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Extraction of starch and proteins from the tubers of large-grain *Cyperus esculentus* L.

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Abstract

The contents of starch in the tubers of Cyperus esculentus L. were determined by the iodine method, and those of proteins were determined by the Coomassie brilliant blue G-250 method. Starch and proteins were simultaneously extracted by the lime water immersion method, and the effects of material-liquid ratio, immersion solution pH value, immersion time and supernatant pH value on the extraction efficiencies were studied. The optimal parameters were obtained by orthogonal experiments. The results show that the contents of starch and protein in large-grain C. esculentus are 35.4% and 6.3%, respectively. The optimal extraction conditions for starch include: immersion solution pH = 11.0, material-liquid ratio = 1:4 (w:v), immersion time = 70 min. The optimal extraction conditions for proteins include: immersion solution pH = 10.5, material-liquid ratio = 1:5 (w:v), immersion time = 50 min. Proteins were precipitated from the supernatant at pH 5.5.

Keywords: *Cyperus esculentus* L.; starch; protein; extraction technology

1. Introduction

Cyperus esculentus L., which is the perennial herb of Cyperaceae, is practically cultivated annually [1]. It has been widely planted in China since the 1950s [2]. *C. esculentus*. is a comprehensively valuable cereal and oil crop rich in oil, starch, sugar, proteins and vitamins [3]. Although the cultivation and oil extraction of *C. esculentus*. have been extensively studied, the starch and proteins extraction technologies have seldom been referred. The carboxymethyl starch paste of *C. esculentus*. can replace gelling agents such as pectin and carrageenin in food industry owing to the excellent freeze-thaw stability. Therefore, studying on the extraction technology of *C. esculentus*. starch is practically feasible [4, 5].

Large-grain *C. esculentus*., which belongs to the common *C. esculentus*., is of large tubers, and high contents of sugar and starch. This paper optimized the extractions of starch and proteins from large-grain *C. esculentus*., which provides evidence for the utilization of *C. esculentus*.

2. Materials and Methods

Materials

The tubers of *C. esculentus*. were planted and harvested from the local coastal sand soils. The tubers were washed, purified, dried and stored in dry places, and those were complete, mildew-free and mothy-free were selected as the raw materials.

Reagents

The main reagents included Coomassie brilliant blue G-250, bovine serum albumin, $Ca(NO_3)_2.4H_2O$, 95% ethanol and soluble starch (all of them were analytically pure).

Apparatus

The main apparatuses included JJ-2B Tissue Mashing Homogenizer (Yusus Electrome-chanical Equipment Co., Ltd.), Q5000 UV spectroscopy (Donglin Changsheng Biotechnology Co., Ltd.), BS21S electronic balance (Yihua Electronics Co., Ltd.), HH-4 digital constant-temperature water bath (Oubei Scientific Apparatus Co., Ltd.), TDL-60B low-speed centrifuge (Ziqi Experimental Equipment Co., Ltd.), pHS-25 pH meter (Jicheng Analytic Equipment Co., Ltd.), and 101AS-3 electric-heating constant-temperature dry box (Huatai Experimental Equipment Co., Ltd.).

Determination of starch and protein contents

The starch contents were measured by the iodine method [6]. The particle sizes of *C. esculentus*. powders were controlled at 95% by a 120 mesh. Starch was extracted by 80% $Ca(NO_3)_2$, and the absorbance of the starch-iodine solution was recorded at 620 nm to calculate the starch content according to the standard curve. The protein contents were measured by the Coomassie brilliant blue method [7]. The experiments were repeated for 5 times, the results of which were averaged.

Extraction procedure

Starch and proteins were separated and extracted by the lime water immersion method. The procedure is schematized in Fig. 1.

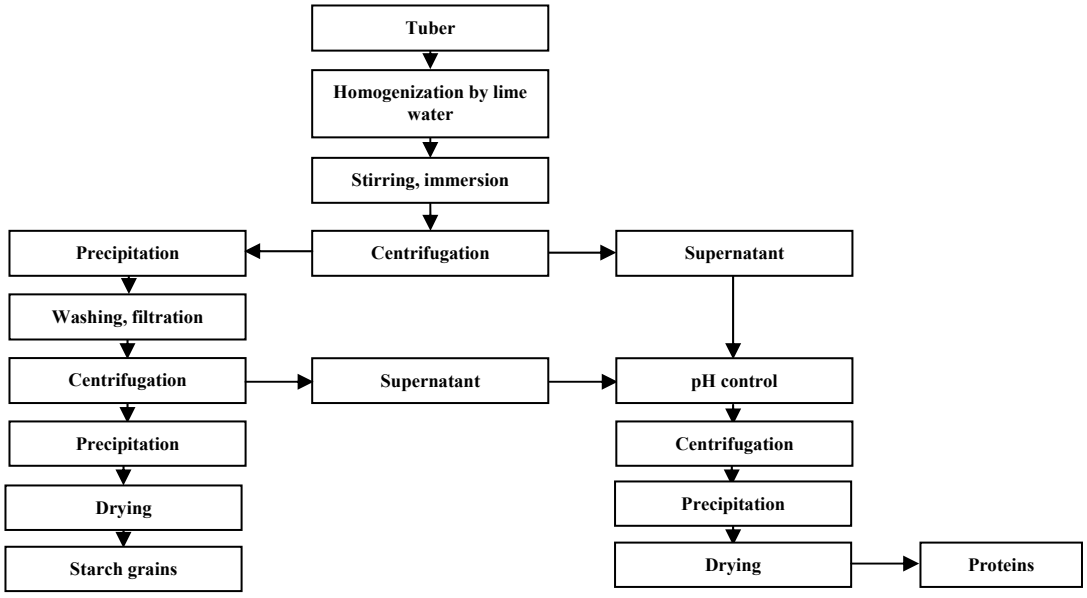


Figure 1. Extraction procedure.

Single-variance analysis

The extraction efficiencies of starch and proteins were mainly affected by material-liquid ratio, immersion solution pH, immersion time, and those of proteins were also affected by supernatant pH. The effects of single variances on the extraction efficiencies of starch and proteins were studied by setting the material-liquid ratio, the immersion solution pH, and the immersion time at 1:1, 1:2, 1:3, 1:4, 1:5, 1:6, 1:7 and 1:8, 8.0, 8.5, 9.0, 9.5, 10.0, 10.5, 11.0, 11.5, 12.0 and 12.5, and 20, 30, 40, 50, 60, 70, 80 and 90 min, respectively. The effects of supernatant pH on the extraction efficiencies of proteins were also studied by being set at 3.5, 4.0, 4.5, 5.0, 5.5 and 6.0. As a result, the extractions conditions were fixed at the material-liquid ratio of 1:5, the immersion solution pH of 9.5, and the immersion time of 60 min. The experiments were repeated for 3-5 times, the results of which were averaged. The differences of significance were analyzed using SPSS 11.5 ($p<0.05$), and the data were subjected to multiple comparisons by the LSD method.

The extraction efficiencies of starch and proteins can be calculated by the following equations.

Starch extraction efficiency = [crude *C. esculentus*. starch mass (g) × crude *C. esculentus*. starch mass fraction (μg/g)/ *C. esculentus*. mass (g) ×*C. esculentus*. starch mass fraction (μg/g)] × 100%.

Protein extraction efficiency = [crude *C. esculentus*. protein mass (g) × crude *C. esculentus*. protein mass fraction (μg/g)/ *C. esculentus*. mass (g) ×*C. esculentus*. protein mass fraction (μg/g)] × 100%.

Orthogonal test

Material-liquid ratio, immersion solution pG, immersion time and supernatant pH were subjected to an L_9 (3^4) orthogonal test (Table 1 and Table 2). The optimal extraction conditions were determined by the range analysis.

Table 1. Orthogonal test

Level	Factor			
	Material-liquid ratio A	Immersion solution pH B	Immersion time min C	Supernatant pH D
1	1:4	10.5	50	4.5
2	1:5	11	70	5
3	1:6	11.5	90	5.5

Table 2. L_9 (3^4) orthogonal test

Test No.	Factor			
	Material-liquid ratio A	Immersion solution pH B	Immersion time min C	Supernatant pH D
1	1	1	1	2
2	1	2	2	3
3	1	3	3	1
4	2	1	2	1
5	2	2	3	2
6	2	3	1	3
7	3	1	3	3
8	3	2	1	1
9	3	3	2	2

Results and Discussions

Determination of starch and protein contents

The contents of starch and proteins in *C. esculentus*. are 35.4% and 6.3%, respectively.

Single-variance test

Effects of material-liquid ratio on the extraction efficiency of starch

Fig. 2 shows that the extraction efficiency of starch increases up to 49.4% with increasing material-liquid ratio to 1:4 and then levels off.

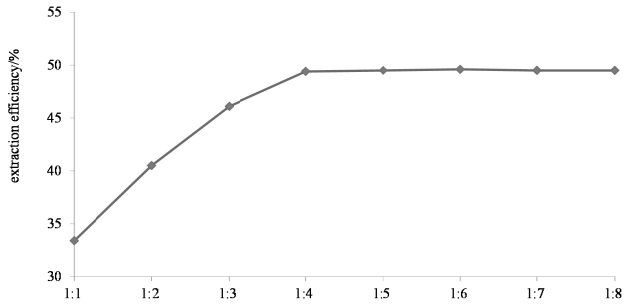
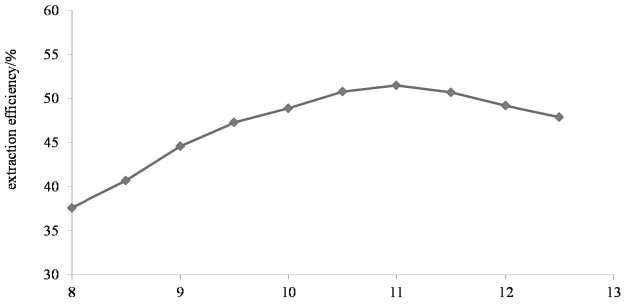


Figure 2. Effects of material-liquid ratio on the extraction efficiency of starch.

Effects of immersion solution pH on the extraction efficiency of starch

Fig. 3 shows that the extraction efficiency of starch increases up to 51.5% with increasing immersion solution pH to 11.0 and then decreases.

Figure 3. Effects of immersion solution pH on the extraction efficiency of starch.



Effects of immersion time on the extraction efficiency of starch

Fig. 4 shows that the extraction efficiency of starch increases up to 51.0% with elapsing immersion time to 70 min and then maintains constant thereafter.

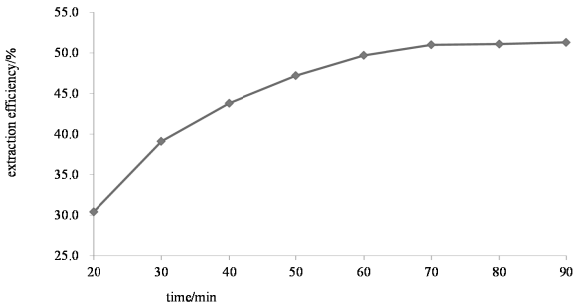


Figure 4. Effects of immersion time on the extraction efficiency of starch.

Effects of supernatant pH on the extraction efficiency of proteins

Fig. 5 shows that the extraction efficiency of proteins reaches up to 68.4% in the case of supernatant pH 5.5. Thus, the isoelectric point of *C. esculentus*. proteins is approximately 5.5. The alkaline environment for starch extraction easily charged proteins negatively, which were then charged neutrally by regulating the pH. Thus, the particles were free from repulsion,

which enabled the formation of most unstable and least soluble large particles that were prone to precipitation.

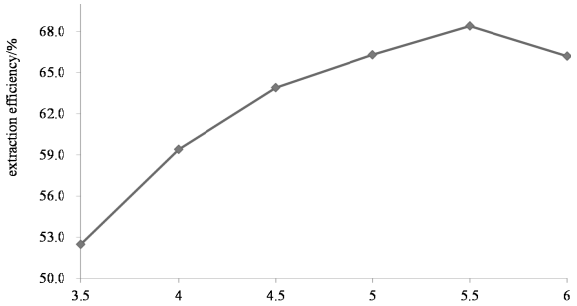


Figure 5. Effects of supernatant pH on the extraction efficiency of proteins.

Orthogonal experiment

The orthogonal experiment results, which are summarized in Table 3, were further subjected to the range analysis based on the starch extraction efficiency (Table 4). The range R values of extraction efficiency range between 0.736-1.623. The impacts of material-liquid ratio (A), immersion solution pH (B) and immersion time (C) follow the descending order of A>B>C. The first level of A and the second levels of B and C yield the highest extraction efficiencies. Therefore, the theoretically optimal condition is $A_1B_2C_2$ (material-liquid ratio = 1:4, immersion solution pH = 11.0, immersion time = 70 min).

Table 3. Orthogonal experiment results

Test No.	Factor					
	Material-liquid ratio A	Immersion solution - pH B	Immersion time - min C	Supernatant pH D	Starch extraction efficiency - %	Protein extraction efficiency - %
1	1:4	10.5	50	5	60.42	74.42
2	1:4	11	70	5.5	64.13	72.63
3	1:4	11.5	90	4.5	62.59	71.84
4	1:5	10.5	70	4.5	62.83	76.38
5	1:5	11	90	5	61.21	72.51
6	1:5	11.5	50	5.5	60.82	74.82
7	1:6	10.5	90	5.5	60.42	72.92
8	1:6	11	50	4.5	61.18	74.85
9	1:6	11.5	70	5	60.67	72.48

Table 4. Range analysis results based on starch extraction efficiency

Level	Factor		
	Material-liquid ratio A	Immersion solution pH B	Immersion time min C
K1	62.380	61.223	60.807
k2	61.620	62.173	61.543
K3	60.757	61.360	61.407
R	1.623	0.813	0.736

The range analysis results based on the protein extraction efficiency are listed in Table 5. The influences of the tested variances follow the order of C>A>B>D. The theoretically optimal condition is $A_2B_1C_2D_3$ (material-liquid ratio = 1:5, immersion solution pH = 10.5, immersion time = 50 min, supernatant pH = 5.5).

Table 5. Range analysis results based on protein extraction efficiency

Level	Factor			
	Material-liquid ratio A	Immersion solution pH B	Immersion time min C	Supernatant pH D
K1	72.963	74.573	74.697	73.137
k2	74.570	73.330	76.450	73.457
K3	73.417	73.047	73.830	74.357
R	1.607	1.526	2.620	1.220

The missing of $A_2B_1C_1D_3$ in this orthogonal experiment was subjected to three repeated tests, exhibiting that the extraction efficiencies of starch and proteins are 62.38% and 80.03%, respectively.

The contents of starch and protein in the tubers of large-grain *C. esculentus*. are 35.4% and 6.3%, respectively. It has been reported that the contents of starch and proteins in the tubers of *C. esculentus*. are 20%-30% and 1%-6%, respectively [8, 9], which may be related to the cultivation environment and the soil administration and are dominated by the type differences. The content of starch in large-grain *C. esculentus*. is commonly higher than that of protein. The results herein are consistent with those reported previously. We hold that the tubers of large-grain *C. esculentus*. are appropriate for the processing of starch, Saccharum Granorum, wine and ethanol, and those of common *C. esculentus,* are proper for oil manufacture and biodiesel transformation [10].

Several starches and proteins have been extracted from plants by the alkaline dissolution method [11, 12], which often increase the extraction quantities of proteins while maintaining the starch quality [13]. Lime water lowers the viscosity and elevates the extraction efficiency of starch owing to the pectins in the tubers of *C. esculentus*. In this study, the optimal extraction conditions for starch include: immersion solution pH = 11.0, material-liquid ratio = 1:4 (w:v), immersion time = 70 min. The optimal extraction conditions for proteins include: immersion solution pH = 10.5, material-liquid ratio = 1:5 (w:v), immersion time = 50 min. Proteins were precipitated from the supernatant at pH 5.5. Starch and proteins were simultaneously extracted due to the high starch content and the low potein content. As an oil crop, *C. esculentus,* is mainly used to prepare greases and develop biodiesel [14]. The conditions concluded herein are also applicable in the extraction of dregs. However, the proteins extracted by alkaline dissolution-acid precipitation are darkly colored, which should be further whitened.

The results in this study are in agreement with those reported in 2009 [15], whereas the effects of immersion solution pH instead of immersion solution concentration were considered herein. Besides, the immersion time was shortened by stirring the lime water. Meanwhile, the simultaneous extractions of starch and proteins from *C. esculentus*. have never been reported.

The hot and cold carboxymethylated starch pastes of *C. esculentus*. are both of good stability, facile pasting ability, high viscosity and transparency, which can thus be applied in food processing [16]. Moreover, the proteins separated from *C. esculentus*. are also superbly effervescent food additives.

3. Conclusions

In summary, starch and proteins were simultaneously extracted by the lime water immersion method [17], and the effects of material-liquid ratio, immersion solution pH value, immersion time and supernatant pH value on the extraction efficiencies were optimized by single-variance analysis and orthogonal experiments. The results provide scientific evidence for the comprehensive utilization of *C. esculentus*.

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