Determining spoilage of whiteleg shrimp (*Litopenaeus vannamei*) during refrigerated storage using colorimetric strips

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ABSTRACT

A reliable spoilage assessment method is needed to ensure sufficient quality control of shrimp. Colorimetric dye-based indicators that change color in response to pH changes can monitor food quality changes in a simple, quick, and accurate way and generate easy-to-interpret results. Significant positive correlations with storage time were observed for the results of the bromophenol blue (BPB) strips ($r = 0.8513$, $p < 0.0001$) and the rose bengal strips ($r = 0.8981$, $p < 0.0001$). The results of both colorimetric methods significantly correlated with sensory and chemical quality indicators, including sensory attributes “salty water-like”, “natto water-like” and “sour milk-like”, and volatile compounds such as 3-methyl-3-butenol, 2-methyl-1-butanol, 3-methyl-1-butanol, hexanol, 2-methyl-1-butanal, and 3-methyl-1-butanal. The BPB strips and rose bengal strips have the potential to be used as objective, accurate, and cost-efficient methods to evaluate shrimp quality and lead to consistent and easy-to-interpret results.

1. Introduction

1.1. Shrimp consumed in the United States

Pacific White Shrimp (*Litopenaeus vannamei*), also known as whiteleg shrimp, is one of the most consumed shrimp species in the U.S. market, other commonly consumed shrimp species are Gulf White Shrimp (*Litopenaeus setiferus*), Brown Shrimp (*Litopenaeus californiensis*), and Gulf Pink Shrimp (*Litopenaeus duorarum*) (FAO, 2019). The per capita consumption of shrimp in the U.S. was approximately 4.7 lbs. (2.1 kg) in 2019 and was estimated to increase by roughly 11% on a year-over-year basis (NMFS, 2021). Shrimp is an abundant source of high-quality fatty acids and protein while low in carbohydrates. Shrimp is also an excellent dietary source of certain vitamins and minerals, such as vitamin B3, folate, calcium, magnesium, phosphorus, and potassium (Wright et al., 2018). Shrimp are an essential part of a well-balanced nutritious diet and should be consumed frequently, especially for people who are looking for a diet high in protein and low in calories.

1.2. Shrimp spoilage

Shrimp is generally high in water, free amino acids, and unsaturated fatty acids, which makes shrimp more prone to degradation than other muscle foods and thus the development of off-flavors and off-odors. The three primary degradation mechanisms of shrimp are enzymatic autolytic action, microbial spoilage, and lipid oxidation (Garcia-Soto et al., 2015). Autolytic changes are mainly caused by proteolytic enzymes, where endogenous enzymes use proteins and peptides as substrates and turn them into nitrogenous compounds and cause tissue softening (Ashie et al., 1996). Autolysis changes happen as soon as the animal dies when there is no preservative treatment applied. Whiteleg shrimp meat exhibited the maximum autolytic activity at 35 °C at acidic pH, and aspartic proteinase was dominate in shrimp muscle autolysis (Eakpetch et al., 2008).

The high amount of unsaturated fatty acids makes seafood highly susceptible to lipid autoxidation compared with other meat products. The ease of formation of fatty acid radicals increases with increasing unsaturation and seafoods typically contain appreciable levels of unsaturated fatty acids. The formation of fatty acid radicals can also be catalyzed by lipoxygenase, which is present in seafood tissues or comes from microbial presence in the food. Hydroperoxides formed from autoxidation itself does not cause off-aromas, but easily decompose to lower molecular weight volatile compounds responsible for off-aromas, including alcohols, aldehydes, and ketones (Damodaran & Parkin, 2017).

After the death of the organism, bacteria start to invade the tissue,
and the metabolites and digestive enzymes that are secreted by bacteria significantly accelerate the spoilage process. Microorganisms that are associated with spoilage processes are often referred to as specific spoilage organisms (SSOs) (Boziaris et al., 2011). SSOs exist in small amounts in the initial stage and dominate the microbial population at the end stage of spoilage (Boziaris et al., 2011). The type of SSOs on seafood is highly dependent on the environment the organism lived in and the storage conditions. In chilled seafood, major SSOs are psychrotrophic gram-negative bacteria, such as Pseudomonas spp., Shewanella putrefaciens, Aeromonas spp., and Photobacterium phosphoreum (Odeyemi et al., 2008). Such bacteria are responsible for the development of compounds that significantly degrade the quality and safety of shrimp. Pseudomonas spp. are found to lead to the formation of volatile sulfides, alcohols (3-methyl-1-butanol and 1-penten-3-ol), and ketones (2-butanone), resulting in stale and putrid off-odors (Odeyemi et al., 2018). Shewanella putrefaciens can produce amines and hydrogen sulfide (Odeyemi et al., 2018).

The production of biogenic amines in spoiled seafood is via the microbial decarboxylation of free amino acids. Histamine, the principle toxin responsible for seafood poisoning, is converted from free histidine by bacterial histamine decarboxylase (Hungerford, 2010). Putrescine and cadaverine are important spoilage indicators of seafood, and are generated from decarboxylation of arginine and lysine, respectively. The generation of histamine-forming bacteria can be inhibited by low temperatures, such as 0 °C, while putrescine and cadaverine-forming bacteria can grow and contribute to amine formation at 0 °C (Lakshmanan et al., 2002). Bacteria belonging to the group of non-fermentative gram-negative rods, including Photobacterium, Aeromonas, and Micrococcus were found to be dominate amine-forming bacteria in shrimp stored on ice for 9–12 days (Lakshmanan et al., 2002).

1.3. Colorimetric methods for the evaluation of food quality

Colorimetric dye-based sensors and indicators that change color in response to pH changes have been investigated for application in monitoring food quality changes. The development of food spoilage sensors and indicators relies on the knowledge of quality-indicating metabolites. For example, lactic acid bacteria ferment glucose and produce organic acids such as acetic acid and lactic acid, as well as ethanol (Wanihsuksombat et al., 2010). Some bacteria grow along with the production of carbon dioxide (Rukchon et al., 2014). Many spoilage bacteria can generate basic volatile nitrogen compounds such as ammonia, dimethylamine, and trimethylamine in food high in protein. These metabolites dissolved in the aqueous environment could lead to changes of the pH of solutions.

Food quality monitoring devices based on the diffusion time of pH-changing compounds in relation with storage time and temperature have been developed. Wanihsuksombat et al. (2010) demonstrated a time-temperature indicator based on the diffusion of lactic acid that could record the effects of both time and temperature during cold storage. The dye mixture solution of bromothymol blue and methyl red was mixed with agar powder to form a gel. In the same device, filter paper absorbed with lactic acid was the source of free lactic acid. The release of lactic acid was a function of both storage time and temperature. The gel responded to the increase of lactic acid and changed color from bright light green to red. Mathematical models were successfully established to demonstrate the relationships between color change, time, and temperature. The indicator could be used for real-time monitoring of quality changes caused by extended storage time and temperature abuse (Wanihsuksombat et al., 2010).

Dräger® tubes which contain pH-sensitive dye was used to determine ammonia concentration in blue crab samples (Sarnoski et al., 2008). Crab samples were homogenized and placed in a sealed glass jar to allow the accumulation of amine volatiles in the headspace. As headspace is pulled through the tubes via vacuum, ammonia caused an increase of pH and led to color changes from yellow to blue of the pH-sensitive dye (Sarnoski et al., 2008). A simplified colorimetric device was reported using bromophenol blue (BPB) absorbed filter paper strips to indicate the freshness of mahi-mahi (Dole et al., 2017). In a later study, Bai et al. (2021) furtherly optimized colorimetric paper strips to improve sensitivity and uniformity of color changes. Miller et al. (2006) stated that the development of colorimetric devices could fulfill the mission of detecting and monitoring food quality changes in a simple, quick, and effective way and generate results that are easily read and understood by the general public. This current study attempts to optimize colorimetric methods based on paper strips coated with pH-responsive dyes for the quality evaluation of shrimp, and to investigate the correlation of the results of colorimetric strips with both sensory and chemical indicators of shrimp spoilage.

2. Materials and methods

2.1. Shrimp sample and preparation

Fresh raw white-leg shrimp were donated by American Mariculture, Inc. (St. James City, FL). Shrimp were packed in ice for same-day transfer to the Aquatic Food Products Laboratory at the University of Florida (Gainesville, FL). The day of arrival was recorded as day 0. Shrimp were then transferred to a 4 °C refrigerator. On day 0, 3, 5, 7, 9, 11 and 14, one seventh of the shrimp were vacuum-sealed in pouches made of metallized laminate to prevent oxygen and light penetration. Pouches of shrimp were stored in a ~20 °C freezer until subsequent analyses. Before the day of analysis, selected frozen shrimp were defrosted overnight in a 4 °C refrigerator. Thawed shrimp were peeled, de-headed and deveined before analyses.

2.2. Determination of volatile profile by SPME-GC–MS

Volatile analysis was performed according to the method published previously (Fan et al., 2020). In brief, seven grams of homogenized shrimp sample was added to a 40 mL amber vial with PTFE silicone septa (Thermo Scientific, Waltham, MA). The amber vial was then filled with 15 mL of saturated NaCl solution, and tightly sealed. The sample vial was placed on a heating block with constant stirring. A DVB/CAR/PDMS fiber (50/30 μm, 2 cm, Supelco, Bellefonte, PA) was inserted into the headspace of the sample, and the extraction was carried out at 60 °C for 25 min. After the headspace extraction, the SPME fiber was withdrawn from the sample vial and transferred to the GC–MS injection port (250 °C) for 1 min. A Shimadzu GCMS-QP2010SE (Shimadzu, Inc., Kyoto, Japan) equipped with a Zebron ZB-5MSplus column (30 m, 0.25 mm ID) was used to separate, identify, and quantify analytes. Chromatographic conditions were as follows: the initial temperature was at 40 °C, and was held for four minutes, the temperature was then increased to 230 °C at 6 °C/min, and was held at 230 °C for 5 min. Analytes were semi-quantified by using 100 ppb internal standard (2-methylpentanal). For each of the seven storage times, the SPME–GC–MS procedures were performed in triplicate, and results were shown as mean values ± standard deviations.

2.3. Descriptive sensory analysis (DA) using chemical references

Descriptive analysis training was performed according to the method published previously (Fan et al., 2021). Briefly, the panel was screened and recruited excluding anyone with seafood allergy or insensitive to seafood sensory characteristics. The training took place over 16 1-hour sessions with two sessions per week. The training had three components. The first component was a one-session introduction to the objective and method. The second major component was 12-sessions of developing chemical reference standards for the five significant aroma attributes. The development of chemical reference standards used volatile compounds that significantly correlated with the intensity ratings of aroma attributes and compounds reported previously to be related with off-
odors in shrimp were also considered. Panelists were presented with chemical references in comparison with the food references and discussed the similarity. Chemical solutions were modified until the panel was satisfied with the chemical reference for each descriptor. After determining the chemical references, panelists practiced rating the intensity of the aroma descriptors using the chemical references and the panel learned to evaluate aromas attributes as a single unit.

The last component of the training was a trial evaluation, where each panelist practiced evaluating the intensities of eight attributes of shrimp samples. The trial evaluation used the same procedures as the final evaluation with a 0–15 point intensity scale on paper ballots. Two-way ANOVA was used to analyze the data to determine if panelists were able to evaluate samples consistently based on the panelists’ sample interaction.

For the final evaluation, panelists received all seven samples (day 0, 3, 5, 7, 9, 11, and 14) at one time and rated samples individually in the same manner as in the trial evaluation. Panelists were not given access to any standard references during the final evaluation session, which is the standard procedure for a final descriptive analysis session.

2.4. Colorimetric strip method

The colorimetric strips were prepared based on the method described in a previous study (Bai et al., 2021). Briefly, the Whatman No. 4 filter papers (Little Chalfont, UK) were cut into squares (1.9 × 1.9 cm) and soaked in a 0.2% BPB solution in 70% ethanol or in 0.24% rose bengal solution in 70% ethanol for 1 min, respectively. Then the strips were dried in a fume hood for 1 h. The rose bengal strips were then acidified by 0.001 M hydrochloric acid (HCl) to convert the color from pink to transparent and were placed in the fume hood for another hour to dry. The dry BPB and rose bengal strips were then ready to measure shrimp samples.

The reaction between shrimp volatiles and colorimetric strips were conducted in sealed 250 mL glass jars where 15 g of homogenized raw shrimp meat was placed at the bottom and a BPB strip or a rose bengal conducted in sealed 250 mL glass jars where 15 g of homogenized raw shrimp samples. The trial evaluation used the same procedures as the final evaluation with a 0–15 point intensity scale on paper ballots. Two-way ANOVA was used to analyze the data to determine if panelists were able to evaluate samples consistently based on the panelists’ sample interaction.

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The reaction between shrimp volatiles and colorimetric strips were conducted in sealed 250 mL glass jars where 15 g of homogenized raw shrimp meat was placed at the bottom and a BPB strip or a rose bengal strip was suspended using a metal clip that hung underneath the cap of the jar. A strip exposed to 15 mL deionized water was used as the blank control for each colorimetric strip method. The BPB or rose bengal strip was allowed to react with the headspace of shrimp sample/DI water for 20 min at room temperature. After the reaction, the strip was removed, and the colors of the four corners and the center of each strip was measured by a colorimeter (Chroma Meter CR-400/410, Konica Minolta, Tokyo, Japan) based on the Hunter L*$a*$b* color system. The color readings of the four corners and the center of each filter paper were averaged. For shrimp of each of the seven storage times, both BPB and rose bengal colorimetric strip methods were performed in triplicate. The absolute differences of color measurements of b* value for BPB strips and a* value for rose bengal strips from the values of blank strips were used to represent color changes after exposure to shrimp samples.

2.5. Statistical analysis

Sensory data was treated as a randomized complete block with replication and was analyzed via the GLM procedure in SAS 9.4. Tukey’s range test (HSD) was performed on the data to determine mean separation for the intensity scores of sensory descriptors, concentrations of volatile compounds, and color readings of colorimetric strips. Pearson correlation analysis was performed between colorimetric data and volatile analysis data, and colorimetric data and sensory data via CORR procedure in SAS 9.4 (SAS Institute Inc., Cary, NC).

3. Results and discussion

To obtain sensitive, high-resolution, and rapid colorimetric strip methods to evaluate the quality of shrimp of the seven storage times, various sample sizes, reaction times, and reaction temperatures were investigated. The optimization of the reaction time, temperature, and sample size started with the conditions used in a previous study where the same types of colorimetric strips were used for the quality evaluation of mahi-mahi and tuna (sample size: 50 g, incubation temperature: 45°C) (Bai et al., 2021). However, both BPB and rose bengal completely changed to the endpoint colors in just a few minutes when reacting with the headspace of moderately spoiled shrimp samples. The colorimetric strips changed colors much faster when reacting with the headspace of shrimp compared to tuna and mahi-mahi, and the differences were likely due to the greater amount of volatile amines released from deteriorated shrimp than from mahi-mahi and tuna. According to Argaiz (1976), trimethylamine was above 300 ppm, and dimethylamine reached to above 60 ppm in raw whole shrimp stored on ice for 7 days. The total amount of biogenic amines calculated in the samples of the lowest FDA/NMFS grades (worst quality) were approximately 39 ppm for mahi-mahi and 24 ppm for tuna (Bai et al., 2019). Therefore, to ensure the development of distinguishable color changes among shrimp of different levels of spoilage within a reasonable amount of time, the sample size, reaction time, and temperature were reduced for spoilage analysis of shrimp compared to that for fish. The optimized conditions were determined to be 15 g of homogenized shrimp meat exposed to freshly-made strips in a 250 mL sealed jar for 20 min at room temperature (25°C).

The mean values ± standard deviation of the colorimetric results are given in Fig. 1. For the BPB strips the b* value in the L*$a*$b* system presents the blue/yellow coordinate, and the greater negative b* value means a bluer hue and a greater positive b* value means a yellower hue. For rose bengal strips the a* value is the red/green coordinate of the L*$a*$b* color system, and a more negative a* value means a greener hue, and the more positive a* value means a redder hue.

BPB is known to be a sulfonated hydroxy-functional triphenylmethylene dye and change colors from yellow to blue when the pH of the environment is higher than the pKa of the dye (Flores, 1978). The BPB loses a proton when dissolved in an aqueous solution with the pH higher than the pKa (4.1) of BPB. The loss of a proton changes the electron distribution within the BPB molecule and shifts the absorption spectra of BPB from UV-blue region to the red region, which leads the visible color changes from yellow to blue (Henari et al., 2012). Miller (2006) patented a colorimetric strip device for monitoring the quality changes of cod based on BPB responding to pH changes, where paper strips were dipped into the BPB solution and allowed to dry, then laminated in a polymer layer, strips were then exposed to a spoiled cod sample headspace. Volatile bases diffused into the indicator strip and changed the color of the strip (Miller, 2006).

Besides BPB, rose bengal is another pH-sensitive dye that has been investigated for the application as a food spoilage indicator (Williams et al., 2006). Rose bengal is in the lactone form and transparent at low pH, and reversibly changes to the quinoid form giving pink color with an increase in pH (Akerlind et al., 2011). Miller et al. (2006) developed a food quality monitoring device where rose bengal can react with volatile amines produced by spoilage bacteria in food and changed its color to indicate the deterioration of food.

BPB and rose bengal change colors in response to a broad range of basic compounds in food. It was reported by Kuchmenko and Mishina (2011) that BPB changed color from blue to yellow when reacting with amines in standard solutions, containing trimethylamine, N,N-dimethylformamide, dimethyl acetel, piperidine, cyclohexylamine, aniline, N,N-dimethylamine, and benzylamine (Bai et al., 2016). It reported that BPB could respond to a broad range of volatile biogenic amines and the color changes had a linear correlation with the concentrations of biogenic amine in mahi-mahi of different quality grades. Rose bengal can be neutralized by basic amines, such as piperidine, diethylamine, triethylamine, and trimethylamine, and changes color from transparent to pink (Paczkowski et al., 1985). Bai et al. (2021) established regression relationships between the results of BPB and rose bengal strips with the concentrations of a biogenic amine cocktail standard solutions with...
acceptance coefficients of determinations ($R^2$), in standard solutions that contained cadaverine, putrescine, histamine, and dimethylamine.

In this study, biogenic amines were not detected in the SPME-GC-MS method used, potentially because of the low affinity of biogenic amines with the stationary phase of the SPME fiber and the GC column. Specifically, the SPME fiber coated with DVB/CAR/PDMS was more suitable for large molecules, while biogenic amines, such as trimethylamine and putrescine, are relatively small molecules that have less affinity with this type of fiber (Fratini et al., 2012; Yu et al., 2008). Additionally, the competitive binding of water molecules for the SPME fiber’s surface likely restricted the extraction of amines. The ZB-5 column used for volatile separation has limited interactions with polar compounds, thus polar and short-chain amine compounds might elute out the column very early before or with the solvent peak (solvent cut time was set at 2.5 min).

The color changes of BPB and rose bengal strips are shown in Fig. 1. When reacting with the headspace of shrimp of increased spoilage level, BPB strips changed colors gradually from yellow to blue, and rose bengal changed gradually from transparent to pink. Correlation analysis also showed that BPB ($r = 0.8513$, $p < 0.0001$) and rose bengal ($r = 0.8981$, $p < 0.0001$) significantly correlated with storage time. Similar results were reported by Bai et al. (2021) that the same BPB and rose bengal colorimetric strips closely correlated with the increasing spoilage grades of mahi-mahi and tuna.

Pearson correlation coefficients ($r$) were also calculated between the colorimetric strip results and results obtained by GC-MS and descriptive sensory analysis to investigate the linear relationship between variables. Since colorimetric strips only respond to pH-changing volatile compounds, the strips are not likely to react with the alcohols, aldehydes, and ketones detected in this study. The significant correlations reflect the accumulation of those compounds may occur concurrently with the production of amines (not detected in this study) and lead to the close positive correlations of alcohols, aldehydes, and ketones with the results of the colorimetric strips. The concurrent accumulation of amines and non-amine volatiles could be due to the diversified microflora that participate in the degradation of seafood. For example, *Shewanella putrefaciens* and *Photobacterium phosphoreum* can produce amines, while *Pseudomonas* mostly generate short-chain alcohols, ketone, and aldehydes in chilled-stored fish (Lougovois and Kyrana, 2005). Additionally, *Shewanella putrefaciens* can produce both heptanone and trimethylamine in chill-stored fish (Lougovois and Kyrana, 2005; Parlapani et al., 2014).

Table 1

| Compounds                        | BPB strip $r$ | P-value | Rose bengal strip $r$ | P-value |
|----------------------------------|--------------|---------|----------------------|---------|
| 3-methyl-3-buten-1-ol            | 0.9148       | 0.0039  | 0.9407               | 0.0016  |
| 2-methyl-1-butanol               | 0.9917       | <0.0001 | 0.9964               | <0.0001 |
| 3-methyl-1-butanol               | 0.7994       | 0.0310  | 0.8475               | 0.0160  |
| hexanol                          | 0.9889       | <0.0001 | 0.9666               | 0.0004  |
| 2,5-dimethyl-3-hexanol           | 0.8763       | 0.0097  | 0.8565               | 0.0139  |
| phenylethyl alcohol              | 0.9017       | 0.0032  | 0.9205               | 0.0033  |
| 2-methyl-1-butanol               | 0.9685       | 0.0003  | 0.9549               | 0.0008  |
| 3-methyl-butanol                 | 0.9830       | <0.0001 | 0.9608               | 0.0006  |
| nonanal                          | 0.9636       | 0.0005  | 0.9410               | 0.0016  |
| decanal                          | 0.9293       | 0.0025  | 0.9172               | 0.0036  |
| hexadecanal                      | 0.8763       | 0.0007  | 0.8565               | 0.0139  |
| 2-pentanone                      | 0.9480       | 0.0012  | 0.9137               | 0.0038  |
| 2-heptanone                      | 0.9296       | 0.0024  | 0.9444               | 0.0014  |
| 2-undecanone                     | 0.8174       | 0.0247  | 0.8553               | 0.0141  |
| 2-tridecanone                    | 0.8763       | 0.0097  | 0.8565               | 0.0139  |
| 5-methyl-2-heptanone             | 0.8764       | 0.0066  | 0.8565               | 0.0135  |
| 6-methyl-5-heptanone-2-one       | 0.8770       | 0.0093  | 0.8565               | 0.0132  |
| tetradecanoic acid               | 0.8763       | 0.0097  | 0.8565               | 0.0139  |
| 3,3’-dimethylbutanoic acid       | 0.8763       | 0.0097  | 0.8565               | 0.0139  |
| acetic acid                      | 0.8598       | 0.0113  | 0.8805               | 0.0089  |
| 3-octen-1-ol acetate             | 0.8398       | 0.0181  | 0.8340               | 0.0197  |

Note: Compounds that significantly correlated with colorimetric results were listed.
colorimetric results were previously reported to be associated with spoilage of seafood. For alcohols, the rapid accumulation of 3-methyl-1-butanol, 2-methyl-1-butanol, and hexanol towards the end of shelf-life was also reported in various seafood (Parlapani et al., 2015; Giri et al., 2010; Duflos et al., 2006), indicating the potential of these compounds being important indicators of spoilage. Phenethyl alcohol has been found in spoiled fish and is produced by gram-negative bacteria of the genus *Achromobacter*. Phenethyl alcohol could impart an odor of rose petal at low concentration and an undesirable odor at high concentration (Feng et al., 2016).

Aldehydes generally have lower odor thresholds than alcohols and therefore have more significant effects on aroma and flavors. Previously, Duflos et al., (2006) reported the increase of 2-methyl-1-butanal and 3-methyl-butanal in spoiled cod, whiting and mackerel. Odeyemi et al. (2018) found the abrupt increase of 2-methyl-butanal to correspond methyl-butanal in spoiled cod, whiting and mackerel. Duflos et al., (2006) reported the increase of 2-methyl-1-butanal and 3-butanone, 2-methyl-1-butanol, and hexanol towards the end of shelf-life decomposed and rejected for entering the U.S. market (NMFS, 2019).

Ketones such as 2-pentanone and 2-heptanone can be produced by *Carnobacterium* species in seafood. *Pseudomonas* and *Shewanella* can also produce 2-heptanone. The compound 2-pentanone was reported to be a spoilage indicator of Atlantic cod stored at 8 °C (Kuiliala et al., 2017). *Pseudomonas* can also cause the production of 2-undecanone in seafood stored in a refrigerator (Parlapani et al., 2017).

For acids, acetic acid is majorly produced by *B. thermosphacta* and lactic acid bacteria. Acetic acid was identified as a potential chemical spoilage index of various seafood (Parlapani et al., 2014; Kuiliala et al., 2018). Another organic acid, tetradecanoic acid, was previously detected in deteriorated raw clam, crab, and prawn meat that were stored at 10 °C (Zhang et al., 2010).

In this study, shrimp samples stored at 4 °C for 0, 3, 5, 7, 9, 11, 14 days were graded into the grades A (good), B (reasonably good), C (slightly decomposed), and D (decomposed) by 15 panelists from the shrimp aroma descriptive analysis (DA) panel using the FDA/NOAA grading standards (Table 2). The results of colorimetric strips were considered to align well with the grading results if the mean separation results are significantly different (without overlapping mean separation letters) between shrimp samples of day 3 and day 5, day 5 and day 7, and between day 9 and day 11, where the grading cutoffs exist (Fig. 1). By comparing the mean separation letters of colorimetric strip results with the grading cutoffs, the results of BPB strips aligned with the grading cutoffs between grades B, C, and D, which indicates a greater resolution of BPB strips towards differentiating the moderate spoiled and highly spoiled shrimp. The results of rose bengal strips aligned with the grading cutoffs between grades A, B, and C, but not between grades B and C, which suggests the rose bengal strips had better resolution for fresh and highly spoiled shrimp samples, while less discriminative for moderate spoiled samples. Overall, the results of rose bengal strips were more consistent with the grading results than the BPB strips, especially among fresh and moderately spoiled shrimp. There was less uniformity of both types of strips at the slightly decomposed stage (grade C) which led to higher standard deviations reported for the a* and b* values and thus likely impacted the discriminative capacity related to mean comparison (Fig. 1). Therefore, it is reasonable to infer that the discriminative capacity of the strips among shrimp of different grades can be potentially improved by reducing the standard deviation of the colorimetric readings. The reduced standard deviation can be achieved by increasing the uniformity of the distribution of colors on the paper squares.

In previous research, “salty water-like” was determined by a trained descriptive sensory panel to be the aroma descriptor of freshness, while “natto water-like” and “sour milk-like” were both spoilage descriptors of whiteleg shrimp (Fan et al., 2021). The results of BPB strips and rose bengal strips negatively correlated with the intensity ratings of descriptors “salty water-like” (BPB: r = -0.674, p = 0.0008; rose bengal: r = -0.718, p = 0.0002), and positively correlated with “natto water-like” (BPB: r = 0.607, p = 0.0035; rose bengal: r = 0.687, p = 0.0006), and “sour milk-like” (BPB: r = 0.688, p = 0.0006; rose bengal: r = 0.701, p = 0.0004) as shrimp became lower in quality. The correlation analysis showed that the colorimetric strip results were consistent with the intensity ratings of the three key aroma descriptors “salty water-like”, “natto water-like” and “sour milk-like”. The correlation analysis also suggested that the results of rose bengal strips were more consistent with the intensity scores of sensory quality indicators than BPB strips as shown by greater Pearson’s correlation coefficients (r) in the ratings across all three key aroma attributes (Table 3).

### Table 2

| Grade                        | Description used to determine defect action levels (NMFS, 2019) |
|------------------------------|---------------------------------------------------------------|
| **Class 1 Passable Grade A** | Good flavor and odor means that the raw product and the cooked product have the normal, pleasant flavor and odor characteristic(s) of freshly caught shrimp that is free from off-flavors and odors of any kind. A natural odor or flavor reminiscent of iodoform is acceptable. |
| **Class 1 Passable Grade B** | Reasonably good flavor and odor means that product may be somewhat lacking in good flavor and odor characteristics of freshly caught shrimp but is free from objectionable off-flavors and off-odors of any kind. |
| **Class 2 Slight Decomposed**| The first stage of definitely identifiable decomposition. An odor is present that, not really intense, is persistent and readily perceptible to the experienced examiner as that of decomposition. |
| **Class 3 Decomposed Grade D**| The product has a very strong odor of decomposition which is persistent, distinct, and unmistakable. |

Note: “Grade C” and “Grade D” used in this study are not real FDA/NOAA grades and equivalent to class 2 and 3 classes in the FDA/NOAA inspection scheme. Class 2 and class 3 shrimp are not furtherly graded and are considered decomposed and rejected for entering the U.S. market (NMFS, 2019).

### Table 3

| Aroma attributes     | BPB strip r | BPB strip P-value | Rose bengal strip r | Rose bengal strip P-value |
|----------------------|-------------|-------------------|---------------------|--------------------------|
| Salty water-like     | -0.674      | 0.0008            | -0.718              | 0.0002                   |
| Natto water-like     | 0.607       | 0.0035            | 0.687               | 0.0006                   |
| Sour milk-like       | 0.688       | 0.0006            | 0.701               | 0.0004                   |
| Dirty sock-like      | 0.092       | 0.6908            | 0.334               | 0.1384                   |
| Fruity-like          | 0.201       | 0.3818            | 0.359               | 0.1995                   |

Note: Aroma attributes significantly correlated (p < 0.05) with colorimetric results are asterisked in the table.
“natto water-like”, and “sour milk-like”. By correlating colorimetric strip results with the GCMS results, there were 6 alcohols, 5 aldehydes, 6 ketones, 3 organic acids, and 1 acetate significantly correlated with colorimetric results. Many of those compounds have been reported to be important chemical spoilage indicators of shrimp, such as 2-methyl-1-butanol, 3-methyl-butanol, hexanal, 2-methyl-1-butanol, 3-methyl-butyl, and acetic acid.

In conclusion, the BBP and rose Bengal strip methods generated results closely correlated with storage time, sensory results, and volatiles associated with shrimp spoilage. Both BBP and rose Bengal colorimetric strips show great potential for being used as simple, cost-effective, and a precise tool that could bring greater convenience to on-site quality assessment of shrimp.

CRediT authorship contribution statement

Ying Fan: Methodology, Validation, Investigation, Writing – original draft, Visualization. Keith R. Schneider: Conceptualization, Writing – review & editing. Paul J. Sarnoski: Conceptualization, Resources, Writing – review & editing, Supervision, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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