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Umami taste components in chicken-spices blends and potential effect of aroma on umami taste intensity

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ABSTRACT: In this study, umami taste intensity (UTI) and umami taste components in chicken breast (CB) and chicken-spices blends were characterized using sensory and instrumental analysis. Our main objective was to assess the aroma-umami taste interactions in different food matrices and reconcile the aroma-taste perception to assist future product development. The impact of key aroma, including vegetable-note “2-pentylfuran”, meaty “methional”, green “hexanal”, and spicy-note “estragole and caryophyllene” on UTI was evaluated in MSG and chicken extract. We found that spices significantly decreased UTI and umami taste components in CB. Interestingly, the perceptually similar odorants and tastantsexhibited the potential to enhance UTI in food matrices. Methional was able to increase the UTI, whereas spicy and green-note components could reduce the UTI significantly. This information would be valuable to food engineers and formulators in aroma selection to control the UTI perceived by consumers, thus, improving the quality and acceptability of the chicken products.

Keywords: Chicken-spices blends, Umami taste components, Aroma-taste interactions, Perceptual similarity, Umami taste intensity

1. Introduction

Chicken is one of the most consumed sources of meat proteins, with high nutritional value, and is welcomed by consumers [1].Sanhuang chicken is a popular native breed in South China and is widely eaten due to its fatty skin, delicious flavour, soft and tender flesh [2]. The taste of chicken meat is further augmented by marination with spices, enhancing the flavour, and working in preservation and salt reduction [3, 4]. Meat marination with spices is most popular in Asia, followed by Europe. Each spice imparts a unique flavour and holds specific functional components that are helpful in lowering cholesterol, mental health, type 2 diabetes, inflammation, and cancer [5]. In order to meet consumers' demands in both flavour perceptions and health

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awareness, a tradition of using blends of spices has been very prevalent since ancient times. Chinese 5-spices blend (CS) and garam masala (GM) are the most popular and routinely used spices in China and Pakistan, respectively. These traditional spices have been proved to hold a considerable number of antioxidants and could be a good source of meat preservation [6, 7]; however, the flavour profile has not been thoroughly investigated. Therefore, in our pre-liminary study, we chose these spices to marinate chicken breast (CB) meat to evaluate the aroma profile analysis. Our results showed a considerable reduction of umami taste volatiles by the addition of spices to the chicken meat [8]. On the other hand, a previous study has marked a significant positive impact on hedonics of chicken broth made-up of the chicken breast after adding non-volatile umami ingredients [9]..

Umami taste, meaning “delicious” or “savoury”, is the 5th basic taste besides salty, sweet, sour, and bitter.The main umami contributing components are peptides (i.e., Asp-Asp, Gly-Glu, Gly-Asp, and Val-Val-Glu),nucleotides(i.e.,inosine monophosphate,adenosine monophosphate, and guanosine monophosphate), organic acids (i.e., lactic and succinic acids), and free amino acids (i.e., glutamate and aspartate) [10]. Chicken meat has been recognized as a good source of these non-volatile umami taste components. The umami taste has been characterised as food palatability which makes it different from the other four basic tastes [11, 12]. In the area of food palatability, the interactions between taste and aroma (particularly retro-nasal aroma) are gaining more attention due to their strong correlation with flavour perception [13]. Gustation and olfaction are physiologically and anatomically distinct objects, where taste and aroma are considered two sense modalities that may process independently. However, the mounting evidence showed that gustation and olfaction, particularly retro-nasal olfaction, interact closely, supporting the concept of a unified perceptual system for perceiving flavour in the mouth [14].

McBurney (1986) proposed that the phenomena of taste enhancement by retro-nasal odour and odour enhancement by taste mainly pertaining to the study of food perception and preferences [15]. A study has described that the simultaneous presentation of odorants and tastants made odours confuse with tastes, leading to increased taste intensity ratings [16]. Frank and Jennifer (1988) reported that such an effect would be due to the perceptual similarity between taste and odour [17]. They suggested that taste enhancement by odour is highly associated with the taste-like qualities of odours. Such as, fruity and strawberry odours have a good association with sweetness but not with wintergreen or peanut butter aroma [18]. Similarly, cheese aroma could enhance umami taste intensity [19], and glutamate imparts a more pleasant taste when presented in combination with savoury odour [20].

Chicken and umami taste are in harmony with each other and prepared together commercially in various products [21]. Research showed theenhancement of umami flavour by the conjunction of glutamate taste and complex savoury vegetable note aroma [20]. However, the individual savoury note has not been thoroughly investigated; thus, we hypothesize that savoury note odorants may enhance the umami taste. Previous studies also indicated the role of meaty note odorants (complex aroma mixture) in the enhancement of umami taste [19, 22]; though the effect of individual meaty odorant on referenced and complex umami taste has not been

examined. In our previous study, the collected aroma profile has shown that the addition of spices (CS and GM) could significantly decrease the umami note volatile compounds in the CB [8]. We, therefore, hypothesize that the spicy note aroma may reduce the umami taste. We are also interested in the impact of the most abundant odorant of the chicken sample having a sharp green note [8], on the umami taste. Based upon literature review and our preceding work [8], several odours that would potentially influence umami taste were selected, including vegetable-like "2-pentylfuran", meaty "methional", green "hexanal", and spicy note "estragole and caryophyllene".

It has been reported that the retro-nasal aroma intensity of a chicken model soup could be considerably increased by umami taste [23]; however, the aroma-taste interaction has not been explored between odorants and the umami taste intensity of chicken. Therefore, our main objective was to evaluate the impact of key aroma components on umami taste intensity in referenced (MSG) and complex (chicken extract "CE") food matrices via retro-nasal interaction. In addition, the proposed work herein would assist food developers and engineers in aroma selection and product design to improve the quality of the relevant products from the flavour and sensory perspectives.

2. Materials and methods

The first goal of this study was to identify the umami taste intensity and characterize the umami taste components in CB and chicken-spices blends (CSBs). The possible approach for enhancing the UTI in CSBs in order to increase food palatability [9] was then explored. Based on our previous work, odorants that exhibited potential impact on umami taste were selected in the current study. The addition of five selected aroma compounds at different levels were mixed with the umami referenced (MSG) and complex (CE) food matrices in terms of identifying the synergistic effect on umami perception. The research methodology flowchart is given in Figure 1. This research was assessed as having no risk of using all food-grade ingredients and followed ethical principles.

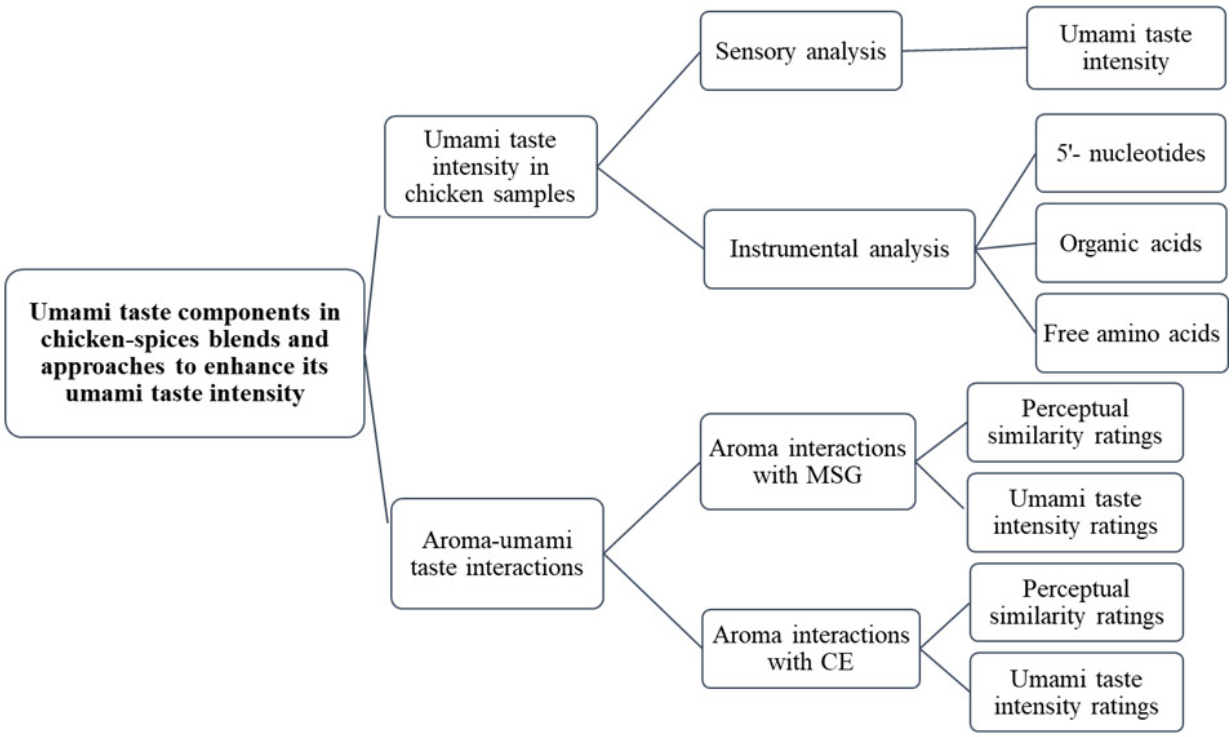

Fig. 1. Methodological flowchart.

2.1. Preparations

2.1.1. Chemicals

5' nucleotides [IMP (≥99%), AMP (≥99%), GMP (≥99%)], organic acids [lactic acid (≥98%) and succinic acid (≥99.5%)], free amino acids [glutamic acid (Glu, ≥99%), aspartic acid (Asp, ≥99%), alanine (Ala, ≥99%), glycine (Gly, ≥99%), serine (Ser, ≥99%), threonine (Thr, ≥98%), histidine (His, ≥99%), leucine (Leu, ≥98%), isoleucine (Ile, ≥98%), phenylalanine (Phe, ≥99%), tyrosine (Tyr, ≥98%), arginine (Arg, ≥99.5%), valine (Val, ≥98%), lysine (Lys, ≥98%), methionine (Met, ≥98%), cysteine (Cys, ≥97%)], potassium dihydrogen phosphate (KH_2PO_4), dipotassium hydrogen phosphate (K_2HPO_4), potassium hydroxide (KOH), phosphoric acid (H_3PO_4), and ethanol (food grade, 95%) were purchased from Sigma-Aldrich Corp., (Shanghai, China). Trichloroacetic acid (TCA) was obtained from Sinopharm Chemical Reagent Co., Ltd., (Shanghai, China), and methanol (CH_3OH) from ANPEL Laboratory Technologies, Inc., (Shanghai, China). Non-iodized salt was purchased from Zhongyan Yangtze River Salinization Co., Ltd., (Yingcheng, China). Monosodium glutamate (MSG, ≥99%) was obtained from Shanghai Yuanye Bio-Technology Co., Ltd., (Shanghai, China). Methional (Food grade, ≥99%), 2-pentylfuran (Food grade, ≥99%), and caryophyllene (Food grade, ≥99%) were purchased from Firmenich Aromatics Co., Ltd., (Zhangjiagang, China). Hexanal (Food grade, ≥99%) was obtained from Chunzhu Aroma Co., Ltd. (Yancheng, China), and estragole (Food grade, ≥99%) was purchased from Wuhan Lullaby Biotechnology Co., Ltd., (Wuhan, China). Ultrapure water was collected from Chengdu Haoneng Technology Co., Ltd., (Chengdu, China).

2.1.2. Materials

Sanhuang chicken breast fillets from freshly slaughtered broilers (Haobang, LinxiaYikelamu Food Co., Ltd., Linxia County, Gansu, China) were transported to the laboratory within one hour in a cooler filled with ice packs. The fillets were washed with ultrapure deionized water, and extra fat was removed with a stainless-steel knife. For umami taste intensity evaluation of chicken samples, meat was cut into 1-2 mm diameter cubes using an odourless stainless-steel knife. For other assessments, it was minced homogeneously using a pulveriser (A11, IKA, Germany). The pre-treated meat was stored at -18 ºC in the odourless sealed aluminium foil bags and thawed at 4 ºC before one day of further use.

2.1.3. Samples

The commonly accepted recipes to blend spices were applied in our study for sample preparation. The Chinese 5-spices blend was made-up of star anise (0.5%), cinnamon (0.5%), Szechwan pepper (0.5%), fennel (0.5%) and cloves (0.1%) (local market, Minhang, Shanghai, China). Ingredients of garam masala were obtained from an international online retailer that imported Pakistani spices to Shanghai, China. Garam masala was make of black cardamom (0.6%), black pepper (0.5%), cinnamon (0.125%), cloves (0.125%) and cumin seeds (1%) [8].

Both spices were used separately for marination at concentrations of 0.5% and 1% salt. The chicken was marinated for 2 hours with spices (0.5%, *w/w*) and salt (1%, *w/w*), while the plain chicken sample was prepared using salt (1%) only. After marination, it was boiled in a stainless-steel pot with 1:1 ultrapure water (*w/v*) at 120 ºC for 20 min on a hotplate (Guangdong Midea Life Electrical Appliances Manufacturing Co., Ltd., Jiaxing, China) to reach an internal temperature of 80-95 ºC monitored using a digital thermometer (VersaTuff Plus 396, Atkins Technical Inc., Gainesville, Fla., U.S.A.). In this study, the plain chicken, chicken with Chinese-5-spices blend, and chicken with garam masala samples are denoted as CB, C+CS, and C+GM, respectively.

2.2. Sensory evaluation

The experimental procedures have been ethically reviewed and approved by the Institutional review board for human research protection, Shanghai Jiao Tong University, Shanghai, China. The sensory evaluation was carried out in the sensory lab in the Department of Food Science and Engineering at SJTU.

2.2.1. Panellists

Panel for participating in our sensory study had to pass an online screener using Questionnaire star software (Changsha Questionnaire Star Network Technology Co., Ltd., Changsha, China) and consent to be a non-drug user, non-pregnant lady, not allergic to chicken or spices, and able to articulate sensory attributes during practices and evaluations. A panel of 10 assessors (5 male, 5 females; aged 23-32 years old), previously trained for Quantitative descriptive analysis (QDA) for boiled chicken meat [24], and Jinhua Ham [25], with umami taste sensory evaluation experience more than a year was selected. They further went through the 3-days training sessions to get more familiar with umami taste categories before the sensory evaluation. In the first session, they were introduced to the use of an 11-point intensity scale that represented “no perception” at

the left side (0) and "most intense" at the right (10). In each session, they were served with four concentrations of umami taste solutions (MSG (mg/mL); 0.0, 2.0, 5.0, 8.0, 10.0). The solutions were served with three random digits and served in a randomized order. They were then expected to rate the strength of the umami taste in each solution accurately using the intensity scale.

2.2.2. Umami taste intensity evaluation:

The evaluation was carried out in a sensory panel meeting room using an 11-point intensity scale. The CB, C+CS, and C+GM cubes were presented in a randomized order in 1 oz white plastic disposable cups with lids (Xiamen Qianniu e-commerce Co., Ltd., Xiamen, China), coded with three random digits, served at 50 ºC to the panellists under the room temperature of (21 ± 2) ℃. The data was compiled by Questionnaire star software. They were required to facilitate with the reference solutions of 2.0, 5.0, 7.0, and 10.0 mg/mL MSG as criteria for intensity ratings and then compare the umami taste intensity of targeted samples with them.

During the test, panellists were asked to chew the sample first, perceive the flavour, and assess the umami taste intensity according to the reference solutions.After perception, they were asked to expectorate samples and clean their palates, followed a rinse protocol of room temperature drinking water and a piece of cucumber to avoid carry-over between each trial. Soon after expectoration, rate the umami taste intensity on the intensity scale. After one test, they need to pause 60-90 sec to relax before initiating the next sample.

2.3. Characterization of umami taste components

2.3.1. 5'-nucleotides

Five grams of aliquot was homogenized (Ultra Turrax homogenizer, IKA Co., Heidelberg, Baden-Württemberg, Germany) with 20-mL 10 ºC cold perchloric acid (chilled at 4 ºC) at 3000 r/min for 2-3 min and centrifuged under 336 x g in a NO.4 rotor (H1850R, Cence®, Changsha, Hunan, China) at 4 ºC for 10 minprior to the repeated step to get a 50-mL supernatant. The collected supernatant was neutralized with 1M KOH (pH 5.8) and validated by a pH meter (Five Easy Plus, Mettler Toledo, Zurich, Switzerland) before being filtered through a 0.22-μm water filter (SCAA-102, Anpel Laboratory Technologies) for sample injection.

The high-performance liquid chromatography (HPLC) (Waters e2695, Waters Technologies Ltd., Shanghai, China) was equipped with a GL Inertsil ODS-3 column (250 mm × 4.6 mm, 5 μm, Anpel Laboratory Technologies). CH_3OH (A) and 20-mmol/L KH_2PO_4:K_2HPO_4 (B; *v/v* = 1:1, pH 5.8) were used as eluting solvents using 1.0 mL/min flow rate at 30 ºC.The gradient elution was programmed using the formulation in the Appendix (Table. A.1). The ultraviolet wavelength was set at 254 nm for the detector. All analyses were carried out in triplicate (n = 3). The retention times and peak areas of nucleotide standards (AMP, at 10, 50, 100, 150, and 200; GMP at 10, 20, 30, 50 and, 100; IMP at 10, 50, 100, 250, and 500 mg/100 mL) were used for identification and quantification of nucleotides [26].

2.3.2. Organic acids

Five grams of aliquot was homogenized with 25-mL ultrapure deionized water for 2-3 min at 3000 r/min and centrifuged under 336 x g at 4 ℃ for 20 min before repeating the same step. The supernatants were combined and passed through 0.45-μm water filters to inject into the HPLC system.

The same column (GL Inertsil ODS-3) was used in organic acids detection. CH_3OH (A) and 0.05% H_3PO_4 (B; *v/v*) were selected as eluting solvents. The gradient elution was programmed using the formulation in the Table. A.1. The flow rate was adjusted at 1.0 mL/min, and the elution peak was set at 215 nm. All analyses were conducted in triplicate. The peak areas and retention times of the standards (succinic acid, at 5, 10, 50, 100, 250, and 500; lactic acid, at 50, 100, 250, 500, 1000, and 2000 mg/100 mL) were used for identification and quantification of organic acids [26].

2.3.3. FAA

Two grams of aliquot was homogenized with 15-mL of 0.1M HCl at 3000 r/min for 2-3 min prior to the placement in the ultrasonic water bath (Shanghai Kudos Ultrasonic Instrument Co., Ltd., Shanghai, China) for 30 min at 25 ºC for extraction. The extracted sample was centrifuged under 672 x g for 10 min at 4 ºC before repeating the same step to get the final volume of 25-mL supernatant. The 10-mL supernatant was mixed with 10-mL of 10% TCA (*w/v*) for one hour, followed by centrifugation under 672 × g for 10 min at 4 ℃. The pH of the supernatant was adjusted with 6M NaOH (pH 2.2) before filtration through a 0.22-μm water filter for injection into the amino acid analyser (L-8900, Hitachi Co., Tokyo, Japan). All analyses were repeated three times (n = 3).

The amino analyser (HITACHI Co., Tokyo, Japan) was equipped with an autosampler, reaction column, injector, detector, and computer (software) for detection based on the ninhydrin reaction. The peak area and retention time of FAAs standards were collected to identify and quantify the FAAs [26].

2.3.4. Taste activity value (TAV)

TAV represented the ratio between the concentrations of taste components in chicken breast fillets and their threshold values. Taste thresholds reported in the literature were applied for calculation, and the compound with TAV value greater than 1 was recognized as taste active [26].

2.3.5. Equivalent umami concentration (EUC)

The EUC is the concentration of MSG equivalent to the umami intensity of the synergy of the umami amino acids and 5'-nucleotides, which has been calculated by the following formula, and expressed as g MSG/100 g [27]:

$$Y = \sum a_i b_i + 1218 \left(\sum a_i b_i\right) \left(\sum a_j b_j\right)$$

where Y is the EUC of the mixture in terms of g MSG/100 g; 1218 is the synergistic constant; a_i is the concentration (g/100 g) of Asp or Glu; $a_j$is the concentration (g/100 g) of IMP or AMP; b_i is the relative umami equivalent concentration (RUC) for each umami amino acid compared to MSG (Glu = 1 and Asp = 0.077), and b_j is the RUC for each umami 5′-nucleotide compared to IMP (IMP = 1 and AMP = 0.18).

2.4. Aroma-taste interaction

2.4.1. Stimuli

The highest umami taste intensity (6.6 mg/mL) in the chicken breast samples has been defined as a reference umami tastant, which is determined by matching its UTI with a serial concentration of MSG taste solutions in ultrapure water. Extract of the chicken breast muscle fillets has been used to validate the umami taste intensity in a complex umami solution, and its threshold in perception (20 mg/50 mL) has been obtained in a preliminary sensory study [28]. Instrumentally pinpointed essential odorants from our previous aroma research [8] were selected to explore their synergistic effects on the umami taste, which may influence UTI in chicken products. The three levels of each odorant selected in this study were all above the thresholds in perception and generally recognized as safe (GRAS) (Table A.2). Consequently, two tastants (MSG and CE) and five odorants (2-pentylfuran, methional, hexanal, estragole, and caryophyllene) with three levels (low "L", medium "M", and high "H") were used as stimuli to assess the impact of odorants at each level on the umami taste intensity of tastants.

Taste stimuli were prepared in fresh for every three days and stored at 4 ºC in air-tight brown containers. Odour stimuli and taste-odour mixtures were prepared on the experiment day to avoid the loss of volatiles; however, the stock solutions of odorants were prepared once in five days and stored at -20 ºC in air-tight glass containers. The odorants and tastants were prepared using ultrapure deionized water since all stimuli were miscible with water. In contrast, caryophyllene is a lipid-soluble compound that needs to be dissolved in 95% food-grade ethanol. The final caryophyllene concentration in ethanol (<0.1%) was unperceivable for the solvent (1.43% ethanol) [29]. Test stimuli were immediately poured into the falcon tubes and sealed tightly with screw-on lids. All stimuli were prepared at room temperature (21 ± 2) ºC.

2.4.2. Procedure

A total of five sessions of sensory evaluation were performed, including two perceptual similarities and three intensity rating sessions. Only one session was carried out each day to ameliorate panel fatigue. All panellists were served individually (one at a time) to maintain samples' serving temperature (50 ºC).

2.4.2.1. Perceptual similarity ratings

The interactions between tastant and each odorant were first assessed to observe the perceptual similarity. One tastant (MSG on the first and CE on the second day) was evaluated with fifteen odorants (five odorants with three levels each) on a single day. It was measured by comparing the similar flavour perception qualities of odorants with the tastants. The samples were provided in pairs, tastant followed by odorant, and panellists were asked to rate how similar the flavour qualities of one object to the flavour qualities of another. After tasting both samples in a pair, panellists need to rate the perceptual similarity of the paired samples on an 11-point line scale, hanging between 0 for "not very similar" to 10 for "very similar".

Initially, an outline of the project was introduced to the sensory panel before delivering samples. The 10-mL stimuli were provided in the disposable falcon tubes and coded with three random numbers. To avoid ortho-nasal inhalation of volatiles, they were asked to pinch their noses with their hands while sipping the solution into the mouth. After sipping, they had to open their noses to breathe naturally to perceive the taste of

the sample for approximately 10 sec using a gentle smacking motion [13, 30]. Right after the expectoration of each sample, they need to rate the perceived similarity using a line-scale on the mobile screen (Questionnaire star software). Panellists had to focus on the maximum similarity of the paired samples perceived from the time of taking stimuli into the mouth to the time of expectoration, abstaining from factoring colour, intensity, and texture within their ratings. After one test, panellists were introduced to 60-90 sec inter-trial intervals and asked to rinse the mouth thoroughly with drinking water three times to remove the lingering mouthfeel given by the previous stimulus. An additional 5-10 min break was provided after every six trials to ameliorate the fatigue between tests. All samples were maintained at 50 ^{o}C until serving to simulate the consumption scenario.

2.4.2.2. Intensity ratings

The impact of the selected odorants on umami taste intensity in MSG and CE was evaluated in this session. Panellists were first facilitated with the reference solutions containing 2.0, 5.0 and 7.0, and 10.0 mg/mL MSG to validate their perceived umami intensity. The test samples were prepared by mixing the odorants' solutions with tastants (6.6 mg/mL of MSG or 20 g/50 mL of CE). The umami taste intensity was then rated using the 11-point intensity scale. The evaluation procedure was similar to the perceptual similarity measurements described previously. All samples were tested in triplicate (n = 3) for sensory study.

2.5. Data analysis

The chemical measurements were analysed using one-way analysis of variance (ANOVA), tracked by post-hoc Duncan test in SPSS (IBM Inc. Chicago, IL, U.S.A). ANOVA trailed by a Tukey's test was performed for the perceptual similarity and intensity evaluation data to ascertain significant differences ($p \leq 0.05$) among test solutions using SPSS. The partial least squares regression (PLSR) was applied to correlate the umami taste intensity perceived by panellists with the effects of additional odorants using XLSTAT 2019 (Addinsoft, New York, U.S.A).

3. Results and discussion

3.1. Sensory evaluation

Table 1 records the perceived umami taste intensity (mg/mL) of the three chicken samples by panellists. The plain chicken registered significantly higher umami taste intensity (CB = 6.6) than the blended chicken samples, though there was no considerable difference between the two blends, C+CS (5.43) and C+GM (5.53). Umami has been reported as a characteristic taste of chicken, mainly comprised of 5'-nucleotides, organic acids, and amino acids [10]. Zhu and others (2022) found a positive correlation between Glu and Asp to umami taste perception [21]. Researchers reported a positive correlation between the overall liking of the chicken product and its umami taste [9], which had also been observed in our preceding study, where umami taste was identified as a driver of liking [31]. Therefore, the umami taste profile of these three samples was first validated instrumentally to assist the understanding of the flavour perception in chicken products.

Table 1. Mean ± standard deviation and TAV values of nucleotides, organic acid, and FAAs in CB and CSBs, and umami taste intensity in chicken breast samples evaluated by sensory panel.

Taste components		Mean ± SD (mg/100g)			TAV			Taste threshold (mg/mL)
		CB	C+CS	C+GM	CB	C+CS	C+GM	
Nucleotides	IMP	83.95±7.36[a]	68.11±3.04[a]	63.86±3[a]	3.36±0.29	2.72±0.12	2.47±0.12	0.23
	AMP	3.1±0.42[b]	0.84±0.09[a]	1.1±0.05[a]	0.06±0.01	0.02±0	0.02±0	0.86
Organic acid	Lactic acid	912.91±7.77[b]	732.58±3.46[a]	840.57±55.27[b]	7.02±0.06	5.64±0.03	6.69±0.43	0.67
FAAs	*Umami-taste*							
	Asp	26.22±1.35[c]	20.09±0.41[b]	9.72±1.28[a]	0.26±0.01	0.2±0	0.1±0.01	0.53
	Glu	42.98±0.55[b]	37.27±0.41[a]	37.03±2.39[a]	1.43±0.02	1.24±0.01	1.23±0.08	0.16
	Sweet-taste							
	Ala	34.14±0.56[b]	30.03±0.27[a]	28.78±1.71[a]	0.57±0.01	0.5±0	0.48±0.03	1.06
	Gly	17.97±0.17[b]	15.86±0.14[a]	15.06±0.88[a]	0.14±0	0.12±0	0.12±0.01	1.87
	Ser	24.91±0.72[b]	21.99±0.11[a]	20.94±1.23[a]	0.09±0	0.08±0	0.08±0	2.62
	Thr	20.04±0.34[b]	17.83±0.13[a]	16.78±1.03[a]	0.05±0	0.04±0	0.04±0	4.16
	Bitter-taste							
	His	13.72±0.35[b]	11.93±0.09[a]	11.58±0.81[a]	0.02±0	0.02±0	0.02±0	6.98
	Ile	15.1±0.25[b]	13.2±0.13[a]	12.81±0.76[a]	0.12±0	0.1±0	0.1±0.01	1.31
	Leu	28.22±0.53[b]	25.18±0.21[a]	24.37±1.49[a]	0.2±0	0.17±0	0.17±0.01	1.44
	Phe	16.44±0.18[b]	14.73±0.13[a]	14.26±0.89[a]	0.02±0	0.02±0	0.02±0	7.43
	Tyr	18.75±0.39[b]	17.03±0.15[a]	16.52±0.93[a]	0.26±0.01	0.24±0	0.23±0.01	0.72
	Bitter/sweet/sulfurous							
	Arg	29.4±0.55[b]	26.25±0.16[a]	25.66±1.65[a]	0.59±0.01	0.52±0	0.51±0.03	13.06
	Val	19.54±0.38[b]	16.96±0.08[a]	16.45±0.98[a]	0.06±0	0.05±0	0.05±0	3.51
	Lys	35.09±0.68[b]	30.54±0.15[a]	29.48±1.78[a]	0.7±0.01	0.61±0	0.59±0.04	0.50
	Met	11.09±0.09[b]	9.52±0.04[a]	9.88±0.6[a]	0.15±0	0.13±0	0.13±0.01	0.74
	Cys	0.38±0.21[a]	0.19±0.05[a]	0.45±0.04[a]	0.02±0.01	0.01±0	0.02±0	0.24
		Umami taste intensity						
		M±SD (mg/mL)			**EUC (g MSG/100g)**			
		6.6±0.43[b]	5.43±0.11[a]	5.53±0.47[a]	4.63	3.23	2.95	

TAV, the ratio between the intensity of taste components and their threshold values. Taste threshold (mg/100 mL) was encoded from the literature [26].Values in each row shared with the same superscript letter are not significantly different from the other sample type(s) at an $\alpha = 0.05$ significance level.

3.2. Umami taste components

3.2.1. 5'-nucleotides

The measured umami taste components of chicken samples are presented in Table 1. Among three types of umami 5'-nucleotides, IMP and AMP were detected in our samples. Overall, the IMP content was substantially higher (far more than ten times) in all three samples than AMP. AMP was significantly higher in the plain chicken with a non-significant difference between CSBs. AMP is found to be able to deliver sweetness and inhibit astringency and bitterness in mushrooms. The ratio between IMP and AMP measured in the chicken breast samples showed a similar trend in previous studies [21, 32], where IMP was much higher than the AMP. Zhang and others (2020) claimed that IMP was positively correlated with the umami taste [33]. IMP could activate the umami receptor that was recognized as the main nucleotide module in chicken [32]. Adenosine 5′-diphosphate (ADP) and adenosine 5′-triphosphate (ATP) are the primary nucleotides in the live chicken, while a majority of ATP decomposes into IMP after slaughtering [34], which has been observed in cooked chicken as shown in our current study as well.

3.2.2. Organic acids

The concentration of lactic acid was significantly higher in CB and C+GM, while the succinic acid was not detected in any chicken sample (Table 1). Lactic acid was the dominant chemical among all umami components (CB = 912.91; C+CS = 732.58; C+GM = 840.57 mg/100 g), with a considerably low value found in C+CS among three samples. A previous study applied a combination of a higher level of lactic acid and a lower concentration of succinic acid in the chicken breast compared with the thigh meat [1]. Compared to their reportedlimited amount of succinic acid, the complete absence of succinic acid in our chicken samples could also be due to the variances in cooking methods, genotypes, sex, and diet of chicken breeds.

3.2.3. FAAs

FAAs contribute to the umami, sweet, sour, or bitter-taste characteristics in foods, which also improves the food palatability and significantly influences the development and growth of organisms [4, 35]. The quantified FAAs in plain chicken and CSBs are shown in Table 1. Each FAA imparts a specific taste perception, based on which they can be classified into certain categories, namely umami, sweet, bitter, and bitter/sweet/sulfurous tastes [26]. The total detectable FAAs were higher in plain chicken than in the CSBs. The enriched FAAs content in the plain sample might be attributed to its higher protein contents. The major FAAs were Glu, Ala, Lys, Asp, Leu, Arg, Ser, and Thr, which accounted for approximately 68% of the total FAAs in all testing samples. Among FAAs, Glu was the most abundant in all chicken samples. The umami note of Glu and Asp could be turned into the perception of 5'-nucleotides and MSG in the presence of sodium salt, contributing a more intense umami taste [27]. The metabotropic glutamate receptors (mGluR1 and mGluR4) located in taste buds have been visualized as being activated by Glu [12]. The detection of other amino acids and umami taste components may entail the other taste receptors articulated in different undiscovered subsets of taste cells. However, the mixture of various umami components has been shown to boost the activation of taste perception regions of the human brain to perceive more intense umami taste [12].

In our study, the highest concentrations of Glu and Ala aligned with previous studies, where Glu and Ala were the abundant FAAs in Chinese chickens [1, 32]. It has been reported that the Gly, Ser, and Ala can elicit umami taste in combination with Glu and Ala. In umami peptides, Glu and Ala have been recognized as key active sites [36], while Arg is responsible for the overall pleasant taste perception of food that plays an essential role in the triggering of umami peptides based on human sensory perception and molecular simulation [37], and their harmonies with the umami taste also need to be further investigated. Our future goal would include the characterization of peptides to assess more complex taste compounds and understand their role in taste perception. Phe is known for its bitter taste; however, a study has discovered that Phe could also be an important fraction of the soy sauce savoury perception in addition to Glu [38].

All FAAs were significantly higher in the CB, except Cys (bitter/sweet/sulphurous), which presented the lowest amount in all samples at parity. Previous research on Sanhuang chicken has also shown a low detectable Cys accounts for 3% of all FAAs [1]. Between CSBs, only Asp was significantly higher in C+CS than the C+GM, while the remaining FAAs were non-significantly different in CSBs.

The present study has shown a considerable reduction in umami taste components in the chicken breast after marination with spices. A similar observation in our preliminary research has also revealed that the umami-note volatiles were decreased in CSBs significantly [8]. One possibility could be the masking effect of spicy notes in the marinated chicken samples. The inhibitory effect of spices on FAAs might be owing to the concentration of spices that should be validated in future studies with different food matrices.

3.2.4. TAVs

TAV is the taste potency value of water-soluble components. TAV >1 indicates the substantial contribution of the substance to food taste perception [10]. The greater the value, the more significant contribution of a specific component to the taste perception. We found that the TAV in the plain chicken decreased after marination with spices (Table 1). Lactic acid, IMP, and Glu played an essential role in the taste perception of chicken, where lactic acid has shown the highest TAVs in all chicken samples (CB = 7.02, C+CS = 5.64: C+GM = 6.69). Similar to the present results, another study found in the literature also reported the highest TAV of IMP and Glu in Chinese yellow-feather chicken [32]. The higher thresholds of umami note components emphasized their contribution to umami taste even as the individual taste component. Other substances might not be very influential when applied individually; however, they could significantly contribute to the taste perception in the synergism of the other components. Researchers also depicted the synergistic effect between Glu and IMP in the chorda tympani nerve in mice [12]. Umami taste could be enhanced by the synergistic effect of umami amino acids (Glu and Asp) and 5'-nucleotides (IMP and AMP) [26]. The binding positions of the taste components to the Venus flytrap (VFT) domain of umami receptor T1R1/T1T3 ensures their synergistic enhancement of umami taste intensity. Glu binds closely to the hinge region of T1R1-VFT, and IMP binds to the adjacent site near the opening of the T1R1-VFT, increasing the sensitivity of T1R1/T1R3 significantly and further stabilizing the closed conformation (active state) of VFT [33]. The sensation of chicken essence as an umami taste matrix by the biomimetic ion nano-channels for sensing umami substances has been verified in previous research. They detected a 47% increase in ionic current by the addition of chicken essence to the electrolyte [39]. The secondary taste cortex “orbitofrontal cortex” (OFC) of the human brain has also shown a significantly higher response to the mixture of IMP and MSG as compared to the individual taste components [12]. Therefore, the taste components could play a substantial role in umami taste enhancement, which also deserves further investigation.

3.2.5. EUC values

EUC (g MSG/100 g) can be used as the standard of sensory evaluation for food containing umami FAAs and 5'-nucleotides [35]. It has been implemented in this study to assess the changes in umami component concentrations in the chicken samples after being marinated with spices. The higher EUC value indicates a more significant contribution to the umami taste. The highest EUC was found at 4.63 g MSG/100 g in CB, showing the intense umami taste of chicken samples. The EUC values were more related to the umami taste perception by sensory evaluation, offering the highest value in CB (4.63) than the CSBs (C+CS = 3.23; C+GM = 2.95). Interestingly, though C+CS was low in AMP, its relatively high content of Asp and Glu could also be

responsible for an increased EUC value. The EUC value determined in our study was greater than previous research using Sanhuang chicken breast [1]. One possible reason for the divergence could be owing to the variances in feed, sex, and cooking methods applied in the study. Conversely, The EUC values of chicken samples in this study were similar to the umami taste intensities reported in the Jinhua ham, which stood out for its umami flavour [21]. We believed Sanhuang boiled breast meat could also be selected as a notable source of umami taste to increase the palatability of various foods. Our previous research has shown a reduction of umami taste volatiles by the addition of spices in the chicken meat [8]; therefore, our future goal would include exploring possible approaches to increase the UTI in the taste models via a controlled aroma-taste interaction.

3.3. Aroma-taste interaction

Literature has shown that the interaction of aroma and taste components depends on the stimulus. This stimulus dependency has been supported by two highly related concepts: congruency and perceptual similarity. Interestingly, both concepts have been used interchangeably, though taste enhancement by odour is highly correlated with perceptual similarity [13]. Therefore, before assessing the influence of odorants on the UTI of tastants, we have evaluated the perceptual similarity between odorants and tastants to obtain some initial understanding of odour-induced taste enhancement. Fig. 2 represents the perceptual similarity of MSG (a) and CE (b) with three levels of five odorants. MSG solution exhibited high perceptual similarity when paired with Meth-H but low with 2PF-L, Hex-L, Est-L, and Cary-H. In addition, CE registered an increased perceptual similarity when paired with higher levels of methional and hexanal but a lower perceptual similarity with the ground level of estragole.

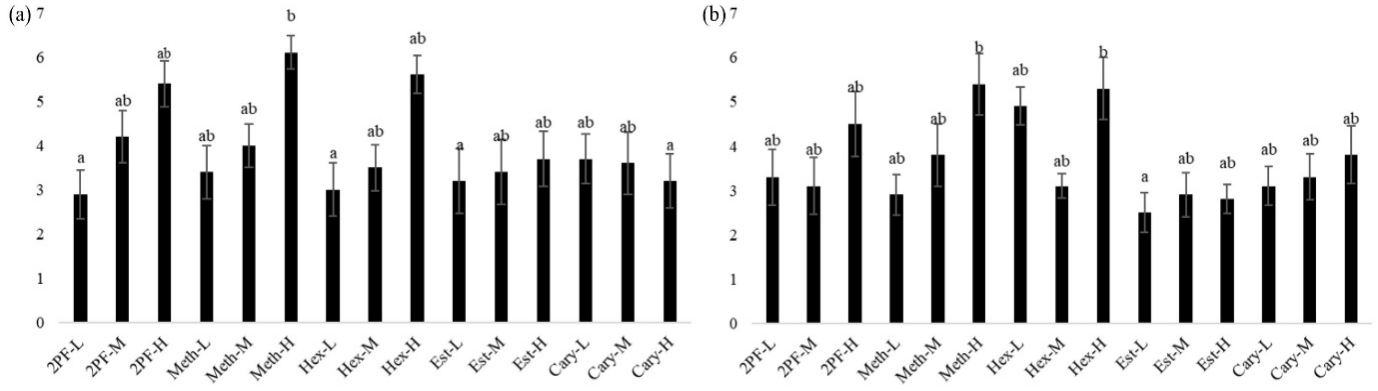

Fig. 2. Mean ratings of perceptual similarity of "MSG" (a) and "chicken extract (CE)" (b) with three levels (L, M, H) of five odorants. The labels on standard error bars shared with the same letter(s) are not significantly different from the other concentration(s) at an $\alpha = 0.05$ significance level.

Overall, our observation showed a clear interrelationship between perceptual similarity and taste enhancement. The impact of each aroma at given levels on UTI in MSG solution is shown in Fig. 3 (a-e). In our study, a high concentration of methional, a sulfur-containing compound with a meaty-note, significantly enhanced the UTI in MSG solution. In contrast, Meth-H non-significantly increased the UTI in CE (Fig. 3g). A previous study has shown the enhancement of umami aftertaste in an umami taste solution by the addition of chicken-note odorants was highly dependent on certain levels of the selected aroma [40]. Similar to our

findings herein, the meaty-note odorant could potentially increase the UTI, and the specific concentration level is the most influencing factor. The varied performances for the same aroma applied to simple and complex umami food matrices established in this study have also emphasized the reaction mechanism and intensity of the aroma-umami taste interactions are food matrix dependent.

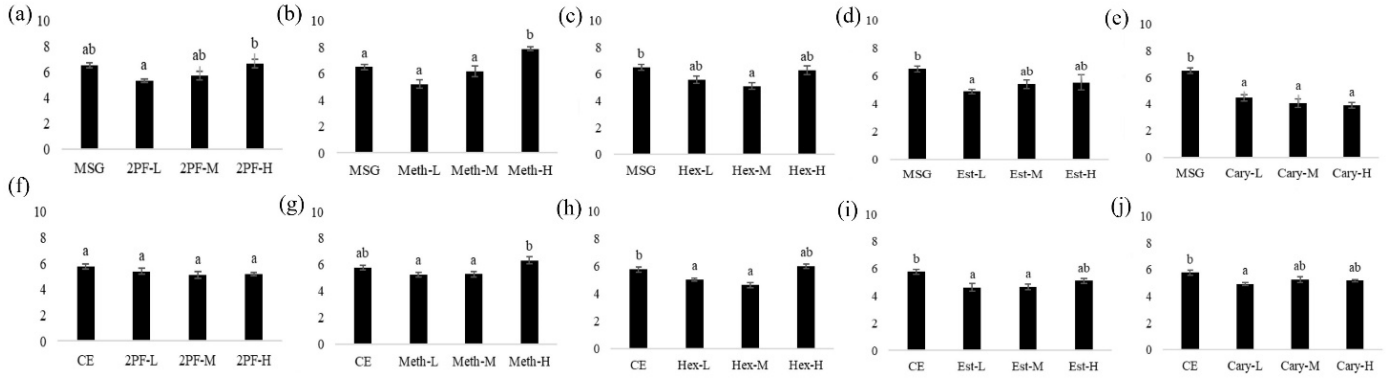

Fig. 3. Mean ratings of perceived intensity of umami in a mixture of MSG (a-e) and CE (f-j) with three levels of odorants. Each first bar represents the tastant (MSG or CE) delivered alone, while the other three bars represent its mixtures with three levels (L, M, H) of odorants. The labels on standard error bars shared with the same letter(s) are not significantly different from the other concentration(s) at an $\alpha = 0.05$ significance level.

Hex-M, Est-L, and caryophyllene at all levels exhibited a significant UTI reducing capability that could lie to the low perceptual similarity between odorants and tastants. These odorants have also demonstrated the UTI reducing capacity in the CE (Fig. 3 f-j), which means they are applicable in both referenced and complex umami food matrices. The reduction of UTI was agreed with the less perceptual similarity between odorants and tastants observed in the study. Our findings were also supported by previous research that demonstrated the strong association of taste enhancement with perceptual similarity [41]. Though hexanal was the most abundant odorant in chicken [8], when added as an individual compound, its green-note aroma could make it more correlated with herbs and spices. In the present study, Hex-M in MSG with both the medium and high levels in CE could all reduce the UTI significantly, showing a reducing capacity. Other researchers have proposed the possibility of employing the green note aroma “butanal” as an umami taste enhancer; however, the green-note given by hexanal is more intense than butanal. Moreover, in their study, the complex food matrices have been applied, which, in turn, could possibly result in an increment of umami taste perception caused by the interaction with other odorants in the system [42]. It has also been reported that the green note aroma “(Z)-3-hexen-1-ol” is able to increase the sweet taste by addition to the apple food matrix [30], which implies the green note odorants might be more related to a sweet taste.

In order to better illustrate the aroma-taste interactions explored in this study, PLSR was implemented to point out the potent contributing odorants that were able to increase the UTI (Fig. 4a-b). PLSR is a commonly used method for correlating two data matrices (X and Y), and uses latent variables to establish the covariance of both patterns. It can analyse data with collinearity, noise, and missing variables in X and Y matrices and has the advantage of no requirement of a higher number of samples than the variables [43]. Overall, PLSR has been used as a compelling method to correlate the instrumental data with the sensory perception of judges for

numerous food items [44], but has been rarely used in non-instrumental statistics [45]. To the best of our knowledge, we are one of the forerunner research groups to apply it to unravel the aroma-taste interactions to assess the impact of odorants on taste intensity. Our PLSR results aligned with UTI analysis using ANOVA, and precisely identified the role of each odorant on umami taste apart from making an intuitive comparison using the plot as a powerful tool to show correlations in aroma-taste interactions.

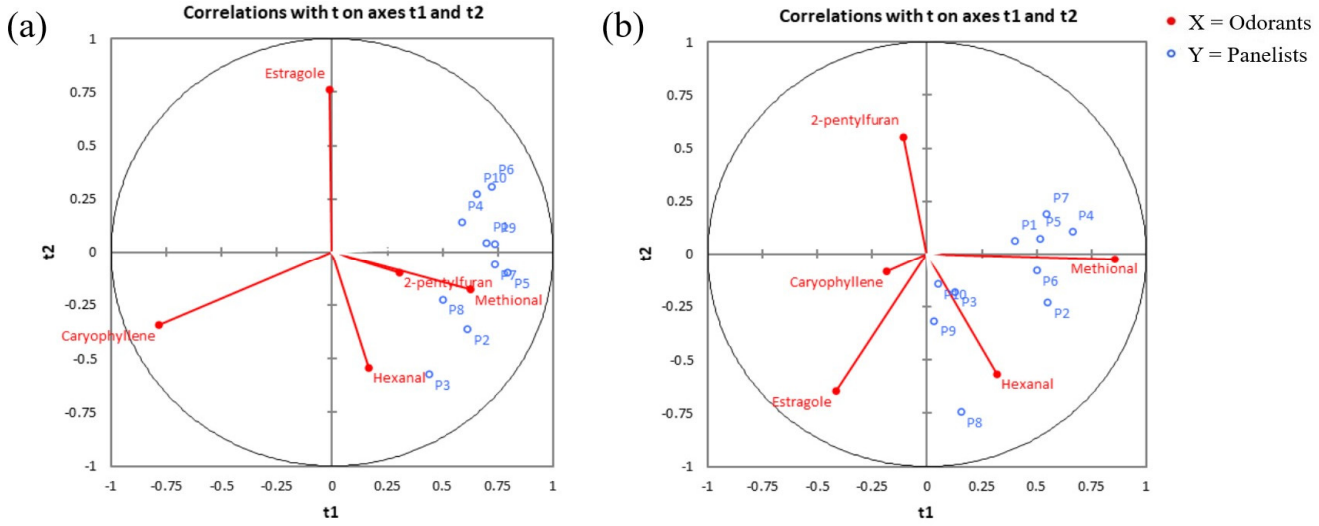

Fig. 4. PLS-R analysis revealed the correlation between the five additional odorants and the UTI evaluated by panelists(P 1-10 represents ten panellists).

In our PLSR plots, all sensory panellists were displayed near the perimeter of the circle, indicating a high correlation to the factors. Their posts clustered on one side of the correlation circle, emphasizing a high consistency in their judgements. The placement of caryophyllene and estragole was the opposite of the sensory judgments, which negatively correlated with umami taste intensity, showing a reducing capability in UTI (Fig. 4a). A similar observation has been displayed in the CE model (Fig. 4b) with a higher UTI reducing competence shown by the addition of estragole. A previous study has reported a reduction in the umami taste perception by adding a spicy-note odorant named “anethole” [40]. Moreover, our preliminary investigation has also shown the inhibition of umami-note odorants after marinating the chicken with spices [8]. We, therefore, hypothesize that spicy-note components can act as anti-umami modules; however, the different locations of caryophyllene and estragole in simple and complex taste models have implied their varied inhibition capacities are matrix-dependent. A similar observation has been discussed when three green-note odorants exhibited diversities in sweet taste enhancement [42]. Hence odorants with a similar note might not impact the tastant in the same way, suggesting that a structure-dependency also needs to be considered. Therefore, our future study will focus on exploring more spicy-note components and other food matrices in terms of obtaining a comprehensive understanding of aroma-umami taste interactions in real food. Moreover, the effect of similar note aroma odorants with different chemical structures on umami taste can be of great interest to explore.

On the other hand, a higher positive correlation between methional to the enhancement of UTI in both taste models has been recorded in our study (Fig. 4a-b). A study has demonstrated that the methional could act

as a positive allosteric modulator for human umami taste T1R1/T1R3 receptors [46]. Umami taste components, including amino acids and nucleotides, can elicit umami taste after binding with T1R1/T1R3 umami taste receptors [33, 36], which has been sensed in the human brain [12]. These findings have shown that methional was able to be perceived as umami taste by human taste receptors. Similar to the synergism that exists in umami taste components, tastants and odorants could also have a synergistic impact on enhancing the taste. Since the umami taste in the chicken product has been reported to be highly preferred by consumers [31], it will be valuable to thoroughly investigate the function of methional as an umami flavour enhancer in other food models to validate its impact on overall acceptability. It is noteworthy that just a high level of methional (1900 μg/L) could augment UTI; however, in general, only the odorant below its safety level of concern would be allowed to use as an additive. Interestingly, the odorants that shared a similar chemical structure have shown a synergistic effect and additive action [47]; therefore, we believed that searching for multiple similar structure meaty-note aroma components might increase the UTI synergistically that could be another possible approach to umami flavour enhancement without exceeding the safety limit for a single component.

In addition, 2-pentylfuran exhibited a low correlation to the enhancement of UTI in MSG solution and the low inhibition capacity of UTI in the CE model. 2-pentylfuran has a vegetable-like and green-note aroma descriptions [48]. Researchers have published that an enhanced pleasant taste could be achieved by combining Glu with savoury odour [20]; however, 2-pentylfuran was explored to be able to reduce UTI in our study. Previous research has also claimed that though odorants were congruent with a sweet taste, not all of them were effective in sweet taste enhancement [49]. Moreover, the reduction of UTI could be due to its green-like aroma description. In the present study, the selected green-note aroma “hexanal” was less correlated with umami taste in both food matrices. The slight increase observed in CE might also be lied to the misalignment among panellists, shown as fewer of them deviated from others in hexanal judgements (Fig. 4b). Our future aim, thus, would also include systematically training sensory panellists to constantly improve their performance in future. Furthermore, a previous study has demonstrated an increase in the sweet taste by green-note odorant [30]. Therefore, the effects of green-note on both umami and sweet tastes need to be taken into consideration in terms of fully understanding their reaction mechanisms while developing a more harmonious taste product with a green note in the future.

4. Conclusions

In this study, we have characterized the UTI in umami referenced and complex food matrices via exploring the aroma-umami taste interactions. The UTI and umami taste components in plain chicken and chicken-spices blends were first measured using our sensory panel and instrumental analysis. The impact of five aroma components with different descriptions was then assessed in MSG and CE via retro-nasal interactions. Significant reduction of UTI and umami taste components in the marinated chicken samples with spices were observed. TAVs study revealed that lactic acid, IMP, and Glu were the most influential contributors to the umami taste in the chicken samples. Most interestingly, we found that the perceptual similarity between aroma and taste components could alter the umami taste perception. The appropriate

amount of methional showed the capability to increase the umami taste significantly. Spicy and green note odorants, on the other hand, exhibited an inhibiting effect on the umami perception in all taste models. For example, the caryophyllene was more powerful in reducing UTI in MSG solution, while the estragole could more profoundly lessen the UTI in CE, a complex food matrix. In addition, PLSR analysis assisted us in interpreting the correlation of the aroma-taste interactions, which might further deliver the information on odorant selections and probe their effects on umami and other taste perceptions.Therefore, our future work will focus on investigating the synergistic effects of multiple odorants to control the umami taste in different food matrices robustly in order to help improve product quality and increase consumers' acceptance.The release of aroma substances from food matrices can also be combined with the present sensory observationto investigate food formulation refinement and flavour blending further. The aroma percentage and its stability in food matrices can then provide the next stage of knowledge for better designing food products. Besides ascertaining the impact of key aroma components on umami taste intensity in different food models, we have also explored possible approaches to boost umami perception via aroma-taste interactions. The current research thus expanded our understanding of implementing a certain strategy in a more complex food system and selecting the formulation to simulate consumers' natural way of consumption to assist product improvement.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

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