INTRODUCTION

Beef is one of the most popular meat with increasing consumption (Asp, Richardson, Collene, Droll, & Belury, 2012). Beef is rich in high-quality protein, minerals, and other highly digestible and absorbable nutrients. In China, beef is very popular and widely consumed though the price of beef is generally higher than pork, fish, and chicken. It is commonly used for preparing traditional dishes such as fried beef with chili, sautéed beef fillet with green pepper, sautéed beef with green vegetable, sautéed beef with scallion, country-style sautéed beef fillet, sautéed sliced beef with ginger and scallion, and sautéed beef slices combined with various food ingredients (Asai et al., 2019).

Raw meat has a blood-like flavor, due to the presence of blood salts and products of pyrolysis and saliva, with some overtones due to species, diet, and environment of the animal. After processing in hot temperature, flavor precursors including free amino acids (FAAs), reducing sugars, 5’-inosine monophosphate (5’-IMP), and polyunsaturated fatty acids in beef undergo a series of thermal reactions, resulting in appealing aroma and taste (Meinert et al., 2009; Mungure, Bekhit, Birch, & Stewart, 2016). The umami has come to be recognized as the “fifth basic taste, after sweet, sour, bitter, and salty” elicited by the common flavor enhancer monosodium glutamate (MSG) along with other amino acids and ribonucleotides (Wang, Tonnis, Wang, Zhang, & Adhikari, 2019). Umami is a basic taste which is associated with the

1 Beijing Advanced Innovation Center for Food Nutrition and Human Health, Beijing Technology and Business University, Beijing, China
2 Beijing Key Laboratory of Flavor Chemistry, Beijing Technology and Business University, Beijing, China
3 Beijing Laboratory for Food Quality and Safety, Beijing Technology and Business University, Beijing, China

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

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Abstract
The objective of this study was to optimize the cooking process of stir-fried beef and investigate the release of taste compounds during cooking by instrumental analysis. The contents of taste compounds in stir-fried beef were determined by high-performance liquid chromatography. The optimum result was: beef 100 g stir-fried 4.00 min with blending oil 25.00 g, sucrose 1.75 g and salt 2.00 g, the content of total umami components showed an increasing trend with the maximum value (2,580.32 µg/g) at 4.00 min cooking and then decreased with the extension of time. The equivalent umami concentration of fried beef normal group (124.29 g MSG/100 g) and fried beef with spice package group (154.54 g MSG/100 g) were far higher than the control group (3.96 g MSG/100 g). It could be an effective method to optimize the cooking method by using instrumental analysis to replace or partly replace sensory evaluation.

Practical applications
In this work, the cooking method of stir-fried beef was optimized. Results elucidated that adding the spices was beneficial for improving the dish flavor. It is an innovative proposal of a good method to replace or partly replace the sensory evaluation with instrumental analysis. Further, it may provide guidance for the cooking process of stir-fried beef.
MSG, 5’-nucleotides, bi-functional acids and certain peptides (Bermas, 2017). Many products made from protein-rich materials exhibited significant umami taste due to the presence of special FAA s (L-glutamic acid and L-aspartic acid) and umami peptides (Rhyu & Kim 2011).

Except for the raw materials, many factors such as age, location, and processing methods also have a significant effect on the quality of meat products. Cooking methods could affect the chemical composition (Vossen, Claeys, Raes, Mullem, & Smet, 2016; Wang et al., 2018), nutrition, taste substances, and finally determines the preferences and acceptability of consumers (Luo, Taylor, Nebi, Ng, & Bennett, 2018; Riyanto et al., 2018). There are many ways of cooking beef in China and in Western countries. In western, the common technique for preparation steak was frying in a pan or barbecue ovens and iron plates. In recent years, vacuum-packaged meat immersed in water bath for longer cooking has been widely used in the western catering industry to provide cooked meat with longer shelf life and better taste (Pourkhalili, Mirlohi, & Rahimi, 2013; da Silva et al., 2017). Comparing with the western cooking methods, the traditional cooking methods of Chinese dishes mainly include stewed and braised, stir-fried, and deep-fried beef, among these cooking types, stir-frying makes the texture of the meat relatively tender.

Literature research used sensory evaluation to enhance the quality of meat products like beef to develop meat products with improved health benefits. Conroy, O'Sulliyan, Hamill, & Kerry (2019) used sensory evaluation to determine assessors’ preferences for sequential reductions in salt concentrations in optimized corned beef products and to determine if preference was affected by the age of the assessors. However, there are some problems during sensory evaluation experiment such as instability, high fatigue degree, high fatigue degree, large individual differences, and personal preference (Wolf et al., 2019).

Therefore, this study aimed to optimize the formula of stir-fried beef based on the results of instrumental analysis: (a) optimization of the adding ingredients by single-factor experiment; (b) monitoring the release of taste compounds by instrumental analysis.

2 MATERIALS AND METHODS

2.1 Materials

Beef (the hind leg of 30 months bull) were purchased from Yonghui supermarket (Beijing, China) and stored at 4 ± 0.5 °C. The sucrose was bought from Beijing Yuli Xing Commerce and Trade Co., Ltd. The scallion (powder), onion (powder), and coriander (powder) were purchased from Fufeng Group Co., Ltd. The cumin was purchased from Hangzhou Wahaha Group Co., Ltd. The lactic acid, citric acid, succinic acid, potassium dihydrogen phosphate (KH₂PO₄), phosphoric acid (H₃PO₄) (all AR grade), and malic acid (BR grade) were obtained from Sinopharm Chemical Reagent Co., (Shanghai, China). Inosine 5’-monophosphate (5’-IMP), adenosine 5’-monophosphate (5’-AMP), guanosine 5’-monophosphate (5’-GMP), and cytidine 5’-monophosphate (5’-CMP) were purchased from Sigma-Aldrich (St. Louis, Mo., USA). Methanol and acetonitrile (ACN) (all HPLC grade) were purchased from Fisher Scientific (Shanghai, China). The ultra-pure water was purchased from Hangzhou Wahaha Group Co., Ltd. (Hangzhou, China). Buffer I (0.01 mol/L KH₂PO₄, pH 2.8) was prepared by dissolving 1.36 g KH₂PO₄ in 1 L water, and then adjusted to pH 2.8 by 1 mol/L H₃PO₄. Buffer II (0.05 mol/L KH₂PO₄) was prepared by dissolving 6.80 g KH₂PO₄ in 1 L water. Durashell AA analytical reagents were purchased from Tianjin Bona Agel Technology Co., Ltd.

2.2 Preparation of stir-fried beef sample

Beef cubes (the hind leg) were cut into pieces with 3 cm × 2 cm × 2 mm, then heated on a dry non-stick pot for 30 s with the power of 1,200 W. The pieces of beef were added to the pot and stir-fried quickly for a time until the blending oil heated to the smoke point, finally cooled down the stir-fried beef to room temperature. Then the sample was prepared as following: (a) 100 g stir-fried beef with 150.00 g water with the beating machine beat for 1 min; (b) the homogenated beef then kept it in the centrifuge for 10 min at 4 °C and 9,600 r/min; (c) removed the oil then obtained the fried beef sample.

2.3 Optimization of the stir-fried beef conditions

Based on the pretreatment of different stir-fried beef samples, the effects of stir-fried time (3.50, 4.00, 4.50, 5.00, 5.50 min), blending oil addition (10.00, 15.00, 20.00, 25.00, 30.00 g), amount of salt addition (0.125, 1.50, 1.75, 2.00, 2.25 g), amount of sucrose addition (0.125, 1.50, 1.75, 2.00, 2.25 g), different formula (fried oil 27.00 g), fried beef control (27.00 g oil, 100.00 g beef), fried beef normal group (27.00 g oil, 100.00 g beef, 1.80 g sucrose, 2.13 g salt), fried spice package group (27.00 g oil, 1.50 g spice package), and fried beef with spice package group (27.00 g oil, 100.00 g beef, 1.50 g spice package) on non-volatile taste components in stir-fried beef were investigated.

2.4 Free amino acid analysis

Stir-fried beef was made into a muddy consistency and was kept in the refrigerator at 4 °C for 12 h, with the broth taken out and the surface oil removed. Then 6.00 g sample was centrifuged at 4 °C with 9,600 r/min for 10 min. The oil was removed and 3.5 mL of sulfo salicylic acid, and 0.1 mol/L of hydrochloric acid solution was added to 10 mL beef broth supernatant. Then the sample solution was filtered by 0.45 μm and 0.22 μm nylon filter membrane (Jinteng, Tianjin). The total amino acid concentration after dilution was 1-2 mg/mL before mixed with an internal standard solution and prepared for the next analysis. The procedure was similar to Wang et al. (2018).

Agilent 1260 high-performance liquid chromatography was used to analyze 17 kinds of FAA s in the standard and sample. Among them,
17 kinds of FAAs were Asp, Glu, Ser, Pro, Gly, Thr, Ala, Val, Met, Ile, Phe, Lys, Leu, Arg, His, Tyr, and Cys-Cys. The determination conditions were as procedure of (Jastrzębska, Piasta, & Szłyk, 2015).

2.5 | Organic acid analysis

The stir-fried beef broth 5.00 g mixed with 150.00 g water was broken into pulp, then centrifuged for 10 min at 4 °C with 9,600 r/min, obtained supernatant which being filtered through a 0.45 μm nylon filter membrane, diluted 20 times to be injected. Six kinds of organic acids were determined by Thermo U3000 UPLC system (Thermo Fisher Scientific Inc., USA), data were collected, processed and analyzed by Chromeleon software (version 7.1, Thermo Fisher Scientific Inc., USA), which following the procedure of Kong et al. (2017). Samples were analyzed by column (Venusil XBP C18, 4.6 mm × 250 mm, 5 μm), the mobile phase was methanol-Buffer salt I (5:95, V:V) at the rate of 1 mL/min, the column temperature was 25 °C, detection wavelength was 205 nm. The mixed organic acid, malic acid, and citric acid were prepared into 4 mg/mL calibration solution with ultrapure water; lactic acid, tartaric acid and succinic acid were prepared into 2 mg/mL calibration solution then diluted into 7 gradients; oxalic acid was prepared into 1 mg/mL calibration solution. According to the concentration range of organic acids in the samples, 7 mixture standard solutions were selected as follows: malic acid and citric acid were 2.67, 2.00, 0.80, 0.40, 0.13, 0.07 mg/mL. Lactic acid, tartaric acid and succinic acid were 1.33, 1.00, 0.40, 0.20, 0.10, 0.07, 0.03 mg/mL. Oxalic acid was 0.67, 0.50, 0.20, 0.10, 0.05, 0.03, 0.02 mg/mL. All organic acids were quantified according to the calibration curve of the standard organic acid.

2.6 | 5′-Nucleotides analysis

The sample pretreatment method is the same as “2.5 Organic acid analysis.” Samples were analyzed by column (Venusil XBP C18, 4.6 mm × 250 mm, 5 μm), the mobile phase was methanol-Buffer salt II (5:95, V:V) at the rate of 1 mL/min, and the detection wavelength was 254 nm. The mixed nucleotides (5′-AMP, 5′-GMP, 5′-IMP, and 5′-CMP) were prepared into 0.1 mg/mL calibration standard solution with ultrapure water, then diluted into seven gradients. According to the concentration range of four nucleotides in the sample, the concentration of 7 standard mixed solutions was selected as follows: 66.70, 40.00, 33.30, 20.00, 10.00, 2.00, and 0.20 mg/mL. Each 5′-nucleotide was quantified according to the calibration curve of the standard 5′-nucleotide.

2.7 | Equivalent umami concentration

EUC value was used to characterize the intensity of food flavor. Specifically, in 100 g of food, the amount of sodium glutamate is used to represent the total amount of umami substances, which is calculated according to the following equation (Chen & Zhang, 2007; Krishnan, Babuskin, Babu, Sivarajan, & Sukumar, 2015).

\[ Y = \sum a_i b_i + 1218 \left( \sum a_i b_i \right) \left( \sum b_i \right) \]

2.8 | Taste activity value

Taste activity value (TAV) is the ratio of the concentrations of substances to their taste threshold values detected in water or other matrices (Schlichtherle-Cerny & Grosch, 1998). The TAVs of FAAs, flavoring nucleotides and organic acids, and the components that contributed significantly to the taste of five samples were calculated. The compounds with TAV >1 (Engel, Nicklaus, Salles, & Quere, 2002) are considered to have a contribution to the taste profile of beef. When the TAV is less than 1, it is considered that the substance does not contribute to the flavor, thus the main taste compounds could be determined (Kato et al., 1989).

2.9 | Statistical analysis

All statistical analyses with the experimental results expressed as means ± SD. One-way ANOVA analysis was performed by SPSS software (version 19.0, SPSS Inc., Chicago, IL, USA). Analysis of variance (p < .05) was used to analyze the data and a Duncan’s multiple range test was used to separate the means.

3 | RESULTS AND DISCUSSION

3.1 | Analysis of the contents of general components

The contents of ash, protein, fat, and sugars (fructose, glucose, sucrose, maltose, and lactose) in beef (lower 0.1%) were determined according to the national standard method (GB/T 22221–2008). The content of water in beef was 70.80 g/100 g, which was determined by the beef, and was heated to constant weight at 105°C, and then the weight of water in the beef was measured. The content of protein was 21.90 g/100 g determined by the Kjeldahl method (Townshend, 1987). Protein was the second-highest component in beef after water. The research showed that beef had high protein, low fat and rich in unsaturated fatty acids (Pereira & Vicente, 2013). The ash (1.00 g/100 g) was determined from the weight after heating at 550 °C for 4 h. The fat content (1.10 g/100 g) was analyzed by the Soxhlet extraction method using diethyl ether as the solvent. These were lower than (Song et al., 2019) reported, may be due to the different locations selected.

3.2 | The optimization of single factors

3.2.1 | The content of tastes in stir-fried beef of different stir-fried time

The contents of these amino acids are shown in Figure 1a. Among the 17 amino acids, Asp and Glu with umami taste (Liu, Liu, He, Song,
FIGURE 1  The contents of free amino acids in stir-fried beef at different conditions (a: stir-fried time, b: amount of blending oil, c: amount of salt, d: amount of sucrose, e: different formula)
& Chen, 2015), were detected in stir-fried beef. The total content of FAAs in stir-fried beef increased initially and decreased with the extension of stir-fried time. The content of bitter amino acids in stir-fried beef increased when processing time was too long, which affected the taste perception quality of stir-fried beef. This would be the precursor substance degraded to FAAs, the consumption of FAAs increased with the time. The total concentration of FAAs reached its maximum value (10,874.39 µg/g) when stir-fried time was 4.00 min. At the same time, the contents of the sweet amino acid were also reached to the maximum value (1,558.39 µg/g). Succinic was reported to be compounds with sour and umami taste (Park et al., 2001). In Figure 2a, the content of succinic decreased with the extension of stir-fried time. The maximum value was 1.02 µg/g when stir-fried time is at 3.50 min. It could be concluded that high-temperature and long-time heating lead to the degradation of succinic. The content of 5’-nucleotide in stir-fried beef with succinic decrease in Figure 2a. It showed that the contents of 5’-AMP, 5’-IMP and 5’-GMP maximum value of 0.03, 0.03 and 0.12 µg/g respectively at 4.00 min, then decreased to 0.01, 0.00, 0.03 µg/g with the increase of stir-fried time. It also indicated that high-temperature stir-frying may lead to the degradation of flavor 5’-nucleotide in beef (Shoji, Satoh-Kuriwada, & Sasano, 2016).

3.2.2 The content of tastes in stir-fried beef with different amount of blending oil addition

The content changes of amino acids are shown in Figure 1b. The total amount of FAAs in stir-fried beef increases initially and then decreased with the extension of oil addition. The maximum value is 10,891.36 µg/g when the oil content is 25.00 g, sweet amino acid and umami amino acid showed significant differences compared with other groups in the oil content. In Figure 2b, the content of succinic decreased with the extension of oil addition. The succinic maximum value was 2.65 µg/g when the blending oil content was 10.00 g. It was indicated that oil addition inhibited the dissolution and promoted the release of succinic. The content of 5’-nucleotide in stir-fried beef with the amount of oil addition is shown in Figure 2b. The content of 5’-IMP maximum value was 0.20 µg/g when the oil was 10.00 g. The maximum value of 5’-GMP decreased then increased with the extension of stir-fried time and reached the maximum value when adding 10.00 g oil.

3.2.3 The content of tastes in stir-fried beef about different salt addition

The content of amino acids is shown in Figure 1c, the total amount of umami amino acids in stir-fried beef showed an increasing trend. The content of umami amino acids in stir-fried beef without salt was 2,580.32 µg/g and the minimum value of umami amino acid was 2,727.00 µg/g after adding 1.25 g salt. It indicated that salt could promote the release of flavor in fried beef (Quelhas et al., 2010).

In Figure 2c, the content of succinic maximum value was 4.01 µg/g which were higher than that in beef without salt (0.62 µg/g). It indcated that NaCl indeed promoted the release of umami organic acid, produced more umami enhancer sodium which could improve the umami of dishes (Broadway, Behrends, & Schilling, 2011).

The main flavor component nucleotide 5’-GMP content did not change significantly with the increase in salt, and the content remained at 0.04 µg/g. The content of 5’-IMP increased with the increase in salt dosage. The maximum value of 5’-IMP was 0.80 µg/g when salt content was 2.50 g. The content of 5’-AMP increased initially and then decreased with the increase in salt dosage. The maximum value was 0.18 µg/g when salt content was 1.50 g. The contents of 5’-GMP, 5’-IMP and 5’-AMP in stir-fried beef with salt increased significantly. Without salt of stir-fried beef, the contents of these three organic acids were 0.03, 0.27, and 0.12 µg/g, respectively. It was confirmed that low concentration of NaCl could promote the release of non-volatile flavor components and other flavor components (Phat, Moon, & Lee, 2016). It showed that salt was beneficial to the release of flavor nucleotides.

3.2.4 The content of tastes in stir-fried beef with different sucrose addition

The contents of taste compounds in stir-fried beef with the amount of sucrose samples are listed in Figure 2d. The content of these amino acids is shown in Figure 1d. In Figure 1d, the umami amino acids aspartic acid and sweet amino acid in stir-fried beef increased with the increase in sucrose. The maximum content was 2,809.57 and 781.14 µg/g, respectively, when the sucrose addition was 2.25 g.

The content of succinic increased with the addition of sucrose. The maximum value was 4.01 µg/g when the sucrose addition was 2.25 g. In Figure 2d, the content of succinic was 0.62 µg/g when fried 4.00 min. It indicated that added sucrose in fried beef could promote the release and dissolution of umami organic acids (Cheled-Shoval, Reicher, Niv, & Uni, 2017).

In Figure 2d, the content of 5’-GMP in stir-fried beef with sucrose content had no significant difference with the increase of sucrose addition, and the content remained at 0.02 µg/g. The effect of 5’-GMP content in stir-fried beef with sucrose is less than that of salt. The content of 5’-GMP in stir-fried beef remained at 0.04 µg/g after adding salt. The content of 5’-IMP and 5’-AMP showed an increasing trend with the increase in sucrose addition, which would be Maillard reaction between amino acid and sugar in beef under high temperature and thus significantly reduced the flavor nucleotide content (Gurikar, Lakshmanan, Gadekar, Sharma, & Anjaneyulu, 2014).

3.2.5 The content of tastes in stir-fried beef with a different formula

The content of FAAs in stir-fried beef with different formulas is shown in Figure 1e. The content of glutamic acid and aspartic acid

& Chen, 2015)
in stir-fried beef between spice-added and non-spiced groups was very significant, which indicated that spices could promote the release of flavor compounds. In Figure 2e, the content of succinic acid in fried beef with spices and without spices was 5.38 and 1.24 µg/g, respectively. It indicated that spices could also enhance the release of flavor compound organic acids. Added spices in stir-fried beef also increased the amount of flavoring nucleotides. The contents of 5’-GMP, 5’-IMP and 5’-AMP in stir-fried beef were 0.07,
3.48, and 0.40 μg/g, respectively, which indicated that stir-fried beef was conducive to the release of flavor nucleotides. This was also consistent with the research of Xu, Zheng, Song, Gong, and Pan (2019).

3.3 Equivalent umami concentration

The EUC values in the fried beef of different formulations included fried oil, fried beef blank control, orthogonal test optimal group fried beef, fried spice package, and orthogonal test optimal group added spice package are shown in Figure 3. The EUC value of stir-frying spice (f-sp) was 8.31 g MSG/100 g, which indicated that stir-frying could increase the release of spice flavor substances. Compared with the beef without spices, the EUC value of fried beef with spices (f-sp-b) was 154.54 g MSG/100 g, while beef without spices (f-b-b) was 124.29 g MSG/100 g, it indicated that spices could improve the flavor of fried beef. Liu, Xu, and Zhou (2007) compared duck with and without spices. The free amino

![Figure 3](image)

**FIGURE 3** The EUC of the stir-fried beef with different formulas samples. (stir-fried oil (f-o); fried beef blank (f-b); fried beef normal group (f-b-b); fried spice group (f-sp); fried beef with spice package group (f-sp-b))

**TABLE 1** The TAV of tastes in stir-fried beef about different formula

| Compound   | Taste threshold(mg/L) | f-o  | f-b  | f-b-b | f-sp  | f-sp-b |
|------------|-----------------------|------|------|-------|-------|--------|
| Asp        | 300                   | ND   | 6.46 | 4.87  | 5.09  | 8.93   |
| Glu        | 50                    | ND   | 5.65 | 3.87  | 3.81  | 2.72   |
| Ser        | 1,500                 | ND   | 0.04 | 0.03  | 0.01  | 0.03   |
| Pro        | 3,000                 | ND   | 0.13 | 0.03  | 0.03  | 0.02   |
| Gly        | 1,300                 | ND   | 0.05 | 0.02  | 0.02  | 0.02   |
| Thr        | 2,600                 | ND   | 0.02 | 0.01  | 0.01  | 0.01   |
| Ala        | 600                   | ND   | 0.43 | 0.65  | 0.40  | 0.52   |
| Val        | 400                   | ND   | 0.07 | 0.16  | 0.25  | 0.58   |
| Met        | 300                   | ND   | 0.27 | 1.05  | 0.13  | 0.13   |
| Ile        | 900                   | ND   | 0.02 | 0.04  | 0.02  | 0.04   |
| Phe        | 900                   | ND   | 0.01 | 0.02  | 0.01  | 0.02   |
| Lys        | 500                   | ND   | 0.12 | 0.06  | 0.06  | 0.05   |
| Leu        | 1,900                 | ND   | 0.02 | 0.03  | 0.01  | 0.02   |
| Arg        | 500                   | ND   | 1.70 | 9.65  | 2.43  | 7.61   |
| His        | 200                   | ND   | 0.05 | 1.15  | 0.15  | 1.01   |
| Tyr        | ND                    | ND   | ND   | ND    | ND    | ND     |
| Oxalic acid| 504                   | ND   | ND   | ND    | ND    | ND     |
| Tartaric acid | 15                | ND   | 1.26 | 2.23  | 1.13  | 3.18   |
| Malic acid | 496                   | ND   | 0.00 | 0.01  | ND    | 0.01   |
| Lactic acid| 1,260                 | ND   | ND   | 0.01  | ND    | 0.01   |
| Citric acid| 450                   | ND   | ND   | 0.00  | 0.01  | 0.00   |
| Succinic acid| 106                | ND   | 0.01 | 0.01  | 0.01  | 0.05   |
| 5'-GMP     | 125                   | ND   | ND   | ND    | ND    | 0.00   |
| 5'-IMP     | 255                   | ND   | ND   | 0.01  | 0.00  | 0.01   |
| 5'-AMP     | 500                   | ND   | ND   | 0.00  | ND    | 0.00   |

Abbreviation: ND, not detected.
acetic acid content in Nanjing duck cooked with spices was higher. Furthermore, the desirable amino acids which possess umami (Asp and Glu) and sweet (Ala, Gly, and Ser) taste in Nanjing duck were significantly greater than the duck without spices, which suggested that spices could be a contributing factor of the delicious taste of Nanjing cooked duck.

### 3.4 | Taste activity value

The TAV in the fried beef of different formulations included fried oil, fried beef blank control, orthogonal test optimal group fried beef, fried spice package, and orthogonal test optimal group added spice package listed in Table 1. The TAV values of Asp and Glu, Arg and tartaric acid in stir-fried spices were all greater than 1, which contributed to their flavor. The TAV values of Asp, Glu, Pro, Ala, Arg, and tartaric acid in orthogonal test optimal group fried beef and spice-fried beef were all greater than 1, indicated that these taste compounds play a key role in the fried beef.

### 4 | CONCLUSIONS

In this work, the optimized cooking method of the stir-fried beef was conducted by instrument analysis. The results of the instrumental analysis indicated that the amount of salt and sucrose added in stir-fried beef could promote the release of flavoring substances. With the prolongation of the processing time, the content of taste substances increased initially and then decreased. The EUC value of stir-fried with spice package was 8.31 g MSG/100 g, which suggested that spices are helpful to enhance the flavor of dishes. It could be an effective method to optimize the cooking method using instrumental analysis to replace or partly replace sensory evaluation.

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### CONFLICT OF INTEREST

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

### ORCID

Yu Yu Zhang https://orcid.org/0000-0003-3095-3083

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