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Evaluation of Post-Harvest Procedures for Quality Enhancement in the Louisiana Commercial Shrimp Industry

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ABSTRACT

The Louisiana commercial shrimp fishery has faced numerous challenges, and the number of fishermen is declining at an alarming rate. The opportunity may exist to add value to the industry by creating a superior product through optimal operating procedures. Post-harvest processes of plate freezing were analyzed to develop a high-quality shrimp product capable of fostering economic growth for fishermen. Sulfite-free melanosis prevention testing used 4-hexylresorcinol treatments in combination with vacuum-packing and modified atmosphere packing to delay the onset of black spot. The physical and chemical properties of plate frozen shrimp were analyzed from collaborating fishermen. Results found 4-hexylresorcinol products successfully inhibited black spot development for up to 10 days. Vacuum-packing and modified atmosphere packing did not have significant effects on melanosis inhibition. The use of plate freezers both onboard and at docks has the potential to improve shrimp quality, as well as advance the economic stability of the industry.

KEYWORDS

Plate freezing; shrimp; post-harvest; best practices; standard operating procedures

Introduction

Shrimp are an extremely important resource economically and culturally to the United States (US). In 2018, the US harvested over 139,000 metric tons of wild caught shrimp, valued at over $500 million (NOAA 2020). Louisiana contributes more shrimp to the domestic market than any other state in the country at approximately 32% of the total landings and 25% of its value (NOAA 2020). Competition with imports has negatively affected the industry and is driving down dockside value (Asche et al. 2012). In addition to rising operating costs, dockside value of shrimp has declined over 30% from the 90s to 2000s in the region (Tabarestani et al. 2017) and 40% between the years 2000 and 2003 alone for the southeastern USA (Keithly and Poudel 2008). There are 50% fewer commercial shrimp fishermen in Louisiana today than there was two decades ago (Bourgeois et al. 2016), and most of the fleet are either inshore day boats with ice or offshore vessels using brine or ice. Very minimal at-sea processing is occurring, and this limits opportunities to increase quality and value. The Louisiana commercial shrimp fishery has faced numerous challenges and is declining at an alarming rate. The opportunity may exist to add value to the industry by creating a superior frozen product through optimal operating procedures.

Options to increase value include the ability to freeze and store catch for periods of time, giving processors a seasonal advantage to hold onto product longer and sell to proper markets at more competitive prices. Plate freezing technology has been used in other fisheries across the globe (Kolbe et al. 2004; Ruiz 2012) but has not been adopted by many in Louisiana. The quality of

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shrimp frozen using plate freezing equipment is considered much greater than other freezing mechanisms. Shrimp retain all appendages and natural color through freezing, creating a visually appealing product.

Improving shrimp quality can also increase product value. Wild caught shrimp are often preferred by consumers (Brayden et al. 2018) and more commonly lack potentially harmful drug residue and antibiotic resistant bacteria found in some imported shrimp (Duran and Marshall 2005; Khan and Lively 2020; Pham et al. 2015). Shrimp quality is determined through the cold-chain management process and handling procedures (Fieger and Friloux 1954). Variability in storage temperatures can determine the shelf life and rates of deterioration of shrimp (Tsironi et al. 2009). The goal for improved quality is to lower the internal temperature of shrimp as fast as possible.

Treatment of shrimp with additives to prevent spoilage or consumer rejection can also affect quality. Shrimp develop black spots, or melanosis, naturally post-mortem, and the inhibition of melanosis is of particular interest to improve quality. Caused by polyphenol oxidase (PPO) activity, numerous factors can affect melanosis severity and PPO activity including both the size and sex of the shrimp and the season they are caught (Bono et al. 2010). While inshore shrimpers typically fish seasonally, some shrimping occurs year-round in Louisiana and is not sex selective, so melanosis prevention is an important consideration year-round. Although melanosis is not harmful to consumers, it often leads to buyer rejection (López-Caballero et al. 2000).

Sulfite products are traditionally used in commercial shrimp fisheries to inhibit the development of melanosis. Residual sulfite in shrimp tissue can trigger asthmatics and cause allergic reactions in sulfite-sensitive individuals. Sulfite-free products have been requested by buyers in higher quality shrimp. 4-Hexylresorcinol (4-HR) products, such as EverFresh ® and Prawnfresh™, have been found to significantly inhibit the development of black spot (Lively, unpublished; Montero et al. 2004; Otwell et al. 1992). Different packaging methods have shown mixed effects in reducing black spot. Vacuum-packaging and modified atmosphere packaging (MAP), which alters the atmospheric gases in the package, have been found effective in various studies (Bono et al. 2012, 2016; López-Caballero et al. 2000). Determining the best post-harvest procedures for melanosis inhibition is essential for a consistent, high-quality product.

Several shrimpers have indicated an interest in adopting plate freezing technology, but no standard operating procedure (SOP) exists for them to create an optimal product. Additionally, there is concern others might thaw and plate freeze imported shrimp, so the collaborators wanted a branded product using the same SOP. The collaborators were also interested in using MAP to increase quality, but immediate use would be restricted due to vessel capacity and could only occur onshore after vessels return to the dock. To create a recognizable plate frozen shrimp brand, an initial SOP was created to maximize quality and consistency in collaboration with several shrimpers using plate freezing technology. The goal of this research was to examine existing plate freezing operating procedures and develop quantitative and qualitative post-harvest procedure guidelines to maximize quality. Objectives were to (1) establish optimal melanosis prevention practices; and (2) quantify quality standards of plate frozen shrimp using existing protocols.

Materials and methods

Melanosis prevention

Shrimp collection

For vacuum-packing, white shrimp, *Litopenaeus setiferus*, were purchased directly from a shrimper in November of 2017, in Bayou Dularge, Louisiana, and treated dockside. The shrimp were caught within the previous 12 hours and known to be free of any post-harvest treatments. This is the realistic time-period shrimp are caught before vacuum-packing and modified atmosphere packing could be applied dockside, as on-board post-harvest treatment is not practical or feasible.

In order to determine the role of MAP packaging and freezing methods on black spot prevention, fresh white shrimp were purchased from a fisherman in November of 2018 and transported to Lafayette, Louisiana, within 18 hours, the standard time for shrimp to reach a packaging location.

Melanosis prevention and vacuum packaging

In order to test melanosis prevention treatment and vacuum-packaging over time, treatments consisted of treated (TR) (a 1× concentration soak (2.11 g/L) of 4-hexyresorcinol product, EverFresh ®, for 2 min (manufacturer recommended protocol) compared to the control replicates (UT), which were not treated or dipped with any additional product. Vacuum-packed (VP) consisted of vacuum-packing the entire box prior to freezing at 99% vacuum in 6×12" OD 3MIL pouches with an oxygen transmission rate (OTR) of 55.3 cc/m^2/day (Acadia Scales and Equipment, Opelousas, LA, USA) (Ultravac vacuum packing machine by Koch Equipment, LLC, Riverside, MO, USA), compared to the box (BX) treatment, which used the standard practice of shrimp in an unsealed bag (oxygen-permeable) within the cardboard box. Three frozen storage periods were chosen (T1: 1 month, T2: 3 months and T3: 6 months). The full combination of melanosis (TR and UT) and packaging treatments (VP and BX) was tested at T1. However, UT shrimp were only evaluated during the first month, as preliminary research indicated untreated shrimp were not acceptable (Table 1). The combination of TR with packaging (VP and BX) was tested at T2 and T3 (Table 1).

Each treatment group was treated and packaged separately. Approximately, 2.04 kg of shrimp was used in each replicate, with three replicates per treatment group. Shrimp were placed into clear plastic bags (oxygen permeable, unsealed), then packaged in individually labeled boxes. All packages were plate frozen on site (Challenger Refrigeration, LeBlanc and Associates, LLC). Once frozen, boxes were returned to the lab and stored in commercial chest freezers (Frigidaire Model #LFFN15M5HWE) at −20°C.

Modified atmosphere packaging and freezing method

All shrimp were dipped using a 1× concentration (2.11 g/L) soak of EverFresh ® for 2 min. Shrimp were packaged in one of the two experimental MAP groups (P5-T MAP Unit from PointFive Packaging, LLC, Schiller Park, IL, USA) or a control (Atmospheric). MAP packages contained approximately 0.45 kg of shrimp, the maximum that fit in each plastic tray (6.5 × 8.5" Crystalline Polyethylene Terephthalate (CPET) trays; PointFive Packaging, LLC) without shrimp rostrums piercing the film (TOPAZ TC B-440 film with an OTR of 1–5 cc/m^2/day; PointFive Packaging, LLC). The two experimental gas combinations used were MAP100: 100% N_2, 0% CO_2 (Nitrogen UHP 200, Airgas) and MAP50:50: 50% N_2, 50% CO_2 (CT 50% NI/CO 200, Airgas). MAP trays were then frozen in walk-in-freezers at −20°C (SF) (Environmental Growth Chambers, Chagrin Falls, OH, USA) and plate frozen (PF) (Custom Plate Freezer by LeBlanc, Houma, LA, USA). A control group consisted of 2.04 kg of shrimp packaged in plastic freezer bags (Ziploc®, S.C. Johnson and Son Inc., Racine, WI,

Table 1. Treatment groups used to test EverFresh ® dips and packaging method on black spot inhibition (n = 3). Untreated replicates of each packaging method acted as controls. UT = Untreated, TR = Treated, VP = Vacuum Packaged, BX = Standard Box Frozen, and the number represents the time interval before testing.

Treatment Name	Melanosis Prevention Method	Packaging	Storage Period
UTVP1	None	Vacuum-pack	1 Month
UTBX1	None	Standard	1 Month
TRVP1	EverFresh®	Vacuum-pack	1 Month
TRBX1	EverFresh®	Standard	1 Month
TRVP2	EverFresh®	Vacuum-pack	3 Months
TRBX2	EverFresh®	Standard	3 Months
TRVP3	EverFresh®	Vacuum-pack	6 Months
TRBX3	EverFresh®	Standard	6 Months

Table 2. Experimental and control treatments used to test modified atmosphere packaging (MAP) on black spot inhibition (n = 3). Control shrimp were not packaged using MAP; therefore, packages contained standard atmospheric gas combinations.

Treatment	MAP Gas Combination	Freezing Type	Frozen Time
MAP100-PF1	100% N_2, 0% CO_2	Plate	1 Month
MAP100-SF1	100% N_2, 0% CO_2	Standard	1 Month
MAP50:50-PF1	50% N_2 50% CO_2	Plate	1 Month
MAP50:50-SF1	50% N_2 50% CO_2	Standard	1 Month
Control-PF1	Atmospheric	Plate	1 Month
MAP100-PF2	100% N_2, 0% CO_2	Plate	3 Months
MAP100-SF2	100% N_2, 0% CO_2	Standard	3 Months
MAP50:50-PF2	50% N_2 50% CO_2	Plate	3 Months
MAP50:50-SF2	50% N_2 50% CO_2	Standard	3 Months
Control-PF2	Atmospheric	Plate	3 Months

USA) and frozen via plate freezing. The control contained more shrimp to emulate the smallest boxes typically used by plate frozen entities. Packages remained in storage for two freezing intervals, 1 month (T1) and 3 months (T2), before being evaluated for black spot (Table 2).

Melanosis evaluation

Shrimps were removed from their package at the end of each frozen storage interval and thawed under cool running water. Shrimp were placed on top of ice in individually labeled coolers and stored in an incubator (Thermo Fisher Scientific – Isotemp) at 4°C for 10 days. For the vacuum-packed trials, coolers were removed at approximately the same time on days 1, 2, 3, 4, 5, 8, and 10 for black spot evaluation. For the MAP trials, only days 1, 3, 5, 8, and 10 were evaluated. MAP packages only contained approximately 25–30 shrimp; therefore, only 5 days of analysis was chosen to ensure enough shrimp were available for each daily evaluation.

Five shrimps were randomly selected daily from each cooler and visually scored by two trained evaluators. Only one side of each shrimp was evaluated and remained consistent throughout experimentation. Each set of five shrimps was photo-documented using a camera (Canon PowerShot SX40 HS) and a light box (32 × 32" Photography Light Box – Amazon). If scores between evaluators differed by more than 1, a third trained evaluator examined the photo as a tie-breaker.

Shrimps were only scored once and discarded after daily evaluation. Coolers were drained and refreshed with ice prior to returning to the incubator. Placement inside the incubator was random and varied across the 10 days to minimize error due to internal temperature variation.

Melanosis scoring

Scores were determined based on a five-point qualitative scale and independently recorded by each evaluator to prevent bias. This scale was designed for statistical analysis purposes and demonstrates approximately equal progression of black spot between each scoring point from 1 to 5. The scale was set as 1 = no black spot; 2 = any black spot beginning on head or body; 3 = objectionable blackspot resulting in defect points; 4 = severe black spot on head, body, or both; 5 = severe black spot on entire exoskeleton. A score of three was set to equal the United States Department of Commerce's Seafood Inspection Manual's (2011) definition of objectionable shrimp.

Plate frozen quality assessment

Ten boxes of plate-frozen shrimp were purchased between November 2017 and November 2018 from three businesses (minimum of 3 per entity) and used for quality analysis of existing protocols. All 10 boxes were available for public retail and selected at random, and shrimp were treated under each business's operating procedure. Application and treatment method of black spot prevention was

Table 3. Information pertaining to the date and size of plate frozen shrimp packages purchased for analysis. Letters represent unique sources. Shrimp size reflects the count or number of shrimp per pound.

Box	Date Purchased	Box Size (kg)	Shrimp Size
A1	5/17/2018	9.07	16/20
A2	5/17/2018	9.07	26/30
A3	5/10/2018	4.54	21/25
B1	9/15/2018	4.54	unknown
B2	9/15/2018	4.54	unknown
B3	8/20/2018	4.54	16/20
C1	11/3/2017	9.07	13/15
C2	11/3/2017	9.07	13/15
C3	11/20/2018	9.07	21/25
C4	11/20/2018	9.07	21/25

unknown for each box prior to evaluation. All were reported to be sulfite-free. All shrimps were transported frozen to Louisiana State University (LSU) for analysis. Boxes were stored in chest freezers (Frigidaire Model #LFFN15M5HWE) at −20°C until analysis (Table 3).

Shrimp packages contained one or two bags of shrimp placed inside a box. The 9.07 kg boxes contained one large bag, while 4.54 kg boxes contained two smaller bags of equal size. The total weight of each package was recorded at the start of the analysis. Shrimp bags were carefully thawed under cool, running water, and the empty bag and box weight was recorded. Species identification was confirmed. An estimated total frozen shrimp weight was determined by subtracting the bag and box weight from the total package weight. Thawed shrimp were sorted by hand, and foreign materials, damaged shrimp, inadvertently peeled shrimp (body segments), inadvertently headed, and heat-abused shrimp were recorded. Foreign material was any incidental matter including bycatch, debris, or unusable shrimp (e.g. size count >100 per pound). Damaged shrimp were broken pieces of shrimp including pieces of tail, body, or head damaged in the freezing process. Heat-abuse was determined by red-orange colored shrimp due to over exposure to heat. Any shrimp damaged during the thawing procedure were not counted. The percentage, by number, of damaged, inadvertently peeled, headed, and heat-abused shrimp was recorded by dividing the total number of each by the estimated total number of shrimp per box. The total number of shrimp was estimated by multiplying the average size count by the number of pounds of shrimp per box. Percentage of foreign material was recorded by dividing total contaminant weight by total shrimp weight.

Following sorting, approximately 40–50 thawed shrimp were randomly pulled from each box, placed on ice in a labeled cooler, and stored in an incubator at 4°C for black spot evaluation using the same method as the melanosis prevention experiments.

At random, 2.5 kg of shrimp were chosen from each box, stored in 7.57 L sealed plastic bags, and placed in chest freezers for future size count analysis. Of the remaining thawed shrimp, an estimated 0.25 kg of shrimp was homogenized (Continental Electric Food Chopper). In boxes that contained two bags, an approximately equal amount from each bag was chosen for melanosis evaluation, size count analysis, and for the homogenized mixture. Samples of the homogenized mixtures were subsequently used for moisture content analysis, salt content analysis, and microbiological analysis. All remaining shrimp and homogenized samples were stored in labeled sealed plastic bags and returned to freezers.

Moisture content analysis

Moisture content was analyzed using the gravimetric method in accordance with AOAC (1984) methodology. Approximately, 3 g of each homogenized shrimp sample was weighed and spread across pre-weighed drying dishes. Samples were placed in a drying oven at 100°C for approximately

18 hours (adequate time until weight did not change). The final weight of the dish and dried shrimp was recorded. Three replicates for each homogenized sample were analyzed, and moisture content was determined by the following equation:

$$\%\text{Moisture} = \frac{\text{Initial Sample Wt.} - \text{Final Sample Wt.}}{\text{Initial Sample Wt.}} \times 100$$

Salt content analysis

The salt content was determined as a percentage of sodium chloride (NaCl), chlorine as sodium chloride, by a volumetric method in accordance with AOAC Official Methods 937.09 of analysis (AOAC 1984). Approximately, 1 g of each homogenized sample was weighed and added to a 100 mL beaker. Exactly 20 mL of silver nitrite ($AgNO_3$, Fisher Scientific 70004) was added, followed by 10 mL of nitric acid (HNO_3, Fisher Scientific R535470025D). The sample was boiled under a fume hood for 10 minutes, allowed to cool, then filtered through a Whatman No. 1 filter paper into a 200 mL Erlenmeyer flask. The filtrate was adjusted to 50 mL using deionized water, and 5 mL of ferric alum indicator solution (Fisher Scientific 305016) was added. The sample was then titrated using potassium thiocyanate (KSCN, Fisher Scientific 658016) until a permanent orange-red color change was achieved. Three replicates from each homogenized sample were analyzed, and the following equation was used to determine salt content:

$\%\text{NaCl} = 5.844 \times [(V_1 \times N_1) - (V_2 \times N_2)]/W$

Where, V_1 = known volume of $AgNO_3$ (mL), N_1 = concentration of $AgNO_3$ (N), V_2 = volume of KSCN used (mL), N_2 = concentration of KSCN (N), and W = sample weight (g).

Microbiological analysis

Immediately after thawing and homogenizing, approximately 100 g of each sample was stored in Falcon tubes and frozen. Frozen samples were delivered to the Food Safety and Food Microbial Testing Laboratory at LSU for aerobic plate count (APC), total coliform, and *E. coli* analysis. AOAC official methods were used to determine bacterial counts, and samples were analyzed in triplicate after thawing overnight at 4°C.

Residual sulfite analysis

Ten shrimps were chosen at random from each sample and screened for residual sulfite using an Alert® for Sulfites in Seafood (Neogen Food Safety, Lansing, MI, USA) dye test. Sulfite content was determined by a color change of the blue dye and is represented by a range of concentrations. No color change indicates <10 ppm residual sulfite, pink/violet indicates between 10 and 100 ppm, and clearly indicates greater than 100 ppm. Shrimps were peeled and one drop of activator solution was added to the second-largest segment of flesh, followed by one drop of dye reagent in the same location. Samples were allowed 1 minute to change color before the final color was visually analyzed against a provided range to determine an estimate of residual sulfite in parts per million.

Size count analysis

The 2.5 kg labeled bags were removed and thawed under cool water. Shrimps from each replicate were spread across a tray, chosen at random, and placed on a scale until a weight of one pound was reached. The number of shrimp contained in each pound was then recorded. The actual average shrimp size was calculated within each box by taking an average of five replicate counts.

Statistical analysis

The effect of treatment on the multinomial response variable, average black spot score, was analyzed using Statistical Analysis Software (SAS Version 9.4, SAS Inc., Cary, NC, USA). The response variable was not normally distributed; therefore, generalized linear mixed models were run using the Glimmix

procedure. Models were fit using a cumulative logit function, and the independent variable, days post thaw, was treated as a covariate when a frozen storage period was included in the analysis. The covariance structure was established as Autoregressive (order 1). The dependent variable, average black spot score, was modeled by the independent treatment groups and months of frozen storage, when necessary. Results are based on the odds ratio of each score being selected. An alpha level of 0.05 was chosen to determine statistical significance.

Results

Melanosis prevention

Treatment and vacuum-packaging

The goal of the first black spot evaluation experiment was to determine the effect of a 4-HR dip and vacuum-packaging on melanosis in plate-frozen shrimp. The average melanosis scores of the treatment groups were significantly different from control groups after 1 month of frozen storage ($F = 60.90$, $P < .0001$). Replicates that were dipped with EverFresh ® (TR) remained below the unacceptable level (<3) for all 10 days, regardless of frozen storage length or packaging type (Figure 1). Control (UT) groups became unacceptable (≥3) by day 3 for both vacuum-packed and standard treatments (Figure 1). The packaging, standard box versus vacuum-packed, did not differ by melanosis score.

The average score of all days combined was 1.6 ± 0.38 for dipped replicates, while control was 3.15 ± 0.81. The frozen storage time was removed from statistical analysis, as it was obvious by inspection that further analysis was not required. None of the daily average scores reached unacceptable levels in any of the dipped experiment groups, regardless of frozen storage time (Figure 1).

Modified atmosphere packaging and freezing method

In order to determine the effect of two MAP gas combinations and the freezing method on melanosis inhibition of plate frozen shrimp, the average melanosis score for treatments was analyzed with the effect of storage period. Days of analysis post thaw were an appropriate covariable, with an estimate of 0.1428 and standard error of 0.2029. All shrimp were 4-HR dipped, and on average, none reached unacceptable (≥3) scores within 10 days of thawing in either frozen storage length (Figure 2). There was no statistical significance of treatment effect ($F = 0.64$, $P = .6372$) or the interaction between treatment and frozen storage length ($F = 1.74$, $P = .1376$) based on generalized linear mixed models. There was a significant difference between month one and three, as melanosis was more likely to develop after 3 months of storage ($F = 36.29$, $P < .0001$) (Figure 3).

After 1 month of frozen storage, MAP50:50-SF had the lowest average score of 1.3 ± 0.48, while the control group had the highest score of 1.7 ± 0.68. After 3 months, the average melanosis score of each treatment increased (Figure 3). MAP50:50-SF, once again, had the lowest average score at 1.4 ± 0.81. The three remaining variables ranged from 1.7 to 1.8 (Figure 3).

Plate frozen quality evaluation

In order to quantify plate frozen shrimp quality, product available for purchase was obtained from the three collaborators and evaluated. Total shrimp weight from all 10 boxes was higher than the labeled package weight. The overall physical condition of plate frozen shrimp remained intact throughout the packaging and freezing process. The majority of boxes had less than 1% by number damaged and inadvertently peeled shrimp. Boxes C1 and C2 contained the most damaged shrimp at 3.2–2.1%, and the most inadvertently peeled between 2% and 3% (Table 4). Foreign material was low

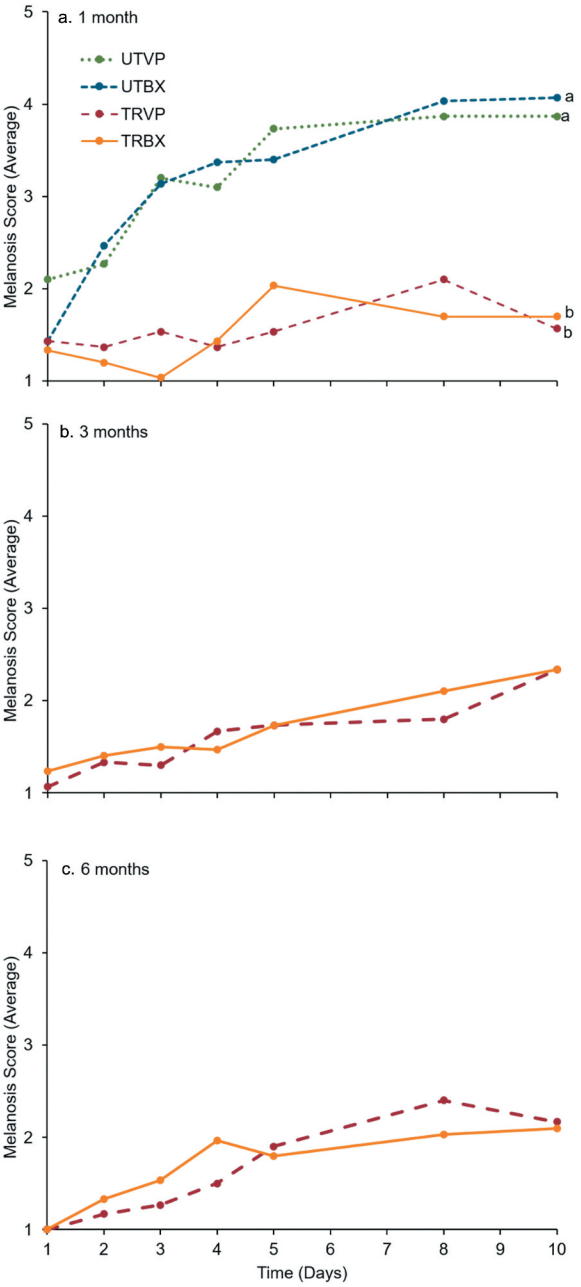

Figure 1. Average melanosis scores per day of plate frozen shrimp at A. 1 month, B. 3 months, and C. 6 months. Shrimp were dipped with Everfresh ® (TR) or untreated (UT) and either vacuum-packaged (VP) or frozen in standard boxes (BX). At 1 month (A), the UT and TR groups were significantly different based on generalized linear mixed models ($F = 60.90$, $P < .0001$), and different letters indicate statistical differences.

in all packages, ranging from 0 to 26 g, with no package exceeding 1% contaminants by weight. Residual sulfite analysis indicated that no samples exceeded 10 ppm residue concentration (minimum detection limit) (Table 4). There was no evidence of heat abuse in any shrimp; therefore, the variable was eliminated.

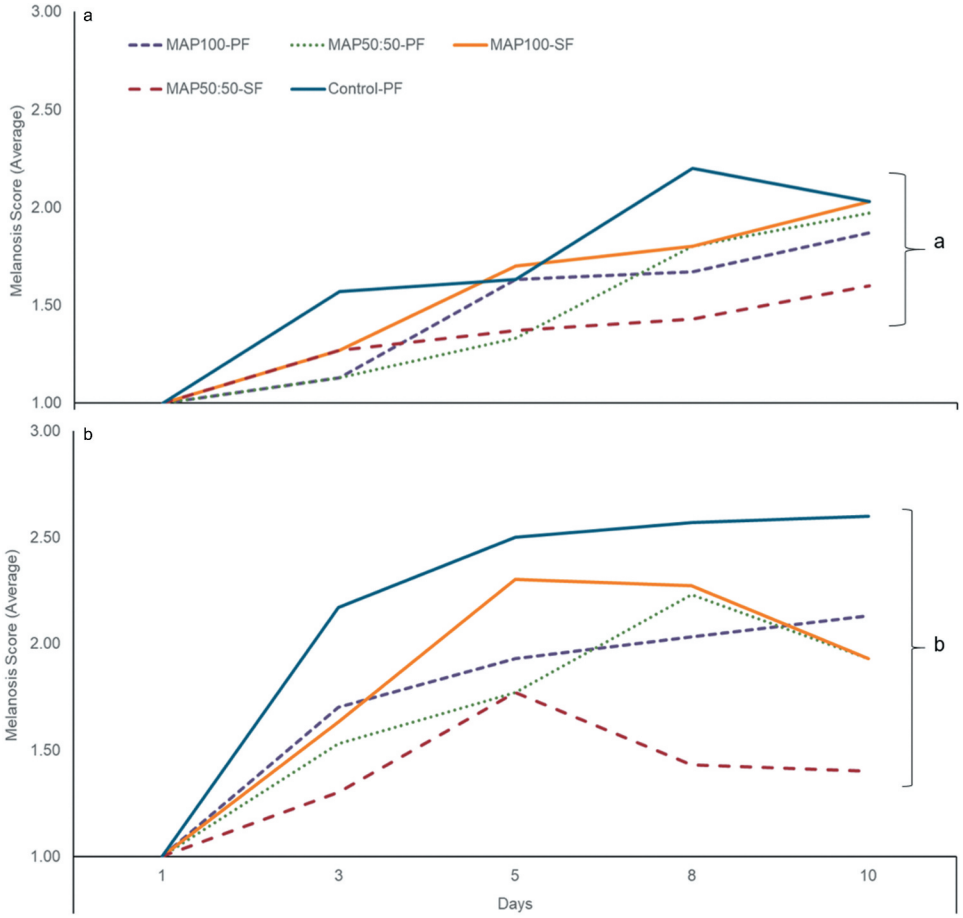

Figure 2. Average melanosis scores per day of modified atmosphere packaged (MAP) shrimp after frozen: A. 1 month and B. 3 months. Months 1 and 3 were significantly different ($F = 36.29$, $P < .0001$). MAP100 = (100% N_2 0% CO_2), MAP50:50 = (50% N_2 50% CO_2, Plate Frozen), SF = (Standard Frozen), PF = (Plate Frozen).

Moisture and salt content analysis

Based on a total of 30 replicates ($n = 30$), three from each of the 10 boxes, salt content ranged from 1.64% to 2.39%, with an average of 2.05 ± 0.29%. Moisture content ranged from 75.6% to 80.7%, with an average of 77.97 ± 1.77%. Shrimp from source A had the lowest average moisture content (76.7%) and the highest average salt content (2.3%). Shrimp from source C had the lowest average salt content of 1.83% (Table 5).

Microbiological analysis

Aerobic plate count, total coliforms, and *E. coli* was determined for samples from each box to ensure shrimp were within acceptable levels. All sample results were within acceptable levels that are considered good quality for shrimp products. APC values ranged from 3.18 to 5.16 Log CFU/g with an average of 4.12 ± 0.61 Log CFU/g. Shrimp boxes from source A had both the highest and lowest APC values (4.35 ± 1.04 Log CFU/g). Samples from source B and C had less APC variation, 4.28 ± 0.24 and 3.85 ± 0.45 Log CFU/g, respectively. The International Commission on Microbiological Specification for Foods sets an upper limit of APC at 6 Log CFU/g for good-quality shrimp, and all samples were below this limit (ICMSF 2011).

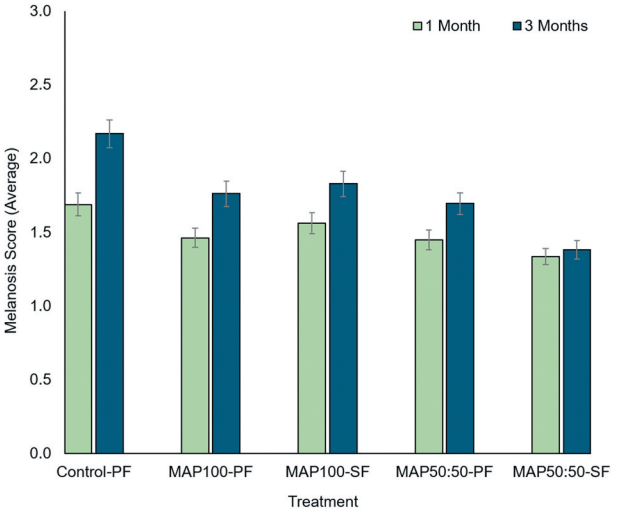

Figure 3. Average melanosis scores per frozen storage period for treatments of modified atmosphere packaged (MAP). Treatment groups were not significantly different based on generalized linear mixed models ($F = 0.64$, $P = .6372$). MAP100 = (100% N_2 0% CO_2), MAP50:50 = (50% N_2 50% CO_2, Plate Frozen), SF = (Standard Frozen), PF = (Plate Frozen) Error bars represent the standard error.

Table 4. Qualitative analysis of plate frozen shrimp quality. Foreign material was recorded as percentage of the box by weight. Damaged and inadvertently peeled shrimp represent percentage of the box by number.

Source	Damaged Shrimp (%)	Inadvertently Peeled (Body) (%)	Inadvertently Peeled (Head) (%)	Contaminants (%)	Residual Sulfite (ppm)
A1	<1.0	0.0	<1.0	<1.0	<10
A2	<1.0	0.0	<1.0	<1.0	<10
A3	<1.0	0.0	<1.0	<1.0	<10
B1	<1.0	0.0	<1.0	<0.1	<10
B2	<1.0	0.0	<1.0	0.0	<10
B3	<1.0	0.0	<1.0	0.0	<10
C1	3.2	<1.0	2.3	<0.1	<10
C2	2.1	0.0	2.7	<0.1	<10
C3	<1.0	0.0	<1.0	<1.0	<10
C4	<1.0	0.0	<1.0	<0.1	<10

Table 5. Moisture and salt content of plate frozen shrimp packages purchased from three sources. All values are an average of three replicates.

Source	Box #	Moisture (%)	Salt Content (%NaCl)
A	1	77.68 ± 0.022	2.39 ± 0.18
A	2	75.69 ± 0.24	2.25 ± 0.086
A	3	76.68 ± 0.23	2.38 ± 0.23
B	1	78.94 ± 0.23	1.88 ± 0.029
B	2	80.71 ± 0.098	1.88 ± 0.077
B	3	77.96 ± 0.24	2.39 ± 0.17
C	1	80.48 ± 0.21	1.64 ± 0.20
C	2	79.21 ± 0.42	1.89 ± 0.10
C	3	75.88 ± 0.33	1.83 ± 0.15
C	4	76.48 ± 0.068	1.96 ± 0.059

Total coliform values ranged from <1 to 2.93 Log CFU/g, and most of the values were <2 Log CFU/g. Samples from box A were again the most variable, while source B was the most consistent at <2 Log CFU/g for each sample. All samples presented undetectable levels for generic *E. coli* (Table 6).

Table 6. Microbiological analysis of plate frozen shrimp boxes from three sources.

Source	APC (Log CFU/g)	Coliforms (Log CFU/g)	*E. coli* (Log CFU/g)
A1	3.18	<1	<1
A2	4.70	<2	<1
A3	5.16	2.93	<1
B1	4.53	<2	<1
B2	4.06	<2	<1
B3	4.26	<2	<1
C1	4.08	<2	<1
C2	3.62	<1	<1
C3	4.35	<2	<1
C4	3.35	<10	<1

Melanosis analysis

Plate frozen shrimp were analyzed for melanosis to determine if proper treatment procedures were being used onboard. Application and treatment method of black spot prevention was unknown for each box prior to evaluation. Black spot score of 3 or greater was considered objectionable or unacceptable. Half of the samples developed objectionable black spot within 4 days (A1, A2, A3, C3, and C4), while the others remained within acceptable levels for all 10 days of analysis (B1, B2, B3, C1, and C2). Shrimps from all the A boxes reached unacceptable levels, while shrimp from all B boxes were acceptable (<3). Box A2 had the highest average black spot score across all days at 4.11 and became objectionable the quickest, within 2 days of thawing. Boxes B1 and C2 had the lowest average black spot score at 1.6 (Figure 4).

Size count analysis

The size count of shrimp was analyzed and compared to the label on each box to determine accuracy in sizing. Shrimps in four of the 10 packages were smaller than the expected minimum size, including one box from each source (Table 7). Box A1 was the only package with an actual average shrimp size that was larger than the size count range.

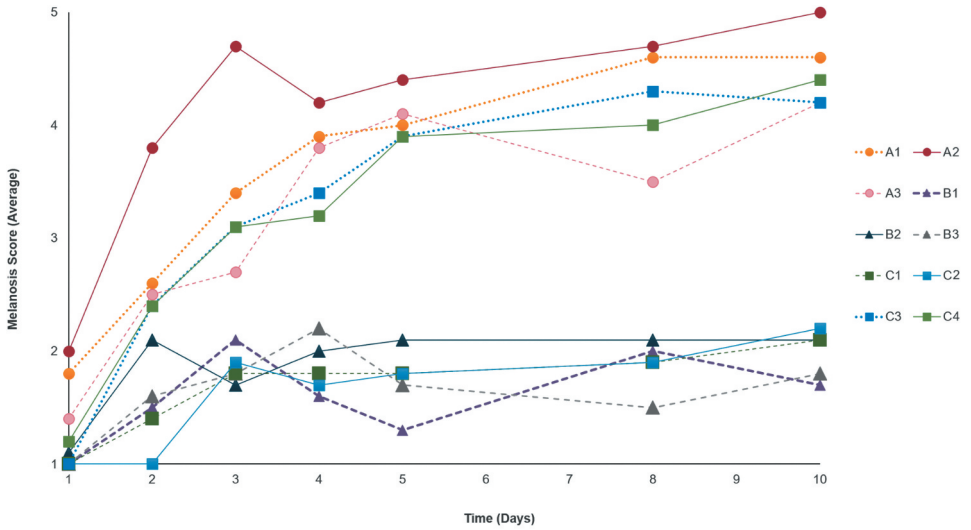

Figure 4. Melanosis scores of plate frozen shrimp from three different sources. Each line represents the average per sample, and similar letters and marker shape represent samples from the same source (Circle = A, Triangle = B, and Square = C).

Table 7. Expected (median size in range) and actual size range of plate frozen shrimp boxes purchased from three sources. Sizes are based on number of shrimp that weigh 1 pound. Values in bold were smaller than indicated. *Shrimp boxes without a labelled size were assumed based on the size counts.

Source	Size Count	Size Range	Expected Average Size	Actual Average Size
A1	16/20	16–20	18	15.6 ± 0.54
A2	26/30	26–30	28	**30.4 ± 1.67**
A3	21/25	21–25	23	24.8 ± 1.30
B1	16/20*	16–20	18	19.6 ± 0.89
B2	21/25*	21–25	23	23.4 ± 1.14
B3	16/20	16–20	18	**20.2 ± 0.45**
C1	13/15	13–15	14	**18.2 ± 0.84**
C2	13/15	13–15	14	**18.0 ± 1.41**
C3	21/25	21–25	23	21.0 ± 0.71
C4	21/25	21–25	23	21.4 ± 0.55

Discussion

The importance of the commercial shrimp industry to Louisiana has been unparalleled in the past century. Shrimp product development through plate freezing shrimp may provide an economic opportunity to help sustain the livelihoods of fishermen but is only possible with quantifiable standards and a realistic standard operating procedure for fishermen to follow. Minimizing the variability observed in post-harvest procedures, improved consistency in handling, and proper treatment with 4-HR could create a premium, plate frozen shrimp product that meets the demand of the high-end seafood market.

Melanosis experiments demonstrated the importance of 4-HR as an alternative additive to reduce black spot. Despite being available for decades, many fishermen are unaware of 4-HR products. Regardless of packaging type, shrimp treated with a 4-HR product remained acceptable for the entire study period. Melanosis analysis was not carried out beyond 10 days post-thaw, as it is unlikely consumers would store raw shrimp for longer periods of time due to spoilage. All untreated samples reached objectionable levels by grading standards within 3 days of thawing (USDC 2011). Our collaborators thought 4-HR products were being used on their boats but still developing black spot. Our results, combined with the box analysis, indicates 4-HR products were not actually being used by the crews. Multiple studies have found similar results. Otwell et al. (1992) found that 4-HR products delayed melanosis for 14 days of ice storage. Iyengar et al. (1991) and Montero et al. (2004) also found that 4-HR inhibited melanosis at low concentrations.

Additionally, there was no effect of vacuum-packaging or MAP in delaying melanosis when used in realistic conditions in the Louisiana shrimp fishery. Although immediate vacuum-packaging and MAP may have yielded different results, neither of these processes are viable on the majority of Louisiana shrimp boats in the industry today. Studies have reported mixed results of packaging style on black spot prevention. The combination of frozen storage and MAP, especially with 100% N_2 gas, inhibited melanosis for 6 months in frozen samples. The same study found vacuum-packaging to be ineffective (Bono et al. 2012). Vacuum-packaging delayed melanosis development in tiger prawns, *Penaeus japonicus*, for up to 21 days sealed in refrigeration (López-Caballero et al. 2000). The effectiveness of packaging in this study, within each storage interval, was likely lost due to the methodology used. Neither packaging technique is feasible on vessel, so there will be a time delay of 12 to 24 hours before shrimp would be vacuum-packed or MAP. When treated within an hour of capture, freezing and MAP was effective at melanosis prevention without a melanosis inhibitor (Bono et al. 2016). Both vacuum-packaging and MAP seals were broken when shrimp were thawed and transferred to coolers for scoring. Although no packaging effect was observed, average melanosis scores were significantly higher between frozen storage periods. This indicates that even while package seals were intact, MAP did not significantly delay black spot formation through frozen storage. MAP machines are expensive, making them an unlikely cost-effective solution and not a viable option for fishermen to use on small in-shore vessels.

The advantages of 4-HR products likely outweigh their disadvantages. 4-HR products are effective at concentrations 250 times lower than sulfites (Iyengar et al. 1991). Functionality does not improve with excess use, and residual compound levels remain low, at 1–2 ppm (Iyengar et al. 1991; Otwell et al. 1992). Sulfites are commonly used in excess, as they have the ability to bleach out black spots that have already formed on shrimp, leading to high residual levels (Iyengar et al. 1991). Sulfite powders release sulfur dioxide fumes when reacted with water (Miget 2010), which has caused death in shrimp holds in the past (Atkinson et al. 1993). For consumers, residual sulfite can elicit reactions in asthmatics and those with food sensitivities (Collins-Williams 1983; Miget 2010). If more than 10 ppm residual sulfite is detected in a product, a label is required on the product declaring that sulfiting agents may be present (FDA 2020). Finally, 4-HR use does not deteriorate shrimp quality. Recent work found that 4-HR application did not affect the proximate composition, microbiological assessment, color analysis, or texture of white or brown shrimp (Khan 2018). A potential deterrent to purchasing 4-HR products is that the compound is slightly more expensive than sulfites. Despite being more expensive, 4-HR is effective at lower concentrations, resulting in less use and lasting longer periods of time. Additionally, while generally considered safer than sulfites by the industry, future research should continue to examine the safety of 4-HR, as some research has indicated it could act as a xenoestrogen (Amadasi et al. 2009), while other research found no xenoestrogen-like behavior for 4-HR in rats (Kang et al. 2020). While not as direct as sulfur dioxide fumes, additional work is needed to understand if there could be health effects on humans working with or ingesting 4-HR.

Analysis of plate-frozen shrimp quality demonstrated its potential to satisfy a premium head-on market. Frozen shrimp from collaborating fishermen were analyzed for physical composition and damage, residual sulfites, microbiology, and melanosis development. The composition of shrimp samples was, on average, 78.0% moisture and 2.05% sodium chloride. These values are comparable to Lee et al. (2002), who found moisture and salt content of *Acetes chinensis* to be 77.5% and 2.3%, respectively. Salt content of shrimp varies greatly in the literature. Studies on cold water shrimp, *Pandalus borealis*, report values of 1.0% (Gudjónsdóttir et al. 2011) and 0.5–2.5% (Zeng et al. 2005). Reported values for farmed Pacific white leg shrimp, *Litopenaeus vannamei*, range from 3–4% in one study (Rattanasatheirn et al. 2008) to 1.0–1.5% in another (Chantarasuwan et al. 2011). Moisture contents are more consistent in the literature, and studies using GOM white shrimp, *Litopenaeus setiferus*, found moisture content to be approximately 77.3% (Khan 2018). It is apparent that seawater, cooling processes, and freezing conditions can affect these values; therefore, results should be interpreted with caution.

The physical condition of shrimp is an extremely important aspect of quality for high-end restaurants utilizing a head-on shrimp product. The shrimp maintained shape and all appendages through the freezing process. Of the physical characteristics analyzed, very few of the packages would result in defect points based on USDC grading standards (USDC 2011). Less than 1% of all shrimps were damaged, inadvertently peeled, or missing heads in most packages. Two packages were slightly more damaged, but they were in frozen storage for over a year, which likely contributed to the decline in quality.

Microbiological analysis also indicated plate frozen shrimp were of high quality. The composition of microorganisms depends on a variety of conditions but is influenced by handling procedures and cold-chain management. High microbial activity is the top cause for quality degradation in shrimp (Yuan et al. 2016), and it is suggested that high bacterial counts in frozen shrimp indicate poor quality at the time of freezing (Yuan et al. 2016). The International Commission on Microbiological Specification for Foods recommends that APC levels should remain below 7 Log CFU/g for good quality (ICMSF 2011). APC values of plate frozen shrimp averaged 4.13 Log CFU/g, with only a sample exceeding 5. An analysis of frozen shrimp purchased across the United States found APC values averaged about 5.48 Log CFU/g (Swartzentruber et al. 1980), nearly double that of the highest value detected in this study. Total coliform values were found to be low in most samples, and averages were comparable to other studies (Swartzentruber et al. 1980). Another study examined microbial quality of retail and wholesale shrimp imported from China, Ecuador, and Mexico and

found the APC values averaged 5.9 Log mean APC/ml across all countries (Berry et al. 1994). The variability of microbiological data within each source is likely a result of packages being purchased at different times, with slightly different handling procedures occurring in the cold-chain management process.

Melanosis results of plate frozen shrimp reflected the lack of consistency in operating procedures observed onboard. Half of the samples developed objectionable black spot by day 3, indicating improper or absence of treatment with a 4-HR product. Although additive type and use was unknown, test results indicated that all plate frozen shrimp samples contained little to no residual sulfites. It is assumed that the other half of samples was properly treated with 4-HR, as they remained at acceptable levels for the duration of analysis. These studies highlight the importance of 4-HR as an effective alternative for sulfite inhibition, as well as the need to standardize protocols on shrimp boats.

Consumer awareness of social and ecological issues associated with fishery products has increased in the past few decades as fisheries are collapsing (Jacquet and Pauly 2007). Plate frozen shrimp fared well in most quality assessments and would be considered a high-quality shrimp product based on USDC/NOAA Seafood Inspection Grades (USDC 2011). The technology gives fishermen the ability to rapidly freeze their catch and hold onto it until prices rise. Despite laws requiring shrimp packages to be labelled with potential sulfite use (FDA 2020), the ability to guarantee a sulfite-free product that will not develop severe melanosis and thaws out still perfect for head-on shrimp dishes could help acquire above-commodity prices and be of interest to consumers and the food industry.

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Data availability statement

Data available upon request.

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