Extraction Technology and Physicochemical Properties of Broccoli Protein

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Abstract: In this study, protein is extracted with broccoli stems and leaves as raw material. Meanwhile, the enzymatic technology-assisted extraction and the physicochemical properties of broccoli protein are investigated. Furthermore, the extraction technology of broccoli stems and leaves is optimized by orthogonal experiment. The results show that, the influence of each factor to extraction rate decreases by temperature > pH > time in order. By calculation, the optimal condition is pH 6, temperature 60 °C, extraction time 20 h, and the water holding capacity, swelling property, and cation exchange capability of broccoli protein are $3.32 \pm 0.10 \text{ mL/g}$, $4.77 \pm 0.59 \text{ g/g}$, $7.52 \pm 0.17 \text{ mmol/g}$ respectively, which are higher than those of soybean protein.

Introduction

Broccoli contains an anti-cancer material called glucosinolates. Thus it is called as healthy vegetable and was planted all over the world [1]. However, environmental pollution is caused since there are no effective methods to handle its leaves and stems after harvest. The leaves and stems can be used as animal feed, and also can be used to extract glucosinolates and plant fiber[2]. But with the ceaselessly increase of broccoli planting quantity in the past few years, it is difficult to find a better application field for them[3]. Thus somebody proposes that the active components in the broccoli leaves and stems can be extracted and applied to food industry [4, 5]. The protein content of broccoli stems and leaves accounts for more than 20% of the dry weight. It is a plant protein resource that has not been fully developed and shows a great developing and utilizing value. In this study, broccoli stems and leaves protein is extracted with broccoli stems and leaves as raw material. Moreover, its functional properties are investigated, expecting that it can be added in food as the natural additive.

Extraction technology of broccoli protein

Broccoli stems and leaves contain large amounts of cellulose binding protein, which can reduce protein solubility. In this experiment, enzymatic technology-assisted extraction is utilized with cellulase as hydrolyzed material to increase the extraction rate of protein. Cellulase activity is 10,000 U/g, and the usage amount of cellulase is 0.5% of the dry weight of material. The extraction process is indicated in the following: 10.0 g fresh broccoli stems and leaves weighted is added in 50 mL water. After milled in grinder, it is put in 200 mL flask and mixed with cellulase. Then it is extracted with water bath heating. Afterwards, the influences of pH value, temperature, and extracting time on extraction rate are studied. Besides, orthogonal experiment is selected as the optimum extraction condition, as shown in table 1. The amount of protein extracted is measured using coomassie brilliant blue; the protein content of raw material are tested by kjeldahl nitrogen determination method.

Extraction rate = amount of protein extracted / protein content in raw material (1)

The influences of pH value on extraction rate mainly include two parts, which are the influences on protein solubility and cellulose activity. Figure 1 shows that, when extraction pH is $3\sim4$, the extraction rate of broccoli stems and leaves protein is highest. And when extraction pH is 6, the leaf protein extraction rate is highest, which is 16.55 ± 0.12 . Temperature also affects protein extraction by influencing protein solubility and cellulose activity. Figure 2 exhibits that, when extraction

temperature is 60 °C, leaf protein extraction rate is highest with a value of $17.2\pm0.25\%$. The influences of extraction time to extraction rate are shown in Figure 3. Where, before 16 h, the extraction rate of broccoli stems and leaves protein gradually increases. Then it declines, which is probably because that some proteins are degenerated due to the overlong extraction time. So in the prospect of extraction efficiency, 16 h is more appropriate.



Fig. 3 Influence of time on extraction rate

Extraction technology is optimized by orthogonal experiment. The factor levels are presented in table 1, and the experimental results are shown in table 2. Where, it is found that, the influences of the three factors on extraction rate decrease by temperature > pH > time in order. By calculating, the optimal condition is A₂B₂C₃, namely, pH 6, temperature 60 °C, time 20 h.

Table 1 Factors and levels of test	Table 1	Factors	and le	evels	of test
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Level	A: pH	B: Temperature	C: Time
1	5	50°C	12h
2	6	60°C	16h
3	7	70°C	20h

Batch No.	А	В	C	Extraction rate
1	1	1	1	15.23
2	1	2	2	17.03
3	1	3	3	16.27
4	2	1	3	17.05
5	2	2	1	17.47
6	2	3	2	16.11
7	3	1	2	16.41
8	3	2	3	15.9
9	3	3	1	14.64
K1j	15.843	15.897	15.780	
K2j	16.727	16.800	16.033	
K3j	15.317	15.190	16.073	
Rj	1.410	1.610	0.643	

Table 2 Results of orthogonal tests

Physicochemical properties of broccoli protein

The water holding capacity, swelling property, emulsibility, and cation exchange capacity of broccoli protein are studied with soybean protein as a comparison as shown in table 3[5-7]. The water holding capacity, swelling property, and cation exchange capacity of broccoli protein are 3.32 ± 0.10 mL/g, 4.77 ± 0.59 g/g, 7.52 ± 0.17 mmol/g respectively, which are all higher than those of soybean protein. But the emulsibility of broccoli protein is far lower than that of soybean protein.

	Water holding capacity (mL/g)	Emulsibility (%)	Swelling property (g/g)	Cation exchange capacity (pH7) (mmol/g)
Soybean protein	2.95±0.05b	91.7±3.12a	4.29±0.45a	6.22±0.08b
Broccoli protein	3.32±0.10a	50.4±4.3b1	4.77±0.59a	7.52±0.17a

Table 3 Physical and chemical properties of Broccoli protein

Conclusion

The extraction technology of broccoli stems and leaves protein is optimized by orthogonal experiment. The results show that, the influence of each factor to extraction rate decreases by temperature > pH > time in order. By calculation, the optimal condition is $A_2B_2C_3$, namely pH 6, temperature 60 °C, time 20 h.

Comparing with soybean protein, the water holding capacity, swelling property, cation exchange capacity of broccoli stems and leaves protein are stronger, which is probably because that broccoli stems and leaves protein contains more hydrophilic groups. So it is indicated that the processing characteristics of broccoli stems and leaves protein is better. But the emulsibility of broccoli stems and leaves protein is poor. If the emulsibility is chemically modified, the processing characteristics can be further improved.

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