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Effects of Heat Stress on the Muscle Meat Quality of Rainbow Trout

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Abstract: The effects of heat stress on aquatic animals are increasingly being discerned, but little is known about the effects of heat stress on muscle meat quality or the flavor of muscle. This study aimed to evaluate the effects of heat stress on the muscle antioxidant properties, structural and physical properties (e.g., pH, muscle color, shear force, and expressible moisture), chemical composition (e.g., nucleotides, organic acids, amino acids, and minerals), and volatile substances of rainbow trout. We observed that the antioxidant capacity of muscle decreased after stress experiments at 22.5 °C for 24 h. The content of inflammatory factors notably increased ($p < 0.05$), the pH value and red value of muscle decreased ($p < 0.05$), the interfiber space increased, and several muscle fibers were broken. Heat stress changed the contents of nucleotides, organic acids, minerals, and amino acids in muscle. The contents of IMP and AMP, which play an important role in the flavor of muscle, decreased ($p < 0.05$). The contents of two amino acids that provide a sweet taste decreased; those of five amino acids that provide a bitter taste increased ($p < 0.05$). Heat stress also affected the amount and type of volatile substances in muscle, which affected muscle odor. These results suggest that heat stress may exert adverse effects on the oxidative stability, structure, meat quality, and flavor of muscle, requiring attention and prevention.

Keywords: rainbow trout; heat stress; antioxidant activity; meat quality; flavor

Key Contribution: This research provides valuable insights into the impact of heat stress on fish muscle mass, offering potential avenues for mitigating or ameliorating its deleterious consequences.

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Citation: Li, Y.; Zhou, C.; Zhang, Y.; Zhao, X. Effects of Heat Stress on the Muscle Meat Quality of Rainbow Trout. *Fishes* **2024**, *9*, 459. https://doi.org/10.3390/fishes9110459

Received: 15 October 2024
Revised: 3 November 2024
Accepted: 6 November 2024
Published: 11 November 2024

1. Introduction

Salmonids are prominent species of cold-water fish widely distributed across freshwater environments throughout the world. The worldwide production of salmonid aquacultures has increased annually, reaching 4243 kilotons in 2022 and accounting for 6% of the annual aquaculture production [1]. Salmonids are typical cold-water fish; thus, their behavior, growth characteristics, and reproduction are significantly affected by climate warming [2]. It has been established that the body temperature of a fish changes with the surrounding water. When water temperatures rise, oxygen levels decrease. This can be lethal for cold-water fish such as salmonids [3,4].

As a major source of animal protein, fish muscle is a primary factor determining economic value and consumer choice. The evaluation of fish quality is mainly based on its nutritional composition, sensory characteristics, and flavor [5]. In terms of nutrition, certain fish are rich in high-quality proteins, vitamins, amino acids, and minerals [6]. In terms of physical characteristics, certain fish are tender in texture and bright in color. In

terms of flavor, certain fish can manifest either a pleasant umami taste or an unpleasant fishy taste because of the presence of free amino acids (FAAs), nucleotides, and volatile substances [6].

High-temperature stress is mainly controlled through the catechin-mediated autonomic nervous system (ANS) and the hypothalamic–pituitary–adrenal (HPA) axis [7]. The initial changes after stress are neuroendocrine manifestations. The release of catecholamines leads to increased respiration, enhanced metabolism, accelerated muscle glycogenolysis, and inhibited energy storage [8]. It has been shown that glucocorticoids increase proteolysis, change lipid metabolism, affect metabolic regulation, and inhibit the production of insulin-like growth factors, thus aggravating the loss of muscle mass [9]. The secondary manifestations of stress are changes in fish osmotic pressure, physiological metabolism, and immunity [8]. Stress can lead to the excess production of reactive oxygen species (ROS), thereby activating the body's antioxidant capacity and, thus, increasing the content of antioxidant enzymes [10]. Oxidative stress activates the phosphatidylinositol-4 signaling pathway to facilitate the production of inflammatory factors, thereby leading to tissue damage [6].

Fish generally accelerate their energy metabolism when subjected to stress, and proteins and lipids are rapidly consumed. Fish are prone to oxidation, with high concentrations of unsaturated fatty acids and amino acids [8]. This abnormal energy metabolism ultimately leads to a poor taste, color, and nutritional value of the fish [8]. Previous studies have shown that stress (such as feeding, temperature, crowding, and salt) in surface aquatic animals can lead to changes in muscle quality (such as fat oxidation, tissue structure, and flavor) [11]. A previous study revealed that chronic heat stress leads to the oxidation of fat and protein in Nile tilapia (*Oreochromis niloticus*); increases water loss from centrifugation and cooking; affects amino acid, lipid, and nucleotide metabolism; and reduces the flavor and nutritional value [11]. Furthermore, chronic heat stress can alter the non-volatile and volatile content of oysters, adversely affecting their nutritional and flavor characteristics [12]. The impact of stress on fish muscle is not always detrimental; artificial stress can be used to enhance the flavor and nutritional value of fish [5].

The flavor of fish is determined by lipids, amino acids, nucleotides, and their precursors. Certain volatile compounds are produced after lipid oxidation and enzyme reactions. Fish contain a significant amount of unsaturated fatty acids, and lipid oxidation can easily produce certain volatile low molecular compounds (such as 1-octene-3-ketone and caprylic aldehyde) [13].

The rainbow trout is a common cold-water fish of the Salmonidae family. Previous research identified that rainbow trout experienced acute heat stress at 22.5 °C for 48 h, leading to a 50% mortality rate [14]. Current research into the effects of heat stress on rainbow trout primarily focuses on growth and disease; little is known about the effects of heat stress on the muscle quality of rainbow trout. Therefore, we investigated changes in the physical properties, nutrition, and flavor of rainbow trout at a heat stress threshold temperature of 22.5 °C to improve the meat quality of rainbow trout and provide technical support for rainbow trout breeding under high water temperatures.

2. Materials and Methods

2.1. Fish and Facilities

Rainbow trout (with an average initial weight of 50 ± 2.8 g) were selected and purchased from Weiyuan Haidianxia Fish Co., Ltd. (Dingxi, Gansu, China). A total of 96 rainbow trout were allocated into either the control group (CO group) or heat stress group (HS group). The 48 fish in each group were then randomly divided into three tanks (1.2 m × 0.40 m × 0.45 m). They were maintained at a water temperature of 16 °C for 2 weeks, during which time the fish were fed with a thickened granule diet (crude protein ≥ 48.0%; crude fat ≥ 10.0%) purchased from Beijing Hanye Technology Co., Ltd. (Beijing, China). After 2 weeks of acclimation, the fish in the control group were continuously fed at a water temperature of 16 °C, and the fish in the HS group were

fed at a water temperature of 22.5 °C for 24 h. A heating rod was used to increase the water temperature from 16 °C to 22.5 °C. Each tank was continuously supplied with oxygen (per 45 W) using an oxygen pump at a maximum concentration of 7 mg/L. The fish were fed twice a day (at 8:00 am and 18:00 pm) before the experiment; this feeding regime was continued to ensure that the rainbow trout received adequate nutrition. One-third of the water in the tanks was replaced daily with deaminated water at 16 °C and 22.5 °C.

2.2. Sample Collection

All fish were anesthetized with 20 mg/L eugenol (Shanghai Plankton Co., Ltd., Shanghai, China) at the end of the heat stress experiment and 8 fish were randomly sampled from both groups. Fish blood was collected from the tail vein using a 5 mL sterile syringe, left at 37 °C for 4 h, and then centrifuged (1000 rpm; 10 min) to obtain serum. Certain back muscles (above the dorsolateral line) were collected for the meat quality analysis, which included the pH, color, and expressed moisture. A portion of the muscle was washed with 0.9% saline, frozen in liquid nitrogen, and stored at −80 °C for the determination of antioxidant enzymes and metabolites.

2.3. Antioxidant Capacity, Inflammatory Factors, and Heat Shock Proteins

Other muscle biochemical indexes were measured using the relevant kits (Jiangsu Memian Industrial Co., Ltd., Nanjing, China). These included malondialdehyde (MDA), glutathione (GSH), total antioxidant capacity (T-AOC), superoxide dismutase (SOD), heat shock protein-70 (HSP-70), heat shock protein-90 (HSP-90), cortisol, catecholamine, interleukin 1β (IL-1β), interleukin 6 (IL-6), interleukin 10 (IL-10), tumor necrosis factor alpha (TNF-α), and transforming growth factor alpha (TGF-α).

2.4. Flesh Quality Measurements

Muscle pH, flesh color, shear force, and expressible moisture were measured using the method of Wu et al. [15]. The pH of the back muscles was measured using a pH meter (S220, Mettler Toledo, Columbus, OH, USA). The muscle shear force was measured using a shear meter (CLM38, College of Engineering, Northeast Agricultural University, Harbin, China). The expressible moisture of the muscle was detected using a texture analyzer (TMS-PRO, FTC, Sterling, VA, USA) at 1000 N compression for 1 min. The muscle color was measured using a portable colorimeter (NR145+, 3nh, China), which included L* (brightness), a* (red value), and b* (yellow value).

2.5. Energy Substances of Metabolism

The detection of nucleotides was conducted following the method of Coulier et al. [16]. In short, 2 g of the muscle sample was placed in a 15 mL centrifuge tube and 10 mL of 10% perchloric acid was added. Another 10 mL of 10% perchloric acid was then added to the centrifuge tube and mixed with the previous supernatant. The pH was adjusted to 6.5 using KOH. After mixing, the sample was filtered through a 0.22 μm microporous membrane to ensure its suitability for the machine analysis. The chromatographic conditions were ascertained using an Agilent C18 column (4.6 mm, 250 mm, and 5 μm) and a DAD detector, with an injection volume of 10 μL, a flow rate of 1.0 mL/min, a column temperature of 25 °C, and a wavelength of 254 nm.

Amino acids in the muscle were analyzed with reference to GB 5009.124-2016 using a Hitachi L-8900 (Hitachi-Hitech, Tokyo, Japan) automatic amino acid analyzer and a sulfonic acid cationic resin separation column. The rainbow trout muscle was weighed in a 10 mL centrifuge tube and dissolved by adding 0.02 mol/L hydrochloric acid to fix the volume. Subsequently, 5 mL methanol and 5 mL water were added to activate the C18 pretreatment column. Next, 2.5 mL of the sample was added, along with 1.5 mL of 0.02 mol/L hydrochloric acid. After passing the column, the sample was fixed at 25 mL with 0.02 mol/L hydrochloric acid and filtered through a 0.45 μm membrane. The moving

phase flow rate was 0.35 mL/min and the column temperature was 35 °C. The wavelength was reduced from 570 nm to 440 nm and the sample size was 20 μL [17].

The contents of potassium (K), sodium (Na), calcium (Ca), magnesium (Mg), iron (Fe), selenium (Se), manganese (Mn), zinc (Zn), and copper (Cu) in the muscle were measured using an inductively coupled plasma spectrometer (ICP-OES optima 8000, Perkin-Elmer, Waltham, MA, USA). The method of Bao et al. was referenced in the assay [18]. The sample (approximately 0.1 g) was weighed and placed in a Teflon digestion tank. Subsequently, 5 mL nitric acid was added to the wave digestion system (TOPEX, Xiamen, China). The parameters and flow rates of the inductively coupled plasma mass spectrometry (ICP-MS) were as follows: RF, 1150 W; sampling depth, 5 mm; auxiliary gas flow rate, 5 L/min; pump rate, 45 rpm; cooler flow rate, 12 L/min.

Six organic acids (including lactic acid) in the muscle were analyzed using HPLC. The supernatants were filtered through a 0.22 μm cellulose membrane before the HPLC analysis. The filtered sample (10 μL) was injected into the HPLC apparatus (Agilent, 1260, Agilent, Santa Clara, CA, USA), which was equipped with an Agilent C18 AQ column (4.6 mm, 250 mm, and 5 μm). Qualitative and quantitative analyses of lactic acid, malic acid, fumaric acid, and citric acid were performed by comparing the retention time and peak area of the standard product (Tan-Mo Technology Co. Ltd., Nanjing, China).

2.6. Headspace Solid-Phase Microextraction Gas Chromatography Mass Spectrometry (HS-SPME-GC-MS) Analysis

Muscle volatile compounds were assayed using HS-SPME, as reported by Yaqub et al. [19]. Muscle (2 g) and an internal standard (2-octanol at a concentration of 500 mg/kg) were added to a 20 mL headspace bottle, followed by the addition of saturated sodium chloride. The mixture was then stored at 80 °C for 30 min. Subsequently, an injection needle was inserted into the headspace bottle and heated for 30 min, then desorbed at 250 °C for 5 min at the gas injection port. The injection port temperature was 250 °C, the transmission line temperature was 280 °C, and the carrier gas flow rate was 1.0 mL/min. The MS conditions were as follows: ion source temperature, 230 °C; quadrupole temperature, 150 °C; mass scanning range, 40–600 m/z.

2.7. Histology Observation

Rainbow trout dorsal muscles were fixed in 4% paraformaldehyde, dehydrated in ethanol, equilibrated in xylene, and then embedded in paraffin. The tissue sections (105 μm) were longitudinally cut using a microtome (Leica, CM3050S, Leica Biosystems Nussloch GmbH, Nussloch, Germany), stained with hematoxylin–eosin (HE), and observed under a light microscope. The images were analyzed using 3DHISTECH's Slide Converter software 2.4.

2.8. Statistical Analysis

Excel 2016 was used to sort the preliminary data. SPSS 22 software was used to analyze the experimental data. The differences in the indicators before and after heat stress were analyzed using an independent sample *t*-test. A p-value < 0.05 indicated a statistically significant difference (*). A p-value > 0.05 indicated that the difference was not significant. The results were expressed as the mean ± SD.

3. Results and Discussion

3.1. Effects of Heat Stress on Muscle Antioxidant Capacity, Inflammatory Factors, and Heat Shock Proteins

The effects of acute heat stress on the muscle antioxidant capacity, the content of heat shock proteins, and the inflammatory factors are presented in Table 1. The results demonstrated a significant increase in cortisol and catecholamine levels ($p < 0.05$) and elevated MDA levels, as well as reduced GSH, T-AOC, and SOD levels, following heat stress exposure. The expression of two heat shock proteins, HSP-70 and HSP-90, was significantly upregulated after heat stress induction ($p < 0.05$). In terms of immune factors,

there was a notable increase in IL-1β, TNF-α, IL-6, and TGF-α levels, whereas the level of IL-10 exhibited a significant decrease after heat stress ($p < 0.05$).

Table 1. Effects of heat stress on antioxidant capacity, inflammatory factors and heat shock proteins in rainbow trout.

C	CO	HS
Cortisol (pg/mL)	806.42 ± 77.63	1000.45 ± 55.88 *
Catecholamine (ng/mL)	250.54 ± 22.96	320.03 ± 20.67 *
MDA (nmol/mL)	2.2 ± 0.24	2.89 ± 0.26 *
GSH (U/mL)	529.1 ± 29.22 *	476.72 ± 23.65
T-AOC (ng/mL)	16.02 ± 0.99 *	14.59 ± 0.99
SOD (U/mL)	193.94 ± 14.03 *	171.06 ± 12.66
HSP-70 (pg/mL)	226.64 ± 29.74	357.06 ± 38.25 *
HSP-90 (pg/mL)	226.59 ± 42.09	320.09 ± 38.56 *
IL-1β (pg/mL)	54.84 ± 7.01	69.5 ± 6.33 *
TNF-α (pg/mL)	170.57 ± 35.83	293.75 ± 29.53 *
IL-6 (pg/mL)	23.77 ± 4.23	36.21 ± 2.56 *
IL-10 (pg/mL)	405.52 ± 38.99 *	272.06 ± 29.1
TGF-α (pg/mL)	107.41 ± 15.73	140.9 ± 18.34 *

Values in the same row with an asterisk were significantly different ($p < 0.05$).

Temperature can affect the mobilization, oxidation, and storage of energy substances in fish. When exposed to heat stress, fish send messages to the hypothalamic–pituitary–mesenchymal (HPI) axis by stimulating receptors, leading to the production of stress hormones such as cortisol and catechin [5]. An increase in temperature results in an increase in ROS production and, consequently, the generation of lipid peroxides (LPO) [5]. MDA is a sensitive marker of intracellular lipid peroxidation. Its content in vivo is related to the degree of cell membrane damage, which is one of the indicators used to evaluate the antioxidant capacity [19]. The presence of antioxidant enzymes is crucial for the detoxification process of superoxide free radicals, which can provide cellular protection against damage caused by these reactive species [7]. GSH is an important antioxidant enzyme that plays a role in protecting biomacromolecules and biofilm oxidation by reducing peroxides to their corresponding alcohols to prevent the generation of free radicals [20]. T-AOC and SOD are also important antioxidant enzymes that can reduce excess ROS produced by oxidative metabolism in fish [20]. Li et al. demonstrated that the SOD, GSH, and MDA contents in the liver of rainbow trout significantly changed under heat stress. The MDA content notably increased under heat stress at 24 °C [21]. These results suggest that high-temperature stress can increase stress hormones in rainbow trout, and antioxidant enzymes in muscle tissue may play a role in removing excess ROS.

Fish increase their expression of heat stress proteins (such as HSP70 and HSP90) to mitigate oxidative damage [6]. This promotes the refolding of stress-denatured proteins, prevents protein aggregation, and assists the folding of newly synthesized proteins, playing a key role in maintaining the stability of the intracellular environment [20]. In a study on heat stress in carp (*Cyprinus carpio*) by Wang et al., both mild and moderate heat stress led to an upregulation of HSP70 expression in hearts and kidneys [22]. The basal expression level of HSP90β in the brains, livers, and hearts of the non-stress group was remarkably higher than that in other tissues. After 12 h of heat stress, the HSP90β levels in the hearts, head kidneys, brains, and gills were 4.76, 4.03, 3.08, and 2.70 times higher, respectively, than in the non-stress group [23].

The expression and secretion of cytokines are regulated by the nuclear transcription factor kappa B (NF-kB) signaling pathway. Heat stress can induce oxidative stress in the body, leading to ROS overgeneration in cells and subsequently activating the NF-kB pathway as a signaling molecule [6]. This may explain the massive secretion of cytokines. A previous study demonstrated that heat stress notably increased the level of proinflammatory cytokines such as TNF-α, IL-1β, and IL-6 and decreased the level of the anti-inflammatory

cytokine IL-10 [24]. Zheng et al. investigated the immune response of *Pelteobagrus pelteobagrus* under transportation stress and observed that the expression of IL-1ß and other inflammatory factors was upregulated after transportation for 16 h [25]. TNF-α is a proinflammatory cytokine that upregulates cytokine production to trigger inflammation [26]. After 7 days of 32 °C heat stress, the TNF-α content in the liver and spleen of largemouth bass (*Micropterus salmoides*) increased, which supports the theory that heat stress can induce inflammation [27]. In this study, heat stress increased the levels of stress hormones, heat shock proteins, and inflammatory cytokines, but decreased the levels of antioxidant enzymes. This suggests that heat stress can affect the muscle function and metabolism of rainbow trout.

3.2. Histology

The effect of heat stress on muscles was observed using HE staining (Figure 1). The results showed that the muscle fibers in the control group were intact and the myoid cell structure was single and dense (1A,B). The myocyte space increased after heat stress, and muscle (1C) and fiber fractures were obvious (1D).

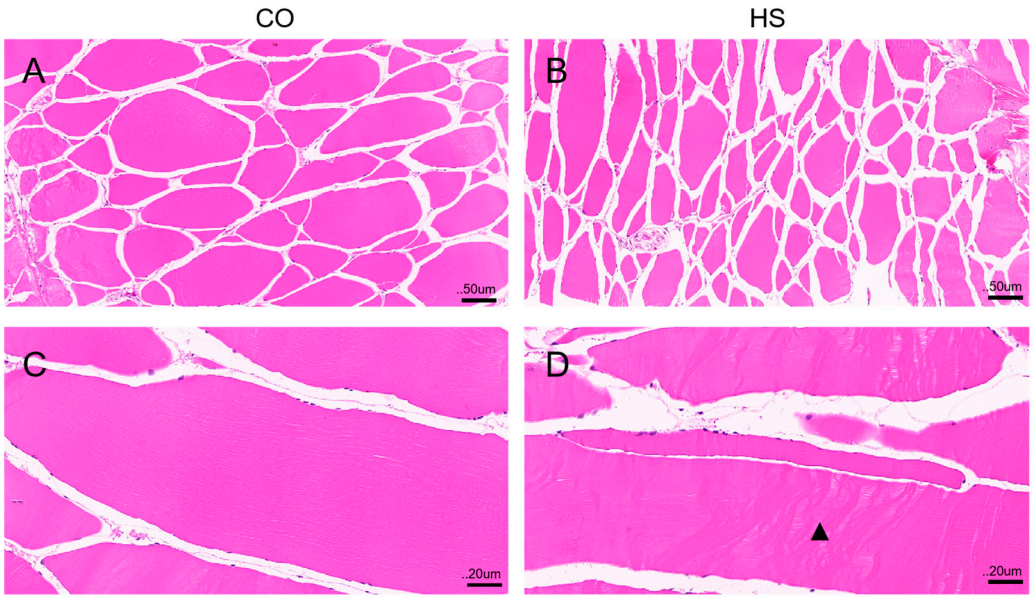

Figure 1. Effects of heat stress on muscle histology (**A**,**B**) muscle transection; (**C**,**D**) longitudinal sectioning of muscle; (**A**,**C**) control group; (**B**,**D**) heat stress group; ▲: rupture of muscle fibers).

ROS produced under high-temperature stress can impair Ca absorption in muscle as well as collagen transformation. Excessive ROS can also degrade myofibrils. Specifically, ROS can induce the release of enzymes that hydrolyze myofibrils, eventually leading to the destruction of muscle fiber machinery [5,28]. A previous study investigated the effects of transport on the muscle texture of Wuchang bream (*Megalobrama amblycephala*). An extension of the transport time resulted in the contraction of the muscle fibers as well as increases in the gaps between the muscle fibers and the number of sarcoplasmic reticulum vesicles in the middle of the fibers [29]. Another study explored abnormal muscle metabolism caused by ammonia stress in rainbow trout. The results revealed that stress induced mitochondrial and muscle fiber damage, muscle fiber atrophy, and muscle space enlargement, but the muscle fiber structure of unexposed tissue remained intact [30].

3.3. Physical Properties of Muscle

The results presented in Table 2 reveal that heat stress notably decreased muscle pH and redness (a*; $p < 0.05$) compared with the control group, but there was no significant effect on shear force, expressible moisture, lightness (L*), and yellowness (b*) ($p > 0.05$).

Table 2. Effects of heat stress on muscle qualities.

Physical Property	CO	HS
Shear force	14.30 ± 2.49	12.57 ± 1.08
Expressible moisture %	21.65 ± 1.41	22.83 ± 1.59
pH	6.52 ± 0.03 *	6.47 ± 0.02
L*	38.45 ± 1.29	37.15 ± 0.78
a*	8.68 ± 0.13 *	8.32 ± 0.12
b*	14.25 ± 0.81	14.93 ± 0.84

L*: lightness; a*: red value; b*: yellow value. Values in the same row with an asterisk were significantly different ($p < 0.05$).

Currently, the evaluation of meat color in fish muscles involves measuring the values of L*, a*, and b* [6]. Modifications to the muscle color primarily result from the presence of pigments within muscle cells. When fish experience stress, the HPI axis is activated. This leads to the secretion of alpha-melanocyte-stimulating hormone (MSH). MSH stimulates melanocytes to produce melanin, which is then transported to and deposited into muscle tissues [6]. This process leads to lighter flesh coloration and decreased L* values. Changes in the redox forms of myoglobin also play a crucial role in determining fish muscle coloration. Stress-induced hypoxia can reduce the levels of oxygen and hemoglobin [6]. Alterations to both pigmentation and the myoglobin status contribute to the impact of stress on the muscle color of fish. Muscle color is a comprehensive manifestation of the muscle myoglobin content and status, which can be influenced by various factors such as stress, pH, and ionic strength [31]. The decrease in a* in this study may have been related to a decrease in myoglobin. The decrease in pH could be attributed to heat stress affecting metabolism to meet energy demands. This may have accelerated oxygen consumption, resulting in a continued decrease in myoglobin content [32]. Wu et al. reported similar findings; rainbow trout experiencing hypoxia stress showed a decrease in myoglobin content, resulting in lower L* and a* values [33]. A previous study compared the a* value of rainbow trout transported under high-temperature conditions and those transported under controlled temperature conditions. The results indicated that the a* value decreased after 24 h of transport, causing in an increase in the muscle pH [15].

3.4. Chemical Composition

3.4.1. Nucleotides and Organic Acids

The composition of nucleic acid compounds in the muscle of rainbow trout is shown in Table 3. The highest concentration of nucleotides was IMP. Notably, the contents of GMP, AMP, and IMP in the back muscle tissue of the HS group decreased ($p < 0.05$), whereas the contents of CMP and UMP significantly increased compared with those in the CO group. Fish under stress can produce changes in energy metabolism and protein catabolism; these metabolites are closely related to the taste of fish. Under stress conditions, ATP is broken down into AMP. AMP is broken down into ADP, and then broken down into IMP and ammonia by AMP deaminase [34]. AMP and IMP (umami enhancers) are the major nucleosides in rainbow trout [13]. The thresholds for AMP and IMP are 50 and 25 mg/100 g, respectively [13]. An AMP content below 100 mg/100 g primarily imparts sweetness rather than an umami taste [35]. IMP can enhance the umami taste of food even at low concentrations while reducing the salt content, thereby showing a salt reduction effect. IMP also enhances food acidity and intensifies the flavor of spices upon ingestion. There is a synergistic effect between AMP and IMP; the presence of IMP significantly amplifies the sweetness provided by AMP [35]. These flavorful nucleotides not only serve as key components in aquatic products, but also are crucial in enhancing the overall taste through synergistic interactions with FAAs [6]. Heat stress significantly reduces the IMP content in muscle, and IMP contents below the threshold no longer provide an umami taste. This confirms that heat stress may adversely affect meat quality.

Table 3. Effects of heat stress on muscle nucleotides and organic acids.

Experimental Group	CO	HS
Nucleotides (mg/100 g)		
CMP	0.66 ± 0.05	0.90 ± 0.06 *
UMP	0.90 ± 0.06	1.36 ± 0.04 *
GMP	3.01 ± 0.15 *	0.31 ± 0.02
IMP	126.39 ± 4.34 *	2.35 ± 0.09
AMP	1.43 ± 0.09 *	0.11 ± 0.01
Organic acids		
Lactic acid	237.39 ± 6.38 *	93.97 ± 0.78
Fumaric acid	0.43 ± 0.01	0.63 ± 0.01 *

Values in the same row with an asterisk were significantly different ($p < 0.05$).

The organic acid findings are presented in Table 3. Only lactic acid and fumaric acid were identified in the muscle tissue of rainbow trout. In response to heat stress, the concentration of lactic acid underwent a marked reduction, whereas the content of fumaric acid significantly increased ($p < 0.05$). Lactic acid, malic acid, citric acid, and succinic acid are the main taste-enhancing organic acids in fish [12,15]. The lactic acid content in muscle before heat stress was higher than the TAV value (126 mg/100 g), proving that lactic acid contributes to muscle flavor. The lactic acid content in muscle after heat stress decreased and no longer contributed to muscle flavor. It is generally believed that organic acids (such as lactic acid) are produced in the process of fatty acid and glucose metabolism [36]. Stress can enhance metabolism and increase the lactic acid content, which leads to a decrease in the muscle pH content. It has been demonstrated that muscle lactic acid level in rainbow trout increased after 3 h of hyperthermic transport [15]. The decrease in muscle lactic acid level in the present study may have been due to compensatory regulatory activity after 24 h of heat stress, together with a decrease in muscle pH caused by other undetected acids.

3.4.2. Mineral Content

The mineral contents in the muscle of rainbow trout are shown in Table 4. Four major elements (Na, Mg, K, and Ca) and five trace elements (Fe, Mn, Cu, Zn, and Se) were detected. The contents of K, Ca, and Mg in the HS group significantly decreased compared with the CO group ($p < 0.05$), whereas the contents of Mn, Cu, Zn, and Na remarkably increased ($p < 0.05$).

Table 4. Effects of heat stress on muscle mineral content (mg/100 g).

Experimental Group	CO	HS
Mn	0.06 ± 0.00	0.12 ± 0.001 *
Fe	1.57 ± 0.08	3.07 ± 0.03 *
Cu	0.116 ± 0.01	0.266 ± 0.00 *
Zn	1.75 ± 0.06	2.17 ± 0.06 *
Se	0.02 ± 0.0	0.02 ± 0.00
K	466.33 ± 3.77 *	406.33 ± 1.53
Ca	15.83 ± 0.35 *	11.97 ± 0.21
Na	24.63 ± 0.38	32.97 ± 0.25 *
Mg	32.53 ± 0.25 *	29.5 ± 0.24

Values in the same row with an asterisk were significantly different ($p < 0.05$).

Aquatic animals can absorb minerals from water and feed; these are related to their growth, metabolism, and disease immunity [37]. Zn, Cu, and Mn are trace elements that may be related to stress relief as they are cofactors of several enzymes (such as superoxide dismutase) that can enhance the body's antioxidant capacity [38,39]. In this experiment, the content of the above-mentioned minerals increased after heat stress. This may have been related to the changes in antioxidant enzymes. Zn also induces the synthesis of metallothioneins, proteins that can effectively reduce hydroxyl radicals and sequester ROS

produced under stressful situations [40].Stress in common carp caused a significant increase in the zinc content of blood and head kidneys, which may have resulted in an increased cortisol content and the upregulation of glucocorticoid receptor protein. The addition of Zn to a *Pangasius hypoophthalmus* diet mitigated the stress effects of exposure to lead and high temperatures on cell metabolism [41]. The addition of Cu to diets has also been shown to exert beneficial effects on oxidative stress and skeletal muscle development [42]. K not only plays a vital role in nerve function, but also affects enzyme activity and the acid–base balance [43]. The decrease in K after heat stress may have been because of the temperature-induced acceleration of metabolism and the requirement for greater K involvement. This could be related to the involvement of minerals in energy metabolism or changes in amino acid contents [44]. A high temperature can directly affect the activity of the sodium potassium pump. Smith et al. showed that an increase in acclimation temperature resulted in a decrease in the specific activity of Na+/K+-ATPase in the intestinal mucosa of goldfish (*Carassius auratus L.*) [45]. Elevated stress levels in salmon or trout muscles disrupt intracellular calcium channels, leading to increased Ca2+ concentrations [46]. This may be because cortisol regulates calcium intake via glucocorticoid and/or salocorticoid receptors (GR and/or MR), as demonstrated using tilapia [46]. Minerals also provide flavor to seafood: Na produces a strong salty taste, and K produces a bitter and salty taste [45]. Our experimental results suggest that the changes in minerals were related to the oxidative stability of the rainbow trout muscles under stress and could also affect the flavor of meat.

3.4.3. Effects of Acute Heat Stress on Amino Acid Content in Muscle

The effects of heat stress on FAAs are shown in Table 5. Fifteen amino acids were detected. Among the key FAAs detected in the rainbow trout, glycine and alanine were the most abundant. The content of the amino acid Glu, which provides the umami taste, decreased after heat stress, as did the contents of the amino acids Ala and Ser, which provide sweetness. The content of five amino acids (Arg, Val, IIe, Leu, and Phe), which provide bitterness, increased. The identification of the flavors of various amino acids was based on the definitions of Duan et al. [13].

Table 5. Effects of acute heat stress on amino acid content in muscle (mg/100 g).

FAA	CO	HS
Asp	8.43 ± 0.81	7.08 ± 0.49
Glu	13 ± 1 *	9.13 ± 0.25
Gly	49.33 ± 2.31	47.2 ± 1.67
Ala	18.73 ± 0.35 *	17.5 ± 0.61
Cys	0.88 ± 0.04	1.02 ± 0.12
Arg	7.63 ± 0.21	9.33 ± 0.5 *
Tyr	9.7 ± 0.26	9.37 ± 0.35
Ser	6.83 ± 0.45 *	4.23 ± 0.38
Val	4.53 ± 1.23	8.13 ± 1.03 *
Lys	14.4 ± 2.76	11.53 ± 0.76
His	3.43 ± 0.21	2.93 ± 0.42
IIe	1.467 ± 0.21	1.92 ± 0.02 *
Leu	4.52 ± 0.32	8.61 ± 1.06 *
Phe	6.1 ± 0.35	11.08 ± 1.11 *
Thr	7.57 ± 0.81	11.47 ± 1.18 *
Total amino acids	156.57 ± 3.77	160.55 ± 4.65

Values in the same row with an asterisk were significantly different ($p < 0.05$).

As components of proteins, amino acids regulate body growth and development [47] and are related to the physiological and behavioral regulation of stress [48]. Glutamate synthesizes the antioxidant glutathione to prevent oxidative damage; it also acts as a major metabolic fuel for other tissues (such as skeletal muscle) and plays a major role in protein synthesis [49]. The glutamate content in black rockfish (*Sebastes schlegelii*) notably increased after high-temperature stress at 27 °C [50]. Threonine can prevent apoptosis;

increases in the threonine content in muscle may be related to the induction of heat shock protein expression [51]. Arginine can repair wounds, inhibit the expression of inflammatory cytokines, and relieve oxidative stress through the nitric oxide pathway [52,53]. A previous study demonstrated that supplementing zebrafish with arginine could enhance their carbohydrate metabolism at 32 °C, promoting better adaptation to high temperatures [54].

Amino acids are important precursors of volatile compounds in seafood [6]. The results of this study indicated that the contents of sweet amino acids all decreased, whereas those of bitter amino acids increased. This indicated that heat stress could reduce the taste of rainbow trout, consistent with the results of Fu et al. for oysters [12]. As a branched-chain amino acid, leucine can serve as a substrate for energy metabolism when carbohydrate is consumed. Heat stress leads to accelerated energy metabolism and, consequently, an increase in the leucine content. This is consistent with the results reported by Wang et al. for goldfish (*Carassius Auratus*) [55]. The interaction of organic acids, inorganic ions, nucleotides, and FAAs generates intricate synergistic effects among these taste substances, thereby enhancing the overall sensory perception. In the absence of Na, the umami taste produced by glutamic acid and aspartic acid rapidly weakens. When Na coexists with nucleotides and FAAs, the umami flavor of aquatic products is more prominent [56]. This suggests that heat stress may affect protein degradation in muscle to alter the type and amount of various amino acids, which adversely affects the taste and flavor of the muscle.

3.5. Effects of Heat Stress on Volatile Substances in Muscle

The structure, receptors, and thresholds of volatile compounds determine the odor of fish meat [6]. Changes in the active compounds of fish odors after stress are primarily associated with lipid peroxidation. Stress induces excessive ROS production in fish. Lipid peroxidation is prone to occur in biological systems containing free radicals. Lipid peroxidation yields lipid hydroperoxides, which can easily be decomposed into compounds such as aldehydes, ketones, alcohols, and esters [57]. Changes in the type and quantity of volatile substances caused by surface stress are mainly related to aldehydes and ketones [6]. In this study, the effects of stress on volatile compounds in rainbow trout were investigated using HS-SPME-GC-MS.

The types and relative contents of the volatile compounds in the CO and HS groups are shown in Figure 2. In total, 54 volatile compounds were identified. A total of 29 volatiles were identified in the CO group; esters (29.32%) had the highest relative content (with four esters detected), followed by other volatiles (21.11%) and alkanes (15.8%). Aldehydes (6.35%) had the lowest content. A total of 35 volatiles were observed in the HS group. Ketones (29.86%) had the highest relative content (five types of ketones were detected), followed by alcohols (26.06%) (three types of substances were detected). Aldehydes (2.99%) had the lowest content. A total of eight substances (such as nonanal) were detected in both the CO and HS groups, but their relative contents significantly changed. Methyl salicylate (with the flavor of asparagus leaf) was the most abundant volatile substance in the CO group, and 5-Hydroxy-7-methoxy-2-methyl-3-phenyl-4-chromenone was the most abundant volatile substance in the HS group. The relative content of esters notably decreased after heat stress, whereas the content of ketones and alcohols significantly increased, demonstrating that heat stress significantly affected the content of volatile compounds in rainbow trout muscle.

The increase in alcohols may have been related to the oxidation of fatty acids; their special flavors (floral and fruity) are related to the flavor of fish [58]. Heat stress increased the content of alcohols, which may have resulted in a decrease in muscle holding power due to the increased ambient temperature, thereby resulting in the release of alcohols [59]. The 3-Ethyl-2-pentanol detected in the HS group has been shown to have a significant effect on flavor [60]. The compound 1-octene-3-ol is a major source of flavor substances in aquatic products. It provides mainly mushroom-like odors and is usually produced by UFA, whose relative content has been shown to decrease after heat stress [61]. Aldehydes are mainly produced through lipid oxidation and degradation reactions, which significantly contribute

to flavor characteristics because of their low odor threshold [62]. The aldehydes detected in this study were at low levels, which was inconsistent with the results of Duan et al. This may have been related to the size of the selected fish. Nonenal is a key component in the production of fresh odors [63], and it randomly accounted for 1.57% and 4.7% of the total volatile compounds. Ketones are produced by the degradation of unsaturated fatty acids and amino acids, which are associated with the fatty aroma of meat [62]. The ketones in the HS and CO groups were mainly caused by 5-Hydroxy-7-methoxy-2-methyl-3-phenyl-4-chromenone, but its specific flavor has not been reported. Hydrocarbon generation is mainly attributed to the homogeneous cleavage of alkoxy groups of fatty acids. Various alkanes (C6 to C19) have been found to be present in the volatile components of salmon and can enhance its overall flavor [64].

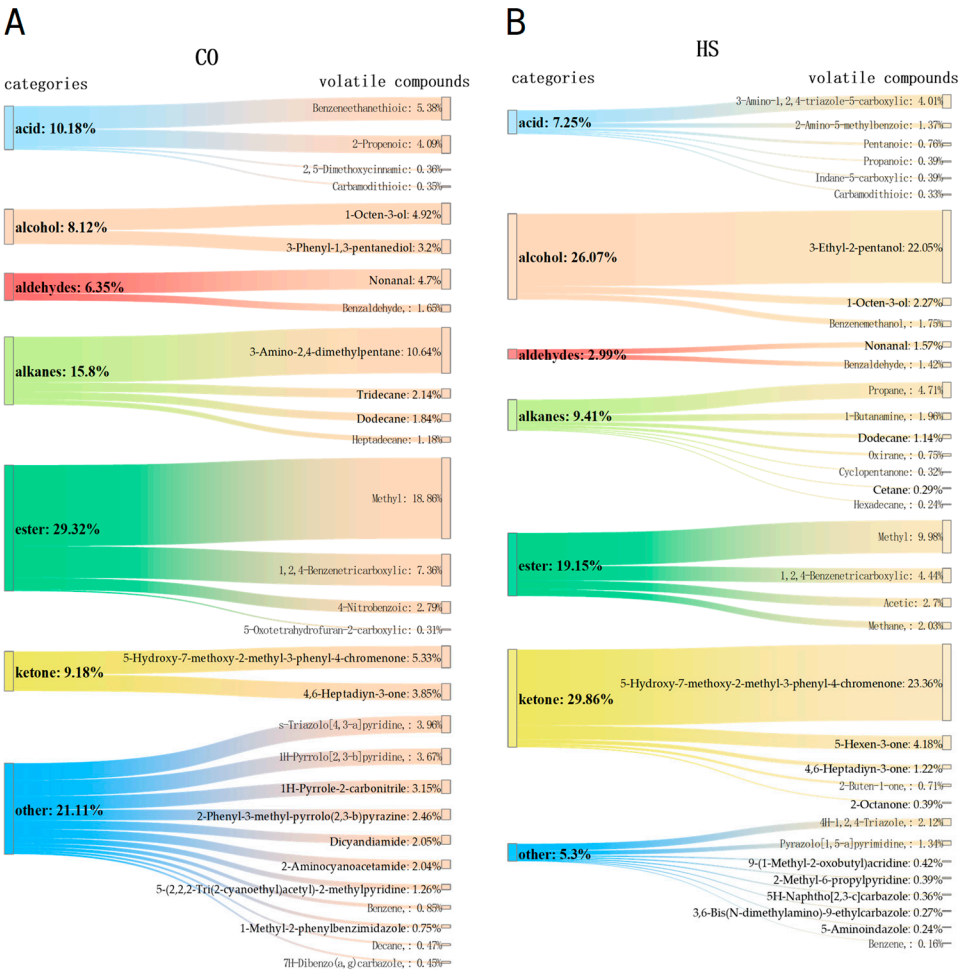

Figure 2. Effects of heat stress on volatile substance content in muscle of rainbow trout. (**A**) Relative volatile substance content in the control group (%); (**B**) relative volatile substance content in the heat stress group (%).

4. Conclusions

In this study, acute heat stress enhanced oxidative stress in the muscles of rainbow trout and promoted the release of inflammatory factors, resulting in elevated levels of stress-related products and structural changes in the muscle tissue. Heat stress also affected the contents of nucleotides, minerals, favorable amino acids, and volatile substances in the muscles. These harmful effects can adversely affect the physical properties, nutritional composition, and flavor of fish muscles. This research provides valuable insights into the effects of heat stress on muscle mass in fish as well as novel ideas to mitigate or improve its harmful consequences.

Author Contributions: X.Z. conceived and designed the experiments. C.Z. and Y.L. performed the experiments. C.Z. and Y.L. performed the data analysis and wrote the manuscript. X.Z. and Y.Z. reviewed and revised the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the Natural Science Foundation of Gansu Province, China (grant number: 23JRRA1067).

Institutional Review Board Statement: The animal study protocol was approved by the Ethics Committee of the School of Life Sciences, Lanzhou University (protocol code EAF2021042; date of approval: 3 May 2021).

Informed Consent Statement: Not applicable.

Data Availability Statement: Data are contained within the article.

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

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