

Literary review and preliminary test design proposal for white shrimp packaging replacement for a shrimp packaging company from USA

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Literary review and preliminary test design proposal for white shrimp packaging replacement for a shrimp packaging company from USA

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Abstract. Shrimps is one of the most consumed seafood products in USA. The shrimp industry faces a big challenge due to short shelf life. One of the main trends in the food industry is the natural products, in which consumers prefer to acquire products that do not use chemical ingredients during their entire process. For this reason, the food industry currently evaluates different types of natural preservatives. One of the natural preservatives that has shown promising results in the meat industry is rosemary extract. In addition, one of the most popular and efficient methods to extend the products shelf life and maintain their quality is Modified Atmosphere Packaging (MAP) and vacuum packaging is one of the most used. A packaging system that includes vacuum atmosphere and rosemary extract is attractive as a fresh product package. During the study, nine different treatments plus three controls were analyzed, rosemary + polybag, rosemary + vacuum packaging, vacuum only and control Polybag in three different white shrimp (*Litopenaeus vannamei*) presentations (Head-on, Headless, Peeled and deveined). The samples were maintained in refrigeration. The analysis results show that for microbiological control (Total plate count, Total coliforms, *Salmonella*, *Staphylococcus aureus*, *E. coli*), rosemary extract + polybag and Rosemary extract + vacuum, showed the lower total coliform count at day 6. For color stability, reducing the color change due to oxidation reaction and quality maintenance, reduce of texture, appearance and odor change, rosemary+ vacuum in headless presentation was the best.

Key words: Extract, natural, preservatives, rosemary, vacuum.

Resumen. El camarón es uno de los productos marinos más consumidos en USA. La industria camaronera se enfrenta a un gran desafío debido a la corta vida útil. Una de las principales tendencias de la industria alimentaria son los productos naturales, en los que los consumidores prefieren adquirir productos que no utilicen químicos durante todo su proceso. Por esta razón, la industria alimentaria evalúa diferentes tipos de conservantes naturales. Uno de los conservantes naturales que ha mostrado resultados prometedores en la industria cárnica es el extracto de romero. Además, uno de los métodos más populares y eficientes para extender la vida útil de los productos y mantener su calidad es el envasado en atmósfera modificada (EAM), el envasado al vacío es uno de los más utilizados. Un sistema de envasado de atmósfera de vacío y extracto de romero es atractivo para un producto fresco. Durante el estudio, se analizaron nueve tratamientos diferentes más tres controles, romero + polietileno, romero + envasado al vacío, sólo al vacío y control en tres presentaciones (con cabeza, sin cabeza, pelado y desvenado) de camarón blanco (*Litopenaeus vannamei*). Las muestras se mantuvieron en refrigeración. Los resultados de los análisis mostraron que para el control microbiológico (Recuento total en placa, Coliformes totales, *Salmonella*, *Staphylococcus aureus*, *E. coli*), extracto de romero + bolsa de polietileno y extracto de romero + vacío tuvieron el menor conteo de coliformes totales en el día 6; para la estabilidad del color, reduciendo el cambio de color por reacciones de oxidación y el mantenimiento de la calidad, reducción de textura, apariencia y el cambio de olor, extracto de romero + vacío en la presentación sin cabeza fue el mejor.

Palabras clave: Extracto, romero, natural, preservantes, vacío.

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1. INTRODUCTION

White shrimp (*Litopenaeus vannamei*) is one of the most important products of the seafood market. According to IMARC Group (2019), the global farmed shrimp production size reached 4626642174 g (5.10 million tons) in 2019. The United States is one of the main shrimp consumers. People in the USA increase their consumption level into 1.99 kg (4.4 pounds) per person per day (NOAA Fisheries 2018). The local market is not supplying the demand of the product. For this reason, the United States has become one of the bigger shrimp importers and producers. USA imports increased by 5.1 percent in 2018 against 2017 according to the FAO (2019) shrimp farming in the USA is increasing, with a shrimp production in 2016 of 1814369.48 kg (4 million pounds), and the marine aquaculture production increased an average of 3.3 percent per year (NOAA Fisheries 2018). White shrimp have become the dominant aquaculture species because of their resistance and survival percentage.

Consumers are demanding natural products. To satisfy these requirements, one of the major challenges in the food industry consists of reducing conventional chemical additives in food formulation (Sánchez-González *et al.* 2011). American Mariculture, Inc. is a shrimp farm that produces fresh shrimp with no chemical ingredients during its growth and processing, but packaging and shelf life has proven more challenging. The shelf life of the shrimp varies due to its presentation. The packed shrimp with a head usually lasts 3-4 days maintaining its organoleptic and microbiological characteristics, headless approximately 5 days and the peeled and deveined shrimp 7-10 days. The shelf life of food depends on different factors; one of the main ones is microbial growth and oxidation. Since consumers expect that, the foods they purchase and consume will be safe and of high quality, microbial contamination plays a major role as a critical quality indicator.

Escherichia coli, *Klebsiella* spp, *Vibrio* spp., *Aeromonas* spp., *Pseudomonas* spp., *Listeria* spp., *Shigella*, *Staphylococcus aureus*, and *Salmonella typhimurium* have been reported as common spoilage and pathogenic bacteria found in shrimp (Rahman *et al.* 2016). “Seafood is also known to have been responsible for a significant percentage of food-borne diseases” (Karunasagar 1994). Costa (2013) recognized the presence of *E. coli* in foods as a potential risk for public health. (Jain *et al.* 2008) reported an outbreak of enterotoxigenic *E. coli* associated with consumption of butterfly shrimp in sushi restaurants in Nevada (USA) in 2004. “Since peeling of shrimp is mostly carried out by hand, it may be assumed that it will be contaminated with *Staphylococcus aureus* of human origin. Enterotoxin production is more often observed in staphylococci of human origin than in those from other sources.” (Beckers *et al.* 1985). A few food-poisoning outbreaks have been ascribed to staphylococcal enterotoxins in cooked peeled shrimp (Gilbert & Wieneke 1973). “Contamination in shrimp and other seafood products poses both a public health risk as well as an economic burden associated with lost productivity due to illnesses and increased resource requirements for monitoring.” (Hamilton *et al.* 2018). *Salmonella* spp was the most frequently reported cause of outbreaks associated with crustaceans from 1998 to 2004 (Hamilton *et al.* 2018). Nowadays many different preservation and storage methods are applied to maintain the shrimp quality (Wan *et al.* 2010). Investigations have proven that natural antimicrobials and antioxidants could be used in food processing. (Pisoschi *et al.* 2018; Preethi 2010). “Several types of Essential oils and their individual components are used as natural antimicrobial compounds in order to reduce

the impact of microbial activities in food products.” (Bhavaniramy 2019). (Helander *et al.* 1998) “Promising results have been obtained with essential oils from herbs and aromatic plants. Such essential oils consist of mixtures of esters, aldehydes, ketones, and terpenes with broad-spectrum antimicrobial activity.” Oils and extracts are now commonly used. Rosemary extract has been highly studied. According to Wang and collaborators (2012), rosemary (*Rosmarinus officinalis*), contains several important compounds such as one, 8-cineole (27.23%), α -pinene (19.43%), camphor (14.26%), camphene (11.52%) and β -pinene (6.71%). Just as it is important to take care of the quality and safety of a food, it is also very important consider its packaging.

Food Industry use a range of packaging attributes, combining, and changing designs, shapes, colors, and symbols (Nancarrow *et al.* 1998). These attract and maintain attention and helps consumers recognize the presented image. Importance of Packaging design and use of packaging as a means of communication and communication the brand is growing (Rettie & Brewer 2000). The packaging is similar to other marketing communication elements. One of the reasons is the fact consumers may not have thought deeply about the brand before entering the store buy. A recent study estimated that 73% of purchase decisions are made in point of sale (Connolly & Davidson 1996). Prendergast & Pitt (1996) define the basic functions of packaging through the role of packaging in logistics or marketing. The logistics function of packaging is mainly to protect the products in the process of moving through distribution channels. This can lead to increase the packaging cost but can reduce deterioration or loss due to theft or misplacement. The second function of packaging is essentially marketing role. Packaging provides an attractive way to convey relevant product attributes to consumers.

Modified Atmosphere Packaging (MAP) is a useful preservation system; it can not only extend the shelf life of food, but also maintain the natural quality of food (Castellanos 2017). MAP has become an effective technology that can meet consumers' demands for more and more natural and fresh foods (Mangaraj & Goswami 2009). One of the most used MAP is vacuum packaging. This system is subjected to an absence of oxygen. According to Berk (2018), vacuum packaging helps to prevent oxidation reactions such as lipid oxidation, loss of certain vitamins, oxidative browning, and loss of pigments. The vacuum also prevents deterioration by aerobic microorganisms and particularly mold (Berk 2018). Vacuum packaging can be supplement to ice or refrigeration to delay spoilage, extend the shelf life of fishery products (Shalini *et al.* 2000)

The objectives of this study were:

- To perform a literature review of natural antimicrobials, antioxidants and packaging systems used in the fresh raw shrimp trade in the USA.
- To conduct a preliminary evaluation of three packaging systems for fresh raw shrimp.
- To determine the analytical color variables that present the highest variation over time with the change of the packaging system for fresh raw shrimp in the company.

2. MATERIALS AND METHODS

Localization of the study

The literature review, quality analysis, samples preparation and experimental design was carried out in the processing plant of American Mariculture, Inc. located in 9703 Stringfellow Rd. St. James City, Florida 33956. The microbial and color analyses were carried out at EMSL Analytical, Inc. 200 Rout 130 North, Cinnaminson, NJ 08077, external laboratory. Statistical analyses were performed in Zamorano.

Literature review

Literature review was done by the method (Templier 2015), this method includes different steps:

1. Formulating the problem: the review's objectives were defined, which justified the need for a review article; 2. Searching the literature: Sources to use were identified as well as the studies that were pertinent for the review; 3. Screening for inclusion: The applicability of the studies was evaluated and then selected or excluded; 4. Assessing quality: the methodological quality of the primary studies was assessed; 5. Extracting data: applicable information of the primary studies included in the review were gathered and 6. Analyzing and synthesizing the data: The information previously extracted were compared, collated, summarized, aggregated and interpreted in order to suggest a new contribution to knowledge.

Literature review was done by research scientific articles, books, manuals, thesis, and internet documents. The information was of reliable sources like ResearchGate, Agris, PubMed, Science direct, Springerlink, and others. The scientific articles used was mostly less than 10 years of publication, some are older than 10 years but contain relevant information for the study.

Preliminary test

Product description. White shrimp (*Litopenaeus vannamei*) is usually commercialized as a refrigerated or frozen raw product. The processing plant processes lots of the same farm. The product processed in this plant is commercialized fresh, refrigerated, never frozen. One of the most important characteristics of the product is that no chemical ingredients are added during their raise, processing, and packaging. The product presentation is the whole shrimp, in three different presentations (head-on, headless, peeled, and deveined).

Packaging. Shrimp is packed in 1.30 or 2.26 kg (3 or 5 pounds) plastic Low-density polyethylene (LDPE) tubs placed in Styrofoam box with gel pack or ice, depending on transportation. Styrofoam box is placed in a corrugated or waxed carton with production traceability code properly indicated.

Preservation method. Cool room 1.1 ± 3 °C (34 ± 3 °F) during plant storage, ice during processing, and gel pack during transportation. Refrigerated during storage.

Temperature. Shipping-storage in a cold room where temperature variate in 1.1 ± 3 °C (34 ± 3 °F).

Organoleptic characteristics. Pieces are selected upon arrival to the plant. All shrimp that fail the quality control is discarded. Defects pieces during shrimp packaging, damages and broken tails need to be < 5% of the total production, discolored shrimp < 10% and melanosis presence in < 2% of the total production.

Flow process. Figure 1 shows the processing lines of the American Mariculture, Inc. plant. The first line is for Head-on shrimp presentation, and the other line is for Headless and Peeled & deveined.

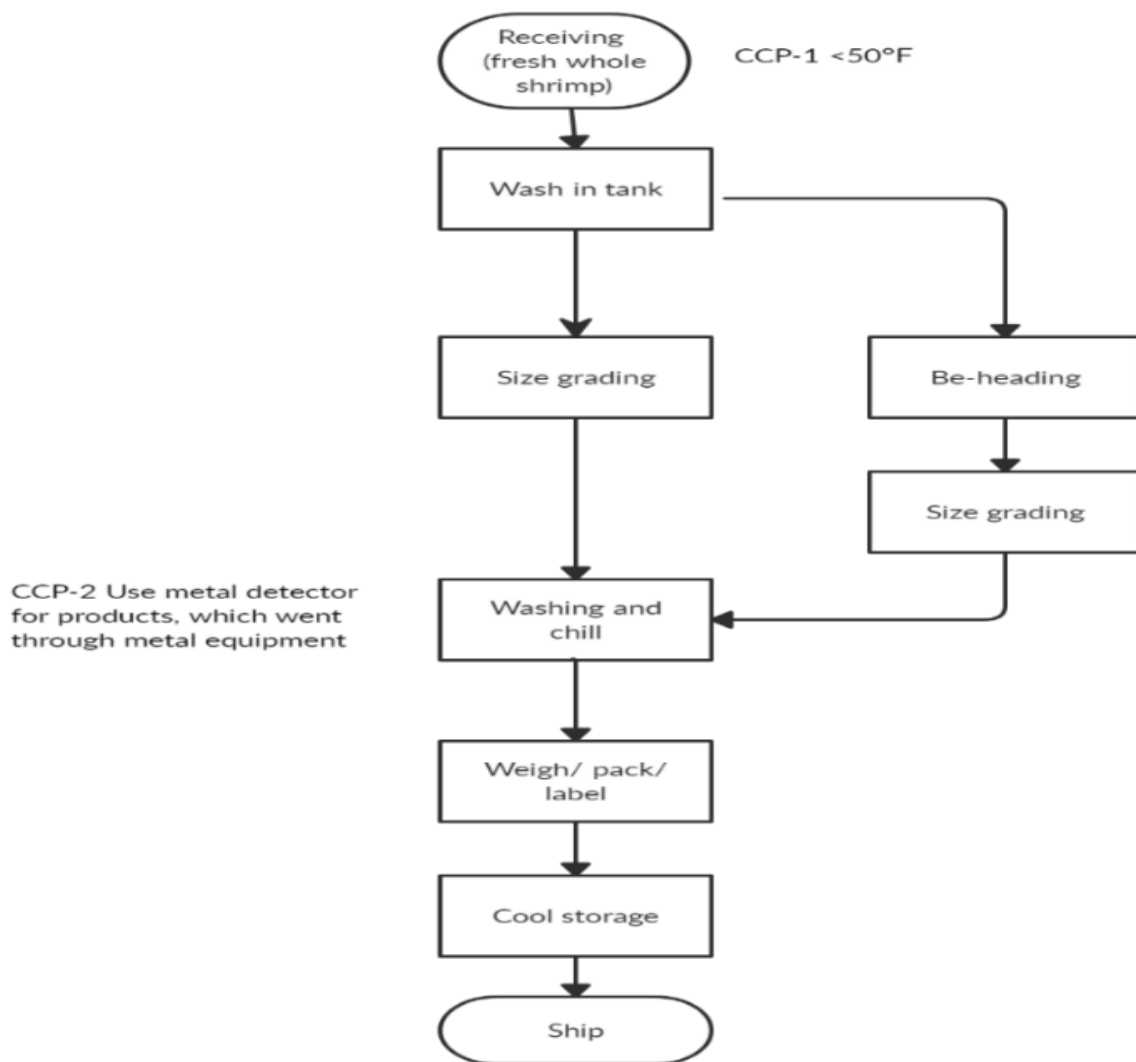


Figure 1. Process flow American Mariculture, Inc.
Source: American Mariculture, Inc., HACCP Plan, 2012.

Receiving. Shrimp is received three or four times a week. Shrimp comes from the production ponds. The company produce white shrimp (*Litopenaeus vannamei*); this is harvested and then sacrificed by ice immersion. The shrimp harvested are transported to the processing plant using a

heister. Shrimp arrive at the plant with a temperature under the 10 °C (50 °F). When the shrimp arrive at the plant, a sample was immediately taken, and the shrimp was measured by counting how many shrimps are into a pound. This measurement was done for the inventory register and to determine the size of the shrimp. In addition, every bin of shrimp that arrive to the plant was marked with a code. This code is used for the product traceability.

Wash in tank. All the shrimp that arrives at the processing plant is washed with clean water, this process is made to eliminate external contaminants like food rests, algae and others. The purpose of washing the shrimp is to assure that only clean product is introduced to the plant. Once the shrimp is washed, depending on the requirements of the consumers and clients, the shrimp can have to different process. Because of the processing plant work under request, shrimp is divided considering this. For head-on shrimp presentation, the next step of the flow process is size grading.

Size grading. For grading the product, a size grading is used, the shrimp pass through a transporting band and through the machine. The size grading machine divide the shrimp into three different sizes small, medium, and large shrimp. Once the shrimp is divided, it is eventually kept into different bins with ice. For shrimps of headless, peeled, and deveined presentation, there is a different step before size grading.

Be-heading. After the shrimp is washed with water, it passes through a transporting band to the be-heading area. This area has the capacity for eight people working. The head is removed by applying manual pressure behind the head. The removed heads are collected in tanks and then discarded to the trash. Once shrimp is be-headed, it passes through the transportation band and then to the size grading machine.

Washing and chill. Once the shrimp is graded by size, it is washed again using water and then chilled. This step is considered the second critical control point. Since the product passes through a metal detector to assure elimination of physical hazard for the consumer.

Weight, packaging, and labeling. The product is weighted depending in the order; usually the shrimp is packaged into 1.36- 2.26 kg (3-5 pounds) Low Density Polyethylene (LDPE) bags. The bags are packed into a secondary pack. The secondary package contains the product information like shrimp presentation, size, and number. The product also is packed into a third pack, where the delivery information, name of the buyer, shrimp presentation and size, is marked.

Cool, storage, and shipping. Shrimp is maintained in a cold room at 0 - 1.1 °C (32-34 °F). Every day the courier used by the company arrive to the processing plant. The products are shipped to the consumers.

Microbiological specifications. The processing plant manage their product with the recommended microbiological specifications by the FDA for raw seafood. Three times per year, samples were analyzed in an FDA approved laboratory to ensure the microbial quality of the product. The limits considered to determine if the product is in good or marginal microbiological quality are found in Table 1.

Table 1. Microbiological limits for raw seafood.

	Good	Marginal
Total plate count	< 1000000 CFU/g	< 5000000 CFU/g
Total coliforms	< 1000CFU/g	< 2400 CFU/g
<i>E. Coli</i>	< 3CFU/g	< 10 CFU/g
<i>S. Aureus</i>	< 200CFU/g	<1000CFU/g
<i>Salmonella</i>	Negative in 25 tests	

Source: American Mariculture, Inc., HACCP Plan, 2012.

Materials

Shrimp. Fresh white shrimp (production code: 3377-2) size 10-15 from the production ponds of the same company were used for this study. The shrimp was harvested the same day of packaging by ice immersion and immediately transported to the processing plant. A total of 36.28 kg (80 pounds) was used.

The shrimp was divided into three groups: 1. Head-on, 2. Headless, 3. Peeled and deveined. After beheading, peeling, deveining, and washing with water, shrimps were ready for the experiment. After the beheading and deveining process, each group weighted 9.07 kg (20 pounds.) with 20 experimental units (EU) per group, each with a weight of 0.45 kg (1 pound).

Rosemary extract. The extract used in this study was the FLAVORSHIELD® Rosemary extract, natural (water-soluble), obtained from Silver Cloud States Company.

Packaging system. The packaging was either vacuum bags or poly bags. 30 samples were packed in poly bags and 30 in vacuum bags. The packaging materials, bags and equipment used for the preliminary test were:

- Five-pound poly bags purchased from Packaging Products Corporation (PPC). Actual packaging of the company American agriculture. (30 units)
- Vacuum bags 0.254 × 0.33 m (10" × 13"). (30 units)
- VACMASTER VP215 vacuum sealer.
- 8 inches, 300W METRONIC Heat sealer.

Methods

Experimental design. A Completely Randomized Design (CRD) with factorial arrangement was used. Three shrimp presentations, four treatments, including the control, with five replicas per treatment for a total of 60 experimental units (Table 2).

Different number of experimental units were used for color and microbiological analysis. Color analysis was done every day in five repetitions of each treatment and presentation. Microbiological

analysis was done at days 0, 6 for Head-on presentation control 1 and TRT1, and for days 0, 6 and 12 for Headless and Peeled and deveined all treatments; the analysis was done with just one repetition, the total of experimental units for color analysis were 60. For microbiological analysis, 34 experimental units were used.

Treatments. Three treatments for three different shrimp presentations plus a control were used for the experiment. Each treatment was a different kind of packaging or a combination of packaging and extract. Table 2 shows the packaging specification of for each treatment.

Table 2. Treatments used for the preliminary test.

Treatments	Shrimp presentation	Packaging specifications
Control 1	Head-on (HO)	Shrimp + polybag packaging (used in the company)
Treatment 1 (TRT1)		Shrimp + rosemary extract + polybag
Treatment 2 (TRT2)		Shrimp + rosemary extract + vacuum packaging
Treatment 3 (TRT3)		Shrimp + Vacuum packaging
Control 2	Headless (HL)	Shrimp + polybag packaging (used in the company)
Treatment 4 (TRT4)		Shrimp + rosemary extract + polybag
Treatment 5 (TRT5)		Shrimp + rosemary extract + vacuum packaging
Treatment 6 (TRT6)		Shrimp + Vacuum packaging
Control 3	Peeled & Deveined (PD)	Shrimp + polybag packaging (used in the company)
Treatment 7 (TRT7)		Shrimp + rosemary extract + polybag
Treatment 8 (TRT8)		Shrimp + rosemary extract + vacuum packaging
Treatment 9 (TRT9)		Shrimp + Vacuum packaging

Rosemary extract solution and samples preparation. The recommended concentration of rosemary extract 0.20% of the total product weight was used. This concentration dosages 100 ppm of carnosic acid into the product. 4.53 kg (10 pounds) of each shrimp presentation were treated with the rosemary extract solution, 13.60 kg (30 pounds) in total. Rosemary solution was done by dilution. The dilution contained 5 L of distilled water and 0.09 kg (0.2 pound) of water-soluble rosemary extract for each presentation process. The total of rosemary extract used for the experiment was 0.27 kg (0.6 pounds).

Shrimps were immersed for 60 minutes in the solution, and then removed and left drained for 30 minutes (Rashidaie *et al.* 2019). Afterwards, all the different treatments of each presentation were packaged, and stored at 0.5 ± 1 °C (33 ± 1 °F). The samples remained at this temperature throughout the experiment.

Microbiological analysis. Microbiological analysis was performed in 34 of the 60 experimental units. Of the five repetitions of head-on presentations for control and TRT1, two repetitions were randomly selected for microbiological analysis. For the rest of the treatments (TRT2 and TRT3) of head-on presentation and for the other two presentations (headless, peeled, and deveined) all treatments, three repetitions were randomly selected for microbial analysis.

Microbiological analysis was done at days 0, 6, and 12. This depended on the presentation. Since the control and TRT1 for the Head-on shrimp presentation presented extended signs of damage (bad odor and extreme purge) after day 6, the samples from that day forward were discarded for the experiment. In Head-on presentation, both control and TRT1 samples were analyzed at days 0 and 6. For Head-on shrimp presentation, TRT2 and TRT3, and the rest of the presentations (Headless, Peeled and deveined) treatments, the analysis was done at days 0, 6 and 12. In the Head-on presentation, control and TRT1 samples were discarded after day 6, for this reason only days 0 and 6 were analyzed. Samples were randomly chosen from the repetitions of each treatment and microbial analysis were performed. The microbiological analysis was performed in EMSL ANALYTICAL, INC. laboratory, the analysis and methods were: *Salmonella*. V-SPT-AOAC 2013.01. VIDAS SPT Assay is an enzyme immunoassay for the detection of Salmonella receptors using the ELFA technique (Enzyme-Linked Fluorescent Assay).

E. coli and Total coliforms. Petrifilm AOAC 991.14. 3M Petrifilm (modified violet-red bile media) contains 2, 3, 5-triphenyltetrazolium chloride and glucuronidase indicator which forms a blue precipitate around any *E. coli* colonies that may be present. Plates are hydrated with sample and gelling agents cause the media to solidify. Gas is formed because of the fermentation of lactose by coliform bacteria (including *E. coli*). Glucuronidase negative bacteria form red colonies as a result of the reduction of 2, 3, 5-triphenyltetrazolium chloride. All blue colonies associated with gas are counted as *E. coli*. Red colonies with gas are non-*E. coli* coliforms. The total coliform count is the sum of red and blue colonies (with gas).

Staphylococcus aureus. Direct plating AOAC 975.55. This method is suitable for the analysis of foods in which more than 100 *S. aureus* cells/g may be expected. For each dilution to be plated, aseptically transfer 1 ml sample suspension to three plates of Baird-Parker agar, distributing 1 ml of inoculum equitably to three plates (e.g., 0.4, 0.3, and 0.3 mL). Spread inoculum over surface of agar plate, using sterile bent glass streaking rod. Retain plates in upright position until inoculum is absorbed by agar (about 10 min on properly dried plates), then count and record colonies.

Total plate count. AOAC 990.12. 3M Petrifilm contains nutrients and 2, 3, 5-triphenyltetrazolium chloride as an indicator of bacterial growth. Reduction of triphenyltetrazolium by bacteria results in red colored colonies. Plates are hydrated with sample and gelling agents cause the media to solidify. The enumeration has different stages: Inoculation, incubation 35 ± 1 for 48 ± 3 hours and interpretation.

Color analysis. The values were measured in CIE L*, a* b* where (L) indicates lightness (0 to 100), (+/-a) the degree of redness or greenness (-60 to 60), and (+/-b) the degree of yellowness or blueness (-60 to 60). The color analysis was done in EMSL ANALYTICS, INC. with Aero's spectrophotometer Dual-beam Non-contact Reflectance Spectrophotometer with the method Port Down; Non-Contact; Rotating platter.

Quality analysis. Analysis was performed every day while the samples were inside the cold room, since the day 0 until the day they were considered not edible. For this test a format was provided by the HACCP PLAN American Mariculture, INC. Product characteristics measured were appearance, head, body-shell color, and firmness. The firmness measurement was done by using the “finger method” (Sigurgisladottir 1999). The linked scale were 1.Good (✓), 2.Intermediate (X✓), or 3.not acceptable (X) and comments were registered for each sample. Comments included odor and melanosis (Black spots caused by enzyme systems present in shrimp.) presence. Once the analysis was done, a numerical score from one to nine were given to each sample every day of its storage. This score was given by using the Shrimp Quality Scale extracted from “Assessing product quality, shelf-life and consumer acceptance for fresh water, farm raises shrimp (*Litopennaeus vannamei*)” Garrido *et al.* 2000. Where class A shrimp means a score of 1-3, class B 4-6 and class C 7-9. My person María Elena Villamarín with the guidance of the Processing Plant Manager did the quality assessing. The processing plant manager manages the company’s quality program.

Statistical analyses. Color (L^* , a^* , b^*) results were analyzed using Analysis of Variance (ANOVA) and Duncan’s mean separation. The results were analyzed in the Statistical Analysis System (SAS version 9.4 ®).

3. RESULTS AND DISCUSION

Literature review

Worldwide shrimp market. Seafood products are one of the most traded and consumed food commodities in the world with an expectation to keep on growing (FAO 2007). Seafood trade has grown up by compound annual growth rate (CAGR) of 4% from 2012 to 2017 (Holland 2019). It was noticed that the seafood trade grows up more in value than volume. This situation may be the result of the high value of the salmon and crustacean trade (Holland 2019).

Shrimp is a small, invertebrate marine animal with a laterally compressed and elongated body. According to IMARC Group's (2019), the global shrimp market reached 4,173,049,804 kg (4.66 million tons) in 2018 and 5.1 million tons in 2019. Due to increased income and healthy lifestyle and other factors, fresh and frozen shrimp products are very popular among consumers. (IMARC group 2019). The increase in production and trade may be related to the health benefits of shrimp consumption, shrimp provides high-quality protein and essential amino acids, minerals and trace elements, fat-soluble vitamins and essential fatty acids, including long-chain n-3 fatty acids (Syama 2013).

“World production of farmed shrimp reached almost 3,628,738,960 kg (4 million tons) in 2018, increased by 3 to 5 percent over 2017.” (FAO 2019). The shrimp market is driven by many factors, such as increasing demand and increasing environmentally friendly production technologies. At present, *Penaeus Vannamei* (white shrimp) is the most popular edible shrimp. Others included *Penaeus monodon* and *Macrobrachium rosenbergii* (Holland 2019).

Nowadays, the shrimp production is dominated by China, continued by India, Indonesia, Vietnam, Thailand and Ecuador (FAO 2019). China shows the major production during the latest years (IMARC group 2019). In the other side, the principal shrimp consumers are United States, China, Europe and Japan. The principal shrimp exporters are India, Ecuador, Vietnam, Indonesia, Argentina, Thailand, China and Mexico on a lesser extent (FAO 2019). According to the FAO (2019), the seven principal markets imported around USD2.7 million tons of shrimp in 2018; this amount shows an increase of 31% since 2017. The imports show a little increase in United States and a decrease in Japan and Australia.

US shrimp market. The US shrimp market reached 691,274.77188 kg (762 tons) in 2019 and is considered the second largest shrimp consumer after China (FAO 2019). In the United States, shrimp is the main seafood consumed product, accounting for 25-30% of the country's total seafood market. Consumers in the United States increased their shrimp consumption to 1.99 kg/day/person (4.4 pound/day/person), 0.45 kg (one pound) more than the second most popular salmon. Due to its health benefits, the consumption of shrimp in the United States has been increasing in recent years. People work hard to consume health products. In the United States, another demand trend is to buy convenience foods, such ready to cook or ready to eat (IMARC 2019). Because of its high demand, the United States needs to import products, which is why it is considered one of the largest shrimp importers. The majority of prawns on the US market are imported from Asia and Latin America.

This increase in demand has caused the US to improve in its local market. The United States is considered a small-scale aquaculture producer. According to NOAA Fisheries (2018), from 2009 to 2014, aquaculture production increased by an average of 3.3% per year and maintained a growth trend. Since many years ago, different states in the United States, such as Georgia, Florida, Alabama, Louisiana, Texas and other areas began to experiment, research and improve shrimp farming. According to Gonzales (2019), Texas is the largest producer of farmed shrimp, with production in 2017 of approximately 3.2 million pounds. Alabama is the second largest farmed shrimp, producing 138,151.1 kg (304,571 pounds) in the same year. Farmed shrimp imports account for most of the shrimp supply in the United States.

Shrimp presentations in US market. The principal shrimp presentations trades in US are Head-on, Headless, Peeled & deveined, Butterfield and EZ peel (Gonzales 2019). In Table 3. are shown the characteristics of different shrimp presentation traded in US like Head-on, Headless, Tail-on, Tail-off, P&D, butterflied and EZ peel.

Table 3. Shrimp presentations in US market.

Shrimp presentation	Characteristics
Head-on	Head, shell, and tailfins on
Headless/Shell-on	Only the head has been removed, leaving the shell and tailfins attached
Tail-on	Headless, peeled & deveined shrimp in which the tail has not been removed. Can be cooked or uncooked.
Tail-off	Headless, peeled & deveined shrimp in which the tail has been removed. Can be cooked or uncooked.
P&D	Peeled & deveined, tail off. All shell and tailfin have been removed, with segments shallowly slit to the largest segment.
Butterflied	The shell and digestive track have been removed and deep cut has been made that "butterflies" the shrimp without splitting it in two pieces.
EZ Peel	Deveined with the shell on.

Source: Seafood of the world, shrimp sizing reference guide 2019, Asche *et al.* 2012

In the shrimp market, size measurement is used for commercialization. The measurement of different sizes depends on how many shrimps are in a pound of sample (commercial numbers are < 21, 21-25, 26-30, 31-40, 41-50, 51-60, 61-70, > 70). The smaller the shrimp, the more shrimp per 0.45 kg (1 pound), for example, shrimp smaller than 21 is the largest, shrimp larger than 70 is the smallest. The 41-50 scale represents the largest market segment, occupying most of the global market share (IMARC group 2019).

US shrimp processing. According to NOAA (2014), in 2012, 15 different companies in Texas processed thirty-one percent (31%) of processed shrimp in the United States. This is approximately equal to the total amount of shrimp processed by 35 different companies in Louisiana, Mississippi and Alabama, accounting for 33% of the total shrimp processed in 2012. Eleven different

processors in Florida accounted for 13% of the shrimp processed that year. Overall, in 2012, shrimp processors in Texas, Louisiana, Florida, Mississippi, and Georgia accounted for 78% of shrimp processing. In other words, the NOAA report (2014) shows that for every 2.26 kg (5 pounds) of shrimp processed in the United States in 2012, 1.81 kg (4 pounds) were processed in the Gulf of Mexico or the South Atlantic.

The US shrimp processing industry has already encountered some economic challenges. The shrimp processing line is its existing business (Kuhar *et al.* 2016), but the increase in imports of processed shrimp products has had a negative impact on the country. Especially in the business of shrimp processors. The processing sector generally welcomes the import of unprocessed shrimp as they represent the source of raw materials for domestic processing activities (Keithly & Poudel 2008).

Packaging alternatives for raw shrimp in US trade. Packaging is an important factor to consider, not only in the extension of the shelf life of fish and fishery products but also improving their marketability (Srinivasa *et al.* 1993). Packaging and combination process for food preservation can be used to improve the quality of conventional products or develop new products. They ensure stability and safety, so that the product has sufficient sensory and nutritional properties (Leistner 1992).

The Food and Drug Administration supervises the production, manufacturing, processing, packaging, and labeling of food and drugs. Food packaging manufacturers must prove to the respective agency (Food and Drug Administration [FDA]) that all materials that encounter food are safe (FDA 2004). For some foods, this does not seem to be that important for example, nuts in the shell, foods that need to be washed before eating. However, for other foods, packaging is essential to ensure that the food is safe to eat (Marinac 2013).

Packaging materials. The most used packaging materials are shown in Table 4 for raw seafood are PET/PVDC/LDPE/LLDPE, PA/PVDC/LDPE/LLDPE, PC/EVOH/EVA, MOPP, and OPP/PVDC, because of its good barrier and resistance (Sharma 2019).

Table 4. Packaging materials commonly used in stand-up pouches packaging for fresh raw shrimp.

Packaging materials	Full name
PET	Polyethylene terephthalate
PVDC	Polyvinyl chloride
LDPE	Low density polyethylene
LLDPE	Linear low-density polyethylene
PA	Polyamide
PC	Polycarbonate
EVOH	Ethylene vinyl alcohol
EVA	Ethylene vinyl acetate
MOPP	Mono-oriented polypropylene
OPP	Oriented polypropylene

Source: Sharma 2019.

Shrimp packaging requirements. Appropriate fresh seafood packaging should keep it moist and prevent dehydration, prevent chemical and bacterial deterioration, provide a barrier for moisture and oxygen, to reduce fat oxidation and prevent external odor penetration (Bindu & Sreejith 2018).

Stand-up pouches. Stand-up pouches are welded bags with a good shape at the bottom and can be placed upright. They are made of various laminates printed on the middle layer, and their multi-color printing is very important for marketing. The retort bag is a type of bag made of laminate, plastic film, and aluminum foil. They can be formed by welding from four sides (pillow-shaped bags), or they can be formed as upright bags with a bottom. (Izdebska 2016). This kind of packaging is one of the most used for fresh and frozen seafood including shrimp. The stand-up pouch also is called barrier bag and Mylar® film bag. This packaging is built by laminating together multiple layers of scientifically formulated film (Marinac 2013). The laminated process results in a puncture and moisture resistant package. Stand up pouches are capable to protect seafood from odor, bacteria, vapor, and oxygen. This kind of packaging reduces the oxygen present in the packaging environment. This oxygen reduces keep safe the product during it travel to the distribution channel and then to home. (Vera *et al.* 2020)

The basic components of most multilayer structures are polyolefin, such as polyethylene (PE) or polypropylene (PP). This is due to its rich content, wide range of uses, flexible processing, moderate price, excellent moisture resistance and chemical inertness. However, these substances have poor barrier properties to oxygen, flavor, and aroma molecules (Vera *et al.* 2020). In addition to other specific barrier effects, the inner and outer layers of the packaging also bear other responsibilities, the inner layer is in direct contact with food ingredients, so it is important that the ingredients are inert and must not react with any food ingredients. The inner layer should also have good sealing properties at lower temperatures. On the other hand, in addition to the barrier function, the outer layer must also provide mechanical stability and printability (Morris 2016).

According to Fredonia group (2017), flexible packaging like stand-up pouches is now used in different kind of food like beverages; candy & snacks; cheese; fresh produce; meat, poultry, & seafood; pet food; processed foods. The demand for stand-up pouches in the U.S. will grow almost 6% year over year to USD2.9 billion by 2022. The high demand of this packaging is due to its multiple benefits (Morris 2016). Stand-up pouches offer many advantages such as product freshness, in some cases clear film that allow consumers to see the product quality for themselves. (Morris 2016; Sharma 2019). This packaging can keep food fresh because it provides an excellent barrier control to prevent the intrusion of elements in the food. Stand up pouches allow using puncture resistant films to protect the product during transportation. The stand-up pouch is very sturdy and can withstand all losses except the most important impact, falling (Marinac 2013).

One of the most important features of this packaging is that it helps to build the company brand in different ways. On the other hand, this packaging can provide free marketing. The company's brand will be enhanced because the stand-up pouch allows printing and design, and manufacturers can add brand and other information about the product. Graphics can be placed on the stand-up pouches make the customer feel an impulse for buying the product (Robat 2017).

MAP. Modified atmosphere packing (MAP) refers the replacing of the air in a food pack with a different mixture of gases (Cann 2001; FAO 2001). Modified atmosphere packaging technology is used since 1930 and has been a critical area of research especially because of the waste and money

lost due to the fast spoilage in fishery products (DeWitt 2016). Along the latest decades, there has been an increase in gas packaged food products in the market. This increase has brought improvements to the packaging industry, which has led to the development of high barrier polymers and thermomould packaging equipment. Gas packaging is considered an extension of vacuum packaging. Food packaging under modified atmosphere use different gases, such as CO₂, N₂, and O₂, with CO₂ (Silliker & Wolfe 1980).

One of the biggest challenges is choosing the right gas mixture. The appropriate MAP must be selected to prevent bacterial growth, because some bacteria are aerobic or facultative under aerobic or anaerobic conditions, while others are anaerobic (Silliker & Wolfe 1980). Soccol (2003) mentioned that the optimal modified atmosphere for packaging of Pacific white shrimp (*Litopenaeus vannamei*) under controlled storage conditions, it is determined to be 75% CO₂, 10% O₂ and 15% N₂. Using this gas mixture, the growth of microorganisms, pH and TVB-N content are reduced. Sufficient gas composition can make the product form the most suitable exudate, reduce the content of TVB-N, and inhibit the growth of microbial flora. In addition, it maintains a high odor and appearance score in the packaged Pacific white shrimp, and the shelf life is extended to 11-12. The results of this study are very similar to those of the FAO (2001), indicating that the recommended mixed gas for white fish, shrimp and scallops is approximately 40% carbon dioxide, 30% oxygen and 30% nitrogen. Flexible and semi-rigid plastics and plastic laminates are the most common materials used for MAP foods. Plastic materials account for approximately one-third of the total materials demand for food packaging applications, and their use is forecast to grow (Mullan 2003).

The three main commercial gases in modified atmosphere packaging are carbon dioxide (CO₂), nitrogen (N₂) and oxygen (O₂) (Giménez *et al.* 2002). Carbon dioxide can inhibit the bacterial deterioration of fish, but a high proportion of carbon dioxide can cause fish schools to collapse and excessive dripping. Oxygen can help prevent color changes, while nitrogen is an inert gas used to dilute the mixture (Cann 2001; FAO 2001). CO₂ is soluble in water and lipids, which is the main reason for the antibacterial effect of MAP. "CO₂ concentration in MAP has been extended the shelf-life of foods by inhibiting the microbial growth of Enterobacteria and H₂S-producing bacteria" (López-Caballero *et al.* 2002). In foods high in fat such as seafood, beef and poultry, excessive absorption of carbon dioxide may lead to a phenomenon known as "package collapse" (Parry 1993). N₂ is an inert gas with low solubility to water and lipids. It is used to replace oxygen in packaging, reduce oxidative rancidity and inhibit the growth of aerobic microorganisms (Farber 1991). Because of its low solubility, it is usually used as a filling gas. Although anaerobic microorganisms have different sensitivity to oxygen, O₂ usually stimulates the growth of aerobic bacteria and may inhibit the growth of only anaerobic bacteria (Farber 1991).

According to Cann (2001) and FAO (2001), MAP has many advantages and disadvantages. Improved atmospheric packaging has some advantages. For example, it extends the storage life. When the modified atmospheric packaging is at 0 °C, the fresh-keeping time of raw shelled shrimp and shrimp is 30% longer than that of other types of packaging. The generation of fast-effect dark spots is suppressed (FAO 2001). The appearance of the packaging is very attractive, because the packaging is transparent, the buyer can clearly see the product, and the MAP is tasteless, easy to label and easy to handle, and the appearance of the packaging is very attractive. Transparent packaging, buyers can clearly see the product (Cann 2001).

MAP shows different shortcomings. For example, this is a relatively expensive technology, about twice as much as vacuum packaging (with regards to packaging material). Equipment is also more expensive, usually fourfold (Mullan 2003). Modified atmospheric packaging is usually heavier than other types of packaging. Therefore, transportation and storage are more complicated, and the packaging walls may collapse due to When the collapsed carbon dioxide content is high, the high carbon dioxide content will cause dripping, and the shelf life may be lost if the cold chain is ignored.

Vacuum Skin Pack (VSP). The technology is used for fresh and processed meats, ready-to-eat meals, poultry, and seafood. This technology tightly wraps the product and couples its shape into a second skin. VSP packaging uses heat and vacuum to tighten the flexible top film to the product and seal it to the tray. Remove all atmosphere from the inside of the package and fix the product in this second skin. By heating the skin to shrink, the formation of air is avoided, the formation of visible exudate is reduced, and the shelf life of microorganisms is extended (Carreira *et al.* 2004). According to Soccol *et al.* (2003), compared with traditional outer packaging, vacuum skin packaging reduces fish rancidity and lipid hydrolysis.

Packaging material. The structure of this packaging consists of three layers: exterior, middle, and interior. The outer layer has strength, heat resistance and printing ability, the middle layer is a barrier layer, and the inner layer has heat sealing, pressure resistance and drip resistance properties. LDPE, PVC and Surlyn ionomers are commonly used for skin packaging; this technology is used in thermoformed containers (Li 2012).

Thermoforming in skin packaging has advantages such as longer shelf life. The shelf life of VSP products is almost twice that of traditional MAP. Retailers will reduce its shrinkage, reduce consumer food waste, present a beautiful appearance, high-definition, and smooth film to focus on the product and make it in Supermarkets stand out, and a small part of commercialization can be purchased at retail (Vasquez *et al.* 2004). The main disadvantage is cost. The smallest continuous thermoforming machines usually round the USD 100,000 or more.

LDPE bags. Usually, the order of packing fresh or frozen seafood starts together is with the main inner packaging and ends with the main packing box or the third packing. The main packaging material in contact with food is usually low-density polyethylene (LDPE). Depending on the product, the packaging can be bag-like or film-like. Usually, the packaged product is about 0.90 – 2.26 kg. (2-5 pounds). The glaze is about 10-20% (Bindu & Sreejith 2018).

LDPE bags are often used for food packaging due to their transparency and water permeability. Although they are not as strong as HDPE, the FDA approved LDPE bags for processing and food packaging (FDA 2004). Low-density polyethylene bags are also commonly used for heat-sealing purposes due to their low melting point. LDPE is chemically resistant, repels microorganisms, and does not leach harmful toxins when storing food at various temperatures (Willige 2010).

LDPE packaging shrimp has many advantages, such as low cost, good flexibility, and melting point: 105 to 115 °Celsius (221 -239 °F) that allows heat sealing, high transparency, high elongation, softness, low water absorption, chemical resistance to alcohol, minerals, and oils, high impact strength at low temperatures, and meets FDA requirements (Bindu & Sreejith 2018; Willige 2010). Some of the disadvantages of LDPE bags are susceptible to stress cracking, highly flammable, high gas permeability, and low strength. When LDPE bags are used as primary

packaging, this is generally wrap into a secondary packaging. The most used secondary packaging is a strong corrugated paper carton box (Bindu & Sreejith 2018).

Corrugated paper carton box. The shape of the box and the materials used allow the use of colors and graphics to promote the company's brand, which helps achieve marketing goals. One of the main reasons why companies use this secondary packaging is low cost. The low cost of packaging is related to the low cost of transportation, which is attributed to the facilities used to deliver products flat, packaging material principally is composed of Paper/PE/paper laminates (Bindu & Sreejith 2018; Mena 2014).

Vacuum packaging. The vacuum packaging process involves evacuating air from the package before sealing (O'Sullivan 2016). Its main purpose is to remove oxygen by pulling the packaging material in contact with the product. According to Berk (2013), vacuum packaging is an ancient technology used for food packaging (especially meat). The main purpose of this packaging is to prevent oxidation reactions, such as lipid oxidation, oxidative browning, pigment loss and certain vitamins. The purpose of vacuum packaging is also to prevent deterioration caused by aerobic microorganisms and molds. The shelf life of vacuum-packed products has been extended by several weeks. Vacuum packaging also offers different advantages, such as reducing the volume of the packaging and increasing its flexibility.

Vacuum packaging is a natural preservative packaging method that can greatly extend the shelf life and overall quality of muscle foods for a long time (Sahoo & Kumar 2005). This is the most feasible packaging method to obtain a longer shelf life (Dey 2003). Vacuum packaging can be supplemented with ice cubes or refrigerated to delay deterioration and extend the shelf life of fishery products (Shalini *et al.* 2000). Keeping food materials under vacuum conditions restricts the use of oxygen for microbial growth and oxidation. This technology will help double the shelf life of products under cold storage conditions. This technique is commonly used for fatty fish. Uncommon odors are usually produced due to fat oxidation. Compared with ordinary air packaging, the shelf life of vacuum-packed refrigerated and refrigerated fish has doubled (Mohan *et al.* 2018).

One of the important aspects of vacuum packaging is to use the right materials with good barrier properties. Usually polyester-polyethylene or nylon-polyethylene laminates are used. Polyester and nylon have good strength and good oxygen resistance. Polyethylene is heat-sealable and resistant to water transmission. Materials for the bags are required to be ones into which air does not penetrate even when they are stored for a long period, and which do not deteriorate or deform under steam of 100 °C. A bag made at a laminated film of polyvinylidene chloride resin layers is preferred, because of its high air shutoff properties and transparency (McElhatton & Marshall 2007).

Vacuum packaging has different advantages, such as reducing fat oxidation, reducing microbial growth, reducing evaporation to reduce the drying of frozen products and burning in the freezer, extending the shelf life, reducing the volume of bulk packaging containing lighter materials. Its transparency makes the product beautiful to the appearance, good quality presentation, and low cost compared with the other MAP (Stammen 1990). Also vacuum packaging technology shows some disadvantages like difficulty in use of products with sharp edges, requires high barrier

packaging material, anaerobic condition caused because of the packaging may allow the growth and toxin production of *Clostridium botulinum* and *Listeria monocytogenes* (Stammen 1990). For refrigerated, reduced-oxygen packaged raw, unpreserved fish and unpasteurized raw fish products, *Clostridium botulinum* type C and non-proteolytic type B and F during the storage and distribution of finished products, the only obstacle to the formation of toxins is cold storage (Cann 2001). These types of *Clostridium botulinum* will grow at temperatures as low as 3.3 °C (38 °F). As mentioned earlier, there is generally no guarantee that the product will remain at a temperature or below after leaving the processor control device. A time temperature integrator on each consumer's packaging may be a suitable method to provide this control (FDA 2001).

A summary of the packaging alternatives used in fresh raw shrimp trade in US are shown in Table 5, the summary includes packaging type, principal characteristics, and the sources of the information. The packaging types described below are Stand-up pouches, MAP, Vacuum Skin Packaging (VSP), LDPE bags, corrugated paper carton box and vacuum packaging.

Table 5. Packaging alternatives for fresh raw shrimp trade in US, summary.

Packaging	Principal Characteristics	Sources
Stand-up pouches	Laminated of multiple layers packaging, can be placed upright, maintain product freshness, reduce cost, build the company brand.	(Izdebska 2016; Marinac 2013; Vera <i>et al.</i> 2020; Morris 2016)
MAP	Use different gas mixtures, extends storage life, attractive appearance, expensive.	(Cann 2001; DeWitt 2016; Silliker & Wolfe 1980)
Vacuum Skin Packaging (VSP)	Forms a second skin, extends shelf life, beautiful appearance, high equipment cost.	(Marcilene <i>et al.</i> 2003; Li 2012; Vasquez <i>et al.</i> 2004)
LDPE bags	Transparency, water permeability, low cost, good flexibility, allows heat sealing.	(Bindu & Sreejith 2018; Willige 2010; FDA 2004)
Corrugated paper carton box	Used as secondary packaging, promotes the company brand, low cost.	(Bindu & Sreejith 2018; Mena 2014; McElhatton & Marshall 2007)
Vacuum packaging	Reduce/eliminate oxygen, prevent oxidation reactions, extends shelf life, laminated multiple layers, reduce microbial growth.	(O'Sullivan 2016; Berk 2013; Sahoo & Kumar 2005; Dey 2003; Shalini <i>et al.</i> 2000; Mohan <i>et al.</i> 2018; McElhatton & Marshall 2007)

Essential oils and extracts for food preservation. Food processors works constantly with food preservatives to extend the shelf life of their products. Consumer demand for natural and safe preservatives to control microbial growth and reduce negative effects on health and environment have increased (Burt 2004). The principal challenges associates with fresh food processing are

focused on the consumers' demand. Consumers insist in safe, long shelf life, and high-quality products (Brul 1999). The use of natural extracts and essential oils provides a potential solution to food processors, due to its antimicrobial properties (Lazar *et al.* 2010).

Plant extracts have been used in seafood to maintain its quality and extend its shelf life by reducing microorganisms and chemical reactions. According to the microbial load, TMA and thiobarbituric acid reactive substances (TBARS), the shelf life of sardine fillets treated with 10% cactus peel extract was extended to 12 days, while the control (untreated sample) was 7 days (Besbes *et al.* 2016). Adding 2% grape seed or 2% clove bud extract can delay lipid oxidation and reduce the brightness (L^*), redness (a^*), salt-soluble protein content and total sulfhydryl changes of silver fish fillets at 4 °C. Under 18 days storage, compared with the control group, the shelf life of fish fillets was extended by 3 days (Shi *et al.* 2014; Hu 2014). Algal extracts have also been used for the shelf-life extension of seafood (El-shemy 2020). According to Li *et al.* (2017), during cold storage, the increase of total volatile basic nitrogen (TVB-N), TBARS and K-value (amount of adenosine triphosphate and related compounds) in Pacific white shrimp treated with algae (*Porphyra yezoensis*) extract (5 g/L) inhibit storage (4 °C). Moreover, compared with the control group, the total survival (TVC) and polyphenol oxidase activity in the treated samples were significantly reduced, and the shelf life of the treated samples was extended to 8 days, which was better than the untreated counterparts were 3 days.

For ease of implementation, plant extracts can be incorporated into ice used to preserve seafood. After the ice melts, the active ingredients will be released from the plant extract, thus preserving the stored seafood. Bensid *et al.* (2014) Store a fish in ice containing thyme (0.04% w/v), oregano (0.03% w/v) and cloves (0.02% w/v) respectively. The shelf life of gastrointestinal and decapitated and fish stored in ice containing a single extract is 12 days, while the shelf life of those fish stored in traditional ice is only 5 days. For fish and meat products, it has been established that different Extracts and oils work as better antibacterial than other preservatives (Tassou *et al.* 1995, Hammer *et al.* 1999). Commonly used extracts for fish preservation are oregano (*Origanum vulgare*), thyme (*Thymus vulgaris*) and rosemary (*Rosmarinus officinalis*) however its efficacy depends on different factors like pH, storage temperature, oxygen presence and the concentration of the extract or oil (Tajkarimi *et al.* 2010). Oregano is one such herb that has been approved in the United States as a spice and natural flavor that can reduce oxidation. A recent study reports that oregano extract can maintain the physical, chemical, and sensory receptivity of lamb meat after freezing for 120 days and reduce its lipid and protein oxidation (Fernandes *et al.* 2017). In addition to maintaining the physical and chemical properties of the meat, consumers have also received good sensory acceptance. These results indicate that oregano prepared using multiple extraction methods may be a promising alternative to synthetic food preservatives (Veenstra *et al.* 2019).

Research on the antibacterial and antioxidant properties of Mediterranean aromatic plants concluded that Thymus (*Thymus vulgaris*) is located between these plants and has an inhibitory effect on the growth of all microorganisms. (Piccaglia & Marotti 1993). Some other researchers (Albarracín *et al.* 2012) It is reported that the concentration of thyme essential oil in water, propylene glycol and emulsifier solutions is low (2.5 and 8% respectively), when used in Nile tilapia fillets, at refrigerated temperature and when the fillets are placed, showing highly effective antioxidants immersed in the solution. By using essential oils, the oxidation process of tilapia fillets is reduced by 5.0 to 96.5%. This shows that it has a high efficacy even at low concentrations (Albarracín *et al.* 2012). Silva *et al.* (2013) recently, the antibacterial activity of thyme against ten

food-borne and degenerative bacteria *Bacillus cereus* was demonstrated. *Clostridium perfringens*; *Enterococcus faecalis*; *Escherichia coli*; *Listeria*; *Pseudomonas aeruginosa*; *Salmonella enterica*; *Staphylococcus aureus*; and *Staphylococcus epidermidis*.

Rosemary extract. Rosemary (*Rosmarinus officinalis*), which originates from the Mediterranean, belongs to the Lamiaceae and is one of the largest and most outstanding flowering plant families, including about 236 genera and 6,900-7200 species worldwide (Laham 2013, Hölihan 1985). The Lamiaceae includes many plants containing phenolic acids, such as rosmarinic acid, which have antibacterial, antiviral, antioxidant, and anti-inflammatory properties (Laham 2013). Murcia (southeast of Spain) is one of the main processors and importers of rosemary. Rosemary is a dense shrub with branches, evergreen, and blue-white flowers, reaching a height of about 1 m (Xinfang 1993; Löliger 1991). In the past 20 years, the number of articles about *R. officinalis* L has increased significantly. The interest in this plant has transformed into many studies conducted since 2010, with an average of 120 times a year, and the number tends to increase.

Rosemary extract has been used due to its hepatoprotective effect (Rašković *et al.* 2014); the therapeutic potential of Alzheimer's disease (Habtemariam 2016) and its anti-angiogenic effect (Kayashima *et al.* 2012) to treat diseases. On the other hand, because they can prevent oxidation and microbial contamination, they are used for food preservation (Djenane *et al.* 2002).

In order to obtain bioactive compounds from rosemary, it is necessary to obtain plant extracts or essential oils and perform phytochemical characterization. Use selective solvents and standard procedures to apply the extraction method to the most active part of the plant (leaf, root, stem or flower) (Aruoma *et al.* 1992). These techniques produce complex mixtures in liquid and semi-solid forms or in dry powder form after solvent removal (Inatani *et al.* 1983). The most important factors affecting the extraction process are related to the nature of the plant, the solvent used, temperature, extraction pressure and extraction time (Inatani *et al.* 1983; Cui 2012). There are classic extraction methods, such as Soxhlet extraction, maceration, decocting and infusion; and modern methods, such as supercritical fluid extraction and solid phase microextraction (Aruoma *et al.* 1992, Cui 2012). After analyzing the collected items, the commonly used extraction method is to extract biologically active compounds from medicinal materials, including maceration, hydrodistillation, distillation and Soxhlet extraction.

The main constituents of the rosemary essential oil are camphor (5.0 – 21%), 1,8-cineole (15 – 55%), α -pinene (9.0 – 26%), borneol (1.5–5.0%), camphene (2.5 – 12%), β -pinene (2.0 – 9.0%) and limonene (1.5 – 5.0%) in proportions that vary according to the vegetative stage and bioclimatic conditions (Gordon 1990, Löliger, 1991). Regarding the extracts, the phytochemicals mainly present in *R. officinalis* are rosmarinic acid, camphor, caffeic acid, ursolic acid, betulinic acid, carnosic acid and carnosol (Gordon 1990). Therefore, *R. officinalis* is mainly composed of phenolic compounds, di- and triterpenes, and essential oils (Wenkert 1965).

Other common compounds in rosemary are terpenes, usually present in essential oils and resins, which include over 10,000 compounds divided into mono-, di-, tri- and sesquiterpenes, depending on the number of carbon atoms and isoprene groups (C₅H₈) (Lovkova *et al.* 2001). It is possible to find in rosemary terpenes such as epirosmanol, carnosol, carnosic acid (tricyclic diterpenes), ursolic acid and oleanolic acid (triterpenes) (Gordon 1990). However, the carnosic acid, which is

converted to carnosol by oxidation, has physicochemical, thermal and photolabile properties, which can be avoided by a supercritical fluid extraction (low temperature operation) (Madsen *et al.* 1998).

The antimicrobial properties of essential oils and extracts are closely related to their chemical composition. Phenolic compounds (such as carvacrol, thymol and eugenol) have the highest antibacterial activity (Lovkova *et al.* 2001). Another class of effective active compounds are alcohols: terpineol-4-ol, gamma terpineol, geraniol, citronellol, menthol and linalool. Plants in the Lamiaceae family synthesize many of them (Arraz 2013). According to Fang (1993), rosemary oil resists the Gram-positive bacteria *Staphylococcus aureus*, *Enterococcus faecalis* molecules and *Listeria monocytogenes*, as well as the Gram-negative bacteria *Escherichia coli*. The antibacterial effects of *Erseria* and *Salmonella* are food-borne bacterial strains. Mihajilov *et al.* (2019) Shows that essential oils and extracts mainly contain carvacrol (67.0%) and -terpinene (15.3%), such as basil and rosemary, which are effective against Gram-negative strains (including *E. coli*).

The antimicrobial effect is the result of the action of the principal rosemary compounds: rosmarinic acid, carnosol, rosmaridiphenol, rosmanol, epirosmanol, and isorosmanol. These compounds interact with the cell membrane, causing changes in the genetic material and nutrients, altering the transport of electrons, leakage of cellular components and changes in fatty acid. In addition, it produced an interaction with the membrane of proteins that produce the loos of membrane functionality and its structure (Arraz 2013). (Vegara *et al.* 2011) reported that the effectiveness of carnosic acid against pathogenic bacteria is superior to that of any other major extra component, including rosmarinic acid. Rosemary was tested in different food studies. Gómez-Estaca *et al.* 2010 reported that rosemary inhibited the growth of common food bacteria contributing to food spoilage.

The antibacterial effect of rosemary has been demonstrated in different food studies: beef meatballs (Fernandez & López 2005), cooked beef (Ahn 2007), Frankfurters (Resurreccion 1990). Also, Rosemary contains several antioxidants, mainly phenolic acids, flavonoids and diterpenoids. Höulihaan *et al.* (1984) and Wu *et al* (1982) determined that the antioxidant properties of rosemary were due to its rich content of isoprenoid quinones, which acted as a chain terminator of free radicals and a chelator of reactive oxygen species (Rosemary Extract). In addition, Gordon pointed out in 1990 that the phenolic compounds present in commercial extracts of rosemary act as the main antioxidants when they react with lipids and hydroxyl radicals to convert them into stable products. According to Löliger (1991), carnosic acid and carnosol act as potent scavengers of peroxy radicals. This fact explains the conclusions obtained by Chen *et al.* 1992, who confirmed that the effect of both compounds on peroxidation of membrane lipids is higher than the effect reported by artificial antioxidants such as BHA, BHT and propyl gallate (Arouma 1992).

One of the most important aspects of the antioxidant activity of rosemary is between diterpene and free radical scavenging activity. In this regard, a (2001) study by Munné-Bosch and Alegre described the antioxidant capacity of diterpenes in rosemary. The most important element in the structure of rosemary is the aromatic ring (C11-C12) in the catechol group and the conjugation of the three basic rings. The catechol group is responsible for scavenging free radical electrons formed by oxidation. The skeleton formed by the three loops allows charge delocalization. The presence of carboxyl groups (in the case of creatine) will increase this conjugation, especially in aqueous systems. However, in a less polar medium such as fat, the lactone structure seems to have greater stability.

Creatine, inositol, rosmannol and epirosmannol are the main phenolic diterpenes responsible for the antioxidant properties of rosemary (Nieto & Castillo 2018). Wijerante *et al.* (2007) reported that creatine and carnosol inhibit lipids, respectively. The percentage of peroxidation is 88-100% and 38-89% under oxidative stress conditions. Generally, the antioxidant effect of natural extracts is higher than synthetic antioxidants, regardless of the medium, which differs in water or oil.

Table 6 shows a summary of the literature review of natural oils and extracts used for seafood preservation. Essential oil or extract name, benefits and the source of the information are presented in the table below. The essential oil/extract presented are cactus peel, grape seed, algal, thyme, oregano, and rosemary.

Table 6. Summary of essential oil/extract used for seafood preservation.

Essential oil / Extract	Benefit as food preservative	Source
Cactus peel	Extends shelf life, reduce TBARS and TMA.	(Besbes <i>et al.</i> 2016; Brul 1999; Lazar <i>et al.</i> 2010)
Grape seed	Delay lipid oxidation, reduce brightness (L*), redness (a*).	(Besbes <i>et al.</i> 2016; Burt 2004; Min hu 2004)
Algal	Extend shelf life; reduce TVC and polyphenol oxidase activity.	(Li <i>et al.</i> 2017; Tassou <i>et al.</i> 1995)
Thyme	Reduce oxidation process, antibacterial properties against the ten food-borne and <i>Bacillus cereus</i>	(Bensid <i>et al.</i> 2014; Piccaglia & Marotti 1993, Albarracín <i>et al.</i> 2012)
Oregano	Maintain lipid and protein oxidation; maintain physical and chemical properties of the product, good sensory acceptance by consumers.	(Tajkarimi <i>et al.</i> 2010; Fernandes <i>et al.</i> 2017; Veenstra <i>et al.</i> 2019)
Rosemary	Prevent oxidation and microbial contamination; resist Gram-positive bacteria <i>Staphylococcus aureus</i> , <i>Enterococcus faecalis</i> molecules and <i>Listeria monocytogenes</i> , as well as the Gram-negative bacteria <i>Escherichia coli</i> .	(Lovkova <i>et al.</i> 2001; Mihajilov <i>et al.</i> 2019; Arraz 2013; Vegara <i>et al.</i> 2011; Fernandez & López 2005; Ahn 2007; Resurreccion 1990)

Preliminary test

Microbiological analysis

Total plate count. For the FDA, 2020. The total number of plates is very important because it not only indicates good manufacturing practices in the feeding, harvesting and processing process, but also indicates the level of viable microorganisms that can produce colonies in the product during analysis (Maturin & Peeler 2001). Total plate count growth of Head-on presentation TRT1, TRT2, TRT3 and control 1 are presented in Figure 2. The results presented for TRT1 and control 1 were of days 0, 6 and for TRT2 and TRT3 of days 0, 6 and 12. Figure 2 shows that all of the treatments plus the control growth in a similar way. TRT1 and Control were analyzed at days 0 and 6 presenting the same count at day 6 of equal or more than 25000 CFU/g, TRT1 and Control were discarded because of extreme damage signs. TRT2 and TRT3 presented the same count at days 6 and 12, the results showed that these two treatments count were equal or more than 25000 CFU/g.

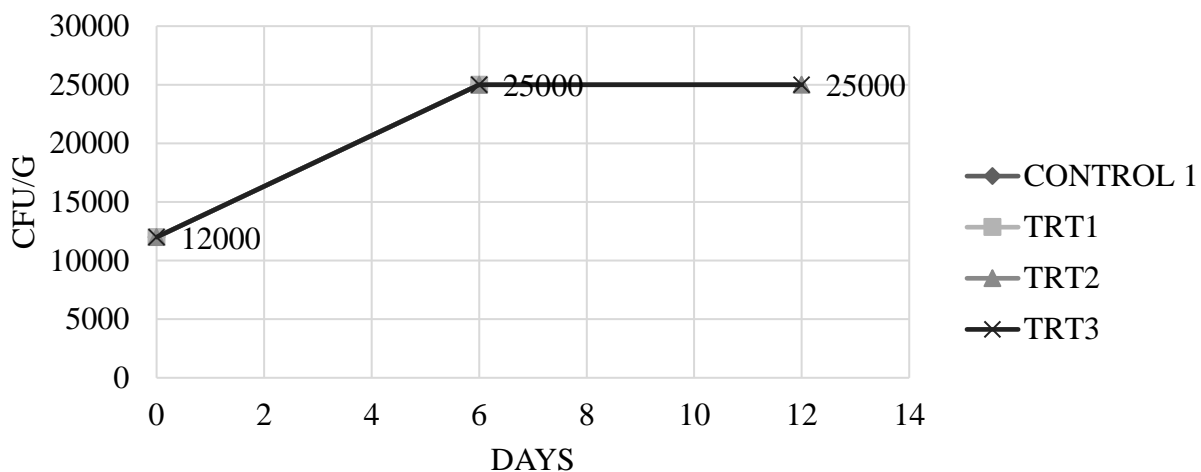


Figure 2. Head-on (HO) presentation, Control 1: Head-on shrimp + polybag, TRT1: Head-on shrimp + rosemary extract + polybag, TRT2: Head-on shrimp + rosemary extract + vacuum packaging, TRT3: Head-on shrimp + vacuum packaging, CFU/g Colonies Forming Units per gram.

Total plate count results of Headless shrimp presentation during 0, 6 and 12 days storage for TRT4, TRT5, TRT6 and Control 2 are presented in Figure 3, It shows that total plate count was maintained equal or more than 25000 CFU/g in all of the treatments including the control in the different days evaluated (0, 6, 12).

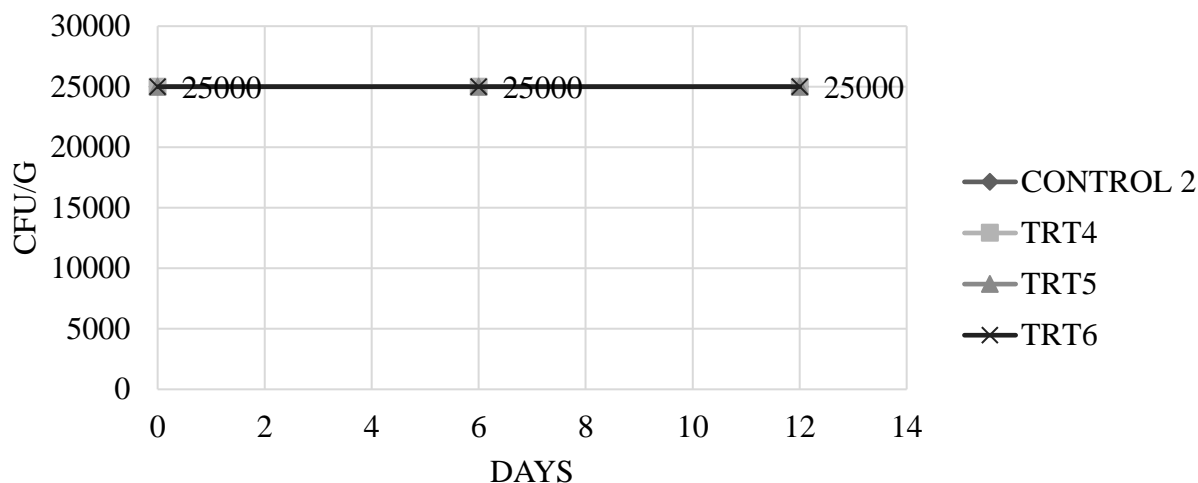


Figure 3. Headless (HL) presentation all treatments, Control 2: Headless shrimp + polybag, TRT4: Headless shrimp + polybag+ rosemary extract, TRT5: Headless shrimp + rosemary extract + vacuum, TRT6: Headless shrimp +vacuum, total plate count during time days (0, 6 and 12), CFU/g Colonies Forming Units per gram.

The total plate count analysis for Peeled and deveined presentation, done at days 0, 6 and 12 for TRT7, TRT8, TRT9 and Control 3 can be found in Figure 4. Figure 4 shows that the control 3, TRT7, TRT8 and TRT9 begun at day 0 with 25000 CFU/g and then maintained a total plate count of equal or more than 25000 CFU/g in all of days 6 and 12.

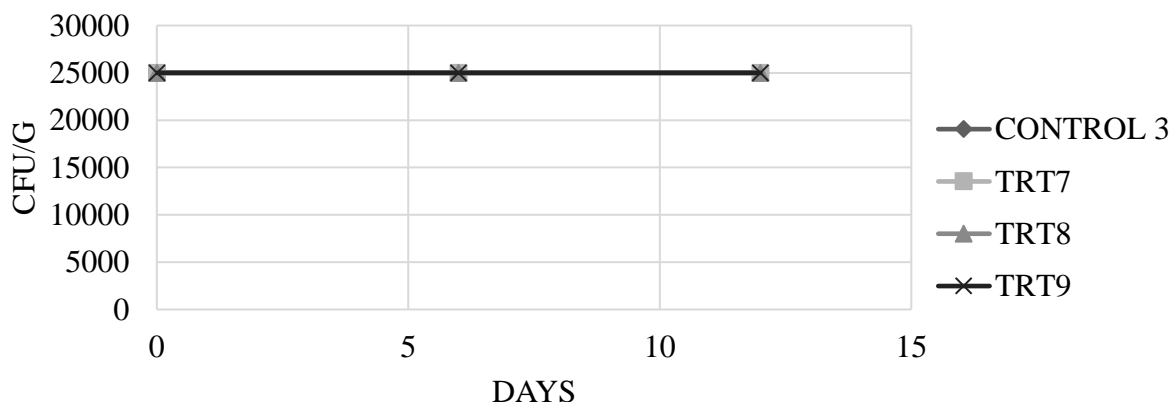


Figure 4. Peeled and deveined (PD) presentation all treatments, Control 3: Peeled and deveined shrimp + polybag, TRT7: Peeled and deveined shrimp + rosemary + polybag, TRT8: Peeled and deveined + rosemary extract + vacuum, TRT9: Peeled and deveined shrimp + vacuum, total plate count during time days (0, 6, and 12), CFU/g: Colonies Forming Units per gram.

All shrimp presentations (Head-on, Headless, Peeled, and deveined) for all treatments Total plate count are presented in Table 7. It can be observed that the total plate number of head-on (HO)

shrimp on day 0 is lower than that of headless (HL), peeled, and deveined (PD) shrimp. This result could be associate with an incorrect handling and processing inside the plant for Headless and Peeled and deveined presentations.

According to the FDA (2019), “a very low “total” count may therefore lead to false conclusions about the hygienic quality of the product”. The TPC test can be used to measure the conditions of raw materials, the effectiveness and sanitary conditions of procedures during processing, the sanitary conditions of equipment and utensils, and the time x temperature curve during storage and distribution (Huss 1994).

Headless and Peeled and Deveined presentations had the same CFU/g at the day 0. For days, 6 and 12 all the presentations and treatments maintained the same count. Is important to mention that during the three days of analysis all the presentations and treatments maintain the limits proposed as good quality by the company HACCP PLAN (<100000CFU/g). In a similar study done by Irkin (2011) in minced beef meat at 4 degrees Celsius, total plate counts in vacuum packaging were found insignificantly ($P > 0.05$) from the control samples. The highest values were obtained for control samples.

Table 7. Total plate count during 0, 6, and 12 days.

Samples	Total plate count CFU/g		
	Day 0	Day 6	Day 12
HO-Control 1	12000	≥ 25000	
HO-TRT1	12000	≥ 25000	
HO-TRT2	12000	≥ 25000	≥ 25000
HO-TRT3	12000	≥ 25000	≥ 25000
HL-Control 2	≥ 25000	≥ 25000	≥ 25000
HL-TRT4	≥ 25000	≥ 25000	≥ 25000
HL-TRT5	≥ 25000	≥ 25000	≥ 25000
HL-TRT6	≥ 25000	≥ 25000	≥ 25000
PD-Control 3	25000	≥ 25000	≥ 25000
PD-TRT7	25000	≥ 25000	≥ 25000
PD-TRT8	25000	≥ 25000	≥ 25000
PD-TRT9	25000	≥ 25000	≥ 25000

HO: Head-on, HL: Headless, PD: Peeled and deveined. Control 1: Head-on shrimp + polybag, TRT1: Head-on shrimp + rosemary extract + polybag, TRT2: Head-on shrimp + rosemary extract + vacuum packaging, TRT3: Head-on shrimp + vacuum packaging, Control 2: Headless shrimp + polybag, TRT4: Headless shrimp + rosemary extract + polybag, TRT5: Headless shrimp + rosemary extract + vacuum, TRT6: Headless shrimp + vacuum, Control 3: Peeled and deveined shrimp + polybag, TRT7: Peeled and deveined shrimp + rosemary extract + polybag, TRT8: Peeled and deveined shrimp + rosemary extract+ vacuum, TRT9: Peeled and deveined shrimp + vacuum, CFU/g: Colony-forming units per gram, ≥: equal or more than.

Total coliforms. Coliform flora, gram-negative, rod-shaped facultative anaerobic or aerobic, non-spore formation and lactose-fermenting bacteria are the commonly used indicators. The total

number of coliforms is the count of coliforms and fecal coliforms. The coliforms are distributed in the environment, while the coliforms are present in the intestines of warm or cold-blooded vertebrates (Tortorello *et al.* 2015). The organisms considered coliforms are *Escherichia coli*, *Enterobacter*, *Klebsiella*, *Serratia*, *Citrobacter* and *Proteus* (Soccol 2014).

In Figure 5 total coliforms for Head-on shrimp presentation TRT1, TRT2, TRT3 and control 1 are presented. The analysis was done at days 0, 6 and 12. Figure 5 shows that at day 6, TRT2 presented the lowest total coliforms count, and Control 1 presented the highest total coliforms count. At day 12, TRT3 and TRT2 presented equal or more than 25000 CFU/g. It is important to mention that Control 1 and TRT1 been analyzed at day 0 and 6, after day 6 the samples were discarded because extreme damage sings.

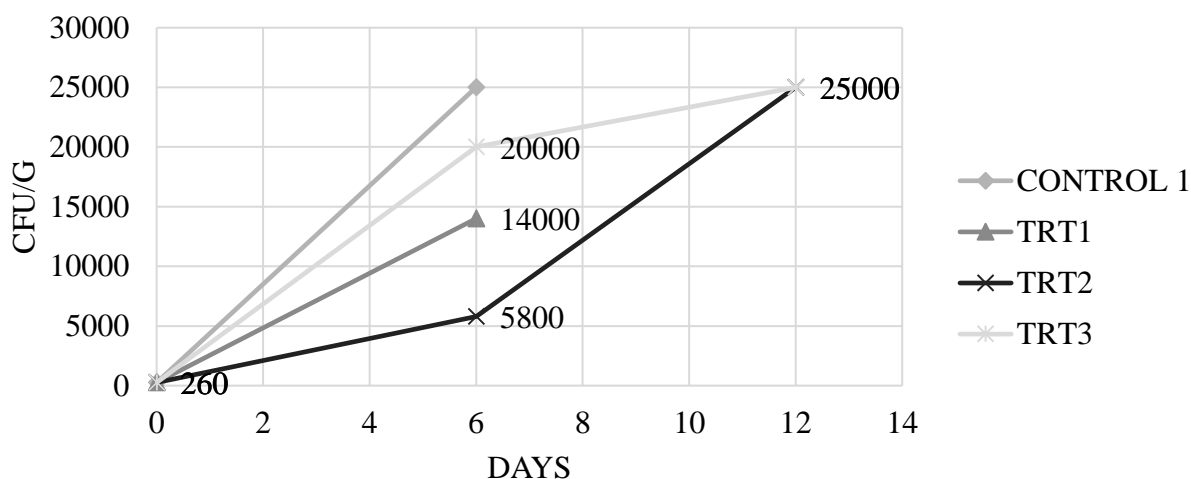


Figure 5. Head-on (HO) presentation all treatments, Control 1: polybag only; TRT1: Head-on shrimp + rosemary extract + polybag, TRT2: Head-on shrimp + rosemary extract + vacuum, TRT3: Head-on shrimp + vacuum, total coliforms count during time days (0, 6 and 12), CFU/g Colonies Forming Units per gram.

Figure 6 shows Headless shrimp presentation Total coliform count. Treatments analyzed were TRT4, TRT5, TRT6 and control 2. The analysis was done at days 0, 6, and 12. Figure 6 shows that the lowest total coliforms count at day 6 was presented by TRT5 and the highest count by TRT6. At day 12, all the treatments plus the control showed more than or equal of 25000 CFU/g.

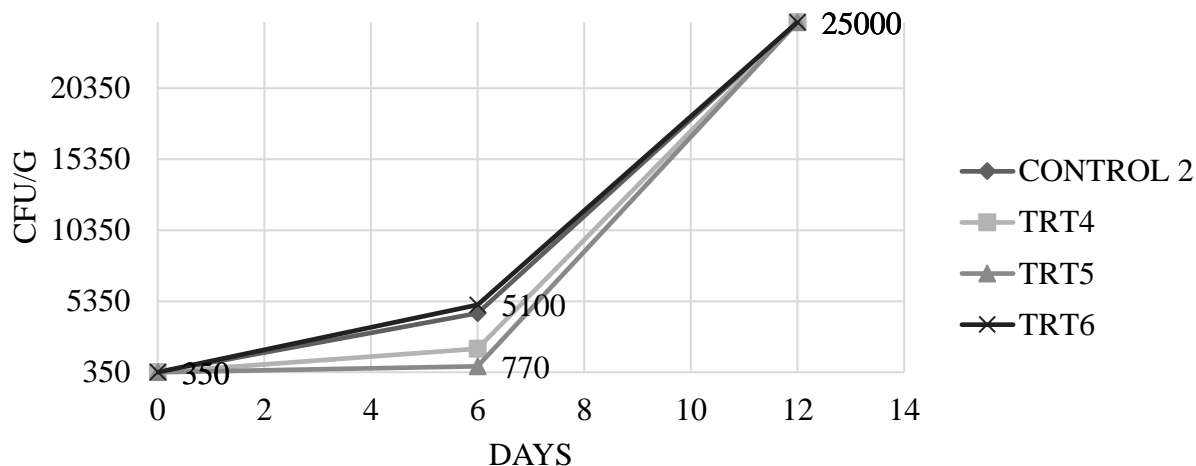


Figure 6. Headless (HL) all treatments, Control 2: Headless shrimp + polybag, TRT4: Headless shrimp + rosemary extract + polybag, TRT5: Headless shrimp + rosemary extract + vacuum, TRT6: Headless shrimp + vacuum, CFU/g Colonies Forming Units per gram.

Figure 7 shows Peeled and deveined shrimp presentation total coliform count during days 0, 6 and 12. In Figure 7. We can observe that all the treatments and control 3 maintained the same count at day 0. TRT7 and TRT9 maintained the same count at day 6 and 12. In addition, TRT7 and control 3 maintained the same count at day 6 and 12. We can observe that at day 6, TRT 7 and control 3 showed the lowest count of total coliforms CFU/g. At day 12, all the treatments (7, 8, 9) and the control 3 showed equal or more than 25000 CFU/g.

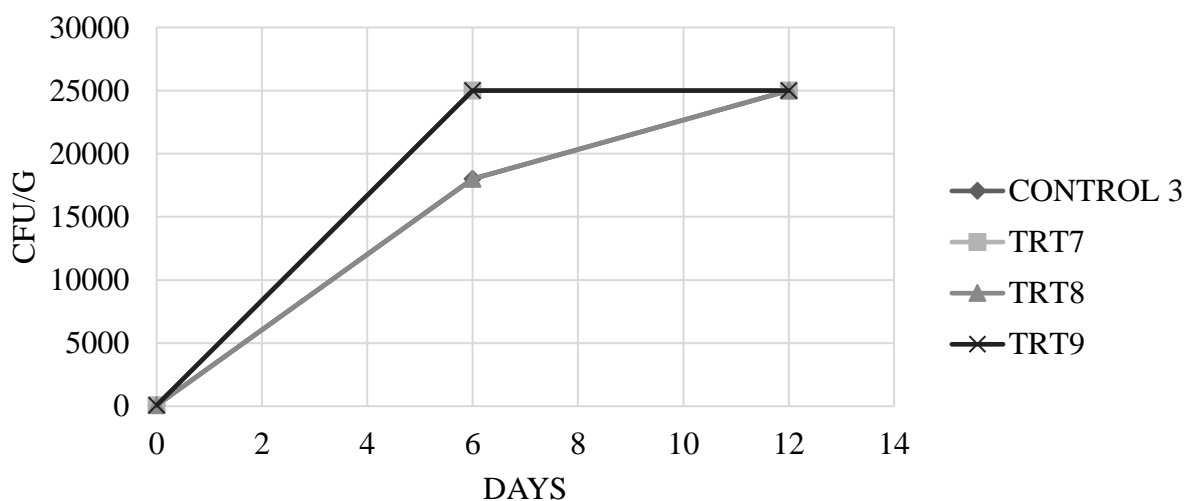


Figure 7. Peeled and deveined (PD) all treatments, Control 3: Peeled and deveined shrimp + polybag, TRT7: Peeled and deveined shrimp + rosemary extract + polybag, TRT8: Peeled and deveined shrimp + rosemary extract + vacuum, TRT9: Peeled and deveined shrimp + vacuum, CFU/g Colonies Forming Units per gram.

All shrimp presentations (Head-on, Headless, Peeled, and deveined) Total coliforms count are presented in Table 8. In all the different treatments it is noticeable the difference in the microbial growth. At day 0, Peeled and deveined presentation showed the lowest count. At day 6 head-on (HO) TRT2 (vacuum + rosemary extract), headless (HL) TRT5 (vacuum + rosemary extract) and peeled and deveined (PD) TR8 (vacuum + rosemary extract) showed the lowest CFU/g values. Then they continue growing up. At day 12 all the treatments of all the presentations, showed equal or more than 25000 CFU/g.

Table 8. Total coliforms count during 0, 6, and 12 days HO: Head-on, HL: Headless, PD: Peeled and deveined.

Samples	Total coliforms CFU/g		
	Day 0	Day 6	Day 12
HO-Control 1	260	≥ 25000	
HO-TRT1	260	14000	
HO-TRT2	260	5800	≥ 25000
HO-TRT3	260	20000	≥ 25000
HL-Control 2	350	4500	≥ 25000
HL-TRT4	350	2000	≥ 25000
HL-TRT5	350	770	≥ 25000
HL-TRT6	350	5100	≥ 25000
PD-Control 3	70	18000	≥ 25000
PD-TRT7	70	≥ 25000	≥ 25000
PD-TRT8	70	18000	≥ 25000
PD-TRT9	70	≥ 25000	≥ 25000

Control 1: Head-on shrimp + polybag, TRT1: Head-on shrimp + rosemary extract + polybag, TRT2: Head-on shrimp + rosemary extract + vacuum packaging, TRT3: Head-on shrimp + vacuum packaging, Control 2: Headless shrimp + polybag, TRT4: Headless shrimp + rosemary extract + polybag, TRT5: Headless shrimp + rosemary extract + vacuum, TRT6: Headless shrimp + vacuum, Control 3: Peeled and deveined shrimp + polybag, TRT7: Peeled and deveined shrimp + rosemary extract + polybag, TRT8: Peeled and deveined shrimp + rosemary extract + vacuum, TRT9: Peeled and deveined shrimp + vacuum, CFU/g: Colony-forming units per gram, ≥ equal or more than.

Rosemary + polybag and rosemary + vacuum in all shrimp presentations were the ones with the slowest microbial growth during all the experiment. These results can be associated with the presence of rosemary extract. The antimicrobial properties of rosemary extract allow controlling the microbial growth during storage. Elżbieta *et al.* (2011) who in their study tittle the effect of rosemary preparations on the microbial quality and TBARS value of model pork batter found that with the use of rosemary as an antimicrobial the coliforms count growth in a slowest way than the control.

Between rosemary + polybag and rosemary + vacuum, the slowest microbial growth was found on (rosemary + vacuum). This result could be associated with the vacuum technology that control different kind of bacteria growth. Similar results were found in the study of filleted rainbow trout and Baltic herring done by Randell *et al.* (1995), they found that vacuum packaging extend slightly

the microbiological shelf life of trout fillets. Other study that showed similar results was in minced beef storage at 4 degrees Celsius done by Irkin (2011); he shows that coliforms count in vacuum samples were different statically from the control samples. TRT2 contains rosemary extract and vacuum, it means that the combination of these two technologies' may be the reason of the slowest coliforms growth but there is no literature that proved this yet. Headless shrimp presentation TRT4 and TRT5 were the only one that maintain the CFU/gr admitted by the HACCP Plan American Mariculture, Inc. until day 6.

E. coli. According to the FDA (2019), “*E. coli* are mostly harmless bacteria that live in the intestines of people and animals and contribute to intestinal health. However, eating or drinking food or water contaminated with certain types of *E. coli* can cause mild to severe gastrointestinal illness”. “Due to its high prevalence in the gut, *E. coli* is used as the preferred indicator to detect and measure fecal contamination in the assessment of food and water safety” FAO (2011).

In Table 9 all shrimp presentations (Head-on, Headless, Peeled and deveined) *E. coli* count are presented. The Analysis was done at days 0, 6 and 12 during storage. Table 9 indicates that all shrimp presentations and all the treatments present < 10 CFU/gr during days 0, 6 and 12. This value is inside the limits approved by the HACCP PLAN American Mariculture, Inc. (< 3 - < 10 CFU/gr).

Table 9. *E. coli* count during days 0, 6, and 12.

Samples	<i>E.coli</i> CFU/g		
	Day 0	Day 6	Day 12
HO-Control 1	<10	<10	
HO-TRT1	<10	<10	
HO-TRT2	<10	<10	<10
HO-TRT3	<10	<10	<10
HL-Control 2	<10	<10	<10
HL-TRT4	<10	<10	<10
HL-TRT5	<10	<10	<10
HL-TRT6	<10	<10	<10
PD-Control 3	<10	<10	<10
PD-TRT7	<10	<10	<10
PD-TRT8	<10	<10	<10
PD-TRT9	<10	<10	<10

HO: Head-on, HL: Headless, PD: Peeled and deveined. Control 1: Head-on shrimp + polybag, TRT1: Head-on shrimp + rosemary extract + polybag, TRT2: Head-on shrimp + rosemary extract + vacuum packaging, TRT3: Head-on shrimp + vacuum packaging, Control 2: Headless shrimp + polybag, TRT4: Headless shrimp + rosemary extract + polybag, TRT5: Headless shrimp + rosemary extract + vacuum, TRT6: Headless shrimp + vacuum, Control 3: Peeled and deveined shrimp + polybag, TRT7: Peeled and deveined shrimp + rosemary extract + polybag, TRT8: Peeled and deveined shrimp + rosemary extract+ vacuum, TRT9: Peeled and deveined shrimp + vacuum, CFU/g: Colony-forming units per gram

These results could be associated with the application of Good agricultural practices, Good manufacturing practices, Good hygiene practices and Hazard analysis critical control point (HACCP) in all the chain. As FAO (2011) mention, “Prevention and control require a multidisciplinary approach in animal and plant production as well as risk-based approaches along the entire food supply chain. These include the application of Good Agricultural Practices (GAP), Good Manufacturing Practices (GMP), Good Hygiene Practices (GHP) and Hazard Analysis Critical Control Point (HACCP) from the farm to the consumer”.

Processed food may be contaminated by raw materials, unsanitary water treatment and treatment, and cross-contamination (FDA 2001). Sales (2020) and Sienkiewics *et al.* (2013) demonstrated in their studies the effectiveness of rosemary extract on *E. coli* control due to its antimicrobial properties. In the other hand, studies shown that vacuum packaging control *E. coli* growth. *E. coli* count is a useful indicator of the quality of vacuum-packed meat. High *E. coli* counts (greater than 100 CFU per g) on stored meat could indicate temperature abuse because *E. coli* does not grow below 7 °C. A high *E. coli* count may also indicate a food safety issue (Egan 1988).

Staphylococcus Aureus. Due to the combination of toxin-mediated virulence and antibiotic resistance, *Staphylococcus aureus* is considered an important pathogen. Table 10 shows *S. Aureus* count during 0, 6 and 12 days of storage for all presentations (Head-on, Headless, Peeled and deveined). Results shows that all the shrimp presentations and treatments maintain a CFU/g of < 10 at days 0, 6 and 12.

Table 10. *S. Aureus* count during days 0, 6, and 12.

Samples	<i>S. Aureus</i> CFU/g		
	Day 0	Day 6	Day 12
HO-Control 1	<10	<10	
HO-TRT1	<10	<10	
HO-TRT2	<10	<10	<10
HO-TRT3	<10	<10	<10
HL-Control 2	<10	<10	<10
HL-TRT4	<10	<10	<10
HL-TRT5	<10	<10	<10
HL-TRT6	<10	<10	<10
PD-Control 3	<10	<10	<10
PD-TRT7	<10	<10	<10
PD-TRT8	<10	<10	<10
PD-TRT9	<10	<10	<10

HO: Head-on, HL: Headless, PD: Peeled and deveined. Control 1: Head-on shrimp + polybag, TRT1: Head-on shrimp + rosemary extract + polybag, TRT2: Head-on shrimp + rosemary extract + vacuum packaging, TRT3: Head-on shrimp + vacuum packaging, Control 2: Headless shrimp + polybag, TRT4: Headless shrimp + rosemary extract + polybag, TRT5: Headless shrimp + rosemary extract + vacuum, TRT6: Headless shrimp + vacuum, Control 3: Peeled and deveined shrimp + polybag, TRT7: Peeled and deveined shrimp + rosemary extract + polybag, TRT8: Peeled and deveined shrimp + rosemary extract+ vacuum, TRT9: Peeled and deveined shrimp + vacuum, CFU/g: Colony-forming units per gram.

This facultative anaerobic Gram-positive bacterium is one of the main causes of hospital infections. *Staphylococcus aureus* is usually found in the nostrils, skin, or hair of warm-blooded animals (Le Loir *et al.* 2003). Results could be associated with the storage temperature 0.55 ± 1 °C (33 ± 1 °F) that is under the temperature that allow *S. aureus* growth. According to (Schmitt *et al.* 1990) *S. aureus* growth temperature, range is from 7 to 48.5 °C (44.6 – 119.3 °F). In addition, the results founded may be associated with the vacuum packaging and rosemary extract use. Christiansen *et al.* (1965) mentioned the growth of *S. aureus* was markedly inhibited by vacuum packaging obtained similar results. In other study, Issabeagloo *et al.* (2012) results founded that rosemary showed synergistic activity against *S. aureus* and *L. monocytogenes*.

Salmonella. According to the FDA (2020), “*Salmonella* is a group of bacteria that can cause gastrointestinal diseases and fever, called salmonellosis. Food handlers who do not wash their hands and/or use surfaces and tools between food preparation steps when people eat *Salmonella* can be spread from raw or undercooked food. *Salmonella* can also be spread from animals to people”. In Table 11 results shows that *Salmonella* was absent for all shrimp presentations (Head-on, Headless, Peeled, and deveined) in all the treatments and controls.

Table 11. *Salmonella* presence/absence during days 0, 6, and 12.

Samples	<i>Salmonella</i> CFU/g		
	Day 0	Day 6	Day 12
HO-Control 1	Absent	Absent	
HO-TRT1	Absent	Absent	
HO-TRT2	Absent	Absent	Absent
HO-TRT3	Absent	Absent	Absent
HL-Control 2	Absent	Absent	Absent
HL-TRT4	Absent	Absent	Absent
HL-TRT5	Absent	Absent	Absent
HL-TRT6	Absent	Absent	Absent
PD-Control 3	Absent	Absent	Absent
PD-TRT7	Absent	Absent	Absent
PD-TRT8	Absent	Absent	Absent
PD-TRT9	Absent	Absent	Absent

HO: Head-on, HL: Headless, PD: Peeled and deveined. Control 1: Head-on shrimp + polybag, TRT1: Head-on shrimp + rosemary extract + polybag, TRT2: Head-on shrimp + rosemary extract + vacuum packaging, TRT3: Head-on shrimp + vacuum packaging, Control 2: Headless shrimp + polybag, TRT4: Headless shrimp + rosemary extract + polybag, TRT5: Headless shrimp + rosemary extract + vacuum, TRT6: Headless shrimp + vacuum, Control 3: Peeled and deveined shrimp + polybag, TRT7: Peeled and deveined shrimp + rosemary extract + polybag, TRT8: Peeled and deveined shrimp + rosemary extract + vacuum, TRT9: Peeled and deveined shrimp + vacuum, CFU/g: Colony-forming units per gram.

Salmonella absence during days 0, 6 and 12 in all treatments and presentations could be associated with the temperature used during storage 0.55 ± 1 °C (33 ± 1 °F) which is under the appropriate conditions for *Salmonella* growth. According to the Appendix 3 (Bacterial Pathogen Growth and

Inactivation) by FDA, the limit condition for *Salmonella* growth is 5.2 - 46.2 °C (41.4 – 115.2 °F) in a facultative anaerobic environment. In addition, studies showed that vacuum packaging and rosemary extract were able to control *Salmonella* growth. Jiyoun K. & Kyung (2004), in their study effect of vacuum packaging on the microbial profile of chilled chicken during storage found that Vacuum packaging effectively retarded microbial growth of total bacteria, *Pseudomonas*, mold and yeast, and *Salmonella*. In the other hand, many authors studied Rosemary extract antimicrobial activity against *Salmonella*. Abramovi *et al.* (2012) in their study titled antioxidant and antimicrobial activity of extracts obtained from rosemary (*Rosemarinus officinalis*) and vine (*Vitis vinifera*) leaves. Confirmed the antimicrobial activity of rosemary extract by the broth microdilution test using minimal inhibitory (MIC) concentrations against gram-positive (*Staphylococcus aureus*, *Bacillus cereus*, *Listeria monocytogenes*) and gram-negative bacteria (*Campylobacter jejuni*, *Salmonella*, *Escherichia coli* O157:H7).

Color analysis

When discussing quality, color is an important feature. The color of the body and exoskeleton depends on different factors, especially the increase in nutrition. Carotenoids are fat-soluble pigments that give shrimps a unique color. Astaxanthin is the main carotenoid responsible for the pigmentation of seafood (Wang *et al.* 2006). Shrimp cannot produce carotenoids by themselves, so they are obtained through nutrition. In the wild and farm-raised environment, shrimp absorb different microorganisms, such as microalgae rich in carotenoids. In addition, carotenoids can be added to the diet of shrimp. To ensure a good color at harvest, 50-100 ppm (mg/kg) of astaxanthin should be included in the finishing diet (Meyers and Latscha 1997; Wan *et al.* 2006). According to (Haard 1992), shrimp carotenoids are sensitive to oxidation, which is why the color disappears and changes significantly in a cold atmosphere.

The color loss occurs due to the oxidation of the unsaturated bonds of astaxanthin (Niamnuy *et al.* 2008). Another important sign that determines damage is the known black spots or melanosis, which is caused by the oxidation of phenolic compounds into quinines because of the phenoloxidase action. This endogenous enzyme can work under cold storage and coagulation conditions. The main signs of melanosis are small black spots around the stomach, feet and head and chest of the shrimp (Haard 1992). The data analyzed presented in Figure 8 correspond to Headless (HL) presentation TRT 4. Results shows that at day 1, variables L*, a* and b* presented variations. In the case of L* value, it maintained with no variation since day 2 until day six. a* and b* maintained their values with no variation until day 5 and at day 6, presented variations.

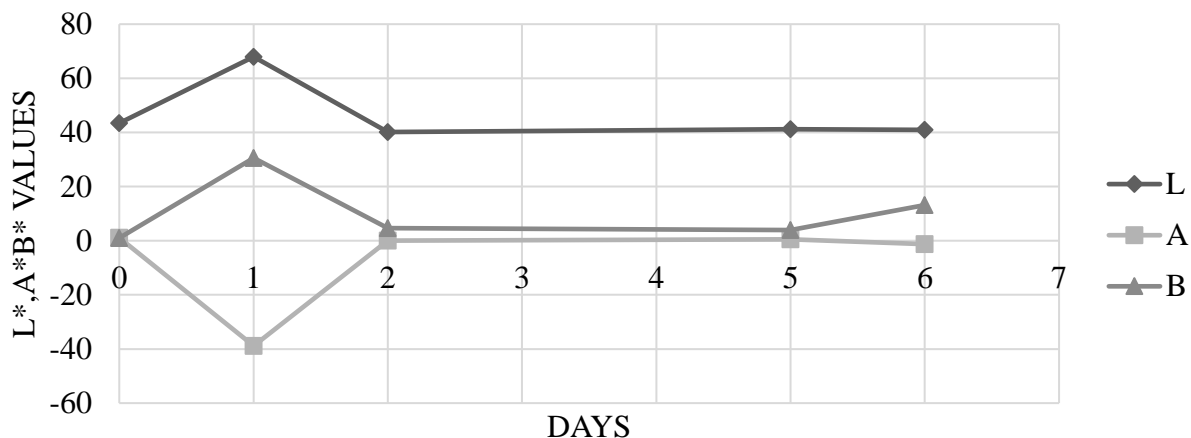


Figure 8. Headless (HL) shrimp presentation, TRT4: rosemary extract + polybag, L*(0 to 100), a*(-60 to 60) b*(-60 to 60) color change between days.

Headless shrimp presentation TRT5 values L*(0 to 100), a*(-60 to 60) b*(-60 to 60) during 6 days of storage are presented (Figure 9). Figure 9 shows that L* value maintained with no variation until day 1, then presented a low increase at day 2 and maintained with the increase until day 5 and finally decrease at day 6. a* value maintained with no variation until day 1 and the showed decrease ad day 2, from day 2 to day 5 showed no changes and then at day 6 showed increase. b* value decrease a little at day 1, then at day 2 presented an increase and from day 2 to day 5 maintained with no changes, finally at day 6 showed a high decrease.

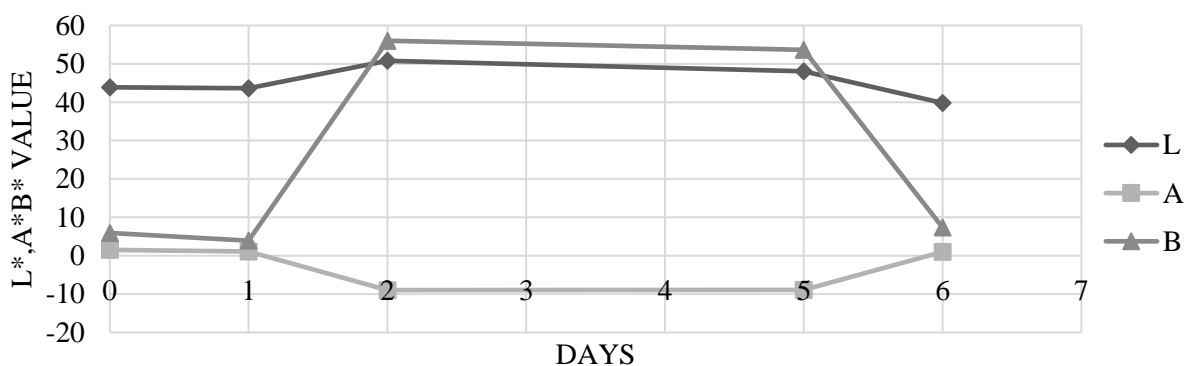


Figure 9. Headless (HL) shrimp presentation, TRT5: Headless shrimp + rosemary extract + vacuum, L*(0 to 100), a*(-60 to 60) b*(-60 to 60) color change between days.

In Table 12. Statistical analysis for L*, a*b*, L*(0 to 100), a*(-60 to 60) b*(-60 to 60) values are presented. Shrimp presentation used was Headless in TRT5: Headless shrimp + rosemary extract + vacuum. Variables L* (Luminosity) and b* (yellow-blue) are the ones that been affected by the treatment.

Table 12. Headless (HL) shrimp presentation TRT4 and TRT5 L*, a*b* statistical analysis.

	R				PROBABILITY				
	F	SQUARE	V.C.	MEAN	MODEL	TRT	REP	DAY	TRT*DAY
L*	1.83	0.397866	6.01 %	43.713	0.076	0.036	0.297	0.298	0.087
a*	0.65	0.190207	2.32 %	-0.280	0.795	0.509	0.240	0.866	0.991
b*	3.95	0.587594	3.93 %	-6.871	0.001	0.000	0.225	0.000	0.761

L* lightness (0 to 100), a* positive value =red, negative value=green (-60 to 60), b* positive value=yellow, negative value=blue (-60 to 60), V.C.: variation coefficient, TRT: treatment, REP: repetitions, TRT*DAY: interaction between treatment and day, PROBABILITY: (P < 0.05), TRT4: Headless shrimp presentation + rosemary extract + polybag, TRT5: Headless shrimp + rosemary extract + vacuum.

Considering that TRT4 (rosemary extract + polybag) and TRT5 (rosemary extract + vacuum) were treated with rosemary, this result means that the different packaging used, polybag and vacuum may be the ones that made L* and b* values change. In addition, it is important to notice that any of the variables (L*, a* b*) been affected by the time storage. Interaction TRT*DAY was no significant for any variable.

Table 13 shows that L* and a* b* of TRT5 (headless shrimp + rosemary extract + vacuum) values haven't show statistically difference and don't presented changes during the 6 days analyzed. In the other hand, TRT4 (rosemary extract + polybag) showed changes in b* value during the days analyzed.

Madhusudana *et al.* (2017) found that the consumers preference for fresh raw shrimp is (L* 37.1 ± 14 a, a* 4.8 ± 0.3 b, b* 10.2 ± 0.5a), in the results, the treatment that maintained similar results with Madhusudana *et al.* (2017) was TRT5 (headless shrimp + rosemary extract + vacuum). This result was similar to Senepati *et al.* (2017) analysis, where yellowness increase (b* 8.93) after 5th day of storage under MAP, also found that the control (no MAP) changed its color in yellow, attributing this change to the oxygen presence.

Table 13. Headless (HL) shrimp presentation TRT4 and TRT5 L*, a* b* Duncan media separation per day.

			Color Analysis L*, a*, b*				
V.C.			Day 0	Day 1	Day 2	Day 5	Day 6
			Mean \pm S.D.	Mean \pm S.D.	Mean \pm S.D.	Mean \pm S.D.	Mean \pm S.D.
TRT4	L*	15.73%	43.74 \pm 6.32 a	43.56 \pm 7.19 a	40.15 \pm 3.52 a	41.07 \pm 6.74 a	41.00 \pm 9.08 a
	a*	772.15%	(0.22) \pm 0.55 a	0.51 \pm 1.63 a	0.12 \pm 1.08 a	0.45 \pm 1.67 a	(0.41) \pm 1.09 a
	b*	24.59%	4.82 \pm 2.24 b	3.95 \pm 1.64 b	4.54 \pm 0.59 b	4.14 \pm 1.24 b	9.29 \pm 0.84 a
TRT5	L*	12.17%	43.80 \pm 3.76 ab	43.50 \pm 6.37 ab	52.08 \pm 3.95 a	49.23 \pm 5.57 a	39.53 \pm 8.11 b
	a*	349.64%	0.69 \pm 0.62 a	0.63 \pm 1.20 a	0.61 \pm 1.76 a	0.25 \pm 1.76 a	(0.1) \pm 1.47 a
	b*	35.34%	9.02 \pm 6.74 ab	5.42 \pm 2.20 b	8.21 \pm 2.23 ab	7.74 \pm 1.55 ab	11.55 \pm 2.09 a

L* lightness (0 to 100), a* positive value =red, negative value=green (-60 to 60), b* positive value=yellow, negative value=blue (-60 to 60), S.D.: Standard Deviation, MEANS with different letters (a-b) in the same row present statistical difference (P < 0.05), V.C.: variation coefficient, numbers in parenthesis () means negative numbers

Quality rating

The quality characteristics of white shrimp (*Litopenaeus vannamei*) are mainly color, flavor, smell, and texture, as well as its hygienic and nutritional qualities (Haard 1992). Quality is a product that must meet the characteristics of consumers (Praxiom 2004). The quality and acceptance of shrimp may be affected by different factors, such as growth conditions, and management during processing according to (Dunajski 1980; Tsuchiya *et al.* 1992).

In Table 14 quality rating results of Headless shrimp presentation Control 2, TRT4 and TRT5 for 6 days storage. As shown in Table 14 during the experiment, the treatments evaluated presented different behavior in their characteristics and damage signs. TRT 5 maintained its quality rating in one value; it means class A product during all the 6 days testing. In the other hand, TRT4 maintained its quality rating in 1 value until day 5 of storage, then begun to show more damage signs and change its quality rating into 2 value. It means that TRT4 also maintain its quality into class A during the 6 days testing but begun to present damage signs earlier than TRT5.

Table 14. Control 2, TRT4 and TRT5 in Headless (HL) presentation quality rating during 6 days of storage.

Treatments	Quality Rating				
	Day 0	Day 1	Day 2	Day 5	Day 6
Headless (HL)-Control 2	1	1	2	2	3
Headless (HL)-TRT4	1	1	1	2	2
Headless (HL)-TRT5	1	1	1	1	1

Control 2: Headless shrimp + polybag, TRT4: Headless shrimp + rosemary extract + polybag, TRT5: headless shrimp + rosemary extract + vacuum packaging, quality rating present different values, 1-3 class A shrimp, 4-6 class B shrimp, 7-9.

According to Garrido *et al.* (2000), the most important characteristics that determine the shrimp quality are odor, texture, color, and melanosis presence. Before determining the quality rating for each treatment, texture, color, odor, and melanosis were measured. During the measurement of this characteristics along the 6 days, some important changes been noticed.

Texture. The postmortem texture change is considered as one of the most unfavorable characteristics in the quality of the product, texture change affect more in seafood than in other kind of meat because of their high endogen enzymatic activity and the low content of collagen in their muscle structure. During texture, analysis is important to mention that the TRT4 and TRT5 showed a better texture maintenance. This result could be associated with the antioxidant properties of rosemary extract. Estévez *et al.* (2005) determine the antioxidant properties of rosemary can avoid or reduce texture changes caused by the direct reaction between enzymatic action and texture changes. Rosemary extract can prevent changes in protein function due to oxidation, which may affect the color and texture characteristics of the meat.

Melanosis. Dark spots or melanosis in shrimps is a harmless but unpleasant discoloration or blackening that occurs mainly on swimmers, heads, tails, and nearby shell areas, and then spreads further along the shell edges and through the body. Black spots are caused by enzyme systems naturally present in shrimp. These enzymes can chemically convert the colorless compounds in the shrimp into complex brown pigments on the surface and near the shell of the shrimp in the air. (Bell 2015).

According to the result obtained during the analysis, TRT4 and TRT5 melanosis appearance was slowest against the control. Also is important to mention that TRT5 melanosis appearance was the slowest in general. This result could be associated with the rosemary extract antioxidant properties and the control of oxidation reactions because of the vacuum packaging. “Antioxidants are used to preserve food, such as delaying rancidity or discoloration, which is result of oxidation” (Gokoglu 2008). Yatmaz & Gokoglu (2016) obtained similar results in his study, where rosemary extract indicated a good protective effect against melanosis compared to green tea extract and showing the lowest values at the end of the storage.

In the other hand, melanosis control also could be the result of the vacuum packaging, due to melanosis is a natural mechanism caused by enzymatic reactions, where the oxygen in the atmosphere can promote the development of melanosis (Gonçalves & Menezes 2016). Kumar *et*

al. (2012), shows similar results in their study effect of antimelanotic treatment and vacuum packaging on melanosis and quality condition of ice stored farmed tiger shrimp (*Penaeus monodon*) where vacuum packaging retard the melanosis appearance in two days against control (bag with air). TRT5 also could has the slowest melanosis appearance due to the combination of rosemary extract and vacuum. According to Kumar *et al.* (2012), the effects of natural extracts and vacuum packaging on storage can control the characteristics of cultured tiger prawns, especially the occurrence of melanosis and the extension of shelf life.

Odor. The two treatments (TRT4 and TRT5) maintained odorless during the day's storage. This result could be associated with the antioxidant properties of rosemary extract and oxidation reactions control of vacuum packaging. Oxidative reactions changes in lipids and proteins in muscles may affect the sensory and the quality of the product, such as smell, rancidity, dehydration, weight loss, color, and texture (Tsironi *et al.* 2009).

Quality. In similar investigations, Peiretti *et al.* (2012), sign that the results of the application of rosemary significantly extend the shelf life. In addition, the presence of terpenoids of rosemary in fish meat improves the quality. As shown in Table 14, TRT5 was the treatment that better maintained the shrimp quality during the time storage. This treatment maintained its quality in a one value during the 6 days storage; it means that this treatment had no bad odor, no melanosis presence and good texture (Garrido *et al.* 2000). This result could be associated with the better maintenance and control of melanosis, odor and texture mentioned before

4. CONCLUSIONS

- A literature review of 100 scientific papers from 46 journals and 10 internet pages from leading institutions was done.
- Rosemary extract solution (0.2%) immersion control the total coliforms growth in fresh raw headless shrimp presentation up to six days; vacuum packaging in combination with rosemary extract solution (0.2%) immersion control coliform growth, maintain color and quality in fresh raw headless shrimp presentation.
- With the use of rosemary extract in a polybag packaging, b^* value presented changes during the days analyzed, with the combination of rosemary extract + vacuum any value (L^* , a^* , b^*) presented changes during the days analyzed.

5. RECOMMENDATIONS

- To determine acceptance and preference of Headless shrimp presentation treated with rosemary extract + vacuum and of Headless shrimp treated with rosemary extract + polybag packaging through a sensory analysis.
- To develop a cost-benefit analysis; to define the benefits will be possible obtained with the packaging change.
- To develop a TBARS test to determine lipid oxidation.
- To analyze Total coliforms in days between 0-6 in Head-on, Headless control and treatment with vacuum only and Peeled and deveined presentations, and between days 6-12 in Headless presentation treated with rosemary and packaging in polybag and vacuum to determine the exact microbial life extension of the product.
- To conduct a quality blind analysis with a group of experts to determine the quality change of fresh raw headless shrimp treated with rosemary and packaging in polybags or vacuum.

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7. APPENDICES

Appendix 1. Quality rating for raw shrimp.

Score	Quality Rating	Description
1	A	Best shrimp - No odor to fresh shrimp odor (Class I Decomposition) Firm texture No melanosis
2	A	Excellent shrimp - very slight noticeable changes in odor, appearance, or texture
3	A	Very good shrimp - some noticeable but not objectionable changes
4	B	Good shrimp - noticeable changes in odor, appearance, and texture, but not objectionable
5	B	Good to fair shrimp - more noticeable changes, with some slightly objectionable, slightly stale or fishy odor, moderate melanosis, or slight soft texture (Class I Low Quality Decomposition)
6	B	Fair shrimp - similar to score 5 yet more pronounced
7	C	Rather poor shrimp - objectionable changes in odor (Class 2 Decomposition), appearance and texture Musty, fishy, "old socks" or ammoniacal odor, heavy melanosis, heat abused appearance, soft texture
8	C	Poor shrimp - most quality attributes objectionable (Class III Decomposition) Putrid, ammoniacal or fecal odor, cooked appearance and/or heavy melanosis, and soft-mushy texture
9	C	Very poor shrimp - very objectionable

Source: Garrido *et al.* 2000.

Appendix 2. Organoleptic analysis used for quality rating during 0, 1, 2, 5, and 6 days.

day 0

American Mariculture, Inc.			
Organoleptic Test		Size intended: <u>10-15</u>	Lbs. sampled: <u>2</u>
Form: Whole <u>X</u>	GH _____	EZ Peel: _____	
PUD _____	P&D _____	T/On _____	
Quality Parameters	Good	Not acceptable	Disposition/Comments
Bag Weight: _____			
Appearance	✓		
Shell color	✓		<i>some with green odor</i>
Shell firmness	✓		
Head color	✓		
Head firmly attached	✓		
Tail firmly attached	✓		
Count / Lb			
Uniformity	✓		
Odor	✓		
Head meat	✓		
Head legs	✓		
Pieces / Lb	<u>15</u>		
Melanosis	✓		
Proper packaging			
Temp. in packaging			
Date: <u>3/18/20</u>	Production code: <u>3377-2</u>	Code: <u>3377-2</u>	
QA Inspector signature: <i>[Signature]</i>	<u>HTD 5.0</u>	Time: <u>12:10 pm</u>	
Procedures: The QA personnel will sample per raceway from the daily production to match the shelf life of the product. This sample will be disposed off according to experienced results. In the case of fresh shrimp, sample will be kept for 5 days for head on and 10 days for headless			

Appendix 2 continuation.

Day 1

AMERICAN MARICULTURE INC.
INTERN PROJECT

DAIRY ORGANOLEPTIC TEST

DATE: 3/19/2020

SAMPLE	APPEARANCE	BODY COLOR	SHELL COLOR	HEAD COLOR	FIRMNESS	COMMENTS
HO-0.1	✓	✓	✓	✓	✓	
HO-0.2	✓	✓	✓	✓	✓	
HO-0.3	✓	✓	✓	✓	✓	2 with orange head
HO-0.4	✓	✓	✓	✓	✓	3 with orange head
HO-0.5	✓	✓	✓	✓	✓	4 with orange head
HO-1.1	✓	✓	✓	✓	✓	2 with orange head
HO-1.2	✓	✓	✓	✓	✓	2 with orange head
HO-1.3	✓	✓	✓	✓	✓	4 with orange head
HO-1.4	✓	✓	✓	✓	✓	last good
HO-1.5	✓	✓	✓	✓	✓	"
HO-2.1	✓	✓	✓	✓	✓	"
HO-2.2	✓	✓	✓	✓	✓	2 orange head
HO-2.3	✓	✓	✓	✓	✓	1 orange head
HO-2.4	✓	✓	✓	✓	✓	3
HO-2.5	✓	✓	✓	✓	✓	1
HO-3.1	✓	✓	✓	✓	✓	1 with orange head
HO-3.2	✓	✓	✓	✓	✓	2 with "
HO-3.3	✓	✓	✓	✓	✓	2 with orange head
HO-3.4	✓	✓	✓	✓	✓	3 with orange head
HO-3.5	✓	✓	✓	✓	✓	2 with orange head
HL-0.1	✓	✓	✓	✓	✓	Great color and firmness
HL-0.2	✓	✓	✓	✓	✓	"
HL-0.3	✓	✓	✓	✓	✓	"
HL-0.4	✓	✓	✓	✓	✓	"
HL-0.5	✓	✓	✓	✓	✓	"
HL-1.1	✓	✓	✓	✓	✓	Really good color
HL-1.2	✓	✓	✓	✓	✓	"
HL-1.3	✓	✓	✓	✓	✓	"
HL-1.4	✓	✓	✓	✓	✓	"
HL-1.5	✓	✓	✓	✓	✓	"
HL-2.1	✓	✓	✓	✓	✓	"
HL-2.2	✓	✓	✓	✓	✓	4 with brown spots (body)
HL-2.3	✓	✓	✓	✓	✓	last good
HL-2.4	✓	✓	✓	✓	✓	"
HL-2.5	✓	✓	✓	✓	✓	"
HL-3.1	✓	✓	✓	✓	✓	"
HL-3.2	✓	✓	✓	✓	✓	"
HL-3.3	✓	✓	✓	✓	✓	"
HL-3.4	✓	✓	✓	✓	✓	"
HL-3.5	✓	✓	✓	✓	✓	"
PD-0.1	✓	✓	✓	✓	✓	good color
PD-0.2	✓	✓	✓	✓	✓	
PD-0.3	✓	✓	✓	✓	✓	
PD-0.4	✓	✓	✓	✓	✓	
PD-0.5	✓	✓	✓	✓	✓	
PD-1.1	✓	✓	✓	✓	✓	best color, firmness, appearance
PD-1.2	✓	✓	✓	✓	✓	"
PD-1.3	✓	✓	✓	✓	✓	"
PD-1.4	✓	✓	✓	✓	✓	"
PD-1.5	✓	✓	✓	✓	✓	"
PD-2.1	✓	✓	✓	✓	✓	"
PD-2.2	✓	✓	✓	✓	✓	"
PD-2.3	✓	✓	✓	✓	✓	"
PD-2.4	✓	✓	✓	✓	✓	"
PD-2.5	✓	✓	✓	✓	✓	"
PD-3.1	✓	✓	✓	✓	✓	"
PD-3.2	✓	✓	✓	✓	✓	"
PD-3.3	✓	✓	✓	✓	✓	"
PD-3.4	✓	✓	✓	✓	✓	"
PD-3.5	✓	✓	✓	✓	✓	"

Mon 3/19/20

Appendix 2 continuation.

Day 2

AMERICAN MARICULTURE INC.
INTERN PROJECT
DAIRY ORGANOLEPTIC TEST 10/12 a.m. DATE: 3/20/2020 10-12 a.m.

SAMPLE	APPEARANCE	BODY COLOR	SHELL COLOR	HEAD COLOR	FIRMNESS	COMMENTS
HO-0.1	✓	✓	✓	X	✓	5 with orange head
HO-0.2	✓	✓	✓	X	✓	7 with orange head
HO-0.3	✓	✓	✓	X	✓	7 with orange head + 1 black head
HO-0.4	✓	✓	✓	✓	✓	5 with orange head
HO-0.5	✓	✓	✓	✓	✓	6 with orange head
HO-1.1	✓	✓	✓	✓	✓	2 with orange head
HO-1.2	✓	✓	✓	✓	✓	3 with orange head
HO-1.3	✓	✓	✓	X	✓	3 with orange head
HO-1.4	✓	✓	✓	X	✓	6 with orange head
HO-1.5	✓	✓	✓	X	✓	3 orange head - looks better
HO-2.1	X	X	X	X	✓	3 orange head + 7 black head
HO-2.2	✓	✓	✓	X	✓	5 orange head
HO-2.3	X	✓	X	X	✓	1 orange head
HO-2.4	✓	X	X	X	✓	3 orange head
HO-2.5	✓	✓	X	X	✓	3 orange head + 1 orange body
HO-3.1	✓	✓	✓	X	✓	3 orange head
HO-3.2	X	✓	✓	X	✓	3 orange head
HO-3.3	✓	✓	✓	X	✓	5 orange head
HO-3.4	✓	✓	✓	X	✓	3 orange head
HO-3.5	✓	X	✓	X	✓	5 orange head
HL-0.1	✓	✓	✓	✓	✓	4 with little brown spot
HL-0.2	✓	✓	✓	✓	✓	4 with black spots in the tail (spot)
HL-0.3	✓	✓	✓	✓	✓	look good
HL-0.4	✓	✓	✓	✓	✓	
HL-0.5	✓	✓	✓	✓	✓	
HL-1.1	✓	✓	✓	✓	✓	1 with black spots in the body
HL-1.2	✓	✓	✓	✓	✓	2 with little black spots in the tail
HL-1.3	✓	✓	✓	✓	✓	more firm than others
HL-1.4	✓	✓	✓	✓	✓	more firm 11 12 with black spots
HL-1.5	✓	✓	✓	✓	✓	4 with black tail
HL-2.1	✓	✓	✓	✓	✓	4 with little black spots in the tail
HL-2.2	✓	✓	✓	✓	✓	look good
HL-2.3	✓	✓	✓	✓	✓	look good
HL-2.4	✓	✓	✓	✓	✓	look good
HL-2.5	✓	✓	✓	✓	✓	look good
HL-3.1	✓	✓	✓	✓	✓	look good
HL-3.2	✓	✓	✓	✓	✓	look good
HL-3.3	✓	✓	✓	✓	✓	look good
HL-3.4	✓	✓	✓	✓	✓	look good
HL-3.5	✓	✓	✓	✓	✓	look good
PD-0.1	✓	✓	✓	✓	✓	look good
PD-0.2	X	X	X	X	X	
PD-0.3	X	X	X	X	X	
PD-0.4	X	X	X	X	X	
PD-0.5	X	X	X	X	X	
PD-1.1	✓	✓	✓	✓	✓	more firm than others, look good
PD-1.2	✓	✓	✓	✓	✓	look good
PD-1.3	✓	✓	✓	✓	✓	look good
PD-1.4	✓	✓	✓	✓	✓	look good
PD-1.5	✓	✓	✓	✓	✓	look good
PD-2.1	✓	✓	✓	✓	✓	look better than others
PD-2.2	✓	✓	✓	✓	✓	look good
PD-2.3	✓	✓	✓	✓	✓	look good
PD-2.4	✓	✓	✓	✓	✓	look good
PD-2.5	✓	✓	✓	✓	✓	look good
PD-3.1	✓	✓	✓	✓	✓	look good
PD-3.2	✓	✓	✓	✓	✓	look good
PD-3.3	✓	✓	✓	✓	✓	look good
PD-3.4	✓	✓	✓	✓	✓	look good
PD-3.5	✓	✓	✓	✓	✓	look good

more firm than others, look good

look better than others

look good

Q45

DAIRY ORGANOLEPTIC TEST

DATE: 3/23/2020

600 400
11/20/2019

Appendix 2 continuation.

AMERICAN MARICULTURE INC.						
INTERN PROJECT						
DAIRY ORGANOLEPTIC TEST						
DATE: 3/24/2020						
SAMPLE	APPEARANCE	BODY COLOR	SHELL COLOR	HEAD COLOR	FIRMNESS	COMMENTS
HO-0.1	X	X	X	X	X 1/2	all with black/orange head.
HO-0.2	X	X	X	X	X 1/2	
HO-0.3	X	X	X	X	X 1/2	
HO-0.4	X	X	X	X	X 1/2	
HO-0.5	X	X	X	X	X 1/2	
HO-1.1	X	X	X	X	X 1/2	soft orange head/white
HO-1.2	X	X	X	X	X 1/2	hard orange head/black
HO-1.3	X	X	X	X	X 1/2	
HO-1.4	X	X	X	X	X 1/2	
HO-1.5	X	X	X	X	X 1/2	
HO-2.1	X	X	X	X	X 1/2	2 black head / 7 orange head
HO-2.2	X	X	X	X	X 1/2	10 orange head / 1 black
HO-2.3	X	X	X	X	X 1/2	17 orange head
HO-2.4	X	X	X	X	X 1/2	1 orange body / 9 orange head
HO-2.5	X	X	X	X	X 1/2	13 orange head
HO-3.1	X	X	X	X	X 1/2	14 orange head
HO-3.2	X	X	X	X	X 1/2	13 orange head
HO-3.3	X	X	X	X	X 1/2	12 orange head
HO-3.4	X	X	X	X	X 1/2	2 black / 10 orange head
HO-3.5	X	X	X	X	X 1/2	13 orange head
HL-0.1	X	X	X	X	X 1/2	7 black spots (body)
HL-0.2	X	X	X	X	X 1/2	9 black spots body
HL-0.3	X	X	X	X	X 1/2	8 black spots body
HL-0.4	X	X	X	X	X 1/2	9 black spots body
HL-0.5	X	X	X	X	X 1/2	5 black spots body / 1 black tail
HL-1.1	X	X	X	X	X 1/2	2 black spots / 3 green spots
HL-1.2	X	X	X	X	X 1/2	3 black spots / 9 green tail
HL-1.3	X	X	X	X	X 1/2	4 black spots / green tail
HL-1.4	X	X	X	X	X 1/2	1 black spot / green tail
HL-1.5	X	X	X	X	X 1/2	5 black spots
HL-2.1	X	X	X	X	X 1/2	lost track
HL-2.2	X	X	X	X	X 1/2	100% brown spot body
HL-2.3	X	X	X	X	X 1/2	1 little black spot body
HL-2.4	X	X	X	X	X 1/2	lost track
HL-2.5	X	X	X	X	X 1/2	
HL-3.1	X	X	X	X	X 1/2	
HL-3.2	X	X	X	X	X 1/2	
HL-3.3	X	X	X	X	X 1/2	
HL-3.4	X	X	X	X	X 1/2	
HL-3.5	X	X	X	X	X 1/2	
PD-0.1	X	X	X	X	X 1/2	1 black spot body
PD-0.2	X	X	X	X	X 1/2	
PD-0.3	X	X	X	X	X 1/2	
PD-0.4	X	X	X	X	X 1/2	
PD-0.5	X	X	X	X	X 1/2	
PD-1.1	X	X	X	X	X 1/2	really firm, lost track
PD-1.2	X	X	X	X	X 1/2	
PD-1.3	X	X	X	X	X 1/2	
PD-1.4	X	X	X	X	X 1/2	
PD-1.5	X	X	X	X	X 1/2	
PD-2.1	X	X	X	X	X 1/2	
PD-2.2	X	X	X	X	X 1/2	
PD-2.3	X	X	X	X	X 1/2	
PD-2.4	X	X	X	X	X 1/2	
PD-2.5	X	X	X	X	X 1/2	
PD-3.1	X	X	X	X	X 1/2	
PD-3.2	X	X	X	X	X 1/2	
PD-3.3	X	X	X	X	X 1/2	
PD-3.4	X	X	X	X	X 1/2	
PD-3.5	X	X	X	X	X 1/2	

Appendix 3. Images of quality change of Headless (HL) shrimp presentation, Control 2, TRT4, TRT5 during days 0, 6 and 12.

DAY 0

DAY 1

DAY 2

DAY 5

DAY 6

HEADLESS (HL) CONTROL 2 (Headless shrimp + polybag)



HEADLESS (HL) TRT 4 (Headless shrimp + rosemary extract + polybag)



HEADLESS (HL) TRT 5 (Headless shrimp + rosemary extract + vacuum)

