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Capturing the impact of oral processing behavior and bolus formation on the dynamic sensory perception and composition of steamed sturgeon meat

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ABSTRACT

The effect of oral processing on flavor release and change in composition of steamed sturgeon meat was investigated. Oral processing caused changes in the concentrations of taste compounds including amino acids, 5′-nucleotides, organic acids, and Na^+. Sensory omics demonstrated that the concentrations of 12 volatile compounds increased significantly ($p < 0.05$) during the initial stage of oral processing. There is no significant difference in microstructure, texture, and particle size of meat bolus. The top fifteen differential lipids which including eight phospholipids in all processed samples significantly ($p < 0.05$) correlated with the flavor release. A total of 589 differential proteins were detected in three samples with different chewing times (0, 12, and 30 s). Analysis of the correlations between odorants and 19 differential proteins was performed. Enriched pathways including fatty acid degradation, valine, leucine and isoleucine degradation, glycine, serine and threonine metabolism, and arachidonic acid metabolism were associated with flavor release during oral processing. This study aimed to investigate potential links between flavor release and biological processes during oral processing from a proteomics perspective.

Introduction

The sturgeon is the largest and longest-lived freshwater fish and has significant biological and economic value. In recent years, the scale of sturgeon farming has continued to increase, especially in China, where the annual output of sturgeon products accounts for more than 80 % of the world total (Wang et al., 2019). The meat of the sturgeon is the primary product. Heat treatment, dry-cured and smoked are common processing methods of most fish products that endow fish meat with an unique aroma and delicious taste (Wu et al. 2021). Compared with other multiple heat treatment methods (microwave heating, baking and frying et al.), steaming and boiling can effectively avoid the excessive oxidation of protein and fat in meat, and the total content of free amino acids in steamed meat is higher than that in boiled meat (Hu, Ren, Shen, Chen, & Ye, 2018).

The oral cavity acts as a complex food processing machine and food oral processing is directly related to the consumption and digestion of foods including preliminary digestion and sensory perception (Chen, 2009). During oral processing, ingested food is first broken down into small particles by chewing (Ployon, Morzel, & Canon, 2017). At the same time, it is enzymatically degraded by enzymes such as α-amylase, lipoxygenase, carbonic anhydrase, and protease in saliva (Salles, Chagnon, Feron, Guichard, Laboure, & Morzel, 2011). Changes in texture and flavor perception are observed during the bolus formation and changes (Devezeaux de Lavergne, van de Velde, van Boekel, & Stieger, 2015). However, variations in oral processing behavior and its influence on sensory perception have been reported mainly for solid foods (Doyennette, Aguayo-Mendoza, Williamson, Martins, & Stieger, 2019). And current studies on flavor release and perception during food oral processing mainly focuses on the law of change. Several studies found that five flavor compounds and six aroma compounds were key contributors to the flavor of white bread during chewing (Pu et al., 2021). Some researchers also took traditional dry-cured pork as the research object to investigate the rate of flavor release during oral

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processing through electronic tongue. The result exhibited that oral processing behavior and saliva release have an important impact on the flavor release of dry-cured pork. The interaction between chewed food and saliva leads to significant changes in sensory perception during oral processing (Tian et al., 2023). Few studies have comprehensively analyzed the mechanism of oral processing with regard to the release of food flavors.

Saliva is crucial to the whole oral process, which not only affects the release and transportation of flavor substances, but also an important factor in food bolus formation and facilitate safe swallowing (Ni, Smyth, Gidley, & Cozzolino, 2021). The key substance in saliva is salivary protein, so it can be considered to study the effect and mechanism of oral processing on flavor release from the perspective of protein. Zhu et al. (2021) firstly identified 11 proteins that could be potential biomarkers for use in quality control of sauced beef by label-free proteomics and reported the association between myosin-2 and myosin heavy chain-4 and the color of sauced beef during processing. Jia, Shi, Zhang, Shi, and Chu (2021) found that the off-flavor produced by irradiated goat meat was associated with protein oxidation and metabolism of cysteine and methionine by high-throughput proteomics. Changes in the coefficient of interaction between saliva and food highlight the effect of protein enrichment on the oral processing of cereal foods (Gibouin, van der Sman, Benedito, & Della Valle, 2022). The interactions between saliva and food components are measured from the aspects of the friction change on the mixture of food and *ex vivo* saliva using tribological method (Fan, Shewan, Smyth, Yakubov, & Stokes, 2021). However, the assessment of interactions between saliva and lipid and protein is still very rare, and most of the existing studies focused on *in vitro* tribology measurements and *in vivo* sensory perception without human saliva. The formation of food bolus and role of the amount of lipids and proteins during the oral processing on flavor release have been seldom reported, especially at the molecular level.

The objectives of the present study were: (1) to determine dynamic changes in flavor compounds and physical properties; (2) to investigate the profiles of differential lipids and proteins during oral processing using lipidomics and TMT proteomics; and (3) to predict the relationships between flavor compounds and lipid and protein communities using bioinformatics analysis. This study laid a foundation for discovering directional control measures to improve the sensory properties of sturgeon meat and has provided an academic reference for improving the adaptability of processing methods.

Materials and methods

Sturgeon meat preparation

Juvenile hybrid sturgeon (*Acipenser baerii* Brandt ♀ × *Acipenser schrenckii* Brandt ♂) with a weight of 1.5 ± 0.5 kg and a length of 65 ± 10 cm were purchased from the Chengyang aquatic products market (Qingdao, Shandong, China), placed in oxygen to keep them alive, and transported to the laboratory within 1 h. The live sturgeon were stunned by a blow to the head, and meat was obtained after removing the head, viscera, and skin. Then the dorsal meat was collected and divided into pieces (2 cm × 2 cm × 1 cm). All the fresh samples were mixed for further use. In addition, the process used for steaming the sturgeon meat was determined by a single-factor experiment in combination with artificial sensory analysis. Finally, all the samples were steamed at 100 °C for 10 min.

Dynamic sensory evaluation

The sensory analysis during the mastication of samples was based on the standard—Sensory analysis-Methodology guidance for establishing a sensory profile (GB/T 39625–2020, China National Standard) and approved by the Ocean University of China human ethics committee (approval reference code: 20220305SPXY01). The chewing time (30 s with a mastication frequency of 2 cycles/s) and optimal amount for consumption (one 2.0 g piece of steamed sturgeon meat) were determined by 50 volunteers (aged 22–28 years). Panellists were master's and doctoral students of Aquatic Product Flavor Chemistry Laboratory of Ocean University of China. The duration of oral processing of the steamed sturgeon meat samples was divided into six time points (0, 6, 12, 18, 24, and 30 s, where 0 s represented the time when the steamed sturgeon meat sample was just placed into the mouth and 30 s represented the swallowing point), which were denoted as C1, C2, C3, C4, C5, and C6, respectively. The recruited participants were informed of the detailed goals and procedures and signed informed consent forms throughout the sensory evaluation trial, which was conducted according to the Helsinki Declaration. Fish meat boluses with different chewing times (6, 12, 18, 24, and 30 s) were collected by 12 panelists (7 females and 5 males, selected from the above 50 volunteers) after spitting out all the chewed fish meat, residue, and saliva completely into a self-sealing plastic bag. The collected boluses were immediately plunged into liquid nitrogen to freeze them. For the 0 s sample, unchewed fish meat was used after being processed by a vacuum freeze-dryer for later experimental use. Each panelist carried out the collection of bolus samples in triplicate.

Temporal dominance of sensation (TDS) and dynamic quantitative descriptive analysis (D-QDA) were used as sensory evaluation methods to appraise the tastes sensed during the oral processing of steamed sturgeon meat. The 12 group members who participated in this sensory evaluation were the same as those mentioned above. All the panelists were required to take part in a two-alternative forced-choice test and a ranking test before the formal sensory evaluation. The specific test procedures followed the method described by Pu, Zhang, Sun, Ren, and Tang (2021) with slight modifications. After they had been trained, the panelists were requested to write down descriptors of the tastes perceived during oral processing of the steamed sturgeon meat. For D-QDA, the 12 participants were asked to score the intensities of the perceived tastes on a scale of 1–9 (1, weak; 5, medium; 9, strong) at every time point (6, 12, 18, 24, and 30 s) while chewing the steamed sturgeon meat (Sensory analysis-Guidelines for the use of quantitative response scales, GB/T 39501–2020/ISO 4121:2003). The sensory evaluation criteria are listed in Table S1. TDS analysis required the participants to select only one dominant taste attribute at each chewing time point without determining its specific intensity.

Determination of taste compounds

Free amino acids

Free amino acids were determined by a modified version of the method described by Yu et al. (2018). A freeze-dried sample (1 g) was homogenized with 15 mL of an HCl solution (0.1 mol/L) for 2 min and then centrifuged at 10,000*g* for 10 min at 4 °C. The extraction and centrifugation were repeated, and the combined supernatant was diluted to a volume of 25 mL with ultrapure water. Equal volumes (10 mL) of the extract solution and trichloroacetic acid (10 g/L) were mixed, allowed to stand for 1 h, and then centrifuged. The pH was adjusted to 2.0 using an NaOH solution (6 mol/L), after which the supernatant was diluted to a volume of 25 mL, filtered through a 0.22 μm membrane, and then injected into an automatic amino acid analyzer (L-8900; Hitachi, Japan).

5′-nucleotides

In addition, 5′-nucleotides were extracted using the method described by Liu, Meng, Tang, Wang, and Zhi (2018) with slight modifications. A 1 g freeze-dried sample was dispersed in 3 mL cold perchloric acid (5 mL/100 mL) and centrifuged at 10,000*g* for 10 min at 4 °C after homogenization. The extraction and centrifugation were repeated once, and then the supernatants were combined. A 5 mol/L KOH solution was dropped into the supernatant to adjust the pH to 6.75, after which the supernatant was diluted to a volume of 25 mL, filtered

through a 0.22 μm membrane, and then injected into a high-performance liquid chromatography (HPLC) system (UltiMate 3000; Thermo Fisher Scientific, Waltham, MA, USA) equipped with a C18 column (5 μm, 4.6 mm id × 250 mm, Agilent Technologies, USA). The mobile phase comprised 98 % NaH_2PO_4 (0.05 mol/L, pH 6.8) and 2 % methanol, with a detection wavelength of 254 nm, a flow rate of 0.6 mL/min, and a column temperature of 30 °C. Standards including adenosine monophosphate (AMP) (≥98 %), guanosine monophosphate (GMP) (≥98 %), and inosine monophosphate (IMP) (≥98 %) (Shanghai Yuanye Bio-Technology Co., Ltd, Shanghai, China) were used.

Organic acids

Organic acids were determined according to the procedure described by Chen and Zhang (2007) with modifications. A 1 g freeze-dried sample was homogenized in 10 mL of a phosphoric acid solution (0.1 mL/100 mL) for 2 min and then centrifuged at 10,000*g* for 10 min at 4 °C. The above procedure was repeated once, and the supernatants were combined and diluted to a volume of 25 mL with the above phosphoric acid solution. The extract solution was filtered through a 0.22 μm membrane prior to HPLC analysis. The HPLC instrument and column used were the same as those mentioned in Section 2.3.2. The HPLC conditions were as follows: the mobile phase comprised 0.1 % H_3PO_4-methanol (v/v = 97:3), the detection wavelength was 210 nm, the flow rate was 1 mL/min, and the column temperature was 30 °C. Each organic acid was analyzed by comparison with the retention times and peak areas of standards, namely, succinic acid (≥98 %), malic acid (≥98 %), and lactic acid (≥98 %) (Beijing Solarbio Science & Technology Co., Ltd).

Sodium ion analysis

The test method was based on the method described by Wu et al. (2021) with modifications. Microwave digestion was used for sample pretreatment. A freeze-dried sample (0.35 g) was accurately weighed into an inner digestion tank, 6.5 mL nitric acid was added, and the outer tank was screwed tight, after which the sample was placed in a microwave digester (Mars6 Xpress, CEM, USA) for digestion. The inner tank was removed after cooling and placed in an acid-driven processor at 120–140 °C until near dryness. The residue was diluted with ultrapure water to a volume of 25 mL for further use. The digestion solution was analyzed by an atomic absorption spectrophotometer (AA-6800, Shimadzu, Japan) at a wavelength of 589.0 nm to quantify sodium ions.

Determination of volatile compounds by headspace gas chromatography–ion mobility spectrometry (HS-GC-IMS) and two-dimensional gas chromatography–time-of-flight mass spectrometry (GC × GC-TOFMS)

The contents of volatile organic compounds (VOCs) in steamed sturgeon meat during oral processing were measured by HS-GC-IMS (FlavourSpec; G.A.S. Dortmund, Dortmund, Germany) in combination with a CTC automatic headspace injector. A freeze-dried sample (2 g) and ultrapure water (1.5 mL) were incubated in a 20 mL headspace vial with stirring at 500 r/min at 60 °C for 20 min. Afterward, a 500 μL headspace sample was automatically injected into a GC column (FS-SE-54-CB, 15 m × 0.53 mm × 0.50 μm), and VOCs were separated. Nitrogen (99.99 % purity) was used as the GC carrier gas with an initial flow rate of 2 mL/min, which was maintained for 2 min. Next, the flow rate was increased to 10 mL/min over 8 min and was then increased to 50 mL/min over 10 min. Finally, the flow rate was increased to 150 mL/min over 10 min. The temperature of the IMS drift tube was 45 °C, and the constant flow rate of the carrier gas (N_2, 99.99 % purity) was 150 mL/min. The analytes were ionized in positive-ion mode, and the ionization source was ^3H (activity of 300 MBq). The retention indices (RIs) of VOCs were calculated using C4–C9 *n*-ketones (Sinopharm Chemical Reagent Co., Ltd, Beijing, China) as external standard references. VOCs were identified by comparing their RIs and drift times with those in the GC-IMS library. GC × GC-TOFMS analysis was performed using an Agilent 7890B gas chromatograph coupled to an Agilent 7200 time-of-flight mass spectrometer (Agilent Technologies, Santa Clara, CA, USA) and the testing conditions were consistent with those reported by Xu et al. (2022).

Granularity analysis

The collected fish boluses (0.5 g) were taken to a 50 mL centrifuge tube and diluted by adding 40 mL of 0.1 g/mL SDS solution. Constant stirring was performed to keep the boluses particles in a stable dispersed state before determination. The particle size and distribution of the boluses were measured by laser light scattering using a laser particle size analyzer (Nano ZS, Malvern Instruments Ltd., Worcestershire, UK).

Texture profile analysis

The textural properties of fish boluses were determined by the texture analyzer (TMS-PRO, Food Technology Corporation, VA, USA). The fish boluses at different oral processing time were collected inside cylindrical glass bottles and made the surface relatively smooth. And then the TPA mode and probe of P50 type were selected. The compression ratio was 75 % with 1 mm/s test speed and 5.0 g trigger point load. The secondary compression interval was 5 s (Khin, Goff, Nsor-Atindana, Ahammed, Liu, & Zhong, 2021).

Microstructure determined by scanning electron microscopy (SEM)

The collected fish boluses were placed at −20 °C for 24 h to stabilize the form. And then the boluses were cut into small pieces by a scalpel and immobilized in a glutaraldehyde solution (2.5 %) for fixation for 24 h at 4 °C. Then, the samples were rinsed with distilled water. Ethyl alcohol solutions (50 %, 70 %, 80 %, 90 % and 100 %, v/v) were used to dehydrate the samples for 15 min each. At last, the dehydrated samples were gold-coated and observed under a SEM (JSM-5800 LV, JEOL, Tokyo, Japan) at an accelerating voltage of 20 kV.

Lipidomics analysis

The method devised by Chen, Nie, Hu, Huang, Huang, and Nie (2020) was used to extract lipids from the samples. Liquid chromatography coupled with quadrupole orbital ion trap mass spectrometry (UPLC-Q-Orbitrap, Thermo, Massachusetts Waltham, USA) was used for lipidomics analysis. The column was a Hypersil Gold C18 reverse-phase chromatography column (100 mm × 2.1 mm × 1.9 μm); mobile phase A comprised 0.1 % formic acid + 10 mmol/L ammonium formate + acetonitrile/water (60:40, *v/v*); and mobile phase B comprised 0.1 % formic acid + 10 mmol/L ammonium formate + isopropanol/acetonitrile (90:10, *v/v*). The lipidomics data were analyzed using MetaboAnalyst 5.0 and the free online platform of Majorbio Cloud Platform (https://www.majorbio.com).

Proteomics analysis by TMT protein labeling

The method devised by Zhu et al. (2021) was used. A frozen sample was taken and transferred to a centrifuge tube, and an appropriate amount of protein lysis buffer (8 mol/L urea + 1 % sodium dodecyl sulfate containing a protease inhibitor) was added. The peptides were redissolved in 2 % acetonitrile (pH 10) and were then fractionated by high-pH reversed-phase liquid chromatography (Vanquish Flex UHPLC, Thermo Fisher Scientific, Waltham, MA, USA) with an Acquity UPLC BEH C18 column (1.7 μm × 2.1 mm × 150 mm, Waters, Milford, MA, USA). The peptides were ionized using a nanospray ionization ion source and analyzed by tandem mass spectrometry (MS/MS) (Q-Exactive HF-X, Thermo Fisher Scientific, Waltham, MA, USA) with an ion source voltage of 2.4 kV. The MS scan range was 350–1300 *m/z*, and the peptides corresponding to the 20 strongest signals among the precursor ions were

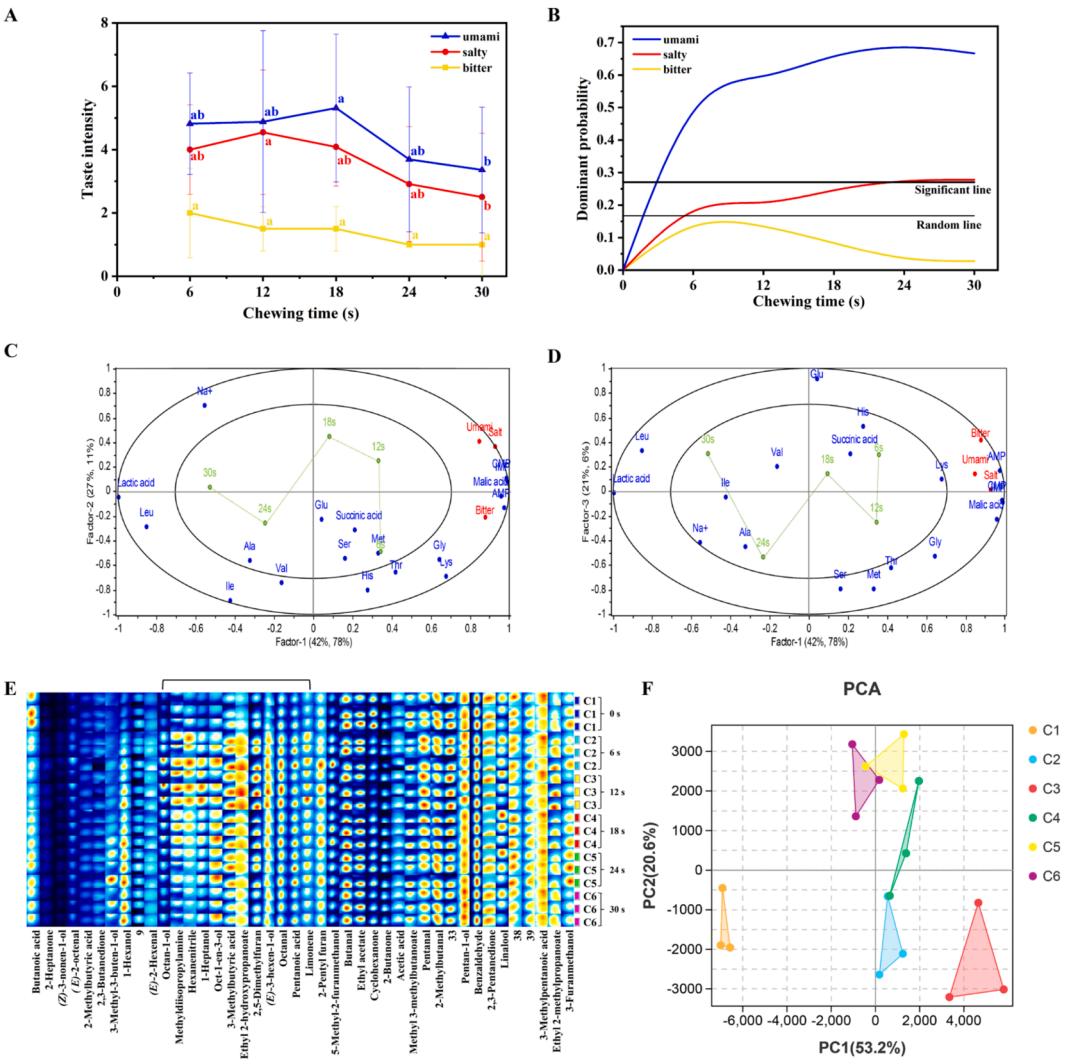

Fig. 1. A and B: Results of dynamic sensory evaluation of tastes perceived during oral processing of steamed sturgeon meat. A: Temporal dominance of sensation. B: Dynamic quantitative descriptive analysis. C and D: The PLSR analysis of the taste compounds to the taste perception with different chewing times. E: Fingerprints of volatile components identified by GC-IMS. F: PCA analysis of volatiles with different oral processing time detected by GC-IMS.

Table 1
The concentrations of amino acids, 5′-nucleotides, organic acids, and Na^+ during oral processing of steamed sturgeon meat.

		TV (mg/100 g)	Concentration (mg/100 g dry weight)					
			0 s	6 s	12 s	18 s	24 s	30 s
Amino acids	Thr	260	26.10 ± 0.41^{a}	25.98 ± 0.41^{a}	25.02 ± 0.40^{a}	22.42 ± 0.35^{b}	25.66 ± 0.42^{a}	23.37 ± 0.396^{b}
	Ser	150	31.30 ± 0.71^{ab}	30.51 ± 0.69^{ab}	32.58 ± 0.73^{a}	25.03 ± 0.58^{c}	29.05 ± 0.67^{b}	26.26 ± 0.605^{c}
	Gly	130	33.54 ± 1.28^{a}	31.42 ± 1.20^{ab}	31.50 ± 1.20^{ab}	29.04 ± 1.11^{b}	31.24 ± 1.19^{ab}	28.43 ± 1.082^{b}
	Ala	60	59.45 ± 0.59^{b}	59.45 ± 0.95^{b}	63.35 ± 1.02^{a}	53.40 ± 0.86^{c}	60.67 ± 0.98^{ab}	54.10 ± 0.875^{c}
	Met	30	5.03 ± 0.05^{a}	4.36 ± 0.04^{b}	3.65 ± 0.02^{e}	3.56 ± 0.03^{e}	4.15 ± 0.04^{e}	3.90 ± 0.049^{d}
	Glu	30	28.15 ± 1.20^{a}	29.81 ± 1.28^{a}	31.77 ± 1.37^{a}	27.22 ± 1.16^{a}	31.28 ± 1.33^{a}	29.79 ± 1.290^{a}
	Val	40	13.07 ± 0.32^{a}	11.81 ± 0.30^{b}	10.67 ± 0.27 ^{cd}	10.13 ± 0.25^{d}	11.68 ± 0.29^{bc}	10.67 ± 0.264 ^{cd}
	Ile	90	10.02 ± 0.26^{a}	8.33 ± 0.21^{b}	7.93 ± 0.20^{bc}	7.41 ± 0.19^{c}	8.23 ± 0.21^{b}	7.80 ± 0.197^{bc}
	Leu	190	16.53 ± 0.23^{a}	13.97 ± 0.19^{b}	13.12 ± 0.18 ^{cd}	12.16 ± 0.17^{e}	13.76 ± 0.19^{bc}	12.92 ± 0.185^{d}
	Lys	50	12.26 ± 0.23^{a}	11.21 ± 0.21^{b}	10.95 ± 0.21^{b}	9.82 ± 0.19^{c}	11.37 ± 0.22^{b}	10.76 ± 0.209^{b}
	His	260	13.96 ± 0.17^{bc}	14.40 ± 0.16^{b}	13.70 ± 0.18 ^{cd}	13.21 ± 0.16^{d}	16.48 ± 0.20^{a}	13.41 ± 0.154 ^{cd}
5′-Nucleotides	GMP	12.5	16.67 ± 0.59^{c}	24.09 ± 4.15^{a}	26.05 ± 0.06^{a}	24.84 ± 0.14^{a}	22.14 ± 0.57^{ab}	19.62 ± 0.091^{bc}
	IMP	25	235.79 ± 4.78^{d}	468.39 ± 14.71^{a}	482.85 ± 9.34^{a}	445.07 ± 1.15^{a}	405.40 ± 6.61^{b}	365.67 ± 17.546^{c}
	AMP	50	23.78 ± 1.49^{b}	52.38 ± 14.17^{a}	49.84 ± 1.61^{a}	46.69 ± 0.01^{a}	42.25 ± 2.86^{ab}	40.11 ± 2.341a^{b}
Organic acids	Malic acid	49.6	302.61 ± 27.52^{a}	361.07 ± 6.47^{a}	361.20 ± 26.63^{a}	348.89 ± 6.89^{a}	337.37 ± 3.29^{a}	299.55 ± 16.607^{a}
	Lactic acid	126.1	1864.85 ± 71.90^{d}	2026.68 ± 48.29^{bc}	2050.47 ± 117.73^{bc}	2129.33 ± 83.50^{bc}	2372.67 ± 121.33^{ab}	2560.39 ± 150.295^{a}
	Succinic acid	10.6	224.75 ± 2.33^{a}	132.25 ± 3.71^{bc}	133.37 ± 0.07^{b}	114.94 ± 2.13^{c}	118.79 ± 10.32^{bc}	131.05 ± 2.619^{bc}
Inorganic ion	Na^+	150	137.00 ± 15.63^{d}	143.72 ± 9.92^{d}	366.16 ± 6.98^{c}	452.34 ± 18.39^{b}	428.33 ± 5.59^{ab}	404.66 ± 8.834^{a}

selected for secondary fragmentation. The data acquisition mode employed a data-dependent scanning program. The proteomics data were analyzed using MetaboAnalyst 5.0 and the free online platform of Majorbio Cloud Platform (https://www.majorbio.com).

Statistical analysis

All experiments were performed in triplicate, and the data were presented as the average value ± standard deviation. IBM SPSS Statistics 25 (SPSS, Inc., Chicago, IL, USA) was used for data processing and analysis. Partial Least Square analysis used The Unscrambler X 13.0 (CAMO software, Oslo, Norway). A value of $P < 0.05$ was considered to indicate a statistically significant difference.

Results and discussion

Dynamic sensory evaluation during oral processing

The results of sensory evaluation indicated that umami and saltiness were the main tastes perceived in steamed sturgeon meat during oral processing (Fig. 1A-B). The TDS results showed that umami taste was above the significance line and bitterness was below the chance line. Saltiness gradually increased in intensity to above the significance line in the final stage. This suggested that umami taste was always present as the dominant taste until the swallowing point throughout the oral processing of steamed sturgeon meat. The results of D-QDA showed that the intensities of umami and saltiness increased significantly ($p < 0.05$) in the early stage of chewing and then decreased and became stable in the last stage. This taste perception pattern was similar to that observed in the oral processing of stewed pork with brown sauce (Mueller, Koehler, & Scherf, 2016). In addition, they found that consumer perception of saltiness and umami during chewing has important correlations with the food matrix. The intensity of bitterness was always at a low level during the whole process, and no significant change was observed.

Release of taste compounds during oral processing

The results for the concentrations of free amino acids in steamed sturgeon meat at different stages of oral processing are shown in Table 1. The major free amino acids in sturgeon meat were alanine (Ala, 53.40–63.35 mg/100 g), glycine (Gly, 28.43–33.54 mg/100 g), serine (Ser, 25.03–32.58 mg/100 g), and glutamic acid (Glu, 27.22–31.77 mg/100 g), of which Ala (taste activity value [TAV] = 0.89–1.06) and Glu (TAV = 0.91–1.06) had a significant impact on taste as their TAVs were close to 1.0. Glu is well known as an umami-associated amino acid that contributes to the associated taste characteristics. During oral processing, the concentrations of valine, isoleucine, and leucine decreased significantly ($p < 0.05$). Free amino acids are generally produced by proteolysis or certain metabolic pathways, and they are important precursors for the formation of flavor substances (Shigematsu, Hayashi, Nakajima, Uno, & Fujii, 2010). The 5′-nucleotides that contribute to flavor include AMP, IMP, and GMP, which are important contributors to umami taste. The results showed that IMP (235.79–482.85 mg/100 g) was the main nucleotide component of steamed sturgeon meat. It has been reported that IMP is conducive to a pleasurable taste and is the reason for taste complexity and sweetness (Chi, Ji, Gao, Lu, & Meng, 2012). All the chewed samples exhibited increased nucleotide concentrations in comparison with the unchewed sample, which may have occurred because chewing can promote the release of substances from a food matrix. The TAVs of malic acid (6.04–6.10), lactic acid (14.79–20.30), and succinic acid (10.84–21.20) were greater than 1, which indicated that these compounds contributed significantly ($p < 0.05$) to the taste of sturgeon meat during oral processing. Lactic acid (1864.85–2560.39 mg/100 g, associated with sour and umami tastes) is the most abundant organic acid in steamed sturgeon meat and is also the main acid present in many aquaculture species (Liu, Meng, Tang, Wang, & Zhi, 2018). Organic acids are closely associated with the synthesis and metabolism of aromatic compounds, amino acids, and esters. With the prolongation of chewing time, the concentration of lactic acid increased gradually, but there was no significant difference between the concentrations of malic acid and succinic acid ($p < 0.05$). Inorganic ions are indispensable cofactors and enhancers of flavor in aquatic products. For example, Na^+ can act synergistically with nucleotides, Glu, etc., and thus make the overall umami taste prominent (Gong et al., 2016). The Na^+ concentration increased from 137.00 mg/100 g to 452.34 mg/100 g within 24 s of oral processing and gradually became stable (404.66–452.34 mg/100 g) at 24–30 s. The result was consistent with the saltiness perception results in the D-QDA. Meanwhile, the pattern of sodium release here was consistent with the results of previous studies (Pu et al., 2021), which indicated that the release of sodium ions gradually reached saturation during the later phase of oral processing.

To interpret variable relationships between taste compounds and attributes, Partial Least Squares regression (PLSR) analysis were explored. Latic acid, Leu, and Na^+ appear to strongly negatively correlated with umami, saltiness, and bitterness. The results showed that the release of umami and saltiness of steamed sturgeon meat was higher than that of 12 s and 18 s when the processing time of steamed sturgeon meat was 6 s, which was consistent with sensory evaluation. The first principal dimension of the analysis indicates thet the perceived levels of umami, saltiness and bitterness were positively associated with nucleotide, malic acid, succinic acid, glycine, lysine. lactic acid, valine, leucine, isoleucine were negatively correlated with the three kinds of taste perception (Fig. 1B and C). It also illustrated that the contribution of these compounds to taste perception is not a single pathway, and there may be a complex synergism effect between them.

Release of volatile components during oral processing

A total of 42 volatile compounds were detected by GC-IMS during oral processing of steamed sturgeon meat. Of these volatile compounds, 38 compounds were identified, namely, 11 alcohols, four esters, six acids, seven aldehydes, five ketones, two furans, and three other compounds. The drift times, retention times, and relative signal intensities (RSIs) are listed in Table S2, and the dynamic changes in each substance were visually revealed in the form of fingerprints (Fig. 1E). PCA analysis of volatiles with different oral processing time detected by GC-IMS was shown in Fig. 1F. Alcohols are generally considered to be important components of meat volatiles and are closely associated with the characteristic fatty flavor of meat (Zhang, Ding, Gu, Zhu, Zhou, & Ding, 2020). During oral processing, (*E*)-3-hexen-1-ol had the highest RSI (7802.06–11837.81). The RSI of 1-hexanol always exhibited an upward trend during chewing, which may have been caused by autoxidation of polyunsaturated fatty acids (Yang, Sun, Pan, Wang, & Cao, 2018). In addition, 1-octen-3-ol has been found to be the main volatile odor-active alcohol in many aquatic products such as fish, clams, crabs, and oysters with grassy, earthy, and mushroom flavors (Han, Zhang, Li, Wang, Chen, & Kong, 2020). As the main products of lipid oxidation, aldehydes have lower olfactory thresholds and distinctive odor characteristics such as rancid, sweet, and floral (Zhang, Ding, Gu, Zhu, Zhou, & Ding, 2020). Saturated linear aldehydes such as octanal have been reported to be detected in products from many different species of fish and have been identified as important volatile flavor components (Moretti, Vasconi, Caprino, & Bellagamba, 2017). Benzaldehyde, which is an aromatic compound with a bitter almond flavor, had the highest RSI (16681.95–18283.88) during chewing (Mohamed, Man, Mustafa, & Manap, 2012). Lipid degradation has been found to promote the formation of certain aliphatic ketones. For example, 2-heptanone, which is mainly produced by the oxidation of linoleic acid, may improve the flavor of meat products (Han, Zhang, Li, Wang, Chen, & Kong, 2020). Moreover, 2,3-butanedione has a strong buttery and creamy flavor and a lower olfactory threshold. Nevertheless, ketones exhibited little change in RSI during oral processing of steamed sturgeon meat, which indicated

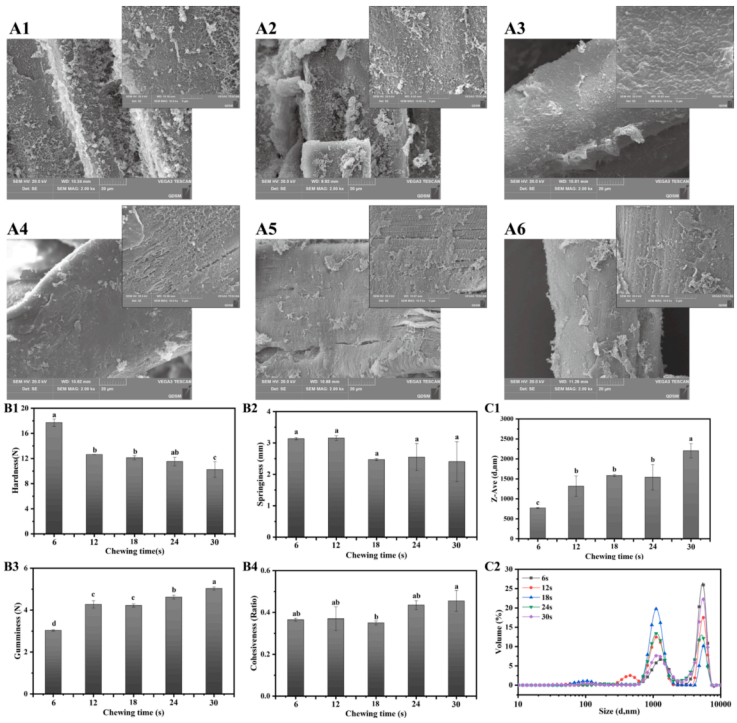

Fig. 2. A: Selected scanning electron microscopic images of the oral bolus of six steamed sturgeon meat samples (A1-A6: thermally processed meat without mastication and chewed for 6 s, 12 s, 18 s, 24 s, and 30 s); B: Instrumental texture analysis data for meat bolus with different chewing time (B1-B4, hardness, springiness, gumminess, and cohesiveness). C: Distribution of particle sizes for different oral processed samples (C1, Z-average particle size, C2, particle size distribution).

that their contribution to flavor release was not significant. Esters can be synthesized from free fatty acids and alcohols (esterification) or from triglycerides and ethanol by transesterification (alcoholysis) (Liu, Holland, & Crow, 2004). Moreover, some typical patterns of changes in volatile compounds were observed during oral processing of steamed sturgeon meat. For instance, the changes in 1-octanol, 1-heptanol, 1-octen-3-ol, (*E*)-3-hexen-1-ol, octanal, ethyl 2-hydroxypropanoate, methyldiisopropylamine, hexanenitrile, 3-methylbutyric acid, 2,5-dimethylfuran, pentanoic acid and limonene exhibited similar tendencies in that their RSIs increased to a maximum at 6–12 s and then decreased steadily until the swallowing point, but their final RSIs were still higher than the initial values. Conversely, the RSIs of ethyl acetate and methyl 3-methylbutanoate continued to decrease and reached a minimum during 0–12 s and then increased gradually until the swallowing point.

Microstructure, texture, and particle size analysis of meat bolus

Fig. 2A shows the microstructural images, viewed under a scanning electron microscope, of the oral boluses of the five samples masticated by the participants with different chewing time (Fig. 2A2-A6) and the sturgeon meat without mastication (Fig. 2A1). There is no remarkable difference in microstructure between samples. The hardness decreased with the increasing chewing time, while the gumminess increased (Fig. 2B1 and 2B3). There was no significant change of the springiness and cohesiveness during the bolus formation (Fig. 2B2 and 2B4). However, there is no significant differences in cohesiveness among all the treatments. The particle size distribution shown by the sturgeon meat bolus during the oral processing was examined (Fig. 2C1 and C2). The results showed that, the sizes of the bolus particles during oral processing had no significant pattern of change, the bolus just before swallowing showed the largest average particle size in all subjects (Fig. 2C1). However, the trend was in an opposite direction from the particle size distribution (Fig. 2C2). The type of matrix and the oral processed time affected the spread of the particle size distribution (Hutchings, Foster, Bronlund, Lentle, Jones, & Morgenstern, 2011). The results showed that the way by which the particle surfaces interact with the substrate could affect the adhesion of the particle to the substrate, and thus affects the difficulty of oral processing of the particle. And also the particle size correlated with chemical changes of the sturgeon meat such as protein and lipid degradation and oxidation.

Lipid deposition analysis during oral processing

High-resolution non-targeted lipidomics was used to perform large-scale characterization of the lipids present in three oral processed sample groups (C1 (0 s), C3 (12 s), and C6 (30 s)). A number of 1216 species of lipid composition were identified and mainly divided into 16 categories, of which 32.54 % for TG (triglyceride), 16.52 % for PE (phosphatidylethanolamine), 9.20 % for PC (phosphatidylcholine), is three major lipids of all categories (Fig. 3a). Fig. 3b lists the dominant eight kinds of lipid composition during oral processing, the number of TG and PC species increased first and then decreased. On the contrary, lipids belonging to PE, FA (fatty acids) and SM (sphingomyelin) showed a trend of decreasing first and then increasing. DG (diacylglycerol) species gradually reduced and the species of LPC (lysophosphatidylcholine) increased during the bolus formation, which suggested that oral processing may cause the mutual transformation of lipid precursors. On the basis of multivariate statistical analysis, the significantly different lipids were selected with variable importance projection (VIP) is greater than or equal to 1 and p value is <0.05, a number of 180 kinds of lipids including 23 PC, 23 LPC, 34 PE, 18 CAR (acylcarnitine), 33 FA, 11 TG, 7 DG, 7 LPE (hemolysis phosphatidylethanolamine) and 6 CL (cardiolipin coenzyme). Fig. 3c showed the top fifteen differential lipids which including eight phospholipids in all processed samples. Cluster analysis was performed on the differential lipids of sturgeon meat with different oral processing time, and the results are shown in the Fig. 3d. The lipid components including FA, DG and PI (phosphatidyl inositol) reduced gradually as the extension of oral processing time, while the

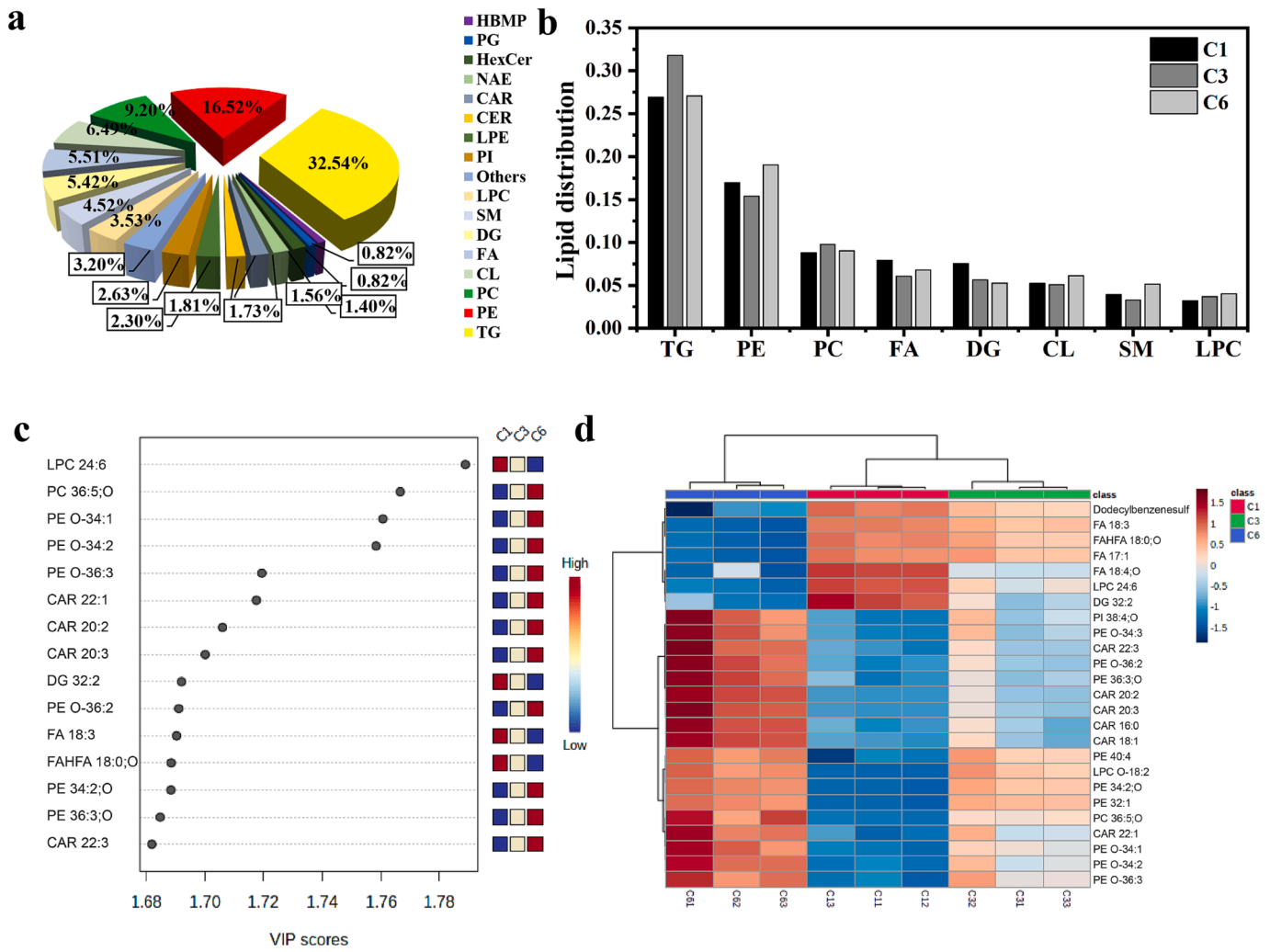

Fig. 3. (a) Total lipid species distribution in all processed samples; (b) Dynamic change of lipid species distribution in the samples with oral processing time of 0 s, 12 s, and 30 s; (c) Top fifteen significantly differential lipids; (d) Cluster heat map of differential lipids with variable importance projection value greater than or equal to 1 (top 25).

relative concentration of PE, PC, and LPC showed a contrast trend. This phenomenon suggested that significant differences in the lipid profiles of bolus formation were associated with oral processing (Pu et al., 2021). Correlation analysis of phospholipids with characteristic odor and taste components is shown in Figure S1. Studies have shown that in addition to product substrates and aroma characteristics, the oral environment also influences the aroma release (Pivk, Godinot, Keller, Antille, Juillerat, & Raspor, 2008). A number of fifteen LPC showed significant positive correlation with eight amino acids such as lysine and methionine and showed negative correlation with 1-heptanol, octan-1-ol, (*Z*)-3-nonen-1-ol. These lipids are to highlight the mechanisms and chemical compounds responsible for meat flavor and off-flavor development (Yang, Sun, Pan, Wang, & Cao, 2018). A conclusion in the literature investigated that linear aldehydes such as hexanal are mainly derived from the peroxidation of unsaturated fatty acids (Chen, 2009). Unsaturated fatty acids can generate different hydroperoxides and produce a large amount of volatile components during lipid oxidation via different decomposition pathways. Various enzymes such as lipoprotein lipases, lipolysis occurs to participate the degradation of phospholipid hydroperoxides, which was closely related to oral processing. Knowing how lipids changed and retained on the oral surface and bolus formation can bring valuable insight in understanding the differences in perception of aroma, texture, or even taste.

Qualitative and quantitative analysis of proteins

Taking into account the results of dynamic sensory evaluation and the release of flavor substances, the samples with oral processing time of 12 s (C3) were selected for proteomic analysis. In the study, a total of 2369 proteins were identified in the samples C1 (0 s), C3 (12 s), and C6 (30 s) by TMT proteomics. Cluster analysis was carried out on the proteins in the three sample groups. C3 and C6 were first aggregated into a cluster while C1 belonged to another cluster, which revealed that the similarity between C3 and C6 was higher than that between either of them and C1. On the basis of all the identified proteins, principal component analysis (PCA) was used to quantitatively distinguish the three groups of samples. A PCA score plot shows that 62.1 % of the variance in the data was explained by the first two principal components, of which the first explained 43.4 % and the second explained 18.7 %. In addition, samples with different chewing times were completely separate and located far apart on the scatter plot. The above phenomena indicated that there were significant differences in proteomes among the three samples, which was consistent with the results of the heat map analysis. The distributions of differential proteins in the samples are illustrated in Fig. 4a. Differential proteins were screened by combining fold change (FC) with Student's *t*-test. Proteins with an FC of >1.20 or <0.83 relative to the control and a two-tailed *P*-value of <0.05 were considered to be significantly upregulated or downregulated,

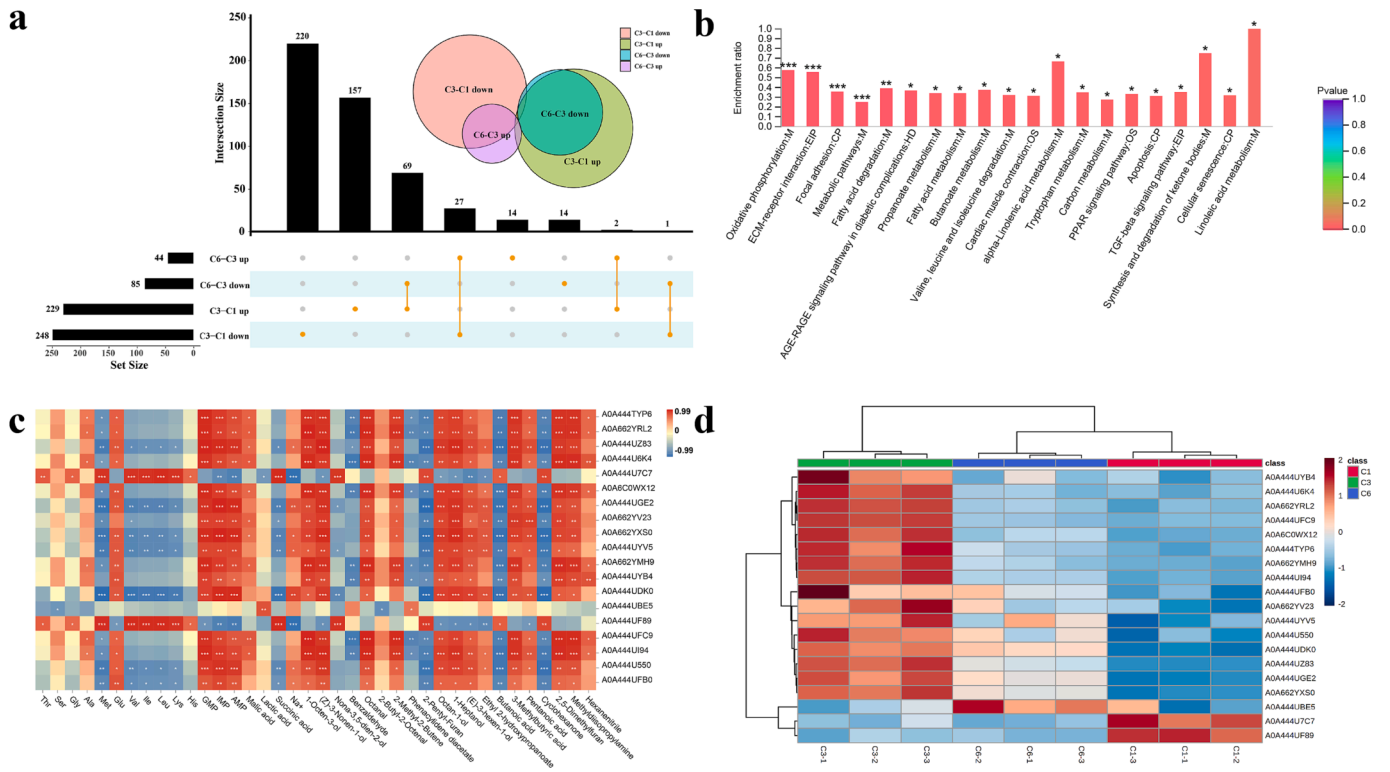

Fig. 4. (a) Upset plot of the distribution of differential proteins in steamed sturgeon meat with different oral processing times. The numbers on the bars represent the differentially abundant proteins belonging to each intersection group. (b) KEGG pathway enrichment analysis of differentially abundant proteins (C3 vs C1 for comparison). The top 20 pathways are listed according to their *P*-values (*$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$); (c) Pearson's correlations between flavor-associated proteins and some flavor substances throughout the oral processing of steamed sturgeon meat (*$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$); (d) Heat map of flavor-associated proteins in steamed sturgeon meat with different oral processing times.

respectively. A total of 589 differential proteins from the three sample groups were screened according to the above criteria, of which 476 (228 upregulated and 248 downregulated) were differentially abundant between C3 and C1 (control) and 127 (43 upregulated and 84 downregulated) were differentially abundant between C6 and C3 (control).

Bioinformatics analysis of differentially abundant proteins

Gene Ontology (GO) is the international standard classification system of gene function. GO analysis has established an information resource library for various species to annotate gene and protein functions, which is divided into cellular component (CC), molecular function (MF), and biological process (BP), respectively. Because of the large number of differential proteins, their bioanalysis was crucial to the proteomics results. Consequently, the biological functions of the differential proteins in the three sample groups of steamed sturgeon meat at different stages of oral processing were annotated by GO analysis. All 589 differential proteins were examined by Fisher's exact test, and it was considered that a GO function was significantly enriched only when the corrected *P*-value was <0.05. The results of GO annotation indicated that all the differential proteins could be classified into three categories in the light of their functional activities, namely, biological process, molecular function, and cellular component. In the earlier phase of oral processing, the vast majority of differential proteins were associated with cellular process (45.17 %) and metabolic process (28.80 %), whereas others were involved in biological regulation (21.22 %), localization (11.13 %), and developmental process (9.88 %) under biological process. Analysis of molecular function showed that the investigated proteins were mainly associated with binding (51.05 %), catalytic activity (42.90 %), and transporter activity (7.56 %). The cellular component functions of the differential proteins were mainly located under cellular anatomical entity (26.27 %) and protein-containing complex (23.53 %). The number of differential proteins produced in the later phase of oral processing decreased drastically, but the distribution of functional annotations did not change overall. KEGG pathway enrichment analysis was performed for the differentially expressed proteins to further analyze the corresponding key metabolic pathways involved in oral processing. Fig. 4b shows the top 20 pathways in terms of enrichment, where the horizontal axis lists the pathway names, the abbreviation before each pathway represents the corresponding category, the vertical axis corresponds to the enrichment rate, and the column color gradient represents the significance of enrichment. The columns corresponding to significantly enriched pathways are marked with asterisks (*$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$). It can be seen that the most highly enriched pathways in C3 in comparison with C1 included oxidative phosphorylation, ECM-receptor interaction, focal adhesion, metabolic pathways, and fatty acid degradation. The most highly enriched pathways in C6 in comparison with C3 included oxidative phosphorylation, cardiac muscle contraction, metabolic pathways, glycerophospholipid metabolism, and propanoate metabolism.

In order to investigate the corresponding relationships between proteins and KEGG pathway annotation and enrichment, a KEGG enrichment chord diagram was created for in-depth analysis. The Z-score indicated whether the corresponding pathway was more likely to be activated (>0) or inhibited (<0). Oxidative phosphorylation had the highest $\log_2$ FC value and Z-score (6.41). Metabolic pathways, fatty acid degradation, and valine, leucine and isoleucine degradation also had high Z-scores: that is, these pathways were more likely to be activated during oral processing. The chord diagram for C6 vs C3 for comparison revealed that the Z-scores of oxidative phosphorylation, metabolic pathways, and valine, leucine and isoleucine degradation were <0. This was due to downregulation of the expression of proteins involved in these pathways, which resulted in inhibition of the reaction pathway.

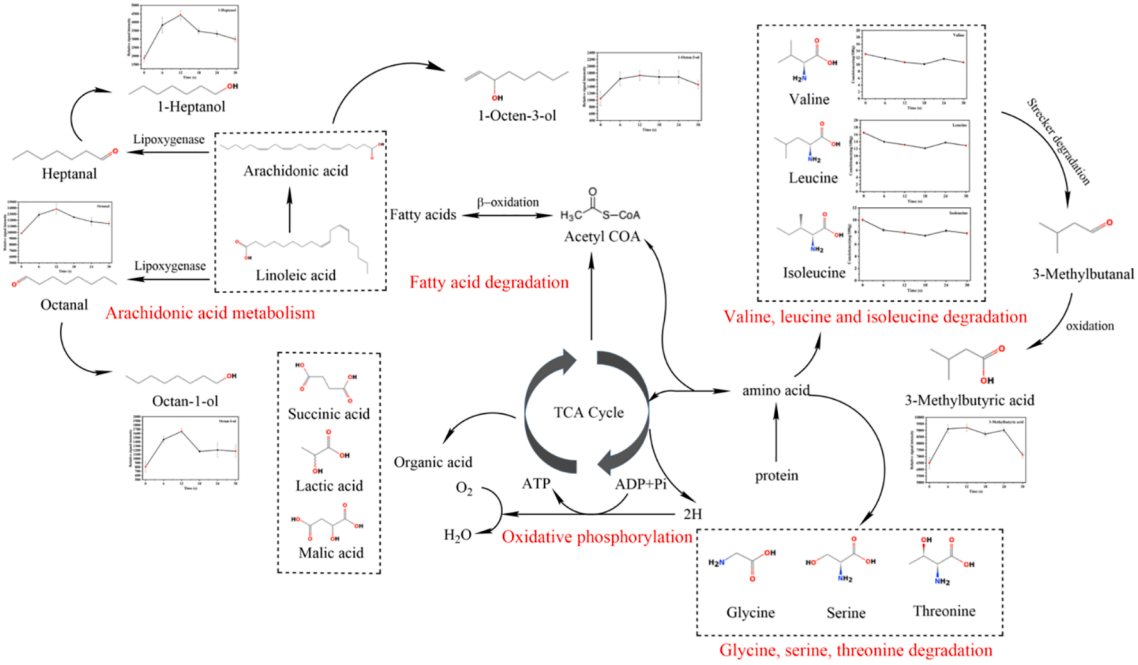

Fig. 5. Schematic diagram of potential sources of representative flavor compounds in steamed sturgeon meat during oral processing based on the KEGG.

Therefore, it could be determined that most of the reactions associated with flavor release occurred in the early stage of oral processing. In continuation, a comprehensive metabolic map was generated using proteins with statistically significantly different abundances to identify key pathways (C3 vs C0). The processes affected during the earlier phase of oral processing were primarily those involved in (1) fatty acid degradation; (2) valine, leucine and isoleucine degradation; (3) glycine, serine and threonine metabolism; (4) oxidative phosphorylation; and (5) arachidonic acid metabolism. The results indicated that fatty acids and amino acids were the main flavor precursors in the oral processing of steamed sturgeon meat.

Relationships between differentially abundant proteins and flavor substances

Steamed sturgeon meat underwent many reactions during oral processing, and hence the formation of flavor compounds was a complex process. Pathways regulated by distinct proteases could potentially be the underlying factors affecting the release of flavor from steamed sturgeon meat. For this reason, 19 differential proteins associated with key metabolic pathways were selected according to the KEGG enrichment chord diagram and metabolic pathways identified by iPath (Table S3).

Fig. 4c showed a heat map of the 19 flavor-associated proteins in steamed sturgeon meat with different oral processing times. The results showed that most of proteins were the highest abundance at 12 s of oral processing. And then the correlations between these proteins and taste and volatile compounds with significant changes in abundance were analyzed by Pearson correlation analysis (Fig. 4b). The results indicated strong positive correlations between volatile compounds and enzymes associated with fatty acid metabolic pathways, such as A0A444TYP6 and A0A662YRL2, which are all key enzymes involved in the β-oxidation of fatty acids. The great majority of the volatile compounds released from steamed sturgeon meat during chewing were the products of lipid degradation. In particular, 1-octanol, 1-heptanol, and other alcohols were principally produced by the action of a specific lipoxygenase on polyunsaturated fatty acids (Jin, Gouda, Jin, & Ma, 2019). A characteristic volatile component of aquatic products, namely, 1-octen-3-ol, was produced by the reaction of alkoxy groups with fatty molecules during the oxidation of lipids (Huang et al., 2021). A0A444U550 and A0A444UFB0 are enzymes involved in the metabolism of arachidonic acid. Arachidonic acid is a product of linoleic acid metabolism, and straight-chain aldehydes such as octanal are mainly generated by the oxidative breakdown of unsaturated fatty acids, for instance, linoleic acid and arachidonic acid (Liu, Wang, Zhang, Shen, Hui, & Ma, 2020).

Amino acids also brought about a pivotal effect in the release of flavor from steamed sturgeon meat. After chewing and immersion in saliva, some free amino acids were released from the food matrix and became important flavor substances. Concurrently, amino acids could also serve as precursors for the Maillard reaction or Strecker degradation reaction and thus were closely related to volatile flavor substances (Zhang, Zhao, Zhao, Yang, & Liu, 2019). Valine, leucine, and isoleucine were significantly ($p < 0.05$) inversely correlated with a protease that is involved in valine, leucine and isoleucine degradation, which suggested that the above three amino acids may be consumed in this pathway. Leucine and isoleucine could be degraded to form 3-methylbutanal, benzaldehyde, etc., and these aldehydes could also further react to generate the corresponding alcohols and acids such as 3-methylbutyric acid (Shi, Li, & Huang, 2019). Besides, leucine can also be degraded to form 2-methylbutyric acid (Apajalahti, Vienola, Raatikainen, Holder, & Moran, 2019), which was also in accordance with the GC-IMS results. There was also a positive correlation between enzymes involved in glycine, serine and threonine metabolism and volatile substances. The combined effect of multiple amino acids and fatty acids made steamed sturgeon meat release a unique flavor during chewing. In addition to the enzymatic catalytic effects of salivary proteins on food, chemical interactions between ingredients were also significant factors that affected flavor release during oral processing. Ions in the food matrix could interact with mucin and other substances in saliva, which would result in interactions between mucin monomers that would change their binding sites with flavor substances and ultimately affect the release of flavor substances (Friel & Taylor, 2001). Thus, it can be seen that the release of flavor substances during oral processing was the result of multiple factors.

Schematic diagram of potential sources of representative flavor compounds in steamed sturgeon meat during oral processing was shown in Fig. 5. The concentrations of 1-heptanol (musty, leafy, violet, herbal, green, sweet, woody, peony), heptanal (fresh, aldehydic, fatty, green,

herbal), octanal (aldehydic, waxy, citrus, orange, peel, green, herbal, fresh, fatty), octan-1-ol (waxy, green, citrus, aldehydic and floral with a sweet, fatty, coconut nuance), 1-octen-3-ol (mushroom, earthy, green, oily, fungal, raw, chicken), 3-methylbutyric acid (cheesy, dairy, acidic, sour, pungent, fruity, ripe, fatty, fruity) was significantly ($p < 0.05$) changed during oral processing which derived from the metabolic pathways of lipids and proteins. Oral processing behavior significantly ($p < 0.05$) alters dynamic mouthfeel and taste perception, which are closely related to changes in food composition (Jia, Shi, Zhang, Shi, & Chu, 2021). To better understand the possible mechanisms and interactions involved in bolus formation, it is important to study the behavior of lipids and proteins in mouth.

Conclusions

Flavor and texture perception were linked with the deposition and retention of lipids and proteins on the tongue and palate. An analysis of lipid and protein correlations with flavor compounds suggests that the release of flavor from steamed sturgeon meat during oral processing is closely associated with fatty acid degradation and amino acid metabolism. The change of lipid and protein deposition on the tongue shows variation and changed with the procedure of bolus formation. The present study elucidated flavor release during oral processing from lipidomics and proteomics perspective, although more work is needed with regard to detailed and specific mechanisms owing to their complexity.

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

No data was used for the research described in the article.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2022.100553.

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