

Study on Extraction of Nutrients from Coffee Production Wastewater

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Abstract: The wastewater from coffee production is rich in nutrients, especially pectin and protein, but there are few studies on the effective extraction and utilization of these resources. The purpose of this experiment is to explore the extraction methods of pectin and protein from coffee wastewater, including acid extraction, enzyme extraction and ultrasonic extraction, and to determine the content of protein and total sugar by photometer. Through experiments, we can effectively extract these valuable nutrients and provide reference for the resource utilization of coffee production wastewater.

Keywords: Coffee Waste Water; Nutrients; Extraction; Waste Recycling.

1. Introduction

Coffee production is an important agricultural product processing industry, however, in the coffee production process, the wastewater is rich in valuable nutrients, including pectin and proteins. At present, domestic and foreign research mainly focus on the treatment of coffee processing wastewater and the utilization of coffee residue resources, but little research pays attention to how to effectively extract these nutrients from coffee wastewater and reuse them. Therefore, this experimental protocol aims to explore the extraction method of pectin and protein from coffee primary processing wastewater, and determine its content, so as to provide strong support for wastewater resource utilization.

2. Materials and Methods

2.1. Materials

In the process of coffee production, wet processing is mainly used, and the water consumption for processing 1 ton of fresh fruit is 5-10 (average 7) cubic meters, 1-2 cubic meters, and very little. At present, Yunnan mainly adopts semi-wet process and water-control wet process (water is not controlled in the early stage, which fluctuates greatly), and a large amount of wastewater and waste residue are produced in the initial processing. Among them, the wastewater mainly comes from mechanical peeling, peeling glue and soaking cleaning processes, and a large number of fruit juice, pectin and pulp are mixed, and the water quality is viscous, so it is inferred that a large number of pectin should be enriched in water. Therefore, acid extraction, enzyme extraction and microwave extraction are used.

2.2. Materials and Instruments

2.3. Pectin Extraction

2.3.1. Acid Extraction Method

Prepare acidic solutions under different acidic conditions, including 3mol/l solutions of citric acid, oxalic acid, malic acid and tartaric acid. The citric acid-sodium citrate buffer solution with pH 5.6 was prepared, and the biuret reagent was prepared.

A. Citric acid extraction: take 100ml of coffee primary processing wastewater, adjust the PH to about 2.5, control the temperature to 80°C, extract for 80min, add 95% ethanol at the ratio of material to liquid of 1:20g/ml for sedimentation, filter and dry after pectin becomes cotton wool.

Table 1. Reagents

Reagent	Grade
Citric acid	AR
Oxalate	AR
malate	AR
Tartrate	AR
Sodium hydroxide	AR
Sodium citrate	AR
Anhydrous copper sulfate	AR
Potassium iodide	AR
Potassium sodium tARtrate	AR
Anhydrous ethanol solution	AR
95% ethanol solution	AR
bovine serum albumin	
glucose	AR
sulfate	
hydrochloride	AR

B. Oxalic acid extraction: 100ml of coffee primary processing wastewater is taken to adjust the PH to about 2.0, the temperature is controlled at 80°C, the extraction time is 80min, and 95% ethanol is added in the material-liquid ratio of 1:20g/ml for sedimentation.

C. Malic acid extraction: take 100ml of coffee primary processing wastewater, adjust the PH to about 2.0, control the temperature at 80°C, and extract for 80min. Other steps are the same as above.

D. Tartaric acid extraction: 100ml of coffee primary processing wastewater is taken to adjust the PH to about 1.5, the temperature is controlled at 80°C, and the extraction time is 100min. Other steps are the same as above.

2.3.2. Enzymatic Extraction Method

Cellulase extraction: adding citric acid-sodium citrate

buffer with pH of pH5.6, adding 2% cellulase, and heating in water bath at 55°C for 2 h Heat in a water bath at 100°C for 20 min to inactivate the enzyme, after cooling, transfer to a centrifugal tube for 20 min at 4000 r/min, collect the supernatant, concentrate it in a water bath to 1 /5 of the volume, then add 4 times of anhydrous ethanol, and let it stand overnight at 4°C to obtain crude polysaccharide.

2.3.3. Ultrasonic Extraction Method

Add 1.0g cellulase into 100ml wastewater from the primary processing of fresh coffee, put it in a 250ml round-bottomed flask, perform enzymolysis for 25min, extract it by ultrasonic at 50°C for 15 min, and filter to obtain the test sample.

2.4. Determination Method

2.4.1. Detection of Protein

A. Preparation of protein standard solution

Transfer 0.5 g of bovine serum protein, add a little water to dissolve it, and then put it into a 50 mL volumetric flask to obtain 10 mg/mL protein standard solution.

B. Preparation of protein standard solutions with different gradients

Measure 0.0 ml, 1.0 ml, 2.0 ml, 3.0 ml, 4.0 ml and 5.0 mL of protein standard solution in the test tube with stopper, number 1-6 respectively, and add water to 5 mL to obtain 0, 2, 4, 6, 8 and 10 mg/mL of protein standard solution.

2.4.2. Determination of Total Sugar

A. Preparation of glucose standard solution

Accurately weigh 50mg of glucose in a 500ml volumetric flask to prepare a standard solution of 0.1mg/ml.

B. Preparation of glucose solutions with different gradients

0.00 0.02 0.04 0.06 0.08 0.01mg/ml were prepared with distilled water. Add 1ml of 5% phenol solution to 1 ml of standard solution in beaker, and quickly add 5ml of concentrated sulfuric acid, let stand for 10min, shake well, let stand at 30°C for 30min, and then determine the OD value at 490nm. With glucose concentration as abscissa and OD value as ordinate, a standard curve was made.

3. Experimental Results and Analysis

3.1. Determination of Soluble Sugar

Table 2. Determination of Soluble Sugar

serial number	1	2	3	4
Coffee wastewater volume /V(mL)	2.00			
Supernatant /V(mL)	1.00			
70% ethanol /V(mL)	4.00			
Anthrone reagent /V(mL)	3.00			
A	0.143	0.126	0.137	0.124
Soluble sugar content (mg/mL)	22.816	20.096	21.856	19.776

Weigh 136g of fresh coffee in a 250mL beaker, soak it in tap water with 70% of the fresh fruit for 1 hour, and then manually peel and degum it to simulate the coffee wastewater processed by a coffee processing factory for later use.

Take 2.00mL of coffee primary processing wastewater diluted 16 times with distilled water in a 10mL beaker, add 4

ml of 70% ethanol, soak in a boiling water bath for 10min, keep stirring, take out, cool, centrifuge for 5min (or filter), and collect supernatant.

Take out 1mL of supernatant in a test tube, add 3mL of anthrone reagent, mix well, bathe in boiling water for 10min, then cool to room temperature in cold water, and measure the absorbance at the wavelength of 620nm.

3.2. Determination of Soluble Protein

Weigh 136g of fresh coffee in a 250mL beaker, soak it in tap water with 70% of the fresh fruit for 1 hour, and then manually peel and degum it to simulate the coffee wastewater processed by a coffee processing factory for later use.

Take 5.00mL of coffee primary processing wastewater in a centrifuge tube, centrifuge it for 15min at the speed of 3200 rpm below 4 degrees Celsius, then take 100uL of supernatant, add 4mL of Coomassie Brilliant Blue, and measure the absorbance at the wavelength of 595nm.

Table 3. Determination of Soluble protein

serial number	1	2	3	4
Coffee wastewater volume /V(mL)	5.00			
Take supernatant (uL)	100			
Coomassie brilliant blue (mL)	4			
A	0.63	0.70	0.62	0.63
Soluble protein content (uL)	1.114	1.4	1.073	1.114

3.3. Determination of Galacturonic Acid

Weigh 196g of fresh coffee in a 500mL beaker, soak it in tap water with 70% of the fresh fruit for 1 hour, and then manually peel and degum it to simulate the coffee wastewater processed by a coffee processing factory for later use.

Take 1.00mL of coffee processing wastewater diluted 250 times in a 10mL beaker, add 3.00mL of sodium tetraborate-sulfuric acid solution in an ice bath, mix well, heat it in a boiling water bath for 5 minutes, immediately cool it to room temperature after taking it out, add 0.10mL of 0.1% carbazole solution, shake it evenly, boil it for 5 minutes, and measure its absorbance at 530nm after cooling it to room temperature.

Table 4. Determination of galacturonic acid

serial number	1	2	3	4
Coffee wastewater /V(mL)	1.00			
Sodium tetraborate-sulfuric acid solution (mL)	3.00			
0.1% carbazole solution (mL)	0.10			
A	0.355	0.331	0.384	0.372
Galactouronic acid content (mg/mL)	8.376	7.804	9.067	1.756

3.4. Determination of Polyphenols (Folin Phenol Method)

Weigh 196g of fresh coffee in a 500mL beaker, soak it in tap water with 70% of the fresh fruit for 1 hour, and then manually peel and degum it to simulate the coffee wastewater processed by a coffee processing factory for later use.

Take 2.00mL of coffee primary processing wastewater, add 3.00mL of 95% ethanol, and extract at 60°C for 2 hours. Centrifuge at 25°C×3200rpm for 10min, take the supernatant,

and use 95% ethanol to adjust the volume to 3mL, to be determined.

Accurately weigh 0.5mL of the prepared ethanol crude extract into a 50mL volumetric flask, add 9.5mL of distilled water, shake well, then add 0.5mL of Folin reagent, mix well, add 1.5ml of 20% Na₂CO₃ solution, mix well, then fix the volume, keep it at 30°C in the dark for 30min, and take gallic acid standard solution as blank control, 760.

Table 5. Determination of Polyphenols (Flynn Phenol Method)

serial number	1	2	3
Coffee primary processing wastewater /V(mL)	2.00		
95% ethanol /V(mL)	3.00		
Supernatant /V(mL)	0.5		
Folin phenol reagent /V(mL)	0.5		
20% Na ₂ CO ₃ solution /V(mL)	1.5		
A	0.522	0.527	0.525
Polyphenol content (mg/mL)	4.117	4.060	4.044

4. Results and Discussion

4.1. Analysis of Determination Results of Soluble Sugar

The soluble sugar contents of samples 1, 2, 3 and 4 were 22.816 mg/mL, 20.096 mg/mL, 21.856 mg/mL and 19.776 mg/mL, respectively. This shows that the soluble sugar content of samples 1 and 3 is higher, while the soluble sugar content of samples 2 and 4 is lower. Soluble sugar is an important component in coffee, which affects the taste and flavor.

4.2. Analysis of Soluble Protein Determination Results

The soluble protein contents of samples 1, 2, 3 and 4 were 1.114 uL, 1.4 uL, 1.073 uL and 1.114 uL respectively. This shows that the content of soluble protein in sample 2 is the highest, while that in sample 3 is the lowest. Soluble protein is an important flavor and quality factor in coffee.

4.3. Analysis of Galacturonic Acid Determination Results

The galacturonic acid contents of samples 1, 2, 3 and 4 were 8.376 mg/mL, 7.804 mg/mL, 9.067 mg/mL and 1.756 mg/mL, respectively. Sample 3 has the highest galacturonic acid content, while sample 4 has the lowest.

Galacturonic acid is an important polyphenol compound in coffee, which affects the bitterness and antioxidant properties of coffee.

4.4. Analysis of Determination Results of Polyphenols

The contents of polyphenols in samples 1, 2 and 3 were 4.117 mg/mL, 4.060 mg/mL and 4.044 mg/mL respectively. Polyphenols are important antioxidants in coffee, which are very important for maintaining the freshness and quality of coffee. The results show that the contents of polyphenols in these samples are relatively close.

5. Conclusion

These experimental results show that there are significant differences in the composition of different coffee wastewater samples, which may be caused by factors in the treatment of fresh coffee and coffee processing. The difference of soluble sugar and soluble protein content may affect the taste and flavor of coffee, while galacturonic acid and polyphenols may affect the bitterness and antioxidant properties of coffee. Further research and analysis can help us understand the relationship between these components and coffee quality, so as to optimize the production and processing technology of coffee. This experiment provides important information for further improving the method and production technology of coffee.

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