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Phytochemical Profiles and Anti-Diabetic Potential of the Extract of *Pueraria lobata* Compound Teabags: Preparation and Optimization Infusion Conditions

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Abstract: A *Pueraria lobata* root compound teabag using *Pueraria lobate* root, *Stigma maydis*, and *Prunus mume* as the main material was prepared in this study, and the brewing technology was optimized using Response Surface Methodology (RSM). The sensory evaluation, total polysaccharide, and phenolic compound content were used as evaluation indicators and the effect of the compound ratio of raw material was studied. As a result, the compound ratio of *Pueraria lobate* root, *Prunus mume*, and *Stigma maydis* was determined to be 2:2:1 (*m:m:m*). To obtain the best brewing conditions for high inhibition rates of α-amylase and α-glucosidase, the teabag brewing process was optimized using RSM equipped with a 3-factor-3-level Box-Behnken (BBD) experimental design. Three independent variables were assigned to brewing water temperature (80-100°C), amount of water addition (150-200 mL), and soaking time (3-9 min) to design an empirical model best fit in the inhibition rates of α-amylase and α-glucosidase. The range of values for each variable was based on the previous one-factor experiment (results not shown). The optimal brewing process for the preparation of the *Pueraria lobata* root compound tea was a brewing water temperature of 90.8°C, an amount of water addition of 155 mL, and a soaking time of 5.9 min. The inhibition rate of α-amylase and α-glucosidase could reach 45.09±1.13 and 60.52±2.47%, respectively. These findings suggested that a *Pueraria lobata* root compound teabag was established, with a unique aroma, a slightly bitter and sweet taste, high polysaccharides and phenolic content, and excellent in vitro hypoglycemic activity.

Keywords: Hypoglycemic Effect, Teabag, Response Surface Methodology, Infusion Process

Introduction

Diabetes Mellitus (DM) is clinically characterized by hyperglycemia that results from insufficient insulin production or defective response of insulin, or both causing blood glucose levels to rise above the normal range, which results in abnormalities in carbohydrate, protein, and fat metabolism over time (Chinsembu, 2019). By 2030, the prevalence of diabetes in adults (20-79 years) worldwide is projected to increase by 7.7%, or 439 million adults (Jo *et al*., 2018). From 2010 to 2030, we expect the number of adults with diabetes in developing countries to increase by 69% and in developed countries by 20%, which is a serious threat to human health (Shaw *et al*., 2010). In type II DM, the hydrolysis of starch by pancreatic α-amylase and the absorption of glucose by intestinal α-glucosidase could lead to postprandial hyperglycemia (Rehman *et al*., 2019). The inhibition of α-amylase is caused by the non-covalent binding interaction of the active substance, such as polysaccharide and phenolic compound, with it, mainly including hydrogen bonding and π-π conjugation (Lo *et al*., 2008; Cao *et al*., 2020), while α-glucosidase inhibitors suppress the postprandial hyperglycemia effect by inhibiting the hydrolysis of disaccharides into glucose monomers, resulting in reduced glucose absorption (Tadera *et al*., 2006). However, synthetic α-amylase and α-glucosidase inhibitors, such as acarbose, voglibose, and miglitol, frequently reported gastrointestinal side effects, including flatulence,

abdominal pain, meteorism, and diarrhea (Derosa and Maffioli, 2012).

Previous studies have shown that plant food, which is naturally rich in polyphenols, polysaccharides, flavonoids, tannins, carotenoids, proteins, vitamin C and alkaloids may have inhibitory activity on α-amylase and α-glucosidase (Gong *et al*., 2020), slowing starch digestion *in vitro* and *in vivo*, thereby reducing the risk of type II DM and its complications. Therefore, efforts have been directed toward searching for natural and safer *α*-amylase and *α*-glucosidase inhibitors and the search for such raw materials that can be eaten and used as medicines in traditional medicinal plants has become more important.

In recent years, natural plants that are defined as homologous to medicine and food have gradually played an important role in medical care due to their low toxicity and side effects, wide material selection, and perfect curative effects. The *Pueraria lobate* root, *Stigma maydis*, and *Prunus mume* are widely distributed in China and approved as both food and medicine on the list by the National Health Bureau (Zhang *et al*., 2021). *Pueraria lobata* (*P. lobata*) is sweet, pungent in taste, and cool in nature, rich in polyphenols and polysaccharides (Wong *et al*., 2015). Research has shown that polyphenols in *P. lobata* root, mainly as puerarin, can reduce blood glucose in STZ-induced diabetic mice accompanied by the expression of insulin (Wu *et al*., 2013). In addition, puerarin may treat diabetic nephropathy by inhibiting most growth factors, blocking protein glycosylation, and reducing the accumulation of extracellular matrix in the kidneys (Xu *et al*., 2016; Zhang *et al*., 2020). *Stigma maydis* (*S. maydis*), also called corn silk, is made from stigmas, the yellowish thread-like strands of the female maize flower. *S. maydis* is rich in phenolic compounds, particularly flavonoids (Hasanudin *et al*., 2012) and studies found that the water extract has the properties of reducing hyperglycemia and can be used as a hypoglycemic food for diabetics (Guo *et al*., 2009). *Prunus mume* (*P. mume*) is an Asian plum species belonging to the *Rosaceae* family, which is one of the most ancient medicinal herbs and health foods commonly used in Asian countries (Cho *et al*., 2019). Modern pharmacological studies have revealed various biological activities and biological activity mechanisms of *P. mume*, including anti-diabetic, hepatoprotective, anti-tumor, anti-inflammatory, and antibacterial activities (Gong *et al*., 2021). Studies have shown that the water extracts of *P. mume* reduced blood glucose levels in a dose-dependent manner, presumably polyphenolic compounds may explain these activities (Lee *et al*., 2016). Therefore, combining *P. lobata* root, *S. maydis* and *P. mume* can not only improve insulin resistance effectively but also help to promote insulin secretion and exert their synergistic function to reduce blood glucose and other active effects.

To date, herbal tea has become a popular beverage worldwide, especially in China, for its aroma, antioxidant properties, therapeutic applications, and other beneficial effects on health (Jin *et al*., 2016). Although there are many previous studies on *P. lobata* root, *S. maydis*, and *P. mume*, this study is the first time to develop a hypoglycemic *P. lobata* root compound tea bag by combining the three raw materials and exerting their synergistic effect. As shown in Fig. 1, we use the sensory test, total polysaccharide, and phenolic content as the inspection indicators to study the formula. Then, the α-amylase and α-glucosidase inhibition rates were used as criteria to evaluate the auxiliary hypoglycemic effect *in vitro* and optimize the brewing process using RSM-BBD 3-factor-3-level experimental design, to guide the optimal selection of brewing conditions for both tea manufacturers and consumers.

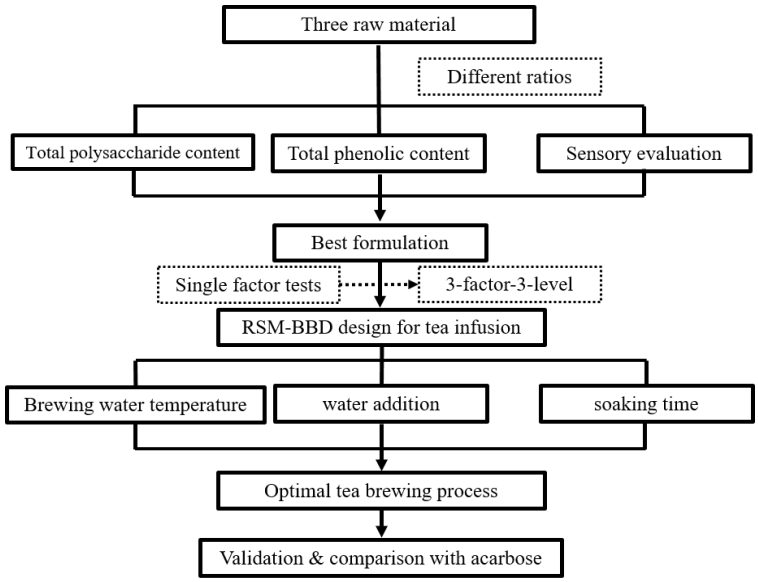

Fig. 1: Experimental design flow chart

Materials and Methods

Materials and Reagents

P. lobata root, *S. maydis*, and *P. mume* were provided by Lejiake Food Co., Ltd. Porcine pancreatic α-amylase (≥5 units mg^{-1}), α-glucosidase (50 units mg^{-1}) and gallic acid (≥98%) were obtained from Shanghai Yuanye Biotechnology Co. (Shanghai, China). Soluble starch, Acarbose (≥95%), 3,5-Dinitrosalicylic acid (≥99%), p-nitrophenyl-α-D-glucopyranoside (pNPG), and other chemicals of analytical grade were purchased from Aladdin Co., Ltd. (Shanghai, China). The water used in this experiment was double-distilled.

Optimization of P. lobata Root Compound Teabag

Based on a large number of preliminary experiments, each sample with a total weight of 12 g was grounded using FSJ-1000A high-speed pulverizer (Xinjiate, China) and passed through an 80-mesh sieve. Six different ratios of *P. lobata* root: *S. maydis*: *P. mume* (1:1:1, 1:2:1, 1:1:2, 2:1:1, 2:1:2, 2:2:1, m:m:m) were compared by taking the sensory test scores, total polysaccharide, and phenolic contents as the evaluation indicators to obtain the best formula. The non-woven fabric was used as tea bag material. Then the brewing water temperature, soaking time, and brewing water volume were selected as single factors to determine the optimal infusion condition of each factor (single-factor results not shown). According to the results, using a 3-factor-3-level RSM - BBD with five replicates at the center point to develop predictive models for different responses, thereby determining the optimal brewing process.

Sensory Evaluation

Twelve trained panelists (six males, six females, 25-50 years old) from the Shandong University of Technology in Food Science and Engineering rated the tea infusions on a scale from 1 to 10. Sensory attributes were assessed as far as sweetness, bitterness, astringency, sourness, and overall flavor of the tea extract. Each of the above features was rated using a 10-point scale (Xu *et al*., 2017), where 8-10 is "Very Strong", 6-8 is "Strong", 4-6 is "Neutral" and 2-4 "Weak" and 0-2 "Very Weak", also scored 0-10 for overall flavor/acceptability.

Total Polysaccharide Content Assay

P. lobata root, *S. maydis*, and *P. mume* possess high anti-diabetes potential arising mainly from their polysaccharide and polyphenol content. The polysaccharide content of samples was measured according to the phenol-sulfuric acid colorimetric method (Gao *et al*., 2021) with some modifications. Briefly, a mixture containing 200 μL tea extract, 200 μL of phenol (freshly prepared, 5%, w/v), and 700 μL of sulfuric acid was mixed and incubated under 80°C for 10 min. After cooling, the absorbance was determined at 490 nm using a UV-2600 ultraviolet spectrophotometer (Shimadu, Japan). The distilled water was used as a blank and glucose as the standard. A series of different concentrations of standard glucose (0, 20, 40, 60, 80, and 100 μg/mL) was constructed for the standard curve.

Total Phenolic Content Assay

The total phenolic content was analyzed according to the protocol described before with some modifications (Almeida *et al*., 2019). 100 μL of the aqueous sample was mixed with 100 μL Folin-Ciocalteu reagent, the reaction mixture was vigorously shaken and left to stand at room temperature for 10 min. 300 μL of Na_2CO_3 solution (2%, w/v) was added and the vial was left in dark for 2 h before the absorbance was measured at 760 nm. Gallic acid was used as standard and the results were expressed in milligrams of gallic acid per gram of sample.

Inhibition Assays of α-Amylase

The inhibition assay of α-amylase was performed by referring to a previously published method (Hussain *et al*., 2018) with some modifications. 100 μL of aqueous tea extract was mixed with 100 μL of 0.02 m sodium phosphate buffer (pH 6.9) containing α-amylase solution (0.5 U/mL) and incubated at 37°C for 10 min. Then, 100 μL of a 1.0% starch solution was added. The reaction mixtures were incubated at 37°C for another 10 min. 200 μL of DNS reagent was added and mixed well, followed by incubation in a boiling water bath for 10 min to generate the color. After cooling to room temperature, the reaction mixture was then diluted by adding 10 mL of distilled water. An optical density of 540 nm was used for the measurement of the absorbance. Acarbose was prepared in sodium phosphate buffer and used as a positive control. The percentage inhibition was calculated using the formula:

$$Inhibition\ rate(\%) = \frac{\left(A_{control} - A_{sample}\right)}{A_{control}} \times 100\%$$

where, $A_{control}$ and A_{sample} are the absorbance of the control and tea extract, respectively.

Inhibition Assays of α-Glucosidase

The inhibitory assay of α-glucosidase was carried out based on previous literature (Zheng *et al*., 2020), with slight modification. As shown in Fig. 2, the α-glucosidase was prepared by dissolving it in 0.05 m PBS (pH 6.9) to obtain 1.0 mg/mL enzyme solution. Then 50 μL of α-glucosidase was mixed with 50 μL sample solution and 50 μL PBS. After shaking and incubation under 37°C at 500 rpm for 10 min. The hydrolysis process was initiated with the addition of 100 μL pNPG solution (6.0 mm) as the substrate at 37°C. After 20 min, the reaction was terminated by adding 1 mL 1.0 M Na_2CO_3. The absorbance of the solution was recorded at 400 nm using

an ultraviolet spectrophotometer. Acarbose was used as a positive control. The inhibition rate of tea extract was calculated based on the following formula:

$$Inhibition\ rate(\%) = \frac{\left(A_{control} - A_{controlblank}\right) - \left(A_{sample} - A_{sampleblank}\right)}{A_{control} - A_{controlblank}} \times 100\%$$

where, $A_{control}$, $A_{control\ blank}$, A_{sample}, *and* $A_{control\ blank}$ represent the optical density of the sample containing enzyme and PBS, PBS without enzyme, sample solution with enzyme, and sample solution, respectively.

Response Surface Experimental Design

In the response surface experiment, RSM using BBD was used to investigate the relationship between the three independent variables (X_1, brewing water temperature; X_2, amount of water addition; X_3, soaking time), which were coded at three levels (-1, 0, 1) and two dependent variables (Y_1, Inhibition rate of α-amylase; Y_2, Inhibition rate of α-glucosidase) in this study (Table 1). The ranges of all parameters in RSM were chosen based on the results of single-factor experiments according to our previous study. The brewing water temperature, amount of water addition, and soaking time varied from 80 to 100°C, 100 to 200 mL, and 3 to 9 min, respectively. The values of the independent variables and the experiment design were shown in Table 2. The complete design was performed in random order, consisting of 17 combinations including 5 center point repeats.

Statistical Analysis

The experimental design and statistical analysis of the results of RSM were executed by Design-Expert Version software (version 8.0.6.1, State-Ease, Inc., Minneapolis, MN, USA) was used for BBD experimental design.

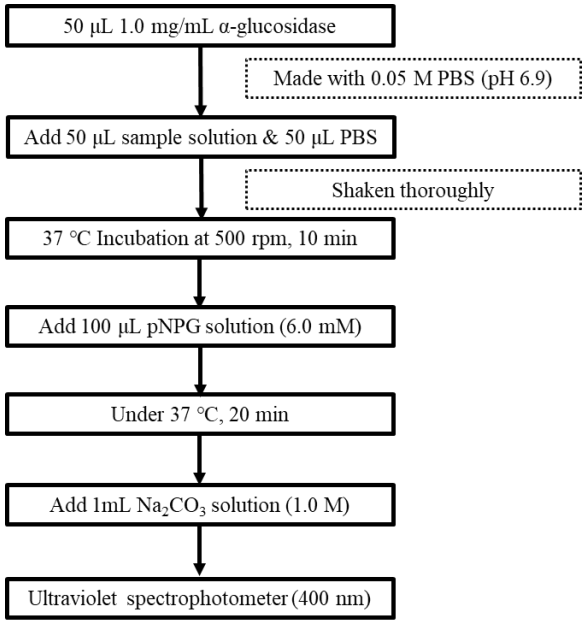

Fig. 2: Flow chart of α-glucosidase inhibition assay

Table 1: Independent variables and their code variable levels used for the Box-Behnken design

		Coded levels			
No.	Independent variables	Symbol	-1	0	1
1	Brewing water temperature (°C)	X_1	80	90	100
2	Amount of water addition (mL)	X_2	100	150	200
3	Soaking time (min)	X_3	3	6	9

Table 2: Box-Behnken experimental design and corresponding response values

Std	Run	X_1 (°C)	X_2 (mL)	X_3 (min)	Y_1 (%)	Y_2 (%)
3	1	80	100	6	36.22	42.02
10	2	90	150	6	45.56	59.26
8	3	80	150	3	37.41	42.52
13	4	100	100	6	30.11	32.86
17	5	90	100	9	38.54	40.88
4	6	80	200	6	30.21	33.78
1	7	100	150	3	42.19	43.02
6	8	90	150	6	45.75	62.22
14	9	100	200	6	42.88	42.08
15	10	90	150	6	47.07	60.43
16	11	90	150	6	47.09	59.84
7	12	90	200	9	38.29	42.89
12	13	90	100	3	33.41	38.62
5	14	90	200	3	41.38	40.03
11	15	100	150	9	36.17	43.07
9	16	90	150	6	47.34	62.97
2	17	80	150	9	36.06	46.32

X_1, Brewing water temperature (°C); X_2, Amount of water addition (mL); X_3, Soaking time (min); Y_1, Inhibition rate of α-amylase (%); Y_2, Inhibition rate of α-glucosidase (%)

Significance analysis and one-way analysis of ANOVA by Duncan's test were conducted with SPSS 22.0 software. The experiments were performed in triplicate and all data were indicated as the means ± Standard Deviation (SD) and differences were regarded as significant at $p<0.05$.

Result and Discussion

Determination of the Best Formula

The final formula of raw material was determined based on the results of sensory evaluation, total polysaccharide content, and total polyphenol content. The sensory attributes of the six different ratios were measured for sweetness, bitterness, astringency, sourness, and overall flavor (Fig. 3). Among six formulas, the one with a 2:2:1 ratio of the three raw materials had moderate bitterness, lower astringency, higher sweetness and the highest in overall flavor, suggesting that the recipe could be better accepted by consumers.

Polysaccharides have relatively high molecular weights and are composed of thousands of monosaccharide molecules. Studies have shown that polysaccharides with hypoglycemic effect can be used for adjuvant therapy of diabetes, which can promote glucose utilization, increase the number of insulin receptors, protect β cells, improve insulin sensitivity to receptors, stabilize insulin and inhibit glucosidase activity (Gong *et al*., 2020). In addition, phenolic compounds are an important class of natural organic compounds that are widely distributed in nature. Phenolic compounds prevent and treat diabetes mainly by affecting islet β cell function and resisting lipid peroxidation.

The total polysaccharide content and the total phenolic content of different formulas are summarized in Fig. 4 and 5, respectively. According to the results, the total polysaccharide content ranged from 524.59±11.23 to 662.12±13.75 μg/mL and the total phenolic content from 164.31±6.48 to 273.57±12.04 GAE mg/g at different raw material ratios, respectively. The polysaccharide and phenolic content of ratio 2:2:1 were significantly higher than that of other ratios ($p<0.05$), indicating that the formula of 2:2:1 possessed the highest amount of polysaccharide and phenolic substances. Therefore, considering all the results above, the formulation with a ratio of 2:2:1 was selected for subsequent further optimization.

The Response Surface Method Fitting Model

α-Amylase and α-glucosidase are important enzymes in the digestive system which hydrolyze dietary carbohydrates and ultimately produce absorbable glucose. Therefore, inhibition of α-amylase and α-glucosidase can reduce and control postprandial blood glucose levels, which is an effective way of relieving and treating diabetes (Pantidos *et al*., 2014). Three-factor-three-level experimental design of RSM-BBD was used to investigate the effects of three variables: Brewing water temperature, amount of water addition, and soaking time on the inhibition ability of α-amylase and α-glucosidase of the aqueous tea extract of *P. lobata* root compound. The design matrix and the corresponding responses are summarized in Table 2. The data was employed for multiple linear regression analysis with a quadratic polynomial model. The results of fitting the quadratic models were shown in Tables 3 and 4. The result showed that the p-values were calculated to be 0.0001 and <0.0001 for the models of two responses (inhibition rate of α-amylase and α-glucosidase), respectively. Both were extremely significant ($p<0.01$), indicating that the models constructed in this study have significant regression. Furthermore, the result showed that p-values for the lack of fit were 0.0669 and 0.9017, respectively. Both were greater than 0.05 and not significant, indicating that the mathematical models were acceptable in predicting the inhibition ability of α-amylase and α-glucosidase of *P. lobata* root compound tea extract. The coefficient of determination (R^2) is the square of the correlation coefficient and accounts for the adequacy of the model. R^2 ranges from 0 to 1. The higher the value of R^2, the better the fit of the model (Hayta and İşçimen, 2017). As shown in Tables 3 and 4, the R^2 values for the two reactions (inhibition rate of α-amylase and α-glucosidase) were 0.9727 and 0.9930, respectively, revealing a close correlation between the predicted and experimental values.

Development of Second-Order Polynomial Mathematical Models

The final regression equations for α-amylase and α-glucosidase inhibition rates were developed respectively as follows:

$$Y_1 = 46.56 + 1.43X_1 + 1.81X_2 - 0.67X_3 + 4.7X_1X_2 - 1.17X_1X_3 - 2.06X_2X_3 - 5.83X_1^2 - 2.78X_3^2 \quad (1)$$

$$Y_2 = 60.94 - 0.45X_1 + 0.55X_2 + 1.12X_3 + 4.37X_1X_2 - 0.94X_1X_3 + 0.15X_2X_3 - 10.07X_1^2 - 13.19X_2^2 - 7.15X_3^2 \quad (2)$$

The experimental data obtained by the Box-Behnken design were fitted to the second-order polynomial mathematical equation by performing multiple regression analysis on the experimental data and an empirical model was established. The model can be fit to the above second-order polynomial Eq. (1)-(2). In the above

formulas, the positive sign in front of the terms (X_1, X_2, and X_3) indicated synergy, and the negative signs indicate antagonism. In addition, the higher the numerical value of the preceding term, the greater the influence of the coefficient (Thakur *et al*., 2017).

Effect of Independent Variables on Inhibition Rates of α-Amylase

α-Amylase is a key carb hydrolyzing enzyme that at first catalyzes starchy parts, delivering reducing sugars, like maltose, maltotriose, maltooligosaccharides, and so forth and these reducing sugars are additionally hydrolyzed by α-glucosidase to glucose that is at long last consumed by enterocytes in the small intestine digestive system. Consequently, repressing the movement of α-amylase by presenting exogenous chemical inhibitors has been viewed as powerful in controlling blood glucose levels after dinners through deferring starch absorption (Warren *et al*., 2015; Sun and Miao, 2020). The inhibition rates of α-amylase concerning brewing water temperature, amount of water addition, and soaking time. Results showed that the inhibition rate of α-amylase of *P. lobata* root compound tea extract varies from 30.11 to 47.34% with the change of different brewing parameters (Table 2). As illustrated in Table 3, coefficients of X_2, X_1X_2, X_1^2, X_2^2, and X_3^2 were found to be extremely significant ($p<0.01$), X_1 and X_2X_3 were significant ($p<0.05$) in α-amylase inhibition activity of *P. lobata* root compound teabag, indicating that a good model was obtained from the above experimental results. However, X_3 and X_1X_3 did not show a significant impact on the inhibition activity, revealing that the interaction effect of brewing temperature and water addition on the α-amylase inhibition activity of tea was not significant. The result in Fig. 6A, is when the brewing water temperature (X_1) moved to the peak value with the amount of water addition (X_2), the inhibition rate of α-amylase increased first and then decreased and the maximum value appeared when the brewing water temperature (X_1) was 90°C and the amount of water addition (X_2) was 150 mL. Moreover, similar trends also appeared in Fig. 6B and C, within certain ranges, with the increase of the two variables (X_1X_3 and X_2X_3) the inhibition rate of α-amylase gradually increased, and when it reached the highest point, it showed a downward trend. It could be due to the long-term high-temperature soaking that will destroy the biological structure of polysaccharides, phenolic compounds, and other active substances, resulting in the oxidative polymerization of polyphenols in the tea soup. Thus, the quality of the tea extract was greatly reduced.

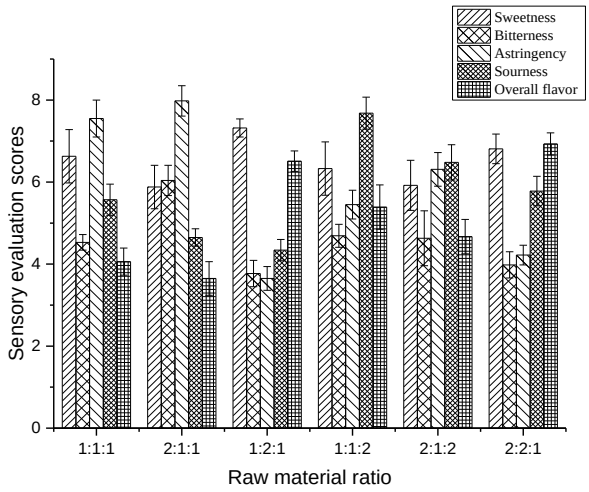

Fig. 3: The effect of different ratios of *P. lobate* root compound tea bag raw materials on the sensory evaluation

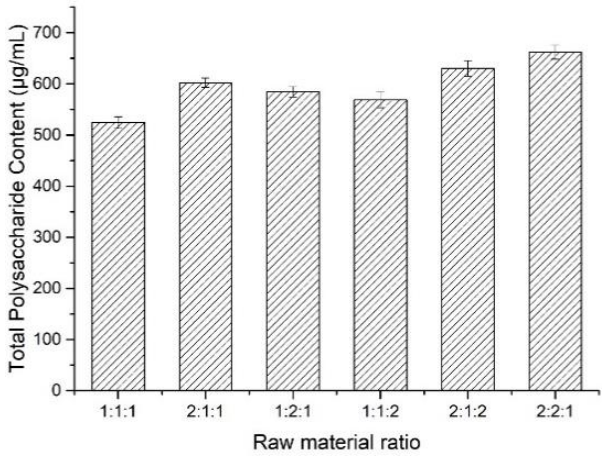

Fig. 4: The effect of different ratios of *P. lobate* root compound tea bag raw materials on the total polysaccharide content

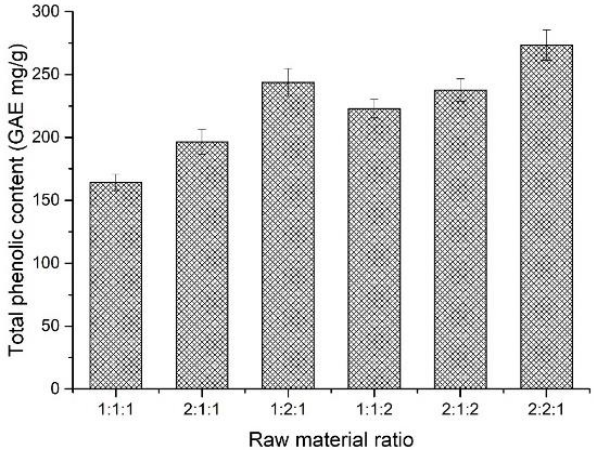

Fig. 5: The effect of different ratios of *P. lobate* root compound tea bag raw materials on the total phenolic content

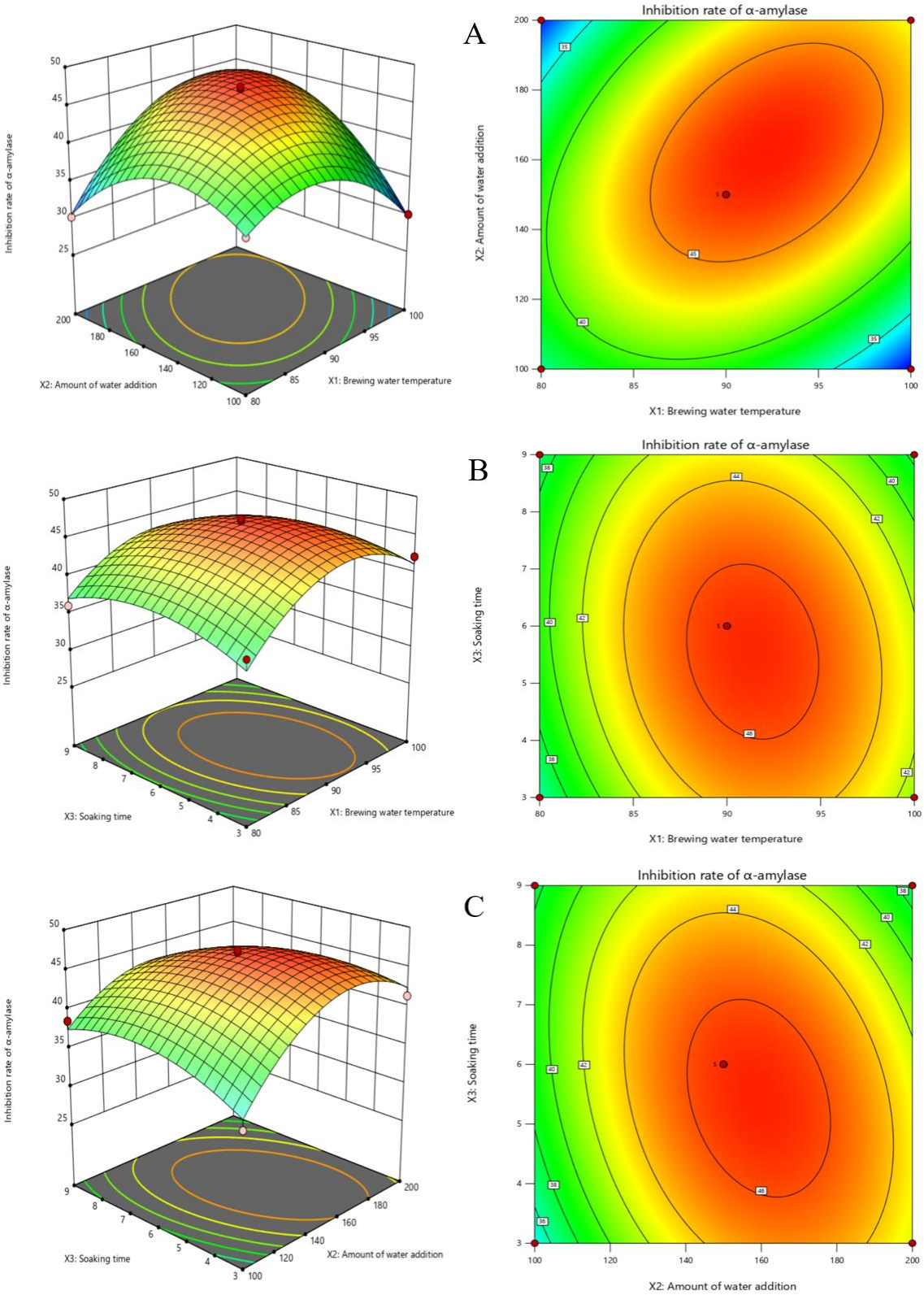

Fig. 6: Response surface plots (3D) and contour plots of inhibition rates of α-amylase as a function of significant interaction between factors. (A) Brewing water temperature and amount of water addition; (B) Brewing water temperature and soaking time; the (C) amount of water addition and soaking time

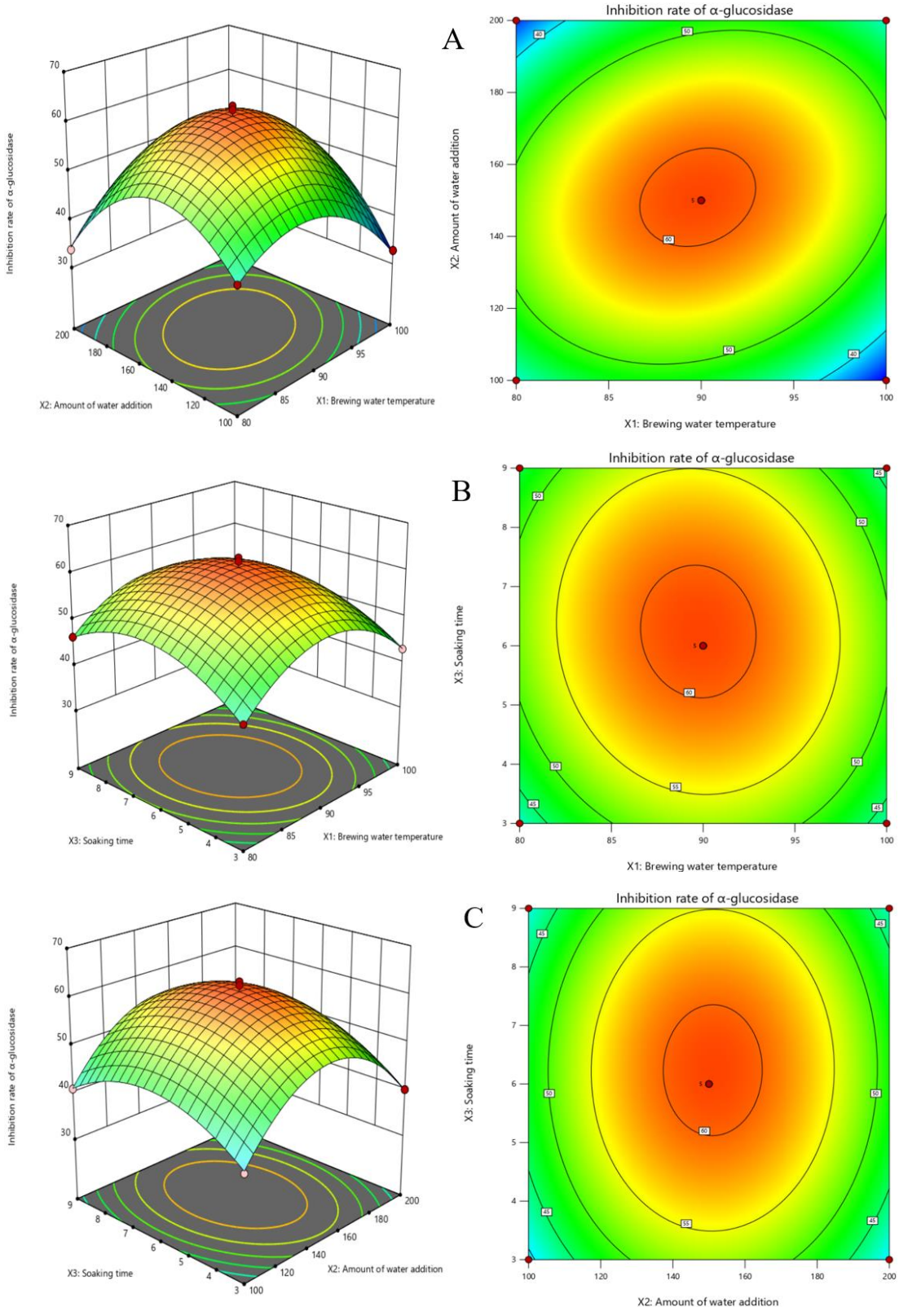

Fig. 7: Response surface plots (3D) and contour plots of inhibition rates of α-glucosidase as a function of significant interaction between factors. (A) Brewing water temperature and amount of water addition; (B) Brewing water temperature and soaking time; the (C) amount of water addition and soaking time

Table 3: Analysis of variance for the fit quadratic model of inhibition rate of α-amylase

Source	Sum of	df	Mean	F-value	p-value	
Model	510.99	9	56.7800	27.72	0.0001	Significant
X_1	16.39	1	16.3900	8.00	0.0255	
X_2	26.21	1	26.2100	12.80	0.0090	
X_3	3.55	1	3.5500	1.73	0.2294	
X_1X_2	88.17	1	88.1700	43.05	0.0003	
X_1X_3	5.45	1	5.4500	2.66	0.1468	
X_2X_3	16.89	1	16.8900	8.25	0.0239	
X_1^2	142.98	1	142.9800	69.80	<0.0001	
X_2^2	145.56	1	145.5600	71.07	<0.0001	
X_3^2	32.48	1	32.4800	15.86	0.0053	
Residual	14.34	7	2.0500			
Lack of Fit	11.53	3	3.8400	5.48	0.0669	Not significant
Pure Error	2.81	4	0.7014			
Cor Total	525.33	16				
	$R^2 = 0.9727$					

X_1, Brewing water temperature (°C); X_2, Amount of water addition (mL); X_3, Soaking time (min), df = Degree of freedom

Table 4: Analysis of variance for the fit quadratic model of inhibition rates of α-glucosidase

Source	Sum of	df	Mean	F-value	p-value	
Model	1617.2100	9	179.6900	109.9100	<0.0001	Significant
X_1	1.6300	1	1.6300	0.9964	0.3514	
X_2	2.4200	1	2.4200	1.4800	0.2632	
X_3	10.0600	1	10.0600	6.1500	0.0422	
X_1X_2	76.2100	1	76.2100	46.6200	0.0002	
X_1X_3	3.5200	1	3.5200	2.1500	0.1860	
X_2X_3	0.0900	1	0.0900	0.0551	0.8212	
X_1^2	426.6100	1	426.6100	260.9400	<0.0001	
X_2^2	732.8900	1	732.8900	448.2900	<0.0001	
X_3^2	215.0000	1	215.0000	131.5100	<0.0001	
Residual	11.4400	7	1.6300			
Lack of Fit	1.3900	3	0.4641	0.1847	0.9017	Not significant
Pure Error	10.0500	4	2.5100			
Cor Total	1628.6600	16				
	$R^2 = 0.9930$					

X_1, Brewing water temperature (°C); X_2, Amount of water addition (mL); X_3, Soaking time (min), df = Degree of freedom

Table. 5: Predicted and experimental response values at optimum conditions

Response	Predicted value	Experimental value (n = 3)	Acarbose (0.02 mg/mL)
Inhibition rate of α-amylase (%)	46.82±3.22	45.09±1.13	45.87±2.04
Inhibition rate of α-glucosidase (%)	60.77±4.66	60.52±2.47	54.92±5.76

Effect of Independent Variables on Inhibition Rates of α-glucosidase

After ingestion of starchy foods, starch is first digested by α-amylase and then the reducing sugars are further hydrolyzed by α-glucosidase at the brush border of the gut, producing glucose that is adsorbed into the portal blood via glucose transporters. Hence, it has been reported that inhibition of carbohydrate hydrolases may regulate starch digestion, thereby controlling blood glucose levels (Warren *et al*., 2015; Sun *et al*., 2019). The inhibition rate of α-glucosidase of *P. lobata* root compound tea extract varied from 32.86 to 62.97% in the RSM experiments (Table 2). As shown in Table 4, except X_1, X_2, X_1X_3, and X_2X_3, the coefficients were all significant at the 95% confidence level affecting the inhibition rate of α-glucosidase of *P. lobata* root compound teabag. It revealed that the equation established by the model had a good fitting, which indicated that the measurement method was reliable and the design of each factor was appropriate and reasonable. Figure 7A-C intuitively showed the 3D and contour analysis plots of the response surfaces for each factor interaction. The contour line under the three interactions (X_1X_2, X_1X_3, and X_2X_3) are all typical ellipses (Fig. 7A-C). In addition, the results of the interaction of the three variables (X_1, X_2, and X_3) showed that there was an extreme value in the selected range, which was not only the highest point of the response surface but also the center point of the contour ellipse. As shown in Fig. 7A, with the increase of brewing

water temperature (X_1) and amount of water addition (X_2), the curve of α-glucosidase inhibition rate showed a trend of increasing first and then decreasing and the interaction of the two variables had an extremely significant impact ($p<0.01$) on the inhibition rate of α-glucosidase of *P. lobata* root compound tea extract, which was consistent with the results of ANOVA analysis. As shown in Fig. 7B and C, there were interactions between X_1X_3 and X_2X_3, but the interactions on the inhibition rate of α-glucosidase were not significant ($p>0.05$).

Optimization and Validation of Brewing Conditions

By optimizing the brewing water temperature, water addition amount, and soaking time, the brewing conditions with the highest inhibition rates of α-amylase andα-glucosidase were optimized by RSM-BBD. Based on the influence of three independent variables on the response value of the two enzyme inhibition rates, the optimal brewing conditions for the *P. lobata* root compound teabag were determined as brewing water temperature of 90.8℃, amount of water addition of 155 mL and soaking time 5.9 min. To verify these predicted brewing conditions, this brewing method was tested in triplicate experiments, which showed that the actual and predicted values for inhibition rates of α-amylase andα-glucosidase were similar (Table 5). The inhibition rate of α-amylase and α-glucosidase were detected to be 45.09±1.13 and 60.52±2.47%, respectively, and no significant differences between the predicted and the experimental values. The inhibitory effect of this tea on α-amylase is similar to that of acarbose with a concentration of 0.02 mg/mL (inhibition rate of 45.87%) without significant difference, while the inhibition rate of α-glucosidase activity is significantly higher ($p<0.05$) than that of acarbose (inhibition rate of 54.92%), indicating that P. *lobata* root compound tea with good anti-diabetic activity.

Conclusion

In this study, the formula of *P. lobata* root compound tea bags was developed by compounding *P. lobata* root, *S. maydis*, and *P. mume* as raw materials. The sensory evaluation, total polysaccharide, and total phenolic content were studied as evaluation criteria to determine the compound ratio of raw materials. The brewing process was optimized using a 3-factor-3-level RSM–BBD experimental design and the optimal infusion parameters for making the teabags were obtained as follows: The brewing water temperature was 90.8℃, the amount of water addition was 155 mL and the soaking time was 5.9 min. Under these conditions, the inhibition rates of α-amylase and α-glucosidase activities were as high as 45.09 and 60.52% and the inhibitory activity of α-glucosidase was significantly better than that of acarbose (54.92%), the inhibition rate of α-amylase activity was not significantly different from that of acarbose (45.87%).

Diet has been recognized as the foundation of diabetes management. Before the introduction of the therapeutic use of insulin, the diet was the main form of treatment for the disease and dietary measures included the use of culinary herbs. The tea bag is convenient to carry, easy to brew, unique in aroma, high in taste acceptance, rich in active ingredients of total polysaccharides and phenolic compounds, and has an excellent hypoglycemic effect. Therefore, this tea bag enriches the types of natural hypoglycemic functional foods and has a broad market prospect. As this study is extensive, in future studies *in vivo* validation experiments will be considered, combined with advanced chromatography techniques, to extract, isolate and purify the active compounds individually from the plants under study.

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Author's Contributions

Qing Liu: Participated in project design, the whole experiment process, and manuscript writing.

Wendy Shi, Futing Xue and Chen Wu: Participated in teabag preparation, indicators determination, and data curation.

Hassan Mohamed: Particiated in manuscript revisement.

Min Li: Participated in sample preparation.

Yuanda Song: Participated in experimental guidance and manuscript revisement.

Ethics

The authors declared no ethical issues that may arise after the publication of this manuscript.

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