Comparative analysis of taste components of three seasoning bases prepared via stir-frying, enzymatic hydrolysis, and thermal reaction

Yan Kong¹* | Chenchen Zhou¹* | Lili Zhang¹,² | Honglei Tian³ | Caili Fu⁴ | Xuepeng Li⁵ | Yuyu Zhang¹

¹Beijing Key Laboratory of Flavor Chemistry, Beijing Technology and Business University, Beijing, China
²College of Food Science and Engineering, Tianjin University of Science and Technology, Tianjin, China
³College of Food Engineering and Nutritional Science, Shaanxi Normal University, Xi’an, China
⁴National University of Singapore (Suzhou) Research Institute, Suzhou, China
⁵College of Food Science and Engineering, Bohai University, Jinzhou, China

Correspondence
Yuyu Zhang, School of Food and Chemical Engineering, Beijing Technology and Business University, Beijing, 100048 China. Email: zhangyuyu@btbu.edu.cn

Funding information
National Key R&D Program of China, Grant/Award Number: 2016YFD0400705

Abstract
Mung bean sprouts have gained the interests of consumers owing to their umami taste and high nutritional value. In this work, high-performance liquid chromatography and liquid chromatography quadrupole time-of-flight mass spectrometry were employed to investigate the taste components of three seasonings prepared via stir frying, enzymatic hydrolysis, and thermal reaction. The results indicated that enzymatic hydrolysis released taste compounds from raw materials more effectively than high-temperature stir frying. The thermal reaction improved the fraction of umami components in the enzymatic hydrolysate, which resulted in the highest equivalent umami concentration and improved the taste contributions of glutamic acid, inosine 5'-monophosphate, and guanosine 5'-monophosphate disodium salt hydrate. Total of 26 peptides were identified, including Ala-Met, Ala-Asp, Glu-Asp, Glu-Ala-Glu, Ala-Pro-Ser, Ala-Glu, Ser-Ala-Ser, Ser-Asp-Ala, His-Ile, Asp-Val, Ala-Asp, Glu-Ala-Ala, Ala-Glu-Asp-Gly-Gly, Ala-Glu-Ser, Glu-Ser-Asp-Val-Ala, Ser-Ser-Ser-His-Phe, Gly-Asp-Cys-Ser-Asp-Asp, Ala-Ala-Lys, Ala-Ser-Tyr, Ser-Ala-Met-Gly, Glu-Ser-Asp-Val-Ala, Thr-Ser-Ser-Ala-Ile-Ser, Ser-Gly-His-Glu-Asp-Glu, Ile-His-Glu-Ala, Ser-Arg-Ser, and Ser-Ala-His-Pro-Gly-Thr. The sweet and umami amino acids were the main residues of the N-terminus positions of peptides. Double and triple continuous umami amino acid sequences could also be important for the umami taste of peptides.

Practical applications
Meat flavoring, which uses animal and plant proteins as precursors, is an important raw material for the industrialization of traditional Chinese food. This study aimed to quantify the taste compounds in three seasonings prepared with mung bean sprouts base, and investigate the relationship between the umami components and the umami taste characteristics of three different seasonings. The results could provide a theoretical basis for the application of mung bean sprouts in food processing.
Mung bean sprouts is one of the most popular sprouts because of their high content of proteins, polypeptides, polysaccharides, and polyphenols. Mung bean sprouts are widely consumed as fresh salad vegetables or common side dishes and also used as health food and medication (Tang et al., 2014). During the germination of mung beans, proteins are converted into free amino acids, which are more easily absorbed by the human body via the action of various enzymes (Nonogaki et al., 2010). Moreover, these free amino acids, which are precursors in Maillard reactions are important for flavor formation in food. Combining the demands of modern consumers for "nutrition, delicacy, and convenience" and the important role of mung bean sprouts for health care, the efficient utilization of mung bean sprouts and industrialization of mung bean sprout products are foreseeable.

Meat flavoring, which uses animal and plant proteins as precursors, is an important raw material for the industrialization of traditional Chinese food (Lieske & Konrad, 1994). Multilevel-targeted enzymatic hydrolysis of proteins during the preparation of meat flavoring is an effective way for improving the utilization ratio of animal and plant proteins. The glutamic acid (Glu) and Glu-rich oligopeptides from the hydrolysis of vegetable proteins present typical umami taste (Sonklin et al., 2011). Sonklin et al. indicated that Glu and aspartic acid (Asp) were the main components of mung bean protein isolate (MPI) (Sonklin et al., 2011). Enzymatic bromelain mung bean meal protein hydrolysate (eb-MPH) produced from MPI presents bouillon, salty, umami, and sweet tastes (Sonklin et al., 2011). Moreover, the amino acid profile of eb-MPH was similar to that of meat, and thus, eb-MPH could be used as a condiment for direct food enhancement or precursor for thermal process flavoring. Mung bean sprouts possess more secondary metabolites than mung beans (Tang et al., 2014). In-depth analysis of taste components, particularly umami taste components of mung bean sprout dishes and their enzymatic hydrolysis products is the key to developing meat flavoring using mung bean sprouts as a plant protein source. However, only few studies have been reported on the taste components of mung bean sprout products.

In this study, three mung bean sprout products were prepared and their taste components were analyzed. The taste components of the three samples were investigated using high-performance liquid chromatography (HPLC) and liquid chromatography quadrupole time-of-flight mass spectrometry (LC-Q-TOF/MS). This work aimed to: (1) quantify the taste compounds from three mung bean sprout product samples, (2) isolate the umami peptides from three mung bean sprout product samples, (3) elucidate the umami and umami-enhancing effect of each fraction separated from the three mung bean sprout product samples, and (4) investigate the relationship between the umami components and umami taste properties of the three mung bean sprout product samples.

2 | MATERIALS AND METHODS

2.1 | Materials and chemicals

Xiaotangshan mung bean sprouts were purchased from Beijing Tian’an Agricultural Development Co., Ltd. (Beijing, China). Hounch pork, fresh Welsh onions, and fresh ginger were purchased from the Yonghui Supermarket (Beijing, China). According to the Chinese Food Composition (2018), 100 g of hounch pork contains 331 kcal of energy, 14.6 g of protein, 30.8 g of fat, and 55.1 g of water. Soy sauce was purchased from Foshan Haitian Flavouring and Food Co., Ltd. (Guangdong, China), vinegar was purchased from Jiangsu Hengshun Group Co., Ltd. (Jiangsu, China), and pig bone oil was purchased from Fushun Dufengxuan Gushen Biotechnology Co., Ltd. (Liaoning, China). Analytical grade hydrochloric acid (HCl), potassium dihydrogen phosphate (KH₂PO₄), phosphoric acid (H₃PO₄), disodium hydrogen phosphate dodecahydrate (Na₂HPO₄·12H₂O), oxalic acid, tartaric acid, lactic acid, acetic acid, citric acid, and succinic acid, and biological reagent grade malic acid were purchased from Sinopharm Chemical Reagent Company (Shanghai, China). Pyroglutamic acid and pyruvic acid were purchased from Macklin Reagent Company (Shanghai, China). A Durashell AA analysis kit, including an internal standard solution (norvaline and sarcosine), was purchased from Agela Technologies (Tianjin, China). Inosine 5’-monophosphate (5’-IMP) derived from Saccharomyces cerevisiae, adenosine 5’-monophosphate monohydrate (5’-AMP), guanosine 5’-monophosphate disodium salt hydrate (5’-GMP), and cytidine 5’-monophosphate assay (5’-CMP) were purchased from Sigma-Aldrich (St. Louis, MO, USA); sodium tetraborate decahydrate (Na₄B₂O₇·10H₂O) was purchased from Alfa Aesar (Ward Hill, MA, USA); HPLC-grade methanol and acetonitrile (ACN) were purchased from Thermo Fisher Scientific (Shanghai, China), and Sephadex G-15 was purchased from Beijing Ruida Henghui Science & Technology Development Co., Ltd. (Beijing, China). Pure water was purchased from Hangzhou Wahaha Group Co., Ltd. (Hangzhou, China).

Buffer salts I and II for the analysis of organic acids and nucleotides, respectively, were prepared according to Kong's method (Kong et al., 2017).

2.2 | Preparation of three seasoning bases

Stir-fried pork with mung bean sprouts (SFPM) was prepared as follows. \( m_{\text{pork}} \cdot m_{\text{mung bean sprouts}} = 3375:2000 \). Pork (337.50 g) was cut into 8 cm long, 0.6 cm wide, and 0.6 cm thick strips. Fresh Welsh
onion and fresh ginger were shredded. After cooking oil (56.00 g) was added to an iron pan and was heated at 160°C, the pork strips were stir-fried for 15 s (the meat could discolor during stir-frying). Subsequently, the mung bean sprouts (200.00 g), shredded fresh Welsh onion (15.00 g), shredded fresh ginger (10.00 g), and salt (9.00 g) were added to the pan and all ingredients were stir-fried for 4.5 min. The dish was cooled to room temperature, mixed with ultrapure water (1:1 w/v), and slurred using a juicer. After four repeated experiments, the prepared SFPM sample was 3,812.54 g, which was used for further analysis.

Enzymatic hydrolysate (EH) was prepared as follows. The ratio of pork and mung bean sprouts was the same as SFPM sample. Ground pork (33.75 g), mung bean sprouts (20.00 g), fresh Welsh onion (1.50 g), fresh ginger (1.00 g), salt (0.90 g), and deionized water (33.75 g) were mixed and hydrolyzed using 0.57 g of an enzyme complex ($m_{\text{neutral protease}} \cdot m_{\text{cellulase}} \cdot m_{\text{pectinase}} = 12:4:3$, activity $10^8$ U/g) at 50°C for 1 hr. After hydrolysis, the system was heated at 90°C for 10 min to inactivate the enzyme, and then it was cooled to 25°C. After six repeated experiments, the prepared EH sample was 877.48 g, which was used for further analysis.

Thermal reaction flavoring (TRF) was performed as follows. The thermal reaction method of Begum et al. (2019) was referred and improved. The optimal TRF process parameters were determined using single factor and orthogonal experiments. Glucose (5.00 g), xylose (1.00 g), EH (37.50 g), HVP (1.35 g), cystine (Cys) (0.73 g), Glu (0.73 g), arginine (Arg) (0.73 g), proline (Pro) (0.73 g), thiamine nitrate ($VB_1$) (0.27 g), disodium 5’-ribonucleotide (I + G) (0.16 g), yeast extract (0.90 g), pig bone oil (7.20 g), spice blend (0.09 g), and soy sauce (2.50 g) were mixed and were subsequently heated at 95°C for 30 min. To prevent the sour taste of vinegar from fading owing to the extensive heating time, vinegar (6.00 g) was added last. After preparation, the TRF sample was cooled to 25°C. After six repeated experiments, the prepared TRF sample was 562.79 g, which was used for further analysis.

2.3 | Free amino acid analysis

The free amino acids in the samples were analyzed according to the method reported by (Wang et al., 2020; Pu et al., 2021a) with some sample preparation modifications. The supernatants of the samples were collected and refrigerated at 4°C for 12 hr. After the oil and fat were removed, the supernatants were centrifuged at 10,000g for 15 min at 4°C. The supernatants (1 mL) were diluted with 0.1 mol/L HCl to prepare samples with a total free amino acid concentration of approximately 1–2 mg/mL. After they were filtered through a 0.22 μm nylon filter membrane (Cleman, Beijing, China), 500 μL of sample and 50 μL of internal standard solution were added to a 2 mL injection vial using a pipette (Eppendorf, Hamburg, Germany) and were subsequently mixed using a MX-5 (DragonLab, Beijing, China) vortex mixer for HPLC analysis. The quantitative analysis of the free amino acids was performed using the internal standard method. All standard curves (five data points, $n = 3$) were linear and their $R^2$ values were higher than 0.999. All samples were analyzed in triplicate.

2.4 | Organic acid analysis

The organic acids in the samples were analyzed according to a previously described method (Kong et al., 2017). Sample pretreatment was performed using the procedure described in Section 2.4. The supernatants were collected and refrigerated at 4°C for 12 hr. After the oil and fat were removed, the supernatants were centrifuged at 10,000g for 15 min at 4°C. The clear supernatant was filtered through a 0.22 μm syringe filter (Cleman, Beijing, China) twice and was diluted before organic acid analysis. The organic acids were quantified using external calibration curves. All standard curves (five data points, $n = 3$) were linear and their $R^2$ values were higher than 0.999. All samples were analyzed in triplicate.

2.5 | Nucleotide analysis

The nucleotides in the samples were analyzed according to a previously described method (Kong et al., 2017). Sample pretreatment was performed using the procedure described in Section 2.4. The nucleotides were quantified using external calibration curves. All standard curves (five data points, $n = 3$) were linear and their $R^2$ values were higher than 0.999. All measurements were performed in triplicate.

2.6 | Separation and purification of umami peptides

Samples were centrifuged as described in Section 2.4. The supernatants of the samples were ultrafiltrated (25°C, 0.2 MPa) using 5k, 3k, and 1kDa molecular weight (MW) membranes (Millipore, Bedford, MA, USA). Four fractions with different MWs were obtained from each sample, and the most intense umami fraction evaluated by sensory evaluation was then lyophilized.

The freeze-dried powder was redissolved in pure water to a concentration of 200 mg/mL. Afterward, 2 mL of solution was filtered through a 0.22 μm syringe filter twice and was loaded onto a Sephadex G-15 (1.6 cm × 100 cm, Qingpuhuixi Instrument Factory, Shanghai, China) gel filtration chromatography (GFC) column at 25°C and a flow rate of 1.0 mL/min with pure water as the eluent. The ultraviolet (UV) absorbance of the effluent was monitored at 220 nm with a sensitivity of 1.0, using an HD-21-2 (Qingpuhuixi Instrument Factory, Shanghai, China) UV detector because the absorbance of the peptide bond yielded the highest sensitivity at this wavelength (Strong et al., 2005). The experiment was repeated 21 times, and the fractions were collected, pooled, and lyophilized for sensory evaluation and subsequent separation. The most intense umami fractions were redissolved to a concentration of 10 mg/mL, and were subsequently separated using an LC3000 HPLC instrument equipped with
a COSMOSIL 5C18-MS-II column (10 mm x 250 mm) at 25°C to obtain several sub-fractions that contained umami peptides. The sub-fractions were analyzed at 214 nm. The mobile phase consisted of ACN/pure water (10:90, V/V) and its flow rate was 1.0 mL/min. The injection volume was 1 mL. Each fraction was collected and freeze-dried for subsequent identification.

The dried HPLC fractions were reconstituted to 1.0 mg/mL using pure water. After they were filtered through 0.22 μm syringe filters, 1 μL of each fraction was injected into an Agilent 6530 Accurate-Mass (Karlsruhe, Germany) Q-TOF LC/MS system equipped with a ZORBAX SB-C18 (2.1 mm x 150 mm, 3.5 μm; Agilent, Beijing, China) C18 column. Gradient elution that consisted of mobile phases A (H2O with 0.1% formic acid) and B (ACN) was used as follows: 5%–40% B from 0 to 15 min, 40%–5% B from 15 to 25 min, and 5% B from 25 to 26 min. The flow rate was 0.3 mL/min. Spectra were recorded in positive ion mode in a mass/charge (m/z) range of 50–1,000 Da. The LC-Q-TOF/MS instrument was run in positive ion and reflectron modes. All peptides eluted from the reverse-phase column were analyzed online via MS, and the peptides of interest were selected for MS/MS sequencing.

### Table 1: Contents of the taste compounds of the stir-fried pork with mung bean sprouts (SFPM), enzymatic hydrolysate (EH), and thermal reaction flavoring (TRF) samples

| Taste compounds | Content (mg/kg) | SFPM | EH | TRF |
|-----------------|----------------|------|----|------|
| Asp             | 1.18 ± 0.03c   | 64.27 ± 1.04b | 157.21 ± 2.25a |
| Glu             | 42.89 ± 1.19c  | 268.49 ± 5.42b | 5,458.86 ± 46.46a |
| Ser             | 33.45 ± 0.68c  | 406.69 ± 12.12b | 481.62 ± 20.96a |
| Pro             | 54.04 ± 3.03b  | 228.82 ± 10.70b | 4,448.15 ± 172.92a |
| Gly             | 12.70 ± 0.37f  | 315.67 ± 22.80b | 1,353.54 ± 47.50a |
| Thr             | 43.23 ± 1.54c  | 185.43 ± 8.90b | 237.84 ± 9.74a |
| Ala             | 99.20 ± 1.41c  | 753.96 ± 32.41f | 628.64 ± 15.19b |
| His             | 38.21 ± 1.14c  | 186.20 ± 4.35b | 424.09 ± 16.37a |
| Tyr             | 20.29 ± 0.24c  | 404.54 ± 8.40b | 656.91 ± 11.02a |
| Val             | 75.81 ± 1.00c  | 382.60 ± 8.98b | 257.97 ± 2.91b |
| Met             | 41.00 ± 2.56c  | 370.06 ± 13.50b | 215.94 ± 7.27b |
| lle             | 47.00 ± 0.54c  | 412.90 ± 15.32a | 303.79 ± 7.16b |
| Phe             | 58.17 ± 0.60c  | 448.43 ± 9.02a | 279.55 ± 4.53b |
| Lys             | 0.89 ± 0.00c   | 780.81 ± 7.19a | 242.86 ± 9.26b |
| Leu             | 45.68 ± 0.74c  | 1,335.98 ± 38.47a | 615.00 ± 17.49b |
| Arg             | 1.25 ± 0.01b   | 10.53 ± 0.24b | 8,495.54 ± 110.26b |
| Cys–Cys         | 6.73 ± 0.04c   | 162.58 ± 3.07b | 425.09 ± 21.25a |
| Total free amino acid | 621.72 | 6,717.96 | 24,682.58 |
| Oxalic acid     | 3.04 ± 0.03c   | 10.90 ± 0.06b | 38.41 ± 0.31a |
| Tartaric acid   | 26.88 ± 0.07c  | 92.95 ± 0.67a | 60.62 ± 1.85b |
| Formic acid     | nd             | 32.62 ± 0.46b | 38.99 ± 1.11a |
| Malic acid      | nd             | 5.59 ± 0.00b | 29.88 ± 0.21a |
| Lactic acid     | 7.56 ± 0.09c   | 36.72 ± 1.30b | 39.37 ± 1.12a |
| Acetic acid     | 2.55 ± 0.04b   | 32.20 ± 0.80a | 32.10 ± 0.95a |
| Pyroglutamic acid | nd             | nd           | 15.83 ± 0.45a |
| Citric acid     | nd             | 26.66 ± 0.00a | 4.03 ± 0.21b |
| Succinic acid   | 2.19 ± 0.02c   | 64.81 ± 0.00a | 50.03 ± 2.47b |
| Total organic acid | 42.22 | 302.45 | 309.26 |
| 5'-CMP          | 21.15 ± 0.87c  | 187.34 ± 4.44b | 644.68 ± 2.75a |
| 5'-AMP          | 1.88 ± 0.01b   | nd            | 15.78 ± 0.67a |
| 5'-GMP          | 13.53 ± 0.35c  | 235.99 ± 1.27b | 3,152.51 ± 46.76a |
| 5'-IMP          | 45.54 ± 1.12b  | 1.16 ± 0.00b  | 2,717.26 ± 44.38a |
| Total 5'-nucleotide | 82.10 | 424.49 | 6,530.24 |

1The contents of compounds were expressed as mean ± standard deviation (mean ± SD mg/kg of raw material of formula in SFPM and EH samples; mean ± SD mg/kg of enzymatic hydrolysate in TRF samples) of triplicate samples; nd: not detectable; Different lowercase letters between columns represent significant differences (p < 0.05).
2.7 | Umami evaluation

Sensory evaluation was performed using the "GB/T 12310-2012 Sensory analysis method-Paired comparison test" with some modifications. The five male and five female (19–28 years old) sensory evaluation panelists were recruited from the Beijing Technology and Business University (Beijing, China). The panelists were trained to distinguish and order umami taste solutions of different concentrations for 20 min three times per week for 3 weeks. The final sensory evaluation criteria were determined via a panel discussion. The umami and umami-enhancing tastes of the samples were scored using a 10-point scale (Wang et al., 2020; Zhuang et al., 2016). Data were expressed as means ± standard deviations (Wang et al., 2020; Zhuang et al., 2016). The standard umami solution consisted of an aqueous solution of salt (0.35% (w/V)) and MSG (0.35% (w/V)) (Su et al., 2012), which was ascribed a score of 5. The lyophilized fraction (1.0 g) was dissolved in 1 L of standard umami solution before tasting to evaluate the umami-enhancing effect.

To obtain taste thresholds, taste dilution analysis was used to determine the taste dilution (TD) factors of each fraction. The lyophilized ultrafiltered and GFC fractions were dissolved in pure water to concentrations of 10 mg/mL, and then, serial dilutions (1:2, 1:4, 1:8, 1:16, 1:32, etc.) were prepared using pure water as solvent. The maximum number of dilutions that still allowed the detection of the umami taste was recorded as the TD value.

All sensory evaluations were performed in a sensory panel room at 25°C and 55% humidity. The panelists were asked to take one sip of the sample, hold it in their mouth for 10 s, and spit it out. To avoid fatigue and the carryover effect, the panelists were asked to rinse their mouths with 50–60 ml of drinking water and take 15 s breaks between samples (Dang et al., 2015). Eating, drinking, or smoking was not allowed for 1 hr before the sensory evaluation testing. All score cards were collected at the end of each session, and the average descriptor values from all 10 judges were used for multivariate statistical analysis.

2.8 | Statistical analysis

Statistical calculations were performed using the SPSS 17.0 (SPSS Inc., Chicago, IL, USA) statistical package for one-way ANOVA (Pu et al., 2021b). Data were expressed as means ± standard deviations of triplicate determinations. The mean values were considered significantly different at p < 0.05.

3 | RESULTS AND DISCUSSION

3.1 | Free amino acids

Free amino acids play a crucial role in food taste. As illustrated in Table 1, the amount of total free amino acids in the SFPM sample was the lowest.

The contents of most free amino acids in the SFPM raw material were increased significantly after enzymatic hydrolysis, and the contents of lysine (Lys), Asp, and leucine (Leu) increased the most (876.31, 53.47, and 28.25 times, respectively). The total content of free amino acids of EH was 10.81 times higher than that of SFPM. These results indicated that enzymatic hydrolysis was more effective for releasing the free amino acids from raw materials than high-temperature stir-frying. Moreover, studies have revealed that blanching can reduce the content of free amino acids of mung bean sprouts (Farhangi & Valadon, 1982). This might be another reason for the content of free amino acids of SFPM being lower than that.

| Taste compound | Taste threshold (mg/L) SFPM | TAV |
|----------------|----------------------------|-----|
| Asp            | 1,000<sup>a</sup>          | 0.00| 0.04| 0.03 |
| Glu            | 300<sup>a</sup>            | 0.17| 0.55| 4.03 |
| Ser            | 1,500<sup>b</sup>          | 0.03| 0.17| 0.07 |
| Pro            | 3,000<sup>b</sup>          | 0.02| 0.05| 0.33 |
| Gly            | 1,300<sup>b</sup>          | 0.01| 1.06| 0.23 |
| Thr            | 2,600<sup>b</sup>          | 0.48| 0.04| 0.02 |
| Ala            | 600<sup>b</sup>            | 2.08| 0.77| 0.23 |
| His            | 200<sup>b</sup>            | 6.21| 0.61| 0.45 |
| Tyr            | –                          | /   | /   | /    |
| Val            | 400<sup>b</sup>            | 0.22| 0.59| 0.14 |
| Met            | 300<sup>b</sup>            | 4.16| 0.76| 0.15 |
| Ile            | 900<sup>b</sup>            | 1.39| 0.28| 0.07 |
| Phe            | 900<sup>b</sup>            | 0.08| 0.31| 0.07 |
| Lys            | 500<sup>b</sup>            | 0.00| 0.96| 0.11 |
| Leu            | 1,900<sup>b</sup>          | 0.03| 0.43| 0.07 |
| Arg            | 500<sup>b</sup>            | 0.00| 0.01| 3.77 |
| Cys-Cys        | –                          | /   | /   | /    |
| Oxalic acid    | 504<sup>c</sup>            | 0.01| 0.01| 0.03 |
| Tartaric acid  | 15<sup>c</sup>             | 2.08| 2.38| 1.43 |
| Malic acid     | 496<sup>c</sup>            | /   | 0.00| 0.02 |
| Lactic acid    | 1260<sup>c</sup>           | 0.01| 0.01| 0.01 |
| Acetic acid    | 120<sup>c</sup>            | 0.03| 0.11| 0.11 |
| Citric acid    | 450<sup>c</sup>            | /   | 0.02| 0.01 |
| Succinic acid  | 106<sup>c</sup>            | 0.02| 0.23| 0.28 |
| 5’-AMP         | 500<sup>c</sup>            | 0.00| /   | 0.01 |
| 5’-GMP         | 125<sup>c</sup>            | 0.14| 0.81| 10.04 |
| 5’-IMP         | 255<sup>c</sup>            | 0.21| 0.00| 3.81 |

Note: –, No threshold was found; /, TAV value could not be calculated because no threshold was found or the compound was below the limit of quantitation in the sample.

<sup>a</sup>Duan et al. (2020).
<sup>b</sup>Kato et al. (1989).
<sup>c</sup>Wang et al. (2020).
The contents of Asp, Glu, serine (Ser), Pro, glycine (Gly), threonine (Thr), histidine (His), tyrosine (Tyr), Arg, and Cystine in the TRF sample prepared using EH as raw material were significantly higher than those of the EH sample, and the contents of Arg, Glu, and Pro increased the most (805.79, 19.33, and 18.44 times, respectively). The most direct method to increase the contents of these three free amino acids was the addition of TRF, including the direct and indirect addition of HVP, yeast extract, spices blend, soy sauce, and vinegar to the formula. After the thermal reaction, the contents of Ala, valine (Val), methionine (Met), isoleucine (Ile), phenylalanine (Phe), Lys, and Leu of the sample decreased significantly. This might be attributed to the consumption of these free amino acids during the thermal reaction being greater than the amounts added to the process formula and thermal reaction. Except for Ala, the other six free amino acids (Val, Met, Ile, Phe, Lys, and Leu) were bitter amino acids, and might contribute to the better taste of the TRF sample.

### 3.2 Organic acids

Organic acids are also important taste compounds. As presented in Table 1, the amount of total organic acids in the SFPM sample was the lowest of all analyzed samples. The contents of organic acids in the SFPM raw material increased significantly after enzymatic hydrolysis, and the contents of succinic, acetic, and lactic acids increased the most (28.59, 11.63, and 3.86 times, respectively). In addition to the five organic acids (oxalic, tartaric, succinic, acetic, and lactic acids) detected in the SFPM sample, formic, malic, and citric acids were also detected in the EH sample. The total organic acid content of the EH sample was 7.16 times higher than that of the SFPM sample. This demonstrated that enzymatic hydrolysis was a more effective way to release organic acid from raw materials than high-temperature stir-frying. After the thermal reaction, the contents of oxalic, formic, malic, lactic, and pyrogulamic acids were significantly increased owing to the introduction of the TRF formula. Tartaric acid, an important plant organic acid,
might be derived from mung bean sprouts particularly after enzymatic hydrolysis. Tartaric acid was also detected in tilapia frame protein hydrolysate, but could not be detected before hydrolysis (Chuesiang & Sanguandeekul, 2015). Because succinic and lactic acids were reported to present sour and umami taste (Park et al., 2001), they could contribute to the umami taste of the EH and TRF samples.

### 3.3 | 5′-Nucleotide

The amount of total nucleotides in the SFPM sample was the lowest of all analyzed samples (Table 1). After enzymatic hydrolysis, the 5′-CMP and 5′-GMP contents of the sample increased significantly (7.86 and 16.44 times, respectively). The content of total nucleotides
of the EH sample was 5.17 times higher than that of the SFPM sample. This indicated that enzymatic hydrolysis was more effective at releasing 5’-CMP and 5’-GMP from raw materials than high-temperature stir-frying. After the thermal reaction, the nucleotide contents significantly increased owing to the addition of TRF, and the contents of 5’-IMP and 5’-GMP increased the most (2,341.47 and 12.36 times, respectively). Because 5’-IMP and 5’-GMP contribute to the umami taste intensity significantly (Yang et al., 2001), they might also contribute to the umami taste of TRF. The 5’-IMP and 5’-GMP nucleotides not only present umami taste but also present a synergistic effect with MSG. It has been reported that the umami taste might be greatly increased owing to the synergistic effect of the MSG-like amino acids and 5’-GMP, 5’-IMP, and 5’-AMP nucleotides (Dermiki et al., 2013).
3.4 | Taste activity value

The taste activity value (TAV) was calculated using the method reported by Schlüchterle-Cerny and Grosch (Schlüchterle-Cerny & Grosch, 1998). The taste thresholds of the free amino acids, organic acids, and 5′-nucleotides in water were retrieved from the literature (Duan et al., 2020; Kato et al., 1989; Schlüchterle-Cerny & Grosch, 1998; Wang et al., 2020). Compounds with TAV greater than 1 were considered to be food taste-active (Engel et al., 2002). As presented in Table 2, five (Ala, His, Met, Ile, and tartaric acid), two (Gly and tartaric acid) and five (Glu, Arg, 5′-GMP, 5′-IMP, and tartaric acid) taste compounds contributed to the taste of the SFPM, EH, and TRF samples, respectively. Among these taste compounds, Glu, 5′-IMP, and 5′-GMP present umami taste; Gly and Ala are sweet; Met, Ile, Arg, and His are bitter; and tartaric acid is sour (Kato et al., 1989; Liu et al., 2015). The EH sample contained less bitter amino acids than the SFPM sample, which indicated that enzymatic hydrolysis increased owing to the release of large amounts of Glu, 5′-GMP, and 5′-IMP from the raw material. The EUC of the TRF sample (2,929.83 g MSG/100 g sample) decreased the contribution of the bitter amino acids to the taste of enhancing TRF.

3.5 | Equivalent umami concentration

The equivalent umami concentration (EUC, g MSG/100 g sample) is the concentration of MSG equivalent to the umami intensity of mixtures of MSG-like amino acids and 5′-nucleotides (Chen & Zhang, 2007). The EUCs of the samples in this study were calculated using the equation reported by Yamaguchi et al. (1971).

The EUCs of the SFPM, EH, and TRF samples were 5.71, 47.54, and 5,698.91 g MSG/100 g sample, respectively. After enzymatic hydrolysis, the EUCs increased owing to the release of large amounts of Glu, 5′-GMP, and 5′-IMP from the raw material. The EUC of the TRF sample was significantly higher than that of the EH sample owing to the addition of large amounts of Glu, 5′-GMP, and 5′-IMP to the TRF formula. The EUC of the TRF sample (2,929.83 g MSG/100 g sample) was much higher than that reported for most commercial soy sauces (Kong et al., 2018).

3.6 | Umami peptides

To investigate the umami and umami-enhancing effect, the samples were partitioned into four peptide fractions using ultrafiltration membranes: U-I (MW < 1 kDa), U-II (MW = 1–3 kDa), U-III (MW = 3–5 kDa), and U-IV (MW > 5 kDa). As depicted in Figure 1a, fraction U-I accounted for the highest mass fractions in the EH and SFPM samples (68.18% and 56.42%, respectively), and only accounted for 28.36% of the TRF sample (the U-II content of the TRF sample was the highest (60.85%)).
For the EH sample, sub-fraction E-2 presented the highest umami fraction, and sub-fraction E-3 presented higher umami-enhancing effect and TD value than subtraction E-2. Therefore, sub-fractions E-2 and E-3 were selected for subsequent separation. Although the mass fraction of sub-fraction F-3 (16.18%) of TRF was not very high, it presented the highest umami level, umami-enhancing effect, and TD value of all TRF sub-fractions. Consequently, sub-fraction F-3 was used for further fractionation using a reverse-phase HPLC system with a preparative C18 column.

The HPLC separation results of sub-fractions C-3, E-2, E-3, and F-3 were depicted in Figure 2d–g (C-3-1–C-3-3 (C-3), E-2-1–E-2-2 (E-2), E-3-1–E-3-3 (E-3), and F-3-1–F-3-4 (F-3)). The amounts of all fractions were listed in Table 3. Due to the problem of collecting the content of each component, priority was given to the study of umami substances in high-content components. The signals of sub-fractions C-3-2, E-2-2, E-3-3, and F-3-3 were higher, and their peaks were relatively independent. Hence, sub-fractions C-3-1, C-3-2, E-2-1, E-2-2, E-3-1, E-3-3, F-3-1, and F-3-3 were collected and lyophilized for LC–MS analysis.

The MWs and peptide sequences of the three samples are presented in Table 4. A total of 26 peptides were identified, and some were umami peptides. Among the seven peptides detected in the SFPM sample, Glu–Asp (Kawai et al., 2002), Ala–Glu (Kirimura et al., 1969), Ala–Asp (Kong et al., 2017), and Glu–Ala–Glu (Nishimura & Kato, 1988) have been reported to present umami taste, and Ala–Met (Sforza et al., 2001) has been reported to be bitter. Of the 11 peptides detected in the EH sample, Asp–Val, which was separated from sub-fraction E-3, has been reported to contribute to the bitter taste of Spanish ham (Sforza et al., 2001). Finally, eight peptides were detected in the TRF sample.

Sentandreu et al. reported that most peptides that contained hydrophobic amino acids, such as Phe, Tyr, and Leu presented the same bitter taste as their monomers (Sentandreu et al., 2003). In addition, peptides with hydrophobic groups, such as Phe and Leu, at the C-terminus position, could provide a strong bitter taste to foods. Sour taste peptides are typically associated with umami. The presence of free and dissociated Glu and Asp conferred sour and brothy/umami taste to foods (Kirimura et al., 1969), and sequences, such as Val–Glu, Asp–Ala, Gly–Asp, Val–Asp, and Gly–Glu, might contribute to the sour and umami taste of dry-cured ham (Sentandreu et al., 2003). Although some dipeptides with L-Glu at the N-terminus positions confer a sour taste.
taste to foods, they could also impart brothy taste in NaCl-containing aqueous solutions at pH 6.0. Thus, sour peptides were considered to be umami peptides (Arai et al., 1973). Ueda et al. reported that Cys-containing peptides extracted from onions imparted kokumi taste to foods because the sulfhydryl group at the side chain of Cys could cause a slight convergence sense on the tongue, and that can significantly increase the kokumi taste (Ueda et al., 1994).

According to related studies, it is important to make some predictions on the unreported peptides analyzed in this study. These peptides mainly consisted of bitter, sweet, and umami amino acids. Because the sweet amino acids were the predominant components, these peptides might present sweet and umami taste, or umami-enhancing effect. A total of 26 peptides were identified in the first-order collected samples (Figure S1). All peptides identified in the SFPM sample were dipeptides or tripeptides. EH consisted mostly of tripeptides and polypeptides with more than three amino acids (72.73%). The peptides identified after the thermal reactions consisted of at least three amino acids. This might be attributed to the cross-linking of peptides and amino acids, or peptides and peptides being promoted during the thermal reaction. The following 16 amino acids Ala, Ser, Glu, Asp, Gly, His, Ile, Val, Met, Pro, Thr, Phe, Cys, Lys, Tyr, and Arg appeared 22, 20, 12, 12, 7, 5, 3, 3, 2, 2, 2, 1, 1, and 1 times in the 26 peptides identified in the three samples analyzed in this study. Among all umami peptides, 18 contained sweet amino acids at their N-terminus positions: (Ala (8), Ser (7), Gly (2), and Thr (1)), 6 contained umami amino acids at the N-terminus positions (Glu (5) and Asp (1)), and 2 contained bitter amino acids at the N-terminus positions (His (1) and Ile (1)). Therefore, the sweet and umami amino acids were the main residues at the N-terminus positions of umami peptides. The Gly–Gly-continuous, Asp–Asp-continuous, Ala–Ala-continuous, and Ser–Ser-continuous amino acid sequences were identified in the polypeptides separated from the EH and TRF samples. The Ala–Ala–Ala-continuous and Ser–Ser–Ser-continuous amino acid sequences were identified in the polypeptides separated from the EH sample. Double and third umami amino acid continuous could be important for the umami taste of peptides.

4 | CONCLUSION

The taste components of three seasonings prepared with mung bean sprout via stir-frying, enzymatic hydrolysis, and thermal reaction were investigated by HPLC and LC-Q-TOF/MS. Enzymatic hydrolysis was more effective at releasing the taste compounds from raw materials than high-temperature stir-frying. The thermal reaction improved the proportion of taste components in the EH, which resulted in the EH sample presenting the highest EUC value of all analyzed food samples, and also improved the contribution of Glu, 5′-IMP, and 5′-GMP to the food taste. The sweet and umami amino acids were the main residues at the N-terminus positions of the 26 peptides identified in the analyzed samples. Furthermore, double and triple continuous umami amino acids could be important for the umami taste of peptides. The aforementioned results not only provide insight into the unique taste of three seasonings prepared with mung bean sprout, but also provide guidelines for the development of umami flavorings.

ACKNOWLEDGMENTS

This work was supported by the National Key R&D Program of China (No. 2016YFD0400705). The authors thank Professor Dejian Huang at the National University of Singapore for extending his help to revise the manuscript.

CONFLICT OF INTEREST

The authors have declared no conflicts of interest for this article.

AUTHOR CONTRIBUTIONS

Yan Kong: Data curation; Formal analysis; Methodology; Software; Visualization; Writing-original draft. Chenchen Zhou: Data curation; Formal analysis; Software; Supervision; Validation. Li Li: Formal analysis; Investigation; Validation. Honglei Tian: Formal analysis; Investigation. Caill Fu: Formal analysis; Investigation. Xuepeng Li: Formal analysis; Investigation. Yuyu Zhang: Funding acquisition; Project administration; Resources; Writing-review & editing.

DATA AVAILABILITY STATEMENT

The data availability statement of “JFPP-08-20-1999.R1, entitled Comparative analysis of taste components of three seasoning bases prepared via stir-frying, enzymatic hydrolysis, and thermal reaction” is as follows: (1) The data that support the findings of this study are available from the corresponding author upon reasonable request. (2) The data that supports the findings of this study are available in the supplementary material of this article.

ORCID

Xuepeng Li https://orcid.org/0000-0002-2445-7158
Yuyu Zhang https://orcid.org/0000-0003-3095-3083

REFERENCES

Arai, S., Yamashita, M., & Noguchi, M. (1973). Tastes of L-glutamyl oligopeptides in relation to their chromatographic properties. *Agricultural and Biological Chemistry*, 37(1), 151-156. https://doi.org/10.1080/00021369.1973.10860638

Begum, N., Raza, A., Song, H. L., Zhang, Y., Zhang, L., & Liu, P. (2019). Effect of thermal treatment on aroma generation from bovine bone marrow extract during enzymatic hydrolysis. *Journal of Food Processing and Preservation*, 43(10), e14105. https://doi.org/10.1111/jfpp.14105

Chen, D., & Zhang, M. (2007). Non-volatile taste active compounds in the meat of Chinese mitten crab (*Eriocheir sinensis*). *Food Chemistry*, 104(3), 1200–1205. https://doi.org/10.1016/j.foodchem.2007.01.042

Chuesiang, P., & Sanguandeeuk, R. (2015). Protein hydrolysatase from tilapia frame: Antioxidant and angiotensin I converting enzyme inhibitor properties. *International Journal of Food Science & Technology*, 50(6), 1436–1444. https://doi.org/10.1111/ijfts.12762

Dang, Y., Gao, X., Ma, F., & Wu, X. (2015). Comparison of umami taste peptides in water-soluble extractions of Jinhua and Parma hams. *LWT - Food Science and Technology*, 60(2), 1179–1186. https://doi.org/10.1016/j.lwt.2014.09.014
Dermiki, M., Phanphenphosophon, N., Mottram, D. S., & Methven, L. (2013). Contributions of non-volatile and volatile compounds to the umami taste and overall flavour of shitake mushroom extracts and their application as flavour enhancers in cooked minced meat. *Food Chemistry*, 141(1), 77–83. https://doi.org/10.1016/j.foodchem.2013.03.018

Duan, W., Huang, Y., Xiao, J. F., Zhang, Y. Y., & Zhang, H. Y. (2020). Comparison of nonvolatile taste components in 18 strong fragrance spices. *International Journal of Food Properties*, 23(1), 340–353. https://doi.org/10.1080/10942912.2020.1720712

Engel, E., Nicklaus, S., Salles, C., & Le Quéré, J. L. (2002). Relevance of amino acids in Parma hams of known cathepsin B activity. *Food Chemistry*, 75(3), 267–273. https://doi.org/10.1016/S0308-8146(01)00224-2

Fonklin, C., Laohakunjit, N., & Kerdzchochen, O. (2011). Physicochemical and flavor characteristics of flavoring agent from mungbean protein hydrolyzed by bromelain. *Journal of Agricultural and Food Chemistry*, 59(15), 8475–8483. https://doi.org/10.1021/jf202006a

**How to cite this article:** Kong Y, Zhou C, Zhang L, et al. Comparative analysis of taste components of three seasoning bases prepared via stir-frying, enzymatic hydrolysis, and thermal reaction. *J Food Process Preserv*. 2021;00:e15652. https://doi.org/10.1111/jfpp.15652

How to cite this article: Kong Y, Zhou C, Zhang L, et al. Comparative analysis of taste components of three seasoning bases prepared via stir-frying, enzymatic hydrolysis, and thermal reaction. *J Food Process Preserv*. 2021;00:e15652. https://doi.org/10.1111/jfpp.15652

**SUPPORTING INFORMATION**

Additional Supporting Information may be found online in the Supporting Information section.