Multiple responses optimization of instant dark tea production by submerged fermentation using response surface methodology

Yuwan Wang¹ · Mingyue Zhang¹ · Zhengzhu Zhang¹ · Jun Jiang¹ ·
Xueling Gao¹ · Pengxiang Yue^{1,2}

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Abstract In this study, submerged fermentation mode for preparing instant dark tea production was developed through utilizing industrial low grade green tea as raw material and *Aspergillus niger* as fermentation microbe starter. The fermentation parameters (inoculum size, liquid–solid ratio and rotation speed) were optimized by using Box–Behnken design and response surface methodology (RSM) with desirability function, the theabrownins content, redness and turbidity value as responses. The optimal conditions were set as follow: inoculum size of 5.3% (v/v), liquid–solid ratio of 27.78 mL/g, and rotation speed of 182 r/min. The optimized conditions model showed a good correlation between the predicted and experimental values. Further, the optimum product of instant dark was achieved in a 3-L laboratory fermenter, and the main parameters of product were theabrownins content of 140.92 g/kg and redness value of 40.78 and turbidity of 90.98 NTU. Sensory evaluation showed that the instant dark tea infusion approached mellow mouthfeel, an aroma of mint and a good overall acceptance.

Keywords Instant dark tea · Submerged fermentation · *Aspergillus niger* · Multiple responses optimization · Scale-up

Introduction

Tea is the second most widely consumed beverage in the world, after water (McKay and Blumberg 2002). In China, tea is usually classified into four categories based on the degree of fermentation: unfermented tea (such as green tea), semi-fermented tea (such as Oolong tea), fully fermented tea (black tea) and post-fermented tea (such as Pu-erh tea, Fu-brick tea and Qingzhuan tea) (Xu et al. 2015). Chinese traditional dark tea belong to post-fermented tea, which usually relate to microbial fermentation process (Li et al. 2017). *Aspergillus niger* as predominant kind of microbes was found in different Chinese dark tea (Zhang et al. 2016). Dark tea not only creates a unique sensory experience, with a brownish-red color, a marked fragrance, and a full-bodied flavor, possibly due to natural fungal inoculation and fermentation (Zhang et al. 2013), but also constituents of dark tea can promote health by improving oral hygiene, lowering hypertension, serving as antioxidants and antibacterial agents, and by countering obesity, hyperlipidemia, hyperglycemia, allergies, and mutagens (Elvira et al. 2009; Zhang et al. 2013; Lee and Foo 2013; Kondo et al. 2015).

Instant teas have been increasingly accepted by consumers due to the simplicity of their diet preparation, and become a fast growing segment of the tea market. Dark tea leaves (dark tea) can be made into an instant dark tea product by extracting and then drying components. Traditional dark tea preparation usually takes 1–2 months, from sun-dried tea leaves via solid-state fermentation by natural

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✉ Xueling Gao
sharling@ahau.edu.cn

✉ Pengxiang Yue
zhonghuajwh11@163.com

¹ State Key Laboratory of Tea Plant Biology and Utilization, Anhui Agricultural University, Hefei 230036, Anhui, China

² Damin Foodstuff (Zhangzhou) Co., Ltd., Zhangzhou 363000, Fujian, China

inoculation (known as a “pile process”) and then dehydration (Zhang et al. 2016). However, dark tea from natural inoculation may pose a potential risk for safety because of microbial contamination (Haas et al. 2013), and most of dark tea process is not automated, resulting in low productivity and low quality consistency. Compared with solid-state fermentation by natural inoculation, submerged fermentation (SF) can be more easily controlled. Additional advantages of SF include a shortened fermentation time, less microbial contamination, and the possibility for automation (Wang et al. 2014). In addition, Chinese green tea ranks as the highest yields in the world, there are a large amount of low-grade green tea and industrial crop waste (trimmed leaves, tea dust and mature leaves), which will be as a cheap material of instant dark tea product by SF.

Consumer satisfaction is generally affected by tea quality, and the appearance of the tea beverage is one of the key factors affecting consumer preference. Dark tea components, such as theabrownins, carbohydrates, and polyphenols, influence the flavor, taste and color of the tea beverage (Xie et al. 2009). Dark tea with a bright and red color is favored, and trials to produce this appearance have used exogenous enzymes, extracting at a low temperature, and different drying methods (Liang and Xu 2001; Kraujalytė et al. 2016). Tea cream, caused by a high level of combination of catechin gallates and theine can have a negative impact on the transparency of tea infusion (Liang and Xu 2001).

Response surface methodology (RSM) as a valuable tool has been widely used to investigate the possible interactions between experimental variables and their measured responses (He et al. 2016). RSM can also evaluate multiple factors and their interactions effectively, it is widely applied in food product design (Yuan et al. 2015; Lu et al. 2016).

Base on our previous study on high theabrownins instant dark tea product (Wang et al. 2017), the present study was focused on the fermentation parameters on influence of tea infusions color, turbidity, chemical components, and sensory characteristics. To acquire the instant dark tea production with a characteristic bright red color, clear appearance and high theabrownins content, response surface methodology (RSM) combined with a desirability function (DF) was used to optimize the parameters for SF in flasks, including inoculum size, liquid–solid ratio, and rotation speed. And the redness, turbidity and theabrownins (TB) content of fermentation tea infusion were investigated. Further, the product of instant dark tea was produced in a 3-L laboratory scale-up of SF, overall satisfaction, the characteristic profiles (redness, turbidity, theabrownins content, flavor and taste) was compared with two instant Pu-erh teas produced via conventional manufacturing method.

Materials and methods

Material and chemicals

Green tea leaves (low-grade, broken, green tea leaves with 5.7% moisture and 26.3% tea polyphenol), and two industrial product samples of instant Pu-erh tea (PET1 and PET2) were provided by Damin Foodstuff (Zhangzhou) Co., Ltd (Fujian, China).

Microorganism

Aspergillus niger (accession number: KY607770) was isolated from Anhua Fu-brick dark tea (Hunan, China). *Aspergillus niger* does not secrete toxins, as determined by a safety assessment using LC–MS (Fig. S1). *Aspergillus niger* was cultured on solid PDA for 3–5 days. The spores were washed by sterile 0.9% (w/v) NaCl solution to prepare a spore suspension. The concentration of spores was counted by a haemocytometer counting chamber and adjusted to 1×10^7 CFU/mL for inoculation of the green tea leaves.

Production of instant dark tea by submerged fermentation

Preparation of the green tea powder was according to our previous study (Wang et al. 2017). Green tea infusion was made by mixing the instant green tea powder and deionized water, in a ratio based on the RSM optimized design, and pasteurization at 80 °C for 30 min. The *A. niger* spore suspension was inoculated into the pasteurized green tea infusion (100 mL) and cultured for 6 days in a shaking incubator at 37 °C. Once fermentation was complete, the fermentation broth was heated to 75 °C for 5 min, filtered (11 µm pore size, Whatman, UK), obtained tea filtrate, then freeze-dried using a vacuum freeze-dryer (GAMMA 1-16 LSC; Germany) to yield the instant dark tea powder.

Experimental design

Response surface methodology (RSM)

In silico experiments of instant dark tea production were performed in flasks using a Box–Behnken design (BBD) with 17 runs. The independent factors were coded as -1 , 0 and $+1$ denoting the low, mid and high level. Inoculum size (x_1 , 3, 5 and 7% v/v), liquid–solid ratio (x_2 , 15, 25 and 35 mL/g), and rotation speed (x_3 , 150, 180 and 210 r/min) were evaluated as independent variables that affect redness

(y_1), turbidity (y_2), and TB content (y_3) of instant dark tea infusion.

The optimization of instant dark tea was modelled using the following quadratic polynomial regression Eq. (1):

$$y = \lambda_0 + \sum_{i=1}^3 \lambda_i x_i + \sum_{j=1}^3 \lambda_{ii} x_i^2 + \sum_{i < j}^3 \lambda_{ij} x_i x_j \quad (1)$$

where y is the experimental response; λ_0 is the intercept term; λ_i , λ_{ii} and λ_{ij} are the linear, quadratic and interactive coefficients, respectively, and x_i and x_j are the levels of the independent variables.

Desirability function

The desirability function (DF) approach is an efficient method used to determine optimum performance levels for one or more responses simultaneously (Mourabet et al. 2012). Redness, turbidity and TB content were positively and significantly correlated with dark tea total quality (Lu et al. 2016; Wang et al. 2016). The desirability function is presented as follows:

Larger-the-better,

$$d_i(y_i(x)) = \begin{cases} 0 & y_i(x) \leq y_i^{\min} \\ \frac{y_i(x) - y_i^{\min}}{y_i^{\max} - y_i^{\min}} & y_i^{\min} \leq y_i(x) \leq y_i^{\max} \\ 1 & y_i(x) \geq y_i^{\max} \end{cases} \quad (2)$$

Smaller-the-better,

$$d_i(y_i(x)) = \begin{cases} 0 & y_i(x) \geq y_i^{\max} \\ \frac{y_i^{\max} - y_i(x)}{y_i^{\max} - y_i^{\min}} & y_i^{\min} \leq y_i(x) \leq y_i^{\max} \\ 1 & y_i(x) \leq y_i^{\min} \end{cases} \quad (3)$$

where $d_i(y_i(x))$ is the desirability value, $d_i(y_i(x))$, $y_{\min}$ and $y_{\max}$ stand for the experimental value, the lowest possible value and the highest value, respectively.

The individual desirability values are combined into an overall desirability function, D , by calculating their geometric mean:

$$D = \left(\prod_{i=1}^n d_i^{w_i} \right)^{1/\sum w_i} \quad (4)$$

where w_i is the relative weight of the response, which reflects the difference in the importance of different responses.

The scale-up experiment

The scale-up fermentations were performed in a 3-L laboratory fermenter (Hanil, Korea) with a 1.2-L working volume. The temperature was set at 37 °C, initial pH of the

medium was natural. Dissolved oxygen (DO) concentration was maintained at 30% of saturated air level by automatically adjusting the aeration rate (Wang et al. 2017; Zheng et al. 2016). Other factors impacting the fermentation were set according to the values predicted by the RSM model of the optimum condition. Grown inoculum (12 h) was transferred to an aseptic flask and further incubated (12 h). The fermentation was conducted for up to 6 days.

Analysis of tea filtrate

Color

The tea filtrate was cooled to room temperature before its color was measured. The color indicators of light–dark (L), red–green (a) and yellow–blue (b), measured in a three-dimensional color coordinate system (Ata et al. 2017), were measured by the Color Quest XE (Hunter Associates Laboratory, Inc., Virginia, USA) in the transmittance mode using the supplied white plate as background.

Turbidity

The tea filtrate was cooled to room temperature before its turbidity was measured using a Turb555/Turb555IR nephelometer (WTW, Munich, Germany). The measure was expressed in nephelometric turbidity units (NTU).

Theabrownins (TB) content

The TB content was determined using a published method (Wang et al. 2017). The total content of TB was calculated according to Eq. (5):

$$TB(\%w/w) = \frac{7.06 \times 2 \times A_{380}}{m(1-w)} \times 0.72 \quad (5)$$

where $m(g)$ was the quantity of the instant green tea and w (% by weight) was the moisture content of the instant green tea. The multiplier of 0.72 corrects for a different ratio of tea leaves and water used here to make the instant tea extracts compared to the values used in the original reference.

Sensory analysis

Sensory analysis was carried out in tea tasting room at the College of Tea and Food Science, Anhui Agricultural University (Hefei, China). Instant dark tea powder (0.5 g) was dissolved in 200 mL of freshly boiled water (80 °C) in a tea glass. Sensory analysis was employed to evaluate each tea infusion sample for taste, fragrance and overall satisfaction, using a 10-point hedonic scale from 10

(excellent, extremely acceptable) to 1 (poor, extremely unacceptable) (Chen et al. 2010). Tea samples were examined and scored by ten well-trained panelists (five males and five females, aged 24–55 years).

Statistical analysis

Results were expressed as the mean $\pm$ SD ($n = 3$) for each analysis. Analysis of variance (ANOVA) was carried out to determine any significant differences ($P < 0.05$). Response surface plots were generated using Statistica 9.0 (Statsoft Inc., NY, USA). All experiments were conducted in triplicate.

Results and discussion

Modeling of the instant dark tea production process

RSM was set up using 3 coded levels and 3 independent variables to predict the production conditions that would yield an instant dark tea with optimal characteristics. The modeling results for redness, turbidity and TB content are shown in Table 1. Across 17 different parameter combinations, the redness ranged from 30.99 to 44.56, the turbidity from 79.98 to 581.51 NTU, and the TB content from 32.97 to 149.52 g/kg.

To evaluate the mutual interactions of the independent and dependent variables, the R^2 values, F -values and P -values were determined from the modeled responses (Table 2). A second-order polynomial model (Eq. 1) fit the experimental data well, with R^2 values of 0.9817, 0.9979 and 0.9919 for redness, turbidity and TB content, respectively, indicating that the total variance could be explained by the model. For both responses, mathematical models were statistically acceptable due to significant regression ($P_m < 0.05$) and non-significant lack of fit ($P_{lf} > 0.05$). Moreover, the coefficient of variation (CV) was low ($CV < 10\%$). The result indicated that the degree of precision was high and the modeled results were predictive. The second-order polynomial Eqs. (7)–(9) described the relationship between inoculum size (x_1), liquid–solid ratio (x_2) and rotation speed (x_3).

$$\begin{aligned} \text{Redness} = & 43.86 + 1.23x_1 + 1.02x_2 + 1.99x_3 + 0.4x_1x_2 \\ & - 1.56x_1x_3 - 1.35x_2x_3 - 5.42x_1^2 - 2.14x_2^2 - 2.98x_3^2 \end{aligned} \quad (7)$$

$$\begin{aligned} \text{Turbidity} = & 90.75 + 33.07x_1 - 123.45x_2 + 62.89x_3 \\ & + 25.46x_1x_2 - 13.77x_1x_3 - 20.17x_2x_3 + 46.71x_1^2 \\ & + 208.27x_2^2 + 66.11x_3^2 \end{aligned} \quad (8)$$

Table 1 Box–Behnken design model design with independent variables and modeled data responses

Run	Independent variables coded and uncoded			Responses		
	x_1	x_2	x_3	y_1	y_2	y_3
1	1 (7)	0 (25)	− 1 (150)	35.69	198.3	107.71
2	0 (5)	0 (25)	0 (180)	43.99	89.09	144.02
3	− 1 (3)	0 (25)	− 1 (150)	30.94	99.56	114.71
4	0 (5)	− 1 (15)	− 1 (150)	35.14	398.89	32.97
5	− 1 (3)	0 (25)	1 (210)	38.34	236.37	102.92
6	1 (7)	1 (35)	0 (180)	39.02	279.9	106.67
7	0 (5)	− 1 (15)	1 (210)	40.59	581.51	51.43
8	− 1 (3)	− 1 (15)	0 (180)	34.37	462.47	39.27
9	0 (5)	1 (35)	1 (210)	39.63	291.02	99.66
10	1 (7)	− 1 (15)	0 (180)	35.9	472.64	44.65
11	0 (5)	0 (25)	0 (180)	44.56	90.98	143.64
12	1 (7)	0 (25)	1 (210)	37.79	280.02	125.15
13	0 (5)	1 (35)	− 1 (150)	39.59	189.08	84.69
14	0 (5)	0 (25)	0 (180)	42.77	97.9	137.09
15	0 (5)	0 (25)	0 (180)	43.99	79.28	144.65
16	− 1 (3)	1 (35)	0 (180)	35.89	167.89	84.44
17	0 (5)	0 (25)	0 (180)	43.97	96.48	149.52

x_1 inoculum size (% v/v), x_2 liquid–solid ratio (mL/g), x_3 rotation speed (r/min), y_1 redness, y_2 turbidity (NTU), y_3 theabrownins (g/kg)

Table 2 ANOVA for response surface quadratic model

Source	F-val	P value	R ²
<i>Redness</i>			
Model	41.73	< 0.0001	0.9817
x_1	16.87	0.0045	
x_2	11.61	0.0113	
x_3	44.58	0.0003	
x_1x_2	0.9	0.3745	
x_1x_3	13.68	0.0077	
x_2x_3	10.28	0.0149	
x_{11}	173.94	< 0.0001	
x_{22}	27.08	0.0012	
x_{33}	52.52	0.0002	
Lack of fit	2.52	0.1967	
CV		2.17	
<i>Turbidity</i>			
Model	364.26	< 0.0001	0.9979
x_1	73.31	< 0.0001	
x_2	1021.59	< 0.0001	
x_3	265.09	< 0.0001	
x_1x_2	21.73	0.0023	
x_1x_3	6.36	0.0397	
x_2x_3	13.64	0.0077	
x_{11}	76.97	< 0.0001	
x_{22}	1530.31	< 0.0001	
x_{33}	154.18	< 0.0001	
Lack of fit	3.77	0.1163	
CV		4.52	
<i>TB</i>			
Model	94.92	< 0.0001	0.9919
x_1	8	0.0255	
x_2	187.05	< 0.0001	
x_3	6.66	0.0365	
x_1x_2	2.48	0.1596	
x_1x_3	7.45	0.0294	
x_2x_3	0.11	0.754	
x_{11}	32.15	0.0008	
x_{22}	532.71	< 0.0001	
x_{33}	39.33	0.0004	
Lack of fit	2.07	0.2463	
CV		5.31	

x_1 , x_2 and x_3 represent inoculum size, liquid–solid ratio and rotation speed, respectively, *SS*, *DF*, *MS* and *CV* represent sum of squares, degree of freedom, mean square, coefficient of variation, respectively

$$\begin{aligned}
 \text{Theabrownins} = & 143.78 + 5.36x_1 + 25.89x_2 + 4.89x_3 \\
 & + 4.21x_1x_2 + 7.31x_1x_3 - 0.87x_2x_3 - 14.80x_1^2 \\
 & - 60.23x_2^2 - 16.37x_3^2
 \end{aligned}
 \quad (9)$$

Effect of independent variables on response values in the RSM model

From the regression evaluation, it could be observed that the three independent variables each has a linear effect on the response values (the redness, turbidity and TB content) within the experimental fermentation range. As shown in Table 2, the redness of the instant dark tea was significantly affected by inoculum size, liquid–solid ratio and rotation speed at the level of $P < 0.05$. All quadratic terms were more significant at $P < 0.01$. The interaction of x_1x_3 and x_2x_3 were highly significant at $P < 0.01$, while x_1x_2 was non-significant ($P > 0.05$). The turbidity of the instant dark tea was significantly affected by inoculum size, liquid–solid ratio and rotation speed at the level of $P < 0.01$. All quadratic terms were more significant at $P < 0.01$. The interaction of x_1x_2 and x_2x_3 were highly significant at $P < 0.01$, followed by x_1x_3 at $P < 0.05$. The TB content of instant dark tea was significantly affected by inoculum size, liquid–solid ratio and rotation speed at the level of $P < 0.01$. All quadratic terms were more significant at $P < 0.01$. The interaction of x_1x_2 and x_2x_3 were non-significant ($P > 0.05$).

The redness response increased as the three fermentation factors increased. Near the midpoint of the response plot, the redness value reached its highest. The redness value was negatively influenced if any of the three parameters were outside of the midpoint (Fig. 1a–c). A high redness value was predicted with a moderate liquid–solid ratio and inoculum size (Fig. 1a). The redness increased and then decreased with increasing rotation speed (Fig. 1b, c).

The turbidity is an important property of tea infusion (Lu et al. 2016). In the model, the turbidity ranged from 79.28 to 581.51 and decreased when any one preparation parameter increased near the midpoint of the response plot. The turbidity was negatively influenced if a parameter passed the midpoint (Fig. 1d–f). A lower liquid–solid ratio and inoculum size would result in a small turbidity value (Fig. 1d). When the microorganisms and nutrients achieve dynamic equilibrium, the microorganisms can secrete abundant enzymes, which leads to low turbidity of a tea infusion (Hama et al. 2015).

The yield for TB content (Fig. 1g–i) was similar to the trends for redness (Fig. 1a–c). The maximum modeled TB yield was 149.52 g/kg at 5% (v/v) inoculum size, a liquid–solid ratio of 25.00 mL/g and a rotation speed of 180 r/min (Table 1). A high rotation speed can accelerate the growth of microbes because of increased oxygen in the broth (Wang et al. 2015; Sharma et al. 2015). However, too high of a rotation speed can cause drastic friction between the fungus and the reactor, which would restrict the fungal growth (Wang et al. 2013).

Fig. 1 Response surface plot for interactions between three independent variables (fermentation parameters) on redness (a–c), turbidity (d–f) and theabrownins content (% w/w, g–i). Two variables were plotted against each other in each panel

The quality of the dark tea infusion (redness value, turbidity and TB content) was mainly associated with secretion of enzymes such as polyphenol oxidase and peroxidase by the microbe (Peng et al. 2013; Wang et al. 2016). Under the appropriate culture conditions, enzyme secretion by *A. niger* would work to oxidize the tea polyphenols (especially catechins) into pigment (Wang et al. 2016), and cause an increase in redness and TB content. In contrast, turbidity of the tea infusion would decline because of a decrease in tea cream content (Ni et al. 2015; Lu et al. 2016).

Optimization of modeled parameters by the desirability function

The desirability function was added to the RSM to simultaneously maximize the redness value and TB content and minimize the turbidity of the dark tea infusion. Limits ($y_i^{\min}$ and $y_i^{\max}$) were set for each parameter that was used to

Table 3 Design of the desirability function

Responses	Symbols	$y_i^{\min}$	$y_i^{\max}$	w_i
Redness	y_1	30	45	1
Turbidity (NTU)	y_2	79	582	1
TB (g/kg)	y_3	30	150	2

$y_i^{\min}$ and $y_i^{\max}$ stand for the experimental value, the lowest possible value and the highest value, respectively; w_i is the relative weight of the response, which reflects the difference in the importance of different responses

achieve optimization of the three responses (Table 3). The composite desirability (D) were plotted in Fig. 2. It directed that for the fermentation process, inoculum size of 5.3% (v/v), liquid–solid ratio of 27.78 mL/g and rotation speed of 182 r/min will yield 44.11 redness, 83.59 NTU turbidity and 147.28 g/kg TB content with overall desirability value of 0.985.

Fig. 2 Individual response and composite desirability of the RSM-modeled optimum conditions for fermentation of instant dark tea. x_1 , x_2 and x_3 represent inoculum size, liquid–solid ratio and rotation speed, respectively. y_1 , y_2 and y_3 represent redness value, turbidity and TB content

Verification of the modeled preparation conditions and scale-up experiment

To confirm the reliability of the models, an experiment was performed under the predicted optimal conditions of 5.3% (v/v) inoculum size, 27.78 mL/g liquid–solid ratio and 182 r/min rotation speed. The resulting tea filtrate had a redness value of 42.35, a turbidity of 86.72 NTU and a TB content of 142.56 g/kg. The errors between the predicted and experimental values were all smaller than 5%, indicating good agreement between the model and the experiment.

Based on the experimental data, the same conditions inoculum size of 5.3% (v/v), liquid–solid ratio of 27.78 mL/g and rotation speed of 182 r/min were used in a

3-L fermenter. The instant dark tea produced in this fermentation reaction yielded a tea infusion with a redness value of 40.78, a turbidity of 90.98 NTU and 140.92 g/kg of TB. This result further demonstrated that additional usage of the desirability function could be an efficient approach to model parameter values for fermentation reactions.

Characteristic profiles of the dissolved instant dark tea

The instant dark tea (IDT) produced by the optimized submerged fermentation protocol was compared to two commercially available Pu-erh teas which are produced by solid state fermentation of green tea leaves (Table 4). The instant dark tea (IDT) produced here in yielded a tea beverage with a higher redness value, clear and transparent, and higher TB content in comparison with the instant Pu-erh tea 1 (PRT1) and the instant Pu-erh tea 2 (PRT2). There were no significant differences between the IDT and PRT1 for aroma and overall satisfaction. The aromas from IDT and PRT2 were significantly different, with the IDT having a marked aroma of mint, while PRT2 emitted an aroma after aging. Taste from IDT significantly differed from that of PRT1 and PRT2. The IDT approached mellow taste, while PRT2 presented a stale and mellow taste. In conclusion, the IDT yielded a beverage that was bright red in color with approach of mellow mouthfeel that carried an aroma of mint.

Conclusion

In this work, RSM combined with desirability function was used to successfully model parameters for the preparation of instant dark tea with a characteristic bright red color, clear appearance and high TB content. The instant dark tea with approach of mellow mouthfeel and aroma of mint, which was produced using the optimized conditions

Table 4 Characteristic profiles and overall acceptability of instant dark tea samples

Tea	Characteristics					Overall acceptability
	Turbidity (NTU)	Redness	TB (g/kg)	Flavor	Taste	
IDT	36.25 ± 0.32 ^a	36.25 ± 0.32 ^a	147.92 ± 0.50 ^a	7.5 ± 0.3 ^a	7.5 ± 0.2 ^a	8.0 ± 0.1 ^a
PRT1	25.61 ± 0.58 ^b	25.61 ± 0.58 ^b	130.12 ± 0.01 ^b	8.0 ± 0.2 ^{ab}	8.5 ± 0.1 ^b	8.2 ± 0.3 ^{ab}
PRT2	30.30 ± 0.01 ^c	30.30 ± 0.01 ^c	136.23 ± 0.01 ^c	8.5 ± 0.3 ^b	8.7 ± 0.3 ^b	8.5 ± 0.1 ^b

Flavor, taste and overall acceptance of tea infusions were evaluated by consumers using a ten-point hedonic scale. Instant dark tea produced through the optimized fermentation (IDT), and two samples of instant Pu-erh tea (PRT1 2) were prepared into beverage by dissolving 0.5 g in 250 mL of distilled water (80 °C). Within the same column, values marked with different superscript letters (a–c) indicate a difference at $P < 0.05$. Data represent the mean ± SD from three beverage mixes for each tea sample

(inoculum size of 5.3% (v/v), liquid–solid ratio of 27.78 mL/g and rotation speed of 182 r/min), appeared a higher redness in color, lower turbidity and a higher TB content compare with commercial instant Pu-erh teas. This study showed that it was feasible to prepare instant dark tea using abundant industrial crop low-grade green tea as raw materials via submerged fermentation.

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References

- Ata GD, Gokay O, Mujdeci A, Kivrak TC, Tavana AM (2017) Effect of various teas on color stability of resin composites. *Am J Dent* 30:323–328
- Chen YS, Liu BL, Chang YN (2010) Bioactivities and sensory evaluation of Pu-erh teas made from three tea leaves in an improved pile fermentation process. *J Biosci Bioeng* 109:557–563
- Elvira GM, Marco VRM, Sirima P (2009) Bioactive components of tea: cancer, inflammation and behavior. *Brain Behav Immun* 23:721–731
- Haas D, Pfeifer B, Reiterich C, Partenheimer R, Reck B, Buzina W (2013) Identification and quantification of fungi and mycotoxins from Pu-erh tea. *Int J Food Microbiol* 166:316–322
- Hama S, Onodera K, Yoshida A, Noda H, Kondo A (2015) Improved production of phospholipase A1 by recombinant *Aspergillus oryzae* through immobilization to control the fungal morphology under nutrient-limited conditions. *Biochem Eng J* 96:1–6
- He B, Zhang LL, Yue XY, Liang J, Jiang J, Gao XL, Yue PX (2016) Optimization of ultrasonic-assisted extraction of phenolic compounds and anthocyanins from blueberry (*Vaccinium ashei*) wine pomace. *Food Chem* 204:70–76
- Kondo M, Nishi K, Sugahara T (2015) Ishizuchi dark tea suppresses IgE-mediated degranulation of RBL-2H3 cells and nasal rubbing behavior of pollinosis in mice. *J Funct Foods* 14:659–669
- Kraujalytė V, Pelvan E, Alasalvar C (2016) Volatile compounds and sensory characteristics of various instant teas produced from black tea. *Food Chem* 194:864–872
- Lee LK, Foo KY (2013) Recent advances on the beneficial use and health implications of Pu-erh tea. *Food Res Int* 53:619–628
- Li Q, Huang J, Li YD, Zhang YY, Luo Y, Chen Y, Lin HY, Wang KB, Liu ZH (2017) Fungal community succession and major components change during manufacturing process of Fu brick tea. *Sci Re* 7:6947
- Liang YR, Xu YR (2001) Effect of pH on cream particle formation and solids extraction yield of black tea. *Food Chem* 74:155–160
- Lu HQ, Yue PX, Wang YW, Fu RY, Jiang J, Gao XL (2016) Optimization of submerged fermentation parameters for instant dark tea production by Eurotium Cristatum. *J Food Process Pres* 40:1134–1144
- McKay DL, Blumberg JB (2002) The role of tea in human health: an update. *J Am Coll Nutr* 21:1–13
- Mourabet M, El Rhilassi A, El Boujaady H, Bennani-Ziatni M, HamriR El, Taitai A (2012) Removal of fluoride from aqueous solution by adsorption on Apatitic tricalcium phosphate using Box–Behnken design and desirability function. *Appl Surf Sci* 258:4402–4410
- Ni H, Chen F, Jiang ZD, Cai MY, Yang YF, Xiao AF, Cai HN (2015) Biotransformation of tea catechins using *Aspergillus niger* tannase prepared by solid state fermentation on tea byproduct. *LWT Food Sci Technol* 60:1206–1213
- Peng CX, Wang QP, Liu HR, GaoB Sheng J, Gong JS (2013) Effects of Zijuan Pu-erh tea theabrownin on metabolites inhyperlipidemic rat feces by Py-GC/MS. *J Anal Appl Pyrol* 104:226–233
- Sharma SK, Gautam N, Atri NS (2015) Optimized extraction, composition, antioxidant and antimicrobial activities of exo and intracellular polysaccharides from submerged culture of *Cordyceps cicadae*. *BMC Complem Altern M* 15:446
- Wang JN, Li A, Yang JX, Wang JH, Guo JB, Ma F, Shi SN, Zhang S, Ren NQ (2013) Mycelial pellet as the biomass carrier for semicontinuous production of bioflocculant. *RSC Adv* 3:18414–18423
- Wang QP, Gong JS, Chisti Y, Sirisansaneeyakul S (2014) Bioconversion of tea polyphenols to bioactive theabrownins by *Aspergillus fumigatus*. *Biotechnol Lett* 36:2515–2522
- Wang L, Zhang B, Han J, Zheng YB, Li JP, Shan AS (2015) Optimization of hydrolysis condition of blood meal by *Bacillus subtilis* with response surface methodology. *Int Biodeter Biodegr* 104:112–117
- Wang QP, Gong JS, Chistic Y, Sirisansaneeyakula S (2016) Production of theabrownins using a crude fungal enzyme concentrate. *J Biotechnol* 231:250–259
- Wang YW, Zhang MY, Zhang ZZ, Lu HQ, Yue PX, Gao XL (2017) High-theabrownins instant dark tea product by *Aspergillus niger* via submerged fermentation: alpha-glucosidase and pancreatic lipase inhibition and antioxidant activity. *J Sci Food Agr* 97:5100–5106
- Xie GX, Ye M, Wang Y, Ni Y, Su MM, Huang H, Qiu MF, Zhao AH, Zheng XJ, Chen TL, Jia W (2009) Characterization of Pu-erh tea using chemical and metabolic profiling approaches. *J Agr Food Chem* 57:3046–3054
- Xu L, Hu FL, Wang W, Wan XC, Bao GH (2015) Investigation on biochemical compositional changes during the microbial fermentation process of Fu brick tea by LC–MS based metabolomics. *Food Chem* 186:176–184
- Yuan J, Huang J, Wu G, Tong JH, Xie GY, Duan JA, Qin MJ (2015) Multiple responses optimization of ultrasonic-assisted extraction by response surface methodology (RSM) for rapid analysis of bioactive compounds in the flower head of *Chrysanthemum morifolium* Ramat. *Int Crops Prod* 74:192–199
- Zhang L, Zhang ZZ, Zhou YB, Ling TJ, Wan XC (2013) Chinese dark tea: post fermentation, chemistry and biological activities. *Food Res Int* 53:600–607
- Zhang W, Yang RJ, Fang WJ, Yan L, Lu J, Sheng J, Lv J (2016) Characterization of thermophilic fungal community associated with pile fermentation of Pu-erh tea. *Int J Food Microbiol* 227:29–33
- Zheng XL, Fang B, Han DF, Yang WX, Qi FF, Chen H, Li SY (2016) Improving the secretory expression of an α -galactosidase from *Aspergillus niger* in *Pichia pastoris*. *PLoS ONE* 11:e0161529