. The product may not have gotten tougher due to reheating to such a low temperature and for such a short time compared to conventional reheating in a 350°F oven for 20-25 minutes. Reheating at a lower temperature for a short time may not have caused the proteins to denature and aggregate, toughening the meat to the same extent as higher temperatures. The reheating method should be trialed in a home setting to take actual consumer practices into account and to ensure the quality of the product. Li et al. (2023) found that when reheating beef, the control that was not reheated received significantly higher acceptability values than the samples that were reheated by open flame or by boiling. One reason for the difference in results could be that the mussels were sous-vide processed when they were first cooked, while the beef was not. Also, beef and mussels are two completely different organisms with different properties. Mussels have more myofibrillar

protein compared to beef and less connective tissue, which could contribute to differences in sensory texture preferences (Nurdiani et al., 2020). On the 9-point hedonic scale, a score of 7 or higher is associated with highly acceptable sensory quality (Tarancon et al., 2021). Despite no significant differences in overall acceptability scores among treatments and no treatments reaching 7 or higher in that category, the flavor of the boil-in-a-bag treatment had a mean score of 7.0 $\pm$ 1.5. Though the difference was not significant, the overall acceptability of the boil-in-a-bag (6.8 $\pm$ 1.7) and reheat-in-a-pan (6.8 $\pm$ 1.5) treatments were slightly higher than the control (6.5 $\pm$ 1.6) treatment (Table 3.3). Arcales and Nacional (2018) marinated green mussels in 2% lactic acid for an overall acceptability score of (5.45 $\pm$ 2.30) and (5.65 $\pm$ 1.94) for the 2% citric acid treatment. The average hedonic score for the control, which was not marinated and just steamed for 18 minutes, was 8.26 $\pm$ 0.95 on a 10 point scale, according to an acceptability panel consisting of 10 panelists. In the current study, all the overall acceptability scores were slightly below the target 7, which was unsurprising, given that the mussels were served at 50°C, which may not be the preferred temperature for all participants. Also, the sensory evaluation center may not have provided participants with the ideal environment in which to enjoy a meal. King et al (2004) reported that when people were served the same meal in different environments (traditional room, social room, restaurant), the hedonic scores differentiated. Specifically, the hedonic scores were significantly higher when eating at a restaurant as opposed to the traditional room, which was the environment most similar to the sensory evaluation center.

Table 3.4. Mean scores for consumer acceptance of sous-vide cooked mussel meats in a marinade on a 9-point hedonic scale

Attribute	Control	Boil-Bag	Pan	*P* value
Appearance	6.5 ± 1.6	6.8 ± 1.5	6.9 ± 1.2	0.16
Aroma	6.4 ± 1.5	6.8 ± 1.3	6.6 ± 1.5	0.11
Texture	6.4 ± 1.8	6.6 ± 1.7	6.6 ± 1.6	0.54
Flavor	6.6 ± 1.7	7.0 ± 1.5	6.9 ± 1.5	0.08
Overall acceptability	6.5 ± 1.6	6.8 ± 1.7	6.8 ± 1.5	0.19

Each value is the mean ± standard deviation (n = 82). There were no statistically significant differences among treatments ($p > 0.05$). 1= dislike extremely and 9= like extremely.

With regard to qualitative data, flavor received the highest number of comments, closely followed by texture (Appendix E). For the control treatment flavor, approximately 36% of comments were positive, while 64% of people stated that the mussel meats were too salty or too bland. Out of all of the comments pertaining to flavor of the boil-in-a-bag sample, more than half were positive. For the reheat-in-a-pan treatment flavor comments, 70% of the comments were positive, and most of the negative comments were focused on the product being too sweet or too savory, overpowering the mussel flavor. With regard to texture, about 65% of the comments for the control treatment were negative, indicating that over half of the participants were not pleased with the texture of the non-reheated product, which was unexpected. Most of the negative comments for the control treatment noted that the mussel meats were either too mushy or too chewy. People seem to have a variety of preferences when it comes to the texture of mussels. The texture of the boil-in-a-bag samples had about half positive and half negative comments. Many people commented that the mussel meats were both mushy and chewy, indicating polarized opinions. Out of all of the reheat-in-a-pan texture comments, 70% were positive.

There were few comments on the aroma or appearance of the samples. The reheat-in-a-pan sample received the most positive comments about flavor (70%), while the boil-in-a-bag sample received the most positive comments about texture (52%). It would be difficult to improve the texture of the mussel meats because of such differing opinions and expectations about mussel texture. Rather than focusing on texture it could be useful to reformulate the flavor of the marinade since many of the comments noted that its flavor overpowered the natural mussel flavor. Surprisingly, the reported frequency of mussel consumption significantly influenced how much participants liked the appearance of the products (Figure 3.4), with those who consume mussels six or more times per year rating product appearance as more acceptable than those who consume mussels fewer times per year. This could be because people who do not consume mussels often may be less familiar with the appearance of shucked meats since most often mussels are served in the shell. Significant effects of mussel consumption frequency on hedonic liking scores were not noted for aroma, texture, flavor, or overall acceptability.

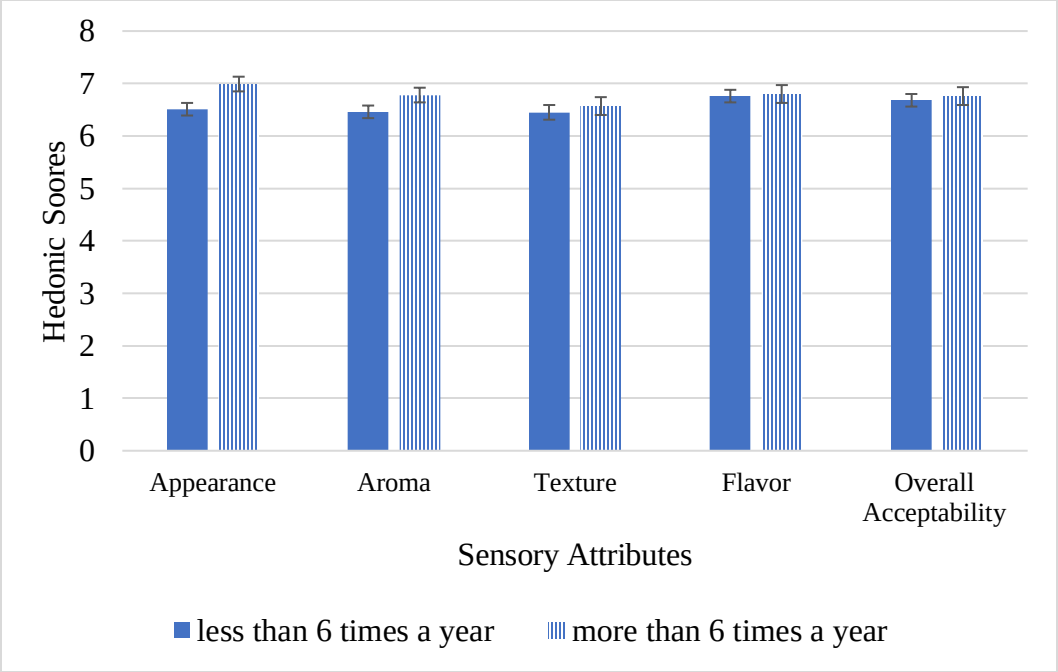

Figure 3.4. Comparison of hedonic scores based on frequency of mussel consumption

(n=153 for less than 6 times a year and n=93 for more than 6 times a year). Each bar represents the mean values ± standard deviation. Treatments not sharing a letter between pairs of columns are significantly different ($p \leq 0.05$) based on an independent t-test.

Correlations among sensory attribute scores revealed that overall acceptability had strong, significant ($p < 0.01$) positive correlations with hedonic scores for both flavor (r = 0.90) and texture (r = 0.75) (Table 3.5). Flavor was significantly ($p < 0.01$) and moderately correlated with texture (r = 0.64), but there was little correlation between overall acceptability and appearance as well as overall acceptability and aroma. The significant correlations indicate that both the texture and flavor attributes of the marinated mussel meats strongly influenced the overall acceptability of the samples. Participants were less concerned with the appearance and aroma of the product. These findings agree with the qualitative comments reported above. Similarly, Humaid (2020) found that when lobsters were sous-vide cooked and HPP processed, their sensory texture and flavor attributes were also highly positively correlated with overall

consumer liking compared to aroma and color attributes. It should also be noted that there were no significant correlations between sensory texture scores and instrumental texture results, with shear force and sensory texture scores having a p-value of 0.102 and r = 0.579, emphasizing the importance of consumer testing.

Table 3.5. Pearson correlation between sensory attributes

	Appearance	**Aroma**	**Texture**	**Flavor**	**Overall acceptability**
Appearance	1				
Aroma	.572 **	1			
Texture	.397 **	.445 **	1		
Flavor	.367 **	.545 **	.643 **	1	
Overall acceptability	.430 **	.551 **	.749 **	.900 **	1

*Correlation is significant ($p < 0.05$). **Correlation is highly significant ($p < 0.01$).

Table 3.6. Pearson correlation between dependent variables of sous-vide mussel meats in a marinade

	Overall acceptability	**pH**	**Moisture content**	**Shear force**	**Positive area**	**Myofibrillar**	**Total soluble**
Overall acceptability	1						
pH	.468	1					
Moisture content	-.517	.080	1				
Shear force	.372	.067	-.361	1			
Positive area	.153	-.120	-.213	.888 **	1		
Myofibrillar	.160	-.617	-.519	.295	.392	1	
Total soluble	.106	-.605	-.396	.276	.408	.981 **	1

*Correlation is significant ($p < 0.05$). **Correlation is highly significant ($p < 0.01$).

Following hedonic testing, the participants were asked to read a product concept statement and answer questions about it. After reading the product concept about the sous-vide

mussel meats in a marinade, 83% of participants answered that they would be at least "somewhat likely" to purchase the product, with 24% specifying that they would be "extremely likely" to purchase (Table 3.7). Only 17% of participants appeared to be indifferent or unlikely to purchase the mussel product. When asked about the price that they would pay for a 4oz bag of the product, the most common responses were $5.99 (29%) and $6.99 (24%), followed by $7.99 (18%) and $8.99 (15%). Only 4% of participants indicated that they would pay more than $8.99 for a 4 oz package of ready-to-eat sous-vide mussel meats in a marinade. A 1-pound mesh bag of in-shell mussels from Hannaford currently costs ~$4.99, and in-shell mussels are about 25% meat by weight. Therefore, in one pound of in-shell mussels, there are approximately 4 ounces of mussel meat. However, there is labor, time, and waste associated with consuming in-shell mussels that can be eliminated with an RTE mussel meat product. Of the participants in the study, more than 60% said they would pay at least $6.99, and 37% said they would pay at least $7.99 for this convenient, value-added product. Those price points would be equivalent to a 40% and a 60% markup, respectively, to cover the costs of the sous-vide equipment, labor, and materials, among other things. Frozen mussel meat products appear to sell for between $10-15 for 8 ounces, with the higher priced products usually containing a sauce or additional ingredients (PanaPesca, USA; Patagonia, USA). About half (46%) of the participants, when asked about their preferred method of home preparation, said that they would reheat the mussel meats and marinade in a saucepan. The rest of the participants were split between reheating by boiling in the vacuum-packaged bag (28%) and not reheating the product at all (20%).

Table 3.7. Participant feedback on product concept*

Question	Response	Percent (%)
Based on the concept statement, how likely would you be to purchase this product?	Extremely likely	24
	Somewhat likely	59
	Neither likely nor unlikely	6
	Somewhat unlikely	9
	Extremely unlikely	2
How much would you be willing to pay for 1/4 pound of ready-to-eat, shucked mussel meats in a marinade?	$4.99	10
	$5.99	29
	$6.99	24
	$7.99	18
	$8.99	15
	More than $8.99	4
If you purchased these mussels at the store, what preparation method would you be likely to use at home?	Chilled without reheating (e.g., on crackers, salad topping, etc.)	20
	Reheat in heat-resistant pouch (in boiling water)	28
	Reheat in saucepan	46
	Another method	6

n=82 participants. *Concept statement is provided on page 74

Panelists were asked at the end of testing to describe anything that they would consider unappealing about the product concept. Approximately 20 comments were received that mainly addressed texture, marinade flavor, and packaging concerns (Table 3.8). The concerns about texture seemed to align with the hedonic samples comments, with most people stating the mussels would be too mushy. A few participants did not like the flavor of the marinade and would have preferred a different flavor profile, but the comments were mixed on whether the marinade was too bland or too flavorful. A few comments were also made about the product packaging, particularly that it might look unappealing to consumers shopping in retail locations. However, the appearance of the package could easily be improved by including a small see-through window in an opaque pouch and placing the pouch in attractive secondary packaging.

Table 3.8. Representative consumer critiques of the mussel meats in marinade

Theme	Comments
Texture	• "Worried the mussels would get too mushy and fall apart, especially if I am going to mix them into a pasta dish" • "The `bellies` were mushy, and wouldn't purchase them a second time." • "I think just finding a way to make sure the texture stays good when reheating." • "Generally gummy and sort of...smears in your mouth" • "The texture felt a bit mushy for my preference."
Marinade Flavor	• "I just cook mussels in a less intensely/heavily spiced/herbed broth." • "I would like other flavor options." • "First two were too bland, but I could fix that at home with additional seasonings"
Packaging	• "Plastic pouch would be scary for me. Would reheating in a pot on the stove change the texture/flavor?" • "Depending on how it looked in the pouch I might be worried about the consistency of the product as I could imagine it resembling something like pickled eggs or other less pleasant food." • "I think the general public would not like the idea of shucked mussels in a bag sitting on the shelf. The visual just doesn`t look good. They would have to be put in a box to catch the consumer`s eye"

3.3.2. pH

Acidity is important when it comes to acceptability testing because it can affect consumer liking of food products. Consumers tend not to enjoy savory foods when they are too acidic. For example, Arcales & Nacional (2018) reported that when acidifying steamed mussels with 2% lactic acid or citric acid, the overall hedonic values dropped by almost half compared to the unacidified control. In the present study it was not expected that reheating would impact mussel pH, rather, pH was measured to compare the mussel pH values of the previous chapter, and the marinade pH was measured to compare to the pH of the lactic acid solutions used in the previous chapter. Treatment did not have a significant effect on the pH value of the mussel meats (Figure

3.5). The average pH value across all treatments was 5.97 $\pm$ 0.04. Minimal research has been conducted on how reheating seafood affects its quality, but when Zhan et al. (2022) sous-vide cooked scallops at 70°C and 75°C, the 70°C cooked scallops had a significantly higher initial pH compared to the control and 75°C processed samples. However, in the present study all of the samples were sous-vide cooked at the same temperature (75°C) just reheated with differing methods. Also, all the samples in our study were treated with a marinade that was inherently acidic (pH = 4.28), keeping the overall pH values of the different mussel meat samples consistent (~pH 6) In comparison, mussels treated with the 1% lactic acid solution in the previous study had an initial pH of ~5.2, while the initial control (non acidified) mussel meats had an initial pH of ~6.5, higher than the marinated mussels. The 0.5% lactic acid solution acidified mussels from the previous chapter had initial pH values of around 5.9. Also, the mean titratable acidity of the marinade was found to be around 1.24, while the titratable acidity of the 1% lactic acid solution was 1.36. Thus, the marinade appears to have been less acidic than the 1% lactic acid treatment but more acidic than the control. The pH of the marinade was significantly higher most likely due to the acids present in its ingredients and its dilution with water, starch, sugars, and spices. In the first study, the lactic acid was not combined with any other ingredients that might interfere with its properties. Han et al. (2022) reported that the organic acids found in white wine in the highest concentration are malic and pyruvic. Malic acid has a pKa of around 3.4, while pyruvic acid has a pKa of around 2.45. Both of these acids have different pKa values and properties compared to the lactic acid (pKa ~3.8) which was applied in the first study. The pH of a vacuum-sealed product must be below a pH of 4.6 to be safe and prevent the production of *Clostridium botulinum* toxin. It would be beneficial to investigate how to lower the pH of the product even further, possibly by using higher concentrations of acids or acids with lower pKa values.

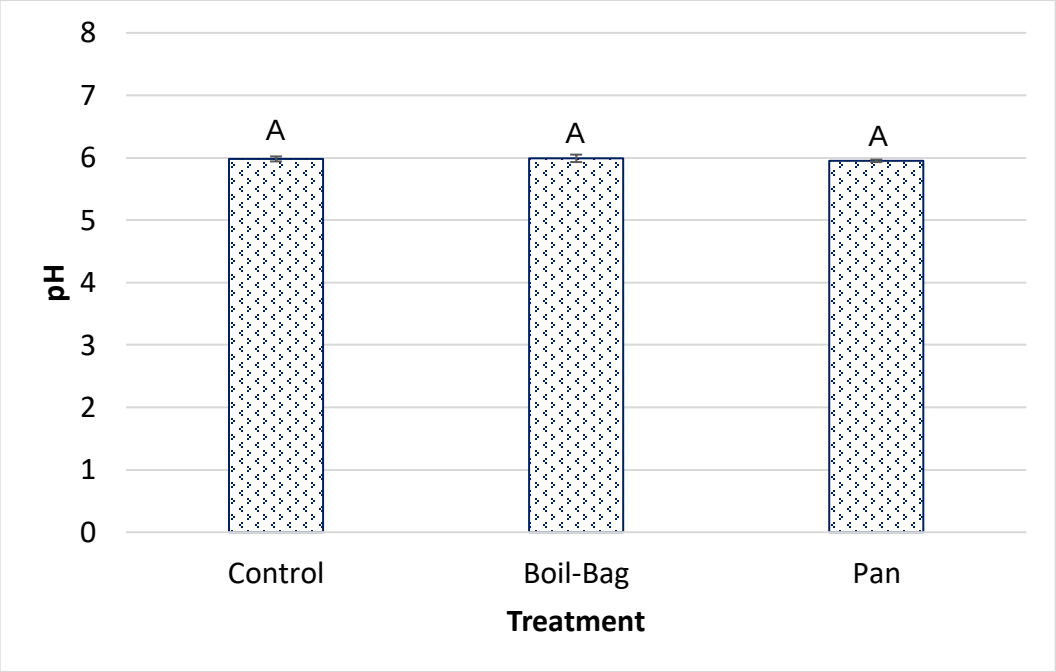

Figure 3.5. pH of mussel meat by treatment
Each bar represents the mean values ± standard deviation (n=3). Treatments not sharing a letter are significantly different ($p \leq 0.05$) based on one-way ANOVA followed by Tukey's HSD post hoc test.

3.3.3. Moisture Content

The moisture content of foods is affected by production, packaging, and storage methods, among other factors, and can, in turn, influence the texture and shelf life of foods (Vera Zambrano et al., 2019). The moisture content of the control treatment (~77.5%) was significantly lower than the moisture content of the boil-in-a-bag treatment (~79.1%) (Figure 3.6). This was unexpected because we would not expect the moisture content to increase after reheating. It would be expected that proteins would denature, and the product would begin to dry out after being exposed to further heat. The reheat-in-a-pan treatment should have had the lowest moisture content values because the product was in direct contact with a heating source. Neither the control nor boil-in-a-bag treatments were significantly different from the reheat-in-a-pan treatment. One reason for the limited significant differences could be because the reheating time

was short (3-3.5 minutes) and at low heat, limiting moisture loss. Castrillon et al. (1997) found that when sardines were reheated, the moisture content was significantly less when the fillets were reheated with a microwave for 6 minutes at 80% capacity (28.2%) compared to when the sardines were reheated in a conventional oven for 19 minutes at 70C (41.1%). There was a significant ($p < 0.01$) negative moderate correlation between moisture content and hedonic flavor (r=-.67). This means that as the moisture content increased, the flavor acceptability scores decreased. Based on the comments and feedback, a higher moisture content in the samples reheated by boiling in a bag could have been associated with the bland or "watered down" flavor that several people found unappealing.

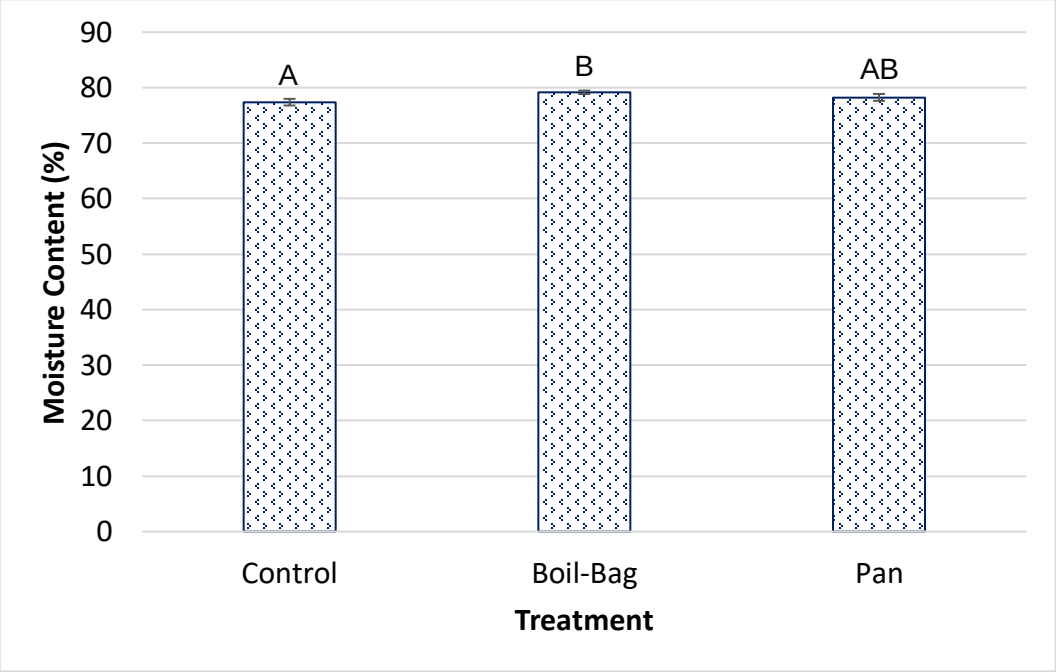

Figure 3.6. Moisture content (%) of mussel meat by treatment
Each bar represents the mean values ± standard deviation (n=3). Treatments not sharing an uppercase letter are significantly different ($p \leq 0.05$) based on one-way ANOVA followed by Tukey's HSD post hoc test.

3.3.4. Texture

Peak shear force is an instrumental measure of the amount of force required to slice through the product. This type of analysis can be relevant to understanding consumer sensory scores related to texture attributes. Treatment did not have a significant effect on the shear force value (N) or area under the curve (N/sec) of the mussel meats (Figure 3.7). There were no significant differences among the control sample, boil-in-a-bag sample, or reheat-in-a-pan sample, which had values ranging from 20.5 N to 21.5 N. This was not expected because, typically, meat that is cooked multiple times would be tougher than meat that cooked only once. We would have expected the reheat-in-a-pan sample to have the highest shear force values because the mussels were in direct contact with the heating source. Brookmire et al. (2013) reported that Atlantic salmon that were pan-fried at 63°C (25.71 N) had significantly higher shear force values than salmon that were steamed (18.79 N). However, as noted previously, in the present study all of our samples were cooked at the same temperature (75°C) they were just reheated using different methods. The reheating methods also used lower temperatures compared to the kind of heat that is produced while pan-frying, usually around 250°C (Brookmire et al., 2013). However, similar to the current study, when mussels were sous-vide cooked at 72°C, 80°C, 90°C, or 100°C, the shear force values were not significantly different immediately post-processing (Russo et al., 2023). The authors speculated that this outcome could have been due to the shear force increases proportionally with temperature to a maximum of 70°C (Russo et al., 2023). Considering the fact that they chose temperatures ranging from 72-100°C, this could make sense and may be a contributing factor to the lack of differences among the present treatments. A lack of differences in texture among reheated samples could be ideal for consumers

since they can choose to prepare the product however, they want, and the quality should not be affected. This could open doors to innovation and different ways to use the mussel product.

The home preparation method also did not have a significant effect on the texture of the marinated mussel treatments as measured by the area under the force curve (N/sec) (Figure 3.8). The positive area values ranged from ~20.2 N/sec to ~23.1 N/sec. This was again unexpected because it was anticipated that the reheat-in-a-pan samples would have significantly higher shear values and the reheated samples would have higher shear values than the control. As previously stated, the main contributor to this not happening was most likely the lack of intensity in the reheating process. Wu et al. (2022) reported that when scallops were sous-vide cooked at both 60°C and 70°C for varying amounts of time, the scallops cooked at 70°C and for longer had overall higher shear force values. This was probably due to the proteins denaturing more extensively at a higher temperature and longer time, then aggregating and becoming tougher. Brookmire et al. (2013) measured (N/sec) also known as texture firmness of shrimp that were boiled and baked. The boiled shrimp had significantly lower firmness values compared to the baked shrimp samples, confirming that direct contact with water has the potential to impact texture values. As observed in the first study (Chapter 2), there was a significant ($p < 0.01$) positive strong correlation between maximum shear force (N) and positive area under the curve (N/sec) (r=.89). This means that as the shear force values increased, so did the area under the curve or work values and vice versa. It should also be noted that there was a lack of correlation between instrumental texture and moisture content meaning that differences in moisture content had no effect on shear force (N) or area under the curve values (N/sec).

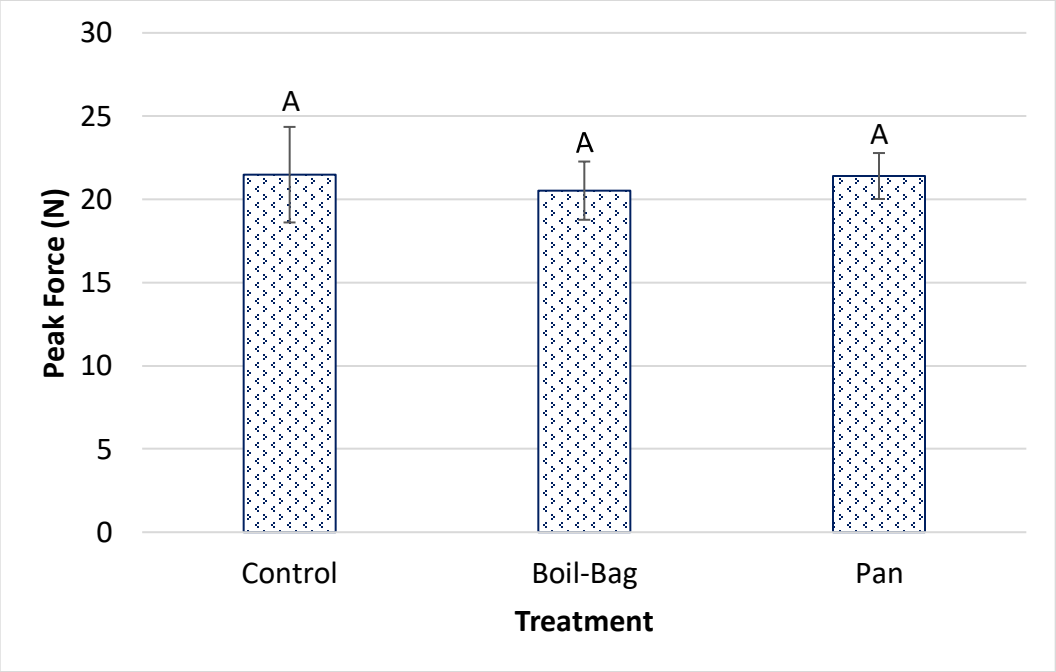

Figure 3.7. Peak force (N) of mussel meat by treatment
Each bar represents the mean values ± standard deviation (n=3). Treatments not sharing an uppercase letter are significantly different ($p \leq 0.05$) based on one-way ANOVA followed by Tukey's HSD post hoc test.

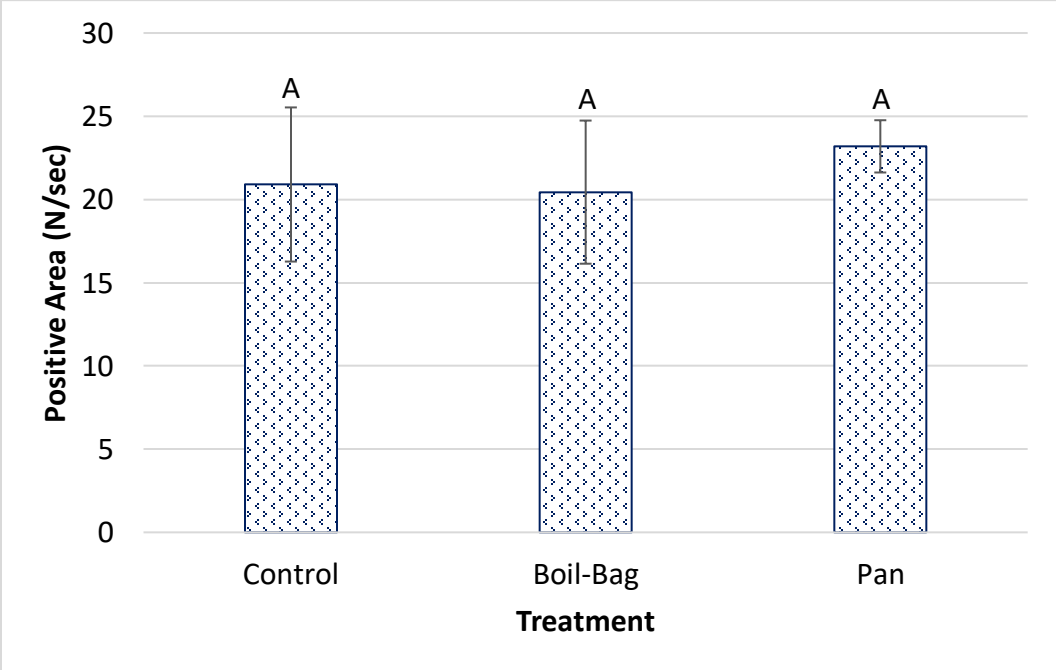

Figure 3.8. Positive area under the curve (N/sec) of mussel meat by treatment
Each bar represents the mean values ± standard deviation (n=3). Treatments not sharing an uppercase letter are significantly different ($p \leq 0.05$) based on one-way ANOVA followed by Tukey's HSD post hoc test.

3.3.5. Protein Solubility

Protein solubility was quantified as a way to indirectly measure the impacts of cooking method on muscle quality. As proteins are denatured and aggregated by thermal processing, their solubility typically decreases (Kong et al., 2008). Myofibrillar proteins are salt soluble and comprise approximately 55-60% of total muscle proteins in land animals, while seafoods have more due to less need for connective tissue (Nurdiani et al., 2020). The proteins in seafood are also generally less thermally stable. The myofibrillar proteins contribute a lot to water-holding capacity and gel-forming ability of foods (Yang et al., 2021), as well as the texture and yield of meat products. The mussel meats reheated in a pan had significantly higher levels of soluble myofibrillar protein (~35 mg /g meat) than the product that was reheated in the bag in boiling water (~29 mg /g meat) (Figure 3.9). This may have been due to the mussels' exposure to lower heat on the stove for 3 minutes compared to boiling water (~100 °C) for 3.5 minutes, although both mussel treatments were heated to achieve the same internal temperature. The temperature has a significant impact on the denaturation and solubility of muscle proteins (Kong et al., 2018), which is why more than twice the amount of myofibrillar protein was extracted from the raw product as compared to the cooked mussel meats. When abalone was boiled for 6, 30, and 240 min at 80°C, the 240-minute treatment had significantly lower myofibrillar protein values, less than half the amount of the 6-minute boiled treatment (Yu et al., 2022). The prolonged heat treatment most likely caused more denaturation of the 240 minutes samples, and exposure of hydrophobic amino acid residues. In the current study, the control treatment did not have significantly higher values when compared to either of the reheating options, which was unexpected. One explanation is that even though the control was not reheated at all and the other samples where they were all initially processed by being sous-vide cooked at 75°C for 30

minutes. In the other study, the abalone were all initially cooked at different times, which was not the case in the present study. The initial 30 min cook time most likely had more impact on denaturation than the brief reheating time.

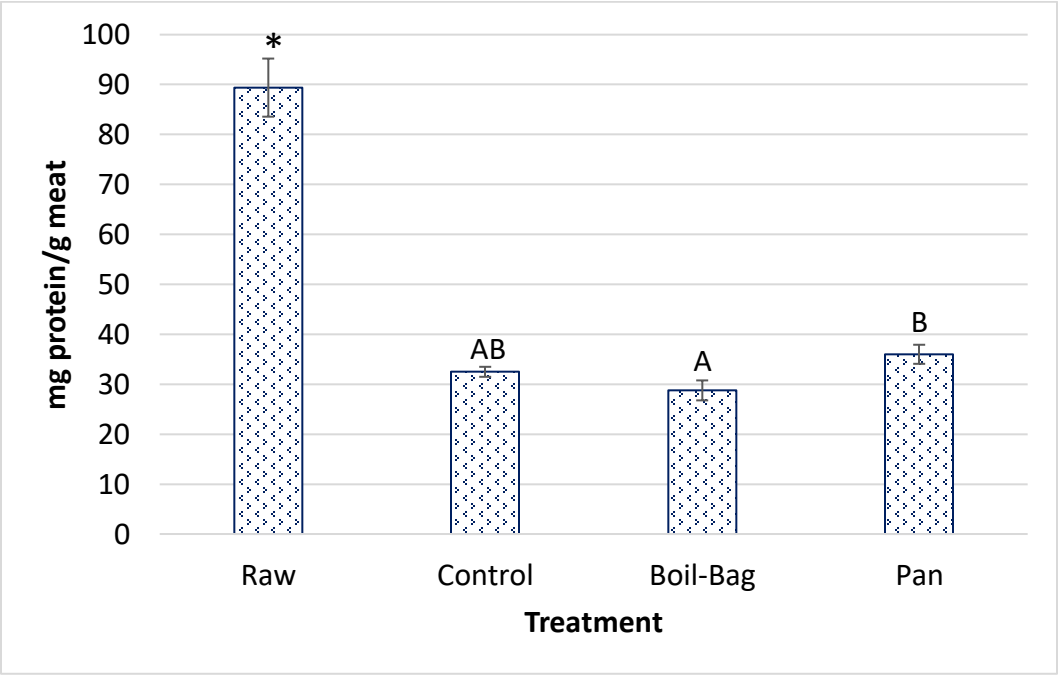

Figure 3.9. Myofibrillar protein (mg / g meat) of mussel meat by treatment
Each bar represents the mean values ± standard deviation (n=3). Treatments not sharing an uppercase letter are significantly different ($p \leq 0.05$) based on one-way ANOVA followed by Tukey's HSD post hoc test.
*Soluble myofibrillar protein of the raw sample was not statistically compared to cooked treatments.

Sarcoplasmic proteins are mostly made up of water-soluble enzymes that participate in cellular metabolism (Warren et al., 2020), and tend to have a lower impact on cooked muscle quality than myofibrillar proteins (Hemung & Chin, 2015). There were no significant differences in sarcoplasmic protein content (data not shown) with regard to the home preparation method. However, the reheat-in-a-pan and boil-in-a-bag treatments had significantly different total soluble protein values from each other, with reheating in a pan resulting in higher levels (Figure 3.10). Much like with the myofibrillar protein results, the treatment reheated in a saucepan had

higher total soluble protein values and appeared to have denatured less than that treatment that was reheated with boiling water. This was confirmed by the significant ($p < 0.01$) high positive correlation between myofibrillar soluble protein and total soluble protein ($r = 0.98$), indicating that almost all the soluble protein present was myofibrillar and not sarcoplasmic protein. For total soluble protein, the sample that was reheated in a pan also had significantly higher values than the control treatment. Typically, it would be expected that the samples that were only heat treated once would be less denatured than samples that were heated more than once and in direct contact with a hot pan. One reason for the unexpected results could be that the control samples were stored in marinade under vacuum packaging overnight before being sous-vide cooked, while the other samples were sous-vide cooked immediately after packaging then stored overnight before reheating. When raw surimi product was treated with 0, 1, 2, 3, or 4 g/ kg citric acid, the acidification decreased the amount of total salt soluble protein extracted (Gu et al., 2021). The marinade that the mussels were in had a pH of 4.28, so it is likely that the acidic environment denatured the raw mussel meats overnight before they were sous-vide cooked, similar to the application of acid marination to denature and soften the texture of tough cuts of meat before cooking (Christensen et al., 2009).

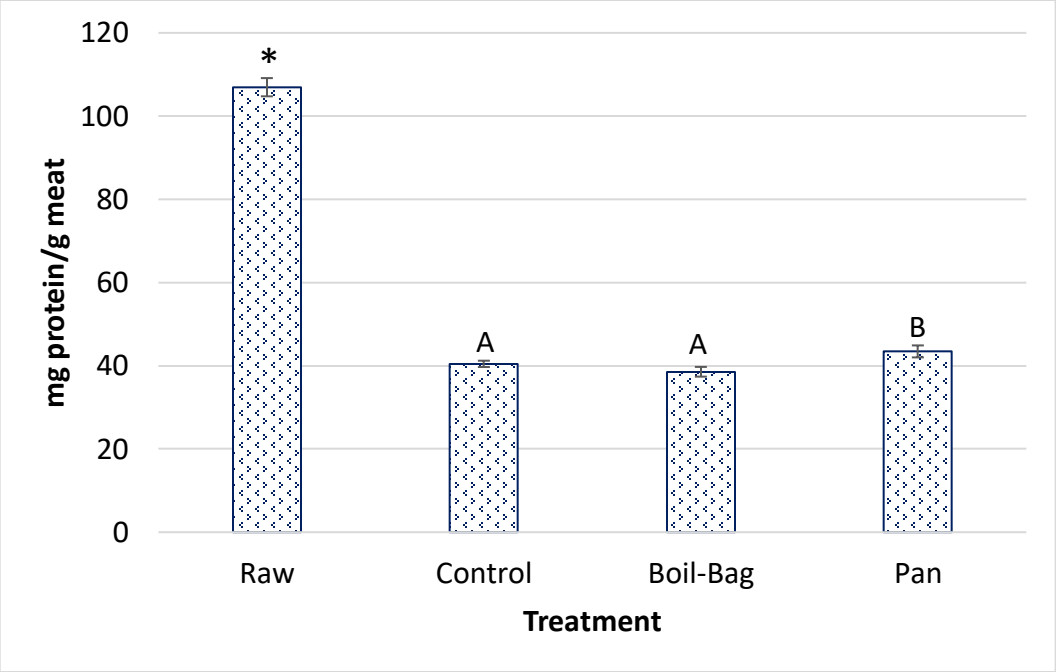

Figure 3.10. Total soluble protein (mg protein/ g meat) of mussel meat by treatment

Each bar represents the mean values ± standard deviation (n=3). Treatments not sharing an uppercase letter are significantly different ($p \leq 0.05$) based on one-way ANOVA followed by Tukey's HSD post hoc test.

*Total soluble protein of the raw sample was not statistically compared to cooked treatments.

3.4. Conclusions

There is sparse published research on how reheating seafood proteins affects their quality, especially when it comes to ready-to-eat (or heat-n-eat) sous-vide products. For sous-vide cooked and marinated mussel meats, the method of home preparation (reheating) did not have a significant effect on the sensory attributes of appearance, aroma, texture, flavor, or overall acceptability. In addition, reheating did not significantly impact the sensory acceptability of the mussels compared to the sous-vide control. Overall acceptability was highly positively correlated with both texture and flavor, indicating that those are the most important sensory attributes of the product for consumers. The most numerous complaints and concerns were related to mussel

texture, marinade flavor, and packaging. Mussels seemed to be a polarizing protein source, meaning that participants either liked them tender or chewy, depending on the individual consumer's preference. Another concern revolved around the appearance of the mussel meats in the packaging.

A majority (63%) of the participants reported purchasing or consuming mussels less than three times per year, showing that this seafood protein source is somewhat under-consumed. Given that 30% of participants said they do not purchase mussels in the store and that convenience in home food preparation was characterized as important to over half of the participants, the availability of ready-to-eat mussel products may promote their consumption. Participants appeared to be receptive to the product concept, with over 80% stating they would be likely to purchase and 61% saying they would pay $6.99 or more for a 4oz package.

Moisture content, myofibrillar protein, and total soluble protein were impacted by the three preparation methods (sous-vide control, boil-in-a-bag reheating, and reheating in a pan), while pH, instrumental texture measurements, and sarcoplasmic soluble protein were not. There was also no significant correlation between instrumental texture and hedonic texture scores, showing the importance of consumer testing. The lack of differences noted between home preparation methods gives a lot of flexibility to the consumer while still allowing them to enjoy the best quality product. Future consumer research should investigate marinade preferences and potentially higher cooking temperatures to firm up the mussel meats, which may be more in line with what consumers expect.

CHAPTER 4

CONCLUSIONS

Overall, these two studies show that sous-vide processing and acidification are promising technologies in the development of value-added seafood products, specifically mussels. Sous-vide cooking has the ability to extend the shelf life of minimally processed and ready-to-eat foods. The technology is particularly good for cooking meats due to the precise temperature control, which results in an ideal texture for consumers. This thermal treatment allows texture and flavors to be retained in food products, which are two sensory attributes of importance for consumers. Acidification of food, especially seafood products, can help to reduce microbial growth and extend shelf life.

However, there are a few downsides to sous-vide processing, including that specific equipment and materials are needed. These include sous-vide bags, a vacuum sealing machine, and immersion cookers. Compared to other commercial thermal heating processes, the equipment needs are not extensive, and most thermal processing facilities still require things like cold storage, which would be required for a sous-vide product. Also, since sous-vide processing is conducted at lower temperatures, potentially less electricity would be required compared to conventional cooking of products. The most significant issue with sous-vide processing is ensuring the safety of the product, especially if it is low acid and ready-to-eat. The safety concerns arise from the vacuum packaged environment because it is anaerobic, allowing *Clostridium botulinum* toxin to be produced if the product is not acidic enough or kept cold enough. A hurdle that can be used to prevent this hazard is making sure that the product temperature is chilled to below 3.3°C immediately after processing and during storage The other main pathogen of concern for ready-to-eat sous-vide products is *L. monocytogenes*, so time-

temperature combinations for both studies were followed to achieve a 6-log reduction of this pathogen. In order for a sous-vide ready-to-eat product to be commercialized, time-temperature indicators (TTI's) would have to be used to ensure that no temperature abuse (i.e., > 3.3C) occurs. This can be costly and hard to maintain for a company but is still doable. There are seafood companies that use TTI's, however, it may be more convenient logistically to control for *C. botulinum* in the product in other ways. For example, a pH of below 4.6 for a product is considered a hurdle to preventing *C. botulinum* toxin production, since the acidity inhibits the spore from producing the toxin. However, some commercially available vacuum packaged smoked salmon products rely on TTI's because they are not acidic enough.

In the first study, the physicochemical and microbial properties of mussel meats sous-vide cooked under two different time-temperature conditions and treated with different concentrations of lactic acid solution were evaluated. There has been no previous published research documenting the effects of sous-vide processing and acidification on mussel meats. All sous-vide cooked treatments remained below the 5 log CFU/g good quality acceptable limit of cooked products over the course of the 35-day study. The most successful combinations at maintaining product quality were sous-vide cooking at 65C or 75°C for 30 minutes with 1% lactic acid treatment. These treatments kept the TVBN content, which is used as an indicator of seafood spoilage, below the 25 mg/100g limit for good quality seafood. The pH values documented for the 65°C/1% and 75C/1% lactic acid treatment were also the lowest, likely contributing to the low TVBN values and microbial counts. However, the overall pH values were not lower than 4.6, meaning that the product was not acidified enough to prevent the growth of *C. botulinum* toxin. In order to bring the mussel meats to a pH of below 4.6 a higher concentration of lactic acid or a more acidic acid with a lower pKa could be investigated. Citric

acid has a lower pKa than lactic acid and has been proven to be effective at acidification. In the present study the acid apparently extended the shelf life in terms of physicochemical and microbial (TPC, psychrotrophs) measurements but may actually have encouraged *C. botulinum* by reducing levels of competing bacteria. TTIs would be required on each retail package as an additional hurdle, along with ensuring the temperature never gets above 3.3°C. This may be difficult to achieve since there are so many steps within the cold chain before the consumer eats the product. A producer who wanted to use this option to control would need to have a reliable cold chain throughout the entire operation and supply chain.

One limitation of the first study was the limited amount of processed sample, which was sufficient for five sampling periods. Because of that, the best days to sample were decided based on collected data from the previous week, since the goal was to achieve at least a 28-day shelf life. Both of the 65°C and 75°C treatments treated with 1% lactic acid solution had a shelf life of 35 days according to TVBN analysis. However, it would have been beneficial to have data on day 28 for all of the analyses conducted. Another limitation was related to microbial analysis. For this study, aerobic plate counts and psychrotrophs were measured, but the sous-vide bag environment was anaerobic. Additional microbial analyses designed to enumerate anaerobic bacteria may have been beneficial. On the final day of storage lactic acid bacteria (LAB) were measured due to a suspicion that LAB growth may have been influencing sensory traits of the product. However, the LAB levels recorded on day 35 did not indicate a spoiled product. For this reason, it may not be necessary to measure LAB counts for the entire study. Instead, it may provide more useful information to monitor *C. botulinum* toxin production during storage.

In the second study, consumer acceptability and physicochemical analysis of the impact of thermal home preparation methods on acidified (marinated) sous-vide mussel meats were

evaluated. Out of 82 participants, 63% reported that they consume/purchase mussels once or twice a year or less. In fact, 7% of the participants noted that they do not consume/purchase mussels at all, showing that mussels may be an under-consumed source of seafood protein. Only 2% of participants stated that convenience when it comes to home food preparation is not important at all. Hedonic scores for flavor and texture attributes of the mussels were found to be highly correlated to their overall acceptability, while those for color and aroma were not. The method of thermal home preparation did not have a significant effect on sensory liking scores, and therefore the perceived quality would not be impacted by how the consumer prefers to prepare the product. There was a lack of correlation between instrumental texture analysis and hedonic texture evaluation, showing the importance of consumer testing, meaning that there is no ideal analytical replacement for consumer feedback. Out of the 82 participants, 83% answered that they would be at least "somewhat likely" to purchase the product. Some participants commented that the texture of the product was too firm or too mushy, that the marinade flavor was too overpowering, and that the packaging might not provide the best appearance of the product.

There were several limitations to this study. One limitation was the number of sensory panelists; 82 participants instead of 100. For hedonic testing with consumers, it is always better to have more participants, specifically people who enjoy the food product and eat it regularly. Another limitation was the number of processing conditions that were tested. Reheating methods (sous-vide not reheated, reheated by boiling in a bag, reheated in a saucepan) that consumers would be most likely to use at home were selected, but there was no prior survey to confirm the choices. Also, the mussel meats were served at 50°C for testing, and people may prefer food to be warmer when it is served in their homes. Choosing different reheating temperatures and times

may have provided greater differences between the samples because of more intense thermal processing. Also, it is important to note that due to the logistics of the sous-vide processing and consumer testing schedule, the control mussels were vacuum packaged in marinade the night before the study. The soaking of the raw mussel meats in an acidic marinade overnight may have contributed to a mushy texture when they were sous-vide cooked the following day.

These two studies have left a lot of room for further research and investigation. With regard to study 1, acidifying the product even further, below 4.6, would reduce the possibility of the production of *C. botulinum* toxin and help ensure the safety of a ready-to-eat product. Literature also suggests that applying the acid in a more concentrated form, such as encapsulated, could be more effective in producing a longer shelf life. It may be useful to investigate the effectiveness of other organic acids such as acetic and citric acid, which may be more common in marinade recipes that consumers are already familiar with.

From the second study, it was determined that consumers would likely be interested in purchasing and consuming the heat-n-eat mussel product. A next step could be to conduct a 6-week shelf-life study with the marinated sous-vide mussel meats, potentially using a trained panel to monitor the sensory quality of the product in addition to physicochemical and microbial analyses. There would also be value in assessing anaerobic microbial populations such as *Lactobacillus* or *Clostridium* species. It was found that some people did not enjoy the flavor of the marinade. This could be a good reason to experiment with other flavor combinations and ingredients. It would be beneficial to investigate reformulating marinade recipes to have a lower pH. This action could reduce the pH of the mussel meats and possibly increase shelf life while ensuring the safety of the product with regard to *C. botulinum*. It would also be useful to understand why American consumers don't eat more mussels. Although two different mussel

consumption questions were asked in the study, neither one addressed what the primary constraints were to consuming mussels more frequently. Having this information could allow for further targeted development and innovation of acidified sous-vide mussel products at the retail level.

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APPENDICIES

APPENDIX A: MODEL EFFECTS FOR TWO-WAY INTERACTIONS BETWEEN STUDY 1 DEPENDENT VARIABLES

Table A. Model effects (p-values) for two-way interactions between project 1 dependent variables

Dependent Variables	Temperature * Acid	Temperature * Day	Acid * Day
pH	0.170	0.998	**<0.001**
Liquid Loss	0.061	0.319	0.400
TVBN	**<0.001**	**<0.001**	**<0.001**
TBARS	0.992	0.883	1.000
Total Plate Count	0.879	0.919	0.284
Psychrotrophs	0.065	**0.027**	**<0.001**
L*	0.723	0.935	0.163
a*	0.531	**0.024**	**0.001**
B*	0.887	**0.016**	**0.034**
Peak Force	0.257	**0.042**	**0.010**
Positive Area	0.731	**0.005**	**<0.001**

APPENDIX B: CONSUMER ACCEPTABILITY OF SOUS-VIDE MUSSEL MEATS IN A MARINADE RECRUITMENT NOTICE

Do you like eating mussels?

Would you like to support the development of a new and convenient seafood product, by trying sous-vide cooked mussels enrobed in a marinade?

If you are at least 18 years old and you like eating mussels, you are invited to evaluate farm raised, sous-vide cooked mussels in a marinade. If you have never eaten mussels, do not like eating mussels, or if you are allergic to shellfish, we request you not to participate in the testing.

Evaluation/testing will take about 15 - 20 minutes. You will be asked to evaluate four different sous-vide cooked mussel preparations. Participants will receive a $5.00 reward for tasting and completing the questionnaire. **The testing will be held on Wednesday June 28th 11-1:30pm and 4-5:30pm at the Sensory Evaluation Center located in Hitchner Hall (Room 158 A and 158 B).**

For more information about the study, please contact Sara Gundermann at sara.gundermann@maine.edu.

To schedule a time to participate, please click the link below or scan the QR code below:

Link to Doodle Poll

APPENDIX C: CONSUMER ACCEPTABILITY OF SOUS-VIDE MUSSEL MEATS IN A MARINADE QUESTIONAIRE

Thank you for participating in this study. Please answer some questions about yourself, then evaluate all four samples from left to right. Make sure that the sample code on the sample you are trying matches the code on the computer screen. Take a sip of water before tasting each sample.

What is your current gender identity?

o Female (Cis or trans)

o Male (Cis or trans)

o Non-binary, genderqueer, or genderfluid

o Prefer not to reply

Please indicate your age.

o 18-25

o 26-35

o 36-45

o 46-55

o 56-65

o 66 years or older

o Prefer not to answer

How do you describe yourself? (Please select all that apply)

o Black or African American

o American Indian/Alaska Native

o Asian

o Caucasian (White)

o Native Hawaiian/Other Pacific Islander

o Prefer not to answer

Approximately how often do you purchase/consume mussels?

o Every week

o Every two weeks

o Once a month

o Every other month

o Once or twice a year

o I do not purchase/consume mussels

Approximately how often do you purchase/consume mussels from the store?

o Every week

o Every two weeks

o Once a month

o Every other month

o Once or twice a year

o I do not purchase/consume mussels from the store

How important is convenience to you as a consumer with regard to home food preparation?

o Extremely important

o Important

o Somewhat important

o Not important

Please evaluate the first sample. Please try a mussel with marinade and answer the questions about the overall product. [Note: These questions will be repeated for each sample.]

How much do you like the overall appearance of this sample?

o Dislike Extremely

o Dislike Very Much

o Dislike Moderately

o Dislike Slightly

o Neither Like nor Dislike

o Like Slightly

o Like Moderately

o Like Very Much

o Like Extremely

How much do you like the aroma of the sample?

o Dislike Extremely

o Dislike Very Much

o Dislike Moderately

o Dislike Slightly

o Neither Like nor Dislike

o Like Slightly

o Like Moderately

o Like Very Much

o Like Extremely

Please take a bite and answer the questions below.

How much do you like the texture of this sample?

o Dislike Extremely

o Dislike Very Much

o Dislike Moderately

o Dislike Slightly

o Neither Like nor Dislike

o Like Slightly

o Like Moderately

o Like Very Much

o Like Extremely

How much do you like the flavor of this sample?

o Dislike Extremely

o Dislike Very Much

o Dislike Moderately

o Dislike Slightly

o Neither Like nor Dislike

o Like Slightly

o Like Moderately

o Like Very Much

o Like Extremely

How much do you like the sample overall?

o Dislike Extremely

o Dislike Very Much

o Dislike Moderately

o Dislike Slightly

o Neither Like nor Dislike

o Like Slightly

o Like Moderately

o Like Very Much

o Like Extremely

Is there anything else that you would like to say about this sample? Please type the sample's three-digit code in your comments.

Comment box

Please read the concept statement below and answer the following questions.
[These questions will only appear once at the end of all of the tasting.]

Concept Statement:
This new product is a pouch of convenient, ready-to-eat, mussel meats in a marinade that would be available in the refrigerated section of your supermarket. The mussels are already sous-vide processed (cooked under vacuum in the heat resistant plastic pouch) to help retain their flavor and ensure meat tenderness. These shucked and cooked mussels can be consumed as is, or briefly re-heated prior to serving.

Based on the concept statement, how likely would you be to purchase this product?

o Extremely likely

o Somewhat likely

o Neither likely nor unlikely

o Somewhat unlikely

o Extremely unlikely

Knowing that a 1 pound mesh bag of in-shell mussels from Hannaford costs ~$4.99, how much would you be willing to pay for 1/4 pound of ready-to-eat, shucked mussel meats in a marinade? (in-shell mussels are about 25% meat by weight)

o $4.99

o $5.99

o $6.99

o $7.99

o $8.99

o More than $8.99

If you purchased these mussels at the store, what preparation method(s) would you be likely to use at home? Please check all that apply.

o I would eat them chilled without reheating (eg. on crackers, as a salad topping, etc)

o I would reheat them in the heat-resistant pouch (in boiling water)

o I would remove them from the pouch and reheat them in a sauce pan

o I would use another method [please specify)]

Is there anything about this product concept that you find unappealing?

Comment Box

[The last screen after all four samples are evaluated and concept questions are filled out.]

Thank you for your time and opinions. Please raise the window slightly to let the kitchen staff know that you are done.

APPENDIX D: CONSENT FORM FOR CONSUMER ACCEPTABILITY OF SOUS-VIDE MUSSELS IN A MARINADE

You are invited to take part in a research project titled **"Consumer Acceptability and Concept Evaluation of a New Sous-Vide Cooked Mussel Product "** by Sara Gundermann, a Master's student in Food Science, and her advisor, Professor Denise Skonberg. The purpose of this research is to evaluate sous-vide cooked mussels in a marinade, and to gain feedback on this new product concept.
You must be at least 18 years old to take part in this project. If you have never eaten mussels, do not like eating mussels, or if you are allergic to shellfish please do not take part.

What will you be asked to do?
If you choose to participate in this study, you will be asked to come to the Sensory Evaluation Center in Hitchner Hall (Room 158 A and 158 B) at the University of Maine Orono campus. You will be asked to answer a few questions about yourself and your mussel consumption habits, followed by tasting four different sous-vide cooked mussel preparations, and to complete a questionnaire about how much you like the samples. You will also be asked to answer some questions about the overall product concept. Testing and evaluation will take approximately 15-20 minutes.

Risks
The risks associated with this testing are minimal with loss of your time and inconvenience.

Benefits
There are no direct benefits involved to you, but you may enjoy eating the mussels.

Confidentiality
Your name and email addresses, collected for time slot organization, will be stored on a password protected computer and deleted by June 30th, 2023. Your answers will be collected anonymously. Your name will not be on any files that contain your answers to our questions. Data will be kept indefinitely on the University's Digital Commons site.

Voluntary
Taking part in this study is voluntary. If you choose to take part in this study, you may stop at any time. However, you must complete the questionnaire to get the compensation.

Compensation
Upon completion of today's evaluation, you will receive $5. No compensation will be provided if you decide not to answer all of the questions.

Contact Information
If you have any questions about this study, please contact Sara Gundermann at sara.gundermann@maine.edu or Dr. Skonberg at denise.skonberg@maine.edu. If you have any questions about your rights as a research participant, please contact the Office of Research Compliance, University of Maine, at (207)581-2657 (or email umric@maine.edu).

APPENDIX E: COMMENTS REPORT FOR SOUS-VIDE COOKING OF MUSSEL MEATS IN A MARINADE

Sample coded 468: Reheat by Boiling in Bag

Sample coded 357: Reheat in a Pan

Sample coded 914: Not Reheated

Reheat by Boil	Really like 468. Surprised to find it so attractive. Not sure if it has to do with the first sample.
	468- This is the first one where I liked the texture of the sample. I left like I could actually taste the mussle and it wasn`t too mushy. I realize the broths are probably the same if not, then very simiiar, but this one tasted better than the other samples. The aftertaste was nice but this one had no smell to it at all, flavoring or mussel wise.
	468 - This had a good taste to it.
	468 - A bit too salty.
	The best sample among all was 914.
	Sample 468- nice color, semi-firm texture, less vinegar taste than first sample, nice sweetness
	This sample had the best texture, in my opinion. Not enough marinade served so the flavor felt more mute in comparison. Code 468
	this one had the best flavor to me but the texture wasnt my favorite. seemed to dissinegrate in my mouth. best flavor over all though.914
	Sample 468 tasted somewhat rubbery to me, tasted fine though.
	468- the dark appearance is a little off putting
	it had a pocket of soft material that i wasnt sure of its nature. not used to that.
	sample 468 was tasty - one little part was a bit tough
	#468 had a sweeter flavor that I didnt care for as much as #914
	468: it tasted a little sweet, I would prefer a more salad flavor.
	468 very gritty
	A bit of an odd aftertaste
	Again nice and soft to begin with then very chewy. Also too much salt. leaves a peppery aftertaste.
	This one was slightly sweet. I guess actual flavor of the mussel came through. The broth is slightly sweet as well.
	The same gummy texture with sample 468 as with samples 914 and 357. I preferred 468 to 357 because, like sample 914, the overall flavor was less briny.
	468 - the sauce was better, not so acidic and a bit more flavorful, sauce was more robust
	468 Marinade was flavorful. My answer regardin appearance is moderated by the fact that all mussels look somewhat strange.
	Sample 468 was a bit chewy, but had fine flavor. This sample and sample 914 had less flavor than sample 357.
	468 a little salty for my taste, but overall a good taste.
	468 was my least favorite
	It really seems to fall apart when I bite into it- almost like it is dissolving. 468. I would say this is similar to what happened with sample 357

	468 The texture was similar to the others, kinda mushy and chewy. I prefer firm mussels. The marinade tastes better and smells better.
	Just by looking, this sample seemed to have more small specs in the liquid and on the mussel (I assume spices). Although it looked slightly less appetizing because of this, it seemed to taste more flavorful than the first (468).
	overall just tasted like an ordinary plain mussel, couldnt taste the spices. (468)
	468 seemssss too heavy on whatever flavorings are in it though it`sss not as bitter aas 357. iff i had to eat a plateful i`d go with the least no goodd #914. All seemed like they were trying too haaard.
	468, I like this sample slightly more than the last one but both were very good
	This mussel was a good size, but the texture varied too much. part of the mussel was mealy and part was more rubbery than I prefer. The taste was good.
	very salty
	Sample 468 seems like the mussel was marinated the longest, it was a little bit salty for me. I enjoyed tasting it anyways.
	468: I prefer the previous examples (357-914).
	not as flavorful
	468 I didn`t really get an aroma, but it was flavorful. It had a nice texture.
	tastes great 468
	Sauce was watery and not very good.
	Sample 468, tray 56. Firmer than prior sample (a good thing). Marinade was very similar, but still bland. Needs a bit more spice or salt or lemon, or something?? Both this sample and the prior one were cool, not warm; so may taste better warm.
	This was my favorite for texture - 468
	Sample one is really very good, I would purchase this from the store or a restaurant!
	Good but something is missing in the sauce. Ratio of salt to sweet. But overall nice
	468 - also slightly mushy but less so than 914. Flavor was good
	468-357-914 to my test it was a little hot.
	468: Seems salty
	468. I like the look of the spices in the marinade. It makes it look more appealing. This one has a nice aroma and flavor.
	Sample 468 was a little better than the first.
	468: does not look appealing, but flavor was good, nice aftertaste, texture still not great
	468 flavor seemed well balnced. Could taste teh mussel. It was a bit mushy in texture.
	468, least favorite of the three, odd taste to it
	468: Similar to the first but the broth being turbid was a bit offputting but overall the flavor was still there.
	Sample 468 has a lovely broth, and aroma. It tastes more like the first sample, a bit less rubbery than the second sample. Good blend of spices. Excellent taste.
	468, it doesn`t taste much different from 914
	sample 468 texture was slightly mushy.
	More tender than expected
	Excellent flavor, somewhat milder aroma than #357. Nice texture, but a little on the soft side. None of the samples looked all that attractive sitting in that much marinade, but this sample #468 looked perfect in the spoon (large and plump looking mussel meat).

Reheat in Pan	357 is not as good as 468.
	357- Broth was a little salty, mussel was very mushy, smelled a little like fishy tomato. Looks wise, the granules of the spices made it seem like it would be gritty but it wasn`t. Aftertaste was good, no bad lingering flavors.
	I don`t care for the tast and the after taste is not something that I personally enjoy.
	357 - This was better than 468. The mussel wasn`t as soft and mushy. Way better texture.
	A bit too salty. 357
	The taste was much better than sample 914.
	Sample 357 strong vinegar taste, not my favorite but still tasty and cooked well.
	This mussel was softer and almost buttery-like. Enjoyed very much. Code 357
	357 enjoyed it very much, would be very please with a plate of these
	nice effort
	sample 357 - tastes good - the marinade was nice - just a slight bitter aftertaste
	I felt that #357 was more chewy in texture than I care for. I felt that the flavor of the sauce was the same or similar as the other two samples but that it was more the texture of the muscle that I didn`t care for.
	357: I felt it more sweet than the 468 and in my opinion I prefer more salad rather than sweet.
	357: Definately my favorite. While the mussell textures were similar to me in all, this marinade I liked the best
	Soft to begin with then very chewy
	Some part of the mussel was really chewy. Couldn`t eat that part, had to spit it out.
	This sample had a better texture. It was not as mushy as the first two samples.
	Texture was odd and kind of gummy.
	357 - This seemed to be the best one, sauce is the most robust in flavor (looks like the sauce is thicker than the others).
	357 This sample was larger than the previous one but with few discernable differences. I enjoyed both,
	#357 -- when looking at the sample, it looks a little bumpy on it, and also the texture seems to be a little `mushier` than the first sample. When looking at all 3 samples this one is not as attractive as the other two.
	914 was suprisingly non rubbery. 357 was a little more rubbery, but I liked its texture more
	I wasn`t expecting it to be cold. 357
	357 texture of mussel was mushy and flavor was good
	This sample (357) seemed to have the darkest liquid, I liked the taste but probebly not as much as my second option (468).
	(357) was very good, had a nice aroma. I liked the texture as it was a little tougher than the previous(468)
	sauce seems ssomewhat bitter as if too much onion or garlic powwder or something else was used, normally mussels taste sweet
	357 This samply texurly wasn`t my favorite however the flavor i think was really good
	The mussel itself was on the small side but seemed to look, smell and taste similar to mussels I`ve eaten in the past
	much too soft - almost mushy in texture

	The mussel 357 was more chewy than sample, and barely andy fish smell.
	Pleasant the taste.
	it was ok
	357 Not a flavor that I am acustomed to, so I didnt`t love it. I liked that the little beard wasn`t there but the texture was really chewy.
	Sample 357, tray 56. Warmer than the other samples, so that may have affected things, but did have more flavor to the marinade. Good mussel size, firm with a soft belly. I like this one the best.
	357 - this sampleis very briney
	Sample three is very appealing.
	357 Nice taste. Not too sweet. nice pairing for seafood.
	357. Flavor isnt good, Doesnt look great
	357 - this was the least mushy, still just a bit softer than I would normally have. Flavor was the best of the three.
	the texture is really good, it does taste more fishy then usual though.
	357. I like the appearance of the marinade. The spices in the juice make it more appealing. Also there is a very faint amount of heat (spice) that I rather enjoy and wouldn`t mind a spicier version.
	Sample 357 was ok-a ltttle bland.
	357: is nicer taste than 914, texture about the same, nicer aftertaste as well
	357 nice combination of flavors
	357, this one was better than the first and less salty
	357: The taste is good the balance of the sweet, salt, and spice is great but there is a bit of an aftertaste that lingers
	357 - although very salty
	357 It has a nice sea coast aroma. Good texture with a clean finish.
	357, this one is delicious, has the best flavor of my 3 samples
	357 - flavor was milder than 468, but still enjoyable
	sample 357 texture was mushiest of all three samples.
	Tougher than previous sample
	Great flavor and aroma, but a little mushy.

Not Reheated	The texure of 914 is better than 357. Not sure if it has to do with individual mussel. Not the favorite taste though.
	914 - The color of the broth was duller. The mussel itself didn`t look that great - the stomach? of it had broken open and there was part of it in the broth. This one tasted a little bit gritty with the spices. Aftertaste was not as nice as the first sample. Flavor was stonger but in a different way than the first one.
	I didn`t like the aftertaste
	914 - This one was ok. The mussel seemed to smush when I bit into it. It left a little of an after taste. 357 is my top choice.
	914 - seemed a bit less salty (perhaps as this was my 3rd sample and my tastes buds have acclimated). Texture seems a bit firmer (best sample, but all good).
	The souce was acceptable but the taste of mussel itself was not desired for me.
	Sample 914- soft meat texture, gritty and sandy, flavor subtle vingegar more heat and spice, unappetizing meat color
	Texture was a bit soft and almost dough/paste like. Not a bad thing. Code 914
	Sample 914 has a nice texture to it and the marinade is tasty, although slightly bitter to my taste.
	914- if I were served these in a restaurant I would not order them again
	better flavor
	good (914) - sorry not discriminating a whole lot between them, but this mussel had spilled its guts a little, though I might have liked the taste best
	Too soft
	914: This was the best because I cannot feel to much sweet.
	914: Pretty good. Marinade might be a little too savory
	914 peppery and broth is not as clear tasting as 357 kind of pasty aftertaste. 357 was the best of these three.
	Mussel is cooked right. I would like some adjustments to the seasoning except salt.
	914 - there was more of that brown green discoloration that mussells sometimes get and that was a little unappitizing
	Texture also seemed a little off on this one, slightly gummy, but the sauce/overall flavor of 914 was less briny than 357 and I overall preferred it.
	914 - the sauce was a bit off, tasted acidic
	914 While this sample was smaller than the previous two (which is why I rated it lower on appearance), the larger creamy element of the mussel (not sure what this is actually called, the large black part--I assume a stomach or something like that) made the texture/flavor unique.
	Sample 914`s flavor was not very bold. The sample`s texture was a bit mealy.
	#914-- the appearance was great, the texture was a little `mushy`on the inside, but a little hard on the edges as compared to #468. #357 was `mushier` throughout the mussel.
	914 It seems like part of the mussel is still something I can bite/chew but part of it feels like a paste.
	914 Texture was more mushy than the first, flavor was more sharp
	Mainly taste the mussel flavor, do not seem to taste much of what herbs may be in the sauce (914)
	This sample was cold and had an after taste that i am still experienceing that can only be described as a slightly spoiled sour (914)
	stilll something slightly off about the sauce flaaavorr though 914 is not as off as 357
	This mussel was easy to chew without being overly mealy or rubbery. the aroma wasn`t as strong as a previous sample but was still pleasant.

	I could not really smell the aroma of the sample. The sauce itself smelled like sea.
	914: I liked this flavor better than the previos one (357).
	yummmm
	914 Not a typical white wine broth, but very tasty
	I liked this one the least. Sauce was too tangy and thick. Mussel was tough.
	Mussel was large, but a bit mushy. Marinade was bland. Sample #914, tray 56.
	Very slimy in texture
	Sample two seems softer, more `squishy` and the aromoa is a bit more `fishy`.
	914 sauce looks a little watery but taste is good. Not too sweet. honnestly can`t find that much difference betwen samples, all are good! This is the kind of pre packagedfood that would be a quick easy addition to a meal
	91 4 - the texture was kind of mushy but the taste was good
	914: texture seemed a little gritty but not bad
	The marinade`s color and transparency is off-putting. 914
	Sample 914 seemed a bit more tender than the other two samples.
	p914: slight bitter taste, maybe too chewy a texture
	914 Kinda blah, not memorable 468 I made a mistake on the texture question. It should be dislike slightly
	914,this was ok, little salty
	914: This one was by far the sweetest but all the other flavors came through as well and it none of the other flavors were overpowered by another
	Sample 914 The liquid/broth was slightly runnier and clearer than the previous sample. It didn`t appear as appetizing as the previous sample. The muscle itself was a little dryer tasting.
	914 - smelled very good, but I was not sure how to place the flavor of this one, though not unpleasant
	sample 914 was the best texture I have had in a mussel.
	Tenderness was abuot in the middle between the other two sample. This one also had a chalky texture.
	Excellent texture and good flavor, but this sample seemed to have a milder aroma than sample#357.

BIOGRAPHY OF THE AUTHOR

Sara Gundermann was born in Palmyra, Pennsylvania on January 12, 2000. She was raised in Palmyra, Pennsylvania, and graduated from Palmyra High School in 2018. She graduated from the University of Maine in 2022 with a B.S. in Food Science and Human Nutrition, with a concentration in Food Science. She worked in many food science labs during her undergraduate and graduate career, including the Food Engineering, Food Analysis, and Seafood Lab. She also served as the food science teaching assistant during graduate school, assisting in classes like Food Microbiology, Sensory Evaluation, and Introduction to Food and Nutrition, among others. She was a member of the food science club, college bowl team, and dance department while at the University of Maine. After receiving her M.S. degree, Sara is excited to continue working in seafood product development as an R&D project manager in Auburn, Maine. Sara is a candidate for the Master of Science degree in Food Science and Human Nutrition from the University of Maine in December of 2023.