Impact of cooking on the sensory perception and volatile compounds of *Takifugu rubripes*

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Abstract

Takifugu rubripes is well-known for the unique flavour but can also develop a putrid off-note. To eliminate off-note and promote desirable flavour, four cooking processes (boiling, steaming, microwave-heating and roasting) were explored to determine their effects on cooked *T. rubripes*. The temperature and water dynamics, physico-chemical properties were analysed and correlated with sensory qualities. The changes of centre temperature dynamics during cooking decreased the water mobility and led to varied sensory properties. Six out of ten orthonasal aroma attributes and four out of five mouthfeel attributes were significantly different among samples ($p<0.05$). Based on Partial Least Squares Regression analysis, orthonasal aroma attributes "roasted" and "earthy/putrid fish" highly correlated with the volatile compounds generated from Maillard reaction and lipid oxidation, respectively; meanwhile mouthfeel attributes of chewy/fibre and tender/juicy were highly associated with water loss and moisture, respectively. This study provides insights for optimising cooking conditions to create desirable fish flavour.

Keywords

Takifugu rubripes, thermal cooking process, sensory, off-note, aroma

1. Introduction

Pufferfish (*Tetraodontidae*), a family of marine and freshwater fish belonging to *Tetraodontiformes*, is well-known for its frightening toxicity all over the world. However, it has been of interest for thousands of years in China, and more recently in other Eastern Asia countries including Japan and the Republic of Korea due to its unique flavour (Zhou & Wang, 2017). *Takifugu rubripes* and *Takifugu obscurus* (the genera of *Takifugu)*, are the only two species of pufferfish that can be cultivated and processed legally in China since 2016, and have been reported as a good source of protein with high nutritional qualities (Tao, et al., 2012). According to Global Statistical Collections statistics from the Food and Agriculture Organization of the United Nations (FAO), 69% of global production and 90% of global aquaculture production occurred in China (2018), and overall production continues to increase (FAO Fisheries and Aquaculture Department, 2020). *T. rubripes* is the main species to be exported in the form of fresh fish or chilled fish (FAO Fisheries and Aquaculture Department, 2020). However, the fresh or chilled pufferfish is extremely prone to become putrid and can develop a rancid off-flavour if not consumed or stored properly. This is a concerning factor for consumers and limits the expansion of the pufferfish industry.

Flavour is one of the important aspects of food quality. Overall, flavour perception is formed and processed by the brain through a series of multiple sensory inputs. These include volatile aroma compounds and non-volatile taste compounds delivered during olfaction and gustation (Reineccius, 2006). Ultimately, food flavour plays an important role in consumers' acceptance. For example, the Equivalent Umami Concentration (EUC), calculated by umami amino acids and the 5′-nucleotides, was positively related to the fish acceptability (Zhang et al., 2019). Meanwhile, slight changes in fish odour were shown to have a great effect on consumer's hedonic responses (Alexi et al., 2018). There are various factors influencing the flavour of fish products, such as the thermal cooking process, food ingredients, *etc.*, of which the thermal cooking process is the first complex factor to be considered. The cooking process can affect the fish aroma and taste by different mechanisms, such as Maillard reaction, lipid oxidation, protein degradation, *etc.* (Reineccius, 2006). Additionally, thermal cooking processing reduces water content and affects protein structure which will also result in different texture properties (Sun et

al., 2020). Therefore, it can be proposed that the appropriate cooking process may help to eliminate off-flavours and be used to design different flavour profiles with different sensory qualities.

Recent research on pufferfish flavour mainly focused on the taste-active compounds investigation (Zhang et al., 2019) and umami characterization (Wang et al., 2021) using chemical analysis and sensory evaluation. However, the overall sensory properties of cooked pufferfish have never been determined. Since off-flavour has always been a concerning problem for pufferfish and the thermal cooking process is known to dramatically change the flavour profile, it is useful to conduct the sensory properties evaluation and correlate this with the instrumental flavour analysis in order to investigate the thermal processing impacts on pufferfish flavours.

Therefore, our hypothesis in this study is that the thermal cooking process can be used to create desirable sensory qualities and mask/decrease any potential off-flavour of pufferfish which is naturally formed through oxidation. The objectives of our study were (i) to establish four thermal process methods (boiling, steaming, microwave-heating and roasting) and monitor the dynamic changes of temperature and water during different cooking processes; (ii) to create and evaluate holistic flavour profiles of cooked pufferfish by sensory evaluation (Quantitative Descriptive Analysis, QDA) and flavour analysis (Electronic nose, E-nose; Gas Chromatography-Time-of-Flight -Mass Spectrometer, GC-TOF-MS); (iii) to correlate sensory attributes with instrumental data by statistical analysis (Principal Component Analysis, PCA; Partial Least Squares Regression, PLS-R and Hierarchical Cluster Analysis Heatmap, HCA heatmap). Achieving these three objectives would generate new knowledge on how thermal processes affect pufferfish flavours, and which method would be the optimum way to improve the flavour profiles of cooked pufferfish, which will directly benefit the expansion and standardization of the pufferfish industry.

2. Material and Methods

2.1 Materials and reagents

The dorsal muscle of *T. rubripes* fillets was purchased from Dalian Tianzheng Industrial Co., Ltd. (Liaoning Province, China). Two-year-old farmed *T. rubripes* was slaughtered according to the

National Standard (GB/T 27624-2011) by the company. After slaughter, the dorsal muscle of fish flesh (fillet) was cut off, packaged, and transported to our lab within 12 hours covered with ice, and frozen in -80 °C freezer until use.

2,4,6-Trimethylpyridine (purity 98%, Ark Pharm, Chicago, USA) was dissolved in methanol (purity≥99.9%, GC standard, Aladdin®, Shanghai, China). C7-C40 Saturated alkanes dissolved in hexane were purchased from Sigma-Aldrich Trading Co., Ltd. (Shanghai, China).

2.2 Preparation of cooked *T. rubripes*

Fillets with similar thickness were thawed in a 4 °C fridge overnight. Before cooking, they were cut into a similar weight (69 ± 4.00 g, 13.7 ± 0.3 cm length, 4.2 ± 0.4 cm width) from the same part. The cooking time for each processing method was defined as the best time when the most pleasant fish aroma was generated. The best time was determined with four specialised sensory assessors in the preliminary experiment by smelling the cooked pufferfish sample with different cooking times. The standardised method for each thermal process was listed below:

Boiled T. rubripes (BTR): fillets were boiled in the boiled water at a ratio of 1:4 (fish/water, w/w) for 10 min without a lid.

Steamed T. rubripes (STR): fillets were placed in a glass plate over the boiled water at a ratio of 1:10 (fish/water, w/w) for 25 min with a lid.

Microwave-heated T. rubripes (MTR): fillets were covered with the baking paper, and cooked for 3.0 min in the 700 W microwave oven (EM7KCGWt3-NR, Midea, Guangdong Province, China).

Roasted T. rubripes (RTR): fillets were covered with the baking paper, and roasted at 200 °C for 20 min on each side in a baking oven (K42, Galanz, Guangdong Province, China).

Four fillets were chosen randomly for each cooking process as replicates. During cooking, the centre temperature of each fillet was detected by a K/J dual input thermometer (UT320D, UNI-Trend, Guangdong Province, China) to guarantee the consistent cooking process. After cooking, each fillet was cooled at room temperature and weighed to calculate the remaining weight % (the weight after cooking divided by the weight before cooking × 100%) and water loss % (100% minus the remaining weight %).

Three sections of each fillet were used for different types of analysis **(Fig. S1**): 15% of the fillet was used for colour and moisture analysis (labelled as part A); 50% at the middle section of the fillet was used for aroma analysis (part B); rest 35% of the fillet was used for water distribution analysis (part C).

2.3 Colour and water content

Colour analysis was conducted on the surface of part A (**Fig. S1**) firstly after cooking. Eight replicates were performed for each cooking process (2 replicates per fillet). L*, a* and b* values were detected with CIE1931 standard colourimetric system by the high-quality portable colourimeter (XD-1055, Modern instruments, Shanghai, China), and represented the lightness, green/red and blue/yellow respectively. The total colour difference (ΔE) was calculated by the following equation compared with the colour of the raw fillet as the control.

$$\Delta E = \sqrt[2]{\Delta L^2 + \Delta a^2 + \Delta b^2}$$

After the colour analysis, the part A of the fillet was ground with a mortar for 1 min to analyse water content following the National Standard (GB 5009.3-2016). Eight replicates were performed for each cooking process (2 replicates per fillet).

2.4 Low-field nuclear magnetic resonance (LF ^{1}H NMR)

One gram sample from part C of **Fig. S1** was taken to perform LF ^{1}H NMR (MesoMR23-060H-I, Niumag, Jiangsu Province, China) measurements at 32 °C with a 60 mm diameter probe coil. The magnetic field strength and proton resonance frequency were 0.5 T and 21 MHz, respectively. The T_2 transverse relaxation time was acquired by Carr-Purcell-Meilboom-Gill (CPMG) sequence with the 90° pulses of 12 μs and 180° pulses of 23 μs. Decay signals were collected with 0.5 ms echo time, 1000 echoes and 8 scan numbers. The decay curves were obtained by the instrument software (Version 2.0, Niumag, Jiangsu Province, China), and multi-exponential fitted by the simultaneous iterative reconstruction technique (SIRT) algorithm. Four replicates were performed for each cooking process.

2.5 Electronic nose (E-nose) analysis

Part B in **Fig. S1** of each fillet was ground with cooled mortars for 1 min. Two grams of each sample were weighed into a 40 mL headspace vial and equilibrated at 50 °C for 20 min. After that, the sensor array unit equipped with 14 metal-oxide semiconductors (MOS) sensors were exposed to the headspace

of samples for 60 s at an air flow rate of 1 L/min. Between samples, the system was cleaned for 60 s twice at the same air flow rate. The signal of each sensor was recorded by the Software (BosinTechNose, Shanghai, China), of which the maximum values of response curves were selected to perform data analysis. Twelve replicates were carried out for each cooking process (3 replicates per fillet).

2.6 Solid-Phase Microextraction (SPME) and Gas Chromatograph-Time-of-Flight Mass Spectrometer (GC-TOFMS)

Three grams of ground fish from Section 2.5 were weighed in a 20 mL headspace vial, cooled down by liquid nitrogen immediately, and stored in -80 °C freezer until GC-MS analysis. Before analysis, 10 μL internal standard (IS) of 2,4,6-trimethylpyridine (6.8 mg/L) was added into the sample vial. Each vial was capped and defrosted at room temperature for 1 h. The analysis condition of SPME-GC-MS has been optimised and performed as the following steps: samples were incubated at 50 °C for 20 min, and volatile compounds in the headspace were extracted by a 2 cm 50/30 µm DVB/CAR/PDMS fibre (Supelco, Sigma-Aldrich, Shanghai, China) at the same temperature for 40 min. The extracted volatile compounds were desorbed in the GC (7890B, Agilent, Beijing, China) inlet at 250 °C for 6 s with a spitless mode, and isolated by a TG-Wax MS column (30 m length, 0.25 mm diameter, 0.25 mm thickness, Thermo, Shanghai, China) with a Helium carrier flow rate of 1 mL/min. The oven temperature started from 30 °C, then increased to 250 °C at a rate of 5 °C/min and held for 3 min. The isolated volatile compounds were then transferred to TOF-MS (LECO, Shanghai, China) to be identified with EI ionisation mode. The temperatures of the transfer line and ion source were set as 250 °C and 200 °C respectively. The full scan was 35-500 m/z with an acquisition rate of 10 spectra/s. All samples were analysed in randomised orders. Four replicates were performed for each cooking process.

The identification of volatile compounds was tentatively determined by comparing MS spectra with the NIST 17 standard reference database (NIST 17 and Version 2.3) and then confirmed from the retention index (Van Den Dool & Kratz, 1963) calculated by C7-C40 saturated alkanes under the same GC-TOF-MS conditions. The semi-quantification analysis of the relative headspace concentration above each

sample was calculated by comparing the ratio of the peak area between the compound of interest and IS, and then multiplied the concentration of the IS.

$$\text{The relative concentration of volatile compounds (μg/kg)} = \frac{\text{Peak area of the volatile compound}}{\text{Peak area of the internal standard}} \times \frac{\text{Amount of the internal standard (μg)}}{\text{Amount of the sample (kg)}}$$

2.7 Sensory evaluation

A quantitative descriptive analysis (QDA) approach was conducted in the standard sensory room (ISO 8589: 2007). Twelve Chinese panellists (four males and eight females, ages 22 to 30 years old) were recruited who had the sensory evaluation experience of 6 months to 3 years. They were trained by four sessions. The first two sessions were carried out to develop vocabularies and reach an agreement on 22 verbal descriptors (**Table S1**)**.** Then, two training sessions were performed to let panellists familiarise scoring scales and sample attributes according to the references shown in **Table S1**. The consensus vocabularies included 10 orthonasal aroma attributes, 10 retronasal aroma attributes, 6 taste attributes, 5 mouthfeel attributes and 9 after-effect attributes, of which after-affect contained the modalities within the aroma, taste and mouthfeel after swallowing samples.

Before each session, *T. rubripes* were cooked on the same day as described in Section 2.2, and 5 g samples were stored in sensory sampling cups with lids at 50 °C and presented to panellists within one hour. Each sampling cup was labelled with three-digit codes and served in randomised orders. During each session, the orthonasal aroma attributes were scored firstly by smelling samples, while retronasal aroma, taste and mouthfeel attributes were scored when chewing samples, and after-affect attributes were scored after swallowing samples in the end. Three repeated sessions were held for scoring samples using a continuously unstructured line scale anchored 0 (left, no perception) to 15 (right, extremely strong perception) as three replicates.

2.8 Statistical analysis

Data were analysed by the Analysis of Variance (ANOVA) using IBM SPSS Statistics software (Version 20) with a Post-hoc Tukey's test at α=0.05. Principal Component Analysis (PCA), Partial Least Squares Regression (PLS-R) and Hierarchical Cluster Analysis Heatmap (HCA heatmap) analysis were achieved by XLSTAT (Annual Version 2020.1.3, Addinsoft Inc.).

3. Results and Discussion

3.1 Centre temperature changes during cooking *T. rubripes*

Diverse temperature evolutions on fish during different thermal cooking processes would result in the distinct physico-chemical and sensory properties of cooked fish. So the centre temperature curves of different cooked *T. rubripes* were monitored and shown in **Fig. 1A**. All centre temperatures increased to near 100 °C, but microwave-heated *T. rubripes* (MTR) took the shortest time (less than 2.5 min) to reach over 90 °C. By contrast, roasted *T. rubripes* (RTR) spent the longest time (more than 10 min) reaching over 90 °C and held for the longest time (25 min) over 90 °C, when compared with those of boiled *T. rubripes* (BTR, 0 min over 90 °C), steamed *T. rubripes* (STR, 10 min over 90 °C) and microwave-heated *T. rubripes* (MTR, 0.5 min over 90 °C).

The microwave-heating process can be the most efficient way to cook food because it penetrates energy into foods by the heat radiation, which converses electromagnetic energy to thermal energy as a result of dipole and ion mechanism (Chandrasekaran, Ramanathan, & Basak, 2013). However, for traditional cooking processes (boiling, steaming and roasting), heat is transferred to the surface of food by the heat convection, and the approximate values of convective heat transfer coefficients were reported as 20-100 (air), 3 000-100 000 (boiling water), 5 000-100 000 (steamed water) for respective free convection (Hanson, 1990). This is consistent with the results on the heating rate of different cooking processes observed in this study, which indicated that the air convection would be the least efficient (i.e., RTR) followed by boiling water (i.e., BTR) and steamed water (i.e., STR). Apart from the heat efficiency, heating time is another variable need to be considered. Increasing the reaction time may not necessarily increase flavour intensity but influence the flavour balance (Reineccius, 2006). Until now, more research has worked on aroma changes from Maillard reactions over time, while very few researches on taste compounds change over time. For example, the kinetic modelling in the potato model system demonstrated that Strecker aldehyde levels decreased on prolonged heating while alkylpyrazine levels increased (Low, Mottram, & Elmore, 2006). This would result in different sensory perception and aroma profiles, which will be discussed in the following sections.

3.2 Water dynamics of cooked *T. rubripes*

With the increase of temperature, water mobility and distribution may change during thermal cooking processes, leading to different sensory qualities. The water mobility reflects the binding relationship between water and protein in the meat system (Zhou et al., 2019). The results of using LF-NMR to compare the water dynamics of raw fish and four types of thermally processed fish were shown in **Fig. 1B**. Three main "peaks" were detected in the range of 0-10 ms, 10-100 ms and 100-1000 ms, which represented bound water (T_{21}) associated with macromolecules tightly, immobilized water (T_{22}) located within the highly organised protein structures, and free water (T_{23}) reflected the extra-myofibrillar water, respectively (Bertram et al., 2001). Compared with RawTR, cooking processes caused the curve shift toward lower relaxation time visually (**Fig. 1B**), which is consistent with the previous report (Sun et al., 2020). The protons with lower relaxation time indicated that the cooking process in this study reduced the water mobility and increased the binding between water and protein backbone. This result is more evident in **Fig. 1C** and **Table S2**, because T_{22} and T_{23} of all cooked samples were lower than raw *T. rubripes* (RawTR) significantly, which indicated a significant lower transverse relaxation time of protein-binding water and free water in all cooked samples ($p<0.05$). During the cooking process, the fibre bundles network was destroyed (Cao et al., 2018), thereby, the immobilised water was transformed into free water firstly and evaporated from the fish system. So, the remaining protons were restrained in the fish system leading to decreased mobility (Sun et al., 2020). Comparing with different thermal processing methods, roasting (RTR) resulted in the lowest T_{22} and T_{23} values, which indicated the most significant reduction in transverse relaxation time ($p<0.05$) linking with reduced water mobility.

Additionally, the changes in the amplitude of peak area proportion at three defined intervals (T_{21}, T_{22}, T_{23}) were summarised in **Fig. 1D** and **Table S2**. The results of P_{21}, P_{22} and P_{23} corresponded to the ratio of bound water, immobilised water and free water, respectively. Despite the sample type, P_{21} counted for 3-8%, P_{22} generally had 88-92%, and the rest of 1-7% was P_{23}. Compared with RawTR, cooking processes led to the increase in P_{21} of all cooked samples, particularly, P_{21} of STR, MTR and RTR were significantly different from RawTR ($p<0.05$). So the proportion of bound water increased significantly ($p<0.05$) in most cooked fish samples except BTR. However, there was no significant difference between

all samples in their P_{22} values. Regarding P_{23} results, only RTR had a significantly different proportion ($p<0.05$), comparing with other cooked samples and RawTR, so apart from roasting, other cooking methods did not significantly change the free water proportion to its original level. Multiple factors of cooking processes would influence the water dynamic, such as the availability of water, heating efficiency or heating time. The partition between water and fish may be easier to reach equilibrium for BTR, leading to the least changes of all relaxation time and proportions values (more similar water dynamics) between BTR and RawTR, while by contrast, it would be harder to achieve balance in MTR and RTR systems. However, it was observed that there was no significant difference between MTR and RTR in terms of all three relaxation times (T_{21}, T_{22}, T_{23}), P_{21} and P_{22} values, which may be caused by the microwave to break hydrogen bonds between water and other macromolecules in an efficiency way (Cao et al., 2018). However, there was not enough time for free water to evaporate from the MTR system, leading to a similar P_{23} value between MTR and RTR.

3.3 Water content and colour of cooked *T. rubripes*

Different in the centre temperature and water dynamics led to diverse physical-chemical properties, so the remaining weight, moisture content and colour were measured (**Table 1**). Comparing the remaining weight of cooked samples, BTR had around 68% remaining weight, which is the significantly highest level ($p<0.05$). Both STR and MTR had around 63% remaining weight, and RTR had the lowest level around 48% which is significantly different from other samples ($p<0.05$). Similarly, RTR had a significantly lower moisture content (57%), which is around 23% lower than its level in RawRT (80%). MTR, STR and BTR had 69-70% moisture content, which is 10-11% significantly lower than RawTR ($p<0.05$). So moisture content reduced significantly for all four types of thermal processing, but the roasting process resulted in the lowest moisture content with the lowest remaining weight, while no significant difference between STR and MTR. BTR was cooked in boiling water, as a result of the highest value of remaining weight and moisture. Compared with MTR, STR was cooked under the steam of boiling water for a longer time, while the more efficient heat transfer for MTR could transform more immobilised water into free water without enough time to evaporate, leading to no significant difference between them.

By comparison of colour results, RTR had the significantly highest a* and b* values ($p<0.05$) among raw and cooked samples, so RTR was significantly more red and yellow. The lightness of raw fish (L*) increased with cooking significantly ($p<0.05$), and comparing with microwave-heating (MTR) with the largest increase, RTR had the least increase. The total colour difference (ΔE) of all cooked samples were calculated based on RawTR data, and their values were similar at 26-28. The colour difference of ΔE ($\Delta E \geq 2.2 \pm 1.3$) corresponded to a Just Noticeable Difference by human eyes (Mahy, Van Eycken, & Oosterlinck, 1994), so the colour difference between raw fish and cooked fish was perceptible by human eyes. As for RTR, a long heating time at high temperature resulted in the brown colour indicating the most intense Maillard reaction, which is the basis for flavour generation in thermal cooking foods, especially some meaty and savour flavour compounds (Parker, Elmore, & Methven, 2015). Colour can influence flavour perception matched with the major components of flavour (olfactory, gustatory and trigeminal sensations) (Bordiga & Nollet, 2019). But previous research (Alexi et al., 2018) indicated that the consumer's hedonic responses for cooked fish were more related to texture and odour/flavour rather than colour. Therefore, the main focus of later sensory evaluation was the flavour and texture of cooked pufferfish samples.

3.4 Perceived sensory attributes of cooked *T. rubripes*

Quantitative descriptive analysis (QDA) was carried out to evaluate the overall sensory profiles of cooked *T. rubripes* and to investigate any perceived differences affected by cooking processes. The sensory quality was defined to measure the orthonasal/retronasal aroma (O/R), taste (T), mouthfeel (M) and after-effect (A) perception. Generally, a total of 40 attributes (22 descriptors, **Table S2**) were selected, including 20 for O/R, 6 for T, 5 for M and 9 for A, and their perceived intensities were calculated (**Fig. 2** and **Table S3**). Among four cooked samples, 16 sensory attributes had significantly different intensities ($p<0.05$), including most of the oronasal and mouthfeel attributes. Among all 40 perceived attributes, the intensities of fishy-O/R and umami-A attributes were at the highest concentrations for all cooked samples (intensities higher than 8.0), which indicated that fishy and umami could be two prominent sensory attributes in this study. Among four cooked samples, RTR had significantly the highest intensities of roasted-O/R, oily-O/R, bitter-T, salty-T, fibre-T, chewy-T, sour-A and astringent-A attributes, and

significantly the lowest scores for the putrid fish-O, earthy-O, soybean-O, dairy-O, juicy-M and tender-M ($p<0.05$). However, these attributes did not change significantly among the other three cooked samples (BTR, STR and MTR), except for the highest intensity of juicy-M attribute for MTR.

Cooking processes affect the sensory quality. Previous studies proved that similar aroma, taste, flavour and texture profiles were obtained under similar cooking methods (steaming at 100 °C for 20 min and roasting at 180 °C for 15 min) (Alexi et al., 2019). In this study, four cooking processes varied a lot, resulting in various sensory qualities. Orthonasal aroma is associated with key aroma-active compounds such as those generated from Maillard reaction, lipid oxidation, *etc.* (Reineccius, 2006), which are delivered to the nose during inhalation directly from the product without consumption, while the retronasal aroma relates to aroma compounds that are delivered to the nasal cavity during chewing and/or swallowing. Comparing orthonasal aroma with retronasal aroma, most aroma attributes (e.g., fishy, roasted, boiled potato, boiled mushroom, boiled vegetable) had no significant difference in their perceived intensities of the same cooked pufferfish sample, which was consistent with the previous report (Du et al., 2020). Taste attributes are associated with key tastants generated from protein degradation, Maillard reaction *etc.* (Reineccius, 2006). Umami, the highest taste intensities characteristic in all cooked samples, is considered as a kind of typical taste characteristic for fish and can interact with other taste substances resulting in the overall taste perception (Wang, Zhou, & Liu, 2020). RTR was perceived as having the significantly highest saltiness and bitterness ($p<0.05$), this was perhaps due to a more intense Maillard chemistry reaction, resulting in the formation of more Maillard chemistry biproducts and a lower moisture content thereby increasing the relative concentration. Besides, salty perception also can be enhanced by umami substances (Wang, Zhou, & Liu, 2020) or salt-related odours (Thomas-Danguin et al., 2019). Furthermore, four mouthfeel attributes exhibited significant difference ($p<0.05$), except for "crumbly", indicating their importance on the overall sensory profiles, which was also highlighted by previous research on the consumers' hedonic responses to fish species (Alexi et al., 2018).

3.5 Orthonasal volatile compounds analysis of cooked *T. rubripes*

Considering that most of the orthonasal sensory attributes differed significantly among cooked samples ($p<0.05$) (**Fig. 2**), orthonasal volatile compounds were further analysed by E-nose and GC-TOF-MS.

3.5.1 Dynamic aroma profile changes of cooked *T. rubripes*

As a quick analytical tool to compare with the sensory analysis, E-nose was applied to rapidly detect aroma profiles of cooked *T. rubripes*. The E-nose results of the maximal corresponding signal values of sensors were summarised in a radar chart (**Fig. 3A)** and a PCA graph (**Fig. 3B**). **Fig. 3A** described the response signal of each sensor corresponded to every cooked sample, and statistical results indicated that all the sensors detected a significant difference among the samples ($p<0.05$). This result indicated the good sensitivity and specificity of MOS sensors, which matched with previous report (Jiang & Liu, 2020). Different sensors could be more sensitive to a specific product, for example, Sensor_1 and Sensor_9 showed their better sensitivity to RTR, and other sensors (Sensor_11, Sensor_12 and Sensor_13) were more sensitive to RawTR/MTR. MOS sensors are widely used in the application of food detection compared with biosensors because of their good performance in complicated food matrices (Jiang & Liu, 2020). Further studies are required to investigate the sensitivity of the MOS sensors for specific groups of aroma compounds.

On the other hand, data from MOS sensors provided some fingerprints to demonstrate the aroma profile differences between cooked pufferfish samples. As shown in the PCA plot (**Fig. 3B**), the first two principal components - PC1 and PC2 explained 48.78% and 41.22% of the variance respectively. Different cooked samples were clustered into different groups: the location of different samples evolved from RawRT at the upper left corner to other cooked samples (MTR, BTR and STR) and RTR samples spread across the 4th quadrant of the PCA. This changing trend indicated that RTR had a distinct aroma profile than RawRT, and the aroma profiles of BTR and STR were more similar, while MTR aroma profiles were the closest one to RawRT. The E-nose technique combined with the data processing method could demonstrate the dynamic change of overall aroma profiles of cooked pufferfish, as well as other aquatic products like the cold-smoked Spanish mackerel (Huang et al., 2019). It could be a powerful tool for rapidly evaluating and monitoring the food aroma changes (Jiang & Liu, 2020).

3.5.2 Orthonasal volatile compounds of cooked *T. rubripes*

Since E-nose could not reveal the change of each volatile compound of cooked *T. rubripes*, SPME-GC-TOFMS was applied to identify and quantify the relevant volatile compounds. A total of 117 volatile

compounds were identified in cooked samples, including 36 hydrocarbons, 11 sulphur-containing compounds, 15 nitrogen-containing compounds, 12 aldehydes, 15 ketones, 18 alcohols and 10 compounds from other chemical families (**Table 2**). Comparing the total number of volatiles detected in different samples, RawTR had the lowest total number (83), and RTR had the largest total number (117), whilst the total number for BTR (94), STR (100) and MTR (98) were in the middle range. The odorants' compositions among BTR, STR and MTR were similar, but their concentrations differed for specific compounds. Despite the number of hydrocarbons accounted for 30% of the total number of volatile compounds (**Table 2**), most of their thresholds are normally high (Shahidi, 1998), so they might not make a great contribution to the overall fish aroma.

Among all sulphur-containing compounds (2 thiols, 1 thial, 2 alkyl sulfides, 2 thiophene and 3 thiazoles), almost no sulphur-containing compounds could be detected in RawTR, while all 11 sulphur-containing compounds were detected in RTR, and most of their concentrations were the highest in RTR significantly ($p<0.05$). Additionally, comparing with other cooked samples, RTR had all 15 nitrogen-containing compounds detected (1 amine, 1 pyridine, 1 pyrrole and 12 pyrazines), and all of them were at the significantly highest levels ($p<0.05$). The reason could be that these heterocyclic compounds are formed through the Maillard reaction (Parker et al., 2015; Reineccius, 2006), so almost none of the heterocyclic compounds were found in RawTR, and the longest heating time in the RTR system led to more heterocyclic compounds generated (such as the presence of methional and much higher level of methanethiol). However, dimethyl disulphide and dimethyl trisulphide showed different trends (STR had a higher level than RTR). Dimethyl disulfide and dimethyl trisulfide are secondary volatiles generated from methanethiol, a product from methional (Strecker aldehydes) which was originally from methionine by Strecker degradation (Schlüter et al., 1999). Different heating methods would result in their varying levels, and one of the reasons could be different physical and chemical interactions between sulphur-containing compounds and proteins in foods during thermal processing. Moreover, trimethylamine (TMA) was also detected in raw fish because it can be generated by trimethylamine oxide (TMAO), a product from soluble sarcoplasmic proteins and nucleotides during alterations of fish post-mortem (Bordiga & Nollet, 2019). It has been reported that the formation of TMA increased as cooking time prolonged (Hughes, 1959), which matched the results of our study that RTR had the highest level.

Although TMA was often regarded as “off-note and fishy” for fish aroma (Shahidi, 1998), it should be considered as an essential important compound that contributes to the aroma of delicious prawn meat (Mall & Schieberle, 2017).

Among 12 aldehydes detected in the samples, 2-methylbutanal and 3-methylbutanal along with furfural and benzaldehyde were the only 4 aldehydes with the significantly highest concentration in RTR ($p<0.05$). However, except nonanal, the concentrations of other aldehydes were the lowest in RTR, despite no significant difference among the other three cooked samples. The first 4 aldehydes mentioned above were generated from Strecker degradation or Maillard reaction, and other aldehydes were generated from lipid oxidation (Shahidi, 1998; Yang et al., 2017). Therefore, the roasting process in our study resulted in the most intense Maillard reaction and generated a higher level of specific aldehydes. Similarly, among all 15 ketones in cooked samples, RTR had the significantly highest concentrations ($p<0.05$) of 2 furanones, 4 hydroxyketones and 1 lactone, which were products of the Maillard reaction. Other ketones were almost generated from lipid oxidation (Shahidi, 1998; Yang et al., 2017), so the concentration of these ketones (methyl ketones,2,3-pentanedione and 3,5-octadienone) showed different patterns. Diones have been reported to be generated by lipid oxidation and further involved in the Strecker degradation and Maillard reaction to form heterocyclic compounds, such as pyrazines and thiazoles (Parker, Elmore, & Methven, 2015), so 2,3-pentanedione may be generated and participated in other chemical reactions during the roasted process leading to the decrease of its concentration in RTR.

Alcohols, derived from lipid oxidation, are considered to contribute to the fresh fish aroma, whereas their contributions are less important due to their high threshold values compared with carbonyl compounds (Shahidi, 1998). Among all cooked samples for all 18 alcohols detected, the concentrations of 15 alcohols were the highest in MTR, which indicated that the more intense lipid oxidation happened in the microwave-heating system. Although microwave-heating is the most efficient way to cook fish, non-uniform temperature distribution may lead to cold and hot spots, therefore, enzymes in the cold spot might continue accumulating the lipid oxidation (Parker, Elmore, & Methven, 2015). Besides, there were 3 esters, 4 furans and 3 acids identified in cooked fish. RTR had the highest level for most esters and furans and the cooked fish samples had a lower level of acids than raw samples.

To visualize the correlations of mass aroma data, Principal Component Analysis (PCA) was applied to

extract information from 15 important volatile compounds with relative OAVs higher than 1 (**Fig. 3C**). The two principal components (PCs) explained 92.00% of the variance, representing the good separation for all samples. RTR and Raw TR were located far from each other, while BTR, MTR and STR were close to each other, whose trend was consistent with PCA from data of E-nose (**Fig. 3B**). BTR, MTR and STR were clustered on the positive side of the PC1 axis, while MTR/STR and BTR were located on the positive and negative of the PC2 axis respectively. Meanwhile, BTR and RawTR were on the negative of PC1, of which RTR and RawTR were separately clustered on the positive and negative of PC2 respectively. As shown in **Fig. 3C**, there were 7 aldehydes, 1 ketone and 2 alcohols with relative OAVs higher than 1, of which only 2-methylbutanal and 3-methylbutanal were closely correlated with RTR (**Fig. 3C**) because of their generations from Strecker degradation. In addition, 2 sulphur-containing compounds (methanethiol and trimethylamine) and both nitrogen-containing compounds (2-ethyl-3,5-dimethylpyrazine and 2-furfurylthiol) were closely correlated with RTR, while another sulphur-containing compound (dimethyl trisulfide) was more related with STR, which was convinced by their concentrations in **Table 2**. Heterocyclic compounds normally play a key role in the meat aroma with low thresholds (Shahidi, 1998), and most of these compounds were generated from Strecker degradation and Maillard reaction, so the cooking process with higher temperature, less water content and longer heating time, would be a good way to drive the pleasant aroma generation.

3.6 Correlation between orthonasal volatile compounds and sensory aroma attributes

Cooking processes can modify food aroma by various chemical reactions, which has been demonstrated by the individual results from sensory analysis and instrumental analysis in this study. To investigate the correlations between them, Partial Least Squares Regression (PLS-R) analysis was performed based on the scores of sensory orthonasal attributes (**Fig. 2** and **Table S3**) and the concentrations of volatile compounds (**Table 2**) as Y quantitative dependent variables and X quantitative explanatory variables, respectively. BTR, STR, MTR and RTR were set as different observation labels and four components were set as a fixed number. As illustrated in **Fig. 4A,** the first two representative components were displayed for the first two axes, and STR/MTR, BTR and RTR were projected into the 2^{nd}, 3^{rd} and 4^{th}

quadrants, respectively. The inner circle and outer circle represent 50% and 100% of the variables' explained variance, while the Q^2, R^2Y and R^2X cumulated index were 0.846, 0.956 and 0.874 respectively. Except for 3 hydrocarbons, benzothiazole and 3 alcohols, all the other Y and X variables were between two circles, so this PLS-R model provided a good quality predictive model.

RTR was positively correlated with orthonasal "fishy, oily and roasted" sensory attributes and those odorants derived from Strecker degradation or Maillard reaction, such as sulphur-containing and nitrogen-containing compounds. Meanwhile, STR and MTR positively correlated with other sensory attributes and odorants generated from lipid oxidation, such as most aldehydes, alcohols and some ketones, while the aroma of BTR was less intense because of limited variables around it. Moreover, the "fishy-O" attribute factors projected on the positive dimension of t1 axes and negatively associated with the "putrid fish-O" attribute projected on the negative dimension of t1 axes. Similarly, the "oily-O and roasted-O" attributes and Maillard-derived odorants negatively related to other sensory attributes (such as boiled vegetable-O and earthy-O) and Lipid-oxidation-derived odorants, which deep-rooted the effect of an efficient Maillard reaction on the orthonasal aroma perception and the generation of heterocyclic compounds. Furthermore, the "roasted-O, oily-O and fishy-O" attributes were significantly positively correlated with heterocyclic compounds, while "boiled vegetable/potato-O, earthy-O, dairy-O" attributes had a significantly positive correlation with most of aldehydes and alcohols from lipid oxidation.

Orthonasal sensory attributes were closely correlated with detected aroma compounds. To some extent, thiazoles and pyrazines can promote similar sensory properties (Reineccius, 2006), which had a significant positive correlation with "roasted-O" sensory attribute. TMA (No. 48) had a significantly positive association with pleasant "fishy-O" rather than unpleasant "putrid fish-O" sensory attributes, imparting the positive contribution with an appropriate amount of TMA. Meanwhile, off-notes like "boiled vegetable-O, earthy-O and putrid fish-O" were highly correlated with most of the aldehydes and cooking processes with STR (mild temperature and abundant water) or MTR (short heating time). As for the higher concentration of aldehydes correlating with off-notes, high moisture in the cooking system might accelerate lipid oxidation leading to the generation of most aldehydes (Damodaran & Parkin, 2017). Or, there might be an irreversible chemical reaction or reversible binding between aldehyde and protein, resulting in the decrease of aldehydes' concentrations in RTR (Reineccius, 2006; Xu et al., 2020).

Therefore, the cooking process with less water, higher temperature and longer heating is more likely to decrease the off-notes and increase roasted pleasant notes. However, more details on the effect of cooking temperature/time and water content on the generation of aroma-active compounds affected by cooking processes still need to be explored by the GC- olfactometry technique.

3.7 Correlation between water properties and sensory mouthfeel attributes

Besides orthonasal sensory attributes, 4 out of 5 mouthfeel attributes had a significant difference ($p<0.05$) among four cooked *T. rubripes* (**Fig. 2** and **Table S3**), which were the most important attribute categories to distinguish four cooked *T. rubripes*. The mouthfeel attributes correspond to the perception of texture properties during chewing (Roberts & Taylor, 2000), which have been reported to correlate with water properties (Sun et al., 2020). Therefore, the relationship between mouthfeel attributes (chewy, fibre, tender, and juicy) and water properties (water loss, moisture content and P_{21}, P_{23}, T_{21}, T_{22}, T_{23} from LF-NMR) were plotted using Hierarchical Clustering Analysis (HCA) and an associated Heatmap (**Fig. 4B)** using the results with the significant difference among the cooked samples ($p<0.05$). Data values corresponded with different colours (blue to red through white) in each rectangular tiling, of which blue to red colour meant the low to high abundance. The dendrogram showed that four cooked samples were separated into three clusters: STR and MTR constituted the first group, which was then clustered as the second group with BTR; while RTR was in an isolated third group. So comparing among different thermal processing methods, the roasting process resulted in products with different water dynamics (higher P_{21}, lower P_{23}, T_{22} and T_{23}) and lower moisture content with higher water loss, which led to more distinct mouthfeels (more chewy and fibrous, less tender and juicy) than other three cooked samples. So, as shown in the correlation between water properties and sensory attributes, two distinctive clusters were identified which exhibited obvious differences amongst cooked samples. Four indexes (water loss, chewy-M, fibre-M and P_{21}) comprised the upper clusters of **Fig. 4B** with the higher values in RTR. Chewy-M and fibre-M attributes were more relevant with the water loss, compared with P_{21}. By contrast, the other 6 indexes were clustered in another group because of lower values in RTR, demonstrating the positive correlation among P_{23}, T_{22}, T_{23}, tender-M, moisture and juicy-M. Within this cluster, one small cluster contained tender-M, juicy-M and moisture, reflecting their higher correlation.

During cooking processes, the fish protein structure denatures in many ways, such as myofibrillar contraction, connective tissues solubilisation and sarcoplasmic protein aggregation (Tornberg, 2005). The transversal and longitudinal shrinkage of fibres can lead to the increased width of the gap between fibres and their surrounding endomysium, and the shortening of sarcomere and fibre, respectively (Tornberg, 2005). As shown in the previous report (Wang et al., 2020), with the increase of the heating temperature on surf clam, the myofibril stretched over to form a streak pattern, and then underwent dilaceration, contraction and separated into the fragmented structure. Meanwhile, with the increase of the cooking time, the gap between fibres was also more apparent under the microscope (Xia et al., 2017), which was also convinced by the instrumental texture profile analysis on the hog maw that the chewiness decreased apparently as cooking time prolonged (Zheng et al., 2021). Therefore, for RTR, the highest temperature and longest heating time produced more fragments with less water, dominated the high values and the positive correlation between chewy, fibre mouthfeel attributes and water loss. By contrast, it is reported that the microwave-heating process with the faster heating rate and less heating time could enhance disulphide bonds but denatured protein inadequately, which result in higher transverse relaxation time values of immobilised water T_{22} and free water T_{23}, a better gel network and tenderness mouthfeel (Wang et al., 2021). This corresponded to the positive correlation among T_{22}, T_{23}, tender-M, juicy-M and moisture indexes for MTR.

4. Conclusion

The sensory and related physico-chemical properties of *T. rubripes* (pufferfish) were investigated when four cooking processes (i.e. boiling, steaming, microwave-heating and roasting) were applied. The changes of centre temperature dynamics led to different water dynamics and the decrease of the water mobility during thermal cooking processes, which finally resulted in different physico-chemical properties and sensory properties of cooked pufferfish. Sensory analysis generated 40 attributes (22 descriptors), including 20 orthonasal/retronasal aroma (O/R), 6 taste (T), 5 mouthfeel (M) and 9 after-effect (A) attributes. The top two sensory properties were orthonasal aroma and mouthfeel, and it was possible to differentiate the four cooked samples. The correlation of orthonasal sensory attributes with

volatile compounds was observed, such as “roasted-O, oily-O and fishy-O” attributes which were highly related with the contribution of most heterocyclic compounds from the Maillard reaction, and off-notes like “boiled vegetable-O, earthy-O and putrid fish-O” highly associated with most aldehydes and alcohols derived from lipid oxidation. The specific mouthfeel attributes of chewy and fibre were highly correlated with the water loss, while the tender and juicy mouthfeel attributes were highly associated with moisture. The cooking process with low water content, high temperature and long heating time (i.e., roasting) might be the best method, which generated pleasant roasted aromas and reduced levels of off-notes with reduced tenderness and juiciness mouthfeel. Overall, the thermal process is an essential step to create a desirable sensory quality of cooked pufferfish with a reduced risk of rancidity compared to the raw fish, and different methods resulted in distinct flavour and texture profiles. The sensory attributes showed good correlations with instrumental analysis, so the instrumental data can be used as markers for the sensory quality evaluation and as tools for the production chain of cooked pufferfish. The combination of sensory and analytical results proposed an optimal cooking process for fish processing companies to meet consumers requirements.

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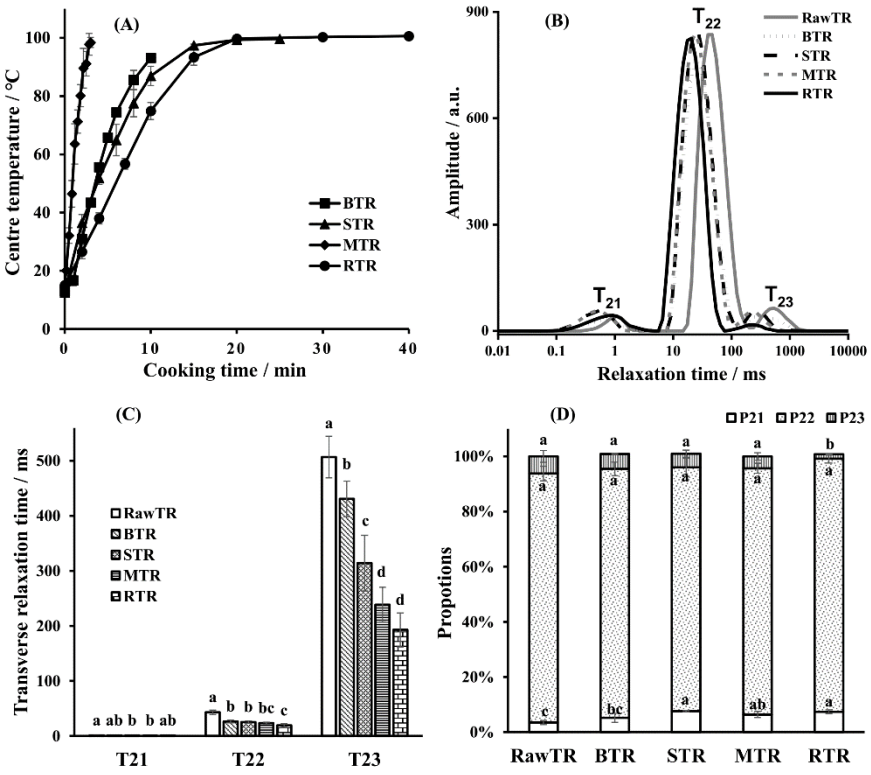

Figure 1 Centre temperature curves (A) and water dynamic of raw and cooked *T. rubripe* by LF-NMR [1, 2]: (B) continuous transverse relaxation time curves; (C) transverse relaxation time (T_{21}, bound water, T_{22}, immobilised water, T_{23}, free water); (D) peak area proportion (P_{21}, P_{22}, P_{23})

1. Different lowercases (a, b, c, d) of each parameter among samples indicated significant difference by ANOVA with a Post-hoc Tukey test at α=0.05. Error bars represented standard deviations of means (n=4).
2. Abbreviations: RawTR, Raw *T. rubripes*; BTR, Boiled *T. rubripes*; STR, Steamed *T. rubripes*; MTR, Microwave-heated *T. rubripes*; RTR, Roasted *T. rubripes*.

Table 1 Water content and colour of raw and cooked *T. rubripes* [1]

Cooked *T. rubripes*	Remaining weight (%)	Moisture (%)	Colour			
			L*	a*	b*	ΔE [2]
Raw *T. rubripes* (RawTR)	-	79.70 ± 0.00 a	54.91 ± 0.76 d	-1.83 ± 0.13 d	-3.90 ± 0.58 d	-
Boiled *T. rubripes* (BTR)	67.92 ± 0.02 a	70.77 ± 0.01 b	77.82 ± 1.11 b	-0.76 ± 0.34 b	8.49 ± 1.23 c	26.07
Steamed *T. rubripes* (STR)	63.14 ± 0.03 b	69.22 ± 0.01 bc	76.85 ± 1.29 b	-0.53 ± 0.14 b	10.93 ± 0.64 b	26.51
Microwave-heated *T. rubripes* (MTR)	62.87 ± 0.01 b	69.00 ± 0.02 c	80.33 ± 1.42 a	-1.11 ± 0.21 c	7.60 ± 1.30 c	27.92
Roasted *T. rubripes* (RTR)	47.80 ± 0.02 c	56.93 ± 0.02 d	65.85 ± 1.58 c	3.36 ± 0.26 a	20.92 ± 1.36 a	27.62

1. The data were presented as Mean ± Standard Deviation (n=8). Different lowercases (a, b, c, d) for each column indicated significant difference by ANOVA with a Post-hoc Tukey test at α=0.05.
2. Reference to Raw *Takifugu rubripes* (RawTR).

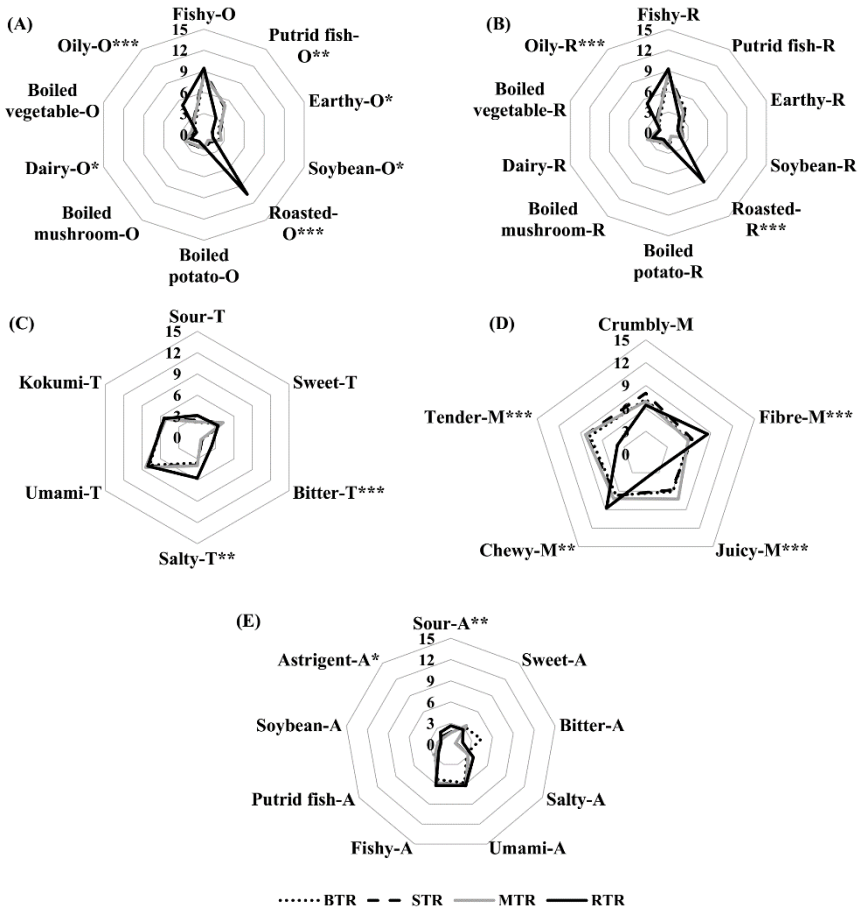

Figure 2 The quantitative descriptive analysis (QDA) results of cooked *T. rubripes* [1,2]: profiles of (A) orthonasal aroma, (B) retronasal aroma, (C) taste, (D) mouthfeel and (E) after-effect

1. *, **, ***: significant at $p<0.05$, $p<0.001$ and $p<0.0001$ respectively (n=3).
2. Abbreviations: BTR, Boiled *T. rubripes (dotted lines)*; STR, Steamed *T. rubripes (dashed lines)*; MTR, Microwave-heated *T. rubripes (grey solid lines)*; RTR, Roasted *T. rubripes (black solid lines)*.

(2E)

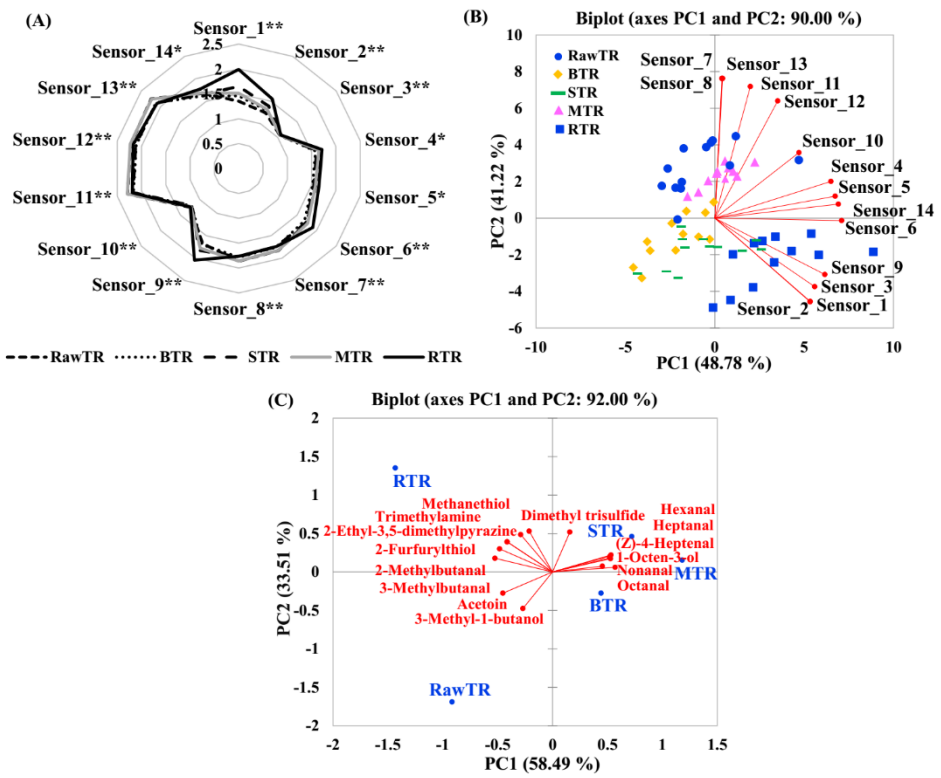

Figure 3 Aroma analysis of cooked *T. rubripes* [1] with a radar chart (A) [2] and Principal Component Analysis (PCA) (B) based on E-nose data and a PCA (C) from the volatile compounds with the relative odour activity value [3] higher than 1 based on GC-TOFMS data and thresholds

1. Abbreviations: RawTR, Raw *T. rubripes*; BTR, Boiled *T. rubripes*; STR, Steamed *T. rubripes*; MTR, Microwave-heated *T. rubripes*; RTR, Roasted *T. rubripes*.
2. *, **: significant at $p<0.001$ and $p<0.0001$ respectively (n=12).
3. Relative odour activity value was calculated as the ratio of the relative headspace concentration to the threshold from Table 2.

Table 2 Volatile compounds and relative headspace concentration of cooked *T. rubripes* (μg/kg) [1]

No.	Volatile compounds [2]	CAS	Threshold (ug/kg) [3]	RI [4]	Concentration (μg/kg)				
					RawTR	BTR	STR	MTR	RTR
	Hydrocarbons (36)								
1	Hexane	110-54-3	-	600	18.35 ± 6.00 b	7.19 ± 3.41 b	13.76 ± 6.22 b	82.90 ± 74.29 a	62.26 ± 11.32 ab
2	Heptane	142-82-5	50000	700	2.42 ± 0.57 b	13.54 ± 2.87 a	18.50 ± 5.87 a	14.06 ± 1.78 a	19.39 ± 5.25 a
3	Ethylcyclohexane	1678-91-7	-	885	-	0.54 ± 0.23 bc	0.83 ± 0.08 ab	0.40 ± 0.09 bc	1.24 ± 0.83 a
4	1,2,3-Trimethylcyclopentene	473-91-6	-	892	3.93 ± 2.24 a	4.32 ± 1.41 a	4.04 ± 0.42 a	4.63 ± 1.13 a	4.81 ± 2.92 a
5	Nonane	111-84-2	10000	900	0.65 ± 0.42 c	1.92 ± 0.80 b	2.72 ± 0.57 b	2.24 ± 0.60 b	4.24 ± 0.66 a
6	2,4-Octadiene	13643-08-8	-	926	1.18 ± 0.62 b	3.18 ± 1.21 a	4.64 ± 0.48 a	3.78 ± 0.82 a	4.79 ± 1.05 a
7	Benzene	71-43-2	72 d	939	1.90 ± 0.38 b	3.65 ± 1.21 a	4.51 ± 0.86 a	3.56 ± 0.53 a	4.44 ± 0.85 a
8	2,2,4,6,6-Pentamethylheptane	13475-82-6	-	961	0.85 ± 0.37 a	0.84 ± 0.21 a	1.27 ± 0.54 a	0.78 ± 0.09 a	0.67 ± 0.62 a
9	Decane	124-18-5	10000 d	1000	0.11 ± 0.06 b	0.13 ± 0.04 b	0.18 ± 0.06 b	0.16 ± 0.03 b	0.27 ± 0.05 a
10	*α*-Pinene	80-56-8	14 d	1014	0.96 ± 0.45 b	1.39 ± 0.20 ab	1.59 ± 0.39 a	1.67 ± 0.32 a	1.75 ± 0.24 a
11	Toluene	108-88-3	527 d	1038	6.21 ± 2.94 b	7.02 ± 1.81 ab	7.20 ± 1.01 ab	6.81 ± 1.47 ab	10.62 ± 2.67 a
12	*β*-Pinene	127-91-3	140 d	1093	0.54 ± 0.11 b	0.83 ± 0.14 ab	1.07 ± 0.21 a	1.26 ± 0.33 a	1.20 ± 0.22 a
13	Undecane	1120-21-4	10000	1100	0.30 ± 0.12 ab	0.20 ± 0.06 b	0.28 ± 0.05 ab	0.25 ± 0.04 b	0.41 ± 0.04 a
14	*β*-Phellandrene	555-10-2	500 d	1107	0.21 ± 0.12 b	1.32 ± 0.60 a	0.91 ± 0.21 a	1.19 ± 0.41 a	0.97 ± 0.18 a
15	Ethylbenzene	100-41-4	2205.25 d	1119	1.75 ± 0.77 a	2.08 ± 0.73 a	2.56 ± 0.56 a	2.09 ± 0.45 a	2.75 ± 0.69 a
16	1,4-Dimethylbenzene	106-42-3	250 d	1128	1.06 ± 0.51 a	1.34 ± 0.44 a	1.51 ± 0.31 a	1.40 ± 0.28 a	1.79 ± 0.51 a
17	1,3-Dimethylbenzene	108-38-3	1000	1134	2.30 ± 1.03 b	3.13 ± 0.76 ab	4.00 ± 0.84 ab	3.64 ± 0.90 ab	4.48 ± 1.06 a
18	3-Methylundecane	1002-43-3	-	1158	0.06 ± 0.03 ab	0.09 ± 0.03 a	0.06 ± 0.03 ab	0.03 ± 0.00 b	0.05 ± 0.01 ab
19	*β*-Myrcene	123-35-3	1.2 d	1156	-	0.15 ± 0.03 b	0.22 ± 0.05 ab	0.22 ± 0.06 ab	0.26 ± 0.05 a
20	D-Limonene	5989-27-5	34 d	1188	0.92 ± 0.43 c	1.36 ± 0.34 bc	3.93 ± 1.09 a	1.60 ± 0.37 bc	2.45 ± 0.75 b
21	Dodecane	112-40-3	10000 d	1200	0.36 ± 0.12 c	0.46 ± 0.06 c	0.91 ± 0.24 a	0.61 ± 0.07 bc	0.84 ± 0.12 ab
22	Propylbenzene	103-65-1	177.12 d	1203	0.06 ± 0.03 b	0.10 ± 0.03 ab	0.12 ± 0.02 ab	0.12 ± 0.03 a	0.15 ± 0.05 a
23	*m*-Ethyltoluene	620-14-4	800 r	1217	0.24 ± 0.12 c	0.40 ± 0.09 bc	0.53 ± 0.13 ab	0.52 ± 0.10 ab	0.71 ± 0.20 a
24	*p*-Ethyltoluene	622-96-8	600 r	1220	1.22 ± 0.75 a	1.42 ± 0.43 a	1.51 ± 0.33 a	1.48 ± 0.27 a	1.86 ± 0.50 a
25	Mesitylene	108-67-8	3 d	1239	0.61 ± 0.30 a	0.73 ± 0.16 a	0.76 ± 0.12 a	0.74 ± 0.11 a	0.90 ± 0.30 a
26	Styrene	100-42-5	3.6 d	1257	0.55 ± 0.26 a	0.68 ± 0.24 a	0.67 ± 0.17 a	0.60 ± 0.12 a	0.80 ± 0.21 a
27	*p*-Cymene	99-87-6	6.2 d	1264	0.38 ± 0.16 b	0.54 ± 0.16 ab	0.76 ± 0.14 a	0.73 ± 0.21 a	0.85 ± 0.17 a
28	Pseudocumene	95-63-6	260 r	1278	0.92 ± 0.43 b	1.55 ± 0.37 ab	1.65 ± 0.30 ab	1.63 ± 0.24 ab	2.03 ± 0.57 a
29	Tridecane	629-50-5	-	1300	0.36 ± 0.08 d	0.69 ± 0.13 c	1.09 ± 0.24 ab	0.83 ± 0.07 bc	1.25 ± 0.21 a
30	4-Propyltoluene	1074-55-1	-	1299	0.26 ± 0.11 c	0.45 ± 0.07 b	0.69 ± 0.10 a	0.56 ± 0.11 ab	0.74 ± 0.08 a
31	2-Ethyl-*p*-xylene	1758-88-9	-	1321	0.18 ± 0.09 b	0.36 ± 0.10 ab	0.36 ± 0.06 ab	0.36 ± 0.05 ab	0.46 ± 0.12 a
32	1,2,3-Trimethylbenzene	526-73-8	-	1334	0.92 ± 0.35 a	0.94 ± 0.24 a	0.94 ± 0.10 a	0.94 ± 0.11 a	1.07 ± 0.31 a
33	Tetradecane	629-59-4	1	1400	0.37 ± 0.10 b	0.36 ± 0.06 b	0.56 ± 0.08 a	0.42 ± 0.03 ab	0.51 ± 0.12 ab
34	2,5-Dimethyl-*p*-xylene	95-93-2	-	1433	0.21 ± 0.08 b	0.39 ± 0.11 a	0.40 ± 0.05 a	0.38 ± 0.04 a	0.46 ± 0.11 a
35	Pentadecane	629-62-9	-	1500	-	-	0.05 ± 0.00 b	0.04 ± 0.00 b	0.08 ± 0.02 a
36	2,6,10,14-Tetramethylpentadecane	1921-70-6	-	1679	0.06 ± 0.02 c	0.08 ± 0.02 bc	0.13 ± 0.01 b	0.08 ± 0.01 bc	0.20 ± 0.06 a
	Sulphur-containing compounds (11)								
37	**Methanethiol**	74-93-1	0.02 d	681	-	12.38 ± 2.96 cd	36.07 ± 4.31 b	18.15 ± 6.92 c	66.87 ± 18.91 a
38	Dimethyl disulfide	624-92-0	1.1 d	1071	-	0.98 ± 0.27 a	0.76 ± 0.19 ab	0.55 ± 0.14 b	0.56 ± 0.22 b
39	3-Methylthiophene	616-44-4	-	1089	-	-	0.18 ± 0.02 b	-	0.75 ± 0.18 a
40	2-Ethylthiophene	872-55-9	-	1168	0.19 ± 0.10 b	0.22 ± 0.07 b	0.37 ± 0.08 b	0.19 ± 0.03 b	0.57 ± 0.17 a
41	4-Methylthiazole	693-95-8	55 d	1288	-	-	-	-	0.16 ± 0.04 a
42	**Dimethyl trisulfide**	3658-80-8	0.1 d	1376	-	0.15 ± 0.03 b	0.27 ± 0.07 a	0.16 ± 0.07 b	0.23 ± 0.06 ab
43	**2-Furfurylthiol**	98-02-2	0.036 d	1438	-	-	-	-	0.24 ± 0.06 a
44	Methional	3268-49-3	0.45 d	1464	-	-	-	-	0.14 ± 0.04 a
45	2-Acetylthiazole	24295-03-2	3 d	1656	-	-	-	-	0.12 ± 0.02 a
46	5-Methyl-2-thiophenecarboxaldehyde	13679-70-4	-	1737	-	-	0.04 ± 0.01 b	-	0.13 ± 0.01 a
47	Benzothiazole	95-16-9	80 d	1966	0.35 ± 0.15 a	0.31 ± 0.06 a	0.24 ± 0.07 a	0.34 ± 0.08 a	0.26 ± 0.08 a
	Nitrogen-containing compounds (15)								
48	**Trimethylamine**	75-50-3	8 d	827	36.67 ± 9.94 c	44.45 ± 11.25 c	94.75 ± 21.81 b	49.82 ± 11.49 c	160.06 ± 24.82 a
49	Methylpyrazine	109-08-0	30000 d	1275	-	0.26 ± 0.06 b	0.10 ± 0.03 b	0.04 ± 0.01 b	5.49 ± 0.89 a
50	2-Ethyl-pyridine	100-71-0	-	1290	-	-	0.21 ± 0.04 ab	0.16 ± 0.03 b	0.22 ± 0.04 a
51	2,5-Dimethylpyrazine	123-32-0	1750 d	1332	-	0.11 ± 0.03 b	0.08 ± 0.01 b	0.12 ± 0.02 b	3.07 ± 0.34 a
52	2,6-Dimethylpyrazine	108-50-9	718 d	1338	-	0.08 ± 0.02 b	0.09 ± 0.02 b	0.12 ± 0.04 b	3.30 ± 0.50 a

53	2,3-Dimethylpyrazine	5910-89-4	800 d	1356	-	-	-	-	1.18 ± 0.18 a
54	2-Ethyl-5-methylpyrazine	13360-64-0	16 d	1392	-	0.12 ± 0.04 bc	0.12 ± 0.03 bc	0.19 ± 0.02 b	1.45 ± 0.22 a
55	2-Ethyl-6-methylpyrazine	13925-03-6	40 d	1398	-	-	-	-	0.87 ± 0.09 a
56	Trimethylpyrazine	14667-55-1	350.12 d	1414	-	-	-	-	4.82 ± 0.10 a
57	2,6-Diethylpyrazine	13067-27-1	6 d	1439	-	-	-	-	0.11 ± 0.02 a
58	3-Ethyl-2,5-dimethylpyrazine	13360-65-1	8.6 d	1452	-	-	-	-	1.40 ± 0.39 a
59	**2-Ethyl-3,5-dimethylpyrazine**	13925-07-0	0.04 d	1468	-	-	-	-	1.25 ± 0.16 a
60	Tetramethylpyrazine	1124-11-4	2525.02 d	1484	-	-	-	-	0.32 ± 0.00 a
61	2,3,5-Trimethyl-6-ethylpyrazine	17398-16-2	-	1520	-	-	-	-	0.19 ± 0.03 a
62	Pyrrole	109-97-7	20000 d	1523	-	0.16 ± 0.01 b	0.14 ± 0.03 b	0.11 ± 0.04 b	0.92 ± 0.32 a
	Aldehydes (12)								
63	**2-Methylbutanal**	96-17-3	1 d	916	8.11 ± 3.69 b	6.81 ± 4.12 b	6.57 ± 0.62 b	6.62 ± 1.40 b	13.92 ± 3.04 a
64	**3-Methylbutanal**	590-86-3	1.1 d	920	19.75 ± 7.80 b	5.14 ± 2.41 c	6.61 ± 0.82 c	8.28 ± 0.77 c	35.66 ± 5.60 a
65	Pentanal	110-62-3	12 d	983	4.08 ± 0.96 b	10.22 ± 2.84 a	11.76 ± 1.51 a	11.57 ± 3.17 a	5.95 ± 1.24 b
66	**Hexanal**	66-25-1	5 d	1083	24.11 ± 8.22 b	52.99 ± 13.00 a	59.70 ± 6.94 a	65.71 ± 8.46 a	35.11 ± 7.63 b
67	**Heptanal**	111-71-7	2.8 d	1186	4.39 ± 0.92 b	6.46 ± 2.02 ab	8.16 ± 2.01 a	7.52 ± 2.10 ab	4.80 ± 1.02 ab
68	**(Z)-4-Heptenal**	6728-31-0	0.0087 d	1244	0.62 ± 0.18 c	1.15 ± 0.37 abc	1.28 ± 0.21 ab	1.60 ± 0.43 a	0.84 ± 0.19 bc
69	**Octanal**	124-13-0	0.587 d	1291	2.62 ± 0.31 b	3.50 ± 0.77 ab	4.03 ± 1.00 a	4.25 ± 0.83 a	2.41 ± 0.54 b
70	**Nonanal**	124-19-6	1.1 d	1396	1.52 ± 0.26 b	1.74 ± 0.36 b	1.97 ± 0.38 b	3.05 ± 0.63 a	1.50 ± 0.17 b
71	Furfural	98-01-1	9562 d	1476	-	-	-	-	0.45 ± 0.17 a
72	(E)-2-Octenal	2548-87-0	3 d	1494	0.23 ± 0.04 ab	0.22 ± 0.08 ab	0.30 ± 0.05 a	0.25 ± 0.06 ab	0.17 ± 0.03 b
73	Benzaldehyde	100-52-7	750.89 d	1532	1.44 ± 0.63 b	1.65 ± 0.19 b	2.60 ± 0.60 ab	2.05 ± 0.51 b	3.67 ± 0.91 a
74	4-Ethylbenzaldehyde	53951-50-1	-	1716	0.24 ± 0.06 b	0.39 ± 0.08 ab	0.50 ± 0.13 a	0.51 ± 0.15 a	0.35 ± 0.08 ab
	Ketones (15)								
75	2-Butanone	78-93-3	35400.2 d	908	3.07 ± 0.46 b	3.32 ± 0.38 b	4.01 ± 1.03 b	3.70 ± 0.69 b	6.58 ± 1.74 a
76	2,3-Pentanedione	600-14-6	20 d	1065	2.49 ± 0.62 b	3.46 ± 0.96 ab	3.87 ± 0.74 ab	4.39 ± 1.32 a	2.25 ± 0.44 b
77	6-Methyl-2-heptanone	928-68-7	8.1 d	1240	0.51 ± 0.22 a	0.38 ± 0.13 a	0.41 ± 0.06 a	0.37 ± 0.05 a	0.30 ± 0.10 a
78	Dihydro-2-methyl-3(2H)-furanone	3188-00-9	-	1270	-	-	-	-	0.38 ± 0.12 a
79	**Acetoin**	513-86-0	14 d	1296	20.09 ± 6.45 a	2.60 ± 0.55 c	6.72 ± 1.28 bc	2.80 ± 0.79 c	11.52 ± 2.45 b
80	1-Hydroxy-2-propanone	116-09-6	80000 d	1313	-	1.31 ± 0.15 c	8.25 ± 1.86 b	1.49 ± 0.37 c	42.16 ± 6.81 a
81	(Z)-6-Octen-2-one	74810-53-0	-	1336	0.26 ± 0.09 b	0.76 ± 0.31 a	0.68 ± 0.10 a	0.78 ± 0.24 a	0.44 ± 0.11 ab
82	6-Methyl-5-hepten-2-one	110-93-0	68 d	1342	0.25 ± 0.09 a	0.31 ± 0.13 a	0.38 ± 0.03 a	0.41 ± 0.09 a	0.43 ± 0.12 a
83	2-Hydroxy-3-pentanone	5704-20-1	-	1368	-	0.33 ± 0.06 b	0.48 ± 0.11 a	0.54 ± 0.07 a	0.49 ± 0.09 a
84	2-Nonanone	821-55-6	41 d	1393	3.21 ± 0.78 b	2.01 ± 0.30 c	2.94 ± 0.22 bc	4.10 ± 0.70 a	2.29 ± 0.28 bc
85	1-(Acetyloxy)-2-propanone	592-20-1	-	1480	-	-	-	-	4.73 ± 1.24 a
86	3,5-Octadienone	38284-27-4	-	1530	1.31 ± 0.13 ab	1.25 ± 0.29 ab	1.41 ± 0.33 ab	1.74 ± 0.36 a	0.97 ± 0.26 b
87	2-Undecanone	112-12-9	5.5 d	1604	0.18 ± 0.03 a	0.12 ± 0.04 a	0.18 ± 0.03 a	0.20 ± 0.05 a	0.15 ± 0.03 a
88	Butyrolactone	96-48-0	20000	1644	0.40 ± 0.14 b	0.28 ± 0.09 b	0.34 ± 0.03 b	0.29 ± 0.06 b	1.34 ± 0.46 a
89	4-Hydroxy-5-methyl-3(2H)-furanone	19322-27-1	2100 d	2144	-	0.08 ± 0.01 b	0.36 ± 0.10 b	0.07 ± 0.00 b	1.58 ± 0.38 a
	Alcohols (18)								
90	1-Propanol	71-23-8	8505.6 d	1046	0.70 ± 0.16 a	0.82 ± 0.21 a	1.06 ± 0.29 a	1.09 ± 0.31 a	0.76 ± 0.15 a
91	1-Butanol	71-36-3	459.2 d	1156	1.27 ± 0.47 a	0.77 ± 0.20 a	0.82 ± 0.22 a	0.74 ± 0.15 a	0.77 ± 0.12 a
92	1-Penten-3-ol	616-25-1	358.1 d	1170	65.83 ± 17.77 b	111.97 ± 18.46 a	121.01 ± 15.23 a	146.94 ± 27.09 a	74.77 ± 15.60 b
93	Eucalyptol	470-82-6	1.1 d	1202	0.47 ± 0.19 b	0.47 ± 0.09 b	0.56 ± 0.07 b	0.99 ± 0.24 a	0.51 ± 0.16 b
94	**3-Methyl-1-butanol**	123-51-3	4 d	1217	6.00 ± 2.59 a	0.91 ± 0.27 b	0.95 ± 0.20 b	1.33 ± 0.26 b	1.23 ± 0.40 b
95	1-Pentanol	71-41-0	150.2 d	1260	1.60 ± 0.48 ab	2.17 ± 0.65 ab	2.40 ± 0.59 a	2.51 ± 0.63 a	1.26 ± 0.26 b
96	(E)-2-Penten-1-ol	1576-96-1	89.2 d	1323	0.22 ± 0.09 b	0.30 ± 0.07 ab	0.32 ± 0.03 ab	0.40 ± 0.11 a	0.24 ± 0.05 b
97	(Z)-2-Penten-1-ol	1576-95-0	720 d	1331	1.30 ± 0.45 b	1.92 ± 0.53 ab	2.10 ± 0.29 a	2.24 ± 0.13 a	1.62 ± 0.31 ab
98	1-Hexanol	111-27-3	5.6 d	1362	2.73 ± 0.47 a	1.20 ± 0.35 b	1.39 ± 0.32 b	1.59 ± 0.50 b	1.08 ± 0.29 b
99	3-Hexen-1-ol	544-12-7	1630 d	1373	0.12 ± 0.06 a	0.13 ± 0.05 a	0.16 ± 0.04 a	0.17 ± 0.04 a	0.13 ± 0.03 a
100	**1-Octen-3-ol**	3391-86-4	1.5 d	1460	6.12 ± 1.10 c	8.53 ± 1.71 bc	10.39 ± 1.16 ab	12.26 ± 2.73 a	7.06 ± 1.64 bc
101	1-Heptanol	111-70-6	5.4 d	1465	2.79 ± 0.65 ab	3.39 ± 0.96 ab	3.68 ± 0.96 ab	4.43 ± 1.50 a	2.20 ± 0.60 b
102	2-Ethyl-1-hexanol	104-76-7	25482.2 d	1499	0.82 ± 0.36 a	0.60 ± 0.18 a	0.56 ± 0.11 a	0.62 ± 0.09 a	0.66 ± 0.16 a
103	1-Octanol	111-87-5	125.8 d	1568	0.59 ± 0.25 a	0.28 ± 0.04 b	0.33 ± 0.08 ab	0.43 ± 0.07 ab	0.27 ± 0.04 b
104	(E)-2-Octen-1-ol	18409-17-1	40 d	1627	0.17 ± 0.03 c	0.21 ± 0.04 bc	0.30 ± 0.08 ab	0.32 ± 0.06 a	0.20 ± 0.06 bc
105	1-Nonanol	143-08-8	45.5 d	1670	0.19 ± 0.08 a	-	0.07 ± 0.01 b	0.08 ± 0.01 b	0.05 ± 0.02 b
106	2-Furanmethanol	98-00-0	4500 d	1677	-	-	-	-	2.55 ± 1.26 a
107	2,7-Octadien-1-ol	23578-51-0		1696	0.66 ± 0.14 c	1.18 ± 0.28 ab	1.25 ± 0.20 ab	1.54 ± 0.35 a	0.86 ± 0.20 bc
	Others (10)								
108	Methyl ethanoate	79-20-9	1500 d	831	2.76 ± 1.22 a	0.21 ± 0.04 c	0.37 ± 0.07 c	0.22 ± 0.04 c	1.73 ± 0.25 b
109	2-Ethylfuran	3208-16-0	-	952	8.34 ± 1.43 b	17.27 ± 4.03 a	20.60 ± 4.05 a	21.14 ± 4.27 a	19.99 ± 4.15 a
110	Methyl butanoate	623-42-7	59 d	989	0.85 ± 0.33 a	0.34 ± 0.08 b	0.33 ± 0.06 b	0.31 ± 0.02 b	0.41 ± 0.12 b
111	2-Pentylfuran	3777-69-3	5.8 d	1228	0.60 ± 0.10 b	1.53 ± 0.39 a	2.32 ± 0.79 a	1.76 ± 0.42 a	2.34 ± 0.36 a

112	(E)-2-(2-Pentenyl)furan	70424-14-5	-	1301	0.17 ± 0.05 c	0.24 ± 0.05 bc	0.32 ± 0.05 ab	0.29 ± 0.06 ab	0.40 ± 0.07 a
113	Methyl 2-hydroxypropanoate	2155-30-8	-	1330	1.94 ± 0.85 a	0.62 ± 0.12 b	0.96 ± 0.24 b	0.90 ± 0.21 b	1.64 ± 0.24 a
114	2-Acetylfuran	1192-62-7	15025.2 d	1516	-	-	-	-	1.94 ± 0.15 a
115	3-Methylbutanoic acid	503-74-2	490 d	1689	0.76 ± 0.56 a	-	0.34 ± 0.01 b	0.31 ± 0.12 b	0.33 ± 0.05 b
116	Octanoic acid	124-07-2	3000 d	2080	1.62 ± 1.03 a	0.44 ± 0.14 b	0.19 ± 0.03 b	0.24 ± 0.09 b	0.17 ± 0.04 b
117	Nonanoic acid	112-05-0	4600	2185	0.64 ± 0.35 a	0.70 ± 0.13 a	0.23 ± 0.03 b	0.45 ± 0.27 ab	0.17 ± 0.03 b

1. The data was were presented as Mean ± Standard Error (n=4). Different lowercases for each row indicated significant differences by ANOVA with a Post-hoc Tukey test at α=0.05.
2. Important volatile compounds, whose relative odour activity values (OAV, concentration in ppb/odour threshold) were higher than the reference thresholds, were marked in bold.
3. Thresholds were collected by previous reports (van Gemert, 2011).
4. RIs were compared with NIST Chemistry Webbook: webbook.nist.gov/chemistry/
5. Abbreviations: RawTR, Raw *T. rubripes*; BTR, Boiled *T. rubripes*; STR, Steamed *T. rubripes*; MTR, Microwave-heated *T. rubripes*; RTR, Roasted *T. rubripes*.

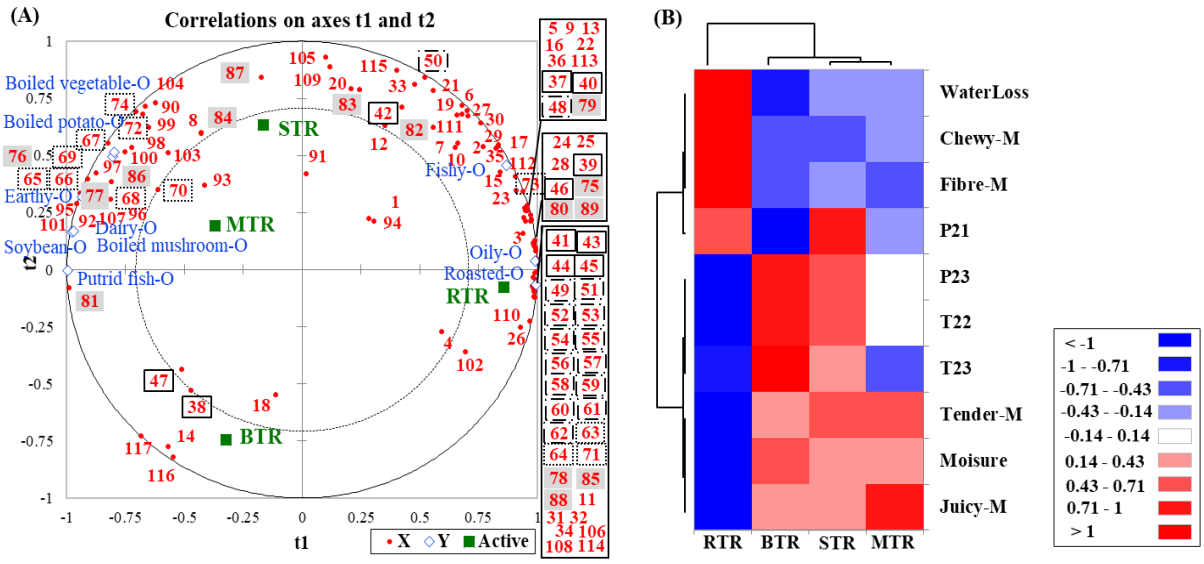

Figure 4 Correlation analysis between sensory attributes and physical-chemical properties of cooked *T. rubripes* [1]: (A) Partial Least Squares Regression (PLS-R) analysis from the data of sensory orthonasal attributes and volatile compounds by GC-TOFMS [2, 3], (B) Hierarchical Cluster Analysis Heatmap (HCA Heatmap) based on the data of water properties and sensory mouthfeel attributes with significant differences among cooked samples [4]

1. Abbreviations: BTR, Boiled *T. rubripes*; STR, Steamed *T. rubripes*; MTR, Microwave-heated *T. rubripes*; RTR, Roasted *T. rubripes*.
2. The first two components: Q^2 cum= 0.846, R^2Y cum=0.956, R^2X cum= 0.874.
3. Volatile compounds corresponded to Table 2: Sulphur-containing compounds (*solid lines*); Nitrogen-containing compounds (*dashed and solid lines*); Aldehydes (*dashed lines*); Ketones (*grey backgrounds*).
4. Red colour represented higher abundance, white represents medium abundance, and blue represented lower abundance.

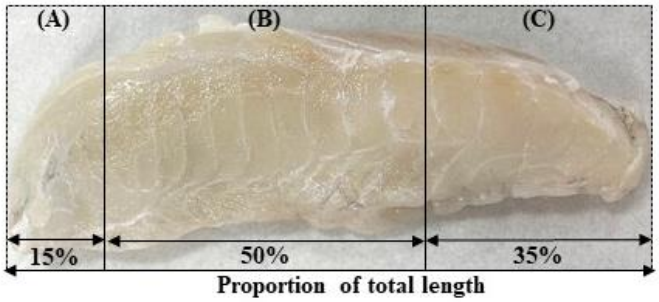

Figure S1 Sampling parts for various analysis of *T. rubripes* fillets: (A) colour and moisture analysis, (B) aroma analysis, and (C) water distribution analysis

Table S1 Sensory attributes and their definition developed by trained panellists

Attributes	Definitions	Reference	Anchors
Fishy	Pleasant aroma associated with fish	5 g Grilled cod fillet (MR.BEAVER'S, China)	nil to extreme
Putrid fish	Unpleasant aroma associated with putrid fish	5 g raw pufferfish at room temperature for 1 h (Tianzheng, China)	nil to extreme
Earthy	Aroma associated with wet soil after rain		nil to extreme
Soybean	Beany aroma associate with soybean products, such as tofu	5 g tofu (Zuming, China)	nil to extreme
Roasted	Aroma associated with roasted peanut, bread, coca powder	5 g roasted Mankattan wheat bread (200 °C, 5 min) (Mankattan, China)	nil to extreme
Boiled potato	Sweet aroma associated with boiled potato	5 g boiled potato (Wumart, China)	nil to extreme
Boiled mushroom	Aroma associated with boiled mushroom	5 g boiled mushroom (Wumart, China)	nil to extreme
Dairy	Sweet odour associated with dairy, such as milk	5 mL SATINE pure milk (Yili, China)	nil to extreme
Boiled vegetable	Aroma associated with boiled green vegetable, such as lettuces	5 g boiled lettuces (Wumart, China)	nil to extreme
Oily	Aroma associated with refined olive oil	5 mL Olivoilà olive oil (Olivoilà, China)	nil to extreme
Sour	Taste associated with organic acid	0.430 mg/mL citric acid	nil to extreme
Sweet	Taste associated with sucrose	5.76 mg/mL sucrose	nil to extreme
Bitter	Taste associated with quinine	0.0325 mg/mL quinine sulphate	nil to extreme
Salty	Taste associated with sodium chloride	1.19 mg/mL sodium chloride	nil to extreme
Umami	Taste associated with sodium glutamate	0.595 mg/mL monosodium glutamate	nil to extreme
Kokumi	Mouth-filling taste associated with chicken broth with glutathione	5.0 mM glutathione	nil to extreme
Crumbly	Easily break the sample into pieces during chewing		regular to irregular
Fibre	Muscle fibre sense of the sample when chewing		nil to extreme
Juicy	Moisture released in the early stage of chewing		nil to extreme
Chewy	Number of chewing times until swallowing		nil to extreme
Tender	Minimum force required to chew samples		higher force to lower force
Astringent	Dryness mouthfeel sensation such as the contraction of the oral mucous surface		nil to extreme

Table S2 Water distribution of cooked *T. rubripes* [1] by LF-NMR

Cooked *T. rubripes*	Relaxation time/ms			Proportions/%		
	T21	T22	T23	P21	P22	P23
Raw *T. rubripes* (RawTR)	1.03 ± 0.22 a	42.93 ± 4.03 a	506.94 ± 37.80 a	3.48 ± 0.01 c	90.33 ± 0.03 a	6.19 ± 0.02 a
Boiled *T. rubripes* (BTR)	0.73 ± 0.19 ab	26.34 ± 2.47 b	430.78 ± 32.12 b	5.16 ± 0.02 bc	90.30 ± 0.02 a	5.45 ± 0.00 a
Steamed *T. rubripes* (STR)	0.58 ± 0.12 b	25.27 ± 2.14 b	313.96 ± 50.49 c	7.59 ± 0.00 a	88.45 ± 0.03 a	4.96 ± 0.01 a
Microwave-heated *T. rubripes* (MTR)	0.52 ± 0.09 b	23.29 ± 1.82 bc	238.54 ± 31.61 d	6.41 ± 0.01 ab	89.30 ± 0.02 a	4.29 ± 0.01 a
Roasted *T. rubripes* (RTR)	0.74 ± 0.23 ab	19.16 ± 3.36 c	192.64 ± 30.98 d	7.39 ± 0.01 a	91.80 ± 0.02 a	1.58 ± 0.00 b

1. The data were presented as Mean ± Standard Deviation. Different lowercases (a, b, c) for each column indicate significant difference by ANOVA with a Post-hoc Tukey test at α=0.05 (n=4).

Table S3 Perceived intensities of sensory attributes of cooked *T. rubripes* [1,2]

No.		Boiled *T. rubripes* (BTR)	Steamed *T. rubripes* (STR)	Microwave-heated *T. rubripes* (MTR)	Roasted *T. rubripes* (RTR)
	Orthonasal/Rethonasal aroma (O/R)				
1	Fishy-O	8.44 ± 0.56 a	9.11 ± 0.55 a	8.58 ± 0.64 a	9.46 ± 0.69 a
2	Fishy-R	8.27 ± 0.86 a	8.77 ± 0.66 a	8.70 ± 0.80 a	9.29 ± 0.77 a
3	**Putrid fish-O**	5.17 ± 0.94 a	4.91 ± 0.84 a	5.09 ± 0.82 a	2.92 ± 0.82 b
4	Putrid fish-R	4.23 ± 0.86 a	4.03 ± 0.86 a	3.71 ± 0.84 a	2.74 ± 0.76 a
5	**Earthy-O**	2.19 ± 0.58 ab	2.52 ± 0.60 a	2.37 ± 0.58 a	1.23 ± 0.31 b
6	Earthy-R	2.15 ± 0.53 a	1.97 ± 0.51 a	1.80 ± 0.45 a	1.44 ± 0.37 a
7	**Soybean-O**	2.26 ± 0.51 ab	2.30 ± 0.51 ab	2.57 ± 0.54 a	1.36 ± 0.35 b
8	Soybean-R	2.12 ± 0.47 a	2.28 ± 0.46 a	2.26 ± 0.48 a	1.82 ± 0.39 a
9	**Roasted-O**	1.03 ± 0.43 b	1.34 ± 0.49 b	0.99 ± 0.28 b	10.46 ± 0.65 a
10	**Roasted-R**	0.65 ± 0.20 b	1.14 ± 0.55 b	0.62 ± 0.21 b	8.83 ± 1.03 a
11	Boiled potato-O	1.91 ± 0.39 a	2.10 ± 0.41 a	1.96 ± 0.30 a	1.70 ± 0.48 a
12	Boiled potato-R	1.64 ± 0.34 a	1.74 ± 0.37 a	2.20 ± 0.46 a	1.79 ± 0.46 a
13	Boiled mushroom-O	1.85 ± 0.45 a	1.92 ± 0.43 a	1.87 ± 0.34 a	1.20 ± 0.32 a
14	Boiled mushroom-R	1.84 ± 0.37 a	1.68 ± 0.39 a	1.70 ± 0.37 a	1.20 ± 0.32 a
15	**Dairy-O**	2.85 ± 0.49 ab	3.14 ± 0.50 a	2.84 ± 0.53 ab	1.98 ± 0.36 b
16	Dairy-R	3.16 ± 0.48 a	2.76 ± 0.46 a	3.20 ± 0.55 a	2.36 ± 0.43 a
17	Boiled vegetable-O	1.35 ± 0.47 a	1.62 ± 0.62 a	1.80 ± 0.63 a	1.11 ± 0.38 a
18	Boiled vegetable-R	1.46 ± 0.58 a	1.38 ± 0.57 a	1.46 ± 0.59 a	1.27 ± 0.47 a
19	**Oily-O**	2.08 ± 0.29 b	2.51 ± 0.33 b	2.39 ± 0.39 b	5.15 ± 0.71 a
20	**Oily-R**	2.15 ± 0.32 b	2.76 ± 0.43 b	2.57 ± 0.35 b	5.21 ± 0.65 a
	Taste (T)				
1	Sour-T	2.18 ± 0.49 a	2.44 ± 0.40 a	2.23 ± 0.45 a	3.13 ± 0.66 a
2	Sweet-T	3.94 ± 0.74 a	4.10 ± 0.82 a	4.22 ± 0.78 a	3.41 ± 0.71 a
3	**Bitter-T**	0.70 ± 0.22 b	0.76 ± 0.21 b	0.59 ± 0.20 b	2.35 ± 0.55 a
4	**Salty-T**	3.59 ± 0.66 b	3.88 ± 0.68 b	3.98 ± 0.73 b	5.74 ± 0.65 a
5	Umami-T	7.80 ± 0.87 a	8.13 ± 0.73 a	8.43 ± 0.85 a	8.07 ± 0.73 a
6	Kokumi-T	5.43 ± 0.81 a	5.46 ± 0.81 a	5.17 ± 0.79 a	5.39 ± 0.87 a
	Mouthfeel (M)				
1	Crumbly-M	7.23 ± 0.96 a	7.99 ± 0.86 a	6.95 ± 0.95 a	6.44 ± 0.95 a
2	**Fibre-M**	5.96 ± 0.78 b	6.40 ± 0.83 b	5.87 ± 0.81 b	8.53 ± 0.86 a
3	**Juicy-M**	6.06 ± 0.71 ab	5.80 ± 0.69 b	7.27 ± 0.87 a	2.52 ± 0.49 c
4	**Chewy-M**	6.64 ± 0.74 b	6.72 ± 0.75 b	7.17 ± 0.78 b	8.75 ± 0.78 a
5	**Tender-M**	7.71 ± 0.95 a	8.26 ± 0.89 a	8.31 ± 0.81 a	3.92 ± 0.59 b
	After-effect (A)				
1	**Sour-A**	1.71 ± 0.33 b	1.75 ± 0.34 b	1.63 ± 0.32 b	2.64 ± 0.55 a
2	Sweet-A	3.18 ± 0.69 a	3.29 ± 0.69 a	3.47 ± 0.59 a	2.72 ± 0.60 a
3	Bitter-A	4.35 ± 6.27 a	0.73 ± 0.23 a	0.65 ± 0.24 a	1.69 ± 0.44 a
4	Salty-A	2.59 ± 0.55 a	2.88 ± 0.54 a	2.84 ± 0.52 a	3.68 ± 0.48 a
5	Umami-A	5.79 ± 0.86 a	6.05 ± 0.88 a	6.24 ± 0.88 a	6.22 ± 0.82 a
6	Fishy-A	5.29 ± 0.83 a	5.82 ± 0.81 a	5.78 ± 0.84 a	6.18 ± 0.89 a
7	Putrid fish-A	2.65 ± 0.71 a	2.37 ± 0.61 a	2.51 ± 0.64 a	1.96 ± 0.59 a
8	Soybean-A	1.62 ± 0.46 a	1.63 ± 0.38 a	1.85 ± 0.46 a	1.40 ± 0.37 a
9	**Astringent-A**	1.51 ± 0.34 b	1.61 ± 0.32 ab	1.36 ± 0.24 b	2.25 ± 0.47 a

1. The data were presented as Mean ± Standard Error (n=3). Different lowercases (a, b, c) for each row indicated significant difference by ANOVA with a Post-hoc Tukey test at α=0.05.
2. Attributes with significant differences were highlighted in bold.