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Title. Black drum shelf life comparing three packaging technologies.

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Title: Black Drum (*Pogonias cromis)* Shelf Life Comparing Three Packaging Technologies

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Abstract:

Seafood is one of the main sources of protein around the world, and its consumption continues to grow annually. Because of its high-unsaturated lipid composition, seafood is highly perishable with a relatively short shelf life. Reduced Oxygen Packaging (ROP) technology is recognized to expand shelf life of fresh products by slowing down lipid oxidation and microbial growth. The main goal of this project was to evaluate the shelf life of Black Drum comparing three different packaging technologies. Fresh Black Drum fillets were purchased direct from the dock within 24 hours of being caught. Fillets were packed using three methods: air packed (AP), vacuum packed (VP), Modified Atmosphere Packaging (MAP) (NC – 50% N_2, 50% CO_2 and NCO – 30% N_2, 40% CO_2, 30% O_2). The packed fish were stored at 0.5±2°C for 20 days. Shelf life was studied in terms of TVB-N, TBARS, pH, color, texture, Aerobic Plate Count (APC), *Enterobactereaceae*, yeast, and mold. Microbiological and physical/chemical evaluations were carried out at day 0 and every 4 days thereafter. A 20-day microbial shelf life based on APC was observed in Black Drum fillets stored in MAP, which was an increase of 8 days compared to AP and VP ($p<0.0001$). Even though MAP showed to extend the shelf life of Black Drum based on APC, there were no significant differences in TVB-N, TBARS, pH, color, and texture analyses. There were also no significant differences between the two MAP gas combinations used during this study

($p<0.6672$). This study demonstrated that MAP is effective in extending the shelf life of Black Drum fillets. Extending fish shelf life allows fishermen and processors to reach larger markets.

Introduction

Black Drum (*Pogonias cromis)* is significant to the Louisiana aquaculture industry, ranking the second largest in the state finfish landings. In 2015, 4,132,204 pounds of Black Drum landings were reported ("National Marine Fisheries Service Annual Landings by Species for Louisiana," 2015). Because of its high perishability, fresh Black Drum has a limited shelf life and must be distributed promptly. There is currently a lack of research on extending the shelf life of Black Drum. Finding opportunities to expand its shelf life will allow fishermen and processors to reach larger markets.

The approximate composition of Black Drum flesh from the Gulf of Mexico is 77- 80.2% moisture, 16.1 – 16.4% protein, 2.2 – 2.8% fat, and 25% Polyunsaturated Fatty Acids (PUFA) (NOAA, 1987). Black Drum's high unsaturated lipid composition makes it prone to lipid oxidation, thus decreasing quality and yielding a short shelf life (Azhar and Nisa, 2006; Dowlati et al., 2013; Rodríguez et al., 2015). The degree of lipid oxidation on fresh fish samples can be measured in means of Thiobarbeturic-acid-reactive-substances (TBARS) (Chen *et al.*, 2016). Quality and shelf life are also affected by microbial spoilage, which includes the growth of bacteria, yeast, and mold on the fish fillets. The degree of fish spoilage can be measured using Total Volatile Basic Nitrogen (TVB-N) (Suvanich *et al.*, 2000; Chomnawang *et al.*, 2007).

Reduced oxygen-packaging technologies such as vacuum pack (VP) and Modified Atmosphere Packaging (MAP) have been shown to expand fish shelf life by decreasing lipid oxidation and microbial activity. Preservation studies on meagre fillets, a fish also in the Sciaenidae family, have found that VP and MAP in combination with chilled storage had a longer shelf life than aerobic packaging (Saez et al., 2014 ; Genc et al., 2013). Similarly, in the study of Silbande et al. (2018), Red Drum fillets in plastic bags had a shorter shelf life than VP or MAP samples. No previous studies have explored the effect of VP or MAP on Black Drum fillets.

Vacuum pack technology applications are becoming more common among small processors in the area. However, vacuum packing may affect physical quality of the seafood products, because of pressure over the tissue during packing. Moreover, MAP is less invasive to the tissue and can provide additional benefits by expanding the shelf life and quality of the seafood products (Saez et al., 2014). Implementing this technology would allow the industry to pursue not only local, but also regional and national markets.

The first objective of this study was to evaluate shelf life and quality of Black Drum comparing three different packaging technologies (air packed, vacuum packing, and MAP). A secondary objective was to compare two MAP gas combinations and their effect on shelf life. Black Drum shelf life measures included physical, chemical, and microbiological evaluation.

Materials & Methods

Black Drum was purchased directly from fishermen at the Port of West St. Mary in Franklin, Louisiana, USA. The fish were transported to the Nutrition and Food Sciences laboratories in Baton Rouge, LA, on ice. Fish were packed within 24 hours of harvest. Each sample contained two fillets that weighed approximately 225 g each. The samples were measured and randomly separated into experimental groups. The control product was air packed (AP) in plastic bags (Ziploc®, S.C. Johnson & Son Inc.). The vacuum samples were packed at 99% vacuum in 3 mil vacuum pack bags. MAP samples were packed in 6.5x8.5" Crystalline Polyethylene Terephthalate (CPET) trays (PointFive Packaging, LLC) and the film oxygen transmission rate (OTR) was 1-5 cc/m2/day (TOPAZ TC B-440, PointFive Packaging, LLC). The MAP samples were separated into two gas combination groups; the first MAP group was a combination mixture of 50% N_2 and 50% CO_2 (NC, AirGas). The second MAP group used a tri-gas mixture of 30% N_2, 40% CO_2, and 30% O_2 (NCO, Praxair). The gas concentration in the package headspace were measured using the Oxybaby 6i O_2/CO_2 headspace analyzer (Witt, GA, USA). The packed fish were stored at 0.5±2°C in a walk-in refrigerated unit. Two samples from each group were tested every 4 days until day 20. Chemical analyses included total volatile basic nitrogen (TVB-N), Thiobarbituric-acid-substances (TBARS), moisture content, and pH. Physical evaluation included texture and color. Microbial stability evaluation included aerobic plate count (APC), coliforms, yeast, and mold.

Chemical Analyses

TVB-N was measured using the Kjeldahl distillation apparatus method (Chomnawang, Nantachai, Yongsawatdigul, Thawornchinsombut, & Tungkawachara, 2007; Suvanich, Jahncke, & Marshall, 2000). The concentration of total volatile basic nitrogen was calculated from volume (V) of sulphuric acid added and its concentration (C), using equation TVB-N (mg/100 g sample) = (V * C * 1 * 100/10). A level of 30 mg N/100 g TVB-N was used as an acceptable limit (Chen, Huang, Deng, & Huang, 2016).

For the TBARS analyses, a standard solution for standard curve determination was prepared. The standard solution helped to create a standard curve by using a spectrophotometer Spectronic 200 (ThermoFisher Scientific, PA, USA). The TBARS values were calculated from the standard curve obtained from working solutions and the values are reported in mg malonaldehyde (MDA) equivalent/Kg of tissue. A level of 8 mg MDA equivalent/Kg was used as an acceptable limit (Chen et al., 2016).

Moisture content was measured using the gravimetric method. The pH was measured pH/mV Meter SX811-SS (Apera Instruments, OH, USA).

Physical Analyses

Color of homogenized fish fillets was measured using Hunter color scale values (L*, a*, and b*) using a Baking meter BC-10 colorimeter (Konica Minolta, NJ, USA). L* measures the lightness on a 0-100 scale (black is 0 and white is 100), a* measures redness (positive value is red and a negative value is green), and b* measures yellowness (positive values are yellow and a negative value is blue) (Saez et al., 2015). Values for a* and b* were reported as Hue. Hue angles (*h*) were calculated as $\tan^{-1}(b^*/a^*)$ (Mclellan *et al.*, 1995). The Texture Profile Analysis (TPA) of fish samples were measured with a TA XT plus texture analyzer (Texture Technologies Corp., MA, USA) with a 2-inch diameter compression cylinder. The TPA was performed using a head test speed of 5 mm/s, a strain of 50%, and a break time between both cycles of 10 s. Samples were taken from a specific portion of the dorsal part of the fillets cut into 1 inch squares before machine measurements. From this analysis, three texture parameters were computed. Hardness in Newtons, defined as the peak force during the first compression cycle (Hallier et al., 2007). Cohesiveness, defined as the ratio of positive force area during the second cycle to that during the first cycle, represents the quantity of deformation that the sample can undergo before rupture (Hallier et al., 2007). Resilience, defined as the ratio of the upstroke area to the downstroke area of the first compression peak, corresponds to the ability of the sample to return to its initial position after the first cycle (Hallier et al., 2007).

Microbial Analyses

For microbial stability, the samples were prepared in Phosphate Buffered Saline (PBS) homogenate followed by serial dilutions and plated in duplicate. Aerobic plate count (APC) was performed using Standard Method Agar (Neogen, MI, USA). Samples were incubated for 48 h at 35±2°C. *Enterobacteriaceae*, yeast, and molds were quantified using 3M™ Petrifilm™ (3M™ Microbiology, St. Paul, MN, USA). *Enterobacteriaceae* samples were incubated for 24 h at 35±2°C, and yeast and mold for 3 to 5 days at room temperature.

Statistical Analysis

Significant differences between treatments and APC, *Enterobacteriaceae*, yeast, and mold counts, pH, color, and texture during 20 day shelf life were determined using ANOVA, followed by the Tukey's test (a = 0.05) using JMP Statistical Discovery software (version 14.1; SAS Institute Inc. Cary, NC, USA).

Results

Chemical changes

The MAP NC and the AP treatments produced similar TVB-N data and each experienced the least amount of nitrogenous buildup. The VP and the MAP NCO samples experienced more change over the 20-day period, and the VP samples produced the most nitrogen by day 20. Overall, there were no significant difference in TVB-N between the four treatments.

The TBARS data for each group were averaged and a threshold of 8 mg N/100 g sample were used. As pictured in Figure 1, none of the samples ever reached the threshold, which indicates that there were low levels of lipid oxidation.

There were no significant differences by day 20 in moisture between all sample groups. However, the AP sample group had a significantly higher moisture content on day 20 compared to VP and MAP NC. Overall, moisture content increased in all samples but not significantly. Moisture ranged from 82% at the beginning of the study to 84% at day 20.

The pH value started at 6.69, showing an increase for AP and VP (6.75/6.87) and a decrease for MAP NC and MAP NCO (6.45/6.39). Figure 2 shows that by day 12, the AP and VP samples had a significantly higher pH, indicating more bacterial growth.

Physical Changes

The data for color shows that the L* and Hue values were similar between groups, indicating no significant difference in color between all samples (Table 1 and 2). The TPA demonstrated no significant difference between treatments based on hardness, cohesiveness, and resilience. However, an increase in hardness was observed during the study in all the treatments (Table 3). Cohesiveness and resilience showed a little reduction (Figure 3 and 4).

Microbial changes

The indicator for spoilage for APC was 6 logCFU/g and above. Figure 5 shows that the VP and AP groups reached the threshold at day 16. Based on APC, the MAP NC group and the MAP NCO group never reached the level of spoilage during the experiment. In addition, at day 20, the AP group had a significantly higher APC than the VP and MAPs treatments. Figure 6 shows that the AP treatment had an overall higher yeast count than the other treatments. This trend was seen in both *Enterobacteriaceae* and mold.

Discussion:

In this study, the different packaging techniques of aerobic packing, vacuum packing, and modified atmosphere packaging were tested over a 20-day period to see how well each technique extended the shelf life of Black Drum. Yeast and mold are associated with spoilage of seafood. During the experiment, the AP and VP samples had higher values for yeast and mold than the MAP NC and MAP NCO samples. This along with our results for APC, indicates that the MAP technology prevented the drum from becoming spoiled for 20 days. The pH values have been correlated with microbial growth and have been used to determine yeast growth on samples. By day 12, pH was significantly higher for AP (6.91±0.04) and VP (6.97±0.03) showing that they had more yeast growth than the MAP samples (6.74±0.08 and 6.67±0.15). Provincial (2010) found similar results for vacuum packed storage of catfish with a pH value of 6.8 on day 16. TVB-N was also used as an indicator of spoilage and was not significantly different between the different packaging methods. VP reached its highest concentration of 16.47±2.49 mg N/100g on day 12 while MAP NC and MAP NCO had maximum values of 13.43±3.68 mg N/100g and14.93±3.81 mg N/100g respectively. These values were lower than the results found by Silbande, who studied a MAP NC storage of Red Drum fillets. They reported a maximum concentration of 18.5±1.0 mg N/100g for VP and a concentration of 16.6± 1.0 mg N/100g for the MAP NC. An increase in hardness through the shelf life study might be due to muscle protein denaturation induced by microbial and enzymatic degradation. Protein denaturation has been associated with hardening of fish fillets (Hallier et al., 2008). TPA showed hardness results with a very high variance (Table 3). A hypothesis that could explain these results is the limitation in control of wild caught fish age and size. This could be due to muscle fiber development throughout the fish growth, which can be decisive in the fish fillet texture (Hallier et al., 2007).

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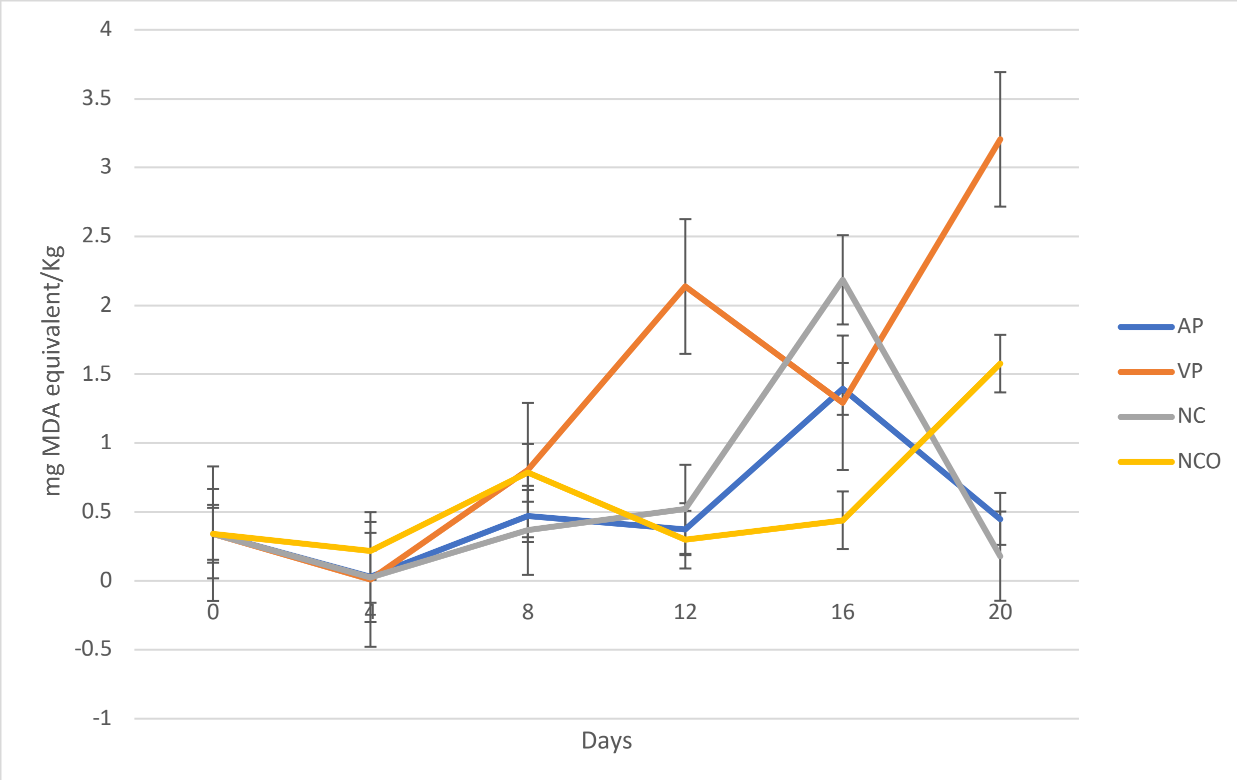

Figure 1. Thiobarbituric-acid-substances (TBARS, mg malonaldehyde (MDA) equivalent/Kg of tissue) values during Black Drum 20-day shelf life study comparing different packaging methods; Air Packed (AP), Vacuum Packed (VP), MAP 50% Nitrogen and 50% Carbon Dioxide (NC), and MAP 30% Nitrogen, 40% CO_2, and 30% O_2 (NCO).

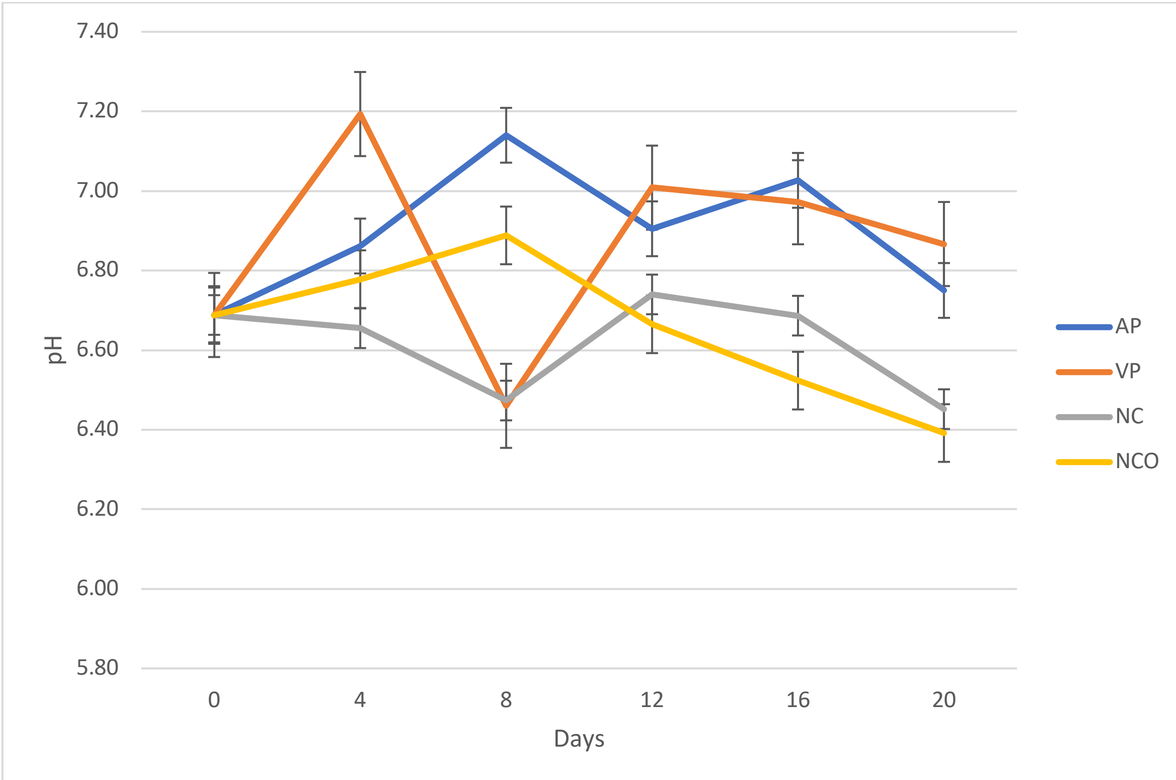

Figure 2. pH values during Black Drum 20-day shelf life study comparing different packaging methods; Air Packed (AP), Vacuum Packed (VP), MAP 50% Nitrogen and 50% Carbon Dioxide (NC), and MAP 30% Nitrogen, 40% CO_2, and 30% O_2 (NCO).

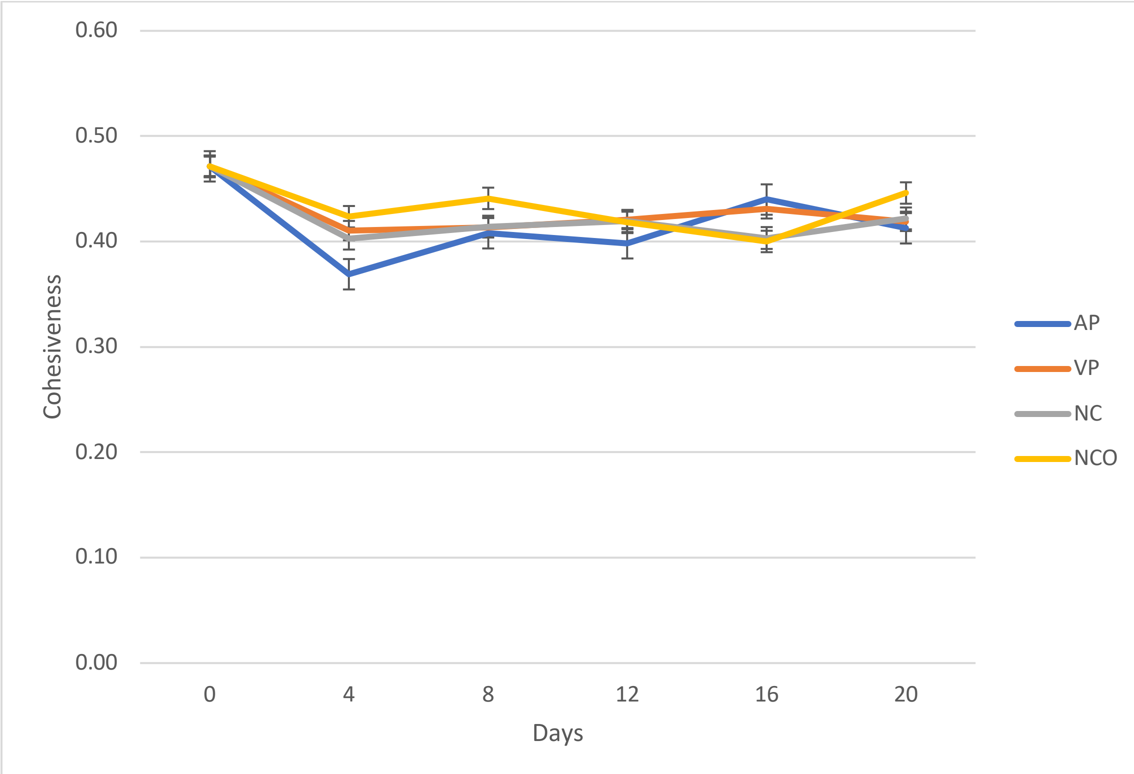

Figure 3. Cohesiveness ratios during Black Drum 20-day shelf life study comparing different packaging methods; Air Packed (AP), Vacuum Packed (VP), MAP 50% Nitrogen and 50% Carbon Dioxide (NC), and MAP 30% Nitrogen, 40% CO_2, and 30% O_2 (NCO).

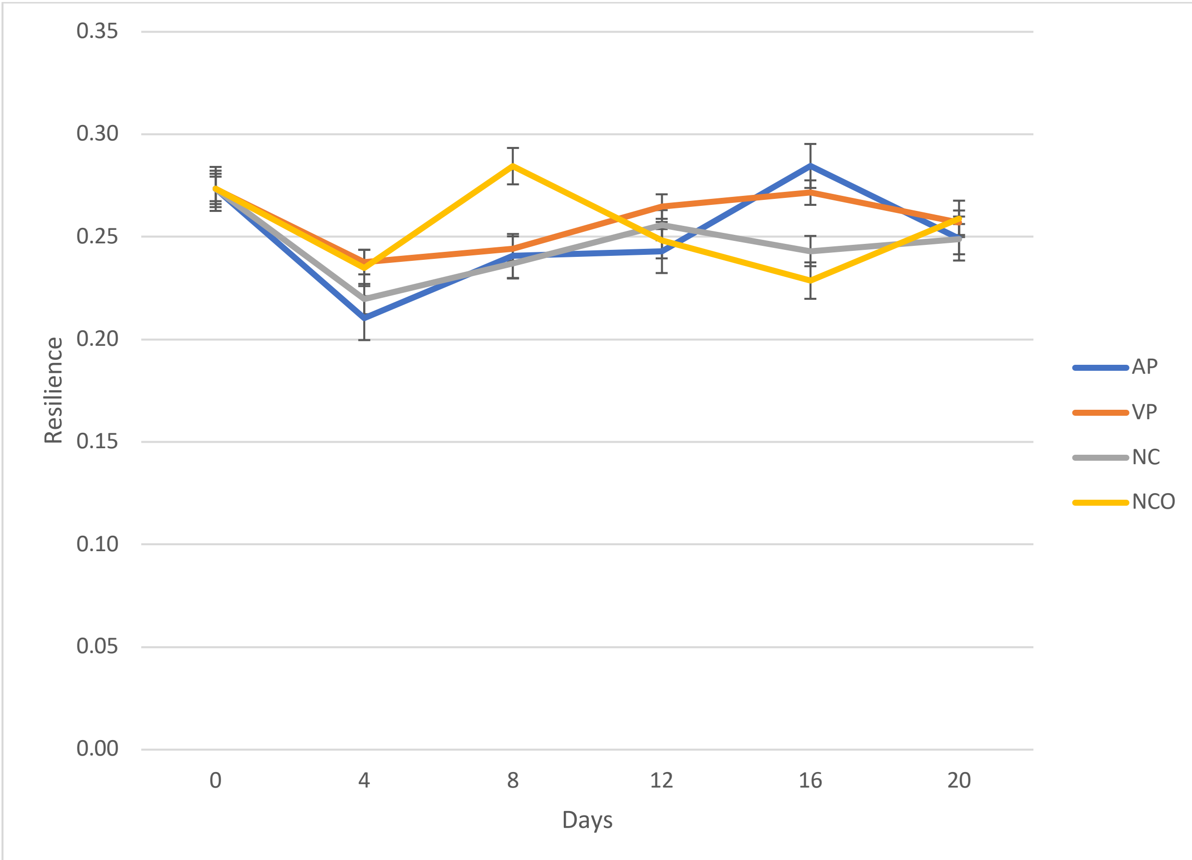

Figure 4. Resilience ratios during Black Drum 20-day shelf life study comparing different packaging methods; Air Packed (AP), Vacuum Packed (VP), MAP 50% Nitrogen and 50% Carbon Dioxide (NC), and MAP 30% Nitrogen, 40% CO2, and 30% O2 (NCO).

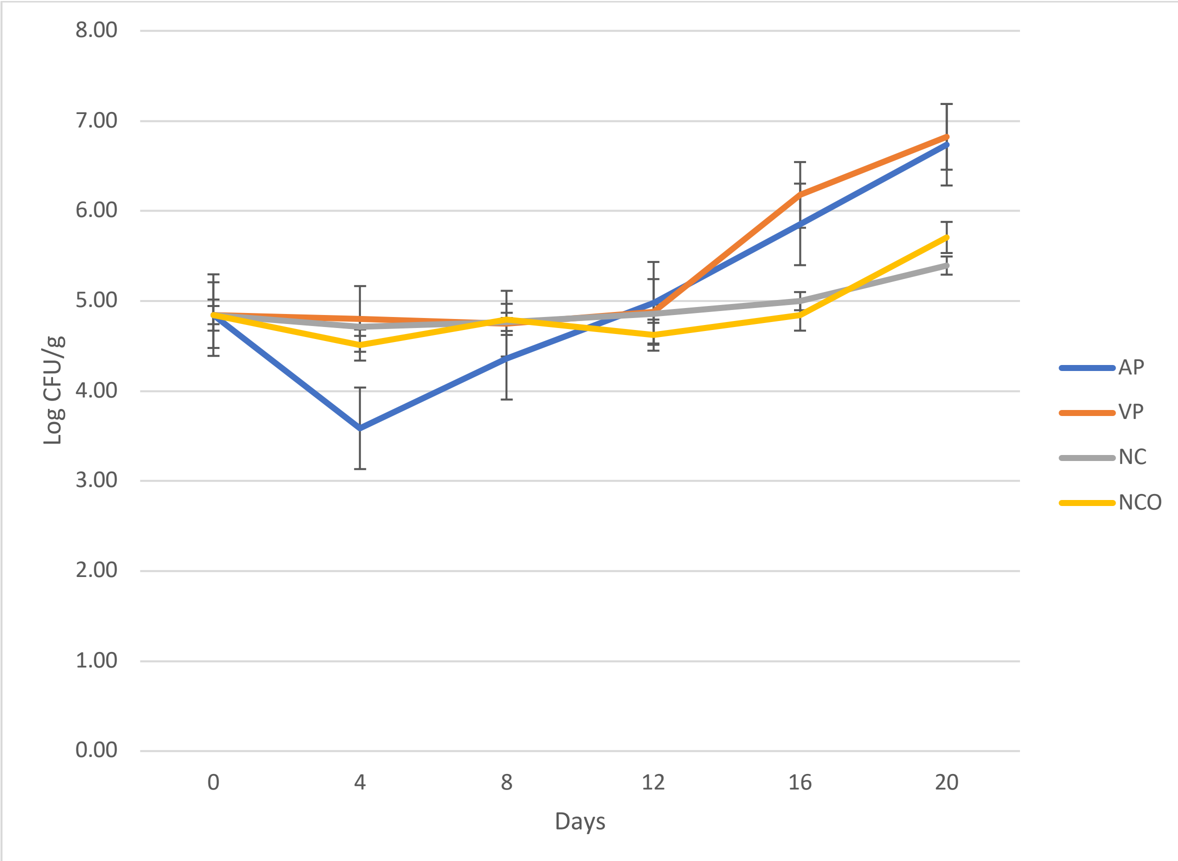

Figure 5. Aerobic plate counts (APC) during Black Drum 20-day shelf life study comparing different packaging methods; Air Packed (AP), Vacuum Packed (VP), MAP 50% Nitrogen and 50% Carbon Dioxide (NC), and MAP 30% Nitrogen, 40% CO_2, and 30% O_2 (NCO).

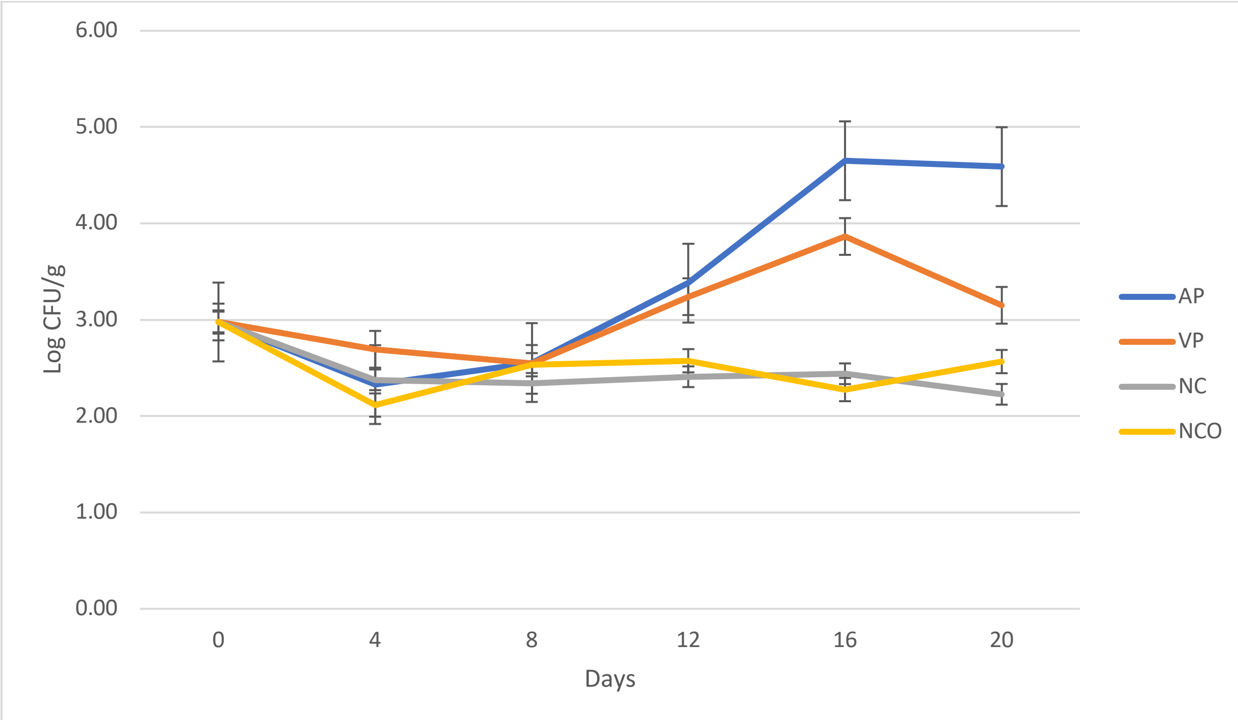

Figure 6. Yeast counts during Black Drum 20-day shelf life study comparing different packaging methods; Air Packed (AP), Vacuum Packed (VP), MAP 50% Nitrogen and 50% Carbon Dioxide (NC), and MAP 30% Nitrogen, 40% CO2, and 30% O2 (NCO).

Table 1. Changes in L* values during Black Drum 20-day shelf life study comparing different packaging methods; Air Packed (AP), Vacuum Packed (VP), MAP 50% Nitrogen and 50% Carbon Dioxide (NC), and MAP 30% Nitrogen, 40% CO2, and 30% O2 (NCO).

Day	AP	VP	NC	NCO
0	65.59±3.61[a]	65.59±3.61[a]	65.59±3.61[a]	65.59±3.61[a]
4	61.90±1.84[a]	62.57±2.30[a]	63.95±2.32[a]	62.17±2.06[a]
8	63.29±1.87[a]	63.41±2.23[a]	64.59±1.00[a]	64.31±2.67[a]
12	62.16±2.40[a]	63.17±2.32[a]	64.72±3.17[a]	63.67±0.92[a]
16	63.77±2.11[a]	63.04±1.89[a]	62.53±1.50[a]	63.00±0.97[a]
20	62.24±3.35[a]	64.26±3.40[a]	65.33±1.78[a]	65.24±2.64[a]

Mean ± Standard Deviation values within each column with different letters are significantly different ($P < 0.05$).

Table 2. Changes in Hue values during Black Drum 20-day shelf life study comparing different packaging methods; Air Packed (AP), Vacuum Packed (VP), MAP 50% Nitrogen and 50% Carbon Dioxide (NC), and MAP 30% Nitrogen, 40% CO2, and 30% O2 (NCO).

Day	AP	VP	NC	NCO
0	0.01±0.60[ab]	0.01±0.60[ab]	0.01±0.60[ab]	0.01±0.60[ab]
4	0.08±0.41[ab]	-0.38±0.67[ab]	-0.15±0.74[ab]	0.47±0.39[a]
8	0.15±0.71[ab]	-0.23±0.51[ab]	-0.01±0.53[ab]	-0.17±0.75[ab]
12	-0.51±0.51[ab]	-0.13±0.38[ab]	-0.22±0.75[ab]	-0.47±0.69[ab]
16	-0.06±0.59[ab]	-0.29±0.87[ab]	-0.18±0.58[ab]	-0.47±0.57[ab]
20	-0.67±0.36[b]	-0.77±0.93[b]	0.05±0.58[ab]	-0.76±0.46[b]

Mean ± Standard Deviation values within each column with different letters are significantly different ($P < 0.05$).

Table 3. Hardness values during Black Drum 20-day shelf life study comparing different packaging methods; Air Packed (AP), Vacuum Packed (VP), MAP 50% Nitrogen and 50% Carbon Dioxide (NC), and MAP 30% Nitrogen, 40% CO2, and 30% O2 (NCO).

Day	AP	VP	NC	NCO
0	33.1±18.9^{cde}	33.1±18.9^{cde}	33.1±18.9^{cde}	33.1±18.9^{cde}
4	32.9±6.8^{cde}	26.0±8.9^{de}	18.9±6.8^{e}	27.5±9.7^{de}
8	69.3±13.9^{ab}	56.8±7.3^{ab}	56.5±15.6^{ab}	67.6±22.5^{bc}
12	68.2±17.9^{ab}	53.4±14.5^{abc}	54.6±16.8^{abc}	52.2±14.0^{ab}
16	74.1±30.4^{a}	62.3±21.0^{ab}	57.2±15.4^{ab}	61.2±17.1^{ab}
20	52.4±4.0^{abc}	54.7±22.4^{abc}	47.9±14.6^{bcd}	66.9±22.7^{ab}

Mean ± Standard Deviation values within each column with different letters are significantly different ($P < 0.05$).