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Effects of Dietary Proanthocyanidins Supplementation on Serum Metabolomics and Muscle Flavor in Common Carp (*Cyprinus carpio*)

Qihao Zhang [1,†], Bing Li [1,2,†], Yiran Hou [1,2], Linjun Zhou [2], Chengfeng Zhang [2], Jian Zhu [1,2,*] and Rui Jia [1,2,*]

[1] Wuxi Fisheries College, Nanjing Agricultural University, Wuxi 214081, China; zhangqihao@stu.njau.edu.cn (Q.Z.); lib@ffrc.cn (B.L.); houyr@ffrc.cn (Y.H.)
[2] Key Laboratory of Integrated Rice-Fish Farming Ecology, Ministry of Agriculture and Rural Affairs, Freshwater Fisheries Research Center, Chinese Academy of Fishery Sciences, Wuxi 214081, China; zhoulinjun@ffrc.cn (L.Z.); zhangcf@ffrc.cn (C.Z.)
* Correspondence: zhuj@ffrc.cn (J.Z.); jiar@ffrc.cn (R.J.)
† These authors contributed equally to this work.

Abstract

Common carp (*Cyprinus carpio*) were fed diets supplemented with 0 (NC), 0.4 (LP), or 0.8 (HP) g/kg PACs for 60 days. Serum biochemical indices, serum metabolites, muscle texture, and muscle volatile compound profiles were then examined. Compared with the NC group, 0.8 g/kg PACs significantly reduced serum glucose (Glu), triacylglycerol (TG), alanine aminotransferase (ALT), and aspartate aminotransferase (AST), while increasing reduced glutathione (GSH). Serum metabolomics identified 2222 metabolites, including 429 differentially abundant metabolites (DAMs) between the NC and HP groups. Pathway analysis revealed that these metabolites were primarily involved in nucleotide metabolism, purine metabolism, phenylpropanoid biosynthesis, biosynthesis of unsaturated fatty acids, and biosynthesis of amino acids. PACs supplementation altered instrumental muscle texture parameters, with decreases in hardness, gumminess, chewiness, and shear force, indicating potential changes associated with muscle tenderness. Furthermore, volatile compound profiling identified 2019 compounds in muscle, of which 178 exhibited significant differences in relative abundance between the NC and HP groups. These compounds mainly included organoheterocyclic compounds, esters, alcohols, aldehydes, and ketones. Supplementation with 0.8 g/kg PACs increased the abundance of several volatile compounds associated with sweet, fruity, and green notes. In contrast, the abundance of several compounds associated with fishy or earthy odors was reduced. Taken together, diets supplemented with 0.8 g/kg PACs positively modulated serum biochemical indices and metabolic profiles and altered the volatile compound profiles of common carp.

Keywords: proanthocyanidins; diet trial; serum metabolomics; muscle texture; flavoromics; flesh quality; common carp

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1. Introduction

Freshwater aquaculture contributes substantially to the supply of edible animal protein, and the quality of farmed fish is therefore closely associated with its nutritional and commercial value [1]. The continued expansion of aquaculture production has been accompanied by the use of intensive farming practices, particularly high stocking densities

[2]. Under such conditions, cultured fish may be exposed to multiple stressors, such as unstable environmental conditions, poor water quality, and nutritional fluctuations. Prolonged or repeated exposure to these stressors can disturb physiological homeostasis and increase oxidative stress, with subsequent effects on immune and metabolic processes [3]. These changes may ultimately be reflected in reduced growth and undesirable alterations in flesh composition and product quality [4]. Nutritional interventions that improve stress resilience and maintain desirable flesh characteristics are therefore of particular interest in intensive aquaculture.

Bioactive compounds of plant origin are increasingly being considered as potential feed additives because of their varied biological activities [5]. Among these, proanthocyanidins (PACs) are naturally occurring polyphenols found in a wide range of plant materials, particularly grape seeds, peanut skins, pine bark, apple peels, and berries [6]. Their phenolic hydroxyl groups are associated with free-radical scavenging and the inhibition of lipid peroxidation [7]. Evidence from feeding studies also points to responses beyond antioxidant activity. In juvenile American eel (*Anguilla rostrata*), PACs extracted from peanut skins improved growth and feed utilization, reduced serum lipid levels, and affected hepatic lipid metabolism [8]. Common carp (*Cyprinus carpio*) *given* PACs showed altered blood glucose and lipid indices and greater antioxidant capacity [9]. Comparable improvements in growth performance, physiological condition, and intestinal health were also observed in juvenile hybrid sturgeon (*Acipenser baeri* Brandt ♀ × *A. schrenckii* Brandt ♂) [10]. Collectively, these studies indicate that these compounds can improve growth performance, antioxidant capacity, and immune function in fish. However, their effects on serum metabolic profiles and muscle flavor characteristics have received comparatively little attention.

Flesh quality is an important factor influencing consumer acceptance and the market value of aquaculture products. It can be evaluated in terms of nutritional composition, textural properties, and flavor characteristics [11]. Fish flesh texture is closely associated with muscle fiber characteristics, collagen content, and water-holding capacity. Its differences may be reflected in hardness, springiness, chewiness, and resilience. Meanwhile, protein and lipid contents, together with amino acid and fatty acid profiles, provide complementary information about nutritional quality [12,13]. Flavor is influenced by both nonvolatile taste-active compounds and volatile aroma compounds. Free amino acids and 5′-nucleotides contribute to taste, whereas aldehydes, alcohols, and ketones are among the volatile compounds associated with aroma. Many of these volatile compounds are generated through lipid oxidation, protein degradation, and amino acid metabolism [14]. An assessment restricted to proximate composition may therefore overlook diet-related changes that are apparent in texture measurements or volatile profiling.

Flavoromics relates chemical profiles of volatile and nonvolatile substances to the flavor characteristics of foods [15]. This approach is increasingly used to compare fish produced under different feeding or husbandry conditions. For example, Chinese longsnout catfish (*Leiocassis longirostris*) raised in different culture systems displayed distinct volatile profiles [16]. In mandarin fish (*Siniperca chuatsi*), 65 differential volatile compounds were identified between fish fed different diets, with 2,3-butanedione, (E)-2-nonenal, and 2-pentylfuran as major contributors [17]. These findings indicate that flavoromics is a useful tool for evaluating diet-induced changes in fish muscle flavor.

Common carp (*Cyprinus carpio*) is widely cultured in China, with its flesh quality contributing to its commercial value [18]. Our previous work indicated that dietary PACs enhanced antioxidant capacity and altered the nutritional composition in common carp muscle [19]. However, the effects of dietary PACs on serum biochemical and metabolic profiles, muscle textural properties, and volatile flavor composition remain unclear. Therefore, this study aimed to evaluate the effects of dietary PACs on the physiological

status and muscle quality of common carp. Specifically, we sought to investigate changes in serum metabolomic profiles, assess alterations in muscle textural properties, and characterize the composition and flavor attributes of muscle volatile compounds. To achieve these objectives, common carp were fed diets containing 0, 0.4, or 0.8 g/kg PACs for 60 days. The findings were expected to provide further insights into the effects of dietary PACs on metabolic responses and flesh quality in common carp.

2. Materials and Methods

2.1. Experimental Fish, Feeding Trial, and Tissue Sampling

Common carp with an initial body weight of 73.62 ± 2.44 g were obtained from the aquaculture facility of the Freshwater Fisheries Research Center (Wuxi, China). Healthy fish showing no signs of disease were acclimated for one week in a recirculating aquaculture system, with water temperature maintained at 24–26 °C, pH at 7.3–7.6, and dissolved oxygen above 5.0 mg/L. Following acclimation, the fish were randomly assigned to three dietary treatments supplemented with 0 (NC), 0.4 (LP), or 0.8 (HP) g/kg proanthocyanidins (PACs) for a 60-day feeding trial. The dietary PACs supplementation levels of 0.4 and 0.8 g/kg were selected based on our previous study in common carp [19], in which these two levels significantly improved growth performance and several antioxidant-related parameters. The experimental diets were prepared using a commercial basal feed (Shunfeng, Jiaxing, China), with guaranteed nutrient levels of crude protein ≥ 28.00%, crude lipid ≥ 2.00%, crude fiber ≤ 12.00%, crude ash ≤ 16.00%, total phosphorus ≥ 0.70%, lysine ≥ 1.00%, and moisture ≤ 12.50%. To prepare the experimental diets, the basal feed was first ground into powder, followed by thorough incorporation of PACs at the designated supplementation levels. The mixtures were subsequently re-pelleted to obtain the experimental diets, which were stored at 4 °C to minimize potential PAC degradation during storage. Each treatment consisted of 36 fish distributed among three replicate tanks, with 12 fish per tank. Fish were fed at 2% of their body weight per day in two meals at 08:30 and 15:00. Grape seed-derived proanthocyanidins (PACs; CAS No. 4852-22-6; purity ≥ 95%) were purchased from Absin Bioscience Inc. (Shanghai, China; catalog No. abs47047427).

At the end of the 60-day feeding period, four fish were randomly sampled from each tank (12 fish per treatment) and anesthetized with 100 mg/L MS-222 (Sigma, St. Louis, MO, USA). Blood was collected for serum biochemical analysis and metabolomic profiling, while muscle samples were collected for texture measurements and flavor–compound analysis. Muscle samples were divided into two portions: one was reserved for texture measurements, while the other was promptly frozen in liquid nitrogen for subsequent flavoromic analysis. For serum biochemical and muscle texture analyses, samples from 12 fish per treatment in the NC, LP, and HP groups (four fish per tank) were analyzed individually. For serum metabolomic and muscle flavoromic analyses, samples from 12 fish per treatment in the NC and HP groups were used. Samples from two fish within the same tank were pooled to generate one composite sample, resulting in two pooled samples per tank and six pooled samples per treatment for subsequent metabolomic and flavoromic analyses. Flavoromic analysis was performed by PANOMIX Biomedical Tech Co., Ltd. (Suzhou, China). Serum metabolomic analysis was conducted by Gene Denovo Biotechnology Co., Ltd. (Guangzhou, China). All procedures complied with animal-welfare guidelines and were approved by the Freshwater Fisheries Research Center. Throughout the feeding trial, one mortality was recorded in each of the NC and HP groups, while all fish survived in the LP group.

2.2. Serum Biochemical Parameter Analysis

Serum was separated from blood by centrifugation at 5000 rpm and 4 °C for 10 min. Glucose (Glu), total protein (TP), albumin (ALB), total cholesterol (TC), triacylglycerol (TG), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) were measured using a Mindray BS-280Vet automated biochemical analyzer (Suzhou, China). Acid phosphatase (ACP) activity and glutathione (GSH) content were measured with assay kits following the manufacturers' protocols. The kits were supplied by Grace Biotechnology Co., Ltd. (G0903W; Suzhou, China), and Nanjing Jiancheng Bioengineering Institute (Nanjing, China), respectively.

2.3. Untargeted Serum Metabolomics Analysis

For metabolite extraction, a 100-μL serum was combined with 400 μL of chilled methanol/acetonitrile (1:1, *v*/*v*) in a microcentrifuge tube. After 30 s of vortexing, proteins were precipitated by keeping the mixture at −20 °C for 30 min. Samples were centrifuged at 14,000 × g and 4 °C for 20 min. The collected supernatant was evaporated to dryness under vacuum prior to LC–MS analysis.

Serum metabolites were analyzed using a Vanquish UHPLC system coupled to an Orbitrap Exploris™ 480 mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA). Separation was performed on a BEH Amide column (2.1 × 100 mm, 1.7 μm) using water containing 25 mM ammonium acetate and ammonium hydroxide (A) and acetonitrile (B) as mobile phases. A gradient elution program was applied over 8 min, with the proportion of mobile phase B varying between 40% and 95%. The column was maintained at 25 °C, with a flow rate of 0.5 mL/min and an injection volume of 2 μL. Mass spectra were acquired in positive and negative ESI modes over an m/z range of 70–1200 at a resolution of 60,000. Pooled quality-control samples were included to monitor analytical stability.

Raw mass spectrometry data were converted to mzML format using ProteoWizard (v3.0.6428), followed by peak detection, alignment, retention-time correction, and peak-area integration using XCMS (v3.7.1). Positive- and negative-ion data were processed separately, and three QC measurements were included in the dataset. Features with more than 50% missing values or a relative standard deviation (RSD) greater than 50% were excluded. Remaining missing values were imputed using the k-nearest neighbors (KNN) method, followed by normalization of peak intensities. Metabolite annotation was performed by matching accurate mass measurements (mass error < 10 ppm) and MS/MS spectra against MassBank, METLIN, MoNA, and an in-house database. Data from both ionization modes were subsequently combined for statistical analysis. OPLS-DA was performed using the ropls package in R. Model performance was evaluated using sevenfold cross-validation (K = 7) and permutation testing with 100 random permutations. During permutation testing, the class labels (Y matrix) were randomly reassigned while the predictor matrix (X matrix) was kept unchanged. A new OPLS-DA model was fitted for each permutation, and the corresponding R^2 and Q^2 values were calculated to assess model stability and potential overfitting. Candidate differential metabolites were screened using a variable importance in projection (VIP) score ≥ 1 and Student's *t*-test $p < 0.05$, followed by metabolic pathway analysis using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database.

2.4. Muscle Texture Measurements

Dorsal muscle was excised from the region above the lateral line and sectioned into 1 cm × 1 cm × 1 cm cubes. Samples were stored at 4 °C after collection, and texture measurements were completed within 2 h of sampling. Hardness, springiness, chewiness, resilience, cohesiveness, gumminess and shear force were measured using a TA.XT Express

490C texture analyzer (Stable Micro Systems, Godalming, UK). For texture profile analysis (TPA), a P/35 cylindrical probe was used, with pre-test, test, and post-test speeds of 1.0, 5.0, and 5.0 mm/s, respectively, a 5.0 s interval between two compression cycles, a compression strain of 30%, and a trigger force of 5 g. Shear force was measured using an A-MORS blade attachment, with pre-test, test, and post-test speeds of 2.0, 5.0, and 10.0 mm/s, respectively, a target strain of 50%, and a trigger force of 5 g. Three technical measurements were obtained for each biological sample (n = 12 fish per group), and their average was used for statistical analysis.

2.5. Muscle Flavoromics Analysis

The internal standard was prepared as 1 mg/L 1-hexanol-d13 in 50% aqueous ethanol (*v*/*v*). The 1000 mg/L n-alkane standard mixture was serially diluted with n-hexane to obtain a 1 mg/L reference solution.

Each 20 mL headspace vial received 0.5 g of sample and 10 μL of the 1-hexanol-d13 internal-standard solution. Following immediate sealing, the vial was equilibrated for 10 min at 80 °C. The solid-phase microextraction (SPME) fiber was conditioned at 270 °C for 10 min before exposure to the sample headspace (80 °C, 25 min). It was then placed in the GC inlet for thermal desorption at 250 °C over 5 min and reconditioned at 270 °C for another 10 min. To calculate retention indices, 10 μL of the n-alkane solution was analyzed in a 20 mL headspace vial using the same equilibration, extraction, and desorption settings.

Analyses used a Pegasus BT 4D GC×GC-TOFMS platform (LECO, St. Joseph, MI, USA) incorporating an Agilent 8890A GC, a split/splitless inlet, and a dual-stage thermal jet modulator. Separation employed a first-dimension DB-Heavy Wax column (30 m × 0.25 mm i.d. × 0.50 μm; Agilent, Santa Clara, CA, USA) and a second-dimension Rxi-5Sil MS column (2 m × 0.15 mm i.d. × 0.15 μm; Restek, Bellefonte, PA, USA). Helium flowed at 1.0 mL/min. The primary oven started at 50 °C (2 min) then ramped at 5 °C/min to 230 °C and remained there for 5 min. The secondary oven was 5 °C above the primary oven; the modulator was 15 °C above the secondary oven. Modulation was set to 6.0 s, with the inlet at 250 °C [20,21]. The TOFMS transfer line and ion source were maintained at 250 °C. Spectra were acquired at 200 spectra/s using 70 eV electron ionization, a 1960 V detector setting, and an m/z range of 35–550 [20,21].

Raw GC×GC-TOFMS data were processed using ChromaTOF v5.51 software, and volatile compounds were tentatively annotated against the NIST 2020 mass spectral library using a minimum spectral match score of 700. Retention indices (RIs) were calculated using C7–C30 n-alkanes and compared with available library reference RI values. According to the study-specific RI-based classification, compounds were categorized as Level 1 when the difference between experimental and reference RIs was less than 20 units, or as Level 2 when reference RI values were unavailable. These categories reflect the availability and agreement of RI information and do not indicate confirmation using authentic standards. PubChem and ClassyFire provided chemical-class assignments [22], from which class counts and relative abundances were calculated. FlavorDB supplied sensory descriptors for comparison of the volatile profiles. We then used igraph in R to connect differential compounds to their FlavorDB descriptors in compound–descriptor networks. Multivariate modeling was conducted with the R package ropls [23], and permutation testing was used to examine model stability and overfitting. Variable importance in projection (VIP) and Student's *t*-test *p*-values informed the selection of differential volatile compounds [24]; the cutoffs were VIP > 1.0 in OPLS-DA and $p < 0.05$.

2.6. Statistical Analysis

Statistical comparisons were conducted in SPSS 26.0. One-way analysis of variance, with Fisher's least significant difference procedure for post hoc comparisons, was applied

to serum biochemical and muscle texture variables. We used $p < 0.05$ as the significance threshold and report measurements as mean ± standard deviation (SD). Plots were generated in Microsoft Excel 2019 and GraphPad Prism 9.1.0.

3. Results

3.1. Alterations in Serum Biochemical Indices

The effects of dietary PACs supplementation on serum biochemical indices are listed in Figure 1. Compared with the NC group, fish given 0.8 g/kg PACs showed significantly lower serum Glu and TG concentrations and lower ALT activity ($p < 0.05$). AST activity was significantly lower in both the 0.4 g/kg PACs and 0.8 g/kg PACs groups than in the NC group ($p < 0.05$). In addition, dietary supplementation with 0.8 g/kg PACs significantly increased serum GSH content relative to the NC group ($p < 0.05$). In contrast, serum TP, ALB, and TC concentrations, as well as ACP and ALP activities, did not differ significantly among the groups ($p > 0.05$).

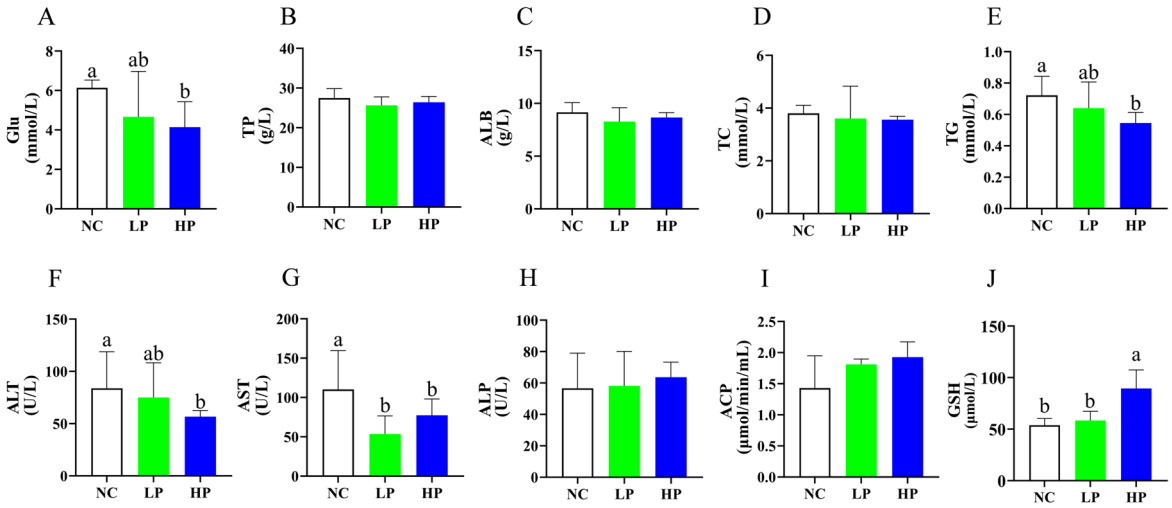


Figure 1. Effects of dietary PACs supplementation on serum biochemical parameters of common carp. (**A**) Glucose (Glu); (**B**) total protein (TP); (**C**) albumin (ALB); (**D**) total cholesterol (TC); (**E**) triacylglycerol (TG); (**F**) alanine aminotransferase (ALT); (**G**) aspartate aminotransferase (AST); (**H**) alkaline phosphatase (ALP); (**I**) acid phosphatase (ACP); and (**J**) glutathione (GSH). NC, 0 g/kg PACs; LP, 0.4 g/kg PACs; HP, 0.8 g/kg PACs. Results are presented as the mean ± SD (three independent replicate tanks per treatment, with four individual fish sampled from each tank; $n = 12$ fish per treatment). Different letters above the bars indicate significant differences among groups for each parameter ($p < 0.05$), whereas the absence of letters indicates no significant differences among groups ($p > 0.05$).

3.2. Changes in Serum Metabolites

3.2.1. Metabolite Identification

The combined-ion dataset contained 2222 annotated metabolites (Figure 2A), with organoheterocyclic compounds (503), lipids and lipid-like molecules (406), organic acids and derivatives (399), and benzenoids (388) representing the major chemical classes. The PCA score plot showed distinct clustering of the NC and HP groups (Figure 2B), and the corresponding OPLS-DA score plot also showed group-specific clustering (Figure 2C). The OPLS-DA model yielded cumulative R^2X, R^2Y, and Q^2 values of 0.391, 0.999, and 0.900, respectively. Cross-validation and permutation testing further confirmed the reliability of the OPLS-DA model (Figure 2D).

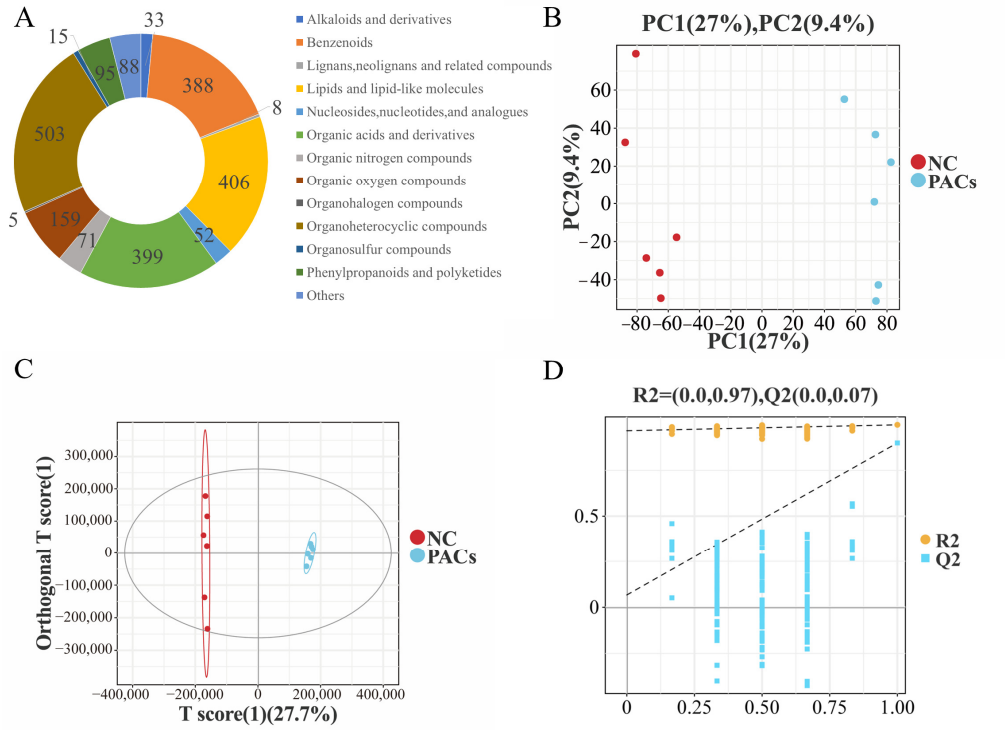


Figure 2. Identification of serum metabolites in common carp. (**A**) Number and classification of metabolites in the combined-ion dataset; (**B**) PCA score plot; (**C**) OPLS-DA score plot; and (**D**) OPLS-DA permutation test. NC, 0 g/kg PACs; PACs, 0.8 g/kg PACs.

3.2.2. Differential Metabolite Analysis

Compared with the NC treatment, the HP treatment group showed marked changes in the abundance of 429 metabolites in the combined-ion dataset, including 124 upregulated and 305 downregulated metabolites (Figure 3A). The differential metabolites were mainly classified as organoheterocyclic compounds (93), organic acids and derivatives (92), lipids and lipid-like molecules (84), benzenoids (61), and organic oxygen compounds (30) (Figure 3B).

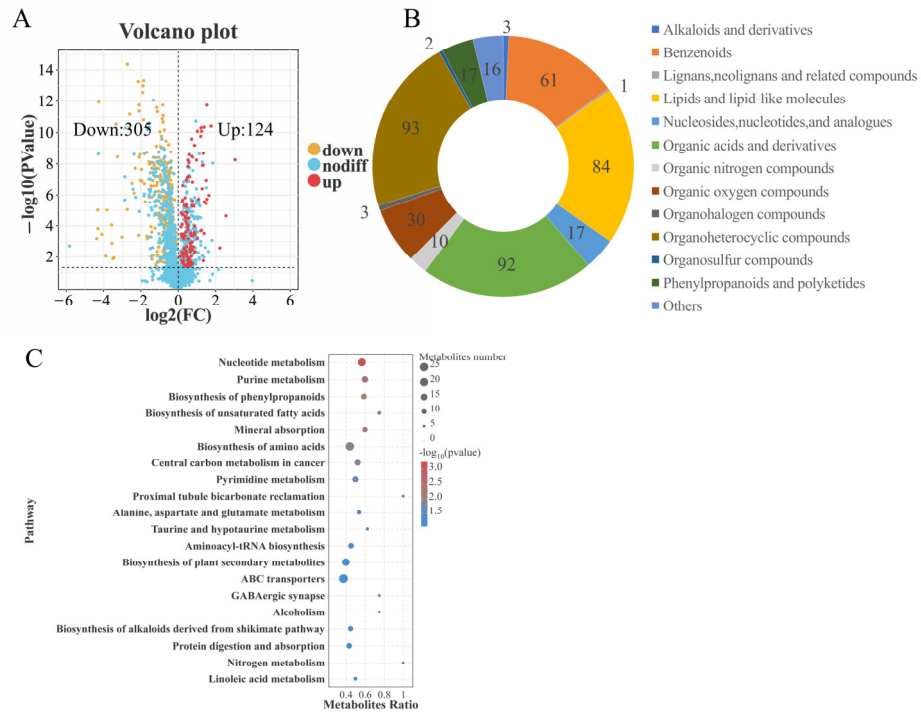


Figure 3. Identification, classification, and enrichment analysis of differential metabolites in carp serum. (**A**) Volcano plot of differential metabolites in the combined-ion dataset; (**B**) number and classification of differential metabolites; and (**C**) major enriched KEGG pathways. NC, 0 g/kg PACs; PACs, 0.8 g/kg PACs.

KEGG mapping of the differential metabolites highlighted nucleotide metabolism (p = 0.0005) and purine metabolism (p = 0.002), followed by biosynthesis of phenylpropanoids (p = 0.006), biosynthesis of unsaturated fatty acids (p = 0.007), and biosynthesis of amino acids (p = 0.011) (Figure 3C; Table S3).

3.2.3. Analysis of Key Metabolites

Comparing the NC group with fish fed 0.8 g/kg PACs, 80 differential serum metabolites belonged to lipid-associated classes: 35 fatty acyls (FAs), 25 glycerophospholipids (GPs), 15 steroids or steroid derivatives (SDs), and 5 prenol lipids (PLs) (Figure 4A). Among the FAs, 22 metabolites exhibited higher relative abundances in the HP group, including oleic acid, (10e,12z)-linoleic acid, palmitoylcarnitine, and oleoyl-L-carnitine. In contrast, the relative abundances of 13 metabolites, including deoxycarnitine and γ-linolenic acid, decreased ($p < 0.05$). Among the GPs, 18 metabolites exhibited significantly increased relative abundances following PACs supplementation, including 1-palmitoyl-2-oleoyl-phosphatidylglycerol, PC (15:0,8,9-epete), and phosphatidylethanolamine. By contrast, 7 GPs, including phosphatidylcholine lyso 20:2, showed significantly decreased relative abundances ($p < 0.05$). Among the SDs, supplementation with 0.8 g/kg PACs decreased the relative abundances of 12 metabolites, including taurochenodeoxycholic acid, but increased the relative abundances of 3 metabolites, including cholesterol 3-sulfate ($p < 0.05$). Notably, PLs exhibited significantly lower relative abundance in the HP group than in the NC group ($p < 0.05$).

A total of 92 amino acids and related derivatives showed significant differences in abundance between the NC and HP groups (Figure 4B). Among them, 77 compounds showed significantly decreased relative abundances, including glutamic acid, glutamine, tryptophan, L-methionine, taurine, and creatinine. In contrast, 15 compounds, including ectoine, Arg-Ala-Lys, and N-methylhistidine, showed substantially higher relative abundance ($p < 0.05$; Figure 4B).

Supplementation with 0.8 g/kg PACs also significantly altered the serum nucleotide and analogue profile (Figure 4C). The relative abundances of 14 compounds, including inosine, guanosine, thymidine, cytidine, hypoxanthine, and uric acid, were significantly decreased. In contrast, adenosine, 2′-deoxyadenosine-5′-diphosphate sodium salt, and 7-methylguanosine showed significantly increased relative abundances ($p < 0.05$; Figure 4C).

We further focused on changes in 19 key differential metabolites. Compared with the NC group, the relative abundances of linoleoylcarnitine, palmitoylcarnitine, oleoyl-L-carnitine, octanoylcarnitine, hexanoyl-L-carnitine, (10e,12z)-linoleic acid, oleic acid, 1-palmitoyl-2-oleoyl-phosphatidylglycerol, phosphatidylethanolamine, and PC (15:0,8,9-epete) were significantly increased in the HP group ($p < 0.05$; Figure 5A). In contrast, the relative abundances of phosphatidylcholine lyso 20:2, glutamic acid, glutamine, L-methionine, taurine, creatinine, inosine, hypoxanthine, and uric acid were significantly decreased ($p < 0.05$; Figure 5B; Table S1).

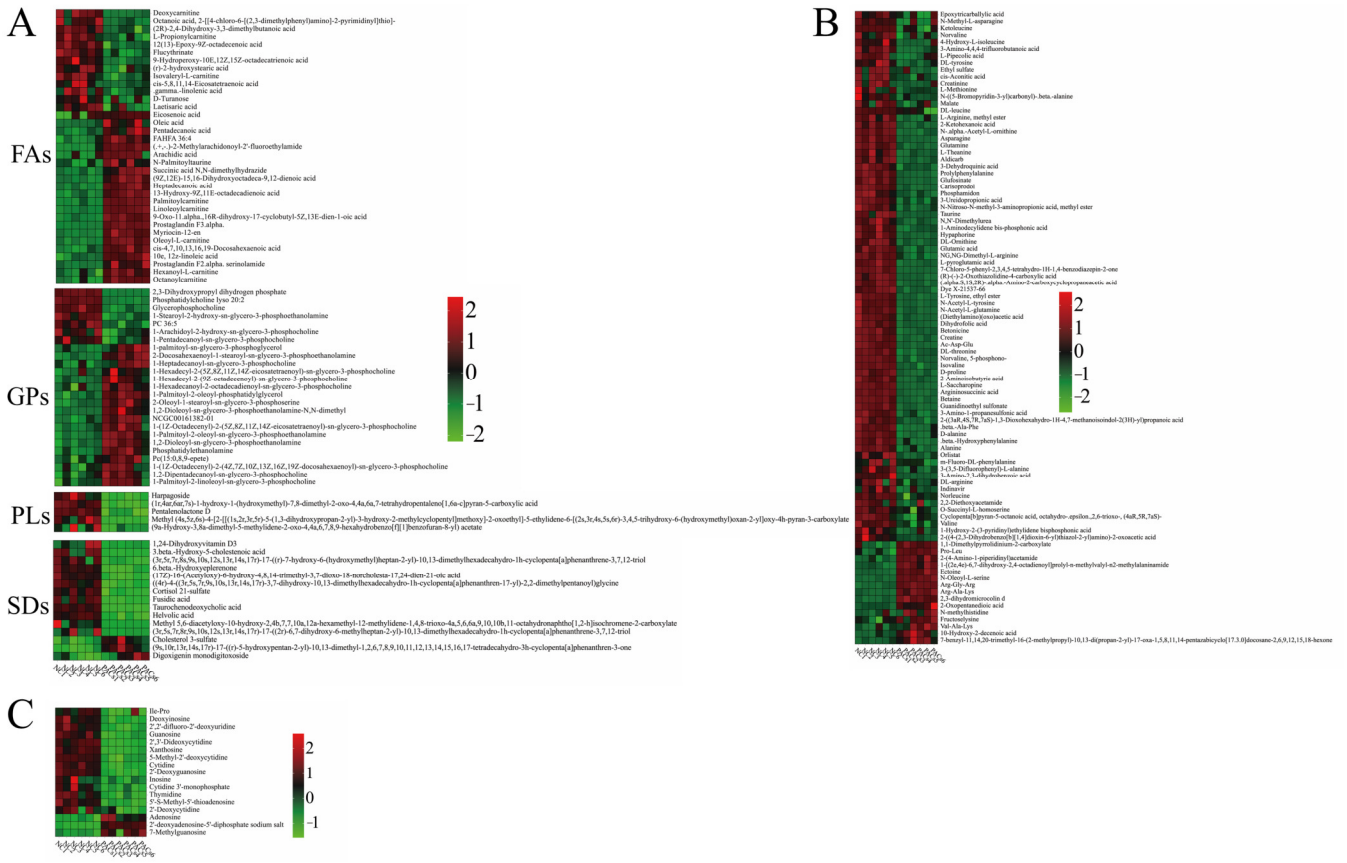


Figure 4. Relative abundances of differential metabolites between NC and 0.8 g/kg PACs groups in common carp serum. (**A**) Differential metabolites related to lipids and lipid-like molecules (FA, fatty acyls; GP, glycerophospholipids; PL, prenol lipids; SD, steroids and steroid derivatives); (**B**) differential metabolites related to amino acids, peptides, and analogues; and (**C**) differential metabolites related to nucleosides, nucleotides, and analogues. NC, 0 g/kg PACs; PACs, 0.8 g/kg PACs.

3.3. Changes in Muscle Texture Properties

As reported in Table 1, both 0.4 g/kg PACs and 0.8 g/kg PACs decreased muscle hardness, gumminess, chewiness, and shear force relative to the NC diet ($p < 0.05$). Springiness, cohesiveness, and resilience did not differ significantly among the three groups ($p > 0.05$).

Table 1. Effects of dietary PACs supplementation on muscle texture properties of common carp after a 60-day feeding.

Group	Hardness (g)	Springiness	Cohesiveness	Gumminess (g)	Chewiness (g)	Resilience	Shear Force (g)
NC	63.08 ± 26.71 ^{a}	0.54 ± 0.07	0.70 ± 0.08	43.43 ± 16.04 ^{a}	23.26 ± 8.15 ^{a}	0.51 ± 0.10	62.31 ± 18.49 ^{a}
LP	23.80 ± 11.53 ^{b}	0.49 ± 0.05	0.63 ± 0.06	15.59 ± 9.02 ^{b}	7.77 ± 5.09 ^{b}	0.47 ± 0.08	40.45 ± 8.87 ^{b}
HP	35.83 ± 14.26 ^{b}	0.51 ± 0.04	0.69 ± 0.04	25.04 ± 11.33 ^{b}	12.95 ± 6.48 ^{b}	0.57 ± 0.12	42.44 ± 8.31 ^{b}

NC, 0 g/kg PACs; LP, 0.4 g/kg PACs; HP, 0.8 g/kg PACs. Results are presented as the mean ± SD (three independent replicate tanks per treatment, with four individual fish sampled from each tank; $n = 12$ fish per treatment). Different superscript letters within the same column indicate significant differences among groups ($p < 0.05$), whereas the absence of superscript letters indicates no significant differences among groups ($p > 0.05$).

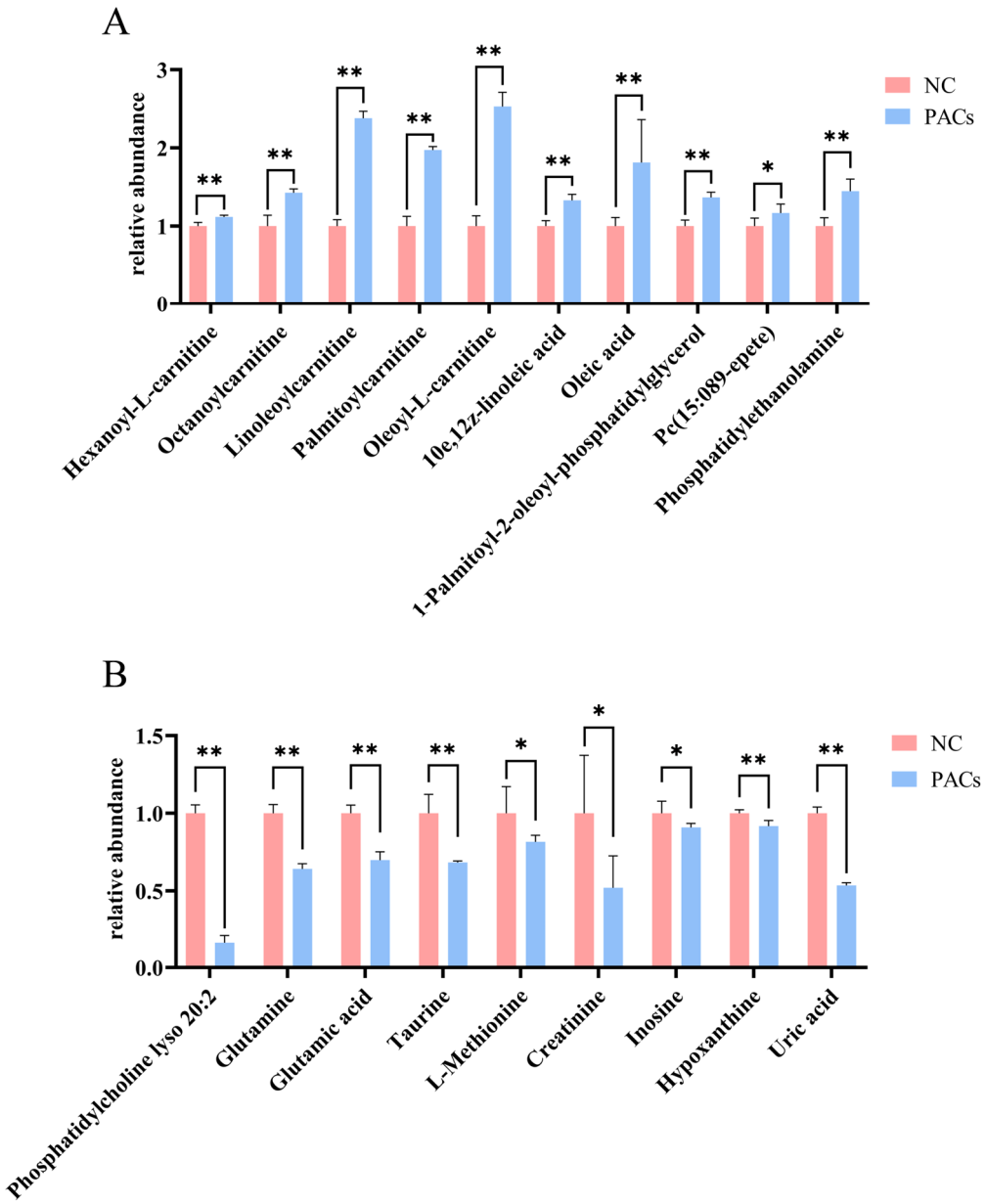


Figure 5. Differential analysis of representative serum metabolites between the NC and 0.8 g/kg PACs groups in common carp. (**A**) Representative metabolites with significantly increased relative abundances in the 0.8 g/kg PACs group compared with the NC group; (**B**) representative metabolites with significantly decreased relative abundances in the 0.8 g/kg PACs group compared with the NC group. NC, 0 g/kg PACs; PACs, 0.8 g/kg PACs. Data are presented as the mean ± SD (six pooled serum samples per treatment, with two pooled samples prepared from each of three independent replicate tanks; each pooled sample comprised serum from two fish). * $p < 0.05$; ** $p < 0.01$.

3.4. Changes in Muscle Flavor Characteristics

3.4.1. Identification of Volatile Compounds

Volatile compounds in muscle from the NC and HP groups were characterized by GC×GC-TOFMS. The two groups formed distinct clusters in the OPLS-DA score plot (Figure 6A). The cumulative R^2X, R^2Y, and Q^2 values of the OPLS-DA model were 0.400, 0.998, and 0.918, respectively. Cross-validation and permutation testing further confirmed the reliability of the OPLS-DA model (Figure 6B). The analysis detected 2019 volatile compounds, with 1307 and 1372 compounds detected in the NC and HP groups, respectively

(Figure 6C). The compounds were mainly classified as organoheterocyclic compounds, hydrocarbons, benzenoids, esters, alcohols, ketones, lipids and lipid-like molecules, heterocyclic compounds, ethers, and carboxylic acids (Figure 6D). Based on their relative abundances, esters, benzenoids, alcohols, and ketones were the predominant compound classes in both groups. In the NC group, these four classes accounted for 38.05%, 34.03%, 6.17%, and 4.71% of the total volatile compounds, respectively. In the HP group, the corresponding proportions were 40.87%, 13.50%, 12.45%, and 9.25%, respectively (Figure 6E). Notably, benzenoids showed a higher relative abundance in the NC group, whereas esters, alcohols, and ketones showed higher relative abundances in the HP group.

As shown in Figure 6F, sweet, fruity, green, and waxy were the predominant database-derived odor descriptors associated with muscle volatile compounds in both groups, with sweet showing the highest frequency. Compared with the NC group, the HP group exhibited higher frequencies of sweet, fruity, green, and fatty odor descriptors, whereas the remaining descriptors showed only minor differences between groups.

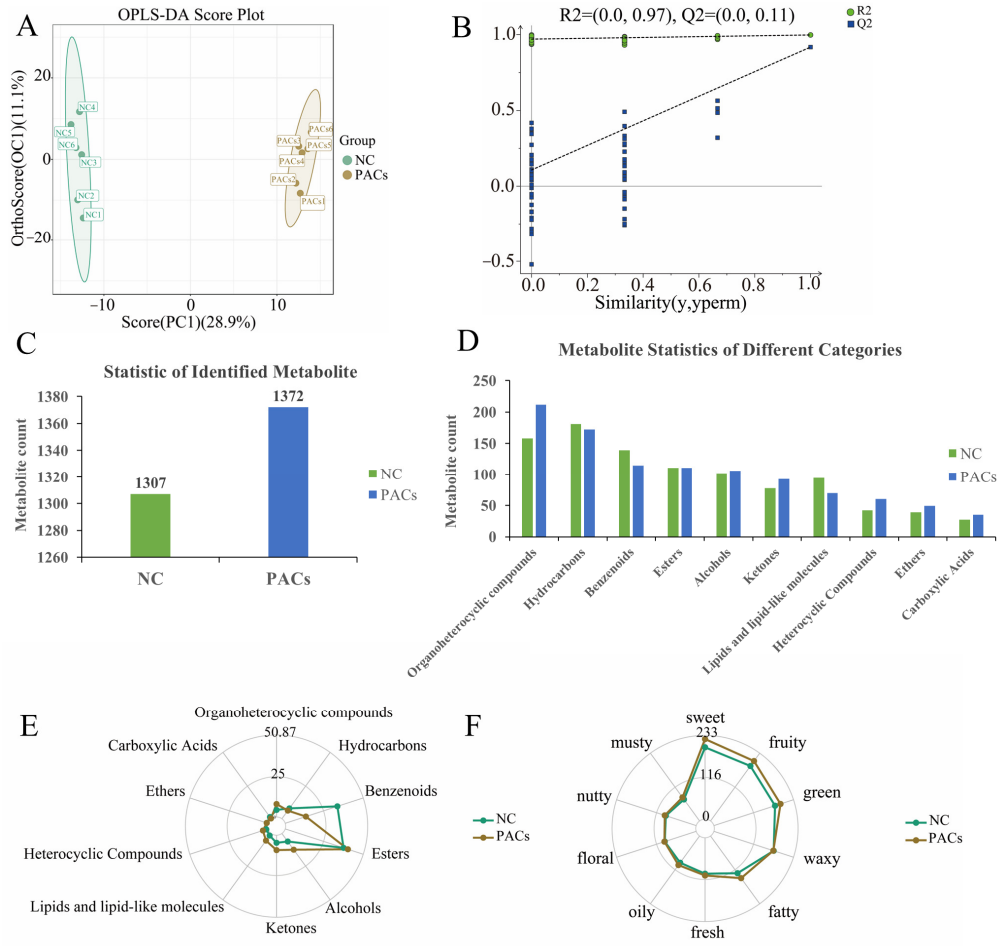


Figure 6. Identification and analysis of volatile flavor characteristics in common carp muscle. (**A**) OPLS-DA score plot; (**B**) OPLS-DA permutation test; (**C**) number of identified volatile compounds; (**D**) numbers of compounds in different chemical classes; (**E**) relative abundance of chemical classes; and (**F**) frequency distribution of odor descriptors based on FlavorDB annotations. NC, 0 g/kg PACs; PACs, 0.8 g/kg PACs.

3.4.2. Analysis of Differentially Abundant Volatile Compounds

A total of 178 differentially abundant volatile compounds were identified between the two groups. In muscle from the 0.8 g/kg PACs group, the abundances of 108 com-

pounds were significantly elevated, while those of 70 compounds were significantly reduced relative to the NC group (Figure 7A). These compounds were classified mainly as organoheterocyclic compounds (37), esters (20), benzenoids (19), alcohols (18), hydrocarbons (15), heterocyclic compounds (14), and aldehydes (11) (Figure 7B). The compounds that were significantly more abundant in the 0.8 g/kg PACs group primarily comprised organoheterocyclic compounds (28), esters (12), heterocyclic compounds (12), alcohols (11), aldehydes (10), and ketones (8) ($p < 0.05$; Figure 7B).

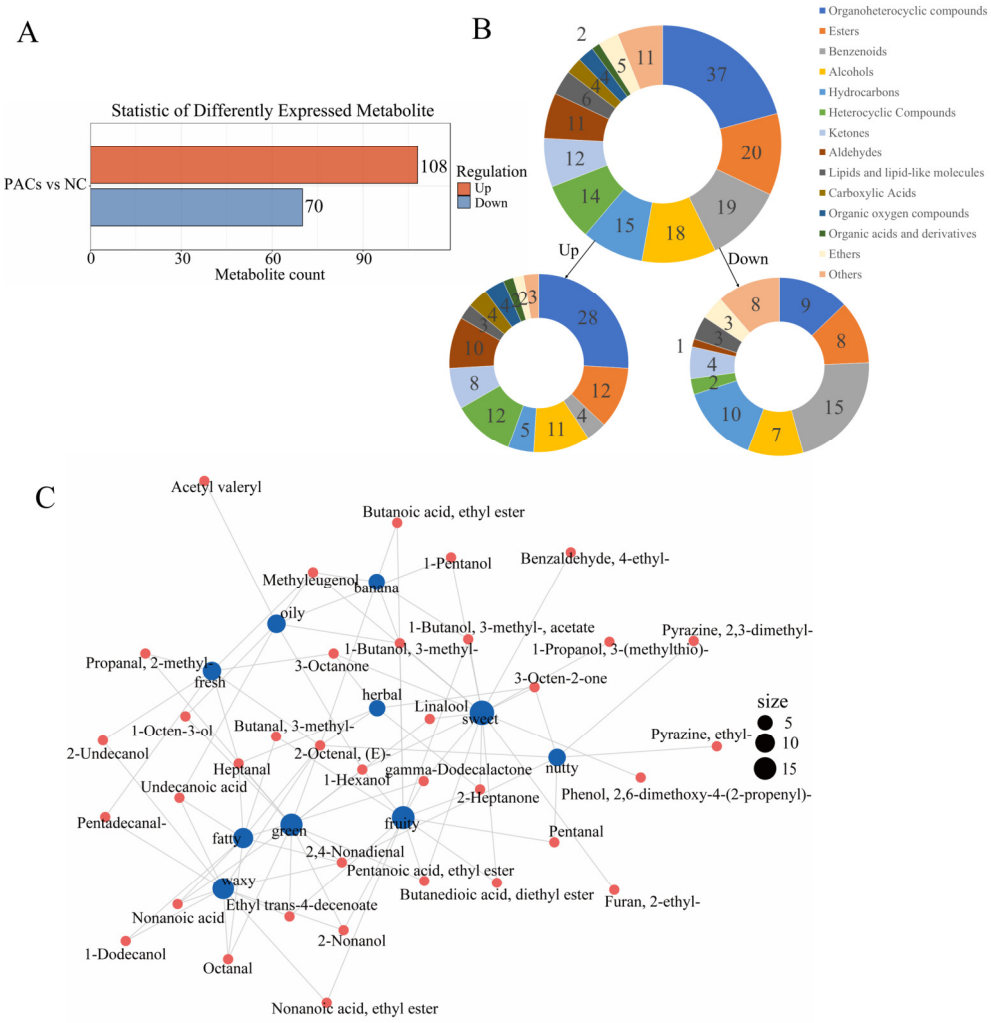


Figure 7. Differential volatile compounds in common carp muscle between NC and PACs groups. (**A**) Number of differential volatile compounds; (**B**) chemical classes and numbers of differential compounds; and (**C**) association network between sensory descriptors and volatile compounds. NC, 0 g/kg PACs; PACs, 0.8 g/kg PACs.

To further explore the relationships between differential volatile compounds and flavor attributes, a compound–attribute association network was constructed. Sweet, fruity, and green emerged as the prominent attributes in the network (Figure 7C), broadly consistent with the flavor profiles presented in the radar chart (Figure 6F). As shown in the association network, the sweet descriptor was linked to 3-methyl-1-butanol, 1-hexanol, and 3-methylbutyl acetate. The fruity descriptor was associated with 3-methyl-1-butanol, 1-hexanol, 2-heptanone, 3-methylbutyl acetate, and 3-methylbutanal, whereas the green descriptor was mainly associated with 1-hexanol, heptanal, octanal, and (E)-2-octenal. Notably, 1-hexanol was associated with all three prominent descriptors: sweet, fruity, and green.

Further analysis focused on 17 key volatile compounds. Among them, 15 showed significantly higher relative abundances in the HP group relative to the NC group (Figure 8A), whereas two showed significantly lower relative abundances (Figure 8B). The compounds with increased relative abundances included ethyl pentanoate, ethyl nonanoate, 3-methylbutyl acetate, 1-octen-3-ol, 1-hexanol, 3-methyl-1-butanol, 1-pentanol, 2-heptanone, 3-octanone, octanal, heptanal, pentanal, 3-methylbutanal, (E)-2-octenal, and (E,E)-2,4-octadienal ($p < 0.05$; Figure 8A). Conversely, the HP group exhibited significantly lower relative abundances of 2,3-octanedione and 2,4-nonadienal ($p < 0.05$; Figure 8B; Table S2).

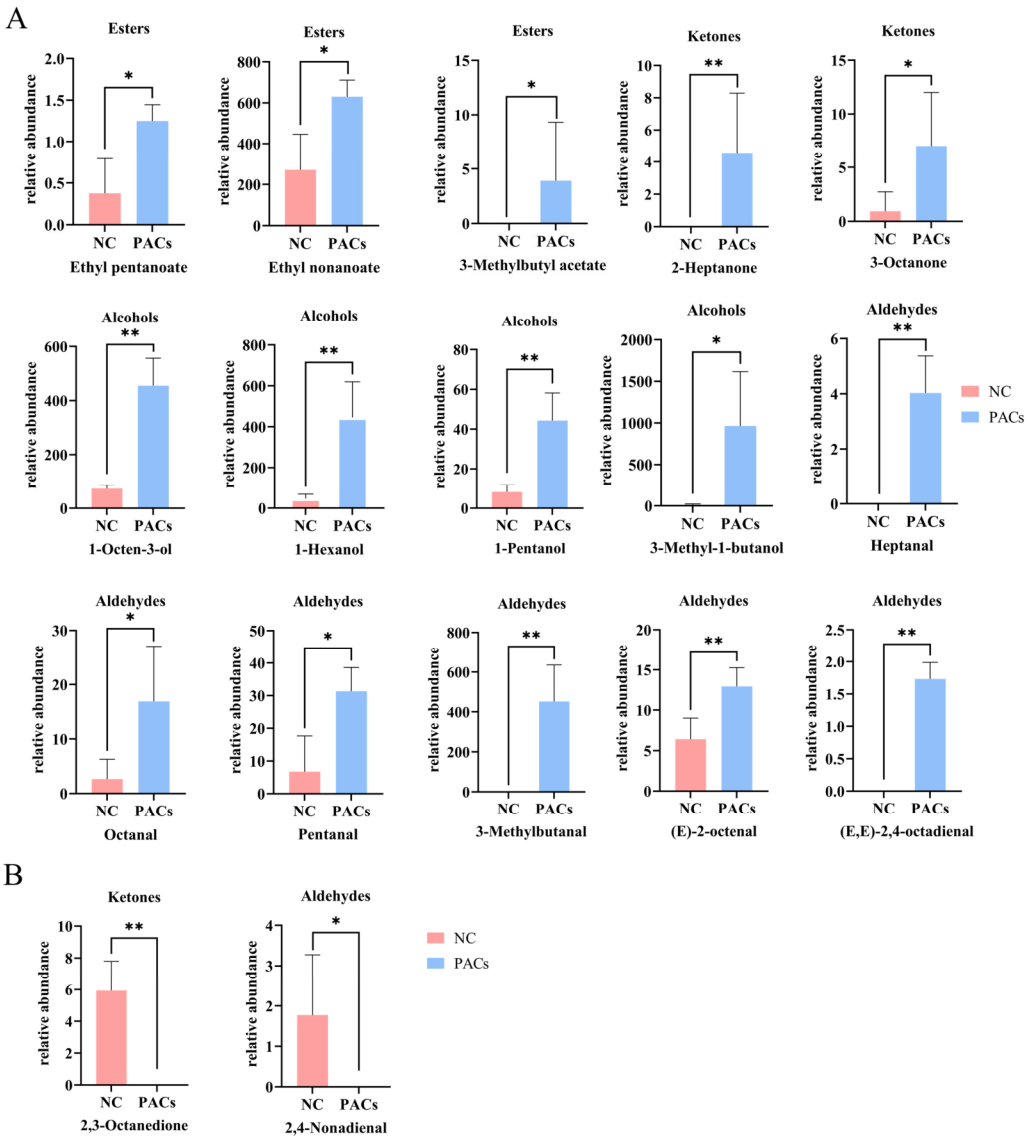


Figure 8. Key differential volatile compounds in the muscle of common carp. (**A**) Representative volatile compounds showing significantly higher relative abundance in the 0.8 g/kg PACs group than in NC; (**B**) compounds with significantly lower relative abundance in the 0.8 g/kg PACs group. NC, 0 g/kg PACs; PACs, 0.8 g/kg PACs. Data are presented as the mean ± SD (six pooled muscle samples per treatment, with two pooled samples prepared from each of three independent replicate tanks; each pooled sample comprised muscle from two fish). * $p < 0.05$; ** $p < 0.01$.

4. Discussion

PACs have attracted increasing attention as plant-derived functional feed additives in aquaculture because of their potential benefits for physiological regulation, metabolic

homeostasis, and flesh quality. Earlier studies have reported improvements in growth performance, antioxidant capacity, immune function, and muscle nutritional quality in aquatic animals following dietary supplementation with PACs [8–10]. However, the physiological and flesh-quality effects of PACs in fish remain poorly understood. Therefore, this study integrated serum biochemical analysis, metabolomics, texture analysis, and flavoromics to assess the responses of common carp to dietary PACs supplementation.

4.1. Effects of PACs on Serum Biochemical Parameters

Common carp fed a diet supplemented with 0.8 g/kg PACs exhibited lower serum Glu levels than those in the NC group. This finding is consistent with previous studies reporting reduced serum Glu levels following PAC supplementation in rainbow trout (*Oncorhynchus mykiss*) and common carp [9,25]. Similarly, the reduction in serum stress-related indicators, including Glu was also observed in Nile tilapia (*Oreochromis niloticus*) fed PAC-supplemented diets [26]. Evidence from previous research suggests that PACs may regulate glucose homeostasis by modulating hepatic glucose metabolism and insulin-related signaling pathways [27]. These mechanisms may partly explain the lower serum Glu level observed in the 0.8 g/kg PACs group.

Serum TG levels were lower in common carp receiving 0.8 g/kg PACs than in the NC group. Reductions in serum TG levels following dietary PAC supplementation have also been reported in GIFT tilapia (*Oreochromis niloticus*) [28] and American eel (*Anguilla rostrata*) [29]. These findings suggest a potential lipid-lowering effect of PACs in fish. This effect may be associated with the regulation of hepatic lipid metabolism. Lu et al. [30] reported that grape seed PACs alleviated high-fat diet-induced increases in serum TG and cholesterol levels in grass carp (*Ctenopharyngodon idella*). This response was accompanied by the downregulation of genes involved in hepatic lipogenesis. Such changes in lipid metabolism may partly explain the lower serum TG levels observed in the 0.8 g/kg PACs group.

ALT and AST are aminotransferases involved in amino acid metabolism in the hepatopancreas. Their activities are normally low in serum but increase when hepatic injury causes their release into the circulation. Therefore, they are commonly used as indicators of liver function [31]. In this study, the inclusion of 0.8 g/kg PACs significantly decreased serum AST and ALT activities. These findings may indicate an improvement in hepatic status and suggest a potential association between PAC supplementation and the maintenance of hepatocellular integrity. In northern snakehead (*Channa argus*), dietary OPC supplementation significantly reduced serum ALT and AST activities and improved hepatic health [32]. Likewise, dietary supplementation with grape seed PACs significantly decreased serum ALT and AST activities and alleviated hepatic injury in hybrid sturgeon (*Acipenser baeri* Brandt ♀ × *A. schrenckii* Brandt ♂) [10] and tilapia [28]. These observations suggest that PACs may help protect the liver from cellular damage in cultured fish.

4.2. Effects of PACs on Serum Metabolites

4.2.1. Effects on Serum Lipid Metabolism

In fish, lipids serve as important sources of metabolic energy and energy reserves while also forming essential components of cellular membranes. In addition to these functions, they are involved in signal transduction and the regulation of immune responses [33]. In this study, supplementation with 0.8 g/kg dietary PACs significantly affected the serum lipid metabolic profile of common carp. Significant enrichment of the unsaturated fatty acid biosynthesis pathway was also observed, indicating that PACs may be involved in the regulation of lipid metabolism in this species.

Linoleic acid and oleic acid are unsaturated fatty acids involved in lipid metabolism. Their regulatory effects may be partly mediated through PPARα-related signaling pathways, which regulate genes associated with fatty acid catabolism and oxidation [34]. In American eel, dietary peanut skin PACs modulated hepatic linoleic acid metabolism, which was proposed to contribute to their hypolipidemic effects [8]. Similarly, procyanidin B2 supplementation increased plasma linoleic acid and oleic acid levels in mice, and these changes were associated with improvements in age-related metabolic disturbances through modulation of fatty acid metabolism [35]. In this work, dietary inclusion of 0.8 g/kg PACs elevated the serum levels of oleic acid and linoleic acid, suggesting that PACs may improve lipid metabolism by modulating unsaturated fatty acid metabolism. In addition, linoleic acid has been reported to enhance serum lipoprotein lipase activity, promote TG hydrolysis, and reduce serum TG levels [36], which may partly account for the reduced serum TG observed in the HP group.

In the present study, dietary supplementation with 0.8 g/kg PACs markedly raised the relative abundances of several acylcarnitines, including linoleoylcarnitine, palmitoylcarnitine, oleoyl-L-carnitine, octanoylcarnitine, and hexanoyl-L-carnitine. Acylcarnitines are important metabolic intermediates related to fatty acid activation, mitochondrial transport, and β-oxidation. Following activation by acyl-CoA synthetase, long-chain fatty acids are converted to long-chain acyl-CoAs. The resulting acyl groups subsequently enter the mitochondrial matrix through the CPT1–CACT–CPT2-mediated carnitine shuttle [37]. Therefore, the increased relative abundances of several long- and medium-chain acylcarnitines observed in the present study may indicate alterations in carnitine-associated lipid metabolism following dietary PAC supplementation. However, elevated acylcarnitine levels do not necessarily indicate enhanced mitochondrial β-oxidation, as they may also reflect changes in fatty acid availability or the accumulation of metabolic intermediates. Similar alterations in carnitine-related metabolism have also been reported following PACs supplementation in mammals. Park et al. [38] observed elevated circulating concentrations of several carnitine-related metabolites following PAC supplementation in mice maintained on a high-fat diet. Similarly, Xiao et al. [35] showed that procyanidin B2 supplementation increased plasma carnitine concentrations in mice, together with improvements in lipid metabolic disturbances. These observations suggest that PACs may influence lipid metabolism through mechanisms associated with carnitine metabolism. Nevertheless, mitochondrial β-oxidation activity was not directly measured in the present study, and further investigations are needed to clarify its potential involvement.

Glycerophospholipids constitute a substantial proportion of biological membranes and are also present in circulating lipoproteins [39]. Among these lipids, lysophosphatidylcholine (LPC) is primarily formed by the phospholipase-catalyzed hydrolysis of phosphatidylcholine (PC) and can be converted back to PC through reacylation during phospholipid turnover [40]. Phosphatidylethanolamine (PE), another abundant glycerophospholipid, participates in phospholipid metabolism and lipid transport [41]. Serum LPC abundance decreased in fish receiving 0.8 g/kg PACs, whereas PE increased. These changes suggest that PACs may modulate circulating glycerophospholipid metabolism in common carp. The decrease in serum LPC may reflect altered PC–LPC turnover, whereas the increase in PE may indicate changes in phospholipid synthesis, transport, or remodeling. Collectively, these findings suggest that dietary PACs may influence systemic phospholipid homeostasis, although the specific metabolic pathways involved remain to be clarified.

4.2.2. Effects on Serum Amino Acid Metabolism

Amino acids contribute to protein accretion, immune and antioxidant functions, and energy metabolism in fish [42]. In this work, the addition of 0.8 g/kg PACs to the diet significantly affected amino acid metabolism in common carp, as reflected by the enrichment of the amino acid biosynthesis pathway. This result suggests that dietary PACs may play a role in regulating amino acid metabolism in common carp.

Glutaminase can convert glutamine to glutamate, which supplies a precursor for glutathione metabolism [43]. Thus, glutamine may contribute indirectly to glutathione metabolism by providing glutamate. Previous research has indicated that PACs can influence glutathione-related antioxidant metabolism. Liu et al. [44] found that procyanidin B2 treatment reduced glutamate levels in the corpus callosum and increased GSH levels in myelin debris in cuprizone-treated mice. Similarly, Yang et al. [45] showed that grape seed PACs increased GSH levels in the cerebral cortex of rats and modulated methylmercury-induced disturbances in glutamate homeostasis. In the present study, dietary supplementation with 0.8 g/kg PACs significantly decreased the serum relative abundances of glutamate and glutamine while increasing serum GSH levels in common carp. These changes may indicate that dietary PACs influence glutamine–glutamate and glutathione metabolism, potentially contributing to improved antioxidant status.

In this study, the addition of 0.8 g/kg PACs to the diet also resulted in a reduction in serum taurine levels. Cysteine is a shared precursor of taurine and GSH, suggesting that their biosynthetic pathways may compete for the available cysteine pool [46]. Chen et al. [47] found that PACs stimulated the Nrf2/ARE signaling pathway and increased the expression of GCLC and GCLM, two genes involved in the rate-limiting step of de novo GSH synthesis, indicating a potential increase in GSH biosynthetic capacity. In support of this interpretation, Rodríguez et al. [48] observed lower hepatic taurine levels in cafeteria diet-fed rats following PAC treatment, along with changes in 5-oxoproline and other metabolites linked to the γ-glutamyl cycle and GSH metabolism. Considering these observations alongside the elevated serum GSH levels found in our study, PACs may influence how cysteine is utilized, potentially directing more of this precursor toward GSH synthesis and limiting its availability for taurine production.

In addition, the present study showed that dietary supplementation with 0.8 g/kg PACs decreased serum creatinine (CRE) levels. As a final product of creatine and phosphocreatine metabolism, CRE is considered an indicator of metabolic status and renal excretory function in fish [49]. Elevated serum CRE concentrations are commonly associated with renal injury. In rats, PAC treatment has been shown to lower serum CRE levels and reduce renal damage, indicating a possible protective effect on kidney function [50]. Similarly, Park et al. [51] also observed a decrease in serum CRE following dietary PAC supplementation in growing pigs (*Sus scrofa*), in agreement with the results of our study. The lower serum CRE levels observed in common carp receiving dietary PACs may therefore indicate a beneficial effect on renal physiological status. However, further research is needed to clarify the mechanisms responsible for this response.

4.2.3. Effects on Serum Nucleotide Metabolism

Purine nucleotide metabolism plays an important role in maintaining cellular energy balance [52]. During adenine nucleotide catabolism, AMP is metabolized to inosine through either the adenosine or IMP pathway. Inosine is subsequently degraded to hypoxanthine, followed by the formation of xanthine and ultimately uric acid [53]. Inosine and hypoxanthine are major products of adenine nucleotide catabolism, and changes in their levels may therefore reflect alterations in ATP degradation [54]. Early reports have demonstrated that energy stress, such as hypoxia, can accelerate ATP depletion and alter purine metabolism [55,56]. PACs supplementation has also been documented to improve

mitochondrial oxidative metabolism and cellular energy status [57]. Consistently, Liu et al. [58] showed that sea buckthorn PACs attenuated H_2O_2-induced reductions in ATP levels and glucose utilization, thereby improving mitochondrial energy metabolism. In the present study, dietary supplementation with 0.8 g/kg PACs significantly reduced serum inosine, hypoxanthine, and uric acid levels in common carp, and the differential metabolites were primarily involved in nucleotide- and purine-metabolism-related pathways. These findings suggest that PACs may modulate purine nucleotide metabolism in common carp. Given the antioxidant and mitochondrial protective properties of PACs, the observed decreases in purine degradation products may be associated with improved cellular energy metabolism and reduced stress-related purine nucleotide degradation.

The reduction in serum uric acid may also be associated with the effects of PACs on xanthine oxidase (XO) activity. During purine catabolism, hypoxanthine is oxidized to xanthine, which is subsequently converted to uric acid, with XO participating in these reactions [59]. Wang et al. [60] found that PACs reduced serum uric acid levels and decreased hepatic XO/XDH activity in potassium oxonate-induced hyperuricemic mice. Similarly, longan flower and seed extracts were reported to reduce plasma uric acid levels in hyperuricemic mice, while procyanidin A2 isolated from longan flowers exhibits potent XO inhibitory activity in vitro [61], providing a possible mechanistic basis for the regulation of uric acid production by PACs. These findings suggest that inhibition of XO-mediated uric acid production may represent one mechanism by which PACs regulates uric acid metabolism. In fish, however, uric acid may undergo further degradation through the action of uricase. Thus, the lower serum uric acid level observed in this study could be associated with changes in uric acid production, subsequent metabolism, or excretion. It remains uncertain whether inhibition of xanthine oxidase (XO) directly contributes to this response in common carp.

4.3. Effects of PACs on Muscle Texture

The textural characteristics of fish muscle can be evaluated using several parameters, such as hardness, adhesiveness, cohesiveness, springiness, gumminess, and chewiness, which reflect its mechanical properties [62]. Tenderness is a key aspect of fish flesh quality, with hardness, chewiness, and gumminess frequently used to assess tenderness-related characteristics [63,64]. Yang et al. [26] observed significant reductions in these three parameters in Nile tilapia fed diets supplemented with PACs, suggesting an improvement in muscle tenderness. Shear force provides another measure of meat tenderness, as lower values are generally associated with more tender muscle [65]. Reductions in muscle shear force following dietary PAC supplementation have also been documented in pigs (*Sus scrofa*) [66,67] and rabbits (*Oryctolagus cuniculus*) [68]. In the present study, feeding common carp diets supplemented with PACs at 0.4 and 0.8 g/kg significantly reduced muscle hardness, gumminess, chewiness, and shear force. Consistent with previous findings, these results suggest that appropriate levels of dietary PACs may influence instrumental muscle texture characteristics related to tenderness in this species.

4.4. Effects of PACs on Muscle Volatile Compounds

Dietary supplementation with 0.8 g/kg PACs altered the volatile composition of common carp muscle, particularly affecting esters, alcohols, ketones, and aldehydes. These compounds play important roles in shaping the aroma of aquatic products and are mainly derived from lipid oxidation, amino acid degradation, and secondary reactions such as esterification [69]. As some of these compounds have low odor thresholds and are associated with distinct aroma characteristics [70], changes in their relative abundances may contribute to variations in the overall aroma profile of common carp muscle.

Esters constituted 40.87% of the total volatile compounds in the 0.8 g/kg PACs group, compared with 38.05% in the NC group, showing a higher relative proportion following dietary PACs supplementation. The 0.8 g/kg PACs group also exhibited higher relative abundances of 3-methylbutyl acetate, ethyl nonanoate, and ethyl pentanoate than the NC group. Among these compounds, ethyl pentanoate has been linked to fruity and apple-like aromas [71], while 3-methylbutyl acetate is associated with fruity and banana-like notes [72]. Ethyl nonanoate has also been reported to exhibit a typical fruity aroma [73]. These findings suggest that dietary supplementation with 0.8 g/kg PACs may shift the potential aroma profile of common carp muscle toward fruity characteristics by increasing the relative proportions of several fruity esters.

Alcohols also showed changes following PACs supplementation. Among them, 1-octen-3-ol has been described as an odor-active compound with fishy, fatty, mushroom-like, and grassy notes [74,75]. Tao et al. [75] identified 1-octen-3-ol as a key odorant in the cooked meat of farmed obscure puffer (*Takifugu obscurus*). Similarly, Liu et al. [76] identified it as a key odorant in enzymatic hydrolysates of Pacific oyster (*Crassostrea gigas*). In this work, the relative levels of 1-octen-3-ol raised in the HP group and were associated with the grassy aroma category in the flavor–compound association network. This association suggests that 1-octen-3-ol may be involved in the altered volatile aroma profile of common carp muscle following PACs supplementation. In addition, the relative abundances of 1-hexanol, 1-pentanol, and 3-methyl-1-butanol were also increased in the 0.8 g/kg PACs group. Previous studies have described 1-hexanol as having fruity notes in blunt snout bream (*Megalobrama amblycephala*) [77] and grassy notes in grass carp [78]. In the present study, 1-pentanol was annotated with sweet and fatty notes, whereas it has been associated with green aroma in tilapia [79], suggesting that its odor characteristics may vary among species and food matrices. 3-Methyl-1-butanol was primarily associated with sweet and fruity notes, partially consistent with the sweet and floral characteristics previously reported in blunt snout bream [77]. Overall, dietary supplementation with 0.8 g/kg PACs resulted in changes in the relative composition of several volatile alcohols in common carp muscle, potentially affecting its aroma characteristics.

A comparable trend was observed for ketones, with their total relative abundance tending to be higher in the 0.8 g/kg PACs group than in the NC group. Notably, 2-heptanone and 3-octanone exhibited significant increases. According to flavor databases, 2-heptanone is mainly associated with fruity and sweet notes, whereas 3-octanone exhibits sweet, fruity, and herbal characteristics. Previous studies have also identified these compounds as contributors to the volatile aroma of aquatic products, although their odor characteristics may vary with species, food matrix, and concentration [70,80,81]. Their increased relative abundances may shift the ketone-associated aroma profile of common carp muscle toward more fruity, sweet, and herbal characteristics following PACs supplementation. In contrast, the relative abundance of 2,3-octanedione decreased in the 0.8 g/kg PACs group. This compound has been associated with fishy, fatty notes in some fish species [82,83]. Reductions in 2,3-octanedione, together with decreases in other off-odor-related volatiles, have been reported to accompany improvements in overall odor quality [84]. Thus, its lower relative abundance may reduce its potential contribution to undesirable odor characteristics.

In the present study, the relative abundances of 10 aldehydes increased, whereas that of one aldehyde (2,4-nonadienal) decreased in the 0.8 g/kg PACs group. Notably, 2,4-nonadienal has been reported to exhibit a relatively high odor activity value in grass carp and is associated with fishy odor [82]. Consistent with this odor description, 2,4-nonadienal was also associated with the fishy aroma category in the present study. Its decreased relative abundance following PACs supplementation may therefore indicate a reduced potential contribution to the fishy aroma of common carp muscle. In contrast, several other

aldehydes showed increased relative abundances. Octanal has been associated with fruity and green notes in aquatic products [75,85], whereas heptanal has been described as having fatty, green, and fishy odor characteristics [86]. Similarly, (E,E)-2,4-octadienal has been associated with grassy notes [17], while (E)-2-octenal has been characterized by citrus, fatty, and green notes [87]. In addition, pentanal has been reported to contribute fruity and malty notes to oysters [88], whereas 3-methylbutanal has been associated with an apple-like aroma in Antarctic krill (*Euphausia superba*) [89]. Collectively, these changes in aldehyde composition suggest that dietary supplementation with 0.8 g/kg PACs may alter the potential aroma characteristics of common carp muscle, with a reduced contribution from fishy-associated aldehydes and increased contributions from aldehydes associated with fruity, grassy, and related aroma notes.

The changes in aldehydes, ketones, and alcohols following 0.8 g/kg PACs supplementation may be related to alterations in fatty acid composition and oxidative metabolism in common carp muscle. In fish muscle, polyunsaturated fatty acids can undergo lipoxygenase (LOX)-mediated oxidation to form lipid hydroperoxides. These unstable intermediates can subsequently decompose into volatile compounds, including aldehydes, alcohols, and ketones [90]. Several volatile compounds such as 1-octen-3-ol, heptanal, and 2-heptanone can be formed through fatty acid oxidation [91]. In tilapia, a high-ALA diet increased muscle n-3 PUFA and n-3 LC-PUFA levels and was accompanied by enhanced LOX activity and increased abundances of several volatile aldehydes and alcohols [92]. These results indicate that the generation of volatile compounds in fish muscle may be influenced by both fatty acid availability and enzyme-mediated lipid oxidation. Consistent with this possibility, our previous study showed that dietary inclusion of 0.8 g/kg PACs significantly elevated the levels of total PUFA, n-3 PUFA, and n-6 PUFA levels in common carp muscle [19], potentially providing more substrates for the formation of lipid-derived volatiles. Meanwhile, PACs possess strong antioxidant activity and can enhance antioxidant capacity. This may reduce MDA formation and suppress free-radical-mediated non-enzymatic lipid peroxidation [93]. Therefore, the changes in aldehydes, ketones, and alcohols observed in the work may be linked to the combined effects of PACs on muscle fatty acid composition and redox status, although the underlying mechanisms require further investigation.

Several limitations of the present study should be acknowledged. (1) Multiple individual or pooled samples were collected from each replicate tank. Although these samples allowed the characterization of biological variation within dietary treatments, observations from the same tank may not be statistically independent. (2) The potential mechanisms discussed in this study, including hepatic protection, mitochondrial β-oxidation, Nrf2 signaling, xanthine oxidase activity, and related enzymatic pathways, were inferred from the observed biochemical and metabolomic changes and previous studies rather than directly verified. Therefore, these mechanistic explanations should be considered hypotheses requiring further experimental validation. (3) The odor descriptors associated with volatile compounds, including sweet, fruity, and green notes, were derived from FlavorDB annotations rather than direct sensory evaluation. Consequently, these associations do not necessarily reflect the actual sensory characteristics of fish muscle. Similarly, although dietary PACs supplementation reduced instrumental muscle texture parameters, including hardness, gumminess, chewiness, and shear force, these changes cannot be taken as direct evidence of improved sensory tenderness without sensory evaluation. Further studies incorporating odor activity value analysis and trained sensory panels are needed to validate the potential sensory implications of the observed changes in volatile compound profiles and muscle texture.

5. Conclusions

In summary, dietary PAC supplementation was associated with reduced serum Glu and TG levels and ALT and AST activities, together with increased GSH levels in common carp, indicating changes in selected serum biochemical and antioxidant-related parameters. Metabolomics revealed that 0.8 g/kg PACs altered the relative abundances of metabolic features associated with nucleotide metabolism, purine metabolism, phenylpropanoid biosynthesis, unsaturated fatty acid biosynthesis, and amino acid biosynthesis. These findings suggest potential associations between PACs supplementation and lipid-, amino acid-, and nucleotide-related metabolic processes, although the underlying mechanisms remain to be established. Dietary PACs supplementation at both 0.4 and 0.8 g/kg reduced muscle hardness, gumminess, chewiness, and shear force, indicating changes in instrumental texture properties associated with tenderness. Furthermore, GC×GC-TOFMS analysis revealed alterations in the relative abundances of muscle volatile compounds, including those associated with sweet, fruity, and green odor descriptors. Overall, the present findings suggest that dietary PACs supplementation may influence serum biochemical and antioxidant parameters, metabolic profiles, and muscle quality-related characteristics in common carp, highlighting its potential application as a functional feed additive in common carp aquaculture.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/antiox15111485/s1, Table S1: FDR-adjusted statistical results of differential serum metabolites; Table S2: FDR-adjusted statistical results of differential muscle volatile compounds; Table S3: FDR-adjusted statistical results of KEGG pathway enrichment analysis.

Author Contributions: Conceptualization, R.J. and J.Z.; methodology, Q.Z. and C.Z.; validation, L.Z.; formal analysis, Q.Z., B.L. and Y.H.; investigation, Q.Z. and C.Z.; resources, L.Z.; data curation, B.L. and R.J.; writing—original draft preparation, Q.Z.; writing—review and editing, Q.Z., B.L., R.J. and J.Z.; visualization, Q.Z. and B.L.; supervision, R.J.; project administration, J.Z.; funding acquisition, J.Z. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: All experimental procedures were conducted in accordance with the guidelines for the care and use of experimental animals of the Freshwater Fisheries Research Center, Chinese Academy of Fishery Sciences, and were approved by the Institutional Animal Care and Use Committee of the Freshwater Fisheries Research Center (LAECFFRC-2025-08-09).

Informed Consent Statement: Not applicable.

Data Availability Statement: All data are contained within the main manuscript and Supplementary Materials.

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

References

1. Obirikorang, K.A.; Quagrainie, K.; Kassah, J.E.; Von Ahnen, M. Editorial: Sustainable aquaculture production for improved food security. *Front. Sustain. Food Syst.* **2024**, *8*, 1485956. https://doi.org/10.3389/fsufs.2024.1485956.
2. Ying, X.; Ying, P. Analysis of Green Development of Aquaculture in China Based on Entropy Method. *Sustainability* **2023**, *15*, 5585. https://doi.org/10.3390/su15065585.

3. Abdul Kari, Z. Nutritional immunomodulation in aquaculture: Functional nutrients, stress resilience, and sustainable health strategies. *Aquac. Int.* **2025**, *33*, 441. https://doi.org/10.1007/s10499-025-02122-5.
4. Zhang, T.; Zhang, L.; Yin, T.; You, J.; Liu, R.; Huang, Q.; Shi, L.; Wang, L.; Liao, T.; Wang, W.; et al. Recent understanding of stress response on muscle quality of fish: From the perspective of industrial chain. *Trends Food Sci. Technol.* **2023**, *140*, 104145. https://doi.org/10.1016/j.tifs.2023.104145.
5. Reverter, M.; Bontemps, N.; Lecchini, D.; Banaigs, B.; Sasal, P. Use of plant extracts in fish aquaculture as an alternative to chemotherapy: Current status and future perspectives. *Aquaculture* **2014**, *433*, 50–61. https://doi.org/10.1016/j.aquaculture.2014.05.048.
6. Nie, F.; Liu, L.; Cui, J.; Zhao, Y.; Zhang, D.; Zhou, D.; Wu, J.; Li, B.; Wang, T.; Li, M.; et al. Oligomeric Proanthocyanidins: An Updated Review of Their Natural Sources, Synthesis, and Potentials. *Antioxidants* **2023**, *12*, 1004. https://doi.org/10.3390/antiox12051004.
7. Patanè, G.T.; Putaggio, S.; Tellone, E.; Barreca, D.; Ficarra, S.; Maffei, C.; Calderaro, A.; Laganà, G. Catechins and Proanthocyanidins Involvement in Metabolic Syndrome. *Int. J. Mol. Sci.* **2023**, *24*, 9228. https://doi.org/10.3390/ijms24119228.
8. Wang, Y.; Chen, X.-H.; Wu, X.-Y.; Cai, G.-H.; Zhai, S.-W. Effects of Dietary Supplementation of Peanut Skin Proanthocyanidins on Growth Performance and Lipid Metabolism of the Juvenile American Eel (*Anguilla rostrata*). *Animals* **2022**, *12*, 2375. https://doi.org/10.3390/ani12182375.
9. Mohammadi, Y.; Bahrami Kamangar, B.; Zarei, M.A. Effects of diets containing grape seed proanthocyanidin extract on the growth and oxidative capacity of common carp (*Cyprinus carpio*). *Aquaculture* **2021**, *540*, 736689. https://doi.org/10.1016/j.aquaculture.2021.736689.
10. Xu, G.; Xing, W.; Li, T.; Yu, H.; Wei, S.; Jiang, N.; Ma, Z.; Luo, L. Dietary grape seed proanthocyanidins improved growth, immunity, antioxidant, digestive enzymes activities, and intestinal microbiota of juvenile hybrid sturgeon (*Acipenser baeri* Brandt ♀ × *A. schrenckii* Brandt ♂). *Aquac. Nutr.* **2021**, *27*, 1983–1995. https://doi.org/10.1111/anu.13334.
11. Xia, B.; Zhang, J.; Li, C.; Wu, S.; Huang, L.; Qin, D.; Hao, Q.; Gao, L. Texture, Nutrition, and Flavor of Different Freshwater Fish Muscles: Comparative Study and Molecular Docking. *Foods* **2025**, *14*, 2258. https://doi.org/10.3390/foods14132258.
12. Dong, M.; Zhang, L.; Wu, P.; Feng, L.; Jiang, W.; Liu, Y.; Kuang, S.; Li, S.; Mi, H.; Tang, L.; et al. Dietary protein levels changed the hardness of muscle by acting on muscle fiber growth and the metabolism of collagen in sub-adult grass carp (*Ctenopharyngodon idella*). *J. Anim. Sci. Biotechnol.* **2022**, *13*, 109. https://doi.org/10.1186/s40104-022-00747-7.
13. Ramasamy, M.; Ramachandran, R.; Partheeban, E.C.; Anbazhagan, V.; Rajendran, R. Proximate analysis, amino acid and fatty acid profiles of edible flatfish collected from Cuddalore coast, Southeast India. *Reg. Stud. Mar. Sci.* **2023**, *66*, 103169. https://doi.org/10.1016/j.rsma.2023.103169.
14. Jin, W.; Fan, X.; Jiang, C.; Liu, Y.; Zhu, K.; Miao, X.; Jiang, P. Characterization of non-volatile and volatile flavor profiles of *Coregonus peled* meat cooked by different methods. *Food Chem. X* **2023**, *17*, 100584. https://doi.org/10.1016/j.fochx.2023.100584.
15. Li, X.; Ma, J.; Chu, Y.; Li, H.; Zhang, Y.; Li, A.; Jia, Y. Multi-Omics Elucidation of Flavor Characteristics in Compound Fermented Beverages Based on Flavoromics and Metabolomics. *Foods* **2025**, *14*, 4119. https://doi.org/10.3390/foods14234119.
16. Zhou, L.; Su, Y.; Yang, D.; Shi, Q.; Yi, T.; Wen, Z. Effects of Two Culture Modes on Muscular Nutrition Content and Volatile Flavor in Chinese Longsnout Catfish (*Leiocassis longirostris*). *Biology* **2025**, *14*, 694. https://doi.org/10.3390/biology14060694.
17. Cheng, Y.; Dong, J.; Zhang, H.; Zhang, Y.; Gao, F.; Wang, F.; Wang, Z.; Ye, X.; Wu, C.; Sun, C. Characterising flavour differences in Mandarin fish muscle under live prey and formulated feed diets using flavouromics and metabolomics. *Food Chem. Mol. Sci.* **2025**, *11*, 100287. https://doi.org/10.1016/j.fochms.2025.100287.
18. Song, Y.; Zhang, W. Balancing Growth and Sustainability in China's Carp Aquaculture: Practices, Policies, and Sustainability Pathways. *Sustainability* **2025**, *17*, 5593. https://doi.org/10.3390/su17125593.
19. Jia, R.; Hou, Y.; Feng, W.; Nomingerel, M.; Li, B.; Zhu, J. Multi-Omics Analysis to Understand the Effects of Dietary Proanthocyanidins on Antioxidant Capacity, Muscle Nutrients, Lipid Metabolism, and Intestinal Microbiota in *Cyprinus carpio*. *Antioxidants* **2023**, *12*, 2095. https://doi.org/10.3390/antiox12122095.
20. Li, H.; Geng, W.; Haruna, S.A.; Zhou, C.; Wang, Y.; Ouyang, Q.; Chen, Q. Identification of characteristic volatiles and metabolomic pathway during pork storage using HS-SPME-GC/MS coupled with multivariate analysis. *Food Chem.* **2022**, *373*, 131431. https://doi.org/10.1016/j.foodchem.2021.131431.
21. Sun, C.; Wang, R.; Wang, T.; Li, Q. Primary evaluation of nine volatile N-nitrosamines in raw red meat from Tianjin, China, by HS-SPME-GC–MS. *Food Chem.* **2020**, *310*, 125945. https://doi.org/10.1016/j.foodchem.2019.125945.

22. Djoumbou Feunang, Y.; Eisner, R.; Knox, C.; Chepelev, L.; Hastings, J.; Owen, G.; Fahy, E.; Steinbeck, C.; Subramanian, S.; Bolton, E.; et al. ClassyFire: Automated chemical classification with a comprehensive, computable taxonomy. *J. Cheminform* **2016**, *8*, 61. https://doi.org/10.1186/s13321-016-0174-y.
23. Thévenot, E.A.; Roux, A.; Xu, Y.; Ezan, E.; Junot, C. Analysis of the Human Adult Urinary Metabolome Variations with Age, Body Mass Index, and Gender by Implementing a Comprehensive Workflow for Univariate and OPLS Statistical Analyses. *J. Proteome Res.* **2015**, *14*, 3322–3335. https://doi.org/10.1021/acs.jproteome.5b00354.
24. Kieffer, D.A.; Piccolo, B.D.; Vaziri, N.D.; Liu, S.; Lau, W.L.; Khazaeli, M.; Nazertehrani, S.; Moore, M.E.; Marco, M.L.; Martin, R.J.; et al. Resistant starch alters gut microbiome and metabolomic profiles concurrent with amelioration of chronic kidney disease in rats. *Am. J. Physiol. Ren. Physiol.* **2016**, *310*, F857–F871. https://doi.org/10.1152/ajprenal.00513.2015.
25. Mousavi, S.; Sheikhzadeh, N.; Tayefi-Nasrabadi, H.; Alizadeh-Salteh, S.; Khani Oushani, A.; Firouzamandi, M.; Mardani, K. Administration of grape (Vitis vinifera) seed extract to rainbow trout (*Oncorhynchus mykiss*) modulates growth performance, some biochemical parameters, and antioxidant-relevant gene expression. *Fish. Physiol. Biochem.* **2020**, *46*, 777–786. https://doi.org/10.1007/s10695-019-00716-4.
26. Yang, H.; Li, Y.; Wang, G.; Xie, J.; Kaneko, G.; Yu, E. Dietary grape seed proanthocyanidin extract improved the chemical composition, antioxidant capacity, myofiber growth and flesh quality of Nile tilapia muscle. *Aquac. Rep.* **2023**, *33*, 101878. https://doi.org/10.1016/j.aqrep.2023.101878.
27. Zhang, Y.; Li, M.; Liu, H.; Fan, Y.; Liu, H.H. The application of procyanidins in diabetes and its complications: A review of preclinical studies. *Front. Pharmacol.* **2025**, *16*, 1532246. https://doi.org/10.3389/fphar.2025.1532246.
28. Zhai, S.-W.; Lu, J.-J.; Chen, X.-H. Effects of Dietary Grape Seed Proanthocyanidins on Growth Performance, Some Serum Biochemical Parameters and Body Composition of Tilapia (*Oreochromis Niloticus*) Fingerlings. *Ital. J. Anim. Sci.* **2014**, *13*, 3357. https://doi.org/10.4081/ijas.2014.3357.
29. Wang, Y.; Chen, X.-H.; Cai, G.-H.; Zhai, S.-W. Grape seed proanthocyanidin extract regulates lipid metabolism of the American eel (*Anguilla rostrata*). *Nat. Prod. Res.* **2022**, *36*, 5889–5893. https://doi.org/10.1080/14786419.2021.2022666.
30. Lu, R.H.; Qin, C.B.; Yang, F.; Zhang, W.Y.; Zhang, Y.R.; Yang, G.K.; Yang, L.P.; Meng, X.L.; Yan, X.; Nie, G.X. Grape seed proanthocyanidin extract ameliorates hepatic lipid accumulation and inflammation in grass carp (*Ctenopharyngodon idella*). *Fish. Physiol. Biochem.* **2020**, *46*, 1665–1677. https://doi.org/10.1007/s10695-020-00819-3.
31. Chen, C.; Huang, F.; Shu, Q.-Y.; Zhang, L.; Zhou, Y.-P. Effects of Dietary Conjugated Linoleic Acids on Growth Performance, Muscle Composition, AST and ALT Activities in Serum in Juveniles Grass Carp (*Ctenopharyngodon idellus*). *Acta Hydrobiol. Sin.* **2010**, *34*, 647–651. https://doi.org/10.3724/SP.J.1035.2010.00647.
32. Liu, X.; Zhang, X.; Zheng, J.; Li, K.; Fang, Q.; Li, X.; An, S.; Li, M.; Kong, Y.; Wang, G. Effects of oligomeric proanthocyanidins on growth performance, antioxidant capability, immunity, liver and intestinal health of *Channa argus*. *Aquaculture* **2025**, *595*, 741504. https://doi.org/10.1016/j.aquaculture.2024.741504.
33. Glencross, B.D. Exploring the nutritional demand for essential fatty acids by aquaculture species. *Rev. Aquac.* **2009**, *1*, 71–124. https://doi.org/10.1111/j.1753-5131.2009.01006.x.
34. Hsu, S.C.; Huang, C.J. Reduced fat mass in rats fed a high oleic acid-rich safflower oil diet is associated with changes in expression of hepatic PPARalpha and adipose SREBP-1c-regulated genes. *J. Nutr.* **2006**, *136*, 1779–1785. https://doi.org/10.1093/jn/136.7.1779.
35. Xiao, Y.; Dong, J.; Yin, Z.; Wu, Q.; Zhou, Y.; Zhou, X. Procyanidin B2 protects against d-galactose-induced mimetic aging in mice: Metabolites and microbiome analysis. *Food Chem. Toxicol.* **2018**, *119*, 141–149. https://doi.org/10.1016/j.fct.2018.05.017.
36. Ma, H. Effects of Linoleic Acid on Lipid Metabolism in High-Fat Diet-Fed Mice and the Underlying Mechanisms. Master's Thesis, Hefei University of Technology, Hefei, China, 2007.
37. Houten, S.M.; Wanders, R.J.A. A general introduction to the biochemistry of mitochondrial fatty acid β-oxidation. *J. Inherit. Metab. Dis.* **2010**, *33*, 469–477. https://doi.org/10.1007/s10545-010-9061-2.
38. Park, S.H.; Park, T.S.; Cha, Y.S. Grape seed extract (*Vitis vinifera*) partially reverses high fat diet-induced obesity in C57BL/6J mice. *Nutr. Res. Pract.* **2008**, *2*, 227–233. https://doi.org/10.4162/nrp.2008.2.4.227.
39. Ali, O.; Szabó, A. Review of Eukaryote Cellular Membrane Lipid Composition, with Special Attention to the Fatty Acids. *Int. J. Mol. Sci.* **2023**, *24*, 15693. https://doi.org/10.3390/ijms242115693.
40. Liu, P.; Zhu, W.; Chen, C.; Yan, B.; Zhu, L.; Chen, X.; Peng, C. The mechanisms of lysophosphatidylcholine in the development of diseases. *Life Sci.* **2020**, *247*, 117443. https://doi.org/10.1016/j.lfs.2020.117443.

41. Calzada, E.; Onguka, O.; Claypool, S.M. Chapter Two—Phosphatidylethanolamine Metabolism in Health and Disease. In *International Review of Cell and Molecular Biology*, Jeon, K.W., Ed.; Academic Press: Cambridge, MA, USA, 2016; Volume 321, pp. 29–88.
42. Wang, L.; Wang, L.; Liu, C.; Ma, F.; Huang, J.; Jin, Z.; Zhang, L.; Feng, D.; Zhang, M.; Yu, M.; et al. Multi-omics reveals the molecular mechanism of muscle quality changes in common carp (*Cyprinus carpio*) under two aquaculture systems. *Comp. Biochem. Physiol. Part D Genom. Proteom.* **2024**, *52*, 101290. https://doi.org/10.1016/j.cbd.2024.101290.
43. Cruzat, V.; Macedo Rogero, M.; Noel Keane, K.; Curi, R.; Newsholme, P. Glutamine: Metabolism and Immune Function, Supplementation and Clinical Translation. *Nutrients* **2018**, *10*, 1564. https://doi.org/10.3390/nu10111564.
44. Liu, J.; Hou, Y.-X.; Chen, Y.; Liang, Y.-J.; Pu, M.; Zheng, L.-L.; Zhang, Z.-W.; Xiao, Y.; Mao, Z.; Ma, C.-G.; et al. Proanthocyanidin B2 Alleviates Cuprizone-Induced Demyelination by Regulating the Astrocytic xCT/GSH/GPX4 Axis. *CNS Neurosci. Ther.* **2025**, *31*, e70598. https://doi.org/10.1111/cns.70598.
45. Yang, H.; Xu, Z.; Liu, W.; Wei, Y.; Deng, Y.; Xu, B. Effect of Grape Seed Proanthocyanidin Extracts on Methylmercury-Induced Neurotoxicity in Rats. *Biol. Trace Elem. Res.* **2012**, *147*, 156–164. https://doi.org/10.1007/s12011-011-9272-x.
46. Kim, S.H.; Seo, H.; Kwon, D.; Yuk, D.Y.; Jung, Y.-S. Taurine Ameliorates Tunicamycin-Induced Liver Injury by Disrupting the Vicious Cycle between Oxidative Stress and Endoplasmic Reticulum Stress. *Life* **2022**, *12*, 354. https://doi.org/10.3390/life12030354.
47. Chen, J.; Chen, Y.; Zheng, Y.; Zhao, J.; Yu, H.; Zhu, J.; Li, D. Neuroprotective Effects and Mechanisms of Procyanidins In Vitro and In Vivo. *Molecules* **2021**, *26*, 2963. https://doi.org/10.3390/molecules26102963.
48. Rodríguez, R.M.; de Assis, L.V.M.; Calvo, E.; Colom-Pellicer, M.; Quesada-Vázquez, S.; Cruz-Carrión, Á.; Escoté, X.; Oster, H.; Aragonès, G.; Mulero, M. Grape-Seed Proanthocyanidin Extract (GSPE) Modulates Diurnal Rhythms of Hepatic Metabolic Genes and Metabolites, and Reduces Lipid Deposition in Cafeteria-Fed Rats in a Time-of-Day-Dependent Manner. *Mol. Nutr. Food Res.* **2024**, *68*, 2400554. https://doi.org/10.1002/mnfr.202400554.
49. Bavia, L.; da Silva, A.P.; Carneiro, M.C.; Kmecick, M.; Pozzan, R.; Esquivel-Muelbert, J.; Isaac, L.; Prodocimo, M.M. Health status biomarkers and hemato-biochemical indices in Nile tilápia. *Comp. Immunol. Rep.* **2024**, *7*, 200168. https://doi.org/10.1016/j.cirep.2024.200168.
50. Zhao, Y.-M.; Zhao, J.-Y.; Gao, L.-P. Protective effects of grape seed proanthocyanidin extract (GSPE) on cis-diamminedichloroplatinum (CDDP)-induced renal oxidative damage and mitochondrial injury in rats. *Mod. Food Sci. Technol.* **2015**, *31*, 6–10+106. https://doi.org/10.13982/j.mfst.1673-9078.2015.1.002.
51. Park, J.C.; Lee, S.H.; Hong, J.K.; Cho, J.H.; Kim, I.H.; Park, S.K. Effect of dietary supplementation of procyanidin on growth performance and immune response in pigs. *Asian-Australas. J. Anim. Sci.* **2014**, *27*, 131–139. https://doi.org/10.5713/ajas.2013.13359.
52. Huang, Z.; Xie, N.; Illes, P.; Di Virgilio, F.; Ulrich, H.; Semyanov, A.; Verkhratsky, A.; Sperlagh, B.; Yu, S.-G.; Huang, C.; et al. From purines to purinergic signalling: Molecular functions and human diseases. *Signal Transduct. Target. Ther.* **2021**, *6*, 162. https://doi.org/10.1038/s41392-021-00553-z.
53. Tasca, C.I.; Lanznaster, D.; Oliveira, K.A.; Fernández-Dueñas, V.; Ciruela, F. Neuromodulatory Effects of Guanine-Based Purines in Health and Disease. *Front. Cell Neurosci.* **2018**, *12*, 376. https://doi.org/10.3389/fncel.2018.00376.
54. Farthing, D.E.; Farthing, C.A.; Xi, L. Inosine and hypoxanthine as novel biomarkers for cardiac ischemia: From bench to point-of-care. *Exp. Biol. Med.* **2015**, *240*, 821–831. https://doi.org/10.1177/1535370215584931.
55. Dahl, H.-A.; Johansen, A.; Nilsson, G.E.; Lefevre, S. The Metabolomic Response of Crucian Carp (*Carassius carassius*) to Anoxia and Reoxygenation Differs between Tissues and Hints at Uncharacterized Survival Strategies. *Metabolites* **2021**, *11*, 435. https://doi.org/10.3390/metabo11070435.
56. Shekhovtsov, S.V.; Bulakhova, N.A.; Tsentalovich, Y.P.; Zelentsova, E.A.; Yanshole, L.V.; Meshcheryakova, E.N.; Berman, D.I. Metabolic response of the Siberian wood frog *Rana amurensis* to extreme hypoxia. *Sci. Rep.* **2020**, *10*, 14604. https://doi.org/10.1038/s41598-020-71616-4.
57. Xianchu, L.; Ming, L.; Xiangbin, L.; Lan, Z. Grape seed proanthocyanidin extract supplementation affects exhaustive exercise-induced fatigue in mice. *Food Nutr. Res.* **2018**, *62*, 1421. https://doi.org/10.29219/fnr.v62.1421.
58. Liu, K.; Li, W.; Yuen, M.; Yuen, T.; Yuen, H.; Wang, M.; Peng, Q. Sea Buckthorn Proanthocyanidins are the Protective Agent of Mitochondrial Function in Macrophages Under Oxidative Stress. *Front. Pharmacol.* **2022**, *13*, 914146. https://doi.org/10.3389/fphar.2022.914146.

59. Wang, X.; Zhao, J.; Lin, Z.; Li, J.; Li, X.; Xu, X.; Wang, Y.; Lv, G.; Lin, H.; Lin, Z. Analysis of Polyphenol Extract from Hazel Leaf and Ameliorative Efficacy and Mechanism against Hyperuricemia Zebrafish Model via Network Pharmacology and Molecular Docking. *Molecules* **2024**, *29*, 317. https://doi.org/10.3390/molecules29020317.
60. Wang, Y.; Zhu, J.X.; Kong, L.D.; Yang, C.; Cheng, C.H.; Zhang, X. Administration of procyanidins from grape seeds reduces serum uric acid levels and decreases hepatic xanthine dehydrogenase/oxidase activities in oxonate-treated mice. *Basic. Clin. Pharmacol. Toxicol.* **2004**, *94*, 232–237. https://doi.org/10.1111/j.1742-7843.2004.pto940506.x.
61. Sheu, S.Y.; Fu, Y.T.; Huang, W.D.; Chen, Y.A.; Lei, Y.C.; Yao, C.H.; Hsu, F.L.; Kuo, T.F. Evaluation of Xanthine Oxidase Inhibitory Potential and In vivo Hypouricemic Activity of *Dimocarpus longan* Lour. Extracts. *Pharmacogn. Mag.* **2016**, *12*, S206–212. https://doi.org/10.4103/0973-1296.182176.
62. Pematilleke, N.; Kaur, M.; Adhikari, B.; Torley, P.J. Relationship between instrumental and sensory texture profile of beef semitendinosus muscles with different textures. *J. Texture Stud.* **2022**, *53*, 232–241. https://doi.org/10.1111/jtxs.12623.
63. Ye, Y.; Ge, Q.; Wu, M.; Yu, H.; Liu, R. Effect of Nitric Oxide on Improving Chilled Pork Quality Based on OPLS-DA and Entropy-Weight Topsis Method. *Food Sci.* **2025**, *46*, 322–336. https://doi.org/10.7506/spkx1002-6630-20241204-024.
64. Li, Y.; Zhang, W.; Huang, H.; Hu, X. Comparative Study on the Texture Characteristics of Three Kinds of Pork. *Food Res. Dev.* **2018**, *39*, 22–27. https://doi.org/10.3969/j.issn.1005-6521.2018.10.005.
65. Dong, X.; Qu, G.; Niu, X.; Chen, X.; Zhang, Y.; Kong, Y.; Li, M.; Wang, G. Comparative analysis of muscle texture characteristics, nutritional composition, and omics characteristics on migratory and landlocked Oncorhynchus masou. *J. Fish. Sci. China* **2025**, *32*, 837–848. https://doi.org/10.12264/JFSC2025-0026.
66. Huang, C. Effects of Dietary Grape Seed Proanthocyanidins on Pork Quality and Antioxidant Capacity of Pork. Master's Thesis, Shanxi Agricultural University, Jinzhong, China, 2021.
67. Xu, M.; Chen, X.; Huang, Z.; Chen, D.; Li, M.; He, J.; Chen, H.; Zheng, P.; Yu, J.; Luo, Y.; et al. Effects of dietary grape seed proanthocyanidin extract supplementation on meat quality, muscle fiber characteristics and antioxidant capacity of finishing pigs. *Food Chem.* **2022**, *367*, 130781. https://doi.org/10.1016/j.foodchem.2021.130781.
68. Wang, S.; Peng, X.; Niu, Y.; Chen, L.; Hu, Y.; Wen, Y.; Mo, S.; Liu, X. Effects of Grape Seed Proanthocyanidin Extracts on Slaughter Performance and Meat Quality of Meat Rabbit. *Acta Vet. Zootech. Sin.* **2014**, *45*, 1135–1141. https://doi.org/10.11843/j.issn.0366-6964.2014.07.015.
69. Xu, J.-X.; Zhang, X.; Fu, B.-F.; Qiao, X.-Y.; Wang, Z.-Y.; Xu, X.-B.; Cheng, S.-Z.; Du, M. Impact of Cooking Processes on Volatile Flavor Compounds and Free Amino Acids in Fish Sauce. *Foods* **2025**, *14*, 586. https://doi.org/10.3390/foods14040586.
70. Fan, W.; Che, X.; Ma, P.; Chen, M.; Huang, X. Aroma Formation, Release, and Perception in Aquatic Products Processing: A Review. *Foods* **2025**, *14*, 2651. https://doi.org/10.3390/foods14152651.
71. Jiang, C.; Liu, Y.; Jin, W.; Zhu, K.; Miao, X.; Dong, X.; Jiang, P. Effects of curing concentration and drying time on flavor and microorganisms in dry salted Spanish mackerel. *Food Chem. X* **2024**, *21*, 101126. https://doi.org/10.1016/j.fochx.2024.101126.
72. Lin, Y.; Zhang, X.; Li, X.; Duan, C.; Ma, F.; Li, D. Analysis of Volatile Flavor Compounds in Three Types of Baked Fish Fillets Using an Electronic Nose Combined with GC-IMS. *Sci. Technol. Food Ind.* **2025**, *46*, 291–301. https://doi.org/10.13386/j.issn1002-0306.2025010150.
73. Corona, O.; Cinquanta, L.; Li Citra, C.; Mazza, F.; Ferrantelli, V.; Cammilleri, G.; Marconi, E.; Cuomo, F.; Messia, M.C. Evolution of Free Amino Acids, Histamine and Volatile Compounds in the Italian Anchovies (*Engraulis encrasicolus* L.) Sauce at Different Ripening Times. *Foods* **2023**, *12*, 126. https://doi.org/10.3390/foods12010126.
74. Pongsetkul, J.; Yongsawatdigul, J.; Boonanuntanasarn, S.; Benjakul, S. Development of Flavor and Taste Components of Sous-Vide-Cooked Nile Tilapia (*Oreochromis niloticus*) Fillet as Affected by Various Conditions. *Foods* **2022**, *11*, 3681. https://doi.org/10.3390/foods11223681.
75. Tao, N.P.; Wu, R.; Zhou, P.G.; Gu, S.Q.; Wu, W. Characterization of odor-active compounds in cooked meat of farmed obscure puffer (*Takifugu obscurus*) using gas chromatography-mass spectrometry-olfactometry. *J. Food Drug Anal.* **2014**, *22*, 431–438. https://doi.org/10.1016/j.jfda.2014.02.005.
76. Liu, L.; Zhao, Y.; Lu, S.; Liu, Y.; Xu, X.; Zeng, M. Metabolomics investigation on the volatile and non-volatile composition in enzymatic hydrolysates of Pacific oyster (*Crassostrea gigas*). *Food Chem. X* **2023**, *17*, 100569. https://doi.org/10.1016/j.fochx.2023.100569.
77. Su, X.-L.; Hou, C.-Y.; Zheng, G.-D.; Zou, S.-M. Evaluation of muscle nutritional value and flavor quality between triploid and diploid blunt snout bream (*Megalobrama amblycephala*): Applications of breeding improvement in fish quality. *Aquac. Rep.* **2024**, *39*, 102493. https://doi.org/10.1016/j.aqrep.2024.102493.

78. Feng, R.; Feng, D.; Wang, L.; Zhang, L.; Liu, C.; Ma, F.; Zhang, M.; Yu, M.; Jiang, H.; Qiao, Z.; et al. Comparative Analysis of Nutritional Quality, Serum Biochemical Indices, and Visceral Peritoneum of Grass Carp (*Ctenopharyngodon idellus*) Fed with Two Distinct Aquaculture Systems. *Foods* **2024**, *13*, 1248. https://doi.org/10.3390/foods13081248.
79. Chen, T.; Xue, Y.; Li, C.; Zhao, Y.; Huang, H.; Feng, Y.; Xiang, H.; Chen, S. Identification of Key Volatile Compounds in Tilapia during Air Frying Process by Quantitative Gas Chromatography–Ion Mobility Spectrometry. *Molecules* **2024**, *29*, 4516. https://doi.org/10.3390/molecules29184516.
80. Jiang, L.; Tang, J.; Huang, B.; Zhang, C.; Jiang, P.; Chen, D. Effect of Vanillin on the Anaesthesia of Crucian Carp: Effects on Physiological and Biochemical Indices, Pathology, and Volatile Aroma Components. *Foods* **2023**, *12*, 1614. https://doi.org/10.3390/foods12081614.
81. Liu, X.-L.; Xie, W.-C.; Yang, X.-H.; Zhang, C.-H. Analysis of Volatile Components in Ostrea rivularis Hydrolysates by SPME-GC-MS. *Food Sci.* **2010**, *31*, 410–414. https://doi.org/10.7506/spkx1002-6630-201024089.
82. Wang, H.; Zhu, Y.; Zhang, J.; Wang, X.; Shi, W. Characteristic volatile compounds in different parts of grass carp by comprehensive two-dimensional gas chromatography/time-of-flight mass spectrometry. *Int. J. Food Prop.* **2020**, *23*, 777–796. https://doi.org/10.1080/10942912.2020.1758715.
83. Fu, C.; Weng, J.; Luo, F.; Liao, M.; Xue, L.; Guo, Z. Elucidating cooking-driven flavor dynamics of paddy field carp (*Cyprinus carpio*) using a combination of E-nose, E-tongue, GC-MS and GC-IMS. *Food Chem.* **2025**, *494*, 146207. https://doi.org/10.1016/j.foodchem.2025.146207.
84. Nilsuwan, K.; Chantakun, K.; Chotphruethipong, L.; Benjakul, S. Development of Hydrolysis and Defatting Processes for Production of Lowered Fishy Odor Hydrolyzed Collagen from Fatty Skin of Sockeye Salmon (*Oncorhynchus nerka*). *Foods* **2021**, *10*, 2257. https://doi.org/10.3390/foods10102257.
85. Sun, W.; Ji, H.; Zhang, D.; Zhang, Z.; Liu, S.; Song, W. Evaluation of Aroma Characteristics of Dried Shrimp (*Litopenaeus vannamei*) Prepared by Five Different Procedures. *Foods* **2022**, *11*, 3532. https://doi.org/10.3390/foods11213532.
86. Song, Y.; Sun, G.; Wei, F.; Wu, Z.; Tian, H.; Meng, Y.; Ma, R. Replacing Fishmeal and Fish Oil with Complex Protein and Canola Oil: Effect on Organoleptic and Nutritional Quality of Triploid Rainbow Trout (*Oncorhynchus mykiss*). *Foods* **2024**, *13*, 1591. https://doi.org/10.3390/foods13111591.
87. Wang, Z.; Yang, Y.; Zhang, D.; Li, J.; Zhang, L.; Zhao, Y.; Zhao, J.; Zhang, J.; Wu, J. Comparative Analysis of Volatile Organic Compounds in Freshwater-Cultured and Saline–Alkaline Selectively Bred Tilapia Using Electronic Nose, GC-IMS, and HS-SPME-GC-MS. *Foods* **2025**, *14*, 3946. https://doi.org/10.3390/foods14223946.
88. Li, T.; Zhao, L.; Wang, S.; Liu, Q.; Cao, R. Changes in Lipids and Volatile Compounds of Pacific Oysters during Live Storage. *Meat Res.* **2024**, *38*, 23–29. https://doi.org/10.7506/rlyj1001-8123-20240618-150.
89. Wei, L.; Shi, W.-z.; Wang, Z.-h.; Zhu, G.-p.; Fang, B.; Xu, N. Changes of Volatile Compounds in Antarctic Krill from March to August. *Sci. Technol. Food Ind.* **2019**, *40*, 213–218+224. https://doi.org/10.13386/j.issn1002-0306.2019.24.035.
90. Shahidi, F.; Hossain, A. Role of Lipids in Food Flavor Generation. *Molecules* **2022**, *27*, 5014. https://doi.org/10.3390/molecules27155014.
91. Liu, Q.; Lei, M.; Zhao, W.; Li, X.; Zeng, X.; Bai, W. Formation of Lipid-Derived Flavors in Dry-Cured Mackerel (Scomberomorus niphonius) via Simulation of Autoxidation and Lipoxygenase-Induced Fatty Acid Oxidation. *Foods* **2023**, *12*, 2504. https://doi.org/10.3390/foods12132504.
92. Chen, F.; He, Y.; Li, X.; Zhu, H.; Li, Y.; Xie, D. Improvement in Muscle Fatty Acid Bioavailability and Volatile Flavor in Tilapia by Dietary α-Linolenic Acid Nutrition Strategy. *Foods* **2024**, *13*, 1005. https://doi.org/10.3390/foods13071005.
93. Li, S.; Xu, M.; Niu, Q.; Xu, S.; Ding, Y.; Yan, Y.; Guo, S.; Li, F. Efficacy of Procyanidins against In Vivo Cellular Oxidative Damage: A Systematic Review and Meta-Analysis. *PLoS ONE* **2015**, *10*, e0139455. https://doi.org/10.1371/journal.pone.0139455.

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