



Comparative evaluation of nutritional quality and flavor characteristics for *Micropterus salmoides* muscle in different aquaculture systems

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ABSTRACT

To investigate the nutritional quality and flavor characteristics of *Micropterus salmoides* muscle cultivated in the pond (P), in-pond raceway (IPRS), and industrial aquaponics (ARAS) systems, we comprehensively analyzed texture properties, nutrient compositions, and volatile compounds. Our results revealed firmer flesh in P-cultured fish due to greater hardness and mastication. ARAS fish exhibited lower crude fat but higher crude protein and muscle glycogen. Notably, recirculating aquaculture significantly elevated total amino acids, minerals, and Σ PUFA/ Σ SFA ratio, enhancing nutritional value. Pyrazine, 2-methoxy-3-(2-methylpropyl)-, and β -ionone were identified as key flavor compounds. Volatile metabolites in all systems were dominated by woody, herbal, and sweet aroma profiles, with ARAS achieving the highest odor activity value, suggesting improved overall flavor. This study underscores the pivotal role of recirculating aquaculture in enhancing *Micropterus salmoides* quality, positioning it as a new quality productive force.

1. Introduction

Micropterus salmoides, belonging to the *Micropterus* genus of the Centrarchidae family within the Perciformes order, originates from North America but was introduced to China in the 1980s (Zhou et al., 2024). Since then, it has emerged as a significant freshwater aquaculture species in China, attributed to its robust adaptability, rapid growth rate, ease of capture, and shortened breeding cycle (Costantini et al., 2023). Consequently, it is widely regarded as the “fifth largest fish” in China (Li et al., 2016). Furthermore, the exceptional fish quality of *Micropterus salmoides*, coupled with its delicious taste and absence of intermuscular spines, makes it a highly desirable choice for consumers, thus boasting immense market potential (Jorge et al., 2022).

Currently, the primary cultivation method for *Micropterus salmoides* revolves around pond culture, albeit with attendant issues such as sub-optimal water quality, diminished fish quality, and augmented labor requirements (Han et al., 2020). Amidst China's current strategic emphasis on green aquaculture development, circular economy

initiatives, and low-carbon emission reduction, recirculating aquaculture systems (RAS) have emerged as a novel mode that is being fervently pursued and advocated (Zhang et al., 2023). Of these systems, the in-pond raceway system represents a cutting-edge pond aquaculture approach that seamlessly integrates pond-recirculating water aquaculture technology, biological water purification methods, and highly efficacious sewage collection techniques (Zhang, Xiao, et al., 2019). Additionally, the industrial aquaponics system constitutes a green, composite production paradigm, encompassing stereo planting and recirculating aquaculture under controlled conditions, fostered by multidisciplinary synergies and industrialized management practices (Goddek et al., 2019). When compared to traditional pond culture methods, employing these two systems for *Micropterus salmoides* cultivation can profoundly diminish nitrogen and phosphorus concentrations in water bodies, thereby enhancing water quality (Ahmed & Giovanni, 2021). Moreover, they offer advantages such as elevated culture density, land, and water conservation, thereby aligning with contemporary consumer preferences and psychologies.

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Amidst the relentless progression of the industry and the growing aspirations of the populace for a superior lifestyle, fish quality has ascended to the forefront of considerations. Fish quality is a complex concept, which is generally reflected in texture, apparent characteristics, nutritional value, and flavor quality (Cortés-Sánchez et al., 2024). “Flavor substances” refer to a class of water-soluble or volatile low-molecular-weight compounds that are discernible by human sensory organs (Lv et al., 2019). Organic substances that have been identified as contributing to the flavor of fish are alcohols, aldehydes, esters, and heterocyclic compounds. Furthermore, certain amino acids and fatty acids in fish can complement flavor compounds to form complex flavor nutrients (Luo, 2021). Extant research has revealed that aquaculture modes significantly influence the growth status, muscle quality, and nutrient composition of various fish species (Lajoie et al., 2019; Smichi et al., 2017), including *Oreochromis spp* (Guo et al., 2021), *Larimichthys crocea* (Zhang et al., 2020), and *Ctenopharyngodon idella* (Kuang et al., 2020). These studies converge on the understanding that fish quality and flavor formation are contingent upon their living environment. Currently, research on the effects of different aquaculture modes on the muscle quality of *Micropterus salmoides* has primarily focused on pond aquaculture (Jia et al., 2022), integrated rice-bass farming systems (Ding et al., 2023), and higher-place pond culture (Hu, 2023). However, there is a paucity of research examining the muscle quality of *Micropterus salmoides* within the in-pond raceway system and industrial aquaponics system modes. Consequently, this study endeavors to analyze and compare the impact of three distinct culturing modes on the muscle quality of *Micropterus salmoides*. To provide a theoretical foundation for the improvement of the quality of the cultured *Micropterus salmoides* as well as the recirculating water aquaculture to become a new quality productivity.

Table 1
Comparison of muscle physical properties and chemical compositions of *Micropterus salmoides* in different culture modes.

Physical characteristics	P	IPRS	ARAS
Hardness (gf)	875.2 ± 26.99 ^a	619.97 ± 31.67 ^b	538.37 ± 65.65 ^{bc}
Adhesion (gf.sec)	-6.83 ± 0.21 ^a	-11.18 ± 0.61 ^a	-9.7 ± 3.72 ^a
Viscosity (gf)	-5.8 ± 0.21 ^a	-7.2 ± 0.2 ^b	-5.3 ± 0.63 ^a
Elasticity	0.52 ± 0.001 ^a	0.51 ± 0.03 ^a	0.49 ± 0.009 ^a
Mastication (gf)	206.12 ± 15.91 ^a	170.12 ± 21.54 ^{ab}	122.06 ± 7.35 ^{bc}
Cohesion	0.46 ± 0.04 ^a	0.54 ± 0.03 ^a	0.47 ± 0.02 ^a
Resilience	0.33 ± 0.02 ^a	0.35 ± 0.02 ^a	0.29 ± 0.02 ^a
Fat loss rate (%)	0.3939 ± 0.009 ^a	0.4079 ± 0.002 ^a	0.4042 ± 0.01 ^a
Water loss rate (%)	0.3955 ± 0.01 ^a	0.3913 ± 0.01 ^a	0.4017 ± 0.007 ^a
Juice loss rate (%)	0.0484 ± 0.001 ^a	0.0491 ± 0.001 ^a	0.0527 ± 0.003 ^a
pH	7.01 ± 0.05 ^a	7.11 ± 0.12 ^a	7.1 ± 0.11 ^a
Chemical composition			
Moisture (%)	0.7653 ± 0.006 ^a	0.7429 ± 0.02 ^a	0.7478 ± 0.01 ^a
Crude fat (%)	0.043 ± 0.001 ^a	0.0472 ^b	0.0412 ^a
Crude ash (%)	0.0121 ± 0.001 ^a	0.0133 ± 0.003 ^a	0.0129 ± 0.002 ^a
Crude protein (%)	0.1949 ± 0.002 ^a	0.1997 ± 0.001 ^a	0.2056 ± 0.001 ^b
Water-soluble protein (mg/g)	55.82 ± 0.97 ^a	58.13 ± 0.85 ^a	61.81 ± 0.88 ^b
Salt-soluble protein (mg/g)	88.67 ± 1.1 ^{ab}	90.75 ± 0.38 ^{bc}	92.99 ± 1.22 ^c
Total hydroxyproline (mg/g)	826.97 ± 11.16 ^a	774.42 ± 7.16 ^b	757.41 ± 1.02 ^{bc}
Alkali-soluble hydroxyproline (mg/g)	591.06 ± 29.95 ^a	568.2 ± 12.89 ^a	557.42 ± 15 ^a
Muscle glycogen (mg/g fresh weight)	1.45 ± 0.02 ^a	1.64 ± 0.08 ^b	1.8 ± 0.04 ^c

2. Materials and methods

2.1. Experimental materials

All experimental *Micropterus salmoides* were adult fish, which were captured in December 2023 from pond mode (body weight: 406 ± 12.3 g, body length: 24.8 ± 2.4 cm), in-pond raceway system mode (body weight: 618.4 ± 25.6 g, body length: 26.8 ± 2.7 cm) and industrial aquaponics system mode (body weight: 529.3 ± 18.7 g, body length: 25.3 ± 1.9 cm). These groups were designated as P, IPRS, and ARAS, respectively. From each cultivation mode, nine healthy *Micropterus salmoides* with no apparent injury or disease and robust bodies were randomly selected. The fish were killed with a lethal dose of 10 g/L MS-222 anesthesia, followed by dissection procedures. The dorsal muscles were excised, and the muscular tissue from three fish was combined into a single sample, which was stored at -80 °C for reserve.

2.2. Experimental methods

2.2.1. Determination of physical properties

In this experiment, a Rapid TA mass spectrometer (Shanghai Tengba Instrument Science and Technology Co., Ltd., the test probe is a P/36 column probe) was utilized for the texture profile analysis of *Micropterus salmoides* to determine the hardness, elasticity, cohesion, mastication, resilience, adhesion, fat loss rate, water loss rate, and juice loss rate of muscle.

2.2.2. Determination of nutrient content

For moisture determination, the GB/T 5009.3, 2016 standard was followed, baking samples in an oven at 105 °C to constant mass, thus assessing moisture loss. Crude protein was quantified using the Kjeldahl method (GB/T 5009.5, 2010). Crude ash content was determined according to GB/T 5009.4-2010, 2010, involving high-temperature burning in a muffle furnace at 550 °C. Crude fat was analyzed via the Soxhlet extraction method (GB/T 5009.6-2010, 2010). pH measurement adhered to GB/T 5009.237-2016, 2016. muscle glycogen, alkali-soluble hydroxyproline, total hydroxyproline, salt-soluble protein, and water-soluble protein were all quantified using colorimetric methods (Nanjing Jiancheng Reagent Kit).

The amino acid content was determined in accordance with GB 5009.124-2016, 2016, utilizing HPLC (HP1260, Agilent, USA). Detection parameters were as follows: Agilent C18 column (250 × 4.6 mm, 5 μm), column temperature of 38 °C, wavelength of 360 nm, flow rate of 1 mL/min, injection volume of 20 μL. Mobile phase A comprised acetonitrile: methanol (90:10), while mobile phase B consisted of 0.02 mol/L sodium dihydrogen phosphate + disodium hydrogen phosphate.

To determine the tryptophan content, we referred to GB 5009.294-2023, 2023 and utilized HPLC (HP1260, Agilent, USA). Detection parameters were set as follows: Agilent C18 column (150 × 2.1 mm, 5 μm), column temperature of 30 °C, wavelength of 280 nm, flow rate of 0.3 mL/min, injection volume of 20 μL, and a mobile phase of 0.02 mol/L sodium acetate (pH = 4.5) and methanol in a ratio of 85:15.

The fatty acid content was determined in accordance with GB 5009.168-2016, 2016 and analyzed using GC6890 (Agilent, USA). Detection parameters included N₂ as the carrier gas, an Agilent DB-23 column (30 × 0.25 mm, 0.25 μm), and flow rates of H₂, N₂, and air set at 30 mL/min, 30 mL/min, and 300 mL/min respectively. The split ratio was 50:1, with an injection volume of 1 μL. The column chamber program started at 50 °C for 1 min, ramped up to 175 °C at 25 °C/min, and then to 230 °C at 4 °C/min, holding for 10 min.

Mineral elements in fish were determined by inductively coupled plasma mass spectrometry (ICP-MS, iCAP RQ, Thermo Fisher, USA) with reference to GB5009.268–2016.

2.2.3. Determination of volatile metabolites

Samples were retrieved from the -80°C freezer, ground in liquid nitrogen, and vortexed for thorough mixing. Each sample (0.2 g) was weighed into a headspace vial, with separate additions of 0.2 g NaCl powder and 20 μL (10 $\mu\text{g}/\text{mL}$) of internal standard solution. Extraction was conducted using fully automated HS-SPME (CTC Analytics AG, Switzerland) for GC-MS (Agilent 8890-7000D, USA) analysis. Raw data underwent qualitative and quantitative analyses using MassHunter software. Relative metabolite content ($\mu\text{g}/\text{g}$) was determined via internal standard semiquantitative method. Statistical analysis included PCA and OPLS-DA, identifying differential metabolites with $\text{VIP} > 1$ and $p \leq 0.05$ for further visualization and analysis.

2.3. Statistical analysis

The experimental data, expressed as mean \pm standard error, were analyzed using One-Way ANOVA in SPSS 27 (IBM, USA), with multiple comparisons via Tukey's method. Significance was set at $p \leq 0.05$. The flavor evaluation relied on the relative odor activity value (rOAV) to assess each substance's contribution to the overall aroma. Compounds with $\text{rOAV} \geq 1$ are odor-active, contributing to the fish's overall flavor, calculated as $\text{rOAV}_i = \frac{C_i}{T_i}$, where rOAV_i is the relative odor activity value of the compound i , C_i is the relative amount of the compound ($\mu\text{g}/\text{kg}$), and T_i is the Threshold value ($\mu\text{g}/\text{kg}$) of the compound (Huang et al., 2022; Xue et al., 2022).

3. Results and discussion

3.1. Analysis of the muscle texture of *Micropterus salmoides* under different aquaculture systems

Table 1 below depicts the physical characteristics of *Micropterus salmoides* muscle cultivated under P, IPRS, and ARAS modes. No significant variations were observed in terms of adhesion, elasticity, cohesion, resilience, fat loss rate, water loss rate, juice loss rate, and pH ($p > 0.05$). The muscle hardness in the IPRS and ARAS groups remained comparable ($p > 0.05$), yet both were significantly lower compared to the P group ($p < 0.05$). The viscosity of the muscle in the P and ARAS groups was indistinguishable ($p > 0.05$), but both were significantly higher than that in the IPRS group ($p < 0.05$). While mastication in the IPRS and P groups, as well as in the IPRS and ARAS groups, showed no significant differences ($p > 0.05$), the P group had a significantly higher score than the ARAS group ($p < 0.05$).

The textural attributes of fish muscle, a pivotal factor in the sensory assessment of fish flesh, significantly influence consumer evaluations. Generally, consumers favor fish with flesh that is elastic and chewable (Luísa et al., 2013). These muscular textural properties are notably influenced by factors such as geographic origin, environmental conditions, specific growth rates, and chemical parameters (Cheng et al., 2013). The current study reveals no significant disparities in terms of muscle adhesion, elasticity, cohesion, resilience, fat loss rate, water loss rate, juice loss rate, and pH among *Micropterus salmoides* muscles cultured in three modes. However, a noteworthy observation is that the P group exhibited significantly higher muscle hardness compared to the IPRS and ARAS groups. Furthermore, adhesion was significantly greater in the P and ARAS groups compared to the IPRS group, while mastication was significantly enhanced in the P group in comparison to the ARAS group. Taking all factors into consideration, it is evident that *Micropterus salmoides* from the P group possess relatively firm and solid muscle texture. This observation is attributed to the significantly higher growth rate of *Micropterus salmoides* in the recirculating aquaculture mode compared to the pond mode. Nevertheless, it is worth noting that flesh firmness is closely correlated with growth rate, and rapid growth tends to diminish the firmness of fish (Folkestad et al., 2008). This is consistent with the results of Xu et al. who studied the effects of RAS and

Table 2

Comparison of amino acid contents of *Micropterus salmoides* muscle in different culture modes.

Amino acid (mg/g)	P	IPRS	ARAS
Aspartic acid (ASP) #	36.16 \pm 0.2 ^a	38.14 \pm 2.68 ^a	37.44 \pm 0.92 ^a
Glutamic acid (GLU) #	36.6 \pm 0.2 ^a	38.44 \pm 2.67 ^a	38.49 \pm 0.89 ^a
Serine (Ser) #	3.82 \pm 0.02 ^a	4.1 \pm 0.29 ^a	4.14 \pm 0.1 ^a
Arginine (Arg) # \times	8.33 \pm 0.09 ^a	8.59 \pm 0.62 ^a	8.7 \pm 0.16 ^a
Glycine (Gly) #	5.57 \pm 0.04 ^a	5.43 \pm 0.38 ^a	5.35 \pm 0.13 ^a
Threonine (Thr) *	3.98 \pm 0.03 ^a	4.21 \pm 0.29 ^a	4.3 \pm 0.09 ^a
Proline (Pro) #	1.77 \pm 0.02 ^a	1.6 \pm 0.11 ^a	1.73 \pm 0.04 ^a
Alanine (Ala) #	10.21 \pm 0.1 ^a	10.75 \pm 0.76 ^a	10.45 \pm 0.25 ^a
Valine (Val) *	5.91 \pm 0.03 ^a	5.78 \pm 0.4 ^a	6.16 \pm 0.15 ^a
Methionine (Met) *	0.94 \pm 0.006 ^a	0.77 \pm 0.06 ^b	0.96 \pm 0.03 ^a
Cystine (Cys) #	3.19 \pm 0.04 ^a	4 \pm 0.31 ^b	4.57 \pm 0.11 ^{bc}
Isoleucine (Ile) *	5.2 \pm 0.04 ^a	5.16 \pm 0.4 ^a	5.48 \pm 0.13 ^a
Leucine (Leu) *	10.17 \pm 0.06 ^a	10.7 \pm 0.78 ^a	10.79 \pm 0.27 ^a
Histidine (His) # \times	20.35 \pm 0.21 ^a	21.2 \pm 1.59 ^a	23.52 \pm 0.58 ^a
Phenylalanine (Phe) *	2.47 \pm 0.03 ^a	2.63 \pm 0.19 ^a	2.7 \pm 0.08 ^a
Lysine (Lys) *	58.38 \pm 1.88 ^a	61.92 \pm 6.18 ^a	60.8 \pm 3.27 ^a
Tyrosine (Tyr) #	6.57 \pm 0.5 ^a	7.26 \pm 1.13 ^a	7.57 \pm 0.81 ^a
Tryptophan*	1.2 \pm 0.003 ^a	1.75 \pm 0.03 ^b	1.65 \pm 0.01 ^c
Nonessential amino acid	132.57 \pm 1.24 ^a	139.51 \pm 10.5 ^a	141.96 \pm 3.95 ^a
Half-essential amino acids	28.69 \pm 0.19 ^a	29.79 \pm 2.21 ^a	32.22 \pm 0.73 ^a
Essential amino	88.23 \pm 2.06 ^a	92.9 \pm 8.24 ^a	92.84 \pm 4 ^a
Total amino acid	220.8 \pm 3.3 ^a	232.41 \pm 8.74 ^b	234.8 \pm 7.95 ^b

Note: #: Nonessential amino acid, \times : Half-essential amino acids, *: Essential amino.

traditional pond aquaculture on the growth of grass carp, i.e., RAS is conducive to promoting fish growth (Xu et al., 2023).

In the chemical composition analysis (Table 1), *Micropterus salmoides* muscle exhibited no significant variations in moisture ($p > 0.05$), crude ash, and alkali-soluble hydroxyproline content across the three culture modes. Crude fat content in the P and ARAS groups was comparable ($p > 0.05$), yet both were lower than in the IPRS group ($p < 0.05$). Crude protein and water-soluble protein contents in the P and IPRS groups were similar ($p > 0.05$), but both were significantly lower than in the ARAS group ($p < 0.05$). Muscle salt-soluble protein contents in the P-IPRS and IPRS-ARAS comparisons showed no significant differences ($p > 0.05$), but the ARAS group had significantly higher levels than the P group ($p < 0.05$). Total hydroxyproline content was comparable between the IPRS and ARAS groups ($p > 0.05$), but both were significantly lower than the P group ($p < 0.05$). Muscle glycogen content varied significantly across culture modes ($p < 0.05$), and from largest to smallest were ARAS>IPRS>P, respectively.

The nutritional worth of fish is predominantly dictated by the protein and fat composition of its muscle tissue, which varies substantially based on the growth environment (Guan, Liu, et al., 2022). The findings of this study indicate that there were no substantial variations in the muscle moisture, crude ash, and alkali-soluble hydroxyproline content of *Micropterus salmoides* across the three culturing modes. However, *Micropterus salmoides* muscle from the ARAS group exhibited a relatively lower crude fat content and higher crude protein and muscle glycogen levels, thus contributing to an enhanced nutritional profile. This phenomenon may be attributed to the higher water velocity in the ARAS group compared to the P and IPRS groups, maintaining a constant low-speed exercise state for the *Micropterus salmoides*. Exercise stimulates the production of fish proteins and hinders fat accumulation (Zhou et al., 2021), while simultaneously promoting the storage of muscle glycogen to cater to prolonged exercise durations. This is also consistent with Liu et al.'s finding that sustained exercise improves *Micropterus salmoides* muscle quality (Liu et al., 2024). Furthermore, the ARAS group also displayed relatively high water-soluble and salt-soluble protein contents, which may be associated with the consumption of high-energy diets. Notably, a higher salt-soluble protein content mitigates muscle water loss (Mørkøre et al., 2002). Hydroxyproline, a crucial component of muscle collagen, serves as a vital indicator of the metabolic status and fibrosis of collagenous tissue (Hu et al., 2021). Our study revealed that

Table 3
Comparison of fatty acid contents of *Micropterus salmoides* muscle in different culture modes.

Fatty acid (mg/g)	P	IPRS	ARAS
Butyric acid (C4:0)	0.065 ^a	0.054 ^b	0.05 ^{bc}
Caproic acid (C6:0)	0.007 ^a	0.013 ± 0.001 ^b	–
Octanoic acid (C8:0)	0.065 ± 0.001 ^a	0.057 ± 0.003 ^b	0.043 ^c
Decanoic acid (C10:0)	0.064 ± 0.002 ^{ab}	0.057 ± 0.003 ^b	0.067 ± 0.003 ^{bc}
Undecanoic acid (C11:0)	0.049 ^a	0.047 ± 0.001 ^a	0.048 ± 0.001 ^a
Lauric acid (C12:0)	0.057 ^a	0.047 ± 0.001 ^b	0.049 ^{bc}
Tridecoic acid (C13:0)	0.047 ± 0.002 ^a	0.039 ± 0.001 ^b	0.038 ± 0.001 ^{bc}
Tetradecanoic acid (C14:1)	0.052 ± 0.002 ^a	0.05 ^a	0.052 ± 0.002 ^a
Pentadecanoic acid (C15:0)	0.08 ± 0.003 ^a	0.062 ± 0.001 ^b	0.068 ± 0.002 ^{bc}
Pentadecenoic acid (C15:1)	0.381 ± 0.01 ^a	0.26 ± 0.009 ^b	0.26 ± 0.02 ^{bc}
Palmitic acid (C16:0)	2.226 ± 0.1 ^a	2.006 ± 0.04 ^a	2.426 ± 0.017 ^b
Heptadecenoic acid (C17:1)	0.069 ± 0.003 ^a	0.076 ± 0.003 ^a	0.065 ± 0.002 ^a
Stearic acid (C18:0)	4.976 ± 0.13 ^a	4.843 ± 0.07 ^a	4.985 ± 0.01 ^a
Elaidic acid (C18:1 Trans)	0.029 ^a	0.029 ^a	0.029 ^a
Oleic acid (C18:1)	13.868 ± 0.2 ^a	11.944 ± 0.09 ^b	9.147 ± 0.05 ^c
Inoleic acid (C18:2 Trans)	0.029 ^a	0.029 ^a	0.029 ^a
Linoleic acid (C18:2)	41.483 ± 0.28 ^a	43.69 ± 0.12 ^b	45.427 ± 0.28 ^c
γ-linolenic acid (C18:3 N6)	0.0343 ^a	0.034 ^a	0.034 ^a
α-linolenic acid (C18:3 N3)	1.095 ± 0.072 ^a	1.436 ± 0.07 ^b	1.398 ± 0.047 ^b
Cis-11-Eicosenoic acid (C20:1 N9)	0.328 ± 0.01 ^a	0.274 ± 0.02 ^a	0.316 ± 0.008 ^a
Arachidic acid (C20:0)	0.29 ± 0.017 ^a	0.253 ± 0.01 ^a	0.291 ± 0.027 ^a
Cis-11,14-Eicosadienoic acid (C20:2)	0.05 ± 0.002 ^a	0.043 ± 0.002 ^a	0.035 ± 0.004 ^b
Heneicosanoic acid (C21:0)	0.083 ± 0.001 ^a	0.05 ± 0.001 ^b	0.044 ± 0.001 ^c
Cis-8,11,14-Eicosatrienoic acid (C20:3 N6)	0.073 ± 0.003 ^a	0.057 ± 0.001 ^b	0.05 ± 0.001 ^{bc}
Cis-11,14,17-Eicosatrienoic acid (C20:3 N3)	0.047 ^a	0.047 ^a	0.047 ^a
Behenic acid (C22:0)	0.023 ^a	0.023 ^a	0.023 ^a
Erucic acid (C22:1)	0.088 ± 0.004 ^a	0.061 ± 0.002 ^b	0.057 ± 0.002 ^{bc}
Cis-13,16-Docosadienoic acid (C22:2)	0.043 ± 0.003 ^a	0.006 ^b	0.007 ± 0.002 ^{bc}
Tricosanoic acid (C23:0)	0.116 ± 0.004 ^a	0.028 ± 0.003 ^b	0.131 ± 0.016 ^a
Lignoceric acid (C24:0)	0.234 ± 0.004 ^a	0.038 ± 0.007 ^b	0.12 ± 0.01 ^c
Saturated fatty acid (SFA)	8.38 ± 0.23 ^a	7.62 ± 0.03 ^a	8.38 ± 0.04 ^a
Monounsaturated fatty acid (MUFA)	14.81 ± 0.17 ^a	12.69 ± 0.08 ^b	9.96 ± 0.03 ^c
Polyunsaturated fatty acid (PUFA)	42.85 ± 0.34 ^a	45.35 ± 0.11 ^b	47.03 ± 0.24 ^c
Total fatty acids	66.05 ± 0.1 ^a	65.66 ± 0.09 ^a	65.37 ± 0.18 ^a
ΣPUFA/ΣSFA	5.12 ± 0.18 ^a	5.95 ± 0.03 ^b	5.61 ± 0.06 ^b

Note: -, not detected.

the total hydroxyproline content in the P group was significantly greater than that in the ARAS and IPRS groups, implying a possible correlation between hardness and total hydroxyproline content. These findings align with previous research on the muscle quality characteristics of *Larimichthys crocea* in diverse culturing modes (Fan et al., 2022).

3.2. Analysis of the amino acid profile of *Micropterus salmoides* muscle under different aquaculture systems

The amino acid concentrations in *Micropterus salmoides* muscle cultivated under P, IPRS, and ARAS modes, are detailed in Table 2. The methionine levels of the P and ARAS groups showed no significant variation ($p > 0.05$), yet both exceeded that of the IPRS group ($p < 0.05$).

The cystine content of the ARAS and IPRS groups was comparable ($p > 0.05$), surpassing the P group's levels ($p < 0.05$). The tryptophan content differed significantly ($p < 0.05$), from largest to smallest were IPRS>ARAS>P, respectively. The total amino acid content of the IPRS and ARAS groups was similar ($p > 0.05$), but both exceeded the P group's content ($p < 0.05$).

The nutritional composition of amino acids in aquatic foods profoundly shapes the level of protein nutrition and serves as a pivotal indicator of nutritional value (Oztekin et al., 2020). The breadth and abundance of the amino acid profile directly correlate with the enhanced nutritional value of the protein (Xing et al., 2023). In analyzing the amino acid profiles, our study detected 18 amino acids in the muscle tissue of *Micropterus salmoides* across all three cultivation modes. With regard to amino acid content, the total amino acid content of both the IPRS and ARAS groups was notably higher than that of the P group, suggesting that the overall nutritional quality of *Micropterus salmoides* cultivated in recirculating aquaculture systems surpasses that of the pond. This disparity may be attributed to the enhanced movement of *Micropterus salmoides* in recirculating systems, leading to adaptive metabolic alterations within the fish. Fish regulate their amino acid composition by modulating the activity of various enzymes and altering the storage of bodily substances (Yuan et al., 2018). Amino acids primarily generate flavor compounds through two metabolic pathways. Firstly, through elimination reactions catalyzed by amino acid lyases, the side chains of amino acids such as tyrosine, tryptophan, and methionine are utilized to release phenol, indole, and methyl mercaptan (Hu et al., 2024). Secondly, amino acids are converted into α-keto acids under the action of microbial transaminases. Subsequently, these α-keto acids are further transformed into flavor compounds such as alcohols, aldehydes, and acids through the actions of decarboxylases, dehydrogenases, and lyases (Wang et al., 2019). The findings of our study align with previous research conducted by Li, demonstrating that an appropriate exercise training intensity can augment the muscle amino acid content in juvenile *Spinibarbus sinensis* (Li, 2013).

3.3. Analysis of the fatty acid profile of *Micropterus salmoides* muscle under different aquaculture systems

Table 3 details the fatty acid composition of *Micropterus salmoides* muscle reared under three modes. A total of 30 fatty acids were identified. The ARAS and IPRS groups exhibited no significant variation of C4:0, C15:0, C15:1, C12:0, C13:0, C20:3 N6, C22:1, and C22:2 ($p > 0.05$), but all were significantly lower than in the P group ($p < 0.05$). No significant difference in C18:3 N3α content and ΣPUFA/ΣSFA was observed between the ARAS and IPRS groups ($p > 0.05$), but both were higher than the P group ($p < 0.05$). C6:0 content was significantly higher in the IPRS group compared to the P group ($p < 0.05$), and undetectable in the ARAS group. Significant differences in C8:0, C18:1, and C21:0 contents ($p < 0.05$) were observed among the three groups, with P > IPRS > ARAS. The C10:0 content was similar between the P-IPRS and P-ARAS comparisons ($p > 0.05$), but the ARAS group had higher levels than the IPRS group ($p < 0.05$). The C16:0 content of the P and IPRS groups was comparable ($p > 0.05$), but both were significantly lower than the ARAS group ($p < 0.05$). Significant variations in C18:2 and polyunsaturated fatty acid contents were noted among the groups ($p < 0.05$), with ARAS > IPRS > P. The C20:2 content was similar between the P and IPRS groups ($p > 0.05$), but both were higher than the ARAS group ($p < 0.05$). The C23:0 content of the P and ARAS groups was comparable ($p > 0.05$), but both exceeded the IPRS group's levels ($p < 0.05$). A significant difference in C24:0 content ($p < 0.05$) was observed among the groups, with P > ARAS > IPRS. The monounsaturated fatty acid content varied significantly among the groups ($p < 0.05$), with P > IPRS > ARAS.

Fatty acids abound in nutrients that are essential to human physiology, and their composition serves as a barometer of the organism's nutritional quality (Cottin et al., 2016). Their content is among the

Table 4

Comparison of mineral element contents of *Micropterus salmoides* muscle in different culture modes.

Mineral elements (μg/g)	P	IPRS	ARAS
Magnesium (Mg)	365.4 ± 3.68 ^a	361.07 ± 3.24 ^{ab}	439.83 ± 2.27 ^c
Phosphorus (P)	1537.67 ± 18.12 ^a	1691.67 ± 14.72 ^b	1802.33 ± 7.69 ^c
Potassium (K)	4086.67 ± 35.08 ^a	4590.67 ± 54.12 ^b	4985 ± 31.5 ^c
Calcium (Ca)	109.46 ± 2.27 ^a	86.83 ± 1.47 ^b	124.8 ± 0.4 ^c
Iron (Fe)	27.5 ± 0.26 ^a	11.39 ± 0.63 ^b	14.59 ± 0.44 ^c
Zinc (Zn)	4.82 ± 0.05 ^a	6.5 ± 0.06 ^b	4.98 ± 0.07 ^a
Selenium (Se)	0.26 ± 0.01 ^a	0.29 ± 0.01 ^{ab}	0.32 ± 0.02 ^{bc}
Iodine (I)	0.14 ^a	0.1 ± 0.005 ^b	0.06 ± 0.004 ^c

paramount factors that influence the flavor of muscle tissue (Fu et al., 2022). In fish flesh, the nutritional significance of fat is contingent upon the type and ratio of fatty acids present. Fatty acids with distinct structures can exert varied physiological functions and effects (Chen & Liu, 2020). It is widely acknowledged that saturated fatty acids, being a crucial energy source, furnish the body with ample energy (Calder, 2015). Unsaturated fatty acids (SFAs) play a pivotal role in regulating lipoprotein homeostasis, diminishing cholesterol levels, preserving the stability of cytokine functions, and combating cardiovascular diseases (Liu et al., 2023). Regarding monounsaturated fatty acids, the muscle content of *Micropterus salmoides* in group P was conspicuously higher than that in groups ARAS and IPRS. This disparity might be attributed to the intricate microenvironment of pond water. Furthermore, mono-unsaturated fatty acids were highest in oleic acid in all three cultivation modes. As a “safe fatty acid,” oleic acid effectively regulates blood lipid levels, aids in reducing serum cholesterol, and lessens blood viscosity (Sales-Campos et al., 2013), thereby constituting a significant indicator of food’s nutritional value (Zhong et al., 2024). This finding suggests that *Micropterus salmoides* serves as a reliable source of safe fatty acids.

Polyunsaturated fatty acids (PUFAs) are essential fatty acids that the human body is unable to synthesize autonomously. Consequently, the PUFA content in fish muscle tissue serves as a pivotal indicator of fish flesh quality (Pyz-Lukasik et al., 2020), and its elevated levels significantly enhance the flavor of the flesh (Wood & Scollan, 2022). Our investigation revealed that the PUFA content of *Micropterus salmoides* muscle was highest in the ARAS group, suggesting that the ARAS culture mode contributes positively to the enhancement of muscle quality and bioefficacy. This finding aligns with the study conducted by Song on the nutritional value of the muscle of red-finned *Puntius schwanenfeldi* (Song, 2008). Furthermore, linoleic acid emerged as the predominant PUFA in all three cultivation modes. Linoleic acid plays a crucial role in maintaining cellular membrane function, serving as a precursor for certain physiological regulatory substances (Mercola & D’Adamo, 2023). This implies that *Micropterus salmoides* muscles possess certain health benefits. Currently, the P/S ratio (Σ PUFA/ Σ SFA) is commonly utilized to assess the nutritional value of fatty acids in fish, where higher values indicate superior nutritional quality. Nutritional guidelines recommend a P/S ratio exceeding 0.4–0.5 (Pyz-Lukasik et al., 2020). In the present study, the P/S ratios of *Micropterus salmoides* muscle exceeded 5 in all three modes, with significantly higher values observed in the ARAS and IPRS groups compared to the P group. It suggests that *Micropterus salmoides* possesses high fatty acid nutritional value, which is further enhanced under recirculating aquaculture conditions. This is consistent with the finding of Guan et al. that RAS significantly elevated muscle fatty acid content in triploid *Oncorhynchus mykiss* (Guan, Xu, et al., 2022).

3.4. Analysis of the mineral element profile of *Micropterus salmoides* muscle under different aquaculture systems

The mineral element contents in *Micropterus salmoides* muscle, cultivated under P, IPRS, and ARAS modes, are presented in Table 4. The

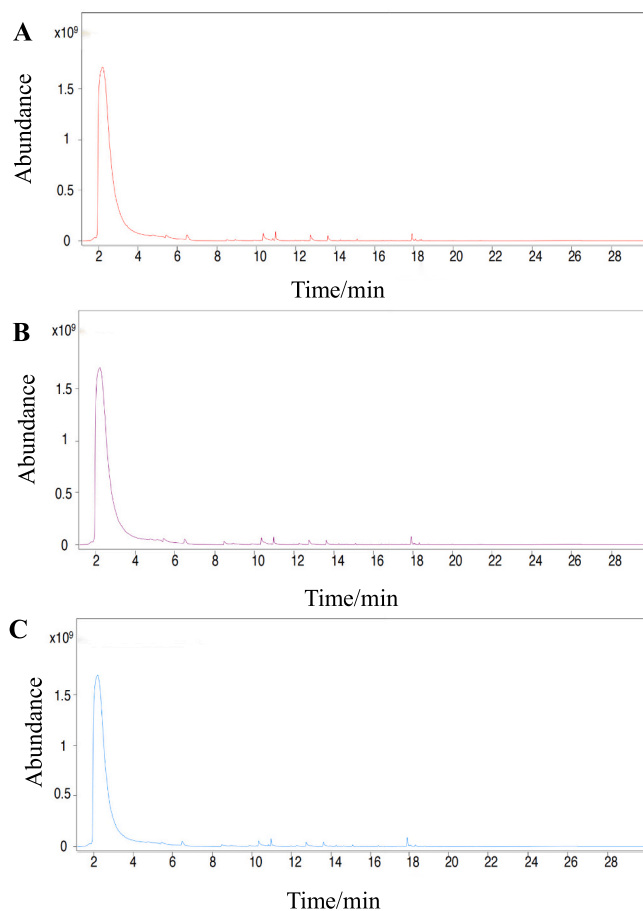


Fig. 1. Total ion flow diagram of volatile components of *Micropterus salmoides* muscle under P (A) IPAR (B) and ARAS (C) modes.

Mg content was similar in the P and IPRS groups ($p > 0.05$), but significantly lower than in the ARAS group ($p < 0.05$). There were significant variations in the P and K contents among the three modes ($p < 0.05$), with ARAS > IPRS > P. The Ca content also varied significantly ($p < 0.05$), with ARAS > P > IPRS. The Fe content differed significantly ($p < 0.05$), and from largest to smallest was P > ARAS > IPRS, respectively. The Zn content in the P and ARAS groups were comparable ($p > 0.05$), but both were significantly lower than IPRS ($p < 0.05$). Regarding the Se content, there were no significant differences between P-IPRS and IPRS-ARAS ($p > 0.05$), but the ARAS group had significantly higher levels than the P group ($p < 0.05$). The I content differed significantly ($p < 0.05$), with P > IPRS > ARAS.

Mineral elements constitute indispensable components in the constitution of human tissues and the sustenance of physiological functions (Lall & Kaushik, 2021), profoundly influencing the nutritional merit of fish, product shelf-life, and flavor (Song et al., 2022). *Micropterus salmoides* harbors not only vital macronutrients such as K, Ca, Mg, and P, but also trace elements like Fe, Zn, and Se (Jia et al., 2022). These trace elements are integral to human growth, development, and metabolic processes (Konikowska & Mandecka, 2018). The current investigation revealed that in the muscle of *Micropterus salmoides*, K exhibited the highest macronutrient content, while Fe held the foremost position among trace elements across all three culture modes. Moreover, substantial variations in mineral composition were observed among different culture methods. With Mg, P, K, Ca, and Se all were the highest in the ARAS mode, whereas Fe and I were the highest in the P mode, and Zn was the highest in the IPRS mode. Taken together, the ARAS mode exhibited a higher diversity and content of mineral elements compared to the other two modes. This might be attributed to the ARAS mode’s

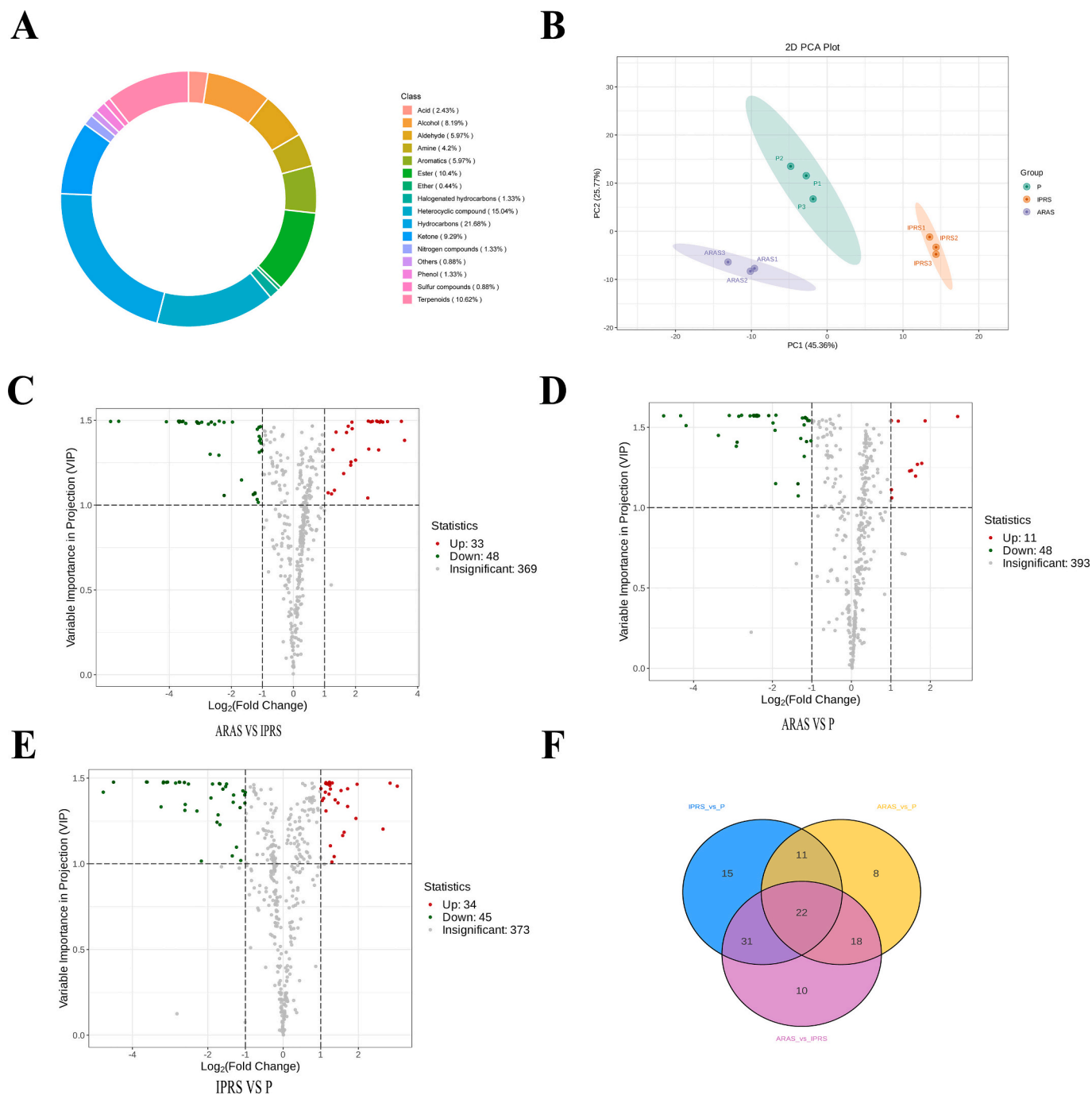


Fig. 2. The differential metabolites in different *Micropterus salmoides* samples. (A) Doughnut chart, (B) Principal component analysis, (C-E) Volcano plots, (F) Venn diagram.

enhanced feed utilization efficiency in *Micropterus salmoides*, ultimately leading to a superior deposition of minerals in their bodies.

3.5. Characterization of the volatile metabolites

To investigate the odor characteristics of *Micropterus salmoides* muscle in different modes, the flavor components and contents were identified and analyzed by HS-SPME-GC-MS. A total of 452 flavor components were identified in the three groups of P, IPRS, and ARAS by analyzing the total ion flow diagrams of the volatile components (Fig. 1A-C) as well as the characterization and categorization of the peaks by spectral library searches. These include hydrocarbons (98), heterocyclic compounds (68), terpenoids (48), esters (47), ketones (42),

alcohols (37), aromatic hydrocarbons (27), aldehydes (27), amines (19), acids (11), phenols (6), halogenated hydrocarbons (6), nitrogen-containing compounds (6), sulfur compounds (4), others (4), ethers (2) (Fig. 2A). PCA analysis showed that the volatile compound profiles of *Micropterus salmoides* muscle from the P, IPRS, and ARAS groups were clearly separated (Fig. 2B). Volcanograms showed that 48 volatile compounds were down-regulated and 33 were up-regulated in the ARAS VS IPRS group (Fig. 2C). 48 volatile compounds were down-regulated and 11 were up-regulated in the ARAS VS P group (Fig. 2D). 45 volatile compounds were down-regulated and 34 were up-regulated in the IPRS VS P group (Fig. 2E). The intergroup distribution of differential compounds is shown in the Venn diagram, with a total of 115 differential metabolites identified in the three group comparisons (Fig. 2F). A

Table 5
The common differential metabolites in the IPRS_vs_P, ARAS_vs_P, and ARAS_vs_IPRS groups.

Category	Compounds	IPRS_vs_P	ARAS_vs_P	ARAS_vs_IPRS
Terpenoids	(1.alpha.,4a.beta.,8a.alpha.)-1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-Naphthalene	down	down	up
	.gamma.-Muuroleone	down	down	up
	Bicyclo[5.2.0]nonane, 4-methylene-2,8,8-trimethyl-2-vinyl-	up	down	down
	[1R-(1.alpha.,3a.alpha.,7a.alpha.)]-1,2,3,6,7,7a-hexahydro-2,2,4,7a-tetramethyl-1,3a-Ethano-3aH-indene	up	down	down
	[1aR-(1a.alpha.,7.alpha.,7a.beta.,7b.alpha.)]-1a,2,3,5,6,7,7a,7b-octahydro-1,1,4,7-tetramethyl-1H-Cycloprop[e]azulene	down	down	up
Heterocyclic compound	4-Quinolinecarboxaldehyde	down	down	up
	4-[2-(2-Propenyl)-1,3-dioxolan-2-yl]-1H-pyrazole	up	down	down
	5-methyl-1H-Indole	down	down	down
	7-methyl-1H-Indole	down	down	down
	Azacyclohexane, 3-[1-pyrrolidyl]-	down	down	up
Ester	2-methyl-Butanoic acid,2-methyl-2-propenyl ester	up	down	down
	4-Ethylbenzoic acid, 2-methylbutyl ester	up	down	down
Nitrogen compounds	methoxy-phenyl-Oxime-	down	down	down
	4-butyl-N,N-dimethyl-Benzamide	up	down	down
Ketone	Benzophenone	up	down	down
	1-[4-(1-methylethyl)phenyl]-Ethanone	down	down	down
Hydrocarbons	(E,E)-2,4-Dodecadienal	up	down	down
	1-Pentadecyne	down	down	up
	Pentadecane, 2,6,10-trimethyl-	up	down	down
Aldehyde	Benzaldehyde, 6-hydroxy-4-methoxy-2,3-dimethyl-	up	down	down
Alcohol	Benzenemethanol,.alpha.-2-cyclohexen-1-yl-	up	down	down
Amines	N1-(4-fluorobenzyl)-N2,N2-dimethyl-1,2-ethanediamine	up	down	down

total of 22 common compounds were found, with a high percentage of terpenoids and heterocyclic compounds (Table 5).

3.6. Analysis of the flavor substances of *Micropterus salmoides* under different aquaculture systems

The contribution of volatile compounds to the flavor of *Micropterus salmoides* muscle was assessed using their rOVA. By applying the criteria of VIP > 1 and rOAV > 1, 41, 39, and 38 volatile compounds were selected from the three groups, respectively. These compounds comprised terpenoids (9), esters (8), alcohols (6), aldehydes (5), ketones (5), heterocyclic compounds (3), sulfur-containing compounds (2), aromatic hydrocarbon (1), phenol (1), and nitrogen-containing compound (1) (Table 6).

The rOVA values of (Z)-2-Octen-1-ol, 2-Octen-1-ol, (E)-,Butanoic acid, butyl ester, and Heptanoic acid,ethyl ester did not significantly differ between the P-ARAS and P-IPRS groups ($p > 0.05$), but the ARAS group were significantly lower than the IPRS group ($p < 0.05$). The rOVA of Pyrazine,2-methoxy-3-(2-methylpropyl), trans-.beta.-Ionone, and β -Ionone did not vary significantly between the P-ARAS and P-IPRS groups ($p > 0.05$), yet the ARAS group were significantly higher than the IPRS group ($p < 0.05$). For 1-Nonanol, 2,6-Octadienal, 3,7-dimethyl-, (E)-, 2-Decanol, and Dimethyl triSulfur compounds, the rOVA differences between P and ARAS were insignificant ($p > 0.05$), but all were notably lower than in the IPRS group ($p < 0.05$). The rOVA of 2(3H)-Furanone,5-butylidihydro- and Cyclohexanone,2,2,6-trimethyl- did not differ significantly between P and IPRS groups ($p > 0.05$), but both were significantly lower than in the ARAS group ($p < 0.05$). The rOVA of 2(3H)-Furanone,5-hexylidihydro- and Dodecanenitrile were not significantly different between ARAS-P and ARAS-IPRS groups ($p > 0.05$), yet the IPRS group was significantly higher than the P group ($p < 0.05$). The rOVA of Anethole in the ARAS and IPRS groups was similar ($p > 0.05$), but both were notably lower than in the P group ($p < 0.05$). The rOVA of o-Cymene and p-Cymene did not vary significantly between the IPRS-ARAS and IPRS-P groups ($p > 0.05$), but the ARAS group was significantly lower than the P group ($p < 0.05$).

Among these varying flavor compounds, ranked by rOAV values, Pyrazine,2-methoxy-3-(2-methylpropyl)-, β -Ionone, trans- β -Ionone, Cyclohexanone,2,2,6-trimethyl-, Dimethyl trisulfur compounds, 2-Octen-1-ol,(E)- (Z)-2-Octen-1-ol, Pyrazine,2-methoxy-3-(1-

methylethyl)-, 2-Octen-1-ol, and 2-methoxy-Phenol (top 10) significantly contribute to the overall flavor of *Micropterus salmoides* muscle from various culture modes. Analysis revealed no significant difference in total muscle odor activity values between *Micropterus salmoides* from the P-ARAS and P-IPRS groups ($p > 0.05$), yet the ARAS group exhibited significantly higher values than the IPRS group ($p < 0.05$).

Based on the screening criteria and annotated sensory flavor characteristics for each differential metabolite in each comparison group, the top 10 sensory flavors with the highest annotations were chosen for the associated network (Fig. 3A-C) and radar chart (Fig. 3D) representation. The findings revealed that volatile differential metabolites in *Micropterus salmoides* muscle exhibited woody, herbal, and sweet flavors under the three cultivation methods.

The aroma profile of fresh fish exhibits profound variations based on species, yet a pervasive sweet and phytoalexin-like scent remains ubiquitous. This scent is readily identifiable and synonymous with the freshness of fish (Morita et al., 2003). The distinctive flavor of fresh fish is attributed to the generation of volatile carbonyl compounds and alcohols, stemming from the enzymatic action of lipoxygenase on polyunsaturated fatty acids present in fish lipids (Dyall et al., 2022). In our study, we observed that hydrocarbons were the most prevalent flavor compounds detected in *Micropterus salmoides* muscle across the three modes. Despite their limited contribution to the overall flavor profile, the branched alkanes present in hydrocarbons exhibit a clean and sweet aroma (Tanchotikul & Hsieh, 1991). In addition, the common differential compounds detected in the three modes were dominated by heterocycles and terpenoids. Among these, N/O/S-containing heterocyclic compounds, albeit present in low concentrations in the flesh, significantly contribute to the flesh flavor due to their low sensory thresholds. These compounds often exhibit flavor characteristics reminiscent of roasted, charred, or nutty aromas (Lin, 2010). Terpenes are a class of secondary plant metabolites, the volatile terpenes mainly contain isoprenoids, mono sesquiterpenes, and their derivatives, which can give a unique flavor to food products (Abbas et al., 2017). The elevated levels of terpenoids detected in this study are likely attributed to the culture feed and environmental conditions. However, it is noteworthy that only a limited number of types of sulfur-containing compounds were identified. These compounds, characterized by odors reminiscent of onion, cabbage, fishy, harsh, or boiled sulfur, and rotten egg, can significantly impact the overall food flavor due to their low thresholds (Lu, 2022).

Table 6
Changes in volatiles of *Micropterus salmoides* muscle rOVA>1 in different culture modes.

Category	Compounds	RI	Odor	Threshold (µg/kg)	rOVA			
					P	IPRS	ARAS	
Terpenoids	.alpha.-Ionone	1435	sweet, woody, floral, violet, orris, tropical, fruity	3.78	3.1 ± 0.07	5.51 ± 0.92	–	
	2,6-Octadienal,3,7-dimethyl-,(E)-	1174	citrus, lemon	28	1.33 ± 0.1 ^a	2.58 ± 0.25 ^b	1.62 ± 0.25 ^{ac}	
	Anethole	1190	sweet, exotic, flowery, stewed	15	2.26 ± 0.14 ^a	1.53 ± 0.11 ^{bc}	1.48 ± 0.1 ^c	
	Geraniol	1255	sweet, floral, fruity, rose, waxy, citrus	6.6	2.21 ± 0.08 ^a	2.19 ± 0.26 ^a	2.54 ± 0.25 ^a	
	Linalool	1100	floral, green	6	101.27 ± 4.95 ^a	100.42 ± 5.85 ^a	99.11 ± 5.68 ^a	
	dl-Menthol	1164	pepperminty, cool, woody	130	1.18 ± 0.09	–	1.2 ± 0.2	
	o-Cymene	1042	gasoline	11.44	1.96 ± 0.1 ^a	1.9 ± 0.24 ^{ab}	1.24 ± 0.23 ^b	
	trans-.beta.-Ionone	1942	dry, powdery, floral, woody, orris	0.2	6076.38 ± 269.94 ^{ab}	5869.09 ± 479.25 ^a	8103.99 ± 899.44 ^b	
	β-Ionone	1457	floral, woody, sweet, fruity, berry, tropical, beeswax	0.007	173,610.95 ± 7712.55 ^{ab}	167,688 ± 13693 ^a	231,542.62 ± 25,698.31 ^b	
	Ester	2(3H)-Furanone,5-butylidihydro-	1261	sweet, coconut, waxy, creamy, tonka, dairy, fatty	17.9	6.77 ± 0.96 ^a	6.11 ± 0.76 ^a	9.01 ± 0.72 ^b
		2(3H)-Furanone,5-hexylidihydro-	1473	fresh, oily, waxy, peach, coconut, buttery, sweet	1.1	6.58 ± 0.27 ^{ac}	9.75 ± 0.83 ^b	8.55 ± 0.78 ^{bc}
2(3H)-Furanone,dihydro-5-(2-octenyl)-,(Z)-		1590	sweet, fatty, waxy, dairy, creamy, fruity	5.4	4.61 ± 0.74 ^a	5.38 ± 0.31 ^a	6.02 ± 0.6 ^a	
2(3H)-Furanone,dihydro-5-pentyl-		1368	coconut, woody	7.9	1.98 ± 0.13 ^a	2.88 ± 0.46 ^a	2.82 ± 0.14 ^a	
2-Propen-1-ol,3-phenyl-,acetate,(E)-		1446	sweet, floral, spicy, balsamic	2	1.58 ± 0.11	2.02 ± 0.28	–	
Butanoic acid,butyl ester		996	fruity, banana, pineapple, green, cherry, tropical fruit, ripe fruit, juicy fruity	28	8.59 ± 0.99 ^{ab}	12.98 ± 1.95 ^a	5.61 ± 0.79 ^b	
Dodecanoic acid,methyl ester		1481	waxy, soapy, creamy, coconut, mushroom	3.5	11.55 ± 0.34 ^a	14.95 ± 1.01 ^a	14.71 ± 1.38 ^a	
Alcohol	Heptanoic acid,ethyl ester	1097	fruity, pineapple, cognac, rummy, wine	2	6.24 ± 0.47 ^{ab}	6.98 ± 0.5 ^a	5.36 ± 0.35 ^b	
	(Z)-2-Octen-1-ol	1067	sweet, floral	25	388.9 ± 18.09 ^{ab}	398.64 ± 4.72 ^a	346.19 ± 16.18 ^b	
	1-Decanol	1271	fatty, waxy, floral, orange, sweet, watery	23	2.27 ± 0.08 ^a	2.43 ± 0.28 ^a	2.7 ± 0.29 ^a	
	1-Nonanol	1170	fresh, clean, fatty, floral, rose, orange, dusty, wet, oily	5.3	2.37 ± 0.1 ^a	3.58 ± 0.24 ^b	2.41 ± 0.27 ^{ac}	
	2-Decanol	1178	anisic, coconut	25	2.87 ± 0.13 ^a	6.7 ± 0.68 ^b	3.88 ± 0.69 ^{ac}	
	2-Octen-1-ol	1067	green, vegetable	50	194.45 ± 9.04 ^{ab}	199.32 ± 2.36 ^a	173.10 ± 8.09 ^b	
	2-Octen-1-ol,(E)-	1067	green, citrus, vegetable, fatty	20	486.13 ± 22.61 ^{ab}	498.3 ± 5.9 ^a	432.74 ± 20.23 ^b	
Aldehyde	(E)-2-Decenal	1263	waxy, fatty, earthy, green, cilantro, mushroom, aldehydic, fried, chicken, fatty, tallow	5	5.05 ± 0.4 ^a	4.68 ± 0.64 ^a	5.6 ± 0.49 ^a	
	1-Cyclohexene-1-carboxAldehyde,4-(1-methylethenyl)-	1207	fresh, green, herbal, grassy, sweet, minty, cumin	30	15.86 ± 1.22 ^a	14.09 ± 0.68 ^a	16.89 ± 0.97 ^a	
	2-Decenal, (Z)-	1252	tallow	50	1.86 ± 0.09 ^a	1.74 ± 0.06 ^a	1.99 ± 0.13 ^a	
	2-Undecenal,E-	1311	fresh, fruity, citrus, orange, peel	0.78	3.25 ± 0.39 ^a	3.65 ± 0.54 ^a	4.18 ± 0.35 ^a	
	Nonanal	1105	aldehyde, citrus, orange peel	1	136.88 ± 38.74 ^a	181.02 ± 57.02 ^a	139.31 ± 29.02 ^a	
	.alpha.-Irone	1490	orris, floral, berry, violet, woody, powdery	2	140.41 ± 5.3 ^a	170.93 ± 11.23 ^a	181.9 ± 17.2 ^a	
	2-Cyclopenten-1-one,3-methyl-2-(2-pentenyl)-,(Z)-	1338	woody, herbal, floral, spicy, jasmin, celery	0.26	3.99 ± 0.21 ^a	5.05 ± 0.29 ^a	5.58 ± 0.73 ^a	
Ketone	2H-Pyran-2-one,tetrahydro-6-methyl-	1095	creamy, fruity, coconut	26.83	2.24 ± 0.09 ^a	2.19 ± 0.14 ^a	2.17 ± 0.4 ^a	
	Cyclohexanone,2,2,6-trimethyl-	1086	pungent, thujone, labdanum, honey, cistus	0.1	3328.33 ± 132.84 ^a	3195.64 ± 176.84 ^a	4013.66 ± 592.86 ^b	
	Isophorone	1123	cool, woody, sweet, green, camphor, fruity, musty, cedarwood, tobacco, leathery	11	3.21 ± 0.15 ^a	2.65 ± 0.32 ^a	3.17 ± 0.19 ^a	
	Indole,3-methyl-	1391	animalic, fecal, indole, civet	0.41	1.71 ± 0.23	–	–	
Heterocyclic compound	Pyrazine,2-methoxy-3-(1-methylethyl)-	1105	beany, pea, earthy, chocolate, nutty	0.002	298.41 ± 42.99 ^a	396.21 ± 39.99 ^a	290.24 ± 37.28 ^a	
	Pyrazine,2-methoxy-3-(2-methylpropyl)-	1204	green bell pepper, pea, galbanum	0.002	2,667,918.9 ± 107,513.53 ^{ab}	2,352,455.89 ± 123,925.15 ^a	2,812,931.37 ± 26,283.66 ^b	
Sulfur compounds	Diallyl Sulfur compounds	860	sulfury, onion, garlic, horseradish, metallic	100	12.16 ± 0.24 ^a	11.58 ± 0.47 ^a	12.25 ± 0.48 ^a	
	Dimethyl triSulfur compounds	972	sulfury, cooked onion, savory, meaty	0.008	2027.93 ± 177.01 ^a	6479.04 ± 710.59 ^b	4666.06 ± 561.21 ^{ac}	
Aromatics	p-Cymene	1025	woody, citrus	11.4	1.97 ± 0.1 ^a	1.91 ± 0.24 ^{ab}	1.24 ± 0.23 ^b	
Phenol	2-methoxy-Phenol	1090	nutty	1.6	143.43 ± 10.09 ^a	154.89 ± 6.03 ^a	151.78 ± 12.75 ^a	
Nitrogen compounds	Dodecanenitrile	1459	citrus, orange, peel, metallic, spicy	0.09	80.02 ± 1.48 ^{ac}	121.15 ± 14.23 ^b	98.63 ± 10.45 ^{bc}	
	Total odor activity value				2,855,052.11 ± 99869 ^{ab}	2,538,045.53 ± 116168 ^a	3,063,296.05 ± 83795 ^b	

Note: Category, primary classification of metabolites; RI, retention indices of metabolites on nonpolar columns; Odor, aromatic description of metabolites; Threshold, thresholds for differential metabolites; -, not detected.

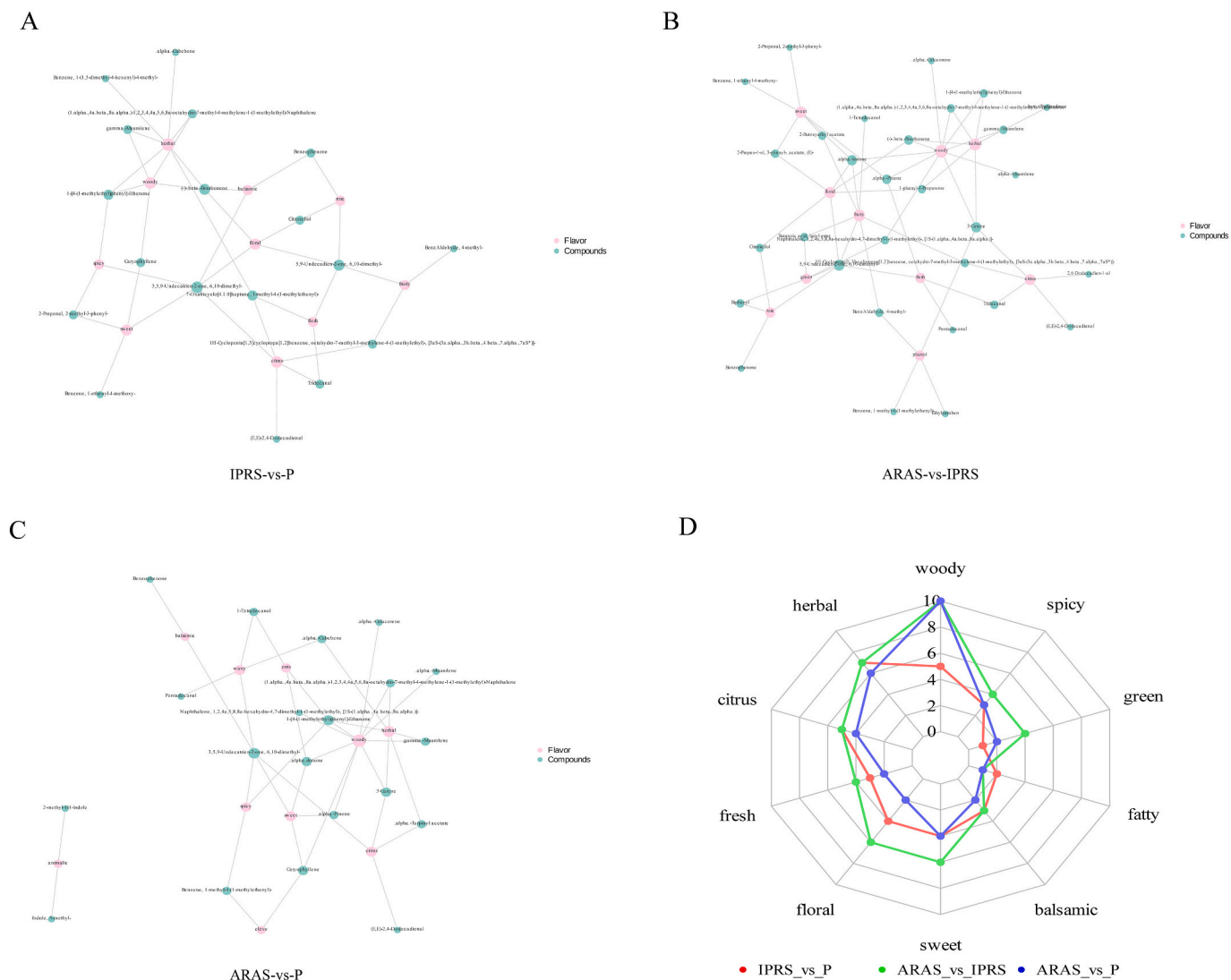


Fig. 3. Sensory flavor characteristics of muscle differential metabolites in *Micropterus salmoides* under different modes. (A-C) Associated network chart, (D) Radar chart.

Note: A-C: The pink circle indicates the organoleptic flavor profile, the green circle indicates the differential metabolite and the line between the two colored circles represents the differential metabolite annotated to the organoleptic flavor profile. D: The name of the outermost circle indicates the organoleptic flavor profile, different colors correspond to different groups, and the numbers corresponding to the color dots indicate the number of occurrences of the corresponding organoleptic flavor profile.

The contribution of volatile flavor compounds to the holistic flavor profile of fish flesh necessitates a joint assessment, encompassing both their relative content and flavor thresholds (Zhang, Zhang, et al., 2019). The relative odor activity value is a commonly used method to objectively describe the contribution value of aroma components to the flavor style of fish flesh based on perception thresholds and relative contents (Mamede et al., 2017). This methodology enables the identification of aroma constituents that exert decisive influences and modifications on the flavor of fish flesh (Li et al., 2021). Furthermore, the integration of the rOAV method with statistical analysis facilitates the precise identification of key flavor compounds in complex matrices that possess distinctive aroma characteristics (Fang et al., 2022). In this study, a total of 41 key odor actives were screened based on the VIP and rOAV values in *Micropterus salmoides* muscle in the three modes. Pyrazine,2-methoxy-3-(2-methylpropyl)-, with its potent aroma of green bell pepper, pea, and galbanum, emerged as the most significant contributor to the overall flavor. Pyrazines, being specific products of the Maillard reaction, possess a distinctive flavor profile (Yang et al., 2019). Furthermore, β-Ionone, renowned for its floral and fruity aroma, contributed

substantially to the comprehensive flavor profile. However, this class of compounds has been predominantly studied in plant aromas (Paparella et al., 2021), with limited research in fish flesh. Notably, the present study revealed that the types of flavor active substances detected were largely consistent across all three modes, albeit with significant variations in their contents. These flavor-active substances predominantly imparted woody, herbal, and sweet flavors for *Micropterus salmoides*. Collectively, the ARAS mode exhibited the highest cumulative odor activity value and crude protein content in the muscle. This may be attributed to the fact that amino acids derived from the degradation of muscle proteins undergo Maillard reactions, generating more volatile compounds (Sohail et al., 2022), which to a certain extent enhance the overall flavor of *Micropterus salmoides* muscle. It is noteworthy that despite the detection of only a few sulfur compounds, Dimethyl trisulfur compounds possessed relatively high rOAV values. Given that sulfur compounds may adversely impact the overall flavor, subsequent elimination studies targeting this specific flavor substance are warranted.

4. Conclusion

This study systematically evaluated the textural properties, nutritional profile, and flavor constituents of *Micropterus salmoides* muscle under the P, IPRS, and ARAS modes. Key findings indicate significant differences in muscle quality among rearing systems, highlighting the unique attributes of each. The P mode exhibited firmer flesh, while the ARAS mode optimized nutritional value by reducing crude fat and enhancing crude protein, muscle glycogen, total amino acids, Σ PUFA/ Σ SFA ratio, and mineral diversity. Furthermore, Flavor analysis identified 452 flavor constituents, with 41 key odor-active compounds. The ARAS mode demonstrated the highest cumulative odor activity value, suggesting enhanced overall flavor component enriched with woody, herbal, and sweet aroma profiles, primarily attributed to Pyrazine, 2-methoxy-3-(2-methylpropyl)- and β -Ionone. In conclusion, the study concludes that the recirculating aquaculture mode, particularly ARAS, plays a crucial role in elevating the nutritional quality and distinct flavor characteristics of *Micropterus salmoides*.

Ethical statement

The proposed methods, use of animals, and research practice (approval number: IACUC-20181015-12) were examined and approved by the Animal Ethics Committee of Southwest University.

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CRedit authorship contribution statement

Zhengxi Wang: Writing – review & editing, Writing – original draft, Methodology, Funding acquisition, Formal analysis, Data curation. **Jishu Zheng:** Writing – review & editing, Writing – original draft. **Decheng Pu:** Writing – review & editing, Writing – original draft. **Peiyuan Li:** Methodology, Investigation. **Xiuli Wei:** Supervision, Project administration. **Dongsheng Li:** Validation, Data curation. **Lihong Gao:** Resources, Funding acquisition. **Xuliang Zhai:** Visualization, Project administration. **Changhua Zhao:** Validation, Supervision. **Yidan Du:** Methodology, Data curation.

Declaration of competing interest

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

Data availability

Data will be made available on request.

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Further-reading

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