Development of Lemon-Flavored Solid Instant Tea

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Abstract [**Objectives**] To investigate the optimal preparation process for lemon-flavored solid instant tea. [**Methods**] The formulation process was optimized through a single-factor combined orthogonal test, utilizing large-leaf Pu'er tea as the primary raw material and perfume lemon as the auxiliary ingredient. Sensory scores and the effectiveness of the spray drying process served as the key indicators for this investigation. [**Results**] The optimal formulation for lemon-flavored solid instant tea was established as follows: 15 g of Pu'er tea, 25 g of perfume lemon, a solid-liquid ratio of 1∶20 (g/mL), an extraction temperature of 90 ℃, an extraction duration of 30 min, and air inlet and outlet temperatures of 200 ℃ and 80 ℃, respectively. This formulation produced the most favorable flavor profile for the perfume lemon-flavored solid instant tea, which was characterized by a stable texture and a sensory evaluation score of 91.97 points. [**Conclusions**] This study has the potential to enhance the development and utilization of large-leaf Pu'er tea and perfume lemon resources.

Key words Large-leaf Pu'er tea, Instant tea, Spray drying, Perfume lemon

1 Introduction

Large-leaf Pu'er tea is produced from the fresh leaves of *Camellia sinensis* var. *assamica* in Yunnan, which undergo a series of processes including fixation, rolling, and sun-drying to create sun-dried green tea. This tea is characterized by a unique processing technology that imparts specific quality attributes[1]. It contains various beneficial compounds for human health, including tea polyphenols, tea polysaccharides, theanine, alkaloids, and other biologically active substances[2]. A substantial body of research has shown that Pu'er tea offers a variety of health benefits, including the alleviation of sensations of fullness and greasiness, stimulation of saliva production and quenching of thirst, reduction of fatigue, support for weight loss and fat reduction, as well as the demonstration of anti-aging, anti-mutagenic, antibacterial, antiviral properties, *etc.*[3]. Currently, Pu'er tea is predominantly available in the market in its traditional pure leaf form, with a notable scarcity of extensively processed products[4]. In recent years, perfume lemon has emerged as a staple fruit for beverages, distinguished by its robust and enduring scent, as well as the complete absence of the sour flavor characteristic of other lemon varieties[5]. Perfume lemons possess significant antioxidant properties attributed to their high vitamin C content[6]. The tea blend was prepared using large-leaf Pu'er tea and perfume lemon, both of which were sourced from Yunnan Province, due to their rich nutritional ingredients and health benefits. The objective is to mitigate the bitterness and astringency of large-leaf Pu'er tea by incorporating the sweetness of perfume lemon, thereby creating a lemon-flavored solid instant tea that possesses a distinctive flavor profile and high nutritional value. Additionally, this work aims to promote the development and utilization of large-leaf Pu'er tea and perfume lemon resources.

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2 Materials and methods

2.1 Materials Large-leaf Pu'er tea was sourced from Kunming Biological Manufacturing Research Institute Co., Ltd.; perfume lemon was obtained from Weishan Jufeng Agricultural Company; sodium hydroxide was provided by Tianjin Beifang Tianji Chemical Reagent Factory; alkaline cupric tartrate liquid A and B was supplied by Beijing Huakesheng Fine Chemical Factory Products Trading Co., Ltd.; hypomethyl blue was acquired from Tianjin Zhiyuan Chemical Reagent Co., Ltd.; and hydrochloric acid was sourced from Chongqing Chuandong Chemical (Group) Co., Ltd. All reagents were of analytical grade purity.

2.2 Equipment and instruments This study utilized a variety of equipment and instruments, including the L720R-3 centrifuge (Zhejiang Saide Instrument & Equipment Co., Ltd.), the SP-1500 spray dryer (Shanghai Shunyi Experimental Equipment Co., Ltd.), the WT2002 electronic balance (Hangzhou Wante Weighing Co., Ltd.), the GMSX-280 vertical autoclave sterilizer (Beijing Yongguangming Medical Instrument Co., Ltd.), the DHG-9140A electrothermal constant temperature blast dryer, and the LRH-150F biochemical incubator.

2.3 Test methods

2.3.1 Process flow. The process flow was delineated as follows: the combination of perfume lemon and large-leaf Pu'er tea→extraction→filtration→centrifugation→spray drying→filling→final product.

2.3.2 Operation points. Large-leaf Pu'er tea and perfume lemon were combined in a mass ratio of 3∶5. The mixture was subjected to extraction by the addition of water, followed by blending, and the soluble solids content of the resulting mixture was measured. The extraction process was conducted using a solid-liquid ratio of 1∶20 (g/mL), at an extraction temperature of 90 ℃, and for a duration of 30 min. This was followed by centrifugation at 4 000 r/min

for 20 min. The extracts were subjected to spray drying at inlet and outlet temperatures of 200 ℃ and 80 ℃, respectively, with a feed rate of 40 mL/h.

2.3.3 Single-factor test. The study examined the effects of various factors on the quality of lemon-flavored solid instant tea. Specifically, the variables investigated included amount of Pu'er tea added (5, 10, 15, 20, and 25 g), amount of perfume lemon added (10, 15, 20, 25, and 30 g), solid-liquid ratios of 1 ∶ 5, 1 ∶ 10, 1 ∶ 15, 1 ∶ 20, and 1 ∶ 25 (g/mL), and air inlet temperatures of 190, 195, 200, 205, and 210 ℃. Sensory scores were utilized as indicators to assess the impact of these factors.

2.3.4 Response surface test. Based on a single-factor test, a response surface optimization test was conducted utilizing the central composite design principle (Box-Behnken) to identify the optimal processing conditions for lemon-flavored solid instant tea. The sensory score of the lemon-flavored solid instant tea served as the response variable. The factors selected for optimization included the amount of Pu'er tea, the amount of perfume lemon, the solid-liquid ratio, and the air inlet temperature, all of which were found to have a significant impact on the sensory scores. The factors and levels for the response surface test are presented in Table 1.

Table 1 Factors and levels for the response surface test

Level	Factor			
	A//g	*B*//g	*C*//g/L	*D*//℃
−1	10	20	1 ∶ 15	195
0	15	25	1 ∶ 20	200
1	20	30	1 ∶ 25	205

NOTE A. Amount of Pu'er tea; B. Amount of perfume lemon; C. Solid-liquid ratio; D. Air inlet temperature.

2.3.5 Sensory evaluation. An evaluation panel consisting of 10 students specializing in food science was established to assess the sensory attributes of lemon-flavored solid instant tea. The evaluation criteria encompassed four dimensions: color, scent, texture, and flavor. Scent and flavor were each assigned a maximum of 25 points, while color and texture were allocated 20 and 30 points, respectively, contributing to a total of 100 points. The final score was determined by calculating the arithmetic mean of the individual assessments. The sensory evaluation criteria for lemon-flavored solid instant tea are presented in Table 2.

Table 2 Sensory evaluation criteria for lemon-flavored solid instant tea

Evaluation indicator	Evaluation criteria	Score//points
Color (20 points)	Faint yellow powder, uniform, glossy	15 – 20
	Faint yellow powder, uniform, with some gloss	10 – 15
	Uneven color, dull or too dark, poor gloss	0 – 10
Scent (25 points)	Strong scent of perfume lemon, very close to the scent of the raw material	20 – 25
	Thin scent of perfume lemon, no peculiar smell	10 – 20
	Very faint scent of perfume lemon	0 – 10
Texture (30 points)	Fine powdery and uniform, no graininess	20 – 30
	Fine powdery and uniform, slightly grainy, particles crushed easily	10 – 20
	Powdery, grainy, particles crushed into powder	0 – 10
Flavor (25 points)	Melting instantly in water, with strong original scent of perfume lemon, and moderate bitterness and astringency of tea	20 – 25
	Easily melting in water, with strong scent of perfume lemon, and slight astringency of tea	10 – 20
	Hardly melting in water, a little grainy, too sweet or too bitter	0 – 10

2.3.6 Physicochemical and microbiological testing. (i) Determination of dissolution time. 250 mL of mineral water at room temperature was measured, and the duration required for the complete dissolution of 1 g of lemon-flavored solid instant tea, following its addition, was recorded.

(ii) Determination of powder yield. The formula for determining the powder yield was as follows:

$$X = (A/B) \times 100$$

where X represents the powder yield, %; A indicates the weight of powder collected after spray drying, g; B refers to the total solids content of the feed solution prior to spray drying, g.

(iii) Determination of physicochemical properties. The physicochemical properties were assessed in accordance with the National Food Safety Standard. The determination of moisture content was conducted following the guidelines outlined in GB 5009.3-2016 *Determination of Moisture in Foods*. The assessment of ash content was based on GB 5009.4-2016 *Determination of Ash in Foods*. The evaluation of reducing sugar content adhered to GB5009.7-2016 *Determination of Reducing Sugar in Foods*. Microbiological testing was performed using the plate colony counting method, in accordance with GB 4789.2-2016 *Microbiological Examination of Food Hygiene—Detection of Aerobic Bacterial Count*, as well as GB 4789.3-2016 *Microbiological Examination of Food Hygiene—Enumeration of Coliforms*.

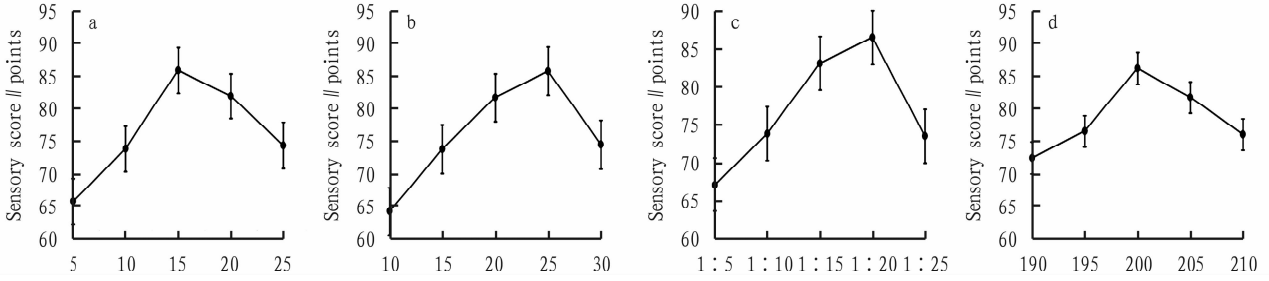
2.4 Data processing Excel 2010 was utilized for the organization of the test data, while the data processing software Design-Expert Version 8.0.6.1 was employed for conducting response surface analysis.

3 Results and analysis

3.1 Results of single-factor test The results of the single-factor test are presented in Fig. 1.

As illustrated in Fig. 1a, the sensory scores for lemon-flavored solid instant tea exhibited a trend of initial increase followed by a subsequent decrease with the incorporation of Pu'er tea. The optimal sensory scores were achieved with an addition of 15 g of Pu'er tea. This quantity served as a critical threshold; when the amount

of Pu'er tea was below 15 g, its flavor was perceived as relatively mild. Conversely, excessive addition of Pu'er tea resulted in an overpowering tea flavor that obscured the flavor of perfume lemon, thereby negatively impacting the sensory scores. Consequently, the range of Pu'er tea addition was determined to be between 10 and 20 g for the subsequent response surface test.



NOTE a. Amount of Pu'er tea (g); b. Amount of perfume lemon (g); c. Solid-liquid ratio (g/mL); d. Air inlet temperature (℃).

Fig. 1 Effect of factors on sensory scores

As illustrated in Fig. 1b, when the amount of perfume lemon was less than 20 g, the texture of the final product exhibited a uniform powdery consistency without any discernible particles; however, the flavor of perfume lemon was relatively mild. Conversely, when the amount exceeded 25 g, the sensory evaluation scores declined, attributed to the overpowering nature of the perfume lemon flavor. Consequently, the optimal quantity of perfume lemon to be added was determined to be 25 g.

As illustrated in Fig. 1c, when the solid-liquid ratio varied between 1∶5 and 1∶20 (g/mL), the increased concentration of the solution adversely affected the spray drying process, resulting in difficulties during the feeding operation. Furthermore, it impeded the complete evaporation of moisture from the sprayed droplets, leading to adherence to the walls of the spray dryer, a phenomenon commonly known as wall sticking, and consequently reduced the yield of the collected powder. Furthermore, an increase in solvent volume led to a decline in sensory scores, likely attributable to an excessively high solid-liquid ratio, which adversely affected the flavor of the lemon-flavored solid instant tea. Therefore, the optimal solid-liquid ratio was determined to be 1∶20 (g/mL).

As illustrated in Fig. 1d, the solubility was observed to be low when the air inlet temperature was below 200 ℃. An increase in the air inlet temperature corresponded with an enhancement in the sensory score; however, excessively high temperatures adversely affected the spray drying process. Consequently, an optimal air inlet temperature was determined to be 200 ℃.

3.2 Results of response surface test In accordance with the findings from the single-factor test, a four-factor, three-level response surface test was employed to optimize the formulation of lemon-flavored solid instant tea. The analysis of the response surface test results was conducted using Design - Expert Version 8.0.6.1 software, with sensory score (Y) designated as the response variable. The four factors selected for the optimization of the processing technology included the amount of Pu'er tea (A), the amount of perfume lemon (B), the solid-liquid ratio (C), and the air inlet temperature (D). The results of the optimization process are presented in Table 3.

3.3 Model establishment and significance test Multiple regression analyses were conducted on the data presented in Table 3 to derive quadratic models for the sensory scores of lemon-flavored solid instant tea (Y) in relation to the following variables: addition amount of Pu'er tea (A), addition amount of perfume lemon (B), solid-liquid ratio (C), and air inlet temperature (D): $Y = 91.65 - 1.17A + 2.36B + 0.56C - 1.24D - 0.23AB - 0.022AC - 0.13AD + 1.78BC - 1.25BD + 0.85CD - 6.25A^2 - 5.31B^2 - 4.72C^2 - 4.57D^2$. The results of the analysis of variance for the regression model are presented in Table 4.

Table 3 Scheme and results of response surface test

No.	A	B	C	D	Y
1	-1	-1	0	0	74.5
2	1	-1	0	0	76.8
3	-1	1	0	0	83.5
4	1	1	0	0	80.8
5	0	0	-1	-1	84.2
6	0	0	1	-1	83.4
7	0	0	-1	1	79.9
8	0	0	1	1	82.5
9	-1	0	0	-1	83.0
10	1	0	0	-1	80.8
11	-1	0	0	1	80.9
12	1	0	0	1	78.2
13	0	-1	-1	0	80.6
14	0	1	-1	0	81.5
15	0	-1	1	0	77.0
16	0	1	1	0	86.3
17	-1	0	-1	0	80.9
18	1	0	-1	0	78.9
19	-1	0	1	0	81.5
20	1	0	1	0	80.7
21	0	-1	0	-1	79.0
22	0	1	0	-1	87.0
23	0	-1	0	1	79.0
24	0	1	0	1	82.0
25	0	0	0	0	91.6
26	0	0	0	0	92.1
27	0	0	0	0	92.2
28	0	0	0	0	91.5
29	0	0	0	0	91.5

NOTE Y denotes the sensory score (points).

Table 4 Analysis of variance of regression model

Origin	Sum of squares	Degree of freedom	Mean square	*F*	*P*	Significance
Model	670.84	14	47.92	106.1	< 0.000 1	* *
A	15.59	1	15.59	34.51	< 0.000 1	* *
B	63.49	1	63.49	140.57	< 0.000 1	* *
C	3.84	1	3.84	8.5	0.011 3	*
D	18.5	1	18.5	40.96	< 0.000 1	* *
AB	0.18	1	0.18	0.4	0.535 9	
AC	2.17E-03	1	2.17E-03	4.80E-03	0.945 7	
AD	0.063	1	0.063	0.14	0.715 5	
BC	13.82	1	13.82	30.59	< 0.000 1	* *
BD	6.25	1	6.25	13.84	0.002 3	*
CD	2.89	1	2.89	6.4	0.024 0	
A^2	253.33	1	253.33	560.92	< 0.000 1	* *
B^2	183.04	1	183.04	405.29	< 0.000 1	* *
C^2	141.55	1	141.55	313.43	< 0.000 1	* *
D^2	132.91	1	132.91	294.3	< 0.000 1	* *
Residual error	6.32	14	0.45			
Lack of fit	5.85	10	0.59	5	0.067 3	
Pure error	0.47	4	0.12			
Total	677.17	28				

NOTE * denotes a statistically significant effect, $P<0.05$; * * indicates an extremely significant effect, $P<0.01$.

As illustrated in Table 4, the significance test for the sensory score regression model yielded a *P*-value less than 0.01, suggesting that the quadratic multiple regression model was extremely significant. Furthermore, the lack of fit test for the sensory score regression model produced a *P*-value greater than 0.05, indicating that the lack of fit for the quadratic regression model of the sensory score of lemon-flavored solid instant tea was not significant when compared to the actual test results. This finding suggested that the model was appropriate for fitting the test data. The coefficient of determination, denoted as $R^2=0.998$, suggested that the regression model demonstrated a strong fit and reliability. Consequently, it was deemed suitable for the sensory evaluation of lemon-flavored solid instant tea. In conclusion, the regression model demonstrated a good fit with minimal experimental errors, enabling accurate analysis and prediction of the sensory scores for lemon-flavored solid instant tea. This model holds significant practical implications. The regression coefficients revealed that the order of influence of the four factors on the sensory scores of lemon-flavored solid instant tea was as follows: the amount of lemon perfume (*B*) had the greatest impact, followed by the air inlet temperature (*D*), the amount of Pu'er tea (*A*), and finally the solid-liquid ratio (*C*). This hierarchy was observed within the specified range of factor levels.

3.4 Analysis of response surface test results The response surface plots illustrating the effects of pairwise interactions on the sensory scores of lemon-flavored solid instant tea are presented in Fig. 2. This analysis employed response surface test to examine the interactions between factors, as described by the regression equation derived from the fitted model for the sensory scores of lemon-flavored solid instant tea. The relative magnitude of the effects of these interactions on the sensory scores was ranked as follows: $BC>BD>CD>AB>AD>AC$.

The response surface test was employed to optimize the processing parameters for lemon-flavored solid instant tea. Adjustments were made in accordance with the equipment utilized, and the optimal processing parameters for the lemon-flavored solid instant tea were subsequently validated. The theoretically predicted values derived from the response surface analysis included 15 g of Pu'er tea, 26 g of perfume lemon, a solid-liquid ratio of 1∶20 (g/mL), and an air inlet temperature of 199 ℃. The sensory evaluation yielded a score of 92.127 6 points for the lemon-flavored solid instant tea. In light of the current circumstances, the experimental verification was conducted under the specified conditions, resulting in the optimal formulation for lemon-flavored solid instant tea. This formulation comprised 15 g of Pu'er tea, 25 g of perfume lemon, a solid-liquid ratio of 1∶20 (g/mL), and an air inlet temperature of 200 ℃. The sensory evaluation yielded a score of 91.97 points for the lemon-flavored solid instant tea, which closely aligns with the theoretically predicted value.

3.5 Physicochemical and microbiological test results The results of the detection indicated that the lemon-flavored instant tea exhibited a dissolution time of (9±3) sec, a powder yield of (19.50±0.34) %, a moisture content of (5.82±0.24) %, an ash content of (14.36±0.43) %, a total sugar content of (38.10±1.47) %, and a total colony count of (163±10) CFU/g. The lemon-flavored solid instant tea was developed through an optimal formulation. Its sensory and physicochemical properties were evaluated in accordance with the national standard GB/T 31740.1-2005 *Tea Products—Part* 1: *Instant Tea in Solid Form*. All evaluated indicators were found to comply with the national standard. The microbiological analysis revealed that the total colony count of lemon-flavored solid instant tea was (163±10) CFU/g, and *Escherichia coli* was not detected, consistent with national testing standards.

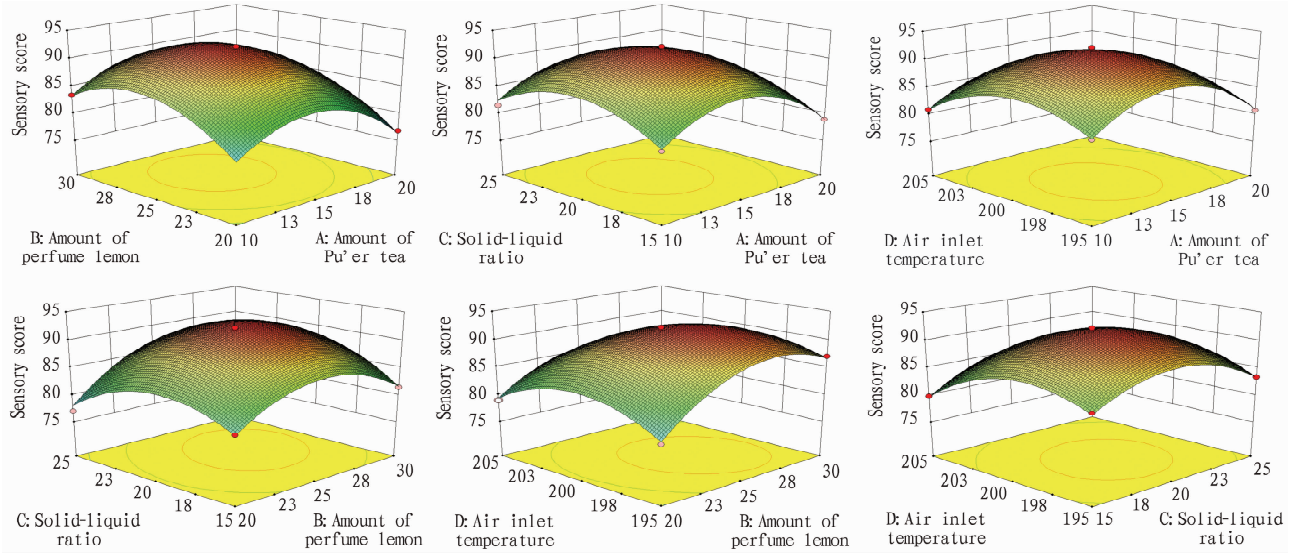


Fig. 2 Optimization results of response surface test

4 Conclusions

In this study, large-leaf Pu'er tea and perfume lemon were utilized as the primary raw materials, and the sensory score served as the benchmark for the development of lemon-flavored solid instant tea. The results indicated that the impact of each factor on the sensory score was ranked as follows: the amount of lemon added > the air inlet temperature > the amount of Pu'er tea > the solid-liquid ratio. Through the validation of the regression model and taking into account practical considerations, the optimal formulation was determined to consist of 15 g of Pu'er tea, 25 g of perfume lemon, a solid-liquid ratio of 1 ∶ 20 (g/mL), and an air inlet temperature of 200 ℃. The lemon-flavored solid instant tea produced under the specified conditions exhibited the most favorable flavor, a stable texture, and the highest sensory evaluation score. The physicochemical properties of the product formulated under these conditions were as follows: moisture content (5.82 ± 0.24) %, total sugar content (38.10 ± 1.47) %, dissolution time (9 ± 3) sec, and powder yield (19.50 ± 0.34) %. These results aligned with the relevant standards.

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have found that small molecules of Radix Bupleuri can bind to multiple targets of hepatitis B virus to inhibit the replication of hepatitis B virus in the liver[7]. Carapax Trionycis is salt in taste, and has the function of tonifying the kidney. Studies have shown that it can inhibit angiogenesis, thus preventing and treating tumors[8].

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