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Effect of brewing conditions on the chemical and sensory profiles of milk tea

Chen Yang [1], Chuanjian Cui [1], Yuanyuan Zhu, Xinyu Xia, Ge Jin, Cunjun Liu, Yeyun Li, Xiuheng Xue [*], Ruyan Hou [*]

State Key Laboratory of Tea Plant Biology and Utilization, School of Tea and Food Science & Technology, Key Lab of Food Nutrition and Safety, Anhui Agricultural University, Hefei 230036, China

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A B S T R A C T

The brewing conditions of beverage milk tea determine the taste of milk tea. This study investigated the changes in sensory characteristics and small molecule compounds in milk tea made from large-leaf yellow tea under different brewing conditions by sensory analysis, colorimeter, and LC-MS. The results show that the tea to milk ratio is the most important process affecting the taste, and the color values of b^* (+yellow, - blue) can be used to evaluate the taste of milk tea made from large leaf yellow tea. The composition of small molecular compounds is affected by tea to milk ratio, which can change the taste of milk tea. L-cysteine and 8-methylsulfinyloctyl glucosinolate are significantly positively correlated with taste by metabolomics analysis. L-cysteine was used to verify the analysis results by LC-MS. The total acceptance of milk tea is improved by adding L-cysteine at a low level (0.025–0.035 mM).

1. Introduction

Tea (*Camellia sinensis*) is a health beverage consumed worldwide (Zhang, Cao, Granato, Xu, & Ho, 2020; Bhardwaj et al., 2021; Bhardwaj et al., 2021). Milk tea, a beverage made from milk and tea infusion, has become increasingly popular among young people around the world (Mao et al., 2021). Milk tea was originally made from dark teas with milk by Tibetans, Uyghurs, and Mongolians in the frontier regions of China (Cao, Zhao, & Liu, 2000). As the demand for milk tea continues to rise (Wingnam, Chongxin, & Pengjun, 2021), more tea varieties are being used, such as green tea, oolong tea, and black tea (Bhagat et al., 2019; Choi & Lee, 2019).

Large-leaf yellow tea (LYT, also called Huang-Da-Cha) is a yellow tea made using coarse, old tea leaves and an intense heating step that is unique to LYT (Guo, Ho, Schwab, Song, & Wan, 2019). This heating procedure imparts a smooth, uniquely toasty and caramel flavor to LYT (Han et al., 2016). The lipid-lowering effects of LYT possess much more potent than green, black, dark, or white teas (Xu et al., 2018). Therefore, LYT may be a premium tea for making milk tea. However, there is no research on the use of LYT for making milk tea.

Generally, milk tea is manufactured by extracting crushed tea under an optimized temperature, time, and tea: water ratio, and then mixing the tea infusion with milk in proportion (Fu & Zhang, 2014). However, there is no objective standard to assist in judging the taste of milk tea products. Much of the knowledge for controlling taste was based on empirical work in the milk tea production. Therefore, milk tea products can vary in quality due to subtle changes in formulation or processing conditions (Fu et al., 2020).

It is difficult to judge taste preferences, but measuring the color and the type of taste-imparting compounds is an objective way to judge the taste of a beverage (Ndom, Elegbeleye, & Ademoroti, 2011; Zhang et al., 2020). Similarity, the tea infusions color can influence the color of milk tea (Mao et al., 2021). In addition, the combination of polyphenols in the tea infusion and milk proteins, which are the major components of milk, will affect the taste of milk tea (Bandyopadhyay, Ghosh, & Ghosh, 2012; Bhagat et al., 2019; Cutrim & Cortez, 2018). However, the relationships between color and taste of milk tea and between the small molecule compounds in milk tea and the taste have not been completely explored.

This study was firstly to compare the flavors of milk tea made with LYT and green, oolong, black, dark and flower tea milk tea. The next aims were to optimize the manufacturing process of LYT milk tea using response surface models and to then explain the relationships between

* Corresponding authors.
E-mail addresses: xuexiuheng@126.com (X. Xue), hry@ahau.edu.cn (R. Hou).
[1] Equally contributing author.

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Table 1
Levels of actual and coded factors used in Box-Behnken design RSM.

	Factors [a]			
	Tea: water (g/mL)	Time (min)	Temperature (°C)	Tea: milk(mL/mL)
Run	A	B	C	D
1	1:50 (0)	4 (-2)	80 (0)	1:1 (0)
2	1:40 (-1)	12 (-1)	70 (-1)	1:2 (-1)
3	1:50 (0)	20 (0)	80 (0)	1:4 (-2)
4	1:60 (1)	28 (1)	70 (-1)	2:1 (1)
5	1:50 (0)	20 (0)	80 (0)	1:1 (0)
6	1:40 (-1)	28 (1)	70 (-1)	1:2 (-1)
7	1:50 (0)	20 (0)	80 (0)	1:1 (0)
8	1:60 (1)	28 (1)	70 (-1)	1:2 (-1)
9	1:60 (1)	12 (-1)	70 (-1)	2:1 (1)
10	1:40 (-1)	12 (-1)	90 (1)	2:1 (1)
11	1:50 (0)	20 (0)	80 (0)	1:1 (0)
12	1:70 (2)	20 (0)	80 (0)	1:1 (0)
13	1:50 (0)	20 (0)	100 (2)	1:1 (0)
14	1:30 (-2)	20 (0)	80 (0)	1:1 (0)
15	1:50 (0)	20 (0)	60 (-2)	1:1 (0)
16	1:60 (1)	28 (1)	90 (1)	1:2 (-1)
17	1:50 (0)	20 (0)	80 (0)	4:1 (2)
18	1:60 (1)	12 (-1)	90 (1)	1:2 (-1)
19	1:40 (-1)	28 (1)	90 (1)	1:2 (-1)
20	1:60 (1)	12 (-1)	70 (-1)	1:2 (-1)
21	1:50 (0)	20 (0)	80 (0)	1:1 (0)
22	1:50 (0)	36 (2)	80 (0)	1:1 (0)
23	1:50 (0)	20 (0)	80 (0)	1:1 (0)
24	1:60 (1)	28 (1)	90 (1)	2:1 (1)
25	1:60 (1)	12 (-1)	90 (1)	2:1 (1)
26	1:40 (-1)	28 (1)	90 (1)	2:1 (1)
27	1:40 (-1)	12 (-1)	90 (1)	1:2 (-1)
28	1:40 (-1)	12 (-1)	70 (-1)	2:1 (1)
29	1:50 (0)	20 (0)	80 (0)	1:1 (0)
30	1:40 (-1)	28 (1)	70 (-1)	2:1 (1)

[a] The factor levels were shown as actual values (outside of the bracket), and coded values (in the bracket).

brewing conditions and the taste of milk tea and its color and small-molecule compounds, which were analyzed by non-targeted metabolomics. This research provides an objective method for evaluating the quality of milk tea products. It also can reveal the taste-related chemical marker compounds and be used to improving the taste of milk tea.

2. Materials and methods

2.1. Materials

The LYT samples were provided by Buoer Zhongxiu Tea Co., Ltd. (Huoshan City, Anhui, China). Details on the other tea samples are shown in Table S5. The whole milk was purchased from Yili Industrial Group Co., Ltd. (Hefei, China). L-cysteine was purchased from Sigma-Aldrich (Sigma-Aldrich, Missouri, USA). All reagents used for the experiments were chromatographically pure (Sigma-Aldrich, Missouri, USA). Water for sample preparation was purified using the Milli-Q water purification system.

2.2. Preparation of milk tea

In order to ensure the homogeneity of the test samples, the teas were ground in a grinding machine (Fan, Zhou, & Huang, 2022) sieved with a 20-mesh sieve. According to the conditions shown in Table 1, the tea powder was weighed into a beaker. Ultra-pure water at the specified temperature was measured into the beaker, and the beaker was placed into a constant-temperature water bath. After extraction, the tea infusion was filtered. The specified amount of milk was added, the tea infusion and the milk were mixed well by vortex shaker. The analysis was repeated three times for each sample.

2.3. Different tea materials for making milk tea

Fourteen raw tea materials, which are commonly used in the market, were screened for making milk tea. The brewing conditions were the same for the different teas: 80 °C, 20 min at temperature, 1:50 g/mL (tea: water ratio), and 1:1 mL/mL (tea: milk ratio). A panel subjectively rated the taste of the milk teas, using a fuzzy mathematical sensory evaluation (Vivek, Subbarao, Routray, Kamini, & Dash, 2020) with the linguistic entities "not satisfactory, medium, good and excellent" to describe the sensory attributes of the milk teas (including aroma, color, taste, total acceptance) (Livio & Hodhod, 2018).

2.4. Response surface test design and analysis

Design Expert 13.0 software (State-Ease, Minneapolis, MN, USA) was applied for the design of experiments, analysis of statistics, and generation of models. Box-Behnken Design (BBD) was used to evaluate the effects of milk tea brewing procedures on the taste profiles and optimize the brewing process parameters of LYT milk tea in this study. The independent variables, including tea: water ratio (X1, g/mL), time (X2, min), temperature (X3, °C), and tea: milk ratio (X4, mL/mL); each variable was set at five levels (Table 1). These ranges of factors were based on preliminary experimental results. The dependent variables were the taste profiles of LYT milk tea (bitterness, sweetness, sourness, astringency, total acceptance). Given that the experiments were repeated, this study used the average values for analysis (Xu et al., 2017).

2.5. Milk tea color determination

The color of milk tea samples was determined in a rectangular transparent unit (dimensions: 50 × 38 mm; optical path: 10 mm) and the colorimeter was used to measure. The values of L^*, a^*, b^* were used for color measurements: L^* values indicated a change in luminosity between black and white (+white, -black); a^* values indicated the difference between the color of red and green (+red, -green); b^* color values indicated the difference between yellow and blue (+yellow, -blue). The color measurement of each sample was repeated 3 times.

2.6. Quantitative descriptive analysis (QDA)

The quantitative description analysis (QDA) of LYT milk tea was performed by ten trained panelists (six females and four males with a normal sense of taste and no smoking habits). Before conducting QDA, the taste attributes of LYT milk tea samples were approved by all panelists through three drinking tests. The taste attributes and total acceptance of milk tea samples were evaluated on a scale of 10-point ('extremely strong' was 8–10, 'strong' was 6–8, 'neutral' was 4–6, 'weak' was 2–4 and 'extremely weak' was 0–2) in accordance with (Xu et al., 2017). The color and taste of milk tea were used to evaluate the total acceptance of samples. Each milk tea infusion was provided at 30 ± 2 °C and assessed 3 times.

2.7. Non-targeted metabolomics of milk tea

2.7.1. Milk tea sample preparation

The milk tea samples were prepared similarly to the sensory evaluation described in 2.2 (Wang, Peng, Bai, Wan, & Li, 2013). Milk tea sample (10 mL) was added with 15 mL of 0.1 % acetic acid ethanol reagent and extracted under ultrasonic wave (200w) for 10 min. The samples were placed in a centrifuge tube to centrifuge at 2500 xg for 5 min. The extracts filtered through 0.22-μm Millipore filters were subsequently injected into LC-MS for metabolomic analysis. Each milk tea sample was independently extracted three times.

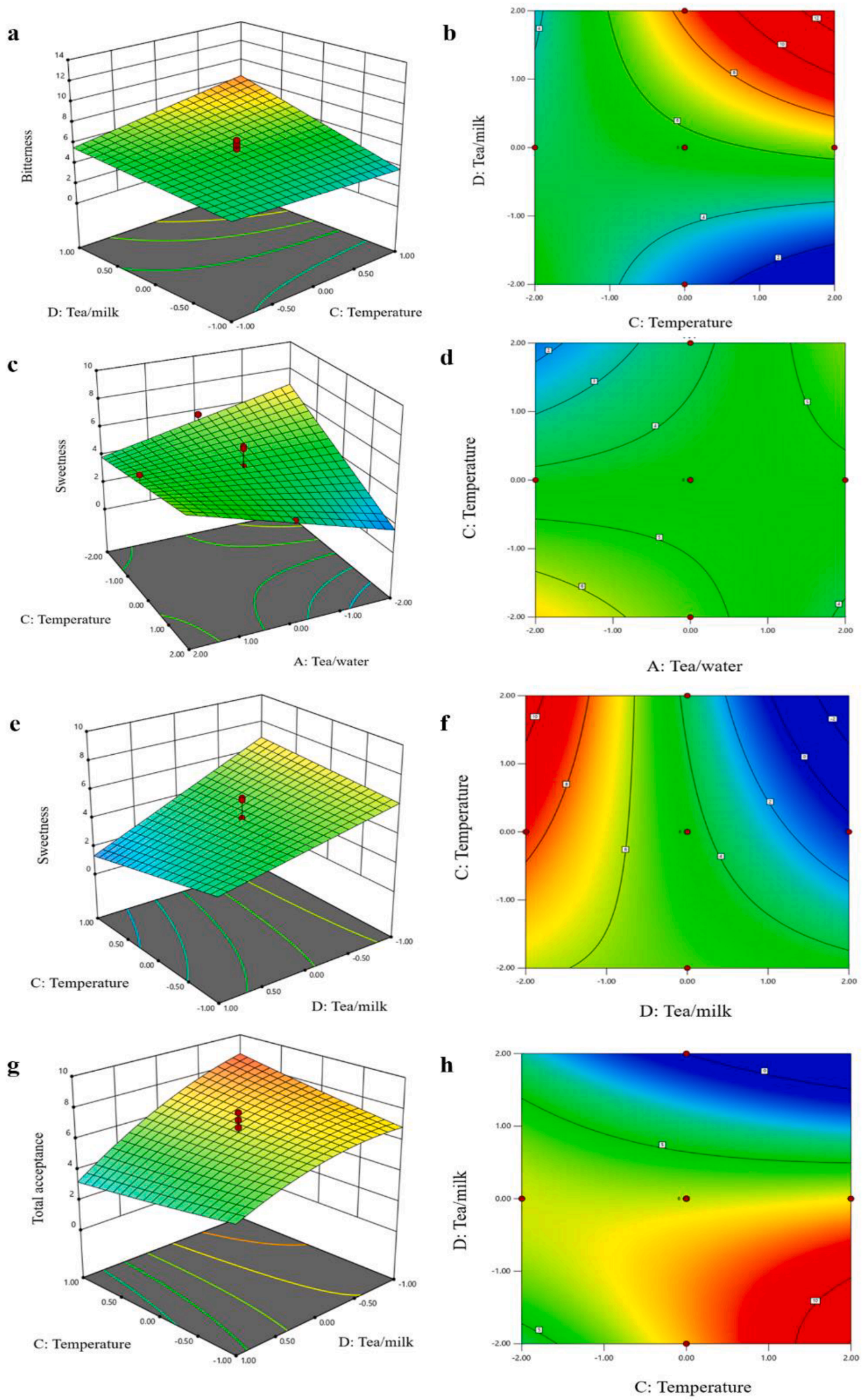

Fig. 1. Surface and contour plots for the effect of different variables on taste profiles with significant ($p < 0.05$). Effects of (a)(b) temperature and tea: milk ratio on bitterness, (c)(d) temperature and tea: water ratio on sweetness, (e)(f) temperature and tea: milk ratio on sweetness, (g)(h) temperature and tea: milk ratio on total acceptance.

2.7.2. LC/Q-TOF-MS analysis

The LC/Q-TOF MS (Agilent, CA, USA) was used to analysis milk tea samples. Separation of milk tea was extracted by ZOBAX SB-C18 column (2.1 × 100 mm, 1.8 μm) at a flow rate of 0.3 mL/min. The injection volume is 2 μL. 0.2 % acetic acid in water (v/v, A) and acetonitrile (B) were the mobile phases. The procedure of elution was: 0–4 min, 95–85 % A; 4–8 min, 85–73 % A; 8–13 min, 73–12 % A; 13–17 min, 12–5 % A; 17–18 min, 5 % A; 18–20 min, 5–95 % A; 20–22 min, 95 % A. The gas temperature and flow were 320 °C and 8 L/min, the sheath gas temperature and flow were 350 °C and 11 L/min, and the nebulizer was set to 35 psig. Full scan mass at *m/z* 100–1500 analysis was used to detect small molecule compounds.

2.7.3. Untargeted metabolomics data processing

MS-Convert converts LC-MS raw data format to mzML, and MS-DIAL (version 4) is used on converted data to filter noise, identify peaks,

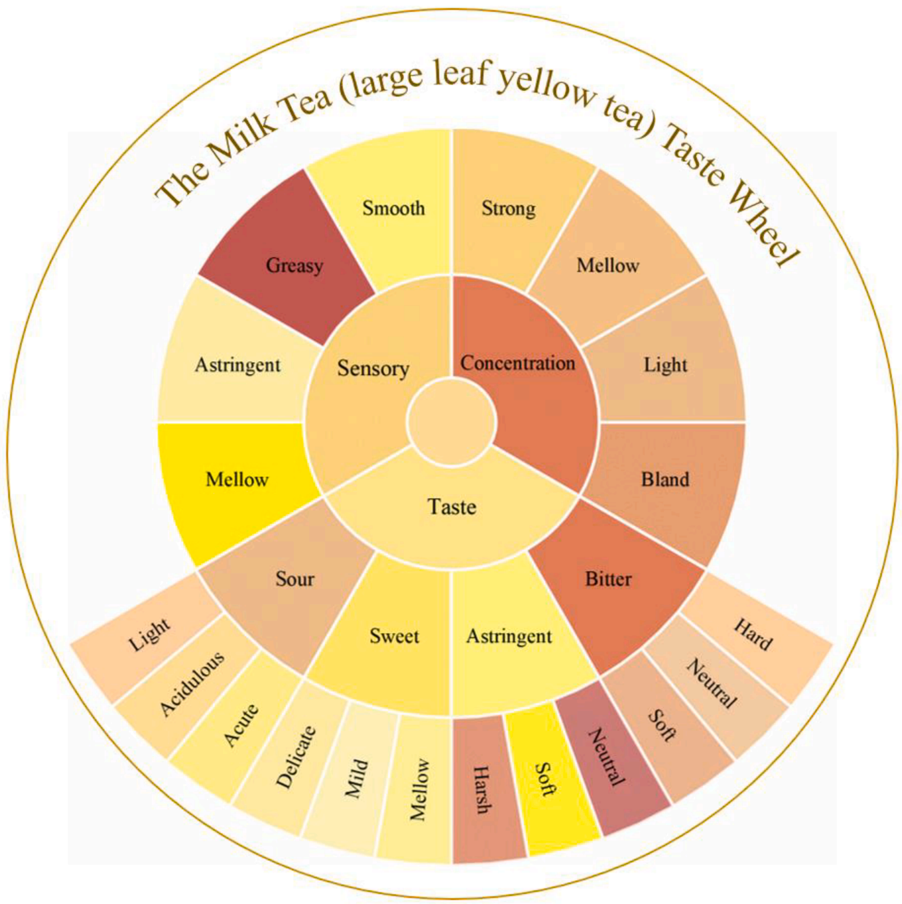

Fig. 2. Taste wheel of milk tea (Large-leaf yellow tea). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

analyze overlapping peaks, align peaks, and fill peaks (Zhou et al., 2018). Principal component analysis (PCA), hierarchical cluster analysis (HCA), and orthogonal partial least squares discriminant analysis (OPLS-DA) were performed after normalizing the data using the metabolome package in R language package 3.6.3 to outline changes in milk tea metabolite patterns under different formulations and to identify differential metabolites. Correlation analysis of differential metabolites with sensory scores was performed using Pearson correlation coefficient.

2.8. Taste compounds addition experiment

Samples were made using the same brewing conditions at 0 levels: 80 °C, 20 min, 1:50 g/mL (tea: water ratio), and 1:1 mL/mL (tea: milk ratio). Five concentrations of L-cysteine (0.025, 0.030, 0.035, 0.040, and 0.045 mM) were added according to the concentration in milk (Hofmann, 1999; Wang et al., 2018). The standards were dissolved in 50 mL tea infusion in a conical flask and using tea infusion dilute to the required concentration. According to this procedure prepare two copies, one for tea infusion taste identification and one for milk tea taste identification by mixing milk in proportion. The same 10 panelists assessed the samples using the same evaluation methods. Each tea infusion and milk tea were provided at 30 ± 2 °C and assessed in triplicate.

2.9. Data analysis and statistics

All samples were replicated 3 times. Data were expressed as mean ± standard deviation (SD). Pearson correlation analysis was used to describe the correlation between sensory profiles and small molecule metabolites in milk tea. All analyses were performed using Sigmaplot (version 12.5) and Origint (version 2019) statistical software.

3. Results and discussion

3.1. Choice of raw tea material for making milk tea

According to the panelists using the fuzzy mathematical sensory evaluation (Table S1.3), the overall ratings of milk tea made from 14 kinds of teas were distributed between “good” and “not satisfactory”. The results showed that the milk tea made from LYT got the highest score of 88.30, due to its unique burnt flavor. The taste of LYT milk tea was novel and mellower compared with the other 13 tea raw materials. Therefore, LYT (large leaf yellow tea) was selected as the material for making milk tea.

3.2. Response surface methodology (RSM) modeling of making milk tea using large-leaf yellow tea

To further investigate the relationship between independent variables (temperature, time, tea: water ratio, and tea: milk ratio) and dependent variables (LYT milk tea taste profiles), Box-Behnken Design (BBD) models were established through the Design-expert software. The results of ANOVA for the models showed all the *p*-values were lower than 0.0001, the R^2 values were greater than 0.9000 and the adjusted R^2 values were higher than 0.8000 (Table S2). These suggested that the models were reliable and the second-order polynomial equation can satisfactorily explain most of the experimental data (He et al., 2021). The range of lack-of-fit value was 0.0682 to 0.7100 which indicates the

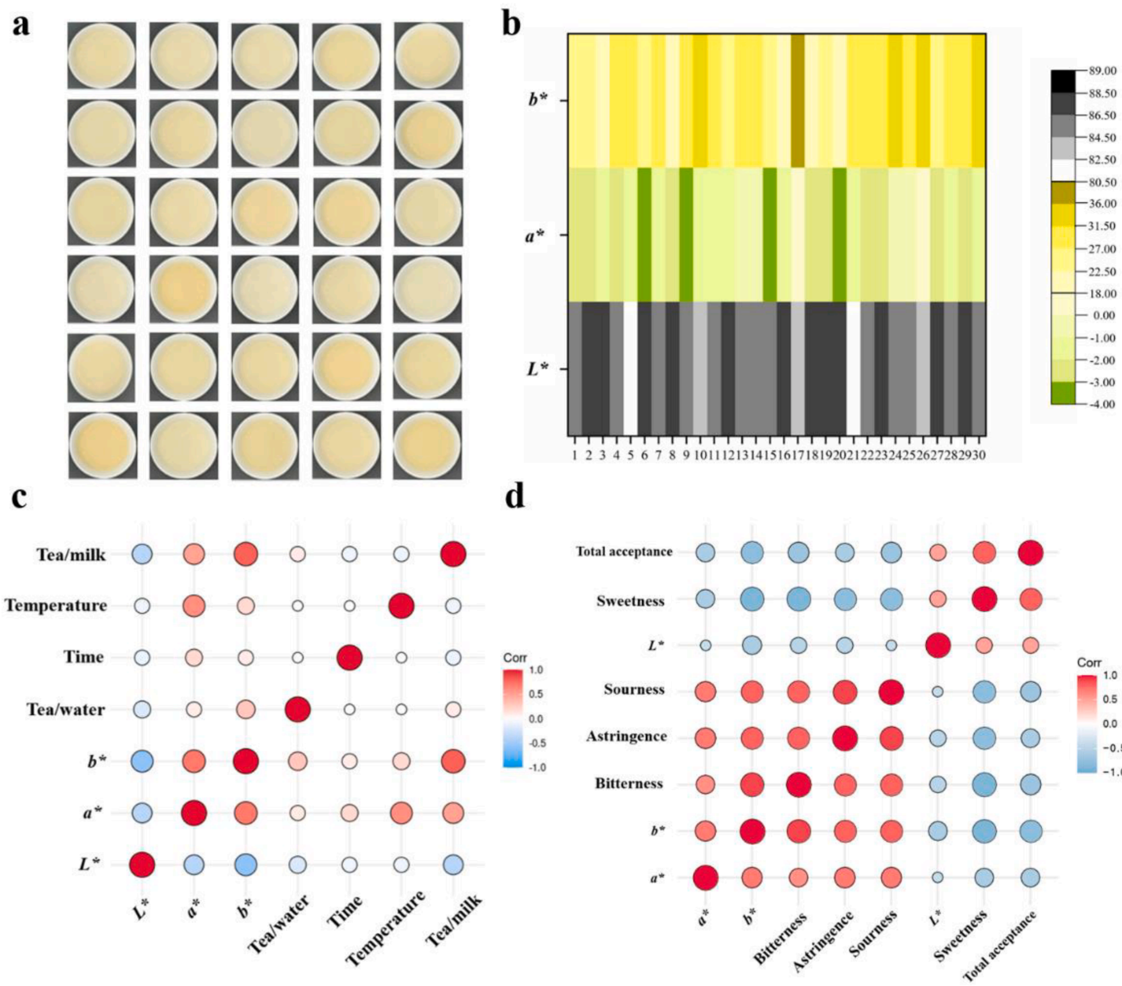

Fig. 3. Effect of different production conditions to the color of milk tea. (a) Images of 30 milk tea samples (from top left Sample 1) down the columns to the bottom right Sample 30). (b) Color of 30 milk tea samples made under different conditions and with different recipes. (c) Effect of different parameters during processing on the color of milk teas. (d) Correlation between taste and color of milk tea.

models have no significant error with the pure error. Therefore, it could be concluded that these models could be suitable for predicting the taste profiles of LYT milk tea (Fig. 1).

Surface and contour plotting of the data showed that the bitterness increased with brewing temperature and with increasing the tea in proportion to the milk (the tea: milk ratio) (Fig. 1a, b). Increased temperature and increased tea in the mix might increase components correlated to bitterness, such as the alkaloids, catechins, anthocyanins, phenolic acids, flavonol glycosides, and theaflavins (Jin et al., 2019; Ye et al., 2022; Zhang et al., 2017). Sweetness tended to be the opposite of bitterness, decreasing considerably when the temperature and tea: milk ratio increased (Fig. 1c, d). Similarly, when the tea: water ratio and temperature increased, the sweetness showed a significant decrease (Fig. 1e, f). This might be due to sweetness being masked by bitterness when bitterness increases at high temperature, high tea: water ratio, or high tea: milk ratio (Xu et al., 2017). When the tea: water ratio was low, the sweetness was enhanced as the temperature for brewing the tea infusion increased. This correlates with another study that showed that when the concentration of tea infusion in milk tea was relatively low, an increase in temperature enhances the sweetness of milk (Francis et al., 2005). The total acceptance increased as the both temperature during brewing and the tea: milk ratio increased (Fig. 1g, h). In brief, milk tea gained a higher consumer acceptance with a balance of bitterness and sweetness, which would form a mellow taste.

Design-expert 13.0 software was used to optimize the brewing conditions by maximizing total acceptance. The optimized results showed that brewing conditions were 89.8 °C, 27.04 min, 1:39.2 g/mL tea: water ratio, and 1:2 mL/mL tea: milk ratio.

The verification tests were prepared under optimized conditions of prediction by RSM. The actual score of LYT milk tea total acceptance was 9.13 ± 0.25. There was no significant difference with the predicted score of milk tea (9.00) at the 95 % confidence level by paired Student's *t*-test analyzed (Table S3). As mentioned above, this optimized protocol could be tested for the industrial production of milk tea (Yu, Liu, Zhang, Luo, & Zeng, 2021).

3.3. Taste wheel during processing of LYT milk tea

During production, the taste of milk tea changed, with each modeled process exhibiting distinct taste characteristics. For instance, as the tea concentration decreased (decreasing ratio of tea: milk), the flavor went from Strong, to mellow, to light to bland. The sensory profile of milk tea included smooth, greasy, astringent and mellow. The taste of milk tea was more complex, with four main tastes, namely bitter, sour, sweet and astringent. The different recipes changed the taste of milk tea (Deng et al., 2021). When the tea and milk were blended in an optimum ratio, the taste of milk tea presented as mellow. We constructed the taste wheel of LYT milk tea (Fig. 2). This taste wheel could be used as a reference to evaluate the taste of LYT milk tea.

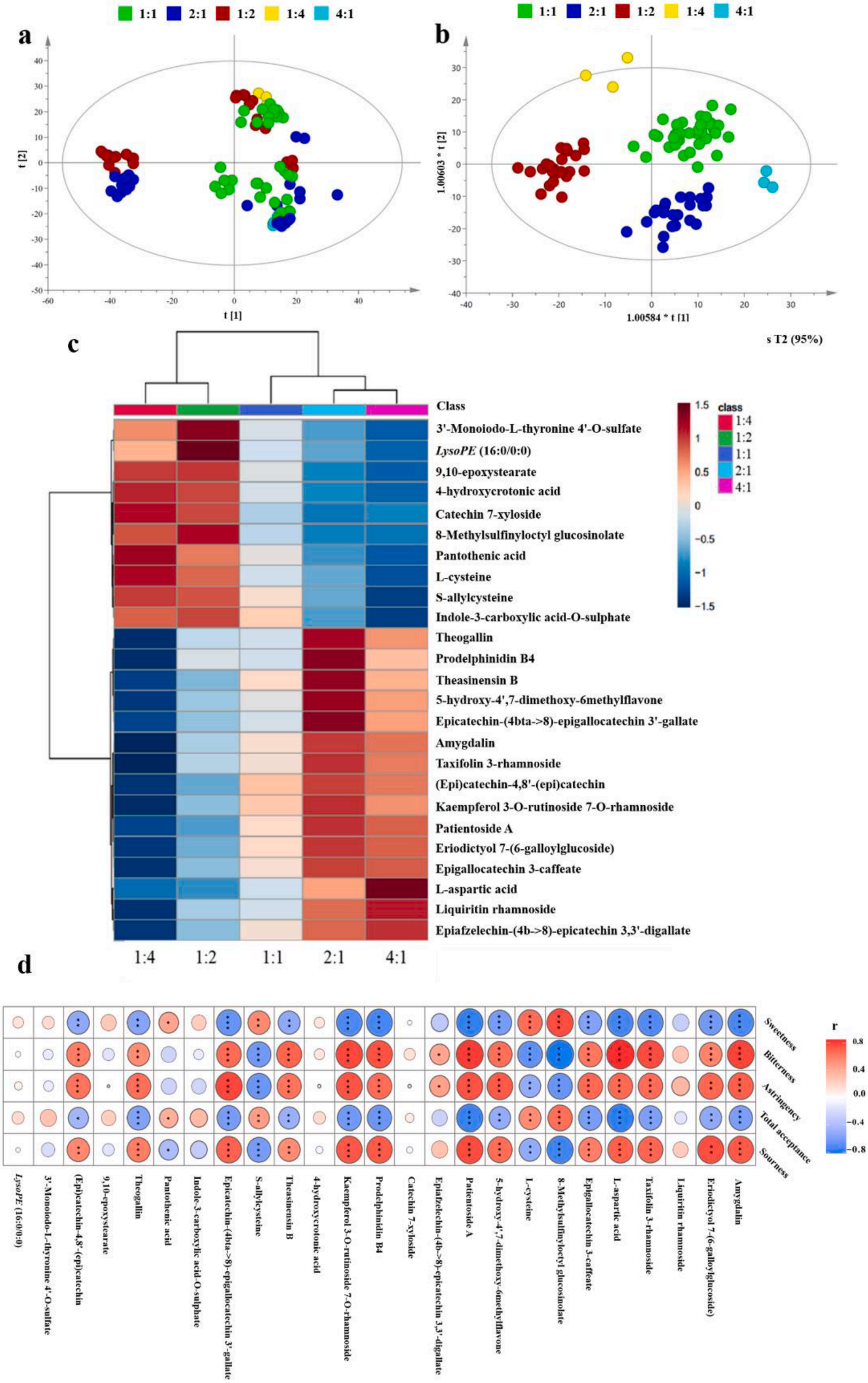

Fig. 4. Multivariate statistical analysis of milk tea mixed with different tea: milk ratios; (a): Principal component analysis score chart (PCA); (b): Orthogonal partial least squares discriminant analysis (OPLS-DA); (c): Heat map and hierarchical clustering of the levels of non-volatile compound in milk tea mixed with different tea: milk ratios; (d): Pearson correlation analysis of chemical composition of major differential metabolites and sensory perception of taste in LYT milk tea.

3.4. Color of LYT milk tea with production variations

The color of the milk tea samples was dark yellow, yellow, light yellow and ivory (Fig. 3a), similar to the previously reported (Mao et al., 2021). Using the chromatic parameters of L^*, a^*, b^* to represent the color of milk tea samples in this study. The ranges of L^*, a^*, b^* were 88.33 ~ 81.00, 0 ~ −3.67, and 35.33 ~ 18.33, respectively (Fig. 3b).

The b^* values that indicated a light-yellow color of the milk tea ranged from 26.33 to 28.33. Milk tea samples with a pleasant light-yellow color included samples 1, 5, 7, 9, 11, 12, 21, 22, 23, 25 and 29 (Fig. 3a). The b^* values that indicated a more yellow color were in the range 29.33 to 31.33. such as the color values for samples 4, 13, 14 and 28. Samples with b^* values <26.33 had a more ivory (yellowish white) color, such as the samples 2, 3, 6, 8, 12, 15, 16, 18, 19, 20 and 27.

The relationship between the color and the recipes of milk tea samples was significant (Fig. 3c). The chromatic parameter b^* (r = 0.768, $p < 0.01$) was significantly correlated with the tea: milk ratio. The chromatic parameter a^* (r = 0.634, $p < 0.01$) was significantly correlated with the temperature. The results proved that when the brewing temperature and the tea to milk ratio increased, the color of the milk tea became darker and the a^*, b^* value increased. Consistent with our results, Mao et al. (Mao et al., 2021) found that the redness of milk tea prepared from black tea was mainly determined by the redness of the black tea infusion. Therefore, the analysis of the color of LYT milk tea showed that the brewing process changed the color of LYT milk tea by changing the color of the tea infusion.

3.5. Correlation between LYT milk tea color and the taste

The relationship between the color and taste of milk tea was subjected to correlation analysis (Fig. 3d). The L^* value ($p < 0.01$) of milk tea had a negative correlation with the bitterness (r = -0.543) and astringency (r = −0.484) of milk tea and a positive correlation with the sweetness (r = 0.516) and total acceptance (r = 0.537). The a^* values ($p < 0.01$) of milk tea had positive correlations with the sweetness (r = −0.625) and total acceptance (r = −0.568) and negative correlations with the bitterness (r = 0.639) and astringency (r = 0.688) (Xu et al., 2017). The b^* values ($p < 0.01$) showed the most significant positive correlation with milk tea bitterness (r = 0.856) and astringency (r = 0.838), and the most significant negative correlations with sweetness (r = −0.858) and total acceptance (r = −0.77). The b^* values of 20–25 showed a high level of overall acceptance (>7.15). The results show that the b^* value can be used as a taste monitoring method when milk tea made from LYT.

3.6. Chemical constituents in LYT milk tea

3.6.1. Metabolomics analysis of LYT milk tea

According to the sensory evaluation, the tea: milk ratio was the principal factor affecting the taste of milk tea. In order to screen for differential substances that were characteristic of milk tea made using different recipes, non-targeted metabolomics analysis by LC-MS was used to detect the changes in the content of small-molecule compounds in milk tea samples with different tea: milk ratios.

PCA analysis was performed on the data matrices of the milk tea samples with different tea: milk ratios (Fig. 4a). The first two major components explained 26.2 % and 14.4 % of the variation. The clusters of 1:2 (red circles) and 2:1 (blue) tea: milk ratios were relatively discrete, indicating that different temperatures, tea concentrations, and brewing times had significant effects on the compounds in milk tea between the same groups.

OPLS-DA was used to classify-five different tea: milk ratios. The OPLS-DA effectively separated the 1:4 tea: milk ratio samples from tea: milk each other and from the 1:1, 1:2 and 1:4 tea: milk ratio clusters (Fig. 4b). These results also demonstrated that the tea to milk ratio might be a key factor in the differentiation of the chemical composition in the milk tea manufacturing process. Next, VIP values were used to screen the main differential compounds between milk teas with different tea: milk ratios. The cross-validation with 200 permutation tests indicated that this OPLS-DA model was reliable (intercepts of $R^2 = 0.399$ and $Q^2 = -0.419$, respectively). Ultimately, 25 characteristic metabolites with VIP values greater than 1 were identified (Table S6).

3.6.2. Heatmap and hierarchical clustering analysis of major differential compounds in LYT milk tea

In order to determine the ability of OPLS-DA to classify milk tea with different tea: milk ratios and to find chemicals to use as process markers, heat mapping and hierarchical clustering were used to analyze the major compounds in milk tea. In the heat map, each row was an OPLS-DA filter of compounds ranked as important, and each column was the average of the category groups (tea: milk ratio) ranked by the cluster analysis. Cluster analysis using Euclidean distance produces a heatmap with trees and key description values as shown in Fig. 4c. According to the tea: milk ratio, the grouping of the different recipes presented two different branches, which was similar with the result of the PCA. The first branch contained low ratios of tea: milk (1:4 and 1:2). The second branch contained tea: milk ratios of 1:1, 2:1 and 4:1, with 2:1 and 4:1 being closer in the same branch. In the heat map, the red boxes indicated compounds with levels above their average, while the blue boxes indicated compounds with levels below their average.

As shown in the Fig. 4c, the main different compounds in milk tea with different tea: milk ratios were amino acids, flavonoids, glycosides, and phenolic acids. L-cysteine is an amino acid contained in milk but not reported in tea (Sahu, Sharma, Kant, Shrivas, & Ghosh, 2021), and its level decreased with increasing tea: milk ratio in LYT milk tea. L-aspartic acid was one of the major free amino acids in tea and was considered to be an important contributor to the freshness of tea infusion (Yang, Baldermann, & Watanabe, 2013), and its level increased with an increasing tea: milk ratio in milk tea. Theogallin, prodelphinidinB4, theasinensin B, 5-hydroxy-4′,7-dimethoxy-6-methylfavone, epocatechin-(4–8)-epigallocatechin 3′-gallate, (*epi*)catechin-4,8′-(*epi*)catechin and epoafzelechin-(4–8)-epicatechin 3,3′-digallate are polyphenols reported in tea, mainly as monomeric phenolic acids and dimers or tannin dimers and multimers (Li, Zhang, Wan, Zhan, & Ho, 2022). These polyphenolic contents increased with an increase in the tea: milk ratio. Most of the glycosides increased with increasing tea: milk ratio, including kaempferol 3-O-rutinoside 7-O-rhamnoside, amygdalin and taxifolin 3-rhamnoside (Zhang et al., 2020). The 8-methylsulfinyloctyl glucosinolate was probably derived from methionine in milk (Bennett, Mellon, & Kroon, 2004). The phenolic glycoside 3′-monoiodo-l-thyronine 4′-O-sulfate has been rarely reported.

3.6.3. Correlation analysis between milk tea taste and its chemical composition

The correlation between milk tea taste and its chemical composition (detected by metabolomics) was subjected to Pearson correlation analysis using the sensory evaluation results and the main differential compounds (Fig. 4d). There were 10 compounds positively correlated with total acceptance, while 15 compounds were negatively correlated.

L-cysteine and 8-methylsulfinyloctyl glucosinolate showed significant negative correlations with the intensity of bitterness, astringency and sourness, and positive correlations with the intensity of sweetness. L-cysteine can conceal bitterness (Hofmann, 1999), while 8-methylsulfinyloctyl glucosinolate, as a derivative of methionine, has not been reported for flavor. These correlations implied that L-cysteine and 8-methylsulfinyloctyl glucosinolate might serve as potential flavoring substances to reduce bitterness and increase total acceptance.

Most of the polyphenols showed significant positive correlations with bitterness, astringency and sourness, and significant negative correlations with sweetness. This is due to the strong bitterness and astringency of phenolic substances. For example, prodelphinidin B4 is a catechin polymer that usually presents a strong astringent taste (Rinaldi,

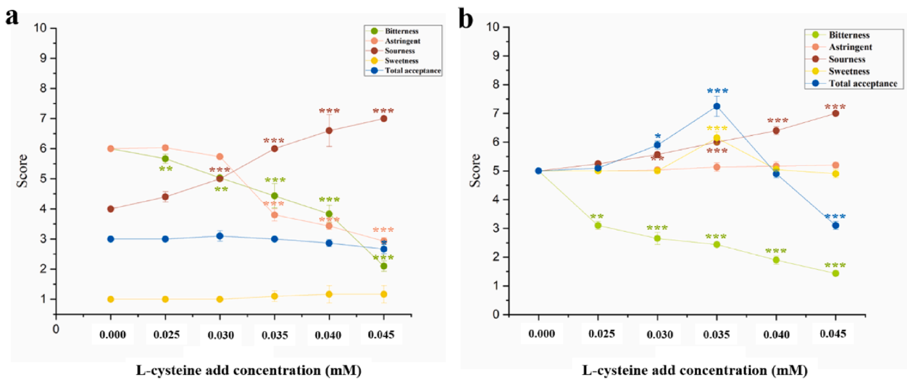

Fig. 5. Taste profile evaluations of samples with additional L-cysteine. (a) LYT tea samples; (b) LYT tea milk tea samples. Points with * are significantly different ($P < 0.05$), ** are extremely different ($P < 0.01$), and without * are not significantly different ($P < 0.05$).

Jourdes, Teissedre, & Moio, 2014). Kaempferol 3-O-rutinoside, 7-O-rhamnoside, and patientoside A are glycosides that usually present a bitter taste and also show potential as bitterness markers in milk tea (Deng et al., 2022). Surprisingly, L-aspartic acid, which contributes freshness in tea, showed a significant positive correlation with bitterness, astringency, and sourness in milk tea. It might be that L-aspartic acid can bind with protein in milk during brewing, resulting in a change in taste presentation. For total acceptance, L-cysteine and 8-methylsulfinyloctyl glucosinolate showed significant positive correlations. This was consistent with the result of the sensory evaluation, which showed that an appropriate decrease in bitterness would increase the total acceptance of the milk tea.

3.7. The effect of L-cysteine on the taste of LYT milk tea

According to the correlation analysis and previous reports, L-cysteine was selected to conduct addition experiments (Chen, Zhou, Yu, Yuan, & Tian, 2021; Xu et al., 2017). The addition of extra taste compounds affected the taste perceptions of both LYT tea and LYT milk tea (Fig. 5). Added L-cysteine had a continuous and significant masking effect on the bitterness in tea and milk tea (Fig. 5). It is probable that L-cysteine can mask the taste presented by bitter components in the tea infusion such as EGCG (Chen, Zhang, Mujumdar, & Liu, 2022). Sourness increased with the addition of L-cysteine, possibly because of the taste of L-cysteine itself. Interestingly, the addition of L-cysteine at 0.35 mM got higher grades on sweetness and total acceptance in LYT milk tea (Fig. 5b). But the total acceptance decreases after the third addition level (0.40 mM L-cysteine). The reason for this might be that the taste of the milk tea lacks balance, causing the scores of sweetness and total acceptance to decline.

4. Conclusion

In summary, large-leaf yellow tea (LYT) was a suitable tea for the making of milk tea and imparting a unique flavor to the final beverage. The tea: milk ratio had the strongest impact on the LYT milk tea (phytochemicals, taste and color). The optimization of the brewing protocol and final mixture were performed through RSM, resulting in an optimal brewing condition of 89.8 °C for 27.04 min at a 1:39.2 g/mL (tea: water ratio) and an optimal mixture of a 1:2 mL/mL (tea: milk ratio). This optimized protocol resulted in the highest total acceptance score for the LYT milk tea. Color analysis was shown to be an effective way to judge the taste of milk tea for industrial production. And metabolomics analysis showed that 25 compounds were related to the milk tea taste. L-cysteine and 8-methylsulfinyloctyl glucosinolate were significantly positively correlated with milk tea taste by correlation analysis. A relatively low-level concentration (0.025 mM–0.035 mM) in L-cysteine to the LYT milk tea, demonstrated that L-cysteine could reduce bitterness and improve milk tea's total acceptance. This study provides numerous reference datapoints and methodologies for further optimizing the industrial production of milk tea beverages.

CRediT authorship contribution statement

Chen Yang: Methodology, Validation, Investigation, Formal analysis, Data curation, Writing – original draft. **Chuanjian Cui:** Writing – review & editing. **Yuanyuan Zhu:** Methodology. **Xinyu Xia:** Methodology. **Ge Jin:** Methodology. **Cunjun Liu:** Methodology. **Yeyun Li:** Writing – review & editing. **Xiuheng Xue:** Writing – review & editing. **Ruyan Hou:** Methodology, Validation, Conceptualization, Formal analysis, Writing – review & editing, Supervision, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The authors are unable or have chosen not to specify which data has been used.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2022.100453.

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