STUDY ON EXTRACTION CONDITIONS AND CHARACTERISTICS OF PROTEIN FROM BURDOCK ROOTS

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Abstract
Burdock (Arctium lappa L.), which belongs to the Compositae family, possesses significant medicinal and nutritional value. Burdock root is abundant in proteins and essential amino acids. In this study, proteins from burdock roots were extracted by alkali extraction and acid sedimentation, and the optimal conditions for protein extraction from burdock roots and protein characteristics were explored. The optimal conditions for protein extraction with a yield rate of 9.03% were 40°C extraction temperature, pH 8.0, a solid–liquid ratio of 1:25 (g/mL), and extraction time of 65 minutes. Single-factor and orthogonal tests on the extraction rate of burdock proteins under different conditions found that the extraction rate of protein from burdock roots were influenced by pH, extraction temperature, solid–liquid ratio, and extraction time, in this order. The solubility and gelation properties of burdock protein were similar to those of soybean protein; however, burdock protein demonstrated 1.2 times the oil absorption capacity and half the water retention capacity of soybean protein. Analysis of the amino acid contents of burdock protein revealed that burdock root contains significant essential and non-essential amino acids, with valine content being the highest.

Keywords: Acid precipitation. Alkali extraction. Amino acid content. Burdock. Protein characteristics. Protein extraction.

1. Introduction

Burdock (Arctium lappa L.) is an edible traditional Chinese medicinal plant that is widely regarded as a healthy and nutritious food in Chinese culture. It has been used therapeutically for centuries in Europe, North America, and Asia, with its roots, leaves, flowers, and fruits used extensively in traditional Chinese medicine (Chan et al. 2011). The medicinal properties of burdock have been documented in the Records of Famous Doctors in the Northern and Southern Dynasties. In recent years, burdock research has primarily focused on its fruits and leaves, with less emphasis on its roots.

The succulent straight root of burdock has detoxifying, detumescent, and analgesic properties (JianFeng et al. 2012; Zhang et al. 2021) and is rich in various active ingredients, including proteins, carbohydrates, burdock acid, aldehydes, polyacetylene substances, and polysaccharides, which are extremely beneficial to health (Chan et al. 2011; Skowrońska et al. 2021; Zhang et al. 2021). These active components have diverse therapeutic effects against numerous diseases and have been used in medicinal treatments, encompassing the anti-inflammatory, antioxidant, lipid-lowering, antidiabetic, anti-atherosclerosis, antimicrobial, and anticancer effects of burdock root (Chan et al. 2011; Mnab et al. 2019;
Moro and Clerici 2020; Oboh 2021; Ha et al. 2021; Herrera-Balandrano et al. 2021; Yosri et al. 2023). Moreover, the medicinal value of burdock roots in managing chronic ailments, such as cancer, diabetes, and AIDS, has been promising (Chan et al. 2011; Predes et al. 2011; Urazova et al. 2011; Prinsloo et al. 2018; Wu et al. 2020; Romualdo et al. 2020; Zhang et al. 2021; Mondal and Eun 2022).

In addition, burdock root is abundant in amino acids, including those essential for human health, such as threonine, methionine, and lysine, as well as a significant quantity of unique amino acids like aspartic acid (Asp) and arginine (Arg) (Lee et al. 2020; Skowrońska et al. 2021; Zhang et al. 2021). Notably, aspartic acid plays a crucial role in regulating the metabolic function of the brain and nerves (Dong et al. 2018), making it highly versatile in the food and medicinal industries. Arginine is an essential amino acid vital for the growth and development of infants and children (Jacobi and Odle 2012) and serves as a key component of sperm protein (Lewis et al. 2004). Thus, burdock root, also a traditional aphrodisiac in China, has been used to address issues of impotence and sterility (JianFeng et al. 2012). Moreover, burdock root is a valuable source of essential macro and trace elements, including calcium, magnesium, iron, manganese, and zinc, which are crucial for health (Chan et al. 2011; Zhang et al. 2021). Researchers have recently leveraged the richness of bioactive compounds in burdock root, particularly polyphenolic compounds, to develop novel biostimulating materials. Such studies greatly facilitate the creation of innovative biological materials and advances the progress of agricultural sustainability (Szparaga et al. 2021).

Proteins are omnipresent in our daily lives and are extensively utilized in the food industry for their high nutritional value. The functional properties of proteins play a crucial role in the food-processing industry, emphasizing the need to determine these characteristics prior to processing. Therefore, the study of the protein extraction process and protein characteristics holds significant research significance. The protein characteristics commonly assessed include solubility, water retention, oil absorption capacity, and gel properties, among others.

Protein solubility is a thermodynamic manifestation of protein–protein and protein–solvent interactions, which establish equilibrium. Solubility denotes the thickening, foaming, emulsification, and gelling properties of proteins, with highly soluble proteins being more suitable for various food-processing applications. Gelation refers to the aggregation of protein molecules to form an organized network structure. This property enables proteins to form solid elastic gels, thicken substances, and enhance water absorption. Protein gelation is extensively applied in the manufacture of dairy products, jellies, coagulated proteins, and other food items (Boukid et al. 2021; Jaeger et al. 2021).

In this study, protein from burdock roots was extracted by alkali extraction and acid sedimentation. The optimal conditions for the extraction and the characteristics of burdock root proteins were explored. The alkali extraction acid sedimentation method for protein extraction was employed. Based on the determination of the burdock protein isoelectric point (pI). The individual effects of temperature, pH, the solid–liquid ratio, and extraction time on the protein extraction rate were investigated, and the orthogonal test was performed to identify the ideal processing conditions for burdock protein extraction. Furthermore, we compared the solubility, gel properties, water retention, and oil absorption ability between burdock protein and soybean protein. These findings provide a solid theoretical foundation for the potential industrial production of burdock root protein.

2. Material and Methods

Chemicals and equipment

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Equipment Co., Ltd.), electric blower dryer (Shanghai Botai Experimental Equipment Co., Ltd.), pH Meter (Aohaus Instrument Co., Ltd.), small ultra-micro grinder (Wenzhou Dingli Medical Device Co., Ltd.).

Plant materials

Fresh burdock roots were washed, cut into pieces, and dried in an electric blower dryer. After drying, the pieces were ground into a powder using a small ultra-micro grinder.

Color retention

Burdock root is abundant in iron, which can oxidize upon exposure to air. To prevent oxidation from affecting the experimental results, we conducted a preliminary study comparing the protective effects of various antioxidants on burdock powder. This step was needed to ensure the smooth progression of subsequent experiments. We prepared a mixture of antioxidants and burdock powder (1:100 ratio, g/g), fully dissolved it in distilled water, and noted the resulting color.

Alkali extraction acid sedimentation

Burdock powder was dissolved, and pH adjusted in sodium hydroxide solution. The mixture was stirred using a temperature-controlled magnetic stirrer for 15 minutes, and the resulting supernatant was collected. The pH of the solution was further adjusted to the isoelectric point (pI). The solution was centrifuged, after which the supernatant was discarded. The resulting precipitate was carefully cleaned and dissolved in water, with the pH adjusted to 7.0. Finally, the precipitate was placed in a freeze-dryer (SCIENTZ-20F/B) for 2 days to yield burdock protein powder.

Protein standard curve

A protein standard curve was prepared to determine the protein yield. Gradient concentrations of bovine serum albumin (BSA) solution were prepared, combined with 5.0 mL Coomassie Brilliant Blue G-250 solution, and incubated at room temperature for 10 minutes. The prepared standard solutions were then analyzed using a UV–visible spectrophotometer (METASH UV-5500) to create a calibration curve.

Determining pI

The pI of a protein refers to the pH at which the protein exhibits the highest precipitation rate. For each research group, three replicates were conducted to determine the pI of burdock protein.

Burdock powder and antioxidant (ratio, 100:1 g/g) were added to 100 mL distilled water and stirred with a magnetic stirrer for 15 minutes. The pH was adjusted to 8.0 with sodium hydroxide solution, and stirring was continued for 30 minutes at 40°C. The supernatant was collected and divided into 11 equal parts, with the pH adjusted to the following values: 3.0, 3.4, 3.6, 3.8, 4.0, 4.2, 4.5, 4.7, 5.0, 5.3, and 5.5. After 20 minutes, centrifugation was performed at 5000 rpm for 15 minutes. The supernatant was collected, and Coomassie Brilliant Blue G-250 was added for spectrophotometric analysis. The pH corresponding to the supernatant with the lowest absorbance was considered the pI of burdock protein.

Single-factor and orthogonal tests

The single-factor tests were used to assess the impact of temperature on the protein extraction rate. For the extraction, the solid–liquid ratio of the burdock aqueous solution was maintained at 1:10 (g/mL), pH at 8.0, and extraction time at 30 minutes. The only variable that was altered was the extraction temperature. Burdock powder and ascorbic acid were dissolved in distilled water, and the mixture pH was adjusted to 8.0. Then, the mixture was magnetically stirred for 30 minutes at 30°C, 35°C, 40°C, 45°C, and 50°C. After 30 minutes, the mixture was centrifuged for 10 minutes, and the supernatant was collected. The supernatant
was diluted twice, and the absorbance was measured by Coomassie Brilliant Blue spectrophotometry. Similarly, the effects of different extraction pH values, temperatures, solid–liquid ratios, and extraction times on the protein extraction rate from burdock roots were investigated. The protein content was determined by spectrophotometry using a standard curve, and the protein extraction rate was calculated as follows:

\[
\text{Extraction rate (\%)} = \frac{\text{Protein (g)}}{\text{Total burdock powder (g)}} \times 100
\]  
(Eq. 1)

Based on the results obtained from the single-factor experiments for burdock root protein extraction, an L\(_9\)(3\(^4\)) orthogonal test was designed to evaluate the influence of temperature, solid–liquid ratio, extraction pH, and extraction time.

**Characteristics of burdock protein**

To further investigate the characteristics of burdock root protein, we examined its solubility, water retention ability, oil absorption ability, and gel properties.

**Burdock protein solubility.** Burdock protein powder was added to distilled water and stirred thoroughly until fully dissolved. The mixture was centrifuged at 5000 rpm for 10 minutes. The supernatant was collected, and the protein content was analyzed using Coomassie Brilliant Blue spectrophotometry. Burdock protein characteristics were analyzed, and its solubility was calculated using the following formula:

\[
\text{Solubility} = \frac{\text{Dissolved protein (g)}}{\text{Total protein (g)}} \times 100\% 
\]  
(Eq. 2)

**Burdock protein water retention.** Burdock protein powder was mixed with distilled water and left at room temperature for 30 minutes. Afterward, the solution was centrifuged at 1500 rpm for 5 minutes, and the upper layer of the solution was discarded. The weight of the precipitate in the lower layer was measured and expressed as the amount of water absorbed per gram of protein sample.

**Burdock protein oil absorption capacity.** Burdock protein powder was weighed and added to salad oil (V). The mixture was stirred thoroughly until homogeneous and then centrifuged for 30 minutes. The volume of the centrifuged salad oil (V') was determined to calculate the oil adsorption ability using the following formula:

\[
\text{Oil absorption capacity}=V-V' 
\]  
(Eq. 3)

**Burdock protein gel property.** Burdock protein solutions were prepared at concentrations of 3%, 6%, and 9%. The solutions were heated in a 95°C water bath for 40 minutes, followed by immediate cooling in a refrigerator at 4°C for 24 hours. The solutions were removed from the refrigerator, allowed to reach room temperature for 20 minutes, and any changes were observed.

**Amino acid content analysis**

The freeze-dried burdock protein powder (30 mg) was mixed with 15 mL of 6 M HCl and subjected to a 24-hour reaction at 110°C. After the reaction, 0.1 mL of the reaction mixture was taken and mixed with 1 mL of water. This mixture was vortexed for 5 minutes and then subjected to ultrasonic extraction for 30 minutes. After extraction, the solution was centrifuged at 4°C and 12,000 rpm for 10 minutes. The supernatant was collected, filtered, and then analyzed for amino acid content.

**Statistical analysis**

All data are expressed as mean and standard deviation. All statistical analyses were performed using GraphPad Prism.

3. Results

**Single-factor and orthogonal tests of the extraction rate of burdock protein under different conditions**

Color protection of burdock powder was performed, and the beaker containing 0.2 g ascorbic acid exhibited the lightest color, indicating that ascorbic acid provided the best color protection for burdock

...
powder among the three additives. Subsequently, we selected 0.02% ascorbic acid as color protectant for burdock powder to control the discoloration reaction in subsequent experiments. BSA solutions with concentrations of 0, 0.2, 0.4, 0.8, and 1.0 mg/mL were prepared. The absorbance of each concentration was measured three times, and the average values were calculated. After plotting the standard curve, we obtained the following equation:

\[ Y = 0.9925X + 0.0135; R^2 = 0.9995 \]  

(Eq. 4)

Based on the designed experimental scheme for measuring pI, the average value of each pH was obtained from three parallel experiments (Figure 1). The absorbance value of the burdock protein supernatant was lowest at pH 4.2. This indicates that the acidification effect on burdock protein as optimal at pH 4.2 and resulted in the highest protein extraction rate. Therefore, pH 4.2 is the pI of burdock protein.

The following experiments were conducted to determine the optimal conditions for protein extraction from burdock roots. The results of the single-factor experiments are presented in Figure 1. The extraction was performed at pH 8.0 using 1 M sodium hydroxide solution with magnetic stirring for 30 minutes at temperatures of 30°C, 35°C, 40°C, 45°C, and 50°C. The maximum protein yield was achieved at 40°C. The extraction rate of burdock protein initially increased and then decreased with increasing temperature (Figure 2A).

The absorbance rate of burdock protein under different pH values was determined under a solid–liquid ratio of 1:10 (g/mL), extraction temperature of 40°C, and extraction time of 30 minutes. As shown in Figure 2B, the extraction rate of burdock protein under pH 6.0, 7.0, 8.0, 9.0, and 10.0 exhibited an initial increase followed by a decrease. The extraction rate of burdock protein peaked at pH 8.0 at 6.84%. Notably, highly alkaline conditions can lead to the degenerative hydrolysis of proteins, resulting in an increase in non-protein content in the extract and consequently reducing the protein extraction rate. Therefore, the optimal extraction pH was determined to be 8.0.

Subsequently, the solid–liquid ratio was varied while maintaining the extraction conditions at pH 8.0, 40°C, and 30 minutes extraction time. The solid–liquid ratios applied were 1:10, 1:15, 1:20, 1:25, and 1:30 (g/mL). The extraction rate reached 7.24% when the solid–liquid ratio was adjusted to 1:25 (g/mL) (Figure 2C).

Similarly, under the extraction conditions of 40°C, pH 8.0, and a solid–liquid ratio of 1:25 (g/mL), the absorbance of the supernatant was measured at extraction times of 20, 35, 50, 65, and 80 minutes. The burdock protein yield exhibited a significant upward trend from 20 to 65 minutes, and then began to decrease. The optimal extraction time for burdock protein was 65 minutes (Figure 2D).
Study on extraction conditions and characteristics of protein from burdock roots

Figure 2. Single-factor tests. (A) Yield of burdock protein under different temperatures, (B) pH values, (C) solid–liquid ratios, and (D) extraction times.

Based on the results of these single-factor tests, an orthogonal test with 4 factors and 3 levels. The four factors investigated were extraction temperature, extraction time, pH, and solid–liquid ratio, each of which was investigated at three different levels to determine their individual influences on the protein extraction rate (Table 1).

Table 1. Orthogonal test design. The L₉(3⁴) orthogonal test was designed to evaluate the influence of temperature, solid–liquid ratio, extraction pH, and extraction time.

<table>
<thead>
<tr>
<th></th>
<th>A (Temperature/°C)</th>
<th>B (Solid–liquid ratio, g/mL)</th>
<th>C (pH)</th>
<th>D (Extraction time/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>35</td>
<td>1:20</td>
<td>7</td>
<td>50</td>
</tr>
<tr>
<td>2</td>
<td>40</td>
<td>1:25</td>
<td>8</td>
<td>65</td>
</tr>
<tr>
<td>3</td>
<td>45</td>
<td>1:30</td>
<td>9</td>
<td>80</td>
</tr>
</tbody>
</table>

Based on the results of the orthogonal test in Table 2, the combination that yielded the highest extraction rate of burdock protein was A₁B₂C₂D₂ (extraction temperature, 35°C; solid–liquid ratio, 1:25 [g/mL]; pH 8.0; extraction time, 65 minutes).

Table 2. Orthogonal test results

<table>
<thead>
<tr>
<th></th>
<th>A (Temperature/°C)</th>
<th>B (Solid–liquid ratio, g/mL)</th>
<th>C (pH)</th>
<th>D (Extraction time/min)</th>
<th>Extraction rate of burdock root protein (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>7.497</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>8.810</td>
</tr>
<tr>
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<td>1</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>8.147</td>
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<tr>
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<td>2</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>8.157</td>
</tr>
<tr>
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<td>2</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td>8.047</td>
</tr>
<tr>
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<td>2</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>8.310</td>
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<tr>
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<td>3</td>
<td>2</td>
<td>7.463</td>
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<td>3</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td>7.640</td>
</tr>
<tr>
<td>9</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>7.957</td>
</tr>
</tbody>
</table>

*All data in the table represent the average of three replicate experiments.
Furthermore, the results of the extreme worst analysis (Table 3) of the orthogonal test shows that the primary and secondary effects on the extraction rate of burdock protein are C > A > B > D. The combination with the highest extraction rate of burdock protein was A2B2C2D2 (extraction temperature; 40°C; solid–liquid ratio, 1:25 (g/mL); pH 8.0; extraction time, 65 minutes).

**Table 3. Extreme worst analysis**

<table>
<thead>
<tr>
<th></th>
<th>K1</th>
<th>K2</th>
<th>K3</th>
<th>R</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8.151</td>
<td>7.706</td>
<td>7.816</td>
<td>7.833</td>
</tr>
<tr>
<td>2</td>
<td>8.171</td>
<td>8.166</td>
<td>8.308</td>
<td>8.194</td>
</tr>
<tr>
<td>3</td>
<td>7.687</td>
<td>8.138</td>
<td>7.886</td>
<td>7.981</td>
</tr>
<tr>
<td>R</td>
<td>0.484</td>
<td>0.460</td>
<td>0.492</td>
<td>0.361</td>
</tr>
</tbody>
</table>

*All data in the table represent the average of three replicate experiments.

A comparative study was conducted to further analyze the combination with the highest yield of burdock protein extraction (Table 4). The optimal combination for burdock protein extraction was a temperature of 40°C, solid–liquid ratio of 1:25 (g/mL), pH 8.0, and extraction time of 65 minutes, resulting in an extraction rate of 9.03%.

**Table 4. Verification experiment**

<table>
<thead>
<tr>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>Extraction rate of burdock roots protein (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>9.030</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>8.757</td>
</tr>
</tbody>
</table>

*All data in the table represent the average of three replicate experiments.

**Analysis of protein characteristics**

Finally, analysis of the characteristics of burdock protein was performed and compared with those of soybean protein (Table 5). The solubility and gel properties of burdock protein were comparable to those of soybean protein. However, the water retention capacity of burdock protein was half that of soybean protein, whereas the oil absorption capacity was 1.2 times that of soybean protein.

**Table 5. Protein characteristics**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Burdock protein</th>
<th>Soybean protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solubility (%)</td>
<td>62.9</td>
<td>60.7</td>
</tr>
<tr>
<td>Water reservation (g/g)</td>
<td>1.5</td>
<td>3.2</td>
</tr>
<tr>
<td>Oil absorption capacity (mL/g)</td>
<td>4.7</td>
<td>3.9</td>
</tr>
<tr>
<td>Gel property</td>
<td>3%</td>
<td>The solution formed a gel</td>
</tr>
<tr>
<td></td>
<td>6%</td>
<td>The solution formed a gel</td>
</tr>
<tr>
<td></td>
<td>9%</td>
<td>The solution formed a gel</td>
</tr>
</tbody>
</table>

*All data in the table represent the average of three replicate experiments.

**Burdock root contains various amino acids**

We analyzed the types and concentrations of amino acids found in burdock root, and the results (Figure 3). Burdock root contains eight essential amino acids: valine (Val; 1354.30 μmol/L), leucine (Leu; 1301.00 μmol/L), isoleucine (Ile; 947.30 μmol/L), phenylalanine (Phe; 545.00 μmol/L), threonine (Thr; 541.10 μmol/L), methionine (Met; 283.20 μmol/L), lysine (Lys; 134.60 μmol/L), and histidine (His; 38.70 μmol/L). Furthermore, burdock also contains 10 non-essential amino acids: Asp (961.10 μmol/L), proline (Pro; 900.40 μmol/L), glutamate (Glu; 881.80 μmol/L), tyrosine (Tyr; 667.90 μmol/L), serine (Ser; 216.10 μmol/L), glycine (Gly; 121.50 μmol/L), Arg (39.50 μmol/L), alanine (Ala; 92.90 μmol/L), cysteine (Cys; 8.20 μmol/L), and tryptophan (Trp; 1.318 μmol/L). Among the non-essential amino acids, Asp, which has multiple medical and food industry applications, has the highest content. For instance, Asp is a primary ingredient in sweeteners, such as aspartame, and is an important component of treatments for diseases such as heart disease, liver disease, and hypertension. Furthermore, Asp can help prevent and recover from fatigue (Ritterhoff et al.
2020; Heinz 2021). Glu is primarily used to treat hepatic coma and enhance the intellectual development of children. It is also a major component of monosodium glutamate, a flavor enhancer (Romero-Gómez 2005). Tyr is a key raw material in melanin production, making it valuable in the development of skin-whitening cosmetics (Li et al. 2023). In addition, for individuals with vitiligo, consuming foods rich in tyrosine can promote melanin formation and alleviate vitiligo symptoms (Ren et al. 2019). Ser is known for its moisturizing and hydrating properties and is a common component of high-end cosmetics (Barua et al. 2017). Gly is used as a food additive, nutritional supplement, such as in flavoring, and preservative. Glycine is also used to treat myasthenia gravis and progressive muscle atrophy (Zheng et al. 2014). Arg is a critical amino acid for the growth and development of infants, as well as in sperm production, providing energy for sperm motility. Furthermore, Arg is involved in anticancer activities and oral health (Eick and Lussi 2021).

Figure 3. The amino acid content in burdock protein. The freeze-dried burdock protein powder was treated with 6M HCl at 110°C for 24 hours to ensure complete hydrolysis, resulting in an amino acid aqueous solution for subsequent analysis.

Economic benefits of burdock protein

Burdock protein possesses numerous medicinal and health benefits. The functional properties and nutritional value of burdock protein make it highly versatile and applicable in various fields.

In the food industry, it can be used as a valuable nutritional ingredient, enhancing the protein content and nutritional profile of products such as protein bars, snacks, beverages, and plant-based meat alternatives (Langyan et al. 2022). Burdock protein can be used in nutraceutical formulations, including dietary supplements and health drinks, providing a natural source of high-quality protein and bioactive compounds that support overall health. Its moisturizing and nourishing properties make it suitable in cosmetic and skincare products, enhancing skin hydration, elasticity, and appearance. In the pharmaceutical industry, the bioactive properties of burdock, such as its antioxidant and anti-inflammatory properties, can be useful in pharmaceutical preparations targeting specific health conditions. Furthermore, these properties make burdock a good candidate for a functional ingredient in drug delivery systems (Kumar et al. 2022).

These potential applications of burdock protein indicate its potential economic benefits. Nevertheless, further research and development are necessary to fully explore and validate the applications of burdock protein in different industries.

4. Discussion

Burdock is a valuable medicinal and edible plant in food and health care. It has been used in China for edible pigments, chlorogenic acid from the leaves, inulin and polyphenols from the roots, dietary fiber from the residue, and volatile oil and lignin from the whole plant (Chan et al. 2011). While these extraction processes have reached relative maturity, the extraction of burdock root protein has posed challenges due to its low content (Zhang et al. 2021). This study determined the optimal extraction conditions for burdock
root protein, which will positively impact the overall extraction process.

In this study, dried and ground burdock roots were used as the material. The protein content was extracted by measuring the absorbance of the supernatant at different pH levels, and a pI of 4.2 for burdock protein was determined. The protein in burdock roots was then extracted by alkali extraction acid sedimentation. Single-factor experiments were performed to investigate the effects of extraction temperature, pH, solid–liquid ratio, and extraction time. Based on these experiments, an orthogonal experimental design of L9(3^4) (4 factors, 3 levels) was constructed. The results of the orthogonal test, along with the extreme worst analysis data, were used to determine the optimal process conditions for burdock protein extraction. The orthogonal test extreme differential analysis revealed that the factors influencing the extraction rate of burdock protein were pH > extraction temperature > solid–liquid ratio > extraction time. The optimal conditions for extracting burdock protein were 40°C extraction temperature, pH 8.0, a solid–liquid ratio of 1:25 (g/mL), and an extraction time of 65 minutes, resulting in a maximum yield of 9.03%.

The comparison of the characteristics of proteins demonstrated that the solubility of burdock protein was similar to those of soybean protein (62.9% and 60.7%, respectively). Gels could be formed when the concentrations of both proteins reached 9% or higher. However, significant differences were observed in water retention and oil absorption capacity between soybean protein and burdock protein. Soybean protein demonstrated twice the water retention capacity of burdock protein, while burdock protein exhibited 1.2 times the oil absorption capacity of soybean protein. Thus, burdock protein is more hydrophobic than soybean protein, making it more prone to binding with molecules, such as lipids and carbohydrates. Consequently, burdock protein is more likely to enhance the body’s absorption and metabolic capabilities, thereby promoting digestion and absorption. This comparison revealed that burdock protein has potentially significant applications in the food-processing industry. The use of burdock protein in food products is anticipated to gradually increase in the future. Amino acid content analysis of revealed that burdock root contains eight essential amino acids and 10 functional amino acids. Evidently, burdock root protein is exceptionally rich in nutritional value.

As the evaluation of the potential applications of burdock indicate, it can be used to develop high-value-added food and health care products with considerable economic and ecological benefits (Moro and Clerici 2020). While the medicinal value of burdock is widely recognized in China, Japan, and other Southeast Asian countries, the consumption of burdock remains limited due to market constraints, as well as the limited dissemination of information regarding its nutritional value. In recent years, with the development of the export industry, efforts have been made to promote the medicinal and edible value of burdock in China, enabling people to gradually understand its culinary applications and expanding the market for burdock products both domestically and internationally. The cultivation of medicinal plants, including burdock, are expected to not only yield significant economic benefits in the near future but also contribute to positive social outcomes (Kumar et al. 2021).

5. Conclusions

The protein from burdock roots was extracted by alkali extraction and acid sedimentation, and the optimal conditions for protein extraction were an extraction temperature of 40°C, pH 8.0, solid–liquid ratio of 1:25 (g/mL), and extraction time of 65 minutes, for a yield rate of 9.03%. The factors affecting the protein extraction rate were ranked as follows: pH, extraction temperature, solid–liquid ratio, and extraction time. The solubility and gelation properties were comparable between burdock protein and soybean protein; however, burdock protein demonstrated 1.2 times the oil absorption capacity and half the water retention capacity of soybean protein. Burdock root protein contained eight essential amino acids (Val, Thr, Phe, Met, Lys, Leu, Ile, and His) and 10 non-essential amino acids (Gly, Tyr, Ser, Pro, Glu, Asp, Arg, Ala, Cys, and Trp). Based on the study findings, burdock has potentially significant economic benefits.

Authors’ Contributions: GONG, X. and ZHANG, G.: collaborated to develop and design the overall experimental plan. GONG, X. and CHANG, F.: worked together to carry out the experiments and obtained the experimental data. YAO, X. and LENG, P. were responsible for analyzing and organizing the data, and they collaborated with MIAO, Y. to draft the initial version of the research paper. Finally, ZHANG, G. reviewed and checked the paper.

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