# Dynamic Release and Perception of Key Odorants in Grilled Eel During Chewing

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**Abstract:** The release mechanism of odorants in the oral cavity during consumption directly affects sensory attributes, consumers' preferences, and ultimately purchase intent. Targets was set to monitor in real-time the key odorants released from grilled eel during mastication via an atmospheric pressure chemical ionization mass spectrometry (APCI-MS) connected with a nose interface. The release and perception of odorants during mastication were divided into three distinct phases. Dimethyl sulfide was the main odorant in the first stage. The release and perception of fishy aromas were predominant in the middle and last stages of mastication contributed by trimethylamine, 1-penten-3-ol, and 2-methyl-1-butanol. Chewing behavior experiments suggested that extending the chewing period to >20 s and having a chewing frequency of 2 cycles/s could enhance the aroma delivery of grilled eel and optimize the consumer experience. Consequently, the results explained the relationship between aroma release and the optimal chewing behavior for grilled eel consumption.

**Keywords:** grilled eel; mastication; APCI-MS; MS-nose; odorants release; dynamic monitoring

## 1 Introduction

Eel is one of the main economic fish in the world. About 300,000 tons of eel are produced every year all over the world, with a value of commodity trade and production of about 3,000,000,000 dollars (FAO, 2021). Grilled eel is the most important consumption form of eel. As a famous traditional fish product, grilled eel is favored by consumers for its unique flavor and complex aroma profile. The aroma perception during consumption directly affects ultimately purchase intent. The composition of food and the way of oral processing directly affect the release and perception of aroma compounds. Therefore, understanding the characteristic aroma release mechanisms that occur during eating grilled eel could further contribute to optimize the product, expand the market, and improve acceptability.

For an aroma compound to be perceived, it first must partition out of the food material. There are two main factors that influence aroma release from food during consumption (Guichard, 2015; Lyu, Chen, Nie, Xu, & Tang, 2021; Ployon, Morzel, & Canon, 2017). The first is the intrinsic physical and chemical properties of the aroma compound, it is important to note that these vary for each individual aroma. Vapor pressure is one of the most important properties that drive aroma partitioning and is a measure of the tendency of a compound to change into the gas state and can be defined as the ratio of the vapor-liquid concentration at equilibrium. Other important properties include the hydrophobicity and molecular polarity (Pérez-Jiménez, Muñoz-González, & Pozo-Bayón, 2021). The second factor is the physicochemical properties of the food matrices, which vary significantly due to different

physical states and compositions (Pu, Duan, Huang, Zhang, Zhang, Sun, et al., 2021). Overall, these two factors interact and together regulate the release of aroma compounds during mastication. However, this is further complicated as both factors will evolve during the process of food oral processing.

Food oral processing involves the breakdown of food using the teeth, mechanical stirring using the tongue, food bolus formation through the addition of saliva and manipulation of the food material in the oral cavity (How, Jones, Morgenstern, Gray-Stuart, Bronlund, Saint-Eve, et al., 2021; Sarkar, Soltanahmadi, Chen, & Stokes, 2021). Within this complex process, several factors directly impact aroma perception, include the length of chewing time, speed of chewing, saliva composition, and the changes in the composition of saliva during chewing. The state of food after oral processing is affected by food properties and consumer characteristics. Consumer characteristics such as age, gender, and ethnicity also impact oral processing behavior (Ketel, de Wijk, de Graaf, & Stieger, 2020). Elderly consumed food with a higher number of chews and longer consumption time than young adults. One study has found that the sensory perception of yogurts about flavor attributes, crumbliness, juiciness, and perceived particle size was similar for healthy young adults and healthy elderly (Aguayo-Mendoza, Santagiuliana, Ong, Piqueras-Fiszman, Scholten, & Stieger, 2020). The analysis of food flavor perception during mastication could explain the general reason for the food choice of consumers to some extent.

Retronasal olfaction is the perception of aroma during consumption by direct delivery to the nasal cavity from the mouth (He, Dukes, & Kay, 2020). It is critical for perception (Wilson, 2021) and drives food choice and acceptability when combined with the other sensory modalities such as taste, trigeminal, and mouthfeel (Duffy, Hayes, & Sharafi, 2020). Ultimately, consumers choose foods based on the quality and balance of food flavor. Therefore, it is very important to understand the real-time perception of key odorants during eating.

There is a complex and dynamic interplay between these three phenomena, 1) the physical and chemical properties of the aroma compound and the food matrix, 2) the dynamic process of food oral processing and 3) retronasal olfaction. For example, the opening and closing of the oral cavity during eating would influence the timing and extent of aroma release via changes in the mechanical transfer of volatile compounds to the nasal cavity (Trelea, Atlan, Déléris, Saint-Eve, Marin, & Souchon, 2008). The first expiration after swallowing delivers the highest amount of aroma to the nose and subsequent exhalations typically deliver less, but also different ratios of aroma compounds are present in the nose due to changes in the aroma profile in the oral cavity. For model cheeses, the total aroma release was positively correlated with the number of swallows (Boisard, Tournier, Sémon, Noirot,

Guichard, & Salles, 2014). Bolus size also impacts aroma release through different dilution rates and Genovese et al. noted that sip volume could affect aroma release with the same beverage/saliva ratio in in-vitro experiments (Genovese, Moio, Sacchi, & Piombino, 2015).

The complex chemistry, fast rates of change, and variable biological events that occur during food oral processing make it very challenging to measure the release of aroma compounds in real-time. Some studies have reported that the changes in aroma perception during drinking wine and eating cheese could be observed by simulating oral chewing in-vitro (Criado, Muñoz-González, & Pozo-Bayón, 2021; Panda, Chen, & Benjamin, 2020; Sharma Khanal, Bhandari, Prakash, & Bansal, 2020). The influences of in-vitro swallowing, in-vitro tooth-breaking, in-vitro tongue stirring, and in-vitro saliva release on the aroma release of food can be effectively analyzed via this in-vitro method. However, there are still marked differences between the in-vitro simulation model and the in-vivo human oral environment. The combination of real-time instrumental analysis of the release of volatile aroma compounds during the eating process can be achieved using atmospheric pressure chemical ionization mass spectrometry (APCI-MS). APCI-MS is well known as a fast real-time analytical technique for volatile compounds and can be used to monitor changes in known volatile compounds in the nasal cavity during eating (Genovese, Yang, Linforth, Sacchi, & Fisk, 2018). APCI-MS has a significant advantage in providing biologically relevant data compared to more static approaches such as gas chromatography mass spectrometer (GC-MS), when determining the influence of various factors on aroma transfer, such as the structure/texture of food matrix (A. Tarrega, Yven, Sémon, & Salles, 2008), oral dynamic processing (Salles, Chagnon, Feron, Guichard, Laboure, Morzel, et al., 2010), and the presence of food ingredients such as fats (Genovese, Yang, Linforth, Sacchi, & Fisk, 2018), proteins (Amparo Tarrega, Yven, Semon, Mielle, & Salles, 2019) and polysaccharides (Su, Festring, Ayed, Yang, Sturrock, Linforth, et al., 2021). The combined benefits of APCI-MS are particularly important for tracking the dynamic release of aroma compounds in the food matrix during food oral processing and retronasal aroma delivery and perception.

Previously we have shown that grilled eels have a strong aroma. However, the perception of the aroma changed during chewing. To the best of the author's knowledge, there is no comprehensive understanding of the release of aroma compounds during food oral processing of grilled eels and how these changes during different chewing behaviors. Therefore, the impact of chewing time, chewing frequency, and saliva dilution on aroma release from grilled eel were investigated in-vivo, using APCI-MS with an MS nose interface. Dynamic descriptions of sensory

and tracking of aroma delivery were then combined to explain the relationship between aroma release and the optimal chewing behavior for grilled eel consumption.

## 2 Materials and methods

### 2.1 Preparation of grilled eel

Fresh eels (*Astroconger Myriaster*) were obtained from Qingdao Lumai Food Co., Ltd in Qingdao, P. R. China. Eels were slaughtered by trained personnel. Fasten the head of eels to the table and crack open its belly from end to end. Finally, eels were boning, eviscerated, cleaned, and cut into 6 cm × 4 cm × 0.7 cm (length × width × thickness) fillets. The fillets were stored at -80 °C no more than three months before grilling. The eel fillets were grilled in accordance with previous studies (Huang, Zheng, Chen, Zhang, Du, Dong, et al., 2019). The grilled eel fillets were vacuum-packed and stored at -80 °C before the experiment. When the experiment was carried out, the roasted eel samples were quickly thawed and reheated at the same conditions. Samples used in the same experiment were all from the same batch of the grilled eel.

### 2.2 Information of chemical reagent

Acetone, trimethylamine, 1-propanol, dimethyl sulfide, 2-methyl-1-propanol, 1-penten-3-ol, (z)-2-penten-1-ol, 3-pentanone, 3-methyl-butanal, 2-methyl-1-butanol, 1-pentanol, 3-pentanol, phenol, methyl-pyrazine, 2-ethyl-furan, 2-furanmethanol, propyl acetate, 1-hexanol, benzyl alcohol, 2,3-dimethyl-pyrazine, 2,5-dimethyl-pyrazine, 1-heptanol, 2-ethyl-5-methyl-pyrazine, phenylethyl alcohol were purchased from Sigma-Aldrich (Shanghai, China). Mequinol, 1-octanol, 2,3-dimethyl-5-ethylpyrazine, 2,6-diethyl-pyrazine, 1-nonanol, acetic acid, propanoic acid, acetoin, 2-vinylfuran, 2-methyl-butanoic acid, styrene, o-xylene, p-xylene were obtained from Aladdin (Shanghai, China). The purity of these flavor standards was more than 99%. The n-alkanes ($C_6$ to $C_{30}$) were acquired from Sigma-Aldrich (Shanghai, China). The isotope internal standards (n-nonan-d20, n-dodecane-d26, n-nonadecane-d40, n-tridecane-d28, n-hexadecane-d34) were purchased from Cambridge Isotope Laboratories, Inc.

### 2.3 Volatile compounds analysis

#### *2.3.1 Extraction method*

The method of Solid phase micro extraction (SPME) was referred to in the previous study about volatiles multiple extraction comparisons (Huang, Zhang, Zhu, Zhou, Du, Zhu, et al., 2021). When extracting volatiles, a 1 g sample was put into the headspace vial for extraction. The vial with the sample was sealed and preheated at 40 °C for 30 min. The length of the SPME fiber was 1 cm with three types of coating (DVB/CAR/PDMS). After the

adsorption process, SPME fiber was immediately desorbed at 250 °C for 2 min in the GC injection port. Then the fiber was desorbed at 250 °C for an additional 10 min via a conditioning port to avoid the carry-over effect.

***2.3.2 GC-MS(O) conditions***

The volatile compounds were detected and identified via GC-MS(O) (ISQ 7000, Thermo Scientific, USA). The polar analytical column was TG-WaxMS (30 m × 250 μm × 0.25 μm). The carrier gas was helium at a constant flow rate of 1 mL/min. Each sample was injected in splitless mode. Injector temperature was kept at 260 °C. The initial oven temperature was held at 35 °C for 3 min. And then the oven temperature was raised at 5 °C/min to 250 °C and held for 10 min. The ion source was EI with 70 eV electron energy and 230 °C source temperature. The scan range was from 30 to 450 m/z. The sniffing port could be connected to the GC and split the eluate for panelist aroma perception during sniffing. When sniffing, the trained panelist felt the aroma by placing his/her nose above the sniffing port. The intensity, odor, and retention time of the chromatographic effluent were recorded by panelists. The number of perception times of each compound by panelists during sniffing was counted for detection frequency calculation (Pollien, Ott, Montigon, Baumgartner, Muñoz-Box, & Chaintreau, 1997). The sniffing experiments were conducted by 10 panelists. Analyses were repeated in duplicate by each panelist.

***2.3.3 Compounds identification***

The volatile compounds identification was referred to in the previous study (Huang, et al., 2019). The NIST14 and Wiley11 library were used for matching the acquired mass spectrogram of volatiles from samples. The RI of volatile compounds was calculated according to the retention time of n-alkanes ($C_6$ to $C_{30}$). The reverse match factors of identified compounds were bigger than 750, whose RI value differ within 5 from those in the database. The volatile standards were used to further confirmed the identified compounds via their RI, mass spectrogram, and odor.

***2.3.4 Quantitation analysis of aroma compounds***

The quantitation analysis of key aroma compounds was carried out by the high-throughput analysis method of multi-isotope internal standards combined with the external standards method. The retention time of the five isotopes n-alkanes internal standards were distributed in stages and covered the retention time range of most of the volatile compounds. The isotope internal standards were used to regression all correct the contents of key aroma compounds and calculate the different extraction and injection efficiency of methods. The standard compounds were used as external standards for quantitative analysis. A high concentration stock mixed standards solution was

prepared with ethyl acetate and finally diluted with ultrapure water to 10 μg/mL. The mixed standards solution was stored at 4 °C. The eight-point calibration curve range from 1 to 100 ng/mL of each standard compound was built and used to calculate the concentration of key compounds.

**2.4 Sensory comparisons of aroma**

The sensory experiment was conducted via the Quantitative Descriptive Analysis (QDA) method referred to in the previous study (Huang, et al., 2021). Twelve panelists (ages ranging from 23 to 35) with both genders were selected from 80 members of the laboratory (students and teachers) via sensitivity test and training. The criteria for sensory assessment of aroma, included descriptive terminology, the aroma judgment, and odor intensity standard, were developed before evaluation. During chewing experiments, panelists rinsed their oral cavity with water between each group interval. After chewing, some of the samples were swallowed and recorded their characteristic, while some of the samples were spit into the plate and left for further observation. The experiments were performed for 6 replicate sessions by 12 panelists.

**2.5 Key odorants dynamic monitoring during chewing**

***2.5.1 chewing method***

The effect of chewing time, chewing frequency, and saliva dilution on the key odorants releasing and perception were investigated in this experiment. When the effect of chewing time was examined, the chewing frequency remained at 2 cycles/s and the chewing time was divided into five groups, each with a difference of 5 seconds, from 5 seconds to 25 seconds. The chewing time of each sample was fixed at 20 s while observing the effect of chewing frequency. The chewing frequencies were range from 1 cycles/s to 3 cycles/s. Distilled water was added during chewing from 2-10 mL to analyze the influence of saliva dilution. In this condition, the chewing time was 20 s and the chewing frequency was 2 cycles/s.

***2.5.2 Odorants dynamic monitoring***

The key odorant dynamic monitoring during chewing was performed with an APCI-MS (Waters Corporation, Milford, MA, USA) connected with an MS nose interface (Micromass, Manchester, UK) (Genovese, Yang, Linforth, Sacchi, & Fisk, 2018). The characteristic monitoring ions of key aroma compounds were screened with standards via full scanning, selective ion recording (SIR), and multiple reaction monitoring (MRM). The standards were put into a headspace vial and directly inhaled at a rate of 30 mL/min. The ions of Mass range were from 30 to 300. The voltage was 30 eV in full scanning mode and SIR mode, while the MRM mode was 10 eV. The source temperature

was 75 °C. The dwell time was 0.02 s. Sampling took place for 10 s, enough for the signal to plateau. When monitoring the key odorants of grilled eel during chewing, the MS nose was connected to the nose of panelists. The panelists chewing the sample and exhaled the aroma gas out of the nose into APCI-MS. Each peak was integrated with Mass Lynx (Waters, UK). The odorants content was determined by relative quantification. The characteristic ion intensity of multi-isotope internal standards and the key odorants were compared to calculate the concentration. The release rate was calculated by the slope of the real-time response curve of the detected characteristic aroma compound. The dominance rate was calculated by the real-time OAV of the characteristic aroma compound during chewing.

### 2.6 Statistical analysis

Microsoft office 2016 (Microsoft, China) was used to data preliminary analysis and combine figures. R Studio (R i386 3.5.0, Free Software Foundation's GNU, USA) was selected to analyze and draw the plot. Analysis of PCA (Principal Component Analysis), heatmap, and correlation was conducted via MetaboAnalyst 5.0 (Xia Lab, USA). Boxplot was made via XLSTAT 2019 (Addinsoft, France). The statistical differences between different data were determined by SPSS v 24.0 (IBM, USA), including ANOVA, repeated ANOVA, t-test, paired t-test. The differences under different times were analyzed by repeated ANOVA. The differences among different methods were analyzed by paired t-test. The differences between two independent groups were analyzed by t-test, while multiple independent groups were analyzed by ANOVA.

## 3 Results and discussion

### 3.1 The identification of key odorants

The volatile compounds of grilled eel after chewing were extracted with SPME and identified by GC-MS. As shown in Figure 1, thirty-seven aroma compounds in the chewed grilled eel sample were identified by GC-MS(O). The results being a little different from those of grilled eel without chewing. These differences will be driven by a range of different factors which include the chemical properties of the volatile compound and the physicochemical properties of the food matrix which would change during chewing (Buettner & Beauchamp, 2010). In the chewed grilled eel sample, alcohols were the main compounds present during retronasal aroma perception during eating. Methyl-pyrazine, 2-furanmethanol, benzyl alcohol, 2,3-dimethyl-pyrazine, 2,5-dimethyl-pyrazine, 2,3-dimethyl-5-ethylpyrazine, and 2,6-diethyl-pyrazine were the main volatile aroma compounds that contributed to the grilled

aroma of eel. However, most of these compounds were released in lower amounts during chewing, resulting in a lower perceived grilled odor to consumers (Table S1).

OAV was introduced to evaluate the odor activity of the key compounds identified. In terms of OAV, all these grilled odor compounds were less than 1 which indicates that these compounds contributed less to the whole aroma during chewing. Six odorants (trimethylamine, dimethyl sulfide, 1-penten-3-ol, 2-methyl-1-butanol, styrene, and 1-heptanol) with OAV > 1 were found during chewing the grilled eel. These odorants could be perceived stably by panelists with the chewed grilled eel sample. At least eight of the twelve people were able to sniff these compounds. Among these six compounds, the OAV of trimethylamine (49.17) and 2-methyl-1-butanol (36.65) were more prominent than other compounds. Both compounds had a fish-like odor. Besides, 1-penten-3-ol were also contributed to fish-like odor with an OAV of 7.18, which indicated that fish-like odor might be the main aroma sense. Dimethyl sulfide and 1-heptanol had a significant impact on the aroma of grilled eel after chewing as well. They had their characteristic odor when they smelled alone. The odor of dimethyl sulfide was like cabbage. Its OAV was 8.21. The OAV of 1-heptanol was the smallest among these six compounds. Its odor was like an herb. Styrene might be a contaminant from the environment in which eels grow or packaging. It had a floral odor with an OAV of 1.79. Although different compounds have their own odor, a new odor would be presented after mixing (Andreas, Martin, Matthias, Bettina, Dietmar, Peter, et al., 2014; Berre, Béno, Ishii, Chabanet, Etiévant, & Thomasdanguin, 2008). All the compounds had different effects on the aroma perception of grilled eel during chewing. How they behave in the mouth during consumption, and ultimately the perception of ‘eating quality’ would drive preference and repeat buying. Further observation on the release and perception of these compounds under different chewing behavior could effectively analyze why consumers prefer to eat grilled eel.

**3.2 Bolus formation during chewing**

The chewing of the mouth is a dynamic process, and it involves many biological changes. In Figure 2-A, morphological changes of grilled eel after different chewing behavior could be found. During grilled eel consumed and masticated, dissolution in saliva leads to a transition in the physical states of food, a semisolid or solid to a liquid state. During oral processing, the grilled eel meat would be formed into a bolus. In this step, the incorporation of saliva was essential for bolus formation. It contributed to the moistening and rheological properties of grilled eel bolus, especially viscosity and spreadability (Ployon, Morzel, & Canon, 2017).

When chewing at a uniform rate, grilled eel meat gradually changed from lumpy to slurry as the chewing time increased. When chewed for up to 25 s, the crushed grilled eel meat condensed into a ball under the action of teeth, tongue, and saliva. When chewed faster, the grilled eel meat crumbled, failed to coalesce, and turned into a semisolid like the slurry state under the effect of saliva. When the speed exceeds two cycles per second, the grilled eel meat became small meat debris after chewing. The ability to chew directly affects the state of the food in the mouth, and thus the subsequent release and perception of aroma (Ketel, de Wijk, de Graaf, & Stieger, 2020). If the saliva was in the appropriate amount, it could moisten or condense the crumbled grilled eel meat. However, when the saliva release was diluted with water, the grilled eel meat would be more dispersed and harder to clump together after chewing, just like small particles dispersed in the liquid. Among them, when 2 mL of water was added to dilute the saliva during chewing grilled eel, the morphology of the grilled eel meat after chewing formed a slurry-like shape, and it was difficult to aggregate into a whole. The influence of saliva on the formation of such a state was mainly directly related to the dilution effect of water (Ployon, Morzel, & Canon, 2017).

**3.3 Establishment of the aroma monitoring method**

APCI-MS connected with an MS-nose has been well used for real-time analysis of the release of volatile aroma compounds during the eating process (Genovese, Yang, Linforth, Sacchi, & Fisk, 2018). Using the methods detailed previously, key aroma compounds were identified and selected for monitoring aroma release during chewing in the breath of panelists using APCI-MS connected with an MS nose interface (Figure 2-C). The results also showed that the known volatile compounds in the nasal cavity during eating grilled eel could be well detected in real-time. Analytical standards of trimethylamine, dimethyl sulfide, 1-penten-3-ol, 2-methyl-1-butanol, and 1-heptanol were used to identify characteristics ions for each analyte using full-scan mode. SIR mode and MRM mode were then used for screening and optimization of the analytical method. The characteristics ions of these five key odorants were 60 m/z, 63 m/z, 87 m/z, 89 m/z, and 117 m/z for trimethylamine, dimethyl sulfide, 1-penten-3-ol, 2-methyl-1-butanol, and 1-heptanol, respectively (Figure 2-B). Identification and quantification of compounds were then carried out using multiple reaction monitoring (MRM) (Table S2) using authentic standards for quantification.

The inter-day precision and within-day precision were measured to analyze the feasibility of this method (Table S3). The real-time monitoring method for odorants during chewing was acceptable with inter-day and within-day RSD both below 5%. However, panelist to panelist variation was present and RSDs were higher. Overall, the

RSDs were still within acceptable limits. The method was suitable to observe the differences in release behaviors of different aroma compounds during chewing.

### 3.4 The odorants monitoring of grilled eel during chewing

#### *3.4.1 Real-time releasing of key odorants during chewing*

Trimethylamine, dimethyl sulfide, 1-penten-3-ol, 2-methyl-1-butanol, and 1-heptanol were selected for monitoring aroma release during chewing and could be grouped into fish odor associated compounds (trimethylamine, 2-methyl-1-butanol and 1-penten-3-ol) and plant odor associated compounds (dimethyl sulfide and 1-heptanol). As chewing time increased, there was a resulting change in aroma concentration (Figure 3-B), rate of release (Figure 3-C), and relative contribution of each aroma compound compared to the overall aroma profile (Figure 3-A).

Trimethylamine, 2-methyl-1-butanol were found in the greatest abundance of all the compounds measured. The concentration of trimethylamine increased significantly over chewing time (Figure 3-B), whereas the concentration of the two other fish odor associated compounds (1-penten-3-ol and 2-methyl-1-butanol), behaved differently to trimethylamine, whilst their concentration increased with chewing time, the maximum concentration peaked at 20 seconds of chewing, the concentration then returned to a lower value with additional chewing, (Figure 3-B), this effect at 20 s was most evident for 1-penten-3-ol.

Whilst the dominance rates of all three fish odor-associated compounds changed over time, trimethylamine and 2-methyl-1-butanol are predicted to have the greatest contribution to the overall odor perception of grilled eel during consumption. The dominance rate of trimethylamine ranged from 0.47 to 0.62, while the dominance rate of 2-methyl-1-butanol and 1-penten-3-ol ranged from 0.25 to 0.35 and 0.02 to 0.07 respectively. It is important to note that changes in concentration were not directly correlated with the dominance rate. It was due to differences in aroma thresholds of the different odorants. The relative contribution of different aroma compounds to the overall aroma of grilled eel during chewing was very a complex dynamic process.

For the three aroma compounds with plant odor sensory attributes, the concentration of 1-heptanol peaked at 20 s of chewing and reduced with additional chewing, this process was like that observed previously for 2-methyl-1-butanol and 1-penten-3-ol. Dimethyl sulfide was present at the lowest concentration and behaved significantly differently to all other compounds, Dimethyl sulfide was present at the highest contraction at 10s of chewing and then reduced significantly with additional chewing time, suggesting a different delivery or loss mechanism. The

296 difference could also be seen from the change of the release rate of each characteristic odorant under different
297 chewing times. In Figure 3-C, the releasing rates of the key odorants under different chewing times were calculated
298 and plotted. In terms of trimethylamine and 2-methyl-1-butanol related to fish-like odor, their releasing rates
299 increased in the first 10 s and then decreased and stabilized to a certain value. In contrast, the release rates of 1-
300 penten-3-ol and 1-heptanol reached their maximum in the late chewing period. The release rate of dimethyl sulfide
301 kept fluctuating after increased to a certain value. According to this result, it could be speculated that dimethyl
302 sulfide in grilled eel was more easily released and maximized after a short chewing period. For grilled eel, most key
303 odor compounds need a long chewing time (20-25 s). The chewing time and the intensity of aroma perception
304 presented a good correlation. The results were consistent with the other related studies that chewing for 15-30 s
305 could obtain a great aroma perception (Muñoz-González, Feron, & Canon, 2021; van Ruth, Frasnelli, & Carbonell,
306 2008).

307 ***3.4.2 The dynamic changes of key odorants under different chewing frequency***

308 The chewing rate directly affected the efficiency of the oral processing of grilled eel. There was a direct impact
309 of chewing speed on aroma concentration (Figure 4-B), rate of release (Figure 4-C), and relative contribution of
310 each aroma compound compared to the overall aroma profile (Figure 4-A).

311 Chewing 1.5 to 2 cycles per second resulted in the highest concentration of aroma release for all five
312 compounds measured and in general the maximum concentration was found at a chewing frequency of 2 cycles/s
313 except 1-heptanol. The releasing amount of 1-heptanol reached the maximum with a chewing frequency at 1.5
314 cycles/s.

315 The dominance rate of most of the characteristic odorants fluctuated with the chewing frequency-changing
316 except dimethyl sulfide which was reasonably stable. The dominance rates of 2-methyl-1-butanol and 1-heptanol
317 behaved similarly with higher dominance rates at 1.5 and 2.5 cycles/s, and a lower rate at 2 cycles/s. Both two
318 compounds behaved inversely compared to trimethylamine which peaked at 1, 2, and 3 cycle/s. The dominance rate
319 of 1-penten-3-ol reached the maximum at 2 cycles/s. The impact of chewing rate on aroma release and dominance is
320 presumably due to changes in the oral processing breakdown pathway and airflow during the chewing process which
321 would directly affect the release, diffusion, and perception of odorants.

322 All 6 key odorants had the greatest releasing rate with chewing frequency ranging from 1.5 to 2.5 cycles per
323 second and a chewing rate of 2 cycles/s was on average most conducive to the effective release of odorants when

eating grilled eel, especially for the fish-like odor. Under different chewing speeds, it was expected that different odorants might have different release rates due to differences in their physicochemical properties. It suggested that 2 cycles/s was an optimum chewing frequency for eating grilled eel, which could be used to design foods with optimum oral processing breakdown pathways or simply by chewing carefully and swallowing slowly the consumer may enjoy a greater flavor of grilled eel. Some studies have also shown that chewing at suitable speed was more conducive to the perception of aroma (Hodgson, Linforth, & Taylor, 2003; Ruijschop, Zijlstra, Boelrijk, Dijkstra, Burgering, Graaf, et al., 2011). The rate of chewing directly affects the shape of food after oral processing and the mixing of saliva. Changes in Bolus morphology directly affect the release of characteristic aroma compounds.

***3.4.3 The dynamic changes of key odorants under different concentrations of saliva***

Saliva plays a significant role in aroma release during oral processing. There is a direct impact of dilution of saliva on aroma concentration (Figure 5-B), rate of release (Figure 5-C), and relative contribution of each aroma compound compared to the overall aroma profile (Figure 5-A).

In Figure 5-A, it could be found that the contribution of each key odorant changed because of the dilution of saliva. In the oral cavity, grilled eel meat, saliva, and air constituted the propagating phases for odorants. The dilution of odorants in saliva directly affects their release. Therefore, the variation of saliva concentration would directly affect the grilled eel characteristic aroma perception of consumers. The dominance rates of trimethylamine and 1-heptanol were greater at lower water dilution. When the panelists consumed grilled eel with 4 mL water to dilute the saliva, the dominance rate of trimethylamine reached its maximum. The dominance rate of 1-heptanol decreased with the increase of water dilution. In contrast, the dominance rates of dimethyl sulfide, 1-penten-3-ol, and 2-methyl-1-butanol were greatest at higher water dilution. When the panelists consumed grilled eel with 6 mL water to dilute the saliva, the dominance rates of 2-methyl-1-butanol increased, while the dominance rate of 1-penten-3-ol increased at 8 mL water dilution. The dominance rate of dimethyl sulfide was almost unaffected by the dilution of less than 6 mL of water during chewing however, when the dilution of water was greater than 6 mL, the dominance rate increased rapidly with the dilution of water. This may suggest that dimethyl sulfide interacted with salivary proteins and bound. Water is the main ingredient in saliva, containing salts and different proteins. The addition of water would change the interaction between salts, proteins, and odorants, which would further change the partitioning of odorants in the food-saliva-air phase (Ployon, Morzel, & Canon, 2017).

Compared to the concentration of key odorants during consumption with different water dilution, it could be found that the variation trend of odorants release was consistent with the variation trend of dominance rates. It indicated that different saliva concentrations had a uniform effect on the aroma perception of grilled eel during eating, which was mainly caused by the different release amounts of the key odorants. When saliva was diluted, the release rates of trimethylamine, and 1-heptanol decreased. The release rates of dimethyl sulfide and 1-penten-3-ol varied little with the dilution of saliva. The release rates of 2-methyl-1-butanol peaked when diluted saliva with 6 mL water, which was different from other key odorants. It suggested that the solubilization of the key odorants into saliva was different. Proper concentration and amount of saliva helped to release key odorants out of grilled eel during chewing.

**3.5 The relationship between different mastication and key odorants**

Multi-dimensional statistical analysis was used to find the relationship between the release of key odorants in grilled eel under different mastication. During consumption, aroma compounds would be released from food matrices. The phenomenon involved a huge diversity of composition, structure, texture, and physicochemical properties. Mastication and salivation worked together to affect their release. As showed in Figure 6-B, chewing time and frequency had similar effects on the release and perception of key odorants, while saliva dilution had different effects. It could be speculated that the mastication corresponded to the breakdown of grilled eel. The time and speed of mastication mainly affected the bolus formation. The process would increase the surface/volume ratio of food particles, improving the transfer of key odorants to saliva and air. While saliva was diluted with water, it may mainly affect the non-covalent or covalent binding, enzymatic reactions or degradation, and solubilization or diffusivity for key odorants in a food matrix, saliva, and air.

In Figure 6-A, the release of key odorants in grilled eel during different chewing processes was compared. The release of trimethylamine, dimethyl sulfide, 1-penten-3-ol, 2-methyl-1-butanol were greatly affected by the dilution of saliva during chewing. The properties of compounds, such as molecular weight, dissociation constant, oil-water partition coefficient, etc. can affect the solubility, the ratio of the vapor-liquid concentration, and intermolecular interactions (Arias-Pérez, Sáenz-Navajas, de-la-Fuente-Blanco, Ferreira, & Escudero, 2021; Buettner & Beauchamp, 2010; van Ruth, Frasnelli, & Carbonell, 2008). The log P, pKa, and molecular weight of the key odorants shown in Figure 2 were factors to investigate the relationship between odorants releasing and their properties. Compared to the properties of the odorants, it could be found that the molecular weight of these compounds was relatively small,

while the molecular weight of 1-heptanol with less influence was relatively large. Meanwhile, 1-heptanol had a bigger log P. The difference in compound polarity significantly affected the spread of key odorants in the phase between food-saliva and saliva-air. On the contrary, the release of 1-heptanol was greatly affected by the chewing time and speed. Long time chewing at a lower frequency may have a similar effect on releasing key odorants as short time chewing at a higher frequency. It suggested that excluding the physical and chemical interference of saliva on the release of key odorants, the mastication was mainly generated by the breakdown and bolus formation of grilled eel meat. Besides, the whole process odor perception of grilled eel consumption could be divided into three stages: the onset, middle, and late mastication, according to the difference of key odorants under different chewing times.

Principal component analysis was conducted on the differences in the release of key odorants during different chewing processes (Figure 6-C). According to the biplot of PCA about key odorants and chewing times, it could also be found that the odorants release and perception of grilled eel could be divided into three stages. Among them, dimethyl sulfide and 1-heptanol were released rapidly in the early stage of consumption. In contrast, other key odorants were mostly released and precepted in the middle and late stages of consumption. As to the chewing frequency and odorants releasing, the chewing rate of 2 cycles/s had a good positive correlation with the release of most key odorants. An appropriate chewing rate could effectively promote the release and perception of key odorants. Besides, 1-heptanol was easier to release and perceive when chewed quickly. In terms of water dilution, its influence on odorants release could be divided into levels, 2 mL, 4 mL, 6 mL, and more than 8 mL according to the biplot of PCA. This phenomenon may be related to the properties, log P and pKa.

The dimethyl sulfide (pKa = -9.9) was close to S10 (diluted with 10 mL water). Most of the compounds with the bigger pKa were close to less water dilution. All above indicated that the release of odor compounds with low pKa in grilled eel was less affected by water dilution during chewing. However, compared to the position of dimethyl sulfide on the biplot, it could be found that when the pKa value was small, the release of the odor compounds with large log P in grilled eel during chewing was greatly affected by water dilution.

Prior to consumption, consumers perceive aroma through orthonasal delivery. During mastication, aroma compounds were released from the oral cavity and delivered to the nasal cavity via retronasal delivery (Figure 6-D), both of which will have a direct impact on aroma perception by consumers (He, Dukes, & Kay, 2020). Through the above mechanisms, the opening and closing of the oral cavity during eating, the size of food after chewing, the

volume of saliva, properties of odorants, etc. would influence the timing and extent of aroma release via changing the transfer of volatiles to the nasal cavity (How, et al., 2021; Salles, et al., 2010). These demonstrated the importance of considering oral processing to understand aroma perception. As showed in Figure 6-E, odorants release and perception can be divided into three stages, onset, middle, and late mastication. There were two main aroma-changing stages. The release and perception of aroma were mainly formed during the late stages of mastication. This was especially evident for the fish-like odor.

## 4 Conclusions

When eating grilled eel, chewing time, chewing frequency, and saliva concentration directly affects aroma release and perception. Different aroma compounds responded to oral processing in different ways, for example, an optimum chewing time of 20 s was observed for most aroma compounds tracked, apart from trimethylamine (30 s) and dimethylsulfide (10 s). The chewing rate directly impacted the release of aroma compounds. The optimum chewing frequency for 1-heptanol was 1.5 cycles per second, both have a higher log P than the other compounds tested. The optimum chewing frequency for trimethylamine, dimethyl sulfide, 1-Penten-3-ol, and 2-Methyl-1-butanol, was 2.0 cycles per second. Suggesting that compounds with higher log P release their aroma more easily during slow chewing processes. Dilution of saliva with water during chewing grilled eel reduced aroma release for all compounds apart from dimethyl sulfide. The impact was related to log p and pKa. Aroma release and perception could be divided into three stages: the onset, middle, and late mastication. Consequently, the release and perception of aroma were mainly formed in the middle and late of mastication, this was especially evident for the compounds associated with a fish-like odor which gave a guiding to the process of fish products. Chewing behavior would affect the releasing rate of aroma and aroma perception which need to be considered during product development and aroma adjustment. Further, these dynamic descriptions of aroma were able to better relate the real feelings of consumers to key aroma compounds and reflect more accurate and comprehensive food flavor perceptions of consumers.

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## FIGURE CAPTIONS

**Figure 1.** The aroma profile of grilled eel after chewing.The radar map was plotted with the OAV. The odor descriptive terminology was come from the panelists and corrected with the Database (Buettner, 2017; Burdock, 2009; Deibler & Delwiche, 2003).

**Figure 2.** Morphological changes and mass spectra of key compounds of grilled eel before and after different chewing. (A) the pictures of grilled eel before and after chewing. (B) the mass spectra detected via a headspace vial and human oral while chewing grilled eel. (C) the information of five key aroma compounds monitored during chewing grilled eel.

**Figure 3.** Dynamic changes of characteristic odorants of the grilled eel under different chewing time. (A) the dominance rates of the key odorants calculated with the aroma intensity and aroma contribution of individual compounds. The solid curves correspond to the primary y-axis while the dotted curves correspond to the secondary y-axis. (B) the concentrations of the key odorants released during different chewing time. (C) the total OAV under different chewing time. (D) the releasing rates of the key odorants during different chewing time. The significance level: * indicated $p<0.05$; ** indicated $p<0.01$; *** indicated $p<0.001$.

**Figure 4.** Dynamic changes of characteristic odorants of the grilled eel under different chewing frequencies. (A) the dominance rates of the key odorants calculated with the aroma intensity and aroma contribution of individual compounds. The solid curves correspond to the primary y-axis while the dotted curves correspond to the secondary y-axis. (B) the concentrations of the key odorants released during different chewing frequencies. (C) the total OAV under different chewing frequencies. (D) the releasing rates of the key odorants during different chewing frequencies. The significance level: * indicated $p<0.05$; ** indicated $p<0.01$; *** indicated $p<0.001$.

**Figure 5.** Dynamic changes of characteristic odorants of the grilled eel under different dilutions of saliva. (A) the dominance rates of the key odorants calculated with the aroma intensity and aroma contribution of individual compounds. The solid curves correspond to the primary y-axis while the dotted curves correspond to the secondary y-axis. (B) the concentrations of the key odorants released during chewing under different dilutions of saliva. (C) the total OAV under different dilutions of saliva. (D) the releasing rates of the key odorants during chewing under different dilutions of saliva. The significance level: * indicated $p<0.05$; ** indicated $p<0.01$; *** indicated $p<0.001$.

**Figure 6.** The statistical analysis of the key odorants releasing under different mastication. (A) the heatmap about the key odorants releasing and different mastication. (B) the correlation heatmaps about different mastication. (C)

563 the biplot for PCA. The symbols, T5-T25, were represented the chewing times, 5-25 s. The symbols, F1-F3, were
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565 mL. (D) the graph about retronasal and orthonasal olfactory perception. (E) the key odor perception changes during
566 eating grilled eel.

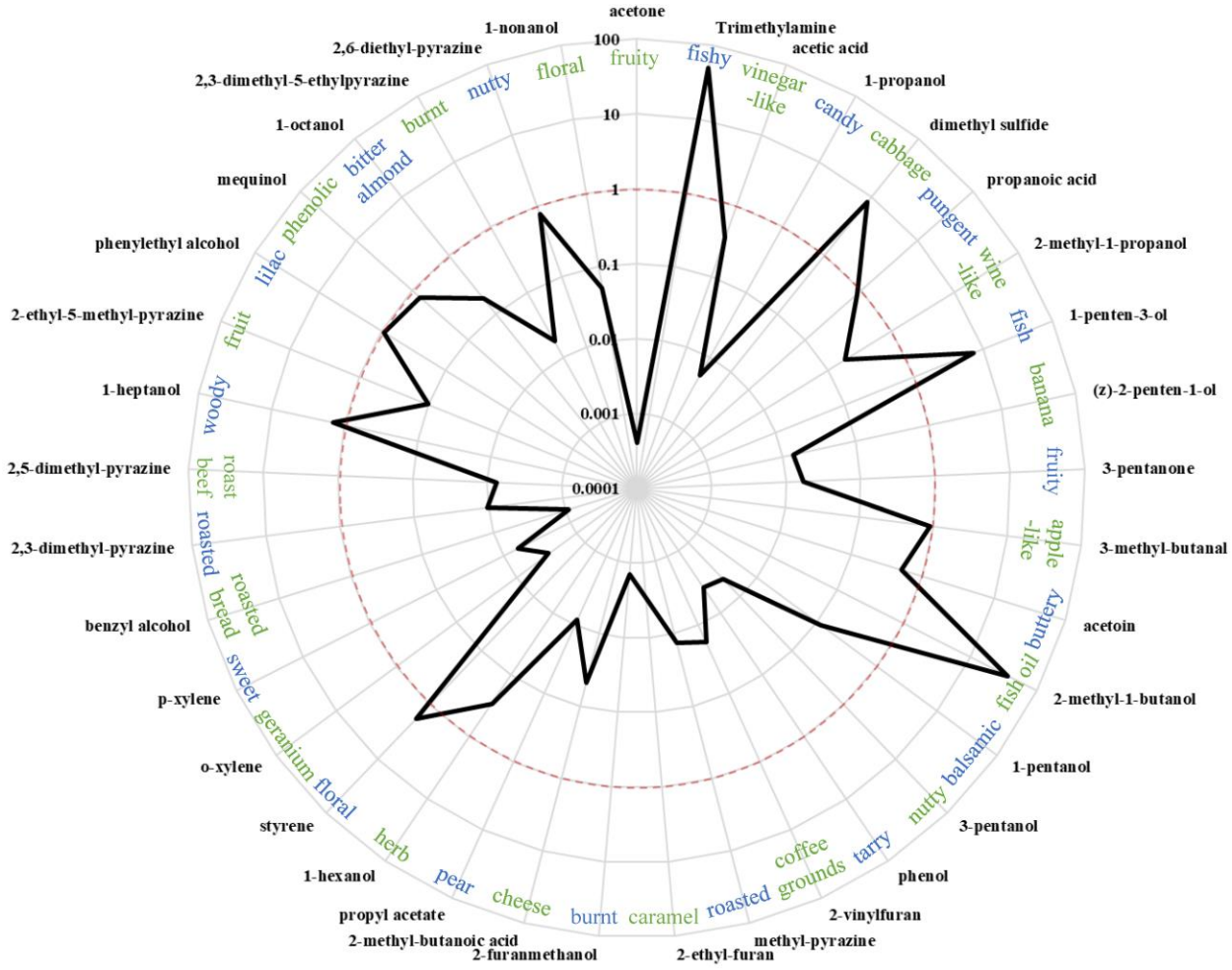


**Figure 1 The aroma profile of grilled eel after chewing.**

The radar map was plotted with the OAV. The odor descriptive terminology was come from the panelists and corrected with the Database (Buettner, 2017; Burdock, 2009; Deibler & Delwiche, 2003).

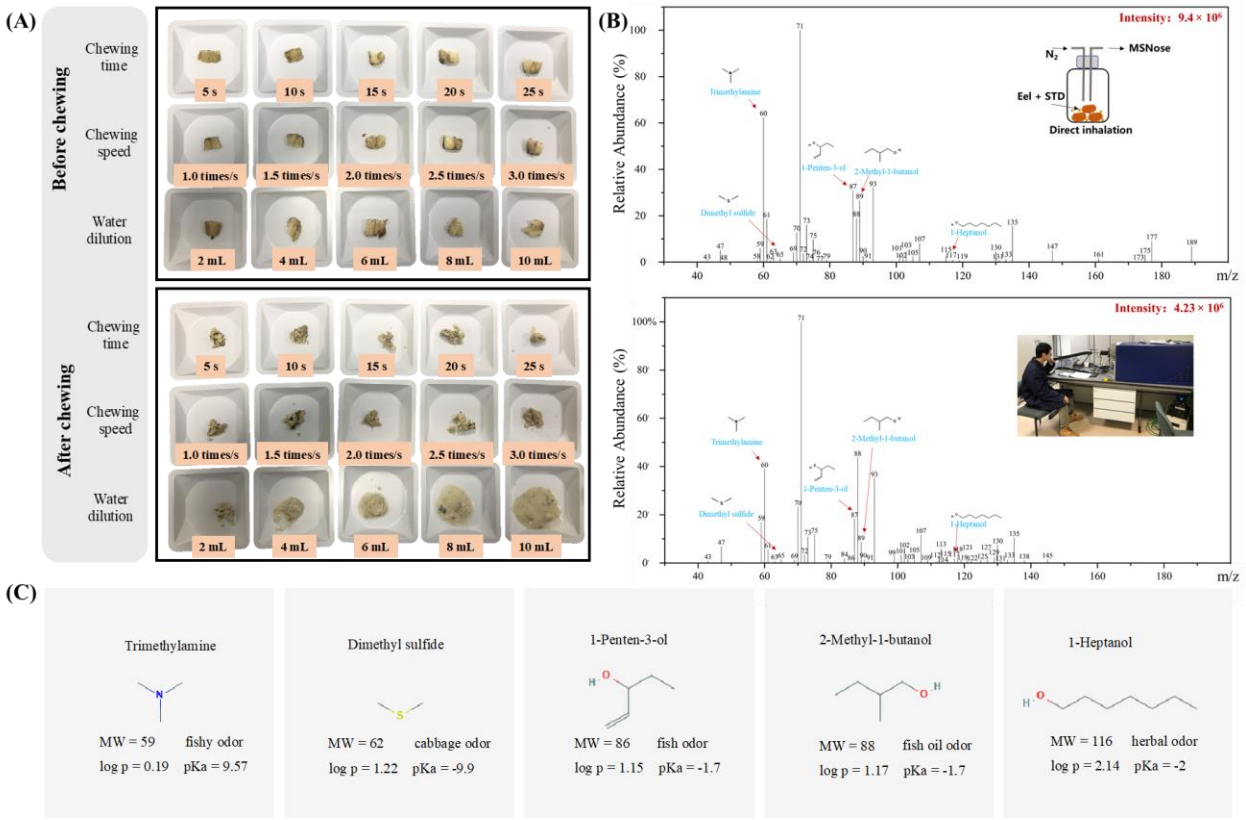


**Figure 2 Morphological changes and mass spectra of key compounds of grilled eel before and after different chewing.**

(A) the pictures of grilled eel before and after chewing. (B) the mass spectra detected via a headspace vial and human oral while chewing grilled eel. (C) the information of five key aroma compounds monitored during chewing grilled eel.

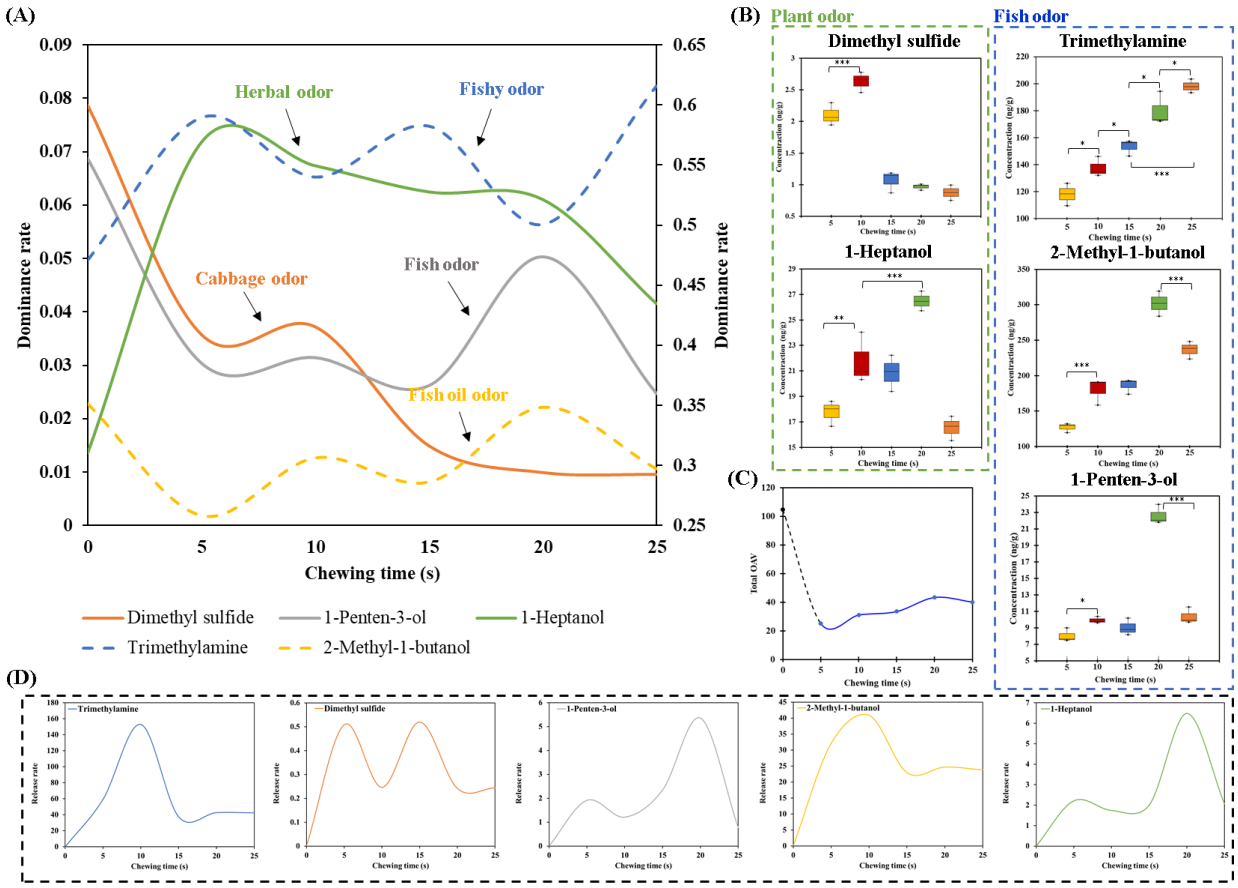


**Figure 3 Dynamic changes of characteristic odorants of the grilled eel under different chewing time.**

(A) the dominance rates of the key odorants calculated with the aroma intensity and aroma contribution of individual compounds. The solid curves correspond to the primary y-axis while the dotted curves correspond to the secondary y-axis. (B) the concentrations of the key odorants released during different chewing time. (C) the total OAV under different chewing time. (D) the releasing rates of the key odorants during different chewing time. The significance level: * indicated $p<0.05$; ** indicated $p<0.01$; *** indicated $p<0.001$.

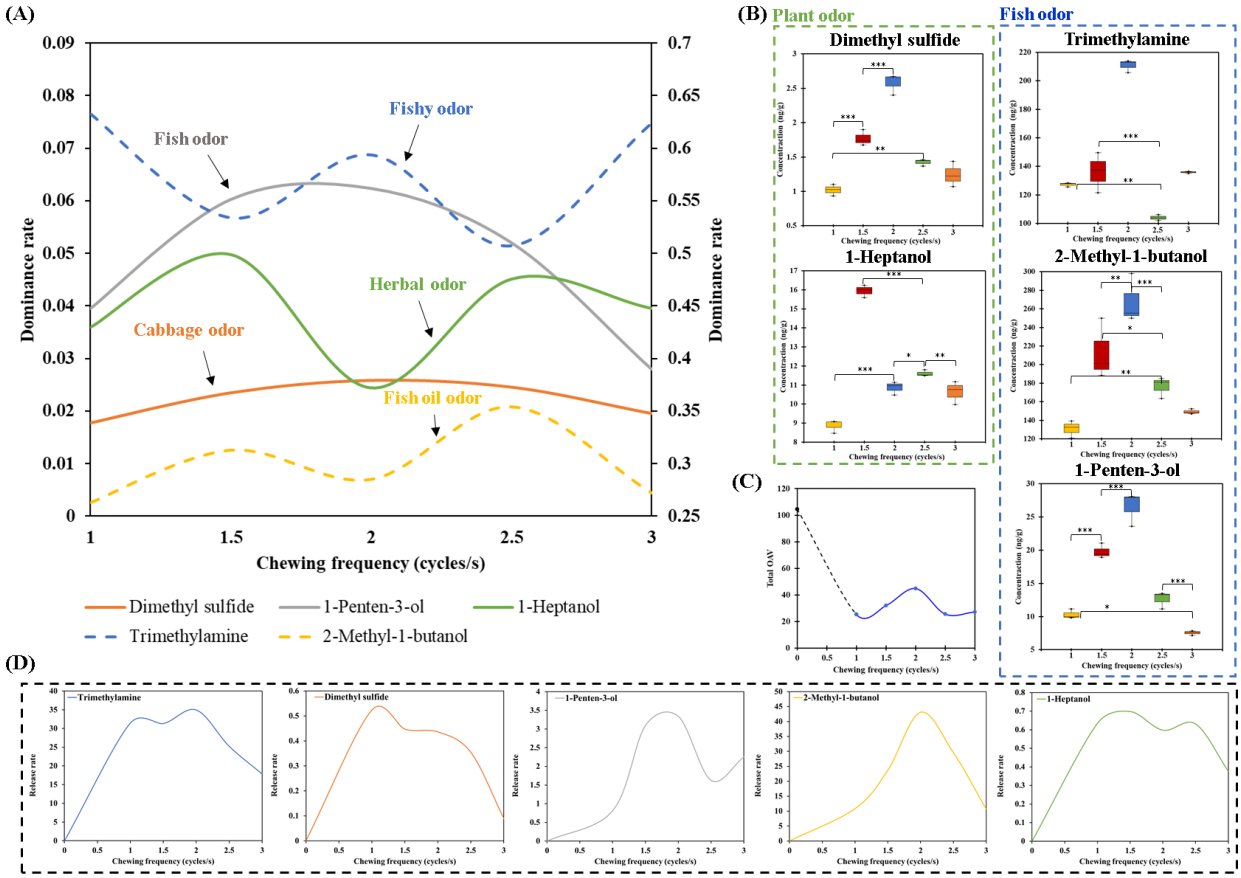


**Figure 4 Dynamic changes of characteristic odorants of the grilled eel under different chewing frequencies.**

(A) the dominance rates of the key odorants calculated with the aroma intensity and aroma contribution of individual compounds. The solid curves correspond to the primary y-axis while the dotted curves correspond to the secondary y-axis. (B) the concentrations of the key odorants released during different chewing frequencies. (C) the total OAV under different chewing frequencies. (D) the releasing rates of the key odorants during different chewing frequencies. The significance level: * indicated $p<0.05$; ** indicated $p<0.01$; *** indicated $p<0.001$.

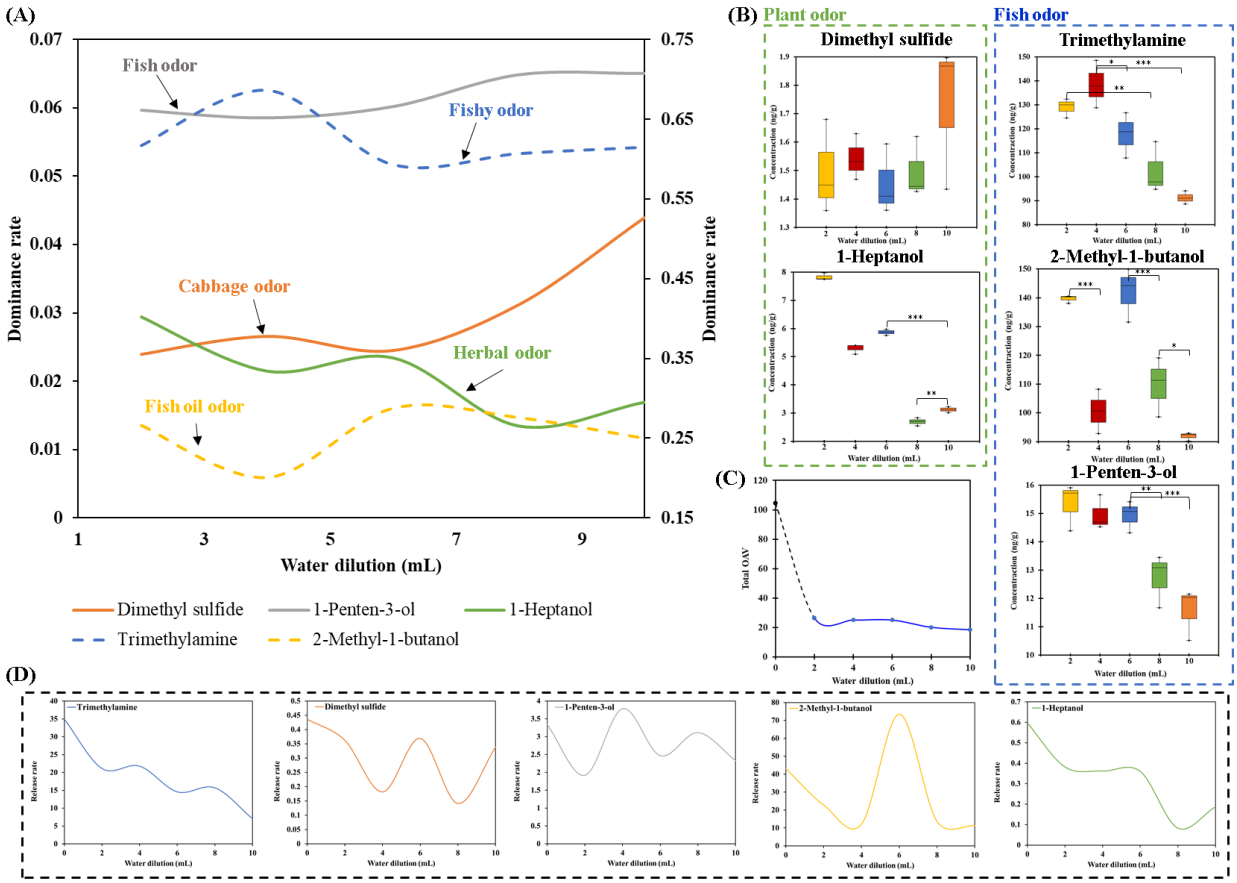


**Figure 5 Dynamic changes of characteristic odorants of the grilled eel under different dilutions of saliva.**

(A) the dominance rates of the key odorants calculated with the aroma intensity and aroma contribution of individual compounds. The solid curves correspond to the primary y-axis while the dotted curves correspond to the secondary y-axis. (B) the concentrations of the key odorants released during chewing under different dilutions of saliva. (C) the total OAV under different dilutions of saliva. (D) the releasing rates of the key odorants during chewing under different dilutions of saliva. The significance level: * indicated $p<0.05$; ** indicated $p<0.01$; *** indicated $p<0.001$.

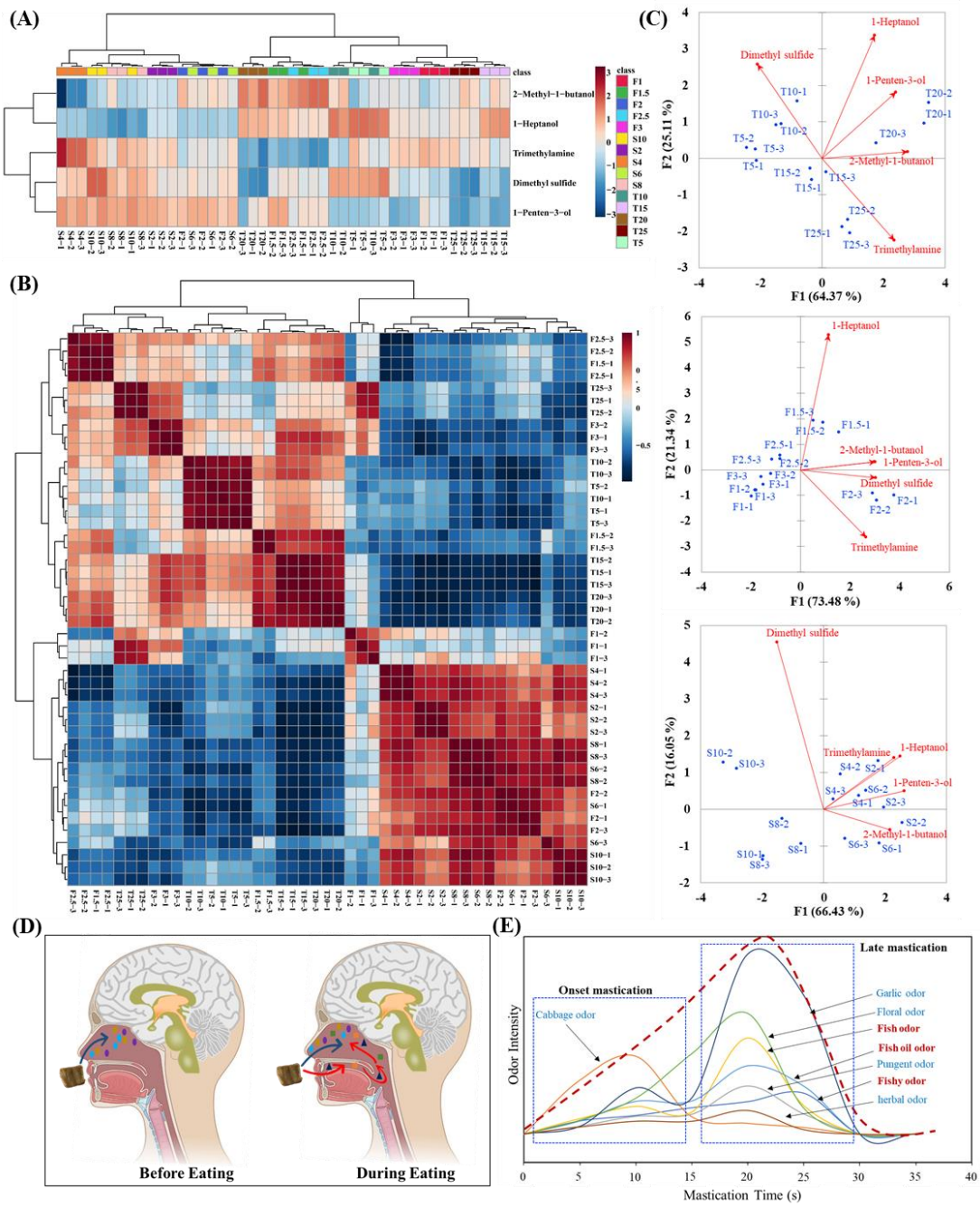


**Figure 6 The statistical analysis of the key odorants releasing under different mastication.**

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