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Effect of 3-Aminobenzoic Acid Ethyl Ester Methanesulfonate (MS-222) on Quality of Marine Cultured Turbot (*Scophthalmus maximus*) during Simulated Transport in Water

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Abstract: This study evaluated the effect of different concentrations (20, 40 and 60 mg/L) of 3-aminobenzoic acid ethyl ester methanesulfonate (MS-222) on the quality changes in turbot during simulated transport in water. The results showed that the ammonia nitrogen content in the transportation water of each sample increased significantly, and the dissolved oxygen level decreased. The dissolved oxygen content in MS-222-treated samples was higher than that of control group (CK) samples. For turbot flesh quality, simulated transport in water led to a decrease in moisture, fat and protein contents in all samples. The MS-222-treated turbot samples showed higher pH values, glycogen contents, springiness and chewiness values and lower lactic acid contents comparing with the CK samples during simulated transport in water. In addition, the fresh and bitter amino acids in the muscle of turbot increased in each treatment group compared to the non-transported fish at the end of the simulated transport. The results showed that MS-222 treatment could retard the turbot transport stress and improve the quality of turbot during simulated transport in water.

Keywords: transport; stress; MS-222; flesh quality; turbot

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1. Introduction

In the aquatic product market, consumers prefer to buy living fish. The transport of living fish is considered one of the most necessary processes that is performed in fish farms. However, fish transport could cause fish stress [1]. Fish are susceptible to shocks and collisions, triggering a series of stress responses, leading to a decline in immune system function, resulting in disease and even death [2]. Factors such as duration, load density and changes in physical and chemical water parameters can also cause stress to the fish [3].

Some studies have investigated alternatives to minimize the stress and contributed to the fish wellbeing, such as density control and the addition of anesthetics to the transport water [4]. Now, anesthesia is one of the important stress mitigation techniques used in a variety of aquaculture operations [5]. During simulated transport in water, anesthetics can keep the fish calm and avoid struggling or trauma, thereby improving the survival rate of fish. Many anesthetics have been used in fish, including 3-aminobenzoic acid ethyl ester methanesulfonate (MS-222), clove oil, 2-phenoxyethanol, CO_2, benzocaine, etc. [6]. MS-222 is an inhalational anesthetic and commonly used in fish transport [7]. It is the only anesthetic used by the USFDA for fish [8]. In addition, Fazio F. et al. used new analytical techniques and found that MS-222 is also used in aquaculture [9].

At present, there are many studies based on fish anesthesia experiments and simulated transport experiments, mainly focused on anesthetic effect on different fishes at physiological and biochemical level [10–13]. However, there are few studies on the effect of MS-222 on fish quality and flavor. Fish flesh is the main edible part, and the deterioration in flesh quality is one of the most important problems in the aquaculture industry [14], especially the pressure during simulated transport [15]. Mohamed et al. indicated that channel catfish quality can be affected by the activity and stresses experienced during transport [14].

Turbot (*Scophthalmus maximus*) is recognized as one of the world's highest quality species of flounder. The flavor and health benefits make the turbot an economically important fish that is in high demand. Thus, developing the methods that help turbot stay alive and maintain quality during transport can be of great economic value. This study aims to investigate the changes in the influence of stress on water quality, the chemical compositions and the changes in turbot flavor quality during simulated transport in water with different concentrations of MS-222 additions.

2. Results

2.1. Water Quality Parameters

The results indicated that the total ammonia nitrogen (TAN) of water was significantly increased (Table 1), while water dissolved oxygen and pH was significantly reduced after simulated transport ($p < 0.05$). After the 24 h of simulated transport, the dissolved oxygen level in the water of each MS-222-treated sample was higher than that of the CK sample. The contents of ammonia nitrogen in MS-222-treated sample was just reversed. It is worth noting that the 60 mg/L MS-222-treated sample had the highest dissolved oxygen level and the lowest ammonia nitrogen content.

Table 1. Water quality parameters during simulated transport.

	Samples	0 h	6 h	12 h	18 h	24 h
Dissolved oxygen ($mg \cdot L^{-1}$)	CK	6.84 ± 0.17^{Aa}	4.37 ± 0.20^{Ab}	3.52 ± 0.13^{Ac}	2.47 ± 0.08^{Ad}	1.18 ± 0.23^{Ae}
	MS-222-20 mg/L	6.81 ± 0.09^{Aa}	5.03 ± 0.26^{Bb}	4.21 ± 0.33^{Bc}	3.37 ± 0.28^{Bd}	2.05 ± 0.17^{Be}
	MS-222-40 mg/L	6.86 ± 0.08^{Aa}	5.15 ± 0.25^{Bb}	4.42 ± 0.16^{Bc}	3.64 ± 0.23^{Cd}	2.65 ± 0.16^{Ce}
	MS-222-60 mg/L	6.83 ± 0.10^{Aa}	5.61 ± 0.14^{Cb}	5.08 ± 0.37^{Cc}	4.32 ± 0.24^{Dd}	3.23 ± 0.19^{De}
TAN ($mg \cdot L^{-1}$)	CK	0.09 ± 0.01^{Aa}	1.83 ± 0.12^{Ab}	2.73 ± 0.10^{Ac}	3.62 ± 0.13^{Ad}	4.14 ± 0.09^{Ae}
	MS-222-20 mg/L	0.10 ± 0.01^{Aa}	1.22 ± 0.09^{Bb}	2.03 ± 0.14^{Bc}	3.02 ± 0.07^{Bd}	3.51 ± 0.08^{Be}
	MS-222-40 mg/L	0.08 ± 0.01^{Aa}	1.14 ± 0.10^{Bb}	1.91 ± 0.18^{Bc}	2.68 ± 0.06^{Cd}	3.09 ± 0.26^{Ce}
	MS-222-60 mg/L	0.09 ± 0.01^{Aa}	0.88 ± 0.55^{Cb}	1.47 ± 0.55^{Cc}	2.27 ± 0.03^{Dd}	2.70 ± 0.08^{De}
pH	CK	7.53 ± 0.13^{Aa}	7.32 ± 0.11^{Ab}	7.14 ± 0.06^{Ac}	6.92 ± 0.12^{Ad}	6.79 ± 0.10^{Ae}
	MS-222-20 mg/L	7.49 ± 0.05^{Aa}	7.40 ± 0.08^{Bb}	7.29 ± 0.12^{Bc}	7.13 ± 0.11^{Bd}	6.98 ± 0.07^{Be}
	MS-222-40 mg/L	7.50 ± 0.11^{Aa}	7.41 ± 0.06^{Bb}	7.31 ± 0.03^{Bc}	7.18 ± 0.04^{Cd}	7.06 ± 0.09^{Ce}
	MS-222-60 mg/L	7.51 ± 0.09^{Aa}	7.44 ± 0.07^{Bb}	7.40 ± 0.08^{Cc}	7.24 ± 0.09^{Dd}	7.13 ± 0.04^{De}

Different capital letters indicate a significant difference in the average value in the same column. Different lowercase letters indicate a significant difference in the average value in the same row ($p < 0.05$). TAN, total ammonia nitrogen ($mg \cdot L^{-1}$). CK, control group.

2.2. Proximate Results of Turbot Flesh

As shown in Table 2, the flesh moisture and fat contents of turbot had a significant decrease after 24 h of simulated transport. However, the protein content increased significantly ($p < 0.05$). The changes in the turbot flesh moisture, fat and protein contents in the MS-222-treated samples were significantly different from those in CK samples ($p < 0.05$). These proximate results of turbot flesh in each MS-222-treated sample did not change significantly from 0 to 12 h. With the simulated transport continued, the proximate results of MS-222-20 mg/L changed significantly; however, there were no significant changes between the MS-222-40 mg/L and MS-222-60 mg/L samples.

Table 2. The effect of simulated transport stress on the chemical composition of turbot flesh.

	Samples	0 h	6 h	12 h	18 h	24 h
Water content (%)	CK	78.29 ± 0.17 ^{Aa}	78.14 ± 0.08 ^{Aa}	78.02 ± 0.10 ^{Aab}	77.85 ± 0.08 ^{Abc}	77.60 ± 0.19 ^{Ac}
	MS-222-20 mg/L	78.29 ± 0.17 ^{Aa}	78.19 ± 0.06 ^{Aa}	78.12 ± 0.04 ^{Aa}	78.01 ± 0.13 ^{ABab}	77.84 ± 0.07 ^{ABb}
	MS-222-40 mg/L	78.29 ± 0.17 ^{Aa}	78.20 ± 0.12 ^{Aa}	78.15 ± 0.05 ^{Aa}	78.09 ± 0.09 ^{Ba}	78.01 ± 0.14 ^{BCa}
	MS-222-60 mg/L	78.29 ± 0.17 ^{Aa}	78.21 ± 0.13 ^{Aab}	78.17 ± 0.10 ^{Aab}	78.12 ± 0.09 ^{Bab}	78.05 ± 0.03 ^{Cb}
Crude fat (%)	CK	1.44 ± 0.02 ^{Aa}	1.26 ± 0.04 ^{Ab}	1.07 ± 0.08 ^{Ac}	0.81 ± 0.07 ^{Ad}	0.67 ± 0.05 ^{Ae}
	MS-222-20 mg/L	1.44 ± 0.02 ^{Aa}	1.32 ± 0.07 ^{Bb}	1.19 ± 0.12 ^{Bc}	0.91 ± 0.12 ^{Bd}	0.77 ± 0.10 ^{Be}
	MS-222-40 mg/L	1.44 ± 0.02 ^{Aa}	1.33 ± 0.03 ^{Bb}	1.22 ± 0.03 ^{Bb}	1.04 ± 0.15 ^{Cd}	0.92 ± 0.07 ^{Ce}
	MS-222-60 mg/L	1.44 ± 0.02 ^{Aa}	1.35 ± 0.03 ^{Bb}	1.23 ± 0.06 ^{Bb}	1.02 ± 0.08 ^{Cd}	0.89 ± 0.04 ^{Ce}
Crude protein (%)	CK	18.53 ± 0.06 ^{Aa}	18.77 ± 0.13 ^{Ab}	18.90 ± 0.07 ^{Ac}	19.19 ± 0.10 ^{Ad}	19.55 ± 0.19 ^{Ae}
	MS-222-20 mg/L	18.53 ± 0.06 ^{Aa}	18.69 ± 0.17 ^{Bb}	18.83 ± 0.21 ^{ABc}	18.98 ± 0.06 ^{Bd}	19.28 ± 0.14 ^{Be}
	MS-222-40 mg/L	18.53 ± 0.06 ^{Aa}	18.61 ± 0.15 ^{Cb}	18.72 ± 0.09 ^{Bc}	19.86 ± 0.12 ^{Bd}	19.15 ± 0.27 ^{Ce}
	MS-222-60 mg/L	18.53 ± 0.06 ^{Aa}	18.62 ± 0.21 ^{Cb}	18.74 ± 0.10 ^{Bc}	18.89 ± 0.17 ^{Bd}	19.12 ± 0.11 ^{Ce}

Different capital letters indicate a significant difference in the average value in the same column. Different lowercase letters indicate a significant difference in the average value in the same row ($p < 0.05$). CK, control group.

2.3. pH Values

The MS-222-treated turbot samples showed higher pH values comparing with the CK samples during simulated transport in water (Figure 1). There were significant differences in the pH values of the MS-222-treated samples and the CK samples at each sampling time ($p < 0.05$); however, no significant differences were found among the MS-222-treated samples during simulated transport ($p > 0.05$).

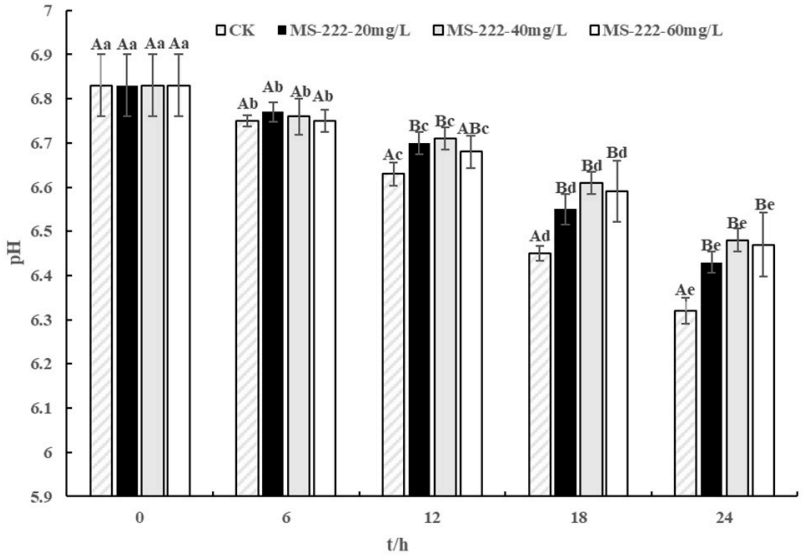

Figure 1. Flesh pH of turbot fillets as affected by simulated transport stress. Different capital letters indicate significant differences in the average between groups. Different lowercase letters indicate significant differences in the average value within each group ($p < 0.05$). CK, control group.

2.4. Texture Profile Analysis (TPA) Results

The results showed that the hardness of all samples was increased during simulated transport in water, and the springiness is decreased (Table 3). However, the hardness of the MS-222-treated samples from 0 to 18 h did not change significantly ($p > 0.05$), and the CK samples and the MS-222-20 mg/L samples had significant changes at the end of simulated transport ($p < 0.05$). The springiness and chewiness of the MS-222-treated turbot samples were significantly improved comparing with the CK samples at the end of simulated transport ($p < 0.05$).

Table 3. Flesh texture of turbot during simulated transport.

	Samples	0 h	6 h	12 h	18 h	24 h
Springiness (%)	CK	0.838 ± 0.013^{Aa}	0.832 ± 0.009^{Ab}	0.821 ± 0.006^{Ac}	0.814 ± 0.011^{Ad}	0.798 ± 0.015^{Ae}
	MS-222-20 mg/L	0.838 ± 0.013^{Aa}	0.835 ± 0.007^{Bb}	0.827 ± 0.004^{Bc}	0.819 ± 0.005^{Bd}	0.812 ± 0.012^{Be}
	MS-222-40 mg/L	0.838 ± 0.013^{Aa}	0.836 ± 0.010^{Bb}	0.830 ± 0.009^{Cc}	0.824 ± 0.009^{Cd}	0.816 ± 0.007^{Be}
	MS-222-60 mg/L	0.838 ± 0.013^{Aa}	0.836 ± 0.003^{Bb}	0.829 ± 0.012^{BCc}	0.822 ± 0.010^{BCd}	0.815 ± 0.005^{Be}
Hardness (g)	CK	4815.13 ± 121.67^{Aa}	4852.71 ± 132.93^{Aa}	4895.40 ± 108.15^{Aa}	4961.82 ± 133.05^{Aa}	5015.46 ± 100.08^{Ab}
	MS-222-20 mg/L	4815.13 ± 121.67^{Aa}	4843.27 ± 160.02^{Aa}	4879.16 ± 152.58^{Aa}	4921.64 ± 138.81^{Aa}	4982.77 ± 108.28^{Ab}
	MS-222-40 mg/L	4815.13 ± 121.67^{Aa}	4838.07 ± 142.66^{Aa}	4864.31 ± 136.14^{Aa}	4900.36 ± 153.76^{Aa}	4944.15 ± 171.84^{Ab}
	MS-222-60 mg/L	4815.13 ± 121.67^{Aa}	4826.78 ± 153.47^{Aa}	4866.52 ± 106.63^{Aa}	4895.48 ± 162.37^{Aa}	4947.89 ± 152.79^{Aa}
Chewiness	CK	477.53 ± 12.17^{Aa}	487.21 ± 16.37^{Aa}	495.83 ± 16.82^{Aab}	504.81 ± 13.17^{Aab}	514.62 ± 12.07^{Ab}
	MS-222-20 mg/L	477.53 ± 12.17^{Aa}	484.14 ± 13.56^{Aa}	490.92 ± 10.43^{Aab}	499.25 ± 14.53^{Aab}	508.74 ± 11.07^{Ab}
	MS-222-40 mg/L	477.53 ± 12.17^{Aa}	482.63 ± 13.91^{Aa}	486.87 ± 9.43^{Aab}	494.26 ± 16.83^{Aab}	502.48 ± 17.63^{Ab}
	MS-222-60 mg/L	477.53 ± 12.17^{Aa}	$480.52 \pm 8.66b^{Aa}$	485.12 ± 12.73^{Aa}	492.53 ± 13.68^{Aa}	499.76 ± 16.89^{Aa}
Cohesiveness	CK	0.726 ± 0.014^{Aa}	0.729 ± 0.005^{Aa}	0.732 ± 0.009^{Aa}	0.735 ± 0.011^{Aa}	0.738 ± 0.012^{Aa}
	MS-222-20 mg/L	0.726 ± 0.014^{Aa}	0.730 ± 0.008^{Aa}	0.731 ± 0.014^{Aa}	0.733 ± 0.005^{Aa}	0.737 ± 0.007^{Aa}
	MS-222-40 mg/L	0.726 ± 0.014^{Aa}	0.727 ± 0.012^{Aa}	0.729 ± 0.006^{Aa}	0.731 ± 0.012^{Aa}	0.734 ± 0.011^{Aa}
	MS-222-60 mg/L	0.726 ± 0.014^{Aa}	0.728 ± 0.011^{Aa}	0.729 ± 0.012^{Aa}	0.732 ± 0.007^{Aa}	0.734 ± 0.009^{Aa}

Different capital letters indicate a significant difference in the average value in the same column. Different lowercase letters indicate a significant difference in the average value in the same row ($p < 0.05$). CK, control group.

2.5. Water Holding Capacity (WHC) Values

From Figure 2, we found that the WHC values of turbot flesh decreased during the simulated transport. The reduction rate of WHC of MS-222-treated samples were significantly lower than that of the CK samples ($p < 0.05$); however, no significant differences were found among the MS-222-treated samples at the end of simulated transport (p>0.05). The decrease rate of MS-222-20 mg/L samples was lower than that of other samples, and the CK samples had the biggest decrease.

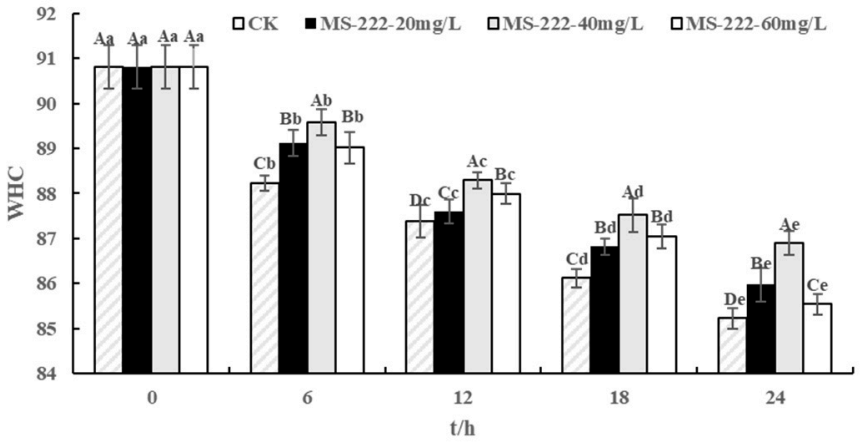

Figure 2. Changes in the water holding capacity (WHC) of turbot flesh during simulated transport. Different capital letters indicate significant differences in the average between groups. Different lowercase letters indicate significant differences in the average value within each group ($p < 0.05$). CK, control group.

2.6. Glycogen and Lactic Acid Changes

The glycogen contents in all turbot samples decreased and the lactic acid contents increased (Figure 3), and the glycogen content in all MS-222-treated samples were higher than that of CK samples and the 60 mg/L MS-222-treated samples from 0 to 12 h was significantly reduced comparing with 20 mg/L MS-222-treated and 40 mg/L MS-222-treated samples.

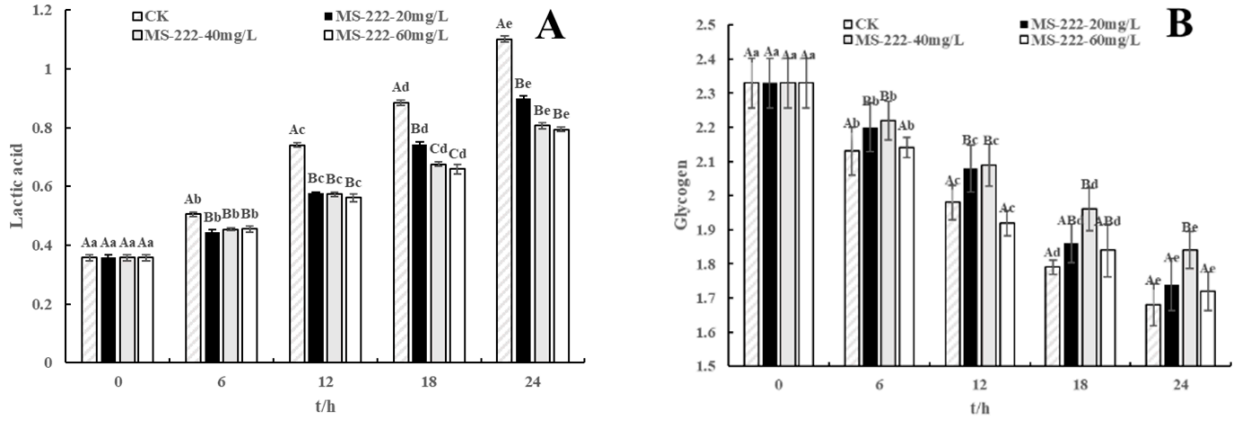

Figure 3. Flesh glycogen (**A**) and lactic acid (**B**) changes in turbot affected by transport stress. Different capital letters indicate that there is a significant difference in the average between the groups. Different lowercase letters indicate significant differences in the average of each group ($p < 0.05$). CK, control group.

2.7. ATP-Related Compounds

Compared with the fish in the control group, the K value of turbot gradually increased during simulated transport (Figure 4A). IMP presents a first increasing and then decreasing trend (Figure 4B). The IMP contents of MS-222-20 mg/L and MS-222-40 mg/L showed increasing trends and the Hx showed a decreasing trend, while CK and MS-222-60 mg/L showed the opposite trends. The contents of Hx and HxR in all turbot samples decreased at first and then increased (Figure 4C,D).

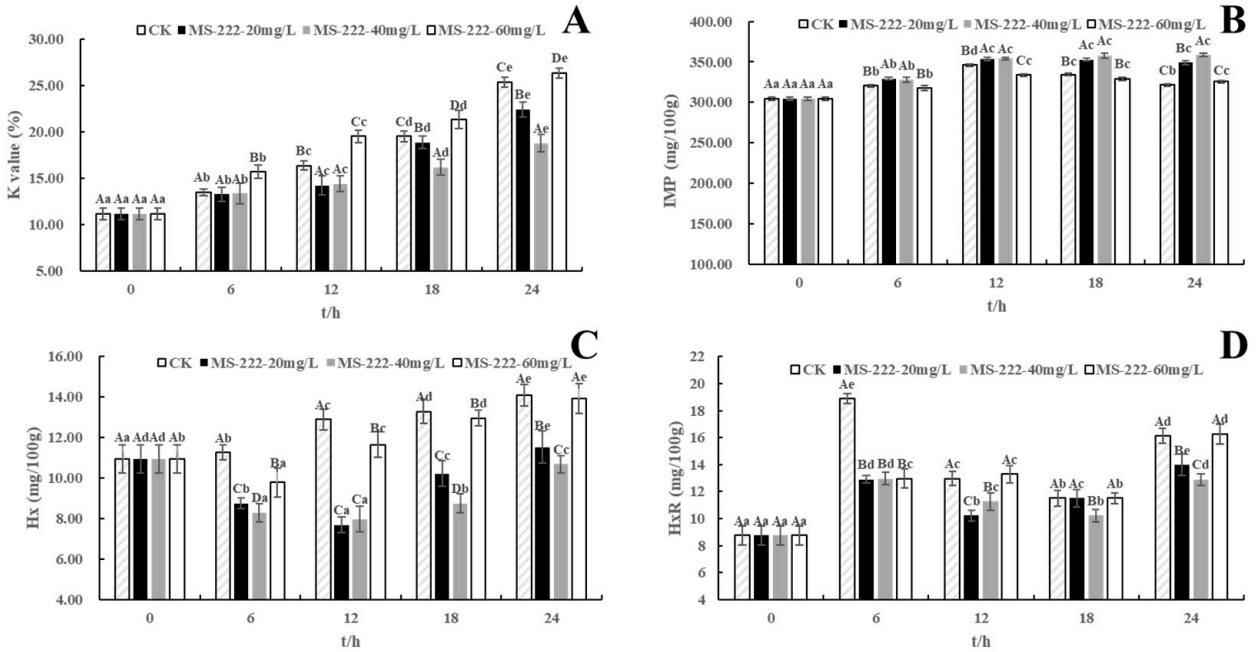

Figure 4. Changes in K values (**A**), inosine 5′-monophosphate (IMP) (**B**), inosine (HxR) (**C**) and hypoxanthine (Hx) (**D**) of turbot (*Psetta maxima*) during simulated transport. Bars represent the standard deviation ($n = 3$). Different capital letters indicate significant differences in the average between groups. Different lowercase letters in-dicate significant differences in the average value within each group ($p < 0.05$). CK, control group.

2.8. Free Amino Acids (FAAs) Analysis

The total amount of FAAs in CK, MS-222-20 mL, MS-222-40 mL and MS-222-60 mL samples increased by 13.62, 13.36, 14.43 and 10.73% at the end compared with the samples on 0 h, respectively, during the simulated transport. The FAAs in Table 4 were divided

into umami and bitter amino acids according to their functional groups. Among them, umami amino acids in CK, MS-222-20 mL, MS-222-40 mL and MS-222-60 mL samples on 24 h increased by 12.64, 14.92, 15.15 and 10.33% comparing with 0 h, and bitter amino acids increased by 14.60, 9.32, 7.67 and 13.70%, respectively.

Table 4. Changes in free amino acids in turbot flesh during simulated transport.

Time	Samples	Aspartic Acid	Threonine	Serine	Glutamic Acid	Glycine
6 h	Not transported	3.03 ± 0.36	15.87 ± 0.75	9.25 ± 0.28	13.02 ± 0.43	70.69 ± 0.49
	CK	3.18 ± 0.13 ^{Aa}	14.74 ± 0.18 ^{Aa}	9.53 ± 0.31 ^{Aa}	14.22 ± 0.26 ^{Aa}	74.43 ± 0.62 ^{Aa}
	MS-222-20 mg/L	3.07 ± 0.22 ^{ABa}	14.93 ± 0.25 ^{ABa}	9.40 ± 0.19 ^{Aa}	13.68 ± 0.18 ^{Ba}	76.03 ± 0.43 ^{Ba}
	MS-222-40 mg/L	2.84 ± 0.14 ^{Ba}	15.13 ± 0.21 ^{Ba}	9.42 ± 0.12 ^{Aa}	13.72 ± 0.34 ^{Ba}	75.29 ± 0.31 ^{Ba}
	MS-222-60 mg/L	2.78 ± 0.22 ^{Ba}	14.69 ± 0.13 ^{Aa}	9.59 ± 0.28 ^{Aa}	14.07 ± 0.21 ^{ABa}	72.64 ± 0.28 ^{Ca}
12 h	CK	3.37 ± 0.19 ^{Aab}	13.95 ± 0.31 ^{Ab}	9.98 ± 0.17 ^{ABab}	15.11 ± 0.23 ^{Ab}	77.19 ± 0.17 ^{Ab}
	MS-222-20 mg/L	3.15 ± 0.07 ^{Aa}	14.42 ± 0.23 ^{BCb}	9.76 ± 0.14 ^{Ab}	14.37 ± 0.19 ^{Bb}	79.62 ± 0.33 ^{Bb}
	MS-222-40 mg/L	2.93 ± 0.13 ^{Bab}	14.76 ± 0.11 ^{Cb}	9.68 ± 0.21 ^{Aab}	14.48 ± 0.25 ^{Bb}	80.39 ± 0.19 ^{Bb}
	MS-222-60 mg/L	3.09 ± 0.23 ^{ABab}	14.08 ± 0.17 ^{ABb}	10.17 ± 0.23 ^{Bb}	15.28 ± 0.32 ^{Abc}	75.57 ± 0.23 ^{Cb}
18 h	CK	3.65 ± 0.26 ^{Abc}	13.36 ± 0.24 ^{Ac}	10.34 ± 0.26 ^{ABbc}	16.24 ± 0.28 ^{Ac}	80.46 ± 0.24 ^{Ac}
	MS-222-20 mg/L	3.37 ± 0.21 ^{ABab}	13.85 ± 0.26 ^{Bc}	10.09 ± 0.13 ^{Ac}	15.12 ± 0.41 ^{Bc}	84.53 ± 0.17 ^{Bc}
	MS-222-40 mg/L	3.10 ± 0.14 ^{Bb}	14.21 ± 0.08 ^{Bc}	10.01 ± 0.24 ^{Ab}	14.93 ± 0.13 ^{Bc}	85.75 ± 0.36 ^{Cc}
	MS-222-60 mg/L	3.28 ± 0.12 ^{ABb}	13.24 ± 0.16 ^{Ac}	10.58 ± 0.17 ^{Bc}	15.84 ± 0.26 ^{Ac}	78.35 ± 0.21 ^{Dc}
24 h	CK	3.98 ± 0.11 ^{Ac}	12.68 ± 0.27 ^{Ad}	10.86 ± 0.31 ^{Ac}	17.05 ± 0.16 ^{Ad}	86.71 ± 0.43 ^{Ad}
	MS-222-20 mg/L	3.61 ± 0.15 ^{Bb}	13.23 ± 0.14 ^{Bd}	10.53 ± 0.18 ^{Ad}	15.89 ± 0.28 ^{Bd}	89.63 ± 0.18 ^{Bd}
	MS-222-40 mg/L	3.27 ± 0.22 ^{Cb}	13.88 ± 0.36 ^{Cd}	10.42 ± 0.13 ^{Ac}	15.56 ± 0.23 ^{Bd}	92.57 ± 0.24 ^{Cd}
	MS-222-60 mg/L	3.63 ± 0.17 ^{Bc}	12.41 ± 0.22 ^{Ad}	10.69 ± 0.23 ^{Ac}	16.43 ± 0.22 ^{Cd}	82.49 ± 0.17 ^{Dd}
Time	Samples	Alanine	Valine	Methionine	Isoleucine	Leucine
6 h	Not transported	28.36 ± 0.62	6.69 ± 0.31	6.07 ± 0.37	4.88 ± 0.34	8.53 ± 0.29
	CK	29.22 ± 0.28 ^{ABa}	6.98 ± 0.21 ^{Aa}	6.15 ± 0.09 ^{Aa}	5.12 ± 0.21 ^{Aa}	8.76 ± 0.17 ^{Aa}
	MS-222-20 mg/L	29.62 ± 0.19 ^{Aa}	6.77 ± 0.13 ^{Aa}	6.22 ± 0.14 ^{Aa}	5.00 ± 0.07 ^{Aa}	8.56 ± 0.09 ^{Aa}
	MS-222-40 mg/L	29.45 ± 0.23 ^{Aa}	6.80 ± 0.11 ^{Aa}	6.26 ± 0.07 ^{Aa}	5.05 ± 0.11 ^{Aa}	8.62 ± 0.11 ^{Aa}
	MS-222-60 mg/L	28.92 ± 0.27 ^{Ba}	6.92 ± 0.06 ^{Aa}	6.14 ± 0.11 ^{Aa}	5.16 ± 0.22 ^{Aa}	8.74 ± 0.05 ^{Aa}
12 h	CK	30.45 ± 0.16 ^{Ab}	7.46 ± 0.23 ^{Ab}	6.28 ± 0.11 ^{Aab}	5.27 ± 0.14 ^{Aa}	8.93 ± 0.14 ^{Aa}
	MS-222-20 mg/L	31.06 ± 0.24 ^{Bb}	7.11 ± 0.17 ^{Bb}	6.37 ± 0.04 ^{Aab}	5.13 ± 0.09 ^{Aab}	8.65 ± 0.06 ^{Ba}
	MS-222-40 mg/L	31.14 ± 0.11 ^{Bb}	7.05 ± 0.09 ^{Bb}	6.42 ± 0.08 ^{Ab}	5.15 ± 0.06 ^{Aab}	8.70 ± 0.08 ^{Ba}
	MS-222-60 mg/L	29.47 ± 0.26 ^{Cb}	7.33 ± 0.13 ^{ABb}	6.26 ± 0.13 ^{Aab}	5.33 ± 0.13 ^{Aa}	8.89 ± 0.16 ^{ABa}
18 h	CK	31.46 ± 0.17 ^{Ac}	8.03 ± 0.19 ^{Ac}	6.40 ± 0.10 ^{Ab}	5.44 ± 0.23 ^{Aab}	9.08 ± 0.22 ^{Aa}
	MS-222-20 mg/L	32.06 ± 0.21 ^{Bc}	7.62 ± 0.22 ^{BCc}	6.58 ± 0.07 ^{Ab}	5.30 ± 0.11 ^{ABb}	8.74 ± 0.13 ^{ABab}
	MS-222-40 mg/L	32.53 ± 0.23 ^{Bc}	7.44 ± 0.14 ^{Cc}	6.61 ± 0.14 ^{Abc}	5.26 ± 0.08 ^{Bb}	8.77 ± 0.11 ^{ABa}
	MS-222-60 mg/L	30.38 ± 0.44 ^{Cc}	7.89 ± 0.11 ^{ABc}	6.43 ± 0.21 ^{Aab}	5.43 ± 0.13 ^{Aa}	9.16 ± 0.07 ^{Bb}
24 h	CK	32.56 ± 0.36 ^{Ad}	8.74 ± 0.08 ^{Ad}	6.51 ± 0.15 ^{Ab}	5.80 ± 0.09 ^{Ab}	9.49 ± 0.14 ^{Ab}
	MS-222-20 mg/L	33.47 ± 0.25 ^{Bd}	8.11 ± 0.33 ^{BCd}	6.72 ± 0.06 ^{ABc}	5.58 ± 0.12 ^{ABc}	8.93 ± 0.21 ^{Bb}
	MS-222-40 mg/L	34.12 ± 0.43 ^{Cd}	7.86 ± 0.21 ^{Cd}	6.80 ± 0.09 ^{Bc}	5.44 ± 0.09 ^{Bc}	8.96 ± 0.06 ^{Bb}
	MS-222-60 mg/L	31.53 ± 0.17 ^{Dd}	8.42 ± 0.27 ^{ABd}	6.58 ± 0.12 ^{Ab}	5.77 ± 0.14 ^{Ab}	9.44 ± 0.12 ^{Ac}
Time	Samples	Tyrosine	Phenylalanine	Lysine	Histidine	Arginine
6 h	Not transported	8.37 ± 0.21	4.36 ± 0.28	38.54 ± 0.54	4.70 ± 0.25	9.59 ± 0.32
	CK	8.51 ± 0.07 ^{Aa}	4.48 ± 0.13 ^{Aa}	38.79 ± 0.06 ^{Aa}	4.83 ± 0.14 ^{Aa}	9.86 ± 0.14 ^{Aa}
	MS-222-20 mg/L	8.40 ± 0.05 ^{Aa}	4.40 ± 0.05 ^{Aa}	38.59 ± 0.11 ^{Aa}	4.75 ± 0.06 ^{Aa}	9.67 ± 0.12 ^{Aa}
	MS-222-40 mg/L	8.38 ± 0.07 ^{Aa}	4.34 ± 0.08 ^{Aa}	38.62 ± 0.07 ^{Aa}	4.77 ± 0.10 ^{Aa}	9.70 ± 0.08 ^{Aa}
	MS-222-60 mg/L	8.47 ± 0.11 ^{Aa}	4.46 ± 0.06 ^{Aa}	38.74 ± 0.14 ^{Aa}	4.92 ± 0.21 ^{Aa}	9.88 ± 0.17 ^{Aa}
12 h	CK	8.59 ± 0.04 ^{Aa}	4.62 ± 0.11 ^{Aab}	38.96 ± 0.10 ^{Ab}	4.95 ± 0.07 ^{ABab}	10.04 ± 0.13 ^{Aa}
	MS-222-20 mg/L	8.47 ± 0.07 ^{Bab}	4.46 ± 0.07 ^{ABa}	38.72 ± 0.07 ^{Bab}	4.82 ± 0.04 ^{Aa}	9.81 ± 0.07 ^{Ba}
	MS-222-40 mg/L	8.42 ± 0.10 ^{Bab}	4.42 ± 0.04 ^{Bab}	38.71 ± 0.16 ^{Bab}	4.83 ± 0.09 ^{Aa}	9.83 ± 0.09 ^{ABab}
	MS-222-60 mg/L	8.58 ± 0.03 ^{Aa}	4.58 ± 0.06 ^{ABa}	39.01 ± 0.13 ^{Aa}	5.03 ± 0.11 ^{Ba}	10.16±0.14 ^{ABab}
18 h	CK	8.66 ± 0.04 ^{Aab}	4.73 ± 0.08 ^{Aab}	39.21 ± 0.14 ^{Ac}	5.14 ± 0.14 ^{ACbc}	10.37 ± 0.11 ^{Ab}
	MS-222-20 mg/L	8.53 ± 0.07 ^{Bbc}	4.55 ± 0.04 ^{Bb}	38.86 ± 0.11 ^{Bb}	4.96 ± 0.05 ^{ABb}	9.99 ± 0.08 ^{Bb}
	MS-222-40 mg/L	8.48 ± 0.05 ^{Bab}	4.49 ± 0.06 ^{Bb}	38.79 ± 0.07 ^{Bb}	4.90 ± 0.07 ^{Bab}	9.94 ± 0.05 ^{Bb}
	MS-222-60 mg/L	8.72 ± 0.12 ^{Aa}	4.80 ± 0.11 ^{Ab}	39.40 ± 0.16 ^{Ab}	5.33 ± 0.18 ^{Cb}	10.42 ± 0.14 ^{Ab}

Table 4. *Cont.*

Time	Samples	Aspartic Acid	Threonine	Serine	Glutamic Acid	Glycine
24 h	CK	8.73 ± 0.08^{Ab}	4.86 ± 0.14^{Ab}	39.52 ± 0.21^{Ac}	5.33 ± 0.12^{ACc}	10.55 ± 0.17^{Ab}
	MS-222-20 mg/L	8.60 ± 0.04^{Bc}	4.67 ± 0.05^{Bc}	39.11 ± 0.09^{Bc}	5.09 ± 0.11^{ABb}	10.12 ± 0.11^{Bb}
	MS-222-40 mg/L	8.54 ± 0.03^{Bb}	4.58 ± 0.07^{Bb}	38.94 ± 0.11^{Bb}	5.01 ± 0.06^{Bb}	10.06 ± 0.09^{Bb}
	MS-222-60 mg/L	8.81 ± 0.11^{Ab}	4.89 ± 0.10^{Ab}	39.52 ± 0.18^{Ab}	5.47 ± 0.23^{Cb}	10.68 ± 0.23^{Ab}
Time	Samples	Proline		Total		
6 h	Not transported	7.09 ± 0.26		239.04		
	CK	7.43 ± 0.17^{Aa}		242.23		
	MS-222-20 mg/L	7.16 ± 0.07^{Ba}		246.61		
	MS-222-40 mg/L	7.05 ± 0.13^{Ba}		245.44		
	MS-222-60 mg/L	7.33 ± 0.14^{ABa}		243.45		
12 h	CK	7.66 ± 0.15^{Ab}		252.81		
	MS-222-20 mg/L	7.29 ± 0.11^{Ba}		253.21		
	MS-222-40 mg/L	7.18 ± 0.09^{Bab}		254.19		
	MS-222-60 mg/L	7.52 ± 0.08^{Aa}		250.35		
18 h	CK	7.90 ± 0.21^{Abc}		260.47		
	MS-222-20 mg/L	7.46 ± 0.15^{Bb}		261.61		
	MS-222-40 mg/L	7.33 ± 0.14^{Bbc}		258.05		
	MS-222-60 mg/L	7.74 ± 0.11^{Ab}		257.09		
24 h	CK	8.23 ± 0.16^{Ac}		271.60		
	MS-222-20 mg/L	7.69 ± 0.22^{Bc}		270.98		
	MS-222-40 mg/L	7.51 ± 0.09^{Bc}		273.52		
	MS-222-60 mg/L	7.94 ± 0.14^{Ab}		264.70		

Among simulated transport groups of different treatments, different capital letters indicate significant differences be-tween different treatment groups at the same time, different lowercase letters indicate significant differences within the same treatment group at different times. ($p < 0.05$). CK, control group.

3. Discussion

During simulated transport, the main cause of stress is the mechanical wear caused by the inevitable contact between fish and fish under high-density conditions [16]. MS-222 reduce swimming activities and breathing intensity of fish and avoid physical injury, which is suitable for long-distance high-density simulated transport. The water quality parameters are essential to improve the efficiency of long distance high-density simulated transport for fish [17]. The marine cultured turbot transport in water could produce the water quality degradation leading to the reduced dissolved oxygen and increased ammonia nitrogen. The concentrations of ammonia nitrogen increased during simulated transport as the fish were confined in plastic bags and the excretion of metabolites [17]. In this study, the ammonia content in the transport water of MS-222 treatment group was found to be lower than that of the control group, indicating that MS-222 was able to reduce ammonia emission from turbot. Similarly, clove oil (10.4 mg/L) reduced the ammonia excretion of *Colossoma macropomum* during 15 h of simulated transport [18]. The water pH value was decreased during simulated transport for turbot mainly for the respiration producing the CO_2 [19]. This finding was also confirmed by the study of Anjos et al. [20].

Loading and simulated transport put tremendous pressure on fish, which induced alterations of moisture, fat and protein contents of the turbot flesh observed in the present study. The decrease in moisture content may be the result of metabolic disorders and enzyme dysfunction during simulated transport. This finding is also consistent with the findings of Jrpeland et al. who found that stressed *Gadus morhua L.* had a significantly reduced moisture content compared with CK samples [21]. Fat is an essential source of energy for fish, and their metabolism may change under stressful conditions. Fish under simulated transport pressure need more energy to cope with the stress, which may lead to fat content changes in metabolism [22]. Changes in fat metabolism were also reported in *Solea senegalensis* [23]. In addition, the change in protein is related to the change in fat, where a negative relationship between protein and fat contents in Nile tilapia, *Oreochromis niloticus* [24]. It was observed in the results of this experiment that the changes in water

content, fat and protein in the muscle of turbot samples in the MS-222 treatment group were less than those in the control group. Among them, MS-222-40 mg/L and MS-222-60 mg/L showed the least changes in muscle chemistry of turbot, indicating that this concentration condition could alleviate the transport stress on turbot.

pH is an important flesh quality parameter [25]. The decrease in dissolved oxygen during simulated transport resulted in an increase in anaerobic metabolism, leading to the conversion of glycogen to lactic acid, which caused the flesh pH to drop rapidly to the point of death [26,27]. The decrease in pH values may cheapen the quality of turbot, such as loss of WHC, and change the texture of flesh [28].

Texture profile analysis (TPA) is a key factor in overall acceptability and consumer satisfaction [29]. The decrease in elasticity due to simulated transport pressure may be attributed to the decrease in flesh pH values, which led to the denaturation of flesh protein and also decreased the springiness [28]. On the other hand, the moisture content decreased during simulated transport resulted in an increase in flesh stiffness, and the relationship between moisture content and hardness is negatively correlated. This is in agreement with the results obtained by Dunajski, who found that fish flesh tissue with a higher moisture and fat contents tend to be softer [30].

Water holding capacity (WHC) is an important quality parameter affecting both profitability and quality [31]. The decrease in WHC value reflects the decrease in water–protein interaction caused by changes in endogenous autolytic enzymes and pH value of fish during simulated transport [32]. This is consistent with the research of MJA den et al. who reported that simulated transport will not lead to higher water loss. Flesh with low WHC is more sensitive to simulated transport vibration than the flesh with high WHC [33].

The glycogen contents in all turbot samples decreased, and the lactic acid contents increased (Figure 3). The decrease in dissolved oxygen during simulated transport leads to an increase in anaerobic metabolism, and lactic acid is the main metabolite. According to Moraes et al. [34], the decrease in oxygen content in water is a common source of stress for fish, resulting in a large amount of muscular lactate output into the plasma. The increase in lactic acid content indicates that the fish cannot maintain the initial homeostasis. Once under stress conditions, flesh glycogen reserves were mobilized to provide energy [35]. The glycogen content of MS-222-60 mg/L was significantly reduced, which could be ascribed to the fish produces stress stimulation to high concentrations of MS-222. Activation of the neuroendocrine system of fish under stress conditions triggers the release of catecholamines and corticosteroids hormones from the interstitial tissues of fish, affecting the storage of carbohydrates and lipids, especially the storage of glycogen [36]. However, 40 mg/L MS-222-treated samples can reduce the consumption of fish flesh glycogen during simulated transport. As shown in previous studies, the effectiveness of anesthetics to limit the stress response during simulated transport mainly depends on the dose administered [37].

ATP-related compounds in fish mainly refer to the degradation products of ATP and ATP. ATP is degraded in the order of ATP → ADP → AMP → IMP → HxR → Hx as shown in Figure 5.

IMP imparts a meaty and sweet flavor contributing to improve the quality of the fish, whereas its transformation in HxR and Hx results in unpleasant bitterness [38]. The IMP contents of MS-222-20 mg/L and MS-222-40 mg/L showed an increasing trend and the Hx showed a decreasing trend, while CK and MS-222-60 mg/L showed the opposite trends. It can be seen that 20 mg/L and 40 mg/L MS-222 additions can better maintain the strong flavor substances of the turbot and greatly reduce the content of bitter substances. HxR and Hx concentrations increased as IMP consumption for turbot samples during simulated transport in water. The experimental results showed that 20 or 40 mg/L MS-222 additions can delay the degradation time of IMP, which has a flavor-enhancing effect for turbot.

The taste characteristics of FAAs are related to the structure of the functional groups and side chain R groups. Most D-amino acids are mainly sweet, Glu and Asp with acidic side chains are mainly sour and umami, and Met, Gly, Thr, Ala and Ser with short side chains are mainly sweet and umami. Tyr, Phe, Ile, Val and Leu with large and long side

chains are mainly bitter, while His, Arg and Lys with basic side chains are bitter and slightly sweet [39]. Simulated transport stress can promote protein degradation, leading to an increase in total FAAs contents [40]. It should be noted that in the simulated transport, the turbot had no food; therefore, simulated transport stress will accelerate the degradation of protein and cause the loss of nutrients. In all samples, the amount of umami and bitter amino acids increased during the simulated transport.

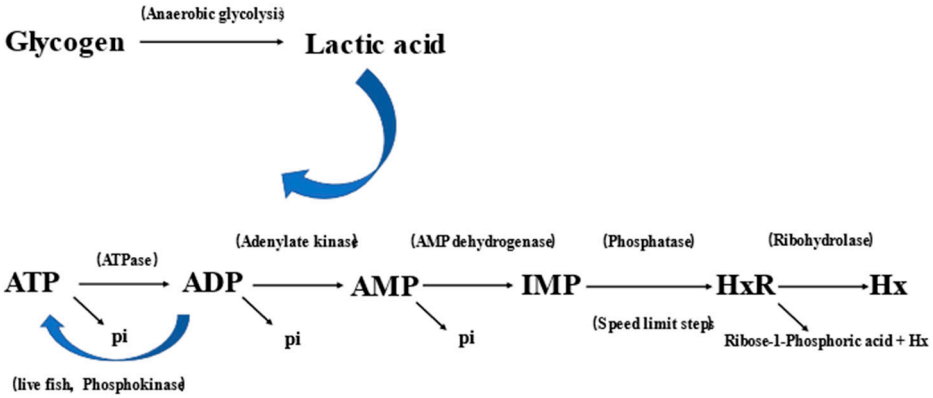

Figure 5. Degradation pathway of ATP in fish flesh.

4. Materials and Methods

4.1. Preparation of Turbot

The experimental protocol was approved by the Institutional Animal Care and Use Committee of Shanghai Ocean University (SHOU-DW-2021-066). A total of 100 live marine cultured turbots (body weight, 600.00 ± 50.00 g) were purchased from a local market in Luchao Port town (Shanghai, China) and then were transported to the laboratory using a truck equipped with an insulated tank. Fish were kept in a prepared polyethylene tank (2.4 × 1.7 × 0.6 m) for 2 days before the experiment, to allow them to adapt to the experimental environment, where the average water temperature was 13 °C, water tank salinity was 30 ‰, the mean pH was 7.5, and the average dissolved oxygen was 6.0 mg·L^{-1}. After two days, the water temperature was adjusted from 13 to 8 °C at a rate of 1 °C/h.

MS-222 (McLean Biochemical Technology Co., Ltd., Shanghai, China) powder and the corresponding weight of sodium bicarbonate ($NaHCO_3$) were dissolved in seawater at concentrations of 0, 20, 40 and 60 mg/L. We set the treatment group without the addition of MS-222 as the CK control group. Then, each fish was packed in a plastic bag containing different concentrations of MS-222 anesthetic solution (fish-to-water ratio was 1:3), with 25 fish for each anesthetic concentration, for a total of 100 fish. Moreover, oxygen was added to transport bags and the content of oxygen reaches more than 80%. Transport of fish was simulated in a vibration conveyor under 100 rpm at 8 °C for 24 h. There was no abnormality observed in fishes during the experimental period. At each time period, three fish samples were randomly selected from each of the treatment groups and analyzed on 6, 12, 18 and 24 h during simulated transport in water, respectively. The samples were anesthetized with 200 mg/L MS-222. Dorsal and abdominal flesh tissue from each fish was sampled and used for biochemical analysis.

4.2. Water Quality Index

The dissolved oxygen of water was measured by JPSJ-605F dissolved oxygen meter (INESA Scientific Instruments Co., Ltd., Shanghai, China). The pH is measured by the PB-10 m (Sartorius Scientific Instruments Co., Ltd., Germany). The total ammonia nitrogen (TAN) in water was measured by GL-200 ammonia nitrogen detector (Green Carey Precision Instrument Co., Ltd., Shandong, China).

4.3. Proximate Analysis of Fish Flesh

Protein content in fish flesh was determined according to Ntzimani et al. [41], using a Kjeldahl apparatus (Kjeltec8400, Foss, Hilleroed, Denmark). Total lipids were determined based on the method reported by Romotowska et al. [42]. Moisture content was determined gravimetrically, by drying at 104 °C for 24 h.

4.4. pH Measurement

The pH of turbot flesh examined in the present study was measured using a PB-10 m (Sartorius Scientific Instruments Co., Ltd., Germany). Ten grams of each turbot sample was diluted in 90 mL Ringer's solution (1:10 dilution), and its pH was recorded.

4.5. Texture Profile Analysis

The texture of the flesh samples was performed following the method described by Zhang et al. [43].The turbot flesh was cut into pieces of uniform size of about 3 × 3 × 3 cm, and the TA.XT Plus texture analyzer (Stable Micro System, UK) was used to analyze the hardness, springiness, chewiness and cohesion. The test rate was 1 mm/s and the degree of compression was 50%. The experiment was repeated eight times for each sample.

4.6. Determination of Water Holding Capacity (WHC)

WHC was determined on the basis of Zang et al. [44]. Three grams turbot flesh from the dorsal part was centrifuged at 5980 rpm for 10 min at 4 °C. The percentage of retained water after centrifugation was expressed as WHC.

4.7. Lactic Acid and Glycogen Determination

The lactic acid and glycogen in turbot flesh were determined using lactic acid and glycogenization kits, respectively, (Nanjing Jiancheng Institute of Bioengineering, Nanjing, China) according to instructions for use.

4.8. Determination of ATP-Related Compounds

ATP-related compounds were analyzed using HPLC (Waters 2695, Milford, MA 01757, USA) proposed by Karim et al. [45].

4.9. Free Amino Acids (FAAs) Analysis

The FAAs were performed with the procedure described by Liu et al. [46] with the use of the automatic amino acid analyzer (L 8800; Hitachi Ltd., Hitachi, Japan).

4.10. Statistical Analysis

All assumptions were met prior to data analysis. The experimental results were statistically analyzed using Microsoft excel 2007 and the 2-way ANOVA procedure in SPSS 26.0 software. The experimental data obeyed a normal distribution and were expressed as mean ± SD, and then, Duncan's multiple range test was used to determine significant differences between treatments ($p < 0.05$).

5. Conclusions

The long-term simulated transport could cause significant changes in turbot quality, such as increased lactic acid content, decreased pH and muscle glycogen levels, and caused the springiness and chewiness deteriorated. In addition, simulated transport also led to the increase in Hx and HxR contents and the increase in bitter amino acids contents, which reduce the quality of turbot. MS-222 treatments reduced the pressure on the turbot during simulated transport in water to improve the quality of turbot flesh. However, a high anesthetic concentration (MS-222-60 mg/L) led to the destruction of turbot quality. Therefore, 40 mg/L MS-222 addition could be considered as a potential for maintaining the quality of turbot by the considerations of economy and the principle of using food additives as little as possible. In order to evaluate the efficacy and human health, we

need to carry out further experiments that relate the different concentration of anesthetic introduced with the feeding of fish.

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Institutional Review Board Statement: In the present study, all procedures were performed in accordance with the "Guidelines for Experimental Animals" of the Ministry of Science and Technology (Beijing, China) and were approved by the Institutional Animal Care and Use Committee of Shanghai Ocean University (SHOU-DW-2021-066).

Conflicts of Interest: The authors declare no conflict of interest.

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