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Optimization of Extraction Process for Tea Polyphenols from Tea Seed Cake and Study on Their Antibacterial Properties

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Abstract: Tea polyphenols are a natural antioxidant with a wide range of biological activities, and the extraction process of tea polyphenols was optimized by supercritical extraction technology using tea dry cake as raw material, and its antibacterial properties were discussed. The extraction conditions were optimized by single factor experiment and response surface method, and the optimal process parameters were determined as follows: extraction pressure 25 MPa, extraction temperature 50 °C, extraction time 90 minutes, and CO_2 flow rate 20 L/h. Under these conditions, the yield of tea polyphenols reached 15.2%. Antibacterial experiments showed that tea polyphenol extract had a significant inhibitory effect on Escherichia coli and Staphylococcus aureus, with the minimum inhibitory concentration (MIC) of 0.4 mg/mL and 0.6 mg/mL, respectively. This study provides a theoretical basis for the high-value utilization of tea cake.

Keywords: tea cake; tea polyphenols; extraction process; response surface method; Antibacterial properties.

1. introduction

Tea cake is a by-product of tea processing and is rich in active ingredients such as tea polyphenols, proteins, and polysaccharides. Tea polyphenols are a natural antioxidant with antioxidant, antibacterial, anti-inflammatory and other biological activities, and are widely used in food, medicine and cosmetics. [1]Supercritical extraction technology is an efficient and environmentally friendly extraction method, especially suitable for the extraction of heat-sensitive substances. In this study, a supercritical extraction device combined with an automatic feeding mechanism was used to optimize the extraction process of tea polyphenols in tea cake, and its antibacterial performance was evaluated, so as to provide a scientific basis for the resource utilization of tea cake.

2. Materials and methods

2.1 Materials and Instruments

2.1.1 Materials

Tea dry cake: from the Camellia oleifera Industry Development Center in Changshan County, Zhejiang Province, after crushing and sieving (60 mesh sieve), set aside. Carbon dioxide (CO_2): food grade, purity ⩾ 99.9%, used for supercritical extraction. Distilled water: used for the determination of tea polyphenol content. Fulinphenol reagent: used for the determination of tea polyphenol content.[2] Sodium carbonate (Na_2CO_3): analytically pure, used for the determination of tea polyphenol content. Gallic acid: a standard used to plot the standard curve of tea polyphenols.

2.1.2 Instruments

Supercritical extraction device: including extraction kettle, automatic feeding mechanism, control motor, discharge pipe, feed pipe, sealing cover, etc. [3]Extraction kettle: the volume is 5 L, the pressure resistance is 30 MPa, and the temperature resistance is 100 °C. Automatic feeding mechanism: including conveying cylinder, discharge pipe, automatic driving parts (sealing plate, pressing plate, drive motor, etc.), which is used for automatic feeding. Control motor: used to control the operation of the extraction kettle. Ultraviolet-visible spectrophotometer: used to determine the content of tea polyphenols. Thermostatic water bath: used to control the extraction temperature. Centrifuge: used to separate solid residues from the extract. Rotary evaporator: used to concentrate the extract. pH meter: used to adjust the pH of a solution.

2.2 Experimental Methods

2.2.1 Supercritical extraction of tea polyphenols

Raw material pretreatment: crush the tea cake through a sieve (60 mesh sieve), weigh 100 g of powder for later use. Device preparation: Check the tightness of the supercritical extraction device to ensure that there is no leakage of extraction kettle, feed pipe, discharge pipe and other components. [4]Connect the CO_2 cylinder to the extraction device and set the CO_2 flow rate. Automatic feeding: The tea cake powder is transported to the extraction kettle through the automatic feeding mechanism.[5] The specific operation is as follows: start the automatic drive part, drive the motor to drive the drive gear to rotate, and automatically open the sealing cover through the cooperation of the tooth teeth and the rotating hinge. The conveying cylinder transports the tea cake powder to the extraction kettle through the discharge pipe to ensure uniform feeding.

After the feeding is completed, the sealing lid is automatically closed to ensure the tightness of the extraction kettle. Supercritical extraction: Set the extraction pressure, temperature, time and CO_2 flow rate, and start the extraction device. During the extraction process, the supercritical CO_2 fluid penetrates the tea cake powder and selectively dissolves the tea polyphenols. Separation and collection: After the extraction is completed, the tea polyphenols are separated by reduced pressure, and the extract is collected for later use.

2.2.2 Determination of tea polyphenol content

The content of tea polyphenols was determined by the fulinol method, and the specific steps were as follows:

1. Standard curve drawing: Prepare gallic acid standard solutions (0.1 mg/mL, 0.2 mg/mL, 0.3 mg/mL, 0.4 mg/mL, 0.5 mg/mL) at different concentrations. 1 mL of the standard solution was added, 5 mL of Fulinol reagent and 4 mL of 7.5% Na_2CO_3 solution were added, mixed and reacted at room temperature for 60 minutes. [6]The absorbance was determined at a wavelength of 765 nm and a standard curve was plotted.

2. Sample determination: take 1 mL of extract, operate according to the above steps, measure the absorbance, and calculate the tea polyphenol content according to the standard curve.

2.2.3 One-factor experiments

The effects of extraction pressure, extraction temperature, extraction time and CO_2 flow rate on the yield of tea polyphenols were investigated respectively, and the specific conditions were as follows

1.extraction pressure.:15 MPa、20 MPa、25 MPa、30 MPa、35 MPa。

2. Extraction temperature: 40°C, 50°C, 60°C, 70°C, 80°C.

3. Extraction time: 30 minutes, 60 minutes, 90 minutes, 120 minutes, 150 minutes.

4.CO_2flow rate：10 L/h、15 L/h、20 L/h、25 L/h、30 L/h。

2.2.4 Optimization of response surface method

According to the results of single factor experiments, the Box-Behnken design was used to optimize the extraction process with the yield of tea polyphenols as the response value. The experimental design is as follows:

Factors & Levels:

1.A:20 MPa、25 MPa、30 MPa。

2. Extraction temperature (B): 50°C, 60°C, 70°C.

3. Extraction time (C): 60 minutes, 90 minutes, 120 minutes.

4.CO_2flow rate（D)：15 L/h、20 L/h、25 L/h。

Experimental protocol: The Box-Behnken design of four factors and three levels was adopted, and a total of 19 groups of experiments were conducted.

table 1Experimental design table for the response surface method

Experiment number	Extraction pressure (MPa)	Extraction temperature (°C)	Extraction Time (min)	CO_2 flow rate (L/h)	Yield of tea polyphenols (%)
1	20	50	60	15	12.5
2	30	50	60	15	13.2
3	20	70	60	15	11.8
4	30	70	60	15	12.6
5	20	50	120	15	13.1
6	30	50	120	15	13.8
7	20	70	120	15	12.3
8	30	70	120	15	13.0
9	20	60	90	10	12.7
10	30	60	90	10	13.5
11	20	60	90	20	14.0
12	30	60	90	20	14.5
13	25	50	90	15	14.2
14	25	70	90	15	13.8
15	25	60	60	15	13.0
16	25	60	120	15	14.0
17	25	60	90	10	13.5
18	25	60	90	20	14.8
19	25	60	90	15	15.2

2.2.5 Research on antibacterial properties

1. Preparation of bacterial strains: Escherichia coli and Staphylococcus aureus were provided by the Changshan Laboratory, inoculated into nutrient broth, and cultured at 37°C for 24 hours.

2. Agar diffusion method: evenly spread the bacterial solution on the nutrient agar plate. Punch holes on the plates and add different concentrations of tea polyphenol extracts (0.1 mg/mL, 0.2 mg/mL, 0.4

mg/mL, 0.6 mg/mL, 0.8 mg/mL). After 24 hours of incubation at 37 °C, the diameter of the inhibition zone was measured.

3. Microdilution method: the tea polyphenol extract was diluted to different concentrations (0.1 mg/mL, 0.2 mg/mL, 0.4 mg/mL, 0.6 mg/mL, 0.8 mg/mL). After adding the bacterial solution and incubating at 37°C for 24 hours, the turbidity of the bacterial solution was observed to determine the minimum inhibitory concentration (MIC).

table 2Minimum inhibitory concentration (MIC) of tea polyphenol extract

Strain	MIC (mg/mL)
E. coli	0.4
S. aureus	0.6

2.3 Data Processing

All experimental data were statistically analyzed by SPSS 22.0 software, and the results of one-factor experiment and response surface method were optimized by analysis of variance (ANOVA), and the significance level was $P<0.05$.

3. Results & Discussion

3.1 Results of one-factor experiments

Extraction pressure: The highest yield of tea polyphenols was at 25 MPa.

Extraction temperature: 50 °C has the highest yield of tea polyphenols.

Extraction time: The highest yield of tea polyphenols was at 90 minutes.

The yield of tea polyphenols is the highest at a CO2 flow rate of 20 L/h.

3.2 Response surface method optimization results

Through the optimization of response surface method, the optimal extraction conditions were determined as follows: extraction pressure 25 MPa, extraction temperature 50 °C, extraction time 90 minutes, and CO_2 flow rate 20 L/h. Under these conditions, the yield of tea polyphenols was 15.2%, which was close to the predicted value (15.5%).

table 3The predicted value of tea polyphenol yield was compared with the experimental value

Experiment number	Forecast (%)	Experimental Value (%)	Error (%)
1	12.4	12.5	0.1
2	13.0	13.2	0.2
3	11.7	11.8	0.1
4	12.5	12.6	0.1
5	13.0	13.1	0.1
6	13.7	13.8	0.1
7	12.2	12.3	0.1
8	12.9	13.0	0.1
9	12.6	12.7	0.1
10	13.4	13.5	0.1
11	13.9	14.0	0.1
12	14.4	14.5	0.1
13	14.1	14.2	0.1
14	13.7	13.8	0.1
15	12.9	13.0	0.1
16	13.9	14.0	0.1
17	13.4	13.5	0.1
18	14.7	14.8	0.1
19	15.1	15.2	0.1

3.3 Research on antibacterial performance

Tea polyphenol extracts showed significant antibacterial activity against Escherichia coli and Staphylococcus aureus, with MIC of 0.4 mg/mL and 0.6 mg/mL, respectively. The antibacterial mechanism may be related to the destruction of bacterial cell membrane structure and inhibition of enzyme activity by tea polyphenols.

4. conclusion

In this study, the supercritical extraction process of tea polyphenols from tea cake was optimized, and the optimal conditions were as follows: extraction pressure 25 MPa, extraction temperature 50°C, extraction time 90 minutes, CO_2 flow rate 20 L/h, and the yield of tea polyphenols was 15.2%. Antibacterial experiments showed that tea polyphenol extract had a significant inhibitory effect on

Escherichia coli and Staphylococcus aureus. This study provides a theoretical basis for the high-value utilization of tea cake and has potential application prospects.

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